

RELATIONSHIP BETWEEN ISCHAEMIA-SELECTIVE DRUG ACTION AND
ANTIARRHYTHMIC EFFICACY

by

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Abstract

This thesis explores the relationship between the electrophysiological actions of drugs on ischaemic myocardial tissue and their effects on arrhythmias induced by ischaemia. Our hypothesis was that drugs with ischaemia-selective electrophysiological actions would provide better antiarrhythmic protection in the setting of acute myocardial ischaemia than those which lacked such selectivity. The actions of a selection of standard antiarrhythmic drugs (quinidine, lidocaine, flecainide and tedisamil) were compared to those of the novel drug RSD1019, under conditions designed to mimic, or produce, myocardial ischaemia in rat hearts. In support of the hypothesis, drugs which exhibited selectivity for the conditions of myocardial ischaemia (i.e., lidocaine and RSD1019) suppressed ischaemia-induced arrhythmias effectively. Drugs that were more potent in normal myocardial tissue, and which lacked such selectivity (i.e., quinidine, flecainide and tedisamil), were less effective for suppression of ischaemia-induced arrhythmias.

Further studies were carried out in order to evaluate the hypothesis using monophasic action potential (MAP) recordings from the epicardium of anaesthetised rabbits before and after induction of myocardial ischaemia. The advantage offered by this preparation was that it allowed the electrophysiological changes caused by ischaemia, and drug effects thereon, to be assessed simultaneously with arrhythmias resulting from myocardial ischaemia. In this preparation, both RSD1019 and lidocaine influenced the electrophysiological properties of ischaemic tissue and arrhythmias but in different ways. Lidocaine exacerbated the electrophysiological derangement caused by ischaemia and had proarrhythmic actions. RSD1019 prevented MAP shortening caused by ischaemia and

arrhythmias. In contrast to RSD1019, the $I_{K(ATP)}$ blocker glibenclamide failed to prevent MAP shortening caused by ischaemia and the antiarrhythmic effects produced by this drug are unlikely to be related to its effects on ischaemic myocardial tissue.

In summary, ischaemia-selective drug actions have the same potential benefits and risks associated with drug action in normal myocardial tissue. The action of a drug on ischaemic tissue can be pro- or antiarrhythmic depending on the nature of the drug's action and other factors that remain to be identified. Prolongation of action potential duration in ischaemic tissue, demonstrated herein for RSD1019, was associated with antiarrhythmic actions. This mechanism represents a novel approach to suppression of ischaemia-induced arrhythmias.

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Some data contained in this thesis has been previously published in abstract form or as a complete manuscript. The title, date, and journal that the material was published in as well as the full list of authors is shown below.

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List of abbreviations.

1,4-piperizine bissulphonic acid	PIPES
Action potential	AP
Analysis of variance	ANOVA
Arachidonic acid-activated K ⁺ current	I _{K(AA)}
Arrhythmia score	AS
ATP dependent K ⁺ channels	K _{ATP}
ATP-dependent K ⁺ current	I _{K(ATP)}
Blood pressure	BP
Calcium current	I _{Ca}
Calcium activated chloride current	I _{Cl(Ca)}
Chloride current	I _{Cl}
Concentration required to produce a 25% change	C _{25%}
Coronary artery disease	CAD
Curve fit co-efficient	r
Delayed after depolarisation	DAD
Dose required to produce a 25% change	D _{25%}
ECG interval from the Q wave to the peak of the T wave	QTa
Early after depolarisation	EAD
Effective dose for a 50% maximum response	ED _{50%}
Effective refractory period	ERP
Heart rate	HR
Inward rectifier K ⁺ current	I _{K1}
Intraperitoneal	ip
Intravenous	iv
Log to the base 10 of the number of PVBs	log PVB
Log to the base 10 of the duration of VT	log VT dur
Log to the base 10 of the duration of VF	log VF dur
Maximum following frequency	MFF
Microamperes	μA
Microlitre	μL
Micromolar	μM
Micromole per kilogram per minute	μmol/kg/min
Milligram per kilogram	mg/kg
Millilitre	mL
Millilitre per kilogram per hour	mL/kg/hr
Millimolar	mM
Millimetres of mercury	mmHg
Millimetres per second	mm/s
Millisecond	ms
Millivolt	mV
Minute	min
Monophasic action potential	MAP
Monophasic action potential duration at 90% repolarisation	MAPD90%
Monophasic action potential foot to peak interval	MAP f-p

Not statistically significant	NS
Norepinephrine activated Cl^- current	$I_{\text{Cl(NE)}}$
Number of animals in the group	n
Number of groups	k
Occluded zone size	OZ%
Percent antiarrhythmic protection	%P
Premature ventricular beat	PVB
Probability of less than 1%	$p < 0.01$
Probability of less than 5%	$p < 0.05$
QT interval corrected for heart rate	QTc
QTa interval corrected for heart rate	QTac
Rapidly activating delayed rectifier K^+ current	I_{Kr}
Rate of action potential shortening	dAPD/dt
Transient outward current	I_{to}
Seconds	s
Slope factor	h
Slowly activating delayed rectifier K^+ current	I_{Ks}
Sodium current	I_{Na}
Sodium activated K^+ current	$I_{\text{K(Na)}}$
Standard deviation	SD
Standard error of the mean	SEM
Sudden cardiac death	SCD
Threshold current for stimulation of the left ventricle	iT
Threshold duration for stimulation of the left ventricle	tT
Unit per millilitre	U/mL
Ventricular tachycardia	VT
Ventricular fibrillation	VF
Ventricular fibrillo-flutter threshold	VFT
Volts per second	V/s

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1.0 Introduction.

1.1 Coronary artery diseases, ischaemia, infarction and "sudden cardiac death."

Epidemiological studies illustrate that coronary artery disease (CAD) continues to be a major health problem for which no complete solution has been found (Kannel & Wilson, 1991; Lopez, 1993; Reddy & Yusuf, 1998). CAD is characterised by atherosclerotic narrowing of the coronary arteries, coronary artery vasospasm, or a combination of both; all of which lead to a temporary or permanent loss of blood flow to a portion of the myocardium. Atherosclerotic plaque rupture or thrombosis can lead to a sudden loss of blood flow. As myocardial oxygen extraction is already maximal under basal conditions, such a restriction to blood flow results in ischaemia. Clinical manifestations of CAD include angina pectoris, cardiac arrhythmias, ischaemic cardiomyopathy and sudden death (Kannel & Wilson, 1991). Longitudinal studies have produced a myriad of risk factors for the development of CAD (see Kannel *et al.*, 1984; Kannel & Wilson, 1991). Foremost among these are male sex, high blood pressure, high serum cholesterol, diabetes, smoking and psychological stress. While recent statistics suggest that death due to CAD is decreasing (Lopez, 1993) it remains at near epidemic proportions (Lopez, 1993; Reddy & Yusuf, 1998). In 1995, 22,000 Canadians died of myocardial infarction, half of these suddenly (Heart & Stroke Foundation of Canada, 1995; Dagenus *et al.*, 1996). Canada is more or less representative of mortality due to CAD and similar statistics can be found for other industrialised nations (Tunstall-Pedoe *et al.*, 1994).

1.1.1 Acute myocardial ischaemia.

The aetiology of CAD and resulting myocardial ischaemia is complex and highly variable between individuals. Atherosclerotic or vasospastic narrowing of coronary arteries is the most common cause of temporary myocardial ischaemia (Roberts, 1990). The hallmark of temporary ischaemia due to a fixed narrowing of a coronary artery is that symptoms of angina pectoris appear at a fixed level of cardiac work. Isolated vasospasm causing myocardial ischaemia has been documented, but is rare (Prinzmetal *et al.*, 1959). Typically, variant angina occurs at rest and is not related to the amount of cardiac work. Intracoronary thrombi can cause sudden and irreversible myocardial ischaemia; however, such events are relatively rare. More commonly, myocardial ischaemia is caused by a complex interplay of atherosclerosis, intracoronary thrombus and/or vasospasm (Gorlin, 1982; Davis, 1992). This condition is known clinically as unstable angina (Braunwald, 1989).

The clinical outcome of myocardial ischaemia is critically dependent on the extent, degree and duration of the ischaemic episode. Biochemical, mechanical and electrical changes occur rapidly after the onset of ischaemia (see Wit & Janse, 1989). Within seconds after its onset, ischaemic tissue ceases to contract, ST shifts appear in the surface ECG and angina pectoris commonly occurs. In general, ST shifts can be observed in ECG recordings before angina occurs (Cohn, 1986). Ischaemic episodes can be "silent" (i.e., painless ischaemia). Silent ischaemia is common in CAD patients (Cecchi *et al.*, 1983) and can occur in asymptomatic patients (Kannel & Abbot, 1984). While ambulatory ECG

recordings and exercise testing can detect silent ischaemia it commonly goes undiagnosed (Cecchi *et al.*, 1983; Amsterdam *et al.*, 1986).

1.1.2 Myocardial infarction.

Clearly, myocardial ischaemia is a reversible process; however, when it is of sufficient degree and duration, infarction (i.e., cell death) results. Acute myocardial infarction is characterised by an increase in non-specific markers of tissue necrosis and inflammation, characteristic changes in the surface ECG and elevated blood concentrations of cardiac specific intracellular enzymes (Baral *et al.*, 1994). Acute myocardial infarction is commonly accompanied by angina pectoris; however, as with myocardial ischaemia, it can also be silent.

1.1.3 Arrhythmias associated with myocardial ischaemia and infarction.

Erichsen (1841, cf: Janse & Wit, 1989) first described occlusion of a coronary artery precipitating fatal ventricular fibrillation (VF). Since then, all types of arrhythmias have been observed to occur with myocardial ischaemia and infarction (see Adgey *et al.*, 1971; Pantridge *et al.*, 1981; Campbell *et al.*, 1981; Sclarovsky *et al.*, 1984; Turitto *et al.*, 1989; Botting *et al.*, 1985). The type of arrhythmia observed depends on the region that was made ischaemic and may include: sinus bradycardia, sinus tachycardia, atrial fibrillation, ventricular premature beats (VPBs), ventricular tachycardia (VT) and VF. Ventricular arrhythmias are the most common (Pantridge *et al.*, 1981). As ventricular arrhythmias are life threatening, and therefore the target for antiarrhythmic drugs, this

thesis will confine discussion of arrhythmias and antiarrhythmic drugs to ventricular tachyarrhythmias. Arrhythmias tend to occur in discrete phases during myocardial ischaemia and infarction (Stevenson *et al.*, 1989; Turitto *et al.*, 1989; Myerburg *et al.*, 1992; Sogaard *et al.*, 1994). Identifying arrhythmias as being caused by acute ischaemia or subsequent infarction is complicated by the heterogeneous nature of the clinical condition and the uncertainty associated with detecting such conditions. The occurrence of such arrhythmias can be divided into four phases depending upon when they occur: acute ischaemia, acute infarction, infarction healing and, the presence of a stable infarct (Botting *et al.*, 1985; Myerburg *et al.*, 1992).

Severe ventricular arrhythmias occur most commonly during the acute ischaemic phase. The highest incidence of VF occurs shortly after the onset of ischaemia (Adgey *et al.*, 1971; Johnson, 1980; Campbell *et al.*, 1981; Pantridge *et al.*, 1981; Mogensen, 1983). For example, Pantridge and co-workers (1981) reported that 19% of patients, in a series of 294 consecutive patients, had VF. Of the total number of patients suffering VF, 53% had the arrhythmia within the first hour. The early arrhythmogenic phase associated with acute ischaemia occurs within the first few minutes to hours after the onset of ischaemia.

The risk of ventricular arrhythmias remains relatively high during the infarction phase although reduced compared to the acute ischaemic phase (Botting *et al.*, 1985; Furakawa *et al.*, 1989). The process of infarct healing takes days to weeks. Mortality in the myocardial infarction and subsequent infarct healing phases represents only a quarter of the total mortality associated with myocardial infarction (Armstrong *et al.*, 1971). In hospital, arrhythmic death during the acute infarction and healing phases has been greatly

reduced by the advent and application of DC cardioversion (Zoll *et al.*, 1956; Reddy & Barby, 1997). The risk of severe arrhythmias and mortality after myocardial ischaemia and infarction decays with time (Furakawa *et al.*, 1989). The cause of sudden cardiac death (SCD) in patients with a healing or stable infarcts is difficult to establish but is commonly attributed to VF (Bayés de Luna *et al.*, 1989). It is not clear if the arrhythmias causing death are due to the infarct itself or an interaction between the presence of an infarct and subsequent ischaemic episodes (Myerburg *et al.*, 1992).

1.2 The problem of sudden cardiac death.

Perhaps the most insidious thing about CAD is that its first sign or symptom is commonly its only and last; sudden death. The term sudden cardiac death (SCD) has been used (and misused) to describe the phenomenon of unexpected death of an otherwise healthy individual within one hour of the onset of symptoms (Roberts, 1988). Assignment of SCD as the cause of death is primarily by default and relies on the fact that few other diseases can kill in a matter of minutes (Kannel & Schatzkin, 1985). It is commonly accepted that SCD is caused by VF. Despite this, it is clear that not all SCD is caused by VF (Roberts, 1990). Death due to aortic dissection, ruptured aneurysm or pulmonary embolism, for example, can also cause SCD while being non-arrhythmic in nature.

ECG records taken at the time when SCD occurs, where they exist, show that VF was the terminal rhythm in ~75% of cases (Cobb *et al.*, 1975; Liberthson *et al.*, 1982; Roelandt *et al.*, 1984; Weaver *et al.*, 1986; Panidis & Morganroth, 1983). In the report by Adgey *et al.* (1969), in which all ECG recordings were obtained within 4 minutes of

collapse, 91% of patients were found to have VF. It is important to note that these recordings were obtained after the event and that VF may give way to bradyarrhythmias or further to asystole (Greene, 1990; Weiver *et al.*, 1986). The observation of a “flat line” (i.e., asystole) in such recordings was directly related to the time between collapse and monitoring (Schaffer *et al.*, 1975) suggesting that asystole occurs secondary to VF.

In cases of patients succumbing to SCD while wearing an ambulatory ECG monitor, similar results were found. A review of 157 cases of Holter documented SCD shows that VF was the terminal rhythm in 70% of patients, with torsade de pointes accounting for 13% (Bayés de Luna *et al.*, 1989). It should be noted that the definition of torsade de point is equivocal and even experienced cardiologists cannot agree on its diagnosis, including Dr. Bayés de Luna (see Clayton *et al.*, 1993).

1.2.1 Is there a relationship between SCD and acute myocardial ischaemia?

It is clear that ischaemia can cause VF and SCD (see Gradman *et al.*, 1977; Savage *et al.*, 1983; Turitto *et al.*, 1989). However, it seems unlikely that all SCD is caused by VF provoked by acute myocardial ischaemia. The question then becomes, what proportion of SCD is caused by myocardial ischaemia? There are a number of lines of evidence which strongly suggest that ischaemia causes or is at least an important antecedent of VF and SCD. These lines of evidence will be discussed in detail below and can be summarised as follows:- First, the occurrence of VF after induction of ischaemia and the occurrence of SCD after the onset of symptoms (Pantridge *et al.*, 1981; Armstrong *et al.*, 1972) are essentially the same. Both occur very rapidly after the initial

event. Second, the predominance of new vascular events involving intracoronary thrombosis or high grade atherosclerotic narrowing in patients succumbing to SCD suggests that ischaemia is a common antecedent (Davis, 1992; Baba *et al.*, 1975; Greene, 1990; Roberts, 1990, Myerburg *et al.*, 1992). Third, documented cases of SCD in patients undergoing ambulatory ECG monitoring which demonstrate the occurrence of acute myocardial ischaemia immediately preceding the event (Gradman *et al.*, 1977; Savage *et al.*, 1983).

It could be argued that myocardial infarction is the cause of SCD. However, a number of studies have shown that only a small proportion (approximately 20%) of patients in which "SCD" was aborted show signs of infarction (Greene, 1990; Liberson *et al.*, 1982). Thus it is not myocardial infarction *per se* that is the cause of SCD but rather the acute myocardial ischaemia which precedes it.

Atherosclerotic narrowing of the coronary arteries is present in a vast majority of persons suffering SCD (Roberts, 1990; Davies, 1992). Studies of the state of the coronary arteries from persons suffering SCD usually show one of two patterns (Davies, 1992). The most common is the occurrence of new vascular events involving coronary thrombosis. The other common pattern is the presence of a chronic high grade stenosis (>75%) with myocardial damage downstream from the stenosis. Davies (1992) suggests that the ratio between the two patterns is 2.7 to 1. Evidence of unstable atherosclerotic plaques was found in 95% of patients suffering SCD (Davis & Thomas, 1984). Attrition of this figure by the occurrence of unstable plaques in normal individuals suggests that 73% of SCDs are associated with an unstable atherosclerotic plaque (Davies, 1992).

While these data support the role of ischaemia in the genesis of VF and SCD, the role of intracoronary thrombosis is controversial. Others report a somewhat lower incidence of intracoronary thrombosis (e.g., Baroldi *et al.*, 1979; also see Collins & Fox, 1990). However, these data clearly show the association between new vascular events, myocardial ischaemia and SCD.

The other common subgroup of patients suffering SCD have chronic high grade coronary stenosis which results in myocardial damage (Roberts, 1990; Davies, 1992). Myocardial damage, such as that seen under conditions of ischaemic cardiomyopathy, prior myocardial infarction or hypertrophy, alters the response to transient ischaemic episodes rendering them much more arrhythmogenic (Myerburg *et al.*, 1992; Luchessi *et al.*, 1993). In this population, SCD is likely to be caused by the interaction between transient ischaemic episodes and the underlying substrate. Occluding thrombi are also expected to occur more readily with the progression of atherosclerotic CAD (Epstein *et al.*, 1989). Thus, while acute ischaemia is not the only factor responsible for precipitating VF in these patients, it is clearly an important component.

The difficulty in detecting myocardial ischaemia is one of the major sources of uncertainty when attempting to relate it to SCD. Indeed, some authors argue that VF and SCD are not caused by ischaemia (e.g., Lerequ *et al.*, 1987; Bayés de Luna *et al.*, 1989). This conclusion is based on data obtained from ambulatory ECG records taken at the time of SCD. Data from Meissner and Morganroth's (1986) review suggest that 30% of patients suffering SCD show ischaemic changes in Holter recordings. It is important to note that many of these studies make no mention of ST segment changes. Also,

ambulatory ECG leads are placed to optimise diagnosis of arrhythmias not myocardial ischaemia. Such recording may fail to detect ischaemia despite its presence (Bayés de Luna *et al.*, 1989). Furthermore, patients wearing such monitors are presumably doing so for a reason (i.e., they have had symptoms of CAD). As only 29-40% of individuals suffering SCD sought a physician's advice within the month before the event (Liberthson *et al.*, 1982; Feinleib *et al.*, 1975) patients wearing a Holter monitor may not be representative of the true population suffering SCD. For example, patients wearing Holter monitors may be taking antiarrhythmic drugs, such as digitalis, quinidine or flecainide, which are known to cause cardiac arrhythmias (Bayés de Luna *et al.*, 1989; Echt *et al.*, 1991; Morganroth & Goin, 1991). Drug-induced arrhythmias would likely be caused by non-ischaemic mechanisms. Similarly, patients wearing Holter monitors are more likely to be seriously ill than those suffering SCD.

These data do not allow an unequivocal conclusion to be drawn about the role of acute myocardial ischaemia in causing SCD as there is considerable censorship and bias in data gathered from Holter recordings at the time of SCD. Clearly, some patients have Holter documented ischaemia as an antecedent to SCD (see Gradman *et al.*, 1977 & Savage *et al.*, 1983), but what proportion can only be guessed. Based on data gathered from ambulatory ECG recordings, between 10 and 30% of patients show signs of ischaemia; however, this method is likely to underestimate the true incidence of ischaemia.

In summary, myocardial ischaemia can cause VF and SCD. The aetiology of SCD often includes myocardial ischaemia. Whether or not ischaemia is the cause of SCD is equivocal; however, ischaemia is a common antecedent of SCD. The clinical data cannot

causally link ischaemia to SCD; however, such data cannot reasonably be expected to provide such a link (depending on your philosophical view point, see Popper, 1972).

1.3 Biochemical changes caused by myocardial ischaemia.

Ischaemia causes profound changes in the metabolism and biochemical status of myocardial tissue. Discussion of the effects of ischaemia on the biochemical homeostasis of the ventricular myocardium will be limited to those produced by complete cessation of blood flow (or perfusing buffer in case of *in vitro* preparations) as these are the most relevant to the occurrence of arrhythmias. After the onset of ischaemia, the availability of oxygen decreases rapidly and metabolism shifts from aerobic to anaerobic. A series of complex biochemical changes occur as a consequence. These changes are confounded by the accumulation of metabolic bi-products; not surprisingly, the effects of ischaemia are greater than those produced by hypoxia alone (Janse & Wit, 1989). In some cases, the biochemical mediators of ischaemic changes may interact and the effects of such combinations are greater than that of either mediator alone. Ischaemia-induced changes in ion homeostasis include, but are not limited to, an increase in extracellular $[K^+]$, catecholamines, lactic acid, intracellular $[Na^+]$ and $[Ca^{++}]$ as well as a decrease in intra- and extracellular pH and intracellular [ATP].

With the onset of ischaemia, oxidative phosphorylation ceases and glycolytic metabolism provides the cell's energy (Dennis *et al.*, 1991). This metabolic pathway only produces a fraction of the ATP produced by oxidative phosphorylation and lactic acid is a by-product. As a result of reduced ATP production, the concentration of high energy

phosphates falls. ATP concentrations remain high enough to support most cellular functions in the early stages of ischaemia (20-30 minutes, Cascio *et al.*, 1995) and cells are viable if the tissue is reperfused (i.e., infarction has not yet occurred).

Extracellular $[K^+]$ increases rapidly after cessation of blood flow (Hirche *et al.*, 1980; Hill & Gettes, 1980; Gasser & Vaughan-Jones, 1990). The increase in extracellular $[K^+]$ is tri-phasic and characterised by an initial increase, a plateau phase and a secondary increase. Extracellular $[K^+]$ increases from its initial value of ~ 3.5 mM to a plateau value of ~ 10 mM within 7 to 10 minutes after the onset of ischaemia. The secondary increase in $[K^+]$ begins approximately 25 minutes after the onset of ischaemia and can bring concentrations in excess of 15 mM. The mechanism(s) which underlie these phases are incompletely characterised but involve increased K^+ efflux (Rau *et al.*, 1977; Vleugels & Carmeliet, 1978; Vleugels *et al.*, 1980; Hill & Gettes, 1980; Shivkumar *et al.*, 1997).

The initial phase appears to be linked to the fall in ATP concentration and has been suggested to occur by at least two mechanisms: increased outward K^+ current(s) (Vleugels & Carmeliet, 1978; Vleugels *et al.*, 1980) and anion linked K^+ efflux (Weiss *et al.*, 1989; Shivkumar *et al.*, 1997). Noma's (1983) description of an ATP regulated K^+ current ($I_{K(ATP)}$) led to the suggestion that it mediated the increased K^+ efflux during ischaemia. This suggestion has been partly born out, but, there are a number of problems with the hypothesis. First, the reductions in $[ATP]$ occurring in the early stages of ischaemia are insufficient to activate $I_{K(ATP)}$ (Jennings *et al.*, 1978; Noma, 1983). This inconsistency might be explained by a number of observations including: the high density of K_{ATP} channels in cardiac myocytes (Nichols *et al.*, 1991), dependence of K_{ATP} channel

activation on the ATP/ADP concentration ratio (Venkatesh *et al.*, 1991) or increased activation of K_{ATP} by hydronium ions (Fan & Makielski, 1993). Despite such explanations, the discrepancy between the reduction in [ATP] caused by ischaemia and activation of $I_{K(ATP)}$ has not been completely explained. Second, blockers of $I_{K(ATP)}$, such as glibenclamide, attenuate but do not abolish K^+ efflux caused by ischaemia (Kantor *et al.*, 1987; Venkatesh *et al.*, 1991). Other K^+ channels may contribute to the K^+ efflux caused by ischaemia. These K^+ currents may include: the rapidly activated delayed rectifier current (I_{Kr}), arachidonic acid activated K^+ current ($I_{K(AA)}$, Kim & Clampham, 1989) and the Na^+ activated K^+ current ($I_{K(Na)}$, Kameyama *et al.*, 1984). Sotalol, a blocker of I_{Kr} and β -adrenoceptors, reduces the rate of ischaemia-induced K^+ efflux from the myocardium independent of β -adrenoceptor blockade (Hicks & Cobbe, 1990). Another factor that might contribute is K^+ -induced K^+ efflux; the K^+ conductance of various K^+ channels is increased by increases in extracellular $[K^+]$ (e.g., Noble & Tsien, 1969; Yang & Roden, 1996).

The mechanism of the plateau phase and secondary increase in extracellular $[K^+]$ are unknown and may be multifactorial. It has been suggested that the reduced rate of K^+ accumulation during the plateau phase is the result of a noradrenaline-induced increase in K^+ uptake by myocardial cells (Warner, 1995) or that K^+ diffuses away from the ischaemic tissue (Coronel *et al.*, 1988). Johnson and colleagues (1988) observed that the ischaemia-induced increases in $[K^+]$ showed "microheterogeneity" that could not be attributed to regional differences in blood flow. This observation illustrates the point that regional differences occur in the response to myocardial ischaemia.

Hydronium ions are produced by a number of metabolic processes in the ischaemic myocardium, although a majority are the product of glycolytic ATP turnover (Dennis *et al.*, 1991). Extracellular pH typically drops to 6.0 within 30 minutes after the onset of ischaemia (Dennis *et al.*, 1991, Yan, 1992). Intracellular pH is reduced less than extracellular pH, an effect that may be due to increased intracellular buffering capacity (Yan, 1992). Carbon dioxide accumulates rapidly in the ischaemic myocardium (Case *et al.*, 1979). Regional differences in CO₂ accumulation due to diffusion across the ischaemic/normal tissue boarder zone, as well as diffusion into the ventricular cavity, cause regional variations in pH (Wilensky *et al.*, 1986; Case *et al.*, 1989; Cascio *et al.*, 1992).

Changes in intracellular Ca⁺⁺ transients can be recorded within seconds after the onset of ischaemia in isolated rabbit hearts (Lee *et al.*, 1988). Not only is the end-diastolic [Ca⁺⁺] increased but the systolic [Ca⁺⁺] peak is increased and prolonged. With longer durations of ischaemia (2-3 minutes), Ca⁺⁺ transients become heterogeneous and beat to beat variations in amplitude and duration are commonly observed (Lee *et al.*, 1988). Other reports suggest that changes in internal [Ca⁺⁺] occur more slowly. For example, NMR studies suggest that changes in intracellular [Ca⁺⁺] do not occur until 5 to 10 minutes after the onset of ischaemia in rat hearts (Steenbergen *et al.*, 1987, 1990). While the rate at which intracellular [Ca⁺⁺] increases may be species or condition dependent, it is generally accepted that [Ca⁺⁺] increases substantially in the early stages of ischaemia (Clusin *et al.*, 1983, 1984).

Depolarisation (January & Fozzard, 1984), hypoxia (Anderson *et al.*, 1990, 1991) ischaemia (Wilde & Kléber, 1986), as well as by-products of ischaemic metabolism

(Undrovinas *et al.*, 1992), all cause increases in intracellular $[\text{Na}^+]$. The time course for the increase varies and may be species dependent (Cascio *et al.*, 1995). Intracellular Na^+ accumulation starts as early as 5 minutes after the onset of ischaemia and continues to accumulate thereafter. The mechanism for this increase may involve reduced activity of the Na^+/K^+ -ATPase or increased activity of the Na^+/H^+ and/or $\text{Na}^+/\text{Ca}^{++}$ antiport, as well as the $\text{Na}^+/\text{HCO}_3^-$ symport (January & Fozzard, 1984; Mitani *et al.*, 1992, Cascio *et al.*, 1995).

Catecholamines have been shown to accumulate in the extracellular space during the early stages of ischaemia (20-30 minutes; Hirche *et al.*, 1980, Carlsson, 1987). Noradrenaline concentrations can reach as high as 1 μM after 20 minutes of ischaemia (Carlsson, 1987). The mechanism of release appears to be non-exocytotic in nature and can be reduced by uptake-1 blockers such as cocaine and nisoxetine (Schömig *et al.*, 1984).

The ischaemic myocardium has been shown to produce amphipathic lipid metabolites including long-chain acylcarnitine, lysophosphatidylcholine and arachidonic acid (Sobel *et al.*, 1978; Corr *et al.*, 1981; Snyder *et al.*, 1981; Povzhnikov *et al.*, 1984; Kiyosue & Arita, 1986; Undrovinas *et al.*, 1992). High concentrations of these compounds cause depolarisation of resting membrane potential and reduce conduction velocity. These metabolites have been suggested to mediate the effects of ischaemia (Sobel *et al.*, 1978; Corr *et al.*, 1981, 1982). The observation that the concentrations of long-chain acylcarnitine and lysophosphatidylcholine required to produce effects on the heart are higher than those found in the ischaemic myocardium has left considerable

question as to the importance of these compounds (Mogelson *et al.*, 1980; Riemersma & Michorowski, 1983).

1.4 Ischaemia-induced electrophysiological changes.

Ischaemia causes profound derangement of the electrophysiological properties of myocardial tissue. Electrophysiological studies, using intracellular and monophasic action potential (MAP) recordings, have shown that a reduction in the maximum rate of rise and action potential (AP) amplitude occurs within minutes, even seconds, of the onset of ischaemia (Kardesch *et al.*, 1958; Prinzmetal *et al.*, 1961; Downar *et al.*, 1977; Lab & Woollard, 1978; Russel *et al.*, 1979; Franz *et al.*, 1984; Inoue *et al.*, 1984). AP duration increases initially but this is quickly (within 1 minute) followed by a sustained shortening (Downar *et al.*, 1977; Inoue *et al.*, 1984; Kurz *et al.*, 1993). In the first minutes of ischaemia, changes in the refractory period parallel changes in AP duration; however, this relationship is not maintained. As the duration of ischaemia increases, cells remain refractory after repolarisation is complete. This phenomenon is referred to as "post-repolarisation refractoriness" (Han & Moe, 1964; Elharrar *et al.*, 1977; Levites *et al.*, 1975). Conduction velocity is initially increased but this is rapidly followed (within minutes) by a progressive slowing (Boineau & Cox, 1973; Waldo & Kaiser 1973; Williams *et al.*, 1975; Downar *et al.*, 1977). The most important feature of the effects of ischaemia on myocardial tissue is the fact that they are not homogeneous. After induction of ischaemia, regional differences in conduction velocity, AP duration and refractory period increase (Isomura *et al.*, 1984; Kléber *et al.*, 1986; Lee *et al.*, 1988). At the interface

between ischaemic and normal tissue (i.e., the border zone) "currents of injury" develop (Nahum *et al.*, 1943; Samson & Scher, 1960; Kléber *et al.*, 1978; Janse *et al.*, 1980). Injury currents can have significant effects on the electrophysiological properties of both ischaemic and normal tissue (Janse *et al.*, 1980).

Reduction in resting membrane potential and AP amplitude caused by ischaemia can be partly, although not completely, explained on the basis of the increase in extracellular $[K^+]$ (Corr *et al.*, 1982; Clusin *et al.*, 1983). In pig ventricular cells, resting membrane potential is progressively reduced from its usual value near -85 mV to -65 or -60 mV within 15-25 minutes after the onset of ischaemia (Kléber *et al.*, 1978). After this time inexcitability results. It appears that the increase in extracellular $[K^+]$ is the predominant mechanism whereby ischaemia causes depolarisation but clearly not the only one. Given that the resting membrane is selectively permeable to K^+ , resting membrane potential is governed by the log ratio of the $[K^+]$ inside and outside the cell (Goldman, 1943; Hodgkin & Katz, 1949). However, the reduction in the membrane potential is greater than that predicted by this relationship (Gettes & Cascio, 1991; Kléber *et al.*, 1986; Buchanan *et al.*, 1985). Blake and colleagues (1988) suggested that altered Ca^{++} homeostasis may contribute to the reduction in membrane potential produced by ischaemia on the basis of their observation that Ca^{++} infusion or pacing augmented ischaemia-induced depolarisation. Acidification, such as that caused by ischaemia, results in depolarisation of resting membrane potential (Campbell & Hemsworth, 1990; Campbell *et al.*, 1991; Ye *et al.*, 1993). Others have suggested that amphipathic lipid metabolites contribute to the depolarisation observed with ischaemia (Corr *et al.*, 1981; Snyder *et al.*,

1981, Kiyosue & Arita, 1986), but this is controversial (see above). Partial recovery of resting membrane potential is commonly observed, particularly in cells near the border zone (Kaplinsky *et al.*, 1979; Meesman *et al.*, 1978). This is thought to be due to diffusion of K^+ to the non-ischaemic tissue (Hirche *et al.*, 1980; Coronel *et al.*, 1988).

Ischaemia-induced changes in AP amplitude and maximum upstroke velocity are partly secondary to the effects on resting membrane potential but clearly other factors contribute. Maximum AP upstroke velocity is transiently increased shortly after induction of ischaemia (30 seconds to 2 minutes; Elharrar *et al.*, 1977; Holland & Brooks, 1976). This effect is most likely mediated by the small degree of depolarisation (5-10 mV) seen in the early stages of ischaemia which brings cells to the threshold voltage for AP firing (Elharrar *et al.*, 1977; Holland & Brooks, 1976). Membrane depolarisation, such as that observed with ischaemia, increases the fraction of fast Na^+ channels in the inactivation state which are then unavailable on the next AP (see Hondeghem & Katzung, 1977, 1984). Suppression of inward Na^+ current leads to a reduction in AP amplitude and maximum upstroke velocity and eventually inexcitability (Wit & Janse, 1989; Kléber *et al.*, 1986). Inexcitability typically occurs at resting membrane potentials of -65 to -60 mV (Kléber *et al.*, 1986). Two to one responses sometimes precede inexcitability (Downar *et al.*, 1977; Kléber *et al.*, 1978; Russel *et al.*, 1979; Barrett *et al.*, 1997), likely due to the time and voltage dependent recovery of inward (and outward) currents.

Controversy exist as to which current is responsible for AP generation in ischaemic tissue. A slow upstroke can be produced by either a reduced fast inward Na^+ current or a slow inward Ca^{++} current (Hauswirth & Singh, 1978; Kupersmith & Cohen, 1980; Arita *et*

al., 1983; Janse & Wit, 1989). The observation that "slow response" APs (so named due to their slow rate of depolarisation) occur in the presence of increased extracellular $[K^+]$ and catecholamine concentrations suggests that APs in the deep ischaemic zone are mediated by the slow inward Ca^{++} current (Cranefield, 1975; Arita *et al.*, 1983). However, it is unlikely that the conditions found in the ischaemic myocardium are extreme enough to allow slow response APs to occur (see Janse & Wit, 1989). The membrane potentials at which ischaemic cells are rendered inexcitable is approximately -60 mV (Kléber *et al.*, 1986). At this membrane potential the fast inward Na^+ current is not completely voltage-inactivated (e.g., Hondeghem & Katsung, 1984). In contrast to those observed in the ischaemic myocardium, slow response APs in partially K^+ -depolarised, epinephrine-treated hearts occurred at resting membrane potentials of -50 mV (Kléber *et al.*, 1986). This suggests that the fast inward Na^+ current, although reduced under the conditions of ischaemia, is the predominant mechanism of AP generation in the ischaemic myocardium (Arita *et al.*, 1983). Pharmacological studies support this observation. Both lidocaine and verapamil depress the upstroke of APs in ischaemic tissue but only lidocaine rendered such tissue inexcitable (Cardinal *et al.*, 1980; Janse, 1982; Arita *et al.*, 1983). Despite this, the potential role of the slow inward Ca^{++} current should not be discounted as ischaemic cells surely have a greater dependence on this current for AP genesis and conduction than do normal cells (Arita *et al.*, 1983; Janse & Wit, 1989).

In pig, dog, rabbit and rat hearts, AP duration increases initially (30 seconds to 2 minutes) after the onset of ischaemia and shortens rapidly thereafter (e.g., Downar *et al.*, 1977; Inoue *et al.*, 1984; Mohabir *et al.*, 1991; Barrett *et al.*, 1997). The initial

prolongation is reported to be due to subepicardial cooling (Janse & Wit, 1989; Kurz *et al.*, 1993). Data from *in vitro* preparations, in which warm blood does not continue to bathe the endocardium, supports this hypothesis (Kurz *et al.*, 1993).

The mechanism of AP shortening caused by ischaemia has been the subject of much investigation. Early studies showed that an increase in outward, time-independent K^+ current was responsible for AP shortening (Vleugels & Carmeliet, 1976; Vleugels *et al.*, 1980; Isenberg *et al.*, 1983). Subsequent work by Noma (1983) and others (Fosset *et al.*, 1988; Sanguinetti *et al.*, 1988; Escande *et al.*, 1989) suggested that activation of $I_{K(ATP)}$ was a likely mechanism for this effect. There is now little doubt that $I_{K(ATP)}$ contributes to the AP shortening observed with ischaemia but this hypothesis is not without flaws (see above) and other ionic currents may contribute (Kim & Clampham, 1989; Kameyama *et al.*, 1984; DeLorenzi *et al.*, 1994; Ruiz-Petrich *et al.*, 1996). AP shortening caused by hypoxia *in vitro* can be partially (e.g., Nakaya *et al.*, 1991; Cole *et al.*, 1991; MacKenzie *et al.*, 1993) or completely (e.g., Gasser & Vaughan-Jones, 1990) prevented by the $I_{K(ATP)}$ blocker glibenclamide. *In vivo* studies involving regional myocardial ischaemia show that ischaemia-induced AP shortening can be blunted by $I_{K(ATP)}$ blockade (e.g., Smallwood *et al.*, 1990; Moritani *et al.*, 1994; Miyoshi *et al.*, 1996). The observation that epicardial APs shorten more than endocardial APs (Taggart *et al.*, 1988) might be explained by differences in the distribution of K_{ATP} channels (Furukawa *et al.*, 1991; Miyoshi *et al.*, 1996).

Evidence, which has largely been ignored, exists as to the involvement of a number of other ionic currents in mediating ischaemia-induced AP shortening. Two high

conductance K^+ currents ($I_{K(Na)}$, Kameyama *et al.*, 1984; $I_{K(AA)}$, Kim & Clampham, 1989) that might be expected to be activated by the conditions of ischaemia have been described. Modifications in the activation of the transient outward K^+ current (I_{to}) might contribute to ischaemia-induced AP shortening (Lukas & Antzelevitch, 1993; DiDiego & Antzelevitch, 1994). The greater density of I_{to} in the epicardium versus endocardium (Litovsky & Antzelevitch, 1988, 1989; Fedida & Giles, 1991; Furukawa *et al.*, 1990) might also explain why epicardial APs shorten more than those in the endocardium (Taggart *et al.*, 1988). Another factor that might contribute to the AP shortening in the ischaemic myocardium is the augmentation of K^+ current carried by I_{Kr} and the inward rectifier (I_{K1}) K^+ currents by increases in external $[K^+]$ (Noble & Tsein, 1969; Yang & Roden, 1996). Thus, the ischaemia-induced increase in extracellular $[K^+]$ may contribute to further K^+ loss and ischaemia-induced AP shortening. Experimental evidence for the above is provided by the observation that blockade of I_{K1} with Ba^{++} significantly attenuates the AP shortening caused by hypoxia (Ruiz-Petrich *et al.*, 1991). Further studies by this group suggest that a catecholamine activated Cl^- current ($I_{Cl(NE)}$) contributes to hypoxia-induced AP shortening (Ruiz-Petrich *et al.*, 1996).

The observation that intracellular Ca^{++} increases quickly after the onset of ischaemia (Lee *et al.*, 1988) hints that a Ca^{++} activated Cl^- current ($I_{Cl(Ca)}$) might also contribute to AP shortening (Zygmunt & Gibbons 1991; Zygmunt, 1994). Acid inhibition of Ca^{++} currents may also contribute to AP changes caused by ischaemia (Kohlhardt *et al.*, 1976; Irisawa *et al.*, 1986). Suffice it to say that the mechanisms of ischaemia-induced AP shortening are complex and not well understood (also see Wilde, 1997). The role of

$I_{K(ATP)}$ may have been overstated while that of other K^+ currents and possibly Cl^- currents may have been underestimated.

A number of investigators have described alternating repolarisation characteristics caused by ischaemia (e.g., Lee *et al.*, 1988; Hirata *et al.*, 1980; Dilly & Lab, 1988; Abe *et al.*, 1989; Surawicz & Fisch, 1992). These disturbances can be measured as alternans in AP duration in intracellular or MAP recordings (Lee *et al.*, 1988; Hirata *et al.*, 1980) or as alternans in the QT interval of the surface ECG (Nearing *et al.*, 1991; Verrier & Nearing, 1994). The term "alternans" refers to the fact that AP duration or T wave morphology is different on every other beat (e.g., the 1st and 3rd beats have the same morphology as do the 2nd and 4th). The work of Clusin's group suggests that alternans in the repolarisation phase of the AP are the result of alternating Ca^{++} transients (Lee *et al.*, 1988). Another important observation made by this group was that alternans can be out of phase at sites which are in close proximity to one and other. Differences in repolarisation on a beat to beat basis creates voltage gradients in the heart which ultimately result in an increase in the heterogeneity of the heart's electrophysiological properties.

In the early stages of ischaemia (~5 minutes), changes in the refractory period parallel changes in AP duration but, with prolonged ischaemia, the difference between the two increases. After the onset of ischaemia the refractory period shortens, likely for the same reasons that AP duration shortens (see above). With longer durations of ischaemia, post-repolarisation refractoriness can develop (Lazzara *et al.*, 1976; Levites *et al.*, 1976; Downar *et al.*, 1977). This occurs more commonly and to a greater extent in the deep ischaemic zone than in the border zone (Janse *et al.*, 1985). The mechanism(s) for post-

repolarisation refractoriness have not been completely delineated but dissociation of gap junctions and voltage-dependent inactivation of Na^+ channels are likely candidates (Janse & Wit, 1989; Smith *et al.*, 1995). Conductance through gap junctions is reduced in a synergistic fashion by increased intracellular $[\text{Ca}^{++}]$ and low pH (Burt, 1987; White *et al.*, 1990). The concentrations required for these effects are similar to those observed after 20-30 minutes of ischaemia; a time which immediately precedes gap junction dissociation (Lee *et al.*, 1988; Dennis *et al.*, 1991). A number of other ischaemia-induced biochemical changes are known to cause gap junction uncoupling including increased concentrations of long-chain acylcarnitines, lysophosphoglycerides and arachidonic acid (Yamada *et al.*, 1994; Owens *et al.*, 1996).

Conduction velocity is transiently increased in the early stages of ischaemia (30 seconds to 2 minutes) after which it is progressively reduced (Gambetta & Childers, 1969; Buchanan *et al.*, 1985). The increase in conduction velocity seen in the early stages of ischaemia parallels the increase in the maximum rate of depolarisation and is likely to be caused by the same mechanism (see above). The reduction in conduction velocity caused by ischaemia is more complex. In normal cardiac tissue conduction velocity is proportional to the square root of the maximum rate of depolarisation (Cascio *et al.*, 1995). While in ischaemic tissue the maximum rate of depolarisation is reduced, conduction velocity is reduced more than predicted by the above relationships (Buchanan *et al.*, 1985). Voltage-dependent inactivation of Na^+ channels causes slowing of conduction; however, other factors also contribute to suppression of conduction caused by ischaemia (Blake *et al.*, 1988). Reduced conductance through gap junctions, a reduced

driving force for Na^+ entry and altered Ca^{++} homeostasis may interact to produce the conduction slowing caused by ischaemia (Clusin *et al.*, 1983; Blake *et al.*, 1988; Burt, 1987; White *et al.*, 1990; Wilde & Kléber, 1986). The slowing of conduction velocity produced by myocardial ischaemia is further exacerbated at higher rates (Carson *et al.*, 1986; Harper *et al.*, 1993).

