# THE ACTIONS OF CALCIUM ANTAGONISTS ON ARRHYTHMIAS AND OTHER RESPONSES TO MYOCARDIAL ISCHAEMIA IN THE RAT

By

MICHAEL JOHN CURTIS

B.Sc. (Hon.), University of London (Chelsea College) 1979

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

Department of Pharmacology and Therapeutics, Faculty of Medicine

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

July 1986

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Pharmacology and Therapeutics

The University of British Columbia
1956 Main Mall
Vancouver, Canada
V6T 1Y3

Date 1 August 1986

ABSTRACT

Studies were carried out in order to examine the actions of calcium antagonists in acute myocardial ischaemia and the mechanism(s) responsible for these actions; the scientific hypothesis under test was that calcium antagonism in the ventricles is antiarrhythmic in acute myocardial ischaemia. In addition, experiments were carried out to investigate the role of the sympathetic nervous system in arrhythmogenesis.

The actions of seven calcium antagonists on responses to myocardial ischaemia were investigated in vivo using the conscious rat preparation. It was found that the drugs with identifiable actions in the heart attributable to calcium antagonism possessed antiarrhythmic activity, whereas the drugs producing only systemic vasodilatation were without tangible antiarrhythmic activity. The results were taken as evidence in support of the main hypothesis (above). In no instance did any of the drugs produce consistent dose-dependent infarct-reducing actions.

It was established from the comparison of the optical enantiomers of verapamil that antiarrhythmic potency corresponded with calcium antagonist potency. It was also shown that these drugs appeared to have no effect on $g_{Na}$ in the heart in vivo (as predicted from work by others, in vitro).

Evidence that arrhythmias were reduced as a result of effects on $i_{si}$ in the ischaemic ventricle was accrued from several studies. In isolated Langendorff-perfused rat ventricles the calcium antagonist activity of the verapamil enantiomers was greatly potentiated by raising $K^+$ concentration to levels seen during acute myocardial ischaemia, whereas nifedipine, which showed little if any antiarrhythmic activity in vivo did not show marked $K^+$-dependent calcium antagonist activity.

In a separate series of experiments it was demonstrated that serial

ablations in the CNS had profound effects on occlusion-induced arrhythmias, but that these effects occurred independently of the level of adrenoceptor activation. It was hypothesised that surgery reduced ischaemia-induced arrhythmias, by virtue of either its effects on serum $K^+$ concentration or its effects on the number of circulating thrombocytes.

TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

LIST OF ABBREVIATIONS

| | |
|---|---|
| action potential | AP |
| action potential duration | APD |
| arrhythmia score | AS |
| calcium | Ca |
| conductance | g |
| conduction velocity | $\theta$ |
| current | i |
| effective refractory period | ERP |
| hour(s) | h |
| infarct zone | IZ |
| length constant | $\lambda$ |
| maximum diastolic potential | MDP |
| membrane potential | $E_m$ |
| minute(s) | min |
| occluded zone | OZ |
| potassium | K |
| premature ventricular contraction | PVC |
| resistance | r |
| second(s) | sec |
| slow inward current | $i_{si}$ |
| sodium | Na |
| sodium current | $i_{Na}$ |
| standard error of the mean | s.e.mean |
| time constant | tau |
| ventricular fibrillation | VF |
| ventricular tachycardia | VT |

ACKNOWLEDGEMENTS

In order to ensure blindness in experimentation, the following people kindly assisted with drug dilution and (occasionally) with drug administration: Kathleen Saint, Tony Au and Michael Walker. Experiments cited in the text as having been carried out by others in the laboratory (measurement of circulating platelets and leukocytes) were carried out by Kathleen Saint. Dr R Wall is thanked for his guidance with the HPLC experiments. The calcium antagonists used in the experiments were requested from and supplied by the following: Dr B. Ljung of Haessle (felodipine); Drs M. Raschack and R Kretzschmar of Knoll (optical enantiomers of verapamil, anipamil and ronipamil); Dr R. Whiting of Syntex (DHM9); Dr Kazda of Bayer (nifedipine).

I am indebted to the Department of Pharmacology and Therapeutics, The University of British Columbia and the B. C. Heart Foundation, all of whom provided me with financial support.

I would like to thank the following people for their support and encouragement concerning the experiments which were carried out, and for their kindness and advice during my time in the department; Dr Bernard MacLeod, Dr Catherine Pang, Dr Morley Sutter, Caroline Bruce and Hari Nair.

I must also thank Tannis for putting up with my work being scattered across the kitchen table, and my bad temper.

Finally, I am indebted to my supervisor, Dr Michael Walker, not only for his encouragement, forthrightness, and for his example as a person searching for the truth (even in the face of adversity), but also for presenting me with a problem to solve, a hypothesis to test, an established preparation with which to begin my studies and an essentially free hand to chose my own direction. One could ask for nothing more.

AUTHORISATION FROM ANIMAL CARE UNIT, U. B. C.

The experiments carried out in partial fulfilment of the requirements for the degree of doctor of philosophy, and described in this thesis, were approved as ethical by the Animal Care Unit of U. B. C. (reference number 511/W8).

# 1 INTRODUCTION

## 1.1 Clinical myocardial ischaemia and infarction

### 1.1.1 Overview

Myocardial ischaemia is defined, simply, as an impairment of coronary blood flow, and myocardial infarction is defined as the necrotic changes resulting from myocardial ischaemia (e.g., Steadman's Medical Dictionary, 1972). However, myocardial ischaemia and infarction is a heterogenous disease, varying from patient to patient in cause, location, severity, time-course and sequelae (Henderson, 1984; Oliver, 1982; Poole-Wilson, 1983; Hillis and Braunwald, 1977; Maseri et al., 1978). It is generally accepted that coronary blood flow insufficiency is the common event which produces the signs, symptoms and sequelae of myocardial ischaemia and infarction.

Ischaemia may involve partial restriction or complete loss of blood flow, and may be permanent or temporary. Temporary ischaemia is associated with reperfusion, which may occur gradually or abruptly (Folts et al., 1982; Maseri et al., 1978). Symptoms of acute myocardial ischaemia include angina pectoris (chest pain) and dizziness, signs include syncope, ventricular arrhythmias, hypotension, S-T segment alterations, a reduction in cardiac output and leakage into the systemic circulation of creatine phosphokinase, and sequelae include ventricular arrhythmias, infarction, a prominant Q wave in chest lead ECGs, heart failure, pulmonary oedema, and hypertension (Henderson, 1984; Oliver, 1982).

The most common presentation of myocardial ischaemia is angina pectoris. Angina of effort is associated with radiating chest pain and S-T segment alterations resulting from an increase in myocardial oxygen demand in the setting of an inadequate oxygen supply, and relief may be obtained by reducing sympathetic drive to the heart with β-adrenoceptor antagonists, or by dilating coronary vessels with organic nitrites (see Poole-Wilson,

1983). Prinzmetal's variant form of angina (Prinzmetal et al., 1959) may occur at rest in the absence of any apparent sympathetic nervous system-mediated increase in myocardial oxygen demand. Angina of effort is generally associated with coronary arteriosclerosis, whereas Prinzmetal's variant is not. The latter is believed to be associated with coronary vasospasm (Hellstrom, 1973; 1977), although this is not yet firmly established.

Ischaemic heart disease can often be attributed to coronary arteriosclerosis. Stenosis (narrowing of vessels) leads to a loss of physiological reserve, resulting in insufficient oxygen supply during periods of increased demand (angina pectoris). Sustained stenosis of sufficient severity may lead to infarction.

Coronary arteriosclerosis is primarily a large vessel disease (Gensini et al, 1971), and mortality resulting from acute coronary occlusion is associated with single vessel disease in 84 % of cases (Liberthson et al., 1982). Thus, the left anterior descending (LAD), left circumflex and right coronary arteries are the most common sites of coronary arteriosclerosis-induced ischaemia and mortality.

In patients with occlusive stenosis in one or more coronary artery, the most important determinant of ischaemia is coronary artery anatomy, which varies with age. Functional collateral anastamoses are rare in young humans, but collaterals become larger and more numerous with age, perhaps in conjunction with (and as a consequence of) the development of arteriosclerosis (Baroldi and Scomazzoni, 1967; Fulton, 1965; Harris et al., 1969; Gensini and Bruto da Costa, 1972; Newman, 1981). It is probable that slowly developing coronary stenosis, from whatever cause, leads to a compensatory development of coronary collateral anastamoses, as has been demonstrated in pigs (Schaper, 1971).

1.1.2 Infarction

Most of our knowledge of the infarction process and its relation to morbidity and survival has come from animal experimentation (see below). For this reason, an exhaustive review of the infarction process and its prognostic significance will not be given here.

It is clearly undesirable for working muscle tissue to die. If sufficient myocardial muscle becomes necrotic and fibrous then the ability of the heart to pump blood will be impaired. Experimental evidence has shown that infarction leads to myocyte hypertrophy in surviving tissue, but that large infarcts preclude complete functional recovery, owing to the survival of an insufficient number of cells; hyperplasia does not occur (Anversa et al., 1984; 1985a; 1985b; 1986).

There are two important points which must be examined when infarct size is considered. Firstly, a clear distinction must be drawn between prevention and delay of necrosis. It is conceivable that necrosis could be delayed by a drug which causes vasodilatation of collateral anastamoses or by a drug which slows myocardial metabolism (reducing the rate of formation of cytotoxic products of anaerobic metabolism). Both types of drug would prevent infarction provided that the stenosis were resolved (by thrombolysis or bypass surgery). It is unfortunate, therefore, that in most clinical studies aimed at reducing infarct size no delineation of the patient population into those with and those without collateral anastamoses is made, and little attempt is made to rationalise the aims of the study in terms of the known pharmacology of the drug under investigation.

Some drugs have been investigated for their ability to reduce infarct size in humans on the basis of their ability to reduce myocardial oxygen consumption or oxygen demand (heart rate, force of contraction, etc.). However, if occlusion is complete, and collateral circulation is minimal or

absent, then no amount of off-loading will salvage the ischaemic myocardium. Attempting to prevent infarction by such a strategy is analogous to attempting to survive in a vacuum by holding one's breath.

It should also be noted that most of the reports in the literature concerning clinical infarct size refer to enzymatically determined infarct size. There are a variety of techniques for measuring infarct size by determining the concentration in the blood of enzymes which are normally only found intracellularly, for example creatine phosphokinase (CPK). It has been shown that in experimental animals the peak levels of serum CPK correlate with infarct size determined histologically following sacrifice (Shell et al., 1971). However, it is not clear whether this relationship still holds under the influence of drugs. It is quite possible that a drug may influence CPK without altering infarct size. The problem clinically, of course, is that one cannot examine a heart histologically unless the patient dies. Therefore one is dependent in most cases on indirect measures of infarct size. There are angiographic techniques for measuring coronary blood flow in-vivo, e.g. using radioactive contrast imaging with xenon$^{133}$ (Pitt et al., 1969), and ultrasound techniques for imaging an infarct (e.g. Mattrey and Mitten, 1984), but these techniques are not normally used in large scale multicentre clinical trials.

1.1.3 Arrhythmias

Clinically, the major type of death in association with myocardial ischaemia is 'sudden'. Sudden death has been defined as death occurring within 1 h of the patient last being seen alive, and has been suggested to occur in approximately 30% of the total patient population with coronary artery disease (Armstrong et al., 1972). Sudden death is the major cause of death in patients with myocardial ischaemia. It is estimated that approximately 400,000 such deaths occur per year in the USA alone (Lown, 1982).

The cause of sudden death is usually uncertain. It has been shown that approximately 43 % of sudden deaths occurring during the 4 weeks following the onset of chest pain occur during the first hour (Armstrong et al., 1972). In addition, it has been estimated that less than 10 % of patients dying during the first hour after the onset of chest pain are seen by a physician (Oliver, 1982). In other words, the majority of patients who die as a result of myocardial ischaemia do so without a definite establishment of the cause of death. In the follow-up reports of the Framingham study of the epidemiology of sudden death, the following comments were made: 'The assignment of sudden deaths to coronary aetiology is largely by inference, since few other diseases can kill in a matter of minutes. Coronary aetiology is assumed when aortic dissection, ruptured aneurysm, and pulmonary embolism are excluded clinically or on postmortem examination. Thus, death within minutes in persons not ill at the time with a potentially lethal illness permits classification as coronary sudden deaths with reasonable certainty' (Kannel et al., 1984).

The cause of sudden death is generally attributed to VF (Oliver, 1972; Campbell, 1983; 1984; etc.). Many clinicians use the terms sudden death and fatal VF interchangeably (e.g., Oliver, 1982). There are several persuasive reasons in support of this, despite the absence of direct evidence in most cases. Firstly, evidence from work using experimental animals (see experimental section of Introduction) clearly demonstrates that VF occurs during the first hour after coronary occlusion, and that this is the major cause of death at this time (cardiogenic shock and pulmonary oedema being relatively rare during the first hour after occlusion). Secondly, if VF is the major cause of sudden death then a reduction in early VF should lead to a corresponding reduction in sudden death. With regard to this point, it was found, in 1956, (Zoll et al.) that VF in humans could be reverted by applying a DC

shock to the chest. This procedure was rigourously applied in Seattle by paramedics in order to defibrillate patients before admission to hospital, and the result was a reduction in out-of-hospital sudden death of approximately 55 % (Cobb et al., 1980), confirming that VF and sudden death were related. In addition, an investigation of the ECGs of patients collapsing outside of hospital and receiving paramedic care within 15 min of collapse has provided a strong suggestion that sudden death and VF are one and the same; of 426 patients, 72 % were in VF when the ECG was first recorded, and of the remainder, only 1 % were not experiencing ventricular arrhythmias (Liberthson et al., 1982). However, it is important to consider that although sudden death is probably caused by VF, there is no established criterion, in the absence of ECG evidence, for categorising a death as having resulted from VF, and that most sudden death occurs out of hospital (Campbell, 1984) in the absence of ECG monitoring.

Irrespective of the exact incidence of fatal VF in clinical myocardial ischaemia and its contribution to the body of 'sudden death', it nevertheless remains that VF can occur during myocardial ischaemia in humans, and that this event is often fatal (e.g., Julian et al., 1964).

The natural history of ventricular arrhythmias during myocardial ischaemia and infarction is not well established in humans. This is understandable since many patients die before admission to hospital. There is very little information concerning the very early phase (first few minutes) of acute myocardial ischaemia in humans. Campbell et al. (1981) evaluated 38 previously unmedicated patients admitted to hospital with 'acute myocardial infarction', and expressed arrhythmia incidence in relation to the onset of the symptoms (not defined, presumably chest pain). It was found that primary VF (defined as VF occurring in the absence of 'shock', heart failure or heart block) occurred principally within the first 4 h after the

onset of symptoms. VF was almost always associated with an initial 'R on T' premature ventricular contraction (defined as a QRS complex falling within 85% of the prevailing QT interval of a normal sinus QRS complex), whereas ventricular tachycardia (VT) was almost never associated with such an occurrence. VF and R on T premature ventricular contractions (PVCs) were almost absent after 4 h. In contrast, non R on T PVCs and VT (defined as 3 or more consecutive PVCs at a rate of 120/min or more) increased in frequency between 4 and 12 h after the onset of symptoms. It is of interest that despite the uncertainty concerning the exact onset of occlusion, the degree of stenosis, the volume of ischaemia, etc., in this study (Campbell et al., 1981), the time distribution of arrhythmias was not grossly different from that reported for dogs (Harris, 1950), and rats (Johnston et al., 1983a).

Adgey et al. (1971) also examined the natural history of arrhythmias occurring during the first few hours after the onset of symptoms. They examined 284 patients with ECG evidence of 'acute myocardial infarction' (S-T segment changes or bundle branch block) and found a 31 % incidence of bradyarrhythmia, 25 % incidence of PVCs, 10 % incidence of VF, 4% incidence of atrial fibrillation and 0.4% incidence of supraventricular tachycardia during the first hour after the onset of symptoms (it must be noted that the above does not include the incidence of fatal ventricular arrhythmias, which were not analysed). Corresponding incidences during the 3rd and 4th hour were 2, 6, 0.7, 2, 0 and 0%, respectively. The decline with time in the incidence of VF corresponds with that reported by Campbell et al. (1981). Adgey et al. (1971) also reported that the incidence of VF 'after 4 h' was low (4%). Since the possibility of a second episode of myocardial ischaemia was not explored, it is possible that the true incidence of VF occurring after 4 h as a result of the initial episode of myocardial ischaemia was less than 4%. It is a problem of most clinical studies that there is gener-

ally little examination of the time course of ischaemia in relation to the possibility of further critical stenosis and occlusion higher up the arteriosclerotic tree.

Recent clinical studies of arrhythmias associated with myocardial ischaemia and infarction have concentrated to a certain extent on the desire to predict sudden death/fatal VF. This is partly a result of the hesitance in prescribing antiarrhythmic agents to all patients at risk of developing myocardial ischaemia and infarction, owing to the occurrence of serious side effects with long-term use (e.g., Kosowsky et al., 1973; Jelineck et al., 1974; Campbell, 1983). In addition, there are no drugs which have been shown to unequivocally reduce clinical life-threatening arrhythmias associated with myocardial ischaemia and infarction (Campbell, 1983; 1984). Therefore there is not sufficient justification, in terms of cost versus benefit, for whole-sale prescription of a particular drug in patients at risk of developing myocardial ischaemia and infarction.

As a result of these considerations, correlations between benign arrhythmias and life-threatening arrhythmias have been sought, under the assumption that a specific patient population 'at high risk' of sudden death can be delineated. In 1967, Lown et al. proposed that certain arrhythmias (for example, R on T PVCs) constituted 'warning arrhythmias'. A relationship between R on T PVCs and sudden death had been speculated earlier (Smirk and Palmer, 1960). For many years, therapeutic decisions were based on the detection of these warning arrhythmias. However, there were contradictory reports (e.g., El-Sherif et al., 1976; Rabkin et al., 1982) which suggested that 'warning arrhythmias' were just as common in patients not subsequently developing VF as in those who did. The latter suggestion has been supported by a recent study in which it was found that a high incidence of complex PVCs (including R on T) and VT in otherwise healthy subjects was not associ-

ated with sudden death during a 10 year period (Kennedy et al., 1985). The natural history study of Campbell (Campbell et al., 1981) showed that whilst R on T PVCs were extremely common in patients during the 10 min preceeding VF, they were almost as common in patients not developing VF. Campbell concluded that 'warning arrhythmias' were of no predictive value for this reason. In addition, it is worth considering that any 'warning' which does not appear until 10 min before a life-threatening event is of little use as a guide to therapy, especially out of hospital.

Part of the confusion concerning 'warning arrhythmias' stems from a fundamental philosphical dichotomy. This concerns the model of arrhythmogenesis in myocardial ischaemia and infarction to which an investigator subscribes. Specifically, is ischaemia more or less important than infarction in arrhythmogenesis?

In experimental preparations, VF occurs before tissue has become infarcted (see experimental section in Introduction). Therefore, when considering myocardial ischaemia and infarction, the principal prerequisite for VF would appear to be myocardial ischaemia.

In support of this premise derived from experimental animal studies are the following pieces of clinical evidence. Firstly, it has been suggested that most patients who die from sudden death do not have myocardial infarction when examined postmortem (Lovegrove and Thompson, 1978), indicating that sudden death may have resulted from myocardial ischaemia, but not myocardial infarction. Secondly, it has been reported that less than 20% of patients resuscitated from VF exhibit ECG signs of infarction such as Q waves in V leads (Cobb et al., 1980). This has lead some clinicians (e.g. Oliver, 1982) to voice the opinion that clinically, VF is associated with ischaemia, whereas electrical instability is not a feature of infarcted tissue, since it is not viable.

Proponents of the 'warning arrhythmia' hypothesis take a different view. The rationale for 'warning arrhythmias' is based on the premise that 'a distinctive myocardial pathophysiologic derangement long preexists in victims of sudden death', and that 'because such deaths are the result of VF, it is not illogical to assume the abnormality to be electrophysiologic and the risk indicator to relate to altered cardiac rhythm' (Lown, 1982). This philosophy is not supported by the evidence described above.

Lown did not include in his scheme any recognition of the duration of symptoms (whether the patient is experiencing ischaemia or infarction), nor did he examine the relative importance of re-infarction versus re-ischaemia. Lown dismisses the evidence which suggests that 'warning arrhythmias' are of no value in predicting VF (see Campbell, 1983), and comments that 'the prevailing pessimism concerning the possibility of protecting a patient who has experienced VF against recurrence is unwarranted' (Lown, 1982). Lown may be overstating his case, because whereas Campbell considers the entire population of myocardial ischaemia and infarction patients, Lown's studies were carried out on a selected subset of myocardial ischaemia/infarction patients, namely those patients 'who have experienced malignant ventricular arrhythmias', and the prognostic criteria which were developed therefore only apply to these patients. As discussed in detail above, this subset of patients (those who have experienced complex VT) are the very patients who are least likely to subsequently experience VF (Campbell et al., 1981). These are the patients who have either survived the VF occurring during the first 3 - 4 h of myocardial ischaemia, or passed through this danger period without experiencing VF. This group, therefore, does not include any of the truly at-risk patients. Indeed, patients who die of sudden death before receiving medical attention were not considered in this article (Lown, 1982). Therefore the statement that 'abolition of advanced grades of ven-

tricular premature beats prevents the recurrence of potentially lethal arrhythmias' (Lown, 1982) does not apply to myocardial ischaemia/infarction patients as a whole. Campbell, referring to all ischaemia/infarction patients, stated that whilst many studies have demonstrated that certain drugs reduce R on T PVCs and complex ventricular contractions, 'no tangible benefit of suppressing these arrhythmias has been detected', (Campbell, 1984). Campbell also stated that 'no current strategy for ventricular arrhythmia prophylaxis or treatment in acute myocardial infarction is satisfactory' (Campbell, 1983).

The failure to distinguish between the various stages and conditions of myocardial ischaemia and infarction, particularly between the initial occlusion and the subsequent course of the disease(s) may be responsible, in part, for the confusion over both prognosis and the assessment of antiarrhythmics. It has been suggested that improvements must be made in this regard in relation to the design of clinical trials, or 'the treatment of these patients will continue to be made from a position of abject ignorance' (Bigger, 1984). In summary, there is no evidence that 'warning arrhythmias' usefully predict sudden death/VF, particularly in patients who have passed through the first few hours of myocardial ischaemia.

The 'warning arrhythmia' concept is contingent upon different arrhythmias being part of a continuum. There is abundant evidence from studies with experimental animals (see experimental sections) which suggests that PVCs, VT and VF do constitute a continuum, either in non-ischaemic preparations (Dresel and Sutter, 1961) or in experimental myocardial ischaemia. Our laboratory has shown that some antiarrhythmics can reduce the incidence of VF without affecting PVCs in rats subjected to coronary artery occlusion (Johnston et al., 1983a).

With regard to variations in the natural history of the disease, in

particular, the incidence of re-ischaemia and re-infarction, there is no reason to believe that the arrhythmias resulting from a first episode of myocardial ischaemia predict in any way the outcome of a subsequent episode of ischaemia. Therefore, in terms of therapy, it would be folly to withold an effective prophylaxis against VF (if such a drug were shown to exist) on the basis of an absence of VF (or R on T PVCs) during a first episode of myocardial ischaemia in one patient, while administering that drug to a second patient with a history of VF (or R on T PVCs) during myocardial ischaemia.

There are strategies which have been used clinically to determine which drug should be prescribed in order to prevent subsequent sudden death in patients who have experienced myocardial infarction, based on responses to drugs. An example is programmed electrical stimulation of the right ventricle (Fisher et al., 1977). The technique is based on the principle that if a premature stimulus is delivered during the terminal phase of repolarisation (the 'vulnerable period') then VT or fibrillation may be elicited (de Boer, 1921; Wiggers and Wegria, 1940). Essentially, the threshold (current or pulse width) for induction of VT or VF is determined before and after the administration of various drugs, with the aim of finding a drug which completely suppresses the induction of the arrhythmia upon stimulation. It has been claimed that a positive response to a drug predicts that treatment with that drug will prevent spontaneous arrhythmias in 90 - 95 % of patients during the following 1 - 2 years (Ruskin et al., 1983). Nevertheless, it should be recognised that 15 - 60 % of patients in the studies reviewed by Ruskin who did not respond to any drug during electrical stimulation went on to experience no VT or VF during the following 1 - 2 years, while receiving apparently 'ineffective' therapy according to the results of the stimulation tests. Therefore the 90-95 % 'success' rate (Ruskin et al., 1983) is perhaps

misleading. The problem with this method is that although it may be reasonably effective in demonstrating that a particular drug may prevent ventricular arrhythmias occurring in the first 2 years following myocardial ischaemia and infarction, it does not benefit the vast majority of patients who suffer sudden death without warning and in whom no programmed stimulation has been undertaken. Also, despite the claimed success of such methods, it remains that there are no effective agents for preventing sudden death (Campbell, 1983; 1984), and that enormous numbers of people still die from sudden death/VF.

If one accepts the premise that the arrhythmias occurring during the first few hours of myocardial ischaemia (Campbell et al., 1981; Adgey et al., 1971) are the major cause of death in patients with myocardial ischaemia and infarction (Oliver, 1982), what are the characteristics and importance of the arrhythmias which begin approximately 4 h after the onset of symptoms (Campbell et al., 1981)? Essentially, late arrhythmias have only been of interest clinically in terms of whether they predict subsequent sudden death (see foregoing paragraphs) or pose a significant health risk in themselves. There are no clinical studies which have attempted to establish the relationship between late ventricular arrhythmias and the onset of ischaemia, the extent of ischaemic muscle mass, the degree of stenosis (residual blood flow), the frequency and extent of re-ischaemia or the relative importance of ischaemia versus infarction. The possible role of arrhythmias occurring more than 24 h after the onset of symptoms in the genesis of sudden death has been discussed, and it appears that there is no prognostic value in the analysis of such arrhythmias (Campbell, 1984). In addition, the poor correlation between the initial episode of ischaemia and late arrhythmias is exemplified by the observation that although the incidence of PVCs and VT occurring during the first 10 h after the onset of

chest pain correlated with infarct size (determined by measuring serum CPK), the rate of PVC measured 1 - 10 months after the onset of chest pain bore no relation to infarct size in the same patients (Roberts, et al., 1975). This implies that it is possible that the so-called late arrhythmias in humans may be a mixture of arrhythmias associated with an old infarct (generated by reentry around the infarct, perhaps; see section on arrhythmogenesis in Introduction), arrhythmias associated with persisting partial ischaemia and arrhythmias associated with new bouts of ischaemia.

In summary, clinical information concerning the aetiology of sudden death and its relationship with VF and other ventricular arrhythmias is incomplete, owing to the fact that the majority of patients dying suddenly do so out of hospital. Clinical information concerning the natural history of arrhythmias and their underlying causes in the min, h, days, weeks and months following the onset of symptoms is confusing, perhaps as a result of the desire to establish an ideal protocol for treatment without having to tediously order and classify each condition and its characteristics. However, such an approach is necessary in order to remove the confusion concerning mechanisms of arrhythmogenesis and the contentious aspects of prognosis. It is clear, however, that the current clinical approach has not provided any effective prophylaxis against sudden death (Campbell, 1983; 1984; Furberg, 1983b).

1.1.4 Therapeutic approaches

Theoretically the primary therapeutic aim would be to prevent myocardial ischaemia from occurring. The Framingham study (Kannel et al., 1984) suggested that in previously healthy humans, the incidence of sudden death increases with increasing age, tripling in males from 2 to 6 per 1000 persons between the age groups 45 - 54 and 65 - 74 years. In addition, the frequency in males is approximately 2 - 3 times that in females. Age and

sex are not treatable conditions at present. However, the Framingham study also identified hypertension, diabetes, cigarette smoking, obesity and the ratio of low versus high density lipoprotein serum cholesterol as important indicators of coronary artery disease and sudden death. While correlation does not prove cause-and-effect, it is generally considered that a healthy lifestyle, a balanced diet and exercise are associated with a low incidence of coronary artery disease and sudden death.

A safe effective prophylaxis against coronary occlusion would be desirable. In this regard, the results of a long term trial of aspirin in apparently healthy volunteers is expected to be published reasonably soon. In theory, thrombolysis will only be of benefit in patients with effective collateral vascularisation or partial occlusion, in whom ischaemia is not complete (in whom the delay between the onset of symptoms and the initiation of thrombolysis is not as critical as it would be expected to be in patients with complete occlusion in the absence of functioning collateral anastamoses). Since this has not been thoroughly investigated in the many clinical studies of thrombolysis (Yusuf et al., 1985), then it is not possible to comment further in this regard.

Since coronary artery disease remains a significant health risk, then it is expedient to limit the sequelae, namely arrhythmias and infarction.

There are at least two ways in which attempts can be made to reduce VF and other arrhythmias. Firstly, automatic defibrillators may be implanted. This has been found to be a successful approach (Echt et al., 1985), but since surgery is required, it is desirable to identify patients most at risk of sudden death; the implantation of defibrillators is not a practice which can be carried out on a very large scale at present. In addition, it has recently been suggested that automatic defibrillators can reduce the quality of life by virtue of the anxiety and fear associated with their use (Cooper

et al., 1985). Alternatively, pharmacological prophylaxis could be administered. Such a strategy would be expected to protect the patient without undue inconvenience in terms of side effects, inconvenient dosing regimen and frequent follow-up. However, a drug suitable for this purpose does not exist, owing to a lack of proven benefit on one hand, or unacceptable side-effects on the other (e.g., Campbell et al., 1984). Along with the search for a cure for cancers, the search for a safe effective agent which may be used in a large number of subjects who might be categorised as at risk of coronary artery disease would appear to be the branch of drug research with the greatest potential clinical impact in terms of prolonging life.

In addition to arrhythmia prevention, attempts should be made to limit infarction. While it would clearly be beneficial to prevent infarction resulting from coronary occlusion, this is not a realistic goal at present, partly because therapy is usually not initiated until hours after the onset of symptoms. Experimental studies in animals without effective collateral anastomoses have illustrated that 15 min of ischaemia will lead to irreversible myocardial cell death, even if complete reperfusion is achieved (Hort and Da Canalis, 1965b). While unequivocal clinical information is lacking, it is reasonable to suppose that in the absence of extensive collateral vascularisation, much the same will occur in humans.

Theoretically, any measure which reduces infarct size is desirable in order to reduce the likelihood of cardiac output failure (Maroko et al., 1971). However, unless an agent is given as prophylaxis, it is difficult to imagine how it might prevent infarction, unless it possesses the capability of either converting fibrous tissue into muscle, stimulating myocardial mitosis, or converting dead cells into living cells. At present, no such drugs exist, and prevention of myocardial infarction with a view to limiting

pump failure and cardiogenic shock (Agress et al., 1952) has not been demonstrated either clinically or experimentally (Reimer and Jennings, 1985). However, it might be hoped that a fast-acting myocardioplastic growth factor might one day be developed.

## 1.2 Experimental myocardial ischaemia and infarction

### 1.2.1 Overview

For an animal model of any human disease to be ideal, it should exhibit the following characteristics. It should:

a. completely mimic at least one aspect of the disease

b. respond in the same manner as humans to drugs

c. have the precision and accuracy of a good bioassay

d. allow various responses to be measured

e. be simple and cost little in terms of apparatus, time, and expertise.

There is no model of myocardial ischaemia and infarction which duplicates the human condition. This was the conclusion of the Coronary Heart Disease Task Group Panel Report of 1973 (see Winbury, 1975). In 1978, the Workshop on More Uniform Animal Models and Protocols for Assessment of Interventions to Protect Ischemic Myocardium held at the NIH recommended the study of 3 coronary occlusion preparations, the rat for 'relatively inexpensive and rapid screening of potentially useful treatments', the anaesthetised dog 'for verification of effectiveness in a more physiological situation' and the conscious dog 'for testing the most promising agents under the most physiological conditions' (see Reimer et al., 1985). The latter recommendations may perhaps appear peculiar when factors other than 'physiological relevance' are considered (see section dealing with choice of species). In addition, it has been suggested that 'no animal heart is truly comparable to that of man' (Sasyniuk and Nattel, 1982) implying that rela-

tive rather than absolute physiological relevance is the issue in question, and as such should not be the primary consideration in the choice of experimental species.

1.2.2 Methods for producing ischaemia

Arteriosclerosis may be induced with a high cholesterol diet. However, with the possible exceptions of the hypercholesterolaemic hare (Pearson et al., 1983), mini-pig (Jacobsson, 1984), and quail (Cheung et al., 1983) this method is not considered to be specific for the coronary vessels. In most investigations, generalised stenosis is produced, therefore this approach is rarely used (Winbury, 1964). Hypercholesterolaemia-induced coronary arteriosclerosis models are also compromised owing to the necessity that the endpoint (development of infarction or arrhythmias) must occur within a prescribed time-frame, or experimentation becomes unacceptably inconvenient. It may be possible to circumvent this problem by increasing oxygen demand by atrial pacing in the setting of a compromised oxygen supply induced by cholesterol feeding, but this method produces only reversible, pacing-dependent ischaemia (demonstrated by S-T segment elevation); arrhythmias and infarction do not occur (Lee and Baky, 1973).

There are several techniques for producing small vessel occlusion and diffuse myocardial ischaemia. By injecting starch suspensions (Roos and Smith, 1948), plastic microspheres (Weber et al., 1972) or lycopodium spores (Guzman et al., 1962), it is possible to produce arteriolar occlusion leading to generalised and diffuse myocardial infarcts. This technique was first tried using powder, wax, oil and ink, more than 100 years ago (Panum, 1862, see Tillmanns et al., 1975). There are many disadvantages of these techniques. Firstly, in order to preclude generalised systemic occlusion, it is necessary to inject the material into a coronary artery. This necessitates either open-chest experimentation, or technically demanding coronary

catheterisation techniques. Secondly, most animals die from cardiac output failure (Weber et al., 1972), which is not the major cause of death in clinical myocardial ischaemia (Campbell, 1983; 1984) or in other models of myocardial ischaemia. Thirdly, arteriolar occlusion and the resultant diffuse ischaemia and infarction differs extensively from clinical occlusion-induced ischaemia which generally results from arteriosclerosis of large coronary arteries (Gensini et al., 1971). While this, in itself, is not necessarily a disadvantage, the diffuse character of the experimental infarct would be expected to be difficult to quantify, thereby making it difficult to determine the effect of a drug on infarct size. In addition, and perhaps more importantly, models of arrhythmogenesis (see elsewhere) are contingent upon the presence of a well defined focus of ischaemia which must be of sufficient volume to serve as a substrate for conduction delays and the generation of injury currents (Gettes, 1974; Janse, 1982; Kleber et al., 1978; etc.). These models of arrhythmogenesis would not apply to the diffuse ischaemia preparations. Indeed, the diffuse ischaemia preparations do not appear to be associated with ventricular arrhythmias at all (see Winbury, 1975).

There are a variety of techniques for producing occlusion of a major coronary artery by embolisation. The 2 major approaches are direct production of an occlusive embolus and the induction of an occlusive thrombus. Direct embolisation may be induced by injecting mercury (Lluch et al., 1969), placing a stainless steel cylinder (Nakhjavan et al., 1968), steel ball bearing (Ribielima, 1964) or detachable catheter tip (Hammer and Pisa, 1962) into a coronary artery, or inflating an intracoronary balloon (Corday et al., 1974). In addition, it has been demonstrated that if a cylindrical magnet is placed around a coronary artery, and small (4 μm diameter) iron particles administered, the particles will be captured by the magnet and

occlude the vessel (Elzinga et al., 1969). These techniques are all capable of producing complete occlusion, although this must be verified by measuring coronary blood flow. Partial occlusion may be brought about via direct embolisation by inserting a cylinder of lead foil into a coronary artery; the cylinder will pass down the artery for a distance governed by its outer circumference, while the degree of stenosis is governed by the inner circumference (Johnsrude and Goodrich, 1969). This technique initially produces a known degree of stenosis, but the device is thrombogenic, and serves to induce secondary progressive stenosis.

Deliberate thrombogenic techniques include electrically-induced thrombogenesis, and placement of a thrombogenic foreign object in a coronary artery. Placement of an electrode in a coronary artery lumen and another on the chest wall can produce thrombi if current is passed between them (Salazar, 1961). This study reported diffuse thrombi distal to the intraluminal electrode. However, Weiss (1971) managed to produce complete occlusion of the circumflex or LAD artery using similar techniques, and used the preparation to evaluate antiarrhythmic drugs. The electrical production of thrombi is used today by Lucchesi for evaluating antiarrhythmic and anti-infarct agents in dogs (e.g., Patterson et al., 1981; 1983). Foreign-body-induced thrombi may be produced by inserting thrombogenic objects into coronary vessels, for example, magnesium alloy or copper helices (Kordenat et al., 1972); the resultant thrombus is generally located at the site of the insertion.

The most common means of producing myocardial ischaemia in use today is occlusion of a major coronary artery by constriction. There are a variety of techniques for producing this end (see below). Occlusion may be gradual or abrupt.

Gradual coronary occlusion may be brought about by the use of aneroids

(Berman et al., 1956), which are rings of hygroscopic material which swell and progressively reduce the lumen of the artery which they have been placed around. Rings of gelatin impregnated with diacetyl phosphate induce the formation of granulomatous tissue which will gradually constrict the encircled vessel (Asada et al., 1962), in a manner analogous with aneroids. A similar result can be produced by the use of pneumatic cuffs (Khouri and Gregg, 1967; Bond et al., 1973) or hydraulic cuffs (Khouri et al., 1968; Hood et al., 1970). A major limitation of these techniques is the requirement for the placement of a coronary flow-probe in order to establish the extent of occlusion and the moment of complete occlusion. Also, the problems associated with the arteriosclerosis models (in addition to the problem of generalised systemic arteriosclerosis) also apply to the gradual coronary occlusion models, namely that production of ischaemia, arrhythmias and infarction does not take place within a convenient time period for experimentation.

Abrupt, complete coronary artery occlusion is perhaps the simplest and most rational technique for investigating myocardial ischaemia. This is because two variables which are difficult to measure and more difficult still to regulate are removed from the experimental arena. These variables are the time course of occlusion and the absolute blood flow. The time course of occlusion is known only for abrupt occlusion techniques, whilst bloodflow in an occluded vessel is only known if occlusion is complete (flow in partially occluded vessels must be measured directly). The major variable in abrupt, complete occlusion preparations is therefore the extent of collateral blood flow. Abrupt occlusion may be produced by clamping an artery with screw clamps (Gregg et al., 1939), bulldog clamps or Goldblatt clamps (Jennings et al., 1960). In small animals (such as rats) with small coronary arteries it is possible to cauterise a major vessel electrically

(Staab et al., 1977; Prum et al., 1984). More simply, a vessel may be ligated.

Coronary ligation was first undertaken by Chirac in 1698, who observed a loss of heart movement as a consequence (see Tillmanns et al., 1975). Interest in the dependence of heart activity on the coronary circulation resumed in the 19th century, when Erichsen (1842) determined the duration of occlusion necessary for producing ventricular standstill in dogs. Subsequently, Cohnheim (1881) ligated dog coronary arteries and developed the hypothesis that the coronary circulation is comprised mainly of end arteries (see Tillmanns et al., 1975 for translation and discussion). In the 20th century, ligation of a coronary artery together with ECG recording was first carried out in 1918 (Smith), but was rarely carried out again until 1935 (Johnston et al.). The reason for the apparent lack of interest in myocardial ischaemia in the early part of this century stems from the general belief at that time that coronary occlusion is a universally fatal event (see Fye, 1985 for review).

In most ligation techniques a simple silk ligature is used. To gain access to the designated artery and tighten the ligation, experiments were initially carried out using open-chest dogs (Townshend Porter, 1894). However, for long term experiments on mortality, chests were subsequently closed and animals allowed to regain consciousness (e.g., Smith, 1918; Le Roy et al., 1942). Most early experiments involved 1-stage coronary ligation. However, an adaptation of a technique designed for partial ligation of a coronary artery was used by Harris to ligate coronary arteries in 2 stages (Harris, 1950). Harris had observed that abrupt occlusion of the left anterior descending coronary artery produced VF in approximately 50 % of dogs (Harris, 1948) within the first 10 min after ligation. Animals surviving this insult experienced few arrhythmias until approximately 4.5 h after

ligation, whereupon PVCs ensued, increasing in intensity with time; by 8 h, frequent VT developed, persisting for a further 2 - 4 days. However, it was reasoned that if the arrhythmias occurring during the first 10 min of occlusion could be circumvented then the frequency of survival would be increased, allowing for a more detailed and specific investigation of the second phase (4.5 - 8 h) and third phase (8 h to 4 days) of arrhythmias. Therefore, an initial partial ligation was undertaken by including a needle in the loop of the ligature, then removing the needle once the ligature had been tightened; the lumen of the artery was therefore stenosed to the diameter of the needle. After 30 min or 1 h, a second ligature was tightened at the position of the first to fully occlude the vessel. The result was the elimination of phase-1 arrhythmias (those occurring during the first 10 min after complete occlusion) without change in phase-2 and phase-3 arrhythmias. It is of interest to note that this much-quoted work of Harris (1950) was in fact pre-empted, to a certain extent, by much earlier work (Michaelis, 1894), in which it was demonstrated that if small coronary artery branches were tied before the larger arteries then cardiac standstill was prevented.

A significant advance was made by the development of a noose-type device for 'atraumatic' occlusion. Fischer and Edwards (1963) threaded a small polythene tube under a coronary artery and then passed both ends through a larger polythene tube, such that traction between the tubes would occlude the vessel. Rushmer et al., (1963) used the same principle, substituting a nylon suture for the small polythene tube, in order to produce coronary occlusion in conscious dogs. This technique allowed arrhythmias and infarction to be investigated in the absence of anaesthetic and acute surgical preparation for the first time. Unfortunately, perhaps, little use of this technique was made for almost 20 years. Coronary occlusion in conscious

animals was not investigated extensively until our laboratory developed a method for coronary occlusion in conscious rats (Au et al., 1979a), using an occluder similar to that used by Rushmer. The use of a 'pre-prepared' animal with a loose occluder implanted around a coronary artery and exteriorised through the skin for induction of occlusion in the absence of anaesthetic and recent surgery may be an extremely important advance, since recent work has suggested that much of the information provided by investigations into ischaemia-induced arrhythmogenesis in anaesthetised, acutely prepared animals is misleading (see section dealing with arrhythmogenesis in Discussion).

1.2.3 Differences between species

1.2.3.1 Infarction. Infarct size reduction has been traditionally investigated in dogs, and therefore the majority of the literature concerning myocardial infarction refers to this species. However, the variability of infarct size in dogs has been suggested to make this species essentially useless for quantitative assessment of infarct size and its modification. This fact was recognised as long ago as 1918, and was attributed to the presence of large and varied collateral anastamoses (Smith, 1918). For this reason, Johns and Olson (1954) developed the rat preparation for assessing infarction, and concluded that any species with undeveloped collateral vascularisation (rats and mice) would be suitable for evaluating infarction, whereas species with variable coronary vasculature (hamsters and dogs) and species with extensive collateral anastomoses (guinea-pigs) would be unsuitable for study. Perhaps surprisingly, this work was completely ignored (see section dealing with coronary occlusion in rats), and studies of myocardial ischaemia and infarction continued to be carried out (almost exclusively) using dogs. Indeed, it was work using dogs which led Braunwald's group (Maroko et al., 1971) to propose the concept of myocardial salvage. It was

suggested that it should be possible to reduce the ultimate size of an infarct by measures 'designed for reduction of myocardial oxygen demands and improvement of coronary perfusion'. It is perhaps significant that these investigators (Maroko et al., 1971) did not in fact demonstrate that any manipulation (ouabain, glucagon, propranolol, haemorrhage, methoxamine or isoproterenol) reduced infarct size as such. The index of infarction used was S-T segment elevation, measured from DC electrograms recorded from the surface of the heart. In only 2 groups was infarct size (at 24 h) recorded, and evidence that infarct size was altered was not convincing.

The many experimental and clinical studies carried out over the following years have not confirmed the hypothesis of Maroko et al. (1971) that infarct size can be reduced in a potentially clinically useful manner by drug treatment, under the condition of irreversible coronary occlusion. Although it is possible to delay both the ECG signs of ischaemia and the development of infarction, the evidence does not suggest that any treatment can prevent death in non-perfused tissue (see Reimer and Jennings, 1984). Certainly, no treatment has yet been claimed to have prevented infarction.

The variable nature of the outcome of coronary occlusion in dogs is exemplified by a study carried out by Sobel and associates (Shell et al., 1971). These authors developed the method of quantification of infarct size based on serum levels of CPK. They found that peak serum CPK correlated linearly with infarct size determined at 24 h by measuring the myocardial content of CPK. However, the actual value of infarct size in 22 dogs at 24 h was highly variable, 21.5 ± 18 (mean ± s.d.) as % ventricular weight, the range of values being 1-55%. These values are typical of published infarct size in dogs. For example, Burmeister and Reynolds (1983) reported that the coefficient of variation (s.d. as a % of the mean) was 23% in mongrel dogs and 73% in beagles, while Miyazaki et al. (1984) reported that

infarct size varied from 0.6 to 46 % of total ventricular weight, with a 60% coefficient of variation in mongrel dogs.

With variability such as this, it is difficult to imagine how so many reports of 'myocardial salvage' have been generated and continue to be generated (e.g., Jugdutt, 1985; Bednar et al., 1985; Tumas et al., 1985). Perhaps the many reports of infarct size reductions in dogs are the result of sampling error, or measurement of 'ultimate' infarct size too soon after occlusion.

1.2.3.2 Arrhythmias. In conscious rats, severe ventricular arrhythmias first occur in the period 4 - 20 min after occlusion, and a second major phase appears after 1 h and lasts for 2 - 6 h (Clark et al., 1980; Johnston et al., 1983a). In dogs, ischaemia-induced arrhythmias were originally described as early, occurring during the first hour, and late, peaking at approximately 24 h after coronary occlusion (Harris, 1950). However, recently the early (phase-1) arrhythmias have been subdivided into phase-1a, occurring during the first 1 - 3 min of ischaemia, and phase-1b, occurring 5 - 20 min after coronary occlusion (Haase and Schiller, 1969; Meesman, 1982). Phase-1a arrhythmias, which usually comprise only of PVC, have only been reported in anaesthetised dogs; information concerning their occurrence in conscious dogs is not available at present. However, the type of anaesthetic used appears to influence the occurrence of phase-1a arrhythmias, in that they are far more frequent in dogs anaesthetised with pentobarbitone than dogs anaesthetised with morphine-chloralose-urethane or nitrous oxide (Meesman, 1982). In addition, phase-1a arrhythmias have been reported to occur in pentobarbitone anaesthetised rats (Fagbemi, 1984), but not conscious rats (Johnston et al., 1983a). Phase-1a arrhythmias have also been reported in pentobarbitone anaesthetised pigs (Bergey et al., 1982); it is not established whether such arrhythmias occur in conscious pigs.

In dogs 24 h after occlusion, episodes of VT and multifocal PVC are commonly observed (Smith, 1918; Harris, 1950). In our laboratory, we have rarely observed VT and never observed VF in rats at 24 h, although almost all 24 h survivors exhibit multifocal PVCs. The reason for this difference between rats and dogs has not been examined, however it is possible to speculate that the presence or absence of collateral anastamoses may govern the severity of 24 h arrhythmias. In this regard, it has been suggested that abnormal automaticity in partially ishaemic Purkinje fibres (maintained by collateral perfusion) is responsible for 24 h arrhythmias in dogs (Friedman et al., 1973; Lazzara et al., 1973). This idea is consistent with the multifocal nature of these arrhythmias. In rats, the relative lack of functional collateral anastamoses compared with dogs (Maxwell et al., 1984; Winkler et al., 1984) may not permit survival of sufficient Purkinje tissue to trigger VT and VF 24 h after occlusion.

In conscious rats, arrhythmias occurring during the first 4 hours after occlusion have been described in detail on the basis of many experiments in our laboratory (Johnston et al., 1983a). Ventricular arrhythmias are extremely common and include PVC, VT and VF. Sinus bradycardia and atrioventricular blocks are much less common. Very occasionally, atrial arrhythmias (fibrilloflutter) and supraventricular tachycardia are seen.

Spontaneous reversion of VT during acute myocardial ischaemia is common, both experimentally and clinically. Spontaneous reversion of VF has been reported in humans (Robinson and Bredeck, 1917; Maseri et al., 1982), but the incidence is considered to be low. However, there is little objective evidence to substantiate this belief. In conscious rats the incidence of VF is high (approximately 90 %), and the incidence of spontaneous defibrillation is correspondingly high (approximately 60 %). However, the incidence of sustained VF is even higher (approximately 90 %), according to experi-

ments from our laboratory (Johnston et al., 1983a). In dogs, there have been occasional reports of spontaneous reversion of VF (e.g., Smith, 1918; Gibson et al., 1986). In pigs, spontaneous reversion of VF has been observed (unpublished observations from our laboratory), although this event is rare. In the absence of any evidence that spontaneously reverting ventricular arrhythmias differ fundamentally in origin maintenance or mechanism from sustained ventricular arrhythmias, there is no reason to believe that spontaneous reversion of VF is an experimental disadvantage. On the contrary, our laboratory has shown that spontaneous defibrillation permits the experimental animal to survive, allowing for an increase in the yield of information from each preparation (Johnston et al., 1983a).

1.2.3.3 Choice of species. The section dealing with clinical myocardial ischaemia and infarction suggested that most deaths in patients with myocardial ischaemia occur from VF, and that the incidence of VF declines exponentially with time following the initial onset of chest pain (Campbell et al., 1981). Therefore it is of great importance to develop treatments for arrhythmias associated with acute myocardial ischaemia. It has been suggested that a drug which can be taken as prophylaxis by patients at high risk of myocardial ischaemia to prevent VF would have far more impact on mortality than therapy initiated after admission to hospital (Campbell, 1984). This implies that arrhythmias occurring during the first few hours, and particularly during the first few minutes of myocardial ischaemia are the arrhythmias which must be investigated in this regard. Clinically, these acute arrhythmias are generally missed; a patient has either died from them, been resuscitated by paramedics, or, perhaps, recovered spontaneously by the time they are admitted to hospital. This leads to a dilemma. One may either investigate early arrhythmias in experimental animals (phase-1 according to Harris, 1950) or late (phase-3) arrhythmias.

Any experimental preparation in which phase-1 arrhythmias are measured cannot be evaluated for its 'clinical relevance', because there is insufficient information concerning these arrhythmias and their susceptibility to drugs in humans to provide a useful template. Therefore it is desirable to use several animal species for such studies.

Alternatively, one may concentrate on phase-3 arrhythmias, under the assumption that drugs which reduce phase-3 arrhythmias will also reduce phase-1 arrhythmias (in other words that common mechanisms of arrhythmogenesis operate for phase-1 and phase-3 arrhythmias).

Essentially, therefore, one must establish one of the two following criteria (depending on which experimental strategy is chosen) in order to justify the choice of model and species.

First of all, if it is decided to investigate phase-1 arrhythmias then the only concern should be the logistic characteristics of the model. It must be established that the model provides accurate and precise information (any debate concerning the clinical relevance of such a model is redundant).

Alternatively, if phase-3 arrhythmias are to be studied as a model of phase-1 arrhythmias then it follows that a drug which is found to inhibit phase-3 arrhythmias should also inhibit phase-1 arrhythmias. Therefore, it is of absolute necessity to establish that all drugs which are effective or ineffective against phase-3 arrhythmias in the chosen preparation possess the same profile of activity against phase-1 arrhythmias in that same preparation. The majority of published reports in which such comparisons have been carried out do satisfy this criterion. It follows, therefore, that the evaluation of drugs for potential clinical use as prophylaxis against phase-1 arrhythmias must not be based upon an extrapolation from activity against phase-3 arrhythmias. Such a strategy is analogous to investigating interventions for preventing fires in the home by measuring the effect of the

experimental intervention on room temperature.

It is, however, permissible to investigate phase-3 arrhythmias for their own sake. In this regard, clinical relevance may be tested directly, because phase-3 arrhythmias are easy to study clinically.

However, whether phase-1 or phase-3 arrhythmias are studied may have important clinical ramifications. With further reference to the analogy of the study of the prevention of fires in the home, if acute out-of-hospital VF is analogous to a fire, it may also be that clinical phase-3 arrhythmias are merely analogous to an increase in room temperature. In other words, while the prevention of out of hospital VF (phase-1 arrhythmias) will have enormous consequences in terms of lives saved, the prevention of phase-3 arrhythmias may have little therapeutic impact.

All the techniques for producing myocardial ischaemia, with the exception of electrocautery, were developed using dogs. Until 1985, dogs were easily the most common species used in studies of myocardial ischaemia and infarction. Recently, the rat has become popular. Popularity, however, does not necessarily imply suitability. There is no concensus at present concerning the ideal choice of species for investigating myocardial ischaemia and infarction. It has been argued that there is no ideal species for such studies, and that the choice depends upon the objects and goals of an investigation (Fozzard, 1975; Harken et al., 1981).

In contrast with animal models of other diseases, such as bacterial infection, in which a high degree of similarity between the animal tissue and human tissue is desirable, it is possible to argue that animals with hearts similar to the human heart are paradoxically unsuitable for studies of myocardial ischaemia and infarction. There are several unrelated reasons for this viewpoint. Firstly, an animal with a heart which resembles the healthy, young human heart in terms of collateral vascularisation is by

definition a poor substrate for investigations concerned with the behaviour of the chronically sick human heart with extensive atheromae and collateral development. Such an animal ought, however, to provide a good model of the young human heart. It has been suggested that young humans are more susceptible to fatal VF than older humans (Morgan Jones, 1969), and it has been argued that this is a result of the relative lack of collateral anastamoses in the younger human (Oliver, 1982). Pig hearts lack collateral anastamoses and have consequently been suggested to resemble young human hearts (Fedor et al., 1978; Verdouw et al., 1983). The problem in this instance is that while such an animal may in theory provide a good model of myocardial ischaemia in otherwise healthy young humans, there is very little information concerning the characteristics of this clinical situation. Moreover, there is essentially no information concerning the effectiveness of drug therapy in such patients. Furthermore, until the improbable eventuality of a large scale clinical study of the effectiveness of a range of drugs in preventing the signs and sequelae of acute myocardial ischaemia in young healthy humans, then such information will remain unavailable. In such a circumstance, any argument that an animal with few effective collateral anastamoses provides a good model of acute myocardial ischaemia in healthy young humans is merely hypothetical.

At the other end of the spectrum, it has been suggested that a species with extensive or varied collateral vascularisation resembles the mature or elderly human heart. The mature human heart may develop collateral anastamoses in response to the slow developmemt of arteriosclerosis (Schaper, 1971). In dogs the development of collateral anastamoses over a period of weeks following coronary artery ligation may represent such a process (Eckstein et al., 1941).

Amongst common experimental species, dog hearts usually have well-devel-

oped collateral anastamoses and may resemble the mature or elderly human heart (Schaper, 1971; 1979). Clearly, if we assume that all other variables are equal, then a species with an extensive coronary collateral circulation is preferred when myocardial ischaemia in the mature human (in whom myocardial ischaemia occurs more frequently than younger humans) is to be modeled.

However, there are other considerations. One important consideration in any scientific investigation is the prior knowledge of the power of the experiment. If one is interested in the effect of a treatment on a Gaussian distributed variable then the sample size, n, is dependent on two independent variables, the effectiveness of the treatment and the variance. If two species differ in terms of the variance of a particular variable, then provided there are no other confounding factors, the preferred species will be the one with the lower variance. Consideration of which of the 2 species is the most closely related to humans becomes of minor importance. There is a tendency, nevertheless, for aesthetic rather than logistic considerations to dictate the choice of species in myocardial ischaemia and infarction studies.

The dog, until recently, has been the most popular species for investigating myocardial ischaemia and infarction. For example, a large general review of models of coronary artery disease published in 1975 is almost exclusively devoted to dog preparations (Winbury, 1975). However, there is an history of evidence which warns that variation in the coronary anatomy of dogs jeopardises myocardial ischaemia studies by constituting a large source of variance (e.g., Smith, 1918). Paradoxically, the dog has been favoured for this very reason; the variable nature of the coronary anatomy of the dog is qualitatively similar to that of the human (see above). However, the question arises: is it desirable to use an animal myocardial ischaemia preparation in which the coronary anatomy parallels the human coronary

anatomy? In this regard it is important to consider the sample size necessary for a clinical study of the effectiveness of a drug in reducing mortality occurring during acute myocardial ischaemia. The problem in this instance is that the required information is not available, since the acute phase of myocardial ischaemia occurs out of hospital in most cases, and has resolved or has lead to death (see preceeding sections). Nevertheless, with regard to the later 'post-infarction' period, in order to show a statistically significant reduction of mortality of 10 - 20% (from approximately 5 - 15% during the first year) one requires a study sample of several thousands (May, 1983a; 1983b). It is therefore undesirable to use a preparation which closely mimics the human situation. As Meesman has clearly demonstrated, the major source of variance in dogs with regard to the outcome of myocardial ischaemia is the extent of collateral vascularisation (Meesman, 1982). Collateral flow correlates not only with arrhythmias (Meesman, 1982) but also with infarct size. It has recently been shown that infarct size in dogs can be predicted on the basis of the extent of collateral vascularisation independently of the size of the occluded bed ('risk zone') (Yellon et al., 1986b). In addition, a recent study showed that whereas simple occlusion of the LAD produced only minor ventricular arrhythmias, LAD occlusion coupled with the obstruction of retrograde collateral blood flow to the occluded bed resulted in severe ventricular arrhythmias in 75% dogs (Shoji et al., 1986). Therefore, a species with little effective collateral perfusion, productive of consistent and predictable regions of ischaemia upon coronary occlusion, would be more appropriate than the dog, in terms of the precision and accuracy for a given to sample size.

The two species in which a well defined and reproducible area of ischaemia following coronary occlusion has been documented are the pig and the rat. Coronary collateral anastamoses connecting to the left anterior

descending coronary artery in the pig are almost absent (Schaper, 1971), leading to a sharp demarcation between the normal and the ischaemic zones following occlusion of this artery (Kleber et al., 1978; Janse et al., 1979). Several reports suggest that collaterals are negligible in the rat ventricle (Johns and Olson, 1954; Selye et al., 1960; Maxwell et al., 1984; Winkler et al., 1984; Schaper et al., 1986). This suggestion is substantiated by the consistent extent of ischaemia and infarction in rat hearts following coronary occlusion (Johnston et al., 1983a; Bernauer, 1982; Lepran et al., 1983; etc.), which compares favourably with the consistency seen in pig studies (Verdouw et al., 1983c; Sjoquist et al., 1983; etc.). There is one report, however, which suggests that there is a difference between rats and pigs in that gradual stenosis induced over a period of 3 months will induce collateral growth in pigs, sufficient to prevent infarct formation upon complete occlusion, whereas rats have end arteries, and are unable to develop collateral anastamoses, even following slow occlusion (Schaper et al., 1986).

In summary, various hypotheses concerning the nature of arrhythmias occurring clinically during and after myocardial ischaemia have translated into a template for determining the 'clinical relevance' of experimental studies (see Reimer et al., 1985). This template may be seriously misleading. It must be remembered that far more is known about the nature of ischaemia-induced arrhythmias and infarction in experimental animals than in humans, particularly during the critical first few hours after occlusion. Clinically, it has been suggested that a lack of scientific basis for the management of ventricular arrhythmias is founded in poorly controlled clinical studies (Campbell, 1983). Clearly, the confusion concerning the nature of risk, cause and the strategy of management of arrhythmias occurring during acute myocardial ischaemia dictates that the clinical concepts of

arrhythmogenesis should follow from studies with experimental animal preparations, rather than govern the acceptability of an experimental approach.

Irrespective of the arguments concerning clinical relevance of different species, until a series of drugs have been characterised for their activity against arrhythmias and infarction in a range of experimental preparations with established precision and accuracy, and until these results have been compared with the analogous condition in humans with clear distinction between complete and partial occlusion, abrupt and gradual occlusion, the time of onset of occlusion, large and small risk zones, the extent of collateral blood supply, the duration of ischaemia in the case of reperfusion, the degree of reperfusion, the extent of pre-existant infarction and the location of pre-existant infarction, then any discussion of clinical relevance is redundant. At present, most experimental preparations have not been characterised satisfactorily, and sources of error variance have not been recognised and eliminated.

The foregoing chapters have alluded to one fact which ought to play the key role in the choice of experimental preparation. That is, without ischaemia there is no infarction, and no ischaemia/infarction related arrhythmias. Therefore, the primary consideration should be the reproducible production of ischaemia of known severity. The most rational means of producing ischaemia of known severity is to abruptly, completely and permanently occlude a coronary artery in a species with minimal collateral vascularisation and little intraspecies variability. In addition, it would be an advantage if the species were small (not requiring technically demanding preparative equipment or inconvenient housing), inexpensive (to allow large sample sizes), robust (to allow rapid recovery from preparative surgery) and 'good natured' (to permit conscious animal experiments). There is a species which fits all these requirements, the common laboratory rat.

1.2.4 A history of coronary occlusion in rats

Coronary occlusion in rats was first undertaken in 1946 by Heimburger. His experiments were prompted by the desire to investigate whether the production of adhesion of the pericardium to the epicardium was capable of preventing infarction (by generation of collateral anastamoses of extracardiac origin). His experiments were a failure, in as much as injections into the pericardial sac of cod liver oil, alcohol, sodium salicylate, urea, sodium stearate, soap and sodium morrhuate failed to prevent infarction. Nevertheless, Heimburger established that ligation of the left coronary artery in rats produced death within 24 h of occlusion in approximately 25 % of animals, and that extensive infarction could be produced. The technique used for occlusion was ligation using a silk suture under positive pressure anaesthesia. The study was essentially qualitative, and no attempt was made to verify occlusion.

Before continuing with the discussion of myocardial ischaemia in rats, the question of the verification of occlusion should be considered. Verification of occlusion is an aspect of experimentation which has been neglected throughout the history of myocardial ischaemia and infarction studies. Without such verification it becomes impossible to distinguish between animals (or humans) in which a treatment has prevented or delayed infarction and animals (or humans) in which occlusion was partial or absent, or present but offset by collateral vascularisation.

Although the nature of collateral vascularisation has been extensively documented in pigs and dogs (e.g. Schaper 1971; Meesman, 1982), very little work has been undertaken in rats. Early work (Johns and Olson, 1954; Selye et al., 1960) showed that the coronary circulation in the rat heart is very uniform, and that the coronary arteries are end arteries (in contrast with guinea pigs in which extensive collateral vascularisation is present).

Recent radiolabeled microsphere studies in rats have shown that the extent of collateral perfusion in the occluded zone (OZ) is negligible, flow being less than 0.01 ml/min/g according to Winkler et al. (1984) and 6.1 ± 0.7% of normal flow according to Maxwell et al. (1984).

It should be mentioned that Kannengiesser et al. (1975) found that residual flow in the OZ in isolated rat hearts was 18% of pre-occlusion flow, as assessed by microsphere techniques. This value is somewhat higher than the values measured in vivo by Winkler et al. (1984) and Maxwell et al. (1984). The higher estimate of flow is likely to be the more accurate, owing to the fact that flow measurements are not dependent simply on the ability of a bed to trap microspheres, as is generally assumed; microspheres tend to distribute preferentially into beds reciving high flow. The result is an under-estimation of flow in beds receiving lower flow.

It has been reported that rats do not develop collateral anastamoses during prolonged and gradual stenosis, in contrast with pigs, and that infarction is inevitable upon complete occlusion in rats, in contrast with guinea pigs in which infarction is prevented by the extensive pre-existant collateral vascularisation (Schaper et al., 1986). Bloor et al. (1967) have also investigated the rat coronary circulation. Although they did not look for collateral anastamoses, they reported that the left ventricle is supplied by a single large coronary artery in more than 90 % of rats in an extremely reproducible and consistent manner. Of the remaining 10 % of rats, almost all received left ventricular perfusion from 2 main arteries arising from separate ostia, and that the second artery was usually an independent septal artery, not supplying the left ventricular free wall.

The work of Heimburger generated no interest for many years. However, in 1954, Johns and Olson decided to undertake a detailed comparison of several small animals for their applicability to the study of myocardial

ischaemia and infarction. The experiments were prompted by the view that the species which had been exclusively used up to that time, the dog, was in essence useless for the study of myocardial ischaemia and infarction; 'as a test procedure, coronary occlusion in the dog produces results so variable as to have only limited statistical usefulness'. In this regard, Johns and Olson were among the first to recognise that logistics play at least as an important part in the choice of a species for study as physiological identity with the human species. Johns and Olson examined the outcome of coronary occlusion in over 500 mice, rats, hamsters and guinea pigs. The experiments were carried out in a manner identical with that used by Heimburger, (1946) except that hearts were exposed via an intercostal incision rather than by thoracotomy.

In preliminary experiments (Johns and Olson, 1954), the coronary circulation was investigated by injecting lead oxide (as a suspension in melted gelatin) into the ascending aorta (briefly clamped to restrict flow to the coronary arteries). The coronary circulation of the rat and mouse were described as similar. Vessels were clearly visible following injection of contrast medium and dissection. The coronary arteries lay just beneath the epicardial surface, and were described as predominantly end arteries (collateral interarterial anastomoses being infrequent). In addition, the left anterior descending coronary artery (LAD) was described as the dominant artery, whereas the circumflex artery was described as merely a small branch of the LAD. For this reason, it is appropriate to describe the artery of occlusion in rats (and mice), that artery which emerges from under the atrial appendage, as the left main coronary artery, although most investigators commonly refer to it as the LAD.

Johns and Olson's studies of the guinea pig (1954) are perhaps worth mentioning, since a comparison of the responses of the guinea pig and rat to

coronary occlusion has been used to investigate the role of collateral circulation in limiting infarction (Winkler et al., 1984). Johns and Olson described the guinea pig as having prominent coronary arteries clearly visible on the epicardial surface. The circumflex was well developed, and both this vessel and the right coronary artery were described as the source of large, prominent and profuse collateral anastomoses feeding into the region supplied by the LAD.

In keeping with the extent and the reproducibility of the collateral vascularisation in rats and mice compared with hamsters and guinea pigs, the respective incidences of infarction were 83, 64, 31 and 25% (Johns and Olson, 1954).

Despite the extent of their study, Johns and Olson (1954) generated certain gross pieces of misinformation. In particular, the authors stated that VF was absent in rats. However, there is a simple reason for this misleading statement. The authors did not record the ECG, and VF was diagnosed by observing 'striking changes in rate and rhythm' of the heart while the chest was open. Since the chest was closed immediately after occlusion, and since later studies (e.g., Johnston et al., 1983a) have clearly shown that ventricular arrhythmias do not occur until several min after occlusion, it is no wonder that the authors did not 'see' VF. Nevertheless, the statement that 'in contrast to dogs, mice and rats are able to survive occlusion of the left main coronary artery; VF does not occur' (Johns and Olson, 1954) undoubtedly served to dissuade researchers from using rats in the study of myocardial ischaemia and infarction, despite the conclusion that 'this method of coronary occlusion produces a test infarct which is more nearly standard than any currently available'.

Before discussing further developments in the history of coronary occlusion in the rat, a comment should be made concerning the smallness of the

coronary arteries of the rat, as described by Johns and Olson (1954). Neither Johns and Olson, nor Heimburger (1946) mentioned that their occlusions must (by virtue of the impossibility of dissecting free almost invisible artery imbedded in the myocardium) have included some myocardium with the ligated artery. It is possible that if the artery is missed and only muscle ligated then spurious 'small infarcts' may form, confounding analysis. However, according to Heimburger (1946), ligation of myocardium alone, or the left coronary vein alone produced no significant sequelae (infarction). In other words, unless the LAD is included in the ligation, no infarction occurs.

From 1954 to 1973 very little work was done using rat occlusion preparations. Bryant et al. (1958) used Johns and Olson's technique (1954) to investigate the infarct process by electron microscopy. They found that gross structural changes in the myocardium did not occur until at least 5 h after occlusion, by light microscopy. However, electron microscopy revealed swelling of the sarcoplasmic reticulum and mitochondrial enlargement after only 1 h of occlusion. By 2 h, deposition of lipid bodies, uniform enlargement of mitochondria and partial disruption of myofibrils were present. Animals sacrificed at later times showed increasingly severe intracellular disruption, such as lipid body formation in cell nuclei (4 h), loss of definition of myofilaments (5 h) general disarray of intracellular order (24 h) and finally extensive infiltration of neutrophils and macrophages, with associated phagocytosis (48 h). The authors made no comments on their reasons for using rats, and gave no indication of the reproducibility of the extent of infarction.

A year later, Kaufman et al. (1959) embarked on a series of studies on infarction in rats (a series which appears to have terminated after 1 publication). Rats were chosen because they were considered to provide 'large

numbers' and 'uniform stock'. Johns and Olson were cited as the source of the technique of occlusion. These investigators (Kaufman et al., 1959) showed that occlusion caused depletion of succinate dehydrogenase which began 4 h after occlusion and slowly progressed over the following 12 h. In confirmation of the results of Bryant et al., Kaufman et al. found that no apparent alterations occurred in gross mycardial structure until at least 6 h after occlusion. However, these authors observed infiltration by mononuclear leukocytes beginning at 18 h after occlusion, reaching 'moderate' proportions by 24 h, at a time when Bryant et al. (1958) detected no such infiltration. The scarring process began 36 h after occlusion with the infiltration of fibroblasts. Kaufman et al. (1959) reported that the decreases in succinate dehydrogenase and other enzymes with time paralleled those reported following occlusion in dogs according to Jennings et al. (1957), and concluded that the rat was a 'satisfactory animal for the study of sequential histochemical and morphologic changes in the myocardium'.

The following year, the much-cited work of Selye et al. (1960) was published. These authors reiterated the view of Johns and Olson (1954) that the dog, by virtue of the variable outcome of occlusion in this species, is unsuitable for the study of myocardial ischaemia and infarction. However, Selye et al. (1960) criticised Heimburger (1946) and Johns and Olson (1954) for their 'complicated' procedure, the high post-operative mortality and the failure to produce an infarct with every occlusion. To be fair to Heimburger (1946) and Johns and Olson (1954), the first 2 criticisms are completely invalid. Firstly, it appears that all 3 groups prepared rats using essentially the same technique. The earlier 2 studies are superior in as much as they both employed artificial respiration. Selye et al. (1960) used no artificial respiration, claiming that they could occlude an artery so quickly that none was required. However, the speed was dependent on the rather

crude expedient of 'exteriorising' the heart, i.e., temporarily 'popping' it through the intercostal incision. Secondly, although it is difficult to make direct comparisons owing to the imprecise declarations of time of death, it appears that peri-operative mortality was 25% in Heimburger's study (1946), 21% in Johns and Olson's (1954), and 10% in Selye's (1960), differences which are not particularly striking. In addition, no distinction was made between death resulting from occlusion and death resulting from bad surgery and anaesthetic overdosage. The myth that rats do not develop VF following occlusion (Johns and Olson, 1954) presumably confounded Selye et al.'s perception of the superiority of their technique, giving rise to the assumption that any peri-operative death occurs as a result of poor technique.

Although Selye et al. (1960) did not record the ECG, they appeared to acknowledge that the extent of occlusion influences survival, since they found that the production of 'smaller' infarcts by deliberately occluding the LAD distal to its origin was associated with a marked reduction in mortality, whereas ligation of the both left and right coronary arteries in the same rat resulted in immediate death in 80% of animals. These authors (Selye et al., 1960) also confirmed the report of Heimburger (1946) that occlusion of the coronary veins alone produced no sequelae (unless the entire coronary sinus was occluded, in which case pericarditis and superficial epicardial calcification developed). The only other point of interest in Selye's work concerns long term survival. It was found that animals surviving the first 24 h after occlusion generally all survived the following month, death generally occurring only as a result of 'accidental infections' (Selye et al., 1960).

Several peculiar publications appeared in the following years. The first report of ECG information following occlusion in rats was given in

1961, when Normann et al., using the technique of Johns and Olson (1954) recorded 5 leads (3 limb and 2 chest) at 2 h after occlusion (presumably during brief ether anaesthesia). Remarkably, no arrhythmias were observed, although ST segment elevation in the anterior chest lead was apparent, and deep Q waves were seen in the same lead at 24 h and 7 days after occlusion. A similar study was performed by Zsoter and Bajusz in 1962, and the only arrhythmias seen were a few PVCs in 1 rat. However in this study (Zsoter and Bajusz, 1962) the ECG was not recorded until 2 days after occlusion. No information concerning survival after occlusion was given. Bajusz, who had previously worked with Selye and Zsoter, reiterated the belief that coronary occlusion causes no arrhythmias in rats, in his book on infarction (Bajusz, 1963).

In 1964 and 1965, 4 detailed and extensive, but completely neglected works were published by Hort's group (Hort et al., 1964; Hort and Da Canalis, 1965a; 1965b; Hort 1965). These authors carried out coronary occlusion in over 1000 rats by 1 stage and 2 stage ligation, with and without reperfusion. According to the English language abstracts following the German text, the critical duration of ischaemia necessary for production of irreversible damage was 13 - 15 min. These authors also stated that infarction could only be delineated sufficiently clearly for analysis, according to the triphenyltetrazolium staining method (Jestadt and Sandritter, 1959), when measured 10 h or more after occlusion, whereas that tissue destined to become infarcted could be differentiated from normal ventricular tissue at only 15 min after occlusion by light microscopy, using the criterion of 'streched' versus 'non-streched' muscle fibres ('stretched' being irreversibly damaged but not yet dead). This criterion was suggested to be potentially useful in the diagnosis of acute myocardial ischaemia in patients who have died from unknown causes (sudden death) without evidence of coronary

thrombosis or infarction; this recommendation has been ignored. The authors concluded that since the standard error of mean infarct size was always less than 10% of the mean, then the coronary-ligated rat prepartion was suitable for measuring the effect of drug treatment on infarct size. In this regard, these authors (Hort and Da Canalis, 1965a;1965b) provided sound quantitative evidence of reproducibility to support the earlier semiquantitative evidence of Heimburger (1946) and Johns and Olson (1954).

Despite the clear exposition of early ventricular arrhythmias in dogs following coronary occlusion, which had been known for decades (e.g., Townshend Porter, 1894; Smith 1918), it was not until 1973 that the myth that rats do not experience arrhythmias following occlusion was dispelled by the simple expedient of monitoring the ECG during and immediately after occlusion. This work was not carried out by any of the investigators who had earlier proclaimed the rat to be superior to the dog for studying myocardial ischaemia and infarction (e.g., Heimburger, 1946; Selye et al., 1960) but by 2 members of the Hungarian Army Medical Corps (Kenedi and Losconci, 1973a).

Kenedi and Losconci recorded the ECG during the 10 min period following occlusion (while the ether anaesthesia used in preparation wore off). Before discussing their results, it is worth mentioning that the technique of Selye et al. (1960) was used in preparation, and that in every case the act of exteriorising the heart through the intercostal incision was reported to produce transient irregular VT, atrioventricular block and sinus bradycardia; the technique of Heimburger (1946) as used by Johns and Olson (1954) would not be expected to lead to arrhythmias in this way, since heart exteriorisation was not employed.

Kenedi and Losconci (1973a) found that during the first 10 min after occlusion, a high incidence of ventricular arrhythmias occurred. Generally, a short run of PVC occurred immediately upon occlusion, but these initial

arrhythmias (later called phase-1a by Fagbemi, 1985) resolved in all cases. However, it was the opinion of these authors that the run of PVC occurring upon occlusion was a result of the mechanical trauma of preparation rather than ischaemia, because these arrhythmias were similar to the pre-occlusion arrhythmias induced by exteriorising the heart through the intercostal incision, rather than the arrhythmias which occurred a few minutes after occlusion. After an unspecified interval, a high incidence of PVC, VT and VF occurred. Unfortunately, the exact incidence and time course of the arrhythmias was not given, although of the 5/20 rats which experienced VF or 'flutter', 2/5 died. In addition to this first exposition of arrhythmias associated with acute myocardial ischaemia in rats, Kenedi and Losconsci (1973a) also noted that all rats surviving 24 h after occlusion exhibited a deep Q wave, and ST segment elevation in lead 1 of the ECG.

Arrhythmias induced by coronary occlusion in rats were not investigated again until 1979, when Szekeres' group (Lepran et al., 1979; Siegmund et al., 1979a; 1979b) and our laboratory (Au et al., 1979a; 1979b) began their series of experiments, which have continued throughout the 1980s, along with work by Parratt's group (Clark et al., 1980; etc.), Winslow (Kane and Winslow, 1980; etc.), Bernauer (1980; etc.) and others. In general, the above Investigators have concentrated on arrhythmias, although our laboratory and Bernauer always measure the OZ and the extent of infarction. Other researchers have concentrated on infarct size reductions in rats, and have completely ignored arrhythmias, for example, the Harvard contingent (MacLean et al., 1976; Kloner et al., 1977; Pfeffer et al., 1979; 1982; 1985), Chiariello's group (Chiariello et al., 1980; 1983; 1984; 1985), Flaim and Zelis (1981) and others. In doing so, these authors ignore censoring associated with VF-induced mortality; in most cases, the numbers of early deaths and their causes are not given.

Independently of whether infarction or arrhythmias are the main subject of study, the history and past literature concerning the use of the rat have been almost universally ignored. For example, the original work of Kenedi and Losconci (1973a) has only been appropriately cited twice (Clark et al., 1980; Abrahamsson and Almgren, 1980). In addition, credit is generally given to Selye (1960) for inventing coronary occlusion in rats, whereas in reality the technique was developed by Heimburger, 14 years earlier.

## 1.3 Ventricular arrhythmias in acute myocardial ischaemia

This chapter is concerned with the genesis of ischaemia-induced ventricular arrhythmias. Many of the concepts associated with arrhythmogenesis, such as reentry (Mines, 1913) have been under investigation for many years. An understanding of what lies behind the gross pathologic expressions of heart disease such as VF would be expected to assist in the prediction of which type of drug might be of benefit. However, it is also worth considering that the cart often comes before the horse, in that many clinically useful drugs have been developed on the basis of a purely empirical approach. In essence, a multilayered approach to the problem of ischaemia-induced arrhythmias is indicated, and it would be foolish to dismiss an empirical approach on the grounds that it does not take sufficient consideration of the underlying pathologic mechanisms. For example, the anti-ulcer $H_2$ receptor histamine antagonists were developed from the premise that histamine was somehow involved in ulcerogenesis via a mepyramine-insensitive mechanism. What that mechanism is, in terms of the biochemical basis for the generation of a gastric lesion and its precise relationship with stomach pH, remains uncertain. Nevertheless, cimetidine and ranitidine have proven to be effective anti-ulcer agents.

This chapter attempts to illustrate what is known and what remains to be ascertained with regard to the electrophysiological basis of arrhythmo-

genesis in acute myocardial ischaemia. In addition, an attempt has been made to examine why and how calcium antagonists might be of benefit in such circumstances. As background information, a brief summary of some of the major features of the electrophysiology of excitable tissue is also given.

1.3.1 The electrophysiology of excitable tissue

Arrhythmogenesis is an electrophysiological phenomenon. In order to understand the electrophysiology of myocardial ischaemia it is necessary to understand normal heart electrophysiology. Normal ventricular function is dependent upon the propagation of depolarisation. The charge is carried, as in the case of conduction in nerves, by ions. The characteristics of conduction are governed in part by the passive electrical properties of the tissue, and in part by the active or dynamic properties of the tissue.

The passive electrical properties may be approximated according to the cable equations developed by Kelvin (1855). If the resistance across the cell membrane is high compared with the intracellular and extracellular resistance, then any transmembrane potential differences and currents can be considered as functions of longitudinal distance and time, only. For such an 'ideal' cable, the change in membrane potential resulting from the focal application of current will decay in distance and time according to:

$$d^2E_m/dx^2 . 1/r_2 = C_m . dE_m/dt + i_i$$

where x is distance along the cable from the current source, $E_m$ is membrane potential, $r_2$ is intracellular fluid resistance, $C_m$ is membrane capacitance, t is time and $i_i$ is the component of the membrane current carried by ions (Hodgkin and Huxley, 1952a).

Rearrangement of the cable equation gives rise to two membrane constants which are often useful in the understanding of electrophysiology. The length constant, $\lambda = \sqrt{r_m/r_2}$ describes the distance along the membrane from a current source at which the resultant potential change has fallen to

1/e (approximately 37%) of that at the point of the current source. The time required for V ($E_m$) to decay from its initial value ($V_o$) to 63% of this value gives the product $R_mC_m$, commonly called the time constant of the membrane ($tau_m$). This arises because the membrane potential at time t after application of a current across the membrane is given by:

$$V_t = V_{max}(1-e^{-t/RmCm}).$$

When t is equal to $R_mC_m$, then $V_t = V_{max}(1-e^{-1})$, and $1-e^{-1} = 0.63$.

The active electrical properties of the tissue depend upon the asymmetrical nature of excitable tissue. The oriented enzyme, $Na^+$-$K^+$-dependent ATPase, the $Na^+$-$Ca^{++}$ exchanger, intracellular $Ca^{++}$ sequestrating proteins such as calsequestrin and other energy-dependent processes serve to generate a chemical potential gradient for several charged entities. In addition, Donnan equilibrium, the presence of a relatively fixed impermeant body of intracellular anionic protein, and the presence of a relatively high resting $K^+$ permeability versus that of other charged entities, leads to the establishment of a membrane potential. Finally, as will be discussed below, $i_i$ does not obey Ohm's Law. This variability in ionic conductance ($g_i$) constitutes the operational mechanism of the active electrical properties of excitable tissue which leads to propagation.

At rest, tissue in the heart (excluding nodal) can be said to approximate a resistance-capacitance circuit with a $K^+$ battery, such that the resulting resting membrane potential is given by the Nernst equation:

$$E_m = RT/F.\ln([K^+]_o/[K^+]_i)$$

where $[K^+]_o$ and $[K^+]_i$ are the local concentrations of $K^+$ on the immediate outside and inside of the membrane, respectively.

The Nernst equation for $K^+$ gives the reversal potential for the $K^+$ current, which is approximately -90 mV under normal physiological conditions ($[K^+]_o$ approximately 2.5 - 4.5 meq/l and $[K^+]_i$ 120 - 140 meq/l), and

this corresponds reasonably well with the resting membrane potential of most non-nodal heart tissue. This model is based on the assumption that at rest, the $K^+$ permeability of the membrane far exceeds that of other charge-carrying species, such as $Na^+$ and $Ca^{++}$.

The active dynamic properties of excitable tissue may be approximated by variations in the equations which were developed by Hodgkin and Huxley (1952a; 1952b; 1952c; 1952d) to explain the rectifying properties of excitable tissue. In this instance, rectification is the deviation of the current-voltage relationship from linear (ohmic) as a result of voltage-dependent changes in ionic conductance. Rectification is explained by invoking the concepts of separate ionic currents associated with relatively ion-selective channels which may vary with membrane potential and/or time (so-called voltage and time dependence). The equations describe the behaviour of these conductances in relation to membrane potential and time.

Rectification has been demonstrated in Purkinje fibres (Deck and Trautwein, 1964). At membrane potentials positive to approximately -30 mV, there is an increase in a conductance which is relatively selective for $K^+$. The properties of this conductance resemble those of a conductance described by Hodgkin and Huxley in the squid axon which activates slowly following step changes in holding potential, and is called, as a consequence, the delayed rectifier (see below). The current associated with this conductance is carried mainly by $K^+$, is outward-going, causes repolarisation and is activated by depolarisation.

There is a second type of rectification in Purkinje tissue associated with a $K^+$ conductance. This conductance is activated by changing holding potential in voltage clamped (see below) tissue from -30 mV to more negative values. Since depolarisation reduces the conductance for this $K^+$current (as opposed to the behaviour of the delayed rectifier), this current is

known as the anomalous rectifier current, with 'inward going' rectification.

It is perhaps important, at this stage, to comment on the use of voltage clamping. When investigating current flowing as a result of a change in membrane potential, it is necessary to eliminate capacitance current ($i_c$). This is done by maintaining membrane potential at a constant value (the holding potential), because $i_c = C_m.dE_m/dt$. Since the value $dE_m/dt$ is zero if $E_m$ is held (clamped) constant, then the term $C_m.dE_m/dt$ disappears from the cable equation, leaving:

$$d^2E_m/dx^2.1/r_2 = i_i$$

In other words, the transmembrane current carried by ions becomes a fairly simple function of membrane potential, under clamped conditions. The principle of voltage clamping was utilised by Hodgkin and Huxley (1952a; 1952b; 1952c; 1952d), in order to measure $i_i$ in the squid giant axon.

According to Hodgkin and Huxley, rectification may be described mathematically by invoking voltage-dependent fitting parameters which govern a conductance channel. In the case of the delayed rectifier, conductance is governed by the parameter, n. For a population of delayed rectifier conductance channels, the total current is dependent on the proportion of channels in the open state. The variable n behaves according to the equation:

$$dn/dt = \alpha_n(1-n)-\beta_n(n)$$

where $\alpha_n$ and $\beta_n$ are voltage dependent rate constants, n is the molar fraction of channels in the open state and 1-n is the molar fraction of channels in the closed state.

Hodgkin and Huxley found that their results best fit a model where $K^+$ conductance (gK) was proportional to $n^4$. The simplest physical conceptualisation of the above relation is that n is a particle which functions to 'gate' the conductance channel and exists either in an open state (n) or a closed state (1-n). The rate constant $\alpha_n$ refers to the rate of conversion

of a channel from the resting (closed) state to the depolarised (open) state, and vice-versa. The voltage-dependence of the channel, which accounts for its rectifying properties, is explained by the voltage-dependence of $\alpha_n$ and $\beta_n$; the value of $\alpha_n$ increases upon depolarisation, while $\beta_n$ decreases upon depolarisation.