The electrophysiological changes caused by ischaemia are heterogeneous in nature (see review by Janse & Wit, 1989). Diffusion of K^+ and CO_2 gives rise to regional differences in the electrophysiological effects of ischaemia (Hill & Gettes, 1980; Cascio *et al.*, 1992; Yan, 1992). Furthermore, by virtue of anatomy and their cellular complement of ion channels, not all cell types respond in the same way to ischaemia. For example, the epicardium and endocardium are differentially influenced by "simulated ischaemic" buffer (e.g., high $[\text{K}^+]$, hypoxic and acidic buffer; Gilmore & Zipes, 1980). Regional differences in the occurrence of alternans in AP duration contribute to the heterogeneity of the ischaemic myocardium (Lee *et al.*, 1988). All this culminates in regional differences in excitability, refractory period and conduction velocity which contribute to the mechanism(s) of arrhythmogenesis in the ischaemic myocardium.

1.5 Mechanism(s) of arrhythmogenesis in the ischaemic myocardium.

The mechanism(s) by which myocardial ischaemia causes arrhythmias has been the subject of much investigation and mechanism(s) have been, at least partly, elucidated. Three mechanisms have been postulated: re-entry, altered automaticity and triggered activity (see Cranefield *et al.*, 1973; Hoffman & Rosen, 1981). Of these, only re-entry has

been demonstrated to occur in intact hearts with some certainty (e.g., Janse *et al.*, 1980; Pogwizd & Corr, 1987; Witkowski *et al.*, 1998; Gray *et al.*, 1998). While methodological difficulties may preclude demonstration of such mechanisms (e.g., the difficulties in recording intracellular potentials in a beating heart) neither have been convincingly linked to the occurrence of arrhythmias in the ischaemic myocardium. To confound these difficulties, these mechanisms are not mutually exclusive and one may initiate the other. Rapid reperfusion of the ischaemic myocardium causes a variety of arrhythmias (see Botting *et al.*, 1985). The importance of these arrhythmias is unknown (Lie, 1993) and they will not be considered further.

A common misconception concerning arrhythmias is that a continuum exists. That is to say that a PVB is a short VT and VF a disorganised VT. This seemingly rational assumption suggests that all arrhythmias share a common mechanism. This assumption appears to be false and may have deadly repercussions (see later and CAST, 1989).

1.5.1 Re-entry.

Re-entry was first postulated by McWilliams over a century ago (1887, cf: Marriott & Conover, 1989). Since then it has been demonstrated in jelly fish excitable tissue (Mayer 1906, 1908: cf Marriott & Conover 1989) and later in cardiac tissue (Mines, 1913, 1914). The basic requirement for re-entry are: a circuit, slow conduction and unequal responses at different sites (Mines 1913, 1914). All of these basic requirements can be fulfilled by the combination of the ischaemic and normal myocardium. There are

two types of re-entry depending on the size of the circuit: micro and macro (Sasyniuk & Mendez, 1971).

The criteria by which an arrhythmia can be reasonably considered to be caused by re-entry were outlined by Mines (1913, 1914) and can be summarised as follows: 1) a uni-directional block of conduction must be demonstrated, 2) the impulse must be shown to be conducted around the site of block and re-excite the tissue proximal to the block and, 3) interruption of the circuit stops the arrhythmia. The first two criteria have been demonstrated by many studies while proof of the third is still lacking in the case of ischaemia-induced arrhythmias (Boineau & Cox, 1973; El-Sherif *et al.*, 1975; Janse *et al.*, 1980; Witkowski, *et al.*, 1998; Grey *et al.*, 1998). In the case of certain cardiac arrhythmias (e.g., supraventricular tachycardia involving the AV node) all three criteria have been reasonably satisfied (Roden, 1996).

The most convincing evidence that re-entry occurs in the ischaemic myocardium is obtained from mapping studies. Durrer and colleagues (1971) were the first to demonstrate the occurrence of re-entry in porcine and canine hearts subject to coronary artery occlusion through the use of an epicardial electrode array. Studies of this type rely on determination of the activation time at various sites; a procedure which can be highly subjective. Moreover, mapping studies are usually confined to the epicardium and the resolution of the data obtained are always limited by the number of recording electrodes. Pogwizd & Corr (1987) elegantly addressed this limitation by constructing activation maps in three dimensions (i.e., electrograms were recorded from epicardial, intramural and endocardial sites) from the ventricle of cats subject to coronary artery occlusion and

reperfusion. The results of their studies suggest that a majority (~75%) of ischaemia-induced arrhythmias are re-entrant in nature. More recently the resolution for mapping of re-entry circuits has been greatly increased through the use of voltage sensitive dyes and high resolution optical equipment (Witkowski *et al.*, 1998; Gray *et al.*, 1998).

The special case of "injury currents" must be considered. Ischaemia-induced changes in APs (e.g., depolarisation, reduced AP amplitude and AP shortening) and the relatively close proximity of ischaemic and normal tissue in the heart causes "injury currents" to flow across the border zone between the two (Hoff *et al.*, 1941; Harris, 1950; Kléber *et al.*, 1978). These injury currents are responsible for the ECG manifestations of ischaemia and may be arrhythmogenic in and of themselves (Janse *et al.*, 1980; Ridley *et al.*, 1992). During diastole injury current flows from ischaemic to normal tissue while the reverse occurs during electrical systole (Kléber *et al.*, 1978). Injury currents flowing during early diastole have been suggested to re-excite normal tissue and cause arrhythmias (Janse *et al.*, 1980). Arrhythmias arising from this mechanism appear to be focal in nature when observed in epicardial mapping studies but arise from mechanisms akin to re-entry. Injury currents may therefore be considered to be a form of re-entry.

Reflection may also be regarded as a form of re-entry (Antzelevitch *et al.*, 1980, 1983). In the case of reflection current flows across an inexcitable gap, which is not required to regain excitability. This differentiates reflection from the classical re-entry model since the classical model requires that the site of re-entry be part of the circuit (i.e., activated by the aberrant impulse). Transmission of an impulse via electrotonic conduction with a sufficiently long delay can re-excite tissue and cause arrhythmias

(Antzelevitch *et al.*, 1979). The issue is complicated by the fact that the tissue on the other side of the block may or may not exhibit automaticity. In the case where it does not, the similarity to re-entry is easily seen. In the case where the tissue does exhibit automaticity (e.g., a Purkinje fibre), the cell can be brought to threshold and an AP fired sooner than it would have otherwise. This scenario essentially describes entrance block and parasystole but with the rate of spontaneous firing accelerated by electrotonic current conduction. In short, reflection might appear to be non re-entrant in nature; however, it likely represents one end of a continuum and may be considered a form of re-entry.

1.5.2 Abnormal automaticity.

A number of cell types normally exhibit automaticity. These include the sinus node, atrio-ventricular node and Purkinje fibres, whereas automaticity can only be induced in ventricular cells by experimental manipulations (Surawicz & Imanishi, 1976). The rate of spontaneous depolarisation differs between cells and the fastest pacemaker prevails. As the sinus node has the fastest rate of spontaneous depolarisation it usually determines heart rate (HR). Abnormal automaticity can then be defined as automaticity which does not originate from the sinus node (Hoffman & Rosen, 1981; Sasyniuk, 1984). One of the hallmarks of automaticity is that it is suppressed by overdrive pacing (Vassalle, 1977).

The relevance of abnormal automaticity to ischaemia-induced arrhythmias is unknown but it is not thought to be a significant mechanism in the acute ischaemic phase (Cranefield *et al.*, 1973; Hoffman & Rosen, 1981). However, it may be more relevant to arrhythmogenesis in the infarction phase (after 24 hours). Abnormal automaticity can be

induced in depolarised Purkinje fibres *in vitro* (Imanishi & Surawics, 1976). However, in other preparations raised extracellular $[K^+]$ markedly suppresses altered automaticity (Katzung *et al.*, 1975).

1.5.3 Triggered activity.

Triggered activity has been described in a number of *in vitro* preparations and comes in two varieties: early and delayed afterdepolarisations (EADs & DADs, respectively). These oscillatory afterpotentials are linked to the previous beat and can be induced by a number of experimental manipulations. EADs are defined as afterdepolarisations which occur during repolarisation before it is complete, while DADs are those occurring after repolarisation is complete or nearly complete (Hoffman & Rosen, 1981). Afterdepolarisations can reach threshold and give rise to VPB, VT or possibly VF (Wit & Rosen, 1986).

EADs can be induced by catecholamines (Hoffman & Crane field, 1960, Priori & Corr, 1990), as well as a combination of acidosis and hypoxia (Adamantidis *et al.*, 1986). Long APs, low heart rates, hypokalaemia and hypomagnesaemia favour the occurrence of EADs while short APs, high heart rates, hyperkalaemia and hypermagnesaemia prevent EADs (Roden & Hoffman, 1985; Davidenko *et al.*, 1989). It seems unlikely that EADs contribute to the occurrence of ischaemia-induced arrhythmias as they are suppressed by hyperkalaemia, a condition known to exist in the ischaemic myocardium. However, the occurrence of EADs may be particularly relevant to arrhythmias occurring during quinidine intoxication and the proarrhythmic actions of any of a number of new Class III

antiarrhythmic drugs (Strauss *et al.*, 1970; Roden & Hoffman, 1985; Carlsson *et al.*, 1993; Antzelevitch & Sicouri, 1994). Based on their positive take-off potential, it has been suggested that the slow inward Ca^{++} current is responsible for EADs (January *et al.*, 1988).

DADs occur under conditions of intracellular Ca^{++} overload. Indeed, DADs are best known for their role in the arrhythmogenic actions of digitalis glycosides such as strophanthidin and ouabain (Ferrier, 1973; Rosen *et al.*, 1973). Other factors known to precipitate DADs include hypercalcaemia, hypomagnesaemia, low pH, a combination of mild hypoxia and acidosis, as well as catecholamines (Adamantidis *et al.*, 1986; Kano & Nishi, 1986). Severe hypoxia prevents the occurrence of DAD (Molina-Viamonte *et al.*, 1991; Coetzee *et al.*, 1987). High heart rates increase the amplitude of DADs and reduce the coupling interval of beats which reach threshold (Ferrier *et al.*, 1973). This positive rate dependence makes DADs an attractive mechanism for causing tachyarrhythmias. DADs are thought to be caused by oscillatory Ca^{++} release from the sarcoplasmic reticulum which activates a transient inward current. The current carrier underlying this transient inward current is not known with certainty but there are two candidates: the electrogenic $\text{Na}^+/\text{Ca}^{++}$ exchanger (Lenderer & Tsien, 1976; Arlock *et al.*, 1985; Lipp & Pott, 1988) or a non-specific cation current (Colquhoun *et al.*, 1981; Shimoni & Giles, 1987).

The potential role of afterpotentials in the genesis of ischaemia-induced arrhythmias is not clear. Their occurrence is favoured by some conditions found in the ischaemic myocardium, but inhibited by others. For example, hypoxia, acidosis,

catecholamines, and myocardial stretch favour the occurrence of EADs. On the other hand, hyperkalaemia, AP shortening and inexcitability can be expected to prevent EADs. Molina-Viamonte and colleagues (1991) demonstrated that afterpotentials can be induced by conditions of simulated ischaemia in canine Purkinje fibres and that their occurrence was dependent on α -adrenergic receptor stimulation. DADs occurring at the border zone may contribute to arrhythmogenesis in the ischaemic myocardium while EADs are not thought to be involved.

1.6 Treatment of acute myocardial ischaemia and infarction.

Ideally, it is preferable to reverse ischaemia before infarction or SCD takes place. Reversal of ischaemia by means of fibrinolytic drugs or angioplasty has been highly successful in reducing mortality (see Hennekens *et al.*, 1995). Aspirin reduces myocardial infarction rates (e.g., Anonymous, 1989) and reduces mortality in patients that do have myocardial infarction (Stein & Fuster, 1989). The beneficial effect of aspirin was thought to be due to its antiplatelet actions (see Stein & Fuster, 1989); however, recent evidence suggests that its beneficial effects might be mediated by its antiinflammatory actions (Ridker *et al.*, 1997). Treatment of myocardial ischaemia is directed towards reduction in the number and duration of ischaemic events. Nitroglycerine, β -adrenergic antagonists and calcium channel antagonists are commonly used for these purposes (Robertson & Robertson, 1996). Despite gains in treatment of ischaemia and infarction, SCD remains a major health problem (see Lopez, 1993; Reddy & Yusuf, 1998). Simply put, patients suffering acute myocardial ischaemia and infarction must survive long enough to reach

hospital in order to be treated. In light of this it seems prudent to prevent the arrhythmias that cause SCD.

1.7 Treatment of arrhythmias.

Due to the large number of deaths attributed to SCD, which are presumably arrhythmic in nature, a great deal of time, effort and money has been spent treating such arrhythmias. In general, there are two basic approaches to the treatment of arrhythmias: prevention or reversion. Discussion of the treatment of arrhythmias is necessarily divided into two sections representing the two populations which require treatment; those suffering acute ischaemia-induced arrhythmias and those with a stable myocardial infarct.

Treatment of arrhythmias occurring in the acute ischaemic or infarction phase is restricted to patients in hospital. This is far from ideal as VF occurs most commonly before patients reach the hospital. As a result, there is little or no data specifically concerned with the treatment of acute ischaemia-induced arrhythmias *per se*. In this case a discussion of arrhythmias associated with acute ischaemia and infarction is more fitting. DC cardioversion, first described by Zoll and colleagues (1956), is very successful in terminating VF when such devices are available (e.g., Reddy & Barby, 1997). Despite the general success of cardioversion it is not possible to revert VF in all patients and it is preferable to prevent VF rather try to revert it. A number of pharmacological agents have been tested for prevention of VF including lidocaine, propafenone, bretylium and, more recently, amiodarone. The clinical data will be briefly considered.

Lidocaine is by far the most common antiarrhythmic drug that has been given to prevent arrhythmias associated with acute ischaemia and infarction. The use of lidocaine for prophylaxis of such arrhythmias is based on Lown's (1967) observation that lidocaine suppressed PVBs. Subsequently, Lie and colleagues (1974) demonstrated lidocaine's antifibrillatory properties. A number of studies (1969 to 1978) were unable to confirm Lie's results (see MacMahon *et al.*, 1988 or Hine *et al.*, 1989). The first systematic review of the use of lidocaine confirmed that lidocaine reduced the incidence of VF (DeSilva *et al.*, 1981). Effects on mortality were not analysed. These investigators concluded that the failure of some studies to show an effect was due to small sample sizes and the use of "inadequate treatment protocols." In 1985, Lie questioned the usefulness of lidocaine on the basis of its potential toxicity, the relatively low incidence of VF associated with the in-hospital phase of acute myocardial infarction and the fact that beneficial effects on mortality had never been demonstrated! Subsequent meta-analysis of fourteen blinded, randomised, controlled clinical trials showed that lidocaine reduced the incidence of VF by about a third; however, there was no effect on mortality (MacMahon *et al.*, 1988). Moreover, subdivision of trials into pre-hospital and in-hospital phases revealed that lidocaine significantly increased mortality during the in-hospital phase (Hine *et al.*, 1989). It was suggested that the apparent lack of a beneficial effect on mortality, despite preventing VF, may be due to an increased incidence of asystole (MacMahon *et al.*, 1988).

While other drugs have been used to prevent arrhythmias, there is relatively little data on the effectiveness of such drugs for preventing arrhythmias due to ischaemia and

infarction. Propafenone has been used to treat drug resistant malignant arrhythmias (i.e., lidocaine resistant; Kowey *et al.*, 1991). Little discrimination as to the presence or absence of an ischaemia substrate was made during these studies. Bretylium has been used as a last chance drug to prevent the recurrence of drug resistant malignant arrhythmias (Bacaner, 1983). It has a unique place among antiarrhythmic drugs as it has been shown to revert VF to sinus rhythm (Nowak *et al.*, 1981) and has been advocated for prophylaxis of VF (Bacaner, 1983). Despite this, bretylium is rarely used (Reiffel *et al.*, 1994). This may be partly due to its complex pharmacology (see Bacaner, 1983). Recently, clinical trials with amiodarone have shown it to be no better or worse than other antiarrhythmic drugs for preventing malignant arrhythmias in hospital (Kowey *et al.*, 1995). Use of amiodarone in the acute setting likely reflects clinician's frustration with the lack of effective drugs and its reputation as the only effective antiarrhythmic drug (Toe *et al.*, 1993).

Treatment of arrhythmias after myocardial infarction has received a great deal of attention. The large number of patients with healing or stable myocardial infarcts attests to the magnitude of the problem (Tunstall-Pedoe *et al.*, 1994). Also, these patients are easily identified as being at substantial risk of SCD. Modes of therapy for post-infarct patients may include implantable cardiac defibrillators, various pharmacological agents, surgical and/or catheter ablation techniques (see Capucci & Boriani, 1993). The implantable cardiac defibrillator, first described by Mirowski and colleagues (1980), is very successful in recognising tachyarrhythmias and reverting them by means of DC shock (see review by Gillis, 1996). Despite reverting almost all episodes of VF, the benefits of

the implantable cardiac defibrillator on mortality have been questioned (see Connolly & Yusuf, 1992; Kim, 1993; Zipes, 1994). This has led to a call for randomised, controlled clinical trials of implantable cardiac defibrillator versus the best medical (i.e., pharmacological) treatment (Connolly & Yusuf, 1992; Kim, 1993; Zipes, 1994). Three such trials have been started (e.g., AVID, see Zipes, 1994; CIDS, see Bigger, 1991, CASCADE, 1991). The recently published results of AVID show that the implantable cardiac defibrillator is more effective than the antiarrhythmic drugs presently available (Anonymous, 1997).

Pharmacological therapy of arrhythmias in post-infarct patients has been much less successful than was hoped (see Reiffel *et al.*, 1994). Several large drug trials in patients at risk of SCD have had disastrous results (CAST Investigators, 1989; Etch *et al.*, 1991; Waldo *et al.*, 1996). The electrophysiological and putative antiarrhythmic actions of the drugs tested in these clinical trials (flecainide, encainide and d-sotalol) are well described in terms of their actions on normal cardiac tissue: an especially interesting nuance with regards to the topic of this thesis. The only antiarrhythmic drugs that have been proven to reduce mortality in post-infarct patients are amiodarone and β -adrenoceptor antagonists (see Teo *et al.*, 1993 & Yusuf *et al.*, 1985). In short, the use of antiarrhythmic drugs after myocardial infarction is restricted to treatment of symptomatic or life threatening arrhythmias (Reiffel *et al.*, 1994). Selection of pharmacological agents intended to prevent VF after myocardial infarction is complex and can be guided by a number of studies including programmed electrical stimulation of the heart, ambulatory ECG monitoring and exercise testing (see Singh, 1991).

1.7.1 Antiarrhythmic drug classification.

A number of classification systems for antiarrhythmic drugs have been proposed (Singh & Vaughan-Williams, 1972; Weirich & Antoni, 1990; Sicilian Gambit, 1991; see Nattel, 1991 for a review). None of these are ideal and antiarrhythmic drug classification is still a matter of some debate. The most commonly used scheme is that proposed by Singh and Vaughan-Williams (1970a, 1970b, 1972). This scheme identifies four classes of antiarrhythmic action within which drugs are classified according to their predominate mechanism of action. The four classes are: local anaesthetic, antiadrenergic, action potential prolonging and calcium channel blockade. Singh and Hauswirth (1974) and later Harrison and colleagues (1981) modified the Vaughan-Williams scheme based on clinical and experimental observations which showed that the actions of Class I drugs on the excitability, conduction velocity, and refractoriness were divergent. This resulted in a tiered sub-classification scheme for Class I drugs whereby drugs which had moderate effects on conduction and prolonged AP duration were grouped together as Class Ia, drugs with limited effects on conduction and shortened AP duration were grouped together as Class Ib, and drugs which markedly suppressed conduction and had little effect on AP duration were grouped together as Class Ic. Despite its nearly universal use, the Vaughan-Williams scheme has been widely criticised (see Sicilian Gambit, 1991). The fact that the system is a hybrid and classifies drug action on the basis of effects on molecular targets as well as functional responses is one of the major criticisms. Secondly, the system

classifies drug actions on the basis of effects on normal myocardial tissue, a condition not associated with the occurrence of arrhythmias.

Observations made by Campbell (1983) were of key importance to our understanding and classification of Class I drugs. Campbell noted that the actions of clinically used Class I antiarrhythmics could be divided into three groups on the basis of the kinetics of onset for depression of the maximum upstroke velocity of guinea pig ventricular APs. Specifically, drugs classed as Ib, Ia and Ic by Harrison and co-workers (1981) were found to correspond to drugs with rapid, intermediate and slow onset kinetics, respectively (Campbell, 1983). These observations provided a rational basis for Harrison's sub-classification of Class I drugs and firmly established the scheme in antiarrhythmic dogma.

An alternative to the Vaughan-Williams classification scheme has been proposed in the form of the Sicilian Gambit (1991), although the authors explicitly deny that they propose to classify antiarrhythmic drugs. The Sicilian Gambit suggests that arrhythmias should be treated by first considering the mechanism of the arrhythmia, identifying the "vulnerable parameter" and selecting a drug which specifically targets that "vulnerable parameter." While this rational approach is very successful in some cases (e.g., AV node-dependent re-entry and the use of adenosine or verapamil) it suffers greatly from a lack of practicality. The crux lies in the identification of the "vulnerable parameter."

1.7.2 Pharmacology of Class I drugs.

Hodgkin & Huxley first described the inward Na^+ current in the squid giant axon as a biophysical construct (1952). Since then, the molecules responsible for the generation of this current have been identified and characterised (e.g., Noda *et al.*, 1986; Kayano *et al.*, 1988; for a review see Catterall, 1992). Sodium channels activate rapidly upon depolarisation and inactivate thereafter (Hodgkin & Huxley, 1952; Noda *et al.*, 1986; Kayano *et al.*, 1988). Both activation and inactivation are time- and voltage-dependent. Sodium channels exhibit inward rectification. Modulation of Na^+ channels by a number of neuronal and hormonal systems has been described. Beta-adrenergic agonists reduce Na^+ current in neuronal (Costa *et al.*, 1982; Costa & Catterall, 1984a) and cardiac tissue (Gordon *et al.*, 1988). Some investigators suggest this effect is due to a reduced number of Na^+ channels rather than a reduction in Na^+ conductance (Taouis *et al.*, 1991). Angiotensin II has been suggested to modulate Na^+ channel activation and inactivation via a protein kinase C-dependent mechanism (Moorman *et al.*, 1989). Acidosis, such as that produced by ischaemia, reduces Na^+ current (Bass & Moore, 1973; Kagiya *et al.*, 1982).

The molecular structure of a number of sodium channels has been described (see Barchi, 1988; Roden & George, 1997). Although simplistic, it is useful to classify Na^+ channels as neuronal, skeletal or cardiac in origin. The pharmacology of cardiac Na^+ channels is different in some ways than that of other Na^+ channels.

Cardiac Na^+ channels are 10 to 100 times less sensitive to tetrodotoxin, a specific Na^+ channel blocker (Narahashi, 1974; Bean *et al.*, 1983; Guo *et al.*, 1987). Moreover,

the actions of tetrodotoxin are rate-dependent on cardiac Na^+ channels but not on those from neuronal or skeletal tissues (Baer *et al.*, 1976; Guo *et al.*, 1987).

Alpert (1989) and co-workers demonstrated the existence of a unique extracellular locus of action for lidocaine, and its permanently charged analogue QX-314, on cardiac Na^+ channels. Thus, in cardiac tissue, local anaesthetics drugs may act not only at an intracellular locus, which is common to all Na^+ channels, but also at a unique extracellular locus.

Single channel studies reveal that cardiac Na^+ channels exhibit a delayed burst of openings after depolarisation exclusive to this type of Na^+ channel (Nilius, 1988; Kirsch & Brown, 1989; Kiyosue & Arita, 1989). These delayed Na^+ channel openings are thought to be responsible for the plateau Na^+ current observed in cardiac tissue (Kirsch & Brown, 1989; Kiyosue & Arita, 1989).

The actions of drugs which block Na^+ channels on the heart (i.e., Class I antiarrhythmics) are relatively well characterised. Class I drugs reduce excitability and conduction velocity in the myocardium (see Singh & Vaughan-Williams, 1971; Singh & Hauswirth, 1974; Harrison *et al.*, 1981; Campbell, 1983; Sicilian Gambit, 1991). Automaticity is reduced at higher concentrations while effects on the refractory period differ between agents (Campbell, 1983; 1987). The modulated receptor hypothesis has been proposed to account for the rate- and voltage-dependence of action exhibited by Class I drugs (Hille, 1977; Hondeghem & Katzung, 1977). It proposes the following: (1) Class I drugs bind to a receptor site on or very close to the transmembrane ionic channel, (2) the affinity of drugs is modulated by channel state (i.e., rested, activated or inactivated)

and/or membrane potential, (3) drug associated channels do not conduct and, (4) the voltage dependence of drug associated channels is shifted to more negative potentials. An alternative model, the guarded receptor hypothesis, has been put forward to explain the rate- and voltage-dependence observed for Class I drugs (Starmer *et al.*, 1983). The guarded receptor hypothesis makes more assumptions for which there is little experimental evidence and, as a result, is not as well accepted as the modulated receptor hypothesis (Hondeghe & Katzung, 1984).

1.7.3 Pharmacology of Class II antiarrhythmics.

While β -adrenoceptor antagonists may have direct antiarrhythmic actions, they have a host of other cardiovascular actions which make it difficult to attribute their beneficial effects in reducing mortality in post myocardial infarction patients to prevention of arrhythmias *per se*. β -adrenoceptor antagonists are commonly used to reduce ischaemia in patients with angina pectoris and have been shown to reduce mortality in post-infarct patients (see Yusuf *et al.*, 1985). While Class II antiarrhythmics are usually assumed to include only β -adrenoceptor antagonists (e.g., Sicilian Gambit, 1991), the original classification includes all antiadrenergic activity (Singh & Vaughan-William, 1970a, 1970b, 1972). Thus, the Vaughan-Williams scheme also includes the antiarrhythmic actions of α -adrenoceptor antagonists (e.g., Moline-Viamonte *et al.*, 1991), bretylium and amiodarone (see Vaughan-Williams, 1992).

1.7.4 Pharmacology of Class III antiarrhythmics.

Class III antiarrhythmic activity is defined as an increase in AP duration without effects on other AP characteristics, or on conduction velocity (Singh & Vaughan-Williams, 1970a & 1970b). Most Class III antiarrhythmic drugs prolong AP duration by blocking outward K^+ currents, although this is not the only possible mechanism. Drugs such as DPI 201-106 and BDF9148 prevent Na^+ channel inactivation and increase AP duration (Hoey *et al.*, 1994, 1994). Ibutilide may prolong APs by this mechanism (Lee *et al.*, 1992), however, others report I_{Kr} blockade as the mechanism of AP prolongation produced by this drug (Yang *et al.*, 1995; Lynch *et al.*, 1995; Hayes, 1995). Reduction of any of the eight known (and still counting) outward K^+ currents might be expected to prolong AP duration. "Specific" blockers of five K^+ currents, namely I_{Kr} , I_{Ks} , $I_{K(ATP)}$, transient outward (I_{to}) and inward rectifier (I_{K1}), have been assessed for their AP prolonging and antiarrhythmic effects.

Irrespective of the mechanism which produces AP prolongation, there are a number of factors that influence the efficacy of Class III drugs for suppression of arrhythmias. These include, but are not limited to: the effects of heart rate on AP prolongation and the homogeneity of AP prolongation. Heterogeneous AP prolongation increases the dispersion of refractoriness and may set the stage for re-entry dependent arrhythmias. Obviously, AP prolongation in tissues, or cell types, that are not the site of the drugs beneficial action can precipitate adverse effects without influencing efficacy. The rate-dependence of Class III action has received a great deal of attention in recent years. The Class III drugs presently available prolong AP duration more at slow heart

rates and have little effect at high heart rates. This phenomenon is called *reverse* use-dependence (Hondeghem & Snyders, 1990). Reverse use-dependent AP prolongation is associated with the potentially lethal polymorphic VT called torsade de pointes (Roden, 1994; Ben-David & Zipes, 1993; Hondeghem & Snyders, 1990; Waldo *et al.*, 1996).

Hondeghem and Snyders (1990, Hondeghem, 1991, 1994) have argued that the ideal Class III drug should selectively prolong AP duration and refractoriness at high heart rates. Such a mechanism can be expected to render VT self-terminating (Hondeghem, 1991) however, it is not clear how effective this mechanism will be for terminating VF. If the wave length of a re-entry circuit is taken as the product of conduction velocity and the refractory period, then prolongation of the refractory period will increase the wave length of the re-entry circuit until there is no longer sufficient tissue to contain it. It follows that more AP prolongation will be required to terminate re-entry circuits in larger hearts and predicts that such drugs will be less effective in man than in small laboratory species.

The characteristics and pharmacology of I_K and I_{to} will be briefly considered as they are particularly relevant to this thesis. Most drugs that prolong AP duration do so by blocking these currents. Also, the species used for these studies rely on I_K and I_{to} for repolarisation. The putative AP prolonging actions of $I_{K(ATP)}$ blockers are best considered in the section discussing the modification of drug action by ischaemia (see later).

I_K is found in cardiac myocytes of a number of species including mouse, guinea-pig, rabbit, cat, dog and sheep (Noble & Tsien, 1969; Sanguinetti & Jurkiewicz, 1990; Liu & Antzelevitch, 1995; Salata *et al.*, 1996). It was first described as a time dependent outward current in Purkinje fibres and dubbed I_{X1} (Noble & Tsien, 1969). I_K is increased

by β -adrenergic stimulation (Bennett *et al.*, 1986) via a protein kinase A-dependent mechanism (Walsh *et al.*, 1988). It is also modulated by protein kinase C-dependent mechanisms (Tohse *et al.*, 1987). Activation kinetics are accelerated by increases in internal $[Ca^{++}]$ and conductance is increased by increases in external $[K^+]$ (Tohse *et al.*, 1987; Shibasaki, 1987; Scamps & Carmeliet, 1989).

I_K displays outward rectification (Noble & Tsien, 1969; Sanguinetti & Jurkiewicz, 1990) and was found to have two components which can be distinguished on the basis of activation potential and time course, as well as by pharmacological means (Sanguinetti & Jurkiewicz, 1990). In isolated guinea-pig ventricular myocytes, I_{Kr} activates between -50 to 0 mV, has an activation time course of hundreds of milliseconds and can be selectively blocked by E-4031. I_{Ks} activates at potentials positive to 0 mV, has an activation time course of several seconds and is not blocked by E-4031.

Blockade of I_K , particularly I_{Kr} , has been suggested to be an ideal target to prolong AP duration due to its time- and voltage-dependence (Courtney *et al.*, 1992). A large number of I_K blockers have been developed which vary in their selectivity for I_{Kr} and I_{Ks} . Dofetilide and E-4031 appear to be highly selective I_{Kr} blockers (Sanguinetti & Jurkiewicz, 1990; Gwilt *et al.*, 1990), whereas azimilide blocks both I_{Kr} and I_{Ks} (Fermini *et al.*, 1995). Unfortunately, azimilide blocks a number of other channels and cannot be used as a selective tool (Fermini *et al.*, 1995). AP prolongation produced by blockade of I_{Kr} has been shown to have antiarrhythmic actions against ischaemia-induced arrhythmias (e.g., Lynch *et al.*, 1990; Chi *et al.*, 1990; Anderson *et al.*, 1992; Rees & Curtis, 1993a). Chromanol 293B has been suggested to prolong APs by selectively blocking I_{Ks} (Busch *et*

al., 1996; Bosch *et al.*, 1998). The antiarrhythmic actions of chromanol 293B have not yet been demonstrated; however, azimilide has been shown to suppresses ischaemia-induced arrhythmias (Black *et al.*, 1993; Brooks *et al.*, 1996).

I_{to} is found in mouse, rat, rabbit, cat, ferret, sheep, dog and human cardiac myocytes (see Coraboeuf & Carmeliet, 1982; Escande *et al.*, 1987; Braun *et al.*, 1990; Dukes *et al.*, 1990; Furukawa *et al.*, 1990). It is found in greater densities in myocytes isolated from the epicardium of the ventricle and the atrium (Litovsky & Antzelevitch 1988, 1989; Braun *et al.*, 1990). I_{to} activates and inactivates rapidly upon depolarisation (Coraboeuf & Carmeliet, 1982; Josephson *et al.*, 1984; Clark *et al.*, 1988; Hiraoka & Kawano, 1989). This current reactivates slowly. Thus with high stimulation rates (or heart rates) the amplitude of I_{to} is reduced. Activation and inactivation kinetics of I_{to} vary with species (compare Josephson *et al.*, 1984; Hiraoka & Kawano, 1989).

Alpha-adrenergic stimulation has been shown to reduce I_{to} and results in AP prolongation (Fedida *et al.*, 1990; Braun *et al.*, 1990). I_{to} appears to be composed of at least two components (Coraboeuf & Carmeliet, 1982; Josephson *et al.*, 1984; Escande *et al.*, 1987; Tseng & Hoffman, 1989; Hiraoka & Kawano, 1989; Clark *et al.*, 1988). The first is a voltage-dependent K^+ current which is sensitive to 4-aminopyridine (e.g., Li *et al.*, 1995). The second was thought to be a K^+ current for some time (Coraboeuf & Carmeliet, 1982) but now appears to be a Ca^{++} -activated Cl^- current (Zygmunt & Gibbons, 1991; Zygmunt, 1994; DiDiego *et al.*, 1996).

Tedisamil blocks I_{to} , as well as I_K , and prolongs AP duration in species that rely on this current for repolarisation (Dukes & Morad, 1989; Dukes *et al.*, 1990; Beatch *et al.*,

1991). This drug has also been shown to block $I_{K(ATP)}$ (Bray & Quast, 1991). In the rat, a species that lacks I_K , AP prolongation and antiarrhythmic actions might be ascribed to blockade of I_{to} (Beatch *et al.*, 1991). Aside from the complication added by $I_{K(ATP)}$ (Bray & Quast, 1991) and I_{Na} (Beatch *et al.*, 1991) blockade, this is controversial (see Rees & Curtis, 1996). Other investigators fail to observe antifibrillatory actions in the rat but instead find only defibrillatory actions (Tsuchihashi & Curtis, 1991; Rees *et al.*, 1993).

1.7.5 Pharmacology of Class IV antiarrhythmics.

Calcium channel blockers have a host of actions on the cardiovascular system and have been used to treat both ischaemia and arrhythmias. All of these drugs relax vascular smooth muscle, reduce the automaticity of the heart, have negative dromotropic actions at the atrio-ventricular node and negative inotropic actions. However, they differ greatly in their relative potency and efficacy for producing these effects. Treatment of ischaemia with Ca^{++} channel blockers will not be considered here, nor will the use of these agents to treat supraventricular tachycardias (Roden, 1996; Robertson & Robertson, 1996). Class IV drugs are not commonly used to treat ventricular arrhythmias although some studies show them to be effective (see Opie, 1992).

Various types of calcium currents have been described (Birnbaumer *et al.*, 1994) and at least two of these can be found in the heart (for a review see Vassort & Alvarez, 1994): L- and T-type Ca^{++} currents. Both activate slowly upon depolarisation and inactivate thereafter. The T-type current inactivates more rapidly than does the L-type. The molecular structure of Ca^{++} channels has been described (e.g., Soong *et al.*, 1993).

Regulation of the L-type Ca^{++} current is relatively well characterised (see Fleckenstein, 1988 or Sperelakis, 1994 for a review). It is increased by β -adrenoceptor stimulation via a protein kinase-A dependent phosphorylation process (Sperelakis, 1994). Activation of muscarinic M2 and adenosine A1 receptors blunts the increase in L-type Ca^{++} current produced by β -adrenoceptor activation (Trautwein & Hescheler, 1990).

The term "calcium channel blocker" was coined by Fleckenstein and co-workers (1964). Since then a number of chemically distinct Ca^{++} channel blockers have been described. These include phenylalkylamines (e.g., verapamil), dihydropyridines (e.g., nifedipine) and benzothiazepines (e.g., diltizem; see Fleckenstein, 1983 for a review). Other types of calcium channel blockers are also known (e.g., Dong *et al.*, 1997). Recently, T-type Ca^{++} channel blockers have been described, with one example being mibefradil (Clozel *et al.*, 1989; Fang & Osterrieder, 1991). However, this drug also blocks L-type Ca^{++} currents (Mehrke *et al.*, 1994; Bezprozvanny & Tsien, 1995) and thus cannot be used as a selective tool.

1.8 Influence of myocardial ischaemia on antiarrhythmic drug action.

The electrophysiology of myocardial tissue can be modified to such an extent by ischaemia that its properties differ in important ways from the properties of non-ischaemic tissue. As a result, the pharmacology of the ischaemic myocardium is different. Components of ischaemia may act directly or indirectly on ion channels to alter their response to drugs. Ischaemia-induced alteration of AP characteristics may influence the interaction of drugs with ion channels by virtue of the voltage- and time-dependence of

such interactions (Hongdehem & Katzung, 1984). Components of ischaemia, such as increased extracellular $[K^+]$, may act directly on ion channels to antagonise the actions of drugs (Baskin & Lynch, 1994; Yang & Roden, 1996; Duff *et al.*, 1997). Another mechanism whereby components of ischaemia may alter drug action is by altering the drug itself. An ischaemia-induced reduction in pH can be expected to increase the fraction of basic drugs in the ionised form (Wendt *et al.*, 1993). If it is assumed that the ionised species is active, this will result in an increase in the apparent potency of the drug.

A number of approaches can provide information about the modification of drug action by myocardial ischaemia. One approach is to examine the effects of drugs on the ischaemic myocardium *in vivo*. However, few studies use this approach due to its inherent difficulty. These difficulties include: the time dependence of ischaemia-induced changes, the time dependence of drug action, the inability to easily explore dose-response relationships, and difficulties associated with investigating the mechanisms of drug action *in vivo*. As a result, a number of investigators have attempted to create a simulated state of ischaemia *in vitro*. The contents of various buffers have been modified so that their constituents resemble those of the ischaemic milieu. Perfusion of cardiac tissue with buffers containing various combinations of increased $[K^+]$, low pH, low oxygen content and low glucose concentration have been used by various groups to examine the potential ischaemia-selective actions of antiarrhythmic drugs (e.g., Campbell & Hemsworth, 1990; Moréna *et al.*, 1980; Lukas & Antzelevitch, 1993; MacKenzie *et al.*, 1993; Barrett *et al.*, 1995). Another approach to simulating ischaemia is to include metabolic poisons such as cyanide or dinitrophenol in the buffer (e.g., Findlay, 1993; Furukawa *et al.*, 1991).

1.8.1 Potentiation of Class I antiarrhythmic activity by myocardial ischaemia.

The actions of all Class I drugs are increased under conditions of myocardial ischaemia but the degree of potentiation varies remarkably among agents. The actions of drugs which bind to the inactivated state are potentiated to a greater degree than those which bind to the activated state of the Na^+ channel (Hondegheem & Katzung, 1984; Campbell & Hemsworth, 1990; Campbell *et al.*, 1991; Ye *et al.*, 1993).

Hondegheem and co-workers (1974) described the selective depression of excitability in guinea-pig papillary muscles, perfused with a hypoxic and glucose deficient buffer, by quinidine, procainamide, lidocaine and diphenylhydantoin. They referred to this effect as “selective” because the same drug concentrations had little or no effect on the excitability of tissue perfused with normal buffer. Further *in vitro* work by Campbell’s group (Campbell & Hemsworth, 1990; Campbell *et al.*, 1991; Ye *et al.*, 1993) showed that drugs which bound preferentially to the inactivated state of the Na^+ channel were potentiated to a greater extent than activated state blockers under high $[\text{K}^+]$, acidotic and hypoxic conditions (i.e., “simulated ischaemic” conditions). Investigations into the rate dependence of drug action under “simulated ischaemic” conditions revealed more similarities between drugs than differences. The usual rate-dependent actions of drugs which bind to the inactivated state were reduced (Campbell & Hemsworth, 1990; Campbell *et al.*, 1991; Ye *et al.*, 1993). The phasic block produced by such drugs (*viz.* block that was produced and declined with every AP) became tonic block (*viz.* block that was present irrespective of the rate of AP firing). The potency for slowing conduction and reduction of the maximum rate of depolarisation of APs for Class Ia and Ic drugs was also

increased under "simulated ischaemic" conditions but to a lesser degree than for Ib drugs. At concentrations which reduce excitability and slow conduction under "simulated ischaemic" conditions, Class Ia and Ic drugs also have these same effects on the normal myocardium. These drugs are thus less selective for the pathological conditions of myocardial ischaemia.

Early *in vivo* studies noted lidocaine's selectivity for depression of conduction velocity in ischaemic myocardial tissue of dogs (Levites *et al.*, 1976). Further studies using electrogram recordings from the epicardium of dogs with recent infarcts showed that lidocaine preferentially suppressed conduction in the surviving tissue over the infarct (El-Sherif *et al.*, 1977). This effect was rate dependent. Moreover, they also observed that administration of lidocaine abolished diastolic bridging (taken to indicate re-entry) and bigeminy at the same time. Conduction in nearby non-infarcted tissue was not influenced by lidocaine. "Lidocaine further depressed, in a selective way, severely depressed cells forming part of the re-entrant pathway, while it had little or no effect on normal or moderately depressed cells," observed El-Sherif and co-workers (1977). Further work by Cardinal *et al.* (1981), in which regional ischaemia was produced in the isolated perfused porcine heart, firmly established the "ischaemia-selectivity hypothesis." They demonstrated that lidocaine preferentially converted areas of slow conduction and unidirectional block to total block. The drug had no effect on conduction or maximum rate of depolarisation of APs in normal tissue.

1.8.2 Alteration of Class II antiarrhythmic activity by myocardial ischaemia.

Since noradrenaline concentrations increase in the ischaemic myocardium (e.g., Carlsson, 1987) blockade of its action on α - and β -adrenoceptors might influence the electrophysiological changes caused by ischaemia and therefore influence arrhythmogenesis in the ischaemic myocardium. The interpretation of the antiarrhythmic effects of antiadrenergic drugs in terms of effects on the ischaemic myocardium is complicated by the possibility of other loci of action (e.g., extracardiac actions), as well as the possibility of other pharmacological actions (Lucchesi *et al.*, 1967; Singh & Vaughan-Williams, 1970b). Thus it is not surprising that experimental evidence does not give a clear picture of the antiarrhythmic actions, nor the mechanism for action, of β -adrenoceptor antagonists.

β -adrenoceptor antagonists have antiarrhythmic actions in the anaesthetised rat (Campbell & Parratt, 1983), however, these effects may not be mediated by cardiac β -adrenoceptors (Paletta *et al.*, 1989). In the dog, propranolol prolonged MAP duration and conduction times in the ischaemic myocardium (Kupersmith *et al.*, 1975).

1.8.3 Attenuation of Class III antiarrhythmic activity by myocardial ischaemia.

In contrast to the potentiation of Class I drugs seen under conditions of ischaemia, the actions of Class III drugs are greatly reduced. One exception to this generalisation is blockade of $I_{K(ATP)}$. As $I_{K(ATP)}$ does not contribute to repolarisation under normal conditions but rather it is activated under conditions of low intracellular [ATP], the effects of blockade of this current can only be observed under the latter conditions (Noma, 1983).

Thus the actions of $I_{K(ATP)}$ blockers are not modified by ischaemia *per se* but rather the current is activated under conditions of ischaemia where after it can be influenced by drugs.

In the rabbit ventricle, the AP prolonging effects of sotalol in the normal myocardium are maintained under conditions of mild hypoxia (Cobbe *et al.*, 1985a) but are lost or even reversed (Cobbe *et al.*, 1985b) under conditions more closely resembling ischaemia *in vivo*. Similar observations have been made in guinea pig myocardial tissue (Culling *et al.*, 1984). Baskin & Lynch (1994) demonstrated that the actions of I_{Kr} blockers were markedly attenuated by increasing extracellular $[K^+]$ from 4 to 10 mM in ferret papillary muscle and suggested that this effect was a common property of I_{Kr} blockers. This has been born out in further experimental studies by Yang & Roden (1996) as well as Duff and co-workers (1997). Yang & Roden demonstrated that the potency of dofetilide for I_{Kr} blockade in a cell expression system was reduced by ~30 times by changing extracellular $[K^+]$ from 1 to 8 mM. Duff and co-workers (1997) later showed that increased extracellular $[K^+]$ reduced the binding of $[H^3]$ dofetilide to the channels that generate I_{Kr} . Thus, not only is K^+ conductance carried by I_{Kr} increased by increases in extracellular $[K^+]$ (Sanguinetti *et al.*, 1992, 1995; Yang & Roden, 1996) but the increase in extracellular $[K^+]$ also reduces the binding of organic blockers to this channel (Duff *et al.*, 1997)!

Bretylum has been shown to reverse AP shortening caused by hypoxia in canine Purkinje fibres (Nishimura & Watanabe, 1983). This effect might be related to its

antifibrillatory actions versus ischaemia-induced arrhythmias in the same species (Holland *et al.*, 1983).

I_{to} has been suggested to contribute to ischaemia-induced AP shortening in the canine heart (Lukas & Antzelevitch, 1993; DiDiego & Antzelevitch, 1994). This suggests that blockade of I_{to} in the ischaemic myocardium with resulting AP prolongation may have antiarrhythmic effects. Interestingly, the $I_{K(ATP)}$ blocker glibenclamide had no effect in this preparation.

1.8.4 ATP-dependent K^+ channels in the ischaemic myocardium.

The ATP-dependent K^+ current was first described by Noma (1983) in guinea-pig ventricular cells. Since then it has been found in many other tissues including the β cells of the Islet of Langerhans in the pancreas, blood vessels and central nervous system (see Edwards & Weston, 1993; Terzic *et al.*, 1994). In the heart, micromolar concentrations of intracellular ATP (e.g., $IC_{50\%}$ 1-580 μ M, Findlay & Faivre, 1991) keep these channel closed (Noma, 1983; Kakei *et al.*, 1985; Deutsch & Weiss, 1993). Other intracellular nucleotides can act as antagonists (e.g., GTP can replace ATP, Kakei *et al.*, 1985), partial agonists (e.g., ADP, Findlay, 1991) or agonists for K_{ATP} channel opening (e.g., UDP causes channel opening, Tung & Kurachi, 1991). Low pH reduces the potency of ATP for closing these channels (Findlay, 1992). Pancreatic $I_{K(ATP)}$ can be modulated by protein kinase A (Ribalet *et al.*, 1989) and protein kinase C (de Weille *et al.*, 1989; Ribalet *et al.*, 1988) which hints that the cardiac channels might also be regulated by these systems. Stimulation of cardiac adenosine A_1 receptors causes $I_{K(ATP)}$ activation via a G protein

coupled mechanism (Kirsch *et al.*, 1990). Arachidonic acid has also been shown to activate $I_{K(ATP)}$ in the heart (Kim *et al.*, 1990).

Once $I_{K(ATP)}$ is activated, it has a large conductance and exhibits outward rectification (Noma, 1983; Findlay, 1987). Rectification is due to internal blockade by Mg^{++} , Ca^{++} and Na^+ ions at positive potentials (Takei *et al.*, 1985; Horie *et al.*, 1987; Findlay, 1987) and not an inherent voltage dependence of the channel (Horie *et al.*, 1987). The current flowing through this channel is essentially time-independent (Noma, 1983; Horie *et al.*, 1987).

K_{ATP} channels in different tissue exhibit different sensitivities to blockers and activators. Known blockers include tedisamil (Bray & Quast, 1991), 5-hydroxydeconoate (Notsu *et al.*, 1992) and sulphonylurea compounds such as glibenclamide and tolbutamine (Sturges *et al.*, 1985; Aguilar-Bryan *et al.*, 1992). K_{ATP} activators (also called potassium channel openers) include cromakalim (Sanguinetti *et al.*, 1988), pinacidil (Arrigoni-Martelli *et al.*, 1980) and nicorandil (Taira *et al.*, 1979). Blockade of $I_{K(ATP)}$ in the heart by glibenclamide requires 100 times higher concentrations than those required in the pancreas (see Edwards & Weston, 1993). Cardiac tissue is less sensitive to the actions of K^+ channel openers than vascular tissue (Edwards & Weston, 1993).

Activation of $I_{K(ATP)}$ has been shown to reduce AP duration under conditions of extreme metabolic stress (Noma, 1983). Activators of $I_{K(ATP)}$ shorten AP duration in cardiac muscle and this can be completely prevented by glibenclamide (Escande *et al.*, 1988 & 1989; Osterrieder, 1988; Sanguinetti *et al.*, 1988; Fosset *et al.*, 1988). These studies coupled with the observation that glibenclamide prevented hypoxia-induced AP

shortening in cardiac muscle (Noma, 1983; Fosset *et al.*, 1988; Cole *et al.*, 1991; Nakaya *et al.*, 1991; MacKenzie *et al.*, 1993) led to the suggestion that activation of $I_{K(ATP)}$ was responsible for AP shortening observed under these conditions. Moreover, such observations also suggested that blocking $I_{K(ATP)}$ may prevent ischaemia-induced AP shortening.

A review of the literature on the sensitivity of ischaemia-induced AP shortening to $I_{K(ATP)}$ blockade yields conflicting results. Interpretation of these studies is complicated by differences in the methods used to produce "ischaemia-like" conditions (see Coetzee, 1992 or DeLorenzi *et al.*, 1994). Therefore results vary depending on the degree of metabolic inhibition, mechanism used to produce metabolic inhibition and species. Glibenclamide and tolbutamide have been reported to completely prevent AP shortening under conditions of simulated ischaemia (e.g., Gasser & Vaughan-Jones, 1990; Furukawa *et al.*, 1991; MacKenzie *et al.*, 1993; Tweedie *et al.*, 1993). Other studies find that glibenclamide only partly inhibits AP shortening under ischaemic conditions (Cole *et al.*, 1991; Nakaya *et al.*, 1991; Furukawa *et al.*, 1991; MacKenzie *et al.*, 1993; Yan *et al.*, 1993; DeLorenzi *et al.*, 1994) while others find no effect until the later stages (>15 minutes) of ischaemia (Ruiz-Petrich *et al.*, 1992).

Assessing the contribution of $I_{K(ATP)}$ to ischaemia-induced AP shortening (and increases in $[K^+]$) is complicated by the observation that $I_{K(ATP)}$ blockers lose their ability to block this current with prolonged metabolic inhibition (Venkatesh *et al.*, 1991; Findlay, 1993). These findings caution against interpreting the apparent failure of $I_{K(ATP)}$ blockers to prevent ischaemia-induced AP shortening (of K^+ efflux) to mean that $I_{K(ATP)}$ does not in

fact underlie this effect (Findlay, 1993). This issue is clearly unresolved (see previous discussion concerning ischaemia-induced K^+ efflux).

Glibenclamide and 5-hydroxydeconoate have been shown to reduce (Moritani *et al.*, 1994; Miyoshi *et al.*, 1996), or even completely prevent (Smallwood *et al.*, 1990), AP shortening caused by regional ischaemia *in vivo*. In the dog, the effects of $I_{K(ATP)}$ modulation appear to be more pronounced in the epicardium than the endocardium (Miyoshi *et al.*, 1996). These findings are not universal however, and negative reports in this species do exist (Smallwood *et al.*, 1990).

Kantor and colleagues (1990) were the first to report the antifibrillatory actions of glibenclamide. Since then, a number of similar studies support this finding (Wolleben *et al.*, 1989; Gwilt *et al.*, 1992; Bellemin-Baureau *et al.*, 1994) while others fail to find such an effect (Bril *et al.*, 1992; Rees & Curtis, 1995; Baczkó *et al.*, 1997). In studies where the incidence of VF was not reduced by glibenclamide, only defibrillatory actions were observed (Bril *et al.*, 1992; Rees & Curtis, 1995; Baczkó *et al.*, 1997). *In vivo* studies with low doses of glibenclamide (0.2 to 3 mg/kg; Chi *et al.*, 1989) fail to find antifibrillatory actions, while those using high doses (10 mg/kg; Billman *et al.*, 1993) clearly show antifibrillatory activity. There are no studies which relate effects on AP duration during ischaemia to the occurrence of arrhythmias.

1.8.5 Modification of Class IV antiarrhythmic activity by myocardial ischaemia.

There are a number of mechanisms whereby Ca^{++} channel blockers might be expected to influence the electrophysiological properties of ischaemic myocardial tissue.

Calcium channel blockade can directly interfere with re-entry dependent mechanisms in the ischaemic myocardium by producing block in areas of slow conduction (Curtis *et al.*, 1984; see Opie, 1992). Calcium channel blockers have been shown to suppress slow response APs which occur with high concentrations of noradrenaline and elevated $[K^+]$ (Arita *et al.*, 1983). It seems unlikely that the conditions in the ischaemic myocardium are altered to the extent required for slow response APs to occur (Cranfield *et al.*, 1973; Janse & Wit, 1989). Despite this, it is likely that ischaemic APs have a greater dependence on inward Ca^{++} currents for propagation and blockade of this current may tip the balance in such a way that conduction through the ischaemic myocardium fails (Thandroyen *et al.*, 1986; Schneider *et al.*, 1975 also see Wit & Janse, 1989 for a review). Ischaemia-induced alterations in Ca^{++} fluxes across the membrane and AP alternans are effectively suppressed by verapamil *in vitro* (Lee *et al.*, 1988). *In vivo*, alternans are effectively suppressed by verapamil (Hirata *et al.*, 1980; Janse, 1982; Wit & Janse, 1989). By acting in this way, Ca^{++} channel blockers might reduce the heterogeneity of the ischaemic myocardium resulting in a reduced risk of arrhythmogenesis.

Calcium channel blockers such as verapamil and diltiazem have been shown to reduce Ca^{++} overload caused by ischaemia (e.g., Clusin *et al.*, 1983, 1984; Lee *et al.*, 1988). This is akin to antiischaemic interventions and cannot be expected to reduce the incidence of arrhythmias (unless the tissue is reperfused) but rather to delay the occurrence of such arrhythmias. A reduction in the ischaemia-induced increase in intracellular $[Ca^{++}]$ may reduce the degree of depolarisation caused by ischaemia (Clusin *et al.*, 1983; Blake *et al.*, 1988), improve conduction in the ischaemic myocardium (Fujimoto

et al., 1981; Peter *et al.*, 1983) and/or prevent Ca^{++} -induced triggered activity (Clusin *et al.*, 1982; Coetzee *et al.*, 1987).

In the rat, Ca^{++} channel blockers reduce the incidence of VF at doses which directly effect the heart (Curtis *et al.*, 1984, Curtis & Walker 1986a-b, 1988; Curtis, 1986; Au *et al.*, 1987; Kinoshita *et al.*, 1988). Even nifedipine, a vascular selective Ca^{++} channel blocker, has antiarrhythmic actions at high, cardio-active, doses (Curtis & Walker, 1986, 1988, Curtis, 1986). Concentrations of verapamil found to have antiarrhythmic actions prevented slow response APs recorded from rat ventricular tissue depolarised with high $[\text{K}^+]$ (Curtis *et al.*, 1984). This suggests that depression of conduction in the ischaemic myocardium is the mechanism for verapamil's antiarrhythmic effects. The antifibrillatory actions of Ca^{++} channel blockers against ischaemia-induced VF have been demonstrated in other species (Billman, 1989, 1991, 1992a; Aupetit *et al.*, 1993; Pugsley *et al.*, 1995). It should be noted, however, that not all studies support the view that Ca^{++} channel blockers have antifibrillatory actions but suggest instead an antiischaemic mechanism. In these studies, the latency to VF was increased but incidence was not reduced (Clusin *et al.*, 1982; Patterson *et al.*, 1983).

The observation of the antifibrillatory properties of Ca^{++} channel blockers led to the suggest that ischaemia-selective Ca^{++} blockers may be ideal for preventing ischaemia-induced VF (Curtis, 1990; Opie, 1992; Heusch, 1994). Mibefradil may be an example of one such drug. This drug binds to L-type Ca^{++} channels at or near the same site as verapamil but is much less potent for producing negative inotropic and dromotropic actions than verapamil (Clozel *et al.*, 1989; Osterrieder & Holck, 1989). Voltage clamp

studies revealed that mibefradil blocks L-type Ca^{++} current more effectively at depolarised potentials (Fang & Osterrieder, 1991; Bezprozvanny & Tsien, 1995). This drug also blocks the T-type Ca^{++} current (Mehrke *et al.*, 1994; Bezprozvanny & Tsien, 1995) which is found in the heart (see Vassort & Alvarez, 1994 for a review). Mibefradil prevents ischaemic-induced VF in dogs (Billman, 1992b, 1996) but has no effect on electrically-induced arrhythmias in the same species (Billman, 1996).

1.9 Rational for this thesis.

The overall focus of studies in the laboratory is to ascertain the pharmacological characteristics of the ideal drug for prevention of ischaemia-induced VF. The laboratory has investigated the electrophysiological and antiarrhythmic actions of selected drugs under conditions designed to mimic or produce myocardial ischaemia. The specific purpose of this thesis was to test the hypothesis that drugs with ischaemia-selective electrophysiological actions provide better antiarrhythmic protection in the setting of acute myocardial ischaemia than those which lack such actions. Experiments were performed in two species; rat and rabbit, in order to evaluate the hypothesis. Two species were used as the occurrence of K^{+} channels is highly species dependent and such differences results in differences in AP characteristics between species. In addition, no one preparation in any species completely mimics the human condition. Attempts were made to delineate, at the whole animal level, the mechanism of action of the putative ischaemia-selective antiarrhythmic drug RSD1019. Figure 1 shows the chemical structure of RSD1019 and the other pharmacological tools used in these studies.

1.10 Experimental plan.

Antiarrhythmic dose-response curves for selected drugs were constructed. Further studies were carried out to assess the electrophysiological actions of these drugs under normal conditions and those designed to mimic or produce myocardial ischaemia. Attempts were made to relate the actions of drugs in ischaemic tissue to their antiarrhythmic actions.

1) The actions of a selection of standard and novel antiarrhythmic drugs (quinidine-Class Ia, lidocaine-Class Ib, flecainide-Class Ic, tedisamil-Class III, and RSD1019) on haemodynamic, electrocardiographic and electrical stimulation variables of the left ventricle were examined in anaesthetised rats. The purpose of these studies was to characterise the action of these drugs on normal tissue. These studies facilitated comparison of drug action under conditions designed to mimic or produce myocardial ischaemia.

2) The same series of drugs were then examined for their ability to slow conduction in the isolated rat heart under normal conditions and those designed to mimic myocardial ischaemia (i.e., "simulated ischaemia" produced by low pH and increased $[K^+]$).

3) The dose-response relationships for suppression of ischaemia-induced arrhythmias in this series of drugs was assessed in anaesthetised rats subject to coronary artery occlusion.

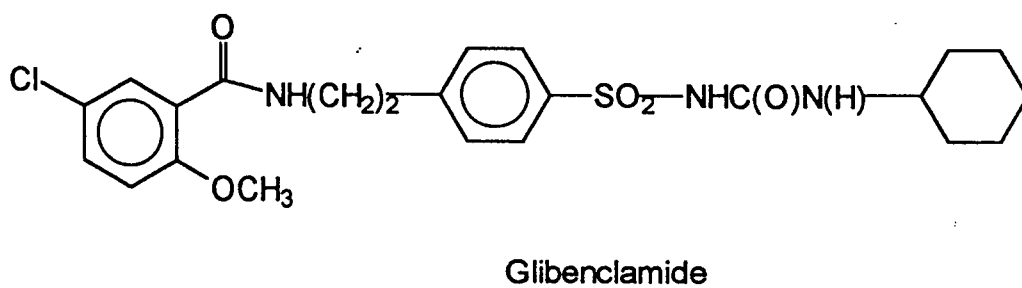
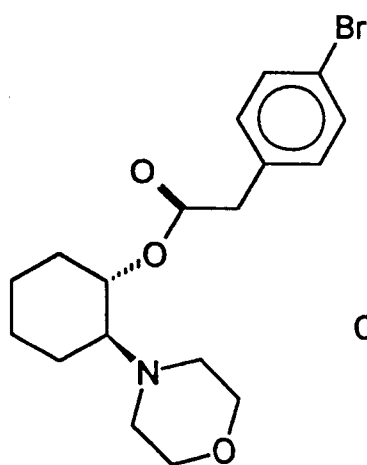
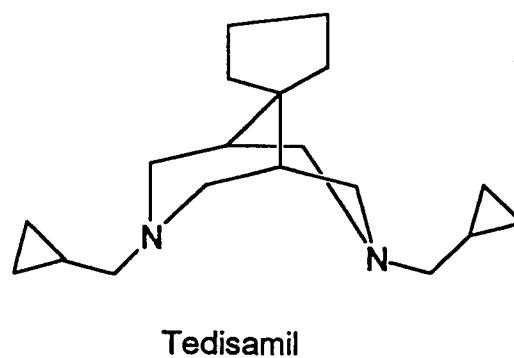
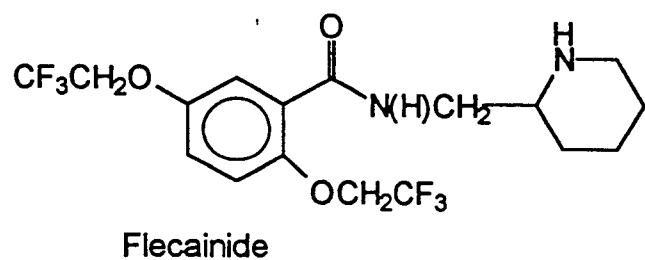
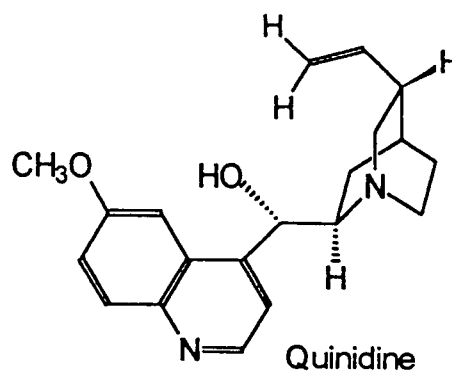
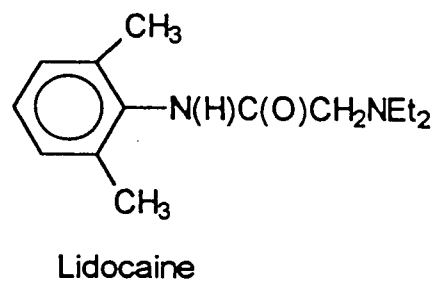


Figure 1. Chemical structures of the drugs used to investigate the relationship between ischaemia-selective drug actions and antiarrhythmic efficacy.

4) A preparation was developed in anaesthetised rabbits which allowed for the simultaneous assessment of ischaemia-induced arrhythmias and electrophysiological changes. Myocardial ischaemia was induced by coronary artery occlusion and electrophysiological effects were assessed via MAP recordings made from the epicardium of anaesthetised rabbits.

5) The putative ischaemia-selective electrophysiological and antiarrhythmic actions of an $I_{K(ATP)}$ blocker (glibenclamide) were investigated in the anaesthetised rabbit preparation.

6) The electrophysiological and antiarrhythmic actions of standard and novel ischaemia-selective drugs (lidocaine, tedisamil and, RSD1019, respectively) were examined in the anaesthetised rabbit preparation.

The remainder of this thesis is divided into two sections which correspond to studies carried out in rats and rabbits, respectively. A discussion of the characteristics of the ideal ischaemia-selective antifibrillatory drug will follow the discussion of studies carried out in rabbits. Finally general conclusions will be drawn.

Part 1. Relationship between ischaemia-selective drug action and antiarrhythmic efficacy in anaesthetised rats.

2.0 Methods.

Rats were housed in the animal care facility in the Department of Pharmacology and Therapeutics with a twelve hour light/dark cycle. Food (standard rat chow) and water were available *ad libitum*. All studies were approved by The University of British Columbia's Animal care committee and animals were cared for according to internationally accepted guide lines.

2.1 Characterisation of drug action on normal tissue.

It was clearly useful to assess the electrophysiological actions of drugs on normal myocardial tissue to allow their actions to be compared to those on ischaemic tissue. Since myocardial ischaemia only occurs *in vivo* (and not *in vitro*), these studies were best conducted in intact animals. The electrophysiological actions of drugs on the heart are best described in terms of the dose-response relationship for drug action on the four basic electrophysiological properties of the heart: automaticity, conduction velocity, excitability and refractoriness. Antiarrhythmic drugs have a characteristic spectrum of action on these properties that can be used to classify drug action. For example, Class I antiarrhythmics decrease the excitability and conduction velocity in the heart and tend to reduce automaticity. These effects can be observed as increases in the threshold current required

to pace the heart, an increase in conduction intervals measured from the ECG and a reduction in HR, respectively. Class III antiarrhythmic drugs increase the refractory period without influencing excitability or conduction velocity. These effects are easily measured as an increase in the effective refractory period and the QT interval of the ECG as well as a reduction in the maximum following frequency. Lack of effect on the indices of conduction velocity and excitability noted above indicates selective Class III action.

Electrical stimulation of the ventricle can precipitate arrhythmias and these techniques can be used to assess the antiarrhythmic actions of drugs. Induction of VF by electrical stimulation techniques in normal myocardial tissue has long been known and a variety of techniques have been developed (see Winslow, 1984). These protocols often involve electrical stimulation during the terminal phases of repolarisation. Electrical stimuli can be timed to occur during the terminal phase of repolarisation or the cardiac cycle can be scanned with high frequency stimuli. Increases in the current required to induce VF is taken as a measure of increased resistance to VF.

The fact that VF is the target for antiarrhythmic drugs makes the above method attractive for assessment of drug action. However, they suffer from a number of drawbacks. Defibrillation may be required if VF does not revert spontaneously. Spontaneous reversion from VF is rare in larger species but occurs commonly in smaller species (Curtis *et al.*, 1987). Repeated episodes of VF, with intervening ischaemia, influence the electrophysiological properties of the heart. This necessitates long rest periods between determinations. Determining VF threshold requires application of relatively large currents to the heart which are known to alter its electrophysiological

properties (Moore & Spear, 1975). Alternatives to this procedure are available. One such procedure is determination of ventricular fibrillo-flutter threshold (Szekeres & Papp, 1971). Ventricular fibrillo-flutter threshold is determined by progressively increasing current output while stimulating throughout the cardiac cycle at a high frequency (20-50 Hz). Ventricular fibrillo-flutter threshold is defined as the occurrence of a VF like wave form in the ECG (Winslow, 1984). Using this end point offers a number of advantages over determination of VF threshold which include the following: defibrillation is not necessary as the arrhythmia is not sustained, long rest periods are not required between determinations, and the current thresholds are much lower (Szekeres and Papp, 1971, suggest 75% lower thresholds).

Electrical stimulation studies were carried out in an anaesthetised rat preparation. This preparation allows drug potency for effects on the four basic electrophysiological properties of the heart, as well as effects on ventricular fibrillo-flutter threshold, to be measured. Drug effects were assessed on excitability and refractory period of the left ventricle through the use of electrical stimulation protocols. The effects of drugs on conduction velocity were assessed in terms of prolongation of ECG intervals (PR and QRS intervals). Reductions in HR were taken as a measure of drug-induced suppression of automaticity. One of the major advantages offered by this preparation was that it allowed cumulative dose-response curves for drug action to be constructed. Also, it facilitated comparison of the electrophysiological actions of drugs in the same species used for other studies.

2.1.1 Preparation of rats for electrical stimulation studies.

Electrical stimulation studies were carried out in anaesthetised rats as previously described (Walker & Beatch, 1988). Rats were anaesthetised with 65 mg/kg pentobarbital ip. The jugular vein and carotid artery were cannulated with polyethylene tubing (PE50, Intramedic) for injection of drugs and measurement of BP, respectively. BP was displayed on a Grass Polygraph utilising a Statham pressure transducer and monitored for the duration of the experiment. A tracheotomy was performed and an endotracheal tube inserted (JelcoTM 14 gage needle sheath). Animals were ventilated with oxygen at a rate of 60 cycles/minute and a volume of 10 mL/kg. This ventilation protocol has been shown to maintain blood gases within physiological limits (MacLean & Hiley, 1988; Milmer *et al.*, 1985). Core body temperature was assessed via rectal thermometer and maintained between 35-36°C through the use of a heating lamp. An ECG was recorded from subcutaneous pin electrodes in approximately a lead V3 configuration. The ECG was displayed on a Honeywell E for M monitor and recorded on a Polygraph chart recorder (Grass 79D).

An incision was made over the fourth intercostal space to expose the chest wall. A pair of TeflonTM coated silver wires were inserted through the chest wall into the left ventricle using a 23 Gage needle as a guide. The end of each wire was stripped of its TeflonTM coating and bent to form a barb. When the needle was withdrawn the electrode was left anchored in the left ventricle. A constant current stimulator (Grass, model SD9) was used to stimulate the left ventricle with square wave pulses.

The end points for electrical stimulation variables were determined from the ECG. Precise definitions of these end points are described in the analysis of data section (see later). All measures were repeated in triplicate before proceeding to the next measurement. Before starting the experiment, the electrical stimulation protocols were repeated at 5 minute intervals until stable values were obtained. In most cases this required three repetitions before starting the experiment. Determination of all electrophysiological variables was completed within two minutes.

2.1.2 Data acquisition.

Electrical stimulation end points were determined from the ECG in all cases. The BP record was used to supplement determination of end points but was not used for diagnosis *per se*. Threshold current (iT) and stimulus duration (tT) for stable capture of the ventricle on a 1:1 basis was determined at a stimulation frequency of 7.5 Hz. The minimum current or duration required to pace the ventricle on a 1:1 basis was taken as the threshold. Stimulus duration was set at 1 ms for determination of iT. Stimulator output was set at twice iT for tT determination and thereafter.

Effective refractory period (ERP) was determined by inserting a single extra stimulus (at the same current strength and duration) at variable coupling intervals from the last beat in the train. The basic stimulation frequency was 7.5 Hz (cycle length 133 ms). Coupling intervals were increased progressively until it was possible to insert an extra beat. The longest coupling interval which failed to produce a propagated response was taken as the ERP. The extra beat was clearly seen on the ECG as well as on the BP

record as a beat with a shorter coupling interval. Maximum following frequency (MFF) was determined by progressively increasing the stimulation frequency until the ventricles failed to follow on a 1:1 basis. This can be seen on the ECG record as a missed beat accompanied by a dip in BP followed by a large pulse. The frequency at which this occurred was taken as the MFF.

Ventricular fibrillo-flutter threshold (VFT) was defined by Winslow (1984) as the occurrence of a "VF-like wave form in the ECG." The Lambeth conventions (Walker *et al.*, 1988) defines VF as an ECG morphology for which individual QRS complexes cannot be distinguished and for which no rate can be determined. The ventricles were stimulated at a frequency of 50 Hz with a pulse width of twice tT . The current required to cause a VF-like wave form in the ECG was recorded. Capture of VFT caused a sustained decrease in BP within which no pulses could be detected.

2.1.3 Drugs and dosing regimen.

Drug or vehicle was infused continuously and the infusion rate doubled ever 5 minutes to achieve a dose doubling regimen. Animals were randomly assigned to receive vehicle or one of the following drugs over the dose range (in $\mu\text{mol/kg/min}$) specified: quinidine 1-16, lidocaine 1-32, flecainide 1-8, tedisamil 0.032-2 and RSD1019 2-64. Lidocaine was dissolved in 10% dimethylsulfoxide, 10% ethanol and 80% distilled water while all other drugs were dissolved in 22% ethanol and 78% distilled water. These solutions were used as vehicle controls for lidocaine and the other drugs, respectively.

Doses were doubled every 5 minutes from the starting dose indicated until the highest dose was reached or until the animal died.

2.1.4 Analysis of data.

Mean arterial BP and ECG intervals were measured from traces taken at 100 mm/s. Measurements were made immediately before starting the electrical stimulation protocol (3 minutes after starting the infusion). ECG intervals were defined as follows: PR interval - the time from the first deflection of the P wave to the peak of the R wave, QRS duration- the time from the first deflection of the Q wave to the peak the S wave, QT interval- the time from the first deflection of the Q wave to the peak of the T wave (for an illustration see Penz *et al.*, 1992; Hayes *et al.*, 1994).

The above definition of the QT interval was adopted due to the problems associated with measuring this interval in the rat (Hayes *et al.*, 1994). In the rat the QT interval is fused to the QRS complex and often appears to have two distinct phases before returning to the isoelectric line. A second peculiarity of measurement of the QT interval in rats is the fact that it does not vary with HR (Hayes *et al.*, 1994). The relationship between the QT interval and heart in other mammals is well known (Bazett, 1920). In most mammals the QT interval is shorter at high HRs and vice versa. Typically, this source of variance is accounted for by correcting the QT interval for HR (Bazett, 1920; Simonson *et al.*, 1962; Hayes *et al.*, 1994). In all species it is difficult to determine the exact point where the T wave returns to the isoelectric line.

The effects of drugs or vehicle on BP, HR, ECG intervals and electrical stimulation variables were analysed. Dose-response curves were fit by least sum of squares regression analysis. A repeated measures ANOVA was used to assess dose-related changes in variables with $p < 0.05$ taken as statistically significant. If the ANOVA indicated that there was a significant effect of drug treatment then the dose required to produce a 25% change from the pre-drug value ($D_{25\%}$) was interpolated. In the case of VFT the dose required to produce a 100% increase ($D_{100\%}$) was determined. Potency values were summarised as the mean \pm SEM, $n=5$.

2.2 Assessment of drug potency for slowing conduction under normal and "simulated ischaemic" conditions.

As discussed, myocardial ischaemia initiates a series of biochemical changes which influence the electrophysiological properties of the heart as well as the action of antiarrhythmic drugs. As ischaemia is a time dependent process, it is not possible to assay for the effects of antiarrhythmic drugs on a **state** of ischaemia. Thus, the intent of these experiments was not to assess drug action on ischaemic tissue but rather to assess drug action under a defined set of conditions similar to those known to occur in the ischaemic myocardium. This method allowed concentration-response curves for drug action to be obtained under ischaemia-like conditions without the added complication of the time-dependent changes which occur in the ischaemic myocardium. We elected to modify the pH and the extracellular $[K^+]$ as these two changes are known to occur under conditions of ischaemia and could reasonably be expected to modify drug action. A Krebs/1,4-

piperazine bissulphonic acid (PIPES) buffer was modified such that it contained a $[K^+]$ 10 mM at a pH of 6.4. PIPES was selected as the buffer because its pKa is between the two pHs of interest and it could be expected to buffer pH effectively in both solutions. The pH and $[K^+]$ used for these studies were selected on the basis of ischaemia-induced changes reported in the literature (see introduction). Similar manipulations have been used by others to assess the actions of drugs under conditions of "simulated ischaemia" (e.g., Hondeghem *et al.*, 1978; Campbell & Hemsworth, 1990; Campbell *et al.*, 1991; Ye *et al.*, 1992).

2.2.1 Characterisation of drug actions under "simulated ischaemic" conditions.

The dose-related action of quinidine, lidocaine, flecainide, tedisamil and RSD1019 on conduction times was examined in isolated rat hearts perfused with normal or "simulated ischaemic" buffer. The isolated heart apparatus described by Curtis *et al.* (1986) was employed. The advantage offered by this apparatus is that it allows perfusates to be switched rapidly; a feature which facilitates gathering cumulative concentration-response data.

Rats were anaesthetised with 70 mg/kg pentobarbital and given 1000 units of heparin ip. Hearts were rapidly excised after animals reached the level of surgical anaesthesia and immediately perfused with ice cold normal Krebs/PIPES buffer. The composition of the normal Krebs/PIPES perfusate (in mM) was: NaCl 123, KCl 3.4, $MgSO_4 \cdot 7H_2O$ 1.2, PIPES 14.4, glucose 11.1, $CaCl_2 \cdot 2H_2O$ 2.5 titrated to pH 7.4 with NaOH. Hearts were then mounted on a Langendorff apparatus and perfused via the aortic

root with Kreb/PIPES buffer. Perfusion pressure was maintained at 100 mmHg throughout the experiment. Buffers were kept at 37°C by a circulating water bath and were continuously bubbled with oxygen.

The left atrium was cut off to allow a low-compliance balloon to be inserted into the left ventricle. End diastolic pressure was maintained at 5 mmHg by filling the balloon with saline. Developed pressure and perfusion pressure were recorded utilising Statham pressure transducers. The first derivative of developed pressure was also recorded. An electromyogram was recorded from silver wick electrodes placed on the right atrium and the left ventricle. The signal was amplified using a Grass amplifier (model 71F). All records were displayed on a Grass Polygraph (model 79D).

Hearts were allowed to equilibrate for 15 minutes before starting the experiment, after which they were randomised to be perfused with either normal or "simulated ischaemic" Krebs/PIPES buffer for 5 minutes before starting drug treatment. The composition of the "ischaemic" perfusate (in mM) was: NaCl 117, KCl 10.1, MgSO₄·7H₂O 1.2, PIPES 15.3, glucose 11.1, CaCl₂·2H₂O 2.5 and titrated to pH 6.4 with NaOH.

2.2.2 Drugs and concentration regimens.

Two separate experiments were conducted. The first with quinidine, lidocaine and flecainide; the second with tedisamil and RSD1019. A vehicle control group was included in each experiment.

Hearts were randomly assigned to either normal or "simulated ischaemic" buffer containing quinidine, lidocaine, flecainide or vehicle. Stock solutions of drugs were made up at a concentration of 10 mM in 10% dimethylsulfoxide, 10% ethanol and 80% distilled water and appropriate dilutions made in Kreb/PIPES buffer. This solution was used as a vehicle control with volumes matched to those used for drug treatment. Cumulative concentration-response curves were constructed for drug action in normal or "simulated ischaemic" buffer over the following concentration ranges (in μM): quinidine 1-64, lidocaine 1-256, or flecainide 1-16 μM . Concentrations were doubled every 5 minutes until the highest concentration was reached. The final concentration of dimethylsulphoxide and ethanol did not exceed 0.3%. Separate hearts were used to study the effects of each drug under normal ($n=5$) or "simulated ischaemic" conditions ($n=5$).

In a second experiment the actions of tedisamil and RSD1019 were examined in normal and "simulated ischaemic" buffer. These experiments were carried out as described above except for the following differences. Data were acquired on a PC computer using customised Lab View software. This system allowed the measurement of all of the variables described above as well as the QT interval of the ECG.

2.2.3 Drugs and concentration regimens.

Hearts were assigned in a random and blind fashion to receive tedisamil, RSD1019 or vehicle in either normal or "simulated ischaemic" buffer. Cumulative concentration-response curves were constructed for tedisamil and RSD1019's action on isolated rat hearts. Concentration increases were carried out in half \log_{10} increments (e.g., 1, 3 and 10

μM) over the concentration range 0.03-30 μM for tedisamil and 0.3-300 μM for RSD1019.

2.2.4 Analyses of data.

Intervals were measured from the electromyogram by using the same criteria used to analyse the ECG (described previously). In isolated heart experiments carried out using the Grass Polygraph the QT interval of the ECG was not analysed. Electromyographic intervals and developed pressure were measured from Grass Polygraph traces taken at 100 mm/s or on a computer screen at a similar time base.

Concentration-response curves were analysed and potency summarised as described for dose-response curves (see section 3.1.4). The ratio of the $C_{25\%}$'s in normal and "simulated ischaemic" buffer were calculated from drug effects on the PR and QRS intervals and reported as the mean \pm pooled SEM, $n=5$.

2.3. Ischaemia-induced arrhythmias in anaesthetised rats.

As a result of the fact that the causes and consequences of myocardial ischaemia and resulting arrhythmias are diverse, variable and incompletely characterised, a large number of animal preparations have been developed to test a variety of hypotheses. These methods have also been used to study the bio-chemical and electrophysiological changes caused by ischaemia. By far the most common technique used to produce ischaemia is occlusion of a large coronary artery. This can be achieved by inflation of a balloon placed inside the artery, inflation of a hydraulic cuff placed around a coronary artery or by simply

ligating the artery with a suture (Winslow, 1984). Two examples of this technique are the two stage occlusion of the left anterior descending artery in the dog developed by Harris (1950) and the single stage occlusion of the same artery in the rat (see Curtis *et al.*, 1987). The selection of technique and test species for the study of arrhythmias is a function of the clinical relevance and the considerations pertinent to all bio-assays.

The clinical relevance of preparations used for the study of arrhythmias is difficult, if not impossible, to assess due to the lack of clinical data on early ischaemia-induced arrhythmias. Arrhythmias occur rapidly after the onset of chest pain in patients and, for the most part, the individuals are dead or have passed through the arrhythmia phase by the time they can be monitored (Oliver, 1981; Adgey, 1982). Similarly, there is little data concerned with the susceptibility of early arrhythmias to drugs and it is not possible to draw conclusions from these limited data. It is clear from these clinical data, however, that the risk of death declines with time after the onset of infarction (Furakawa *et al.*, 1989). Thus, despite the lack of data on early arrhythmias in man, a large number of techniques have been developed for the study of these arrhythmias due to their possible importance in being the cause of SCD.

Although the absolute clinical relevance of preparations used to study arrhythmias cannot be defined, a number of factors related to the similarities (or dissimilarities) of the test species to man are relevant to the discussion. These factors include, but are not limited to: differences in HR, diversity of K^+ channels in the heart and resultant differences in AP morphology, breadth of data in the literature on the species in question, the use of young animal hearts for the study of arrhythmias, as well as the degree of coronary

collateral circulation. The modulation of drug action by HR and the differences in drug selectivity for the plethora of K^+ channels in the heart have already been discussed (see introduction). Smaller species have higher HRs; a factor that has been associated with a higher incidence of arrhythmias (Bernier *et al.*, 1989). The use of young, healthy hearts for the study of arrhythmias is a dubious practice since arrhythmias are known to occur much more commonly in older, diseased hearts. The reason for the use of young animals is mostly a practical matter related to the ease of experimentation and the fact that the variability in the coronary collateral circulation increases with age. There is, however, some evidence that young human hearts are more likely to fibrillate after an ischaemic episode than older hearts. This may be due to the absence of coronary collaterals in young hearts and/or the effects of ischaemic preconditioning in older hearts (see Morgan Jones *et al.*, 1969; Oliver 1982).

Ideally, a bio-assay allows the determination of the drug's maximum response and potency as well as the slope of the dose-response relationship. Such a bio-assay must have a high level of precision and accuracy to test a hypothesis with a relatively small number of animals. Curtis *et al.* (1987) have argued persuasively that the considerations given to a good bio-assay should dictate the choice of methods for the study of arrhythmias rather than the undefinable clinical relevance of such methods. This is based on the observation that any preparation (or species) which accurately mimics the clinical situation fails, by definition, to meet the basic requirements of a bio-assay. This is illustrated by the observation that Harris' (1950) two stage coronary artery occlusion technique in the dog was thought to best parallel the clinical situation and the subsequent

demonstration by Meesman (1982) that the method produced highly variable results. To produce useful data an excessively large number of animals would be required to show the effectiveness of a drug, thus rendering the method useless as a bio-assay. From the above it follows that reducing the variance in the arrhythmic response is essential to the utility of such techniques. This is best achieved by standardisation of the arrhythmogenic stimulus.

2.3.1 The use of rats for the study of ischaemia-induced arrhythmias.

The use of the rat for the study of ischaemia-induced arrhythmias has been advocated by a number of laboratories (Clark *et al.*, 1980; Winslow, 1984; Curtis *et al.*, 1987; Brooks, 1989; Opitz *et al.*, 1995). One of the major advantages offered by this species is the fact that the degree of coronary collateral circulation is uniformly low (Maxwell *et al.*, 1984) and occlusion of the left anterior descending artery produces an occluded zone which is consistent in size between animals. Occluded zone size has been shown to correspond well to the occurrence of arrhythmias in this species (Austin *et al.*, 1982; Bernauer *et al.*, 1982; Curtis *et al.*, 1987). The rat preparation is also relatively cheap, in terms of expense and time, which allows the full dose-response relationship for drug action to be explored.

Despite these advantages the rat is not adored by all and the use of this preparation has been criticised on the basis of the rat's high heart rate and short AP duration. The short AP duration of the rat results from an increased reliance on I_{to} for repolarisation (see Coraboeuf & Nargeot, 1993 for a comparison to human cardiac electrophysiology). Some researchers prefer the canine heart for the study of ischaemia-induced arrhythmias (e.g.,

Lucchesi *et al.*, 1993; Billman *et al.*, 1993). The reasons for this are likely related to the similarities between the canine and human heart in terms of size, and AP morphology.

2.3.2 Preparation of anaesthetised rats for the study of ischaemia-induced arrhythmias.

Myocardial ischaemia was induced by occlusion of the left anterior descending artery in rats weighing between 200-350 g (see Curtis *et al.*, 1987). Rats were anaesthetised with 65 mg/kg pentobarbital ip. A tracheotomy was performed to allow artificial ventilation with oxygen at a rate and volume previously demonstrated to maintain blood gases within physiological limits (60 cycles/minute and 10 mL/kg; Milmer *et al.*, 1985; MacLean & Hiley, 1988). Core body temperature was assessed via a rectal thermometer and maintained at 35-37°C through the use of a heating lamp. The jugular vein and carotid artery were cannulated with polyethylene tubing (PE50, Intramedic) for injection of drugs and measurement of BP, respectively. BP was displayed on a Grass Polygraph (model 79D) utilising Statham pressure transducer and monitored for the duration of the experiment.

The chest was opened at the fourth intercostal space and a pericardial sling made. A specially constructed snare was loosely tied around the proximal left anterior descending artery. The snare was made prior to the experiment from polyethylene tube (PE10, Intramedic) in which a shoulder had been introduced by carefully melting the tubing. A cuff was formed at the distal end of the tubing where it would contact the heart. A polypropylene suture (5-0 Ethicon) was threaded through the tubing and one end melted to form a retaining ball. After placing the snare the chest was closed and an

attempt was made to reduce the pneumothorax by evacuating the chest. A 5 mL syringe was used to draw the air out of the cavity while the chest was closed with a purse string suture (3-0 silk, Ethicon). An ECG was recorded using subcutaneous pin electrodes in an approximately lead V3 configuration. The ECG was recorded on a Grass Polygraph (model 79D, pre-amplifier model 7P1F) and displayed simultaneously on a Honeywell E for M monitor (model PM-2A). Rats were allowed to recover for 15 minutes before starting the experiment.

Vehicle or test drug was infused continuously (Harvard infusion pump) starting 5 minutes before occlusion. Thereafter coronary artery occlusion was performed by tightening the snare. Arrhythmias were diagnosed on the E for M monitor and the Polygraph trace for 20 minutes following coronary artery occlusion (paper speed not less than 50 mm/s; Re: Lambeth Conventions, Walker *et al.*, 1988).

Blood $[K^+]$ was measured before and 20 minutes after occlusion, wherever possible. Blood $[K^+]$ was determined using an K^+ -selective electrode (Ionetics) which was calibrated with three standards (2.00, 4.00 & 8.00 mM, Ionetics K^+ standards). Arterial blood samples, ~300 μ L, were heparinised with ~50 μ L of 1000 U/mL heparin sulfate (Fisher Scientific). After completing the experiment, the occluded zone size (zone at risk) was measured as follows. The heart was excised and perfused via the aortic root with saline at approximately 100 mmHg followed by saline containing cardiogreen (0.5 mg/mL). Occluded zone size was defined as the percentage of the ventricular mass not dyed green. This technique has been thoroughly reviewed previously (see Curtis, 1986; Curtis *et al.*, 1987).