Although this model was developed for nerve, manipulation of the values of $\alpha_n$ and $\beta_n$ can provide theoretical conductance-voltage relations which correspond well with experimentally-derived data in Purkinje tissue (Noble, 1960; Noble 1962).

It is suspected that there are many different conductances in the heart which show a range of properties. These properties include ion selectivity, activation by changes in membrane potential, inactivation by changes in membrane potential, inactivation with time (see below) and reactivation. In addition, under pathological conditions such as myocardial ischaemia, some of these properties may be influenced by changes in pH, cyclic AMP, temperature, etc. (see Hauswirth and Singh, 1978).

The delayed rectifier is voltage-dependent, since $\alpha_n$ and $\beta_n$ vary in size with membrane potential. However, the $Na^+$ conductance ($g_{Na}$) in the squid axon (and the similar 'fast inward current' in the ventricle) shows an additional property, time dependence. This refers to the observation that the increase in $g_{Na}$ which occurs upon depolarisation is transient, switching off (inactivating) with time. This phenomenon was explained by the invocation of two gating variables (in contrast with the single species for the delayed rectifier), one of which, m, behaves like n of the delayed rectifier, shifting to the open channel state upon depolarisation, while the second, h, behaves in the opposite manner, shifting to the closed state upon depolarisation. If either n or h is in the closed state, then that particular channel is closed (conductance for that channel is minimal), since, in

analogy with genetics, the open state is recessive while the closed state is dominant.

In order for $g_{Na}$ to first increase upon depolarisation, then decrease, it is necessary that the rate constant, $\alpha_m$, which governs the rate of conversion of m from the resting (closed) state to the open state, be larger than $\alpha_h$, the rate constant for the conversion of h from the resting (open) state to the closed state, both of which reactions occur upon depolarisation.

The interaction between the fast inward current ($i_{Na}$) and the delayed rectifier in time governs the ability of an AP to occur, and in space, to propagate, in many excitable tissues. Depolarisation serves to increase $g_{Na}$ via effects on $\alpha_m$. This effect leads to more depolarisation. However, depolarisation also serves to reduce $g_{Na}$ via effects on $\alpha_h$, and also serves to increase gK via effects on $\alpha_n$. These latter effects serve to repolarise the membrane. However, since the maximum value of $\alpha_m$ is much larger than that of $\alpha_n$ or $\alpha_h$, then provided depolarisation is large enough (at or above 'threshold') and occurs quickly (so as to preclude steady-state elevation of $\alpha_h$ leading to 'inactivation') then positive feedback of depolarisation will occur before the repolarisation processes are activated, producing the AP. The AP is terminated by a combination of inactivation of $g_{Na}$ and activation of the delayed rectifier.

The assymetry of excitable cells leads to the possibility of propagation, since Kirchoff's law is satisfied by the induction of outward current some distance away from the inward current, linked to the inward current by longitudinal (intracellular and extracellular) current, which is induced in turn by the longitudinal potential gradient resulting from focal depolarisation. Amplification may occur in this circuit as a result of similar processes which lead to the AP, as follows. If within a region of membrane depolarisation is sufficiently large, and occurs sufficiently quickly, then

$i_{Na}$ will swamp repolarising $K^+$ current, leading to further depolarisation at points further and further away from the initial point of depolarisation in a manner described by the cable equation. This manifests as a wave of depolarisation (the 'propagated AP'). The threshold for generation of a propagated AP depends upon $\lambda_m$ and $tau_m$.

A propagated AP passes as a wave through excitable tissue, leading to depolarisation-linked events, such as neurotransmittor release, or in the case of ventricular tissue, coordinated muscle contraction. The gating properties of the conductance channels confer direction to conduction in ventricular tissue. This is because the propagating wave of excitation leaves behind a band of tissue in which $g_{Na}$ is inactivated. The inactivation of $g_{Na}$ is a consequence of depolarisation-induced increases in $\alpha_h$. This inexcitability is known as 'refractoriness'. The duration of refractoriness under normal circumstances is dependent on repolarisation, since repolarisation serves to increase $\beta_h$ and reduce $\alpha_h$, leading to a shift in the equilibrium of h to the open-state. With the passing of time, and the re-establishment of polarisation, as more and more channels become re-available for opening, the tissue is said to pass from the stage of absolute refractoriness to relative refractoriness, and finally to the fully excitable state.

The conduction velocity ($\theta = x/t$) of a propagated AP can be derived in terms of the cable equation:

$$\theta^2 = (\lambda^2.d^2V/dt^2)/(tau_m.dV/dt + V)$$

This derivation predicts that $\theta$ is directly proportional to $\lambda$, and inversely proportional to the square root of $tau_m$. In addition, $\theta$ is proportional to the square root of the maximum rise rate of the AP ($dV/dt_{max}$). Under normal circumstances, $dV/dt_{max}$ is the major determinant of $\theta$, with $\theta$ almost linearly related to the square root of $dV/dt_{max}$. Manipulations

which either prevent channels from opening (by fixing the channel in the inactivated state, preventing activation or physically blocking the channels), or slow the kinetics of opening by shifting the relationship between $\alpha_m$ and $E_m$ to the right (to more positive values of $E_m$) will reduce $\theta$. In most nerve tissue and normal ventricular tissue, $dV/dt_{max}$ is governed by $i_{Na}$. In ischaemic ventricular tissue, it is possible that $dV/dt_{max}$ is dependent on currents other than the normal fast inward current (see below).

1.3.2 The electrophysiology of the normal ventricle

Variations in the active and passive electrical properties of ventricular tissue between different regions of the ventricle govern the shape of the propagating AP. These variations are brought about by variations in the dimensions of the cells in specific regions and also variations in the conductance channels present in the different regions.

By manipulation of extracellular ion composition (for example, replacement of $Na^+$ with choline or $Li^+$), addition of substances shown to selectively inhibit specific conductances, such as tetrodotoxin which selectively blocks $g_{Na}$ (Moore et al., 1967), and by application of various techniques for clamping voltage or current such as the recently-introduced patch-clamp technique (Lee et al., 1980), it has been possible to describe some of the conductance systems which contribute to the propagated AP of the heart. While it is possible to study isolated heart cells (despite their small size) by patch clamping (Lee et al., 1979), it is difficult to study heart cells in vivo because of movement (the organ beats!), and unfavourable cellular and intercellular geometry (Attwell et al., 1979). Nevertheless, although some conductances have yet to be fully characterised, it is generally accepted that the shape of the ventricular AP is mainly governed by three current systems, $i_{Na}$, the outward repolarising currents (carried

mainly by $K^+$) and the slow inward current ($i_{si}$, carried mainly by $Ca^{++}$).

In ventricular tissue, $i_{Na}$ with its rapid opening and closing kinetics dominates the initial rise of the AP, and governs the $dV/dt_{max}$ of depolarisation (Beeler and Reuter, 1970a). This in turn governs the conduction velocity. The proportion of $i_{Na}$ channels available for opening governs the excitability of the tissue. Ventricular $i_{Na}$ is similar to the $Na^+$ current described by Hodgkin and Huxley for the squid giant axon, with minor differences (such as the voltage-dependence of the inhibitory action of tetrodotoxin in the ventricle).

Repolarisation is associated with several $K^+$ currents including a current similar to the delayed rectifier current in the squid axon, called $i_{K2}$ (McAllister and Noble, 1966), which is governed by the gating variable s (analogous to n). Several other outward currents may also contribute to repolarisation, for example, mixed ion currents (predominantly $K^+$) called $i_{x1}$ and $i_{x2}$ (Noble and Tsien, 1969). The gK systems influence the AP duration, since inhibition of gK delays repolarisation by prolonging the tail of the AP. If the total duration of the AP (often measured as the time for 90 % repolarisation, or APD90) is increased, then the refractory period is increased. This is because the membrane potential must remain sufficiently negative for a time sufficient for conversion of the h variables from their closed to their open state (reactivation) for excitability to be restored. In this regard, if Purkinje tissue is clamped at -70 mV then the $g_{Na}$ system is 50 %inactivated (Weidmann, 1955a).

The third major current in ventricular tissue is the slow inward current ($i_{si}$). This current is carried mainly by $Ca^{++}$, and is the major determinant of the plateau of the AP in ventricular tissue (Mascher and Peper, 1969; Beeler and Reuter, 1970a; 1970b; New and Trautwein, 1972). As such,

$i_{si}$ serves to govern the AP duration, in conjunction with the repolarising outward $K^+$ current systems described above. The latter may themselves be partly governed by $i_{si}$, since $i_{si}$ causes a transient rise in unbound intracellular $Ca^{++}$ concentration which has been shown to enhance some $K^+$ conductances (Isenberg, 1977b; 1977c). In the ventricles, restoration of normal excitability after the propagation of an AP requires that $i_{si}$ as well as $i_{Na}$ recovers from inactivation. Since the duration of the AP is governed by $i_{si}$ to a large extent, then refractoriness is also dependent on $i_{si}$. Inhibition of $i_{si}$ will shorten AP duration at 25% repolarisation (APD25), but APD90 may either increase or decrease, presumably according to the dependence of the tail of the repolarising current on free intracellular $Ca^{++}$ (this may vary from one type of ventricular tissue to another, and may also vary from species to species). It is possible that during myocardial ischaemia, $i_{si}$ plays an important role in additional aspects of conduction in the ventricular tissue, as will be discussed in detail below.

Just as $i_{Na}$ possesses two gating variables, $i_{si}$ has been shown to be governed by two variables, denoted as d and f, analogous to m and h, respectively (Reuter, 1973). The voltage-dependence of $\alpha_d$ is such that the threshold for $i_{si}$ is approximately -50 mV, slightly more positive than that for $i_{Na}$. For f, the rate constant for conversion to the open state ($\beta_f$) far exceeds the rate constant for conversion to the closed state ($\alpha_f$) at membrane potentials more negative than -60 mV. The rate constants for activation ($\alpha_d$) and inactivation ($\alpha_f$) are much larger (indicating slower kinetics) than corresponding rate constants for $i_{Na}$. There is some species variation, $\alpha_f$ being 80–200 msec in cat and dog ventricular tissue (McDonald and Trautwein, 1978; Reuter and Scholz, 1977), and 10–30 msec in rat ventricular tissue (Isenberg and Klockner, 1980). In the latter

species, the fast kinetics of $i_{si}$ probably account for the abbreviated plateau of the AP (compared with the cat, dog, pig and human ventricular AP), which is associated with a shorter APD90 of 100 instead of 300 msec (Langer, 1978), and accords with the high resting heart rate of 350-450 beats/min.

Since ventricular cells are small compared with, for example, the squid giant axon, and are part of a functional syncytium, it is difficult to investigate their electrophysiology in a manner which provides unequivocal information (Beeler and McGuigan, 1978; McDonald, 1982). Furthermore, beating cells are difficult to impale with microelectrodes. Patch clamp studies are favoured at present for their power to reveal the properties of ventricular conductances. Nevertheless, as one removes cells or membranes from their physiological environment one may introduce factors which confound investigation. For example, $i_{si}$ is enhanced by pharmacological and physiological manipulation which increases cyclic AMP (cAMP). It is thought that cAMP triggers phosphorylation of the f 'particle', leading to slowing of inactivation of $i_{si}$ and an enhancement of peak $i_{si}$ (Bean et al., 1984). Therefore, since the basic properties of conductance channels in ventricular tissue remain to a certain extent unclear, it is possible to suggest many explanations for the mechanism by which a drug influences ventricular electrophysiology, whether tissue is normal or abnormal (ischaemic, for example).

1.3.3 Electrophysiological changes caused by myocardial ischaemia

The study of the electrophysiology of myocardial ischaemia is confounded by the dynamic unstable characteristics of myocardial ischaemia. The electrophysiological properties of the ischaemic ventricle vary from one region to another, and also from time to time, as intra- and intercellular biochemistry varies from one region to another, and changes from time to time.

Furthermore, the electrophysiological changes vary according to whether blood supply is completely or partially reduced. In short, these considerations dictate that 'the ischaemic myocardium' is not a single entity.

The electrophysiology of the squid giant axon is considered to be well understood, that of the normal heart less so, and that of the heart under the influence of regional ischaemia even less. Attempts to correlate microscopic changes in electrophysiology during acute myocardial ischaemia with the macroscopic consequences of such changes - arrhythmias - have yet to provide a consensus. This is partly a result of the difficulties in studying the electrophysiology of the heart and partly a result of the variability in experimental preparations and approach. Nevertheless, despite a lack of convincing correlation between specific electrophysiological changes in myocardial ischaemia and arrhythmias, much has been speculated concerning mechanisms of arrhythmogenesis.

Recently, it was demonstrated, using anaesthetised pigs, that the maximum diastolic potential (MDP) in subepicardial muscle cells begins to fall precipitously within seconds of occlusion (Downar et al., 1977b; Kleber et al., 1978). Simultaneously, the AP amplitude, $dV/dt_{max}$ and APD90 all decrease.

The decrease in $dV/dt_{max}$ and APD90 are considered to be secondary to the fall in MDP, since, as discussed in detail previously, steady state diastolic depolarisation leads to inactivation of $g_{Na}$. However, it is possible that ischaemia directly reduces $g_{Na}$ independently of MDP changes, although it is not necessary to postulate such a mechanism. As a consequence of the reduction in APD90, the effective refractory period (ERP) decreases, since reactivation of $g_{Na}$ occurs more quickly. However, this situation is not maintained. As MDP continues to fall ERP lengthens, despite a maintained narrow APD90. Since ERP continues beyond the point of

full repolarisation, this phenomenon is known as post-repolarisation-refractoriness (Lazzara et al., 1978). Since post-repolarisation-refractoriness can be induced by depolarisation alone in isolated ventricular tissue (Inoue et al., 1984), it follows that it may be possible to explain this phenomenon in terms of the normal ventricular conductance channels (see below).

It is important to note that the electrophysiological changes described are not uniform in time or space. For example, adjacent cells with very similar resting $E_m$ may vary in ERP from 180 to 500 msec in pig ischaemic subepicardial muscle (Downar et al., 1977b).

As a result of the fall in $dV/dt_{max}$, $\theta$ falls, producing characteristic slow conduction in the ischaemic tissue. In addition, depolarisation to between -55 and -60 mV is associated with complete inactivation of $g_{Na}$, and under these circumstances complete conduction block may occur.

The phenomena described above were recorded in vivo from subepicardial tissue (Downar et al., 1977b; Kleber et al., 1978). It is not possible to record from deeper layers in vivo, owing to the limitations of intracellular recording electrodes (electrodes are too fragile to be plunged deep into the myocardium and penetrate cells). It is possible to record from isolated strips of subendocardial tissue in vitro, but it is unclear to what extent the experimental technique influences the variables under investigation (not least because the tissue is generally superfused, in which case it is difficult to regulate the experimental ischaemia). A compromise preparation is the isolated Langendorff-perfusion rat heart in which careful incision exposes the sub-endocardial tissues, while coronary occlusion may be undertaken to produce regional ischaemia. Experiments carried out in our laboratory using this preparation have revealed similar changes to those described above for subepicardial pig tissue (Inoue et al., 1984). In addition, it was shown that the changes were more severe and less reversible

with time in the deeper layer cells versus surface cells. The heterogeneity of ischaemic electrophysiology in time and space was clearly demonstrated in these experiments (Inoue et al., 1984).

Qualitatively, it is clear that the active and passive electrical properties of ventricular tissue may change with time in a fairly reproducible manner during myocardial ischaemia, and attempts have been made to relate this information to theoretical mechanisms of arrhythmogenesis, including reentry, automaticity and triggered automaticity.

1.3.4 Models Of Arrhythmogenesis

1.3.4.1 Reentry. A brief description of some models of arrhythmogenesis follows. Reentry was originally described as reexcitation of the myocardium via a circular route (Mines, 1913). The prerequisite for reentry is one-way block of propagation in one limb of the reentrant circuit. The nature and mechanism of reentry, and the roles of refractoriness, conduction velocity and duration of the 'wave of excitation' were explicitly described by Mines (1913) using the tortoise heart, and reiterated by Schmitt and Ehrlanger (1928) using a turtle heart preparation. The mechanism requires that the normal wave of excitation brings $E_m$ in the blocked limb of the circuit closer to threshold for conduction, such that the normal wave can then travel retrogradely up this limb and reenter its previous pathway. For this to happen, the tissue of the anterograde pathway must be excitable at the time the wave front reenters. This criterion will be met if one of two mechanisms operate. Firstly, if the circuit is long enough it will allow sufficient time to elapse for the anterograde pathway to recover excitability. This type of reentry is called macroreentry.

The pathway will be functionally long if conduction velocity is reduced, either in the anterograde or retrograde limb. Alternatively, if the refractory period in the anterograde pathway is very short (excitability being

restored quickly), then the requirement for a prolonged interval between excitation and re-excitation is reduced. In this case the reentry circuit may be extremely short, perhaps only a few mm (Sasyniuk and Mendez, 1971). Indeed, transmural (epi- to endocardial) reentry has recently been reported (Kramer et al., 1985). This type of reentry is called microreentry.

Acute myocardial ischaemia provides all the theoretical requirements for reentry: slow conduction, dispersion of refractoriness and areas of 2-way conduction block around which a reentrant impulse may encircle, and of 1-way block through which retrograde conduction may occur (e.g., Janse, 1982).

How does one recognise reentry experimentally ? The techniques employed for investigation in open-chest animals have not been successfully applied to clinical or closed-chest animal studies. In open-chest animals an array of DC electrodes may be placed on the epicardium for mapping the activation pathways. By this method, arrhythmias may be visualised (see paragraphs dealing with automaticity, below). It may be possible, in the future, to prepare experimental animals with in-dwelling DC electrode arrays. In the clinical situation, or in closed-chest animals, standard 12 lead ECGs do not permit one to distinguish between reentry and automaticity (see below).

Reentry, theoretically, may give rise to PVCs or VT. VT may degenerate to VF, in which case the mechanism of initiation of the arrhythmia will be lost to analysis, since VF is usually defined as uncoordinated chaotic electrical activity (Moe et al., 1964), without recognisable QRS complexes (Bigger, 1980).

In closed-chest experimental animals undergoing repeated episodes of VT, it has been suggested that the delivery of a premature electrical stimulus to the ventricle can terminate or initiate the arrhythmia, if the mechanism is reentry (Bigger and Goldreyer, 1970). The premature stimulus presumably either conducts through the one-way blocked tissue to initiate VT, or depol-

arises the one-way blocked tissue allowing anterograde conduction of the normal impulse to terminate VT. The use of premature stimulation does not permit the recognition of reentrant PVCs, only relatively sustained arrhythmias, such as VT.

Trains of high frequency ventricular stimulation will often initiate or terminate reentrant VT by the same mechanism as premature stimulation. Such stimulation will initially capture the reentrant circuit, then, as frequency is increased, the ventricle will fail to follow. Once the stimulus is terminated, sinus rhythm will resume. However, since the underlying condition is not changed, the arrhythmia will shortly resume. Unfortunately, this response is not unlike the post-overdrive suppression response seen with automaticity (see below). Therefore, in closed chest animals it is difficult to prove reentry as the cause of VT. Furthermore, it is impossible to prove reentry as the underlying trigger for VF, according to the above considerations. In open-chest animals, using epicardial mapping techniques, the situation is different (see below).

1.3.4.2 Abnormal Automaticity. The understanding of abnormal automaticity as a mechanism of arrhythmogenesis requires the understanding of normal automaticity. During diastole, the membrane potential in normal ventricular muscle is almost constant. However, in the sinus node a slow spontaneous diastolic depolarisation precedes and triggers the propagated AP. Other tissues in the heart (for example the atrioventricular node and the Purkinje fibres) also show this slow depolarisation, but $dV/dt_{max}$ is greatest in the sinus node. As a result, the frequency of triggering of propagated APs is highest in the sinus node. Consequently the propagated AP originating from the sinus node overdrives the spontaneous depolarisations in other regions of the heart, and dictates the heart rate. This constitutes the pacemaker property of the sinus node. The current responsible for

the spontaneous diastolic depolarisation is known as the pacemaker current. In the sinus node the pacemaker current has recently been shown to result from the interaction of two currents (Shibata and Giles, 1985), the decay of a delayed rectifier $K^+$ current called $i_K$, and the activation of an inward current carried by $Ca^{++}$, called $i_{Ca}$. The decay of $i_K$ is highly voltage-dependent over the range -80 to -55 mV. The activation of $i_{Ca}$ occurs at between -60 and -55 mV with peak current at 0 mV.

Abnormal automaticity simply means that heart tissue other than the sinus node has taken over the pacemaker role of the sinus node (Hoffman and Dangman, 1982; Sasyniuk, 1984). Usually, in order for this to occur, the rate of firing of the abnormal pacemaker region must exceed that of the sinus node and overdrive the heart (but see comments concerning parasystole, below). The anatomical source of this pacemaker activity is known as the ectopic focus. Abnormal pacemaker activity may occur as a result of an increase in the steepness of the slope of the diastolic depolarisation in latent pacemaker tissue, or a reduction in the threshold for generation of the (abnormal) propagated AP. The latter may result from either a reduction in the absolute threshold in mV, or from a shift in the maximum diastolic potential (MDP) to a more positive value.

The difficulties in recognising reentry in closed-chest animals also apply to the recognition of abnormal automaticity. Automaticity may be associated with intermittant exit block whereby the ectopic impulse fails to propagate (Fisch et al., 1971). This will occur if the ectopic focus discharges just after the normal wave of excitation has passed by (leaving a band of refractory tissue). Under such circumstances one will see, perhaps, only PVC in the ECG. Alternatively, the ectopic focus may be protected, by entrance block, from overdrive suppression by the normal wave of excitation (Wennemark and Bandura, 1974), in which case automatic arrhythmias may occur

even when the cycle length of the abnormal pacemaker is longer than the sinus cycle length. Theoretically, entrance block may be caused by a band of tissue which possesses one-way blocking properties, or by anatomical abnormalities. Conceivably, entrance block may also result from the induction of a band of refractory tissue by the previous ectopic impulse. If the normal sinus frequency and the entrance-block-protected ectopic frequency are similar, the normal and abnormal pacemakers may alternate in their dominance, producing parasystole.

It has been suggested that abnormal automaticity and reentry may be differentiated by studying the effects of overdrive pacing (see Bigger and Goldreyer, 1970; Vasalle, 1977). If an automatic arrhythmia is overdriven by pacing, then following termination of overdrive one will see a delay (post-overdrive suppression), then a resumption of the automatic arrhythmia. Reentrant arrhythmias do not show typical post-overdrive suppression.

The mechanism of post-overdrive suppression has been studied in dog Purkinje fibres (Vasalle, 1977). Early during the onset of overdrive pacing, MDP falls. Providing that stimulation does not exceed a critical frequency, maximum diastolic depolarisation then rises and the slope of the pacemaker potential is reduced. Immediately upon cessation of overdrive, the ectopic frequency is lower than it had been before overdrive pacing, as a result of the effect of overdrive pacing on the slope of the pacemaker potential. Over a short priod of time the original electrophysiological characteristics of the ectopic focus return, and the arrhythmia resumes. In the case of reentry there is generally no post-overdrive suppression, and the reentrant arrhythmia resumes after an unpredictable interval. Nevertheless, it is possible that reentrant arrhythmias may resume, following overdrive, after an interval similar to that seen following post-overdrive suppression of abnormal automaticity. Therefore a reentrant arrhythmia may

be incorrectly classed as automatic. In addition, parasystolic automaticity, protected from overdrive by entry block, will not exhibit post-overdrive suppression, and may therefore be incorrectly classed as reentrant.

As discussed above, overdrive techniques are only useful for assessing relatively sustained arrhythmias. However, it is possible to gain insight into arrhythmogenic mechanisms (including mechanisms of PVC induction) from ECG recording, coupled with simple physiological manipulations. In this regard, reentrant PVCs are triggered initially by the normal wave of propagation. Therefore, by analysing the frequency of PVC in relation to heart rate, it may be possible to establish a relationship. Furthermore, heart rate may be slowed or stopped by stimulating the vagal efferents, or accelerated by administering atropine (etc.), such that PVC incidence may be measured over a wide range of heart rate. Theoretically, PVCs resulting from automaticity should not vary in frequency with changes in heart rate, whereas the frequency of reentrant PVCs should increase with tachycardia, and fall with bradycardia. However, the study of the effect of changes in heart rate on PVC incidence is not an infallible means of diagnosis, owing to the possibility of entry- and exit-block. In addition, it is necessary to ensure that the manipulation designed to alter heart rate does not influence other variables, directly or indirectly, leading to an alteration in PVC frequency. It is almost impossible to control for this possibility.

It is possible to make certain speculations concerning the mechanism of arrhythmogenesis of PVCs by simply observing the ECG without other manipulation. In this regard, sustained fixed coupling of PVCs (of consistent QRS configuration) with the normal sinus beats is regarded as indicative of reentry rather than automaticity (Langendorf and Pick, 1967). The reason for this is that the frequency of an ectopic arrhythmia would not be expected to be the same as the sinus frequency, whereas a reentrant arrhythmia

should be linked to the sinus cycle in a fixed manner.

It is questionable whether any of the tests and experiments described are useful for distinguishing between reentry and automaticity during acute myocardial ischaemia, despite their possible usefulness in other circumstances. During the development of myocardial ischaemia, the electrophysiological characteristics of a hypothetical reentrant circuit will change with time, causing changes in coupling interval. Therefore, reentry in acute myocardial ischaemia may be incorrectly described as automaticity if the criterion for diagnosis is a fixed coupling interval. In addition, it is possible that parasystolic (entry-blocked) foci may be influenced by the wave of normal excitation via electrotonic intercourse (reflection) across an area of entry block (Jalife and Moe, 1976). Finally, in this regard, the functional distinction between reentry and automaticity becomes questionable when the phenomenon of triggered automaticity is considered.

1.3.4.3 Triggered Automaticity. Triggered automaticity has only been demonstrated in vitro. The reason for this is quite simple. In order to demonstrate triggered automaticity it is necessary to stimulate quiescent tissue, and it is not possible to have quiescent ventricles in vivo. Since triggered automaticity is, by definition, automaticity which is initiated by an exogenous source, and the exogenous source theoretically includes the normal wave of depolarisation, then it is possible that abnormal automaticity in vivo may be triggered by the sinus beat (Spear and Moore, 1982). In such a case the frequency of automatic PVC would correlate with heart rate. Therefore, it is not possible to distinguish between automaticity and reentry on the basis of the relationship between heart rate and PVC frequency unless triggered automaticity is ruled out. However, it would be necessary to induce asystole in order to test for triggered automaticity in vivo, and this is not feasible.

1.3.5 Epicardial Activation Mapping

Although it is not possible to prove whether an arrhythmia is automatic or reentrant in closed-chest animals, the technique of epicardial mapping allows for visualisation of conduction pathways in anaesthetised open-chest animals and in-vitro. Epicardial mapping has been carried out in conjunction with simultaneous intracellular recording in acute myocardial ischaemia, and attempts have been made to relate the pattern of abnormal electrogenesis and conduction with the underlying electrophysiology.

How do the electrophysiological changes in acute myocardial ischaemia correlate with arrhythmogenic mechanisms visualised by epicardial mapping ? In coronary-occluded blood-perfused dog and pig hearts, records of reentry, but not automaticity, were observed (Janse et al., 1980; Janse and Kleber 1981; Janse, 1982). Most of these arrhythmias arose from the normal tissue close to the occluded bed within the first few minutes of occlusion. The time course of arrhythmias corresponded reasonably well with the time course of ischaemia-induced arrhythmias in-vivo in dogs and pigs (e.g., Bergey et al., 1982; 1984).

Interestingly, the initial trigger for the reentrant VT was an impulse which arose from the normal tissue. The mechanism proposed to account for this phenomenon was as follows. By comparing the AP of normal cells with that of ischaemic cells it was found that many ischaemic cells were in the plateau phase of the cycle when normal cells were at the resting repolarised phase. This situation occurred as a result of the heterogeneity of conduction velocity in the ischaemic tissue versus the non-ischaemic tissue, in association with slow conduction in the ischaemic tissue. The consequences of this situation included a large potential difference between the normal and the ischaemic tissue. It was proposed that a flow of injury current between the depolarised ischaemic tissue and the polarised non-ischaemic

tissue may have been sufficient to re-excite the non-ischaemic tissue. Alternatively, since the non-ischaemic tissue could be in the plateau phase of the AP when the ischaemic tissue was repolarised, injury current may have flowed in the opposite direction to re-excite the ischaemic tissue.

For this mechanism to operate, it is necessary that a layer of cells exists (separating the current source and sink) which is inexcitable, at least temporarily, and capable of permitting electrotonic coupling between the source and sink (Janse and Kleber, 1981).

Whatever the mechanism of initiation, activation maps revealed fragmentation and fusion of wavefronts, one- and two-way block and reentry. Reentry was associated with VT. VF was associated with multiple wavelets traveling slowly along tortuous routes among multiple islets of conduction block which changed position and magnitude from moment to moment. VT either terminated as a result of major single wavefronts arriving at a large area of inexcitable tissue, or degenerated to VF as major wavefronts split into subsidiary wavefronts. VF did not terminate spontaneously (within the 10 sec allowed before defibrillation was applied in this study). It was suggested that this was because VF was associated with more wavefronts than VT, therefore if some wavefronts terminated, others remained to perpetuate the arrhythmia. This is a possible explanation for the higher frequency of spontaneously reverting VF in small animals such as rats (Kenedi and Losconci, 1973a; Clark et al., 1980) compared with larger animals such as dogs (Townshend Porter, 1894; Smith, 1918). Presumably the larger hearts have more wavefronts in VF than smaller hearts, and consequently express a lower probability of spontaneous defibrillation.

It was found that stimulation of the left stellate ganglion improved conduction in the ischaemic tissue, and increased the incidence of VT and VF. It is possible that this enhanced sympathetic activity increased exci-

tability in the ischaemic tissue, facilitating the conduction of PVCs and their degeneration into VT and VF (Janse and Kleber, 1981). High concentrations of lidocaine increased the proportion of the ischaemic tissue which was inexcitable at any given moment, thereby causing termination of VT and prevention of progression to VF.

To summarise, of the major theoretical mechanisms of arrhythmogenesis, only reentry has been demonstrated in acute myocardial ischaemia. This evidence was provided from epicardial mapping experiments. However, reentry appeared to be triggered in many cases by an ectopic injury current arising from normal Purkinje fibres. The prerequisite for induction of an ectopic impulse appeared to be a dispersion of refractoriness between the normal and the ischaemic tissue. This heterogeneity was produced by the slow conduction in the ischaemic tissue. Abnormal slow conduction and variable conduction block in the ischaemic tissue appeared to be responsible for the continuation of the arrhythmias and their progression into VT and VF. Conduction block was made worse by lidocaine, such that arrhythmias terminated soon after initiation, while left stellate ganglion stimulation improved conduction (lessened block) and worsened the arrhythmias.

Do these phenomena occur in rats following coronary occlusion? Our laboratory has demonstrated effects of occlusion in vitro on intracellular potentials (Inoue et al., 1984) similar to those demonstrated in other species. In addition, indirect evidence in vivo such as a reduction in refractory period (Northover, 1986) and a lack of effect of vagal stimulation-induced bradycardia on arrhythmia incidence (Mertz and Kaplan, 1982) suggests that the arrhythmias occurring during the 30 min period after occlusion are more likely to be reentrant than automatic.

A question which arises is, to what extent does $i_{Na}$ contribute to arrhythmogenesis compared with $i_{si}$? The effects of lidocaine and left

stellate stimulation (Janse, 1982) suggest that both may be involved.

1.3.6 The slow inward current and arrhythmogenesis in acute ischaemia

1.3.6.1 Introduction

As discussed previously, in normal ventricular tissue the initial upstroke of the AP (phase-0) is governed by $i_{Na}$, and the plateau by $i_{si}$. The changes in phase-0 and resting $E_m$ produced by myocardial ischaemia, as described above, may represent one of four electrophysiological phenomena. Firstly, the reduction in $dV/dt_{max}$ may represent a severely depressed $i_{Na}$. This is because the depolarisation associated with acute myocardial ischaemia will cause inactivation of $i_{Na}$. Secondly, the upstroke may represent an enhanced $i_{si}$. This is because depolarisation to -60 to -65 mV will bring $E_m$ closer to the threshold for $i_{si}$ without inducing steady-state inactivation of $i_{si}$ ($\alpha_f$ should be similar to that at an MDP of -90 mV), whereas $i_{Na}$ will be almost completely inactivated at this voltage. Thirdly, the upstroke may involve both depressed $i_{Na}$ and enhanced $i_{si}$. Finally, the upstroke may be associated with an abnormal current system which possesses elements of $i_{Na}$ and/or $i_{si}$, but perturbed by factors of ischaemia other than depolarisation, such as increases in pH (Iijima et al., 1986) and the products of anaerobic metabolism (Corr et al., 1984).

1.3.6.2 How might $i_{si}$ contribute to arrhythmogenesis ? The electrophysiological changes produced by acute myocardial ischaemia (Downar et al., 1977a; 1977b; Kleber et al., 1978), and the attendant hypothetical arrhythmogenic mechanisms (reentry and automaticity, etc.) reveal that $i_{si}$ may play an important role in arrhythmogenesis (Hauswirth and Singh, 1978). The principal reasons for such a suggestion are as follows.

a. Acute myocardial ischaemia is associated with depolarisation (Downar et al., 1977b; Kleber et al., 1978; Inoue et al., 1984) to $E_m$

values close to those producing complete inactivation of $i_{Na}$ (Beeler and Reuter, 1970a). However, from the known voltage-dependence of $i_{si}$ (Reuter, 1973), it would be expected that this depolarisation should not reduce $i_{si}$, but rather enhance it by virtue of a reduction in threshold for activation.

b. Acute myocardial ischaemia is characterised by slow conduction in the ischaemic tissue (Downar et al., 1977b; Kleber et al., 1978). This slow conduction can be accounted for, almost completely, by the fall in $dV/dt_{max}$ of the upstroke of the ischaemic AP (Dodge and Cranefield, 1982); the increase in longitudinal intercellular resistance associated with increases in intercalated disc resistance may also contribute (Weidmann, 1982). Slow conduction in depolarised Purkinje tissue has been shown to be dependent on $Ca^{++}$ (Cranefield et al., 1972), From considerations of resting $E_m$, as discussed above, the current responsible for the upstroke of the slow conduction AP may be $i_{si}$. In this regard, slow conduction in Purkinje tissue in vitro has been shown to be inhibited by (±)-verapamil at concentrations below those which inhibit $i_{Na}$ (Cranefield et al., 1974).

c. Acute myocardial ischaemia is associated with a large increase in extracellular $K^+$ concentration ($[K^+]_o$) (Hill and Gettes, 1980; Hirche et al., 1980). While this phenomenon may contribute to the depolarisation occurring in acute myocardial ischaemia and its attendant consequences (see above), it may also serve to release noradrenaline from sympathetic nerve endings (Hirche et al., 1985). By activating $\beta_1$ adrenoceptors, noradrenaline inhibits inactivation of $i_{si}$ (Bean et al., 1984). This arrhythmogenic mechanism is highly speculative, however, and recent evidence from our laboratory (Botting et al., 1983) and elsewhere (Daugherty et al., 1986) does not support a major role for catecholamines in arrhythmogenesis in acute myocardial ischaemia. Therefore, if $i_{si}$ is involved in arrhythmo-

genesis, this involvement is essentially independent of the modulating influence of adrenoceptor activation and antagonism on $i_{si}$.

d. Although current evidence does not support a role for automaticity in arrhythmogenesis in acute myocardial ischaemia (Janse, 1982; Janse and Kleber 1981; Mertz and Kaplan, 1982; Northover, 1986), this possibility is not precluded. If abnormal automaticity is the expression of enhanced latent pacemaker currents in Purkinje tissue (Spear and Moore, 1982), then it is possible that $i_{si}$, or a similar current plays an important role in arrhythmogenesis. This is because the pacemaker current in the sinus node comprises, in part, of $i_{Ca}$, a $Ca^{++}$ current similar to $i_{si}$ (Shibata and Giles, 1985), and phase-0 depolarisation is carried almost exclusively by $i_{si}$ (Yangihara et al., 1980; Brown, 1982).

e. Activation mapping experiments have shown that a disparity in AP phase between the normal and ischaemic tissue generates injury currents which trigger reentry (Janse, 1982; Janse and Kleber 1981). The principal determinant of such triggering is the occurrence of an AP plateau adjacent to excitable tissue. The plateau of the AP in normal (and probably also in ischaemic) ventricular tissue is governed by $i_{si}$ (Reuter, 1973). The AP plateau in non-ischaemic tissue is much longer than the plateau in ischaemic tissue (Downar et al., 1977b; Kleber et al., 1978; Inoue et al., 1984). It follows that the longer the plateau in non-ischaemic tissue, the higher the probability of triggering an electrotonic re-excitation of the ischaemic tissue. Therefore, $i_{si}$ may be involved in arrhythmogenesis by virtue of its role in creating a dispersion of AP plateau (and consequently a dispersion in refractoriness) between the ischaemic and non-ischaemic tissue.

f. It is possible to hypothesize other mechanisms by which $i_{si}$ is instrumental in initiating or maintaining arrhythmias in acute myocardial ischaemia. Only one further possibility will be discussed here, and this

concerns the hypothetical mechanism of arrhythmogenesis known as triggered automaticity. From in vitro studies, triggered automaticity has been linked to the occurrence of oscillatory afterpotentials (Cranefield, 1977), also known as early or delayed after-depolarisations (DADs), depending on whether they occur before or after full repolarisation, respectively. While there is no evidence to support a role for triggered automaticity in arrhythmogenesis in acute myocardial ischaemia, it has been suggested that DADs and triggered activity occur in surviving Purkinje tissue excised from the occluded ventricular regions of dog hearts following the development of infarction (El Sherif et al., 1982). The involvement of $i_{si}$ in DADs has been suggested by the observations that DADs are enhanced by raising extracellular $Ca^{++}$, occur in depolarised tissue (especially tissue depolarised by cardiac glycosides), and that DADs are inhibited by compounds which inhibit $i_{si}$ (Ferrier and Moe, 1973). However, other evidence suggests that DADs are generated by the transient inward current (Lederer and Tsien, 1976), a current which appears to be distinct from $i_{si}$.

Recently it was shown that simulated ischaemia (16.2 meq/l $K^+$, low pH, lactate and hypoxia) abolished DADs induced by high frequency pacing in Purkinje tissue in vitro (Opie et al., 1986; Coetzee et al., 1986), suggesting that this mechanism of arrhythmogenesis in acute myocardial ischaemia may not be important.

## 1.4 The pharmacology of calcium antagonists

### 1.4.1 Definition

Calcium antagonists were originally described as drugs which produce their pharmacological effects predominantly by inhibiting voltage-activated entry of $Ca^{2+}$ in a manner which can be inhibited by $Ca^{2+}$ (Fleckenstein et al., 1969). This definition has been extended to include drugs which inhibit $Ca^{2+}$ entry resulting from the opening of $Ca^{2+}$ channels coupled

with drug receptors (see Janis and Triggle, 1983), and drugs which inhibit intracellular $Ca^{2+}$-dependent processes such as binding with calmodulin (Rahwan, 1983; Lynch and Rahwan, 1982). The expansion of the definition has occurred in conjunction with the introduction of new terms such as 'slow channel blockers' and 'calcium entry blockers' (see Nayler, 1983). The term calcium antagonist is used here in accordance with Fleckenstein's original definition (see above and Fleckenstein, 1983 for review).

1.4.2 Pharmacology of phenethylalkylamines and 1,4-dihydropyridines

Phenethylalkylamine calcium antagonists include verapamil, gallopamil, bepridil, tiapamil, anipamil and a variety of trial preparations such as D888. 1,4-dihydropyridines include nifedipine, felodipine, nimodipine, and nitrendipine. Verapamil, gallopamil and nifedipine have been studied in the most detail (see Henry, 1979; 1980; Triggle, 1981; Fleckenstein, 1983; Nayler and Horowitz, 1983).

Phenethylalkylamines have been suggested to possess a higher affinity for the channel associated with $i_{si}$ when the channel is in the open or inactivated state (Kohlhardt and Mnich, 1978; Pelzer et al., 1982; Lee and Tsien, 1983), whereas 1,4-dihydropyridines are equipotent in rested and open channels (Lee and Tsien, 1983). This difference may account for the well-known frequency-dependence of phenethylalkylamines (Sanguinetti and West, 1982) compared with 1,4-dihydropyridines, which possess little or no frequency-dependence, but considerably more resting block (Woods and West, 1983; 1985; Hachisu and Pappano, 1983). Both 1,4-dihydropyridine and phenethylalkylamine calcium antagonists have been suggested to act by slowing the recovery from inactivation of the $i_{si}$ channel (Lee and Tsien, 1983).

Studies with permanently charged analogues of gallopamil (Hescheler et al., 1982), and studies carried out using 'skinned' (plasmalemma-free) smooth-muscle and cardiac tissue (Fleckenstein, 1977; Kreye et al., 1983;

Itoh et al., 1984) have indicated that both phenethylalkylamine and 1,4-dihydropyridine calcium antagonists produce their pharmacological effects via an action on the inner surface of the plasmalemma, and that intracellular actions such as inhibition of $Ca^{2+}$ binding with calmodulin (Silver at al, 1984) do not contribute to the effect of these drugs on excitable tissue.

Both phenethylalkylamines and 1,4-dihydropyridines possess a wide spectrum of pharmacological properties, such as inhibition of $i_{Na}$ (Bayer et al., 1975a; Nawrath et al., 1981; Yatani and Brown, 1985), inhibition of corticosteriod release from the adrenal cortex (Costa et al., 1983), inhibition of myosin light chain kinase activity (Movsesian et al., 1984), etc. However, consideration of the concentrations required to produce these effects (generally $10^{-6}$ M or more) has lead most investigators to regard these effects as relatively unimportant compared with effects on vascular smooth-muscle and myocardial tissue resulting from inhibition of voltage-operated calcium entry (see Nayler and Horowitz, 1983; Fleckenstein, 1977; 1983; Henry, 1979; Triggle, 1981; 1982).

It is of interest to determine the mechanism of any action of a drug. In order to ascribe an effect of a drug to calcium antagonism a variety of approaches may be taken, since it is often impossible to directly measure calcium entry through voltage-operated channels at the same time as other variables, especially in-vivo. Of fundamental importance is information concerning the concentration range over which calcium antagonism is produced; ideally one would like to know $EC_{50}$ values. For example, if one wished to know whether the antiarrhythmic actions of verapamil and nifedipine were produced by calcium antagonism in the ventricles, one might approach the problem by comparing $ED_{50}$ values for reductions in arrhythmias with $ED_{50}$ (or $EC_{50}$) values for calcium antagonism in ventricles. In order to support this information it might be considered of interest to

investigate the relative calcium antagonist potency of these drugs in a variety of tissues under a variety of conditions. There are a number of studies which have compared several calcium antagonists for their relative potencies in vascular and cardiac muscle, in vivo and in vitro. In order to illustrate some of the problems inherent in determining whether the effect of a drug may be attributed to calcium antagonism, some studies in which nifedipine and (±)-verapamil were compared are summarised. $EC_{50}$ or $ED_{50}$ values were either taken directly from the text of the publications or approximated by interpolation (or extrapolation) of the data presented.

$EC_{50}$ values for inhibition of depolarisation/$Ca^{2+}$-dependent contraction in vascular smooth muscle range from $3 \times 10^{-7}$ M (Kenakin and Beek, 1985) to $2.4 \times 10^{-8}$ M (Millard et al., 1983) for (±)-verapamil, and from $1.5 \times 10^{-8}$ M (Millard et al., 1983) to $6.3 \times 10^{-9}$ M (Kenakin and Beek, 1985) for nifedipine. In all of the studies considered (Fleckenstein-Grun et al., 1976; Lee et al., 1983; Kenakin and Beek, 1985; Millard et al., 1983; Nakayama et al., 1985) nifedipine was more potent than (±)-verapamil. However, the difference in potency varied considerably, from 1.7:1 (Lee et al., 1983) to 350:1 (Fleckenstein-Grun et al., 1976). This may relate to the variety of preparations, test conditions and methods used.

In heart preparations (ventricles and atria) a similar survey reveals an even greater variation. $EC_{50}$ values for (±)-verapamil have been reported to range from $10^{-5}$ M (Nabata, 1976; Raschack, 1976a; Briscoe and Smith, 1982) to $1.5 \times 10^{-8}$ M (Clarke et al., 1984a; 1985). Corresponding $EC_{50}$ for nifedipine range from $7.2 \times 10^{-6}$ (Briscoe and Smith, 1982) to $5.8 \times 10^{-9}$ M (Nabata, 1976). In atria and ventricles nifedipine has been reported to be as much as 62.5 times as potent as (±)-verapamil (Raschack, 1976a), but other investigators have found (±)-verapamil to be up to 5 times as potent as nifedipine (Clarke et al., 1985). Nifedipine was found to be

more potent than (±)-verapamil in 4 studies (Clarke et al., 1984; 1985; Lee et al., 1983; Millard et al., 1983), whereas (±)-verapamil was found to be more potent than nifedipine in 7 studies (Raschack, 1976a; Nabata, 1976; Briscoe and Smith, 1982; Winslow et al., 1983; Kenakin and Beek, 1985; Nakayama et al., 1985).

The data discussed above illustrates that it is dangerous to rely on published reports of the potency and relative potency of calcium antagonists with regard to the investigation of the mechanism of action of calcium antagonists (as antiarrhythmics, for example).

## 1.5 Aims of studies

### 1.5.1 The action of calcium antagonists in acute myocardial ischaemia

The pharmacologist functions to examine and describe the properties of drugs, and then account for the mechanism(s) underlying these effects. In acute myocardial ischaemia, the effects of calcium antagonists (Fleckenstein, 1969) are not well characterised.

The first report of the actions of a calcium antagonist in acute myocardial ischaemia appeared in 1968, when Kaumann and Aramendia demonstrated that 0.79 mg/kg (±)-verapamil abolished arrhythmias and prevented death when administered 10 min before coronary occlusion in anaesthetised dogs. Since this time there have been a number of reports confirming the antiarrhythmic activity of (±)-verapamil and diltiazem in dogs, although nifedipine has generally not been found to be antiarrhythmic (e.g., Guelker et al., 1983; Kobayashi et al., 1983; Clusin et al., 1984). Most of these studies used low doses of calcium antagonists (0.1 - 0.5 mg/kg (±)-verapamil, 0.04 - 0.08 mg/kg nifedipine). The results were highly variable between studies, and a clear exposition of the antiarrhythmic actions of calcium antagonists, and possible mechanism(s) of action remained elusive.

In the development and characterisation of the conscious rat preparation

by our laboratory, it was found that (±)-verapamil and quinidine appeared to have antiarrhythmic activity (Johnston et al., 1983a). It was decided, therefore, to investigate in more detail the classes of antiarrhythmics of which these drugs represent, beginning with class-4 (calcium antagonist) antiarrhythmics such as (±)-verapamil (Singh and Vaughan Williams, 1972), using the conscious rat preparation.

The experiments with calcium antagonists described in the Methods section were designed to answer the following questions:

a. Do calcium antagonists reduce arrhythmias induced by acute myocardial ischaemia in conscious rats?

b. Do calcium antagonists reduce infarct size following permanent coronary occlusion?

c. Do these effects (if any) occur as a result of calcium antagonism?

d. What factors determine these effects?

It was expected that by carrying out experiments blind and assembling dose-response curves for a range of variables, using several preparations (in vivo and in vitro) it would be possible to provide unequivocal answers to questions a and b. In addition, by considering the dose-dependence of the effects of different types of calcium antagonists on the same variables, it was hoped that circumstantial evidence in support of the hypothesis described in question c, or unequivocal disproof of this hypothesis would be provided. By considering the overall results of the experiments, speculative hypotheses concerning question d were expected to be generated.

1.5.2 Arrhythmogenesis in acute myocardial ischaemia

The introduction has broached the subject of arrhythmogenesis in acute myocardial ischaemia. An understanding of the mechanism(s) of arrhythmogenesis is a necessary adjunct to the understanding of the mechanism of action of beneficial drugs. The experiments carried out as part of this thesis

were not designed to provide a definitive statement concerning the determinants of arrhythmogenesis in acute myocardial ischaemia. However, in view of the conflicting evidence concerning the role of the autonomic nervous system in arrhythmogenesis, and the relationship between $i_{si}$ and adrenoceptors (Bean et al., 1984), an attempt was made to resolve this particular question by carrying out a series of graded ablations in the CNS.

In rats, evidence concerning the role of the sympathetic nervous system in arrhythmogenesis is contradictory. Campbell and Parratt (1983) found that a variety of β-adrenoceptor antagonists reduced arrhythmias induced by occlusion in anaesthetised rats. Marshall et al. (1981a) found that isoprenaline infusion increased the severity of arrhythmias while adrenaline and noradrenaline reduced the severity. However, in conscious rats, our laboratory found that propranolol, labetalol and chemical sympathectomy did not influence arrhythmias (Botting et al., 1983). Therefore experiments were carried out in order to answer the following questions:

a. Does the autonomic nervous system play an independent role in arrhythmogenesis following coronary occlusion in rats?

b. Is the role of the autonomic nervous system of sufficient magnitude for it to constitute a major determinant of arrhythmogenesis?

The use of serial ablation in the CNS coupled with selective replacement of catecholamines was chosen as a unique method for answering the questions outlined without resorting to the use of drugs as tools, a practice which often involves unjustified assumptions concerning the specificity and selectivity of drug action. If the autonomic nervous system (particularly the sympathetic nervous system) plays a negligible role in arrhythmogenesis it is important to establish this fact. Such a result has important consequences, since it would be difficult, in this event, to justify the use of β-adrenoceptor antagonists as antiarrhythmics in the prophylaxis of sudden death.

# 2 METHODS

## 2.1 Coronary occlusion in rats

### 2.1.1 Overview

A brief history of coronary occlusion in rats was given in the Introduction. A conscious animal preparation was developed by our laboratory in order to remove the potentially confounding influences of anaesthesia and recent major surgery from the experimental arena (Johnston et al., 1983a). It is our opinion that when investigating the actions of drugs or the nature of a disease process, the initial experiments should be carried out using conscious unrestrained preparations, free from 'artificial' constraints such as anaesthesia. In the case of the conscious rat model of myocardial ischaemia, this approach is consistent with the fact that the human conditions of myocardial ischaemia generally occur in the absence of anaesthetic and concurrent surgery.

The choice of animal species in ischaemia studies and the human spectrum of disease were discussed in some detail in the Introduction. It was stressed that clinically, information concerning the critical first h after the onset of symptoms (when sudden death and ventricular arrhythmias are at a premium), and information concerning the relationship between the extent of ischaemia, its time-course and its sequelae (particularly arrhythmias) are both incomplete at best. Therefore, arguments concerning clinical relevance are essentially pointless, since no human template of myocardial ischaemia and infarction exists.

The philosophy behind the use of the conscious rat preparation is based on a simple premise, namely that the principal aim of a myocardial ischaemia model should be the reproducible and unequivocal production of myocardial ischaemia of known severity and reproducible sequelae, in a manner which can be manipulated simply, whereby the preparation serves as a bioassay. The

rat may be the only species which meets all these requirements (dogs, hamsters and guinea-pigs are too variable, pigs are too large, primates are too expensive and mice are too small).

The following chapters attempt to collate all the methods which have been described, in part, in various publications from our laboratory (Au et al., 1979a; 1979b; Johnston et al., 1983a; 1983b; Botting et al., 1983; Curtis et al., 1984; 1985a; 1985b; 1986a; 1986b; Curtis and Walker, 1986a; 1986b), in an explicit manner which would permit the reader to reproduce the techniques without further reading or assistance.

2.1.2 Preparation

Before preparation, all instruments, leads, lines and occluders were placed in a bath of 70% ethanol in distilled water for antiseptic purposes. In addition hands were carefully washed in soapy water and rinsed in 70% ethanol. As a further precaution against infection, finger nails were cut as short as possible.

All experiments were carried out using male Sprague Dawley or Wistar rats, 230 - 350 g. A glass gas jar was equilibrated with 5 % halothane in oxygen delivered via a vapourisor (Fluotec). For the last 300 - 400 preparations, a humidifier was included in the anaesthetic circuit. This comprised simply of a conical flask containing a saturated saline solution through which the anaesthetic gas was bubbled. The saline prevented the growth of algae and yeasts for periods of at least 9 months. Each rat was placed in the jar 2 - 3 min after halothane had been introduced. Experience had shown that if a rat was placed in the jar before equilibration with halothane, or if a lower concentration of halothane (1 - 3 %) was used, then rats passed through the excitatory plane of anaesthesia more slowly, and exhibited coprophagic behaviour, which compromised the subsequent stage of preparation (intubation).

Induction of anaesthesia took approximately 1 min, but rats were kept in the gas chamber for a further 1 - 2 min to induce deep anaesthesia (exemplified by slow, shallow respiration). Following induction, each rat was intubated as follows. The mouth was fixed open by temporarily attaching the upper jaw to the bench, and a 14 gauge human intravenous catheter (Jelco) was inserted into the trachea with the aid of a paediatric laryngoscope. The sharp point of the metal insert of the catheter had been previously blunted and smoothed for atraumatic location of the catheter. The vocal cords, visible as two white bands on the left and right side of the entrance to the trachea, were used as landmarks. The paediatric laryngoscope had been filed on one side in order to permit atraumatic entry into the small buccal cavity of the rats.

Intubation was verified by observing the condensation produced when the exhaled air was directed onto the surface of the bench. The rats were transferred to a small square metal operating table, maintained on 1% halothane, and prepared with an occluder, intravascular lines and ECG leads, as follows. Anaesthesia was adjusted during preparation such that movement was just prevented.

2.1.2.1 Occluders. In contrast with earlier work in rats in which coronary occlusion was brought about using silk thread (Heimburger, 1946; Johns and Olson, 1954; etc.), our laboratory uses a snare device made from nylon and polythene (Au et al., 1979a; 1979b; Johnston et al., 1983a). The manufacture of the occluder has never been described in detail. Essentially, a guide was made from an 11 cm length of PE10 polythene tubing. One end was flared by brief exposure to heat from a soldering iron, whilst 1 cm from the other end, a flange was made by briefly melting the tubing by rotating it in front of a jet of hot air. The hot air jet was also used in the manufacture of blood pressure and intravenous lines (see below), and was created simply

by passing pressurised air through a thin copper tube over a Bunsen burner. The occluder was made complete by threading a 5.0 gauge atraumatic nylon suture (Ethicon) through the polythene guide such that the needle end of the suture appeared at the flared end of the guide.

The occluder was implanted as follows. A 1 cm skin incision was made over the 4th to 6th ribs on the left thorax. This was enlarged by blunt dissection (Spencer Wells forceps). The forceps were then inserted under the Pectoralis muscle, which was gently separated from the underlying Rectus Abdominus, exposing the Intercostal muscles beneath. Artificial respiration was immediately initiated. Using a Palmer pump, or equivalent, at a stroke volume of 4 ml per rat and a stroke rate of 54/min, the anaesthetic regimen described above was delivered, and controlled using the same criteria described above. Voluntary respiration was switched to artificial by the simple redirection of gas flow through the plumbing of the anaesthesia set-up. The 5th or 6th intercostal space was then punctured using the Spencer Wells forceps. This incision was enlarged by blunt dissection. The choice of 5th versus 6th intercostal space was arbitrary, and generally not noted. If the heart was exposed unfavourably for placement of the occluder, then a second intercostal incision was made. This was necessary in less than 5 % of preparations. The intercostal incision was widened by inserting 4 aluminium retractors attached to braided silk thread or lengths of PE90 polythene tubing. The aluminium retractors were placed carefully in order to avoid damaging the left lung, and were fixed by attachment to the small stainless steel operating table (via slits in its corners). The retractors could be easily moved and repositioned. In later experiments, all retractors were made with polythene attachments, since these were easier to replace (if they broke during surgery) than the braided silk attachments.

By applying gentle pressure under the right thorax, and replacing 1 of

the retractors under the thymus (which is large and overlies the heart in most rats), it was a simple matter to expose the left ventricle and atrial appendage. Using a pair of small blunt forceps, the thin pericardial membrane was retracted at a position overlying the junction between the left atrial appendage and the left ventricle, at which point it could be manipulated easily. Using a second pair of small blunt forceps, a small tear in the pericardium was created. The aluminium rib retractors were then moved, one after another, by inserting their tips through the small pericardial tear, such that the pericardium was now included with the retracted tissue. As a result, the heart was lifted toward the intercostal incision by this pericardial cradle, facilitating subsequent surgery.

Without disturbing the heart by gripping it between the thumb and forefinger (Johns and Olson, 1954), or exteriorising it through the intercostal incision (Selye et al., 1960), the nylon suture of the occluder was sewn under the left coronary artery (generally referred to as the LAD, see Introduction) by inserting the needle into the left ventricle under the overhanging left atrial appendage, and bringing it out high on the pulmonary conus. The atrial appendage was displaced slightly for this purpose by gentle manipulation with a small pair of blunt forceps. The LAD is described as invisible to the naked eye in rats by some Authors (Heimburger 1946; Johns and Olson 1954). These Authors located the LAD using the highly visible coronary veins as landmarks. In our experiments, although the veins were found to be visible, it was usually possible to see the artery quite clearly as a thin pink line emerging from under the atrial appendage. The stitch of the suture was made wide, such that the needle entered and left the myocardium approximately 2 mm either side of the artery. As Heimberger (1946) and Selye et al. (1960) have pointed out, unless the artery is included in the ligation there are no sequelae of consequence. This whole process usually

produced no blood loss. Occasionally (in less than 10% of preparations) there was a little bleeding, amounting to less than 1 ml of blood. If this occurred, 5 min was allowed for clotting, then the thoracic cavity was gently cleared with gauze in order to restore a blood-less field.

Following the sewing of the suture under the left coronary artery, the suture was sewn through the flared end of the guide tubing. Therefore, the suture emerged from the guide tubing, passed into the myocardium, under the LAD, out of the myocardium and back through the tip of the guide tubing, making a loose loop or snare. The needle of the suture was then cut off, and the remaining length of nylon carefully melted down to form a small ball, which prevented the suture from being pulled back through the flared tip of the guide tubing. The nylon suture extended the full length of the guide tubing. The size of the loop at the end proximal to the heart was adjusted by pulling on the suture at the end distal to the heart. It was found in some early experiments that occlusion sometimes resulted in part of the thymus being caught in the occluder. This could be completely prevented by making the loop small, such that the flared tip of the guide tubing was positioned adjacent to, or just underneath the atrial appendage. Once the size of the loop had been adjusted, the distal length of suture was melted down to a small ball adjacent to the distal tip of the guide tubing, in a manner similar to that by which the proximal end of the suture had been affixed.

The Pectoralis muscle was sutured lightly to the Rectus Abdominus muscle with silk. The loose ends of the silk suture were then tied to the occluder to fix it in position in the thorax. In all but the last 50 - 60 preparations, the pneumothorax was evacuated at the time the chest was closed by inserting a length of PE90 polythene tubing through the intercostal incision, and applying negative pressure to the thorax as the chest was closed. This

precaution was recently successfully replaced by hyperinflation of the lungs by transiently increasing stroke volume to 6 ml per rat as the chest was closed. This modification reduced the time taken to prepare the rats.

As soon as the chest was closed, the halothane anaesthetic was replaced with 100 %oxygen. The occluder was then fed subcutaneously to the subscapular region using a stainless steel trocar. The occluder was exteriorised between the shoulder blades, and a silk suture was attached to the occluder just below the flange at the distal end (relative to the heart). This piece of silk was designed to serve as a 'flag' to assist in the location of the occluder on the day of occlusion. The length to which the occluder projected from the subscapular region was limited to no more than 1 cm, in order to reduce the likelihood of the occluder being chewed or tugged at by the rat during the week of recovery from surgery. This was arranged by pulling on the occluder with a pair of small forceps. In later experiments the occluder was allowed to slip just beneath the skin, with only the silk 'flag' exposed. The rat was then turned onto its back, and the chest wound was infiltrated with Cicatrin (bacitracin, neomycin and streptomycin) powder, and Marcaine (1% bupivacaine).

The skin over the Pectoralis muscle was closed with a purse-string suture of silk. The rat was disconnected from the respiratory pump, and allowed to breath spontaneously. The interval between closing the thoracic incision and closing the skin incision was generally approximately 2 - 3 min, and since the rat was receiving only oxygen during this time, sufficient halothane was expired to permit the respiratory centre to override the artificial respiratory rhythm, such that once the rat was disconnected from the respiratory pump, it immediately began to breath spontaneously. However, the rat generally remained immobile for a further 2 - 5 min. During this time, Cicatrin and Marcain were infiltrated into the subscapular wound, and the

other exteriorised leads and lines (see below) were tidied up.

The location of the occluder was the last stage in preparation, but was described first since it is the focus of the experiment. After the induction of anaesthesia, the first stage of preparation was the location of the blood pressure and intravenous lines.

2.1.2.2 Leads and lines. Blood pressure and intravenous lines were made from PE polythene tubing, using the hot air welding technique described for manufacture of the occluder. A 14 cm length of PE50 tubing was welded to a 10 cm length of PE10 tubing by rotating and melting their tips, then pressing them together. This process was carried out after the lengths of tubing had been threaded onto a length of thin wire, in order to prevent the welding process from occluding the lumen. The seal between the tubings was tested by closing the open end of the PE10 tubing, attaching a needle and air-filled syringe to the open end and applying pressure to this, ostensibly closed system, while it was submerged in distilled water. An incomplete seal was revealed by bubbles emerging from the junction of the tubings. A curl was put into the PE10 end of the line, by looping it around a glass rod and submerging it in boiling water for 3 sec. The loop was fixed by submerging it in ice cold water.

The lines were implanted using a modification of the method developed by Weeks (Weeks and Jones,1960; Weeks, 1981). A midline laparotomy was performed, extending from approximately 1 cm posterior to the xiphoid process to the region overlying the bifurcation of the common iliac vessels. Using fingers only, the connective tissue overlying the abdominal aorta and vena cava was cleared. A silk thread was then placed round these vessels, using a pair of small forceps, in the standard manner. The thread was usually positioned in the region of the bifurcations of the renal veins and arteries, at which point the small forceps passed under the aorta and vena cava most

easily. A more posterior approach occasionally produced haemorrhaging from the vena cava. Whilst this was rarely fatal, and usually resolved without sequelae by the time of occlusion and sacrifice, it was deemed inconvenient (5 min was allowed for clotting if ever haemorrhaging occurred, and this, naturally, delayed surgery). Therefore in most of the last 500 - 600 preparations the aorta and vena cava were accessed from the former, anterior approach.

Once the aorta and vena cava had been located, these vessels were separated using small forceps. Again, this procedure was most easily accomplished when the more anterior approach was made. The thread was divided such that tension on one or the other of the resultant ties would stop flow in either the aorta or the vena cava. The lines themselves were then located in the rat as follows. Using the same trocar which would subsequently be used for locating the occluder, a channel was created through the Psoas muscle and subcutaneously to the subscapular region. With the trocar in place, two lines were threaded into this channel, and the trocar was then removed, leaving the lines in place. A syringe full of saline (without heparin) was attached to each of the lines at their exit point between the shoulder blades (note that this exit point was used subsequently for exteriorising the occluder). The abdomen was then retracted on the left side (using two retractors in the same manner as for the placement of the occluder).

Both the lines at the end proximal to the blood vessels (the PE10 ends) were severed, using a pair of sharp scissors, to produce a pointed bevel to facilitate the subsequent insertion into the vessels. Usually, the vena caval line was located first. The air in the line was displaced with saline, and the line was lightly held with a pair of small forceps. The vena cava was temporarily occluded with the silk thread, and a tiny hole was created

distal to the occlusion (proximal to the heart), just anterior to the renal veins. The line was then inserted into the hole, and the occlusion removed. The process was repeated for the placement of the aortic line, with the exception that the hole, distal to the occluder, was also distal to the heart. In both the case of the vena caval and also the aortic line, the PE10 tubing was inserted 2 - 3 cm into the vessels, pointing towards the heart.

The lines, and their placement, were designed in order that blood pressure recording and drug administration could be undertaken via functioning (non-occluded) vessels. The holes in the vessels were made using a 27 gauge hypodermic needle which had been bent to an angle of approximately 120 °. The hole was of a smaller diameter than the PE10 tubing, such that the lines could be moved freely without blood leakage. In addition, the small diameter of the PE tubing did not occlude the aorta or vena cava; this was obviously crucial, since these were recovery rats. The aorta and vena cava were used because they are large, easily accessible vessels, suitable for cannulation with chronic indwelling non-occluding lines.

The abdomen was dusted with Cicatrin, and the body wall closed with a running stitch. The skin was then closed, also with a running stitch, and the wound was infiltrated with Cicatrin and Marcaine. Following placement of the occluder by the method described above, the exteriorised ends of the cannulae were treated as follows. Silk flags were attached to each cannula in the same way that a flag was attached to the occluder. Approximately 0.3 ml of saline was then injected into the vena caval line, which was abruptly clamped with a pair of Spencer Wells forceps, the tips of which had been made atraumatic by encasement in soft polythene tubing. The open end of the exposed PE50 portion of the cannula was then sealed by melting, using an ordinary cigarette lighter. The same process was carried out for the aortic

line. The melted seals were flared such that their diameters were approximately double that of the PE50 tubing. The lines were allowed to extend from between the rats' shoulder blades by about 1 cm. As in the case of the occluder, the lines sometimes slipped beneath the skin during the interval between preparation and occlusion; the silk flags permitted easy location of the lines, and their position under the skin was considered acceptable, since the cannulae were therefore out of the reach of the rats. In general, however, the flared, melted ends of the cannulae prevented them from slipping completely under the skin.

The rats appeared to be unconcerned by the presence of the exteriorised cannulae and the occluder. There was an extremely low incidence of infection around the exit site (fewer than 1 % of rats had pus exudation from the wound), presumably as a result of the prophylaxis with Cicatrin.

ECG leads were prepared from teflon-coated stainless steel wire. Approximately 1 cm of one end of each lead was de-insulated using an ordinary cigarette lighter flame. The lead was approximately V3. The chest lead was implanted with the occluder, as follows. A tight ball was made in one end of the lead by wrapping it around a 21 gauge hypodermic needle. This end was then positioned underneath the Pectoralis muscle, using the 21 gauge needle as a trocar. The distal end of the chest lead was exteriorised with the occluder between the shoulder blades. The large trocar used for this purpose was directed through the hole made earlier for the exteriorisation of the intravascular cannulae.

The limb leads were implanted on the day of occlusion. They were made of the same material as the chest lead, but were positioned in a slightly different manner (see below).

After preparation, the animals were placed in individual cages and given tap water and Purina rat chow ad libitum. The body weight of each rat was

recorded before preparation. The procedures described usually took approximately 25 - 40 min to complete, from induction of anaesthesia to recovery of consciousness.

2.1.3 Coronary occlusion

This section deals with the experiment proper, which was carried out approximately 7 days after preparation (range 4 - 15 days). The sequence of events is described below. The actual process of occlusion was as follows. The occluder was gripped by the Spencer Wells forceps with the atraumatic tips (see above) just above, and adjacent to the flange located approximately 1 cm from the end distal to the heart. The purpose of the flange was to provide a stock for the forceps which would permit the outer guide of the occluder to be held firm without excessive clamping, such that the inner snare of the occluder remained free to move within the outer guide. Using fingers, the small bobble on the distal end of the inner snare of the occluder was pulled from the adjacent outer guide tubing to the extent of approximately 2 mm. A second pair of forceps was then used to firmly grip the exposed inner snare of the occluder. Traction was then applied smoothly between the inner snare and the outer guide tubing to produce occlusion. Successful occlusion occurred when the outer guide tubing became crinkled and it was no longer possible to move the inner snare in relation to the outer guide. This moment was designated time zero. The atraumatic forceps were clamped down firmly on the occluder at this moment, and the length of exposed inner snare of the occluder was melted down with a soldering iron to form a bobble adjacent to the distal end of the outer guide tubing, fixing it in place.

Time zero was accompanied in most cases by a sudden change in the ECG, but this was not taken as a criterion for occlusion, since it was possible that drug treatment could delay the ECG changes caused by occlusion. Never-

theless, in the present experiments it was found that an absence of ECG changes within 15 min of occlusion was associated in every instance with an absence of an occluded zone (measured upon sacrifice, see below), indicating that occlusion had been unsuccessful. This event occurred in less than 5% of rats.

2.1.3.1 Sequence of events. The sequence of events on the day of occlusion is outlined in Figure 1. Rats were anaesthetised with halothane in the manner described above, and allowed to breathe 1.5% halothane through a small face mask. The limb leads of the ECG (made in the same way as the chest lead) were then placed as follows. Each wire was threaded through a 23 gauge hypodermic needle, such that the non-insulated tip projected approximately 0.5 cm from the sharp end of the needle. The wire was then bent back cleanly, forming a barb. The needle, together with the electrode was passed subcutaneously, either to the elbow region of the forelimb (dorsal aspect) for the 2 forelimb leads, or to the right anterior dorsal flank region for the right hind limb lead. The entry point was the same for all three leads, namely approximately 0.5 cm anterior to the exit point of the occluder and intravascular lines. The ECG leads were disconnected from the hypodermic needle by pinching the skin around the needle tip and carefully withdrawing the needle. As the needle was withdrawn, the electrode remained in place, owing to the action of the barb.

In approximately the first 50 preparations, the limb leads were implanted on the day of preparation. Although they did not appear to cause discomfort, they were sometimes removed by the rat during the interval between preparation and occlusion. Therefore, it was considered sensible to implant limb leads on the day of occlusion in every case. In this way an element of consistency was introduced, which was absent if some rats were used with fresh electrodes placed on the day of occlusion, and some not.

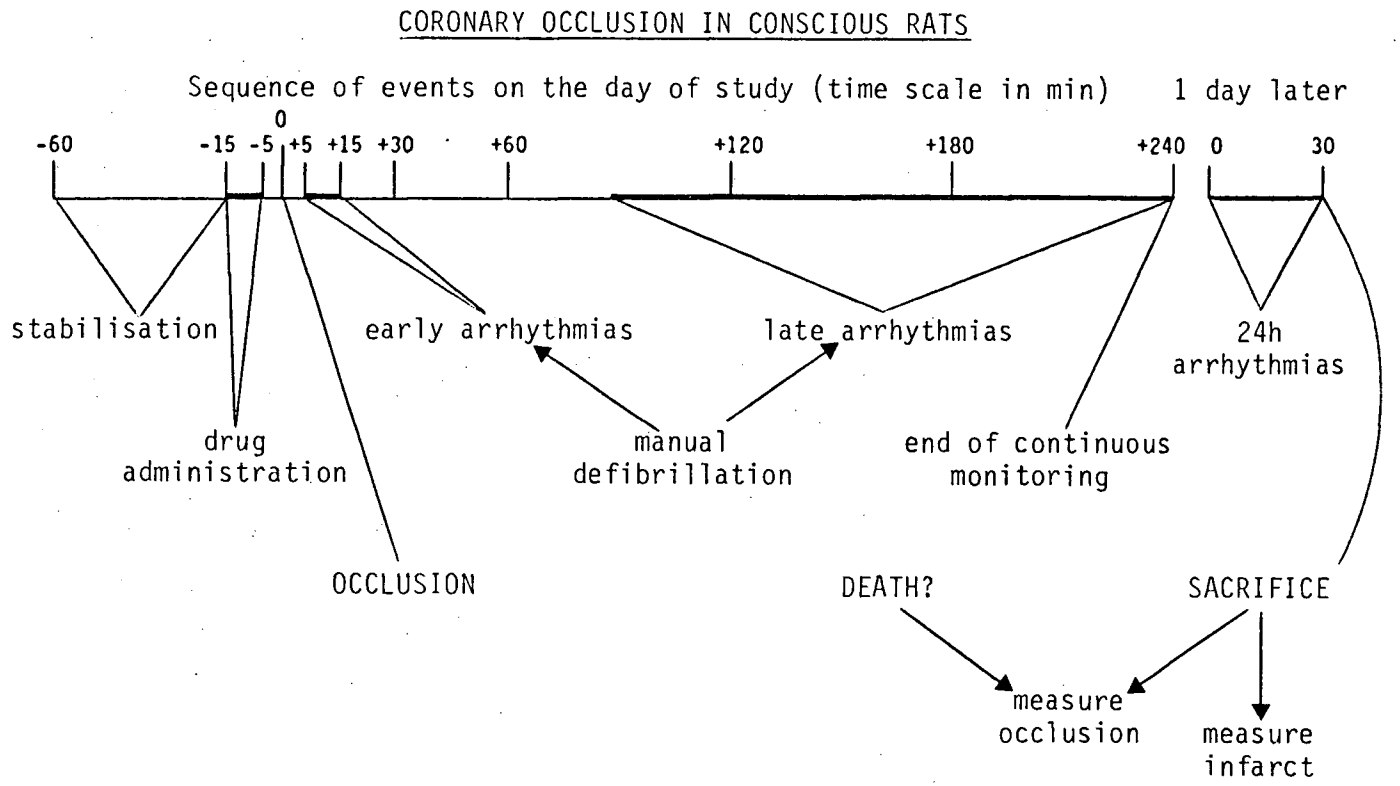



Figure 1. Sequence of events on the day of occlusion following connection of leads and lines

The 3 limb leads were connected to one another by simply twisting their exposed ends together, forming a composite whole body lead, which was trimmed to approximately 2 cm length using a pair of scissors. The teflon was removed from the end of the composite lead as described above. It should be noted that the anaesthetic mask was temporarily moved well away from the rat during this procedure. The un-insulated leads were connected to 1 of 2 lengths of enamel-coated stainless steel wire by twisting the wires together. The tips of the wires had been scraped with a scalpel blade to remove the insulating enamel. The connections were isolated by applying a small piece of surgical tape to each. The tape was attached in such a way that it could easily be removed at a later time. Additionally in this regard, when the leads and wires were connected, the twist was always clockwise, facilitating subsequent disconnection.

Whilst the rat was still anaesthetised with halothane, the blood pressure line was connected to a pressure transducer via a length of PE tubing and a blunted 23 gauge needle tip. The intravenous line was connected to a syringe full of saline in a similar manner. Both lines were flushed with approximately 0.3 ml saline. The intravenous line was indistinguishable from the arterial line by visual inspection, but once the melted tip of the line had been snipped off, the lines were easily distinguishable in most cases by the fact that blood immediately filled the aortic line, but not the vena caval line. In the infrequent event that blood did not flow back through either line, both lines were flushed with saline until a distinction could be made. The aortic line was kept open by attaching a leak pump in series with the line, according to the method of Weeks (1981). In at least 75% of cases, negative pressure on the vena caval line caused dark venous blood to appear in the line. In the remaining 25% of cases, the venous line was tested by the injection of a small amount of adrenaline which elicited a small pressor

response when the line was patent.

The placement of the ECG leads and the connection of the blood pressure and intravenous lines to the transducer and syringe of saline, respectively, took no more than 10 min. Rats regained consciousness upon termination of anaesthetic generally within 30 sec, and were fully alert within approximately 2 - 5 min. Blood pressure and the ECG were displayed on a Grass Polygraph (4 channel, model 7), and ECG was also displayed on a delayed loop oscilloscope with a 4 sec delay and a 4 sec real time display (Honeywell Type E for M). The latter was particularly useful for the diagnosis of arrhythmias (see below). The ECG channel was calibrated once per week; little adjustment was ever required. The blood pressure channel was calibrated every time it was used.

After approximately 1 h, the administration of drugs, if any, and coronary occlusion were carried out while the rat was fully conscious and not restrained in any way. The procedure was as follows. Intravenously administered drugs were injected slowly over a 10 min period. A fast (100 mm/sec chart speed) record of ECG and blood pressure was taken before drug administration and 4 min after completion of injection. One min after the second fast recording of blood pressure, the occluder was tightened, as described above. Occlusion did not appear to cause the rats any distress. No changes in behaviour ever occurred during the first few min after occlusion, unless blood pressure fell precipitously following occlusion (in which case the animal became subdued), or unless acute pulmonary oedema, (characterised by laboured respiration) developed. In general, the first behavioural response to occlusion was sudden convulsive-type behaviour which occurred in conjunction with VF (see below).

The rats were monitored for at least 4 h following occlusion. Fast speed polygraph recordings were made every min for the first 15 min, every 5 min

for the following 15 min, then every 15 min. If the rat survived for 4 h, it was disconnected from the ECG wires and blood pressure transducer by reversing the processes used for connecting the animal to these recording devices. The animal was conscious and not restrained during this process. Usually the rat was subdued at this time as a result of the sequelae of occlusion (see below) and was submissive and compliant enough for this disconnection to be completed without any difficulty.

After 24 h, if the rat was still alive, it was reconnected to the blood-pressure and ECG recorders and carefully monitored for a further 30 min, whereupon it was sacrificed. Reconnection to the recording devices was carried out in the same way as the initial connection the previous day, with the exception that the rat was not anaesthetised. Instead, it was placed on the bench and wrapped in a lab coat, with only the subscapular region exposed. Rats, like many rodents, do not struggle when they cannot see. In this manner the lines and leads were reconnected without incident. It is worth mentioning that in early experiments, when the limb leads were implanted on the day of preparation along with the occluder and blood pressure line, etc., it was routine to connect all rats to the blood pressure and ECG recording devices in this manner (i.e., when the rat was conscious) on the day of occlusion.

Upon death or sacrifice, the heart was excised and the occluded zone and infarct size were determined (see below), and a general postmortem examination was performed (the appearance of the lungs, liver, kidneys, spleen, bladder and snout were noted, and any gross abnormalities were recorded). Exclusion criteria (see below) were considered at each stage of experimentation.

2.1.3.2 Monitoring of responses to occlusion. As described above, the ECG and blood pressure were recorded continuously for 4 h following

occlusion, and again at 24 h after occlusion. Behavioural changes were noted in a general way, and any peculiarities were recorded. Most of the techniques described below have been described in brief previously (Au et al., 1979; Johnston et al., 1983a; Curtis et al., 1984; 1985b). All information concerning each rat was recorded permanently on individual analysis sheets.

2.1.3.3 Occluded-zone (OZ). The OZ was recorded for every rat. In 24 h survivors, sacrifice was undertaken by delivering a blow to the head with a metal cosh. No anaesthetic was used. The heart was excised and perfused via the aorta according to Langendorff (1895) with saline (0.9 %). Once blood was no longer present in the perfusate, the saline was replaced with saline containing Indocyanine (Fast Green dye, BDH) 0.5 g/l. Approximately 20 - 50 ml of this solution was allowed to pass through the coronary circulation. The delivery of the 2 solutions, saline and Fast Green dye was controlled using a T-tube device connected to 2 large reservoirs containing each solution, and was regulated with a simple glass 2 by 2-way stopcock.

In the case of animals which died overnight, care was taken not to dislodge the stasis thrombi present in the left ventricle. Perfusion with approximately 20 ml Fast Green dye sometimes took 5 - 10 min in such hearts, compared with the 2 - 3 min in the case of hearts from freshly dead animals. However, generalised clotting in the coronary circulation had probably not occurred, because the hearts from rats dying overnight provided OZs as well defined and of a similar extent to those from freshly dead animals. It is more likely that perfusion rate was reduced as a result of the contracture which was a common feature of hearts removed from rats dying overnight since, with reference to the effect of systole, coronary perfusion is highly sensitive to the contractile state of the myocardium.

After perfusion, the heart was removed and processed. First of all, the atria, aorta and pulmonary vessels were removed and discarded. Delineation

of the remaining ventricular myocardium into normal and occluded tissue was made by visual inspection. The two zones were almost invariably well differentiated with a clear-cut border, as might be expected on the basis of the reported lack of collaterals in rat hearts (Johns and Olson, 1954; Maxwell et al., 1984; Winkler et al., 1984). Using a small pair of scissors the perfused (green) and non-perfused (pink) tissues were separated, lightly blotted and weighed.

2.1.3.4 Infarct zone (IZ). The infarct size was only determined for animals which survived for 24 h. In this regard, it has been shown that an interval of at least 10 h must be allowed after occlusion for infarct size (measured in the manner described below) to be sufficiently developed for quantification in rats (Hort and Da Canalis, 1965a). Infarct size was determined by a modification of the method of Jestadt and Sandritter (1959), based on the production of formazan red by the reduction of 2,3,5-triphenyl-tetrazolium (TTZ) by $H^+$ emanating from NAD- or NADP-linked dehydrogenases. The normal and occluded tissues were cut into strips approximately 2 mm wide and incubated with TTZ (Fischer Scientific) 0.1 g in 10 ml phosphate buffer at 37 °C. The buffer was made by dissolving 25 g $Na_2HPO_4$ and 0.5 g $NaH_2PO_4$ in 2 l distilled water (pH adjusted to 8.5 - 8.6 with 0.1 M NaOH). Incubation was carried out for approximately 10 min (incubation was terminated when the strips of non-occluded tissue had turned deep purple from the formazan red reaction, rather than after a specific time).

In later experiments, the OZ tissue was cut not into strips, but into 2 discs by slicing through the subendocardium. The resultant discs were no more than 2 mm thick (by visual inspection) at any point. The rationale for this approach was to investigate whether the endocardial and epicardial surfaces were salvaged to any extent as has been suggested by Hort and Da Canalis (1965b) and others. Although no quantitative data was assembled, it

appeared that the endocardial surface was usually not infarcted (white) at 24 h, and occasionally parts of the epicardial surface were also not white, although a gentle scrape with a pair of sharp scissors was always sufficient to remove this 'salvaged' tissue. In addition, by separate weighing of this tissue it was found that its presence changed the infarct size by only 2 to 3 %.

Infarct size was quantified by separating the white and purple tissues and weighing them. However, the tissue was first fixed for 2-3 days in 10 ml formal saline (made by dissolving 3.56 g NaCl and 125 ml 40% formaldehyde in 375 ml distilled water). It has been shown that the infarct, as detected by this method, corresponds with the histologically identified infarct (Fishbein et al., 1981).

2.1.4 Definition of occlusion-induced arrhythmias

2.1.4.1 Introduction. In order to investigate the effects of treatments on a variable, an unequivocal definition of that variable is necessary. In the case of occlusion-induced arrhythmias, it is generally the case that variations exist in definitions. While these differences may not necessarily be important in themselves, they make direct comparison between studies difficult to interpret in some cases. Although there is variation in the distinction between a run of PVC and VT, and differences in the quantification of PVC, the main source of contention lies in the distinction between VT (particularly that of the torsade de pointes variety) and VF.

VF was first described by Erichsen (1842) in terms of the contractile behaviour of the myocardium (this was more than 60 years before the development of the ECG). VF has been defined in various ways. Moe et al. (1964) defined VF as chaotic, asynchronous fractionated electrical activity. Bigger (1980) gave an operational definition, based on the characteristic ECG

pattern of 'the absence of QRS complexes and T waves and the presence of low-amplitude baseline undulations'. These definitions are not amenable to quantitative differential diagnosis because they are subjective definitions, and they do not differentiate between abrupt VF and VF occurring after a run of VT. This is not really a serious problem clinically; the distinction between irregular torsade de pointes, ventricular flutter and VF is, perhaps academic, since all 3 arrhythmias are associated with cardiac output below that necessary for the maintenance of life, and the primary concern is the prevention of mortality. However, more rigorous definitions are necessary experimentally.

Abrahamsson's group who induce myocardial ischaemia in rats define VF as asynchronous disorganised electrical activity of at least 5 sec duration (Abrahamsson et al., 1985). While this definition introduces an important caveat, namely that some indication concerning the duration of the phenomenon may be useful in diagnosis, it nevertheless fails as a truly objective definition, since how does one define 'asynchronous' and 'disorganised' ? Opie's group have defined VF as 'total irregularity of morphology of the repetitive ectopic complexes for at least 6 cycles' (Lubbe et al., 1978), introducing once more the notion that VF is only VF if disorder is present for an arbitrary but specified duration. Clearly there is a subjective element in the diagnosis and definition of VF. This is an important point and attempts to deal with this problem are discussed below.

It is unfortunate that many researchers do not appear to have a rigid set of criteria for defining arrhythmias. A glance through the literature will reveal that most researchers do not define what they mean by VT and VF. In particular, it is often unclear by what criteria torsade de pointes and VF are differentiated, and how many consecutive PVCs constitute a run of VT. If PVC, VT, torsade de pointes, flutter and VF represent a continuum,

as was once believed (e.g., Harris, 1950), then the distinction between these arrhythmias would not be seriously important; a drug which reduces PVC would be expected to reduce VT and VF as well. However, there is some indication from studies with animals (Dresel and Sutter, 1961) and from follow-up investigations in healthy humans with a high frequency of PVC (Kennedy et al., 1985), that PVC, VT and VF are not necessarily part of a continuum. Therefore the possibility exists that a drug may influence selectively one type of ventricular arrhythmia but not another. The latter issue is neither proven nor disproven at present, and wholesale re-classification of drugs as 'classical antiarrhythmic agents' and 'antiarrhythmic/antifibrillatory agents', with selectivity for VF versus PVCs (Anderson, 1984) is somewhat premature.