Inclusion criteria were applied to ensure that experiments were comparable with regards to arrhythmogenic stimulus and quality of the preparation. The inclusion criteria applied were: 1) mean arterial BP greater than 80 mmHg before starting the experiment, 2) mean BP not less than 25 mmHg for more than 2 minutes after occlusion, except when caused by arrhythmias, 3) atrio-ventricular block did not occur except if preceded by prolonged global ischaemia caused by arrhythmias, 4) occluded zone size between 25 and 50% of the ventricular mass (assessed post mortem) and, 5) blood $[K^+]$ less than 3.5 mM before starting the experiment.

2.3.3 Drugs and dosing regimens.

The antiarrhythmic actions of quinidine, lidocaine, flecainide, representing Class Ia, Ib and Ic antiarrhythmic drugs respectively, as well as a representative Class III antiarrhythmic, tedisamil, were assessed and compared to the actions of the novel drug RSD1019. All drugs were administered as a continuous infusion for 25 minutes starting 5 minutes prior to occlusion. A random and blind experimental design was used. The infusion rate was the same in all animals (3.3 mL/Kg/hr) while the concentration of the infused solution was varied so as to achieve the desired infused dose. The following doses (in $\mu\text{mol/kg/min}$) were tested: quinidine 0.2, 0.5, 1, 2 and 4; lidocaine 1, 2, 4, 8 and 16; flecainide 0.01, 0.03, 0.1, 0.375, 0.75, 1.5 and 3; tedisamil 0.25, 0.5, 1, 2 and 4; RSD1019 0.5, 1, 2, 4 and 8. The group size was 7 for all doses of each drug except tedisamil where the group size was 5.

2.3.4 Analysis of data.

The Lambeth conventions were used as guidelines for the study of arrhythmias in ischaemia, infarction and reperfusion (Walker *et al.*, 1988). In short, these conventions provide definitions of arrhythmias based on ECG evidence and state that experiments for the study of arrhythmias should be done in a random and blind fashion. These conventions have been adopted for this thesis with one exception. The occurrence of bigeminy was not quantified separately from the occurrence of VPBs. VPBs were counted as such regardless of whether they occurred as bigeminy or as a single isolated VPB.

The number, occurrence and duration of arrhythmias were quantified from the ECG and summarised using an arrhythmia score (score A, Curtis & Walker, 1988). Although the arrhythmia score was originally designed to summarise the arrhythmic history of conscious rats, it may also be used in the anaesthetised rat preparation. It may be particularly useful for constructing dose-response curves for the actions of antiarrhythmic drugs since it transforms arrhythmia data, which is typically not Gaussian distributed, into a distribution that is approximately Gaussian. This allows the use of more powerful parametric statistical tests.

An attempt was made to fit the dose-response curves to a logit function. To do this the percent protection (%P) provided by each dose was calculated from the arrhythmia score in the vehicle group as shown in Equation 1.

$$1) \%P = 100 - 100 \times (AS_{\text{test}} / AS_{\text{control}})$$

Where AS_{test} and $AS_{control}$ are the arrhythmia scores in the test group and vehicle control group, respectively.

Data were fitted to Equation 2 using the curve fitting function in Slidewrite version 2 software.

$$2) \%P = 100 * (D^h / D^h + ED_{50\%}^h)$$

Where %P is the percent protection defined above, D is the infused dose of the drug, h is the slope factor and $ED_{50\%}$ is the dose required to produce 50% of the maximum effect. The arrhythmia score for each individual experiment was used for curve fitting; averaged data were not used. The maximum response was defined as 100% protection and curves were forced to pass through this point. The effect of infusions on BP, HR and ECG variables including: PR, QRS and the QT interval, were measured immediately before occlusion. Occlusion-induced maximum R wave and "ST" segment elevation, as well as the time at which they occurred, were recorded.

2.4 Assessment of acute central nervous system toxicity of Class I drugs.

Antiarrhythmic doses of the standard Class I drugs were infused into acutely prepared conscious rats. The right jugular vein was cannulated under halothane anaesthesia and the cannula tunnelled subcutaneously to the back of the head. Wound sites were infiltrated with bupivacaine and animals allowed 3 hours to recover from surgery. Drug infusion was maintained for 25 minutes while the animal was observed for signs of

toxicity including: ataxia, sedation, collapse or convulsions. When convulsions did occur the animal was immediately sacrificed by an iv overdose of pentobarbital.

2.4.1 Drugs and dosing regimens.

Only the two most effective doses of each of the standard Class I antiarrhythmic drugs were tested in this protocol. These doses were (in $\mu\text{mol/kg/min}$): quinidine 2 and 4, lidocaine 8 and 16, and flecainide 1.5 and 3. The infusion protocol was the same as those tested in the occlusion studies.

3.0 Results

3.1 Overview.

The dose-related effects of the antiarrhythmic drugs: quinidine, lidocaine, flecainide and tedisamil, were compared to those of the putative antiarrhythmic drug RSD1019 for action(s) on BP, HR, ECG intervals, electrical stimulation variables, as well as the threshold current for induction of ventricular fibrillo-flutter. The purpose of these studies was to characterise the action of these drugs on normal cardiac tissue *in vivo*. These data allow comparisons between drugs in terms of their effectiveness and potency for effects in normal cardiac tissue. These data could then be compared to the actions of the same drugs under conditions designed to mimic myocardial ischaemia. Comparison of the doses required to increase VFT to those required to prevent ischaemia-induced VF provides a measure of ischaemia-selectivity.

3.2 Drug effects on Haemodynamics.

All drugs tested significantly reduced HR as compared to pre-drug values and vehicle control. The bradycardia produced was dependent on the drug and dose considered. Figure 2 compares the bradycardic actions and Table 1 summarises the potency ($D_{25\%}$) for drug action on all variables. Tedisamil was the most potent bradycardic agent and RSD1019 the least.

BP was reduced by all of the drugs tested except tedisamil which caused an increase (Figure 2 & Table 1). Quinidine, lidocaine and flecainide reduced BP over the entire dose range tested while RSD1019 only had hypotensive effects at the highest dose tested ($64 \mu\text{mol/kg/min}$). Increases in BP produced by tedisamil were accompanied by an increase in pulse pressure.

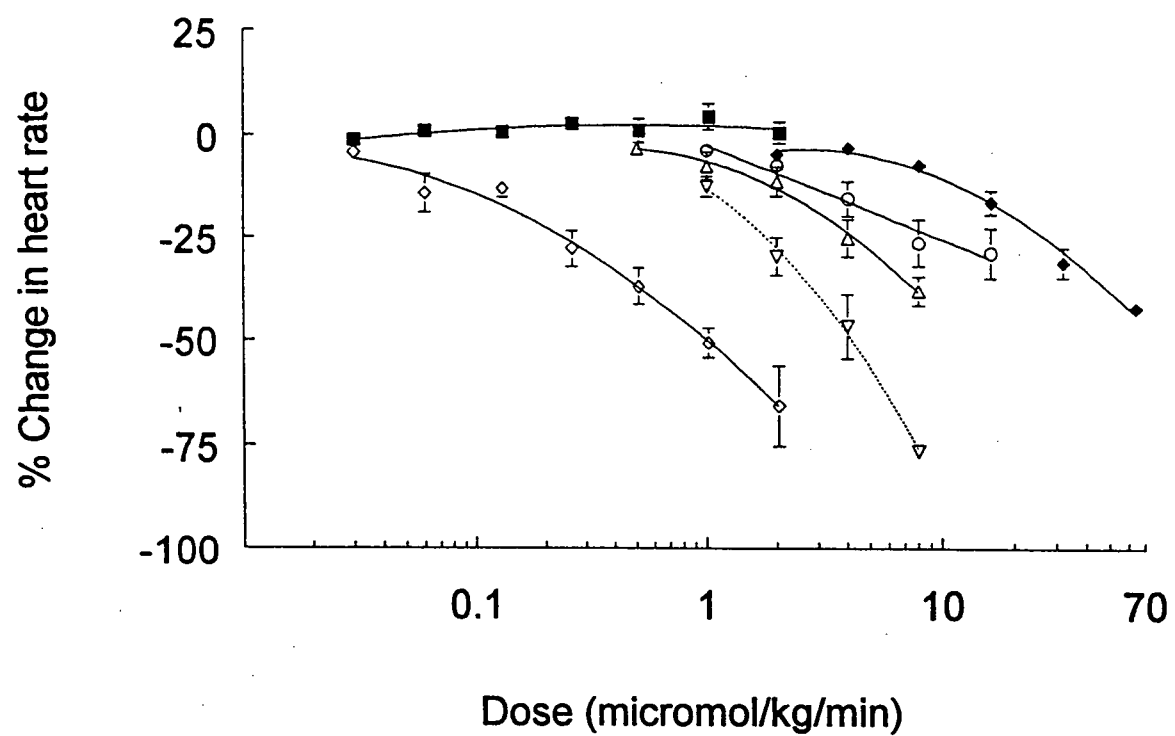
3.3 Drug effects on ECG intervals.

Quinidine, flecainide and tedisamil caused dose-related increases in PR, QRS and QT intervals of the ECG (Figure 3 & Table 1). Tedisamil was particularly efficacious in prolonging the QT interval. At a dose of $2 \mu\text{mol/kg/min}$ tedisamil increased the QT interval by over 200 times! In contrast, to the actions of the other drugs, lidocaine and RSD1019 had no effect on ECG intervals (Figure 3).

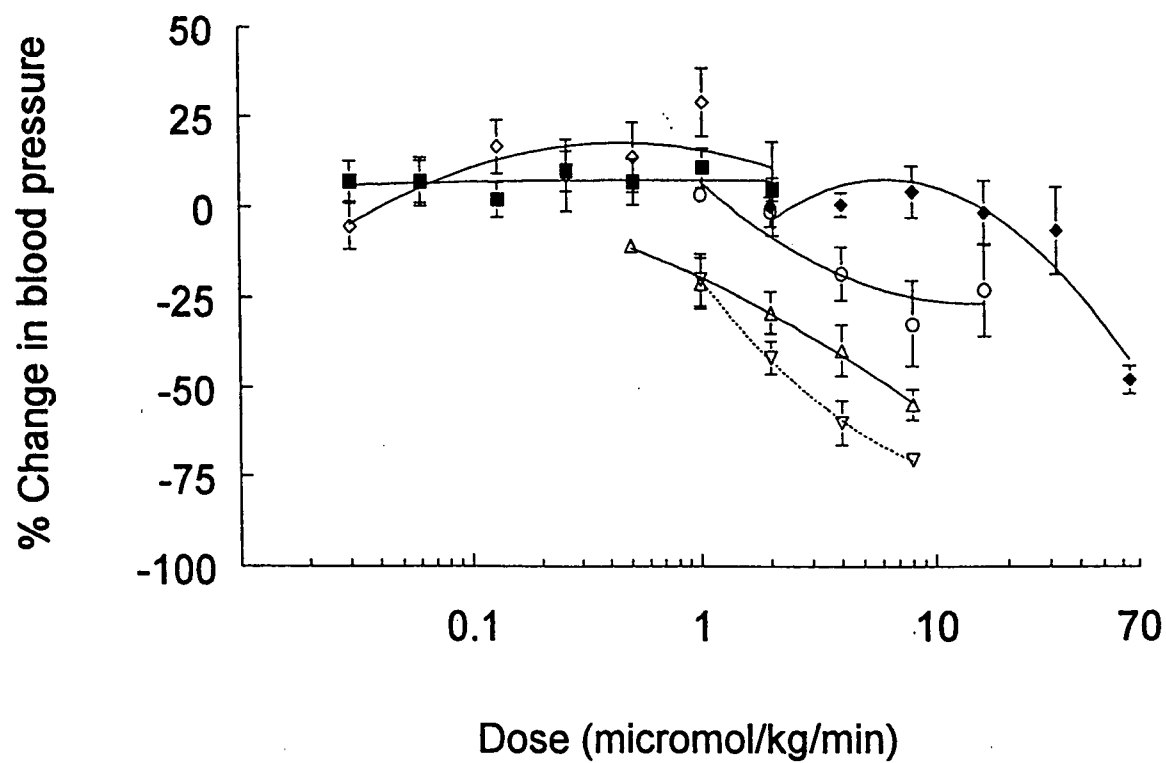
3.4. Drug effects on electrical stimulation variables.

Quinidine, flecainide and tedisamil increased iT in a dose-related fashion (Figure 4 & Table 1). Lidocaine increased iT but only to a small degree while RSD1019 had no

Figure 2. Heart rate and blood pressure responses to various infused doses of standard and putative antiarrhythmic drugs in pentobarbital anaesthetised rats. Percent changes from pre-drug HR and BP caused by infusion of quinidine (Q, open triangles), lidocaine (L, open circles), flecainide (F, inverted triangles), tedisamil (T, open diamonds), RSD1019 (R, filled diamonds) and vehicle control (C, filled squares) are shown as the mean \pm SEM (n=5). Responses were measured 3 minutes after starting infusions and the infused dose was doubled at 5 minute intervals. Pre-drug HR and BP did not differ significantly between groups, nor from vehicle control ($p>0.05$ by ANOVA). Group mean pre-infusion HR for all rats was 414 ± 6 beats/minute (mean \pm SD, n=35, range 340 to 517 beats/minute) while BP was 130 ± 4 mmHg (range 70 to 172 mmHg). The lines represent the best fit to these data by a second order polynomial (Slidewrite software). For clarity the curve for flecainide is shown as a dotted line.



Δ Q \circ L ∇ F \diamond T \blacklozenge R \blacksquare C



Δ Q \circ L ∇ F \diamond T \blacklozenge R \blacksquare

Figure 3. Changes in the PR, QRS and QT intervals of the ECG caused by various infused doses of standard and putative antiarrhythmic drugs in pentobarbital anaesthetised rats. Percent changes from the pre-drug values are shown as the mean \pm SEM (n=5). The symbols are the same as those used in Figure 2. Pre-drug PR, QRS and QT intervals did not differ significantly between groups, nor from vehicle control (p>0.05 by ANOVA). The group mean, pre-infusion PR, QRS and QT intervals for all rats were 56 \pm 1.0 ms (mean \pm SD, n=35, range 42 to 68 ms), 29 \pm 0.4 ms (range 23 to 32 ms) and 37 \pm 0.6 ms (range 27 to 42 ms), respectively. The lines represent the best fit to these data by a second order polynomial (Slidewrite software). For clarity the curve for flecainide is shown as a dotted line.

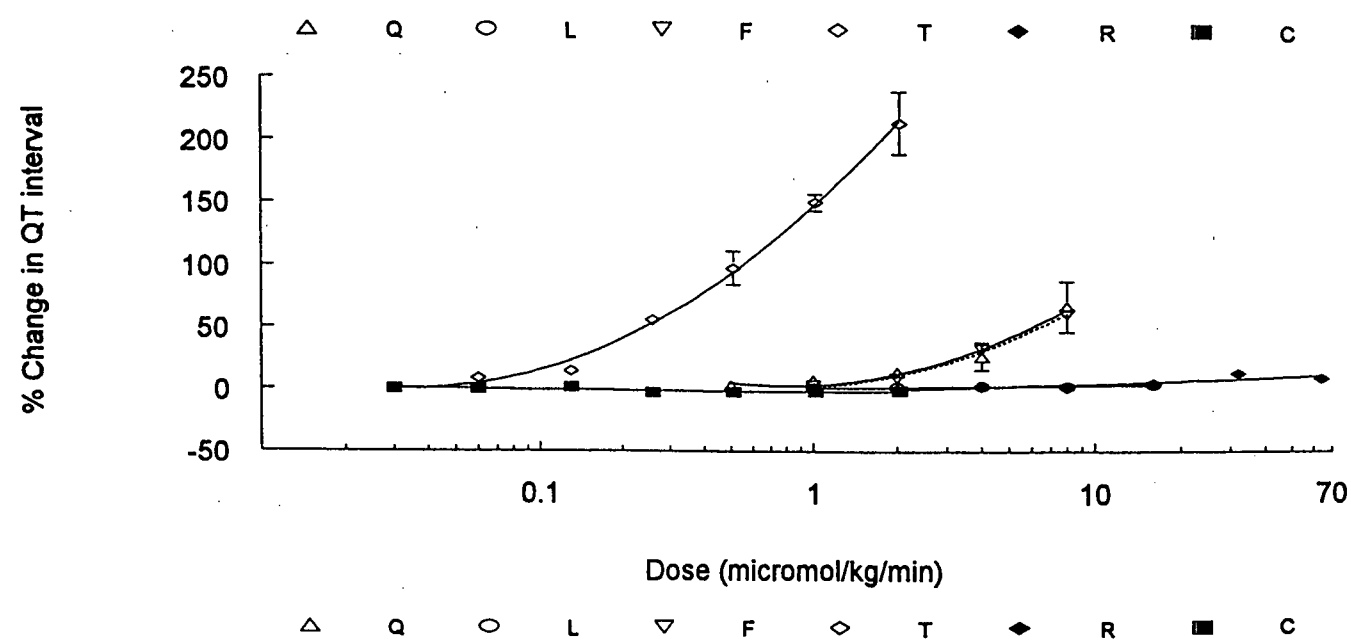
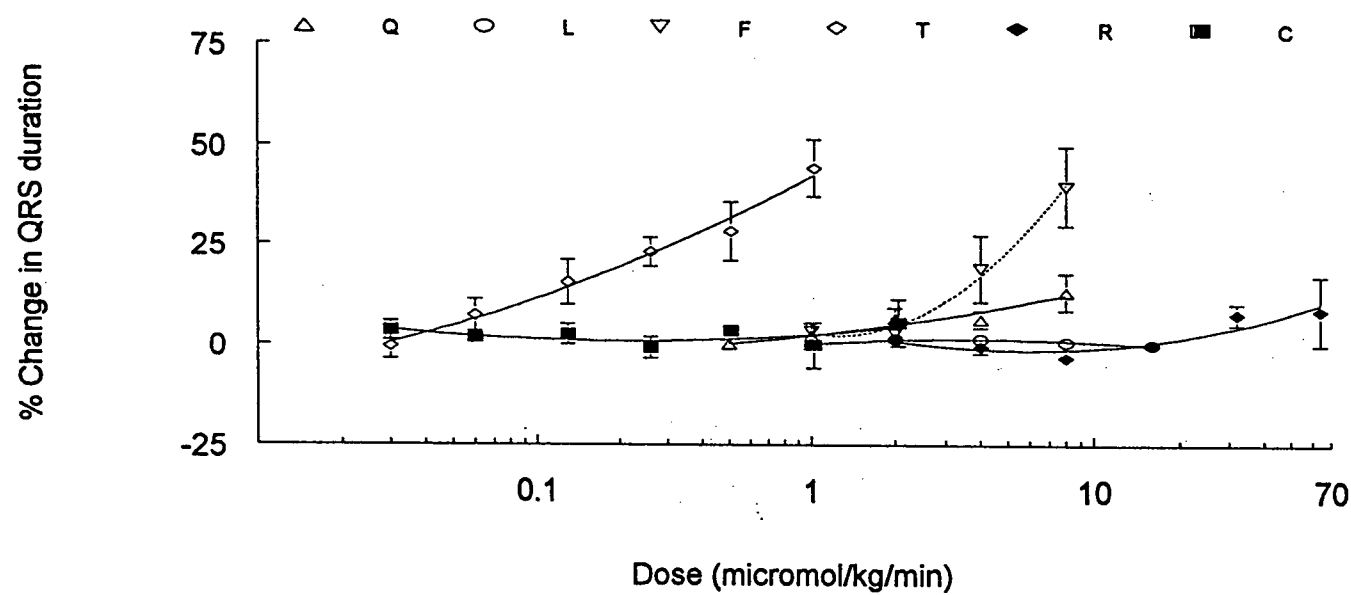
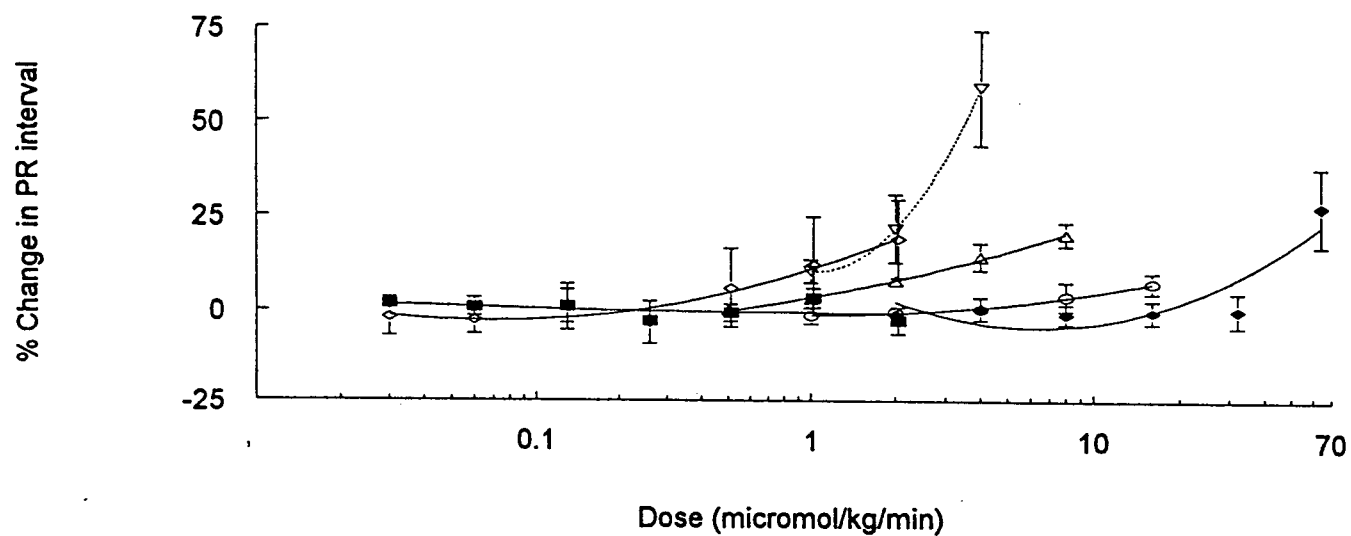
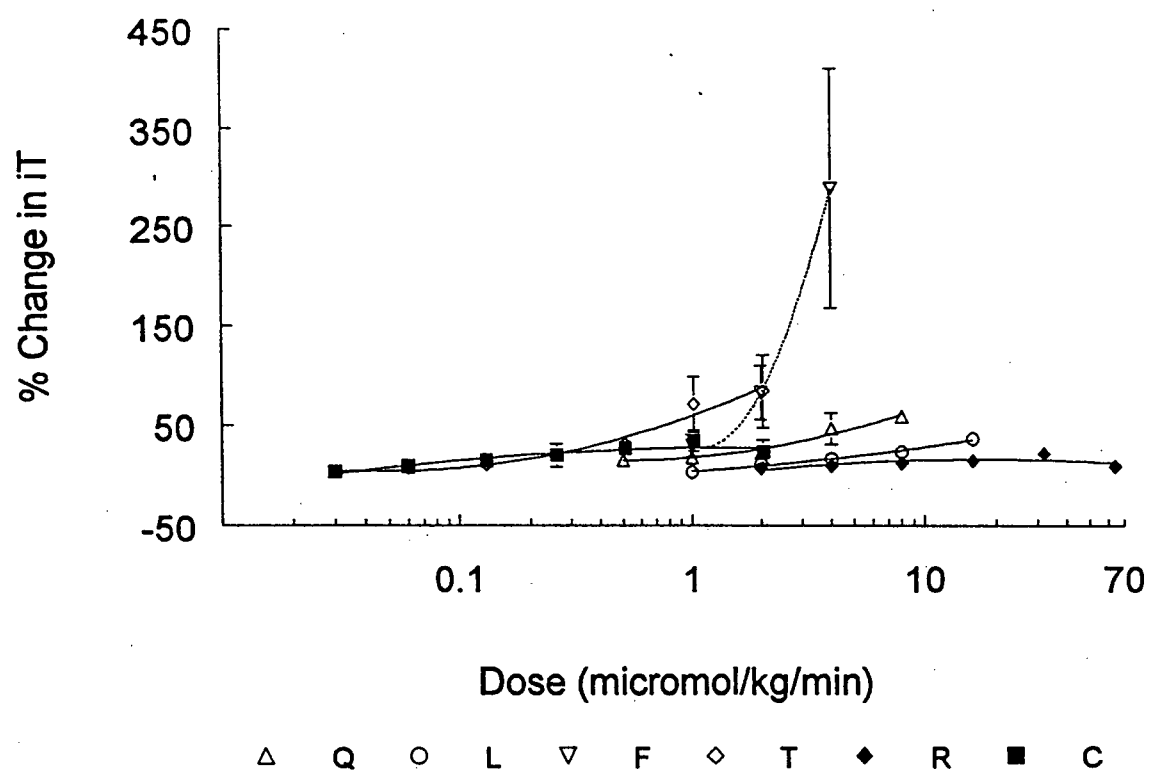


Figure 4. Changes in the threshold current for capture (iT) caused by various infused doses of standard and putative antiarrhythmic drugs in pentobarbital anaesthetised rats. Percent changes from pre-drug iT caused by each of the drugs is shown as the mean \pm SEM (n=5). The symbols are the same as those used in Figure 2. Pre-drug iT did not differ significantly between groups nor from vehicle control (p>0.05 by ANOVA). The group mean, pre-infusion iT for all rats was 105 \pm 8 μ A (mean \pm SD, n=35, range 37 to 260 μ A). The lines represent the best fit to these data by a second order polynomial (Slidewrite software). For clarity the curve for flecainide is shown as a dotted line.



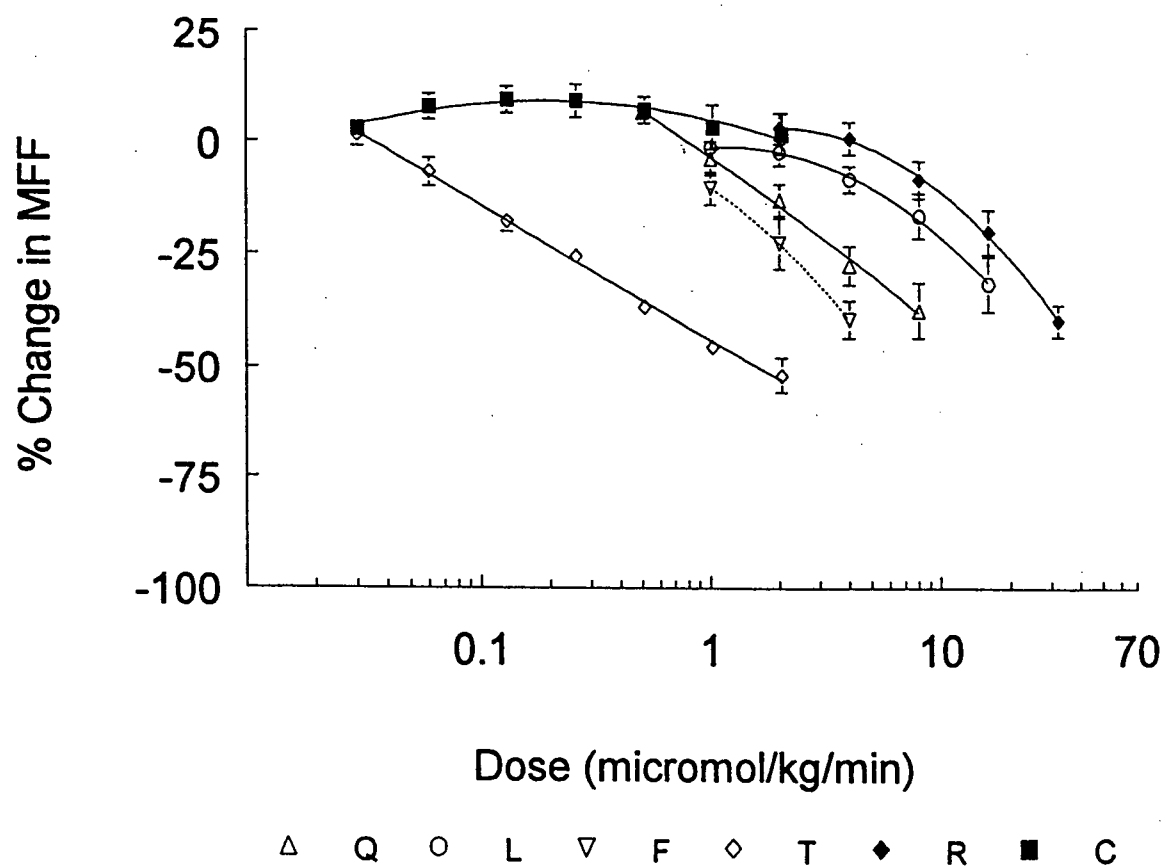
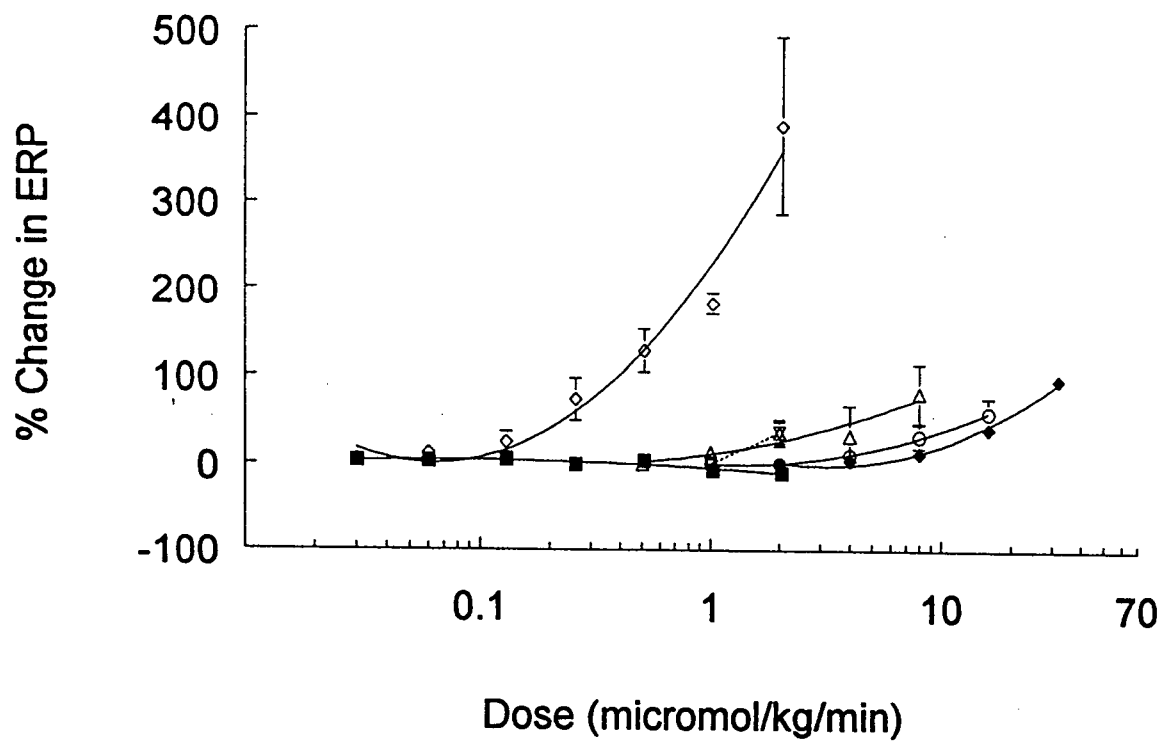
effect up to the maximum dose tested. It is clear from Figure 4 that the extent of drug induced changes in iT differed greatly among drugs. Tedisamil caused a relatively small increase in iT (maximum response $84 \pm 36\%$), while flecainide caused an enormous increase ($289 \pm 122\%$). The large error associated with the maximum effect of flecainide on iT reflects the fact that it was not possible to pace all hearts with the maximum output of the stimulator ($1000 \mu\text{A}$).

All of the drugs tested had significant effects on ERP and MFF. Drug-induced changes in ERP and MFF were reciprocal with all drugs producing an increase in the former and a decrease in the latter (Figure 5 & Table 1). Consistent with effects on the QT interval of the ECG, tedisamil was the most potent and efficacious drug in terms of effects on ERP and MFF. RSD1019 and lidocaine were the least potent and were approximately equally effective in their effects on ERP and MFF.

3.5 Drug effects on VFT.

Quinidine, lidocaine, and flecainide increased VFT in the left ventricle of anaesthetised rats (Figure 6 & Table 1). Tedisamil did not increase VFT *per se*, however, it did prevent induction of ventricular fibrillo-flutter at doses above $0.5 \mu\text{mol/kg/min}$. In these rats, only VT occurred. This observation coupled with the large increase seen in the QT interval of the ECG and ERP has important mechanistic implications (see discussion). In contrast, it was still possible to induce ventricular fibrillo-flutter despite infusion of maximum tolerated doses of RSD1019.

Figure 5. Changes in effective refractory period (ERP) and maximum following frequency (MFF) caused by various infused doses of standard and putative antiarrhythmic drugs in pentobarbital anaesthetised rats. Percent changes from pre-drug ERP and MFF caused by each of the drugs is shown as the mean \pm SEM (n=5). The symbols are the same as those used in Figure 2. Pre-drug ERP and MFF values did not differ significantly between groups nor from the vehicle control ($p>0.05$ by ANOVA). The group mean, pre-infusion ERP for all rats was 40 ± 2 ms (mean \pm SD, n=35, range 20 to 60 ms) while for MFF it was 15.4 ± 0.3 ms (range 13 to 20 ms). The lines represent the best fit to these data by a second order polynomial (Slidewrite software). For clarity the curve for flecainide is shown as a dotted line.



The magnitude of the drug-induced elevation in VFT varied depending on the drug considered. Infusion of lidocaine increased VFT ($116 \pm 77\%$) less than quinidine and flecainide (both greater than 250%). RSD1019 appeared to increase VFT somewhat ($25 \pm 5\%$) at the highest dose tested, however, this was not statistically significant. The rank order of potency for drug-induced elevation of VFT was flecainide > quinidine > lidocaine >>> RSD1019.

3.6 Summary of drug effects in normal tissue.

All of the drugs tested caused bradycardia and reduced BP in anaesthetised rats except tedisamil which caused bradycardia and tended to increase BP.

Lidocaine and RSD1019 had no effects on ECG intervals and had lesser effects on electrical stimulation variables as compared to quinidine, flecainide and tedisamil. Quinidine and flecainide depressed conduction velocity and excitability, as assessed by changes in ECG intervals and electrical stimulation variables, to a greater extent than the other drugs tested. All of the drugs tested increased ERP and reduced MFF although the extent of these effects were agent specific. Tedisamil was more potent and effective, than the other drugs tested, for prolonging AP duration and refractoriness as assessed by effects on the QT interval of the ECG as well as ERP and MFF.

Greater differences between compounds were observed for their effects to elevate VFT. Both qualitative and quantitative differences in drug action were observed. Tedisamil did not increase VFT but prevented the induction of ventricular fibrillo-flutter at high doses. Lidocaine and RSD1019 increased VFT in a statistically significant, dose-

Figure 6. Changes in VFT caused by various infused doses of standard and putative antiarrhythmic drugs in pentobarbital anaesthetised rats. Percent changes from pre-drug VFT caused by infusion of each drug are shown as the mean \pm SEM (n=5). The symbols are the same as those used in Figure 2. Pre-drug VFT did not differ significantly between groups nor from vehicle control ($p>0.05$ by ANOVA). The group mean, pre-infusion VFT was 221 ± 20 μ A (mean \pm SD, n=35, range 50 to 540 μ A). The lines represent the best fit to these data by a second order polynomial (Slidewrite software). For clarity the curve for flecainide is shown as a dotted line.

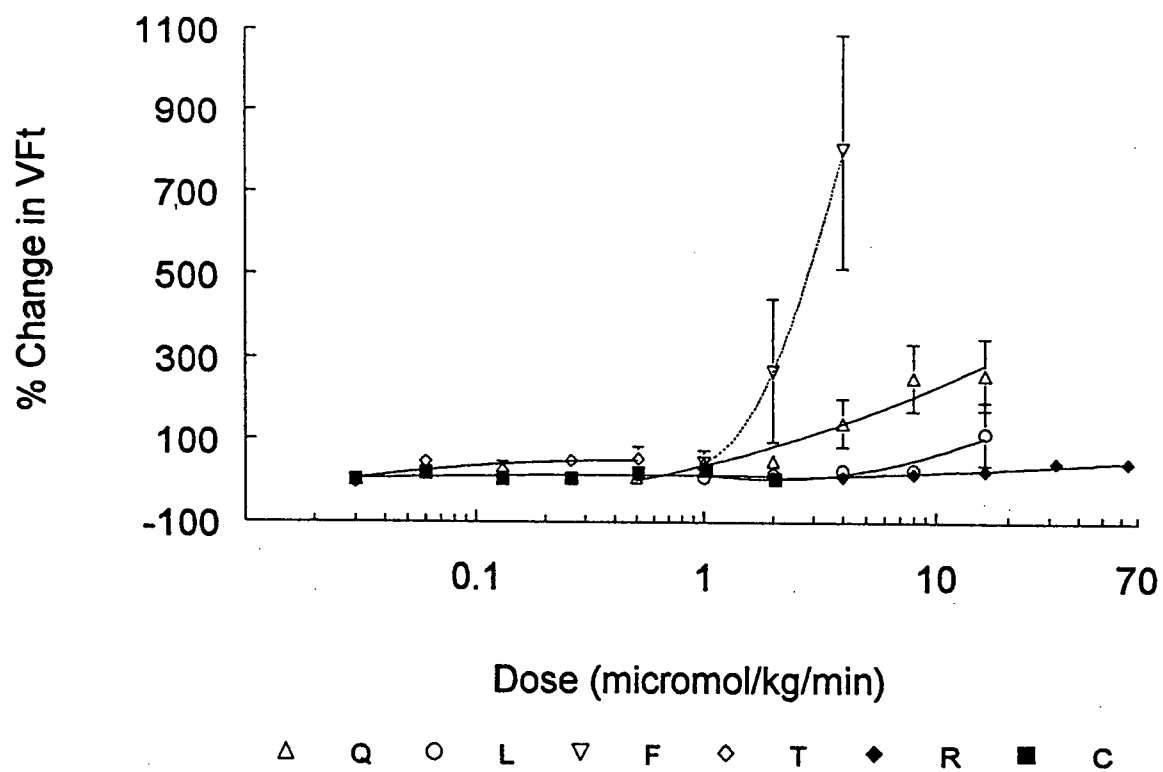


Table 1. Summary of potencies of selected drugs for effects on normal myocardial tissue in anaesthetised rats.

	Q	L	F	T	R
BP	1.9 ± 0.6	7.4 ± 2.5	1.2 ± 0.2	$\uparrow 0.7 \pm 0.4$	$45 \pm 9.4^*$
HR	3.3 ± 0.9	11 ± 3.2	1.8 ± 0.2	$0.26 \pm 0.05^*$	$27 \pm 7.2^*$
PR	$(9.5 \pm 2.4)^*$	NE*	1.8 ± 0.2	(3 ± 1)	$(75 \pm 10)^*$
QRS	> 8.0	NE*	5.0 ± 1.2	0.33 ± 0.02	$> 64^*$
QT	3.0 ± 1.0	NE*	2.5 ± 0.4	$0.13 \pm 0.02^*$	$> 64^*$
iT	2.4 ± 0.6	$11 \pm 1.1^*$	1.2 ± 0.2	0.3 ± 0.2	$> 64^*$
ERP	1.1 ± 0.2	$6.6 \pm 1.5^{\#}$	2.0 ± 0.4	$0.1 \pm 0.04^*$	$9.3 \pm 2.1^*$
MFF	4.3 ± 1.4	10 ± 1.3	2.6 ± 0.6	$0.2 \pm 0.02^*$	$19 \pm 4^*$
VFT	2.3 ± 1.3	14 ± 3.0	1.4 ± 0.4	NE*	$> 64^*$

Potency of quinidine (Q), lidocaine (L), flecainide (F), tedisamil (T) and RSD1019 (R) on BP, HR, ECG intervals and electrical stimulation variables in anaesthetised rats. The values represent the dose (in $\mu\text{mol/kg/min}$) required to produce a 25% change from the respective pre-drug value ($D_{25\%} \pm \text{SEM}$, $n=5$) for all variables except VFT. For VFT the dose required to produce a 100% increase was determined ($D_{100\%} \pm \text{SEM}$, $n=5$). NE indicates the drug had no effect. In the case where the drug had a statistically significant effect but did not cause a 25% increase, the value has been entered as maximum dose tested. Values shown in brackets were extrapolated just beyond the dose range tested. Statistical significance was tested using a single factor ANOVA ($p < 0.05$) followed by a Tukey test for differences. The symbol (*) indicates a significant difference from all other drugs, while the symbol (#) indicates that lidocaine was significantly less potent for effects on ERP than quinidine and tedisamil. The abbreviations are the same as those used in the text.