Irrespective of whether ischaemia-induced ventricular arrhythmias represent a continuum or not, it is nevertheless important in any experiment to define endpoints. Since arrhythmia definitions vary from one investigator to another, all one can aim for is internal consistency at present. Before describing the criteria which were used for defining ventricular arrhythmias produced by coronary occlusion in rats, the subjective nature of classification of arrhythmias must be reiterated. No matter what definition is chosen for VF and VT, there remains an element of subjectivity, particularly with regard to differentiation between torsade de pointes and VF.

A simple way around the problem of subjectivity is to carry out studies using a blind and random protocol. In this manner, inconsistencies in definition should be spread randomly and evenly between study groups, and conscious and unconscious bias should be eliminated. It must be stressed that attempts were made to carry out all the experiments described in a blind and random manner, where possible.

The following definitions were used in the diagnosis of arrhythmias following coronary occlusion in rats.

2.1.4.2 Premature ventricular contractions (PVC). PVC were defined as premature QRS complexes occurring independently of the P wave. PVC were generally accompanied by a transient drop in aortic blood pressure. Owing to the high frequency filtering of the ECG by the Grass polygraph, no attempt to measure coupling intervals was undertaken. Measurement of coupling intervals is believed to give an indication of whether the PVC is reentrant or automatic (see Introduction). However, it was considered that the equipment available was not amenable to the routine measurement of coupling intervals, since measurement of such by hand would be extraordinarily time consuming. Only singlets, doublets (bigemini) and triplets were counted as PVCs. Longer runs were recorded as VT (see below). It is noteworthy that other workers include every deflection in a run of VT as a PVC (Kane and Winslow, 1980; Clark et al., 1980). This manoeuvre is based on the assumption that PVC and VT are identical in mechanism and drug sensitivity. While our definition implies that singlets, doublets and triplets represent the 'same' arrhythmia, we only associate these arrhythmias for analytical convenience; since the incidence of doublets and triplets is variable and lower than the incidence of singlets, a useful investigation of the effects of a drug on these individual types of arrhythmia would require an increment in the study group size. It was considered that the cut-off value of 4 PVCs, which was low, removed the possibility of spurious amalgamation of arrhythmias which are not necessarily the product of a common electrophysiological mechanism (namely short runs of VT and long runs of PVCs). In other words, in the absence of clear evidence concerning the point at which a run of PVCs ceases to be PVCs and becomes VT, it was decided to artificially segregate PVCs and VT. Essentially, since one cannot be sure whether VT and PVCs are

expressions of the same arrhythmia or not, it is sensible to separate them for analysis by generating an arbitrary cut-off point.

2.1.4.3 Ventricular tachycardia (VT). VT was defined as a run of 4 or more consecutive PVC. No restriction was made on the associated rate. This definition differs from that of other workers who measure ischaemia-induced arrhythmias in rats. VT has been defined as 7 or more PVCs at a rate of > 600/min (Kane et al., 1980), heart rate exceeding 500 beats/min (Lepran et al., 1981b), 7 or more PVCs, no limitation on rate (Fagbemi and Parratt, 1981b; McLennan et al., 1985) and 5 or more PVCs (Mertz and Kaplan, 1982; Manning et al., 1984; Daugherty et al., 1986). Clinically, VT has been defined as 3 or more consecutive PVCs (Lown et al., 1973), and this definition has been adopted by Verdouw's group in their work with pigs (Verdouw et al., 1978).

2.1.4.4 Ventricular fibrillation (VF). VF was defined as disorder in the ECG accompanied by a precipitous fall in blood pressure. In addition, since defibrillation was undertaken in all rats experiencing 10 sec of VT or VF, a time constraint was imposed in order to differentiate between 'primary' VF and VF 'secondary' to VT, namely that disorder had to have begun within 10 sec of the last sinus beat for the arrhythmia to be classed as VF, otherwise the arrhythmia was classified as VT. Disorder was defined as an irregular cycle length with no identifiable QRS complex. As discussed above, the word 'disorder' lends an element of subjectivity to the definition. Bias was eliminated as far as possible by blind and random experimental design.

In the introduction to this subsection, a variety of operational definitions of VF were given, and it was suggested that the timing and duration of 'disorder' needs to be defined, as well as the actual nature of 'disorder'. Some Authors have avoided the question of the definition of VF altogether. Winslow initially reported the incidence of VF in rats following occlusion

(Kane and Winslow, 1980), but in later studies (Marshall et al., 1981a; 1981b; 1981c; Marshall and Winslow, 1981; etc.) VT and VF were combined, and the arrhythmia was called 'fibrilloflutter'. Northover has adopted a similar strategy, avoiding 'arbitrary and subjective distinctions' between VT and VF by simply recording the time spent in the 'combined forms' (Northover, 1985). Other groups have taken a completely different approach, defining VF in absolute terms. For example, Harron et al. (1985) defined VF as a run of ectopic beats with a rate of 720/min or more.

It is evident that there is a lack of consistency between research groups in terms of definition of VF. The method used in the current experiments is simply a definition of VF which was felt to be reasonably consistent with clinical definitions, and more importantly, amenable to routine use, internally consistent, but not obsessively unequivocal to the point of absurdity (see the definition used by Harron et al., 1985, above).

Despite the different classifications used, the results obtained by various groups with $Na^+$ channel blocking drugs (see Discussion) are extremely consistent, all studies demonstrating antifibrillatory activity for quinidine and Org-6001, for example, suggesting that the variability in arrhythmia classification appears not to compromise investigations, in terms of the recognition of antifibrillatory drugs. However, the variability in control incidence of VF within research groups suggests that there may be an element of internal inconsistency. This may relate to the fact that apart from ourselves, research groups do not appear to carry out their studies using blind and random protocols. This may give rise to some misleading information, particularly concerning drugs with weak antiarrhythmic actions, as a result of the loss of precision of endpoints.

2.1.4.5 Other arrhythmias. Although VF, VT and PVC are the arrhythmias of major interest and importance, all arrhythmias following

occlusion are recorded. The incidence of non-ventricular (and non-nodal) arrhythmias is, however, extremely low in control rats, and it is not considered worthwhile investigating them in a quantitative manner. Atrio-ventricular (AV) blocks occur from time to time, particularly in association with pulmonary oedema-induced gasping. Anecdotal observations have strongly suggested that gasps in rats with severe pulmonary oedema are coupled, 1:1 with Moebitz Type-2 AV block. Presumably the AV block is the result of a vagal reflex.

All types of AV block and dissociation have been observed. Third degree AV block is usually only seen in rats experiencing persistent and severe respiratory distress (exudation of sputum), and according to the exclusion criteria (see below) are not usually included in studies.

Sinus bradycardia and tachycardia do not occur very often during the first 4 h after occlusion, but both have been observed in 24 h survivor rats.

Atrial arrhythmias are extremely rare. It is difficult to distinguish between atrial flutter and fibrillation using the V3 lead, but some sort of atrial 'fibrilloflutter' has been observed (in fewer than 1% of rats).

2.1.4.6 Rationale for defibrillation. VF is a serious arrhythmia which is life-threatening if it does not spontaneously revert. It is well established that rats can spontaneously defibrillate (e.g., Johnston et al., 1983a). However, between approximately 25% (Kenedi and Losconci, 1973a) and 95% (Siegmund et al., 1979b) of control rats which experience VF die from VF (values being dependent on whether conscious or anaesthetised animals are used, and on how VF is defined). Therefore, it is expected in any study that the sample size will vary with time, as animals die, and will also vary between groups. This means firstly that ultimate group sizes will vary, compromising the value of statistical tests, secondly that group size may be so small in controls that meaningful comparisons cannot be made, and thirdly

that much information concerning the time course and the interrelationships of variables is lost as animals die during the course of the study period. This censoring can only be eliminated if the animals are all kept alive by defibrillating VF as it occurs.

In all rats an attempt was made to revert all episode of VT and VF lasting longer than 10 sec by thump-version. VT was reverted as well as VF because many (but not all) episodes of VT reduce blood pressure to close to zero in a manner analogous to that seen with VF; it was considered that in order to preclude the generation of arbitrary definitions concerning the 'severity' of VT, all VT would be reverted after 10 sec in the manner used for VF. The procedure was as follows.

After 10 sec of continuous VF or VT the rat was lifted out of the home cage by the tail. Within a few seconds convulsive-type behaviour (see section concerning preliminary screen) ceased and syncope ensued, whereupon the chest was flicked with the index finger. Usually only 1 or 2 flicks were required to revert the arrhythmia to sinus rhythm. If reversion did not occur immediately then the flicks were continued. Reversion was diagnosed by the combination of a sudden return to consciousness, a sudden increase in aortic blood pressure and the termination of the arrhythmia (as seen on the ECG record).

Because 10 sec was the maximum time allowed before defibrillation was initiated, differentiation between VT and VF was only attempted during the 10 sec period of the arrhythmia. Of course, if VT were allowed to continue for longer than 10 sec, it may possibly degenerate to VF. Therefore our method of diagnosing VF may lead to an underestimation of VF compared with other workers who do not use defibrillation. To our knowledge, only 1 other group attempts thump-version, Charnock's group (McLennan et al., 1985), using our technique.

Although thump version greatly reduces censoring due to arrhythmia-induced mortality, it introduces a new form of censoring, namely that hitting the chest may 'change things' compared with rats which have not experienced VF. This possibility was examined by our laboratory (unpublished observations) and it was found that defibrillation reduced the incidence of mortality from VF, but did not influence the incidence of VF itself. In other words, defibrillation does not influence the likelihood of subsequent VF. Therefore it was considered that the benefits of thump-version outweighed the possible disadvantages.

Thump-version creates 2 classes of VT and VF, that which spontaneously reverts to sinus rhythm within 10 sec of onset (SVT and SVF) and that which does not spontaneously revert before thump-version (NVT and NVF). The generation of subclasses of VT and VF may be irrelevant in terms of analysis; while we continue to note the incidence and $\log_{10}$ number of episodes of VT, SVT, NVT, VF, SVF and NVF, we have found that drugs which influence SVF also influence NVF, and drugs which influence SVT also influence NVT. Therefore it is reasonable to assume that SVT and NVT are identical in terms of mechanism of generation; the same can be said for SVF and NVF. This is an important point because it implies that spontaneous reversion of VF to sinus rhythm in rats is not a disadvantage of the preparation. Moreover, the occurrence of SVF, and the readily revertable nature of NVF (see results) means that most animals will survive the period of continuous monitoring such that censoring produced by early mortality is reduced.

2.1.4.7 Arrhythmia scores. In quantifying arrhythmias, many workers record their incidence, number, type and duration. Such information has limited value since the number of episodes of VT and VF are $\log_{10}$-normally distributed variables (Johnston et al., 1983a); this fact is not taken into consideration by other workers in the field. In addition, thump-version may

influence the duration of VT and VF compared with the values found by other investigators. In the hands of workers who do not revert VT and VF, the mean duration of VF is dominated by the terminal event. Intuitively, such measures of duration of VF should be $\log_{10}$ normally distributed for this reason. However, correction of data is never undertaken (Clark et al., 1980; Kane and Winslow, 1980; etc.). In our laboratory, it was found that even with thump-version, the durations of VT and VF were $\log_{10}$ normally distributed (Johnston et al., 1983a). Nevertheless, since thump-version clearly censors the durations of VT and VF, it is not reasonable to completely rely upon such variables for antiarrhythmic quantification. There is another reason for this conclusion; if a drug abolishes VF then there is no mathematical value for $\log_{10}$ VF duration. If an arbitrary value of 1 sec is given to rats not experiencing VF then the result is a standard deviation of zero in the group in which VF is absent, creating a variance inhomogeneity which invalidates the use of standard parametric statistical tests (although the non-parametric Mann-Whitney U test may be used). In summary, measuring arrhythmia duration alone is not an acceptable means of gauging the severity of arrhythmias.

An alternative approach is to generate a number scale which can be used to summarise and grade all the arrhythmias following occlusion in terms of incidence and severity. This scale (arrhythmia score) should accomodate and summarise complex arrhythmia data sets. Arrhythmia scores should be normally distributed (to permit parametric statistical testing) and linearly additive. The arrhythmia score which has been used for occlusion studies in the conscious rat in our laboratory has been shown to be Gaussian distributed in control rats (Johnston et al., 1983a) and amenable to modified t tests.

This score is as follows:

0 = No more than 49 PVCs,

1 = 50 - 499 PVC,

2 = No more than 1 episode of SVT or SVF and/or > 499 PVC,

3 = More than 1 episode of VT and/or VF < 60 sec total duration,

4 = VT and/or VF of 60 - 119 sec total duration,

5 = VT and/or VF of > 119 sec total duration,

6 = Fatal VF occuring 15 min - 4 h after occlusion,

7 = Fatal VF occurring 4 min - 14 min 59 sec after occlusion,

8 = Fatal VF occurring 1 min - 3 min 59 sec after occlusion,

9 = Fatal VF occurring before 1 min after occlusion.

This score has been adopted by Charnock's group for studies in anaesthetised rats (McLennan and Charnock, 1984; McLennan et al., 1985; McLennan, 1986).

Other arrhythmia scores have also been developed. Martinez and Crampton (1981) have used a score based on the product of arrhythmia duration and arrhythmia type (the latter being graded from 1 to 5 according to severity). However, this score was found not to be amenable to parametric statistical testing, since drug treatments altered the variance. Mueller et al. (1984) have used a simple 3 point score which they sum for each group and submit to the $chi^2$ test for analysis. Woodward's group have used a group arrhythmia score, whereby arrhythmias for a whole group are summed, and a score assigned to the group (Daugherty et al., 1986). However, the object of having an arrhythmia score seems to have been forgotten by this group, since this particular score does not seem to be amenable to any statistical test.

The ideal arrhythmia score should be amenable to parametric statistical tests (analysis of variance followed by modified t tests for multiple comparisons, such as Tukey's test). In this manner, arrhythmia score may be plotted against log dose in order to estimate $ED_{50}$ values for overall

antiarrhythmic actions (see section concerned with statistics).

At least 6 other arrhythmia scores were investigated during the course of experimentation, since it was considered worthwhile to investigate whether more closely Gaussian-distributed scores could be invented.

2.1.5 ECG changes produced by occlusion

Coronary occlusion produces characteristic S-T segment elevation (Pardee, 1920) and changes in R wave size. Since these effects are produced by occlusion, and are presumably the result of myocardial ischaemia, it was considered of interest to measure and record these variables. Apart from ourselves (Johnston et al., 1983a; etc.), only Bernauer (1982; 1983; 1985) has attempted to measure and quantify the ECG changes produced by occlusion in rats. The optimum method for measuring and expressing such changes is therefore not well established. A variety of techniques have been considered as follows. Figure 2 illustrates the changes in ECG configuration with time following occlusion.

2.1.5.1 'S-T' segment elevation. The position of the T wave of the ECG in rats is not as clear-cut as it is in other species. In most chest leads (including the V3 lead which we use), the T wave is superimposed upon the terminal portion of the QRS complex, indicating that repolarisation is beginning before depolarisation is complete (Cooper, 1969). In order to measure S-T segment elevation, an arbitrary, but standardised position for the T wave was determined. Prior to occlusion, a sample of approximately 50 ECGs recorded at fast chart speed (100 mm/sec) were inspected, and the position of the S wave in relation to the Q wave was measured. This was found to be approximately 30 msec (3 mm on the fast speed record). Therefore, as an index of S-T segment elevation, the height of this S wave position above isoelectric was measured before ($S_0$) and after occlusion ($S_t$).

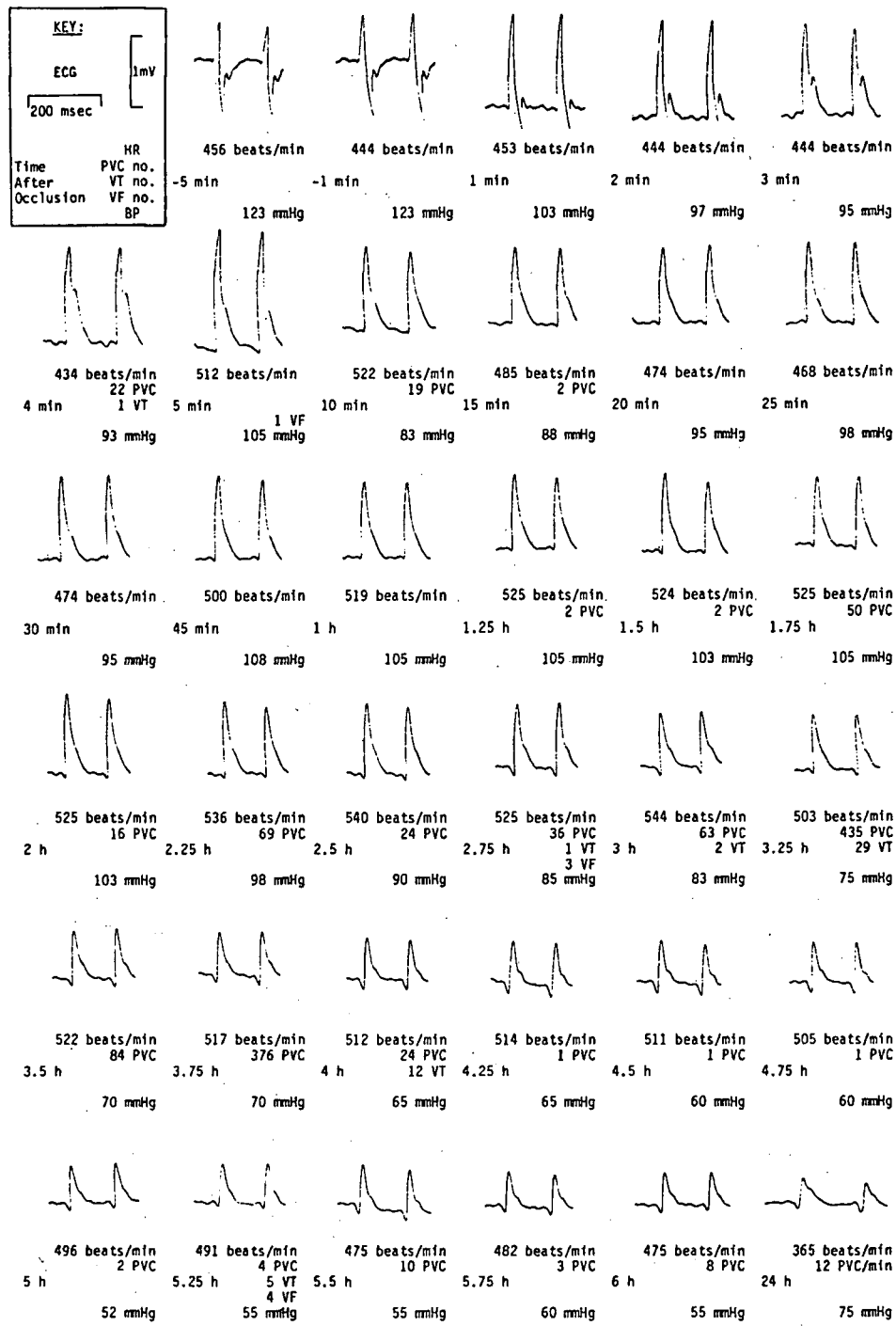



Figure 2. Anecdote of ECG changes caused by occlusion in a conscious rat

Isoelectric was defined as the voltage at the foot of the P wave of the preceding beat. Before occlusion, values are negative, since the S wave position is negative to isoelectric. 'S-T' segment elevation begins immediately upon occlusion, and in order to simplify subsequent analysis, all negative (pre-occlusion) values are assigned the value zero.

'S-T' segment elevation was expressed in a variety of ways. The variable originally used in our laboratory was the dimensionless dSTR. This was determined by measuring the R wave amplitude (see below) before occlusion ($R_o$) and at times after occlusion ($R_t$), and was calculated as $(S_t-S_o).(R_t/R_o)$. In other words, 'S-T' segment was corrected for the change in R wave amplitude following occlusion as a function of pre-occlusion R wave. Other measures of 'S-T' segment elevation have been subsequently evaluated, as follows;

Uncorrected elevation, referred to as ST, calculated as $S_t$ mV.

Elevation corrected for amplidude as a % of R wave amplitude, referred to as $ST^2/R$, calculated as $(S_t)^2/R_t$ mV.

Elevation as a % of R wave amplitude, referred to as ST%, calculated as $(100)(S_t)/R_t$ (dimensionless).

The criterion for determining which measure was most useful was simply to determine which variable produced the largest coefficient of variation (defined as mean divided by standard deviation). It was found during the course of experimentation that ST% was consistently the most precise variable. The coefficients of variation for ST% were approximately double those found for dSTR.

The variable dSTR has been shown to be Poisson distributed (Johnston et al., 1983a). The problem with all measures of S-T segment elevation is that the variable regresses with time in a non-linear manner. An attempt was made to account for this by measuring the maximum value of S-T segment

elevation and the time at which this occurred. Since the development of S-T segment elevation is not strictly a mono-exponential process (see results) it was decided to measure the maximum value of S-T segment elevation (rather than the half-maximum value). This was carried out according to the following criteria.

S-T segment elevation was measured at regular intervals after occlusion from 100 mm/sec chart speed records. The maximum value of S-T segment elevation was determined by considering each time point consecutively and applying an arbitrary '5% rule' in the case of ST%, or a '0.05 mV' rule in the case of dSTR. This rule operates as follows (using the derivative ST% as the example). The first value is initially classified as the maximum. This is superceded by the first subsequent value which exceeds it by 5% or more. In the example below, each successive apparent maximum has been underlined. The final underlined value, 85% was the value taken as the actual maximum according to the criteria outlined. The time at which the maximum occurred, 120 min, was taken as the 'time of maximum ST%'

| Time (min) | 1 | 2 | 5 | 10 | 15 | 30 | 60 | 120 | 180 |
|---|---|---|---|---|---|---|---|---|---|
| ST% | 6 | 15 | 17 | 66 | 68 | 80 | 82 | 85 | 88 |

Of course, if the relationship between S-T segment elevation and time after occlusion were a simple saturating monoexponential function of the form:

$$\text{'S-T' elevation} = 1/(1 + \text{tau}/t)$$

where t is time after occlusion and tau is the time constant (defined here as the time at which 'S-T' segment elevation is half maximal),

then tau would be the ideal variable to record. However, when 'S-T' elevation is plotted against time (e.g., Johnston et al., 1983a) then it is clear

that a simple monoexponential equation will not fit the relationship. Indeed, the relationship does not fit any simple model.

Since the action of drugs on 'S-T' segment elevation was not the primary concern, it was not considered worthwhile elaborating upon the analysis of the variable beyond using the semi-empirical approach outlined above.

2.1.5.2 Pathological R-waves. Coronary occlusion in rats produces an initial increase in the amplitude of the R wave, followed by a gradual decline in amplitude to values smaller than those seen before occlusion. An attempt was made to quantify R wave changes using a similar approach to that used in assessing S-T segment elevation.

R wave amplitude was easier to measure than S-T segment elevation, since the peak of the R wave is clearly visible on fast chart speed recordings (100 mm/sec). R wave amplitude (mV) was taken as the deflection of the peak of the R wave above the isoelectric point. As in the case of S-T segment elevation, R wave does not correlate with time after occlusion in a simple manner, and maximum R wave amplitude (using a 0.05 mV rule) and the time at which this occurred were determined in a manner analogous to the determination of maximum S-T segment elevation.

2.1.5.3 Pathological Q waves. The chest lead ECGs of normal rats exhibit no Q wave, presumably because the rat heart frontal plane axis is perpendicular to the horizontal plane, according to ECG vector analysis (Cooper, 1969). However, coronary occlusion produces a deep Q wave in the chest leads which appears at approximately 2 h after occlusion, and persists for at least 11 days (Normann et al., 1961; Zsoter and Bajusz, 1962). If lead-I is recorded, a Q wave appears within 10 min of occlusion in approximately 30% of rats, long before it is present in the chest leads (Kenedi and Losconci, 1973a). Using a V3 lead we rarely see a Q wave before 1 h after occlusion, but its presence is almost universal in rats surviving 24 h.

Recently, attempts have been made to quantify Q wave development by measuring the time at which a significant Q wave is present in the ECG. A significant Q wave was defined as a downward deflection from isoelectric equal to approximately 10% of the R wave amplitude.

2.1.6 ECG changes produced by drugs

Since the ECG is always recorded before drug administration and again 1 min before occlusion, there is an opportunity for assessing the electrophysiological effects of drugs in the same rats in which antiarrhythmic/anti-infarct activity is assessed. This is achieved by measuring P-R interval and QRS interval.

2.1.6.1 P-R interval. P-R interval reflects the duration of conduction from high in the atrium through to the AV junction and bundle branches (see Horan and Flowers, 1980). Since conduction velocity in the AV node is much slower than conduction velocity in the atrium, in accordance with the lower $dV/dt_{max}$ of the upstroke of the action potential and the lower excitability (Merideth et al., 1968, and see Introduction), then P-R interval is essentially a reflection of conduction through the AV node. As such, drugs influencing P-R interval therefore influence AV conduction. The P-R interval is fairly easy to record in rats, although the foot of the P wave can be somewhat indistinct on occasions, particularly when recorded on a Grass Polygraph. P-R interval was measured according to the standard definition (from the foot of the upstroke of the P wave to the start of the QRS). The effects of drug treatment on P-R interval were determined by comparing values 15 min before occlusion (pre-drug) with those 1 min before occlusion (4 min after drug administration).

2.1.6.2 QRS interval. The QRS interval reflects ventricular depolarisation (see Horan and Flowers, 1980). If the QRS is widened, this reflects a dispersion, or delay in ventricular conduction, and reduction

in conduction velocity. The QRS interval was not measured in the conventional manner (from the beginning of the Q wave to the end of the S) because it was considered that the position of the downward going peak of the S was more amenable to measurement than the less well-defined terminal end of the S wave. The effects of drug treatment on QRS interval were determined by comparing values 15 min before occlusion (pre-drug) with those 1 min before occlusion (4 min after drug administration).

In some experiments, an attempt was made to measure QT interval. The problems involved in measuring QRS were even more of a confounding factor here, since the T wave is the least well-defined of the ECG waves in the rat. The T wave reflects ventricular repolarisation, and is therefore dependent on action potential duration. Therefore, QT interval reflects a combination of ventricular conduction velocity (governed almost exclusively by $dV/dt_{max}$), ventricular action potential plateau (governed by $i_{si}$) and ventricular repolarisation (governed by inactivation of $i_{si}$ and activation of repolarising $K^+$ currents, see Introduction). It can be seen that QT interval is therefore a rather non-specific variable. In addition, in rats the T wave is superimposed on the QRS (Cooper, 1969; Driscoll, 1980). This makes measurement of QT interval particularly difficult.

2.1.7 Measurement of serum $K^+$ concentration

Serum $K^+$ was measured in recent experiments as a consequence of the results of investigations into mechanisms of arrhythymogenesis (see below). Since it has been shown that extracellular $K^+$ rises in the ischaemic tissue with a time course corresponding with that of early occlusion-induced arrhythmias (Hirch et al., 1980), and since the incidence of arrhythmias following myocardial ischaemia is inversely proportional to serum $K^+$ clinically (Nordrehaug and Von der Lippe, 1983; 1985) and experimentally in coronary-ligated rat hearts in vitro (Lubbe et al., 1978; Daugherty et al.,

1981), it was decided to monitor serum $K^+$ in order to determine whether the antiarrhythmic actions of drugs were related to alterations in serum $K^+$.

A blood sample (0.5 - 0.8 ml) was withdrawn from the aortic blood pressure line at approximately 2 h after occlusion. The sample was spun at 10000 x g for 2 min, using an Eppendorf centrifuge (Model 3200). The plasma was removed using a small pipette with a rubber bulb attached, and allowed to clot. The serum was then pipetted into a 1 ml stoppered vessel (occasionally a second spin was required to separate the serum from the clot). The $K^+$ concentration was determined in the analytical laboratory of the Acute Care Hospital (by their technicians), using a $K^+$-selective electrode (Kodak Ektachem).

2.1.8 Exclusion criteria

It is of paramount importance, when investigating myocardial ischaemia, to ensure that animals are included into the study only when the coronary artery has been occluded and ischaemia has been produced. This is ensured by measuring the OZ, ex-vivo, according to the technique described above. However, there are other less obvious sources of variance which may jeopardise the precision and accuracy of an experiment. Over the years, a set of exclusion criteria has been developed which is designed to reduce the variance not attributable to treatment. It must be stressed that the exclusion criteria must be treated in the same manner as all other aspects of experimentation and analysis, namely that it must be applied blindly.

The exclusion criteria are tiered in as much as rats must be excluded before, during or after occlusion according to a definite chronological sequence. In other words, pre- or post-occlusion exclusion criteria must not be applied post-hoc (except when postmortem verification is required).

A. The following action is taken in relation to the following pre-occlusion abnormalities, with the object of excluding rats in which either the occluder has been accidentally tightened during the interval between preparation and experimentation, or in which infections are present:

a. The presence of a Q wave, as defined above, dictates obligatory exclusion, on the grounds that a Q wave is only seen in chest leads in rats following coronary occlusion (Normann et al., 1961; Zsoter and Bajusz, 1962), and that a Q wave is associated in our experience only with an infarct or scar tissue.

b. If more than 5 PVC occur during the 15 min period prior to drug administration then the rat should be excluded on the grounds that there is a lesion of some sort in the myocardium which may influence the outcome of occlusion.

c. If there has been > 25% weight loss between preparation and occlusion, rats are only excluded if there are other signs of illness, such as diarrhoea and/or inflammation associated with surgical wounds, and/or pre-drug mean arterial blood pressures of 85 mmHg or less (> 2 s.d. from mean pre-occlusion values).

d. If there are signs of lung infection, such as exudate around the snout and/or noisy respiration, the rat is excluded. In this regard, the sensitivity of rats to lung infections is well known. Evans et al. (1985) reported that male Sprague Dawley rats have often been received from the supplier with 'a pulmonary infection characterised by wheezing', and that the mortality in these rats following coronary occlusion was increased. In early experiments in our laboratory it was probable that some rats were included despite having an underlying pulmonary infection. During the last 2 years the problem of respiratory infection became so severe on

occasions that rats died even before preparative surgery had been carried out. Mortality associated with pneumopathy after occlusion is characterised by extensive exudation of sputum, often bloody, during the first 5 - 15 min after occlusion, indicative of severe pulmonary oedema. Postmortem examination reveals mottling and occasionally haemorrhaging of the lungs, and severe pulmonary oedema. Often the thorax is full of serum, and the lungs have a similar appearance to the liver (uniformly red). Lung swabs and sputum swabs have been examined (by Charles Ford in Medical Microbiology, UBC) and found to consistently grow monocultures of Psuedomonas aeruginosa. Currently, attempts have been made to remove this problem by slightly acidifying the drinking water, and allowing an interval of at least a week between arrival of the rats from the supplier and surgical preparation. These manoeuvres, in conjunction with regular disinfection of the laboratory with bleach appear to have reduced the incidence of life-threatening pneumopathy.

e. We have had no reason to exclude rats from study on the grounds of low blood pressure. Parratt's group require such exclusion criteria since they use acutely prepared anaesthetised animals (Clark et al., 1980), which can be obtunded by preparative surgery.

B. The following action is taken in the event of the following post-occlusion abnormalities, with the object of excluding rats with incomplete or non-existant occlusions and rats which experience reperfusion as a result of the loosening of a defective occluder:

a. If there is no increase in R wave, and/or no ST elevation following occlusion, the rat is excluded:

i. if postmortem examination reveals an unacceptably small OZ or IZ (see below),

ii. if the rat dies within 4 hr of occlusion and is found to have inflammation or scarring in the heart,

iii. if postmortem examination reveals that the occluder is loose,

b. If, at some time after occlusion, the ECG returns to the pre-occlusion configuration, the rat is excluded:

i. if the OZ and/or IZ are unacceptably small (see below),

ii. if the ligator is found to be loose.

c. If the rat dies within the first 10 min after occlusion in association with immediate calamatous hypotension, the rat is excluded if the thorax is found to contain blood. It is possible that overtightening of the occluder can cause haemorrhaging from the coronary vessels, perhaps as a result of roughness in the region of the flared outer guide tubing. This particular problem is seen in less than 2% of rats.

d. Animals experiencing fatal or non-fatal cardiogenic shock are not excluded from studies. Cardiogenic shock has been defined as a fall in blood pressure of at least 30%, maintained for at least 30 min, associated with ECG signs of ischaemia, but not caused by arrhythmias (Agress et al., 1952). This definition adequately describes the syndrome which we recognise as cardiogenic shock in rats subjected to coronary artery occlusion.

C. The following action is taken in the event of the following postmortem abnormalities, in order to exclude animals with inadequate or abnormal occlusions:

a. Rats are excluded if they are found to have an inappropriately small OZ, defined as < 25 % ventricular weight (more than 2 s.d. from the mean).

b. Rats having inappropriately small IZ, defined as < 50% of the OZ are excluded. This criterion is somewhate arbitrary, and is based on the assumption that since the rat has no functional collaterals capable of providing 'myocardial salvage' (Johns and Olson, 1954; Selye et al., 1960; Maxwell et al., 1984; Winkler et al., 1984; Schaper et al., 1986), then the IZ at 24 h should be a reasonably fixed % of the OZ. This appears to be the case, since to date, our laboratory has not found any drugs which consistently reduce IZ, which may mean that 'myocardial salvage' is impossible in rats (see Discussion). The arbitrary nature of this exclusion criterion is not really a problem, since no rats have ever been excluded from a study solely on the basis of this criterion.

c. If infarcted tissue or scarring in the heart is found in a rat which dies before 4 h after occlusion, the rat is excluded, on the grounds that infarction is not detectable using TTZ until at least 6 h after occlusion, and is not well demarcated until at least 10 h after occlusion (Hort and Da Canalis, 1965a). Early scarring is indicative of infarction produced during or shortly after preparation. Less than 1% of rats have been excluded on this basis.

d. If pus is found at the occlusion site, the rat is excluded.

Occasionally, acutely prepared animals are used for experimentation. The exclusion criteria outlined above are used except where they are obviously inappropriate. In accordance with Parratt's criteria (Clark et al., 1980), rats with pre-drug pre-occlusion mean arterial blood pressures of < 70 mmHg are excluded (in the present experiments no rats actually failed this test; pre-occlusion blood pressure was always > 80 mmHg).

2.1.9 Statistics

In most experiments, a control group of rats was compared with at least two treated groups. The control group was defined as a group of animals treated with drug vehicle. The variables compared were either Gaussian (normally) distributed or binomially distributed (Johnston et al., 1983a). In all comparisons, the limit of 'statistical significance' was defined as $p < 0.05$. In accordance with the requirements for undertaking the types of statistical tests described below, randomisation to treatment and blind analysis of records were carried out. Whenever possible, treatment was also given blind. Obviously a procedure such as decerebration or pithing (see below) could not be carried out blind. The group size was kept to a minimum, and was based loosely on the minimum sample size required to reveal a 50 % reduction in VT and VF. The control incidence of VT and VT during the 0 - 4 h period after occlusion in conscious rats is approximately 90 - 100 % (Johnston et al., 1983a; etc.). In a 1-tailed $chi^2$ test, the minimum group size to reveal a 50 % reduction in VT and VF is 9 (Mainland et al., 1956).

2.1.9.1 Normalisation procedures. In order to carry out parametric tests, such as Duncan's multiple range test, it was necessary to ensure that the variable was Gaussian distributed. It has been shown by our laboratory (Johnston et al., 1983a) that many of the variables measured are $\log_{10}$-Gaussian distributed. Therefore, in order to compare means, the $\log_{10}$ values were calculated. This manoeuvre was carried out for the following variables: PVC, number and duration of VT and VF and time of maximum R wave and S-T segment elevation.

Corrections for co-variance by the use of normalization procedures were carried out, in certain instances, to improve precision. For example, it has been shown that the arrhythmia score (AS) correlates linearly with $\sqrt{OZ}$ (Johnston et al., 1983a). Therefore AS can be expressed as a function of

OZ. There is a major problem associated with the relationship between AS and OZ, namely that $AS/\sqrt{OZ}$ becomes meaningless if AS is zero. This is never the case in controls, but in drug-treated groups this is often the case. An attempt was made to overcome this problem by developing an ischaemia score (ASMC), which functions in the same way as the AS, but includes scores for signs of ischaemia (R wave size, etc.). The fact that AS correlates with $\sqrt{OZ}$, but cannot easily be corrected for $\sqrt{OZ}$ was also approached by adding 1 to each AS value, then correcting this value for $\sqrt{OZ}$, giving the variable $(AS + 1)/\sqrt{OZ}$. However, the precision of OZ is high, such that in practice $\sqrt{OZ}$ and AS are not linearly correlated but are instead scatter values around a single point. Only when small OZs are deliberately produced (Curtis et al., 1984) is the relationship between $\sqrt{OZ}$ and AS apparent. When large OZs only are produced there is no need to correct AS for $\sqrt{OZ}$.

2.1.9.2 Censoring. Censoring as a result of death was discussed in the section concerned with thump-version defibrillation. Censoring cannot be avoided and its effect can only be minimised. The type of censoring which jeopardises an experiment must be identified and appropriate action taken. The censoring introduced by thump-version was considered to jeopardise the experiment less than the censoring associated with allowing animals to die.

2.1.9.3 Statistical tests. For binomially distributed variables $chi^2$ tests were used. Mainland's contingency tables of minimum contrasts were used for $chi^2$ testing (Mainland et al., 1956). For Gaussian-distributed variables, analysis of variance (ANOVA) was carried out, using a U.B.C. statistics program (Gregg and Osterlin, 1977). Only if treatment constituted a significant source of variance according to an F test were means compared (using Duncan's multiple range test). This test was appropriate since the number of groups exceeded two in every study. In no

instance was a simple t test used for comparing means, since the t test is only appropriate when two groups are compared; if a 1 in 20 probability is set as the limit of chance, and 20 groups are compared with a control group, then the probability approaches 100% that at least one group will be found to be different from controls, according to the t test. Modified t tests, such as Duncan's multiple range test, avoid this pit-fall, because the number and type of comparisons are accounted for.

## 2.2 Calcium antagonist studies in coronary-occluded rats

### 2.2.1 General experimental design

Randomisation to treatment was carried out by assembling a table of lines. Each line was assembled by drawing playing cards numbered according to the number of groups in each study. Drug stocks were prepared, and appropriate dilutions made by various members of the laboratory according to specific instructions. The stock solutions were coded. Therefore the person preparing a syringe of drug for a particular rat was also blind to treatment. Lines were chosen in random order by the person preparing the injection. This person prepared a second table in which the rat code (assigned on the day of preparation) and the coded treatment were noted.

In the case of rats treated with unstable drugs (felodipine, nifedipine and DHM9), the person making up the drugs was instructed to weigh out a specified amount of drug for each rat, and was therefore not blind to the treatment. However, the person administering the drug, occluding the coronary artery, monitoring responses and analysing the records remained blind to the treatment. All other aspects of study were as outlined in previous chapters.

Up to 4 rats were occluded on each day of experimentation, with a 15 min interval between each occlusion. In this manner, rat-1 had passed through the phase of early arrhythmias (Clarke et al., 1980; Johnston et al., 1983a)

by the time rat-2 was due for occlusion; rat-2 received its drug treatment while rat-1 was being monitored for arrhythmias, and so on.

For each rat a complete history was kept, both in the laboratory day book and also on individual analysis sheets. The analysis sheets contained all information concerning haemodynamic, ECG and arrhythmia data, as well as OZ, IZ, serum $K^+$, body weight, date of preparation and occlusion, general comments concerning preparation, behavioural responses to drug treatment and occlusion and postmortem findings. When the study was complete, the codes were broken and the results analysed. According to the principle of randomisation to lines it is statistically acceptable to break the code upon the completion of each line. However, this practice was avoided.

2.2.2 Phenethylalkylamines

2.2.2.1 Anipamil and Ronipamil. The effects of anipamil and ronipamil on responses to coronary occlusion were investigated because these analogues of verapamil differ in respect to their calcium antagonist activity, and were therefore considered to be valuble tools for testing the hypothesis that calcium antagonism in the ventricle is antiarrhythmic during acute myocardial ischaemia.

Anipamil and ronipamil are analogues of verapamil. In the case of anipamil the main chain of verapamil has been extended by 10 C-atoms and the methoxy substituents removed from the 4 position on the phenyl rings, leaving 1,7-bis-(3-methoxyphenyl)-3-methylaza-7-cyano-nonadecane. Ronipamil resembles anipamil except for a lack of methoxy substituents on the phenyl rings, i.e it is 1,7-bisphenyl-3-methylaza-7-cyano-nonadecane. Both drugs have been demonstrated to have anti-ischaemic activity in a variety of preparations (Kovach, 1984; Kretzschmar and Raschack, 1984; Raschack 1984; Urbanics and Kovach 1984).

Both anipamil and ronipamil were administered by the p.o. route. The

doses chosen were 50 and 150 mg/kg, based on preliminary toxicity studies in which it was found that administration of up to 300 mg/kg was non-toxic (higher doses produced death in approximately 50 % of rats given anipamil; the cause of death was unclear, since it occurred overnight in every instance, but was considered to be the result of cardiovascular depression, since the drug caused a progressive decrease in blood pressure with time in these rats). Both drugs were dissolved in distilled water and administered at a volume of 0.25 ml/kg body weight. Suspensions of 20 or 60 mg/ml of either drug were prepared and gently heated to 60 °C to facilitate dissolution, then allowed to cool slightly before administration. Control animals received an equivalent volume of distilled water at the same temperature as the drug suspensions. Oral administration was carried out 4 h before occlusion during a period of extremely brief anaesthesia (1 min) with halothane. An intragastric tube for drug administration was created by simply fixing a 12 cm length of PE90 tubing to a 3 ml syringe. The treatments were administered slowly in order to preclude accidental administration into the trachea. The possibility of aspiration pneumonitis was discounted since rats lack a well-developed vomit reflex (Briggs and Oehme, 1980). Drugs were made up fresh approximately once per week, refrigerated and stored in light-proof containers.

2.2.2.2 (+)- And (-)-verapamil. The optical enantiomers of verapamil were compared for their actions on the responses to coronary occlusion in order to test the hypothesis that calcium antagonism in the ventricles is antiarrhythmic during acute myocardial ischaemia, and also to examine the complementary hypothesis that the antiarrhythmic action of (±)-verapamil in conscious rats (Curtis et al., 1984) occurred by virtue of calcium antagonism in the ventricles (see Introduction). Both hypotheses predict an antiarrhythmic potency ratio equal to the calcium antagonist potency ratio in

the ventricular myocardium, based on the reported potency difference between the enantiomers for calcium antagonism (e.g., Bayer et al., 1975b; 1975c; Nawrath et al., 1981; Ferry et al., 1985).

Drugs were dissolved in saline and administered at a volume of 0.25 ml/kg by slow i.v. injection over 10 min, beginning 15 min before coronary occlusion, according to the protocol outlined in the general methods section (see Figure 1). Ten groups of rats (n = 9 per group) were used. Two groups received saline (controls). The remaining groups received either (-)-verapamil (0.2, 0.6, 2 or 6 mg/kg) or (+)-verapamil (0.4, 4, 8 or 12 mg/kg). The doses studied were chosen on the basis of the earlier experiment with (±)-verapamil (Curtis et al., 1984), in which 6 mg/kg was found to be the $ED_{50}$ for reducing arrhythmia score (AS).

Drug stocks were made up in advance of the study by dilution, and were refrigerated and stored in light-proof vessels, coded to ensure double-blindness.

2.2.3 1,4-Dihydropyridines

2.2.3.1 Felodipine. Felodipine was evaluated on the basis of its selectivity as a calcium antagonist for the vasculature versus the myocardium (e.g., Au and Sutter, 1984). The hypothesis that calcium antagonists reduce ischaemia-induced arrhythmias via an action in the ventricles predicts that felodipine (and other 1.4-dihydropyridine calcium antagonists) will only reduce arrhythmias at high doses. In addition, a calcium antagonist which shows marked selectivity for the vasculature may precipitate life-threatening reductions in blood pressure at doses below those producing blood levels sufficient to reduce $i_{si}$ in the ventricles and reduce the incidence and severity of ischaemia-induced arrhythmias. Therefore, it was predicted that felodipine would have little if any antiarrhythmic activity at doses which produced large reductions in blood pressure.

Eight groups of rats were used (n = 9 per group). In addition to the standard large OZ (LOZ) rats (5 groups), 3 groups of small OZ (SOZ) were prepared and subjected to coronary occlusion by Kathy Johnston. The LOZ rats received 1 of 3 doses, 0.2, 2.6 or 12.2 μmol/kg, according to the schedule outlined in the general methods. These doses were 0.08, 1 and 4.68 mg/kg, respectively, and have been coded as L, I and H (low, intermediate and high, respectively) for the purpose of labeling the figures in the Results section. Only the lower and higher doses were given to the SOZ rats. Felodipine was dissolved in 20% ethanol in saline and administered at a volume of 0.25 ml/100g body weight. Care was taken to protect all syringes from direct sunlight/fluorescent room lighting before administration. Twenty seven control rats were used (18 LOZ rats and 9 SOZ rats). Control rats received 0.25 ml/100g body weight of 20% ethanol in saline. The LOZ and SOZ rats were analysed separately, since treatment was the only known source of variance within the LOZ and SOZ series, but operators were a source of variance between the LOZ and SOZ series. Drugs stocks were made up in advance of the study and were refrigerated and stored in light-proof vessels.

2.2.3.2 Nifedipine and DHM9. These 1,4-dihydropyridines were studied in order to supplement the work with felodipine. DHM9 has been reported to possess a selectivity of action for ventricular tissue versus vascular smooth muscle, in contrast with all other 1,4-dihydropyridine calcium antagonists (Clarke et al., 1984b). Therefore, according to the hypothesis that calcium antagonism in the ventricles is antiarrhythmic during acute myocardial ischaemia, it was expected that DHM9 should be more effective in reducing ischaemia-induced arrhythmias than nifedipine at doses producing a similar degree of blood pressure lowering.

Five groups of rats were studied (n = 9 per group). Drugs were dissolved in 20% ethanol in saline and administered at 0.25 ml/100g body

weight in accordance with the schedule outlined in the general methods section. Controls received the ethanol vehicle alone. The doses were 0.5 and 2 mg/kg nifedipine and 5 and 20 mg/kg DHM9. These doses appear to be large, but they were based on a preliminary toxicity study in which it was found that conscious rats could tolerate at least 16 mg/kg nifedipine. Although not investigated in any detail, it was considered that the dose-limiting factor was in fact the ethanol vehicle rather than the drug itself. Preliminary investigations with DHM9 showed that no haemodynamic effects occurred at all at cumulative doses well in excess of 30 mg/kg. In accordance with the general method, stock solutions were prepared in advance of the study, coded, refrigerated and stored in light-proof containers. Care was taken to prevent exposure of the drug-containing syringes to light before administration. True blindness could not be achieved in this study, since the yellow colouration of the drugs could be seen in the thin PE tubing entering the subscapular region of the rats at the highest doses (both DHM9 and nifedipine are vivid yellow in colour). However, all records were analysed blind.

## 2.3 Arrhythmogenesis and the role of the CNS

### 2.3.1 Introduction

The experiments outlined here were not designed to answer all questions concerning arrhythmogenesis in rats, but were intended to examine the role of the CNS and the sympathetic nervous system. Our laboratory has carried out preliminary experiments for this purpose in the past (Botting et al., 1983).

The strategy was to remove, by surgery, the autonomic nervous system in a graded manner in some groups of animals, and replace it by infusion of catecholamines in others. In addition, the extent of surgery was considered as a possible source of variance. In conjunction with this latter consider-

ation, the effects of pithing (the most extensive surgical ablation) on possible mediators and modulators of arrhythmias (serum $K^+$, leukocytes and thrombocytes) were determined in a separate series of experiments.

All groups in this study consisted, as usual, of 9 rats. Occlusion was carried out as described above, and blood pressure was recorded via the aorta, except when stated otherwise. Since there were many groups, and preparation was complicated, a summary table has been prepared which lists all the groups (Table 1).

2.3.2 Preparation

2.3.2.1 Pithing. Male Sprague Dawley rats were lightly anaesthetised with halothane (4% in oxygen) and intubated. The rats were prepared for occlusion in the normal manner, with an aortic blood pressure line, jugular intravenous line and $V_3$ ECG leads. These rats were allowed to recover from preparative surgery for approximately 7 days. This group was subsequently pithed, as described below, and constituted the chronically prepared (c) pithed (P) group (i.e., cP).

A second group was prepared with occluders, $V_3$ ECG leads, carotid blood pressure lines and femoral intravenous lines (by standard catheterisation techniques using PE tubing). This group constituted the acutely prepared (a) pithed (P) group (i.e., aP), since they were pithed immediately after preparation.

Pithing was carried out as follows. A stainless steel rod (3 mm diameter) was passed through the orbit and down the spinal cord during light anaesthesia (1% halothane in oxygen). Immediately afterwards, artificial respiration with 100% oxygen was instigated (stroke volume 4 ml per 300 g body weight, 54 strokes/min). This artificial respiration regimen has been shown to produce blood gas and blood pH levels within the normal range in pithed rats (Milmer and Clough, 1985). The rats were then mounted vertically

with the head pointing downward, by securing the tail and the pithing rod in 2 clamps attached to a retort stand. The blood pressure transducer was elevated at this time in order that it should remain in the same horizontal plane as the heart. A rectal thermocouple was connected via an Indicating Controller unit (YSI Model 73ATA) to a 100 W light bulb, placed 20 cm from the rat, and a sheet of polythene was placed over the entire preparation, to maintain body temperatute at 37 - 38 °C. Saline (1 ml per 100 g body weight) was then injected i.v. in an attempt to compensate for the initial blood-pressure lowering effect of pithing. Coronary occlusion was carried out 30 min after pithing. Rats were monitored for 4 h, then sacrificed (exsanguination). All variables were measured in the usual way (see general Methods section). Infarct size (IZ) was not measured, since at least 10 h is required for quantifiable infarct development according to the TTZ staining technique (Hort and Da Canalis, 1965b).

2.3.2.2 Spinalisation. Rats were prepared and occluded in a manner identical with that described for acutely prepared pithed rats (the aP group) with the exception that instead of pithing, the rats were spinalised. This was achieved by inserting a steel rod into the skull through the foramen magnum at the level of C1, advancing it rostrally and rotating it laterally to mascerate the brain. This group constituted the acutely prepared (a) spinalised (S) group (i.e., aS). As in the case of the aP group, 30 min was allowed between ablation in the CNS and occlusion. At the time of occlusion, spinal reflexes (foot withdrawal to pinching) were returning. This group received similar cardiovascular and respiratory support to the pithed rat groups. This group was also monitored for 4 h then sacrificed.

2.3.2.3 Decerebration. Rats were prepared and occluded in a manner identical with the acutely prepared pithed and spinalised groups (aP and aS respectively) with the exception that the ablation carried out was decere-

bration. Decerebration was achieved by removing the brain rostral to the midcollicular level using the blunt end of a spatula following craniotomy. The empty space was packed with gel foam. A 30 min interval was allowed between ablation and occlusion. In contrast with the previous groups, no cardiovascular or respiratory support was required. After preparation the rats in this group breathed spontaneously and maintained a high blood pressure while horizontal. This group constituted the acutely prepared (a) decerebrate (D) group (i.e., aD).

2.3.3 Other manipulations

The groups described above received ablations in the CNS at different levels. The pithed groups received complete CNS ablation, the spinalised rats were intact distal to C1, and the decerebrate group possessed intact respiratory and vasomotor centres as well as spinal reflexes.

In a supplementary group of acutely prepared pithed rats, an attempt was made to restore the effects of the sympathetic nervous system by infusing a mixture of noradrenaline and adrenaline. This infusion was begun 15 min before occlusion and was designed to elevate blood pressure to levels seen in conscious rats (mean of approximately 100 - 110 mmHg; Johnston et al., 1983a). The mixture was 4:1 noradrenaline:adrenaline (on a weight basis), and the infusion rate was varied from 0.2 to 5 µg/kg/min noradrenaline. Infusion volume was kept below 10 ml/kg/h. This group constituted the acutely prepared (a), pithed (P) noradrenaline/adrenaline treated (N) group (i.e., aPN). Respiratory and cardiovascular support and all other aspects of experimentation were identical with those used for the aP and aS groups.

Four groups of 'control' rats were used. A standard group of conscious chronically-prepared rats were prepared and occluded in the manner described in the general methods section. This group constituted the chronically prepared (c) conscious (C) group (i.e., cC) and served as a control group

free from recent surgery and ablations in the CNS.

A second, similar group received an infusion of noradrenaline/adrenaline mixture identical with that administered to the aPN pithed group. This group of conscious rats constituted the chronically prepared (c) conscious (C) noradrenaline/adrenaline (N) treated group (cCN), and served as control for the effects of the catecholamine infusion in the absence of ablations in the CNS.

A third group of rats was prepared in the usual way, but instead of 7 days, these rats were allowed only 1 h to recover from preparative surgery before occlusion. This group constituted the acutely prepared (a) conscious (C) group (i.e., aC), and served as a control for recent surgery in the absence of surgical ablation in the CNS.

Finally, a group of rats was prepared for occlusion according to Clark et al. (1980) using pentobarbitone (60 mg/kg i.p.) anaesthesia, with the minor exception that our standard occluder (not the silk type of Clark et al.) and our V3 ECG leads (not lead 2) were used. Blood pressure was recorded from the left carotid artery, and a cut-down tracheotomy was performed (for delivery of artificial respiration according to the regimen used for the pithed and spinalised groups). Occlusion was carried out 30 min after preparation, and rats were sacrificed 4 h after occlusion. This group constituted the acutely prepared (a) barbiturate anaesthetised (B) group (i.e., aB), and served as a control for recent minor surgery in the absence of surgical ablation in the CNS but in the presence of chemical ablation in the CNS (anaesthesia).

Table 1. Summary of Groups in the CNS Ablations Study

| Acutely Prepared Groups | Code |
|---|---|
| Conscious | aC |
| Anaesthetised | aB |
| Pithed | aP |
| Pithed plus catecholamine infusion | aPN |
| Spinalized | aS |
| Decerebrate | aD |
| **Chronically Prepared Groups** | **Code** |
| Conscious | cC |
| Conscious plus catecholamine infusion | cCN |
| Pithed | cP |
| **Others** | **Code** |
| Isolated perfused hearts | I |

Details of the surgical preparation are given in the text.

An additional group was included in this study, a group of isolated hearts perfused via the aorta at 37 °C with a modified Krebs Henseleit buffer containing 5.3 meq/l $K^+$. This group was prepared and occluded by Reza Tabrizchi according to the method of Kannengeisser et al. (1975). This group constituted the isolated heart (I) group, and served as a general reference group.

2.3.4 Statistics

Variables were measured and analysed as described for the standard occlusion preparation. The full data set for this study is extremely complicated, and it is possible to discuss at great length the possible implications of the results. In order to simplify matters somewhat, statistical significance has only been noted between the chronically prepared conscious group (cC) and the other groups.

## 2.4 Preliminary Screen for drug activity in acute ischaemia

2.4.1 Introduction

Although the conscious rat preparation for investigation of myocardial ischaemia and infarction is capable of providing up to 10 data points per variable per week, it was decided to develop a preliminary screen for assessing new drugs with which 4 times as much information could be generated in the same time. This preparation was designed simply to provide information concerning mortality and infarct size. However, assessment of animal behaviour was also used, in order to attempt to delineate between mortality resulting from ventricular arrhythmias (VF) and other causes.

2.4.2 Preparation

Male Sprague Dawley rats were prepared for occlusion in the manner described in detail in preceding chapters. No intravascular catheters and ECG leads were implanted, only the occluder. By this technique, 16 rats could be prepared daily, with ease. The rats were housed individually and

allowed approximately 7 days before being subjected to coronary occlusion in the usual manner.

2.4.3 Experimental endpoints

Following occlusion, rats were carefully monitored by observation alone. The behaviour of each rat was recorded on individual analysis sheets. All observation and analysis was carried out blind.

2.4.3.1 Definitions. Certain behaviours were carefully categorised for each time interval following occlusion (the time intervals corresponded with those used for the standard occlusion preparation). The time of death was noted, and OZ was measured in the usual manner. In rats surviving 24 h, the IZ was also measured.

Behaviour was classified and assessed according to the following subjective criteria. Morbidity was graded according to the presence or absence of the following 3 behaviours, respiratory distress (which was defined as laboured breathing), head-down posture, and prone posture. These 3 endpoints were considered to be signs of cardiogenic shock (Agress et al., 1952). If nothing more serious than minor panting was present, the rat was classed as normal.

Severe ventricular arrhythmias were diagnosed on the basis of sudden 'convulsive-type' behaviour. This was defined as sudden frenzied attempts to climb out of the home cage accompanied by convulsive-type limb movements and a sudden blanching of the ears and eyes. This behaviour is entirely characteristic of VF (or severe torsade de pointes) lasting longer than 10 sec in the standard instrumented rat preparation, and has never been observed in association with other sequelae of coronary occlusion such as AV block or cardiogenic shock.

Occasionally, rats develop fatal pulmonary oedema following occlusion. The associated behaviour is different from that caused by VF. Fatal pulmon-

ary oedema in conscious rats is associated with frenzied behaviour, but is characterised by the absence of paling of the ears and eyes and the presence of expectoration of copious sputum (often bloody). Furthermore, convulsive-type behaviour resulting from VF lasts for less than 10 sec, since syncope rapidly follows the loss of cardiac output, whereas the frenzied behaviour associated with acute pulmonary oedema generally lasts for more than 30 sec, since cardiac output is maintained. Acute pulmonary oedema is always fatal; the death throes are associated with serious expectoration and an unpleasant gagging sound. In contrast, sudden convulsive-type behaviour resulting from ventricular arrhythmias is never associated with sudden expectoration, and is not invariably fatal; often a rat will loose consciousness, then suddenly the ears and eyes will turn pink and the rat will regain consciousness without apparent ill effect. Finally, acute pulmonary oedema is generally associated with a highly pathognomic prodroma, involving the signs of morbidity outlined above. Sudden convulsive-type behaviour is not associated with any prodroma.

2.4.3.2 Validation of behaviour end-points. The behaviours described above were tested in a blind study in which 12 standard fully-instrumented rats were prepared and occluded in the usual manner, while an observer recorded behaviour in the manner described. These particular rats were not defibrillated if VF developed, but were allowed to die (or spontaneously defibrillate) and were therefore identical to rats subsequently used for comparing the verapamil enantiomers.

2.4.4 Comprison of (+)-, (-)- and (±)-verapamil

As part of the investigations of the actions of verapamil in myocardial ischaemia and infarction, a study was carried out with the optical enantiomers and the racemate, using the above-described technique. Since the precision of this method was unknown, and since up to 16 rats could be

occluded and observed per day, it was decided to use large group sizes (n = 25 per group).

Rats were prepared and allowed to recover from surgery as described. On the day of study, up to 16 rats were stationed on a single laboratory bench. Drugs were administered via a superficial tail vein using a butterfly hypodermic needle while each rat was temporarily restrained in a perspex restrainer. Drugs were injected over a 10 min period, and 5 min was then allowed before occlusion, in a manner analogous to the method used for studies in standard fully-instrumented rats. The cycle time (interval between successive occlusions) was therefore 15 min. This interval was designed such that close attention could be given to the behaviour of each rat during the crucial first 5 - 10 min after occlusion (when the highest incidence of VF and VT occurs). Rats were continuously monitored for at least 4 h after occlusion.

The following treatments were administered: saline (0.25 ml/100 g body weight), (+)-verapamil (6 mg/kg), (±)-verapamil (6 mg/kg) and (-)-verapamil (6 mg/kg). The dose of 6 mg/kg was chosen in accordance with the previously determined $ED_{50}$ for (±)-verapamil in fully-instrumented conscious rats (Curtis et al., 1984).

The object of the study was essentially identical with the object of the study with the enantiomers in fully-instrumented rats described previously, namely to test the hypothesis that the antiarrhythmic actions of (±)-verapamil occur by virtue of calcium antagonism. The hypothesis predicts that the incidence of convulsive-type behaviour should be reduced more by (-)-verapamil than by (+)-verapamil, with (±)-verapamil having intermediate activity. In addition, the experiments were also carried out in order to examine and characterise this new method of assessing drug activity in acute myocardial ischaemia and infarction on the basis of a combination of object-

ive (OZ, IZ and mortality) and subjective (behavioural) end-points.

2.4.5 Statistics

The incidence of mortality and VF are binomially distributed in conscious rats following occlusion (Johnston et al., 1983a). Therefore, the incidence of sudden convulsive-type behaviour, mortality and morbidity were analysed using $chi^2$, in the manner described previously. The results were categorised in terms of the first 0.5 h and the 0.5 - 4 h periods after occlusion, in accordance with the bimodal distribution of ischaemia-induced arrhythmias with time in conscious rats (Johnston et al., 1983a).

## 2.5 Electrically-induced arrhythmias in conscious rats

2.5.1 Introduction

The optical enantiomers of verapamil are known to block $i_{Na}$ in ventricular muscles at high concentrations (Nawrath et al., 1981). These concentrations are 50 - 150 times in excess of those necessary to abolish $i_{si}$ and contractility in the normal ventricle (Nawrath et al., 1981). Therefore, the possibility that blockade of $i_{Na}$ (sodium channel blockade) contributes to the pharmacological actions of (±)-verapamil and its enantiomers in vivo seems highly unlikely. However, this possibility was nevertheless examined by comparing the actions of the enantiomers for their ability to influence arrhythmias induced by electrical stimulation of the left ventricle in conscious rats. It was previously shown that quinidine influenced electrically induced arrhythmias in conscious rats (Curtis et al., 1984) at the same dose which reduced occlusion-induced arrhythmias (arrhythmia score) by 50% (Johnston et al., 1983a), whereas (±)-verapamil had no such actions at the dose reducing arrhythmia score by 50% (Curtis et al., 1984). Therefore the assessment of electrically-induced arrhythmias was considered to be useful for differentiating between $Na^+$ channel blockers and calcium antagonists.

2.5.2 Preparation

Male Sprague Dawley rats were prepared in a manner identical with that for rats used for coronary occlusion studies, with 1 exception. Instead of a coronary occluder, 2 teflon-coated stainless steel wire electrodes were implanted approximately 3 mm apart into the left ventricle, in approximately the centre of the left ventricular wall. This was achieved by plunging the ethanol-sterilised leads into the ventricle, using a 23 gauge hypodermic needle as a locator. All leads and lines were exteriorised in the subscapular region, and approximately 7 days was allowed for recovery from this preparative surgery. On the day of study, the rats were connected to the standard devices for intravenous drug administration, and blood pressure and ECG recording.

2.5.3 Experimental end-points

The variables described below were each measured 3 times every 5 min. Their measurement has been described previously, but not in great detail (Curtis et al., 1984). Stimulation of the left ventricle with square wave pulses was undertaken using a Grass stimulator (Model SD9), which was calibrated using a standard voltmeter (Beckman Model 3020B). A permanent record of the ECG and blood pressure was made using the standard Grass polygraph, while the ECG was also continuously monitored using a delayed loop oscilloscope (Honeywell Type E for M). Discrimination of end-points was carried out using the oscilloscope.

2.5.3.1 Maximum following frequency. Maximum following frequency was defined as the frequency at which the ventricle failed to follow the stimulus on a 1:1 basis. Failure to follow was accompanied by a characteristic blood pressure change; the dropped-beat produced a drop in blood pressure, and the subsequent beat produced a large pulse as a result of the increase in filling time and end-dioastolic pressure. The blood pressure

record could be used for discrimination of the end-point, but in this series of experiments the oscilloscope alone was used. Maximum following frequency was determined at 0.8 msec pulse width and twice threshold voltage, by slowly increasing the stimulation frequency from a baseline of 5 Hz.

2.5.3.2 Threshold voltage. The threshold voltage for inducing VT was determined at 50 Hz and 0.8 msec by the same principle used for determining maximum following frequency. The high frequency was used in order to maximise the probability of delivering a pulse during the vulnerable period, the terminal portion of the QT (de Boer, 1921; Wiggers and Wegria, 1940). In addition to the appearance in the ECG of VT, the threshold voltage for VT was characterised by a drop in blood pressure. VT was almost always non-sustained.

2.5.3.3 Threshold pulse width. The threshold pulse width for inducing VT was determined from the oscilloscope according to the same criteria as those used for measuring the threshold voltage. This variable was measured at 50 Hz and twice threshold voltage.

2.5.4 Comparison of (+)- and (-)-verapamil

In the earlier study with (±)-verapamil, it was found that 6 mg/kg had no effect on the variables in question in conscious rats (Curtis et al., 1984). Therefore, it was decided to evaluate only the equivalent of the highest doses of the enantiomers administered to coronary-occluded rats, since it was not expected that the lower doses would influence the variables. Therefore either 8 or 12 mg/kg (+)-verapamil or 2 or 6 mg/kg (-)-verapamil (n = 6 per group) were administered via the vena caval i.v. line, over a 10 min period. The stimulation variables were recorded 1 min before the start of drug administration and 5 min after completion of drug administration. The %changes in the variables were recorded and analysed. The values for the variables were noted on the ECG traces, and were analysed blind.

Mean %changes in the variables were compared by ANOVA and Duncan's multiple range test.

## 2.6 Haemodynamic and ECG effects of calcium antagonists in pithed rats

### 2.6.1 Introduction

In previous sections, the procedures for assessing the antiarrhythmic action of the enantiomers of verapamil were described. The premise was that (-)-verapamil is more potent as a calcium antagonist than (+)-verapamil. It was decided that rather than rely on literature values for the relative potency of the enantiomers of verapamil (Bayer et al., 1975b; 1975c; Ferry et al., 1985; Nawrath et al., 1981; Raschack 1976b; Echizen et al., 1985), which give disparate values ranging from 4 to over 100 in favour of (-)-verapamil, various attempts should be made to gauge the relative potency of the enantiomers ourselves. The first method was to examine the effects of the enantiomers on blood pressure, heart rate, P-R interval and QRS interval in conscious rats. This particular experiment constituted a portion of the occlusion study with the enantiomers, since a comparison of values before and after drug administration provided this information. However, it was decided to supplement this information with other studies.

Earlier work with pithed rats (see section concerned with arrhythmogenesis in myocardial ischaemia) had shown that it was possible to produce a pithed rat preparation with a high mean blood pressure of at least 60 mmHg, which remained viable for well over 4 h. It was decided to use this preparation to investigate the actions of the verapamil enantiomers in the absence of autonomic tone and reflexes. This information was considered to be of interest, not least because there appears to be a large disparity between the potency of (±)-verapamil in anaesthetised versus conscious rats (Curtis et al., 1984).

2.6.2 Preparation

The pithed rat preparation was essentially the same as that described previously, with the exception that in every instance blood pressure was recorded from the aorta, and drugs were administered into the vena cava. Each preparation was allowed to stabilize for at least 1.5 h before study, and mean aortic blood-pressure and the ECG were periodically recorded during this time.

2.6.3 Variables measured

Mean blood pressure, heart rate, P-R interval and QT interval were recorded. The ECG variables were measured at a chart speed of 100 mm/sec, using standard ECG criteria (Horan and Flowers, 1980) as modified by Driscoll (1980) for the rat. Reductions in blood-pressure and prolongation of P-R interval are effects characteristically produced by calcium antagonists, and are considered to be the result of calcium antagonism (see Nayler and Horowitz, 1983).

2.6.4 Comparison of (+)- and (-)-verapamil

Once mean blood pressure had reached a stable level (no more than a 5 mmHg variation over a 10 min period), increasing doses of either (+)-verapamil (0.4, 0.8, 4 and 8 mg/kg) or (-)-verapamil (0.02, 0.06, 0.2 and 0.6 mg/kg) were administered (n = 6 per drug). Each successive dose was administered as a slow i.v. injection over a 10 min period. It was observed in preliminary experiments that the peak blood-pressure lowering effect of the enantiomers occurred during the first 30 sec after finishing an injection. In the present experiments, all variables were recorded 10 - 15 sec after administering each dose, in order to measure the peak effect. An interval of 15 min was allowed between completing the first injection and starting the second, and so on.

2.6.5 Statistics

The 25 min cycle time used in the current experiments permitted recovery of mean blood pressure to pre-drug values by the time the subsequent dose was administered, justifying the expression of the data as single point dose response curves, rather than cumulative dose response curves. Variables ($ED_{50}$ or $ED_{25}$ values) were compared, where appropriate, using ANOVA and Duncan's multiple range test.

## 2.7 Actions of calcium antagonists in isolated perfused rat ventricles

2.7.1 Introduction

The following experiments were carried out in order to characterise further the calcium antagonist potency ratio of the optical enantiomers of verapamil, and to investigate the actions of nifedipine and DHM9. It was considered worthwhile to have a measure of calcium antagonist potency in rat ventricular tissue, since this was the tissue in which the antiarrhythmic actions of the calcium antagonists were suspected of being mediated.

In addition, verapamil was recently reported to exhibit steroselective plasma protein binding and hepatic metabolism in humans (Echizen et al., 1985; Eichelbaum et al., 1984; Vogelgesang et al., 1984). If the same phenomena occur in rats there is a possibility that calcium antagonist potency ratios in vivo and in vitro may be different. Therefore the potency ratios of the enantiomers in vivo (P-R interval prolongation and reductions in blood pressure in conscious and pithed rats) were compared with those obtained in vitro (negative inotropic actions) in isolated ventricles perfused via the aorta by the method of Langendorff (1895).

The experiments were also designed to investigate the possibility that extracellular $K^+$ plays a role in governing calcium antagonist potency and the locus of antiarrhythmic activity of verapamil and other calcium antagonists. Since extracellular $K^+$ concentration has been shown to rise rapidly

following coronary occlusion (Hirche et al., 1980; Hill and Gettes, 1980), experiments were carried out over a range of buffer $K^+$ concentration.

2.7.2 Perfusion apparatus

The experiments were carried out using a perfusion apparatus which has recently been designed in the laboratory. The apparatus was designed with a small dead-space for rapid switching between different solutions in order to permit the generation of dose-response data. In brief, 9 cylinders of 70 ml capacity were machined into a block of plexiglass which contained channels for circulating warm water (37 °C). Drug solutions flowed from each cylinder via separate silastic tubes (2 mm outer diameter, 1 mm inner diameter) to meet in a common manifold with a low volume (less than 0.1 ml) dead-space. The isolated ventricle preparation (see below) was attached to the apparatus here.

The flow from each cylinder to the appropriate silastic feed line passed through machined Teflon taps such that flow could be switched between cylinders by simply opening 1 tap and closing another. The contents within any cylinder could be changed via a second Teflon tap at the opposite side of the cylinder. The perfusion apparatus was pressurised to drive the drug solutions though the coronary circulation, oxygenate each solution and regulate pH. The gas used was 5% $CO_2$ in oxygen. A single gas inflow branched at an exterior manifold on the perspex box to give 9 separate silastic tubes which served to deliver gas to each cylinder. All cylinders were sealed with a common perspex lid. The lid was tightened by brass screws closing on a rubber gasket. The atmosphere above each cylinder was common, and was bled via a common exit through a regulating 'pop-off' pressure-relief valve. The pressures in the apparatus and at the aortic root were monitored by a manometer (anaeroid).

Oxygen delivery in this system was improved because of the design which reduces loss of oxygen to the dead space between the reservoir and the heart, and because the apparatus oxygenates preheated buffer, rather than heating pre-oxygenated buffer. A thermocouple inserted in the pulmonary artery was used to monitor temperature.

2.7.3 Specifications for Langendorff perfusion

Hearts were excised from male Sprague Dawley rats (250 - 320 g) following cervical dislocation and exsanguination. The perfusion pressure was set at 95 mmHg. The atria were removed and the ventricles were paced (300 stimuli/min) with square wave pulses at 4 V and 1 msec (supramaximal threshold voltage and pulse width) via teflon-coated stainless steel plunge electrodes, using a Grass stimulator (Model SD9).

The perfusing solution was a modified Krebs-Henseleit buffer comprising (in mM) $CaCl_2$ 0.7, NaCl 118, KCl 1.8 - 8.8, $MgCl_2$ 1.2, $KH_2PO_4$ 1.2, $NaHCO_3$ 25 and dextrose 11, at 37 °C and pH 7.4. We used 0.7 mM $CaCl_2$ because in previous studies the $EC_{50}$ for positive inotropism in perfused rat ventricles was found to be 0.5 ± 0.1 mM (Curtis et al., 1984). KCl was varied at the expense of NaCl to produce $K^+$ concentrations from 3 to 10 meq/l in order to simulate $K^+$ elevation seen in the extracellular fluid during the early period of myocardial ischaemia (Hirche et al., 1980; Hill and Gettes, 1980).

2.7.4 Variables measured

2.7.4.1 Isochoric left ventricular developed pressure. A small compliant, but non-elastic, balloon was made from plastic wrapping film (Stretch n' Seal). The balloon was connected to a transducer via a 30 cm length of PE50 tubing. The balloon was attached by tying a suitable section of film to the end of the PE tubing with silk thread, and was then filled with water and stretched by excess pressure to triple its original volume.

The balloon so-formed was non-elastic and yet compliant enough to fill the cavity of the left ventricle without distorting its shape. Left intraventricular pressure was recorded after inserting the balloon into the left ventricle via a left atrial incision. The pressure within the balloon was adjusted to give an end diastolic pressure of 15 mmHg.

2.7.4.2 Coronary blood flow. The perfusion apparatus was graduated to indicate the volume of fluid in each chamber. Coronary flow was estimated by measuring the volume of perfusion fluid passing from the graduated chambers, under the assumption that if end diastolic pressure remained constant when perfusion pressure was varied from 80 to 140 mmHg, then the aortic valve was competent. This relation was tested during the stabilisation period.

2.7.4.3 Ventricular excitability. Preliminary experiments showed that the threshold voltage and pulse width for capture of ventricles were approximately 0.6 V and 0.1 msec at 5.9 meq/l $K^+$. The experiments were carried out at well above threshold (4 V and 1 msec), but at well below the threshold for inducing unwanted effects such as noradrenaline release and heating of the myocardium (as judged from the responses of hearts to large variations in stimulation variables in preliminary experiments). It was considered inappropriate to examine the strength-duration relationship in these particular ventricles, since it was possible that such an experiment may jeopardise the subsequent viability of the preparations. Nevertheless, the threshold voltage (at 1 msec) and the threshold pulse width (at 4 V) for capture were determined in most experiments as an index of excitability.

2.7.5 Comparison of (+)- and (-)-verapamil

Following 10 - 20 min stabilisation, the ventricles were perfused with (+)- or (-)-verapamil (in half $\log_{10}$ increments) and steady state developed pressures were recorded. The enantiomers were loaded into the chambers of

the perfusion apparatus before each experiment from stock solutions (prepared by serial dilution) designed such that 0.2 ml of stock per 70 ml buffer gave the desired concentration within each cylinder. The stock solutions were refrigerated and stored in light-proof containers. Each preparation was used for a single drug at a single $K^+$ buffer concentration (3, 5.9, 8 or 10 meq/l); therefore 8 groups of preparations (n = 6 per group) were used. Records were analysed blind. Concentration-response curves were constructed and $EC_{50}$ and slope values were estimated for individual preparations. Potency ratios at each $K^+$ concentration were determined by contrasting every $EC_{50}$ for (+)-verapamil with every $EC_{50}$ for (-)-verapamil.

2.7.6 Comparison of nifedipine and DHM9

Nifedipine and DHM9 were compared in exactly the same manner as (+)- and (-)-verapamil. However, special precautions were taken to protect these drugs from ultraviolet light. The experiments were carried out in a darkened room, illuminated only by a 40 W bulb. The lamp was wrapped in red plastic to filter out short wave-lengths. In addition, the fluid circulating and warming the perfusion apparatus was stained with red dye for the same purpose. The effectiveness of these procedures was exemplified by the reproducibility of the results with time (see Results). The stock solutions for both drugs used 50% ethanol in saline as the solvent. When the drugs were added to the reservoir chambers of the perfusion apparatus, dilution reduced the final ethanol concentration to 0.25%.

2.7.7 Statistics

Slopes, $EC_{50}$ values and potency ratios were compared by ANOVA and Duncan's multiple range test.

## 2.8 Metabolism of racemic verapamil in acute myocardial ischaemia

### 2.8.1 Introduction

The following experiment was the first which was carried out as part of this thesis. This experiment was part of the initial investigation of (±)-verapamil (Curtis et al., 1984). It had been shown by others in the laboratory that (±)-verapamil reduced arrhythmias induced by coronary occlusion in conscious rats. As part of the attempt to investigate the mechanism of action of (±)-verapamil, it was decided to measure the amount of (±)-verapamil in the blood, the normal ventricular tissue and the OZ of rats subjected to coronary occlusion.

### 2.8.2 Animals

Male Sprague Dawley rats were prepared for coronary occlusion as described previously. The occluder was placed 3 mm below the atrial appendage in order to produce small OZs. Approximately 7 days were allowed for the animals to recover from preparative surgery.

### 2.8.3 Drug administration and tissue samples

On the day of study, 6 mg/kg (±)-verapamil was administered, either before occlusion, according to the usual protocol, or over a 10 min period starting immediately after occlusion. It had been shown that (±)-verapamil was approximately equally effective as an antiarrhythmic when given after occlusion as when given before occlusion, and the $ED_{50}$ for reducing arrhythmia score (AS) was approximately 6 mg/kg in both cases. Therefore it was of interest to determine whether the drug was capable of distributing into the OZ when given after occlusion, in relation to the possible locus of action within the myocardium.

Rats were monitored for 30 min after occlusion. Episodes of VF were thump-verted as required, in the usual manner. After 30 min the rats were quickly anaesthetised with halothane (5 %), using a face mask, and the aorta

was exposed by midline laparotomy for blood sampling. At least 10 ml of blood was taken and stored temporarily in an iced heparinised test tube (Kymax). The heart was then removed. In several experiments, other tissues (skeletal muscle and liver) were also sampled.

2.8.4 Extraction

The following procedures were carried out in order to prepare samples for injection onto an HPLC separation system. All the test tubes and syringes used were made of glass.

a. Blood was divided into 3 samples. One ml of whole blood was immediately pipetted into a Dreyer test tube and spiked with 16 µl of 1 µg/µl gallopamil (internal standard for the HPLC and detector). Extraction of (±)-verapamil was carried out using a modification of the method of Cole et al. (1981). The sample was made basic (approximately pH 9) with approximately 250 µl of 4 M NaOH for extraction into organic solvent, since the pKa of (±)-verapamil is approximately 8.5 (Dorrscheidt-Kafer, 1977). The organic solvent was 1 - 2 ml methyl-tert-butylether (MTBE). In preliminary experiments it was found that increasing MTBE volume beyond the recommended 1 ml did not influence yield. The test tube was vortex-mixed for 30 sec and centrifuged at 9950 x g for 2 min in order to separate the organic and aqueous phases. Using a 1 ml syringe, most of the upper organic phase was removed (the exact amount was immaterial, see below for explanation). The MTBE was then evaporated by blowing a jet of $N_2$ into the Dreyer tube, and the residue was reconstituted in 75 % acetonitrile in distilled water. This constituted the injection sample.

A sample of plasma was prepared from the remainder of the blood, and 1 ml was processed in the same way as the whole blood sample, above. The remainder of the plasma was removed, using a glass syringe (Hamilton Rheodyne), and centrifuged at 900 x g for 40 - 60 min through a re-usable Amicon

centriflow filter (type CF50A), in order to produce a sample of plasma water free of $\alpha_1$-acid glycoprotein, with which (±)-verapamil has been reported to be approximately 90% bound (Schomerus et al., 1976; McAllister et al., 1983). The Amicon filter retains more than 95% of molecules > 50 kD, and more than 97% of serum protein. The filter was prepared by soaking in distilled water for 3 h, followed by 10 min centrifugation at 900 x g in order to remove excess water. The filter was cleansed after use by soaking in 0.1 M NaOH. The volume of the ultrafiltrate was measured, and this sample of free plasma water was then processed in the same way as the plasma and whole blood samples.

b. Ventricular tissue was processed according to a modification of the technique of McAllister and Howell (1976). Samples of normal and occluded ventricular tissue were taken after blotting the tissue dry. Using small surgical scissors, the tissue was subdivided on the basis of the known location of the OZ in rats, by visual inspection. A transmural sample of the centre of the OZ was taken, as well as the entire OZ and a large sample of normal ventricle. All 3 samples were weighed, then processed as follows.

The tissue was minced using small surgical scissors and tissue forceps, and mixed in a glass homogenistation tube with 3 ml 0.1 M HCl and 16 µl of 1 µg/µl gallopamil (internal standard). This acidification was carried out to encourage the (±)-verapamil to partition out of the tissue into the aqueous melieu by promoting ionisation of this weakly basic drug. The tissue was then homogenised using a pestle which fit tightly in the homogenisation tube. The tube was kept cool by constant immersion in a slurry of ice. The contents of the tube were washed into a test tube, which was vortex-mixed for 2 min and then centrifuged at 10,000 x g for 30 min. The aquum was decanted into a Kymax test tube, and the pellet was resuspended in 1 ml 0.1 M HCl and reprocessed. The final extract was titrated to pH 8.5 with

20 % $Na_2CO_3$ (approximately 400 µl) in order to encourage subsequent partition into MTBE. The cautious adjustment of pH was carried out in order to preclude the development of a soapy scum, which was associated with the use of NaOH in preliminary experiments. Subsequent processing steps were identical to those used for blood matrix samples.

2.8.5 HPLC

Separation of (±)-verapamil and gallopamil was carried out by reverse-phase high pressure liquid chromatography (HPLC, SP8000), using MOS Hypersil as the packing material and an Altex injection device. The running solvent was a 50:50 mixture of distilled water and acetonitrile containing 500 µl of sodium octylsulphate and 0.355 g of $Na_2HPO_4$ per litre. The pH of the aqueous component was adjusted to 4.6 with 0.1 M phosphoric acid before mixing with acetonitrile. Flow rate was set at 1 ml/min using a Micrometrics pump. Injection of samples into the column was carried out using a 25 µl Hamilton Rheodyne glass syringe.

2.8.6 Calculation of verapamil concentration

(±)-Verapamil and gallopamil were detected using a fluorimeter (Schoeffel FS970). The optimum excitation wavelength was found in preliminary experiments to be 203 nm, and an ordinary glass detection filter was used. A permanent record of peaks was generated using a Rikadenki B-107 chart recorder. (±)-Verapamil concentration was calculated under the assumption that the extraction processes extracted (±)-verapamil and gallopamil equally. This assumption was tested and found to be the case in preliminary experiments. The column was calibrated daily by injecting 20 ng (±)-verapamil and 20 ng gallopamil simultaneously onto the column, and measuring the ratio of peak heights and the retention times. The retention times were used to locate (±)-verapamil and gallopamil on the traces from blood and tissue samples. The ratio of heights was used to calculate the concentration of

(±)-verapamil in the samples, according to the following formula:

$$\text{Concentration (mol/l)} = (Vh_s/Dh_s)(16/491080)(R/Y)$$

Where $Vh_s$ and $Dh_s$ are the peak heights for (±)-verapamil and gallopamil from the blood matrix samples, respectively, R is the ratio of the peak heights of the (±)-verapamil:gallopamil standards, Y is the volume of blood matrix in ml, and 16 and 491080 are constants to account for the weight of gallopamil used to spike each sample (16 µl) and the molecular weight of (±)-verapamil (491.08). In the case of tissue samples, Y refers to g weight, and the units of (±)-verapamil concentration are mol/kg.

The above calculation was based on preliminary experiments which showed that the yields of (±)-verapamil and gallopamil from a sample spiked with known amounts of the drugs were similar (ratio of recovery of (±)-verapamil:gallopamil 1.003 ± 0.059 according to peak height, mean ± s.e.mean, n = 14), and experiments which showed that the ratio of peak heights, R, was constant over at least 3 orders of magnitude of drug concentration.

# 3 RESULTS

## 3.1 Metabolism of (±)-verapamil in acute myocardial ischaemia

This study was carried out in conjunction with an investigation into the effects of (±)-verapamil on the responses to coronary occlusion in conscious rats. It had been demonstrated that (±)-verapamil possessed dose-dependent antiarrhythmic effects when administered before occlusion and when administered after occlusion. Therefore, it was of interest to examine the concentrations of (±)-verapamil in the blood matrices after coronary occlusion, and also to examine whether the drug penetrated into the occluded zone. The latter consideration was expected to shed light on the question concerning the site of antiarrhythmic action of the drug, since an absence of (±)-verapamil in the occluded zone would suggest that the antiarrhythmic effects were produced via an action elsewhere. The results of this study have been published (Curtis et al., 1984).

### 3.1.1 Concentration of (±)-verapamil in blood matrices

The concentration of (±)-verapamil in whole blood, plasma and plasma ultrafiltrate (PU) is shown in Table 2. It can be seen that concentrations were slightly higher following post-occlusion administration compared with pre-occlusion administration, although the differences were statistically significant only for the PU. Since drug administration took approximately 10 min, whereas all rats were sacrificed at 30 min after occlusion, then the difference between pre- and post-occlusion administration in terms of blood matrix (±)-verapamil concentration reflects the short distribution half life and the rapid hepatic metabolism of the drug (Schomerus et al., 1976; McAllister, 1982). The concentration of (±)-verapamil in the PU was in the same range as the concentration reported to inhibit $i_{si}$ but not $i_{Na}$ in ventricular tissue (Kohlhardt et al., 1972; Nawrath et al., 1981).

The extent of plasma-protein binding was 82 ± 4 % in rats given (±)-vera-

pamil after occlusion, and 84 ± 4% in rats given the drug before occlusion. These values were calculated by contrasting the plasma concentrations with the PU concentrations. They compare favourably with published values for humans (Schomerus et al., 1976; Eichelbaum et al., 1984).

3.1.2 Concentration of (±)-verapamil in the ventricular myocardium.

The amounts of (±)-verapamil in the normal non-occluded ventricular tissue (NZ), the occluded zone (OZ) and the centre of the OZ ($OZ_c$)are shown in table 3. Values from rats in which the drug was administered before occlusion indicate that (±)-verapamil appeared to accumulate in the ischaemic tissue. The $OZ_c$, which was presumably the most ischaemic tissue (or the portion of the OZ least contaminated with NZ) contained the most amount of drug ($p < 0.05$). The amount of (±)-verapamil in the NZ in rats given the drug after occlusion was approximately 3 times the value found in rats administered the drug before occlusion (although the difference was not statistically significant). In this regard, NZ values varied according to the time of administration in a qualitatively similar manner to levels in the blood matrices.

It was of considerable interest to find that (±)-verapamil was present in the OZ (and $OZ_c$) of rats administered the drug after occlusion. However, unlike the case in which (±)-verapamil was given before occlusion, the levels in the OZ and $OZ_c$ were not disproportionately high, compared with the NZ. Indeed, there were no significant differences between the levels in the NZ, OZ and $OZ_c$ following post-occlusion administration. Although the amount of (±)-verapamil in the NZ was greater following post-occlusion administration, the amount in the $OZ_c$ was less, indicating that although the drug did partition into the ischaemic tissue even when given after ligation, the ability of the ischaemic tissue to accumulate the drug was diminished. This presumably reflected the fact that whereas (±)-verapamil had

Table 2. Concentration of (±)-verapamil in blood matrices

| Administration | n | Whole Blood | Plasma | Ultrafiltrate |
|---|---|---|---|---|
| Pre-occlusion | 6 | 2.4 ± 0.8 µM | 2.7 ± 1.1 µM | 0.24 ± 0.04 µM |
| Post-occlusion | 6 | 3.2 ± 0.5 µM | 3.6 ± 0.8 µM | 0.57 ± 0.10 µM* |

The dose of (±)-verapamil was 6 mg/kg. Values are mean ± s.e.mean. * Indicates $p < 0.05$ versus the value in the pre-occlusion-administration group (unpaired 2-tailed t test).

Table 3. Distribution of (±)-verapamil in the ventricles

| Administration | n | Non-occluded | Occluded | Occluded centre |
|---|---|---|---|---|
| Pre-occlusion | 6 | 5.7 ± 0.7 | 8.4 ± 0.8 | 22.1 ± 3.1 |
| Post-occlusion | 6 | 15.8 ± 5.6 | 11.0 ± 2.3 | 13.9 ± 1.4 |

The data is expressed exactly in the same way as the data in Table 2, with the exception that values are in μmol/kg. There were no statistically significant differences between the pre- and post-occlusion administration groups (unpaired 2-tailed t test).

free access to the ischaemic tissue when given before occlusion, and indeed was probably trapped there in high concentrations (occlusion took place immediately after drug administration in these rats), it only had access to the ischaemic tissue via the veins which drain over the OZ in the rat heart (Taira et al., 1985) when given after occlusion.

## 3.2 Arrhythmogenesis and the role of the CNS

### 3.2.1 Overview

In view of the contradictory evidence regarding the importance of the autonomic nervous system in arrhythmogenesis in the rat (Botting, et al., 1984; Siegmund, et al., 1979; Szekeres, 1979; Au, et al., 1983; Kenedi and Losconci, 1973a; 1983b; Campbell and Parratt, 1983; Marshall, et al., 1981), and contradictory evidence regarding the role of the sympathetic nervous system in arrhythmogenesis in general (e.g., Harris, et al., 1951; Schaal et al., 1969; Gillis, 1971; Myers et al., 1974; Hope, et al., 1974; Fowliss, et al., 1974; Pantridge, 1978; Johnston et al., 1983a), a systematic investigation of the importance of adrenoceptor activation and the central nervous system was carried out, using a series of ablations in the CNS combined, in some cases, with catecholamine infusions. The groups in this study are summarised in Table 1. The results of this study have been published (Curtis et al., 1985b).

### 3.2.2 Occluded zone (OZ)

OZ size (Figure 3) ranged from 33 ± 3 ( % ventricular weight) in aS rats to 45 ± 2 in cP rats. Differences in mean OZ size between groups were not statistically significant (except for the aS group) and therefore did not account for variations in blood pressure, heart rate or arrhythmias (described below).

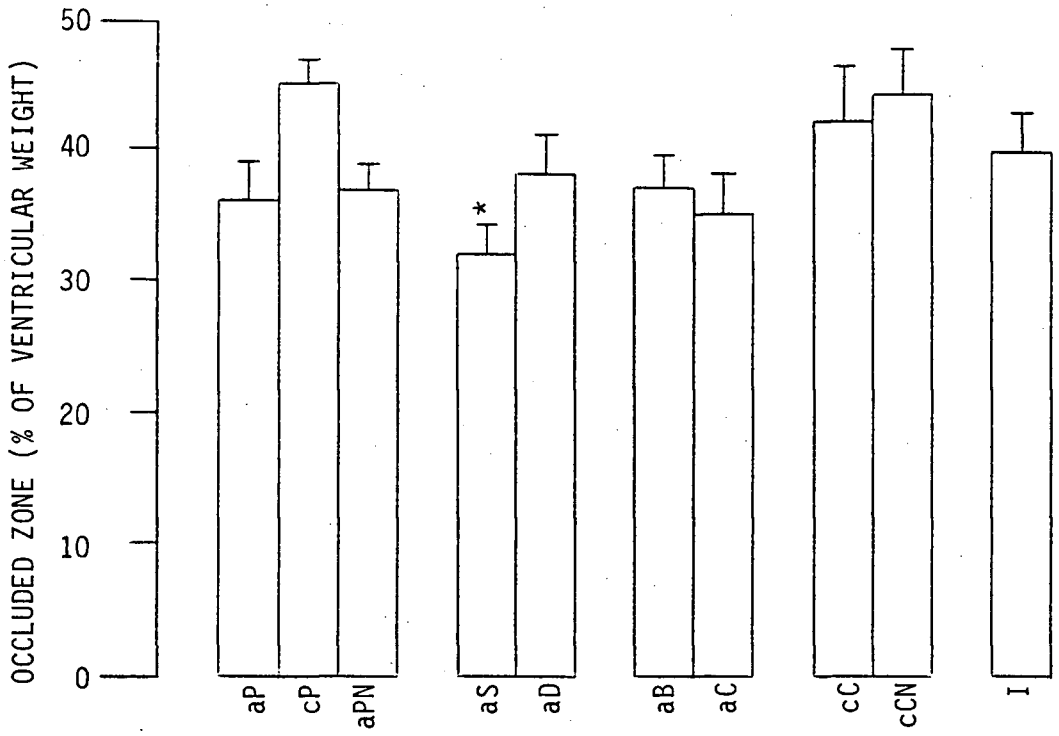



Figure 3. Each histogram represents mean ± s.e.mean OZ size as % ventricular weight. The groups are indicated by symbols defined in the text and in Table 1. * Indicates $p < 0.05$ versus cC.

3.2.3 Arrhythmias

3.2.3.1 Arrhythmia scores. There were considerable differences between the groups with regard to arrhythmias, which are summarized (Figure 4) as mean arrhythmia scores for the early (0 - 0.5 h) and late (0.5 - 4 h) periods. The most important findings were in the 0.5 - 4 h post-occlusion period. A dramatic fall in arrhythmia score was seen in all acutely prepared animals, in isolated hearts, and also in chronically prepared, but acutely pithed, rats. In most pithed and spinalized rats not even a single PVC was seen in the late (0.5 - 4 h) period. Only one isolated heart had VF (at 3.75 h after occlusion).

3.2.3.2 VT and VF. VF in the 0.5 - 4 h period was absent in all pithed rats including those receiving catecholamine infusions (Figure 5b). VF was also absent or of low incidence in the aS, aD, aB, and aC groups. The differences in VF incidence between the groups during the early period (Figure 5a) were qualitatively similar to the differences during the late period, although less pronounced.

Changes in the incidences of VT (Figure 6) resembled the changes seen in the incidences of VF. The most marked changes were seen in the late period during which the incidence was low in all acutely prepared rats and absent in all pithed groups (aP, cP and aPN) including the group receiving the catecholamine infusion (aPN).

3.2.3.3 PVC. $Log_{10}$ PVC number in the early period (Figure 7) was similar in all groups. Values were highest in the two acutely prepared non-ablated groups (aB and aC). In the late period (0.5 - 4 h) $log_{10}$ PVC was reduced by all types of surgical ablation in the CNS. PVC were in fact absent in the aP group. $Log_{10}$ PVC number was also slightly lower in the aB group versus conscious controls (cC).

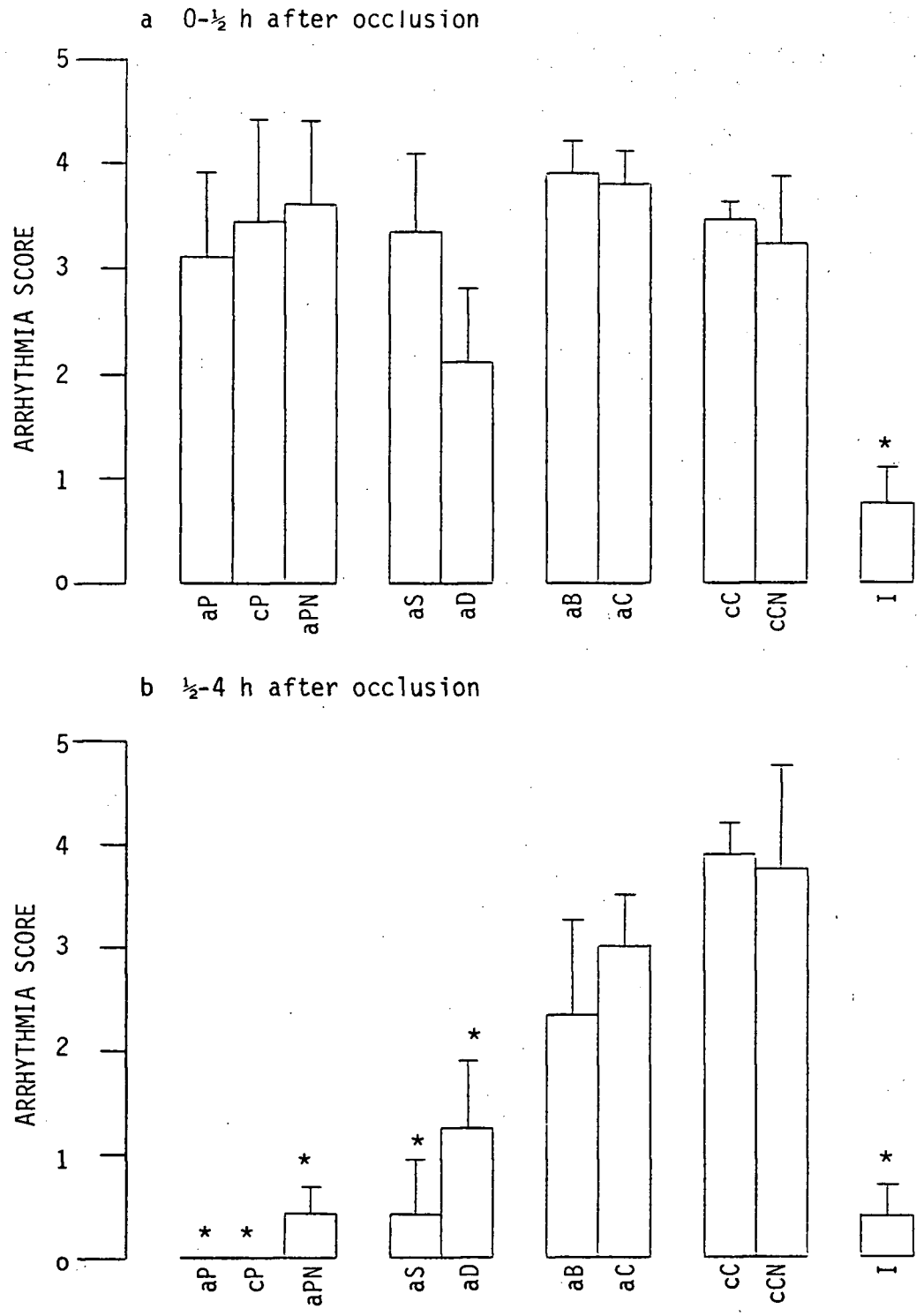



Figure 4. Arrhythmia scores (mean ± s.e.mean). The groups are the same as in figure 3. * Indicates $p < 0.05$ versus cC.

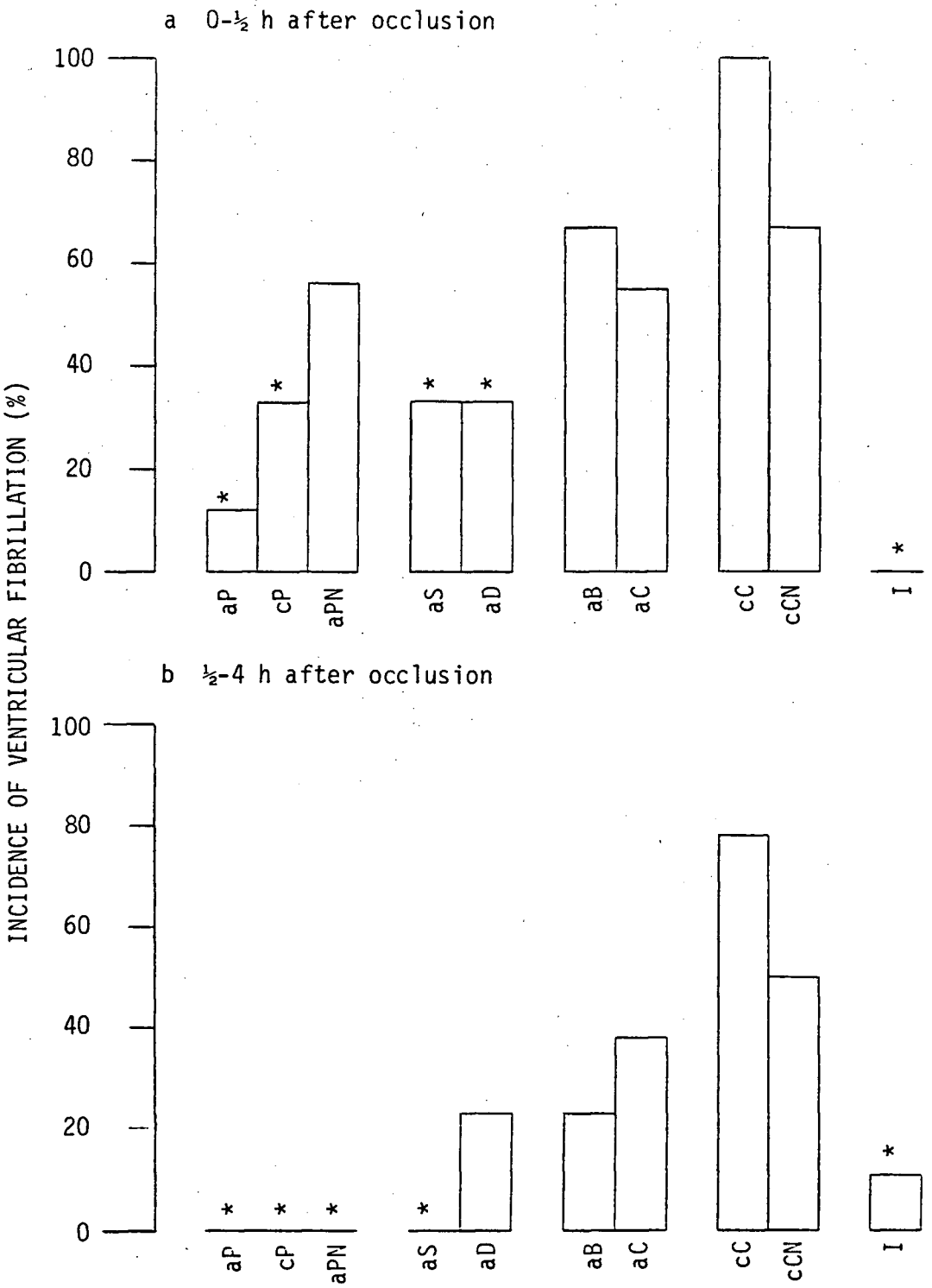



Figure 5. Incidence of VF. The groups are indicated by symbols defined in the text and in Table 1. * Indicates $p < 0.05$ versus cC.

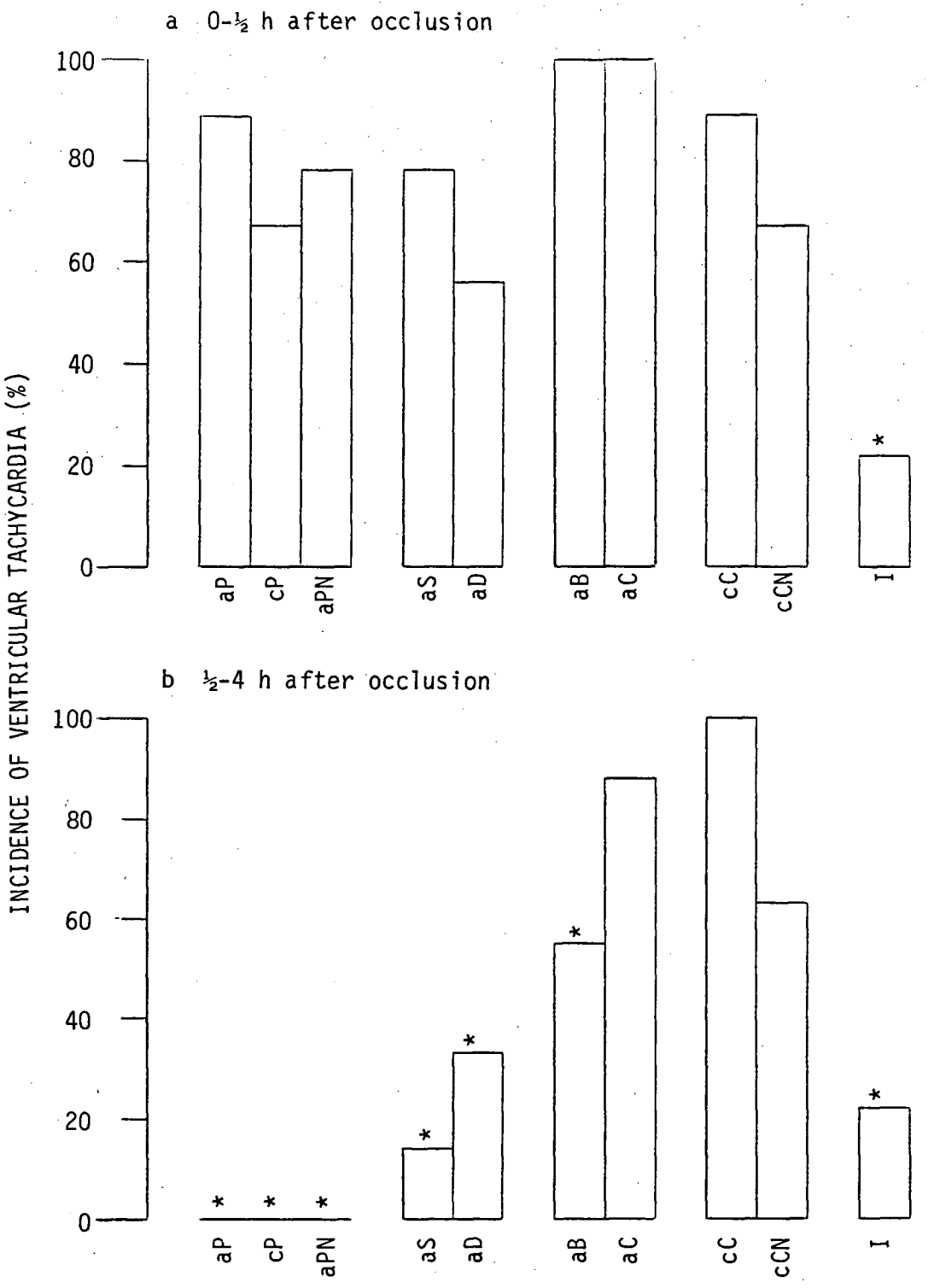



Figure 6. Incidence of VT. The groups are indicated by symbols defined in the text and in Table 1. * Indicates $p < 0.05$ versus cC.

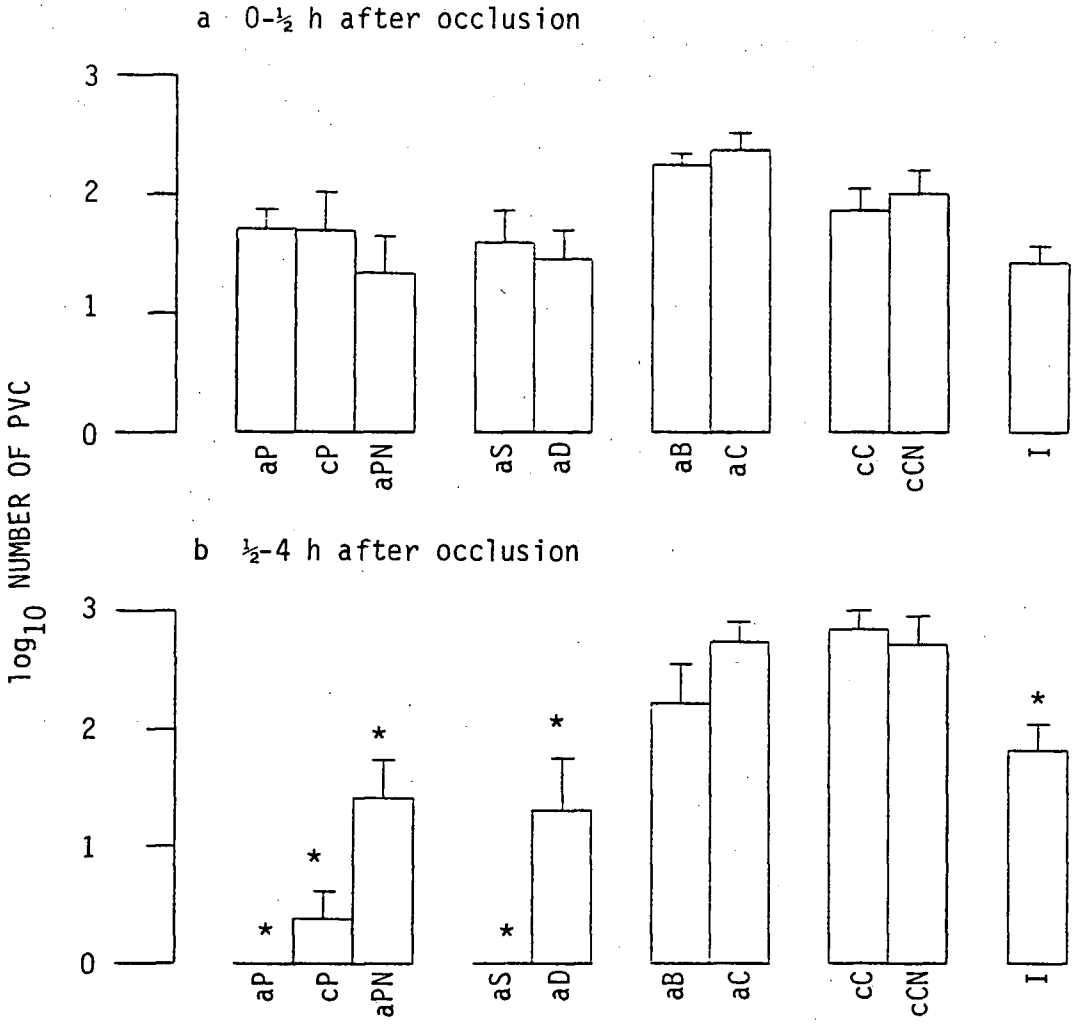



Figure 7. The $\log_{10}$ number of PVC (mean ± s.e.mean). The groups are indicated by symbols defined in the text and in Table 1. * Indicates $p < 0.05$ versus cC.

3.2.4 Haemodynamic variables

The effects of occlusion on heart rate and blood pressure are summarized in Figure 8a and Figure 8b respectively. Before occlusion, heart rate and blood pressure were generally lowest in pithed and spinalized animals, as was expected. The highest pressures and heart rates were seen in the decerebrate (aD) and conscious catecholamine-infused (cCN) groups. In the aPN pithed group, catecholamine infusion restored blood pressure and heart rate to close to levels seen in the conscious control group (cC). In isolated hearts (I), left ventricular systolic pressure was 130 ± 8 mmHg at a diastolic pressure of 10 - 15 mmHg (not shown in Figure 8).

Occlusion produced definite falls in blood pressure (Figure 8b) only in rats without surgical ablations in the CNS (aB, aC, cC, cCN). The effect of occlusion on heart rate (Figure 8a) was generally slight in all groups (except the cCN group, in which marked tachycardia occurred after occlusion). Heart rate in isolated hearts (not shown) was 168 ± 14 beats/min before occlusion and 160 ± 13 beats/min 30 min after occlusion. Corresponding left ventricular systolic pressures were 96 ± 7 and 57 ± 7 mmHg.

3.2.5 ECG changes

The only statistically significant difference between the groups in terms of ECG changes following occlusion was a delay in the development of maximum S-T segment elevation in the aP and cP groups versus all other groups (Figure 9). No other obvious trends were apparent. Since some rats (the pithed and spinalised groups) were mounted vertically, posturally-induced shifts in the position of the heart in the thorax with respect to the chest lead of the ECG make direct comparison of the ECG data between the groups difficult.

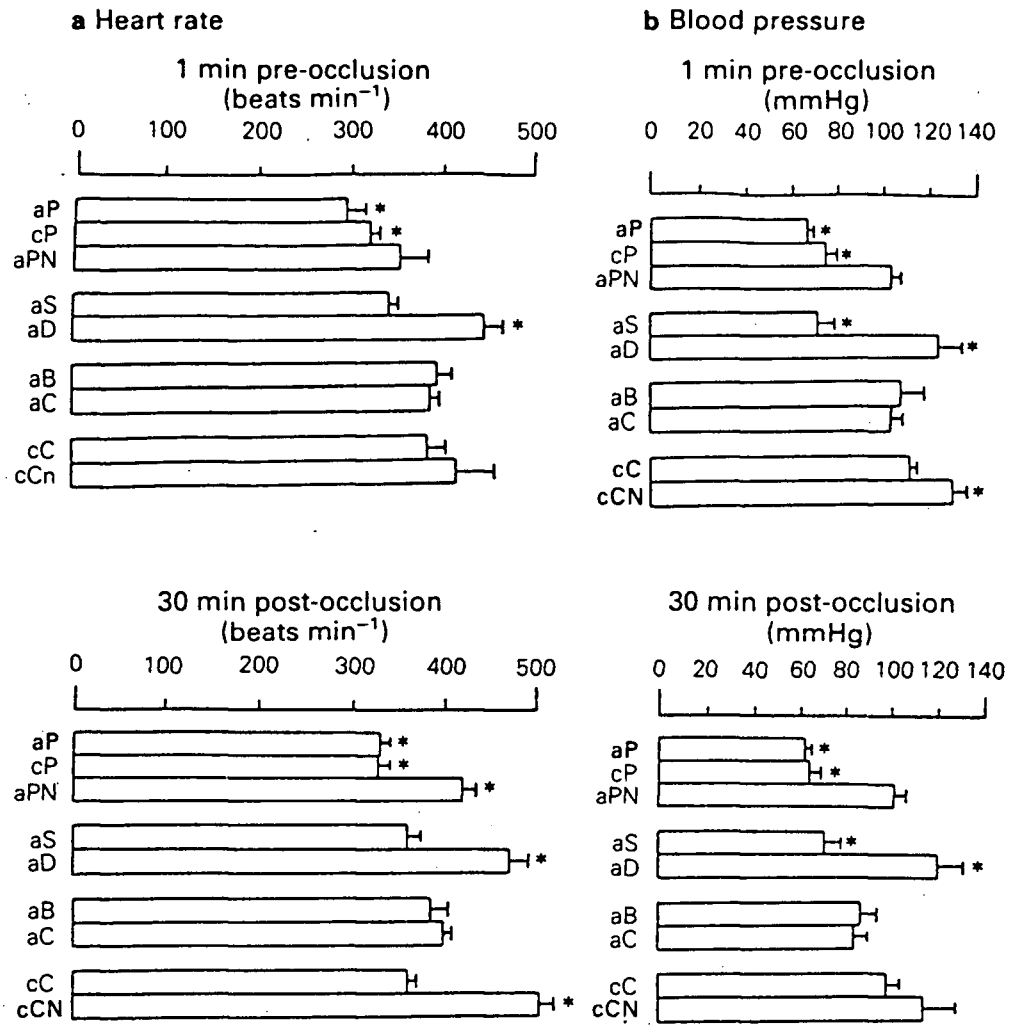



Figure 8. Heart rates (a) and blood pressure (b) before (-1 min) and after (30 min) coronary occlusion. Each value is mean ± s.e.mean. The groups are indicated by symbols defined in the text and in Table 1. * Indicates $p < 0.05$ versus cC.

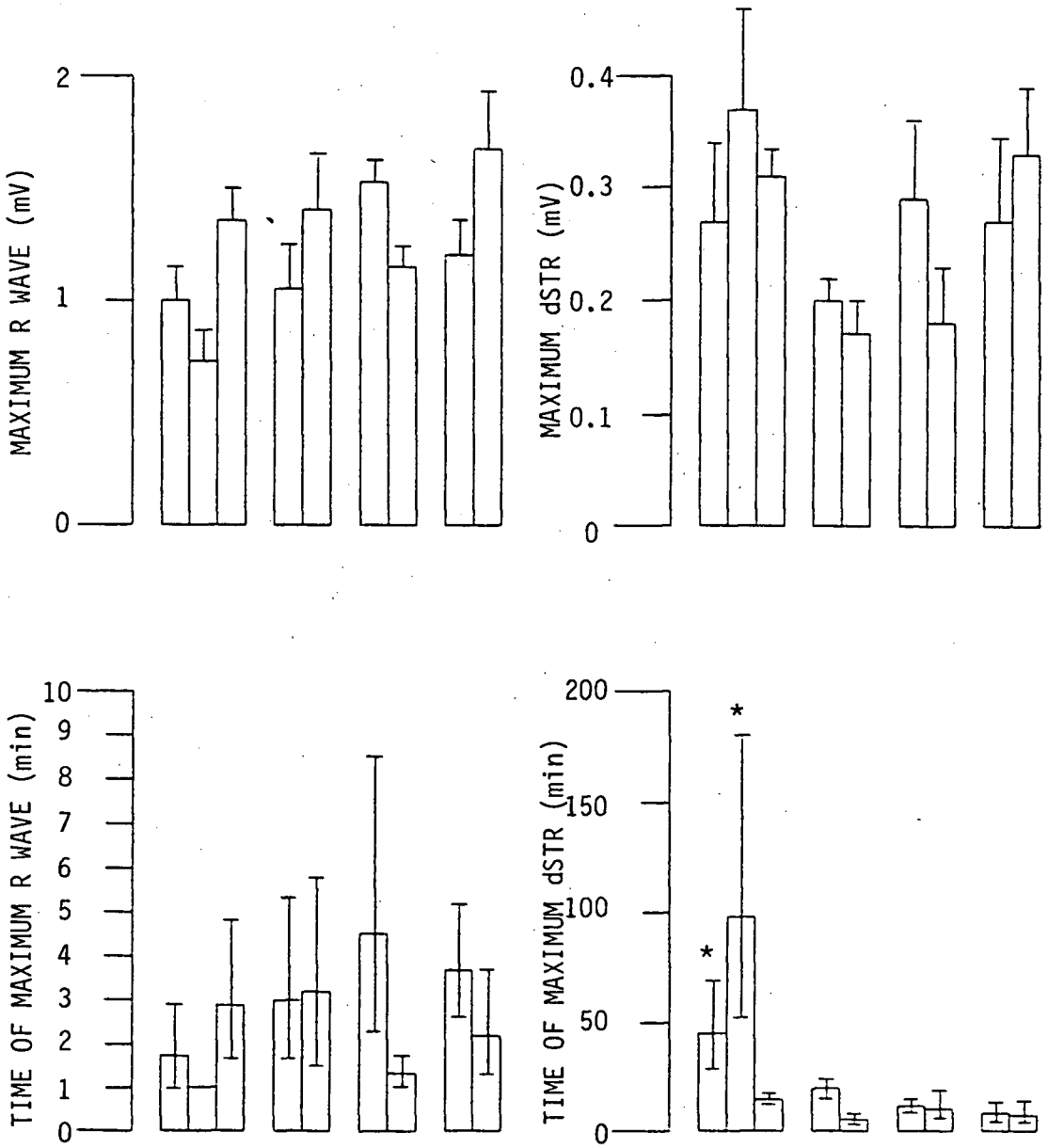



Figure 9. ECG changes following coronary occlusion. In each part of the figure, the groups (from left to right) are aP, cP, aPN, aS, aD, aB, aC, cC and cCN (symbols defined in Table 1). * Indicates $p < 0.05$ versus cC.

3.2.6 Thrombocytes, leukocytes and serum $K^+$

These variables were measured in an ancillary group of acutely prepared pithed rats only. The most striking finding was an elevation in serum $K^+$ following pithing (Figure 10). At the same time periods the thrombocyte and leukocyte counts fell. The leukocyte count returned toward the pre-pithing value by 4 h after occlusion, whereas the reduction in thrombocyte count was maintained throughout the observation period (Figure 10). Arrhythmias in this ancillary group (not shown) were almost identical with those seen in the original aP group.

3.2.7 Summary

The role of the CNS in arrhythmogenesis following coronary occlusion was investigated in rats by use of CNS ablations and noradrenaline/adrenaline infusions. All procedures involving acute surgical preparation reduced the incidence and severity of the arrhythmias induced by occlusion (Figures 4 - 7). Such reductions were most marked in the late (0.5 - 4 h period) after occlusion. The observed reductions in arrhythmias could not be explained in terms of involvement of the CNS or adrenoceptor activation. When circulating leukocytes, thrombocytes and serum $K^+$ were measured in a group of pithed rats before and after occlusion, reduced levels (20-50 %) of both leukocytes and thrombocytes occurred while serum $K^+$ levels rose by 50-100 %.

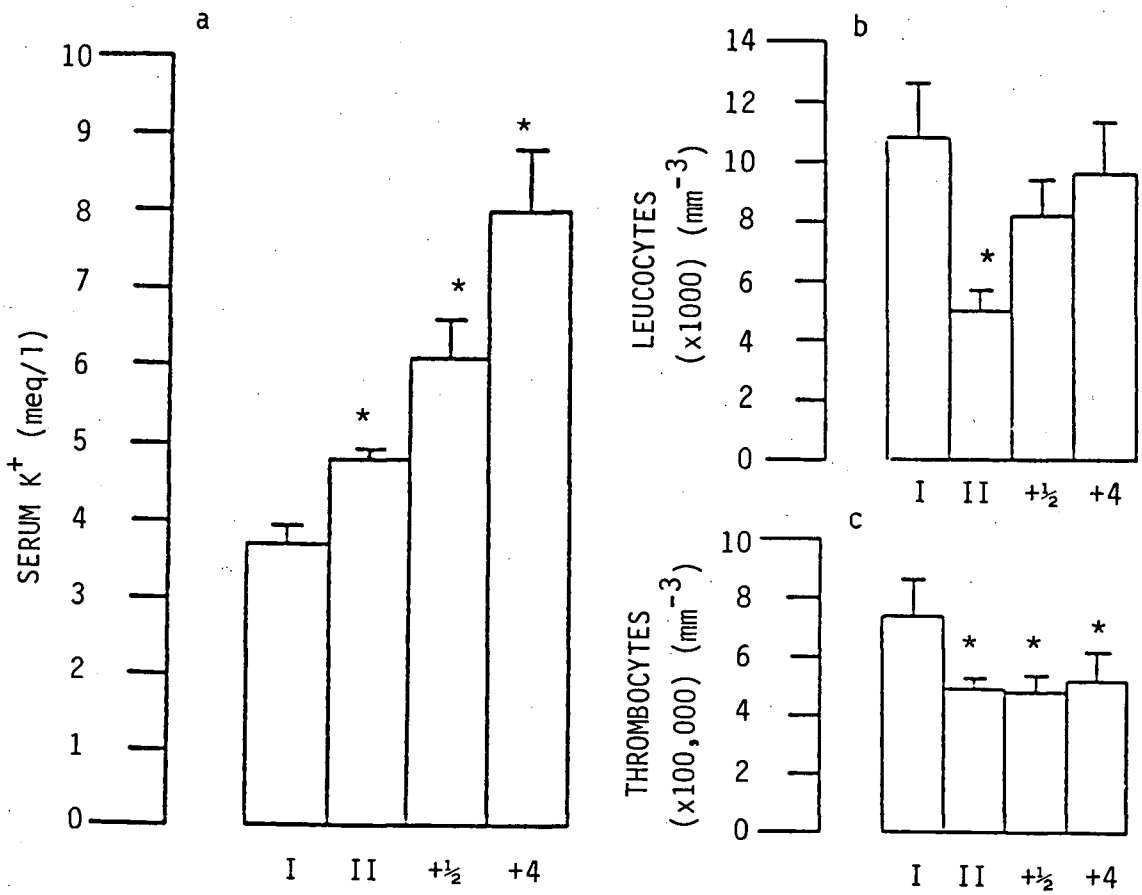



Figure 10. Changes in serum $K^+$ concentration (part a), leukocytes (part b) and thrombocytes (part c) in pithed rats before and after coronary occlusion. The variables were measured immediately following preparative surgery but before pithing (I), after pithing but 1 min before occlusion (II), 1/2 h after occlusion (+1/2) and 4 h after occlusion (+4). Each value is mean ± s.e.mean (n = 9). *Indicates $p < 0.05$ compared with values before pithing (I).

## 3.3 Actions of anipamil and ronipamil in acute myocardial ischaemia

### 3.3.1 Overview

The hypothesis that the antiarrhythmic activity of (±)-verapamil (demonstrated previously in our laboratory; Curtis et al., 1984) occurred by virtue of calcium antagonism in the ventricular myocardium predicts that all calcium antagonists with such an action should be antiarrhythmic. Preliminary experiments (Kretzschmar, personal communication) have suggested that anipamil is a calcium antagonist with relative selectivity for the myocardium, whereas ronipamil, a close structural analogue is relatively inactive. Therefore, it was of interest to determine whether these verapamil analogues could reduce ischaemia-induced arrhythmias at doses below those producing vasodilatation-induced hypotension, in a manner consistent with their calcium antagonist profile. Based on the limited information concerning the pharmacological properties of these drugs, it was predicted that anipamil should reduce ischaemia-induced arrhythmias, whereas ronipamil should be relatively inactive. In the figures, C refers to controls, R = ronipamil, A = anipamil, L = low dose (50 mg/kg, p.o.) and H = high dose (150 mg/kg, p.o.). Therefore the 5 groups are C, RL, RH, AL and AH. This study has been published (Curtis et al., 1986b).

### 3.3.2 OZ and infarct zone (IZ)

The size of the OZ was not found to be statistically significantly altered by treatment. The mean ± s.e.mean OZ size (expressed as % ventricular weight) was 42 ± 4, 41 ± 4, 40 ± 2, 39 ± 4 and 36 ± 3 % in the control, RL, RH, AL, and AH groups, respectively.

The mean IZ size in the AH group was 29 ± 4 % of total ventricular weight which was comparable with values in previous control groups (Curtis et al., 1984). We were unable to obtain enough estimates of IZ size in the other groups to permit useful comparisons.

3.3.3 Arrhythmias

Arrhythmias following occlusion in control rats have been shown by our laboratory to be bi-modally distributed with time (Johnston et al., 1983a). Peaks occur at approximately 10 min and 2 h after occlusion, with a quiescent interval lasting from approximately 20 min to 1.5 h after occlusion. As arrhythmia incidence within groups was similar during both the 0 - 0.5 h, and 0.5 - 4 h periods after occlusion, arrhythmia data is presented for the overall 0 - 4 h period. The arrhythmia scores for the different groups are shown in Figure 11. Both doses of anipamil statistically significantly reduced arrhythmia score, whereas ronipamil had no significant effect.

With regard to individual types of arrhythmias, the incidence of VT was high in all groups. In those rats having VT, the $\log_{10}$ duration of such events (Figure 12a) was not statistically significantly reduced by either drug. The $\log_{10}$ number of episodes of VT in those rats having VT was 1.5 ± 0.2 (mean ± s.e.mean) in controls. This value was reduced to 1.4 ± 0.2, 1.1 ± 0.2, 1.1 ± 0.2 and 1.0 ± 0.2 by RL, RH, AL, and AH treatment, respectively.

More importantly, anipamil dose-dependently reduced both the incidence (Figure 12b) and duration (Figure 12c) of VF. $\text{Log}_{10}$ number of PVC, however, was not reduced to a statistically significant degree by either anipamil or ronipamil (Figure 12d).

In summary, although anipamil was an effective antiarrhythmic, particularly against VF, ronipamil was far less effective and failed to produce any statistically significant antiarrhythmic effects.

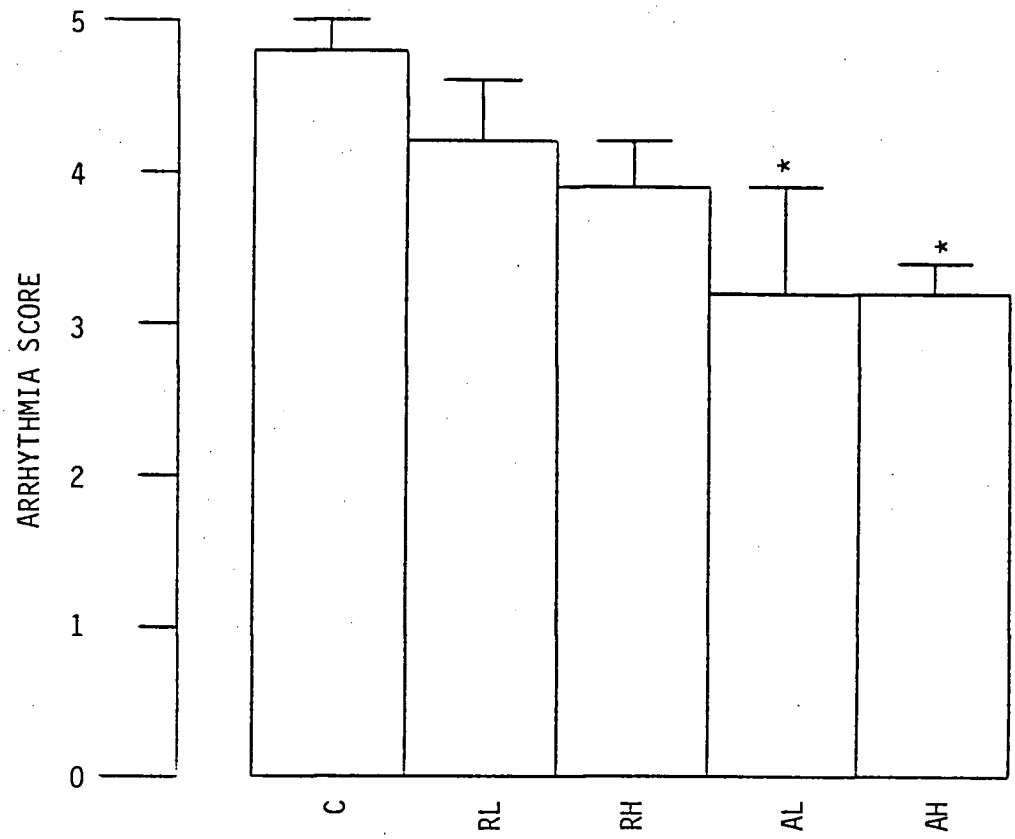
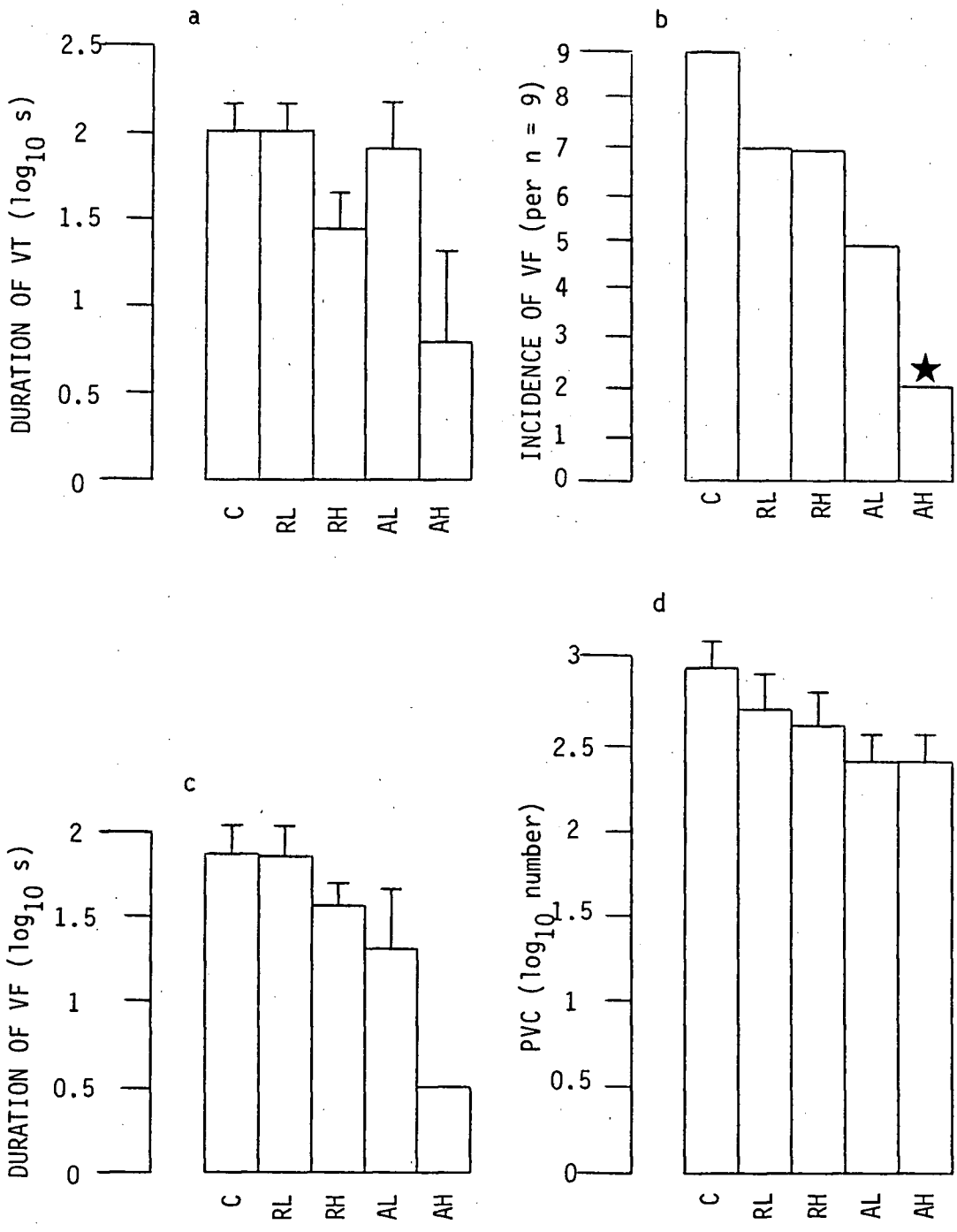



Figure 11. Effect of ronipamil and anipamil on arrhythmia score. The mean (± s.e.mean) arrhythmia scores are shown for the 4 h period following occlusion. The groups are: controls (C), 50 mg/kg ronipamil (RL), 150 mg/kg ronipamil (RH), 50 mg/kg anipamil (AL) and 150 mg/kg anipamil (AH); n = 9 for each group. * Indicates $p < 0.05$ versus controls.

Figure 12. Effect of ronipamil and anipamil on the incidence and duration of various arrhythmias during the first 4 h following coronary occlusion. The groups are: controls (C), 50 mg/kg ronipamil (RL), 150 mg/kg ronipamil (RH), 50 mg/kg anipamil (AL) and 150 mg/kg anipamil (AH); n = 9 for each group. Part a illustrates duration ($\log_{10}$ sec) of VT (mean ± s.e.mean) in those animals having this arrhythmia. The number of rats per group which had VF is given in Part b. Part c illustrates duration ($\log_{10}$ sec) of VF (mean ± s.e.mean) in those animals having this arrhythmia. The s.e.mean data is omitted where n was less than 5. Part d shows the mean (± s.e.) of $\log_{10}$ PVC. * Indicates $p < 0.05$ versus controls.


a
DURATION OF VT ($log_{10}$ s)
2.5
2
1.5
1
0.5
0
C
RL
RH
AL
AH
b
INCIDENCE OF VF (per n = 9)
9
8
7
6
5
4
3
2
1
0
★
C
RL
RH
AL
AH
c
DURATION OF VF ($log_{10}$ s)
2
1.5
1
0.5
0
C
RL
RH
AL
AH
d
PVC ($log_{10}$ number)
3
2.5
2
1.5
1
0.5
0
C
RL
RH
AL
AH

3.3.4 Mortality

Despite a high incidence of VF, particularly in control rats, all episodes of VF lasting longer than 10 s were successfully defibrillated by thump-version, thus all deaths occurring during the 4 h period following occlusion were associated with hypotension (cardiogenic shock) or severe pulmonary oedema. In an earlier study in our laboratory (Curtis et al., 1984) it was found that 20 mg/kg (±)-verapamil exacerbated the mortality associated with cardiogenic shock during the 4 h period following occlusion. In the present study however, drug treatment did not increase the number of such deaths. The number of deaths (out of n = 9) which had occurred during the 4 h period after occlusion was 1, 5, 1, 1, 1 in the control, RL, RH, AL and AH groups, respectively. The high value seen with the 50 mg/kg dose of ronipamil (RL) was not statistically significantly different from that for the other groups. By 24 h post-occlusion, mortality (out of n = 9) was 5, 6, 5, 5, and 3 respectively.

3.3.5 Haemodynamic variables

Mean arterial blood pressure and heart rate changes at various times pre- and post-occlusion are shown in Table 4. Before drug administration, the mean ± s.e.mean heart rates ranged from 376 ± 19 beats/min in the control group to 432 ± 20 beats/min in the AH group. The corresponding mean arterial blood pressures ranged from 103 ± 9 to 117 ± 4 mmHg. There were no statistically significant differences between these means.

Immediately before occlusion, and for 4 h after drug administration, neither drug, at either 50 or 150 mg/kg, had marked actions on heart rate or blood pressure when compared with the untreated animals. The small reductions in blood pressure seen prior to occlusion (expressed in Table 4 as % changes from pretreatment values) were not statistically significantly different. In the animals treated with anipamil, heart rate and blood

Table 4. Haemodynamic Effects of Anipamil an Ronipamil Before and After Occlusion

| Group | Percentage change in MAP | | | | | | Percentage change in HR | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | -30min | -1min | +1min | +30min | +1h | +4h | -30min | -1min | +1min | +30min | +1h | +4h |
| C | 0±3 | 3±3 | -5±7 | -10±5 | -8±5 | -17±6 | -1±2 | 2±3 | 9±4 | -4±4 | -5±4 | -1±6 |
| RL | 4±4 | 5±4 | -3±5 | -4±6 | -7±6 | -9±8 | 0±6 | 2±7 | 3±5 | 0±6 | -4±5 | -15±9 |
| RH | 2±6 | 4±4 | -15±7 | -15±4 | -16±4 | -24±4 | -5±6 | -5±5 | 1±6 | -13±5 | -16±5 | -14±5 |
| AL | -5±4 | -7±3 | -22±6 | -22±6 | -22±5 | -41±4* | -5±5 | 1±4 | 8±4 | -1±5 | -7±6 | -18±4 |
| AH | -6±3 | -5±2 | -21±7 | -22±4 | -21±5 | -36±4* | -5±4 | -4±4 | 2±6 | -12±7 | -15±9 | -19±10 |



The effects of treatment on mean arterial pressure (MAP) and heart rate (HR) are shown. Control values were recorded at the time of drug administration (4 h before coronary occlusion), and the percentage changes in these values (mean ± s.e.mean) are shown for various time points before (-30 and -1 min) and after (+1 min to +4 h) occlusion. The symbol * indicates $p < 0.05$ versus the control group (C), at a particular time point, by ANOVA and Duncan's range test. The groups are C = control, R = Ronipamil, A = Anipamil, L = low dose (50 mg/kg) and H = high dose (150 mg/kg.

pressure were reduced by occlusion to a greater degree than occurred in control rats, although the only statistically significant differences were for mean blood pressure at 4 h after occlusion (owing to the large variance in each group). Thus ronipamil had no marked actions on blood pressure and heart rate while anipamil had limited actions to lower heart rate and blood pressure.

3.3.6 ECG changes

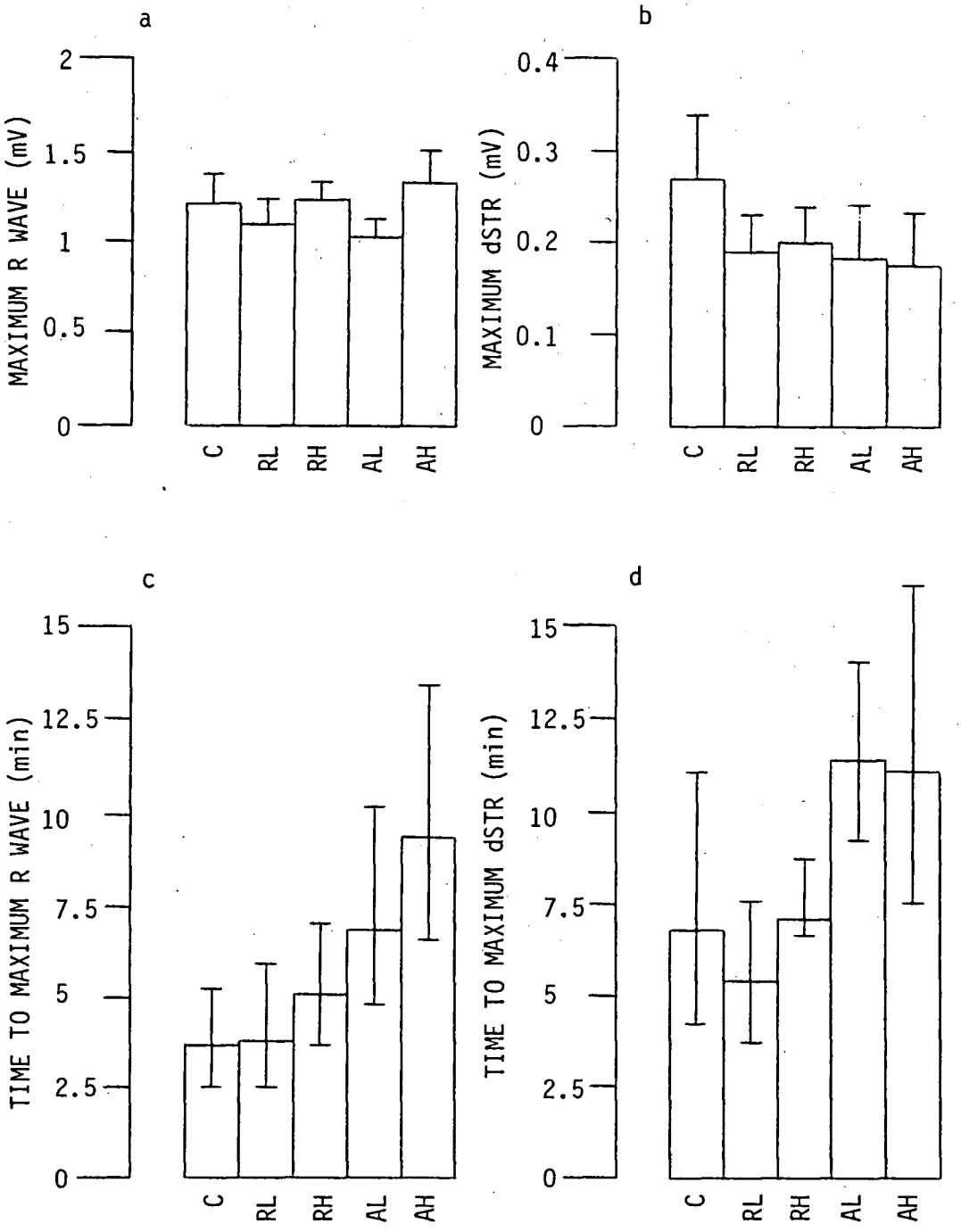
There were some effects of treatment on the ECG changes produced by coronary occlusion (S-T segment elevation and R-wave amplitude increases) (Figure 13). It appeared that anipamil reduced the rate (Figure 13c and 13d) at which both changes occurred while both anipamil and ronipamil reduced maximum S-T segment elevation (Figure 13c). However, the maximum R-wave amplitude (Figure 13a) was the similar in all groups.

In Figure 13, S-T segment elevation is expressed as dSTR. The elevation of the S-T segment was also reduced by anipamil and ronipamil if changes in were expressed as %R-wave amplitude (ST% ) in the manner used by Bernauer (1982).

3.3.7 Plasma concentrations of anipamil

To ensure that the drugs were being absorbed following administration, plasma anipamil levels were measured (by Dr. Brode, Knoll A.G., Ludwigshafen, using a technique developed in his laboratory) in separate groups of rats (n = 5 per dose). Pooled plasma concentrations in rats given 50 mg/kg anipamil p.o. were 3.2, 4 and 2.4 µg/ml at 1, 3 and 5 h after administration, respectively. Concentrations at 1, 3 and 5 h after administration of 150 mg/kg were 5, 8.5 and 3.4 µg/ml, respectively.

Figure 13. Effect of ronipamil and anipamil on occlusion-induced ECG changes (R-wave and "S-T" segment elevation). Part a shows maximum R-wave amplitudes (mean ± s.e.). Part c shows the times at which maximum R-wave amplitudes were reached. Parts b and d show corresponding values for dSTR. Values in parts c and d were calculated as $\log_{10}$ min, but are illustrated as antilogs ± 1 s.e.mean. The groups are as indicated in figure 12. * Indicates $p < 0.05$ versus controls.


a
MAXIMUM R WAVE (mV)
2
1.5
1
0.5
0
C
RL
RH
AL
AH
b
MAXIMUM dSTR (mV)
0.4
0.3
0.2
0.1
0
C
RL
RH
AL
AH
c
TIME TO MAXIMUM R WAVE (min)
15
12.5
10
7.5
5
2.5
0
C
RL
RH
AL
AH
d
TIME TO MAXIMUM dSTR (min)
15
12.5
10
7.5
5
2.5
0
C
RL
RH
AL
AH

3.3.8 Summary

Ronipamil and anipamil (2 analogues of verapamil), were administered p.o. to conscious rats which were subjected to coronary occlusion. Only anipamil (50 and 150 mg/kg) statistically significantly reduced arrhythmias (Figures 11 and 12); it was more effective against VF than VT or PVCs. Ronipamil at the same doses had little antiarrhythmic activity. Only anipamil delayed the development of ECG signs of ischaemia, whereas both drugs reduced the extent of such changes (Figure 13). The apparent delays in the development of ischaemia produced by both drugs were not associated with delays in the onset of arrhythmias. The results appeared to conform with the hypothesis that ischaemia-induced arrhythmias are reduced by calcium antagonism in the ventricles.

## 3.4 Actions of felodipine in acute myocardial ischaemia

3.4.1 Overview

Felodipine, a calcium antagonist with selectivity for vascular smooth muscle versus the myocardium (Au and Sutter, 1984), was evaluated for activity against responses to coronary occlusion under the assumption that compared with (±)-verapamil, little antiarrhythmic activity would be seen, as predicted from the hypothesis that calcium antagonism in the ventricular myocardium is the principal determinant of the antiarrhythmic activity of calcium antagonists during acute myocardial ischaemia. In the figures, C refers to controls, L = low dose (0.2 μmol/kg), I = intermediate dose (2.6 μmol/kg) and H = high dose (12.2 μmol/kg). The results of this study have been published (Curtis et al., 1985a).

3.4.2 OZ, IZ and mortality

There were no statistically significant differences between control groups and felodipine-treated groups with regard to OZ or IZ size, within the subgroups of rats with deliberately-produced large (LOZ) or small (SOZ)

OZs (Figure 14a). Owing to the high mortality rate during the first 4 h following coronary occlusion in LOZ rats (Figure 14b), too few IZ measurements were taken to warrant useful comparison, thus IZ values have been omitted for LOZ rats. With regard to SOZ rats, when IZ was expressed as% OZ weight, the extent of infarction was 88 ± 13, 71 ± 14 and 79 ± 10% in control, low dose and high dose rats, respectively, indicating slight myocardial salvage ($P < 0.05$ versus control for the low dose only). Mortality during the first 4 h following occlusion was low (0 - 11%) in all SOZ groups, and higher (33 - 67%) in all LOZ groups (Figure 14b). There were no obvious dose-related effects of felodipine on mortality, whether resulting from VF or cardiogenic shock.

3.4.3 Arrhythmias

In SOZ rats, felodipine produced a small reduction in the incidence of VF and VT (Figure 15). This effect was reflected in the arrhythmia score (Figure 16), but only the reduction in VT incidence produced by the high dose during the first 30 min following occlusion was statistically significant. The $\log_{10}$ number of PVC was reduced ($P < 0.05$) by high and low doses of felodipine only during the first 30 min following occlusion, the effect being lost when the entire 4 h period was evaluated (Figure 17).

In LOZ rats, felodipine had no effect on the incidence of VT or VF (Figure 15), $\log_{10}$ number of PVC (Figure 17) or arrhythmia score (Figure 16).

The weak antiarrhythmic activity of felodipine is exemplified by the arrhythmia scores (Figure 16) and by the incidence of serious arrhythmias (VT and VF). In SOZ rats, 100% of controls experienced either VT or VF during the 4 h following occlusion. This incidence was reduced to 67% by both high and low doses of felodipine (not statistically significant). In LOZ rats, all rats in all groups experienced either VT or VF.

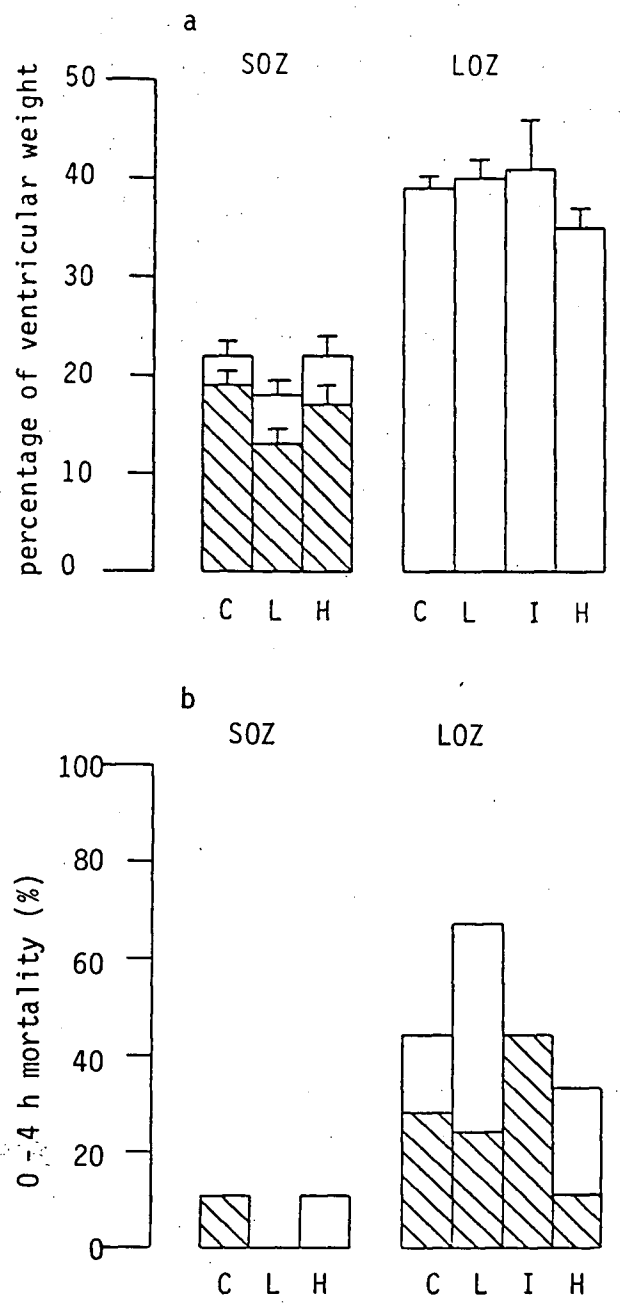



Figure 14. Effects of felodipine on OZ (unhatched) and IZ (hatched) (part a), and total (unhatched) and arrhythmia-induced (hatched) mortality during the 0 - 4 h period following occlusion (part b). The groups are indicated by: C = controls, L = 0.2, I = 2.6 and H = 12.2 μmol/kg felodipine. * Indicates $p < 0.05$ versus the appropriate small (S) or large (L) OZ control group.

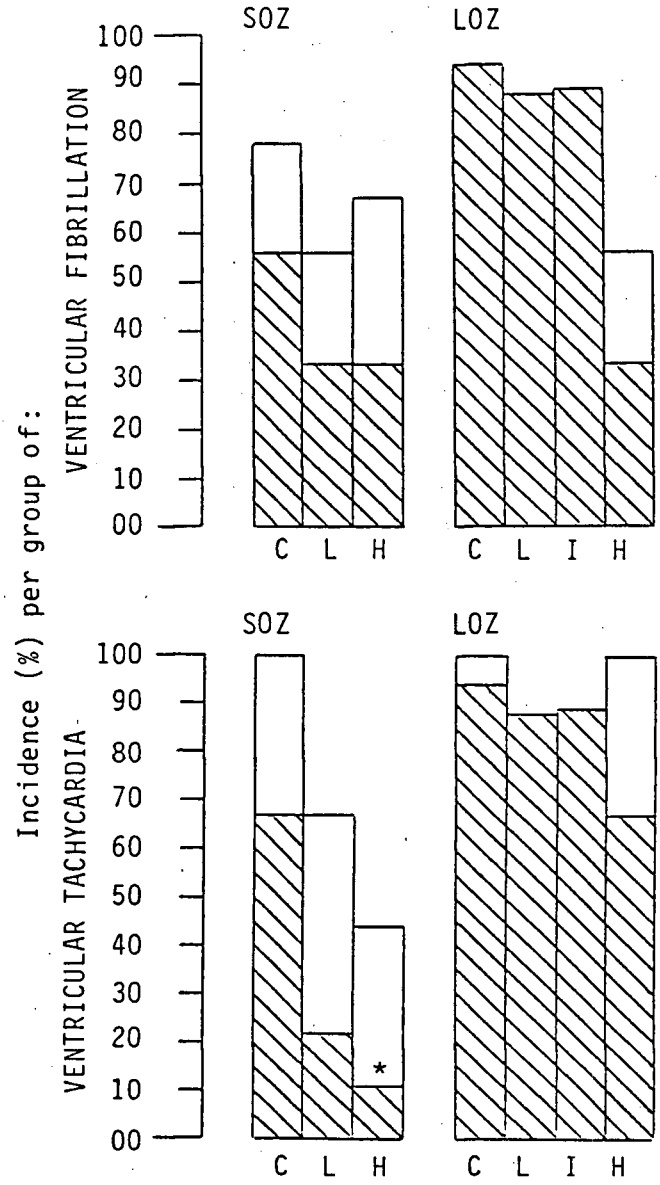



Figure 15. Effects of felodipine on the incidence of VF and VT during the 0 - 30 min (hatched) and 0 - 4 h (unhatched) periods following occlusion. Groups and statistical significance symbols are as indicated in figure 14.

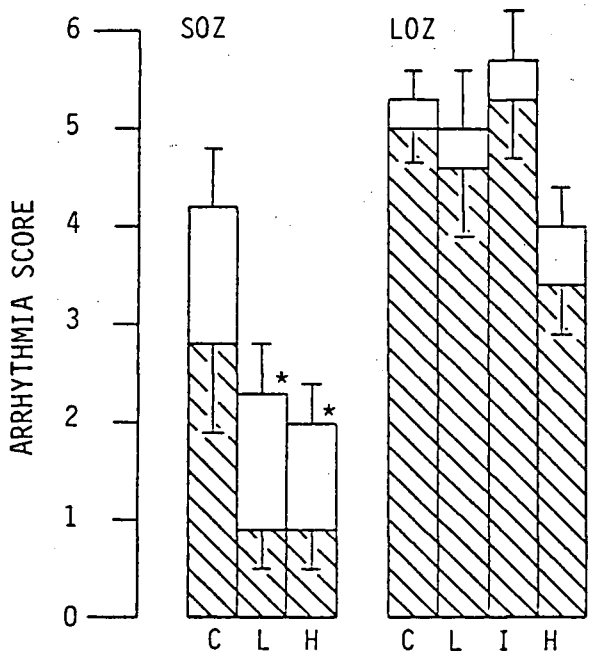



Figure 16. Effects of felodipine on arrhythmia score for the 0 - 30 min (hatched) and 0-4 h (unhatched) periods following occlusion. Values are mean ± s.e.mean. Groups and statistical significance symbols are as indicated in figure 14.

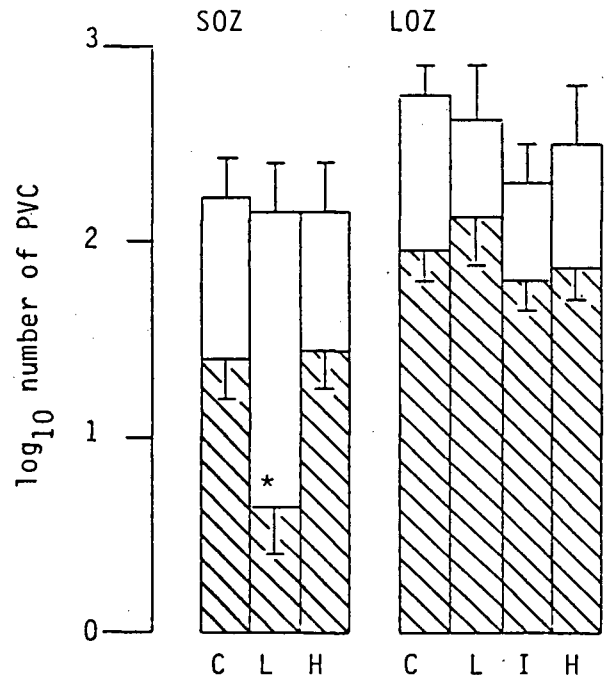



Figure 17. Effects of felodipine on $\log_{10}$ number of PVC during the 0 - 30 min (hatched) and 0 - 4 h (unhatched) periods following occlusion. Values are mean ± s.e.mean. Groups and statistical significance symbols are as indicated in figure 14.

3.4.4 Haemodynamic variables

Felodipine produced a large dose-dependent reduction of blood pressure before and after coronary occlusion (Figure 18). Heart rate changes produced by felodipine were negligible (Figure 18). Heart rates 1 min before occlusion were 447 ± 13 and 430 ± 10 beats/min in SOZ and LOZ control rats, respectively. These values were both slightly higher than historical control values of 392 ± 10 beats/minute (Johnston et al., 1983). Coronary occlusion reduced blood pressure, particularly in LOZ rats. The effect of occlusion on blood pressure was neither enhanced nor attenuated by felodipine (Figure 18). In this respect, felodipine does not resemble (±)-verapamil (Curtis et al., 1984) which enhanced the fall in blood-pressure produced by coronary occlusion in previous studies in our laboratory.

3.4.5 ECG changes

There were no obvious drug effects on either maximum R wave amplitude nor the time at which this occurred (not shown). However, felodipine delayed the time at which maximum S-T segment elevation occurred, whether expressed as maximum dSTR or ST %, in both SOZ and LOZ rats, particularly at the high dose ($P < 0.05$). In Figure 19, maximum ST % and the time at which this occurred are shown. It can be seen that the maximum ST% values were higher in all LOZ rats versus SOZ rats, and that felodipine did not influence this variable in any predictable manner.

3.4.6 Summary

Felodipine was not a consistently effective antiarrhythmic agent (Figures 15 - 17) at doses which reduced blood pressure (Figure 18). Although the development of ECG signs of ischaemia was delayed (Figure 19), IZ size was not correspondingly reduced (Figure 14).

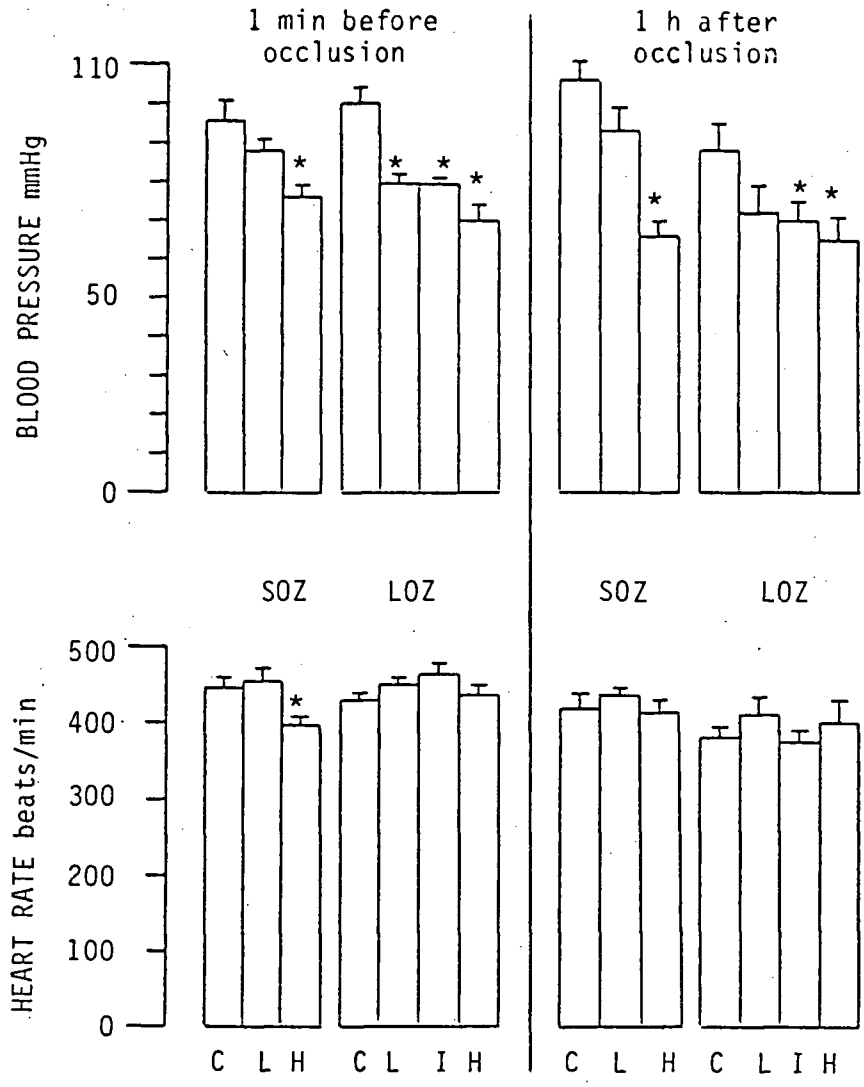



Figure 18. Effects of felodipine on blood-pressure (upper parts) and heart rate (lower parts) 1 min before and 1 h after occlusion. Values are mean ± s.e.mean. Groups and statistical significance symbols are as indicated in figure 14.

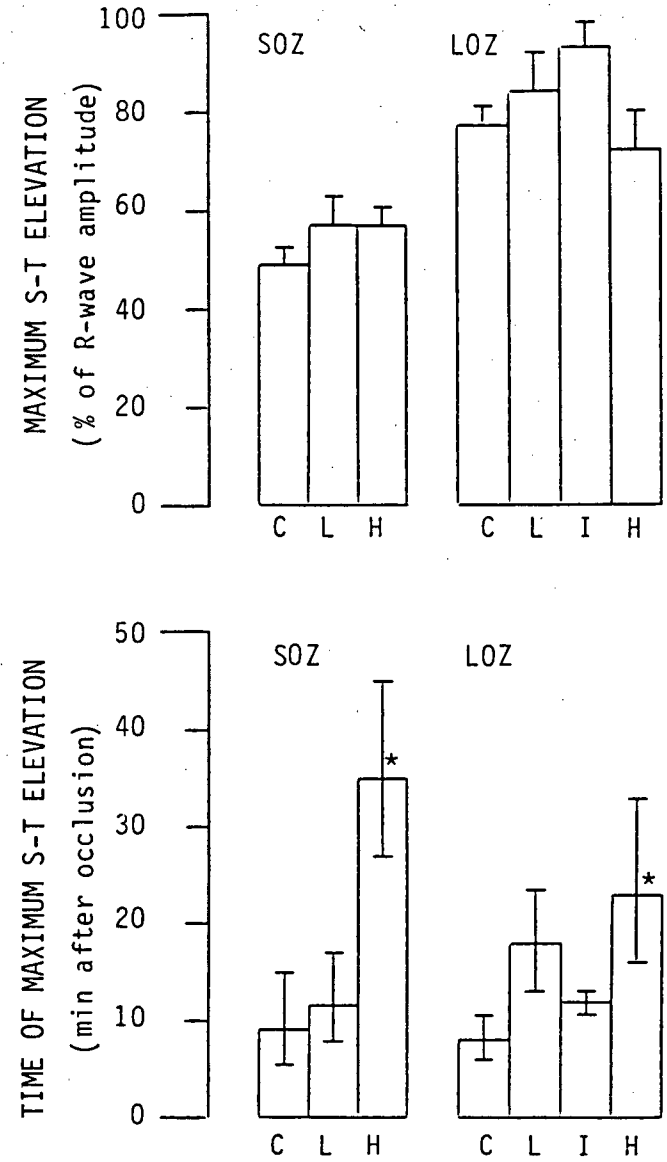



Figure 19. Effects of felodipine on maximum S-T elevation (upper part), and the time at which maximum S-T elevation occurred (lower part). The latter values were calculated as $\log_{10}$ min, but are expressed in antilog form (min). Values are mean ± s.e.mean. Groups and statistical significance symbols are as indicated in figure 14.

## 3.5 Actions of verapamil enantiomers in acute myocardial ischaemia

### 3.5.1 Overview

In our laboratory, (±)-verapamil has been shown to reduce in a dose-dependent manner the arrhythmias resulting from permanent coronary occlusion in conscious rats (Curtis et al., 1984). To test the hypothesis that the antiarrhythmic actions of (±)-verapamil occurred by virtue of calcium antagonism in the ventricles, the antiarrhythmic actions of the optical enantiomers of verapamil were compared. One of 4 doses of each enantiomer was administered to conscious rats before coronary occlusion. The hypothesis predicts that the antiarrhythmic potency ratio should be equal to the calcium antagonist potency ratio in the ventricular myocardium, based on the reported potency difference between the enantiomers for calcium antagonism (Bayer et al., 1975; Nawrath et al., 1981; Ferry et al., 1985; etc.).

The optical enantiomers of verapamil have been previously shown to be equipotent blockers of $i_{Na}$ (Kohlhardt et al., 1972; Nawrath et al., 1981), but effective concentrations were much greater than those required for reducing ventricular contractility to below 50 % (Nawrath et al., 1981). If the antiarrhythmic actions of (±)-verapamil are a result of $Na^+$ channel blocking properties, then the optical enantiomers should be equally antiarrhythmic. As an indirect measure of the $Na^+$ channel blocking potency of the optical enantiomers of verapamil, we measured the effects on the QRS of the ECG, in order to supplement the information provided from the literature (Nawrath et al., 1981) with independent information from the same rats in which occlusion-induced arrhythmias were studied. This study has been accepted for publication (Curtis and Walker, 1986a).

3.5.2 Effects of enantiomers before occlusion

3.5.2.1 P-R and QRS intervals. The effects of the enantiomers on P-R and QRS intervals are shown in Figure 20 (parts a and b, respectively). There were no significant differences between the groups before drug administration; mean ± s.e.mean values in controls were 44 ± 1 msec (P-R interval) and 28.3 ± 0.4 msec (QRS). Only the high doses of (+)- (12 mg/kg) and (-)-verapamil (6 mg/kg) prolonged P-R interval immediately before occlusion ($P < 0.05$). Neither enantiomer influenced QRS interval.

3.5.2.2 Blood pressure and heart rate. The effects of the enantiomers on blood pressure and heart rate immediately before coronary occlusion are shown as $\log_{10}$ dose-response curves in Figure 21. Pre-drug values were not statistically significantly different from one another; values in the control group were 111 ± 2 mmHg and 398 ± 12 beats/min. Blood pressure was reduced dose-dependently by both enantiomers (Figure 21a); only 0.2 mg/kg (-)- and 0.4 mg/kg (+)-verapamil did not produce statistically significant reductions compared with controls. (-)-Verapamil was approximately 4 times as potent as (+)-verapamil (Figure 21a). Both enantiomers elicited a small increase in heart rate at low doses, and a dose-dependent decrease in heart rate at higher doses (Figure 21b). The heart rate changes between the enantiomers occurred in parallel with an approximate potency ratio of 4 in favour of (-)-verapamil.

3.5.3 OZ, IZ and mortality

Treatment did not influence OZ size, or IZ size (whether expressed as a percentage of ventricular weight or as a percentage of OZ) (Table 5). Mortality (Table 6) was low during the 4 h period following occlusion (12 deaths out of 90 rats) and occurred as a result of severe pulmonary oedema or cardiogenic shock. Manual defibrillation always resulted in the restoration of sinus rhythm. There were no differences in mortality between the groups.

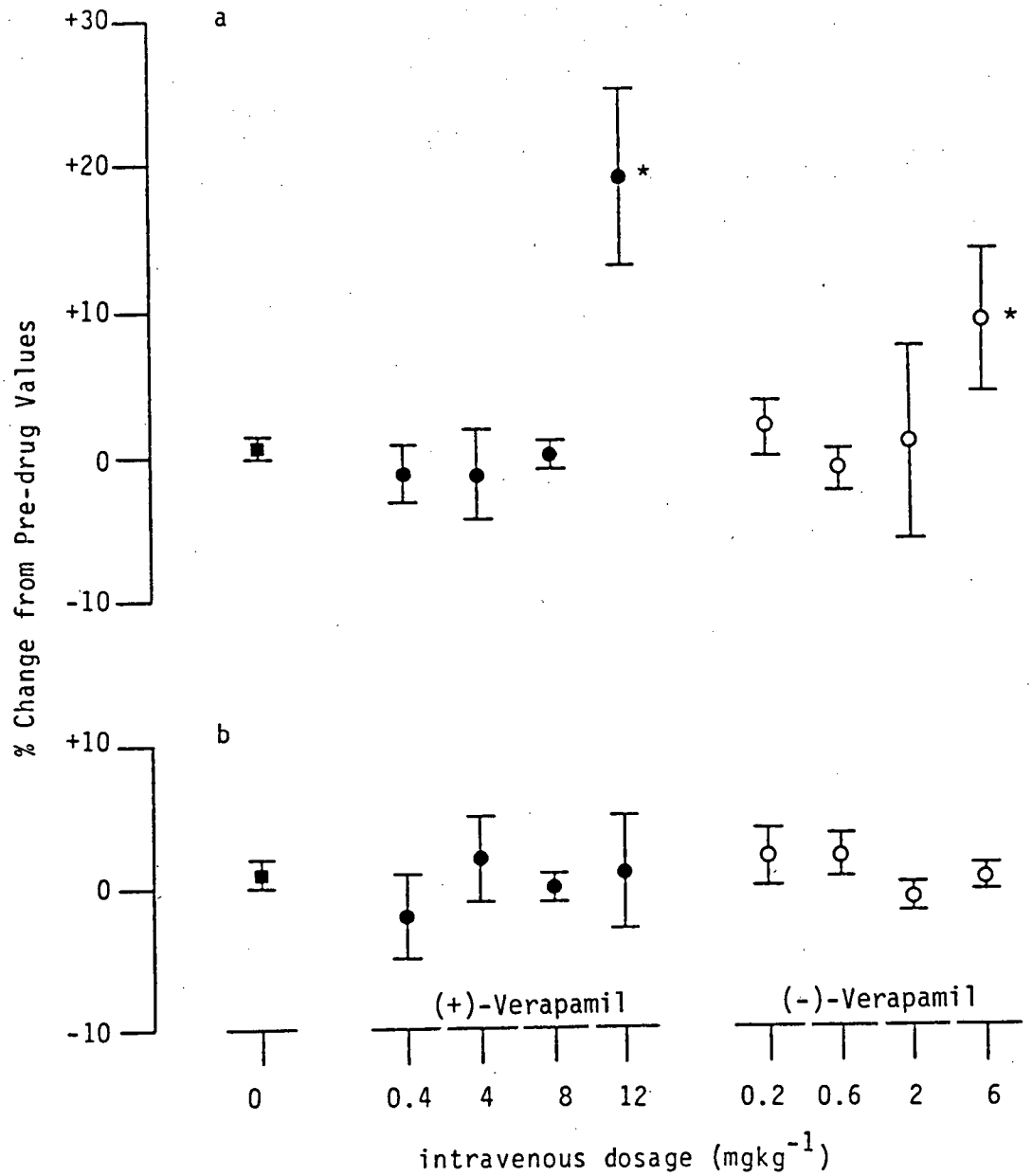



Figure 20. Changes in P-R (a) and QRS (b). Values were measured 1 min before occlusion and expressed as % change from pre-drug values (measured 15 min before occlusion). Symbols are: (■) controls, (●) (+)-verapamil, (○) (-)-verapamil, and * $P < 0.05$ versus controls. The bars are ± s.e.mean.