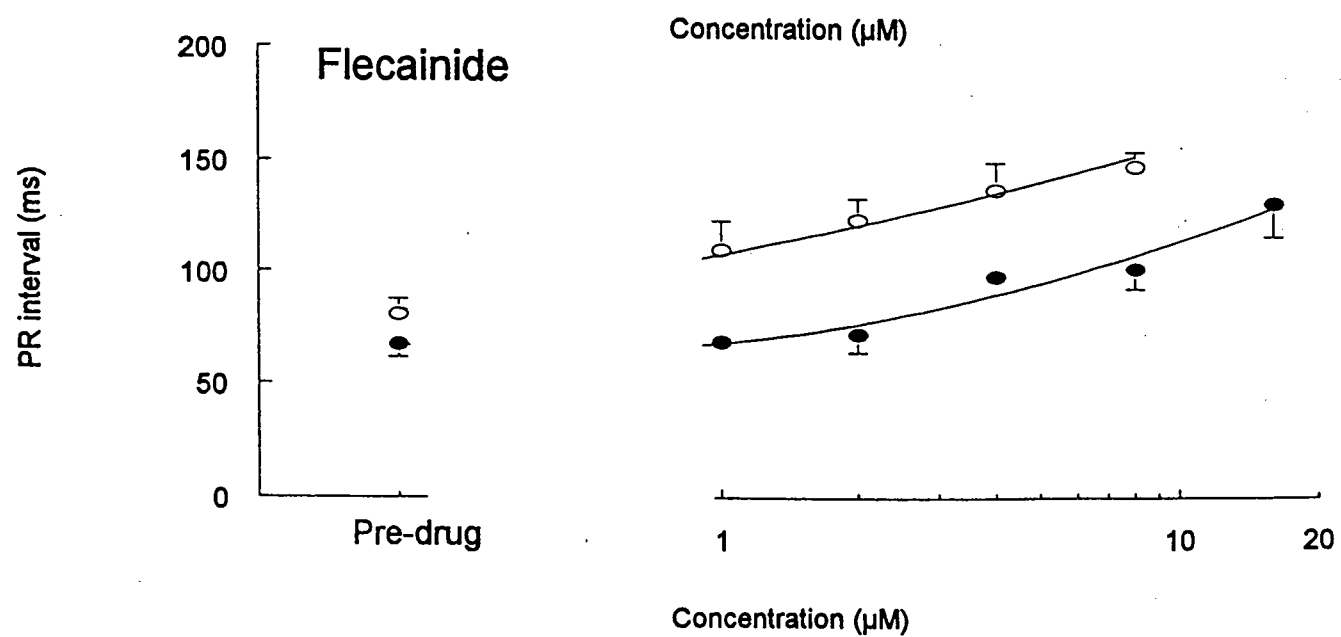
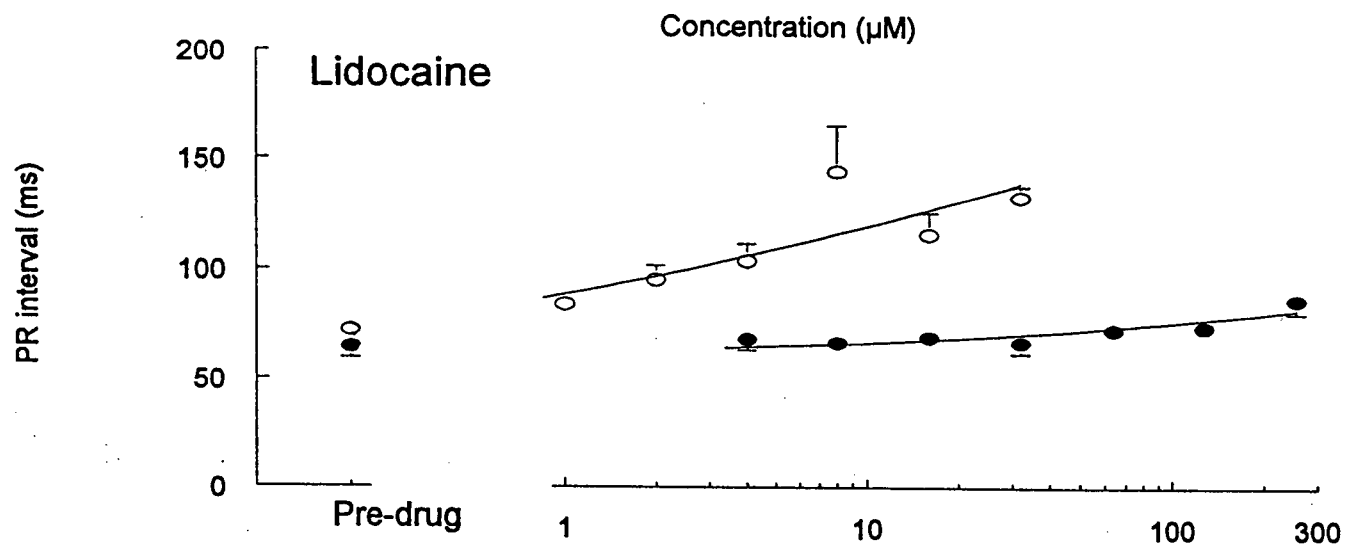
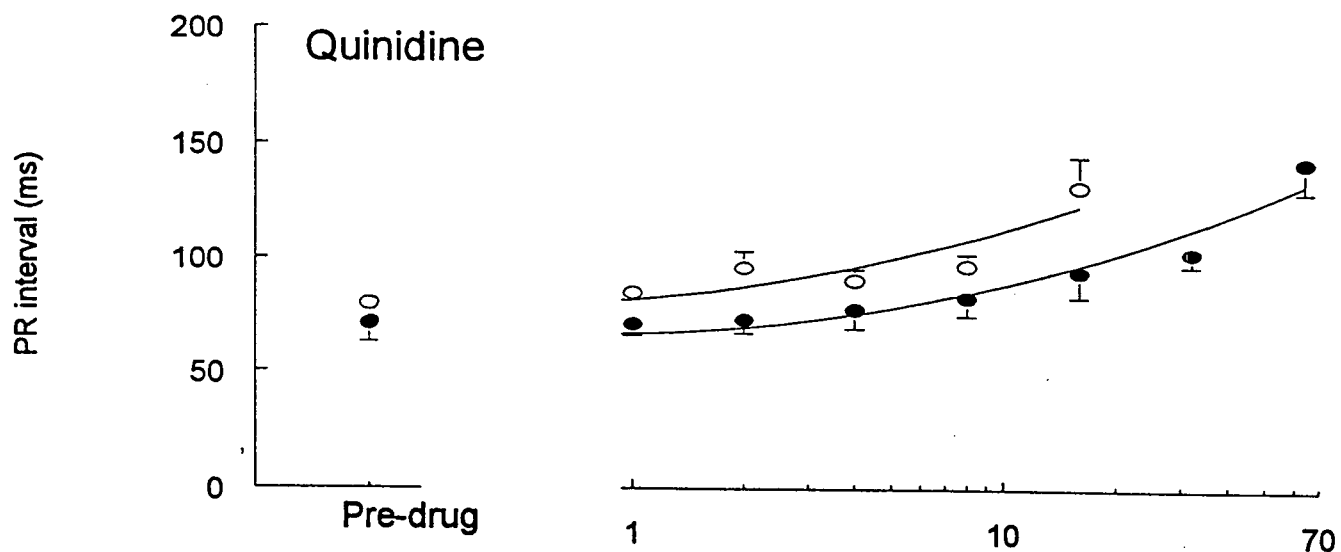
related fashion but only to a small extent. In contrast, quinidine and flecainide caused large dose-related increases in VFT.

3.7. Increased potency for slowing conduction under "simulated ischaemic" conditions.

Perfusion with "simulated ischaemic" buffer caused bradycardia and increased the PR and QRS intervals of the ECG. No differences were found between groups before drug treatment; hence all pre-drug data were grouped together for the purposes of presentation (Figures 7-8). The pre-drug HR in hearts perfused with normal buffer was 237 ± 46 beats/min (mean \pm SD, $n=35$) while in hearts perfused with "simulated ischaemic" buffer it was 179 ± 35 beats/min ($p < 0.05$ from normal buffer). In the time matched vehicle control groups the PR and QRS intervals did not change significantly over the course of the experiment (4 to 12% change). In the first series of experiments (quinidine, lidocaine & flecainide) the group mean pre-drug PR and QRS intervals in normal and "simulated ischaemic" buffers were 63 ± 3 ms versus 76 ± 3 ms (mean \pm SD, $n=20$) and 32 ± 3 ms versus 40 ± 5 , respectively. In the second series of experiments (tedisamil & RSD1019) the pre-drug PR, QRS and QT intervals in normal buffer were 72 ± 3 ms, 21 ± 3 ms, 49 ± 4 ms (mean \pm SD, $n=15$) while those in "simulated ischaemic" buffer were 90 ± 4 ms, 40 ± 4.5 ms and 69 ± 5 ms, respectively.

The drugs were found to be differentially influenced by the conditions of "simulated ischaemia." The conduction slowing actions of quinidine, flecainide and tedisamil in the ventricular myocardium, as assessed by their ability to increase the PR and QRS intervals of the ECG, were increased by 1 to 4 times by perfusion with "simulated

Figures 7-8. Concentration-response curves for increases in the PR interval in isolated rat hearts produced by standard and putative antiarrhythmic drugs, under normal and "simulated ischaemic" conditions. Concentration-response curves for normal and "simulated ischaemic" conditions are shown in open and filled circles, respectively. Figure 7 shows concentration-response curves for quinidine (top), lidocaine (middle) and flecainide (bottom). Figure 8 shows concentration-response curves for tedisamil (top) and RSD1019 (bottom). Each point represents the mean \pm SEM (n=5). Cumulative concentration-response curves were constructed. Responses were measured after 3 minutes of perfusion with the drug containing solution. Pre-drug ECG values did not differ significantly between groups nor from control for either experiment (ANOVA). The lines represent the best fit to these data by a second order polynomial (Slidewrite software).



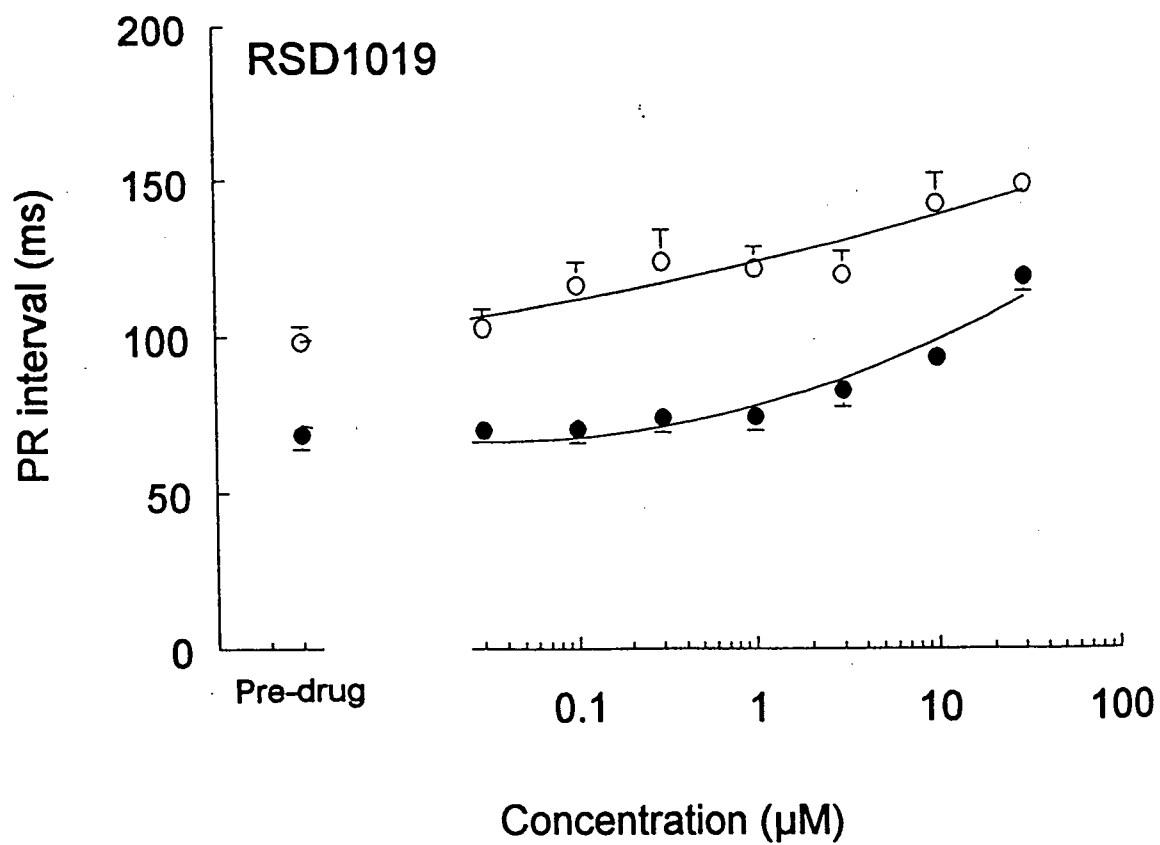
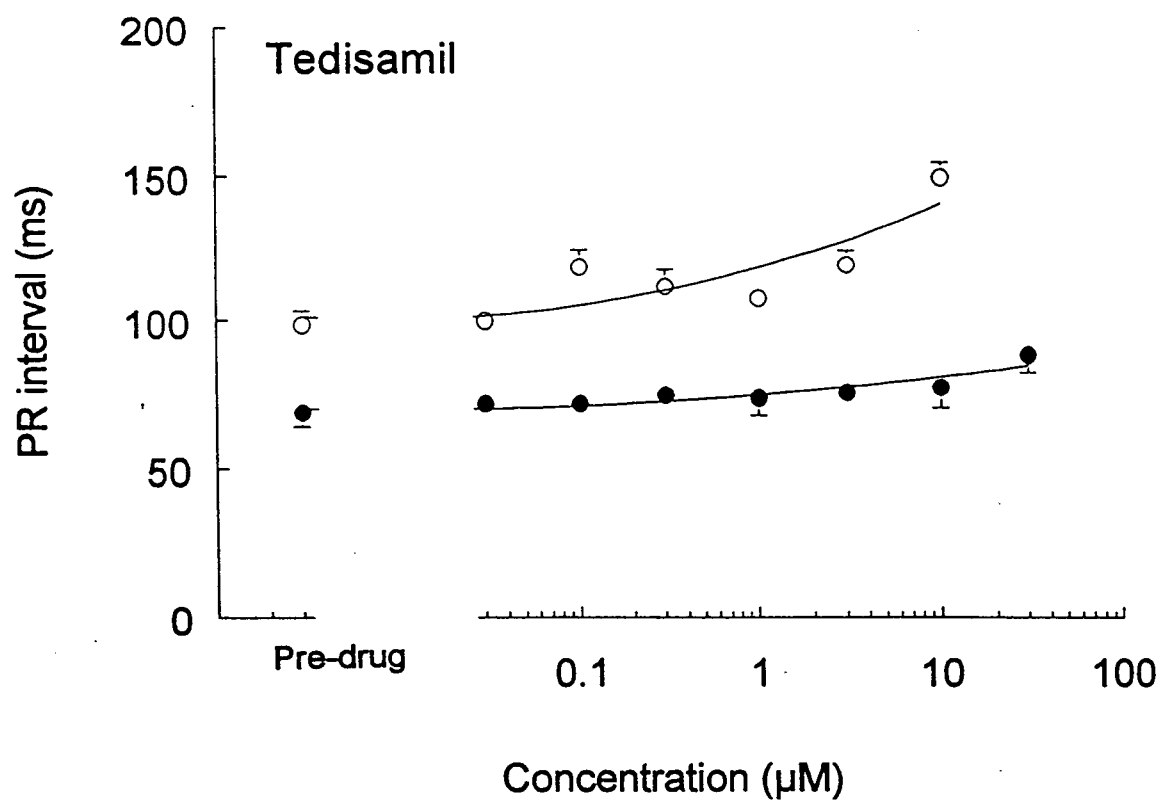


Table 2. Influence of "simulated ischaemic" buffer on drug-induced increases in ECG intervals.

C_{25%} values for drug-induced increases in ECG intervals.

Drug	PR-N	PR-I	QRS-N	QRS-I	QT-N	QT-I
C _{25%} (μM)						
Quinidine	7.9±2	4.0±2	25±1 ⁺	6.3±1	NM	NM
Lidocaine	316±3*	1±2	79±2 [%]	0.8±2	NM	NM
Flecainide	2±1	0.8±1	5±1 ⁺	2±2	NM	NM
Tedisamil	3.6±0.7	4.9±1	3.9±0.2	1.1±2	0.6±0.1	2.2±0.4
RSD1019	540±7 [#]	20±2 [%]	105±3 [#]	34±5 [#]	34±8 [#]	38±1 [#]

Potency ratio for effects in hearts perfused with normal and "simulated ischaemic" buffer.

Drug	C _{25%} Ratio (normal/ischaemic)		
	PR	QRS	QT
Quinidine	2.0±1.1	3.9±1.1	NM
Lidocaine	340±1.8*	105±1.6*	NM
Flecainide	2.9±0.8	2.6±1.3	NM
Tedisamil	0.7±1.1	3.5±1.6	0.3±0.4
RSD1019	22±3.0 [#]	3.1±4.5	0.9±2.8

The top panel shows the concentration required to produced a 25% change from the pre-drug value (C_{25%}) for ECG intervals in isolated rat hearts perfused with normal (N) or "simulated ischaemic" buffer (I). Each value represents the C_{25%}±SEM (n=5). The ECG intervals (PR, QRS & QT) are indicated at the top of the column. In the first series of experiments the QT interval was not measured (NM). The lower panel shows the ratio of the C_{25%} values obtained in hearts perfused with normal or "simulated ischaemic" buffer. Each value is the mean± pooled SEM, n=10.

Statistical significance was tested by ANOVA at a significance level of p<0.05 followed by a Tukey test for differences. The asterisk (*) indicates a significant difference between lidocaine and the other drugs; (#) indicates the same thing for RSD1019; (%) indicates a difference between lidocaine and RSD1019; while (°) indicates that quinidine and flecainide were significantly different than lidocaine and tedisamil.

ischaemic" buffer (Figures 7-8, Table 2). However, the prolongation of these intervals produced by lidocaine was greatly increased by perfusion with "simulated ischaemic" buffer. The potency of lidocaine was increased by 105 to 340 times, depending on the measure considered. The ischaemia-selective actions of RSD1019 were found to be intermediate between those of lidocaine and those of quinidine, flecainide and tedisamil.

The modification of drug action on the QT interval of the ECG by perfusion with "simulated ischaemic" buffer was only assessed for tedisamil and RSD1019. Perfusion with "simulated ischaemic" buffer prolonged the QT interval. The pre-drug QT interval was 113 ± 27 ms (mean \pm SD, $n=15$) in hearts perfused with normal buffer while in hearts perfused with "simulated ischaemic" buffer it was 147 ± 21 ms ($p < 0.05$). Perfusion with "simulated ischaemic" buffer did not influence the QT widening produced by RSD1019; however, it reduced the potency of tedisamil (Table 2).

During the course of these experiments it was noted that lidocaine caused asystole at high concentrations in 3 of 5 hearts perfused with normal PIPES/Krebs buffer. None of the other drugs tested had this effect.

3.8 Suppression of ischaemia-induced arrhythmias in anaesthetised rats.

All of the drugs tested had statistically significant antiarrhythmic actions against ischaemia-induced arrhythmias in anaesthetised rats (Figures 9-13, Table 3-4). However, the potency and efficacy of quinidine, lidocaine, flecainide, tedisamil and RSD1019 for suppression of ischaemia-induced arrhythmias differed considerably. Examination of the dose-response relationships for effects on BP, HR and ECG intervals in comparison to

their antiarrhythmic actions reveals further differences between drugs and possibly provides information concerning the mechanism(s) of drug action (Figures 9-13; Table 4).

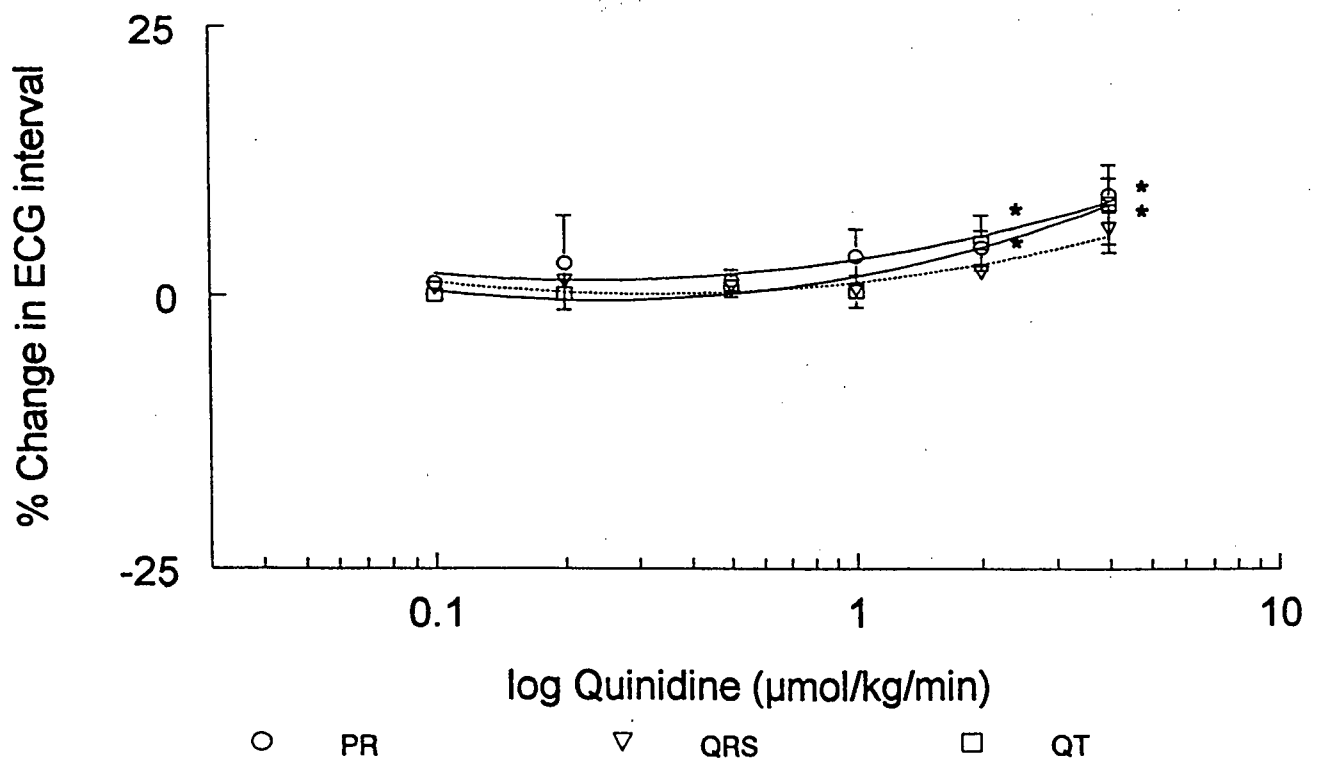
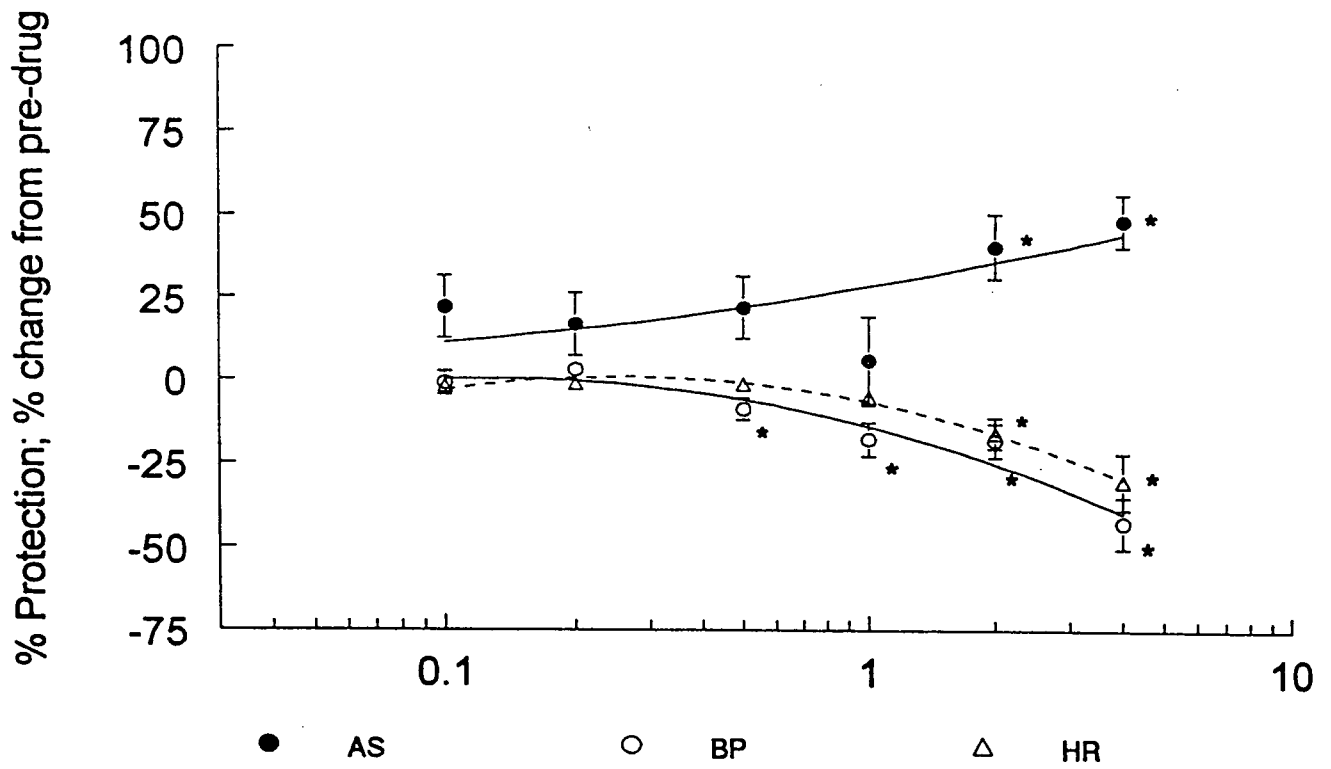
Quinidine had no effect on the occurrence of VT but suppressed VF at high doses. At the highest dose tested quinidine also significantly reduced the \log_{10} number of PVB. The arrhythmia score was reduced in a dose-related fashion; however, the maximum antiarrhythmic protection was limited. The relatively shallow dose-response curve for antiarrhythmic protection produced by quinidine reflects the fact that quinidine was unable to suppress ischaemia-induced VT. BP and HR were reduced in a dose-related fashion by quinidine. At doses which had significant antiarrhythmic actions quinidine increased the PR and QT intervals of the ECG.

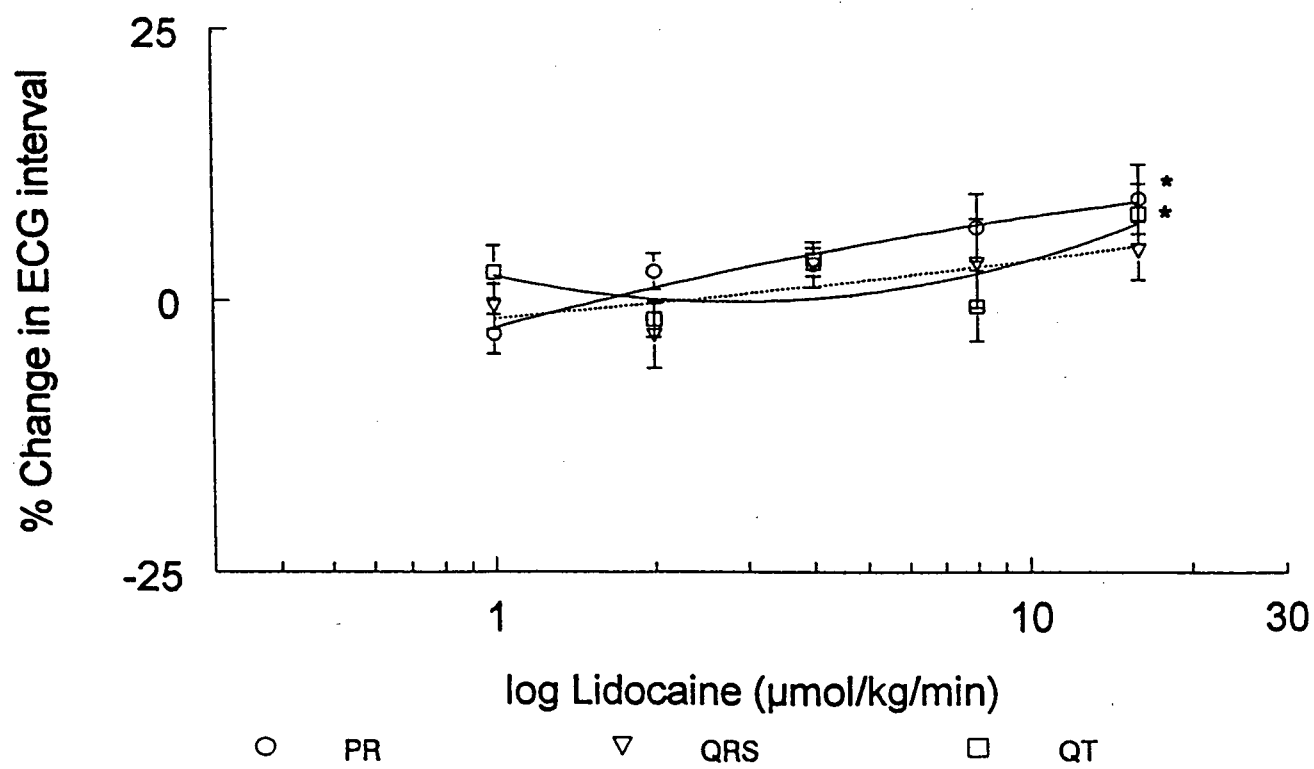
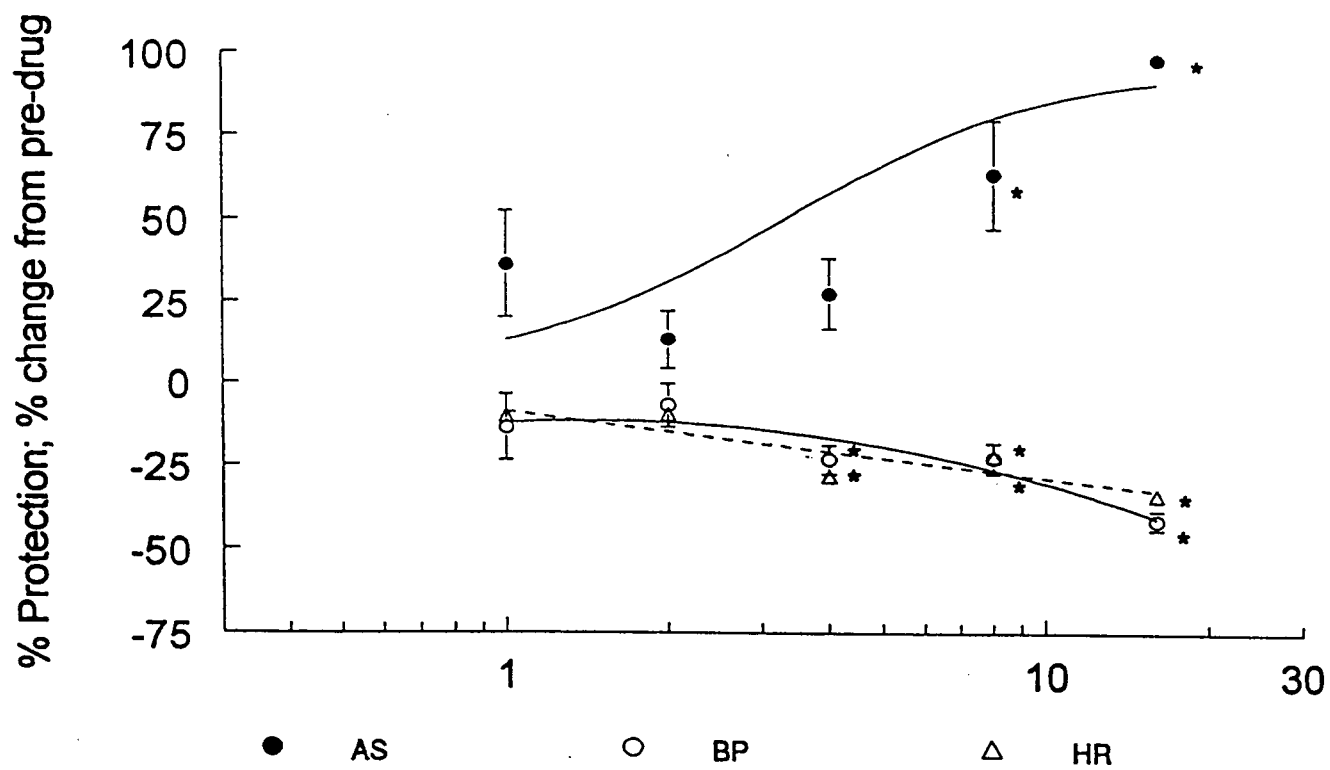
Over the dose range tested, lidocaine was the only drug tested that completely suppressed all ischaemia-induced arrhythmias in anaesthetised rats. It is also noteworthy that the dose-response curve for antiarrhythmic protection was relatively steep compared to that produced by quinidine, flecainide and tedisamil. Lidocaine produced bradycardia and hypotension in a dose-related fashion. The PR interval and, paradoxically, the QT interval of the ECG were increased by the highest dose of lidocaine. This dose also completely prevented ischaemia-induced ST-segment elevation while none of the other drugs had any effect on this variable.

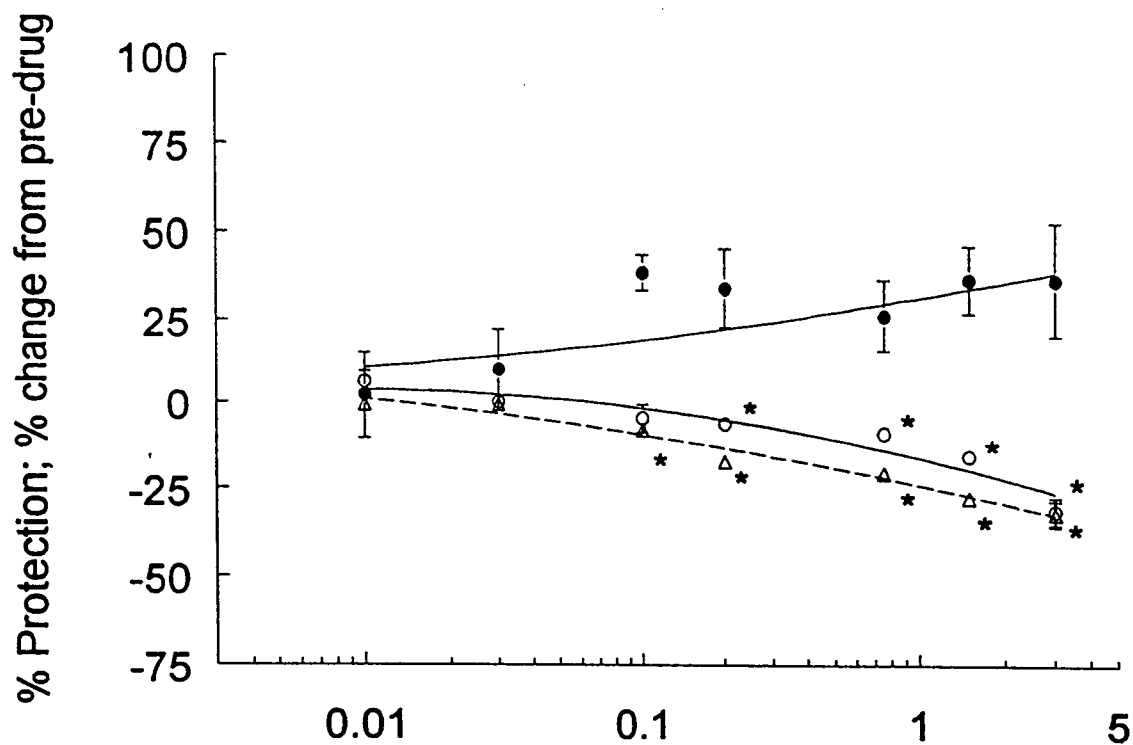
The suppression of ischaemia-induced arrhythmias produced by flecainide stands in contrast to that of the other drugs tested. Only flecainide failed to completely suppress the occurrence of VF at some dose. It also had limited effects on the occurrence of VT.

Figures 9-13. Dose-response curves for the action of standard and putative antiarrhythmic drugs on ischaemia-induced arrhythmias, blood pressure, heart rate and ECG intervals in pentobarbital anaesthetised rats. Each value represents the mean \pm SEM, $n=7$ except for tedisamil (Figure 12) where $n=5$. Drugs were infused continuously starting 5 minutes before performing coronary artery occlusion. BP, HR and ECG response were measured immediately before occlusion. Arrhythmia score data were expressed as the percent protection, as compared to the vehicle control group, and fit to a logistic function as described in the text. The antiarrhythmic curve $ED_{50\%}$, Hill co-efficient (h) and curve fit co-efficient " r " are summarised in Table 4. Drug effects on BP, HR and ECG intervals are expressed as the percent change from the pre-drug. The lines represent the best fit to these data by a second order polynomial (Slidewrite software). All abbreviations are those used in the text with the addition of AS = arrhythmia score.

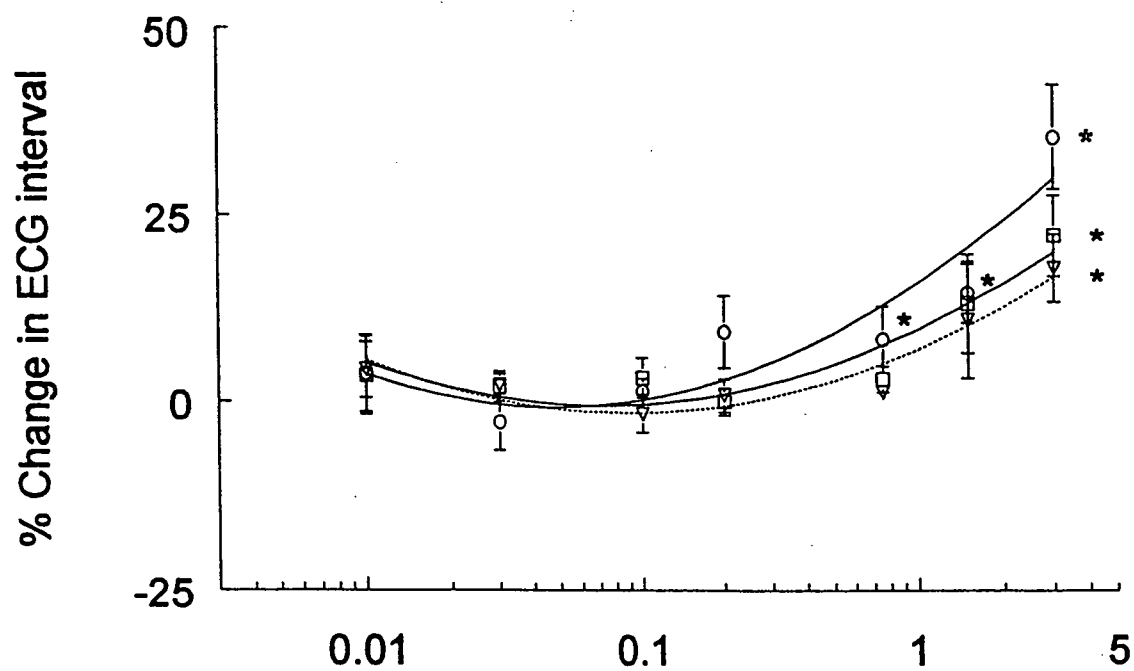
No differences were found between pre-drug data, occluded zone size nor blood $[K^+]$ (ANOVA, $p>0.05$). The group mean occluded zone size was $36\pm6\%$ (mean \pm SD, $n=263$, range 25-52) and the blood $[K^+]$ was 3.4 ± 0.5 mM ($n=195$, range 2.2-5.4 mM). The group mean, pre-drug BP and HR were 108 ± 19 mmHg (range 74-165 mmHg) and 354 ± 42 beats/min (range 221-472 beats/min). The group mean pre-drug PR, QRS and QT intervals were 62 ± 5 ms (range 48-85 ms), 27 ± 7 ms (range 11-60 ms) and 37 ± 6 ms (range 21-50 ms), respectively.





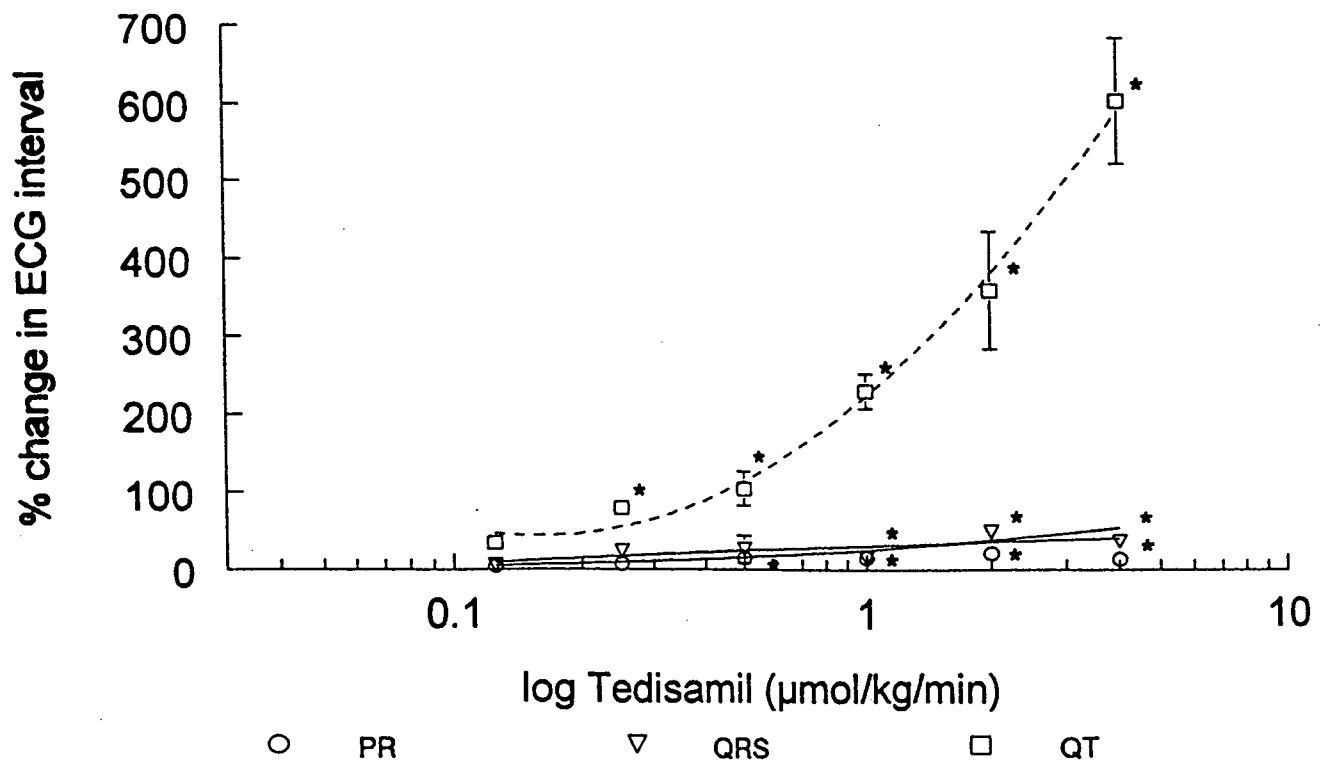
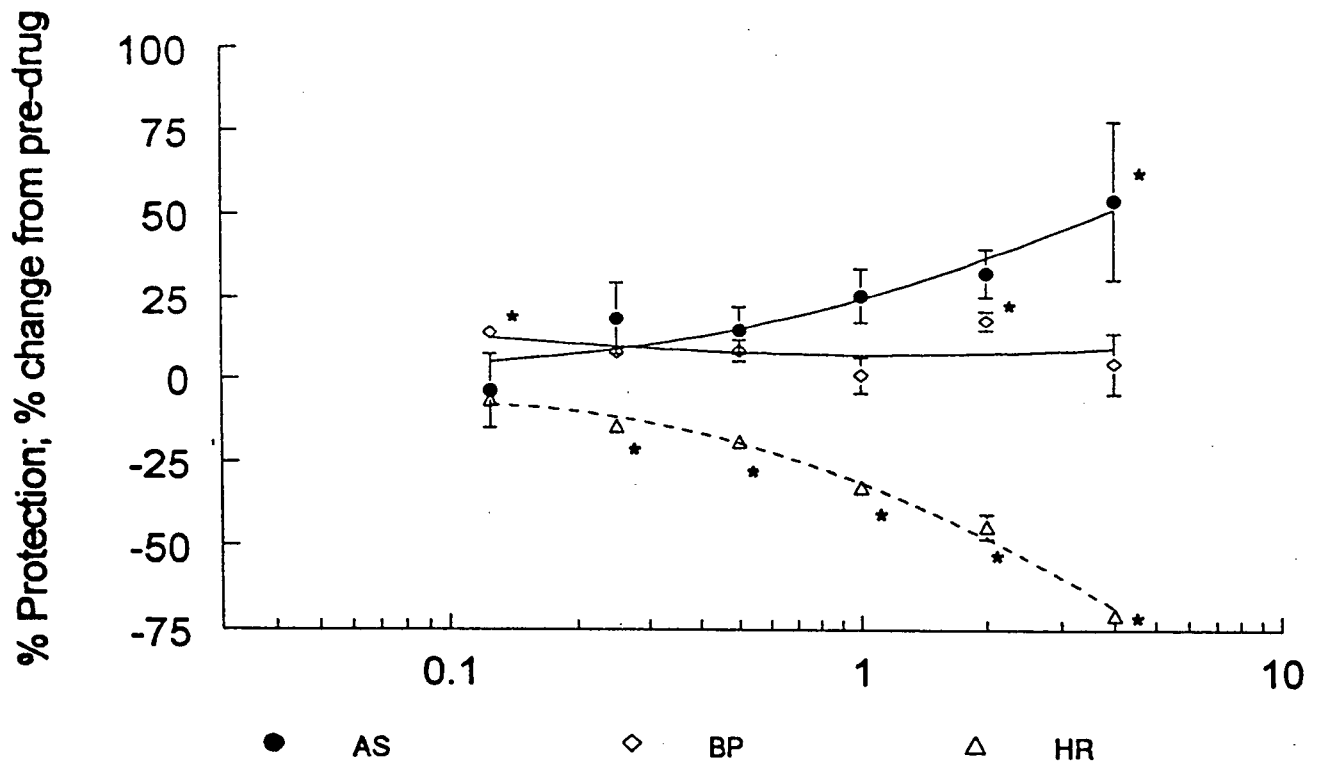


● AS ○ BP △ HR



○ PR ▽ QRS □ QT

log Flecainide ($\mu\text{mol/kg/min}$)



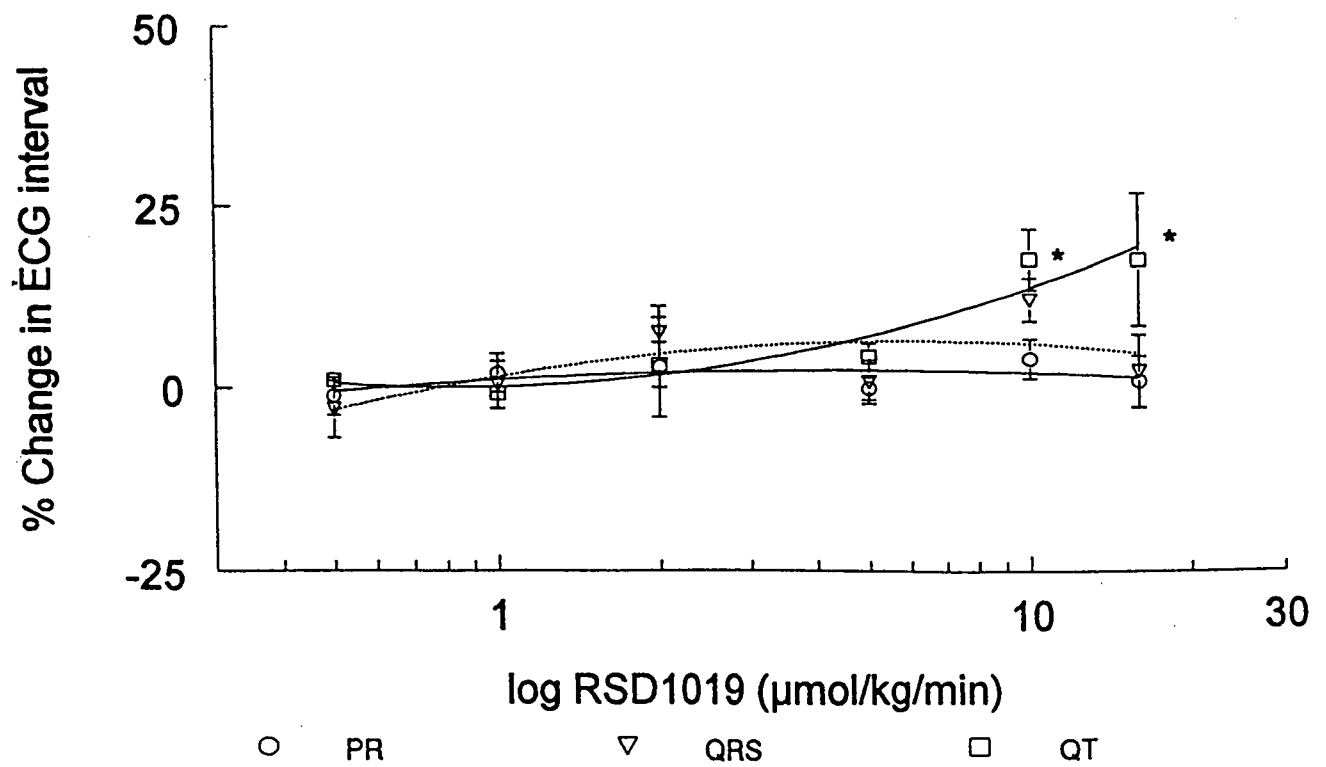
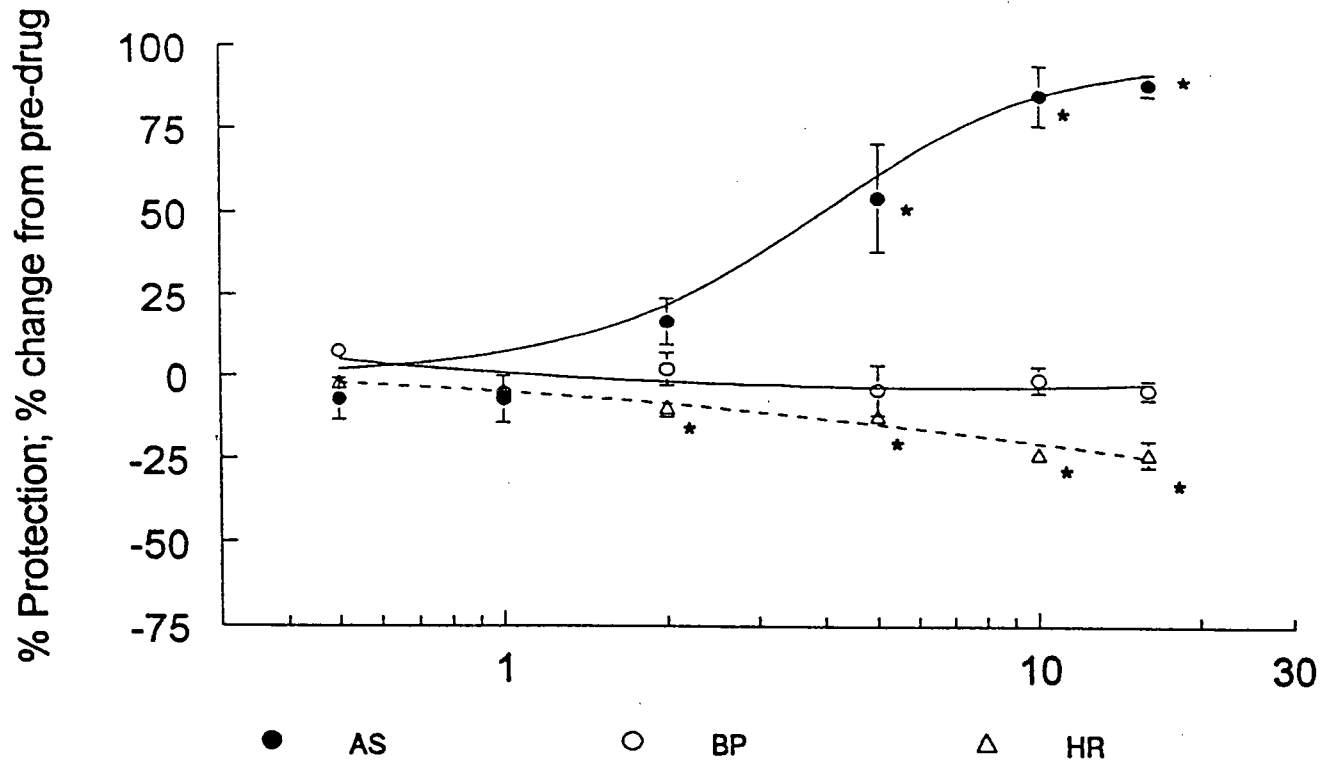


Table 3. Antiarrhythmic actions of quinidine, lidocaine, flecainide, tedisamil and RSD1019 in pentobarbital anaesthetised rats after occlusion.

Dose	PVB	log # PVB	VT	log VT dur.	VF	log VF dur.
C	65/65	1.8±0.5	65/65	1.6±0.5	48/65	1.7±0.5
Q 0.1	7/7	2.1±0.1	7/7	1.8±0.1	4/7	1.1±0.1
Q 0.2	7/7	2.2±0.2	7/7	1.9±0.1	5/7	1.6±0.2
Q 0.5	7/7	2.0±0.2	7/7	1.7±0.1	4/7	1.3±0.3
Q 1	6/7	1.9±0.3	7/7	1.7±0.3	5/7	1.6±0.3
Q 2	7/7	1.7±0.1	6/7	1.7±0.3	0/7*	/
Q 4	7/7	1.3±0.3*	6/7	0.6±0.2	0/7*	/
L 1	7/7	2.2±0.1	4/7	2.1±0.2	4/7	1.1±0.3
L 2	7/7	2.3±0.1	6/7	1.6±0.1	4/7	1.2±0.4
L 4	7/7	2.1±0.2	7/7	2.0±0.2	5/7	1.2±0.1
L 8	7/7	1.2±0.4	2/7*	2.0±0.1	1/7*	2.0
L 16	0/7*	/	0/7*	/	0/7*	/
F 0.01	7/7	1.8±0.2	7/7	1.6±0.2	7/7	1.7±0.2
F 0.03	7/7	1.8±0.1	7/7	1.7±0.2	5/7	1.4±0.3
F 0.1	7/7	2.0±0.2	7/7	1.7±1.3	2/7*	1.6±0.4
F 0.375	7/7	2.2±0.2	7/7	1.5±0.2	2/7*	1.7±0.4
F 0.75	6/7	2.6±0.1	7/7	1.8±0.3	1/7*	2.0
F 1.5	7/7	2.3±0.1	6/7	1.8±0.3	2/7*	1.5±0.5
F 3	5/7	1.7±0.4	5/7*	1.3±0.5	2/7*	1.4±0.7
T 0.125	5/5	2.0±0.1	5/5	2.0±0.2	5/5	2.0±0.1
T 0.25	5/5	2.2±0.1	5/5	1.9±0.3	4/5	1.9±0.1
T 0.5	5/5	2.1±0.1	5/5	1.9±0.2	4/5	1.9±0.1
T 1	5/5	2.1±0.1	5/5	1.5±0.3	4/5	1.3±0.3
T 2	5/5	1.7±0.2	5/5	1.3±0.3	2/5	1.0±0.2
T 4	5/5	1.3±0.3	3/5*	1.3±0.5	0/5*	/
R 0.5	7/7	1.8±0.1	7/7	2.0±0.2	7/7	1.8±0.2
R 1	7/7	1.9±0.2	7/7	1.7±0.2	7/7	1.9±0.1
R 2	7/7	2.3±0.1	7/7	1.8±0.2	7/7	1.6±0.2
R 5	7/7	1.9±0.2	4/7*	1.5±0.1	4/7	1.6±0.3
R 10	7/7	1.6±0.2	1/7*	1.9	1/7*	2.0
R 16	6/7	1.3±0.3	0/7*	/	0/7*	/

Table 3 shows the effects of quinidine (Q), lidocaine (L), flecainide (F), tedisamil (T) and RSD1019 (R) on ischaemia-induced arrhythmias in pentobarbital anaesthetised rats. Arrhythmias occurrence data are summarised as the number of rats having the arrhythmia over the number in the group. Data in the control group are shown as the mean±SD, n=65 and mean±SEM, n=7 or n=5 (tedisamil) for treated groups. The abbreviations are the same as those used in the text with the addition of the following: log # PVB = log₁₀ of the number of PVB, log VT dur. and log VF dur. = log₁₀ of VT and VF duration, respectively. The asterisk (*) indicates p<0.05 from control by ANOVA followed by a Tukey test for differences. Arrhythmia occurrence data were tested for statistical significance using Fisher's exact test with p<0.05 taken as statistically significant.

Table 4. Potency ratios for suppression of ischaemia-induced arrhythmias versus drug effects on normal tissue.

	Quinidine	Lidocaine	Flecainide	Tedisamil	RSD1019
Part A Potency for suppression of ischaemia-induced arrhythmias					
ED _{50%}	5.8±4.0	5.8±5.2	13.3±24	3.5±0.9	3.9±0.5
h	0.5±0.2	1.8±0.6	0.3±0.1	0.9±0.7	1.9±0.4
r	0.53	0.66	0.51	0.90	0.98
Part B Potency ratio for suppression of ischaemia-induced arrhythmias versus drug effects on normal tissue.					
BP	3.1	0.8	11	5.0	0.1
HR	1.8	0.5	7.4	14	0.1
PR	0.6	0.4	7.4	1.2	0.1
QRS	<0.7	0.4	2.7	11	<0.1
QT	1.9	0.4	5.3	27	<0.1
iT	2.4	0.5	11	12	<0.1
ERP	5.3	0.9	6.7	35	0.4
MFF	1.3	0.6	5.1	18	0.2
VFT	2.5	0.4	9.5	1.8	<0.1
N/I	2.9	0.02	5.0	5.0	0.2

Part A summarises the antiarrhythmic ED_{50%} (μmol/kg/min), h and curve fit co-efficient for Figures 9-13. These data are shown as the mean±SEM (n=30-42, total number of doses = 5-7).

Part B summarises the ratio of the antiarrhythmic ED_{50%} to doses which influenced haemodynamic or electrophysiological variables in normal tissue (AA ED_{50%}/D_{25%} or AA ED_{50%}/D_{100%} for VFT). The abbreviations are those used in the text. Large numbers indicate that the drug was more potent for effects on normal myocardial tissue than for suppression of ischaemia-induced arrhythmias; small numbers indicate that the drug was more potent for suppression of ischaemia-induced arrhythmias than for effects on normal myocardial tissue.

This resulted in a shallow antiarrhythmic dose-response curve. Flecainide reduced BP and HR in a dose-related fashion while the PR, QRS and QT intervals of the ECG were increased.

The antiarrhythmic actions of tedisamil were similar to those produced by quinidine. While tedisamil effectively suppressed VF at high doses it did not prevent VT. The resulting antiarrhythmic dose-response curve was shallow and did not reach 100%. Tedisamil was unique among the antiarrhythmic drugs tested in that it produced enormous increases in the QT interval of the ECG. The PR and QRS intervals were also increased over the dose range tested. HR was reduced in a dose-related fashion while effects on BP did not appear to be dose-related.

RSD1019's antiarrhythmic profile was similar, although not identical, to that of lidocaine. RSD1019 completely prevented the occurrence of VT and VF. However, in contrast to lidocaine, it did not prevent or even reduce the occurrence of ischaemia-induced PVBs (at the doses tested). The antiarrhythmic dose-response curve produced by RSD1019 was relatively steep and the drug provided 100% protection against ischaemia-induced arrhythmias. The haemodynamic effects of RSD1019 were limited to bradycardia while its electrophysiological effects were confined to a small increase in the QT interval.

3.9 Effects of Class I drugs in conscious rats.

The convulsogenic actions of antiarrhythmic doses of the standard Class I drugs were assessed in conscious rats. Quinidine (2 & 4 $\mu\text{mol/kg/min}$) caused sedation and ataxia while only the highest dose of flecainide tested (1.5 & 3 $\mu\text{mol/kg/min}$) had these

effects. At an infusion rate of 4 $\mu\text{mol/kg/min}$ quinidine caused all 3 rats tested to collapse. Lidocaine caused sedation and ataxia in all rats and caused convulsions in 1 of 3 and 3 of 3 rats infused with 8 and 16 $\mu\text{mol/kg/min}$, respectively.

4.0 Discussion.

4.1 Overview of studies in rats.

The first section of the discussion deals with characterisation of the electrophysiological actions of standard and putative antiarrhythmic drugs on non-ischaemic rat ventricular tissue. The mechanism(s) of drug action will be discussed and interpreted with reference to the literature. The second section is concerned with the influence of "simulated ischaemic" conditions on the myocardium and the modification of drug action by these conditions. Potential factors and mechanism(s) by which ischaemia or ischaemia-like conditions might selectively increase the potency of some drugs are discussed. Finally, the antiarrhythmic actions of drugs against ischaemia-induced arrhythmias in anaesthetised rats will be discussed. The site of drug action, i.e., ischaemic versus normal tissue, and the mechanism(s) of action also will be considered.

4.2 Electrical stimulation studies in intact rats.

The actions of a series of drugs on non-ischaemic rat cardiac tissue were assessed by analysing their effects on electrical stimulation variables and changes in ECG intervals. Characterisation of drug effects on normal myocardial tissue has been used to classify

antiarrhythmic drug action (Singh & Vaughan-Williams 1970a, 1970b, 1972; Sicilian Gambit, 1991) and serve as a starting point for a comparison of the mechanism(s) whereby such drugs prevent arrhythmias. These studies demonstrate that the actions of lidocaine and RSD1019 were different than those of quinidine, flecainide and tedisamil. Quinidine, flecainide and tedisamil were potent and efficacious for altering the electrophysiological properties of normal myocardial tissue, while lidocaine and RSD1019 were not.

Class I antiarrhythmic's (i.e., local anaesthetics) reduce conduction velocity and excitability of the myocardium (Singh & Vaughan-Williams, 1970a, 1970b, 1972). Class I drugs slow conduction in the heart which results in an increase in the PR and QRS intervals of the ECG (e.g., Harrison *et al.*, 1981). The PR interval is also sensitive to the effects of Class IV antiarrhythmics. While the PR interval is a relatively non-selective indicator of Class I antiarrhythmic activity, the QRS duration and iT are more selective indicators. The effects of Class I antiarrhythmics depend on HR and the selectivity of the drug for different states of the Na⁺ channel (see Hondeghem & Katzung, 1984). Drugs with slower onset rates and those which block open Na⁺ channels have the greatest effects on the conduction in the normal myocardium (Harrison *et al.*, 1981; Campbell, 1983). The rank order of potency for indices of Na⁺ channel blockade in normal tissue (e.g., increases in PR and QRS intervals of the ECG and iT) among the drugs tested was flecainide >> quinidine > tedisamil >>> lidocaine > RSD1019. Where data are available, this order is consistent with what is reported in the literature (e.g., Harrison *et al.*, 1981; Campbell, 1983; Chi *et al.*, 1996).

Due to the nature of repolarisation in rat ventricular tissue, assessing the Na^+ channel blocking properties of tedisamil by its effects on the QRS duration is likely to over-estimate its potency (see Figures 3-5, 8, 12). This is because rat ventricular APs are very short (Josephson *et al.*, 1984; Inoue *et al.*, 1984; Hayes *et al.*, 1996) and, as a result, the T wave of the ECG is tightly fused to the QRS complex (Curtis *et al.*, 1987; Hayes *et al.*, 1994). Large increases in the QT interval, such as those produced by tedisamil (see Figures 3, 12 & Beatch *et al.*, 1991), cause the S wave, which was partly obscured by the T wave, to drop closer to the isoelectric line. This appears as an increase in the QRS duration but is partly a function of the narrow band width of the instrument used for these studies (Grass Polygraph, 1/2 amp frequency 40 Hz). When the ECG is recorded using a wider band width (e.g., as in the occlusion studies) the increase in the QRS duration produced by tedisamil were not as large (compare Figure 3 with Figure 12). Tedisamil increased the PR interval and iT to a lesser extent than would be expected from the increase in the QRS duration observed (compare with flecainide, Figures 3 & 11). Evidence from voltage clamp studies shows that tedisamil does not block Na^+ channels in concentrations up to 20 μM (Dukes & Morad, 1989; Dukes *et al.*, 1990). Taken together, these data suggest that tedisamil blocks Na^+ channels only at high doses/concentrations. While the same argument might apply to the QRS prolonging effects of quinidine and flecainide, it is unlikely to be of major importance as these two drugs were much less effective in prolonging the QT interval.

Class III antiarrhythmic action is defined as an increase in AP duration and refractoriness (Singh & Vaughan-Williams, 1970a, 1970b, 1972). In the experiments

reported here, such changes can be measured as an increase in the QT interval of the ECG and ERP, as well as a reciprocal reduction in MFF. Tedisamil was very potent and efficacious for prolonging the QT interval and produced large increases in ERP and reciprocal reductions in MFF. In the rat ventricle, I_{to} is the predominant current responsible for repolarisation (Josephson *et al.*, 1984). Tedisamil blocks I_{to} (Dukes & Morad, 1989; Dukes *et al.*, 1990) which results in prolongation of ventricular APs and the QT interval (Dukes & Morad, 1989; Dukes *et al.*, 1990; Beatch *et al.*, 1991). Quinidine and flecainide were approximately equi-potent for effects on the QT interval, ERP and MFF. As both of these drugs block I_{to} at concentrations likely to be achieved by the infusion regimens used (Slawsky & Castle, 1994; Yamashita *et al.*, 1995; Wang *et al.*, 1995) the mechanism whereby these drugs increase refractoriness is likely due to an increase in AP duration. Campbell (1983) has argued persuasively that the actions of quinidine and flecainide on refractoriness are primarily due to their AP prolonging effects (e.g., Class III antiarrhythmic actions) with only a small contribution from Na^+ channel blockade. This is due to their selective blockade of activated Na^+ channels (Hondegheem & Katzung, 1977 & 1984) and relatively slow onset kinetics (Campbell, 1983).

The increase in refractoriness produced by lidocaine, despite its having no effect on the QT interval and hence AP duration, can be explained on the basis of its blockade of inactivated state Na^+ channels and its kinetics (Hondegheem & Katzung, 1977 & 1984; Campbell, 1983). Binding to the inactivated state of the Na^+ channel prolongs refractoriness by delaying their recovery after cells have repolarised.

The mechanism whereby RSD1019 prolonged refractoriness has not been characterised; however, some speculations can be made based on its pharmacological profile in this preparation. RSD1019 prolonged the QT interval to a small degree, suggesting that AP duration was increased. In support of this is the observation that RSD1019 blocks I_{to} ($IC_{50} \sim 5 \mu M$, Dr. J.G. McLarnon, personal communication). However, the small increases in the QT interval produced by RSD1019 cannot account for the increase in refractoriness observed (compare with tedisamil Figures 3, 12 & 13; Table 4). RSD1019 also increased the threshold current for electrical stimulation of the left ventricle and produced a small increase in the PR and QRS duration of the ECG, effects which suggest that RSD1019 blocks Na^+ channels. The fact that RSD1019's profile was similar to that of lidocaine further suggests that RSD1019 blocks Na^+ channels in the inactivated state. If this is so, the combination of inactivated state Na^+ channel block and AP prolongation might explain the increases in refractoriness produced by RSD1019. Over all, the rank order of potency for prolongation of refractoriness was tedisamil >>> quinidine \equiv flecainide > lidocaine > RSD1019.

Threshold current for electrical induction of ventricular fibrillo-flutter has been used as a surrogate measure for susceptibility to VF (Szekeres & Papp, 1971, cf: Winslow, 1984; Moore & Spear, 1975; Winslow, 1984). While this method is useful in assessing the effects of Class I and III drugs, it is relatively insensitive to Class II and IV drugs (Winslow, 1984). The rank order of potency and efficacy for drug-induced increases in VFT was flecainide > quinidine > lidocaine >>> RSD1019. Drug-induced increases in VFT paralleled increases in indices of Na^+ channel blockade measured at sinus

heart rates (e.g., conduction times measured from the ECG and iT). Therefore, despite the fact that ventricular fibrillo-flutter is an inherently high rate phenomenon, drugs which exhibit marked use-dependent Na^+ channel blockade (e.g., lidocaine) are less effective than drugs which exhibit less use-dependence (e.g., quinidine & flecainide). Over all, it appears that drug-induced changes in VFT parallel changes in excitability rather than susceptibility to VF.

The actions of tedisamil on ventricular fibrillo-flutter were unique among the drugs tested. While tedisamil did not increase VFT, it prevented induction of ventricular fibrillo-flutter at high doses ($>0.5 \mu\text{mol/kg/min}$). Instead of ventricular fibrillo-flutter only VT occurred. This observation is constant with that of Adaiakan and co-workers (1992) and others (Janse, 1992; Chay, 1995). VF, and by analogy ventricular fibrillo-flutter, is characterised by the presence of multiple re-entry circuits in the ventricle (Sasyniuk & Mendez, 1971; Gray *et al.*, 1998; Witkowski *et al.*, 1998). Given that re-entry cycle length is the product of conduction velocity and refractory period, increases in the refractory period will prolong cycle length (see Adaiakin *et al.*, 1992 or Janse, 1992). In a heart of a given size, large increases in re-entry cycle length reduce the number of circuits that can occur. High frequency stimulation still elicits APs at the same current threshold, but only one circuit can be induced.

4.3 Drugs effects on BP and HR.

In contrast to the increase in BP produced by tedisamil, all of the other drugs tested reduced BP. The rank order for reduction in BP was quinidine = flecainide >

lidocaine > RSD1019. Class I drugs have been reported to have direct negative inotropic actions (Honerjäger *et al.*, 1986; Matsumoto *et al.*, 1993), while Class III drugs have no effect, or increase, the inotropic state (Abrahamsson *et al.*, 1993). Several actions of Class I drugs could result in negative inotropism (Honerjäger *et al.*, 1986; Matsumoto *et al.*, 1993). Blockade of Na⁺ channels by the specific Na⁺ channel blocker tetrodotoxin, produced less negative inotropy than equivalent Na⁺ channel blocking concentrations of Class I drugs (Honerjäger *et al.*, 1986). This observation illustrates that Class I drugs possess negative inotropic actions not related to Na⁺ channel blockade. In this regard it is noteworthy that high concentrations (50 µM) of quinidine are known to block Ca⁺⁺ influx into the heart (Nawrath, 1981). Obviously, the negative inotropic actions of Class I drugs are not desirable in patients who commonly have compromised cardiac function (Greene, 1991). Some authors suggest that the positive inotropic actions of Class III drugs are advantageous (Abrahamsson *et al.*, 1993); however, a drug with no effect on inotropy should be preferred. The actions of antiarrhythmic drugs on vascular smooth muscle may also contribute to reductions in BP (Aberg & Wahlstrom, 1972).

All the drugs tested had dose-related bradycardic actions in anaesthetised rats. The rank order of potency for bradycardic effects was tedisamil >>> flecainide > quinidine = lidocaine > RSD1019. Bradycardia is likely due to direct action on the sino-atrial node but indirect actions may also contribute. Blockade of outward K⁺ currents in sino-atrial tissue can be expected to prolong AP duration and cycle length (Brown, 1982). This is likely to be the mechanism whereby tedisamil produces bradycardia. Paradoxically, Class I drugs also prolong AP duration in the sino-atrial node of the guinea pig (Campbell, 1987).

Thus, prolonging repolarisation time of the sino-atrial node is likely to be the mechanism whereby Class I drug produce bradycardia. Only class Ib drugs reduce the rate of phase 4 depolarisation in the sino-atrial node (Campbell, 1987). This suggests that the bradycardic actions of lidocaine are mediated by a different mechanism than those of the other class I drugs. The mechanism for the bradycardic actions of RSD1019 can only be speculated on. The hypotensive actions of the drugs tested would be expected to counteract bradycardia via baro-receptor reflexes. In the case of the tedisamil, its tendency to increase BP might contribute to its bradycardic actions.

Quindine has atropinic actions (Mirro *et al.*, 1981) which would be expected to increase HR by relieving vagal tone to the sino-atrial node. Sinus tachycardia was not observed, perhaps due to the predominance of sympathetic tone in pentobarbital anaesthetised acute preparations (see Lazzara *et al.*, 1978) and the direct actions of quinidine on the sino-atrial node.

4.4 Modification of drug action by "simulated ischaemic" conditions.

As outlined in the introduction, the actions of antiarrhythmic drugs are known to be modified by the conditions of myocardial ischaemia. A buffer containing a $[K^+]$ and a pH which are known to occur in the ischaemic myocardium was selected as a **surrogate state** for myocardial ischaemia. Concentration-response curves were constructed for the conduction slowing effects of selected drugs in isolated rat hearts perfused with either normal or "simulated ischaemic" buffer.

4.5 Effects of "simulated ischaemic" buffer alone.

As expected, perfusion of isolated rat hearts with "simulated ischaemic" buffer caused increases the PR and QRS intervals of the ECG. Increased $[K^+]$ can be expected to reduce resting membrane potential and cause voltage-inactivation of fast inward Na^+ channels (see Hondeghem & Katzung, 1984). Reduction in the availability of Na^+ channels slows conduction in the heart. Campbell's group (1991) reports that resting membrane potential depolarised from -88 to -75 mV for a similar change in $[K^+]$ to that used in this study. Decreased extracellular pH causes a small reduction in resting membrane potential (Kagiyama *et al.*, 1982; Campbell & Hemsworth, 1990; Campbell *et al.*, 1991; Ye *et al.*, 1993) and a corresponding reduction in conduction velocity. The combination of raised extracellular $[K^+]$ and low pH has been shown to reduce conduction velocity more than expected from simple addition of the two effects (Moréna *et al.*, 1980; Gettes & Cascio, 1991).

4.6 Modification of drug action by "simulated ischaemic" buffer.

Perfusion of isolated rat hearts with "simulated ischaemic" buffer increased the potency of lidocaine considerably. Concentrations of lidocaine that have no effect in hearts perfused with normal buffer markedly depressed conduction in hearts perfused with "simulated ischaemic" buffer. This result can be explained in terms of the modulated receptor hypothesis (Hondeghem & Katzung, 1977 & 1983). Lidocaine binds to inactivated Na^+ channels and $[K^+]$ -induced depolarisation increases the fraction of Na^+ channels in the inactivated state. Due to the voltage-dependent increase in the number of

binding sites available, the actions of lidocaine are increased by $[K^+]$ -induced depolarisation (Hondeghe & Katzung, 1977; Bean *et al.*, 1983).

As lidocaine is a weak base with a pKa of 7.9 (Courtney, 1987), reduction in the pH will increase the concentration of the cationic species in solution. The percent change in the ionised species caused by a reduction in pH from 7.4 to 6.4 can be calculated from the Henderson-Hasselbach equation. For lidocaine, this change in pH increases the proportion of the ionised species from 68 to 97%. The net effect of this manipulation is difficult to predict as both the charged and the uncharged forms of lidocaine are thought to be active (Hille, 1977; Hondeghe & Katzung, 1984). The uncharged species is thought to block inactivated state Na^+ channels while the charged species blocks activated state Na^+ channels. An increase in the relative concentration of the cationic species can be expected to reduce the inactivated state block while increasing the activated state block (Hondeghe & Katzung, 1984).

The affinity of local anaesthetics for Na^+ channels is increased at low pH (Grant *et al.*, 1980; Wendt, 1993), an effect that is primarily due to a slower dissociation rate constant from Na^+ channels. Nattel *et al.* (1981) demonstrated that the potentiation of lidocaine's action by small reductions in pH is independent of changes in the fraction of the ionised species.

Other investigations with inactivated state Na^+ channel blockers, such as lidocaine (Chen *et al.*, 1975; Bean *et al.*, 1983) and amiodarone (Mason *et al.*, 1983), illustrate the ischaemia-selectivity exhibited by such drugs (Campbell & Hemsworth, 1990; Campbell *et al.*, 1991; Ye *et al.*, 1993). The effects of lidocaine and amiodarone on maximum AP

upstroke velocity were further increased in tissue perfused with high $[K^+]$, hypoxic, and acidic buffer (i.e., "simulated ischaemic" buffer). Studies with the separate components of this "simulated ischaemic" buffer showed that increased $[K^+]$ was the most important factor in determining lidocaine's "ischaemia-selectivity" while low pH had only a small effect (Ye *et al.*, 1993). Interestingly, the phasic block produced by lidocaine under normal conditions was converted to tonic block in "simulated ischaemic" conditions. Campbell's group also noted that *perfusion* of cardiac tissue with hypoxic buffer had little influence on the potency of lidocaine (Ye *et al.*, 1993).

The conduction slowing actions of RSD1019 were marginally increased by perfusion with "simulated ischaemic" buffer. RSD1019 has been shown to block cardiac Na^+ channels ($IC_{50} > 30 \mu M$, Dr. J.G. McLarnon, personal communication) but its affinity for various states of the Na^+ channel has not been determined. RSD1019 is a weak base with a pK_a of 6.1 (Dr. R.A. Wall, pK_a determined by titration method, personal communication). A reduction in pH from 7.4 to 6.4 will increase the fraction of the charged species from 5 to 50%. If the cationic form is the active species, this might contribute to the increased potency under "simulated ischaemic" conditions. In short, RSD1019 was less "ischaemia-selective" and less potent than lidocaine for slowing of conduction.

The actions of quinidine and flecainide were only marginally increased in hearts perfused with "simulated ischaemic" buffer. This result is expected on the basis of the modulated receptor hypothesis and is consistent with other experimental data. The modulated receptor hypothesis (Hondegheem & Katzung, 1977 & 1984) predicts that the

actions of open channel blockers such as quinidine (Hondegheem & Katzung, 1977; Colatsky, 1982) and flecainide (Campbell & Vaughan-Williams, 1983) will not be influenced to a large degree by depolarisation of the myocardium. Experimental evidence shows that the actions of flecainide were only modestly increased by perfusion with either "simulated ischaemic" or high $[K^+]$ buffer (Campbell *et al.*, 1991; Campbell & Hemsworth, 1990). There is no comparable data in the literature for quinidine.

While a number of studies suggest that tedisamil blocks Na^+ channels (Beatch *et al.*, 1991; Wallace *et al.*, 1995; Chi *et al.*, 1996), the state dependence of blockade has not been investigated. A close congener to tedisamil, KC8851, has been shown to block activated (open) Na^+ channels (McLarnon & Xi, 1997). Although tedisamil is a less potent Na^+ channel blocker than KC8851 (McLarnon & Xi, 1997), its mechanism of block is likely to be similar. Assuming that tedisamil does block activated Na^+ channels, this might explain the lack of "ischaemia-selectivity" seen in these experiments.

An increase in the fraction of the charged species might account for the small increase in potency seen for quinidine, flecainide and tedisamil under "simulated ischaemic" conditions, however, this seems unlikely. Quinidine, flecainide and tedisamil are all weak bases. The pKa values are reported to be 8.3 (Courtney, 1987), 9.0 (Campbell, 1983) and 12.7 (Dr. J. Schmidt, pKa determined by a pH titration method, personal communication), respectively. For quinidine, a decrease in pH from 7.4 to 6.4 can be expected to increase the percentage of the charged species from 87 to 99%. The significance of this change in the fraction of the ionised species for quinidine is controversial. Grant and co-workers (1982) find no effect while, Nattel *et al.* (1981)

found that the actions of quinidine were increased at low pH. For the above stated change in pH, the fraction of flecainide present as the charged species is increased from 97 to 99%, while tedisamil is essentially completely ionised at both pHs (>99.9% at either pH). If it is assumed that the charged forms of flecainide and tedisamil are the active species, the fact that the fraction of ionised drug does not change over the relevant pH range might also account for the lack of significant "ischaemia-selectivity."

Both RSD1019 and tedisamil prolonged the QT interval of the ECG in the isolated rat heart. In normal buffer, tedisamil was much more potent in this regard while its potency in "simulated ischaemic" buffer was reduced. RSD1019 was equi-potent in "simulated ischaemic" and normal buffer. This suggests that the AP prolonging effects of RSD1019 might be maintained under conditions of myocardial ischaemia. Two factors complicate interpretation of drug effects on the QT interval in the isolated rat heart under "simulated ischaemic" conditions. As previously noted the T wave of the rat is tightly fused to the QRS complex making measurement of the QT interval more difficult in this species. For this reason it is not commonly measured (e.g., Rees & Curtis, 1993 a-c). The second problem is that the "simulated ischaemic" buffer increased the QT interval. This effect may be due to acid-induced AP prolongation (Poole-Wilson & Langer, 1975; Spitzer & Hogan, 1979). Increased extracellular $[K^+]$ would be expected to have the opposite effect (Carmeliet, 1989). Despite these complications, it is remarkable that the QT prolonging effects of RSD1019 were better maintained in "simulated ischaemic" buffer than those of tedisamil.

4.7 The use of ECG intervals to assess drug effects.

While changes in ECG intervals have been used to assess drug effects clinically and experimentally, they are not without flaws. Measurement of conduction intervals assumes that the path of conduction is the same before and after drug treatment. This may not be true, especially when conduction is slow (e.g., with ischaemia or Class I drugs). Measurement of conduction velocity is thus always riddled by this flaw. However, there are better techniques to measure conduction velocity in the heart (see Nattel & Jing, 1989; Kottkamo *et al.*, 1994). Some rely on a larger number of electrodes to trace the wave of excitation across the heart and can take into account the anisotropic nature of cardiac conduction.

Measurement of repolarisation times from the surface ECG is similarly problematic. Measurement of the QT interval has plagued antiarrhythmic research for years (see Botstein, 1993; Moss, 1993; Surawica & Knoebel, 1984). Indeed, as it is defined, the QT interval cannot be measured but only approximated. Moreover, measurement of the QT interval does not give any information about changes in AP morphology which may be of major importance to the mechanism(s) of drug action. The T wave of the ECG is thought to be generated by regional differences in AP duration and morphology which give rise to repolarisation gradients in the heart (see Franz *et al.*, 1991). Changes in AP morphology, such as those caused by drugs or various pathological conditions, can modify these repolarisation gradients. The observed changes in the QT interval cannot be easily interpreted nor predicted.

4.8 Ischaemia-selective drug actions.

In summary, the rank order of "ischaemia-selectivity" for drug-induced conduction slowing was lidocaine >>> RSD1019 > quinidine \cong flecainide \cong tedisamil. This order approximately parallels the increase in the fraction of the ionised species of the drug calculated from the Henderson/Hasselbach equation. However, it is not clear if changes in the fraction of the ionised species are an important determinant of "ischaemia-selectivity." The greater "ischaemia-selectivity" shown by lidocaine appears to be related to its blockade of inactivated-state Na^+ channels. The relative lack of "ischaemia-selectivity" shown by quinidine and flecainide is likely to be related to the selective blockade of activated-state Na^+ channels. Blockade of activated-state Na^+ channels might also explain why tedisamil exhibited little "ischaemia-selectivity." RSD1019 was less potent than the other drugs tested and exhibited "ischaemia-selectivity" that was intermediate between the two groups.

Increased potency under "simulated ischaemic" conditions can be expected to translate into increased potency under conditions of real ischaemia *in vivo*. It seems likely that the conditions used to simulate ischaemia in these studies underestimate the increase in potency that occurs in the ischaemic myocardium *in vivo*. For example, other alterations in the ischaemic myocardium such as altered Ca^{++} fluxes (Clusin *et al.*, 1982, 1983) and production of fatty acid metabolites (Sobel *et al.*, 1978) cause depolarisation independent of changes in $[\text{K}^+]$. Furthermore, the effects of ischaemia are more than the sum of their parts (see Moréna *et al.*, 1980). However, some ischaemia-induced changes that are not simulated in this assay may counteract the increase in potency that would

occur *in vivo*. One example is ischaemia-induced AP shortening which can be expected to reduce the number of inactivated-state Na^+ channels available and thus reduce the effects of drugs which bind to the inactivated-state (Hondeghe & Katzung, 1984).

4.9 Antiarrhythmic actions versus ischaemia-induced arrhythmias.

While all of the drugs tested in anaesthetised rats suppressed ischaemia-induced arrhythmias, their potency and efficacy differed greatly. Comparison of their electrophysiological and antiarrhythmic effects also suggests fundamental differences in the antiarrhythmic mechanism(s) of action for quinidine, lidocaine, flecainide, tedisamil and RSD1019. As the primary mechanism of arrhythmogenesis for ischaemia-induced arrhythmias is thought to be re-entry (see introduction), the results of these studies will be interpreted in terms of this mechanism. Reference will be made to other mechanisms where relevant. The antiarrhythmic mechanism(s) will be considered for each drug in turn with reference to the literature. The characteristics of the ideal antiarrhythmic drug for preventing ischaemia-induced arrhythmias will be considered later in the thesis.

Quinidine effectively prevented ischaemia-induced VF at the highest dose tested but had no effect on VT and was thus unable to provide 100% antiarrhythmic protection. Examination of the electrophysiological effects of quinidine show that it slowed conduction and the prolonged refractory period of ventricular tissue. This is consistent with the electrophysiological effects of quinidine in other species (Chen *et al.*, 1975; Colatsky, 1982; Roden & Woosley, 1983). The conduction slowing effects of quinidine were increased somewhat under "simulated ischaemic" conditions. Thus, the Class I

actions of quinidine may be expected to convert unidirectional block occurring in the ischaemic myocardium to bi-directional block. In this way re-entry can be prevented (see Cardinal *et al.*, 1980; Janse, 1991). However, quinidine's conduction slowing effects were relatively non-specific for ischaemic over normal tissue. This lack of selectivity may actually contribute to arrhythmogenesis (Morganroth & Horowitz, 1984; Dhein *et al.*, 1993; Starmer *et al.*, 1991; Shaw & Rudy, 1995; see Levine *et al.*, 1989 for a review). Quinidine's Class III antiarrhythmic actions can be expected to prolong the cycle length of re-entry circuits, an effect which may also contribute to its antiarrhythmic actions.

Lidocaine provided dose-related antiarrhythmic protection and could completely prevent ischaemia-induced arrhythmias. Comparison of lidocaine's effects in the electrical stimulation and "ischaemia-selectivity" studies strongly suggests that its antiarrhythmic actions were mediated by its effects in ischaemic tissue. The electrical stimulation studies showed that lidocaine prolonged refractoriness in normal tissue but only to a small extent. In contrast, lidocaine was potent and efficacious for suppression of conduction under "simulated ischaemic" conditions. The ischaemia-selective negative dromotropic and antiarrhythmic actions of lidocaine have been noted by others *in vitro* (e.g., Hondeghem *et al.*, 1974; Hondeghem & Cotner, 1978; Cardinal *et al.*, 1981; Uematsu *et al.*, 1986; Campbell & Hemsworth, 1990; Quinteiro *et al.*, 1990; Campbell *et al.*, 1991; Ye *et al.*, 1993; Wolk *et al.*, 1998) and *in vivo* (El-Sherif *et al.*, 1977; Uematsu *et al.*, 1986). The study by Davis *et al.* (1985) demonstrated that the concentration of lidocaine in the ischaemic myocardium correlated best with its antiarrhythmic actions, whereas its concentration in blood or non-ischaemic myocardium did not.

An elegant study by Cardinal's group (Hélie *et al.*, 1995) demonstrated that lidocaine caused conduction block in ischaemic subepicardial tissue found over top of a recent infarct. Conduction block was most likely to occur in the ischaemic myocardium and the likelihood of it occurring was increased by increasing concentrations of lidocaine. They also noted that the concentrations of lidocaine which produced this effect were high. These observation parallels those made in the present study. Since antiarrhythmic doses of lidocaine caused convulsions in conscious rats these high doses could not be used. A similar observation has been made with mexiletine (Igwemezie *et al.*, 1992) suggesting a mechanism that is common to Class Ib drugs. Clinical studies have shown that high doses of lidocaine effectively prevent ischaemia-induced VF and that these doses are associated with central nervous system toxicity (Lie *et al.*, 1974; for reviews see MacMahon *et al.*, 1988 and Hine *et al.*, 1989).

The observation that lidocaine suppressed all ischaemia-induced arrhythmias and ST segment elevation at the same dose is intriguing. Tetrodotoxin had these effects in a similar preparation (Abraham *et al.*, 1989). ST segment elevation is caused by the flow of injury current across the ischaemic/normal tissue border (see Moréna *et al.*, 1980; Coronel *et al.*, 1988). A Na⁺ channel blocking drug could only be expected to prevent ST segment elevation and ischaemia-induced VF at the same dose if it rendered ischaemic tissue inexcitable. If this were the case, ischaemic tissue could not participate in the flow of injury currents nor in arrhythmogenesis. It should be noted that the presence of an ischaemic zone was confirmed in both studies.

Taken together, these observations suggest that lidocaine prevents ischaemia-induced arrhythmias by converting uni- to bi-directional block in the ischaemic zone. A more detailed discussion of the characteristics of ischaemia-selective Na⁺ channel blockade and the characteristics of the ideal ischaemia-selective antiarrhythmic drug will be presented later in the thesis.

Flecainide reduced the occurrence of ischaemia-induced VF but was unable to completely prevent it despite being potent and highly effective in slowing conduction and reducing excitability in the normal myocardium. However, its conduction slowing actions were not potentiated to a large degree under "simulated ischaemic" conditions. These results illustrate that flecainide, unlike lidocaine, is not selective for ischaemic tissue but rather produces similar effects in ischaemic and normal tissue. The mechanism whereby flecainide produces its antiarrhythmic effects is likely to be related to a general reduction in excitability and conduction velocity of the myocardium. Reducing excitability in the ischaemic myocardium can be expected to cause conduction block, however, the doses required severely impair conduction in the normal myocardium. This effect limits the maximum doses that can be tested. Moreover, the slowing of conduction produced by flecainide in the normal myocardium (or border zone) can be proarrhythmic (Stramba-Badiale *et al.*, 1994; Heisler & Ferrier, 1996).