Figure 21. The effects of (-)-verapamil ( ○ ) and (+)-verapamil ( ● ) on blood pressure (part a) and heart rate (part b) at 1 min before occlusion in conscious rats. Statistical significance symbols have been omitted for clarity. Only the effects of the lowest dose of (+)- (0.4 mg/kg) and (-)-verapamil (0.2 mg/kg) on blood pressure were not statistically significant, whereas only the bradycardia induced by the highest dose of (+)- (12 mg/kg) and (-)-verapamil (6 mg/kg) was statistically significant.

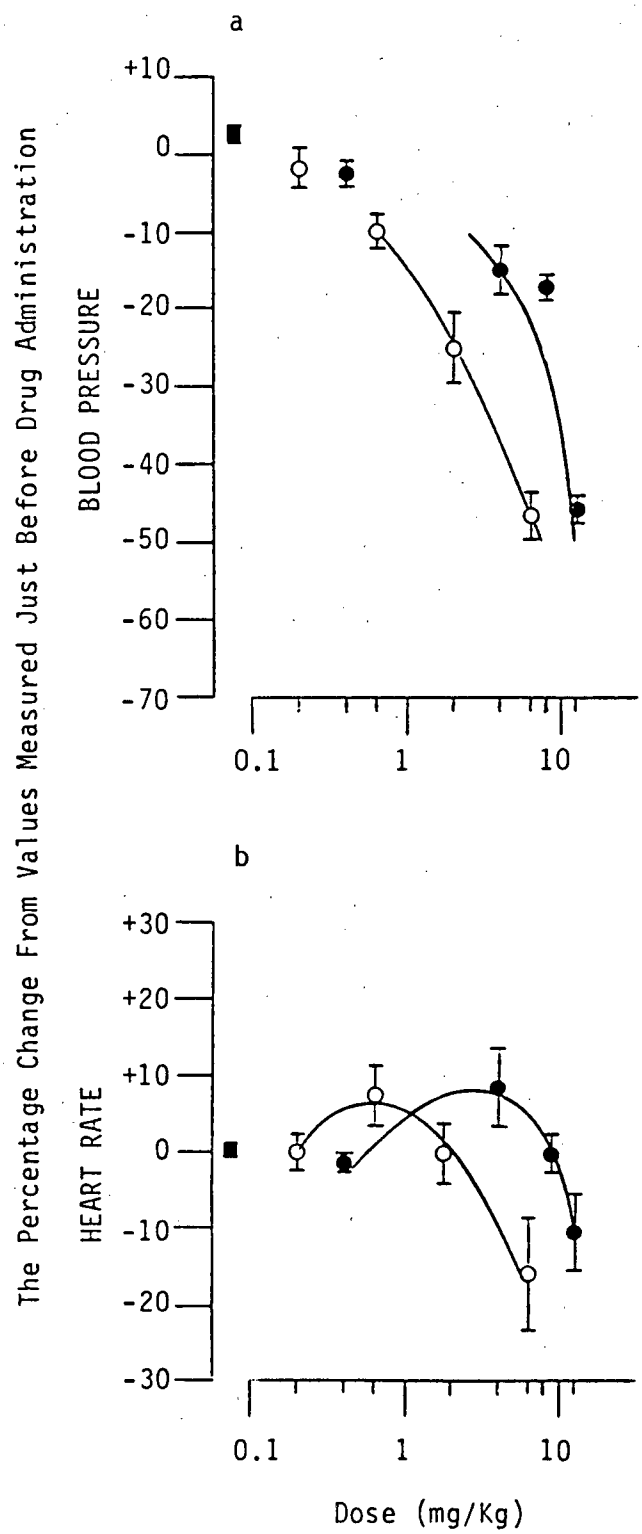

a
+10
0
-10
-20
-30
-40
-50
-60
-70
BLOOD PRESSURE
0.1
1
10
b
+30
+20
+10
0
-10
-20
-30
HEART RATE
0.1
1
10
Dose (mg/Kg)
The Percentage Change From Values Measured Just Before Drug Administration

Table 5. Extent of ischaemia, infarction, and ECG changes after occlusion: effects of verapamil enantiomers

| Dose (mg/kg ) of verapamil | | OZ % ( % ventricular weight) | IZ % | IZ (% OZ) | max ST % ( % of R) | time of max ST % (min) | Max R (mV) | time of max R (min) | time of Q-wave (h) |
|---|---|---|---|---|---|---|---|---|---|
| Controls | n=18 | 39±1 | 31±1 | 81±4 | 68±4 | 8 (7-9) | 1.15±0.07 | 3 (2-4) | 1.9 (1.7-2.2) |
| 0.4 + | n=9 | 37±2 | 32±1 | 86±3 | 74±5 | 11 (9-13) | 1.02±0.09 | 3 (2-5) | 2.6 (2.3-2.9) |
| 4.0 + | n=9 | 40±2 | 29±2 | 75±4 | 74±7 | 9 (8-11) | 1.31±0.12 | 5 (4-7) | 1.7 (1.4-2.0) |
| 8.0 + | n=9 | 39±3 | 22±4 | 59±8 | 89±10 | 11 (9-14) | 1.13±0.14 | 4 (3-7) | 2.4 (2.1-2.7) |
| 12.0 + | n=9 | 35±2 | 29±2 | 88±6 | 87±4 | 33 (24-46)* | 1.47±0.16 | 4 (3-5) | 2.8 (2.3-3.3) |
| 0.2 - | n=9 | 34±3 | 29±3 | 88±7 | 64±5 | 11 (9-13) | 1.08±0.08 | 7 (5-10) | 1.8 (1.4-2.3) |
| 0.6 - | n=9 | 37±2 | 25±4 | 70±10 | 79±5 | 12 (9-16) | 1.06±0.09 | 10 (9-11) | 1.5 (1.3-1.9) |
| 2.0 - | n=9 | 38±3 | 27±1 | 79±6 | 72±7 | 25 (18-36)* | 1.00±0.13 | 10 (6-16) | 2.4 (2.0-2.9) |
| 6.0 - | n=9 | 39±3 | 27±1 | 79±4 | 70±7 | 28 (16-49)* | 0.98±0.07 | 8 (5-13) | 3.2 (2.7-3.7) |

The OZ size is expressed as % ventricular weight. The IZ is expressed as % ventricular weight and also as % OZ weight. Times to max ST%, max R and Q-wave development were calculated in $\log_{10}$ time, but are expressed as mean ± 1 s.e.mean of real time for clarity. Other variables are mean ± s.e.mean. * Indicates $P < 0.05$ versus controls.

3.5.4 Arrhythmias

Arrhythmias were reduced in incidence and severity in a dose-dependent manner by both enantiomers.

3.5.4.1 VT and VF. The incidences per group of spontaneously reverting VF (SVF) and non-spontaneously reverting VF (NVF) are shown in Table 6. Both enantiomers dose-dependently reduced SVF and NVF. Since the control incidence of SVF was low, particularly during the first 30 min after occlusion, the reductions were statistically significant for NVF but not SVF, in general. The effects of the enantiomers on both types of VF (considered together) and on VT are also shown in Table 6. Both VT and VF incidence were reduced dose-dependently during the 0 - 0.5 h and 0 - 4 h periods following occlusion. (-)-Verapamil reduced VT and VF incidence more potently than (+)-verapamil, (potency ratio approximately 4).

3.5.4.2 PVC. In contrast with the large reductions in VT and VF, the enantiomers produced only small reductions in $\log_{10}$ PVC during the 0 - 0.5 h period following occlusion, and this effect was lost when the entire 0 - 4 h period following occlusion was considered (Table 6). Neither enantiomer appeared to delay the onset of arrhythmias, which exhibited the classic bimodal time-frequency pattern in all groups, (Figures 22 - 24), in the manner reported previously (Johnston et al., 1983a).

3.5.4.3 Arrhythmia scores. Arrhythmias are summarised in terms of arrhythmia score in Figure 25. Arrhythmia score was regressed with $\log_{10}$ dose, assuming linearity, parallelism and a potency ratio of 4, whether 0 - 0.5 h or 0 - 4 h data were considered. $ED_{50}$ values were determined by interpolation. During the 0 - 0.5 h period following occlusion (Figure 25a) $ED_{50}$ values were 2.4 mg/kg for (-)- and 9.6 mg/kg for (+)-verapamil. Corresponding $ED_{50}$ values for the 0 - 4 h period (Figure 25b) were 5 and 20 mg/kg, respectively.

Figure 22. The effects of (-)-verapamil (upper figure) and (+)-verapamil (lower figure) on the incidence of PVC in relation to the time after occlusion are shown. Each histogram represents the % incidence per group of PVC during the interval indicated. In each figure the histograms in the back row are control values. In the front row are the values for the highest dose (12 mg/kg (+)- or 6 mg/kg (-)-verapamil), and successive rows represent decreasing doses of (+)- (8, 4 and 0.4 mg/kg) or (-)-verapamil (2, 0.6 and 0.2 mg/kg) in the lower and upper figure, respectively.

INCIDENCE OF PREMATURE VENTRICULAR CONTRACTIONS

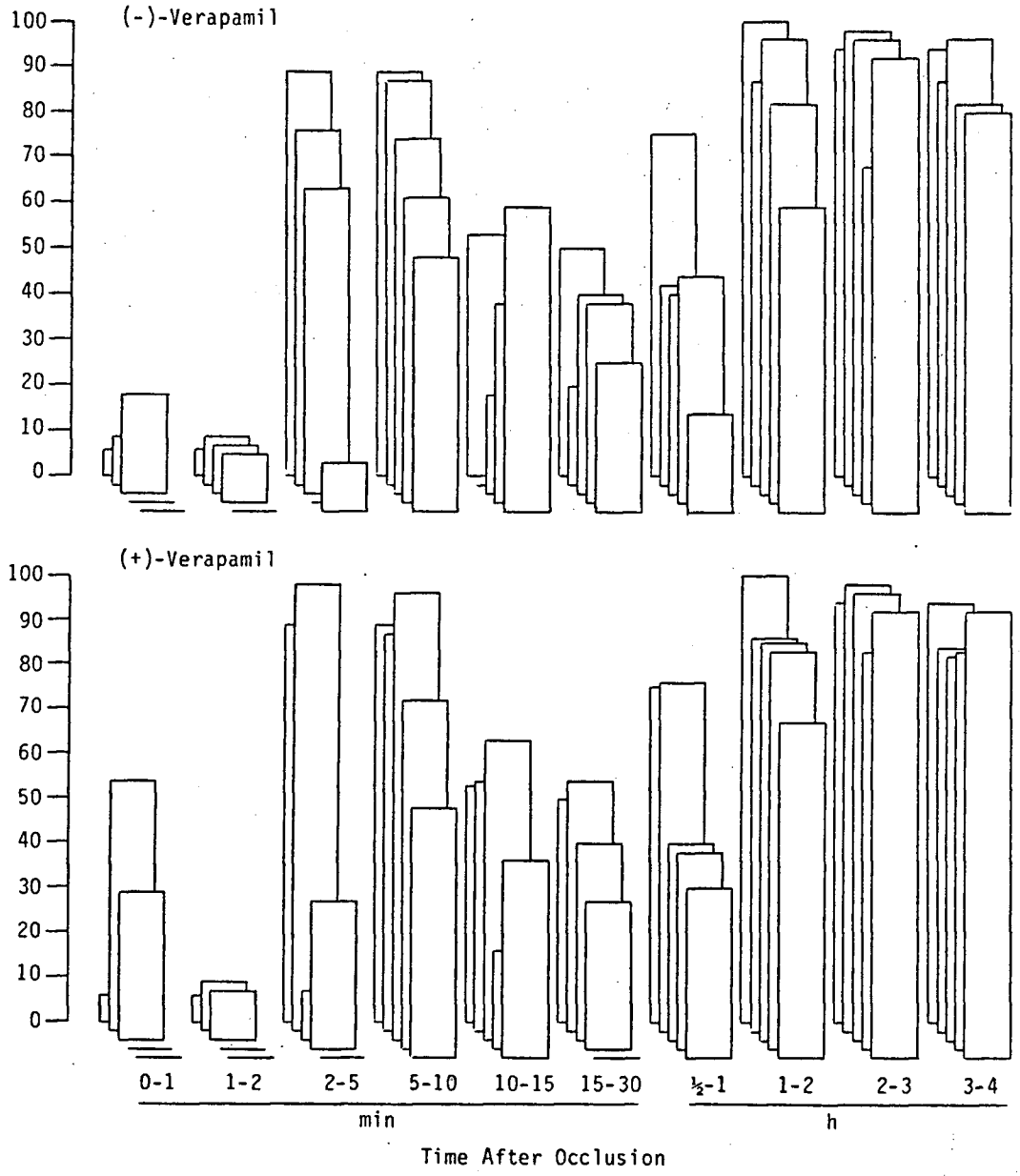
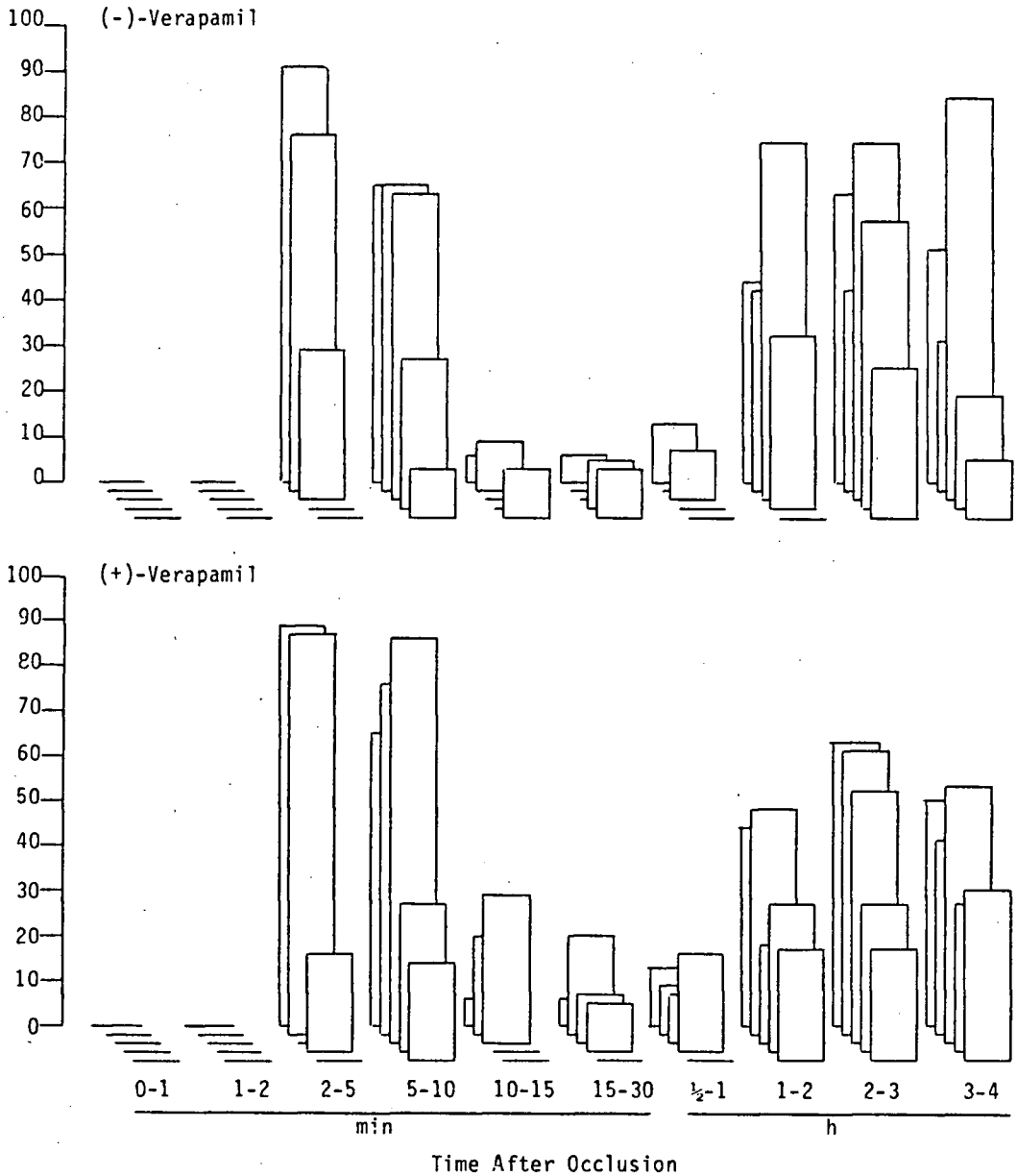


Figure 23. The effects of (-)-verapamil (upper figure) and (+)-verapamil (lower figure) on the incidence of VT in relation to the time after occlusion are shown. The format of this figure is identical with that of figure 22.

INCIDENCE OF VENTRICULAR TACHYCARDIA



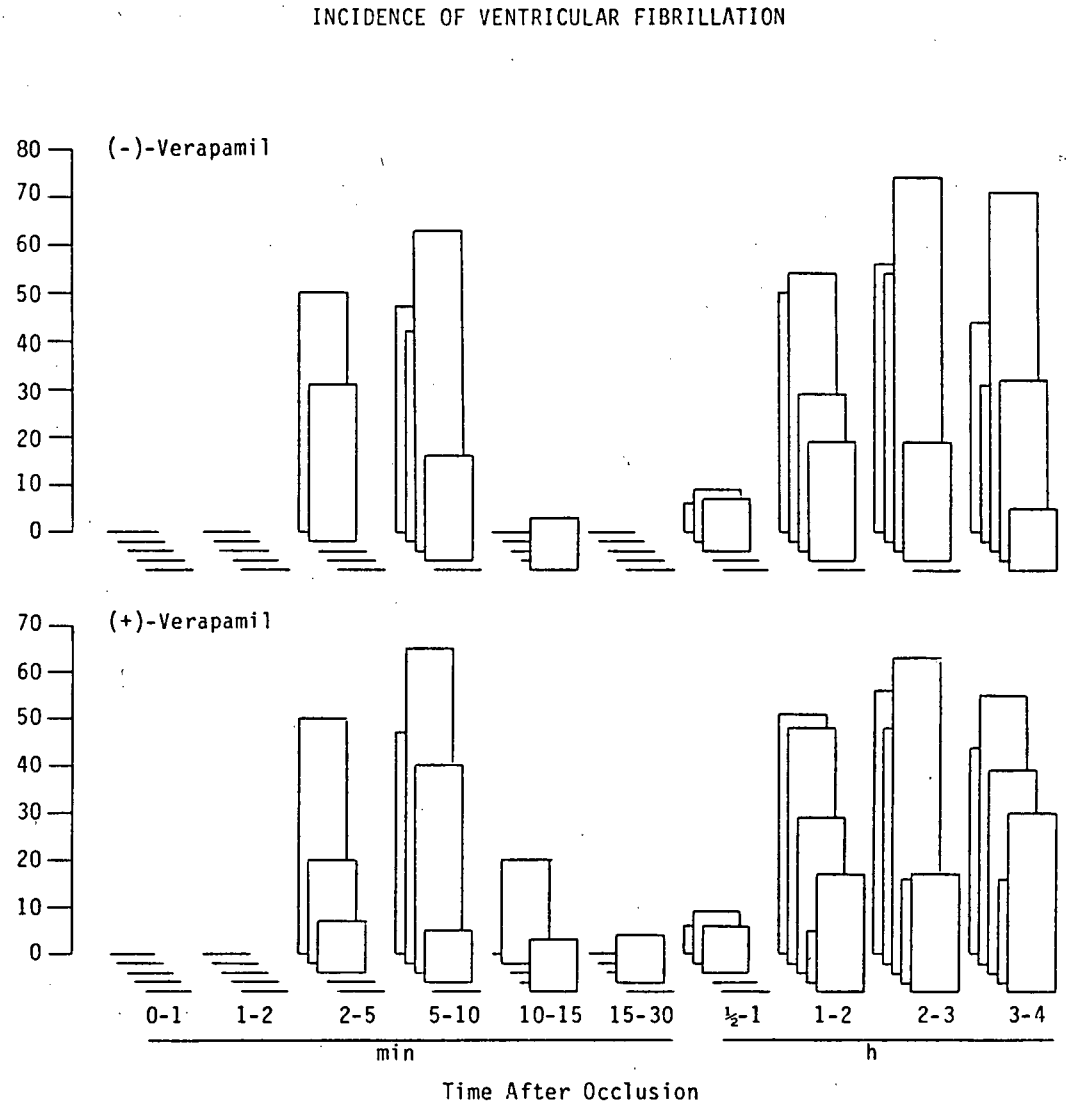



Figure 24. The effects of (-)-verapamil (upper figure) and (+)-verapamil (lower figure) on the incidence of VF in relation to the time after occlusion are shown. The format of this figure is identical with that of figure 22.

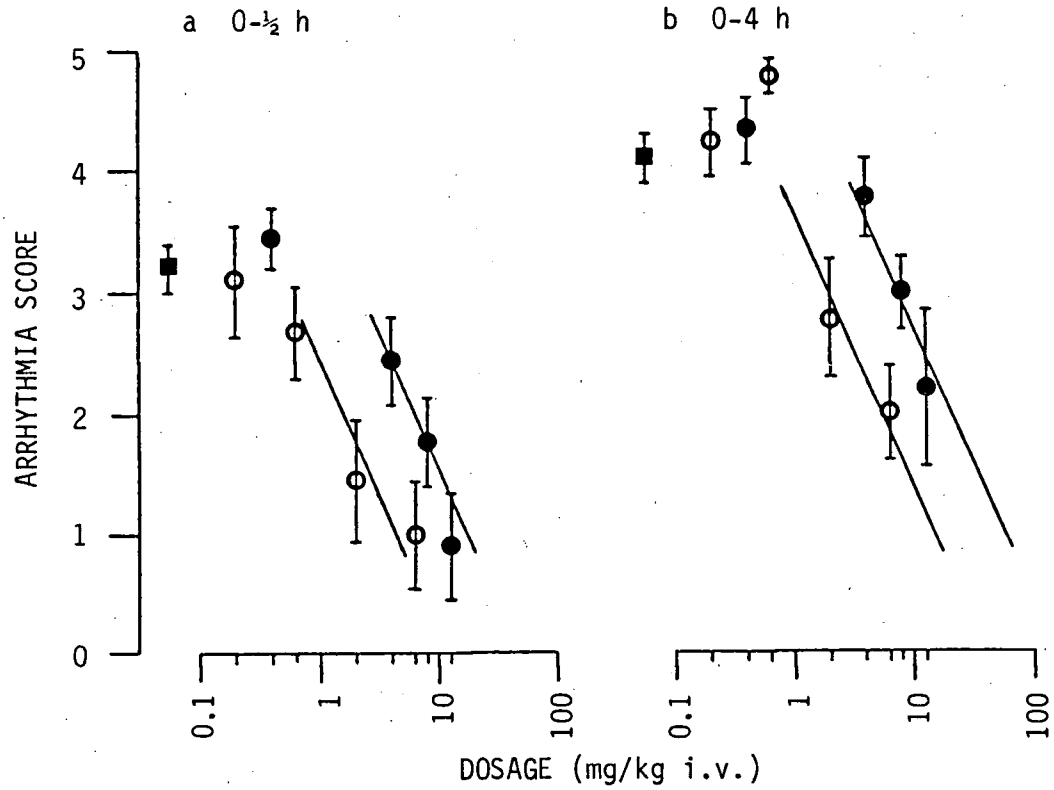



Figure 25. Mean arrhythmia scores for the 0 - 0.5 h (a) and 0 - 4 h (b) periods following coronary occlusion are shown. Bars are ± s.e.mean. The abscissa gives dosage in $\log_{10}$ scale. Symbols are ( ■ ) controls, ( ● ) (+)-verapamil and ( ○ ) (-)-verapamil. Scores during both periods were significantly reduced ($P < 0.05$) versus controls for the 2 highest doses of both enantiomers (not shown for clarity). The 4 parallel lines were constructed assuming a potency ratio of 4 in favour of (-)-verapamil in order to estimate $ED_{50}$ values.

Table 6. Arrhythmias and mortality following coronary occlusion: effects of verapamil enantiomers

| Dose (mg/kg ) of verapamil | | VT Incidence 0-½h | VT Incidence 0-4h | VF Incidence 0-½h | VF Incidence 0-4h | SVF Incidence 0-½h | SVF Incidence 0-4h | NVF Incidence 0-½h | NVF Incidence 0-4h | $\log_{10}$ PVC 0-½h | $\log_{10}$ PVC 0-4h | Mortality 0-4h | Mortality 0-24h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| controls | n=18 | 17/18 | 17/18 | 14/18 | 18/18 | 5/18 | 12/18 | 13/18 | 18/18 | 1.8±0.1 | 2.8±0.2 | 2/18 | 8/18 |
| 0.4 + | n=9 | 9/9 | 9/9 | 8/9 | 8/9 | 3/9 | 6/9 | 8/9 | 8/9 | 1.8±0.2 | 2.8±0.2 | 3/9 | 5/9 |
| 4.0 + | n=9 | 8/9 | 9/9 | 5/9 | 8/9 | 1/9 | 5/9 | 4/9 | 8/9 | 1.5±0.1 | 2.7±0.2 | 2/9 | 4/9 |
| 8.0 + | n=9 | 4/9* | 8/9 | 2/9* | 4/9* | 1/9 | 2/9 | 1/9* | 3/9* | 1.4±0.3 | 2.6±0.2 | 0/9 | 1/9 |
| 12.0 + | n=9 | 2/9* | 4/9* | 1/9* | 3/9* | 0/9 | 3/9 | 1/9* | 3/9* | 0.8±0.2* | 2.3±0.3 | 1/9 | 1/9 |
| 0.2 - | n=9 | 8/9 | 9/9 | 6/9 | 9/9 | 3/9 | 7/9 | 5/9 | 8/9 | 1.5±0.2 | 2.5±0.3 | 0/9 | 4/9 |
| 0.6 - | n=9 | 7/9 | 9/9 | 6/9 | 9/9 | 1/9 | 8/9 | 6/9 | 8/9 | 1.5±0.2 | 2.9±0.1 | 1/9 | 5/9 |
| 2.0 - | n=9 | 4/9* | 7/9 | 2/9* | 5/9* | 1/9 | 5/9 | 1/9* | 4/9* | 1.3±0.3 | 2.3±0.1 | 1/9 | 2/9 |
| 6.0 - | n=9 | 3/9* | 5/9 | 1/9* | 2/9* | 1/9 | 1/9* | 0/9* | 1/9* | 0.9±0.2* | 2.1±0.2 | 2/9 | 5/9 |

Values are incidence (out of n) of mortality, VT, VF, spontaneously reverting VF (SVF) and non-spontaneously reverting VF (NVF), and mean ± s.e.mean for other variables. PVC are expressed as $\log_{10}$PVC number. * Indicates $P < 0.05$ versus controls.

3.5.4.4 Arrhythmias at 24 h. In 24 h survivors (10/18 controls, 25/36 (+)-verapamil-treated, 19/36 (-)-verapamil-treated), frequent multifocal PVCs (but no VT or VF) were observed. The incidences of PVCs were 18/18, 19/25 and 15/19 in controls, (+)- and (-)-verapamil-treated groups, respectively (no statistically significant differences).

3.5.5 Haemodynamic variables

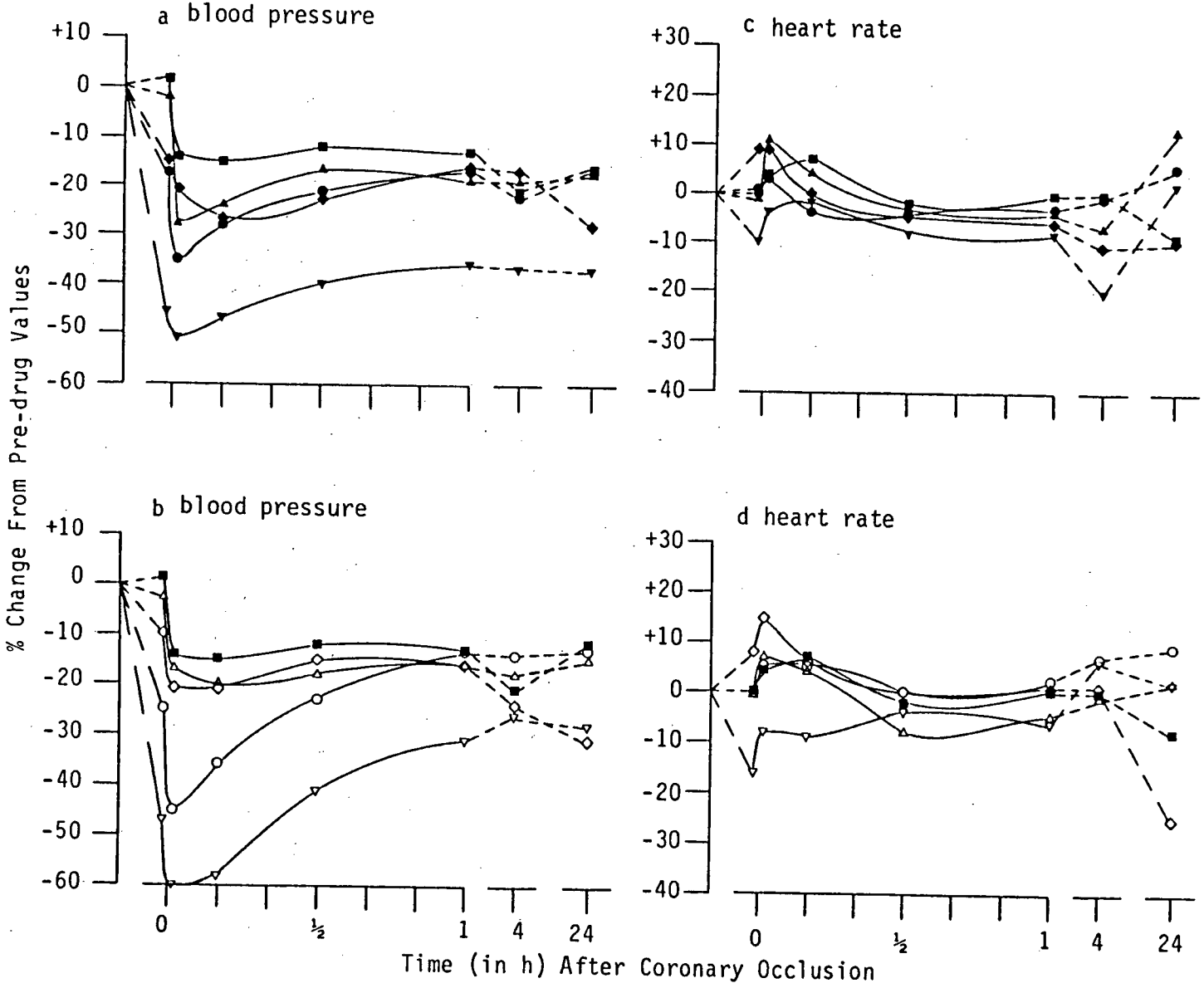
Immediately following occlusion, blood pressure fell (Figures 26a and b) and heart rate increased (Figures 26c and d) in all groups. Changes were essentially additive with the changes seen immediately before occlusion. However, at no time after occlusion were the differences in heart rate between the groups statistically significantly different. By 0.5 h after occlusion blood pressure remained low only with 12 mg/kg (+)-verapamil (Figure 26a) and 6 mg/kg (-)-verapamil (Figure 26b) (the highest doses).

3.5.6 ECG changes

In addition to the antiarrhythmic actions of the enantiomers, the ECG changes caused by coronary occlusion were delayed (Table 5). Both enantiomers delayed the development of S-T segment elevation (max ST %), elevation in R-wave amplitude (max R) and the development of a Q-wave following coronary occlusion (Table 5), although the effects were only statistically significant in the case of time to max ST%. Neither maximum R-wave amplitude following coronary occlusion nor max ST% were statistically significantly altered by either enantiomer (Table 5).

It was stated in the Introduction that ECG changes in response to occlusion are not related to time in a manner permitting simple statistical analysis (hence the use of the artificial variables, maximum R wave and maximum S-T segment elevation). This can be clearly seen if the mean ± s.e.mean values for ST% (Figure 27) and R wave amplitude (Figure 28) are plotted in relation to the time after occlusion.

Figure 26. Mean percent changes in blood pressure (a and b) and heart rate (c and d) from pre-drug values in response to (+)- or (-)-verapamil. The s.e.mean values have been omitted for clarity. The abscissa indicates time (h) in relation to coronary occlusion. Broken lines indicate time discontinuity. The effects of (+)-verapamil (solids) are shown in parts a and c, and the effects of (-)-verapamil (open symbols) are shown in parts b and d, while control values (■) are shown in all parts. The symbols correspond with dose (in mg/kg); for (+)-verapamil (▲) 0.4, (◆) 4, (●) 8, (▼) 12, and for (-)-verapamil (△) 0.2, (◇) 0.6, (○) 2, (▽) 6. Statistical significance symbols have been omitted for clarity.


a blood pressure
c heart rate
b blood pressure
d heart rate
% Change From Pre-drug Values
+10
0
-10
-20
-30
-40
-50
-60
+30
+20
+10
0
-10
-20
-30
-40
0
½
1
4
24
Time (in h) After Coronary Occlusion

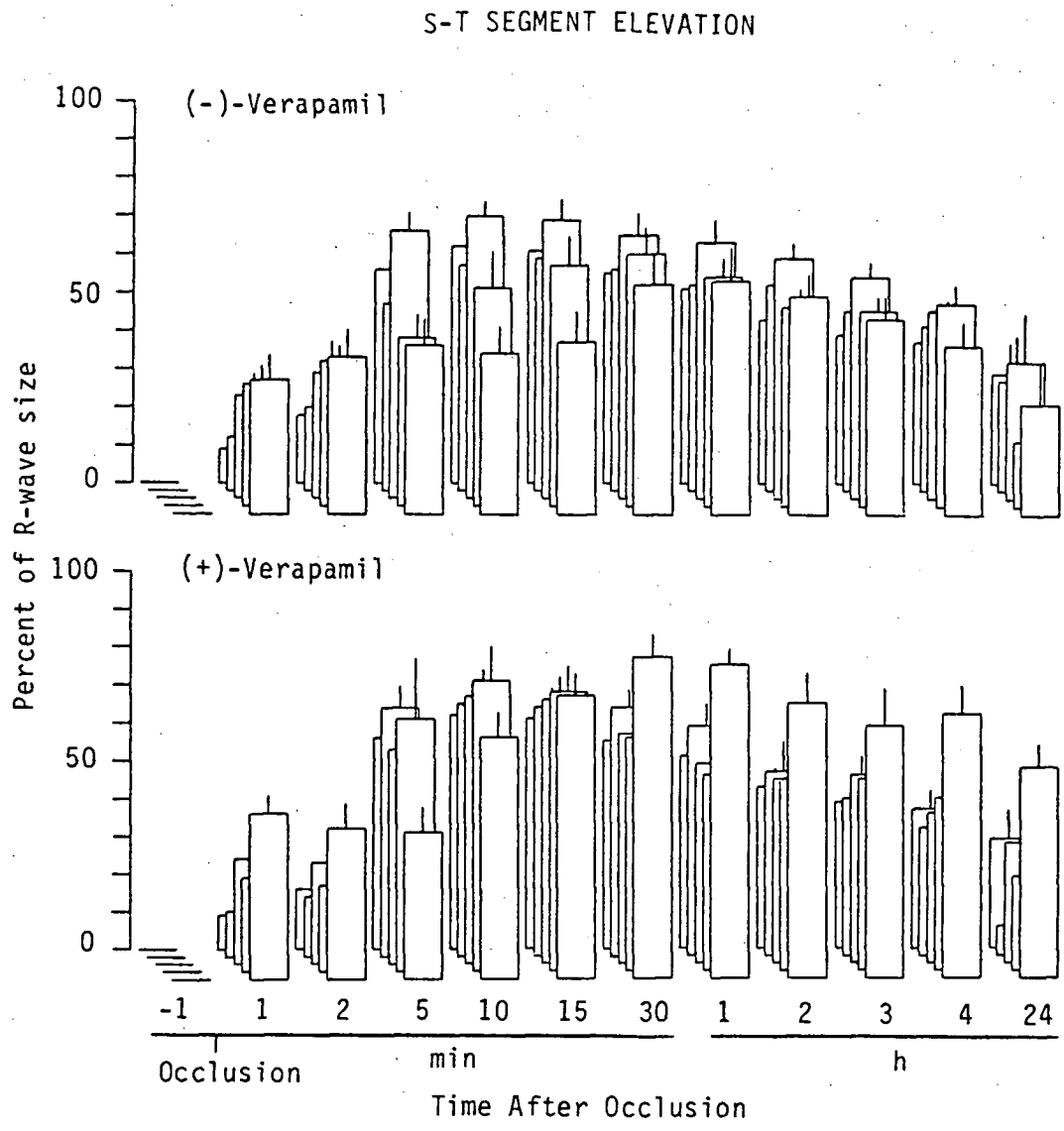



Figure 27. The effects of (-)-verapamil (upper figure) and (+)-verapamil (lower figure) on S-T segment elevation (expressed as % R wave amplitude) at various times before and after occlusion. Each histogram represents the mean ± s.e.mean value at the time indicated. In each figure the histograms in the back row are control values. In the front row are the values for the highest dose (12 mg/kg (+)- or 6 mg/kg (-)-verapamil), and successive rows represent decreasing doses of (+)- (8, 4 and 0.4 mg/kg) or (-)-verapamil (2, 0.6 and 0.2 mg/kg) in the lower and upper figure, respectively.

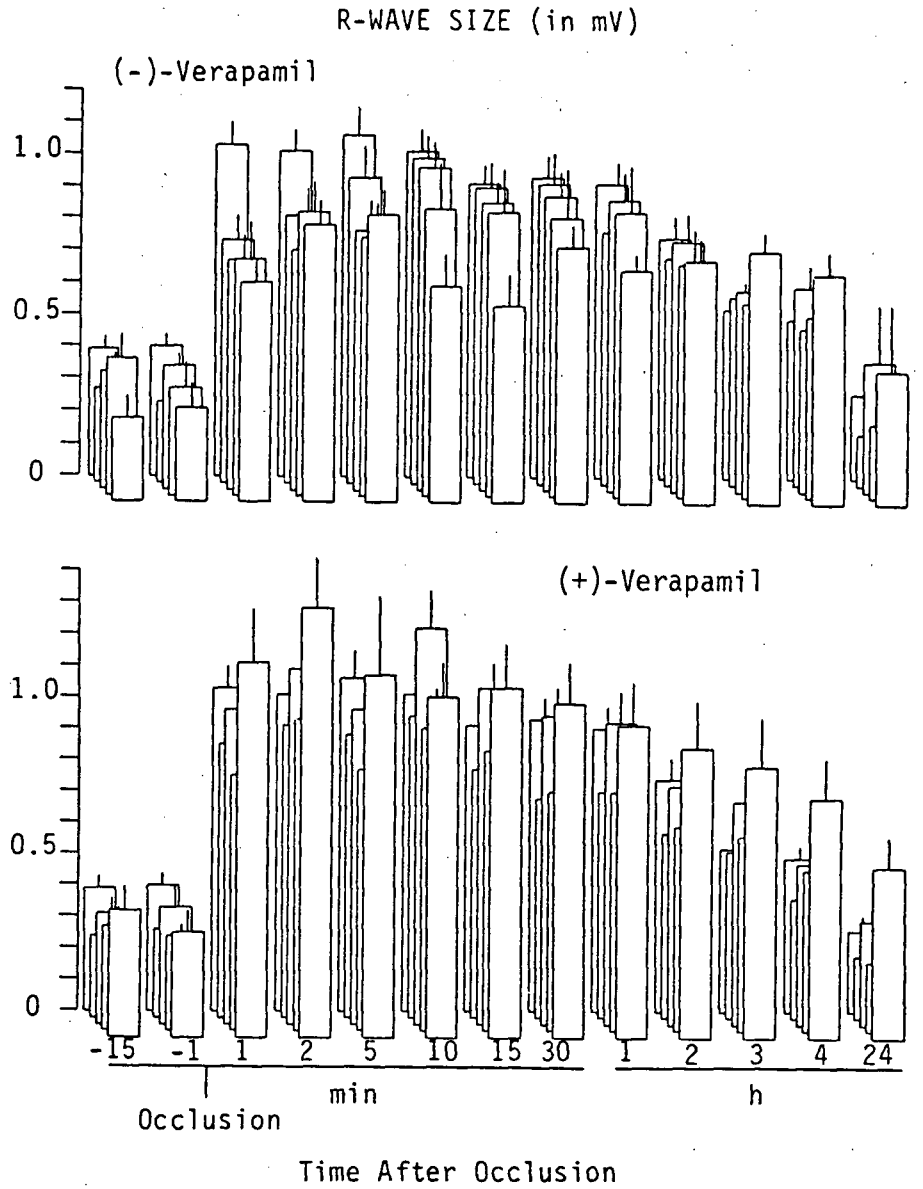



Figure 28. The effects of (-)-verapamil (upper figure) and (+)-verapamil (lower figure) on R-wave amplitude (in mV). The format for this figure is identical with that used for figure 27, with the exception that the values at 15 min before occlusion are included in this figure and not in figure 27.

3.5.7 Summary

Both (+)- and (-)-verapamil dose-dependently reduced arrhythmias during acute myocardial ischaemia (Figure 25, Table 6) without reducing IZ size (Table 5). (-)-Verapamil was approximately 4 times as potent as (+)-verapamil in its ability to reduce arrhythmia score. The development of ECG signs of ischaemia appeared to be delayed by both enantiomers (Table 5). Both enantiomers prolonged P-R interval at high doses, but QRS interval was not affected (Figure 20). Although both enantiomers slowed heart rate this effect was lost after occlusion, and reductions in blood pressure produced by the enantiomers were also not maintained throughout the entire period over which antiarrhythmic effects were seen (Figure 26).

## 3.6 Actions of nifedipine and DHM9 in acute myocardial ischaemia

3.6.1 Overview

The effects of nifedipine and DHM9 were examined in conscious rats as part of the studies to investigate the activity of calcium antagonists in acute myocardial ischaemia, and the mechanism by which their actions are mediated. Nifedipine was examined because it is the prototype 1,4-dihydropyridine calcium antagonist (Fleckenstein et al., 1972), and DHM9 was studied because preliminary reports suggest that it is a cardioselective calcium antagonist, albeit with low potency (Clarke et al., 1984b).

3.6.2 Effects of nifedipine and DHM9 before occlusion

3.6.2.1 P-R and QRS intervals. There were no differences in P-R interval (43 ± 1 msec in controls) and QRS interval (29 ± 1 msec in controls) between the 5 groups before drug administration. P-R interval was not significantly affected by the drug solvent or by DHM9, but P-R interval was shortened by 0.5 mg/kg nifedipine (by 6 ± 2 %) and by 2 mg/kg nifedipine (by 4 ± 1 %) (Figure 29a). QRS interval was not influenced by any treatment (Figure 29b).

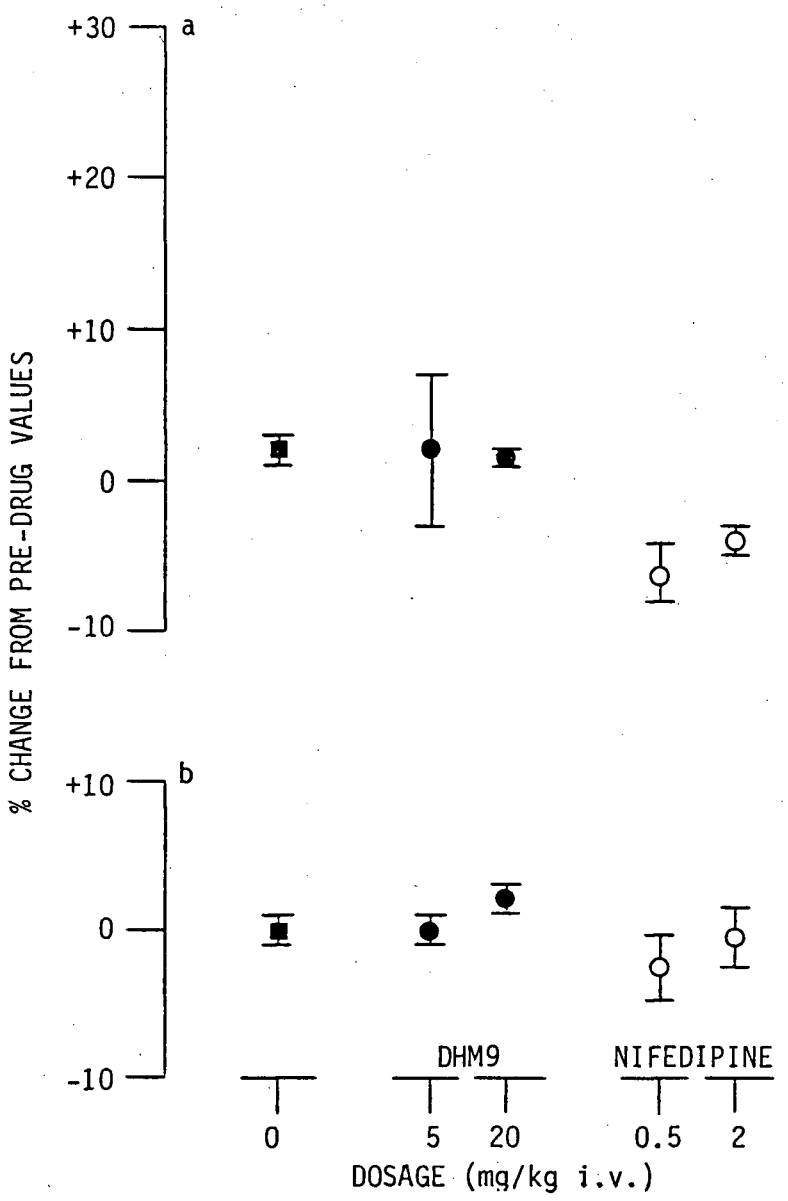



Figure 29. The effects of nifedipine (○), DHM9 (●) and vehicle (■) on P-R (a) and QRS (b) intervals. Values were measured at 1 min before occlusion and expressed as %of pre-drug values (15 min before occlusion). Treatment was not a significant source of variance. The bars are ± s.e.mean.

3.6.2.2 Blood pressure and heart rate. There were no differences between the 5 groups for mean arterial blood pressure (109 ± 2 mmHg in controls) and heart rate (401 ± 17 beats/min in controls) before drug administration. Blood pressure was dose-dependently reduced by nifedipine (Figure 30b) and heart rate was increased (Figure 30d) compared with controls ($p < 0.05$). DHM9 did not influence these variables (Figures 30a and c).

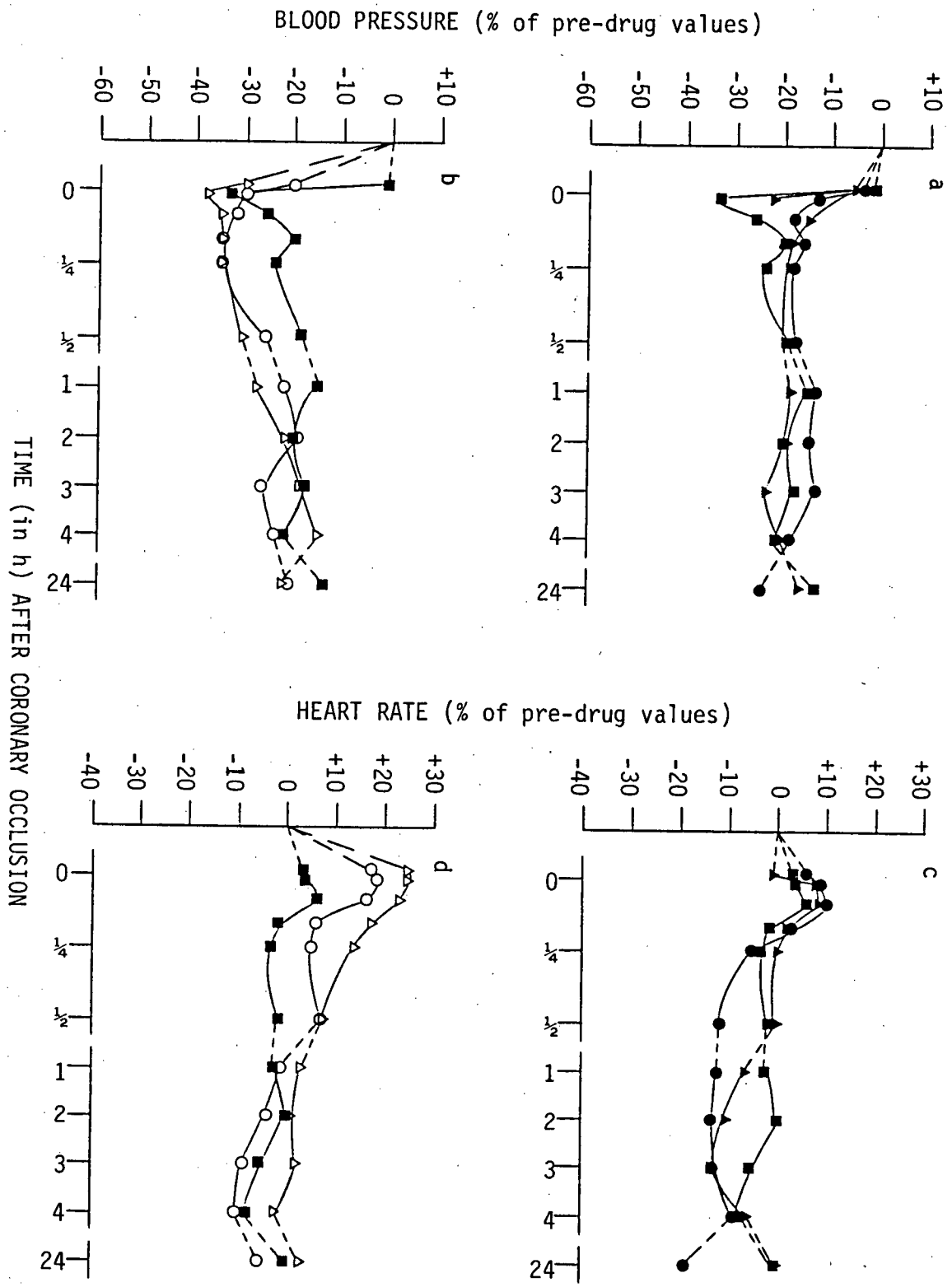
3.6.3 OZ, IZ and mortality

In Table 7 it can be seen that there were no significant effects of treatment on the size of the OZ or the extent of infarction (whether expressed as a % of the OZ or as a % of the ventricular weight). The mortality rate during the 4 h after occlusion was extremely low (1 death out of 45 rats). Thump-version defibrillation was successful in every instance of VF lasting longer than 10 sec. The 24 h survival rate was also high (Table 8), facilitating the assessment of IZ size (which is only measured in 24 h survivors in our laboratory) (Table 7).

3.6.4 Arrhythmias

The incidences of VT and VF and the $\log_{10}$ number of PVC were not statistically significantly reduced by either drug, in either the 0 - 0.5 or 0 - 4 h periods after occlusion (Table 8). The arrhythmia score, correspondingly, was also not affected by either treatment (Figure 31). Almost all rats which survived for 24 h had PVCs at this time, but the incidence and $\log_{10}$ number were not different between the groups.

Figure 30. Mean percent changes in blood pressure (a and b) and heart rate (c and d) from pre-drug values in response to DHM9 or nifedipine. The s.e.mean values have been omitted for clarity. The abscissa indicates time (h) with respect to coronary occlusion. Broken lines indicate time discontinuity. The effects of DHM9 (solids) are shown in parts a and c, and the effects of nifedipine (open symbols) are shown in parts b and d, while control values (■) are shown in all parts. The symbols correspond with dose (in mg/kg); for DHM9 (●) 5, (▲) 20, and for nifedipine (○) 0.5, (△) 2. Statistical significance symbols have been omitted for clarity.


BLOOD PRESSURE (% of pre-drug values)
-60 -50 -40 -30 -20 -10 0 +10
a
b
HEART RATE (% of pre-drug values)
-40 -30 -20 -10 0 +10 +20 +30
c
d
0 ¼ ½ 1 2 3 4 24
TIME (in h) AFTER CORONARY OCCLUSION

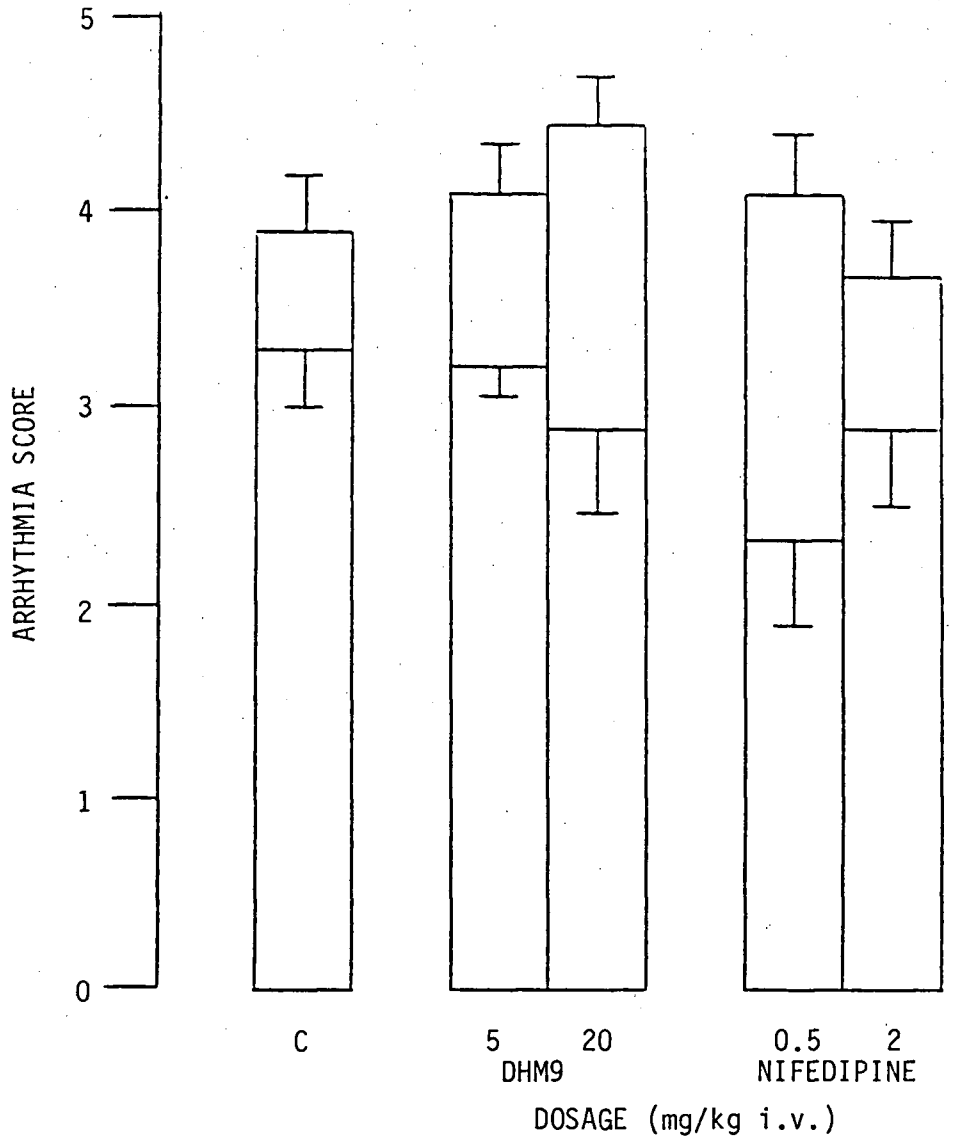



Figure 31. Mean arrhythmia scores for the 0 - 0.5 h and 0 - 4 h periods following coronary occlusion (downward-going s.e.mean bar and upward-going s.e.mean bar, respectively) are shown. There were no significant differences between the groups.

Table 7. Extent of ischaemia, infarction, and ECG changes after occlusion: effects of nifedipine and DHM9

| Dose (mg/kg ) | | OZ% (% ventricular weight) | IZ% | IZ (%OZ) | max ST% (% of R) | time of max ST% (min) | Max R (mV) | time of max R (min) | time of Q-wave (h) |
|---|---|---|---|---|---|---|---|---|---|
| Controls | n=9 | 39±3 | 32±2 | 81±4 | 77±4 | 9 (8-11) | 1.12±0.06 | 5 (3-9) | 2.1 (1.7-2.5) |
| 5.0 D | n=9 | 35±2 | 30±3 | 86±8 | 82±8 | 6 (5-7) | 1.29±0.08 | 7 (5-10) | 1.7 (1.4-2.2) |
| 20.0 D | n=9 | 35±2 | 25±3 | 72±5 | 63±7 | 11 (8-15) | 1.42±0.15 | 14 (9-22) | 1.7 (1.3-2.2) |
| 0.5 N | n=9 | 37±3 | 29±3 | 80±7 | 85±6 | 11 (9-14) | 1.26±0.13 | 6 (4-11) | 1.7 (1.4-2.1) |
| 2.0 N | n=9 | 38±2 | 25±3 | 70±10 | 74±6 | 13 (10-18) | 1.06±0.09 | 7 (4-12) | 2.8 (2.4-3.2) |

Treatments were DHM9 (D) and nifedipine (N). The OZ was expressed as % ventricular weight. The IZ was expressed as % ventricular weight and also as % OZ weight. Times to max ST%, max R and Q-wave development were calculated in $\log_{10}$ time, but expressed as mean ± 1 s.e.mean of real time for clarity. Other variables are mean ± s.e.mean. * Indicates $P < 0.05$ versus controls.

Table 8. Arrhythmias and mortality following coronary occlusion: effects of nifedipine and DHM9

| Dose (mg/kg ) | | VT Incidence | | VF Incidence | | SVF Incidence | | NVF Incidence | | $\log_{10}$ PVC | | Mortality | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0–½h | 0–4h | 0–½h | 0–4h | 0–½h | 0–4h | 0–½h | 0–4h | 0–½h | 0–4h | 0–4h | 0–24h |
| controls | n=9 | 9/9 | 9/9 | 6/9 | 8/9 | 3/9 | 5/9 | 5/9 | 7/9 | 1.7±0.1 | 2.8±0.2 | 0/9 | 3/9 |
| 5.0 D | n=9 | 9/9 | 9/9 | 8/9 | 8/9 | 1/9 | 6/9 | 8/9 | 8/9 | 1.9±0.1 | 2.9±0.2 | 0/9 | 2/9 |
| 20.0 D | n=9 | 8/9 | 9/9 | 5/9 | 7/9 | 1/9 | 5/9 | 5/9 | 7/9 | 1.7±0.2 | 2.6±0.2 | 0/9 | 2/9 |
| 0.5 N | n=9 | 7/9 | 9/9 | 3/9 | 8/9 | 1/9 | 4/9 | 3/9 | 8/9 | 1.7±0.1 | 2.8±0.1 | 0/9 | 2/9 |
| 2.0 N | n=9 | 7/9 | 8/9 | 4/9 | 7/9 | 1/9 | 3/9 | 4/9 | 6/9 | 1.8±0.4 | 2.7±0.3 | 1/9 | 2/9 |

Treatments were DHM9 (D) and nifedipine (N). Values are incidence (out of n) of mortality, VT, VF, spontaneously reverting VF (SVF) and non-spontaneously reverting VF (NVF), and mean ± s.e.mean for other variables. PVC are expressed as $\log_{10}$PVC number. * Indicates $P < 0.05$ versus controls.

3.6.5 Haemodynamic variables

The effects of treatment on the haemodynamic consequences of coronary occlusion are shown in Figure 30. Blood pressure fell considerably in all groups by 1 min after occlusion. However, the dose-dependent reduction in blood pressure produced by nifidepine before occlusion was no longer apparent, values being no different from control values. This did not reflect rapid metabolism of nifedipine, however, since blood pressure recovered to a certain extent in controls and DHM9-treated rats during the following min, whereas it remained depressed in the nifedipine-treated rats, such that differences in blood pressure were again statistically significant by 10 min after occlusion. After 10 min, however, treatment was no longer a significant source of variance, once again. Blood pressure values in the DHM9 treated rats appeared to be higher than the values in control rats, however the differences were not statistically significant (Figure 30a).

Coronary occlusion had no effect on heart rate in control rats, except that values appeared to fall at approximately 2 h after occlusion (although there was much variability at this time). DHM9 did not influence heart rate, compared with control rats (Figure 30c). The dose-dependent tachycardia produced by nifedipine was still apparent 1 min after occlusion, and occlusion neither added to or subtracted from this effect (Figure 30d). However, by 5 min after occlusion there were no significant differences between the groups (this situation persisted for the remainder of the experiment).

3.6.6 ECG changes

Table 7 shows the effects of nifedipine and DHM9 on the ECG changes produced by occlusion. There were no statistically significant effects of either drug on S-T segment elevation, giant R wave size or the time at which these events reached maximal proportions. In addition, the time at which a pathological Q wave became evident was also not affected.

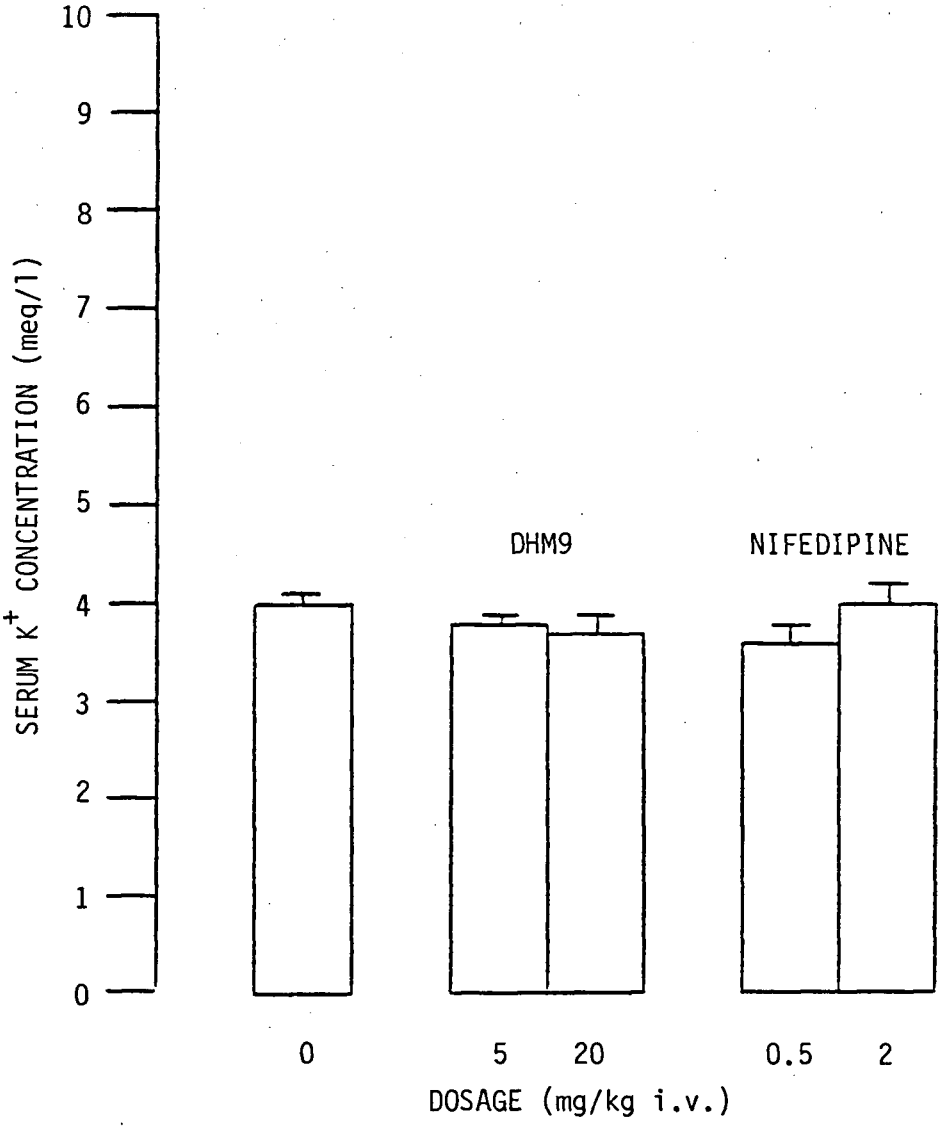



Figure 32. The effects of DHM9 and nifedipine on serum $K^+$ concentration. Values are mean ± s.e.mean, and were measured 2 h after coronary occlusion. There were no statistically significant differences between the groups.

3.6.7 Serum $K^+$ concencentration

There were no differences between the 5 groups in terms of serum $K^+$ concentration measured 2 h after occlusion (Figure 32). Values were similar to those found previously in our laboratory for control rats following coronary occlusion.

3.6.8 Summary

Although nifedipine lowered blood pressure in a dose-dependent manner (Figure 30), neither this drug nor DHM9 had any influence on the outcome of coronary occlusion, in terms of arrrhythmias (Table 8 and Figure 31), infarction, ECG changes (Table 7) and survival (Table 8).

## 3.7 Preliminary Screen for drug activity in acute ischaemia: verapamil enantiomers

3.7.1 Overview

The following experiments were carried out for 2 purposes. Firstly, it was decided to attempt to develop a new simple coronary occlusion preparation in conscious rats in order to produce large group sizes in a short time. In order to do this, preparative surgery was limited to the implantation of an occluder, and animal monitoring following occlusion was restricted to an assessment of behaviour and a post-mortem examination. The validity of behaviour as a subjective measure of VF was assessed in a preliminary investigation. Secondly, the optical enantiomers of verapamil were compared in order to provide information concerning their relative activity in reducing occlusion-induced arrhythmias and IZ size, and in order to test the new experimental preparation.

3.7.2 Validation of behaviour endpoints

In the preliminary study in which convulsive-type behaviour was recorded as a subjective estimate of VF and compared with objective evidence of VF (from polygraph records), every episode of fatal VF was successfully detected

by observation. In addition, all non-fatal episodes of VF lasting longer than 10 sec were also correctly detected by observation. One episode of torsade de pointes lasting 14 sec was also detected. Atrioventricular block and all other arrhythmias were never associated with convulsive-type behaviour.

3.7.3 OZ and IZ

The mean OZ size was approximately equal in all groups (Figure 33A). No statistically significant effects of treatment on IZ size occurred (Figure 33B). This was so whether IZ size was expressed as % of ventricular weight, or %of OZ (Figure 33C).

3.7.4 Mortality and morbidity

Mortality during the first 4 h after occlusion was expressed both as total mortality (Figure 34A) and also convulsive-type mortality (Figure 34B). Total mortality was only reduced by (±)-verapamil (Figure 34A). However, convulsive-type mortality was reduced by both (-)- and (±)-verapamil when compared with both (+)-verapamil and controls ($p < 0.01$). If groups were ordered according to their reported potencies as calcium antagonists (+ < ± < -) then the overall reductions from control in the incidences of convulsive-type behaviour (15, 69 and 77 %, respectively) were highly significant ($p<0.005$). There was a similar graded reduction in the incidence of fatal plus non-fatal convulsive-type behaviour of 24, 43, and 71% by (+)-, (±)- and (-)-verapamil, respectively ($p<0.005$). The control incidence of fatal plus non-fatal convulsive-type behaviour was 84%. Only (±)- and (-)-verapamil produced morbidity before occlusion (Figure 34C). Morbidity was increased in all groups by occlusion, particularly during the first 0.5 h, when the incidences were 19, 50, 70 and 80% in the control, (+)-, (±)- and (-)- groups, respectively. During the 0.5 - 4 h period, corresponding morbidity incidence fell to 13, 10, 33 and 38%.

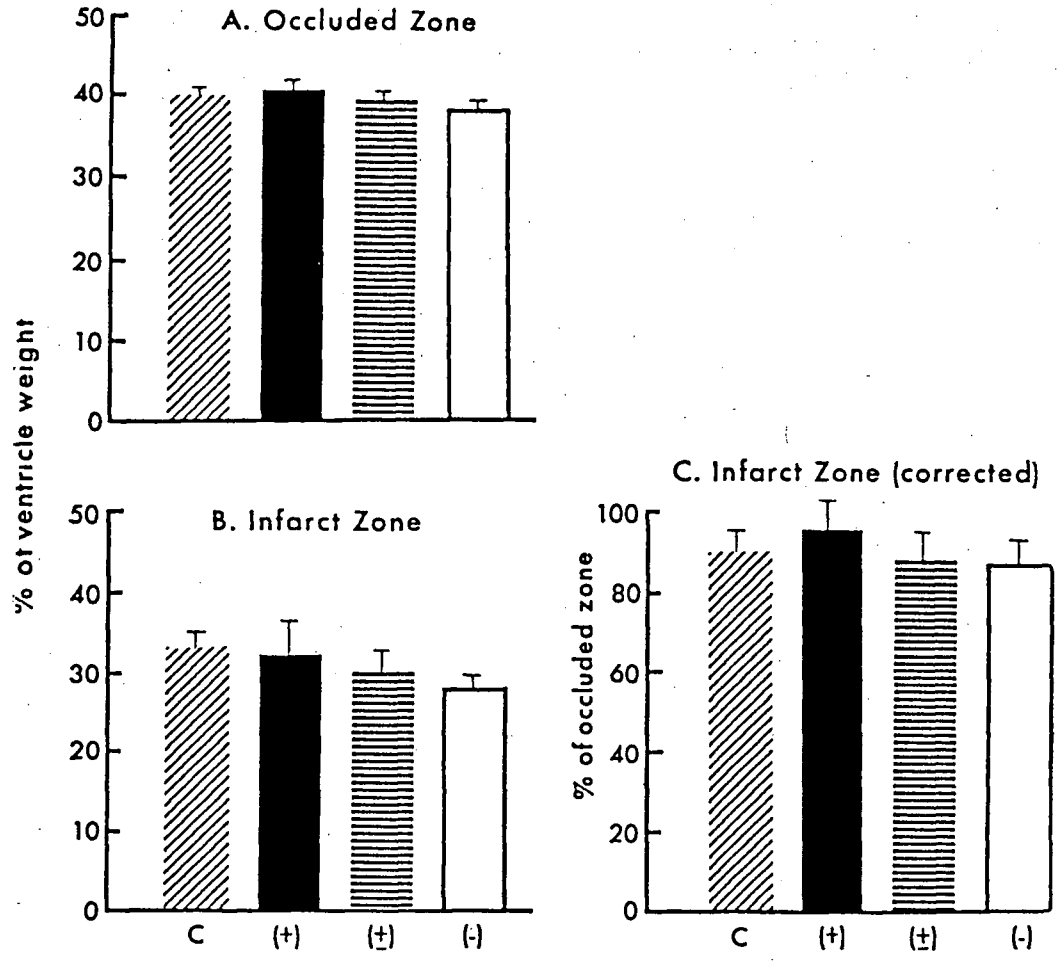



Figure 33. Effects of (+)-, (±)- and (-)-verapamil (6 mg/kg i.v.) on OZ size (part A) and IZ size (parts B and C). Values are expressed as% total ventricular weight (parts A and B), or% of OZ (part C), and are mean ± s.e. The letter C refers to the control group. There were no statistically significant differences between the groups.

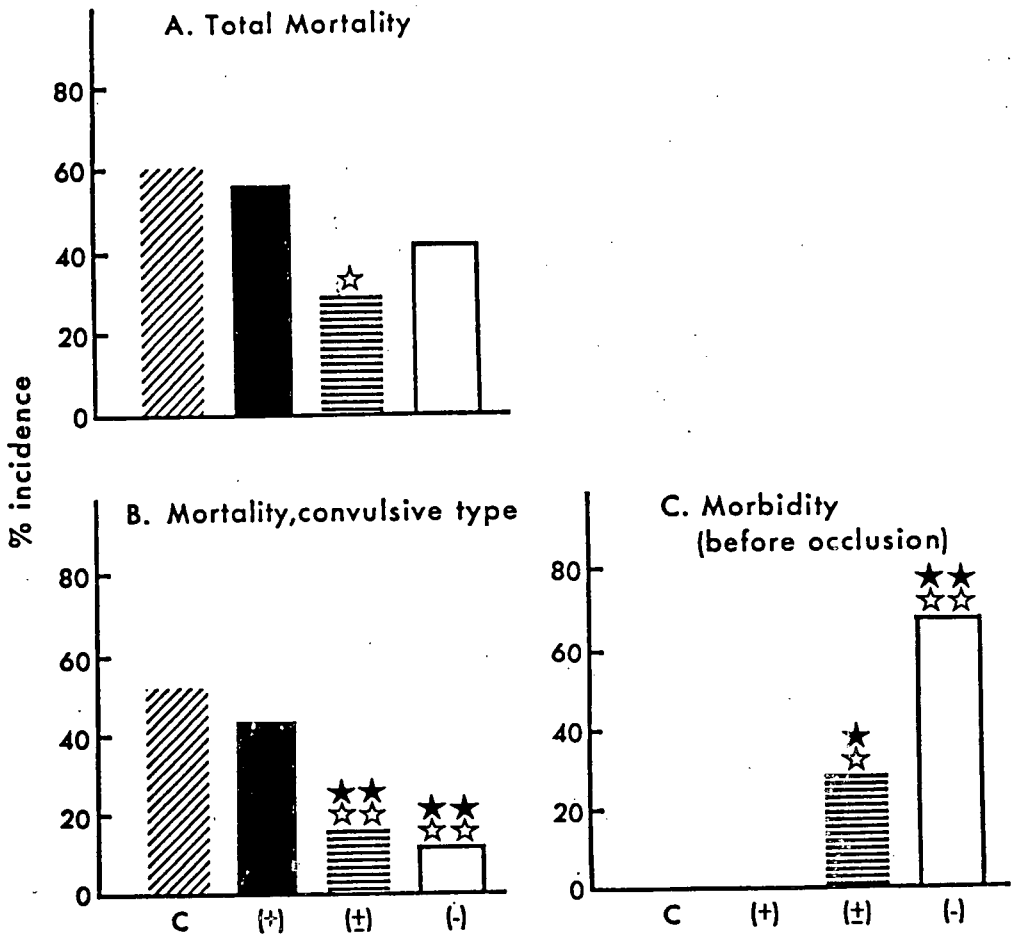



Figure 34. Effects of (+)-, (±)- and (-)-verapamil on total mortality (part A) and mortality associated with sudden convulsive-type behaviour (part B) during the 4 h period following occlusion, and morbidity during the 5 min interval between drug administration and coronary occlusion (part C). Values are % incidence (n = 25 per group). 1 Star = $p < 0.05$, 2 stars = $p < 0.01$ (open stars versus controls, filled stars versus the (+) group).

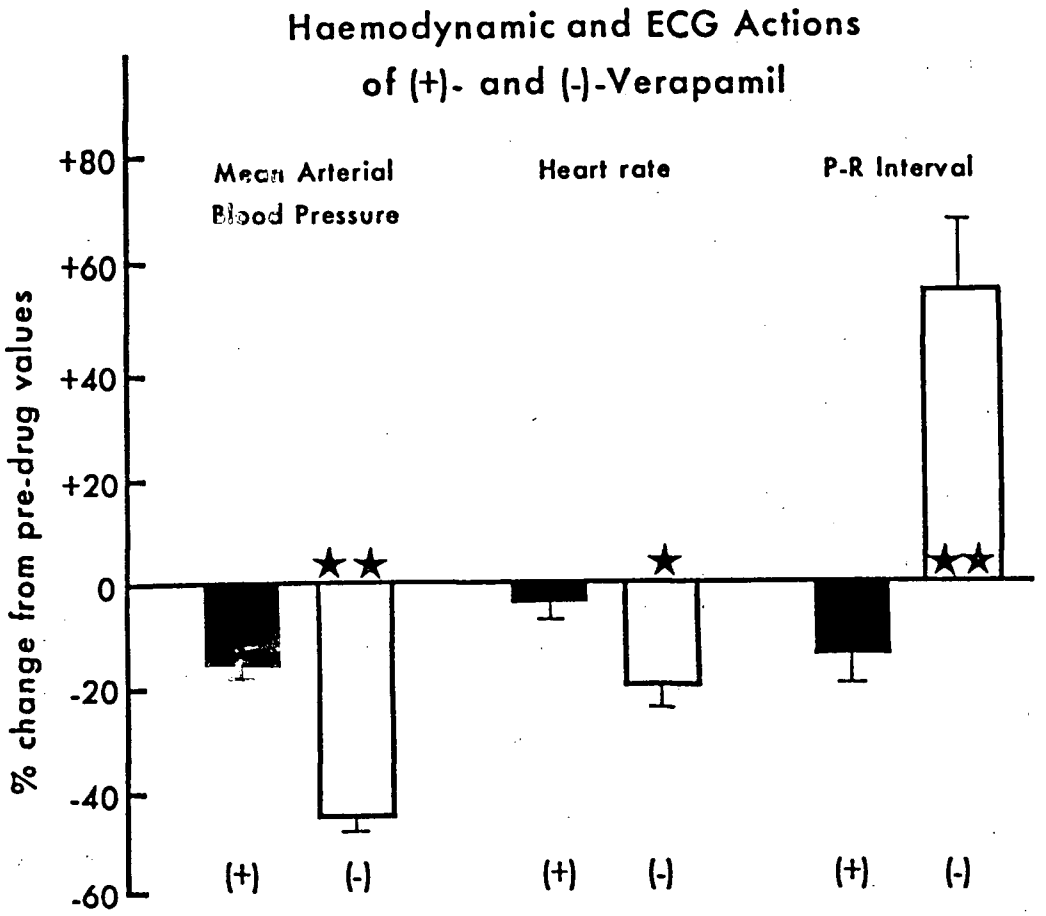



Figure 35. Effects of (+)- and (-)-verapamil (6 mg/kg i.v.) on mean aortic blood pressure, heart rate and P-R interval. Values are mean ± s.e.mean % of pre-drug values, measured 5 min after drug administration. Pre-drug blood pressures were 115 ± 5 and 117 ± 4 mmHg, heart rates were 414 ± 15 and 402 ± 18 beats/min, and P-R intervals were 47 ± 3 and 44 ± 3 msec, in the (+) and (-) groups, respectively. 1 Star = $p < 0.05$ and 2 stars = $p < 0.01$.

A good concordance was seen when morbidity before occlusion (Figure 34C) was compared with blood pressure and ECG reponses to the isomers, recorded in a separate group of rats (Figure 35). The high incidence of morbidity in the rats given (-)-verapamil corresponded with P-R prolongation and hypotension, which greatly exceeded that produced by (+)-verapamil (Figure 35).

3.7.5 Summary

In a new behavioural model of acute ischaemia-induced VF and sudden death the antiarrhythmic profile of (+)-, (±)- and (-)-verapamil (Figure 34) corresponded qualitatively with their relative calcium antagonist activity. Infarct size was not reduced by any treatment, however (Figure 33).

## 3.8 Electrically-induced arrhythmias in conscious rats

3.8.1 Overview and summary

As an indirect measure of the $Na^+$ channel blocking-dependent antiarrhythmic potency ratio of the optical enantiomers of verapamil, their effects on electrically induced fibrillo-flutter and maximum following frequency were measured in conscious rats. These variables have previously been show by our laboratory to be sensitive to $Na^+$ channel blocking agents such as quinidine (Curtis et al., 1984). The results of this study have been accepted for publication (Curtis and Walker, 1986a).

At 5 min after drug administration, neither (+)-verapamil (8 and 12 mg/kg), nor (-)-verapamil (2 and 6 mg/kg) produced statistically significant alterations in threshold voltage or pulse width for induction of fibrillo-flutter, or maximum following frequency in conscious rats during electrical stimulation of the left ventricle (Figure 36).

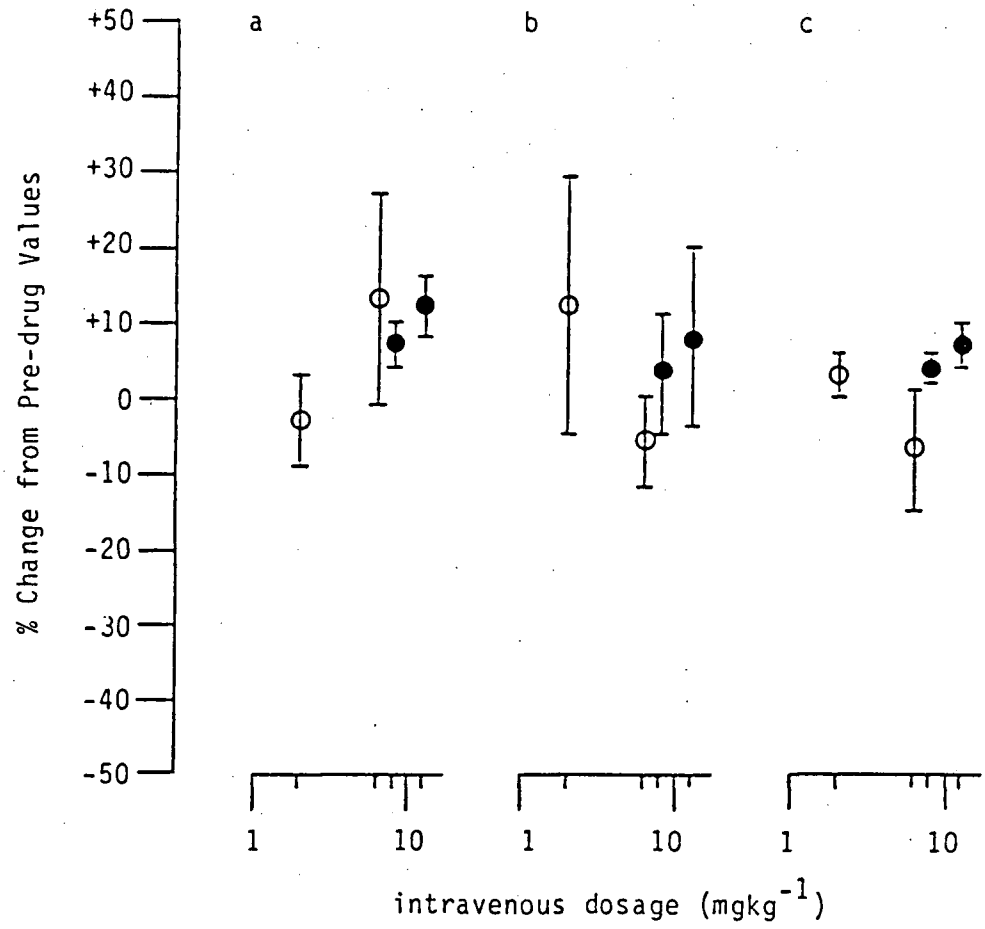



Figure 36. Effects of (+)-verapamil (●) and (-)-verapamil (○) on threshold voltage (a) and threshold pulse width (b) for induction of fibrillo-flutter, and maximum following frequency (c), in conscious rats subjected to electrical stimulation of the left ventricle. Values were measured 5 min after drug administration and expressed as% of pre-drug values. Bars indicate ± s.e.mean. The abscissa gives dose in $\log_{10}$ scale. Treatment was not a statistically significant source of variance.

## 3.9 Haemodynamic and ECG effects of verapamil enantiomers in pithed rats

### 3.9.1 Overview

The pithed rat preparation has been used for many years in order to evaluate the haemodynamic pharmacology of drugs in the absence of autonomic reflexes (Gillespie and Muir, 1967). The preparation is compromised, however, since in the absence of autonomic reflexes, blood-pressure is low and the preparation is unstable (Gillespie and Muir, 1967). In addition, and as a consequence, drugs which lower blood-pressure cannot be evaluated directly, rather blood-pressure must first be elevated by administration of a pressor agent, or by electrically stimulating the sympathetic efferents (Gillespie and Muir, 1967). A simple pithed rat preparation which permits the investigation of the pharmacology of blood-pressure-lowering drugs in the absence of pressor agents was therefore developed. This preparation was used to compare the potencies of the optical enantiomers of verapamil. The results of this study have been accepted for publication (Curtis and Walker, 1986b).

### 3.9.2 Stability of the preparation

The stability of the pithed rat preparation is demonstrated in Figure 37. Part A shows that pithing caused an initial and transient pressor response which was followed by a sharp fall in mean arterial blood pressure. During the following 90 min, mean arterial blood pressure gradually increased by approximately 10 mmHg. Heart rate (Figure 37, Part B) was also significantly slowed 5 - 15 min after pithing, but heart rate recovered to pre-pithing values by 60 min after pithing. The first min after pithing was generally associated with a multiphasic heart rate response involving sinus tachycardia, bradycardia and atrioventricular nodal block, consistent with profound sympathetic and parasympathetic discharge. For this reason it was considered inappropriate to present heart rate values for the first min following pithing (Figure 37, Part B).

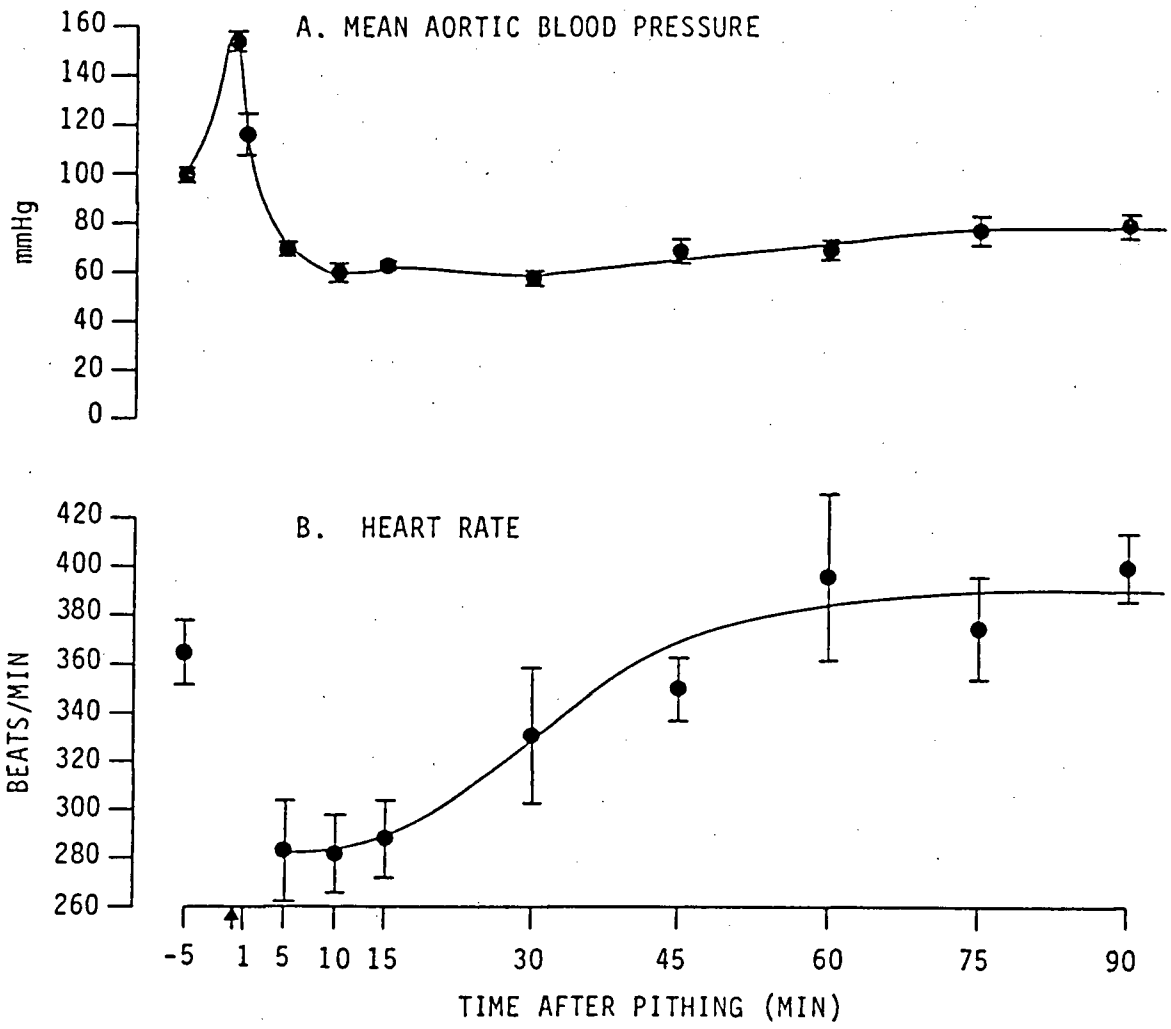



Figure 37. The time course of changes in mean ± s.e.mean aortic blood-pressure (MAP) and heart rate following pithing are shown in parts A and B, respectively (n = 14). The time of pithing is indicated by the arrow.

3.9.3 Effects of verapamil enantiomers

3.9.3.1 Blood pressure and heart rate. The haemodynamic actions of the optical enantiomers of verapamil are shown in Figure 38. Both (-)- and (+)-verapamil reduced mean arterial blood pressure in parallel, in a dose-dependent manner (Part A). (-)-Verapamil was approximately 15 times as potent as (+)-verapamil. However, heart rate (Part B) was only reduced from pre-drug values by high doses (4 and 8 mg/kg) of (+)-verapamil. The effects of the enantiomers on heart rate appeared to occur in series, suggesting that the enantiomers were equipotent in this regard. Pre-drug values of mean arterial blood pressure and heart rate are shown in Table 9.

3.9.3.2 P-R and QT intervals. The ECG effects of the enantiomers are shown in Figure 39. Neither (+)- nor (-)-verapamil influenced QT interval (Part A). However, P-R interval (Part B) was dose-dependently prolonged by both enantiomers, (-)-verapamil being approximately 4 times as potent as (+)-verapamil. If P-R interval was expressed as % change from pre-drug values instead of absolute P-R interval, then (-)-verapamil appeared to be even more potent than (+)-verapamil (approximately 10 times). Pre-drug values of P-R and QT intervals are shown in Table 9.

3.9.4 Summary

Conventional pithed rat preparations are often associated with pre-drug mean arterial blood-pressures of approximately 25-50 mmHg (Gillespie and Muir, 1967). These low values are not conducive to the evaluation of blood-pressure lowering agents. Generally, either a pressor agent is administered, or sympathetic efferents are electrically stimulated before evaluation of a blood-pressure-lowering agent (Gillespie and Muir, 1967; de Jong et al., 1983), in which case, the actions of the drug are contingent upon the nature of the pressor agent used, or the degree of sympathetic discharge; the effects of the latter can be highly variable (Gillespie and Muir, 1967).

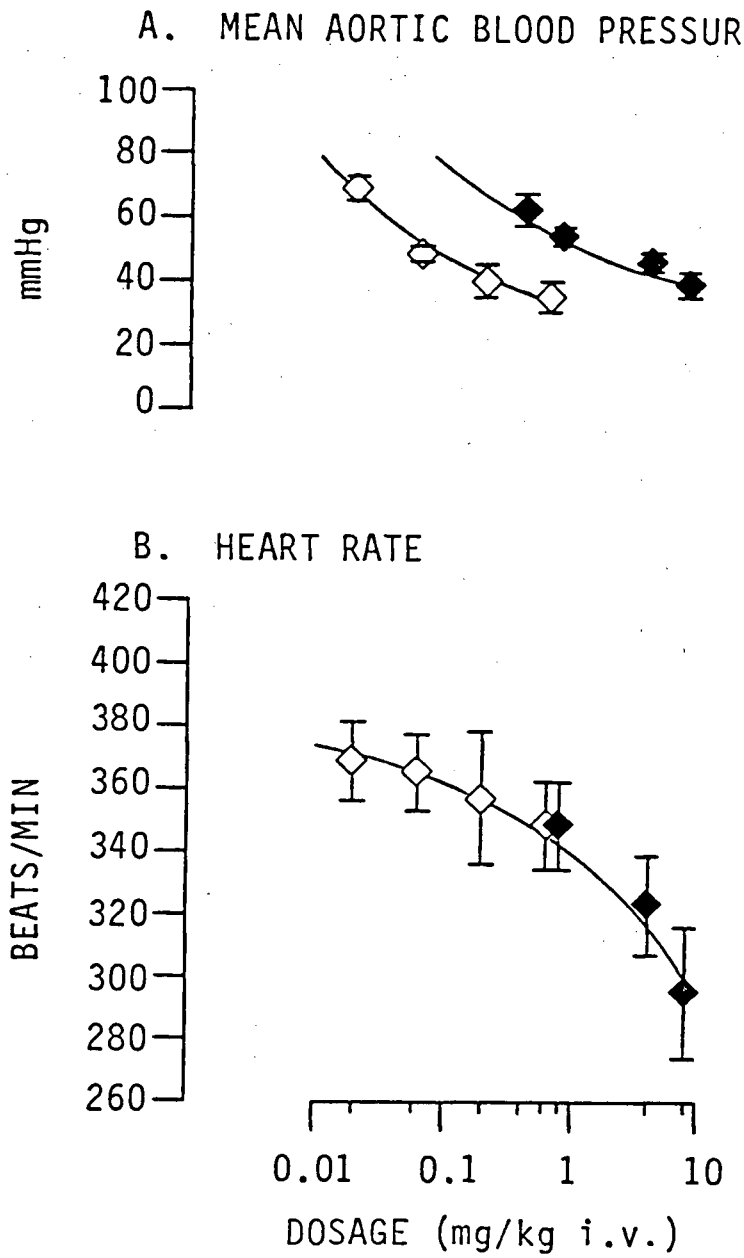



Figure 38. The effects of (-)-verapamil (open symbols) and (+)-verapamil (filled symbols) on blood pressure (Part A) and heart rate (Part B) are shown. Values are mean ± s.e.mean of peak effects measured 10-15 sec after administration, n = 6 per group. Pre-drug values are given in Table 9.

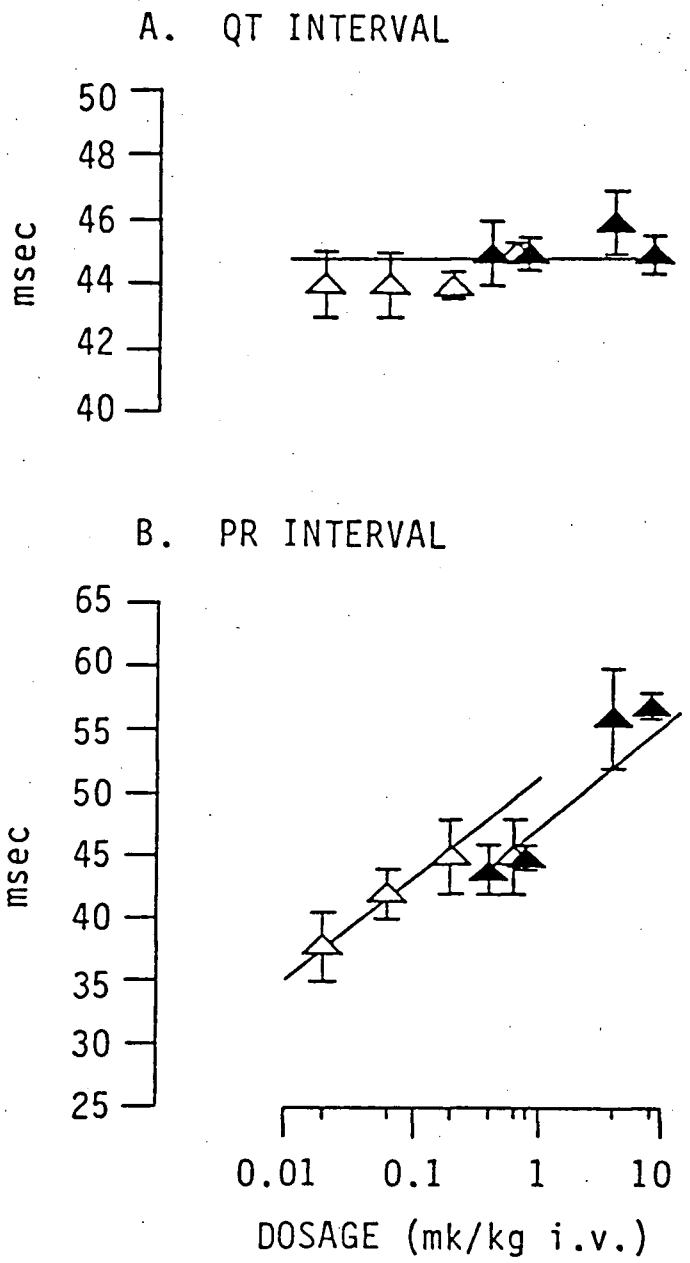



Figure 39. The effects of (-)-verapamil (open symbols) and (+)-verapamil (filled symbols) on QT interval (Part A) and P-R interval (Part B) are shown. Values are mean ± s.e.mean of peak effects measured 10-15 sec after administration (n = 6 per group). Pre-drug values are given in Table 9.

Table 9. Pre-drug values for haemodynamic and ECG variables in pithed rats.

| Group | n | MAP | Heart Rate | QT | P-R |
|---|---|---|---|---|---|
| (-)-Verapamil | 6 | 69 ± 4 | 376 ± 15 | 44 ± 1 | 37 ± 2 |
| (+)-Verapamil | 6 | 85 ± 8 | 412 ± 14 | 44 ± 1 | 41 ± 1 |

Values are mean ± s.e.mean. The units for the variables are mmHg for MAP, beats/min for heart rate and msec for QT and P-R. There were no statistically significant differences between the groups for any variable (t-test).

In the present experiments, the requirement for a pressor agent was eliminated by the simple expedient of mounting the pithed rat preparation vertically, with the head pointing downward. In this manner, the effects of the optical enantiomers of verapamil were evaluated in the absence of sympathetic reflexes with a minimum of artificial constraints such as the presence of pressor agents. The results show that such a manoeuvre lead to the gradual recovery of blood-pressure and heart rate to values amenable to the evaluation of the verapamil enamtiomers (Figure 38).

(-)-Verapamil was found to be more potent than (+)-verapamil in reducing blood-pressure and prolonging P-R interval. (-)-Verapamil was approximately 4-15 times as potent as (+)-verapamil in influencing these variables, depending on how the data was expressed. This potency ratio is similar to the calcium antagonist potency ratio determined from inhibition of $i_{si}$ in ventricular tissue (Nawrath et al., 1981; Ferry et al., 1985) and binding studies (Ferry et al., 1985).

It is of interest to compare the results of the present study in pithed rats with data derived from the occlusion study of the enantiomers in conscious rats. In conscious rats, the relative potency of (-)- versus (+)-verapamil was similar to the relative potency in pithed rats for effects on mean arterial blood pressure. However, conscious rats were far less sensitive to the effects of the enantiomers than pithed rats with regard to effects on P-R interval and blood-pressure. In this regard, when higher doses of (-)- (2 mg/kg) and (+)-verapamil (12 mg/kg) (which produced only moderate blood-pressure lowering in conscious rats) were administered to the pithed rats, such severe blood-pressure reductions were produced that cardiovascular collapse and death ensued in at least 50% of preparations. This strongly suggests that sympathetic reflexes offset the actions of the enantiomers of verapamil in conscious animals.

In contrast with the effects of the enantiomers on blood-pressure and P-R interval, the QT interval was not affected by either enantiomer. Since QT interval is a measure of ventricular conduction velocity and repolarisation, then the results suggest that the enantiomers had little or no effect on these variables at doses which caused severe hypotension and P-R prolongation. In this regard, our results are consistent with the reported pharmacological profile of (±)-verapamil, in that effects in the heart *in vivo* are essentially restricted to inhibition of $i_{si}$, manifest as a narrowing of action potential plateau in the working heart, and a depression of $dV/dt_{max}$ and conduction velocity in parts of the AV node, whereas $i_{Na}$- and $i_K$-dependent events are not affected (e.g., Singh, 1975; 1979; 1982).

## 3.10 Actions of verapamil enantiomers in perfused, paced rat ventricles

### 3.10.1 Overview

In order to supplement the literature concerning the potency of the optical enantiomers of verapamil as calcium antagonists in ventricular tissue, experiments were carried out using isolated, perfused rat ventricles. This information was required in order provide a template for assessing the mechanism of the antiarrhythmic actions of the enantiomers. Verapamil was recently reported to exhibit stereoselective plasma protein binding and hepa- tic metabolism in humans (Echizen *et al.*, 1985; Eichelbaum *et al.*, 1984; Vogelgesang *et al.*, 1984). If the same phenomena occur in rats there is a possibility that calcium antagonist potency ratios *in vivo* and *in vitro* may be different. Therefore, the potency ratios of the enantiomers *in vivo* (reductions in blood pressure, and P-R interval prolongation) were compared with those obtained *in vitro* (negative inotropic actions) in isolated Langendorff-perfused ventricles. Since extracellular $K^+$ concentration has been shown to rise rapidly following coronary artery occlusion (Hill and

Gettes, 1980; Hirche et al., 1980), in vitro experiments were carried out over a range of buffer $K^+$, in order to examine the dependence of absolute and relative potencies of the enantiomers on $K^+$ concentration. The results of this study have been accepted for publication (Curtis and Walker, 1986a).

3.10.2 Isochoric left ventricular developed pressure

Both enantiomers dose-dependently reduced contractility in paced rat ventricles. The dose-response curves for negative inotropism at 5.9 meq/l $K^+$ are shown in Figure 40. The slopes for both enantiomers were not significantly different from 1.0 at any $K^+$ concentration. However, $EC_{50}$ values were highly dependent on buffer $K^+$ concentration (Figure 41). At 3 meq/l $K^+$, mean $EC_{50}$ (and ± 1 s.e.mean from antilog) were 2.8 (2.4 - 3.3) μM for (+)- and 0.16 (0.13 - 0.2) μM for (-)-verapamil. At 10 meq/l $K^+$, (+)-verapamil was 70 times as potent as it had been at 3 meq/l $K^+$, while the potency of (-)-verapamil was increased 33-fold. Raising buffer $K^+$ concentration increased the potency of (+)- more than (-)-verapamil, such that the potency ratio of (-)- to (+)- fell from 21.3 ± 2.6 at 3 meq/l $K^+$, to 17.4 ± 1.1, 8.6 ± 0.4 and 8.3 ± 0.7 at 5.9, 8 and 10 meq/l $K^+$, respectively. Ratios at 8 and 10 meq/l $K^+$ were significantly smaller than those at 3 and 5.9 meq/l $K^+$ ($p < 0.05$). For both enantiomers, the relationship between $EC_{50}$ and $K^+$ concentration was approximately linear between 3 and 8 meq/l, whereupon the effect appeared to saturate (Figure 41).

3.10.3 Ventricular excitability

Before the application of drugs, threshold voltage (at 1 msec) and pulse width (at 4 mV) for capture of the ventricles were both elevated 3 - 4 fold (from 0.5 ± 0.04 mV and 0.068 ± 0.009 msec to 1.8 ± 0.1 mV and 0.31 ± 0.04 msec, respectively, $p < 0.05$) by elevating $K^+$ from 3 to 8 meq/l.

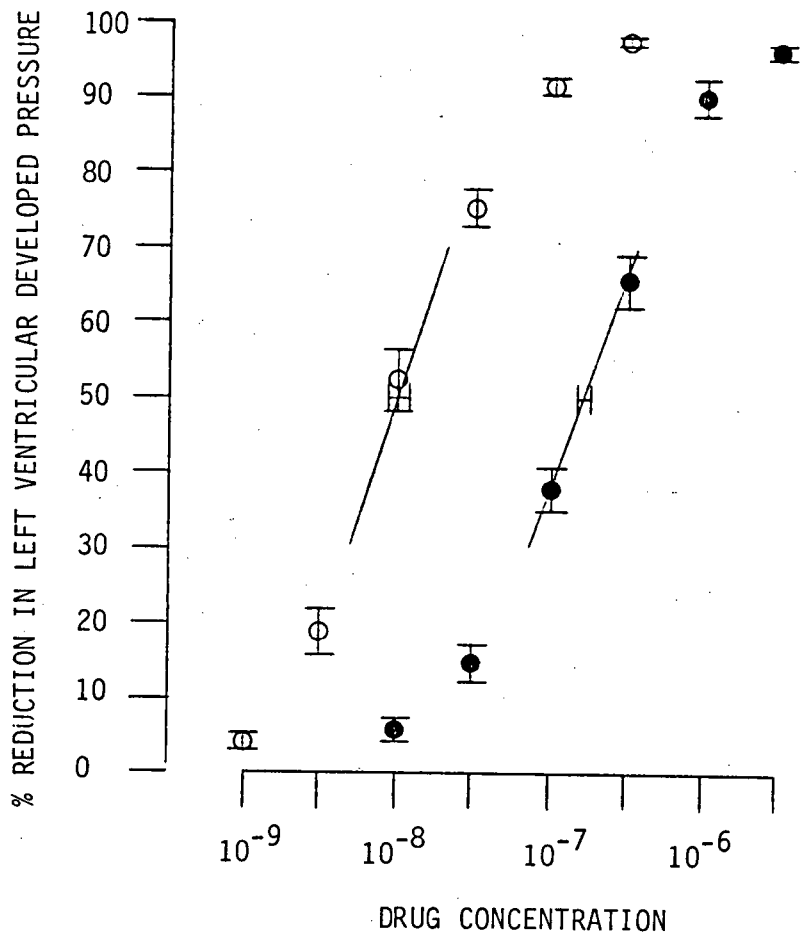



Figure 40. Dose-response curves for the effects of (-)- (open symbols) and (+)-verapamil (solids) on developed pressure in Langendorff-perfused rat ventricles paced at 300/min in vitro. The $K^+$ concentration was 5.9 meq/l in the Krebs-Henseleit buffer, and the data qualitatively resembles that at other $K^+$ concentrations. Each point is mean ± s.e.mean of 6 values. Shown also are the mean slopes (drawn between $EC_{30}$ and $EC_{70}$) and the mean ± s.e.mean $EC_{50}$ values (horizontal lines with verticle error bars).

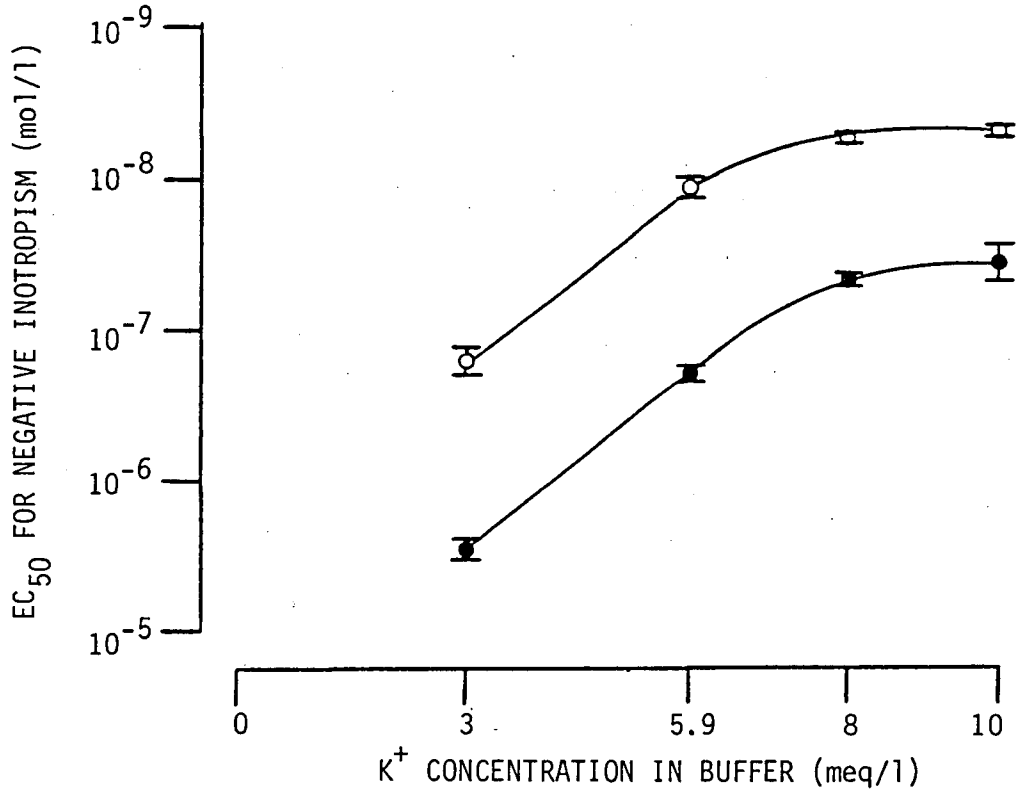



Figure 41. The relationship between negative inotropic potency of (-)- verapamil (open symbols) and (+)-verapamil (solids) and $K^+$ concentration in isolated Langendorff-perfused rat ventricles. The abscissa is $K^+$ concentration (meq/l) in the perfusion, while the ordinate is mean $EC_{50}$ for negative inotropism (calculated as $\log_{10}$ mol/l but shown as antilog). Bars indicate ± s.e.mean for n = 6 preparations.

Table 10. Incidence of PVC in isolated ventricles: effects of verapamil enantiomers

| | Drug Concentrations | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1nM | 3nM | 10nM | 30nM | 100nM | 300nM | 1μM | 3μm | 10μm |
| | | | | | (-)-verapamil | | | | | |
| Buffer $K^+$ | | | | | | | | | | |
| 3 meq/l | 6 | – | – | 6 | 6 | 4 | 0* | 0* | 0* | – |
| 5.9 meq/l | 6 | 6 | 5 | 2 | 0* | 0* | 0* | – | – | – |
| 8 meq/l | 0 | 0 | 0 | 0 | 0 | 0 | 0 | – | – | – |
| 10 meq/l | 0 | 0 | 0 | 1 | 0 | 0 | 0 | – | – | – |
| | | | | | (+)-verapamil | | | | | |
| 3 meq/l | 6 | – | – | – | 6 | 6 | 6 | 2 | 0* | 0* |
| 5.9 meq/l | 6 | – | – | 6 | 6 | 4 | 2 | 0* | 0* | – |
| 8 meq/l | 0 | – | – | 0 | 0 | 0 | 0 | 0 | 0 | – |
| 10 meq/l | 0 | – | – | 0 | 0 | 0 | 0 | 0 | 0 | – |

Incidence (out of n = 6) of spontaneous PVC in isolated rat ventricles perfused with 0.7mM $CaCl_2$ at 300 beats/min under various buffer $K^+$ conditions. * Indicates $p < 0.05$ versus pre-drug incidence.

3.10.4 Additional comments

Neither control developed pressure nor control coronary flow correlated with $K^+$ concentration, although both tended to be lower at 3 meq/l $K^+$ versus the other $K^+$ concentrations. Pulmonary artery temperature ranged from 36.8 ± 0.3 to 37.3 ± 0.3 °C, and remained constant throughout each experiment. At 3 and 5.9 meq/l $K^+$, episodes of PVC were observed (Table 10). PVC were absent at 8 and 10 meq/l $K^+$. Both enantiomers dose-dependently reduced and abolished these PVC (Table 10), (-)-verapamil being much more potent than (+)-verapamil in this regard. In addition, the potency of both enantiomers for this antiarrhythmic action was enhanced by elevating $K^+$ from 3 to 5.9 meq/l (Table 10). We were unable to extend the study to $K^+$ values above 10 meq/l since the ventricles either failed to capture, or only captured at unacceptably high voltages (> 20 V) under these conditions.

3.10.5 Summary

(-)-Verapamil was more potent than (+)-verapamil in reducing the ventricular developed pressure in paced ventricles. The potency of both enantiomers varied considerably with the concentration of $K^+$ to which the preparation was exposed (Figure 41). In addition, (-)-verapamil was more sensitive to the potentiating effect of $K^+$ than (+)-verapamil.

3.11 Actions of nifedipine and DHM9 in isolated perfused rat ventricles

3.11.1 Overview

The actions of these 1,4-dihydropyridines in isolated ventricles were investigated for the same reason that the verapamil enantiomers were studied (see above), namely to provide an independent estimate of calcium antagonist activity in ventricular tissue in order to support explanations for the actions (or lack of actions) in the ischaemic heart in vivo.

3.11.2 Isochoric left ventricular developed pressure

DHM9 at up to $3 \times 10^{-5}$ M had no effect on left ventricular developed pressure, irrespective of the $K^+$ concentration in the perfusing buffer.

Figure 42 shows the relationship between the negative inotropic activity of nifedipine and the concentration of $K^+$ present. Increasing $K^+$ caused a small increase in $-\log_{10} EC_{50}$, and the relationship appeared to be linear over the range 3 - 10 meq/l $K^+$. Despite the fact that increasing $K^+$ concentration from 3 - 10 meq/l only increased the potency of nifedipine approximately 4 fold, each $-\log_{10} EC_{50}$ value was statistically significantly different from each other value, illustrating that variance was low.

In contrast with the enantiomers of verapamil (see above), the slope for the negative inotropic actions of nifedipine was significantly greater than 1 at 3, 5.9 and 8 meq/l $K^+$ (values being $1.46 \pm 0.14$, $1.7 \pm 0.11$ and $1.39 \pm 0.12$, respectively. However, at 10 meq/l $K^+$, the slope was not different from 1 (it was $1.01 \pm 0.03$). Therefore it is not strictly appropriate to extrapolate from the $-\log_{10} EC_{50}$ data and state that $K^+$ had changed 'potency'. Nevertheless, the differences in slope were not particularly large (slope values were always less than 2), and variance values for $-\log_{10} EC_{50}$ were always small.

Nifedipine caused increases in coronary flow which were not clearly concentration-related nor related to the $K^+$ concentration. Since nifedipine is relatively selective in its actions on vascular smooth muscle compared with the heart (see Discussion), then it may be argued that the lowest concentrations applied probably produced the maximum coronary vasodilation possible with this drug, and that subsequent variations were a reflection of the influence of other variables such as intraventricular pressure. Pre-drug flows were $9.3 \pm 0.6$, $11.3 \pm 1.2$, $8.9 \pm 0.9$ and $11.2 \pm 0.7$ ml/min in the 3, 5.9, 8 and 10 meq/l $K^+$ groups respectively.

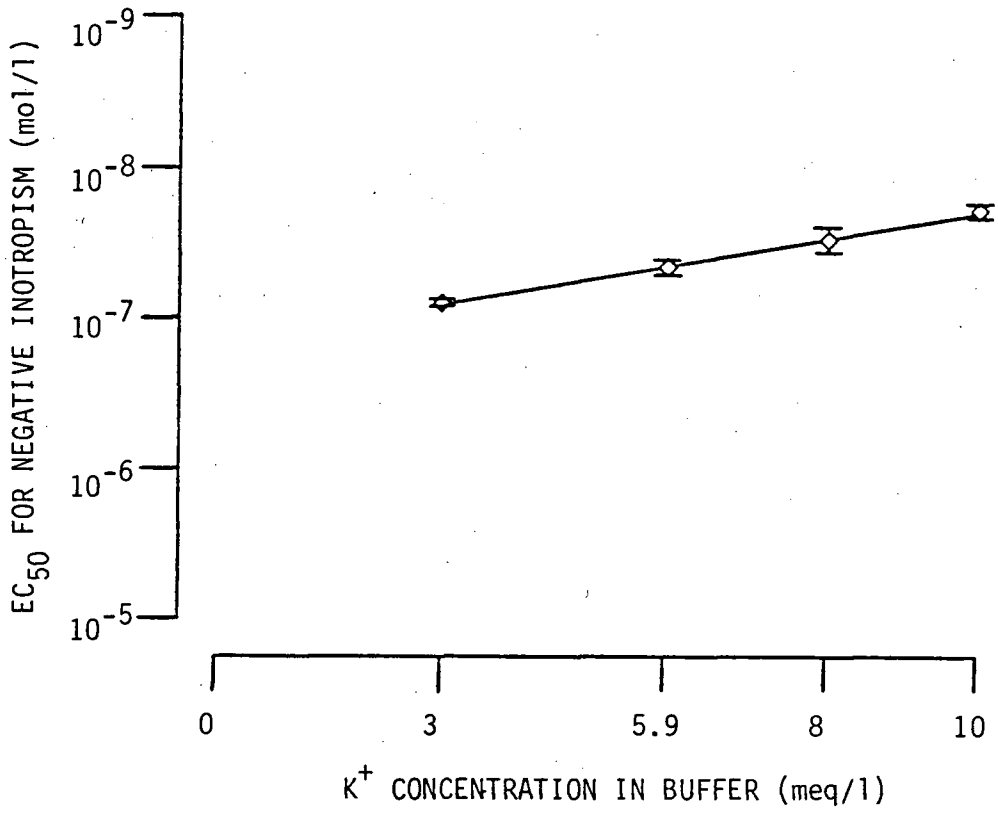



Figure 42. The relationship between negative inotropic potency of nifedipine and $K^+$ concentration in isolated Langendorff-perfused rat ventricles is shown. The abscissa is $K^+$ concentration (meq/l) in the perfusion, while the ordinate is mean $EC_{50}$ for negative inotropism (calculated as $\log_{10}$ mol/l but shown as antilog). Bars indicate ± s.e.mean (n = 6 preparations).

3.11.3 Ventricular excitability

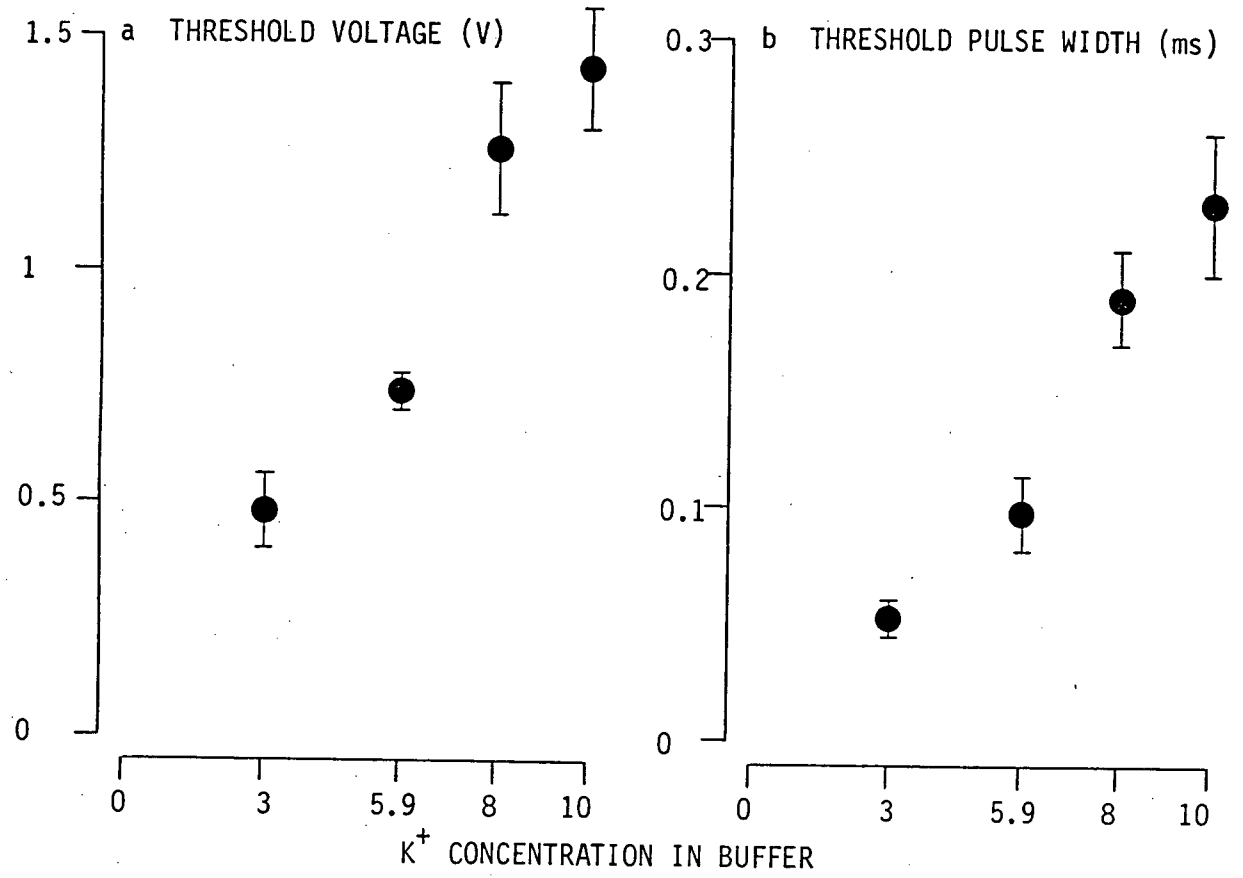
Preliminary evidence from the previous study with the enantiomers of verapamil (see above) suggested that increasing the $K^+$ concentration reduced the excitability of the ventricles (as determined by sampling the strength/duration curve at 4 V and 1 msec). This effect was confirmed quite explicitly in the present experiment (Figure 43). Raising $K^+$ concentration caused a large increase in the threshold voltage for capture at 1 msec and the threshold pulse-width for capture at 4 V. This effect appeared to be linear over the $K^+$ range studied, but there was a suggestion that this effect was beginning to saturate; with only 4 points it was impossible to distinguish between the 2 models, therefore the points have not been connected in the figure. Experiments at higher $K^+$ concentrations were not carried out because previous work with the enantiomers of verapamil had suggested that it was not possible to pace the ventricles efficiently when $K^+$ concentration was more than 10 meq/l.

3.11.4 Summary

DHM9 was without effect on isochoric left ventricular developed pressure, even at high concentrations. Nifedipine was an effective negative inotropic agent (Figure 42). Increasing buffer $K^+$ concentration caused a small but statistically significant increase in the potency of nifedipine, and a large reduction in excitability (Figure 43).

As a general comment (which also applies to the in vitro work with the enantiomers of verapamil) it will be of interest to examine the potency of nifedipine in the perfused ventricle preparation using another approach, by keeping the calcium antagonist concentration constant and constructing dose-response curves for $Ca^{2+}$. Such curves would give an estimate of drug affinity for its 'receptor' at different $K^+$ concentrations.

Figure 43. The effect of different buffer $K^+$ concentrations on the threshold voltage (at 1 msec pulse width, part a) and threshold pulse width (at 4 V, part b) for capture of the left ventricle in the Langendorff-perfusion rat ventricle preparation (stimulation frequency 300/min). Values are mean ± s.e.mean and were recorded after 10 - 15 min stabilisation. Each preparation was subsequently used to measure the negative inotropic activity of nifedipine (see figure 42). The values for both variables at 3 and 5.9 meq/l $K^+$ were significantly different from each other and from the values at 8 and 10 meq/l $K^+$ ($p < 0.05$).


a THRESHOLD VOLTAGE (V)
1.5
1
0.5
0
b THRESHOLD PULSE WIDTH (ms)
0.3
0.2
0.1
0
0
3
5.9
8
10
K+ CONCENTRATION IN BUFFER

# 4 DISCUSSION

## 4.1 The conscious rat preparation for myocardial ischaemia studies

### 4.1.1 Overview

The rat has been used sporadically since 1946, and intensively since 1979 for investigating myocardial ischaemia and infarction. The rat is used because its lack of coronary collateral anastamoses (Johns and Olson, 1954; Selye, et al., 1960; Maxwell et al., 1984; Winkler et al., 1984; Schaper et al., 1986) allows for reproducible and uniform infarcts (Johns and Olson, 1954; Selye et al., 1960; Bajusz, 1963; Hort and Da Canalis, 1965a; 1965b; MacLean et al., 1976; Bernauer, 1980; Johnston et al., 1983a), and reproducible ventricular arrhythmias (Clark et al., 1980; Kane and Winslow, 1980; Coker and Parratt, 1981; Mertz and Kaplan, 1982; Lepran et al., 1983; Johnston et al., 1983a; 1983b; MacLeod et al., 1983). The preparation is used to investigate arrhythmias (Kenedi and Losconci, 1973a; 1973b; Szekeres et al., 1980; Clark et al., 1980; Kane and Winslow, 1980; Bernauer 1980; Clark et al., 1980; Mueller and Wilsmann, 1981; Martinez and Crampton, 1981; Bergey et al., 1982; Au et al., 1983; 1986; Johnston et al. 1983a; 1983b; etc.) and infarction (Bryant et al., 1958; Zsoter and Bajusz, 1962; Hort and Da Canalis, 1965a; 1965b; MacLean et al., 1976; 1978; Pfeffer et al., 1979; 1985a; 1985b; Bernauer 1980; 1982; Innes and Weisman, 1981; Flaim and Zelis, 1981; de Jong-Koster and van Zwieten, 1981; Byrne et al., 1982; Edoute et al., 1983; Au et al., 1983; 1986; Johnston et al. 1983a; 1983b; Manning et al., 1983a; Godfraind and Saleh, 1984; etc.).

### 4.1.2 Precision of variables

4.1.2.1 Occluded zone (OZ) and infarct zone (IZ). Drugs which reduce the extent or degree of ischaemia can be expected to be antiarrhythmic. Therefore such actions must be considered when assessing antiarrhythmic actions. The quantification of ischaemic and infarcted tissue size as

well as arrhythmias in the same animals provides a series of checks and balances by which it can be ascertained that reductions in arrhythmias occurred as a result of treatment rather than as a result of 'missed' occlusion. In addition, ischaemia and infarction can be studied in their own right. The results of studies of infarct size in rats (see below) should be considered within the wider context of whether infarct size reduction is actually possible in any species (Hearse and Yellon, 1984). It is believed by Hearse's group that animals with little coronary collateral anastamosis development such as the rabbit (Dennis et al., 1986) and rat (Hearse et al., 1986) are unsuitable for assessing potential infarct-limiting drugs since, in the absence of early reperfusion, all the non-perfused tissue is destined to become necrotic. Even in dogs which often possess extensive collateral vascularisation (Schaper et al., 1967; Schaper 1971; Meesman, 1982), it has recently been suggested that although drug treatment may reduce infarct size when assessed 24 h after occlusion, the effect is lost if infarct size is measured 48 h after occlusion (Yellon et al., 1986a), illustrating that care must be taken in distinguishing between prevention of necrosis and delays in the development of necrosis.

With regard to the relationship between collateral anastamoses and infarction, Hearse's group recently reviewed their infarct size data and found that for control animals infarct size could be predicted accurately from the extent of collateral flow, independent of the 'risk zone' size (Yellon et al., 1986b). It is possible, therefore, that following permanent coronary occlusion the ultimate infarct size is a fixed function of the extent of collateral anastamoses. The present results suggest that this appears to be the case for rats.

For species such as dogs with variable collateral development (Meesman, 1982) the possibility exists that a combination of infarct development

delays and sampling error (an excess of animals with well developed collaterals in the drug-treated group) has given rise to the many reports of 'reductions in infarct size' in this species. Unless an experiment is carried out blindly and randomly with control for variations in collaterals, and unless at least 3 doses of a drug are investigated, then the results of infarct limitation studies in dogs should be treated with scepticism. In species (such as the rat) with poorly developed collaterals it is difficult to imagine how IZ size (expressed as % of OZ size) can be reduced by treatment under any experimental circumstance. Nevertheless, many reports of infarct size reduction in rats have been published (see below).

4.1.2.2 Arrhythmias. Direct comparisons of arrhythmia incidence between various rat models is not strictly possible owing to the lack of a common method for diagnosis and quantification of arrhythmias. Despite this, there appears to be a good concordance within and between groups of investigators and for different models. For example, the mean ± s.d. incidences of VT and VF for conscious rats calculated from the experiments of Szekeres' laboratory (82 ± 7, and 89 ± 10 %, respectively) correspond well with the incidence found in the present experiments during the 0 - 0.5 h period after occlusion.

4.1.2.3 ECG changes. In rats there is very little information concerning the pattern of ECG changes following coronary occlusion, since most investigators use the ECG merely to diagnose arrhythmias. The ECG was first recorded in rats following coronary artery occlusion by Normann et al. (1961). S-T segment elevation, giant R waves and pathological Q waves were shown to be present by 2 h after occlusion. By 24 h, deep Q waves and abnormally small R waves were present. These findings are characteristic of the changes described in the present studies.

Of some interest is a set of experiments carried out in dogs in which

the ECG effects of coronary occlusion were compared with the effects of haemorrhage (Prinzmetal et al., 1961). It was shown that whereas occlusion induced changes in the ECG of similar nature to those described in rats following coronary occlusion, haemorrhage characteristically resulted in immediate S-T segment depression and a reduction in R wave size. This effect of haemorrhage in dogs corresponds with similar findings in rats (Cooper, 1969), and also corresponds with findings in the present series of experiments. In this regard, it was found that in rats in which occlusion caused haemorrhaging in the thorax (demonstrated by post-mortem examination) the ECG was characterised by S-T segment depression rather than elevation, and tiny R waves were present at the time that the R wave is usually abnormally large.

4.1.3 Responses to drugs

4.1.3.1 Overview. Many drugs have been investigated for actions against ischaemia-induced arrhythmias using rat preparations, both in vivo and in vitro, and the pattern of outcome is not consistent for all drugs. In in vivo studies, some of the variations in outcome may be a result of the low doses used in some studies (e.g., Fagbemi et al., 1984; Mueller and Wilsmann, 1982) since, if clinical dose regimens are given to rats, ineffectively low blood concentrations may result owing to the rapid metabolic rate of rats. Unfortunately, blood concentrations have only been determined in conjunction with the assessment of antiarrhythmic activity in rats on rare occasions (Kane et al., 1982; Curtis et al., 1984; Hashimoto et al., 1986).

It is important to examine the dose-dependence of the effects of a drug. In many studies only one or two doses of a drug have been administered. Multiple dose studies generate dose-response curves which are one of pharmacology's more powerful tools for data analysis. Therapeutic ratios can be calculated, and the curves may offer insight into mechanisms of drug

action. Clinically it is well established that a therapeutic window exists for many drugs, whereby insufficient amounts of drug will be ineffective, whereas high concentrations will be toxic. Antiarrhythmic agents, by their very nature, have significant influence on cardiac electrophysiology in most instances. Therefore it is not surprising to find that antiarrhythmic agents can precipitate or exacerbate arrhythmias during therapy. Since arrhythmogenic properties have been reported for most types of antiarrhythmic drugs including lidocaine, quinidine, procainamide, bethanidine, lorcainide, flecainide, verapamil, bepridil and ethmozin both clinically and experimentally (see Torres et al., 1985), consideration of dose-response relationships in the study of antiarrhythmic agents cannot be over-emphasized.

Since, in general, insufficient consideration has been given to the above factors and to other important aspects concerning use of rat models (see later), it is clear why contradictory reports may be found in the literature concerning the effectiveness of drugs as antiarrhythmics and infarct limiting agents (this applies equally well to results from studies using other species).

4.1.3.2 Class I antiarrhythmics. With the exception of lidocaine (see below) all the Class I antiarrhythmics which have been tested (quinidine, disopyramide, procainamide, Org 6001, etc.) are effective in all models, and in all studies (e.g. Johnston et al., 1983a; Marshall et al., 1981b; 1982; Marshall and Winslow, 1982; Winslow, 1980; Winslow et al., 1983; Woodward, 1981). At effective antiarrhythmic doses most of these drugs reduce blood pressure, except for disopyramide which raises it (Marshall et al., 1982; Johnston et al., 1983a). Effective doses of Class I antiarrhythmics for intact rats are generally higher than those for dogs and pigs, as a consequence of differences in the rate of metabolism of drugs

between these species. The mechanism of action of Class 1 drugs, in general terms, is most likely related to inhibition of $g_{Na}$, since in isolated perfused hearts the concentrations of Class I drugs which are antiarrhythmic are similar to the concentrations which inhibit ventricular $g_{Na}$ *in vitro* (Grant *et al.*, 1984; Weidmann, 1955a).

There are reports that lidocaine is antiarrhythmic in anaesthetised rats (Clark *et al.*, 1980; Bergey *et al.*, 1982; Lepran *et al.*, 1983), however there are contradictory reports which suggest that the antiarrhythmic effectiveness of lidicaine is lower than that of Class Ia drugs such as quinidine (Mertz and Kaplan, 1982; Johnston *et al.*, 1983a). Lidocaine has been used clinically for many years, particularly as a consequence of a highly encouraging report in the early 1970s (Lie *et al.*, 1974). However, as with all currently available drugs (Campbell, 1984), the clinical effectiveness of lidocaine is not established (Pentecost *et al.*, 1981; May *et al.*, 1983). Recently it was shown that lidocaine dose-dependently increased the incidence of VF following coronary occlusion in pigs in association with a slowing of conduction in the ischaemic and normal myocardium (Carson *et al.*, 1986). By slowing conduction without prolonging refractoriness, lidocaine would be expected to facilitate reentry (Mines, 1913). High concentrations of lidocaine *in vitro*, however are capable of converting unidirectionl block to bi-directional block (Cardinal *et al.*, 1981; Janse 1982) The example of lidocaine illustrates the importance of consideration of dosage, since in isolated hearts in which side-effects of drugs (such as lidocaine-induced convulsions) are not a consideration, it may be possible to demonstrate an antiarrhythmic action of a drug which would be impossible to produce *in vivo*.

4.1.3.3 *Class 2 antiarrhythmics*. This section should be considered in conjunction with the section concerned with the role of the sympathetic nervous system in arrhythmogenesis. Results obtained with β-adrenoceptor

antagonists have to be considered within the context of dose (or concentration) and the associated pharmacological profile, since the positive antiarrhythmic actions reported for some β-adrenoceptor antagonists only occur in vivo at concentrations above those necessary for effective (dose ratio for isoprenaline of > 10) β-adrenoceptor antagonism (Campbell and Parratt, 1983). Parratt's group (Campbell and Parratt, 1983; Campbell et al., 1984; Hughes et al., 1984) have found that many β-adrenoceptor antagonists possess antiarrhythmic properties in anaesthetised rats. On the other hand, others (including our laboratory) have found only marginal antiarrhythmic actions of β-adrenoceptor antagonists in anaesthetised rats (Kenedi and Losconci, 1973b; Au et al., 1979a; 1979b; 1983), and an absence of antiarrhythmic activity of β-adrenoceptor antagonism and sympathectomy in conscious rats (Botting et al., 1983), and non-stereoselective antiarrhythmic actions of β-adrenoceptor antagonists in perfused rat hearts (Daugherty et al., 1986). β-Adrenoceptor antagonists are effective against reperfusion-induced arrhythmias in vitro only at concentrations which inhibit $i_{Na}$ (Rochette et al., 1984).

An explanation for the contradictory reports of the effectiveness of β-adrenoceptor antagonism in vivo is given in the section dealing with the role of the autonomic nervous system. Briefly, β-adrenoceptor antagonism in vivo only seems to be effective in reducing arrhythmias in acutely prepared animals. Since surgery elevates serum $K^+$, and elevated serum $K^+$ reduces the incidence of arrhythmias during acute myocardial ischaemia (Lubbe et al., 1978; Daugherty et al., 1981; Nordrehaug and von der Lippe, 1983; 1985; Solomon, 1984), and since catecholamines lower serum $K^+$ whereas β-adrenoceptor antagonists inhibit the effect of catecholamines to lower serum $K^+$ (Brown et al., 1983), then β-adrenoceptor antagonists may inhibit ischaemia-induced arrhythmias in acutely-prepared rats by simply reinforcing

the effect of surgery to raise serum $K^+$ (by inhibiting the $K^+$-lowering effect of the sympathetic nervous system and circulating catecholamines).

4.1.3.4 Class 3 antiarrhythmics. This class of drugs has not been studied extensively in rats. Mertz and Kaplan (1982) reported that 5 mg/kg bretylium had no significant action on VF in anaesthetised rats. Sotalol (50 mg/kg) and melperone (5 and 10 mg/kg) have been shown to prevent coronary occlusion-induced falls in the threshold current required for induction of VF in anaesthetised rats (Marshall et al., 1983). Amiodarone (2.5 - 20 mg/kg i.v., or pretreatment with 25 or 50 mg/kg/day, p.o. for 2 weeks) has been found to dose-dependently reduce and abolish VF in anaesthetised rats (Schoenfeld et al., 1984). However, using the same preparation, Winslow et al. (1984) found that 21 days of pretreatment with 20 mg/kg amiodarone p.o. had no effect on occlusion-induced arrhythmias. Doses of amiodarone which failed to influence arrhythmias were found to prolong action potential duration in isolated non-ischaemic ventricular tissue, but were found to have no influence on this variable in ischaemic tissue (Winslow et al., 1984). However, meobentine and bethanidine, at doses which prolonged the effective refractory period in the normal ventricular myocardium were found to be effective in reducing occlusion-induced arrhythmias in anaesthetised rats (Northover, 1985). In summary, it is not established whether Class 3 antiarrhythmic drugs are effective antiarrhythmics in acute myocardial ischaemia in rats. In studies in which these drugs have been shown to be antiarrhythmic, the mechanism of action is unclear. There have been no investigations into the effect of Class 3 antiarrhythmics on infarct size in rats.

4.1.3.5 Class 4 antiarrhythmics. Drugs which reduce the duration of the plateau of the cardiac action potential (calcium antagonists) are classified as Class-4 antiarrhythmics (Singh and Vaughan Williams, 1972).

The antiarrhythmic activity of a variety of calcium antagonists has been investigated in acute myocardial ischaemia in rats. The results are discussed in detail in subsequent sections, with particular reference to the current investigations. As an overview, it was found in the present series of experiments that phenethylalkylamine calcium antagonists (verapamil and anipamil) were very effective antiarrhythmics in conscious rats, whereas 1,4-dihydropyridines (felodipine and nifedipine) had little activity. In contrast, in anaesthetised acutely prepared animals (in which much lower doses of drugs were given) other investigators found that (±)-verapamil was only marginally antiarrhythmic (Fagbemi et al., 1984), whereas 1,4-dihydropyridines were very effective (Fagbemi and Parratt, 1981a; 1981b; 1981c). Differences in the results may reflect differences in drug dosage in relation to the cardiovascular and neuronal status of the animals used. Dosage restrictions depend on whether conscious or anaesthetized preparations are used, since much higher doses of calcium antagonists have been found to be tolerated in conscious versus anaesthetised animals (Curtis et al., 1984).

With regard to studies in vitro, it must be remembered that calcium antagonists possess a myriad of 'non-specific' actions at high concentrations (see Henry, 1980; Nayler and Horowitz, 1983) which may confound the interpretation of results. In particular, many 'interesting' effects of (±)-verapamil have been reported at concentrations of $10^{-4}$ M, such as stimulation of noradrenaline release from sympathetic nerves (Karaki et al., 1984), inhibition of $Ca^{2+}$-dependent ATPase activity (Davis et al., 1984), inhibition of plasminogen activator secretion by aortic endothelial cells (Kant et al., 1985) etc. Such effects cannot possibly contribute to the antiarrhythmic effects of (±)-verapamil in vivo, since the associated effective concentrations are at least 100 times greater than those which abolish contraction in the heart (see the present results of studies with isola-

ted ventricles).

Nifedipine is antiarrhythmic *in vitro* only at concentrations producing calcium antagonism in the myocardium (Thandroyen, 1982; Opie and Thandroyen, 1983). In intact animals the evidence suggests that even following high doses, there are no direct effects of 1,4-dihydropyridines on the myocardium (e.g., Raschack, 1976a). Therefore, it is difficult to imagine how 1,4-dihydropyridines can reduce ischaemia-induced arrhythmias *in vivo*.

If 1,4-dihydropyridine calcium antagonists elevate serum $K^+$, this action would account for the antiarrhythmic effects observed in acutely prepared animals (Fagbemi and Parratt, 1981a; 1981b; 1981c). However, it has been shown that nifedipine has no effect on adrenaline-induced hypokalaemia (Struthers and Reid, 1983). In addition, the present experiments showed that nifedipine did not influence serum $K^+$ following coronary occlusion in conscious rats.

Calcium antagonists have been extensively tested for infarct-reducing actions in the rat. The general comments concerning infarct size outlined previously apply to these results. Experiments carried out by ourselves and others have revealed no infarct size reductions with (±)-verapamil (Johnston *et al.*, 1983a; Curtis *et al.*, 1984; Baur *et al.*, 1984; Evans *et al.*, 1985) and nifedipine (Leinot *et al.*, 1983). Others have reported infarct size reductions with diltiazem (Flaim and Zelis, 1981; Richard *et al.*, 1984; Leinot *et al.*, 1983), bepridil (Richard and de Leiris, 1983; Leinot *et al.*, 1983), nifedipine (Richard *et al.*, 1984), (±)-verapamil (Richard *et al.*, 1984) and lidoflazine (Leinot *et al.*, 1983).

4.1.3.6 *Arachidonic acid metabolites and antiphlogistics*. It has been suggested that eicosanoids influence ischaemia-induced arrhythmias in the rat (Coker, 1982; 1983; Martinez and Crampton, 1981), although studies in our laboratory have shown that that eicosanoids have little effect on

ischaemia-induced arrhythmias in conscious rats (Au et al., 1979a; 1980; 1981; 1983). Alterations in prostaglandin production by non-steroidal antiinflammatory agents have also been reported to reduce arrhythmias (Coker and Parratt, 1981a; Fagbemi, 1984; 1985; Lepran et al., 1981a; 1985; Fiedler, 1983) in anaesthetised rats. However, in conscious rats, 2 different aspirin regimens were found to possess little antiarrhythmic actions in our laboratory (Johnston et al., 1983b).

Various investigators (Koltai et al., 1982; 1983; Lepran et al., 1982b) have suggested that leukocytes may, perhaps, be involved in arrhythmogenesis via production of arrhythmogenic arachidonic acid metabolites (see above). Provisional results from experiments from our laboratory suggest that depletion of thrombocytes and leukocytes with antisera do not reduce arrhythmias occurring during the 0 - 0.5 h period after occlusion, however, complete data for the 0.5 - 4 h period is not yet available. The present experiments with pithed rats suggested that reductions in circulating thrombocyte levels may be associated with a lower incidence of occlusion-induced arrhythmias. Drugs affecting the eicosanoid system have been reported to reduce infarct size (Fiedler, 1983; Lepran, et al., 1985), although studies from our laboratory do not support these claims (Johnston et al., 1983b, Au et al., 1983).

4.1.3.7 Other entities. α-Adrenoceptor antagonists have been reported to be antiarrhythmic in cats (Sheridan et al., 1980). There is much disagreement concerning antiarrhythmic effectiveness of α-adrenoceptor antagonists, however. Questionable selectivity of action of many α-adrenoceptor antagonists complicates the interpretation of most studies. If large doses are required for antiarrhythmic effects, pharmacological actions other than α-adrenoceptor antagonism may be involved. Both in vivo (Daugherty et al., 1986) and in vitro (Bralet et al., 1985) studies have shown that whereas some α-adrenoceptor antagonists reduce occlusion-induced arrhythmias

(e.g., phentolamine and prazosin) others (phenoxybenzamine and trimazosin) are without effect. Therefore the antiarrhythmic effects of α-adrenoceptor antagonists appear to bear no relation to their activity as α-adrenoceptor antagonists. α-Adrenoceptor antagonists are discussed again in relation to the antiarrhythmic activity of verapamil, below.

Most anaesthetics have little effect on ischaemia-induced arrhythmias in rats. Halothane has consistently been reported to have a marked antiarrhythmic activity, whereas other halogenated hydrocarbons have no such activity (Au et al., 1983; MacLeod et al., 1983). Chloroform and enflurane may have weak antiarrhythmic actions (Jang et al., 1983), whereas pentobarbitone, fentanyl and nitrous oxide have no influence on arrhythmias (Au et al., 1983). Anaesthetics do not appear to have infarct-reducing actions in rats (Au et al., 1983; Macleod et al., 1983; Jang et al., 1983), although there is one positive report for halothane (Kissin et al., 1981), and one report suggesting that morphine increases infarct size (Markiewicz et al., 1982).

4.1.4 The conscious rat versus other preparations

The rationale for the use of the conscious rat has been discussed in detail in the Introduction. There are a few additional points to be made, however.

Firstly, the conscious, chronically-prepared preparation (Johnston et al., 1983a; 1983b; Curtis et al., 1984) has an advantage over acutely-prepared preparations (Clark et al., 1980; Kane et al., 1980; Lepran et al., 1983) as a result of the higher degree of precision of variables.

Secondly, and more importantly, some drugs (notably β-adrenoceptor antagonists and calcium antagonists) appear to possess quite different effects on arrhythmias in acutely-prepared versus chronically-prepared animals, as discussed above.

The reasons for the differences between acutely prepared and chronically prepared rats have not been elucidated. Various possibilities may be speculated, for example, differences in serum $K^+$ may alter both the incidence of arrhythmias and responses to drugs (see elsewhere). However, since the doses of drugs given to anaesthetised rats are generally much lower than those given to conscious rats, it is perhaps premature to propose a hypothesis to account for differences between acutely prepared and chronically prepared animals which is testable by merely surveying the literature.

Coronary occlusion in isolated rat hearts has been carried out for many years (Kannengiesser et al., 1975). There are, unfortunately, no definitive descriptions of this preparation, in terms of precision and reproducibility. It has recently been shown that PVCs peak at 14 - 16 min after occlusion in the isolated rat heart preparation (Daugherty et al., 1986). However, the time-course of VF in this preparation has not been adequately described. The incidence of arrhythmias in isolated rat hearts has been show to depend absolutely on the concentration of $K^+$ in the perfusion fluid (Daugherty et al., 1981).

4.1.4.1 Advantages and disadvantages of rats. The relative merit of ischaemia preparations using rats compared with other species has been considered in the Introduction. The rat is similar to other species in terms of sensitivity to electrical stimulation-induced and drug-induced arrhythmias (Winslow, 1984), including those induced by ouabain (Tepper et al., 1985; Wilson and Hewick, 1985). In summary, the advantages lie in:

a. the reproducibility and ease of production of ischaemia
b. the small size, low cost and ready availability
c. the large, well-defined data-base (Johnston et al., 1983a)
d. the large physiological data-base (Farris and Griffith, 1967; Petty, 1982).

Disadvantages of the preparation are equivocal.

a. The rat has a high resting heart rate. However, in conscious rats, heart rate does not correlate with arrhythmias (Johnston et al., 1983a) unlike the situation with dogs (Kaplinsky et al., 1981).

b. Ventricular action potentials in the rat myocardium are different from those found in many laboratory species in that they are of short duration with a narrow plateau phase (Langer, 1978; Inoue et al., 1984). However, the plateau phase of the ventricular action potential is governed in rats in a similar manner to that of other mammals (Reuter, 1979) by the slow inward current (Payet et al., 1980a; 1980b). The narrow action potential of the rat ventricle may relate to the fast heart rate, in that one may argue taeliologically that whereas high heart rate in small animals compensates for the low pumping efficiency of small hearts, a narrow action potential (and its associated low absolute refractory period) reduces the probability of the occurrence of unidirectional block and reentry in normal circumstances. How (or whether) such differences influence ischaemia-induced arrhythmias is unknown.

c. The rat has a higher rate of drug metabolism than larger animals. This is a disadvantage only in that dose regimens for humans cannot be determined directly from studies using rats.

## 4.2 Important determinants of arrhythmogenesis in acute myocardial ischaemia

### 4.2.1 Role of OZ size

According to previous studies in our laboratory, arrhythmias (incidence and duration) depend on the size of the ischaemic (occluded) zone such that arrhythmia score (AS) is linearly correlated with the square root of the OZ (Johnston et al., 1983a). The interpretation of this finding is that there

is a linear relationship between arrhythmias and the interface area between the ischaemic and normal tissue. It has been suggested from studies with pigs and dogs that this border zone is the site of arrhythmogenesis (Brofman et al., 1956; Beck, 1958; Janse et al., 1979; 1980). Indeed, global ischaemia, which is characterised by the absence of a border zone, is associated with comparatively few arrhythmias (Beck, 1958). This suggests that it is not ischaemia per se, rather the presence of both normal and ischaemic tissue which is necessary for arrhythmogenesis.

Regardless of the implications of the above relationship, OZ size data can be used to normalise arrhythmia scores and thereby reduce variance. The usefulness of such normalization has been tested in our laboratory by deliberately producing either large or small OZs and observing the associated arrhythmias (e.g., Curtis et al., 1984). In the present study with felodipine, arrhythmia scores for the large and small OZ control groups were almost identical after normalization for OZ (10.9 ± 2.5 and 9.1 ± 0.6, respectively) according to the $(AS + 1)/\sqrt{OZ}$ method (see Methods section). Correlations between incidences of major arrhythmias and OZ size are non-linear (Johnston et al., 1983a), therefore normalization is more complex. Sigmoid-shaped correlations have been shown for dogs (Austin, 1982).

Irrespective of the exact relationship between the amount of ischaemic tissue and the consequences of myocardial ischaemia, it nevertheless remains that some relationship must exist, therefore the OZ should be measured in all rats in order to verify that occlusion has taken place. The necessity for this has been emphasized by Bernauer (1982) who showed that post-occlusion survival depends on OZ size in rats. Bernauer (1980; 1982; 1983a; 1983b; 1985) corrects survival and ECG changes following occlusion for OZ size assuming a direct correlation. This may not be appropriate (Johnston et al., 1983a). However, irrespective of whether the sequelae of acute

myocardial ischaemia are proportional to the OZ or the square root of the OZ, it may not be necessary to correct variables for OZ size, provided the variance for OZ size is small. The results of extensive studies with rats in our laboratory suggest that this is the case (Johnston et al., 1983a; 1983b; Au et al., 1983; MacLeod et al., 1983; Jang et al., 1983; Curtis et al., 1984; etc.).

4.2.2 Role of the autonomic nervous system

Arrhythmogenesis following the onset of myocardial ischaemia is believed to result from disordered electrophysiology which in turn depends primarily on ischaemia. Secondary factors which may or may not act independently, namely the activity of the sympathetic system, heart rate and blood pressure are also suspected to be involved in arrhythmogenesis in acute myocardial ischaemia by some investigators (Harris, et al., 1951; Scherlag et al., 1970; Hope, et al., 1974; Fowliss, et al., 1974 Gillis et al., 1976; Hirche et al., 1980; Kaplinsky et al., 1981;).

Some of these factors have been investigated in rats subjected to coronary artery occlusion in our laboratory. Analysis of covariance in over 250 conscious rats showed no correlation between arrhythmias and blood pressure and/or heart rate (Johnston et al., 1983a). In addition, in conscious rats, propranolol (acute or chronic treatment), labetalol, and 6-OHDA plus adrenalectomy did not influence arrhythmias produced by coronary occlusion (Botting, et al., 1984). However, others, using conscious rats, found that chronic β-adrenoceptor antagonist treatment did reduce arrhythmias (Siegmund, et al., 1979).

In acutely prepared anaesthetised rats propranolol (Au, et al., 1983) practolol and pindolol (Kenedi and Losconci, 1973b) were found to have very weak antiarrhythmic activity. However, Campbell and Parratt (1983) demonstrated more powerful antiarrhythmic effects with β-adrenoceptor antagonists

in anaesthetised rats. In addition, β-receptor activation has been found to exacerbate arrhythmias in anaesthetised rats (Kenedi and Losconci, 1973a; Marshall, et al., 1981a). Therefore the arrhythmogenic role of β-adrenoceptor activation remains contentious, although it appears to be more important in anaesthetised rather than conscious rats.

In view of the contradictory evidence regarding the importance of the autonomic nervous system in arrhythmogenesis in the rat, a systematic investigation was carried out, using a series of ablations in the CNS combined, in some cases, with catecholamine infusions. The results of this study have been outlined and published (Curtis et al., 1985b).

It was found that ablations in the CNS greatly reduced arrhythmias following coronary occlusion in rats, almost abolishing the late arrhythmias (those occurring between 0.5 h and 4 h after occlusion). However, a simple relationship between the functional status of the CNS and the arrhythmia incidence was not demonstrated. When CNS integrity, and/or peripheral adrenoceptor activity was systematically altered in a graded manner the arrhythmias induced by occlusion were not similarly graded. According to blood pressure and heart rate data, sympathetic activity was relatively normal in the aS group (see Table 1), above normal in the aD group, and absent in aP and cP rats, whereas arrhythmias were reduced in all 4 groups. An infusion of noradrenaline and adrenaline sufficient to restore blood pressure and heart rate to normal in a group of pithed rats (aPN) did not restore arrhythmias. Likewise, catecholamine infusions in intact conscious chronically prepared rats (cCN) did not increase arrhythmias. When the CNS was obtunded by pentobarbitone anaesthesia (aB) there was no reduction in arrhythmias compared with conscious animals (aC).

In contrast with the lack of correlation between CNS ablations and arrhythmias and between adrenoceptor activation and arrhythmias, there

appeared to be an inverse relationship between the extent of acute surgery and arrhythmias. Possible mechanisms to account for this relationship are discussed in the sections concerned with the role of serum $K^+$, and the role of leukocytes and thrombocytes in arrhythmogenesis.

Regardless of the mechanisms by which acute surgery lowered the incidence of arrhythmias following coronary occlusion, the results of this study may have important implications regarding the suitability of acute myocardial ischaemia models in which acute surgical preparation is used. In this regard, even acutely prepared conscious (aC) and pentobarbitone anaesthetised (aB) rats had fewer episodes of VF and VT than conscious chronically prepared (cC) animals. Therefore larger group sizes would be required if acutely prepared animals were used for drug studies. In addition (as discussed previously) some antiarrhythmic drugs such as β-adrenoceptor antagonists and calcium antagonists produce different effects in acutely prepared versus chronically prepared animals. Why might β-adrenoceptor antagonists reduce arrhythmias only in acutely prepared animals? It is known that catecholamines reduce serum $K^+$ (Brown et al., 1983; Vincent et al., 1985) and that this effect is inhibited and reversed by β-adrenoceptor antagonists (Vincent et al., 1985). Therefore, β-adrenoceptor antagonists may reduce arrhythmias in acutely prepared rats by simply exacerbating the effect of acute surgery to raise serum $K^+$. This is because the incidence of arrhythmias during acute myocardial ischaemia appears to be critically dependent on serum $K^+$ (see section discussing the role of $K^+$ in arrhythmogenesis, below).

In summary, acute surgery appears to reduce arrhythmias (particularly those occurring between 0.5 h and 4 h) after coronary occlusion in a manner graded with the degree of surgery, and this effect is independent of, and little affected by, the extent of adrenoceptor activation. In contrast, the

functional status of the autonomic nervous system does not appear to influence ischaemia-induced arrhythmias in the rat.

4.2.3 Role of $K^+$

In contrast with the lack of correlation between CNS ablations, adrenoceptor activation and arrhythmias in the present studies, there appeared to be an inverse correlation between the extent of acute surgery and the arrhythmias produced by occlusion. If such a correlation exists, what might be the mechanism?

Elevations in serum $K^+$ are known to occur following acute surgery, and were observed in the CNS ablation study following pithing. Serum $K^+$ elevation may contribute to the results of this study. Elevation of serum $K^+$ is associated with a fall in the incidence of VF in patients with acute myocardial infarction (Nordrehaug and von der Lippe, 1983; Solomon, 1984). In isolated perfused hearts, elevation of the $K^+$ concentration of the perfusate drastically reduces arrhythmias induced by coronary occlusion (Lubbe et al., 1978; Daugherty et al., 1981). In the isolated heart group of the present CNS ablation study, a low incidence of arrhythmias was seen. This was not unexpected in view of the above, since the hearts were exposed to 5.3 mM $K^+$ in the perfusing solution. In addition, preliminary results of experiments (carried out by others in our laboratory) in which KCl infusion and other treatments were used to alter serum $K^+$, large changes in VF incidence were produced. The incidence of VF was 100%, 77%, 44% and 11% in groups of rats whose serum $K^+$ were in the ranges 3.0 - 3.9, 4.0 - 4.9, 5.0 - 5.9 and > 5.9 meq/l, respectively. Therefore, serum $K^+$ appears to govern the probability of the manifestation of arrhythmias during acute myocardial ischaemia, such that mild hyperkalaemia can almost abolish ventricular arrhythmias. If acute surgery elevates serum $K^+$, as was demonstrated in pithed rats, then the low incidence of arrhythmias is ex-

plained.

What might be the mechanism by which hyperkalaemia protects against ischaemia-induced arrhythmias? Occlusion in perfused rat hearts induces intracellular potential changes which include partial depolarization and action potential narrowing (Inoue et al., 1984). These ischaemia-induced effects may result in part from elevations in extracellular $K^+$ in ischaemic tissue, since the latter have been shown to occur at the same time as the electrophysiological changes which coincide with the onset of ventricular arrhythmias (Hirche et al., 1980; 1981; 1982; Hill and Gettes, 1980). In addition, many of the electrophysiological and electrocardiographic changes associated with acute myocardial ischaemia can be simulated by elevating extracellular $K^+$ in ventricles; such changes include slow conduction, uni-directional block and reentry (Schmitt and Erlanger, 1928), depolarisation and S-T segment elevation (Prinzmetal et al., 1961), slowly developing reductions in intracellular $Na^+$ concentration (Wild and Kleber, 1986) and slowly developing inexcitability (Kleber, 1986).

The electrophysiological disparity between ischaemic and normal tissue observed in several species (Cinca et al., 1980; Downar, 1977a; 1977b; Janse et al., 1980; Janse and Kleber, 1981; Janse, 1982; Kleber et al., 1978) including rats (Inoue et al., 1984) might then be expected to be reduced by elevations in serum $K^+$ concentration (which elevate extracellular $K^+$ concentration in the normal myocardium) since such an increase would be expected to cause the non-ischaemic myocardium to resemble the ischaemic tissue, electrophysiologically. Alternatively, or in addition, if the threshold for electrical excitation is raised by elevated serum $K^+$, the normal myocardium would be protected from invasion by aberrant (arrhythmogenic) impulses emanating from the ischaemic tissue. Preliminary results of studies with isolated ventricles showed that the strength-duration relation-

ship for excitation was shifted in the direction of reduced excitability by increased perfusate $K^+$ concentrations (see Figure 43).

4.2.4 Role of thrombocytes and leukocytes

An alternative explanation for the effects of surgery on arrhythmias produced by acute myocardial ischaemia relates to possible involvement of leukocytes and thrombocytes in arrhythmogenesis.

Possibly, acute surgical preparation and CNS destruction releases an antiarrhythmic substance into the circulation, or removes an arrhythmogenic substance from the circulation. In this regard, pithing was found to deplete circulating thrombocytes and leukocytes in the present studies. A fall in circulating thrombocytes following pithing has also been observed by others (Botting, personal communication to our laboratory). There have been many reports suggesting that interference with the properties of thrombocytes and leukocytes may reduce both ischaemia-induced myocardial tissue damage and also arrhythmias (Romson, et al., 1983; Mullane, et al., 1984; Fiedler, 1983; Fagbemi, 1984; 1985; Cahn and Borzeix, 1983; Coker and Parratt, 1981; 1983a; 1983b; Coker et al., 1981; Jolly and Lucchesi, 1983; Lucchesi, et al., 1983; Lepran, et al., 1981b; 1985). Therefore, leukocytes, and/or thrombocytes, may play an important role in governing arrhythmias and other responses of the myocardium to ischaemia.

If thrombocytes, and/or leukocytes, trapped within (Leinberger et al., 1979) or migrating to the ischaemic zone (Mullane et al., 1984) are partly responsible for arrhythmogenesis in acute myocardial ischaemia, particularly in the late (0.5 - 4 h) period, then it is possible that acute surgery produces tissue damage which acts as a trap for leukocytes, and/or thrombocytes, thereby reducing their availability for participating in ischaemia-induced arrhythmogenesis. Adjuvant arthritis, which may cause similar trapping, has been shown to reduce arrhythmias in conscious rats (Koltai,

et al., 1982). It is not yet clear by what mechanism leukocytes and/or thrombocytes influence arrhythmogenesis. However, it may be speculated that some component of the inflammatory process may be involved, arachidonic acid metabolites for example.

4.2.5 Role of arachidonic acid metabolites

The role of thromboxanes, prostaglandins and leukotrienes in arrhythmogenesis was not investigated in the current series of experiments, and will be discussed only in brief. Experiments carried out (in a variety of experimental preparations) with non-steroidal anti-inflammatory agents (Cahn and Borziex, 1983; Fagbemi, 1984; Coker and Parratt, 1981a; 1983a; 1983b; Coker et al., 1981; Coker, 1982; 1983; Lepran et al., 1981c; 1985; Fiedler, 1983) and arachidonic acid metabolites (Coker, 1983; Coker and Parratt, 1981; 1983a; 1983b; Martinez and Crampton, 1981) support the hypothesis that thromboxane is arrhythmogenic during acute myocardial ischaemia and that other prostaglandins may also play a role. However, our laboratory (Au et al., 1979a; 1980; 1983; Johnston et al., 1983b) has found that aspirin and a variety of prostaglandins have little influence on occlusion-induced arrhythmias. Once again, most of the positive reports originate from studies using acutely prepared anaesthetised animals, whereas the negative reports were generated from studies using conscious animals which had recovered from surgery.

4.2.6 Role of lipid metabolites

As in the case of arachidonic acid metabolites, the role of lipid metabolites in arrhythmogenesis was not investigated in the current studies. Long-chain acyl carnitines (for example palmitylcarnitine), and lysophospholipids (for example lysophosphatidylcholine) are amphipathic metabolites of unesterified free fatty acids (FFA) which may increase in concentration in myocytes during ischaemia, owing to limitations in aerobic oxidation of FFA

brought about by the lack of oxygen. Some of these compounds, by virtue of their ability to partition into the sarcolemma, are capable of producing electrophysiological changes, and even arrhythmias under some experimental conditions. In particular, palmitylcarnitine at high concentrations ($3 \times 10^{-4}$ M) can depolarise ventricular muscle, abbreviate refractoriness and action potential duration (Matsui et al., 1985), effects which all occur following coronary cocclusion during the period of early arrhythmias (e.g., Inoue et al., 1984). However, it is not established whether any of these compounds play a role in arrhythmogenesis (see Corr et al., 1984 for review).

4.2.7 Role of heart rate and blood pressure

In conscious rats, the frequency and incidence of occlusion-induced arrhythmias are not related to blood pressure or heart rate according to correlation matrix analysis (Johnston et al., 1983a). Correlations between heart rate or blood pressure and arrhythmias have been reported in larger species (e.g., Scherlag et al., 1970). With regard to the role of heart rate, it has been shown that vagal stimulation in anaesthetised rats does not unmask PVCs during the first 30 min after coronary occlusion, suggesting that it is more likely that early arrhythmias following occlusion in rats are reentrant rather than due to automaticity (Mertz and Kaplan, 1982). This data is consistent with the findings of studies using epicardial mapping techniques in isolated perfused pig hearts (Janse, 1982, etc.), and explains why heart rate would be expected to have little influence on the incidence of arrhythmias during acute myocardial ischaemia.

4.2.8 Role of the fast and slow inward currents

The possible mechanisms by which the slow inward current ($i_{si}$) may be involved in arrhythmogenesis in acute myocardial ischaemia were outlined in the Introduction. Both normal and abnormal cardiac rhythms are dependent on the characteristics of propagation of the myocardial action potential. This

is dependent on the behaviour of ionic conductances. If conduction in the ischaemic tissue could be abolished by selective inhibition of the currents responsible for such conduction then arrhythmias might be abolished. In this regard it has been suggested that chemical destruction of the ischaemic endocardium by local injection of phenol can abolish arrhythmias in dogs following coronary occlusion (Chilson et al., 1983).

As discussed in the introduction, myocardial ischaemia is characterised by depolarisation and a depression in the rise rate of the upstroke of the action potential. The consequence of this change is a slowing of conduction through the ischaemic tissue. Studies in vitro have shown that elevated $K^+$ concentrations in combination with adrenaline are capable of generating action potentials of which the depolarising current is $i_{si}$ (Carmeleit and Vereecke, 1972), but it is not clear how relevant this observation is to acute myocardial ischaemia. Theoretically, consideration of the relationship between the resting membrane potential and percentage of openable channels does not really help in determining which conductance is the most important in arrhythmogenesis, since resting membrane potential does not remain constant during acute myocardial ischaemia (Janse, 1982), and since depolarisation is not the only consequence of coronary occlusion which produces electrophysiological changes. For example, the fall in extracellular pH (Hirch et al., 1980) may alter both the kinetics of $i_{si}$ (Iijima et al., 1986) and the responses to drugs which interfere with $i_{si}$ in ventricular tissue (Briscoe and Smith, 1982). With regard to arrhythmogenesis in-vivo, it is unknown whether the depolarising inward current in ischaemic tissue is carried by a depressed $i_{Na}$, by $i_{si}$ or a combination of the two.

It is not technically possible at present to determine which conductances are responsible for arrhythmogenesis in acute myocardial ischaemia in

conscious animals (or humans) by electrophysiological methods. Nevertheless, there is abundant evidence that drugs which inhibit $i_{Na}$ (see above) and $i_{si}$ (see below) can reduce arrhythmias induced by coronary artery occlusion. Although this evidence is not definitive proof of an involvement of either of these conductances in arrhythmogenesis, it is highly suggestive. Pharmacological studies designed to rule out the involvement of subsidiary properties of these drugs will assist in removing confusion concerning mechanisms of arrhythmogenesis.

As a general comment, it is unlikely that arrhythmogenesis in acute myocardial ischaemia is governed by a myriad of biochemical, physiological and electrophysiological mechanisms. It is possible to hypothesise that abnormal conduction in acute myocardial ischaemia is dependent primarily on the fast and slow inward currents, and that many drugs which have been reported to be antiarrhythmic are so by virtue of inhibition of one or the other of these currents. If rats alone are considered, antiarrhythmic actions have been reported for d and l propranolol (Daugherty et al., 1986), antihistamines (Dai, 1984), meptazinol (Fagbemi et al., 1983) indomethacin (Fagbemi 1984), halothane (MacLeod et al., 1983) and other drugs, all of which can inhibit either the fast or slow inward current in addition to their 'major' actions. For example, d- and l-propranolol inhibit $i_{Na}$ in ventricular muscle (Pollen et al., 1969), SKF-93479 (an antihistamine), and meptazinol produced effects consistent with inhibition of $i_{Na}$ (Dai, 1984; Fagbemi et al., 1983), and indomethacin (Northover, 1977) and halothane (Lynch et al., 1981) have been reported possess calcium antagonist activity. In addition, quinidine, which is an effective antiarrhythmic in acute myocardial ischaemia (e.g., Johnston et al., 1983a) possesses calcium antagonist activity (Spedding, 1983) in addition to its well known ability to inhibit $i_{Na}$.

With regard to calcium antagonists and the role of $i_{si}$ in arrhythmogenesis, many of the current experiments were designed to examine the mechanism of antiarrhythmic action of calcium antagonists. The results support the hypothesis that $i_{si}$ is important in arrhythmogenesis (see below).

## 4.3 Mechanism of action of calcium antagonists in acute myocardial ischaemia

### 4.3.1 Role of calcium antagonism

4.3.1.1 Overview. (±)-Verapamil has been shown to be highly effective in reducing occlusion-induced arrhythmias in dogs (Kaumann and Aramendia, 1967; Kroll and Knight, 1984), rats (Johnston et al., 1983; Mertz and Kaplan, 1982; Bernauer 1982; Fagbemi et al., 1984) and pigs (Bergey et al., 1984). The mechanism of action of (±)-verapamil in these studies has not been proven. In the introduction it was explained why it is difficult to prove a mechanism of antiarrhythmic action in closed-chest experimental animals, or in the clinic. By studying activation maps in open-chest animals, it should be possible to test whether (±)-verapamil inhibits arrhythmia generation or maintainance. As yet, such studies have not been carried out. Nevertheless, even if (±)-verapamil were to be shown to inhibit, for example, reentry in the ischaemic tissue in association with the conversion of slow conduction to complete inexcitability, this would not prove that inhibition of $i_{si}$ was the mechanism of action unless it could be established that the abnormal conduction was dependent on $i_{si}$.

It is difficult (perhaps impossible, depending on one's philosphical approach) to establish a mechanism of action by experiment. It has been argued that one can only disprove a hypothesis by experiment, and that failure to disprove a hypothesis does not influence the probability of that hypothesis being correct. At present there is no convincing evidence to disprove that (±)-verapamil inhibits ischaemia-induced arrhythmias by inhib-

iting $i_{si}$. This consideration applies to other calcium antagonists such as anipamil.

Proponents of the hypothesis that calcium antagonists possess antiarrhythmic actions have developed a hypothetical concept to account for the reported antiarrhythmic actions of calcium antagonists, such as (±)-verapamil, called the modulated receptor hypothesis (see Hondeghem and Katzung, 1984) (this concept applies equally well to $i_{Na}$ blockers). This hypothesis essentially attempts to incorporate the voltage-dependence of the inhibitory actions of drugs such as quinidine on $i_{Na}$ (Weidmann, 1955b) and (±)-verapamil on $i_{si}$ (Ehara and Kaufmann, 1968) into a mass-action model by proposing that the affinity of a drug for a receptor associated with a conductance channel is dependent on whether the channel is rested, activated or inactivated. This model predicts, on the basis of relative voltage-dependence, that (±)-verapamil should selectively reduce high frequency arrhythmias (VT and VF) compared with PVC, and select between tissues on the basis of $E_m$ and APD90. The results of the present experiments are consistent with the former prediction, while the predicted selectivity of (±)-verapamil for tissue with a low (relatively positive) resting $E_m$ may account for the ability of (±)-verapamil to inhibit atrioventricular conduction at doses below those influencing cardiac output (selectivity for the 'depolarised' atrioventricular node versus the 'fully polarised' ventricles) (Singh 1975; Singh et al., 1978; Nayler and Horowitz, 1983; etc).

The sections below discuss the results of present studies with (+)-, (±)- and (-)-verapamil, anipamil, ronipamil, felodipine, nifedipine and DHM9, and attempt to explain the mechanism of action of calcium antagonists in acute myocardial ischaemia.

4.3.1.2 Anipamil versus ronipamil. Anipamil and ronipamil are analogues of verapamil. At present, little is known about the pharmacology

of these drugs. Preliminary evidence suggests that anipamil appears similar to verapamil in having calcium antagonist activity in the myocardium, whereas ronipamil has little activity (Raschack, 1984; Kretzschmar and Raschack, 1984; Kretzschmar, personal communication to the laboratory), in accordance with the known structural requirements of verapamil analogues for calcium antagonism (Mannhold et al., 1978; 1981). The evidence suggests that anipamil is a calcium antagonist with selectivity for the myocardium versus the vasculature, whereas ronipamil appears to be relatively inactive. Most calcium antagonists including (±)-verapamil have selectivity for vascular smooth-muscle versus the myocardium and produce profound hypotension at doses below those affecting $i_{si}$-dependent processes in the heart (Briscoe and Smith, 1982; Millard et al., 1983; Lee et al., 1983; Van Zwieten and Timmermans, 1983; Kenakin and Beek, 1985; etc.). However, anipamil may be different in that it appears to reduce cardiac output at doses below those influencing systemic blood-flow.