Modelling flecainide's antiarrhythmic actions on the basis of depressing excitability in normal tissue yields similar expectations of limited efficacy. Reducing excitability will increase the current required to initiate VF and can be expected to reduce the incidence of ischaemia-induced VF but not completely prevent it. With sufficient current the

excitability barrier will be overcome and VF initiated. The only way to completely prevent VF by this mechanism would be to render the heart entirely inexcitable!

Tedisamil effectively prevented ischaemia-induced VF at the highest dose tested. Its electrophysiological profile suggests that its antiarrhythmic mechanism is unique among the drugs tested. Tedisamil reduced MFF and produced enormous increases in the QT interval and ERP, indicating that the drug increased AP duration. AP widening can be expected to prolong the cycle length of re-entry and, in a heart of a given size, prevent induction of multiple re-entry circuits (Adaikin *et al.*, 1992; Janse, 1992; Chay, 1995). Thus, it seems likely that the mechanism whereby tedisamil prevents ischaemia-induced VF and electrically induced ventricular fibrillo-flutter are the same (see Beatch, 1991; Adaikin *et al.*, 1991).

Various investigators have demonstrated tedisamil's AP prolonging (Dukes & Morad, 1989; Dukes *et al.*, 1990; Beatch *et al.*, 1991; Wallace *et al.*, 1995) and antifibrillatory actions (Beatch *et al.*, 1991; Wallace *et al.*, 1995; Chi *et al.*, 1996). Tedisamil's antifibrillatory actions were unaffected when heart rate was held constant which demonstrates that bradycardia does not mediate its antifibrillatory actions (Adaikin *et al.*, 1991). It should be noted that other investigators, using an *in vitro* model, fail to find antifibrillatory actions but report instead defibrillatory activity for tedisamil (Tsuchihashi & Curtis, 1991; Rees *et al.*, 1993). The difference is likely a function of the dose / concentration tested. With higher concentrations it is likely that true antifibrillatory actions would be observed.

The novel ion channel blocker RSD1019 suppressed ischaemia-induced VT and VF. The electrophysiological and antiarrhythmic profile of RSD1019 was similar in some aspects to that of lidocaine. Like lidocaine, RSD1019 effectively suppressed ischaemia-induced arrhythmias and had minimal effects on the electrophysiological properties of normal myocardium tissue. The actions of RSD1019 on normal tissue cannot account for its antiarrhythmic actions. Other agents tested were more potent and effective for modulation of the electrophysiological properties of normal myocardial tissue but were less effective for suppression of ischaemia-induced arrhythmias.

In contrast to lidocaine, the conduction slowing actions of RSD1019 were only modestly increased under "simulated ischaemic" conditions. Despite this, RSD1019 was epi-potent and nearly as effective as lidocaine in suppressing ischaemia-induced arrhythmias. If the actions of lidocaine under "simulated ischaemic" condition are taken as a benchmark for the degree of conduction slowing required to suppress ischaemia-induced arrhythmias, it is clear that RSD1019 falls shy of this mark. While an "ischaemia-selective" suppression of conduction may contribute to RSD1019's antiarrhythmic actions, these data suggest that another, yet undefined, mechanism(s) contributes to the antiarrhythmic actions of RSD1019 in the rat.

These studies do not define the mechanism(s) whereby RSD1019 suppresses ischaemia-induced arrhythmias, however, some speculations can be made. Higher doses/concentrations of RSD1019 prolonged AP duration, as assessed by increases in ERP and the QT interval. RSD1019 has been shown to block I_{to} in rat ventricular myocytes ($IC_{50} \sim 5 \mu M$, Dr. J.G. McLarnon, personal communication). Some investigators

have suggested that this current mediates ischaemia-induced AP shortening (Lukas & Antzelevitch, 1993; DiDiego & Antzelevitch, 1994). As a working hypothesis I suggest that RSD1019 may prolong AP duration in ischaemic tissue and that this may contribute to its antiarrhythmic actions in the rat (see later).

4.9 Conclusions from studies in rats.

The effectiveness of drugs which act predominately on normal tissue (e.g., quinidine, flecainide and tedisamil) for suppression of ischaemia-induced arrhythmias in anaesthetised rats was found to be limited. Drugs which act more selectively in ischaemic tissue (e.g., lidocaine) were more effective. In other words, drugs which interfere with the fundamental mechanisms of arrhythmogenesis in ischaemic tissue were more effective in preventing ischaemia-induced arrhythmias. The utility of available ischaemia-selective drugs is limited by their mechanism based toxicity (e.g., convulsions) and other toxicity (e.g., bradycardia and hypotension). RSD1019 effectively prevented ischaemia-induced arrhythmias in anaesthetised rats and its actions in normal tissue are insufficient to mediate such actions. These studies hint, but do not demonstrate, that the electrophysiological actions of RSD1019 in ischaemic tissue mediate its antiarrhythmic actions.

Part 2. Relationship between ischaemia-selective drug action and antiarrhythmic efficacy in anaesthetised rabbits.

5.0 Methods.

5.1 The use of rabbits for the study of ischaemia-induced arrhythmias.

The use of anaesthetised rabbits for the study of ischaemia-induced arrhythmias and antiarrhythmic drug action was first described by Cooker (1989). Since then, at least two other groups have employed the technique, or variations on it, to study arrhythmias (Holbrook & Cooker 1991; Chakrabarty *et al.*, 1991; Bril *et al.*, 1991).

The use of the rabbit offers similar, although somewhat less favourable, characteristics to those of the rat with regard to the uniformity of the ischaemic/arrhythmogenic insult caused by coronary artery occlusion. The advantage of using rabbits over rats are two fold; the HR and ventricular AP morphology of the rabbit is closer to that of humans.

5.2 Preparation of anaesthetised rabbits for the study of ischaemia-induced arrhythmias.

Male rabbits (2-3.5 kg) were used for the study. Rabbits were kept in house for at least a week before the day of the experiment. During this time they had access to standard rabbit chow and water *ad libitum*. On the day of the experiment a marginal ear vein was cannulated and animals were given 10 mL/kg of 5% dextran in saline before inducing anaesthesia with 50 mg/kg pentobarbital iv. After the corneal reflex was

abolished, a tracheotomy was performed and animals were ventilated with oxygen at a rate of 25 breaths/minute and 10 mL/kg. The jugular vein and left carotid artery were cannulated for injection of drugs and measurement of BP, respectively. Additional pentobarbital was given as required to maintain anaesthesia. Body temperature was monitored with a rectal thermometer and maintained between 36-40°C using a heating lamp.

A mid-line sternal opening was made and a pericardial cradle formed by suturing the pericardium to the chest wall. Two sutures (5-0 polypropylene) were placed in the heart for traction purposes so as to obtain an unobstructed view of the coronary arteries. The first of these was placed near the apex of the left ventricle while the other was passed through the margin of the left atrium.

Three occlusion sites were tested, the left anterior descending artery, left branch of the coronary artery, and both of these arteries. A snare was loosely placed around the coronary artery(ies) in question. The left branch of the coronary artery was generally visible immediately below the left side of the atrium (see Bellemin-Baurreau *et al.*, 1994 for a description of the coronary vasculature of the rabbit). Although the left anterior descending artery was not usually visible it could be occluded by placing a snare encircling the region where this artery was found. Arteries were not dissected free of the ventricular muscle prior to implanting snares, instead, a portion of ventricular muscle was encircled by the suture. Coronary artery occlusion was performed by tightening the snare around the artery.

ECG and BP were recorded as described for studies in rats and will only be described briefly here. A lead II ECG was obtained using subcutaneous pin electrodes. The ECG and BP records were displayed simultaneously on a Honeywell E for M monitor and on a paper trace using a Grass (model 79D) chart recorder. During experiments, the chart speed was not less than 50 mm/s to allow the occurrence of arrhythmias to be captured in detail (see Walker *et al.*, 1988).

Blood $[K^+]$ was measured before and 30 minutes after occlusion using a K^+ selective electrode (Ionetics) as described for the rat occlusion studies. Arterial blood samples, ~500 μ L, were drawn from the rabbits corotid artery and heparinised with ~50 μ L of 1000 U/mL heparin sodium (Fisher Scientific). In six rabbits the arterial blood $[K^+]$ was measured in conscious rabbits and after establishing anaesthesia.

Rabbits were allowed 15 minutes to stabilise before performing coronary artery occlusion. Occlusion was performed in animals in which mean arterial BP was greater than 50 mmHg and HR was greater than 180 beats/minute. BP and HR were monitored throughout the experiment.

The size of the occluded zone (zone at risk) was measured by the dye exclusion technique described for rats (see Curtis, 1986; Curtis and Walker, 1987). The heart was excised at the end of the experiment and perfused by the Langendorff technique with saline followed by a dye containing saline solution (0.5 mg/mL of Cardiogreen). The perfusion pressure was 100 mmHg. The occluded zone size was defined as the percentage of ventricular tissue not dyed green.

5.2 Analysis of data.

Animals were excluded from analysis if mean arterial BP fell below 25 mmHg for more than 2 minutes after occlusion or if atrio-ventricular block occurred except after sustained hypotension caused by arrhythmias.

BP, HR and the following ECG intervals were measured: PR, QRS, QTa and QT. The ECG intervals have their usual meaning except for those defined below. QTa was the QT interval measured from the Q wave to the peak of the T wave while the QT interval is the QT interval measured from the Q wave to the point where the T wave returns to an isoelectric line. To correct for the possible confounding effects of HR on QT intervals Bazett's formula was used (Bazett, 1920).

5.2.1 Analysis of arrhythmias.

Arrhythmias were quantified from the ECG according to the Lambeth conventions (Walker *et al.*, 1988) as previously discussed for rats (see above). An attempt was made to summarise arrhythmias using an arrhythmia score (score A, Curtis & Walker, 1988).

The occurrence of VPBs, VT and VF were recorded over one minute intervals after occlusion and a time of arrhythmias occurrence profile was constructed. The occurrence of one type of arrhythmia did not exclude the occurrence of another type (e.g., PVB and VT could occur in the same minute).

A sub-group of rabbits was analysed with respect to the suitability of the preparation for testing antiarrhythmic drugs. In this group both the left anterior descending and left branch of the coronary artery were ligated. For inclusion in this

group, preparations were selected which had an occluded zone size greater than 30% of the ventricular mass and blood $[K^+]$ less than 3.0 mM before occlusion.

5.3 Relationship between occluded zone size and ischaemia-induced arrhythmias.

In an attempt to better define the characteristics of the preparation which determined arrhythmia incidence, the relationship between occluded zone size and the arrhythmia score was explored. In all studies subsequent to this one both the left anterior descending and left branch of the coronary artery were occluded.

5.4 Antiarrhythmic actions of RSD1019 in rabbits.

The antiarrhythmic actions of RSD1019, at a dose of 8 $\mu\text{mol/kg/min}$, were assessed in the anaesthetised rabbit preparation. This dose was selected on the basis of rat studies (see section 3.8) as a dose which completely prevented the occurrence of ischaemia-induced tachyarrhythmias. Rabbits were anaesthetised and prepared as described above. The occurrence of arrhythmias was documented for 30 minutes of occlusion.

5.4.1 Dosing regimen.

Rabbits were assigned in a random and blind fashion to receive either RSD1019 at 8 $\mu\text{mol/kg/min}$ or vehicle control (22% ethanol, 78% in distilled water) as a continuous infusion (1 mL/hr/kg). Test and vehicle control groups each contained 7 rabbits.

Infusions were started 5 minutes before coronary artery occlusion was performed and was continued for the rest of the experiment (30 minutes of occlusion).

5.4.2 Analysis of data.

The effects of RSD1019 on BP, HR, ECG intervals as well as ischaemia-induced arrhythmias were assessed and summarised as detailed above.

The effects of RSD1019 on BP, HR, ECG intervals, as well as arrhythmia score were compared to the vehicle control using an unpaired t-test at a significance level of $p < 0.05$. Data were summarised as the group mean \pm SEM, $n=7$, except for arrhythmia occurrence data which were summarised as the number of rabbits which had the arrhythmia over the total number in the group. Statistical significance of arrhythmia occurrence data were assessed by Maitland contingency tables with $p < 0.05$ taken as a statistical significant difference.

5.5 Development of a preparation that allowed ischaemia-induced electrophysiological changes and arrhythmias to be assessed simultaneously.

In order to investigate the possible ischaemia-selective actions of antiarrhythmic drugs, a preparation was developed which allowed ischaemia-induced changes in electrophysiological variables and arrhythmias to be assessed simultaneously. Rabbits were selected as a test species for the reasons previously noted, as well as the fact that the size of the rabbit heart facilitates measurement of its function. Epicardial MAPs were

recorded from a site expected to become ischaemic after coronary artery occlusion and the electrophysiological changes observed correlated to the occurrence of arrhythmias.

MAPs have been used by a number of groups to investigate the electrophysiological changes caused by myocardial ischaemia (e.g., Dilly & Lab, 1987; Franz *et al.*, 1991; Timor *et al.*, 1991; Schmitt *et al.*, 1992). The reason for the popularity of this technique relates to the specificity of MAPs for a relatively small number of cells, the ease of recording, the stability of such records, and the fidelity of these records compared to intracellular recordings (Levine *et al.*, 1986; Mohabir *et al.*, 1991). There are, however, a number of limitations to the technique. These include the fact that MAPs do not provide any information about the resting membrane potential and that the usefulness of the upstroke velocity as a measure of conduction velocity is questionable. Since MAPs record potentials from many cells, upstroke velocity is much slower than that of intracellular recordings and cannot be interpreted in the same way as the maximum rate of depolarisation of a single cell. However, comparative studies have shown that manipulations that influence the maximum rate of depolarisation in intracellular recordings have similar effects on MAPs (Duker, 1988; Franz *et al.*, 1991). The upstroke velocity of MAPs has been suggested to be a measure of the synchronicity of depolarisation of the myocardium beneath the electrode (Levine *et al.*, 1986). The major advantage of MAP recordings in these studies is the ability of MAPs to record the time-dependent electrophysiological changes caused by ischaemia and allow drug effects thereon to be assessed.

5.5.1 MAP electrodes.

MAP electrodes were modelled on those described by Franz (1991). A MAP electrode was developed that could be sutured to the heart from the epicardial surface and which allowed the contact pressure between the MAP electrode and the heart to be easily adjusted. Figure 14 is a schematic of the electrode.

A sintered silver/silver chloride electrode (E225 wire electrode, In Vivo Metrics) was encased in a polypropylene guide (PE 90) which had one end flared to form a cuff. The electrode tip extended just beyond the end of the polypropylene guide (approximately 0.5 mm). A second electrode was prepared in the same way except the electrode was allowed to extend further beyond the cuff (approximately 2.0 mm). The bodies of the two electrodes were tied and glued together so that the longer electrode was slightly recessed from the other. This allowed the longer electrode to remain in loose contact with the heart while the other more shielded electrode was pressed tightly into the muscle.

A snare was passed through the polypropylene cuff of the electrode, through the ventricular muscle and back through the cuff on the other side of the electrode. The MAP electrode was connected via gold pin connectors to leads suspended over the heart. This allowed the MAP electrode to maintain contact with the heart without bearing the full weight of the electrode resting on the heart. This configuration also allowed the electrode to remain perpendicular to the heart, a factor which is important for recording high quality MAPs (see Franz, 1991). The contact pressure between the MAP electrode and the heart could easily be controlled by adjusting the amount of traction on the suture. The tension on the MAP electrode could be maintained by clamping the suture in place.

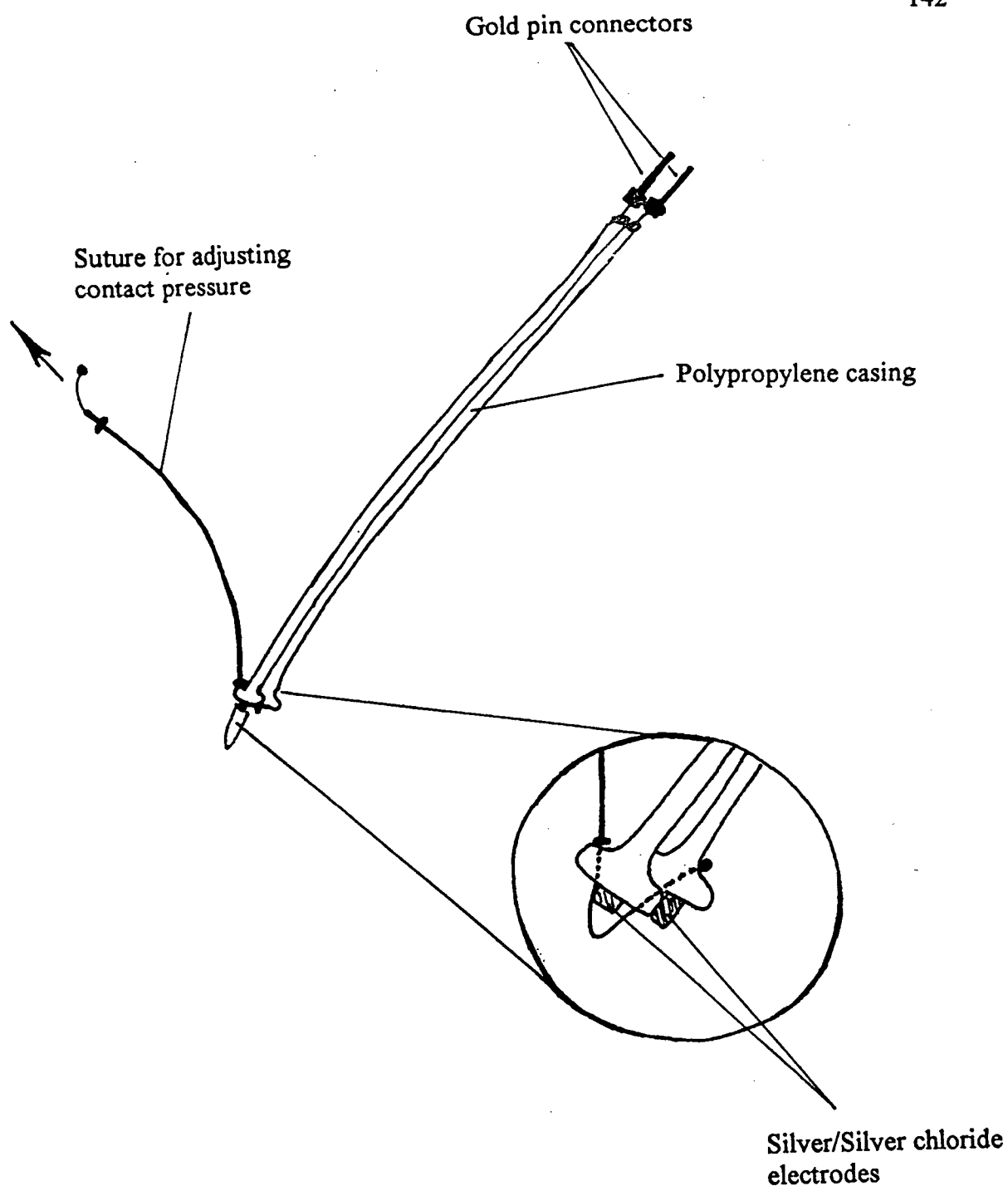


Figure 14. Schematic representation of the contact electrode developed for recording epicardial MAPs.

5.5.2 MAP recording.

MAPs were recorded using a Philbrick instrumentation amplifier (model P65AU) configured in the differential mode. The recording was then amplified 100 times to facilitate analysis. No filters were used to "condition" the signal. MAPs were displayed on a Tektronics oscilloscope (model R5013N) and recorded digitally (OT2831G-Data Translations A/D board) on a personal computer (486, Trison PC). Global Lab software was used to record and analyse MAP recordings. A sampling rate of 5 kHz was used (see Franz, 1991). MAP recordings were also archived on video tape (NEC video recorder) after conversion by a pulse code modulator (Medical Systems Corporation PCM-4/8 CO).

Stable MAP recordings were obtained and the following inclusion criteria were used to ensure the quality of the records: 1) a flat base line, 2) amplitude greater than 15 mV before starting the experiment, 3) a clear sharp rising phase, 4) a clearly distinguishable plateau phase, 5) stable morphology, and 6) the MAP electrode was within the ischaemic zone, not closer than 5 mm to the normal tissue (assessed post mortem). The stability of MAP recordings was assessed by visual inspection for 3 to 5 minutes before starting the experiment.

5.5.3 Sham occlusion experiments; stability of MAP recordings.

In a separate group of 6 rabbits the stability of MAP recording was tested. Rabbits were anaesthetised and prepared exactly as described for the study of ischaemia-induced arrhythmias except coronary artery occlusion was not performed. In this group, and all subsequent experiments, a MAP electrode was sutured over the apex. In these

experiments the MAP electrode was used as a lever to manoeuvre the heart to obtain a clear view of the coronary arteries and allow placement of sutures around the coronary arteries. MAPs were recorded for 35 minutes.

5.6 Assessment of the effects of ischaemia on MAPs.

Rabbits were anaesthetised and prepared as described above for the study of ischaemia-induced arrhythmias.

5.6.1 Analysis of MAPs.

MAPs were analysed off-line using MAPPER© software (Dickenson *et al.*, 1997). Data were periodically checked by cursor measurements in Global Lab to ensure that accurate results were obtained. MAPPER© software measures the following conventional MAP characteristics for every beat: amplitude, maximum upstroke velocity and AP duration at 25, 50, 75 and 90% repolarisation. For the purposes of data analyses, time effect curves were constructed for each variable in every rabbit. For such plots data were averaged over 10 beats. Values at specific times were interpolated from these curves and data were averaged across the number of rabbits in the group to give a group mean. Initial analysis included times up to 15 minutes after coronary artery occlusion. However, subsequent analysis of data was confined to 5 minutes after coronary artery occlusion for the reasons described below.

Analysis of conventional MAP variables was restricted to comparisons made 5 minutes after occlusion. The reason for this is related to ischaemia-induced deterioration

of APs. Since ischaemia is a time dependent process and not an equilibrium state, the selection of a single time point for analysis is necessarily arbitrary. However, analysis of conventional MAP variables 5 minutes after occlusion offers a number of advantages. MAP morphology changes with time after occlusion and summarising these records in terms of conventional variables becomes progressively more difficult. Furthermore, results obtained from such records are difficult to interpret. Five minutes after occlusion, MAPs in the ischaemic zone had a morphology which resembled that recorded from normal myocardial tissue and thus records could easily be analysed. Also, MAP variables could be measured in all rabbits at this time. With longer durations of ischaemia it was no longer possible to measure conventional MAP variables in all rabbits and thus exposing data sets to undesirable statistical censoring.

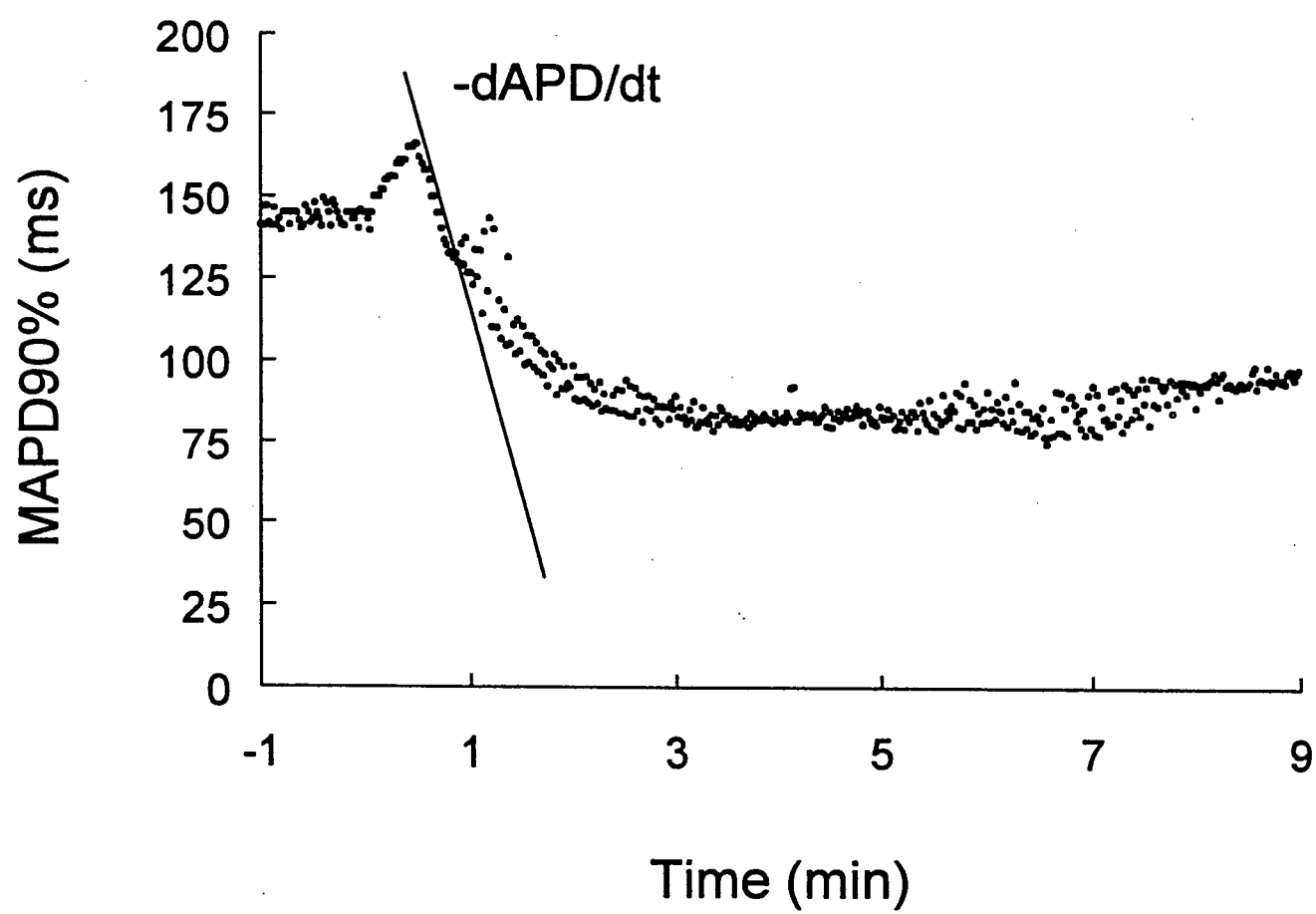
The initial rate of MAP shortening was measured as a possible index of Class III antiarrhythmic activity in ischaemic myocardial tissue. The average MAP duration at 90% repolarisation was taken from ten beats and plotted at the time that the first beat occurred. A tangent was drawn to the initial rate of ischaemia-induced MAP shortening as shown in Figure 15. The slope of the line was taken as the rate of MAP shortening.

5.7 Actions of glibenclamide in the anaesthetised rabbit preparation.

The actions of the $I_{K(ATP)}$ blocker glibenclamide on ischaemia-induced MAPs changes and arrhythmias were investigated in anaesthetised rabbits. Rabbits were anaesthetised and prepared as described above.

Arterial blood glucose concentration was measured using a commercial glucose

Figure 15. The effect of time on ischaemia-induced MAP shortening and the method used to measure the rate of MAP shortening. MAPD90% was plotted against time and a tangent was drawn to the initial phase of ischaemia-induced MAP shortening. The slope of the line was taken as the rate of MAP shortening ($-dAPD/dt$).



monitor (One Touch Basic) before dosing, before occlusion (20 minutes after dosing), and 30 minutes after occlusion. Arterial blood $[K^+]$ was measured immediately before and 30 minutes after occlusion. BP and HR were monitored after injection of test solution and the peak response documented. MAPs were recorded continuously throughout the experiment starting 3 minutes prior to occlusion. Coronary artery occlusion was performed 20 minutes after dosing and the occurrence of ischaemia-induced arrhythmias and MAP changes were monitored for 30 minutes.

5.7.1 Dosing regimen.

A random and blind experimental design was used. Rabbits were randomised to receive varying doses of glibenclamide (3, 6, 12 and 24 mg/kg iv bolus, n=7) or vehicle control (5% dimethylsulfoxide: 95% polyethylene glycol-400, 0.2 mL/kg, n=14). Test solutions were administered as a bolus 20 minutes before performing coronary artery occlusion. Doses were selected on the basis of a previous literature report which showed that 3 mg/kg glibenclamide iv prevented ischaemia-induced AP shortening in rabbits (Smallwood *et al.*, 1990).

5.7.2 Analysis of data.

The effects of treatment on BP, HR and ECG variables were assessed before and 20 minutes after administration of test solution as described previously. The effects of glibenclamide or vehicle on MAPs were assessed before and 5 minutes after occlusion and the incidence of arrhythmias were documented for 30 minutes after occlusion.

Statistical comparisons were made by ANOVA with $p < 0.05$ taken as statistically significant. Dunnett's test for differences was used for multiple comparisons. Statistical comparisons for arrhythmia occurrence data were made using Maitland contingency tables. An arrhythmia score was used which transforms data into an approximately normal distribution and allows the use of parametric statistics (score A, Curtis & Walker, 1988). Data for this control group were defined as the population. As such, population statistics were used to describe it (e.g., mean \pm SD, $n=14$). Data for treated groups were summarised as the mean \pm SEM, $n=7$. Arrhythmia occurrence data were summarised as the number of rabbits having the arrhythmia over the total number in the group.

5.7.3 Actions of a low dose of glibenclamide in anaesthetised rabbits.

Further studies were carried out to assess the action of a lower dose of glibenclamide in the anaesthetised rabbit preparation. A dose of 0.3 mg/kg (iv bolus) was selected as one that is commonly used in cardiovascular studies to assess the role of $I_{K(ATP)}$ in cardiovascular function (e.g., Yao & Gross, 1994). The study was performed exactly as described above with 18 rabbits being randomised to receive either 0.3 mg/kg glibenclamide or vehicle control iv.

Arterial blood gases and pH were measured to assess the adequacy of ventilation. An AVL OPTI 1 blood gas analyser was used to measure blood pH, PCO_2 and PO_2 . An arterial blood sample ($\sim 500 \mu\text{L}$) was drawn into a syringe which contained 50 μL of 1000 U/mL heparin sodium (Fisher Scientific). Results for blood gas partial pressures were adjusted for the temperature of the blood sample. Tidal volume was adjusted as required

to maintain blood pH and PCO₂ within physiological limits. Typically, little adjustments had to be made. Since animals were ventilated with oxygen, and were all hyperoxaemic, PO₂ was not used to assess the adequacy of ventilation.

5.7.4 Assay for the glucose lowering actions of glibenclamide in anaesthetised rabbits.

The glucose lowering actions of 3 mg/kg glibenclamide iv were investigated in pentobarbital anaesthetised rabbits. Rabbits were anaesthetised with pentobarbital (50 mg/kg iv). A tracheotomy was performed and artificial respiration with oxygen instituted at a rate of 20 breaths/min and approximately 10 mL/kg. Blood pH and gases were monitored and ventilation adjusted to maintain values within physiological limits. The carotid artery and jugular vein were cannulated to allow measurement of BP and injection of drugs, respectively. A lead II ECG was recorded from subcutaneous pin electrodes and displayed on a Grass Polygraph alongside the BP record.

The BP, HR and ECG intervals were also measured as previously described. Blood [K⁺] was measured before administration of glibenclamide and after completing the experiment. Blood glucose concentration was measured before, as well as 20, 50, 80, 110, 140 and 170 minutes after administration of vehicle or glibenclamide.

5.7.5 Dosing regimen.

Glibenclamide, 3 mg/kg, or vehicle control, 5% dimethylsulfoxide: 95% polyethenglycol-400, was administered as a bolus. The total volume administered was 0.2 mL/kg. For this study a group size of 3 was used.

5.7.6 Analysis of data.

BP, HR and ECG variables were analysed as previously described. Where no statistical significant differences were found between treated and untreated groups, data were grouped together and expressed as the mean \pm SEM, n=6. These data were used to summarise the stability of the preparation.

Statistics were performed using an un-paired t-test for the differences between treated and control rabbits. A probability value of $p < 0.05$ was considered statistically significant. For assessment of the glucose lowering effects of glibenclamide, the maximum response produced by 3 mg/kg glibenclamide was compared to the time matched control using an un-paired t-test.

5.8 Actions of lidocaine, tedisamil and RSD1019 in the anaesthetised rabbit preparation.

The actions of the two reference compounds, lidocaine and tedisamil, were compared to those of RSD1019 in the rabbit. Lidocaine was taken as a representative ischaemia-selective antiarrhythmic drug (see introduction). Doses of lidocaine and RSD1019 were selected on the basis of studies in rats. Tedisamil was selected as a Class III antiarrhythmic since it prolongs AP duration in both rats and rabbits. The laboratory also has extensive experience with tedisamil. Doses of tedisamil were selected to bracket the AP widening produced by RSD1019 in normal tissue. Rabbits were anaesthetised and prepared as described previously.

5.8.1 Dosing regimens.

Rabbits were randomly assigned to receive a continuous infusion of lidocaine, tedisamil, RSD1019, or vehicle, starting 5 minutes before coronary artery occlusion. Test solution or vehicle control (10% dimethylsulfoxide, 20% ethanol, 70% distilled water) was infused at a rate of 0.016 mL/kg/min. The following doses (in $\mu\text{mol/kg/min}$) were tested: lidocaine 2.5, 5 and 10; tedisamil 0.063, 0.125 and 0.25; RSD1019 2, 4 and 8.

5.8.2 Analysis of data.

The effects of treatments on BP, HR, ECG intervals and MAP variables were assessed as previously described. ANOVA, followed by Dunnett's test for differences, was used to test for statistical significance. Maitland contingency tables were used to test the significance of arrhythmia occurrence data. A probability value of $p < 0.05$ taken as statistically significant.

For the purpose of presentation, the vehicle control group was defined as the population and measures of population variance were used. Sample statistics were not used to summarise results in the control group as the large size of the control group ($n=42$) yielded what appeared to be a very small variance. It was reasoned that this apparently small variance in the control group may mislead readers. In the control group mean \pm SD ($n=42$) was used to summarise these data while in the treated groups mean \pm SEM ($n=7$) was used. Drug effects in normal tissue were assessed 5 minutes after starting infusions.

5.8.3 Analysis of ischaemia-induced changes in MAP morphology.

In addition to analysis of conventional MAP variables (e.g., MAPD90%), ischaemia-induced changes in MAP morphology were assessed. Figure 18 (presented later in the thesis) illustrates typical ischaemia-induced changes in MAP recordings and illustrates the effects of ischaemia on MAP morphology. Ischaemia-induced by coronary artery occlusion caused characteristic changes in MAP morphology (Lab & Dilly, 1988; Franz, 1984). These morphology changes included activation alternans and multiple wave forms. Activation alternans were defined as repetitive morphology changes which occurred on every other beat. For example, AP amplitude of the 1st beat was larger than that of the 2nd beat with the 3rd beat having an AP amplitude similar to that of the 1st beat and the 4th beat being similar to the 2nd. The term "multiple wave forms" was used to describe the occurrence of more complicated patterns than activation alternans in which a repetitive sequence was not apparent. The occurrence of changes in MAP morphology were noted and summarised for each minute after occlusion (Figure 21).

An attempt was made to measure changes in the time between the foot and the peak of the MAP (MAP f-p interval) at various times after induction of ischaemia. The foot of the MAP is seen as a small deflection (~0.5 mV) preceding the MAP upstroke. The peak of the MAP was taken as the most positive value. The interval was measured using on-screen cursor measurements. Figure 18 (see results section) shows how this interval was measured before and after induction of ischaemia. MAP upstroke velocity has been suggested to reflect the homogeneity of conduction beneath the electrode (Levine *et al.*, 1986), a factor that is expected to be reduced by myocardial ischaemia.

To assess the stability and utility of the MAP f-p interval for assessment of ischaemia-induced changes, it was measured in sham-occluded rabbits as well as those subject to myocardial ischaemia, with and without drug treatment. As previously described, MAP morphology was altered after induction of ischaemia. Statistics analysis was not carried out beyond 5 minutes after occlusion.

5.8.4 Sham occlusion experiments.

To better assess the effects of drugs on normal tissue (without ischaemia), sham occlusions were carried out with the highest doses of the drugs tested. Experiments were carried out exactly as described above except occlusion was not performed. Vehicle control and the following doses (in $\mu\text{mol/kg/min}$) were tested: 10 of lidocaine; 0.25 of tedisamil; and 8 of RSD1019. Each group contained 5 rabbits. Drugs were infused continuously for 35 minutes. The effects of treatments were summarised 5 and 35 minutes after starting infusions. Data were analysed as described above.

6.0 Results.

6.1 Ischaemia-induced arrhythmias in anaesthetised rabbits.

Coronary artery occlusion was performed in rabbits to assess the utility of the anaesthetised rabbit preparation for the study of arrhythmias. Data have been summarised for those rabbits in which the left anterior descending and left branch of the coronary artery were successfully occluded. This protocol was used in the other experiments.

6.1.1 Exclusions.

A number of rabbits were excluded from subsequent analysis since they showed signs of bundle branch block (5 cases), atrio-ventricular block (5 cases) or reduced cardiac output resulting in severe hypotension (9 cases). The occurrence of bundle branch block, atrio-ventricular block and severe hypotension was usually associated with large occluded zone sizes and occlusion of the septal artery. Reduced cardiac output could occur with occluded zone of average size, although this was uncommon.

6.1.2 Haemodynamic response to coronary artery occlusion.

Mean BP decreased by $24 \pm 4\%$ from the pre-occlusion BP of 82 ± 4 mmHg (mean \pm SEM, $n=21$) within the first minute after occlusion. BP was maintained at this reduced level throughout the rest of the experiment. Pre-occlusion HR in these rabbits was 248 ± 8 beats/minute. Occlusion did not appear to alter HR.

6.1.3 Electrocardiographic changes induced by coronary artery occlusion.

Immediately after induction of ischaemia, changes in the ECG were apparent. Similar changes were observed in all animals although the time course over which such changes occurred between animals. Within seconds after the induction of ischaemia, the S wave of the ECG moved towards the isoelectric line and a transient decrease in the amplitude of the R wave was observed. The initial decrease in the R wave size soon gave

way to a progressive increase. This progressive R wave increase was observed after occlusion in all but 2 of a total of 21 rabbits.

Changes in the ST-T wave morphology typical of myocardial ischaemia were apparent shortly after the onset of ischaemia. ST segment elevation followed one of two patterns. The first was characterised by an initial ST segment elevation which partially resolved before a progressive increase. The secondary elevation in the ST segment started about 5 minutes after induction of ischaemia and peaked at approximately 9 minutes. The second pattern was characterised by progressive ST segment elevation starting at approximately 5 minutes. In these rabbits little or no ST segment changes were observed immediately after induction of ischaemia.

Alternans in T wave morphology were commonly observed over the course of the experiment. Generally, such alternans were observed within the first 10 minutes and occurred more commonly within the first 3 minutes after the onset of ischaemia.

6.1.4 Ischaemia-induced arrhythmias.

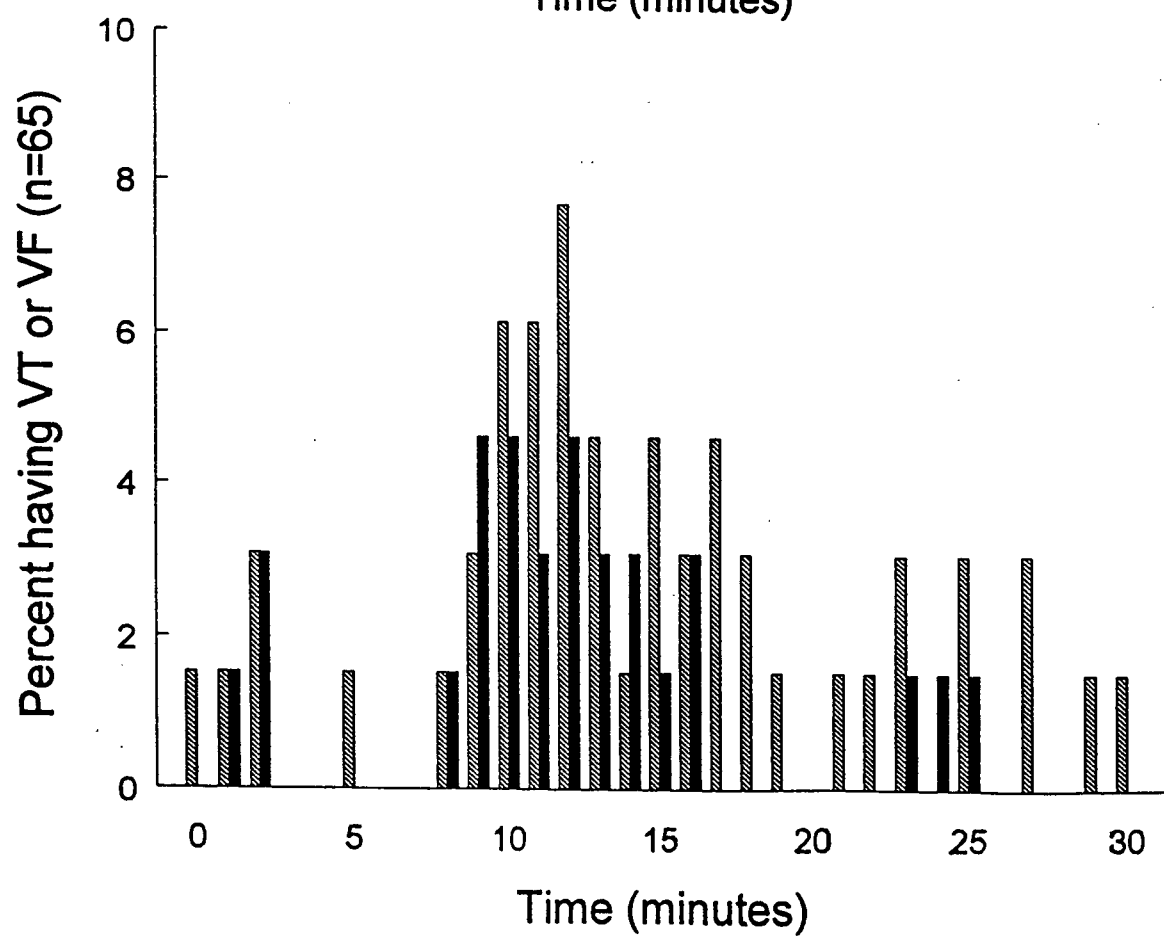
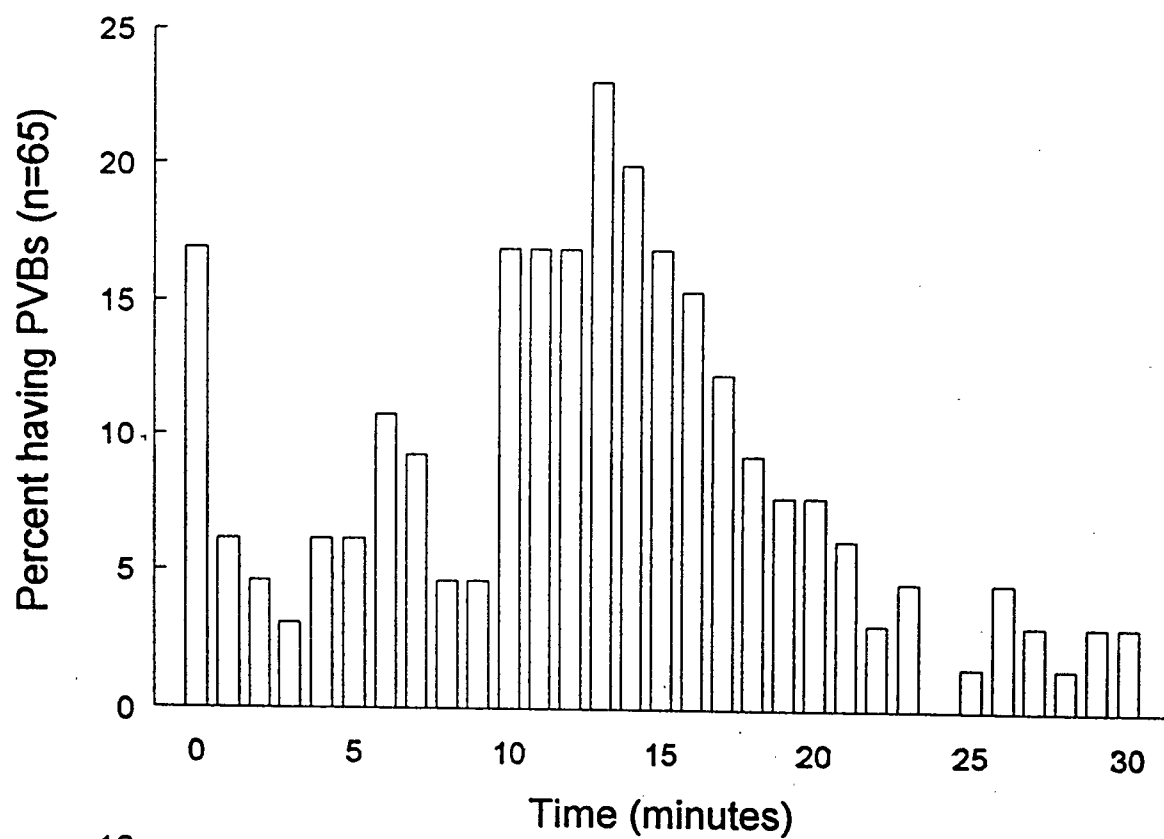
Coronary artery occlusion and resulting myocardial ischaemia caused cardiac arrhythmias in anaesthetised rabbits. Typically, less severe arrhythmias preceded more serious ones (e.g., PVBs preceded VT and VT preceded VF); however, this was not always the case. VF could occur before either PVBs or VT. Of 21 rabbits in which the left anterior descending and the left branch of the coronary artery were occluded, 20 (95%) had PVBs and 14 (67%) had ventricular tachyarrhythmias. Of those rabbits having tachyarrhythmias, 8 (38%) had VT while 10 (48%) had VF. VF was often spontaneously

reversible and was rarely fatal. One rabbit spontaneously reverted from VF after having the arrhythmia for more than 3 minutes! Rabbits in which VF occurred commonly had multiple episodes of VF. The average number of PVBs in this group of rabbits was 80 (range 0-193). The average duration of VT and VF was 25 s (range 1 to 88 s, $n=8$) and 73 s (range 4 to 206 s, $n=10$), respectively. An arrhythmia score (score A, Curtis and Walker, 1988) was used to summarise the occurrence, severity and duration of arrhythmias induced by ischaemia. The arrhythmia score for this group of rabbits was 3.0 ± 0.5 (mean \pm SEM, $n=21$).