Anipamil and ronipamil were developed as verapamil analogues with a long duration of action. The lipophilicity conferred by the straight 10 C-chain extension on C-9 may be responsible for this. Both compounds have been demonstrated to delay the depletion of myocardial enzymes in rat hearts induced by a period of hypoxia (Kretzschmar and Raschack, 1984; Raschack, 1984). In other tests similar anti-ischaemic or anti-hypoxic actions have been observed (Kovach, 1984; Ferrari et al., 1984; Urbanics and Kovach, 1984).

In agreement with these previously reported actions, it was found in the present studies that both anipamil and ronipamil moderated the ischaemia induced by occlusion in terms of reductions in the maximum values of S-T segment elevation. Anipamil also delayed the development of S-T segment elevation and R-wave changes. However, there were no differences in OZ size

between the groups. Therefore differences in the patterns of ECG changes associated with drug treatment appeared to reflect actions in the ischaemic tissue, rather than actions to limit the size of the zone of ischaemia.

Regardless of possible anti-ischaemic actions, it was apparent that only anipamil possessed significant antiarrhythmic activity. Anipamil appeared to be a selective antiarrhythmic against VF compared with both VT and PVC. This result does not support the concept of an arrhythmia continuum whereby PVCs 'cause' VT, and VT causes or 'degenerates to' VF (e.g., Harris et al., 1950). Anipamil 150 mg/kg p.o. reduced the incidence of VF from 9/9 in controls to 2/9. With respect to such a specific antifibrillatory action, it has been suggested by our laboratory (Curtis et al., 1984) that a selective antifibrillatory drug would have frequency-dependence leading to electrophysiologic actions only at high frequencies (fibrillation). This property can also be described in terms of a selectivity for the inactivated state of the $i_{si}$ channel according to the modulated receptor hypothesis (Hondeghem and Katzung, 1984). A description of the frequency-dependence of the action of anipamil on ventricular tissue is not, at present, available.

Neither ronipamil nor anipamil increased the incidence of cardiogenic shock in the present study. However, 20 mg/kg i.v. (±)-verapamil, in an earlier study from our laboratory (Curtis et al., 1984) caused cardiogenic shock. It was of interest, therefore, to compare anipamil with (±)-verapamil for antiarrhythmic versus cardiovascular activity. In this regard, anipamil reduced VF by about 50% and 80 % at 50 and 150 mg/kg, respectively, doses which reduced blood pressure just before occlusion by only 7% and 5%, respectively. In contrast, (±)-verapamil, at a dose reducing the incidence of VF by 50%, reduced blood pressure by 23%. Thus, when one considers the haemodynamic and antiarrhythmic effects in tandem, it follows that the therapeutic ratio of anipamil is higher than that of (±)-verapamil.

With regard to the antiarrhythmic actions of anipamil versus ronipamil in relation to their pharmacological properties, the major known difference between them appears to be in their ability to reduce cardiac output (anipamil being much more potent in this respect than ronipamil). Kretzschmar (personal communication to the laboratory) has suggested that the effect of anipamil to lower cardiac output in dogs can be reversed by the injection of bolus doses of $Ca^{2+}$. Therefore, it is tentatively concluded from this study that the antiarrhythmic actions of anipamil may be dependent on calcium antagonist activity. A more firm conclusion can be made with regard to the relationship between the ECG signs of ischaemia and arrhythmias. There does not appear to be a linear correlation between these variables, since anipamil reduced maximum S-T segment elevation to the same degree as ronipamil, but was much more effective than ronipamil as an antiarrhythmic. Therefore the mechanisms responsible for the ECG signs of ischaemia and those responsible for arrhythmogenesis may different.

4.3.1.3 (+)- Versus (-)-verapamil. Before considering the actions of (+)- and (-)-verapamil following coronary occlusion in conscious rats, it is of interest to consider their calcium antagonist potencies in terms of effects on heart rate and blood pressure *in vivo* in conscious rats (from the occlusion study) and pithed rats, and on left ventricular developed pressure *in vitro*. These effects probably depend on inhibition of the transmembrane flux of $Ca^{2+}$ (Nayler and Horowitz, 1983).

Without exception, (-)-verapamil was the more potent enantiomer. However, both the absolute and relative potencies of the enantiomers varied from one test situation to another. In conscious rats the (-):(+) potency ratios were the lowest. In pithed rats, higher potency ratios were observed, while absolute potencies were also increased. In isolated ventricles both the absolute potencies and the (-):(+) potency ratios were highly

dependent on $K^+$ concentration.

The 'true' potency ratio is best estimated from studies *in vitro*. Therefore, deviation *in vivo* from values measured *in vitro* probably result from the ability of the body to 'filter' the drug. The present results suggest that the calcium antagonist potency of the verapamil enantiomers is governed, *in vivo*, by at least 3 factors, namely stereoselective pharmacokinetics, sympathetic tone and ambient $K^+$ concentration.

In conscious humans the potency of (-)-verapamil is reduced compared with that of (+)-verapamil as a result of stereoselective hepatic metabolism (Vogelgesang *et al.*, 1984; Eichelbaum *et al.*, 1984). The low potency ratio of (-)- to (+)-verapamil in conscious rats is consistent with stereoselective metabolism. In pithed rats it is possible that the greater difference in potency between the enantiomers (compared with that found in conscious rats) results from a reduction in hepatic blood flow in pithed rats, serving to limit the influence of stereoselective metabolism.

There is evidence of a 'functional' antagonism (i.e., not competitive) between (±)-verapamil and the sympathetic nervous system (Tung *et al.*, 1985). The enhanced absolute potencies of both enantiomers in pithed versus conscious rats may therefore result from the lack of sympathetic reflexes in pithed compared with conscious rats.

In isolated ventricles, where potential influences of pharmacokinetic factors and the sympathetic nervous system are removed, an important influence of extracellular $K^+$ on potency was revealed. The mechanism by which elevation of $K^+$ increased the calcium antagonist potency of the enantiomers while reducing the difference in potency between them is uncertain (see below).

The results have implications regarding the hypothetical role of calcium antagonism in governing the antiarrhythmic action of (±)-verapamil during

acute myocardial ischaemia. Firstly, extracellular $K^+$ is known to rise (within min) to 8 - 15 meq/l in the ischaemic tissue (Hirche et al., 1980; Hill and Gettes, 1980). Therefore, according to the hypothesis that calcium antagonists reduce ischaemia-induced arrhythmias as a result of calcium antagonism in the ventricles, (-)-verapamil should be no more than 8 times as antiarrhythmic as (+)-verapamil if, as the present experiments suggest, extracellular $K^+$ concentration governs the calcium antagonist potency of phenethylalkylamines at the cellular level in the ischaemic ventricular tissue. Secondly, stereoselective hepatic metabolism may occur in conscious rats as it does in humans (Vogelgesang et al., 1984; Eichelbaum et al., 1984), in which case the antiarrhythmic potency ratio would be expected to be less than 8.

The actions of the enantiomers of verapamil during acute myocardial ischaemia were first examined using a simple new conscious rat preparation. The end-points used were morbidity, death, OZ and IZ size. Death was categorized according to the behaviour pattern which accompanied it. In separate experiments it was established that convulsive-type behaviour always and only occurred after a minimum of 10 sec of VF or VT, and that morbidity was associated with hypotension and cardiogenic shock. The preparation was devised on the basis of considerable experience with coronary-occluded conscious rats, and was designed to provide information concerning the antifibrillatory and infarct-reducing actions of drugs rapidly and simply.

The results of this study were in agreement with those reported previously by the laboratory for (±)-verapamil (Curtis et al., 1984). The incidence of convulsive-type behaviour (indicative of VF) during the first 4 h after occlusion in the present study was reduced from 84 to 43 % by 6 mg/kg (±)-verapamil. In the previous study the incidence of VF was reduced from 89 to 45% by the same dose (Curtis et al., 1984). Mortality not associated

with convulsive-type behaviour was increased from 8 to 12% in the present study. This increase compares favourably with the increase from 11 to 22% found in the previous study (Curtis et al., 1984).

The order of effectiveness of the treatments (i.e., - > ± > +) in reducing behaviour indicative of VF suggests that calcium antagonism, rather than sodium channel blockade, accounted for the antiarrhythmic action. This is because (-)-verapamil is more potent than (+)-verapamil as a calcium antagonist (Bayer et al., 1975b; 1975c; Raschach, 1976; Nawrath et al., 1981; Gloor and Urthaler, 1983; Ferry et al., 1985; Echizen et al., 1985) whereas the isomers are equipotent as sodium channel blockers (Nawrath et al., 1981). The order of effectiveness (- > ± > + > saline) of the treatments in producing morbidity and cardiovascular depression also corresponded with the calcium antagonist potency order. However, since this initial study used only single doses it would be premature to rule out other possible mechanisms of action in the absence of full dose-response data.

We found that (+), (±)- and (-)-verapamil did not reduce infarct size. This agrees with previous findings in rats for (±)-verapamil (Baur et al., 1984; Evans et al., 1985), including those from our laboratory (Curtis et al., 1984). Since rats have few effective collaterals, drug-induced reductions in infarct size may not be possible in this species (Schaper 1984; Hearse et al., 1986), as discussed previously.

In summary, (±)-verapamil was more effective than (+)-verapamil and less effective than (-)-verapamil in reducing behaviour consistent with VF following permanent coronary artery occlusion in conscious rats. The order of potency corresponded qualitatively with the calcium antagonist potency order. Infarct size was not reduced.

Following this preliminary investigation with the enantiomers of verapamil, an extensive dose-response study was carried out, in which blood

pressure and the ECG were monitored in the usual manner. In these 'fully-instrumented' conscious rats, both (+)- and (-)-verapamil possessed dose-dependent antiarrhythmic activity against coronary occlusion-induced arrhythmias. VT and VF appeared to be reduced more effectively than PVC. (-)-Verapamil was consistently 4 times more potent in this respect than (+)-verapamil. The antiarrhythmic potency ratio corresponded with that for effects on heart rate and blood pressure seen immediately before (but not after) coronary occlusion. The potency ratio for the antiarrhythmic actions corresponded reasonably well with recent estimates of the calcium antagonist potency ratio for the enantiomers in ventricular muscle, based on pharmacological and radioligand binding studies (Ferry et al., 1985).

In order to supplement data from the literature concerning the relative potencies of the optical enantiomers of verapamil as calcium antagonists, experiments were carried out using isolated rat ventricles and pithed rats (as discussed above). In isolated rat ventricles (-)-verapamil was more potent than the (+)-verapamil, and the difference in potency was dependent on the $K^+$ concentration in the perfusing buffer. At high $K^+$ concentrations, the relative potency of the enantiomers resembled the antiarrhythmic potency following coronary occlusion. The antiarrhythmic potency ratio also corresponded with the potency ratio for effects on blood pressure and heart rate in vivo, reductions in which presumably occurred as a result of calcium antagonism (Nayler and Horowitz, 1983).

We consider the above to support the hypothesis that the antiarrhythmic actions of both enantiomers during acute myocardial ischaemia occurred by virtue of calcium antagonism, rather than via quinidine-like $Na^+$ channel blockade, a property which is shared equally by both enantiomers and which is only manifest at concentrations in excess of those required to abolish $i_{si}$ (Nawrath et al., 1981). The possible role of $Na^+$ channel blockade

in determining the antiarrhythmic activity of the enantiomers is discussed further, below. In support of the present conclusion is the experiment of Kaumann and Serur (1975) in which it was found that both enantiomers of verapamil reduced arrhythmias induced by acute coronary occlusion in dogs, and that (-)-verapamil appeared to be the more potent enantiomer (the group size was described by the Authors as being too small for a meaningful assessment of potency differences between the enantiomers, however).

The experiments carried out in pithed rats were interesting, but they did not assist in supporting or disproving the hypothesis that (±)-verapamil reduces ischaemia-induced arrhythmias by virtue of calcium antagonism. This was because the sensitivity of the pithed rats to the effects of the enantiomers was greatly enhanced compared with conscious rats, making interpretation of the results difficult. The increased sensitivity of pithed rats to the enantiomers compared with conscious rats suggests that the actions of the enantiomers are offset in conscious animals by the sympathetic nervous system. However, since the potency ratio of (-)- to (+)-verapamil in pithed rats for effects on blood pressure was not very different from the corresponding potency ratio in conscious rats, then it appears that if the sympathetic nervous system does offset the effects of the enantiomers in conscious rats then this effect is not selective for one enantiomer versus the other. With regard to the effects of the sympathetic nervous system on responses to calcium antagonists, it has recently been suggested that a similar effect occurs to limit the effects of some 1,4-dihydropyridine calcium antagonists in conscious animals (Chelly et al., 1985).

There were 2 differences between the present occlusion study with the optical enantiomers of verapamil and the previous study with (±)-verapamil from our laboratory (Curtis et al., 1984). Firstly, in the present study arrhythmia-induced mortality was abolished by improving the defibrillation

technique, allowing for improved precision of variables (the previous control incidence of VF-induced death was 30% ). Secondly, the incidence of mortality associated with cardiogenic shock was lower in the present study. In the previous study such deaths mainly occurred with 20 mg/kg (±)-verapamil, and the doses used in the present study were lower. These small differences appear to be unimportant since, if the $ED_{50}$ for (±)-verapamil (determined from the previous study) during the 0-30 min period (6 mg/kg) is compared with that for (-)-verapamil (2 mg/kg) and (+)-verapamil (10 mg/kg), it is apparent that the order of potency (- > ± > +) corresponds with that expected if calcium antagonism alone, rather than $Na^+$ channel blockade alone, or a combination of the 2 properties was responsible for the antiarrhythmic actions observed.

In summary, the antiarrhythmic actions of the optical enantiomers of verapamil during myocardial ischaemia corresponded with their calcium antagonist potency ratios in isolated ventricles under conditions of raised $K^+$. In addition, (±)-verapamil was found to distribute into the ischaemic tissue, and appeared to accumulate there. It is not possible to estimate the proportion of drug available at this site for effects (much may have been bound 'non-specifically' to the tissue). However, the current experiments did establish the presence of (+)-verapamil in the ischaemic tissue. Therefore, calcium antagonism in the ischaemic ventricular myocardium appears to be the most likely explanation for the antiarrhythmic actions of (±)-verapamil in acute myocardial ischaemia. However, alternative possibilities exist, and these are discussed in subsequent chapters.

4.3.1.4 Felodipine, Nifedipine and DHM9. Nifedipine is the prototype 1,4-dihydropyridine calcium antagonist (Fleckenstein et al., 1972; Kohlhardt and Fleckenstein, 1977). Nifedipine inhibits $i_{si}$ in ventricular muscle (Kohlhardt and Fleckenstein, 1977) in a manner which is essentially

independent of stimulation frequency according to most (Bayer et al., 1977; Hachisu and Pappano, 1983) but not all (Woods and West, 1983) studies. In this regard, nifedipine differs from (±)-verapamil, which shows marked frequency-dependence (Sanguinetti and West, 1982; Hachisu and Pappano, 1983). In addition, nifedipine does not appear to affect the inward sodium current in ventricular tissue (Bayer et al., 1977), unlike (±)-verapamil which can block this current at high concentrations in vitro (Bayer et al., 1975a; Nawrath et al., 1981).

DHM9 is a metabolite of an analogue of nifedipine (nicardipine) which, in contrast with both phenethylalkylamines such as verapamil and 1,4-dihydropyridines such as nifedipine, appears to inhibit $i_{si}$-dependent events selectively in ventricular muscle versus vascular smooth muscle (Clark et al., 1984b), although it is in fact less potent than both nifedipine and verapamil in ventricular tissue.

Felodipine is a 1,4-dihydropyridine vasodilator with structural similarity to nifedipine. Felodipine interacts with calmodulin (Bostrom et al., 1984), and has been reported to increase calcium uptake by the sarcoplasmic reticulum (Movsesian et al., 1984). However, even at high concentrations ($0.8 \times 10^{-4}$M), felodipine does not inhibit calmodulin-dependent calcium-stimulated contraction in chemically skinned rabbit renal arteries (Kreye et al., 1983). It appears from the above that felodipine dilates smooth muscle via an action on the plasma membrane. Owing to the close structural similarities between felodipine and 1,4-dihydropyridines such as nifedipine, nisoldipine, niludipine and PY-108-068, it seems likely that the major pharmacological actions of felodipine, which are shared with other 1,4-dihydropyridines, occur as a result of calcium antagonism. In this regard, felodipine has been shown to possess calcium antagonist activity in rat aortic strips, portal veins, atria and ventricles (Au and Sutter, 1984). In

ventricles, and $K^+$ depolarized vessels, felodipine resembled nifedipine more than (±)-verapamil (Au and Sutter, 1984).

In view of the above, the effects of felodipine, nifedipine and DHM9 were evaluated in conscious rats subjected to coronary artery occlusion, in order to test the hypothesis that calcium antagonism in ventricular muscle is responsible for the antiarrhythmic actions of calcium antagonists. None of these drugs would be expected to inhibit $i_{si}$ in ventricular tissue in vivo at doses producing moderate reductions in blood pressure, owing to their vascular selectivity (nifedipine and felodipine) or low potency (DHM9).

In the earlier study (Curtis et al., 1984) with (±)-verapamil, it was found that by deliberately producing a smaller area of ischaemia, the antiarrhythmic actions of high doses of (±)-verapamil were more clearly demonstrated, since fewer animals died from cardiogenic shock. Since 1,4-dihydropyridines are even more selective systemic vasodilators than (±)-verapamil (Van Zwieten and Timmermans, 1983; Briscoe and Smith, 1982), the technique of production of large and small OZs was used again in order to offset possible cardiogenic shock of felodipine. Since cardiogenic shock was not, in fact, found to be a problem with felodipine, the subsequent studies with nifedipine and DHM9 were carried out using large OZ rats only.

Felodipine, at doses which produced profound hypotension in conscious rats, possessed only weak antiarrhythmic actions following coronary occlusion. These actions were really only apparent in rats with small OZs (SOZ). Our results are not entirely in agreement with those of Verdouw and Wolffenbuttel (1983), who showed that 10 nmol/kg felodipine abolished VF during the 10 min period immediately following coronary occlusion in pigs. However, these Authors found that after a 20 min period of reperfusion, a second 10 min occlusion produced fatal VF in 5/9 pigs, which was not different from the control incidence. The size of the OZ was not determined in

the above study (Verdouw and Wolffenbuttel, 1983); since we demonstrated in the present study a small antifibrillatory action of felodipine in rats with small OZs, the difference between this, and the present study may relate to the size of the ischaemic area produced. An alternative explanation, also relating to the extent of ischaemia, is provided by the observation that felodipine markedly elevates blood flow in the border of the ischaemic zone in pigs (Sjoquist et al., 1983). In the latter study (Sjoquist et al., 1983) the extent of subsequent infarction was unfortunately not determined; an increase in blood flow to the border of the ischaemic tissue implies a potential degree of salvage. In the present study we were able to demonstrate a small degree of myocardial salvage with felopine (reduction in IZ as a percentage of OZ). However, this action was not dose-related and only occurred in one group of small OZ rats. In addition, since rats, like pigs, do not possess a well developed collateral circulation (Johns and Olson, 1984; Selye et al., 1960; Schaper 1983; Hearse 1983; Verdouw et al., 1983c; Maxwell et al., 1984; Winkler et al., 1984) it seems unlikely that an increase in blood flow to the ischaemic tissue could produce a sufficient reduction in the size of the ischaemic area to greatly reduce arrhythmias.

The present work has secondary implications, in that secondary properties of felodipine such as its ability to reduce afterload (Verdouw et al., 1983a), to bind with calmodulin (Bostrum et al., 1981), to inhibit calmodulin-sensitive phosphodiesterase activity (Norman et al., 1983), to interfere with calcium uptake by the sarcoplasmic reticulum (Movsesian et al., 1984) and to influence calcium-ATPase (Wang et al., 1984), which may or may not occur over the dose range studied here, do not appear to confer antiarrhythmic activity following occlusion-induced myocardial ischaemia in conscious rats.

Nifedipine and DHM9 were both without antiarrhythmic activity. In the

case of DHM9, this was not unexpected, since this drug was without pharmacological activity of any kind, both in conscious rats and in isolated perfused rat ventricles at up to $3 \times 10^{-5}$ M. Nifedipine produced dose-dependent reductions in blood pressure (as did anipamil, (+)- and (-)-verapamil in the previously-described studies), but increased heart rate and shortened P-R interval, effects presumably mediated by sympathetic reflexes. These results show that the 1,4-dihydropyridines did not appear to have any direct effects on the electrophysiology of the heart.

In summary, the 1,4-dihydropyridine calcium antagonists did not reduce occlusion-induced arrhythmias, nor did they prolong P-R interval, cause atrioventricular block or slow heart rate. These actions contrast with the actions of (+)- and (-)-verapamil and anipamil (the significance of which is discussed below).

4.3.2 Role of calcium antagonism in the myocardium

By integrating the results of the antiarrhythmic studies with the pharmacological profile of the 1,4-dihydropyridine and phenethylalkylamine calcium antagonists, it is possible to suggest explanations, both for the difference in antiarrhythmic activity of the two classes of drugs, and also for the site of action of the phenethylalkylamines. Phenethylalkylamine calcium antagonists (anipamil and the optical enantiomers of verapamil) were found to possess antiarrhythmic activity in the present studies. These results are consistent with previous findings from our laboratory concerning the antiarrhythmic activity of (±)-verapamil, (Johnston et al., 1983a; Curtis et al., 1984). Ronipamil (a close structural analogue of verapamil and anipamil without calcium antagonist activity) had no antiarrhythmic activity. (±)-Verapamil may have antiarrhythmic activity in anaesthetised rats (Fagbemi et al., 1984), and has been shown to possess marked antifibrillatory activity in dogs (Kaumann and Aramendia, 1968; Bren et al., 1982)

and pigs (Kroll and Knight, 1984; Bergey et al., 1984) subjected to coronary artery occlusion. It has also been suggested that (-)-verapamil is more potent than (+)-verapamil as an antiarrhythmic in anaesthetised dogs (Kaumann and Serur, 1975).

With regard to 1,4-dihydropyridine calcium antagonists, nifedipine, nisoldipine and niludipine have been reported to abolish VF in rats (Fagbemi and Parratt, 1981) but not pigs (Bergey et al., 1984) following coronary artery occlusion. Dihydropyridine calcium antagonists, until now, have not been evaluated in conscious rats following coronary occlusion, and felodipine has not been tested in conscious or anaesthetised rats. Felodipine and nifedipine were evaluated for antiarrhythmic activity in order to assess whether relatively vascular selective calcium antagonists possess antiarrhythmic activity in acute myocardial ischaemia in conscious rats. DHM9 was studied on the basis of its unusual property of myocardial selectivity compared with other 1,4-dihydropyridines; however, the results suggest that DHM9 is in fact completely inactive as a calcium antagonist, at concentrations < 30 μM.

The lack of antiarrhythmic action of felodipine, nifedipine, ronipamil and DHM9 compared with anipamil and the verapamil enantiomers was predicted from the hypothesis that calcium antagonists reduce ischaemia-induced arrhythmias via an action in the ventricles. If the actions of the 1,4-dihydropyridine calcium antagonists are compared with those of the phenethylalkylamines, some notable differences, which exemplify the above statement, may be seen.

Firstly, the antiarrhythmic phenethylalkylamines enhanced the fall in blood pressure produced by occlusion, whereas the 1,4-dihydropyridines did not. Secondly, the 1,4-dihydropyridines, unlike high doses of (±)-verapamil (Curtis et al., 1984) did not increase the number of deaths attributable to

cardiogenic shock. Thirdly, all the antiarrhythmic phenethylalkylamines reduced pre-occlusion heart rate at doses which subsequently reduced arrhythmias, whereas the 1,4-dihydropyridines all increased heart rate. Finally, (+)- and (-)-verapamil caused dose-dependent P-R prolongation at doses which reduced arrhythmias, whereas nifedipine caused dose-dependent shortening of P-R interval. Therefore, anipamil and the verapamil enantiomers produced definite actions attributable to direct effects in the heart, and reduced arrhythmias, whereas the 1,4-dihydropyridines appeared to have no direct actions in the heart, and did not reduce arrhythmias.

It is clear that 1,4-dihydropyridine calcium antagonists possess a greater selectivity for the systemic vasculature versus the ventricular myocardium than phenethylalkylamines. Several investigators have directly compared the potencies of nifedipine and (±)-verapamil in vascular smooth muscle and cardiac tissue (Fleckenstein-Grun, et al., 1976; Raschach, 1976a; Nabata, 1976; Briscoe and Smith, 1982; Winslow et al., 1983; Millard et al., 1983; Lee et al., 1983; Clarke et al.; 1984a; 1985; Kenakin and Beek, 1985; Nakayama et al., 1985). By comparing these studies it is possible to calculate mean potency differences in vascular and cardiac tissue for nifedipine and (±)-verapamil. It was calculated that nifedipine is approximately 43 times as potent as (±)-verapamil as a calcium antagonist in vascular smooth muscle, whereas nifedipine is only 10 times as potent as (±)-verapamil in cardiac tissue. According to the hypothesis that calcium antagonists reduce ischaemia-induced arrhythmias via calcium antagonism in the ventricles, the following predictions are made on the basis of the calculated potency ratios. Firstly, it was found previously that the antiarrhythmic $ED_{50}$ for (±)-verapamil was 6 mg/kg (Curtis et al., 1984). Therefore, the antiarrhythmic $ED_{50}$ for nifedipine should be 0.1 times this dose: 0.6 mg/kg. However, in terms of actions in vascular smooth muscle, 0.6 mg/kg

nifedipine is equivalent to 0.6 x 43 = 26 mg/kg (±)-verapamil. This dose, 26 mg/kg of (±)-verapamil cannot be tolerated in conscious rats following coronary occlusion. Indeed, in the earlier study (Curtis et al., 1984) 20 mg/kg of (±)-verapamil was found to cause cardiogenic shock in 6/9 rats within 30 min of occlusion. Therefore these calculations predict that nifedipine should not be capable of reducing arrhythmias in-vivo, owing to its selectivity for vascular smooth muscle.

These calculations were based on the assumption that nifedipine and (±)-verapamil are pharmacologically equivalent. This is probably not the case; differences exist, for example, in frequency-dependence (see below). Differences in pharmacokinetics are also apparent. The higher degree of plasma-protein binding with nifedipine (Hermann and Morselli, 1985) compared with (±)-verapamil would be expected to reduce the apparent potency of nifedipine relative to (±)-verapamil in-vivo. These factors may account for the fact that doses exceeding 0.6 mg/kg nifedipine could be given to conscious rats without adverse (or antiarrhythmic) effect.

If felodipine resembles nifedipine in terms of calcium antagonism and relative tissue selectivity, then it is clear why felodipine, also, had little antiarrhythmic activity; sufficient concentrations of felodipine to affect calcium currents in the ventricles were probably not achieved. In common with nifedipine, doses of felodipine sufficient to affect the ventricular myocardium probably greatly exceed those given in the present studies.

There are additional factors to consider with regard to the difference between 1,4-dihydropyridines and phenethylalkylamines as antiarrhythmics. Of particular interest is frequency-dependence. Nifedipine has been reported to possess little (Woods and West, 1983; 1985) if any (Kohlhardt and Fleckenstein, 1977; Hachisu and Pappano, 1983) frequency-dependence in

cardiac tissues. However, phenethylalkylamine calcium antagonists possess marked frequency-dependence (Ehara and Kaufmann, 1978; McDonald et al., 1980; Sanguinetti and West, 1982; Woods and West, 1985). If phenethylalkylamines possesses pronounced frequency-dependence such that calcium antagonist activity is potentiated at fast heart rates, but 1,4-dihydropyridines do not, this may partly account for the relative lack of antifibrillatory activity of 1,4-dihydropyridines versus phenethylalkylamines, as has been suggested previously (Woods and West, 1985).

With regard to the relationship between frequency-dependence and antiarrhythmic activity, it is worthwhile to consider the possible mechanisms by which frequency-dependence operates.

Frequency-dependence in heart tissues can refer to actions on $i_{si}$ and actions on force of contraction. It is not clear to what extent frequency-dependent reductions in $i_{si}$ (McDonald et al., 1980) correlate with frequency-dependent inhibitions of inotropism (Mannhold et al., 1981). However, it is clear that frequency-dependent negative inotropic actions of phenethylalkylamines are not due to depletion of intracellular $Ca^{2+}$ or non-specific myocyte fatigue (Bayer and Ehara, 1979) since the effects of alterations in stimulation frequency are rapidly reversible on resumption of control stimulation frequency.

In attempting to understand the mechanism of frequency-dependence, it is important to consider the phenomenon of voltage-dependence (Weidmann, 1955a). It is possible that frequency-dependence and voltage-dependence are manifestations of the same phenomenon, namely an effect of depolarisation to alter the apparant affinity of a drug for its receptor. This may occur via an increase in the probability of the $i_{si}$ (or $i_{Na}$) channel being in the open or inactivated state (Lee and Tsien, 1983), whereby the affinity of certain drugs for the channel increases (Hondeghem and Katzung, 1984). This

is the 'modulated receptor hypothesis' which predicts that the affinity of a drug for the receptor via which it interferes with channel function is voltage-dependent. However, the modulated receptor hypothesis does not adequately account for the observation that a drug which exhibits voltage-dependent behaviour may not necessarily exhibit frequency-dependent behaviour. Although most studies suggest that 1,4-dihydropyridines do not possess marked frequency-dependent behaviour, (Hachisu and Pappano, 1983; Kohlhardt and Fleckenstein, 1977), it has recently been shown that nifedipine shows marked voltage-dependent behaviour, inhibiting $i_{si}$ with an $IC_{50}$ of 20 nM at -40 mV holding potential, and an $IC_{50}$ of 700 nM at -50 mV, and producing negative inotropism in ventricular tissue with an $IC_{50}$ of 480 nM at 5.9 meq/l $K^+$ and an $IC_{50}$ of 0.63 nM at 37 meq/l $K^+$ (Holck and Osterrieder, 1985).

An alternative hypothesis, 'the guarded receptor hypothesis' of Starmer et al. (1984) suggests that the affinity of a channel-modulating drug for its receptor is constant, whereas access to the receptor is voltage-dependent and determined by the gating mechanism of the channel. The latter hypothesis appears to be flawed, however, because it predicts that the rate of change of apparant affinity of a drug for its receptor is independent of the drug and the receptor, and is constant. The fact that (±)-verapamil exhibits frequency- and voltage-dependence but nifedipine only exhibits voltage-dependence appears to disprove the guarded receptor hypothesis.

The difference between the results of frequency-dependence and voltage-dependence studies with nifedipine may be explained by invoking a modulating influence which restricts the access of nifedipine to its receptor in or adjacent to the $i_{si}$ channel, but which is independent of the gating of the channel. If sustained depolarisation removes this modulating influence, whereas brief depolarisation does not, then the difference between the

effects of changing frequency compared with changing resting membrane potential on responses to nifedipine can be explained. This explanation is contingent on the presence of separate receptors for 1,4-dihydropyridines and phenethylalkylamines. It is possible to speculate that the modulating influence may simply represent a voltage-dependent interaction between the drug and its receptor, whereby depolarisation causes the affinity of the receptor for the ligand to increase, but slowly. This hypothesis side-steps the modulated receptor hypothesis in that voltage directly modulates the drug receptor, independently of whether the channel is rested, open or inactivated. If verapamil and nifedipine interact with different receptors within or in association with the $i_{si}$ channel, then the most simple explanation for the difference between nifedipine and verapamil in their frequency- and voltage-dependent properties is that the affinity of the verapamil receptor for verapamil changes rapidly (time constant of msec) with depolarisation, whereas the affinity of nifedipine for its receptor changes slowly (time constant of sec) with depolarisation. The presence of different receptors for verapamil and nifedipine is supported by extensive radioligand binding studies (Glossman et al., 1985) and some pharmacological evidence (Spedding and Berg, 1984).

There is a limited amount of evidence which suggests that the frequency-dependence of verapamil is stereoselective. Bayer and Ehara (1979) found that whereas the negative inotropic effects of (-)-verapamil were enhanced by increasing stimulation frequency from 6 to 60 per min, the negative inotropic effects of (+)-verapamil were inhibited. Although it is difficult to suggest an explanation for this which is consistent with all that is known concerning the pharmacology of the enantiomers, this observation may partly explain the results of the present studies with the enantiomers in isolated ventricles, since raising $K^+$ did not increase the negative ino-

tropic potency of (+)-verapamil to the same extent as it did for (-)-verapamil. However, raising $K^+$ concentration in the present experiments did not actually reverse the effects of (+)-verapamil, therefore it would be premature to suggest that the results of the present experiments are entirely consistent with stereoselective frequency- or voltage-dependence, although the possibility cannot be ruled out.

With regard to the hypothetical voltage-dependent modulating influence, the existence of such an entity may explain the difference between the $K_a$ for dihydropyridines determined by measuring a physiological response such as ventricular muscle contraction at different stimulation frequencies (generally between $10^{-7}$ and $10^{-9}$ M, see Nayler and Horowitz, 1983) and the $K_D$ according to radioligand binding studies (generally no more than $10^{-9}$ M, see Glossman et al., 1982), since binding studies are often carried out using tissue depolarised in high $K^+$.

At present the mechanism of voltage-dependent interactions between drugs and ion channels is uncertain. This discussion is somewhat speculative, however it may bear some relation to the discussion of the effects of $K^+$ changes on the actions of calcium antagonists (see below).

In summary, 1,4-dihydropyridine calcium antagonists were ineffective as antiarrhythmic agents, probably because concentrations sufficient to produce calcium antagonism in the heart were not reached, despite evidence of systemic vasodilatation. In contrast, phenethylalkylamines were effective antiarrhythmic agents at doses which produced effects consistent with calcium antagonism in the heart.

4.3.3 Role of calcium antagonism in the ischaemic ventricle

The studies with the verapamil enantiomers, nifedipine and DHM9 in isolated ventricles under conditions of varied $K^+$ concentration were designed to examine the possibility that some calcium antagonists may

possess a selectivity of action for ischaemic versus non-ischaemic tissue, by virtue of the differences in extracellular $K^+$ between ischaemic and non-ischaemic tissue (Hill and Gettes, 1980; Hirche et al., 1980). Such a site-selectivity of action may account in part for differences in antiarrhythmic activity of different calcium antagonists.

The potency ratio of (-)- to (+)-verapamil determined in isolated ventricles was highly dependent on the concentration of $K^+$ in the perfusing buffer. The mechanism for this effect is unclear. Elevation of $K^+$ from 5.4 to 8 meq/l has been previously shown to produce only a small depolarisation of resting membrane potential in ventricular tissue (Buchanan et al., 1985) of insufficient magnitude to affect steady state activation and inactivation of $i_{si}$ (Reuter, 1979). Therefore the $K^+$ ion appeared to alter the potency of the (±)-verapamil enantiomers independently of gating. However, it may be the case that the small depolarisations which are predicted to have occurred with raised $K^+$ were responsible for increases in the affinity of the verapamil receptor for the enantiomers, in accordance with the proposed mechanism of frequency- and voltage-dependence discussed above. Experiments are currently being carried out in the laboratory to examine whether the potency changes associated with $K^+$ concentration changes correlate with changes in resting membrane potential, by constructing isobolograms. Preliminary information suggests that this may be the case, although the relationship between ventricular excitability and $K^+$ concentration in the present experiments did not appear to be identical with the relationship between $K^+$ concentration and negative inotropic potency of the enantiomers of verapamil, suggesting that the relationship between resting membrane potential and the affinity of verapamil for its receptor may be complex.

The effect of elevations in $K^+$ to reduce the ratio of potency between

(-)- and (+)-verapamil (from 21 to 8) may have important implications concerning the site of action of antiarrhythmic phenethylalkylamines, since in raised $K^+$, the potency ratio was closer to the antiarrhythmic potency ratio (4) during myocardial ischaemia. In this regard, it is important to consider that within 5 - 10 min of coronary occlusion, extracellular $K^+$ rises to 8 - 15 meq/l in the ischaemic tissue (Hill and Gettes, 1980; Hirche et al., 1980). The present in vitro results predict, therefore, that in ischaemic ventricular tissue, the calcium antagonist potency ratio of (-)- to (+)-verapamil should be 8 or less. It follows from this that the antiarrhythmic potency ratio of (-)- to (+)-verapamil should be no more than 8, if these phenethylamines reduce ischaemia-induced arrhythmias via calcium antagonism. In addition, the calcium antagonist potency ratio in vivo may be even less than 8 in the ischaemic myocardium, in terms of dose, if pharmacokinetic factors are considered (see above). In human tissue the potency ratio of the enantiomers in vivo for prolonging P-R interval, in terms of unbound plasma concentration (Echizen et al., 1985), and the negative inotropic potency ratio in vitro (Ferry et al., 1985) have been reported to be 4.5 and 8 in favour of (-)-verapamil, respectively. These values correspond almost exactly with the antiarrhythmic and in vitro negative inotropic potency ratios in raised $K^+$, respectively, reported here.

Consideration of the antiarrhythmic potency ratio of the verapamil enantiomers, and their effects on isolated rat ventricles in the presence of raised $K^+$ concentrations suggests an explanation for the site of action of the enantiomers, and explains how $i_{si}$ might be abolished by the enantiomers in the ischaemic tissue while $i_{si}$ in the non-ischaemic tissue remains relatively unaffected. Since the enantiomers of verapamil clearly did not abolish conduction in the non-ischaemic ventricular tissue in vivo, a selective action in the ischaemic tissue leading to a reduction in arrhythmias is

an obvious hypothesis. The high extracellular $K^+$ concentration seen in acutely ischaemic tissue is therefore a candidate for mediation of such selectivity of action, provided that the actions of (±)-verapamil on arrhythmogenesis during ischaemia depend on $K^+$ in a similar manner to the actions of (±)-verapamil on the force of contraction in isolated ventricles.

It is of interest to note that the effect of $K^+$ on the activity of nifedipine was quite different from that of (-)- and (+)-verapamil. Increasing $K^+$ from 3 to 10 meq/l increased the potency of nifedipine only 4 fold. Therefore, $K^+$ did not confer site-selectivity of action on nifedipine to the same extent that was seen in the case of the verapamil enantiomers. Qualitatively, therefore, calcium antagonists which show great potency changes with small changes in $K^+$ concentration (verapamil enantiomers) possess potent antiarrhythmic activity in acute myocardial ischaemia, whereas calcium antagonists without such a property (nifedipine) have little or no antiarrhythmic activity. It will be of interest to examine whether this pattern is also a feature of other calcium antagonists.

The spontaneous occurrence of PVC, and inhibition of such by the verapamil enantiomers in isolated ventricles were 2 phenomena which were $K^+$-dependent. To our knowledge, such PVC have not been previously investigated. (-)-Verapamil was more potent than (+)-verapamil in reducing these PVC, in concordance with reductions of ischaemia-induced arrhythmias. It is possible, therefore, that these PVC share a common mechanism with ischaemia-induced PVC. However it is premature to reach such a conclusion at present. In the study of nifedipine and DHM9 in isolated ventricles the control incidence of such PVC was much lower than that in the verapamil enantiomer study, and therefore it was not possible to state whether nifedipine reduced these arrhythmias.

4.3.4 Role of other pharmacological properties

Although the present results support the hypothesis that the antiarrhythmic action of calcium antagonists occurs as a result of calcium antagonism, other explanations should be considered. Phenethylalkylamine and 1,4-dihydropyridine calcium antagonists possesses, in addition to their $i_{si}$ blocking properties (Fleckenstein et al., 1969; Kohlhardt and Fleckenstein, 1977; etc.), numerous other actions (see Nayler and Horowitz, 1983). It may be possible to hypothesize that some or all of these actions contribute to the antiarrhythmic actions described in the present experiments. However, close examination of the data reveals that most of these non-specific actions occur at concentrations greater than those required for inhibition of $i_{si}$ (see below, and Nayler and Horowitz, 1983).

4.3.4.1 Inhibition of the fast inward current. (+)-, (±)- And (-)-verapamil inhibit $i_{Na}$ in ventricular tissue in vitro (Bayer et al., 1975a; 1975b; 1975c), but only at 100-150 times the concentration required to inhibit $i_{si}$ (Nawrath et al., 1981). Therefore, it would seem unlikely that such actions contribute to the antiarrhythmic actions of (±)-verapamil, since doses effective in inhibiting $i_{Na}$ would produce complete inhibition of $i_{si}$, leading to atrioventricular dissociation and almost complete uncoupling of excitation and contraction in the heart and vascular smooth muscle. In the present studies, and in antiarrhythmic studies carried out by other investigators (Kaumann and Aramendia, 1968; Kaumann and Serur, 1975; Kroll and Knight, 1984; etc.) this was clearly not the case.

Although it is generally believed that 1,4-dihydropyridines do not affect $i_{Na}$ (e.g., Kohlhardt and Fleckenstein, 1977), it was recently found that nitrendipine blocked $i_{Na}$ in neonatal rat heart cells (Yatani and Brown, 1985). However, as in the case of (±)-verapamil (Nawrath et al., 1981), this effect only occurred with concentrations greatly in excess of

those required to abolish $i_{si}$. Therefore, if it were possible to administer massive doses of 1,4-dihydropyridines sufficient to inhibit ischaemia-induced arrhythmias, inhibition of $i_{Na}$ could not account for such an action.

Our laboratory previously found that quinidine at doses reducing ischaemia-induced arrhythmias by 50 % (20 mg/kg) protected against electrically induced arrhythmias, and increased maximum following frequency and QRS interval (Curtis et al., 1984). In the present studies, neither (+)- nor (-)-verapamil affected these variables at doses which protected against ischaemia-induced arrhythmias. In addition, Raschack (1976) showed that in anaesthetised rats, (+)-verapamil reduced aconitine-induced arrhythmias, whereas high doses of (-)-verapamil (which caused second degree atrioventricular block) were ineffective. This suggests that plasma concentrations of (-)-verapamil which are effective in reducing the $i_{Na}$ (and associated aconitine-induced arrhythmias) cannot be reached in vivo.

In summary, it is extremely unlikely, if not impossible, for $i_{Na}$ blockade to be a contributary factor in the antiarrhythmic activity of 1,4-dihydropyridine and phenethylalkylamine calcium antagonists.

4.3.4.2 Blockade of α-adrenoceptors. There have been suggestions that many calcium antagonists inhibit responses to α-adrenoceptor agonists. However, current evidence does not support the suggestion (Motulsky et al., 1983) that α-adrenoceptor antagonism contributes to the antiarrhythmic activity of calcium antagonists, for the following reasons.

Firstly, with regard to the antiarrhythmic activity of (+)- and (-)-verapamil, it is unclear whether (±)-verapamil truly influences α-adrenoceptors. While it has been claimed that (±)-verapamil is a selective antagonist of $\alpha_2$-adrenoceptors, having little effect on $\alpha_1$ responses (Timmermans et al., 1983; Cavero et al., 1983; Haeusler, 1985), it has also been

claimed that the opposite is the case (Vanhoutte 1982; Holck and Gerrold, 1986). Proponents of the $\alpha_2$-selective hypothesis suggest that the antagonist action of (±)-verapamil is non-competitive, and report that (±)-verapamil has almost negligible affinity for the $\alpha_2$ receptors themselves (Van Meel et al., 1981). It was also suggested that while (±)-verapamil inhibited α-adrenoceptor agonist-induced inotropic responses in atria, the effect was not mediated by α-adrenoceptor antagonism (Tung et al., 1985).

Secondly, it is doubtful whether α-adrenoceptor activation is of pathological importance in arrhythmogenesis during acute myocardial ischaemia. In non-ischaemic heart tissue, pharmacological and electrophysiological studies revealed that α-adrenoceptor agonism is mediated essentially by the $\alpha_1$ subtype and is expressed as a prolongation of action potential duration (Dukes and Vaughan Williams 1984; Black et al., 1985). It was argued that, with regard to reentry, this electrophysiological effect should be antiarrhythmic rather than arrhythmogenic (Dukes and Vaughan Williams 1984) owing to the dependence of reentry on the presence of a relatively narrow action potential and abbreviated refractoriness (Mines 1913). In addition, in ventricular muscle the effects of α-adrenoceptor agonism have been shown to be extremely small; maximum positive inotropic effects of phenylephrine were only 15 % of the maximum effects of isoprenaline, and the changes in action potential configuration caused by α-adrenoceptor agonists were not significant (Nawrath and Rupp, 1986). Studies with perfused rat hearts have shown that (-)-verapamil and nifedipine are equipotent in their ability to inhibit adrenaline-induced falls in VF threshold (Higginson et al., 1983). It is difficult to reconcile data such as this with the results of the present studies and, at the same time, hypothesize an involvement of cardiac α-adrenoceptors in arrhythmogenesis. Other studies in vitro have shown that (-)- but not (+)-verapamil inhibits release of noradrenaline from the heart

caused by global ischaemia (Nayler and Sturrock, 1983). However, this effect of (-)-verapamil was associated with a bell-shaped dose-response curve, whereas the antiarrhythmic dose-response curve generated by the present studies (Figure 25) appeared to be a saturating sigmoidal function.

Thirdly, studies of the effect of α-adrenoceptor agonism and antagonism during myocardial ischaemia have produced conflicting results. α-Adrenoceptor antagonism was reported to reduce arrhythmias in cats (Sheridan et al., 1980), but identical treatment was ineffective in dogs (Bolli et al., 1984). In addition, the reported antiarrhythmic actions of α-adrenoceptor antagonists in isolated ischaemic rat hearts is not attributable to α-adrenoceptor antagonism (Daugherty and Woodward, 1982; Daugherty et al., 1986), rather to 'membrane stabilising' effects (Bralet et al., 1985). Our laboratory has shown that neither combined α- and β-adrenoceptor blockade nor sympathectomy reduce arrhythmias in rats (Botting et al., 1983). In addition, the present studies showed that graded ablations in the CNS and catecholamine infusions did not influence arrhythmias in a manner consistent with a role for α- (or β-) adrenoceptors in arrhythmogenesis during acute myocardial ischaemia.

In summary, it appears to be highly improbable that (+)- or (-)-verapamil reduced ischaemia-induced arrhythmias in conscious rats via myocardial α-adrenoceptor antagonism.

4.3.4.3 Indirect actions. It may be suggested that the verapamil enantiomers exerted their antiarrhythmic actions indirectly by reducing afterload or heart rate. However, this may be ruled out for the following reasons.

Firstly, our laboratory has shown that there is no correlation between either blood pressure or heart rate and arrhythmias in conscious rats (Johnston et al., 1983a).

Secondly, the antiarrhythmic effects of the calcium antagonists studied in the current series of experiments did not correlate with effects on blood pressure or heart rate. Felodipine and nifedipine produced large reductions in blood pressure without concomittant reductions in arrhythmias. Anipamil, which appears to be a calcium antagonist with selectivity for the myocardium versus vascular smooth muscle, reduced arrhythmias without causing significant reductions in blood pressure. The enantiomers of verapamil reduced arrhythmias both at doses with and without effects on blood pressure. Although heart rate was reduced by antiarrhythmic doses of the verapamil enantiomers and increased by the dihydropyridines at the time of occlusion, these effects were not sustained, and during the periods of arrhythmias the effects of treatment on heart rate did not correlate with arrhythmias.

It may be suggested that the antiarrhythmic actions of the enantiomers of verapamil and anipamil occurred as a result of their apparent anti-ischaemic actions, since these drugs delayed the development of ECG indices of ischaemic such as S-T segment elevation. However, these effects did not appear to correlate with arrhythmias. The onset of arrhythmias was not delayed by the verapamil enantiomers or anipamil. In addition, felodipine delayed S-T segment elevation in but did not reduce arrhythmias during myocardial ischaemia. It is perhaps worth commenting that delays in ECG signs of ischaemia did not correlate with infarct size, either. Infarct size was not reduced by any treatment.

## 4.4 General conclusions

### 4.4.1 Arrhythmogenesis in acute myocardial ischaemia

The experiments with calcium antagonists described here do not contradict the hypothesis that $i_{si}$ is involved in arrhythmogenesis in acute myocardial ischaemia. On the contrary, they support the hypothesis. There are many theoretical reason why $i_{si}$ may be involved in arrhythmogenesis.

Slow conduction in the ischaemic tissue, which occurs during acute myocardial ischaemia and is predicted to predispose to reentry (Mines, 1913) may depend on $i_{si}$, since $i_{Na}$ may be completely inactivated by depolarisation. Of the hypothetical mechanistic models of arrhythmogenesis, only reentry has been shown by activation mapping to occur during acute myocardial ischaemia. The results of the current experiments are consistent with a role for $i_{si}$ in the ischaemic tissue in arrhythmogenesis in acute myocardial ischaemia.

Experimental evidence from the present studies and other published work has not yet disproven a role for $i_{si}$ in arrhythmogenesis. However, until a definitive link is made between $i_{si}$ (or other currents) and reentry (or other mechanistic models of arrhythmogenesis) in myocardial ischaemia, it will not be possible to state with certainty by what mechanism an arrhythmia arises or is maintained in acute myocardial ischaemia, in terms of conductances.

The current experiments involving ablations in the CNS showed that the autonomic nervous system, heart rate and blood pressure are not significant determinants of arrhythmogenesis in acute myocardial ischaemia. However, acute surgery, or a factor produced by acute surgery, is capable of abolishing arrhythmias resulting from acute myocardial ischaemia. The identity of this variable is at present unknown. However preliminary results suggest that this factor may be either hyperkalaemia or leukopaenia.

4.4.2 Action of calcium antagonists in acute myocardial ischaemia

The study of the effects of drugs on ischaemia-induced arrhythmias has lead to a certain amount of tautology. Some researchers use drugs as tools to probe for mechanisms of arrhythmogenesis, whereas others consider the underlying electrophysiology of ischaemia while investigating the pharmacology of these same drugs in an attempt to explain their mechanism of anti-

arrhythmic action. Neither approach has yet yielded a safe prophylactic agent for prevention of arrhythmias in acute myocardial ischaemia.

The current studies showed that some, but not all, calcium antagonists are capable of reducing ischaemia-induced arrhythmias. The differences between (+)- and (-)-verapamil on one hand, and anipamil and ronipamil on the other suggest that the mechanism of antiarrhythmic action of calcium antagonists is calcium antagonism (inhibition of $i_{si}$, in electrophysiological terms). The differences between phenethylalkylamines and 1,4-dihydropyridines suggest that the site of action of antiarrhythmic calcium antagonists is the ventricular tissue. Consideration of the relationship between calcium antagonist activity and extracellular $K^+$ concentration suggests that the site of antiarrhythmic action of calcium antagonists is the ischaemic ventricular tissue. The results of the present studies with (±)-verapamil suggest that phenethylalkylamine calcium antagonists can certainly penetrate into the ischaemic tissue, even when administered after occlusion.

It is not possible to account for the antiarrhythmic actions of calcium antagonists, as catalogued in the present experiments, by invoking alternative known actions of the drugs in question. It is quite possible, of course, to account for the results by invoking some as yet unknown action.

With regard to possible therapeutic applications of the current crop of calcium antagonists, it appears that most, if not all, do not possess the necessary pharmacological profile for prophylaxis of arrhythmias associated with acute myocardial ischaemia. According to the results of the present experiments, the ideal antiarrhythmic calcium antagonist, should:

a. Have no action on vascular smooth muscle

b. Have no action in non-ischaemic cardiac tissue at normal heart rates

c. Abolish conduction in ischaemic tissue.

Currently, the drug which appears to best fit this profile is anipamil. Recent experiments in our laboratory suggest that anipamil is relatively cardioselective compared with other calcium antagonists but, unlike verapamil, does not cause profound atrioventricular block.

Consideration of the pharmacological profile of calcium antagonists suggests that there is much to be learned with regard to their mechanism of action and the processes which govern their apparent tissue selectivity, frequency-dependence, voltage-dependence and subsidiary properties. A fuller understanding of these caveats may facilitate the design of therapeutically useful prophylaxis against ischaemia-induced arrhythmias.

## 5 REFERENCES

ABRAHAMSSON, T., ALMGREN O. Ventricular fibrillation following coronary artery ligation in the rat. In: Budden, R., Detweiler, D. K., Zbinden G. (Eds.), The rat electrocardiogram in pharmacology and toxicology, pp. 239-241, Oxford, Pergamon Press (1980).

ABRAHAMSSON, T., ALMGREN, O., CARLSSON, L., SVENSSON, L. Antiarrhythmic effect of reducing myocardial noradrenaline stores with α-methyl-meta-tyrosine. J Cardiovasc Pharmacol 7, (Suppl 5), S81-S85 (1985).

ADGEY, A. A. J. Initiation of ventricular fibrillation outside hospital. In: Adgey A. A. J. (Ed.), Acute phase of ischaemic heart disease and myocardial infarction, pp. 67-76, Martinus Nijhoff, Hague/Boston/London (1982).

ADGEY, A. A. J., ALLEN, J. D., GEDDES, J. S., JAMES, R. R. G., WEBB, S. W., ZAIDI, S. A., PANTRIDGE, J. F. Acute phase of myocardial infarction. Lancet 501-504 (1971).

AGRESS, C. M., ROSENBERG, M. J., JACOBS, H. I., BINDER, M. J., SCHNEIDERMAN, A., CLARK, W. J. Protracted shock in the closed chest dog following coronary embolization with graded microspheres. Am J Physiol 170, 536-549 (1952).

ANDERSON, J. L., Antifibrillatory versus antiectopic therapy. Am J Cardiol 54, 7A-13A (1984).

ANVERSA, P., BEGHI, C., KIKKAWA, Y., OLIVETTI, G., Myocardial infarction in rats. Infarct size, myocyte hypertrophy and capillary growth. Circ Res 58, 26-37 (1986).

ANVERSA, P., BEGHI, C., KIKKAWA, Y., OLIVETTI, G., Myocardial response to infarction in the rat. Morphometric measurement of infarct size and myocyte cellular hypertrophy. Am J Pathol 118, 484-492 (1985a).

ANVERSA, P., BEGHI, C., MCDONALD, S. L., LEVICKY, V., KIKKAWA, Y., OLIVETTI, G., Morphometry of right ventricular hypertrophy induced by myocardial infarction in the rat. Am J Pathol 116, 504-513 (1984).

ANVERSA, P., LOUD, A. V., LEVICKY, V., GUIDERI, G. Left ventricular failure induced by myocardial infarction. I. Myocyte hypertrophy. Am J Physiol 248, H876-H882 (1985b).

ARMITAGE, P. Tests for linear trends in proportions and frequencies. Biometrics 11, 375-386 (1955).

ARMSTRONG, A., DUNCAN, B., OLIVER, M. F., JULIAN, D. G., DONALD, K. W., FULTON, M., LUTZ, W., MORRISON, S. L. Natural history of acute coronary heart attacks. A community study. Br Heart J 34, 67-80 (1972).

ASADA, S., CHIBA, T., OSAWA, K., NAKAMURA, K., MURAKAWA, S. Experimental studies of the effect of long-term oral administration of persantin. Jap Circ J 26, 849-855 (1962).

ATTWELL, D., EISNER, D., COHEN, I. Voltage clamp and tracer flux data: effects of a restricted extracellular space. Quart Rev Biophys 12, 213-261 (1979)

AU, T. L. S., COLLINS, G. A. HARVIE, C. J., WALKER, M.J.A. The actions of prostaglandins $I_2$ and $E_2$ on arrhythmias produced by coronary occlusion in the rat and dog. Prostaglandins 18, 707-720 (1979a).

AU, T. L. S., COLLINS, G. A., MACLEOD, B. A., WALKER, M. J. A. Effects of prostaglandin $E_2$, propranolol, and nitroglycerine with halothane, pethidine or pentobarbitone anaesthesia on arrhythmias and other responses to ligation of a coronary artery in rats. Br J Pharmacol 79, 929-937 (1983).

AU, T. L. S., COLLINS, G. A., HARVIE, C. J., WALKER, M. J. A. Actions of prostaglandins $I_2$ and $E_2$ on coronary occlusion induced arrhythmias in the rat. Advances in Prostaglandin and Thromboxane Research, Vol 7, (Eds.) Samuelsson, B., Ramwell, P.W., Paoletti, R. Raven Press, New York, pp 647-649, (1980).

AU, T. L. S., COLLINS, G. A., MACLEOD, B. A., and WALKER, M. J. A. Actions of nitroglycerin (N) and propanolol (P) in rats after coronary artery ligation. Pharmacologist 21 (Part 3), 274 (1979b).

AU, T. L. S., CURTIS, M. J., WALKER, M. J. A. Effects of the Isomers of Verapamil on Coronary Occlusion-Induced Mortality and Infarct size. Submitted for publication in: Journal of Cardiovascular Pharmacology (1986).

AU, T. L. S., HARVIE, C. J., JOHNSTON, K., MACLEOD, B. A., WALKER, M. J. A. The effect of Prostaglandin infusions on arrhythmic and other responses in coronary artery ligation. In: Prostaglandins and Thromboxanes, (Ed.) Forster, W., Sarembe, B., Mentz, P. Jena, Gustav Fischer Verlag, pp. 37-53, (1981).

AU, T. S. L., SUTTER, M. C., The inhibitory effects of four calcium antagonists on rat aortic strips, portal veins and hearts. IUPHAR 9th Int Congr Pharmacol Abstracts, 413 (1984).

AUSTIN, M., WENGER, T. L., HARRELL, F. E., LUZZI, F. A., STRAUSS, H. C. Effect of myocardium at risk on outcome after coronary occlusion and release. Am J Physiol 243, H340-345 (1982).

BAJUSZ, E. Conditioning factors for cardiac necrosis. pp. 250-271, Verlag S. Karger AG, Basel, (1963).

BAROLDI, G., SCOMAZZONI, G. Coronary circulation in the normal and the pathologic heart. U. S. Government Printing Office, Washington, D. C. (1967).

BAUR, H. R., CALDERON, M., PIERACH, C. A. Failure of verapamil to reduce infarct size in the rat. Eur Heart J 5, Suppl 1, 167 (1984).

BAYER, R., EHARA, T. VII. Comparative studies on calcium antagonists. Progr Pharmacol 2 (1) 30-37 (1978).

BAYER, R., HENNEKES, R., KAUFMANN, R., MANNHOLD, R. Inotropic and electrophysiological actions of verapamil and D600 in mammalian myocardium. I. Pattern of inotropic effects of the racemic compounds. Naunyn Schmiedeberg's Arch Pharmacol 290, 49-68 (1975a).

BAYER, R., KAUFMANN, R., MANNHOLD, R. Inotropic and electrophysiological actions of verapamil and D600 in mammalian myocardium. II. Pattern of inotropic effects of the optical isomers. Naunyn Schmiedeberg's Arch Pharmacol 290, 69-80 (1975b).

BAYER, R., KALUSCHE, D., KAUFMANN, R., MANNHOLD, R. Inotropic and electrophysiological actions of verapamil and D-600 in mammalian myocardium. III Effects of the optical isomers on transmembrane action potentials. Naunyn Schmiedeberg's Arch Pharmacol, 290, 81-97 (1975c).

BAYER, R., RODENKIRCHEN, R., KAUFMANN, R., LEE, J. H., HENNEKES, R. The effects of nifedipine on contraction and monophasic action potential of isolated cat myocardium. Arch Pharmacol 301, 29-37, (1977).

BEAN, B. P., NOWYCKY, M. C., TSIEN, R. W. Beta-adrenergic modulation of calcium channels in frog ventricular heart cells. Nature 307, 371-375 (1984).

BECK, C. S. Coronary artery disease. Am J Cardiol 1, 38-45 (1958).

BEDNAR, M., SMITH, B., PINTO, A., MULLANE, K. M. Nafazatrom-induced salvage of ischemic myocardium in anesthetized dogs is mediated through inhibition of neutrophil function. Circ Res 57, 131-141 (1985).

BEELER, G. W. Jr., MCGUIGAN, J. A. S. Voltage clamping of multicellular myocardial preparations: capabilities and limitations of existing methods. Proc Biophys Mol Biol 34, 219-254, (1978).

BEELER, G. W. Jr., REUTER, H. Voltage clamp experiments on ventricular myocardial fibres J Physiol (Lond) 207, 165-190 (1970a).

BEELER, G. W. Jr., REUTER, H. Membrane current in ventricular myocardial fibres J Physiol (Lond) 207, 191-209 (1970b).

BERGEY, J. L., NOCELLA, K., MCCALLUM, J. D. Acute coronary artery occlusion-reperfusion induced arrhythmias in rats, dogs and pigs: antiarrhythmic evaluation of quinidine, procainamide and lidocaine. Eur J Pharmacol 81 205-216 (1982).

BERGEY, J. L., WENDT, R. L, NOCELLA, K., MCCALLUM J. D. Acute coronary artery occlusion-reperfusion arrhythmias in pigs, antiarrhythmic and antifibrillatory evaluation of verapamil, nifedipine, prenylamine and propranolol. Eur J Pharmacol 97, 95-103 (1984).

BERMAN, J. K., FIELDS, D. C., JUDY, H., MORI, V., PARKER, R. J. Gradual vascular occlusion. Surgery 39, 399-410 (1956).

BERNAUER, W. Effects of glucocorticosteroids in rats with acute myocardial infarction. Arch Int Pharmacodyn Ther 244, 86-99 (1980).

BERNAUER, W. Comparative investigation of the effects of metoprolol, propranolol, practolol and verapamil in the acute phase of experimental myocardial infarction. Klin Wochenschr 60, 87-96 (1982).

BERNAUER, W. The metabolic role of endogenous catecholamines in acute myocardial infarction: effects of reserpinization and of infused noradrenaline. Eur J Pharmacol 88, 161-168 (1983).

BERNAUER, W. The effect of β-adrenoceptor blocking agents on evolving myocardial necrosis in coronary ligated rats with and without reperfusion. Naunyn-Schmiedebergs Arch Pharmacol 328, 288-294 (1985).

BIGGER, J. T. Jr. Mechanism and diagnosis of arrhythmias. In Braunwald, E. (Ed.), Heart disease: a textbook of cardiovascular medicine, vol 1. pp. 630-690, Saunders, Philadelphia/London/Toronto (1980).

BIGGER, J. T. Jr. Identification of patients at high risk for sudden cardiac death. Am J Cardiol 54, 3D-8D (1984).

BIGGER, J. T., GOLDREYER, B. N. The mechanism of supraventricular tachycardia. Circulation 42, 673-695 (1970).

BLACK, J. W., GERSKOWITCH, V. P., HULL, R. A. D, LEFF, P. Concentration-ratio analysis of the inotropic effects of phenylephrine in guinea-pig left atrial muscle. Br J Pharmacol 85, 258P (1985).

BLOOR, C. M., LEON, A. S., PITT, B. The inheritance of coronary artery anatomic patterns in rats. Circulation 36, 771-776 (1967).

BOER, S. DA. On the fibrillation of the heart. J Physiol 54, 400-409 (1920/1).

BOLLI, R., FISHER, D. J., TAYLER, A. A., YOUNG, J. B., MILLER, R.R. Effect of alpha adrenergic blockade on arrhythmias induced by acute myocardial ischemia and reperfusion in the dog. J Mol Cell Cardiol 16, 1101-1117. (1984)

BOND, R. F., MANNING, E. S., GONZALEZ, R. R. Jr. Methods for chronically implanting coronary artery blood flow probes, arterial occluders and coronary sinus catheters. In: McCutcheon, E. P. (Ed.), Chronically implanted cardiovascular implantation. pp. 281-228, Academic Press, New York (1973).

BOSTRUM, S. L., LJUNG, B., MARDH, S., FORSEN, S., THURLIN, E. Interaction of the antihypertensive drug felodipine with calmodulin. Nature 292, 777-778 (1981).

BOTTING, J. H., JOHNSTON, K. M., MACLEOD, B. A., WALKER, M. J. A. The effect of modification of sympathetic activity on responses to ligation of a coronary artery in the conscious rat. Br J Pharmacol 79, 265-271 (1983).

BRALET, J., DIDIER, J., MOREAU, D., OPIE, L. H., ROCHETTE, L. Effect of α-adrenoceptor antagonists (phentolamine, nicergoline and prazosin) on reperfusion arrhythmias and noradrenaline release in perfused rat heart. Br J Pharmacol 84, 9-18 (1985).

BREN, G., VARGHESE, P. J., LEIBOFF, R., ROSS, A. Electrophysiologic mechanism for the antifibrillatory action of verapamil. Am J Cardiol 49, 1043, (1982).

BRIGGS, G. B., OEHME, F. W. Toxicology. In: Baker, H. J., Lindsey, J. R., Weisbroth, S. H. (eds.), The laboratory rat, vol II, pp. 104-167. Academic Press, New York/London/Toronto/Sydney/San Francisco (1980).

BRISCOE, M. G., SMITH, H. J. Sensitivity of cat papillary muscles to verapamil and nifedipine: enhanced effects in acidosis. Cardiovasc Res 16, 173-177 (1982).

BROFMAN, B. L., LEIGHNINGER, D. S., BECK, C. S. Electric instability of the heart: the concept of the current of oxygen differential in coronary artery disease. Circulation 13, 161-77 (1956).

BROWN, H. F. Electrophysiology of the sinoatrial node. Physiol Rev 62, 505-530 (1982).

BROWN, M. J., BROWN, D. C., MURPHY, M. B. Hypokalemia from beta$_2$-receptor stimulation by circulating epinephrine. New England J Med 309, 1414-1419 (1983).

BRYANT, R. E., THOMAS, W. A., O'NEAL, R. M. An electron microscopic study of myocardial ischemia in the rat. Circ Res 6, 699-709 (1958).

BUCHANAN, J. W. Jr., SAITO, T., GETTES, L. S. The effects of antiarrythmic drugs, stimulation frequency, and potassium-induced resting membrane potential changes on conduction velocity, and $dV/dt_{max}$ in guinea pig myocardium. Circ Res 56, 696-703 (1985).

BURMEISTER, W. E. REYNOLDS, R. D. Comparison of infarct size produced by coronary artery occlusion in purebred beagle and mixed-breed dogs. Can J Physiol Pharmacol 61, 672-675 (1983).

BYRNE, J. E., WALTERS, J. S., DEITCHMAN, D. An acute rat model for assessing anti-ischemic drug activity. Abstract Pharmacologist 24, 210 (1982).

CAHN, J., BORZIEX, M. G. The effect of ketoprofen, an anti-inflammatory drug upon early ventricular arrhythmias resulting from acute myocardial ischaemia. Arch Pharmacol 324 (Suppl), R39 (1983).

CAMILION DE HURTADO, M. C., CINGOLANI, H. E. Interaction between calcium and slow channel blocking drugs on atrial rate. Arch Pharmacol 322, 65-71, (1983).

CAMPBELL, C. A., PARRATT, J. R. The effect of beta-adrenoceptor blocking agents, with differing ancillary properties, on the arrhythmias resulting from acute coronary artery ligation in anaesthetized rats. Br J Pharmacol 79, 939-946 (1983).

CAMPBELL, C. A., PARRATT, J. R., KANE, K. A., BULLOCK, G. Effects of prolonged administration of oxprenolol on severity of ischaemic arrhythmias, enzyme leakage, infarct size and intracellular cardiac muscle action potentials. J Cardiovasc Pharmacol 6, 369-377 (1984).

CAMPBELL, R. W. F. Prophylactic antiarrhythmic therapy in acute myocardial infarction. Am J Cardiol 54, 8E-10E (1984).

CAMPBELL, R. W. F. Treatment and prophylaxis of ventricular arrhythmias in acute myocardial infarction. Am J Cardiol 52, 55C-59C (1983).

CAMPBELL, R. W. F., MURRAY, A., JULIAN, D. G., Ventricular arrhythmias in the first 12 hours of acute myocardial infarction. Natural history study. Br Heart J 46, 351-357 (1981).

CARDINAL, R., JANSE, M. J., EEDEN, I. VAN, WERNER, G. NAUMANN D'ALONCOURT, C. DURRER, D. The effects of lidocaine on intracellular and extracellular potentials, activation and ventricular arrhythmias during acute regional ischemia in the intact isolated porcine heart. Circ Res 49, 792-806 (1981).

CARMELEIT, E., VEREECKE, J., Adrenaline and the plateau phase of the action potential. Importance of $Ca^{++}$ $Na^{+}$ and $K^{+}$ conductance. Pflug Arch ges Physiol 313, 300-315 (1972).

CARSON, D. L., CARDINAL, R., SAVARD, P., VASSEUR, C., NATTEL, S., LAMBERT, C., NADEAU, R. Relationship between an arrhythmogenic action of lidocaine and its effects on excitation patterns in acutely ischemic porcine myocardium. J Cardiovasc Pharmacol 8, 126-136 (1986).

CAVERO, I., SHEPPERSON, N., LEFEVRE-BORG, F., LANGER, S. Z. Differential inhibition of vascular smooth muscle responses to $\alpha_1$ and $\alpha_2$ adrenoceptor agonists by diltiazem and verapamil. Circ Res 52 (Suppl 1), 69-76 (1983).

CHARLON, V., PESTRE, S., LEIRIS, J. DE. Effect of chronic administration of L-carnitine on myocardial infarct size in the rat. J Mol Cell Cardiol 18 (Suppl 1), 354 (1986).

CHELLY, J., DOURSOUT, M. F., HYSING, E., HARTLEY, C. Role of the autonomic nervous system in the haemodynamic properties of nicardipine. J Mol Cell Cardiol 17 (Suppl 1), 19 (1985).

CHEUNG, W., MAROKO, P. R., HASTIE, R., DAY, C. Bepridil, a new calcium antagonist reduces sudden death in the atherosclerotic quail model. Circulation 68 (Suppl 1), 271 (1983).

CHIARIELLO, M., AMBROSIO, G., CAPPELLI-BIGAZZI, M., PERRONE-FILARDI, P., TRITTO, I., MARONE, G., CONDORELLI, M. Mepindolol reduces myocardial necrosis in rats with coronary artery occlusion. J Cardiovasc Pharmacol 7, 525-531 (1985).

CHIARIELLO, M., BREVETTI, G., AMBROSIO, G., CATAFFO, A., POLLICE, U., CONDORELLI, M. Long-term protection of ischaemic myocardium by nitroglycerin ointment. Cardiovasc Res 18, 321-325 (1984).

CHIARIELLO, M., BREVETTI, G., GENOVESE, A., CATAFFO, A., AMBROSIO, G., CONDORELLI, M. Dilazep-induced reduction of ischemic necrosis in rats with coronary artery occlusion. Internat J Cardiol 2, 339-45 (1983).

CHIARIELLO, M., BREVETTI, G., DE ROSA, G., ACUNZO, R., PETILLO, F., RENGO, F., CONDORELLI, M. Protective effects of simultaneous alpha and beta adrenergic receptor blockade on myocardial cell necrosis after coronary artery occlusion in rats. Am J Cardiol 46, 249-254 (1980).

CHILSON, D. A., PEIGH. P., MAHOMED, Y., WALLER, B. F., ZIPES, D. P., Endocardial application of phenol ablated ventricular tachycardia in the dog. Circulation 68 (Suppl III), 584 (1983).

CINCA, J., JANSE, M. J., MORENA, H., CANDELL, J., VALLE, V., DURRER, D. Mechanism and time course of the early electrical changes during acute coronary artery occlusion. An attempt to correlate the early ECG changes in man to the cellular electrophysiology in the pig. Chest 77, 499-505 (1980).

CLARK, C., FOREMAN, M. I., KANE, K. A., MACDONALD, F. M., PARRATT, J.R. Coronary artery ligation in anesthetized rats as a method for the production of experimental dysrhythmias and for the determination of infarct size. J Pharmacol Methods 3, 357-368 (1980).

CLARKE B., PATMORE, L., WHITING. R. L. Comparative inotropic effects of calcium modulators on guinea pig and rat myocardium. Proc Br Pharmacol Soc April, P50 (1985).

CLARKE, B., DUNCAN, G. P., PATMORE, L., WHITING, R. L. Measurement of calcium antagonist potency using cardiac cell cultures. Br J Pharmacol 83, 365P (1984a).

CLARKE, B., EGLEN, R. M., PATMORE, L., WHITING, R. L. Cardioselective calcium entry blocking properties of nicardipine metabolite. Br J Pharmacol 83, 437P (1984b).

CLUSIN, W. T., BUCHBINDER, M., ELLIS, A. K., KERNOFF, R. S., GIACOMINI, J., HARRISON. D. C. Reduction of ischaemic depolarization by calcium channel blocker diltiazem. Correlation with improvement of ventricular conduction and early arrhythmias in the dog. Circ Res 54, 10-20 (1984).

COBB, L. A., WERNER, J. A., TROBAUGH, G. B., Sudden cardiac death. Mod Con Cardiovasc Disease 49, 31-42 (1980).

COETZEE, W. A., DENNIS, S. C., YON, E., OPIE, L. H. Reperfusion electrical instability: are free radicals involved? J Mol Cell Cardiol 18 (Suppl 1) 115 (1986).

COETZEE, W. A., OPIE, L. H. Are early ischaemic arrhythmias caused by delayed afterdepolarizations? J Mol Cell Cardiol 18 (Suppl 1) 262 (1986).

COHNHEIM, J. SCHULTHESS-RECHBERG, A. VON. Uber die folgen der kranzarterienverschliessung fur das herz. Virchows Arch Pathol Anat 85, 503-537 (1881).

COKER, S. J. Early ventricular arrhythmias arising from acute myocardial ischaemia; possible involvement of prostaglandins and thromboxanes. In: Parratt, J. R. (Ed.), Early Arrhythmias Resulting from Myocardial Ischaemia, pp. 219-237, Macmillan Press, London (1982).

COKER, S. J., PARRATT, J. R. The effects of prostaglandins $E_2$, $F_{2\alpha}$, prostacyclin, flurbiprofen and aspirin on arrhythmias resulting from coronary artery ligation in anaesthetized rats. Br J Pharmacol 74, 155-159 (1981).

COKER, S. J., PARRATT, J. R. Nafazatrom, prostacyclin and ZK36374 suppress reperfusion induced ventricular fibrillation in anaesthetized greyhounds. Br J Pharmacol 79, 237p, (1983a).

COKER, S. J., PARRATT, J. R. Different effects of intravenous and intracoronary prostacyclin and ZK36374 on cardiac arrhythmias in anaesthetized dogs. Br J Pharmacol 79, 31p (1983b).

COKER, S. J., PARRATT, J. R., LEDINGHAM, I. M., ZEITLIN, I. J. Thromboxane and prostacyclin release from ischaemic myocardium in relation to arrhythmias. Nature 291, 323-324, (1981).

COLE, S. C. J., FLANAGAN, R. J., JOHNSTONE, A., HOLT, D. W. Rapid high-performance liquid chromatographic method for the measurement of verapamil and norverapamil in blood plasma or serum. J Chromatog 218, 621-629 (1981).

COOPER, D. K. C. Electrocardiographic studies in the rat in physiological and pathological states. Cardiovasc Res 3, 419-425 (1969).

COOPER, D., LUCERI, R. M., THURER, R. J., CASTELLANOS, A., MYERBERG, R. J., The impact of the automatic implantable defibrillator on quality of life. Circulation 72 (Suppl), 93 (1985).

CORDAY, E., LANG, T. W., MEERBAUM, S., GOLD, H., HIROSE, S., RUBINS, S., DALMASTRO, M. Closed chest model of intracoronary occlusion for study of regional cardiac function. Am J Cardiol 33, 49-59 (1974).

CORR, P. B., GROSS, R. W., SOBEL, B. E. Amphipathic metabolites and membrane dysfunction in ischaemic myocardium. Circ Res 55, 136-154 (1984).

COSTA, G., SAIJA, A., CAPUTI, A. P. Effect of nimodipine, a new calcium antagonist, on ACTH and corticosterone release 'in-vivo' and 'in-vitro'. Res Comm Chem Pathol Pharmacol 41, 355-367 (1983).

CRANEFIELD, P. F. Action potentials, afterpotentials and arrhythmias. Circ Res 41, 415-423 (1977).

CRANEFIELD, P. F., ARONSON, R. S., WIT, A. L. Effect of verapamil on the normal action potential and on a calcium dependent slow response of canine cardiac Purkinje fibers. Circ Res 34, 204-213 (1974).

CRANEFIELD, P. F., WIT, A. L., HOFFMAN, B. F. Conduction of the cardiac impulse. III. Characteristics of very slow conduction. J Gen Physiol 59, 227-246 (1972).

CROME, R., HEARSE, D. J., MANNING, A. S., Reperfusion arrhythmias and time-response relationships: modification by drugs in the anesthetized rat. Br J Pharmacol 86, 418P (1985).

CURTIS, M. J., JOHNSTON, K. M., MACLEOD, B. A., WALKER, M. J. A. The actions of felodipine on arrhythmias and other responses to myocardial ischaemia in conscious rats. Eur J Pharmacol 117, 169-178 (1985a).

CURTIS, M. J., MACLEOD, B. A., WALKER, M. J. A. Antiarrhythmic actions of verapamil against ischaemic arrhythmias in the rat. Br J Pharmacol 83, 373-385 (1984).

CURTIS, M. J., MACLEOD, B. A., WALKER, M. J. A. The effects of ablations in the central nervous system on arrhythmias induced by coronary occlusion in the rat. Br J Pharmacol 86, 663-670 (1985b).

CURTIS, M. J., MACLEOD, B. A., TABRIZCHI, R., WALKER, M. J. A. An Improved Perfusion Apparatus for Small Animal Hearts. J Pharmacol Methods, 15, 87-94, (1986a).

CURTIS, M. J., WALKER, M. J. A. The Mechanism of Action of the Optical Enantiomers of Verapamil against Ischaemia-Induced Arrhythmias in the Conscious Rat. Br J Pharmacol (1986a).

CURTIS, M. J., WALKER, M. J. A., YUSWACK, T. Actions of the Verapamil analogues, Anipamil and Ronipamil, Against Ischaemia-Induced Arrhythmias in Conscious Rats. Br J of Pharmacol (1986b).

CURTIS, M. J., WALKER, M. J. A. An improved pithed rat preparation: actions of the optical enantiomers of verapamil. Asia Pacific J Pharmacol (1986b).

DAI, S. Effects of SK& F 93479 on experimentally induced ventricular arrhythmias in dogs, rats and mice. Agents and Actions 15, 131-136 (1984).

DAUGHERTY A., FRAYNE K. N., REDFERN W. S., WOODWARD B. The role of catecholamines in the production of ischaemia-induced ventricular arrhythmias in the rat in vivo and in vitro. Br J Pharmacol 87, 265-278 (1986).

DAUGHERTY, A., MOHAMED, O. Y., WOODWARD, B. Effect of potassium on coronary artery ligation induced ventricular arrhythmias in the isolated rat heart. J Physiol (London) 340, 66P (1981).

DAUGHERTY, A., WOODWARD, B. Lack of adrenergic influences on ventricular arrhythmias in coronary artery ligated isolated rat hearts. Br J Pharmacol 76, 182P (1982).

DAVIS, F. B., DAVIS, P. J., BLAS, S. D., MYLOTTE, K. M. Inhibition by calcium channel blockers of thyroid hormone-responsive $Ca^{2+}$-ATPase activity in human red blood cells. Ann New York Acad Sci 435, 402-404 (1984).

DECK, K. A., TRAUTWEIN, W. Ionic currents in cardiac excitation. Pflug Arch Ges Physiol 280, 63-80 (1964).

DENNIS, A. R., HEARSE, D. J., YELLON, D. M. Lack of spontaneous or verapamil-induced salvage of potentially necrotic myocardium following 24 hours of coronary artery occlusion in the rabbit. J Mol Cell Cardiol 18 (Suppl 1), 202 (1986).

DODGE FA, CRANEFIELD PF. In: Paes de Carvalho, A., Hoffman, B. F., Lieberman, M. (Eds.) Normal and abnormal conduction in the heart. Futura (1982).

DORRSCHEIDT-KAFER, M. The action of D-600 on frog skeletal muscle; Facilitation of excitation contraction coupling. Pflug Arch Ges Physiol 369, 259-267, 1977.

DOWNAR, E., JANSE, M. J., DURRER, D. The effect of "ischemic" blood on transmembrane potentials of normal porcine ventricular myocardium. Circulation 55, 455-462 (1977a).

DOWNAR, E., JANSE, M. J., DURRER, D. The effect of acute coronary artery occlusion on subepicardial transmembrane potentials in the intact porcine heart. Circulation 56, 217-24 (1977b).

DRESEL, P., SUTTER, M. C. Factors modifying cyclopropane-epinephrine cardiac arrhythmias. Circ Res 9, 1284-1290 (1961).

DRISCOLL, P. The normal rat electrocardiogram. In: Budden, R., Detweiler, D. K., Zbinden, G. (Eds.), The rat electrocardiogram in pharmacology and toxicology. pp. 3-15, Pergamon Press, Oxford (1980).

DUKES, I. D., VAUGHAN WILLIAMS, E. M. Effects of selective $\alpha_1$-, $\alpha_2$-, $\beta_1$-, and $\beta_2$-adrenoceptor stimulation on potentials and contractions in the rabbit heart. J Physiol (London) 355, 523-546 (1984).

ECHIZEN, H., BRECHT, T., NIEDERGESASS, S., VOGELGESANG, B., EICHELBAUM, M. The effect of dextro-, levo-, and racemic verapamil on atrioventricular conduction in humans. Am Heart J 109, 210-217 (1985).

ECHT, D. S., ARMSTRONG, K., SCHMIDT, P., OYER, P. E., STINSON, E. B., WINKLE, R. A. Clinical experience, complications, and survival in 70 patients with the automatic implantable cardioverter/defibrillator. Circulation 71, 289-296 (1985).

ECKSTEIN, R. W., GREGG, D. E., PRITCHARD, W. H. Magnitude and time of development of collateral circulation in occluded femoral, carotid and coronary arteries. Am J Physiol 132, 351-363 (1941).

EDOUTE, Y, SANAN, D., LOCHNER, A., GRANEY, D., KOTZE, J. C. N. Effects of propranolol on myocardial ultrastructure, mitochondrial function and high energy phosphates of isolated working rat hearts with coronary artery ligation. J Mol Cell Cardiol 13, 619-639 (1981).

EDOUTE, Y., MERWE, E. L. VAN DER. SANAN, D., KOTZE, J. C. N., NIEKERK, I, LOCHNER, A. Normothermic ischaemic cardiac arrest and reperfusion of the isolated working heart: effect of chlorpromazine on functional, metabolic and morphological recovery. J Mol Cell Cardiol 15, 603-620 (1983).

EHARA, T, KAUFMANN R. The voltage and time-dependent effects of (-)-verapamil on the slow inward current in isolated cat ventricular myocardium. J Pharmacol Exp Ther 207, 49-55 (1968).

EICHELBAUM, M., ENDE, M., REMBERG, G., SCHOMERUS, M., DENGLER, H. J. The metabolism of DL-($^{14}$C)verapamil in man. Drug Metab Disp 7, 145-148 (1979).

EICHELBAUM, M., MIKUS, G., VOGELGESANG, B. Pharmacokinetics of (+)-, (-)- and (±)-verapamil after intravenous administration. Br J Clin Pharmacol 17, 453-458 (1984).

EL-SHERIF, N., MEHRA, R., GOUGH, W. B., ZEILER, R. H. Ventricular activation patterns of spontaneous and induced ventricular rhythms in canine one-day-old myocardial infarction. Circ Res 51, 152-166 (1982).

EL-SHERIF, N., MYERBURG, R. J., SCHERLAG, B. J., ELFELLAH, B., ARANDA, J. M., CASTELANOS, A., LAZARRA, R. Electrocardiographic antecedants of primary ventricular fibrillation. Br Heart J 38, 415-422, (1976).

ELZINGER, W., KAUFMAN, W., UPRIGHT, D., POWELL, D. A new animal model of myocardial infarction. In: Hegyeli, R. (Ed.), Artificial heart program conference procedings. pp. 689-699, Natl Heart and Lung Inst, US Dept Health Education and Welfare, Bethesda, Md. (1969).

ERICHSEN, J. E. On the influence of the coronary circulation on the action of the heart. Med Gazette 30, 561-564 (1842).

EVANS, R. G., VAL-MEJIAS, J. E., KULEVICH, J., FISCHER, V. W., MUELLER, H. S. Evaluation of a rat model for assessing interventions to salvage ischaemic myocardium: effects of ibuprofen and verapamil. Cardiovasc Res 19, 132-138 (1985).

FABIANI, J. N., DELOUCHE, J. P., CAMILLERI, D., JOSEPH, M., SCHLUMBERGER, A., CARPENTER, A., DUBOST, C. Etude experimentale de la microcirculation dans l'infarctus du myocarde. Coeur et Medicine Interne 15, 543-555 (1976).

FAGBEMI, O, and PARRATT, J. R. (1981c) Marked suppression by nifedipine and nisoldipine of ventricular fibrillation resulting from acute myocardial ischaemia. Br J Pharmacol, 74, 830P-831P.

FAGBEMI, O., KANE, K. A., MCDONALD, F. M., PARRATT, J. R., ROTHAUL, A. The effects of verapamil, prenylamine, flunarizine and cinnarizine on coronary artert ligation induced arrhythmias in the anaesthetised rat. Br J Pharmacol 83, 299-304 (1984).

FAGBEMI, O., LEPRAN, I., PARRATT, J. R., SZEKERES, L. Naloxone inhibits early arrhythmias resulting from acute coronary ligation. Br J Pharmacol 76, 504-506 (1982b).

FAGBEMI, O., PARRATT, J. R. Suppression by orally-administered nifedipine, nisoldipine and niludipine of early, life-threatening ventricular arrhythmias resulting from acute myocardial ischaemia. Br J Pharmacol 74, 12-14 (1981a).

FAGBEMI, O., PARRATT, J. R. Calcium antagonists prevent early post-infarction ventricular fibrillation. Eur J Pharmacol 75, 179-185 (1981b).

FAGBEMI, S. O. The effect of aspirin, indomethacin and sodium meclofenamate on coronary artery ligation arrhythmias in anaesthetized rats. Eur J Pharmacol 97, 283-87 (1984).

FAGBEMI, S. O. The effects of the combined administration of β-adrenoceptor antagonists and non-steroidal anti-inflammatory drugs on ligation-induced arrhythmias in rats. Brit J Pharmacol 85, 361-365 (1985).

FARRIS, E. J., GRIFFITH, J. Q. (Eds.) The Rat in Laboratory Investigations. Hafner Press, N.Y. (1967).

FEDOR, J. M., MCINTOSH, D. M., REMBERT, J. C., GREENFIELD, J. C. Coronary and transmural myocardial blood flow response in awake pigs. Am J Physiol 235, H435-44 (1978).

FERRARI, R., RADDINO, R., CECONI, C., CURELLO, S., VISIOLI, O. Protective effect of anipamil pretreatment during myocardial ischaemia and reperfusion. In: Calcium entry blockers and tissue protection (International symposium proceedings, in press) Rome (1984).

FERRIER, G. R., MOE, G. K. Effect of calcium on acetylstrophanthidin-induced transient depolarizations in canine Purkinje tissue. Circ Res 33, 508-515 (1973).

FERRY, D. R., GLOSSMANN, H., KAUMANN, A. J. Relationship between the stereoselective negative inotropic effects of verapamil enantiomers and their binding to putative calcium channels in human heart. Br J Pharmacol 84, 811-824 (1985).

FIEDLER, V. B. Reduction of myocardial infarction and dysrhythmic activity by nafazatrom in the conscious rat. Eur J Pharmacol 88, 263-267 (1983).

FISCH, C., GREENSPAN, K., ANDERSON, G. Exit block. Am J Cardiol 28, 402 (1971).

FISCHER, S. III., EDWARDS, W. S. Tissue necrosis after temporary coronary artery occlusion. Ann Surg 29, 617-619 (1982)

FISHBEIN, M. C., MEERBAUM, S., RIT, J., LANDO, U., KANMATSUSE, K., MERCIER, J. C., CORDAY, E., GANZ, W. Early phase acute myocardial infarct size quantification: validation of the triphenyltetrazolium chloride tissue enzyme staining technique. Am Heart J 101, 593-600 (1981).

FISHER, J. D., COHEN, H. L., MEHRA, R., ALTSCHULER, H., ESCHER, D. J. W., FURMAN, S. Cardiac pacing and pace,akers. II. Serial electrophysiologic - pharmacologic testing for control of recurrent tachyarrhythmias. Am Heart J 93, 658-668 (1977).

FLAIM, S. F., ZELIS, R. Diltiazem pretreatment reduces experimental myocardial infarct size in rat. Pharmacology 23, 281-86 (1981).

FLECKENSTEIN, A. History of calcium antagonists. Circ Res 52, 1-16 (1983).

FLECKENSTEIN, A. Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. Ann Rev Pharmacol Toxicol 17, 149-166 (1977).

FLECKENSTEIN, A., TRITTHART, H., FLECKENSTEIN, B., HERBST, A., GRUN, G. A new group of competitive Ca antagonists (iproveratril, D600, prenylamine) with highly potent inhibitory effects on excitation-contraction coupling in mammalian myocardium. Pflug Arch Ges Physiol 307, R25 (1969).

FLECKENSTEIN, A., TRITTHART, H., DORING, H. J., BYON, K. Y. Baya 1040 - ein hochaktiver inhibitor der elektro-mechanischen koppelungsprozesse im warmbluter-myokard. Arzniem Forsch 22, 22-33 (1972).

FLECKENSTEIN-GRUN, G., FLECKENSTEIN, A., BYON, Y. K., KUN, K. W. Mechanism of action of $Ca^{2+}$ antagonists in the treatment of coronary disease with special reference to perhexiline maleate. In: Perhexiline maleate, procedings of a symposium. pp. 140-150, Excerpta Medica, Amsterdam (1976).

FOLTS, J.D., GALLAGHER, K., ROWE, G.G. Blood flow reductions in stenosed canine coronary arteries: vasospasm or platelet aggregation. Circulation 65, 248-259 (1982).

FOWLISS, R. A. F., SANG, C. T. M., LUNDY, P. M., AHUJA, S. P., COLHOUN, H. Experimental coronary artery ligation in conscious dogs six months after bilateral cardiac sympathectomy. Am Heart J 88, 748-757 (1974).

FOZZARD, H. A. Validity of myocardial infarction models. Circulation 52 (Suppl III), 131-146 (1975).

FRIEDMAN, P. L., FONOGLIS, J. J., WIT, A. L. Time course for reversal of electrophysiological and ultrastructural abnormalities in subendocardial Purkinje fibres surveying extensive myocardial infarction in dogs. Cardiol Res 37, 127 (1975).

FRIEDMAN, P. L., STEWART, J. R., WIT, A. L., Spontaneous and induced cardiac arrhythmia in subendocardial Purkinje fibers surviving extensive myocardial infarction in dogs. Circ Res 33, 612-626 (1973).

FULTON, W. F. M. The coronary arteries. Charles C. Thomas, Springfield, Ill. (1965).

FURBERG, C. D. Beta blocker heart attack trial. Drugs 25, 314, (1983a).

FURBERG, C. D. Effect of antiarrhythmic drugs on mortality after myocardial infarction. Am J Cardiol 52, 32C-36C (1983b).

FYE, W. B., The delayed diagnosis of myocardial infarction: it took half a century! Circulation 72, 262-271 (1985).

GENSINI, G. G., BRUTO DA COSTA, B. C. The coronary collateral circulation in living man. Am J Cardiol 24, 393-400 (1972).

GENSINI, G. G., KELLY, A. E., COSTA, B. C. B. DA, HUNTINGTON, P. P. Quantitative angiography: the measurement of coronary vasomobility in the intact animal and man. Chest 60, 522-530 (1971).

GETTES, L. S. Electrophysiologic basis of arrhythmias in acute myocardial ischemia. In: Oliver, M. D. (Ed.) Vol 3 Modern Trends in Cardiology, pp. 219-44, London, Butterworth (1974).

GIBSON, J. K., PATTERSON, E., LUCCHESI, B. R. Electrophysiologic, antiarrhythmic and cardiovascular actions of UM301, a quaternary ammonium compound. J Pharmacol Exp Ther 237, 318-325 (1986).

GILLESPIE, J. S., MUIR, T. C. A method of stimulating the complete sympathetic outflow from the spinal cord to blood vessels in the pithed rat. Br J Pharmacol Chemother 30, 78-87 (1967).

GILLIS, R. A., CORR, P. B., PACE, D. G., EVANS, D. E., DIMICCO, J., PEARLE, D.L. Role of the Nervous System in Experimentally Induced Arrhythmias. Cardiol 61, 37-49 (1976).

GLOOR, H. O., URTHALER, F. Differential effect of verapamil isomers on sinus node and AV junctional region. Am J Physiol 244, H80-H88 (1983).

GLOSSMAN, H., FERRY, D. R., LUEBBECKE, F., MEWES, R., HOFMANN, F. Calcium channels: direct identification with radioligand binding studies. Trends In Pharm Sci 1, 431-437 (1982).

GLOSSMAN, H., FERRY, D. R., GOLL, A., STRIESSNIG, J., ZERNIG, G., Calcium channels and calcium channel drugs: recent biochemical and biophysical findings. Arz Forsch 35, 1917-1935 (1985).

GODFRAIND, T., SALEH, M. M. Action of creatinol-o-phosphate on the contractility changes evoked by hypoxia and ischemia in rat isolated heart. Arzneim Forsch 34, 968-72 (1984).

GRANT, A. O., STARMER, C. F., STRAUSS, H. C. Antiarrhythmic drug action. Blockade of the inward sodium current. Circ Res 55, 427-439 (1984).

GRASS, A. O., LEW, M. F. DE, CINGOLANI, H. E., BLESA, E. S., Adrenergic beta-blockade and changes in plasma potassium following epinephrine administration. Eur J Pharmacol 15, 209-213 (1971).

GREGG, D. E., THORNTON, J. J., MAUTZ, F. R. The magnitude, adequacy and source of the collateral blood flow and pressure in chronically occluded coronary arteries. Am J Physiol 127, 161-175 (1939).

GREGG, M., OSTERLIN, D. UBC ANOVAR. Vancouver, UBC Computer Centre Publications. (1977).

GUELKER, H., THALE, J., HEUER, H., BENDER, F. Antifibrillatory actions of diltiazem, verapamil and nifedipine following acute coronary artery occlusion and reperfusion. Naunyn Schmiedeberg's Arch Pharmacol 324 (Suppl) 152 (1983).

GUZMAN, S. V., SWENSON, E., MITCHELL, R. Mechanism of cardiogenic shock. Circ Res 746-752 (1962).

HAASE, M., SCHILLER, U. Zur zeitlichen Parallelitat zwischen der Aktivitat ektopischer Schrittmacher und dem Eintritt von Kammerflimmern nach Ligatur eines Hauptkoronarastes beim Hund. Acta Biol Med Germ 23, 413-22 (1969).

HACHISU, M., PAPPANO, A. J. A comparative study of blockade of calcium-dependent action potentials by verapamil, nifedipine and nimodipine in ventricular muscle. J Pharmacol Exp Ther 225, 112-120 (1983).

HAEUSLER, G. Contraction of vascular muscle as related to membrane potential and calcium fluxes. J Cardiovasc Pharmacol 7 (Suppl 6), S3-S8 (1985).

HAMMER, J., PISA, Z., A method of isolated, gradual occlusion of a main branch of a coronary artery in closed chest dogs. Am Heart J 64, 67-70 (1962).

HARKEN, A. H., SIMPSON, M. B., HASELGROVE, J., WETSTEIN, L., BARLOW, C. H. Early ischemia after complete coronary ligation in the rabbit, dog, pig and monkey. Am J Physiol 241, H202-210 (1981).

HARRIS, A. S. Delayed development of ventricular ectopic rhythms following experimental coronary occlusion. Circulation 1, 1318-28 (1950).

HARRIS, A. S. Potassium and experimental coronary occlusion. Am Heart J 71, 797-802 (1966).

HARRIS, A. S. Terminal electrocardiographic patterns in experimental anoxia, coronary occlusion, and hemorrhagic shock. Am Heart J 35, 895 (1948).

HARRIS, A. S., ESTANDIA, A., TILLOTSON, R. F. Ventricular ectopic rhythms and ventricular fibrillation following cardiac sympathectomy and coronary occlusion. Am J Physiol 165, 505-512 (1951).

HARRIS, C. N., KAPLAN, M. A., PARKER, D. P., ARONOW, W. S., ELLESTAD, M. H. Anatomic and functional correlates of intercoronary collateral vessels. Am J Cardiol 30, 611-614 (1969)

HARRON, D. W. G., BREZINA, M., LILLIE, C., KOBINGER, W. Antifibrillatory properties of alinidine after coronary artery occlusion in rats. Eur J Pharmacol 110, 301-308 (1985).

HASHIMOTO, Y., HORI, R., OKUMURA, K., YASUHARA, M. Pharmacokinetics and antiarrhythmic activity of ajmaline in rats subjected to coronary artery occlusion. Br J Pharmacol 88, 71-77 (1986).

HAUSWIRTH, O., SINGH, B. N. Ionic mechanisms in heart muscle in relation to the genesis and the pharmacological control of cardiac arrhythmias. Pharmacol Rev 30, 6-63 (1978).

HEARSE, D. J. Models and problems in the study of myocardial ischemia and tissue protection. Eur Heart J 4 (Suppl C), 43-48 (1983).

HEARSE, D. J., DENNIS, S. C. Myocardial ischaemia and metabolic changes associated with the genesis of ventricular arrhythmias. In: Parratt, J. R. (Ed.) Early Arrhythmias Resulting from Myocardial Ischaemia, pp. 315-35, Macmillan Press, London (1982).

HEARSE, D. J., RICHARD, V., KINGMA, J., YELLON, D. M. The rat: an unsuitable model for the assessment of the pharmacological limitation of infarct size? J Mol Cell Cardiol 18 (Suppl 1), 201 (1986).

HEARSE, D. J., YELLON, D. M. Why are we still in doubt about infarct size limitation ? The experimentalist's viewpoint. In: Hearse, D. J., Yellow, D. M. (Eds.), Therapeutic approaches to myocardial infarct size limitation, pp. 17-41, Raven Press, New York (1984).

HEIMBURGER, R. F. Injection into pericardial sac and ligation of coronary artery of the rat. Arch Surg 52, 677-689 (1946).

HELLSTROM, H. R. The injury-vasopasm hypothesis of ischemic heart disease, revisited. Am Heart J 94, 642-648 (1977).

HELLSTROM, H. R., Vasospasm in ischaemic heart disease - a hypothesis. Perspect Biol Med 16, 427-440 (1973).

HENDERSON, A. H. The Problem and the question: A clinician's viewpoint. In: Hearse, D.J., Yellon, D.M. (Eds.) Therapeutic approaches to myocardial infarct size limitation, pp. 1-16, Raven Press, New York (1984).

HENRY, P. D. Calcium antagonists: mechanisms of action with special reference to nifedipine. In: Kharasch, N. (Ed.), Trace Metals In Health And Disease, Raven Press pp. 227-233 (1979).

HENRY, P. D. Comparative pharmacology of calcium antagonists: nifedipine, verapamil and diltiazem. Am J Cardiol 46, 1047-1058 (1980).

HERMANN, P., MORSELLI, P. L. Pharmacokinetics of diltiazem and other calcium entry blockers. Acta Pharmacol et Toxicol 57 (Suppl II), 10-20 (1985).

HESCHELER, J., PELZER, D., TRAUBE, G., TRAUTWEIN, W. Does the organic calcium channel blocker D600 act from inside or outside on the cardiac cell membrane? Pflugers Arch 393, 287-291, (1982).

HIGGINSON, L. H., THANDROYEN, F. T., OPIE, L. H. Role of calcium in ventricular arrhythmias. J Mol Cell Cardiol 15, 184, (1983).

HILL, J. L., GETTES. L. S. Effect of acute coronary artery occlusion on local myocardial extracellular $K^+$ activity in swine. Circulation 61, 768-778 (1980).

HILLIS, L. D., BRAUNWALD, E. Myocardial ischemia. N Engl J Med 296, 971-978 (1977).

HIRCHE, H. J., BISSIG, R., FRIEDRICH, R., KEBBEL, U., ZYLKA, V. Measurement of myocardial extracellular $Na^+$, $K^+$, $Ca^{2+}$ and $H^+$ using ion-selective electrodes during ischemia. In: Lubbers, D. W., Acker, H., Buck, R. P., Eisenman, G., Kessler, M., Simon, W. (Eds.) Progress in Enzyme and Ion-selective Electrodes, pp. 164-70, Springer Verlag, Berlin (1981).

HIRCHE, H. J., FRANZ, C., BOS, L., BISSIG, R., LANG, R., SCHRAMM, M. Myocardial extracellular $K^+$ and $H^+$ increase and noradrenaline release as possible cause of early arrhythmias following acute coronary artery occlusion in pigs. J Mol Cell Cardiol 12, 579-593 (1980).

HIRCHE, H. J., FRIEDRICH, R., KEBBEL, U., MCDONALD, F., ZYLKA, V. Early arrhythmias, myocardial extracellular potassium and pH. In: Parratt, J. R. (Ed.) Early arrhythmias resulting from myocardial ischaemia, pp. 113-124 MacMillan, (1982).

HOCK, C. E., BREZINSKI, M. E., LEFER, A. M. Anti-ischemic actions of a new thromboxane receptor antagonist, SQ-29,584, in acute myocardial ischaemia. Eur J Pharmacol 122, 213-219 (1986).

HODGKIN, A. L., HUXLEY, A. F. Currents carried by sodium and potassium ions through the membrane of the giant axon of Loglio. J Physiol (Lond) 116, 449-472 (1952a).

HODGKIN, A. L., HUXLEY, A. F. The components of membrane conductance in the membrane of the giant axon of Loglio. J Physiol (Lond) 116, 473-496 (1952b).

HODGKIN, A. L., HUXLEY, A. F. The dual effect of membrane potential on sodium conductance in the giant axon of Loglio. J Physiol (Lond) 116, 497-506 (1952c).

HODGKIN, A. L., HUXLEY, A. F. A quantitative description of membrane current and its application to conduction and excitation in nerve. J Physiol (Lond) 117, 500-544 (1952d).

HOFFMAN BF, DANGMAN KH in: Paes de Carvalho, A., Hoffman, B. F., Lieberman, M. (Eds.) Normal and abnormal conduction in the heart. Futura (1982).

HOLCK, M., OSTERRIEDER, W., Potential-dependent modulation of cardiac Ca channels by dihydropyridines (DHP). Experientia 41, 821 (1985).

HONDEGHEM, L. M., KATZUNG, B. G. Antiarrhythmic agents: the modulated receptor mechanism of action of sodium channel and calcium channel blocking drugs. Ann Rev Pharmacol Toxicol 24, 387-423 (1984).

HOOD, W. B., JOISON, J., KUMAR, R., KATAYAMA, I., NEIMAN, R. S., NORMAN, J. C. Experimental myocardial infarction. I. Production of left ventricular failure by gradual coronary occlusion in intact conscious dogs. Cardiovasc Res 4, 73-83 (1970).

HOPE, R. R., WILLIAMS, D. O., EL-SHERIF, N., LAZZARA, R., SCHERLAG, B. J. The efficacy of antiarrhythmic agents during acute myocardial ischemia and the role of heart rate. Circulation 50, 507-514 (1974).

HORAN, L. G., FLOWERS, N. C. Electrocardiography and vectorcardiography. In: Braunwald, E. (Ed.), Heart disease: a textbook of cardiovascular medicine. pp. 198-254, Saunders, Philadelphia/London/Toronto (1980).

HORT, W. Ventrikeldilatation und muskelfaserdehnung als fruheste morphologische befunde beim herzinfarkt. Virchows Arch Path Anat 339, 72-82 (1965).

HORT, W., CANALIS, S. DA., JUST, H. J. Untersuchungen bei chronischem experimentellen herzinfarkt der ratte. Arch Kreisl Forsch 44, 288-299 (1964)

HORT, W., CANALIS, S. DA. Untersuchungen an rattenherzen mit dauerligatur der linken kranzarterie unter besonderer berucksichtigung der infarktgrosse. Virchows Arch Path Anat 339, 53-60 (1965a).

HORT, W., CANALIS, S. DA. Untersuchungen an rattenherzen nach temporarer unterbindung der linken kranzarterie. Virchows Arch Path Anat 339, 61-71 (1965b).

HUGHES, B., KANE, K. A., MCDONALD, F. M., PARRATT, J. R. Aspects of the cardiovascular pharmacology of exaprolol J Pharm Pharmacol 36, 597-601 (1984).

HUME, J. R. Specificity of three organic calcium channel antagonists in single isolated frog atrial cells. Pharmacologist 25, 216, (1983).

HUMPHREY, P. P. A., ROBERTSON, M. J. Functional characteristics of verapamil and nitrendipine interactions at calcium channels in smooth muscle. Br J Pharmacol 80, 502P, (1983).

IIJIMA, T., CIANI, S., HAGIWARA, S. Effects of the external pH on Ca channels: experimental studies and theoretical considerations using a two-site, two-ion model. Proc Nat Acad Sci USA 83, 654-658 (1986).

INNES, I. R., WEISMAN, H. Reduction in the severity of myocardial infarction by sulfinpyrazone. Am Heart J 102, 153-157 (1981).

INOUE, F., MACLEOD, B. A., WALKER, M. J. A. Intracellular potential changes following coronary occlusion in isolated perfused rat hearts. Can J Physiol Pharmacol 62, 658-664 (1984).

ISENBERG, G. Cardiac Purkinje fibres. The slow inward current component under the influence of modified $[Ca^{2+}]_i$. E J Physiol 371, 61-69 (1977a).

ISENBERG, G. Cardiac Purkinje fibres. $[Ca^{2+}]_i$ Controls steady state potassium conductance. E J Physiol 371, 71-76 (1977b).

ISENBERG, G. Cardiac Purkinje fibres. $[Ca^{2+}]$ Controls the potassium permeability via the conductance components $g_{K1}$ and $g_{K2}$. Pflug Arch Ges Physiol 371, 77-85 (1977c).

ISENBERG, G., KLOCKNER, U. Glycocalyx is not required for slow inward current in isolated rat heart myocytes. Nature 284, 358-360 (1980).

ITO, S., SURAWICZ, B. Effect of tetraethylammonium chloride on action potential in cardiac Purkinje fibers. Am J Physiol 251, H139-H144, (1981).

ITOH, T., KANMURA, Y., KURIYAMA, H., SUZUKI, H. Nisoldipine-induced relaxation in intact and skinned smooth muscles of rabbit coronary arteries. Br J Pharmacol 83, 243-258 (1984).

JACOBSSON, L., LUNDHOLM, L., WINGREN, G. Sudden death related to advanced coronary atherosclerosis in mini-pigs: influence of some drugs. Acta Pharmacol et Toxicol 55, 1748-182 (1984).

JALIFE, J., MOE, G. K. Effect of electrotonic potentials on pacemaker activity of canine Purkinje fibers in relation to parasystole. Circ Res 39, 801 (1976).

JANG, T. L., MACLEOD, B. A., WALKER, M. J. A. Effects of halogenated hydrocarbon anesthetics on responses to ligation of a coronary artery in chronically prepared rats. Anesthesiology 59, 309-315 (1983).

JANIS, R. A., TRIGGLE, D. J. New developments in $Ca^{2+}$ antagonists. J Med Chem 26, 775-785 (1983).

JANSE, M. J. Electrophysiological changes in the acute phase of myocardial ischaemia and mechanisms of ventricular arrhythmias. In: Parratt J. R. (Ed.) Early arrhythmias resulting from myocardial ischaemia, pp. 57-80, MacMillan (1982).

JANSE, M. J., CAPELLE, F. J. L. VAN, MORSINK, H., KLEBER, A. G., WILMS-SCHOPMAN, F., CARDINAL, R., D'ALINONCOURT, C. N., DURRER, D. Flow of "injury" current and patterns of excitation during early ventricular arrhythmias in acute regional myocardial ischemia in isolated porcine and canine hearts. Circ Res 47, 151-165, (1980).

JANSE, M. J., CINCA, J., MORENA, H., FIOLET, J. W. T., KLEBER, A. G., PAUL De VRIES, G. P., BECKER, A. E., DURRER, D. The 'border zone' in myocardial ischemia. An electrophysiological, metabolic, and histochemical correlation in the pig heart. Circ Res 44, 576-88 (1979).

JANSE, M. J., KLEBER, A. G. Electrophysiological changes and ventricular arrhythmias in the early phase of regional myocardial ischaemia. Circ Res 49, 1069-1081 (1981).

JELINECK, M. V., LOHRBAUER, L., LOWN, B. Antiarrhythmic drug therapy for sporadic ventricular ectopic arrhythmias. Circulation 44, 659-665 (1974).

JENNINGS, R. B., KALTENBACH, J. P., SMETTERS, G. W. Enzymatic changes in acute myocardial ischemic injury. AMA Arch Pathol 64, 10-21 (1957).

JENNINGS, R. B., SOMMERS, H. M., SMYTH, G. A., FLACK, H. A., LINN, H. Myocardial necrosis induced by temporary occlusion of a coronary artery in the dog. Arch Pathol 70, 82-92 (1960).

JESTADT, R., SANDRITTER, W. Erfahrungen mit der TTC (Triphenyltetrazoliumchlorid) reaktion fur die pathologische-anatomische diagnose des frischen herzinfarktes. Z Kreislaufforsch 48, 802-809 (1959).

JOHNS, T. N. P., OLSON, B. J. Experimental myocardial infarction. 1. A method of coronary occlusion in small animals. Annals of Surgery 140, 675-682 (1954).

JOHNSRUDE, I. S., GOODRICH, J. K. An experimental partial occlusion device for vessels delivered by arterial catheter. AM Heart J 77, 805-808 (1969).

JOHNSTON, F. D., HILL, I. G. W., WILSON, F. N. Form of electrocardiogram in experimental myocardial infarction: early effects produced by ligation of anterior descending branch of left coronary artery. Am Heart J 10, 889-902 (1935).

JOHNSTON, K. M., MACLEOD, B. A., WALKER, M. J. A. Responses to ligation of a coronary artery in conscious rats and the actions of antiarrhythmics. Can J Physiol Pharmacol 61, 1340-1353 (1983a).

JOHNSTON, K. M., MACLEOD, B. A., WALKER, M. J. A. Effects of aspirin and prostacyclin on arrhythmias resulting from coronary artery ligation and on infarct size. Br J Pharmacol 78, 29-37 (1983b).

JOLLY, S. R., LUCCHESI, B. R. Effect of BW755C in an occlusion-reperfusion model of ischemic myocardial injury. Am Herat J 106, 8-13 (1983).

JONG, A. DE, SANTING, P. N., TIMMERMANS, P. B. M. W. M., ZWIETEN, P. A. VAN. Effect of age on the postjunctional α-adrenoceptor-mediated feedback in the heart of spontaneously hypertensive rats and normotensive Wistar Kyoto rats. Evidence for a physiological role of prejunctional $\alpha_2$-adrenoceptors. Naunyn Schmiedeberg's Arch Pharmacol 323, 33-36 (1983).

JONG-KOSTER, M. C. DE, ZWIETEN, P. A. VAN Experimental myocardial infarction in the rat. Prog Pharmacol 4, 79-88 (1981).

JUGDUTT, B. I. Delayed effects of early infarct-limiting therapies on healing after myocardial infarction. Circulation 72, 907-914 (1985).

JULIAN, D. G., VALENTINE, P. A., MILLER, G. G. Disturbances of rate, rhythm and conduction in acute myocardial infarction. Am J Med 37, 915-927 (1964).

KANE, K. A., MCDONALD, F., PARRATT, J. R., TIMMER, C., VINK, J. Anti-arrhythmic effects of Org 6001 in rats: correlation with plasma and tissue drug concentrations. Br J Pharmacol 75, 319-323 (1982).

KANE, K. A., MORCILLO-SANCHEZ, E. J., PARRATT, J. R., RODGER, I. W., SHAHID, M. The relationship between coronary artery occlusion-induced arrhythmias and myocardial cyclic nucleotide levels in the anaesthetized rat. Br J Pharmacol 84, 139-145 (1985).

KANE, K. A., PARRATT, J. R., WILLIAMS, F. M. An investigation into the characteristics of reperfusion-induced arrhythmias in the anaesthetized rat and their susceptibility to antiarrhythmic agents. Br J Pharmacol 82, 349-357 (1984).

KANE, K. A., WINSLOW, E. Antidysrhythmic and electrophysiological effects of a new antianginal agent, bepridil. J Cardiovasc Pharmacol 2, 193-203 (1980).

KANNEL, W. B., MCGEE, D. L., SCHATZKIN, A. An epidemiological perspective of sudden death. 26-Year follow-up in the Framingham study. Drugs 28 (Suppl 1), 1-16 (1984).

KANNENGIESSER, G. J., LUBBE, W. F., OPIE, L. H. Experimental myocardial infarction with left ventricular failure in the isolated perfused rat heart. Effects of isoproterenol and pacing. J Mol Cell Cardiol 7, 135-151 (1975).

KANT, K. S., SEXTON, J., POLLAK, V. E., GLAS GREENWALT, P. Calcium channel blockers inhibit plasminogen activator secretion by endothelial cells. Clin Res 33, 283A (1985).

KAPLINSKY, E., OGAWA, S., MICHELSON, E. L., DREIFUS, L. S. Instantaneous and delayed ventricular arrhythmias after reperfusion of acutely ischemic myocardium: evidence for multiple mechanisms. Circulation 63, 333-40 (1981).

KARAKI, H., NAKAGAWA, H., URAKAWA, N. Effect of calcium antagonists on release of $^{3}$H-noradrenaline in rabbit aorta. Eur J Pharmacol 101, 177-183 (1984).

KASS, R. S., LEDERER, W. J., TSIEN, R. W., WEINGART, R. Role of calcium ions in transient inward currents and aftercontractions induced by strophanthidin in cardiac Purkinje fibres. J Physiol (Lond) 281, 187-204 (1978).

KAUFMAN, N., GAVAN, T. L., HILL, R. W. Experimental myocardal infarction in the rat. A M A Arch Pathol 67, 482-488 (1959).

KAUMANN, A. J., ARAMENDIA, P. Prevention of ventricular fibrillation induced by coronary ligation. J Pharmacol Exp Ther 164, 326-332 (1968).

KAUMANN, A. J., SERUR, J. R. Optical isomers of verapamil on canine heart. Prevention of ventricular fibrillation induced by coronary artery occlusion, impaired atrioventricular conductance and negative inotropic and chronotropic effects. Naunyn Schmiedeberg's Arch Pathol 291, 347-358 (1975).

KELVIN, W. T. On the theory of the electric telegraph. Proc Royal Soc (Edinburgh) 7, 382-399 (1855).

KENAKIN, T. P., BEEK, D. The activity of nifedipine, diltiazem, verapamil and lidoflazine in isolated tissues: an approach to the determination of calcium channel blocking activity. Drug Dev Res 5, 347-358 (1985).

KENEDI, I., LOSCONCI, A. Effect of beta adrenergic blocking on the arrhythmias following coronary ligation in rat. Acta Phys Acad Sci Hung 44, 247-257 (1973b).

KENEDI, I., LOSONCI, A. Arrhythmia after left coronary ligation and the effect of isoproterenol in the rat. Acta Physiol Acad Sci Hung 43 133-141 (1973).

KENNEDY, H. L., WHITLOCK, J. A., SPRAGUE, M. K., KENNEDY, L. J., BUCKINGHAM, T. A. GOLDBERG, R. J. Long-term follow up of asymptomatic healthy subjects with frequent and complex ventricular ectopy. New Eng J Med 213, 193-197 (1985).

KENT, K. M., EPSTEIN, S. E. Neural basis for the genesis and control of arrhythmias associated with myocardial infarction. Cardiol 61, 61-74, 1976.

KHOURI, E. M., GREGG, D. E. An inflatable cuff for zero determinations in blood flow studies. J Appl Physiol 23, 395-397 (1967).

KHOURI, E. M., GREGG, D. E., LOWENSHON, H. S. Flow in the major branches of the left coronary artery during experimental coronary insufficiency in the unanaesthetised dog. Circ Res 23, 99-109 (1968).

KINOSHITA, K., HEARSE, D. J., BRAIMBRIDGE, M. V., MANNING, A. S. Beneficial effect of diltiazem on ischemia- and reperfusion-induced arrhythmias: studies in the conscious rat. J Mol Cell Cardiol 18 (Suppl 1) 266 (1986).

KISSIN, I., STANBRIDGE, R., BISHOP, S. P., REVES, J. G. Effect of halothane on myocardial infarct size in rats. Can Anaesth Soc J 28, 239-243, (1981).

KLEBER, A. G. Extracellular $K^+$ and $H^+$ shifts in early ischemia in relation to ventricular arrhythmias. J Mol Cell Cardiol 18 (Suppl 1) 76 (1986).

KLEBER, A. G., JANSE, M. J., VAN CAPELLE, F. J. L., DURRER, D. Mechanism and time course of S-T and T-Q segment changes during acute regional myocardial ischaemia in the pig heart determined by extracellular and intracellular recordings. Circ Res 42, 603-613 (1978).

KLONER, R. A., FISHBEIN, M. C., MACLEAN, D. BRAUNWALD, E., MAROKO, P. R. Effect of hyaluronidase during the early phase of acute myocardial ischemia: an ultrastructural and morphometric analysis. Am J Cardiol 40, 43-49 (1977).

KOBAYASHI, A., SUZUKI, Y., MASUMURA, Y., KAYASHI, H., ABE, M., NISHIHARA, K. Antiarrhythmic and myocardial metabolic effects of verapamil during coronary arterial reperfusion. J Mol Cell Cardiol 15 (Suppl 5), 5 (1983).

KOHLHARDT, M., FLECKENSTEIN, A. Inhibition of the slow inward current by nifedipine in mammalian myocardium. Arch Pharmacol 298, 267-272, 1977.

KOHLHARDT, M., MNICH, Z. Studies on the inhibitory effect of verapamil on the slow inward current in the mammalian ventricular myocardium. J Mol Cell Cardiol 10, 1037-1052, 1978.

KOLTAI, M., LEPRAN, I., NEMECZ, G., SZEKERES, L. Experimental myocardial infarction in adjuvant arthritic rats. J Cardiovasc Pharmacol 4, 363-369 (1982).

KOLTAI, M., LEPRAN, I., NEMECZ, G., SZEKERES, L. The possible mechanism of protection induced by dexamethasone against sudden death due to coronary ligation in conscious rats. Br J Pharmacol 79, 327-329 (1983).

KORDENAT, R. K., KEZDI, P., STANLEY, E. L. A new catheter technique for producing experimental coronary thrombosis and selective coronary visualization. Am Heart J 83, 360-364 (1972).

KOSOWSKY, B. D., TAYLOR, J., LOWN, B., RITCHIE, R. F. Long term use of procainamide following acute myocardial infarction. Circulation 47, 1204-1209 (1973).

KOVACH, A. G. B., Protective effects of gallopamil and new verapamil derivatives against brain and other vital organ damage developing during anoxia and haemorrhagic shock. In: Calcium entry blockers and tissue protection (International symposium proceedings, in press) Rome (1984).

KRAMER, J. B., SAFFITZ, J. E., WITKOWSKI, F. X., CORR, P. B. Intramural reentry as a mechanism of ventricular tachycardia during evolving canine myocardial infarction. Circ Res 56, 736-754 (1985).

KREYE, V. A. W., RUEGG, J. C., HOFMANN, F. Effects of calcium antagonist and calmodulin antagonist drugs on calmodulin-dependent contractions of chemically skinned vascular smooth muscle from rabbit renal arteries. Naunyn Schmiedeberg's Arch Pharmacol 323, 85-89 (1983).

KROLL, D. A., KNIGHT, P. R. Antifibrillatory effects of volatile anesthetics in acute occlusion/reperfusion arrhythmias. Anesthesiol 61, 657-661 (1984).

KUWADA, M., TATEYAMA, T., TSUTSUMI, J. Simultaneous determination of verapamil and its seven metabolites by high performance liquid chromatography. J Chromatog 222, 507-511 (1981).

LANGENDORF, R., PICK, A. Parasystole with fixed coupling. Circulation 35, 304 (1967).

LANGENDORFF, O. Untersuchungen am uberlebenden saugetierherzen. Pflug Arch Ges Physiol 61, 291-295 (1895).

LANGER, G. A. Interspecies variation in myocardial physiology: the anomalous rat. Environ Health Perspect 26, 175-179 (1978).

LAZZARA, R., EL-SHERIF, N., HOPE, R. R., SCHERLAG, B. J. Ventricular arrhythmias and electrophysiological consequences of myocardial ischaemia and infarction. Circ Res 42, 740-749 (1978).

LAZZARA, R., EL-SHERIF, N., SCHERLAG, B. J. Electrophysiological properties of canine Purkinje cells in one day old myocardial infarction. Circ Res 722-734 (1973).

LEDERER, W. J., TSIEN, R. W. Transient inward current underlying arrhythmogenic effects of cardiotonic steroids in Purkinje fibres. J Physiol 263, 73-100 (1976).

LEE, J. Y., KRUEGER, A. D., WALSH, G. M. Hemodynamic effects of intravenous verapamil and nifedipine in spontaneously hypertensive rats. Fed Proc 46, 1085 (1983).

LEE, K. S., AKIAKE, N., BROWN, A. M. The suction pipette method for internal perfusion and voltage clamp of small excitable cells. J Neuroscience Methods 2, 51-78 (1980).

LEE, K. S., TSIEN, R. W. Mechanism of calcium channel blockade by verapamil, D600, diltiazem and nitrendipine in single dialysed heart cells. Nature 302, 790-794, (1983).

LEE, K. S., WEEKS, T. A., KAO, R. L., AKAIKE, N., BROWN, A. M. Sodium current in single heart muscle cells. Nature 278, 269-271, (1979).

LEE, R. J., BAKY, S. H. The influence of nitroglycerin and dipyridamole on pacing-induced ST segment depression in unanaesthetised, atherosclerotic rabbits. J Pharmacol Exp Ther 184, 205-212 (1973).

LEINBURGER, H., SUCHIRO, G. T., MCNAMARA, J. J. Myocardial platelet trapping after coronary ligation in primates (Papio anubis). Platelet trapping in infarct marginal zone. J Surg Res 27, 36-40 (1979).

LEINOT, M., BASIEZ, M., TISNE VERSAILLES, J., LAMAR, J. C. Le modele des "rats ligatures": un test de choix pour le screening des antiarrhythmiques et/ou anti-ischemiques. J Pharmacol 14, 238-239 (1983).

LEIRIS, J. DE, PESTRE, S., BERNIER, M. Pharmacological protection against myocardial ischemia in the rat. Abstract Naunyn-Schmiedebergs Arch Pharmacol 324, Suppl., R9 (1983).

LEPRAN, I., KOLTAI, M., SZEKERES, L. Effect of non-steroid anti-inflammatory drugs in experimental myocardial infarction in rats. Eur J Pharmacol 69, 235-238 (1981a).

LEPRAN, I., KOLTAI, M., SIEGMUND, W., SZEKERES, L. Coronary artery ligation, early arrhythmias and determination of the ischemic area in conscious rats. J Pharmac Methods 9, 219-230 (1983).

LEPRAN, I., KOLTAI, M., SZEKERES, L. Effect of actinomycin D and cycloheximide on experimental myocardial infarction in rats. Eur J Pharmacol 77, 197-199, (1982).

LEPRAN, I., NEMECZ, Gy., KOLTAI, M., SZEKERES, L. Effect of a linoleic acid-rich diet on the acute phase of coronary occlusion in conscious rats, influence of indomethacin and aspirin. J Cardiovasc Pharmacol 3, 847-853 (1981b).

LEPRAN, I., PARRATT, J. R., SZEKERES, L., WAINWRIGHT, C. L. The effects of metoprolol and dazmegrel, alone and in combination, on arrhythmias induced by coronary occlusion in conscious rats. Br J Pharmacol 86, 229-234 (1985).

LEPRAN, I., SIEGMUND, W., SZEKERES, L. A method of acute coronary occlusion in anaesthetized closed chest rats. Abstract Acta Physiol Hung Acad Sci 53, 190 (1979a).

LEPRAN, I., SZEKERES, L. Effect of prolonged treatment with antiarrhythmic and antianginal drugs on the incidence of ventricular fibrillation and death in the acute phase of myocardial infarction in awake rats. 3rd Congr Hung Pharmacol Soc 59-62 (1979a).

LIBERTHSON, R. R., NAGEL, E. L., RUSKIN, J. N. Pathophysiology, clinical course and management of prehospital ventricular fibrillation and sudden cardiac death. In: Adgey, A. A. J. (Ed.), Acute phase of ischaemic heart disease and myocardial infarction. pp. 165-182. Martinus Nijhoff, Hague /Boston/London (1982).

LIE, K. I., WALLENS, H. J., CAPELLE, F. J. VAN, DURRER, D. Lidocaine in the treatment of primary ventricular fibrillation. New England J Med 291, 1324-1325 (1974).

LLUCH, S., MOGUILEVSKY, H. C., PIETRA, G., SHAFFER, A. B., HIRSCH, L. J., FISHMAN, A. P. A reproducible model of cardiogenic shock in the dog. Circulation 39, 205-218 (1969).

LOVEGROVE, T., THOMPSON, P. The role of acute myocardial infarction in sudden cardiac death - a statistician's nightmare. Am Heart J 96, 711-713 (1978).

LOWN, B. Management of patients at high risk of sudden death. Am Heart J 103, 689-697 (1982).

LOWN, B. TEMTE, J. V., ARTER, W. J. Ventricular tachyarrhythmias: clinical aspects. Circulation 47, 1364-1381 (1973).

LOWN, B., FAKHRO, A. M., HOOD, W. B. Jr., THORN, G. W. The coronary care unit. New perspectives and direction. J Am Med Assoc 199, 188-198 (1967).

LUBBE, W. F. Influence of potassium on arrhythmogenesis. J Mol Cell Cardiol 18 (Suppl 1), 185 (1986).

LUBBE, W. F., DARIES, F. S., OPIE, L. H. Ventricular arrhythmias associated with coronary artery occlusion and reperfusion in the isolated perfused rat heart: a model for assessment of antifibrillatory action of antiarrhythmic agents. Cardiovasc Res 12, 212-220 (1978).

LUCCHESI, B. R., ROMSON, J. L., JOLLY, S. R., KUNKEL, S., ABRAMS, G. Role of inflammation in experimental canine myocardial infarction. J Mol Cell Cardiol 15 (Suppl 4), 32 (1983).

LYNCH, J. J., RAHWAN, R. G. Comparison of the characteristics of the negative inotropic actions of dinitrophenol, rotenone, antimycin A and the intracellular calcium antagonist, propyl-methylenedioxyindene. Gen Pharmacol 14, 437-443, (1983).

LYNCH, J. J., VOGEL, S., SPERELAKIS, N. Halothane depresesion of myocardial slow action potentials. Anesthesiol 55, 360-368, (1981)

MACLEAN, D., FISHBEIN, M. C., MAROKO, P. R., BRAUNWALD, E. Hyaluronidase-induced reductions in myocardial infarct size. Science 194, 199-200 (1976).

MACLEOD, B. A., AUGEREAU, P., WALKER, M. J. A. Effects of halothane anesthesia compared with fentanyl anesthesia and no anesthesia during coronary ligation in rats. Anesthesiology 58, 44-52 (1983).

MAINLAND, D., HERRERA, L., SUTCLIFFE, M. I. Statistical tables for use with binomial samples - contingency tests, confidence limits and sample size estimates. Department of medical statistics publications, New York medical college of medicine, New York (1956).

MANDERS, T. W., VATNER, S. F. Effect of sodium pentobarbital anesthesia on left ventricular function and distribution of cardiac output in dogs. Circ Res 39, 512-517 (1976).

MANNHOLD, R., STEINER, R., HAAS, W., KAUFMANN, R. Investigations on the structure-activity relationships of verapamil. Arch Pharmacol 302, 217-226, (1978).

MANNHOLD, R., ZIERDEN, P., BAYER, R., RODENKIRCHEN, R., STEINER, R. The influence of aromatic substitution on the negative inotropic action of verapamil in the isolated cat papillary muscle. Arzneim Forsch 31, 773-780 (1981).

MANNING, A. S. Reperfusion-induced arrhythmias: a possible role for xanthine oxidase-derived free radicals. J Mol Cell Cardiol 18 (Suppl 1), 197 (1986).

MANNING, A. S., COLTART, D. J., HEARSE, D. J. Ischemia and reperfusion-induced arrhythmias in the rat. Effects of xanthine oxidase inhibition with allopurinol. Circ Res 55, 545-548 (1984).

MARKIEWICZ, W., FINBERG, J. P. M., LICHTIG, C. Morphine increases myocardial infarction size in rats. Anesth Analg 61, 843-846 (1982).

MAROKO, P. R., KJEKUS, J. K., SOBEL, B. E., WATANABE, T., COVELL, J. W., ROSS, J. Jr., BRAUNWALD, E. Factors influencing infarct size following experimental coronary artery occlusions. Circulation 43, 67-82, 1971.

MARSHALL, R. J., MUIR, A. W., WINSLOW, E. Development of a severe model of early coronary artery ligation-induced dysrhythmias in the anaesthetized rat. Br J Pharmacol 73, 951-959 (1981a).

MARSHALL, R. J., MUIR, A. W., WINSLOW, E. Comparative antidysrhythmic and haemodynamic effects of orally or intravenously administered mexiletine and Org 6001 in the anaesthetized rat. Br J Pharmacol 74, 381-388 (1981b).

MARSHALL, R. J., MUIR, A. W., WINSLOW, E. The effects of postligation administration of Org 6001 and disopyramide on early ischaemia-induced arrhythmias in the anaesthetized rat. Br J Pharmacol 76, 501-503 (1982).

MARSHALL, R. J., MUIR, A. W., WINSLOW, E. Effects of antiarrhythmic drugs on ventricular fibrillation thresholds of normal and ischaemic myocardium in the anaesthetized rat. Br J Pharmacol 78, 165-171 (1983).

MARSHALL, R. J., MUIR, A. W., WINSLOW, E. A comparison of the intensity and duration of the antidysrhythmic effects of mexiletine and Org 6001 in anaesthetized rats. Br J Pharmacol 73, 199p-200p (1981c).

MARSHALL, R. J., WINSLOW, E. The antidysrhythmic and cardiovascular effects of the aminosteroid, Org 6001. Gen Pharmacol 12, 315-322 (1981).

MARSHALL, R. J., WINSLOW, E. The effects of sodium channel inhibitors on early arrhythmias associated with acute myocardial ischaemia. In: Parratt, J. R. (Ed.) Early arrhythmias resulting from myocardia ischaemia, mechanisms and prevention by drugs, pp. 251-294 MacMillan Press, London (1982).

MARTINEZ, T. T., CRAMPTON, J. M. Prostaglandins show marked differences when tested against early ischemic dysrhythmias in rat. Life Sci 29, 1449-56 (1981).

MASCHER, D., PEPER, K. Two components of inward current in myocardial muscle fibers. Eur J Physiol 307, 190-203, 1969.

MASERI, A., SEVERI, S., MARZULLO, P. Ann NY Acad Sci 382, 204-216 (1982).

MATTREY, R., MITTEN, R. Enhancement of myocardial infarctions with perfluorocarbon compounds; documentation. Invest Radiol 19, S48 (1984).

MATZUI, K., NAKAZAWA, M., TAKEDA, K., IMAI, S. Effect of l-carnitine chloride and its acetyl derivative on the electrophysiological derangement induced by palmityl-l-carnitine in isolated canine ventricular muscle. Jap J Pharmacol 39, 263-270 (1985).

MAXWELL, M. P., HEARSE, D. J., YELLON, D. M. The coronary collateral circulation during regional myocardial ischemia: a comparative study in eight species. J Mol Cell Cardiol 16, Suppl. 2, 22 (1984).

MAY, G. S. A review of acute-phase beta-blocker trials in patients with myocardial infarction. Circulation 67, 21-25 (1983a).

MAY, G. S. A review of long-term beta-blocker trials in survivors of myocardial infarction Circulation 67, 46-49 (1983b).

MAY, G. S., FURBERG, C. D., EBERLEIN, K. A., GERACI, B. J. Secondary prevention after myocardial infarction: a review of short term acute phase trials. Prog Cardiovasc Disease 25, 335-359 (1983).

MCALLISTER, R. E., NOBLE, D. The time and voltage dependence of the slow outward current in cardiac Purkinje fibres. J Physiol (Lond) 186, 632-662 (1966).

MCALLISTER, R. G. Jr., HAMANN, S. R., PIASCIK, M. T. Aspects of the clinical pharmacology of verapamil, a calcium-entry antagonist. Biopharm Drug Dispos 4, 203-231 (1983).

MCALLISTER, R. G. Jr., HOWELL, S. M. Fluorometric assay of verapamil in biological fluids and tissues. J Pharmaceut Sci 65, 431-432, (1976).

MCDONALD, T. F. The slow inward calcium current in the heart. Ann Rev Physiol 44, 425-434 (1982).

MCDONALD, T. F., PELZER, D., TRAUTWEIN, W. On the mechanism of slow calcium channel block in heart. Pflugers Arch ges Physiol 385, 175-179 (1980).

MCDONALD, T. F., TRAUTWEIN, W. Membrane currents in cat myocardium: separation of inward and outward components. J Physiol (Lond) 274, 193-216 (1978).

MCLENNAN, P. L. Cardiac arrhythmias and myocardial infarction: influence of dietary fat. J Mol Cell Cardiol 18 (Suppl 1), 31 (1986).

MCLENNAN, P. L., ABEYWARDENA, M. Y., CHARNOCK, J. S. Influence of dietary lipids on arrhythmias and infarction after coronary artery ligation in rats. Can J Physiol Pharmacol 63, 1411-1422 (1985).

MCLENNAN, P. L., CHARNOCK, J. S. Dietary lipids alter susceptibility to cardiac arrhythmias and infarction in rats. IUPHAR 9th Internat Cong of Pharmacol, 489P (1984).

MEEL, J. C. A. VAN, JONG, A. DE, WILFFERT, B., KALKMAN, H.O., TIMMERMANS, P. B. M. W. M., ZWIETEN, P. A. VAN. Organic and inorganic calcium antagonists reduce vasoconstriction in-vivo mediated by post-synaptic $\alpha_2$-adrenoceptors. Naunyn Schmiedeberg's Arch Pharmacol 316, 228-293 (1981).

MEESMANN, W. Early arrhythmias and primary ventricular fibrillation after acute myocardial ischaemia in relation to pre-existing coronary collaterals. In: Parratt, J. R. Early arrhythmias resulting from myocardial ischaemia, pp. 93-112 MacMillan Press, London, (1982).

MERIDETH, J., MENDEZ, C., MUELLER, W. J., MOE, G. K. Electrical excitability of atrioventricular nodal cells. Circ Res 23, 69-85, (1968).

MERTZ, T. E., KAPLAN, H. R. Pirmenol hydrochloride (C1-845) and reference antiarrhythmic agents: effects on early ventricular arrhythmias after acute coronary artery ligation in anesthetized rats. J Pharmacol Exp Ther 223, 580-586 (1982).

MICHAELIS, M. Uber einige ergbnisse derligatur der kranzarterien des herzens. Z Klin Med 24, 270-294 (1894).

MILLARD, R. W., GRUPP, G., GRUPP, I. L., Di SALVO, J., De POVER, A., SCHWARTZ, A. Chronotropic, inotropic, and vasodilator actions of diltiazem, nifedipine, and verapamil. A comparative study of physiological responses and membrane receptor activity. Circ Res 52, Suppl. 1, 29-39 (1983).

MILMER, K. E., CLOUGH, D. P. Optimum ventilation levels for maintenance of normal arterial blood $pO_2$, $pCO_2$ and pH in the pithed rat preparation. J Pharmacol Methods 10, 185-192 (1985).

MINES, G. R. On the dynamic equilibrium in the heart. J Physiol 46, 350-383, (1913).

MOE, G. K., ABILDSKOV, J. A., HAN, J. Factors responsible for the initiation and maintenance of ventricular fibrillation. In: Surawicz, B., Pellegrino, E. D. (Eds.) Sudden cardiac death. Grune and Stratton, New York (1964).

MOORE, J. W., BLAUSTEIN, M. P., ANDERSON, N. C., NARAHASHI, T. Basis of tetrodotoxin's selectivity in blockage of squid axons. J Gen Physiol 50, 1401-1411 (1967).

MORGAN JONES, A. Preclinical coronary heart disease. In: Morgan A. Jones (Ed.) Modern Trends in Cardiology, Vol 2 pp. 36-45 Butterworth, London (1969).

MOTULSKY, H. J., SNAVELY, M. P., HUGHES, R. J., INSEL, P. A. Interaction of verapamil and other calcium channel blockers with $\alpha_1$- and $\alpha_2$-adrenergic receptors. Circ Res 52, 226-231 (1983).

MOVSESIAN, M. A., ADELSTEIN, R. S., AMBUDKAR, I. S., SHAMOO, A. E. Stimulation of cardiac sarcoplasmic reticulum calcium uptake by dihydropyridine calcium antagonists. Clin Res 32, 474A (1984).

MUELLER, B., SCHNEIDER, J., HENNIES, H. H., FLOHE, L. Cardioprotective action of the new stable epoprostenol analogue, CG 4203 in rat models of cardiac hypoxia and ischemia. Arzneimittel Forschung 34, 1506-1509 (1984).

MUELLER, B., WILSMANN, K. Effects of the optical isomers of D 600 on cardiovascular parameters and on arrhythmias caused by aconitine and coronary artery ligation in anaesthetised rats. J Cardiovasc Pharmacol 4, 615-621 (1982).

MULLANE, K. M., READ, N., SALMON, J. A., MONCADA, S. Role of leucocytes in acute myocardial infarction in anaesthetized dogs: relationship to myocardial salvage by anti-inflammatory drugs. J Pharm Exp Ther 228, 510-522 (1984).

NABATA, H. Effects of calcium antagonistic vasodilators on myocardial contractility and membrane potentials. Jap J Pharmacol 27, 239-249 (1976).

NACHLAS, M. M., SHNITKA, T.K. Macroscopic identification of early myocardial infarcts by alterations in dehydrogenase activity. Am J Path 43, 379-405, 1963.

NAKAYAMA, K., KURIHARA, J., MIYAJIMA, Y., ISHII, K., KATO, H. Calcium antagonistic properties of nicardipine, a dihydropyridine derivative, assessed in isolated cerebral arteries and cardiac muscle. Arz Forsch 35, 687-693 (1985).

NAKHJAVAN, F. K., SHEDROVILZKY, H., GOLDBERG, H. Experimental myocardial infarction in dogs. Description of a closed chest technique. Circulation 38, 777-782 (1968).

NAWRATH, H. RUPP, J. Adrenoceptor-mediated changes of action potential and force of contraction in human ventricular heart muscle. J Mol Cell Cardiol 18 (Suppl 1), 325 (1986).

NAWRATH, H., BLEI, I., GEGNER, R., LUDWIG, C., ZONG, X. No stereospecific effects of the optical isomers of verapamil and D-600 on the heart. In: Zanchetti, A., Krikler D. M. (Eds.), Calcium antagonists in cardiovascular therapy, pp. 52-63, Excerpta Medica, Amsterdam-Oxford-Princeton (1981).

NAYLER, W. G. The heterogeneity of slow channel blockers (calcium antagonists). Int J Cardiol 3, 391-400 (1983).

NAYLER, W. G., HOROWITZ, J. D. Calcium antagonists: a new class of drugs. Pharmacol Ther 20, 203-262 (1983).

NAYLER, W. G., STURROCK, W. J. An inhibitory effect of verapamil and diltiazem on the release of noradrenaline from ischaemic and reperfused hearts J Mol Cell Cardiol 16, 331-344 (1983).

NEW, W., TRAUTWEIN, W. The ionic nature of the slow inward current and its relation to contraction. Pflug Arch Ges Physiol 334, 24-38 (1972).

NEWMAN, P. E. The coronary collateral circulation: determinants and functional significance in ischemic heart disease. Am Heart J 102, 431-445 (1981).

NOBLE, D. A modification of the Hodgkin-Huxley equations applicable to Purkinje fibre action and pacemaker potentials. J Physiol 160, 317-352 (1962).

NOBLE, D. Cardiac action and pacemaker potentials based on the Hodgkin-Huxley equations. Nature 188, 495-487 (1960).

NOBLE, D., TSIEN, R. W. Outward membrane currents activated in the plateau range of potentials in cardiac Purkinje fibres. J Physiol (Lond) 200, 233-254 (1969).

NOMA, A., MORAD, M., IRISAWA, H. Does the "pacemaker current" generate the diastolic depolarization in the rabbit SA node cells? Pflug Arch 397, 190-194 (1983).

NONEMAN, J. W., ROGERS, J. F. Lidocaine prophylaxis in acute myocardial infarcton. Medicine 57, 501-15 (1978).

NORDERHAUG, J. E., LIPPE, G. VON DER. Hypokalaemia and ventricular fibrillation in acute myocardial infarction. Br Heart J 50, 525-529 (1983).

NORDREHAUG, J. E., LIPPE, G. VON DER. Independent inverse association between serum potassium concentrations and ventricular arrhythmias in acute myocardial infarction. Circulation 72 (Suppl II), 1441 (1985).

NORMAN, J. A., ANSELL, J., PHILLIPS, M. A. Dihydropyridine $Ca^{2+}$ entry blockers selectively inhibit peak 1 cAMP phosphodiesterase. Eur J Pharmacol 93, 107-112 (1983).

NORMANN, S. J., PRIEST, R. E., BENDITT, E. P. Electrocardiogram in the normal rat and its alteration with experimental coronary occlusion. Circ Res 9, 282-287 (1961).

NORTHOVER, B. J. A comparison of the effects of bethanidine, meobentine and quinidine on the electrical activity of rat hearts in vivo and in vitro. Br J Pharmacol 84, 755-763 (1985).

NORTHOVER, B. J. Indomethacin - a calcium antagonist. Gen Pharmacol 8, 293-296 (1977).

NORTHOVER, B. J. A protective effect of sulphinpyrazone against coronary occlusion-induced shortening of myocardial refractory periods in the rat. Br J Pharmacol 88, 141-148 (1986).

NYBORG, N. C. B., MULVANY, M. J. Effect of felodipine, a new dihydropyridine vasodilator, on contractile responses to potassium, noradrenaline and calcium in mesenteric resistance vessels of the rat. J Cardiovasc Pharmacol 6, 449-457 (1984).

OLIVER, M. F. Sudden cardiac death - an overview. In: Parratt, J. R. (Ed.) Early arrhythmias resulting from myocardial ischaemia, pp. 1-14 MacMillan Press, London (1982).

OPIE, L. H., COETZEE, W. A., YON, E. Y. Components of ischaemia: effects on delayed afterdepolarizations and arrhythmogenesis. J Mol Cell Cardiol 18 (Suppl 1), 74 (1986).

OPIE, L. H., THANDROYEN, F. T. Calcium antagonists, ventricular fibrillation, and enzyme release in ischemic rat hearts Fed Proc 42, 2465-2469 (1983).

PANTRIDGE, J. F. Autonomic disturbance at the onset of acute myocardial infarction. In: Schwartz, P. J., Brown, A. M., Malliani, A., Zanchetti, A. (Eds.), Neural mechanisms in cardiac arrhythmias, pp. 7-17, New York, Raven Press (1978).

PARDEE, H. E. B. An electrocardiographic sign of coronary artery obstruction. Arch Int Med 26, 244-257 (1920).

PATTERSON, E., ELLER, B. T., LUCCHESI, B. R. Effects of diltiazem upon experimental ventricular dysrhythmias. J Pharm Exp Ther 225, 224-233 (1983).

PATTERSON, E., GIBSON, J. K., LUCCHESI, B. R. Postmyocardial infarction re-entrant ventriculat arrhythmias in conscious dogs: suppression by bretylium tosylate. JPET 216, 453 (1981).

PAYET, M. D., SCHANNE, O. F., RUIZ-CERETTI, E. Frequency dependence of ionic currents determining the action potential repolarization in rat ventricular muscle. J Mol Cell Cardiol 13, 207-215 (1981).

PAYET, M. D., SCHANNE, O. F., RUIZ-CERETTI, E. Competition for slow channel of $Ca^{2+}$, $Mn^{2+}$, verapamil, and D-600 in rat ventricular muscle. J Mol Cell Cardiol 12, 635-638 (1980a).

PAYET, M. D., SCHANNE, O. F., RUIZ-CERETTI, E., DEMERS, J.-M. Inhibitory activity of blockers of the slow inward current in rat myocardium, a study in steady state and of rate of action. J Mol Cell Cardiol 12, 187-200 (1980b).

PEARSON, A., DILLMAN, J. M., HEPTINSTALL, R. H. The hypercholesterolemic hare: a promising new model of human atherosclerosis. Circulation 68 (Suppl II), 303 (1983).

PELZER, D., TRAUTWEIN, W., MCDONALD, T. F. Calcium channel block and recovery from block in mammalian ventricular muscle treated with organic channel inhibitors. Pflugers Arch ges Physiol 394, 97-105 (1982).

PENTECOST, B. L., GIOVANNI, J. V. DE, LAMB, P., CADIGAN, P. J., EVEMY, K. L., FLINT, E. J. Reappraisal of lignocaine therapy in management of myocardial infarction. Br Heart J 45, 42-47 (1981).

PEON, J., GONZALEZ, E., HONG, E., Effect of ropitoin on cardiac arrhythmias and infarct size induced by coronary artery ligation in the rat. Pharmacologist 27 (Part 3), 113 (1985).

PETTY, C. (Ed.). Research techniques in the rat, Charles C. Thomas, Springfield, Ill. (1982).

PFEFFER, J. M., PFEFFER, M. A., BRAUNWALD, E. Influence of chronic captopril therapy on the infarcted left ventricle of the rat. Circ Res 57, 84-95 (1985).

PFEFFER, J. M., PFEFFER, M. A., MIRSKY, I., STEINBERG, C. R., BRAUNWALD, E. Progressive ventricular dilatation and diastolic wall stress in rats with myocardial infarction and failure. Circulation 66, Suppl. II, 66 (1982).

PFEFFER, M. A., PFEFFER, J. M., FISHBEIN, M. C., FLETCHER, P. J., SPADARO, J., KLONER, R. A., BRAUNWALD, E. Myocardial infarct size and ventricular function in rats. Circ Res 44, 503-512 (1979).

PITT, A., FRIESINGER, G. C. ROSS, R. S. Measurement of blood flow in the right and left coronary artery beds in humans and dogs using the $^{113}$xenon technique. Cardiovasc Res 3, 100-106 (1969).

POLLEN, D. W., SCOTT, A. C., WALLACE, W. F. M. A comparison of the direct effects and adrenergic blocking activity of D/L- and D-propranolol on the electrical and mechanical behaviour of isolated frog ventricles. Cardiovasc Res 3, 7-13 (1969).

POOLE-WILSON, P. A. Angina - pathological mechanisms, clinical expression and treatment. Postgraduate Med J 59 (Suppl 3), 11-21 (1983).

PRINZMETAL, M., KENNAMER, R., MERLISS, R., WADA, T., BOR, N. Angina pectoris I. A variant form of angina pectoris (preliminary report). Am J Med 27, 375 (1959).

PRINZMETAL, M., TOYOSHIMA, H., EKMEKCI, A., MIZUNO, Y., NAGAYU, T. Myocardial ischemia. Nature of ischemic electrocardiographic patterns in the mammalian ventricles as determined by intracellular electrography and metabolic changes. Am J Cardiol 8, 495-503 (1961).

PRUM, I. A., LISHMANOV, Y. B., SLEPUSHKIN, V. D. Influence of enkephalins on vasopressin and aldosterone levels in experimental acute myocardial ischemia (translation from Russian). Byull Eksp Biol Med 97, 18-19 (1984).

RABKIN, S. W., MATHEWSON, F. A. L., TATE, R. B. The electrocardiogram in apparently healthy men and the risk of sudden death. Br Heart J 47, 546-552 (1982).

RAHWAN, R. G. Mechanisms of action of membrane calcium channel blockers and intracellular calcium antagonists. Med Res Rev 3, 21-42 (1983).

RASCHACK, M. Anipamil, a novel calcium antagonist with long-lasting oral efficacy, as assessed against hypoxic and ischaemic damage of heart and kidney. In: Calcium entry blockers and tissue protection (International symposium proceedings, in press) Rome (1984).

RASCHACK, M. Differences in the cardiac actions of the calcium antagonists verapamil and nifedipine. Arzniem Forsch 26, 1330-1333 (1976a).

RASCHACK, M. Relationship of antiarrhythmic to inotropic activity and antiarrhythmic qualities of the optical isomers of verapamil. Naunyn-Schmiedeberg's Arch Pharmacol 294, 285-291 (1976b).

REIMER, K. A., JENNINGS, R. B., COBB, F. R., MURDOCK, R. H., GREENFIELD, J. C. Jr., BECKER, L. C., BULKLEY, B. H., HUTCHINS, G. M., SCHWARTZ, R. P. Jr., BAILEY, K. R., PASSAMANI, E. R. Animal models for protecting ischemic myocardium: results of the NHLBI cooperative study. Comparison of unconscious and conscious dog models. Circ Res 56, 651-665 (1985).

REIMER, K. A., JENNINGS, R. B. Effects of calcium channel blockers on myocardial preservation during experimental acute myocardial infarction. Am J Cardiol 55, 107B-115B (1985).

REUTER, H. Divalent cations as charge carriers in excitable membranes. Prog Biophys Mol Biol 26, 1-43 (1973).

REUTER, H. Properties of two inward membrane currents in the heart. Ann Rev Physiol 41, 413-424 (1979).

REUTER, H., BEELER, G. W. Jr. Calcium current and activation of contraction in ventricular myocardial fibers. Science 163, 399-401 (1969).

REUTER, H., SCHOLZ, H. A study of the ion selectivity and the kinetic properties of the calcium dependent slow inward current in mammalian muscle. J Physiol (Lond) 264, 17-47 (1977).

RIBIELIMA, J. Selective embolization of the coronary arteries. A haemodynamic, metabolic and radiologic study. Proc Soc Exp Biol Med 117, 367-369 (1964).

RICHARD, V., PESTRE, S., LEIRIS, J. DE. Effect of four calcium antagonists, bepridil, diltiazem, nifedipine and verapamil on infarct size following a 48 hours permanent coronary occlusion in the rat. J Mol Cell Cardiol 16 (Suppl 2), 162 (1984).

ROBERTS, R., HUSAIN, A., AMBOS, H. D., OLIVER, G. C., COX, J. R., SOBEL, B. E. Relationship between infarct size and ventricular arrhythmias. Br Heart J 37, 1169-1175 (1975).

ROCHETTE, L., DIDIER, J. P., MOREAU, D., BRALET, J., OPIE, L. H. Role of beta-adrenoceptor antagonism in the prevention of reperfusion ventricular arrhythmias: effects of acebutolol, atenolol, and d-propranolol on isolated working rat hearts subject to myocardial ischemia and reperfusion. Am Heart J 107, 1132-41 (1984).

ROMSON, J. L., HOOK, B. G., RUIKEL, S. L., ABRAMS, E. D., SCHONK, M. A., LUCCHESI, B. R. Reduction of the extent of ischemic myocardial injury by neutrophil depeletion in the dog. Circulation 67, 1016-1023 (1983).

ROOS, A., SMITH, J. R. Production of experimental heart failure in dogs with intact circulation. Am J Physiol 153, 558-566 (1948).

ROY, G. V. LE, FENN, G. G., GILBERT, N. C. The influence of xanthine drugs and atropine on the mortality rate after experimental occlusion of a coronary artery. Am Heart J 23, 637-643 (1942).

RUSHMER, R. F., WATSON, N., HARDING, D., BAKER, D. Effects of acute coronary occlusion on performance of right and left ventricles in intact unanesthetized dogs. Am Heart J 66, S22-S31 (1963).

RUSKIN, J. N., SCHOENFELD M. H., GARAN, H. Role of electrophysiologic techniques in the selection of antiarrhythmic drug regimens for ventricular arrhythmias. Am J Cardiol 52, 41C-46C (1983).

SALAZAR, A. E. Experimental myocardial infarction. Induction of coronary thrombosis in the intact closed-chest dog. Circ Res 9, 1351-1356 (1961).

SANGUINETTI, M. C., WEST, T. C. Comparison of papaverine and verapamil on frequency-dependent changes in $V_{max}$ of K-depolarized ventricular tissue. J Cardiovasc Pharmacol 4, 791-802 (1982).

SASYNIUK, B. I. Concept of reentry versus automaticity. Am J Cardiol 54, 1A-6A (1984).

SASYNIUK, B. I., NATTEL, S. Lidocaine - problems in understanding an antiarrhythmic drug. TIPS July 1982, 297-300 (1982).

SASYNIUK, B., MENDEZ, C. A mechanism for reentry in canine ventricular tissue. Circ Res 28, 3-15 (1971).

SCHAAL, S. F., WALLACE, A. G., SEALY, W. C. Protective influence of cardiac denervation against arrhythmias of myocardial infarction. Cardiovasc Res 3, 241-244 (1969).

SCHAPER, W. Collaterals in chronic coronary occlusion. In: Schaper, W. (Ed.) Pathophysiology of Myocardial Perfusion, pp. 415-70 Elsevier, North Holland, Amsterdam (1979).

SCHAPER, W. Comparative angiography of the collateral circulation. In: Black, D.A.K. (Ed.) The Collateral Circulation of the Heart. North Holland Co., Amsterdam (1971).

SCHAPER, W. Experimental myocardial infarction. Triangle 19, 3-9 (1980).

SCHAPER, W. Experimental Infarcts and the Microcirculation. In: Hearse, D.J., Yellon, D.M. (Eds.), Therapeutic approaches to myocardial infarct size limitations, pp. 79-90, Raven Press, N. Y. (1984).

SCHAPER, W. The role of animal models for pharmacologic interventions aimed at reduction of infarct size. Naunyn Schmiedeberg's Arch Pharmacol 324 (Suppl) R8 (1983).

SCHAPER, W., JAGENEAU, A., XHONNEUX, R. The development of collateral circulation in the pig and dog heart. Cardiologia 51, 321-335 (1967).

SCHAPER, W., SCHAPER, J., WINKLER,B. The collateral circulation of the heart. J Mol Cell Cardiol 18 (Suppl 1), 60 (1986).

SCHERLAG, B. J., HELFANT, R. H., HAFT, J. I., DAMATO, A. N. Electrophysiology underlying ventricular arrhythmias due to coronary ligation. Am J Physiol 219, 1665-1671 (1970).

SCHMITT, F. O., ERLANGER, J. Directional differences in the conduction of the impulse through heart muscle and their possible relation to extrasystolic and fibrillary contractions. Am J Physiol 87, 326-347 (1928).

SCHOENFELD, P., BROTELLE, R., BRUYNINCK, C., FEYS, G., CHATELAIN, P. Efficacy of amiodarone pretreatment on coronary artery ligation dysrhythmias in rats. IUPHAR 9th Internat Cong of Pharmacol, 424P (1984).

SCHOMERUS, M., SPIEGELHALDER, B., STIEREN, B., EICHELBAUM, M. Physiological disposition of verapamil in man. Cardiovasc Res 10, 605-612, 1976.

SCHWEITZER, P., MARK, H. Delayed repolarization syndrome. Am J Med 75, 393-401 (1983).

SELYE, H., BAJUSZ, E., GRASSO, S., MENDELL, P. Simple techniques for the surgical occlusion of coronary vessels in the rat. Angiology 11, 398-407 (1960).

SHERIDAN, D. J., PENKOSKE, P. A., SOBEL, B. E., CORR, P. B. Alpha adrenergic contributions is dysrhythmias during myocardial ischemia and reperfusion in cats. J Clin Invest 65, 161-171 (1980).

SHIBATA, E. F., GILES, W. R. Ionic currents that generate the spontaneous diastolic depolarization in individual cardiac pacemaker cells. Proc Natl Acad Sci USA 82, 7796-7800 (1985).

SIEGMUND, W., LEPRAN, I., SZEKERES, L. Effect of prolonged beta blocking treatment alone and in combination with a parasympatholytic blocker on early arrhythmia arising after sudden coronary occlusion in conscious rats. Acta Physiol Acad Sci Hung 53, 209 (1979a).

SIEGMUND, W., LEPRAN, I., SZEKERES, L. Effect of pentobarbitone anesthesia, age and sex in the acute phase of myocardial infarction in rats. 3rd Congr the Hung Pharmacol Soc 63-66 (1979b).

SILVER, P. J., AMBROSE, J. M., MICHALATE, R. J., DACHIW, J. Effects of felodipine and W7 on arterial myosin phosphorylation, actin myosin interactions and contraction. Eur J Pharmacol 102, 417-426 (1984).

SINGH, B. N. An overview of slow channel blocking drugs: Pharmacological basis for therapeutic applications. Introductory lecture. Cardiology 69 (Suppl 1), 2-25 (1982).

SINGH, B. N. Verapamil and the heart: pharmacological and therapeutic considerations. N Z Med J 82, 339-343 (1975).

SINGH, B. N. Verapamil: mechanisms of pharmacologic actions and therapeutic applications. In: Kharasch, N. (Ed.) Trace Metals In Health And Disease, pp. 235-250 Raven Press, New York (1979)

SINGH, B. N., VAUGHAN WILLIAMS, E. M. A fourth class of antidysrhythmic action? Effect of verapamil on ouabain toxicity, on atrial and ventricular intracellular potentials, and on other features of cardiac function. Cardiovasc Res 6, 109-119 (1972).

SJOQUIST, P. O., ADLER, G., DUKER, G., ALMGREN, O. Effects of felodipine, a new dihydropyridine vasodilator, on regional myocardial blood-flow during acute coronary occlusion in the pig. J Cardiovasc Pharmacol 5, 202-206 (1983).

SMIRK, F. H., PALMER, D. G. A myocardial syndrome with particular reference to the occurrence of sudden death and/or premature systoles interrupting anticedant T-waves. Am J Cardiol 6, 620-631 (1960).

SOLOMAN, R. J. Ventricular arrhythmias in patients with myocardial infarction and ischaemia. Drugs 28 (Suppl 1), 66-76 (1984).

SPEAR, J. F., MOORE, E. N. Mechanisms of cardiac arrhythmias. Ann Rev Physiol 44, 485-497 (1982).

SPEDDING, M. Inhibitory effects of Local Anaesthetics on Calcium Channels in Smooth Muscle. Br J Pharmacol 79, 421p (1983).

SPEDDING, M., BERG, C. Interactions between a "calcium channel agonist", Bay K 8644, and calcium antagonists differentiate calcium antagonist subgroups in $K^+$-depolarized smooth muscle. Naunyn Schmiedeberg's Arch Pharmacol 328, 69-75 (1984).

STAAB, R. J., PAUL LYNCH, V. DE, LAU-CAM, C., BARLETTA, M. Small animal model for myocardial infarction. J Pharm Sci 66, 1483-1485 (1977).

STARMER, C. F., GRANT, A. O., STRAUSS, H. C. Mechanisms of use-dependent block of sodium channels in excitable membranes by local anesthetics. Biophys J 46, 15-27 (1984).

STEADMAN'S MEDICAL DICTIONARY, 22 edit. Williams and Wilkins, Baltimore, Md. (1972).

STRUTHERS, A. D., REID, J. L. Nifedipine does not influence adrenaline induced hypokalaemia in man. Br J Clin Pharmacol 16, 342-343 (1983).

STRUTHERS, A. D., REID, J. L., WHITESMITH, R., RODGER, J. C., Effect of intravenous adrenaline om electrocardiogram, blood pressure, and serum potassium. Br Heart J 49, 90-93 (1983).

TAIRA, Y., KANAIDE, H., NAKAMURA, M. Coronary venous perfusion of the ischaemic myocardium during acute coronary artery occlusion in isolated rat hearts. Circ Res 56, 666-675 (1985).

TEPPER, D., REICHMANN, P., SOMBERG, J. C., CAPPASSO, J. M., SONNENBLICK, E. H. Ouabain cardiotoxicity in the rat; a new model for studying arrhythmogenicity. Clin Res 33, 232A (1985).

THANDROYEN, F. T. Protective action of calcium channel antagonist agents against ventricular fibrillation in the isolated perfused rat heart. J Mol Cell Cardiol 14, 21-32 (1982).

TILLMANNS, H., IKEDA, S., BING, R. J. Production of myocardial infarcts in animal experiments. Handbuch Exp Pharm 16, 117-130 (1975).

TIMMERMANS, P. B. M. W. M., MEEL, J. C. A. VAN and ZWIETEN, P. A. VAN. Calcium antagonists and alpha receptors. Eur Heart J 4 (Suppl C), 11-17 (1983).

TORRES, V., FLOWERS, D., SOMBERG, J. C. The arrhythmogenicity of antiarrhythmic agents. Am Heart J 109, 1090-1097 (1985).

TOWNSHEND PORTER, W. On the results of ligation of the coronary arteries. J Physiol 15, 121-138 (1894).

TRIGGLE, D. J. Calcium antagonists: Basic chemical and pharmacological aspects. In: Weiss, G. B. (Ed.), New perspectives on Calcium Antagonists, pp. 1-18 (1981)

TRIGGLE, D. J. Chemical pharmacology of calcium antagonists. In: Rahwan, R. G., Witiak, D. T. (Eds.), Calcium Regulation by Calcium Antagonists, 17-34 (1982).

TUMAS, J., DETH, R. C., KLONER, R. A. Effects of nisoldipine on myocardial infarct size, hemodynamics, and myocardial performance. Clin Res 31, 673A (1983).

TUMAS, J., DETH, R., KLONER, R. A., Effects of nisoldipine, a new calcium antagonist, on myocardial infarct size and cardiac dynamics following acute myocardial infarction. J Cardiovasc Pharmacol 7, 361-367 (1985).

TUNG, L. H., LOUIS, W. J., RAND, M. J. Cardiac alpha adrenoceptors. In: Receptors, membranes and transport mechanisms in medicine. (Eds.) Doyle, A. E., Mendelsohn, F. A. O. pp. 41-54 Elsevier Science Publishers B.V., (1985).

URBANICS, R., KOVACH, A. G. S. Microcirculatory effects of the calcium antagonists gallopamil and fedopamil in cat cortex under normoxia and hypoxia. In: Calcium entry blockers and tissue protection (International symposium proceedings, in press) Rome (1984).

VANHOUTTE, P. M. Heterogeneity of postjunctional vascular α-adrenoceptors and handling of calcium. J Cardiovasc Pharmacol 4, Suppl 1, S91-S96 (1982).

VASALLE, M. The relationship among cardiac pacemakers. Overdrive suppression. Circ Res 41, 269-277 (1977).

VERDOUW, P. D., SCHAMHARDT, H. C., REMME, W. J., DE JONG, J. W. Antiarrhythmic metabolic and haemodynamic effects of Org 6001 (3α-amino-5α-androstan-2β-ol-17-one-hydrochloride) after coronary flow reduction in pigs. J Pharmacol Exp Ther 204, 634-644, (1978).

VERDOUW, P. D., WOLFFENBUTTEL, B. H. R., SCHEFFER, M. G. Cardiovascular actions of the calmodulin inhibitor felodipine. Naunyn Schmiedeberg's Arch Pharmacol 323, 350-354 (1983a).

VERDOUW, P. D., WOLFFENBUTTEL, B. H. R., TEN CATE, F. J. Nifedipine with and without propranolol in the treatment of myocardial ischemia: effect on ventricular arrhythmias and recovery of regional wall function. Eur Heart J 4, Suppl C, 101-108 (1983b).

VERDOUW, P. D., WOLFFENBUTTEL, B. H. R., VAN DER GIESSEN, W. J. Domestic pigs in the study of myocardial ischaemia. Eur Heart J 4 (Suppl C), 61-67 (1983c).

VERDOUW, P. D., WOLFFENBUTTEL, B. H. R. The effect of felodipine on ventricular fibrillation after coronary artery ligation in the anaesthetised pig. Br J Pharmacol 79, 6-8 (1983).

VINCENT, H. H., MAN IN'T VELT A. M., BOOMSMA, F., SCHALEKAMP, M. A. D. H. Prevention of epinephrine-induced hypokalemia by non-selective beta blockers. Am J Cardiol 56, 10D-14D (1985).

VOGELGESANG, B., ECHIZEN, H., SCHMIDT, E., EICHELBAUM, M. Stereoselective first-pass metabolism of highly cleared drugs: studies of the bioavailability of L and D-verapamil examined with a stable isotope technique. Br J Clin Pharmacol 18, 733-740 (1984).

WANG, T., TSAI, L. I., SCHWARTZ, A. Effects of verapamil, diltiazem, nisoldipine and felodipine on sarcoplasmic reticulum. Eur J Pharmacol 100, 253-261 (1984).

WEBER, K. T., MALININ, T. I., DENNISON, B. H., FUQUA, J. M., SPEAKER, D. M., HASTINGS. F. W. Experimental myocardial ischaemia and infarction. Production of diffuse lesions in unanaesthetised calves. Am J Cardiol 29, 793-820 (1972).

WEEKS, J. R. Cardiovascular techniques using unanaesthetized and freely moving rats. Upjohn Brochure No. 1011, Kalamazoo, Michigan (1981).

WEEKS, J. R., JONES, J. A., Routine direct measurement of blood pressure in rats. Proc Soc Exp Biol Med 104, 646-652 (1960).

WEIDMANN, S. Effects of calcium ions and local anaesthetics on electrical properties of Purkinje fibres. J Physiol (Lond) 129, 568-582 (1955b).

WEIDMANN, S. Low resistance contacts and the propagation of the cardiac action potential. In: Paes de Carvalho, A., Hoffman, B. F., Lieberman, M. (Eds.) Normal and abnormal conduction in the heart, pp. 43-51, Futura (1982).

WEIDMANN, S. The effect of the cardiac membrane potential on the rapid availability of the sodium carrying system. J Physiol (Lond) 127, 213-224 (1955a).

WEISS, A. B., MOSCHOS, C. B., PASSANNANTE, A. J., KHAN, M. I., REGAN, T, J, Relative effectiveness of three anti-arrhythmic agents in the treatment of ventricular arrhythmias in experimental acute myocardial ischaemia. Am Heart J 81, 503-510 (1971).

WENNEMARK, J. R., BANDURA, J. P. Microelectrode study of Wenckebach periodicity in canine Purkinje fibres. Am J Cardiol 33, 390-398 (1974).

WIGGERS, C. J., WEGRIA, R. Ventricular fibrillation due to single localised induction and condensor shocks applied during the vulnerable phase of ventricular systole. Am J Physiol 128, 500-505 (1940).

WILDE, A. A. M., KLEBER, A. G. The combined effects of hypoxia, high $K^+$, and acidosis on the intracellular sodium activity and resting potential in guinea pig papillary muscle. Circ Res 58, 249-256 (1986).

WILSON, S. A., HEWICK, D. S. A strain dependent sex difference in ouabain-induced cardiotoxicity in rats. J Pharm Pharmacol 37, 671-672 (1985).

WINBURY, M. M. Experimental coronary disease-models and methods of drug evaluation. Handbuch Exp Pharmacol 16, 1-69 (1975).

WINBURY, M. M. Experimental approaches to the development of antiinginal drugs. In: Garratini, S., Shore, P. A. (EDs.), Advances in pharmacology. pp. 1-82, Academic Press, New York (1964).

WINKLER, B., SASSM S., BINZ, K., SCHAPER, W. Myocardial blood flow and myocardial infarction in rats, guinea pigs and rabbits. J Mol Cell Cardiol 16 (Suppl 2), 48 (1984).

WINSLOW, E. Methods for the detection and assessment of antiarrhythmic activity. Pharmac Ther 24, 401-433 (1984).

WINSLOW, E., CAMPBELL, J. K., MARSHALL, R. J. Antiarrhythmic and electrophysiological effects of prolonged dosing with disopyramide or amiodarone in normokalaemic and hypokalaemic rats. IUPHAR 9th Internat Cong of Pharmacol 476P (1984).

WINSLOW, E., KELLY, M. E., LAW, E. A. Effect of Org 6001 on reperfusion dysrhythmias and lactate dehydrogenase release in isolated rat hearts: comparison with propranolol and lignocaine. J Cardiovasc Pharmacol 2, 425-434 (1980).

WOOD, M. A. DE, SPORES, J., HENSLEY, G. R., SIMPSON, C. S., EUGSTER, G. S., SUTHERLAND. K. I., GRUNWALD, R. P., SHIELDS, J. P. Coronary arteriographic findings on acute transmural myocardial infarction. Circulation 68 (Suppl 1), 139-149 (1983).

WOODS, J. P., WEST, T. C. Frequency-dependent effects of nifedipine in cardiac tissue. Pharmacologist 25, 217 (1983).

WOODS, J. P., WEST, T. C. Frequency-dependence of $V_{max}$ in K-depolarized guinea pig ventricle: effects of nifedipine and verapamil. J Cardiovasc Pharmacol 7, 197-204 (1985).

WOODWARD, B., ZAKARIA, M. N. M. Effect of some free radical scavengers on reperfusion induced arrhythmias in the isolated rat heart. J Mol Cell Cardiol 17, 485-493 (1985).

YELLON, D. M., KUDOH, Y., MAXWELL, M. P., YOSHIDA, S., HEARSE, D. J., DOWNEY, J. M. Does nifedipine cause a sustained limitation of infarct size during permanent coronary artery occlusion in the dog ? J Mol Cell Cardiol 18 (Suppl 1), 204 (1986a).

YELLON, D. M., MIURA, T., HEARSE, D. J., DOWNEY, J. M. Factors influencing infarct size: is coronary collateral flow the key determinant ? J Mol Cell Cardiol 18 (Suppl 1), 101 (1986b).

YUSUF, S., COLLINS, R., PETO, R., FURBERG, C., STAMPFER, M. J., GOLDHABER, S. Z., HENNEKENS, C. H. Intravenous and intracoronary fibrinolytic therapy in acute myocardial infarction: overview of results on mortality, reinfarction and side-effects from 33 randomized controlled trials. Eur Heart J 6, 556-585 (1985).

ZIMMER, H. G., Metabolic and functional recovery following myocardial ischaemia and infarction. J Mol Cell Cardiol 18 (Suppl 1), 195 (1986).

ZOLL, P. M., LINENTHALL, A. J., GIBSON, W., PAUL, M. H., NORMAN, L. R. Termination of ventricular fibrillation in man by externally applied electric countershock. New England J Med 254, 727-732 (1956).

ZSOTER, T., BAJUSZ, E. Electrocardiograms in surgically induced cardiopathies of the rat. Cardiologia 40, 290-297 (1962).

ZWIETEN, P. A. VAN, TIMMERMANS, P. B. M. W. M. Calcium entry blockers: a review with an attempt at classification. E Heart J 4 (Suppl C), 1-10 (1983).