Occluded zone size and blood $[K^+]$ were measured in order to define the arrhythmogenic conditions which evoked the above arrhythmias. Occluded zone size was $44 \pm 2\%$ (mean \pm SEM, $n=21$) and blood $[K^+]$ was 2.5 ± 0.1 mM.

Figure 16 shows the time of occurrence of arrhythmias for 65 rabbits in which coronary artery occlusion was performed. This analysis includes all rabbits irrespective of which coronary artery(ies) were occluded and resulting occluded zone size. Ischaemia-induced arrhythmias appeared to occur in 2 phases. The first phase of arrhythmias occurred within the 2 minutes of the onset of ischaemia. VF was rare during this phase but VT and PVBs were more common. The second phase started 8 minutes after induction of ischaemia and lasted approximately 10 minutes. More severe arrhythmias (VT and VF) tended to occur during this phase. The two arrhythmic phases were separated by a short time during which arrhythmias occurred less commonly. Arrhythmias did not usually occur between 3 and 8 minutes after the onset of ischaemia. Similarly, few

Figure 16. The temporal distribution of arrhythmias after coronary artery occlusion in the rabbit. The top panel shows the percentage of rabbits in which PVB occurred while the lower panel shows the same for the occurrence of VT (cross hatched bars) and VF (filled bars). Arrhythmias were quantified from the ECG as suggested by the Lambeth conventions (Walker *et al.*, 1988). The total number of rabbits included in this analysis was 65.



arrhythmias occurred between 20 and 30 minutes after induction of ischaemia. The occurrence of two arrhythmia phases parallels changes in the ST segment of the ECG.

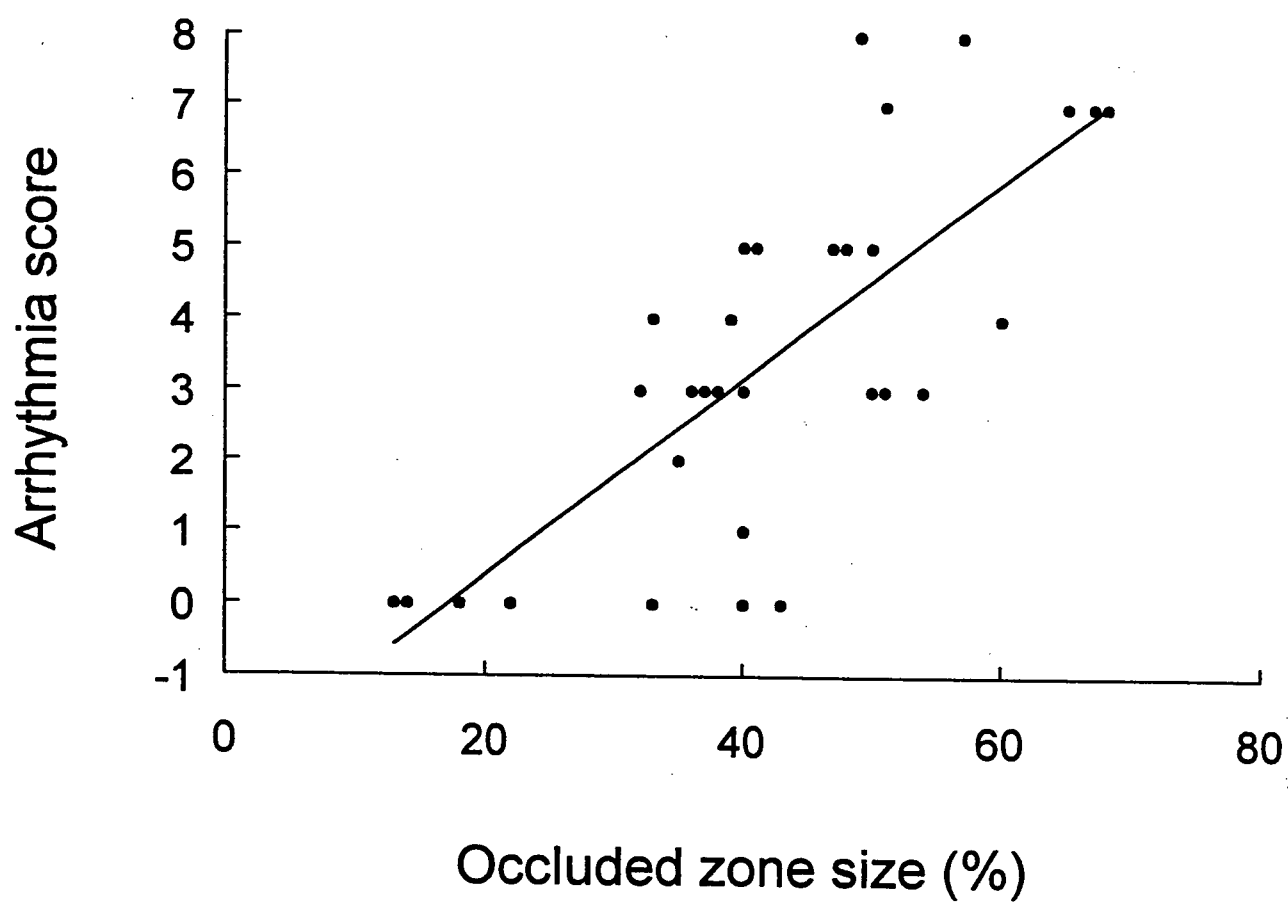
6.1.5 Investigations into the possible determinants on ischaemia-induced arrhythmias.

In 39 rabbits in this series ($n=65$) in which the occluded zone size was measured, a direct correlation was found between the occluded zone size and the arrhythmia score ($r=0.61$, $p<0.01$; Figure 17). An occluded zone of 40% appeared to be the minimum size required to reliably induce arrhythmias in this species. No relationship was found between the pre-occlusion blood $[K^+]$ and the arrhythmia score ($r=0.13$, $n=39$, $p>0.5$). Pre-occlusion heart rate was found to have no relationship to the arrhythmia score ($r=0.14$, $n=39$, $p>0.5$). Thus, the only factor identified as being associated with ischaemia-induced arrhythmias was a large occluded zone size. It should be noted, however, that a large range of values for blood $[K^+]$ and heart rate were not tested, nor were they systematically varied.

6.1.6 Influence of anaesthesia and ventilation on blood $[K^+]$.

In a sub-group of rabbits arterial blood $[K^+]$ was measured before and after establishing anaesthesia and artificial ventilation. In the conscious state rabbits had a blood $[K^+]$ of 4.0 ± 0.4 mM (mean \pm SEM, $n=6$) while under pentobarbital anaesthesia and artificial ventilation blood $[K^+]$ was 2.6 ± 0.2 mM ($p<0.01$, paired sample t-test).

Figure 17. Relationship between occluded zone size and the arrhythmia score in pentobarbital anaesthetised rabbits. The line represents the sum of least squares fit to a straight line ($r=0.61$, $n=39$, $p<0.01$).



6.2 Effects of RSD1019 in anaesthetised rabbits.

RSD1019, at a dose of 8 $\mu\text{mol/kg/min}$ for 5 minutes, increased mean arterial BP to 100 ± 5 mmHg from the pre-drug value of 85 ± 4 mmHg (mean \pm SEM, $n=7$, $p<0.05$). Conversely, heart rate was reduced to 215 ± 6 beats/minute from the pre-drug value of 240 ± 10 beats/minute ($p<0.05$ from pre-drug value). No significant effects on the PR or QRS intervals of the ECG were observed. The QTa interval of the ECG was prolonged from 105 ± 5 ms to 135 ± 4 ms ($p<0.05$) by RSD1019 while the QT interval was prolonged from 146 ± 10 ms to 169 ± 15 ms (NS). Pre-treatment values for vehicle and RSD1019 treated rabbits did not differ significantly.

6.2.1 Antiarrhythmic effects of RSD1019.

RSD1019 reduced the occurrence of ischaemia-induced tachyarrhythmias compared to vehicle controls and significantly reduced the arrhythmia score (Table 5, $p<0.05$).

No significant differences were found between groups in terms of occluded zone size or blood $[\text{K}^+]$. Occluded zone size was $54 \pm 3\%$ and $47 \pm 3\%$ (mean \pm SEM, $n=7$, NS) in controls and RSD1019 treated rabbits, respectively. Blood $[\text{K}^+]$ before occlusion was 2.8 ± 0.2 in controls and 2.9 ± 0.1 mM ($p>0.05$, NS) in RSD1019 treated rabbits.

6.3 Sham occlusion experiments.

In sham occluded rabbits, HR and BP did not change significantly over the course of the experimental observation period. No cardiac arrhythmias occurred. Mean arterial

Table 5. Antiarrhythmic actions of RSD1019 in pentobarbital anaesthetised rabbits.

Dose	Arrhythmia score	PVB (x/n)	VT (x/n)	VF (x/n)
Control	2.9 ± 0.6	7/7	5/7	4/7
8 µmol/kg/min	0.4 ± 0.2*	4/7	0/7*	0/7*

Table 5 shows the antiarrhythmic effects of RSD1019 in pentobarbital anaesthetised rabbits. RSD1019, at a dose of 8 µmol/kg/min, or vehicle control was infused continuously commencing 5 minutes before induction of ischaemia. The arrhythmia score data are expressed as the mean±SEM, n=7, while the incidence of PVB, VT and VF are shown as the number of rabbits having the arrhythmia over the total number in the group. Statistical significance for the arrhythmia score data were tested using an unpaired t-test while arrhythmia occurrence data were tested for statistical significance using Maitland contingency tables. The symbol (*) indicates a statistically significant result at $p < 0.05$.

BP was 81 ± 3 mmHg (mean \pm SEM, $n=5$) before and 81 ± 7 mmHg 30 minutes later (NS, paired sample t-test). HR was 252 ± 13 beats/min at the start of the experiment while 30 minutes later it was 240 ± 12 (NS, paired sample t-test).

6.3.1 Stability of MAP recordings.

Only minor changes in MAP characteristics were observed in sham-occluded rabbits. The primary change was a decrease in MAP amplitude. However, the reduction in MAP amplitude did not influence measurement of MAP duration. MAP amplitude decreased by $30 \pm 5\%$ from an initial value of 15 ± 3 mV (mean \pm SEM, $n=5$, $p < 0.05$, paired sample t-test). MAPD90% duration did not change significantly over the course of the experiment (145 ± 7 ms before and 146 ± 6 ms 30 minutes later, NS). MAP duration was more stable at later stages of repolarisation; early stages of repolarisation showed more variability. For example, the coefficient of variation for MAP duration at 90% and 25% repolarisation was typically 2% and 9%, respectively. For this reason, changes in MAP duration have been summarised using MAPD90%. Maximum upstroke velocity did not change significantly over the course of the experiment (NS, paired sample t-test); however, some variation was seen. Before starting the experiment the maximum upstroke velocity was 10.7 ± 1 V/s while 30 minutes later it was 11.7 ± 3 V/s. The mean difference in maximum upstroke velocity, expressed as a percent of the initial value, was $-0.3 \pm 14\%$.

Mechanical disruption of the electrodes, such as that produced by pulling the snare to produce ischaemia, had little effect on MAP characteristics; any effects that were observed were small and rapidly reversed. Neither activation alternans nor multiple wave

forms were observed in MAPs recorded during the sham occlusion experiments. Over the course of these experiment, alternans in MAP duration were observed in normal tissue only on one occasion (n=105).

6.4 Ischaemia-induced changes in MAPs.

MAP recordings showed time dependent changes after coronary artery occlusion which were typical of myocardial ischaemia (Figure 18). These changes could be described in terms of changes in conventional AP variables (e.g., AP maximum upstroke velocity, amplitude, and duration) as well as MAP morphology (see Figure 19). MAP duration increased in the first minute after induction of ischaemia and then shortened rapidly (Figure 20). The amplitude of MAP recordings decreased progressively with time after induction of ischaemia and maximum upstroke velocity was rapidly reduced (Figure 20). A secondary recovery in maximum upstroke velocity was not observed. With periods of ischaemia longer than 15-20 minutes, MAP morphology changed to such an extent that recordings were no longer recognisable as MAPs (Figure 21). Such ischaemia-induced changes in MAPs rendered measurement of conventional MAP variables impossible. In all rabbits it was possible to measure conventional MAP variables 5 minutes after induction of ischaemia while it was not possible to do so in any rabbit after 20 minutes.

Figure 18. Typical ischaemia-induced changes in MAPs recorded from the epicardium of a rabbit. Recordings are labelled according to the time they were taken after starting the experiment (e.g., "+5' Control" indicates that the recording was taken 5 minutes after starting the experiment, before induction of ischaemia). The arrows show how the MAP foot to peak (MAP f-p) interval was measured.

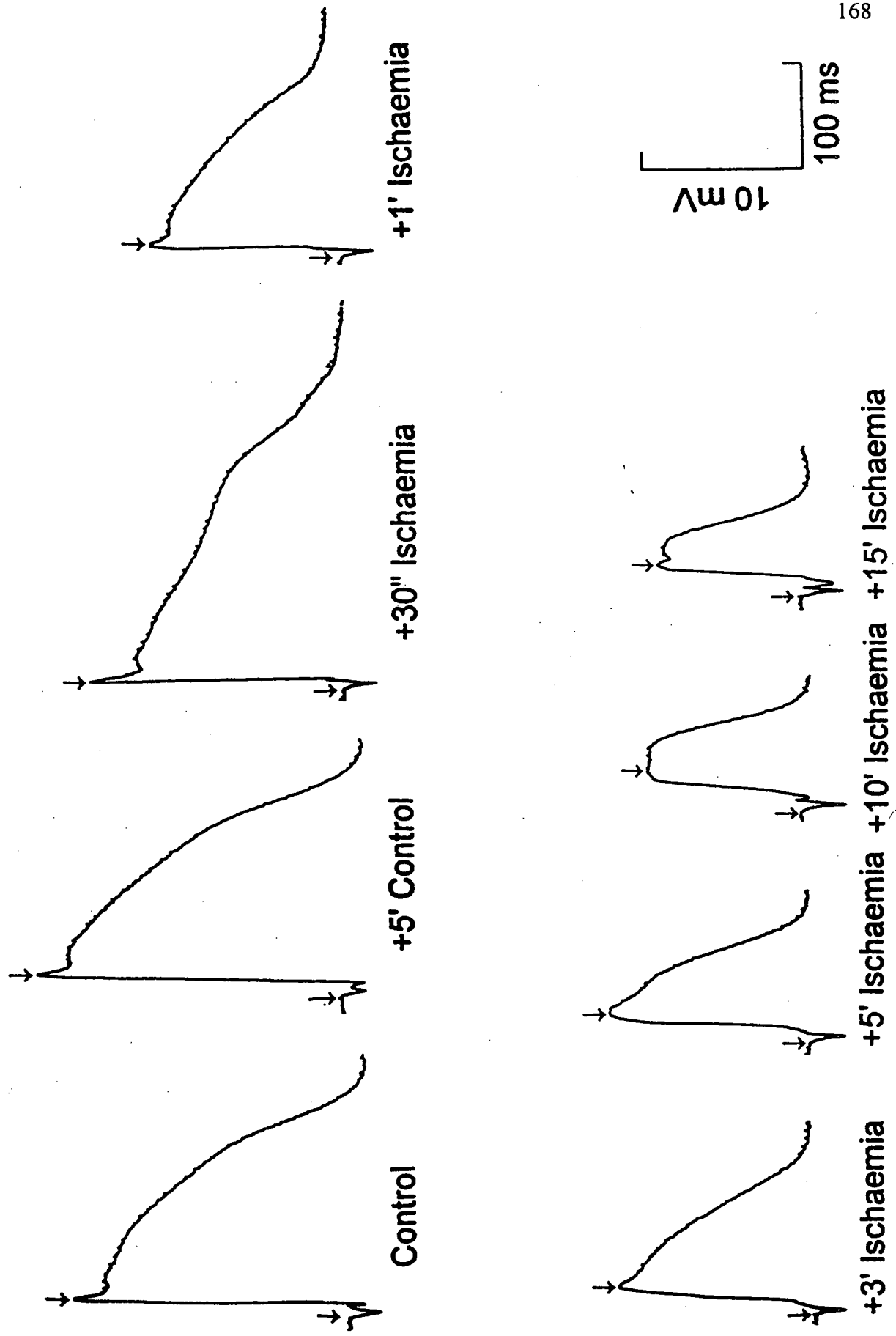
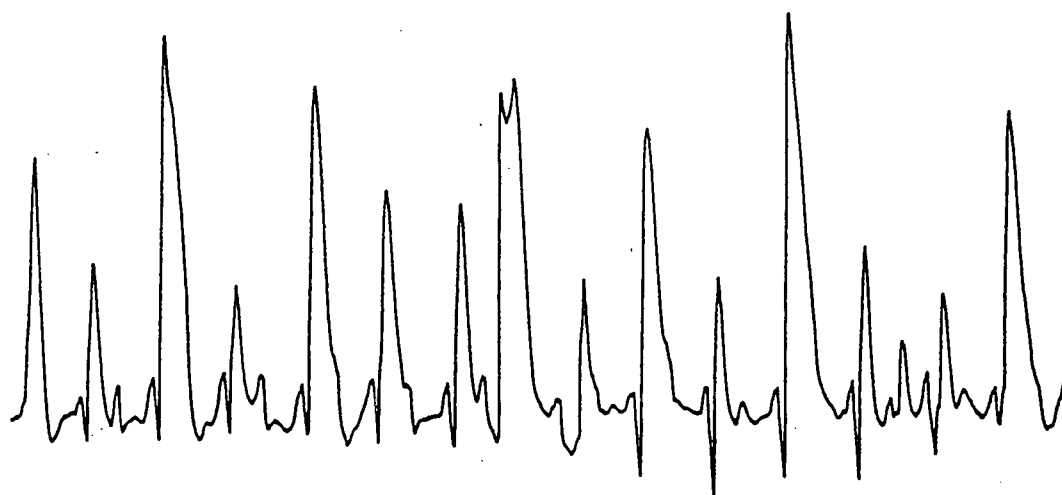
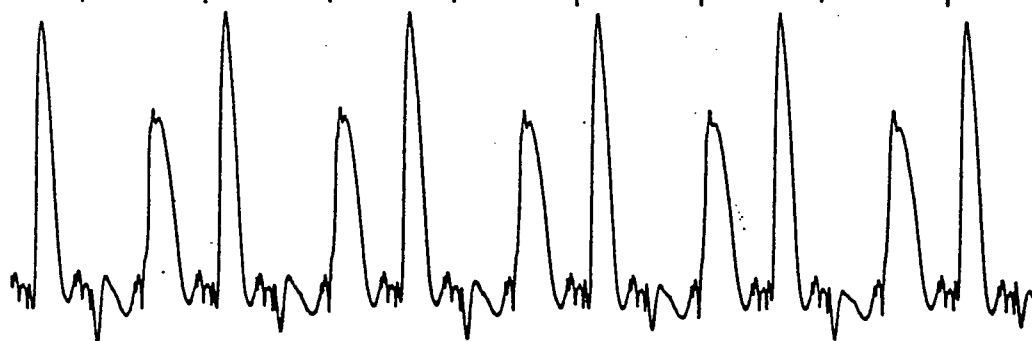
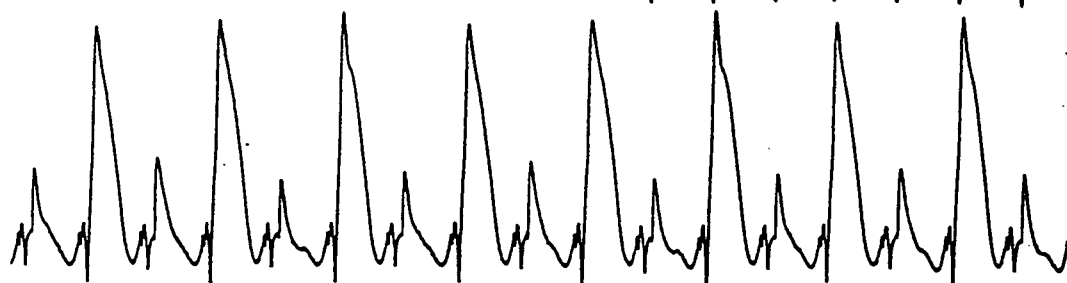
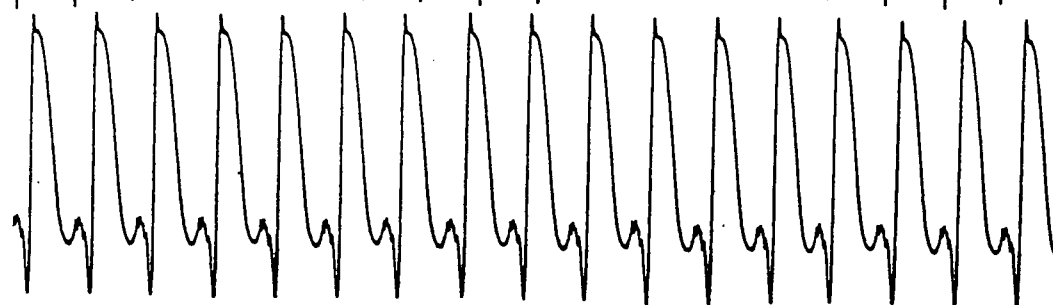
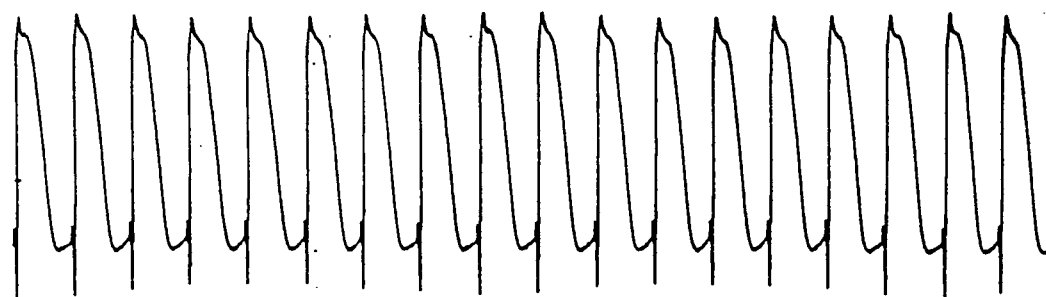


Figure 19. Typical activation alternans and multiple wave forms in MAPs recorded from ischaemic myocardial tissue. Recordings are labelled according to the time after occlusion which they were taken. The first recording labelled "Control" was taken immediately before occlusion. All traces were taken during normal sinus rhythm except for the final trace which was taken during VF. The trace taken 5 minutes after occlusion shows typical activation alternans while the trace taken 7 minutes after occlusion shows multiple wave forms. The trace taken 7 minutes after occlusion is somewhat misleading in that the pattern of multiple wave forms was more regular than was typically observed. When multiple wave forms occurred, MAP morphology could change on a beat to beat basis and repeating sequences were not commonly observed. At times, MAP records similar to those seen during VF were observed while the animal was still in sinus rhythm. This rabbit was given 5 $\mu\text{mol/kg/min}$ lidocaine starting 5 minutes before induction of ischaemia (see later).



10 mV
500 ms

Figure 20. Changes in conventional MAP variables recorded from ischaemic myocardial tissue in pentobarbital anaesthetised rabbits after coronary artery occlusion. MAPs recorded from ischaemic myocardial tissue are shown as the mean \pm SEM (n=14, filled circles) while MAPs recorded from normal tissue are shown as the mean \pm SEM (n=5, open circles). The recordings from normal tissue were obtained in sham occlusion experiments. The top, middle and lower panels show changes in MAP amplitude, maximum upstroke velocity (Vmax) and duration at 90% repolarisation (MAPD90%), respectively.

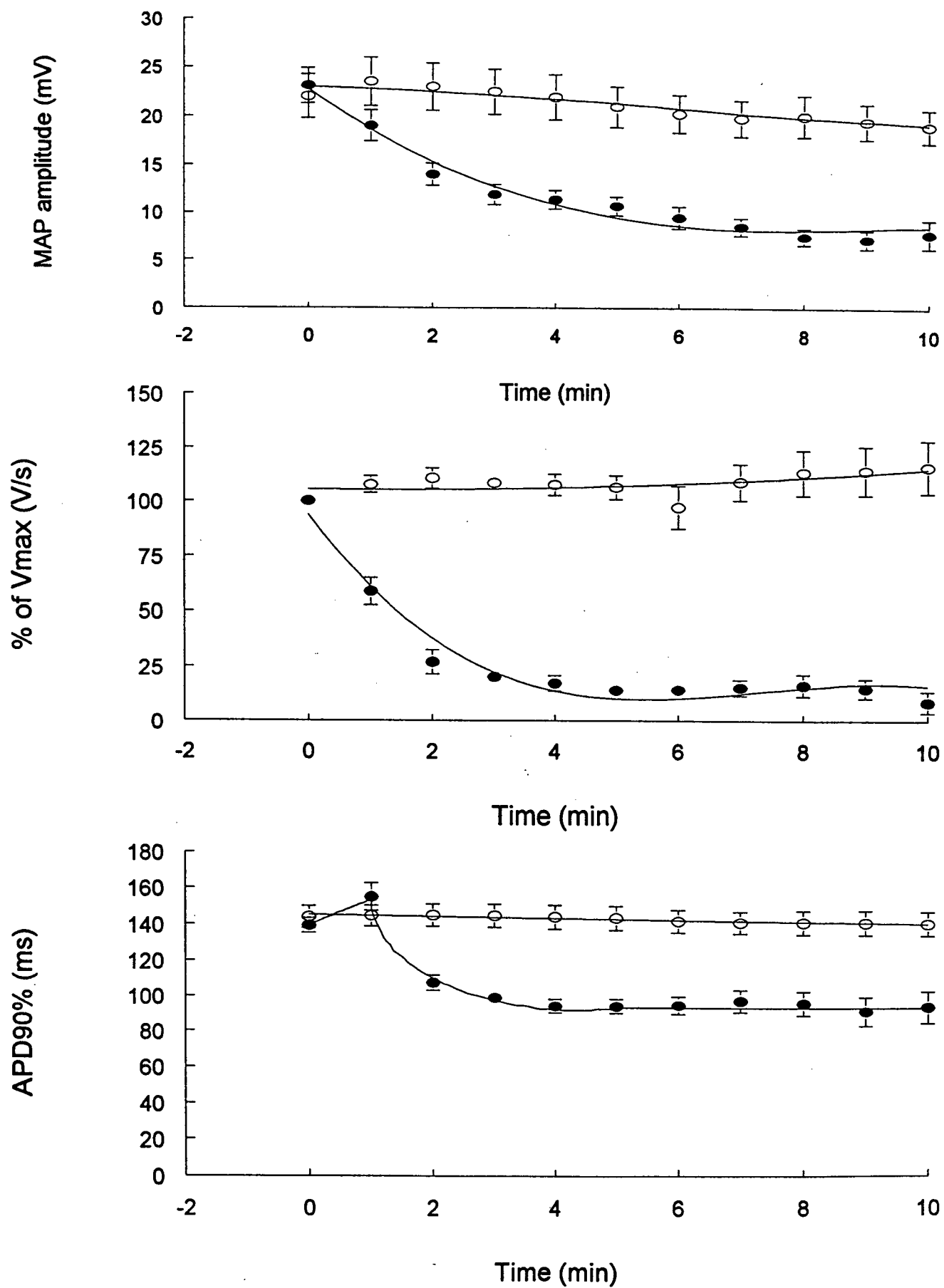
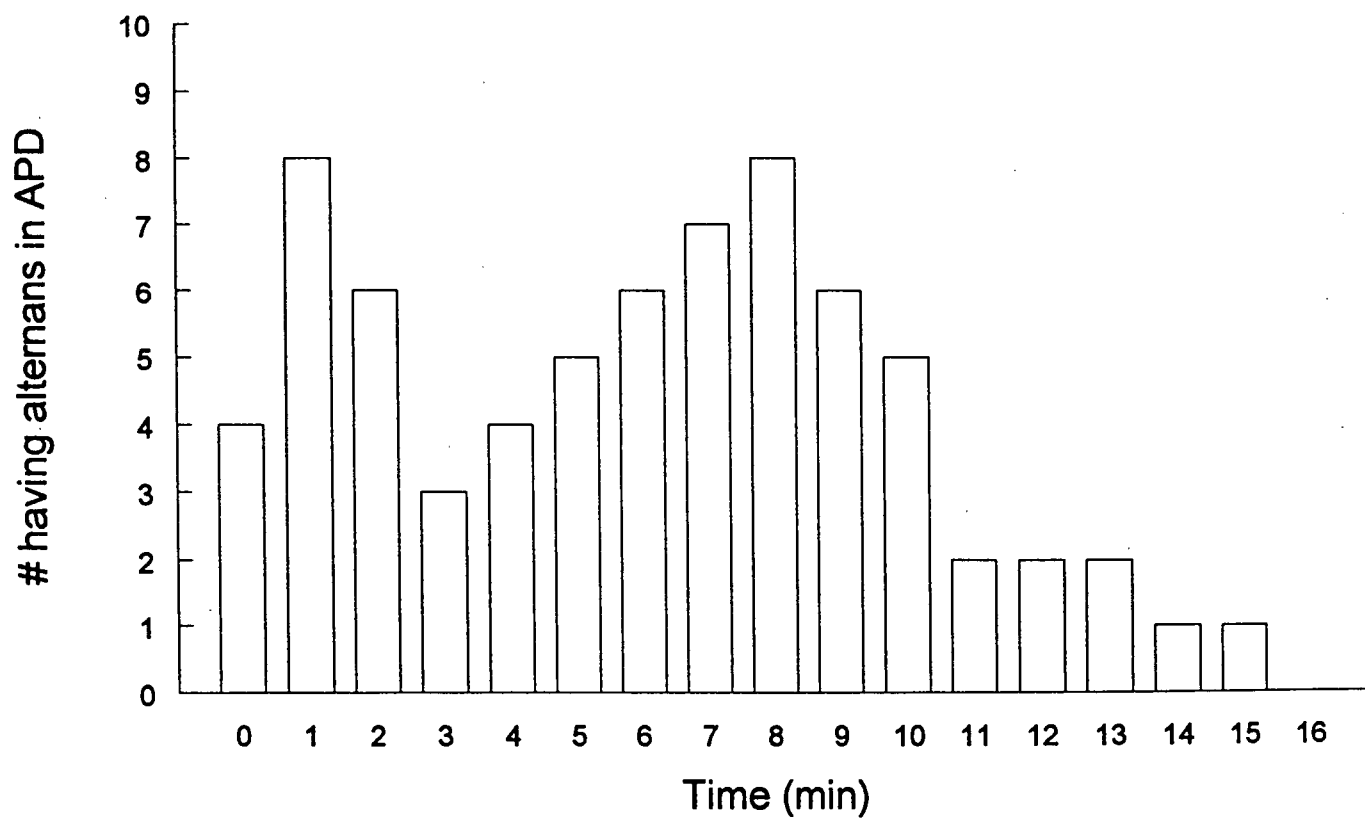
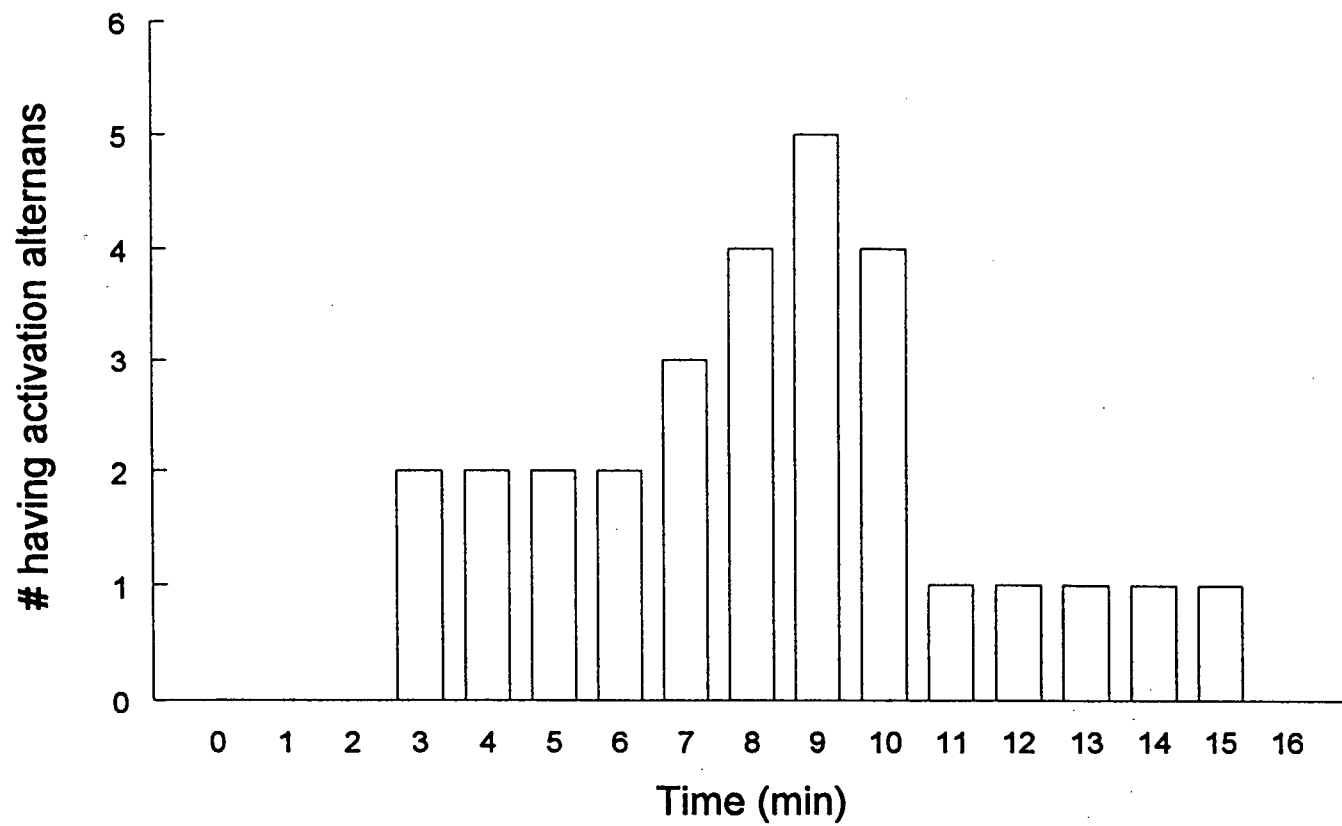


Figure 21. Temporal distribution of activation alternans in MAP recordings after induction of ischaemia. The top panel shows the distribution of alternans in MAP duration while the lower panel shows the distribution for activation alternans. Alternans in MAP duration, shown in top panel, included isolated repolarisation alternans and those which occurred subsequent to activation alternans (see text for further details). Activation alternans are defined in the text and shown in Figure 19.



Activation alternans were observed in a time dependent pattern after induction of ischaemia (Figure 21). MAP morphology changed such that two morphologies were observed, each on successive beats (see Figure 19). Isolated alternans in MAP duration were observed predominately within the first 3 minutes after induction of ischaemia. Activation alternans, such as those shown in Figure 19, did not usually occur until after 3 minutes of ischaemia. More complex sequences of alternans in MAP morphology (defined as multiple MAP wave forms, see methods) were observed, although these were rare. When these sequences were observed they typically followed persistent activation alternans and preceded MAP recordings becoming unrecognisable as such.

Isolated alternans in MAP duration should be distinguished from those occurring secondary activation alternans (or multiple wave forms). In the case of isolated alternans in MAP duration, changes were apparent only in the terminal repolarisation phase (late phase 2 and phase 3). In contrast, with activation alternans changes occurred during all phases; activation alternans always caused alternation in MAP duration.

6.5 Relationship between ischaemia-induced changes in MAPs and arrhythmias.

Alternans in MAP duration and activation alternans occurred in two distinct phases in a similar fashion to ischaemia-induced arrhythmias (compare Figures 16 & 21). Alternans in MAP duration were coincident with the first phase of arrhythmias while activation alternans preceded the second phase of arrhythmias. Alternans were not observed in all rabbits which had arrhythmias (irrespective of the type of arrhythmia) nor did arrhythmias occur in all rabbits in which alternans were observed.

6.6 Actions of glibenclamide in anaesthetised rabbits.

Glibenclamide transiently increased mean BP immediately after injection. BP in the control group 1 minute before the start of the experiment was 81 ± 3 mmHg (mean \pm SEM, $n=14$). The increase in mean BP caused by glibenclamide was not dose-related and administration of 3, 6, 12 and 24 mg/kg glibenclamide caused an increase of 15 ± 3 , 16 ± 4 , 18 ± 5 and 15 ± 5 mmHg, respectively (mean \pm SEM, $n=7$, $p < 0.05$ for each value vs. pre-drug and control). Vehicle control had no effect on BP (2 ± 1 mmHg, NS vs. pre-drug). The effect of glibenclamide on BP was transient and there were no significant effects compared with controls 1 minute before the start of ischaemia. The average mean arterial BP immediately before induction of ischaemia was 80 ± 2 mmHg (mean \pm SEM, $n=42$). Glibenclamide had no effect on HR. The group mean HR before occlusion was 256 ± 5 beats/minute. ECG intervals were not effected by treatment with glibenclamide (except as noted below).

The highest dose of glibenclamide (24 mg/kg) altered T wave morphology of the ECG and 1 animal had VPBs immediately after drug administration. Five of 7 rabbits treated with 24 mg/kg glibenclamide suffered severe hypotension approximately 10 minutes after the start of ischaemia despite having the same occluded zone size as vehicle treated rabbits (see Table 6). In these animals electromechanical dissociation, severe hypotension or reduced cardiac output, occurred. Electrophysiological data could be obtained only during the first 5 minutes after the start of ischaemia in this group.

Table 6. Effect of glibenclamide on ischaemia-induced arrhythmias in pentobarbital anaesthetised rabbits.

Glibenclamide	[K ⁺]	OZ (%)	AS	VPB	VT	VF
Vehicle	2.5±0.4	49±9	3.4±3.0	13/14	5/14	8/14
3 mg/kg	2.6±0.1	53±5.1	2.4±0.9	7/7	3/7	1/7
6 mg/kg	2.3±0.1	47±5.5	2.3±0.6	5/7	4/7	1/7
12 mg/kg	2.1±0.3	43±2.1	2.9±0.5	7/7	5/7	3/7
24 mg/kg	2.9±0.3	50±4.6	ND	6/7	3/7	2/7

Table 6 shows the effects of glibenclamide on ischaemia-induced arrhythmias in pentobarbital anaesthetised rabbits. Blood [K⁺], occluded zone size (OZ%) and the arrhythmia score data are expressed as the mean±SD, n=14, for the vehicle control group and then mean±SEM, n=7, for treated groups. Blood [K⁺] and occluded zone size were not significantly different between groups (ANOVA, p>0.05). Arrhythmia occurrence data were tested for statistical significance using Maitland contingency tables. While no significant antiarrhythmic effects were found for any single dose, VF was significantly reduced when all treated groups are compared to the vehicle control (p<0.05). The arrhythmia score was not determined (ND) for the 24 mg/kg glibenclamide group since 5 of 7 animals suffered severe hypotension after occlusion.

6.6.1 Effects of glibenclamide on blood glucose concentration.

The glucose lowering actions of glibenclamide were observed only at the highest dose tested. In all other groups, including controls, blood glucose concentrations increased as a function of time. In controls, blood glucose concentration increased by 60% over the course of the experiment from the pre-drug value of 5.0 ± 0.4 mM (Table 7). Paradoxically, glibenclamide appeared to potentiate this unexpected increase in blood glucose concentration. The highest dose of glibenclamide (24 mg/kg) prevented the time dependent increase in blood glucose, but did not lower blood glucose below normal physiological concentrations (blood glucose concentration 7.4 ± 1.4 mM, $n=7$, 50 minutes after dosing).

6.6.2 Influence of glibenclamide on MAPs.

Glibenclamide had no effect on MAP duration before the start of ischaemia (Figure 22). After induction of ischaemia MAP duration showed bi-phasic changes with an initial prolongation followed by a rapid and sustained shortening (see Figures 15, 18 & 19). Glibenclamide dose-dependently decreased the rate of ischaemia-induced MAP shortening (Figure 23). However, this effect was not maintained and there were no differences in MAP duration 5 minutes after the start of ischaemia (Figure 22).

Ischaemia-induced change in MAP amplitude and maximum upstroke velocity were similar for all groups. Glibenclamide had no effects on MAP amplitude, nor on maximum upstroke velocity, before or after induction of ischaemia. As no differences between groups were found, data were grouped together for the purposes of presentation. MAP amplitude decreased progressively during ischaemia from the pre-

Table 7. Summary of the effects of glibenclamide on blood glucose concentration in pentobarbital anaesthetised rabbits included in the arrhythmia study.

Dose (mg/kg)	Pre-drug	Pre-isch.	+30' isch.
Vehicle control	5.1±1.5	5.9±1.5	8.2±3.0*
Glibenclamide-3	5.6±0.5	6.1±0.5	11.3±1.7* [#]
Glibenclamide-6	5.4±0.6	8.2±1.3* [#]	13.0±2.5* [#]
Glibenclamide-12	6.4±0.1	6.9±0.6	14.5±1.4* [#]
Glibenclamide-24	6.4±1.2	9.3±2.2* [#]	7.4±1.4

Table 7 shows blood glucose concentrations before administration of vehicle control or various doses of glibenclamide (pre-drug), 20 minutes after administration but before ischaemia (pre-isch.) and 30 minutes after induction of ischaemia (+30' isch.). The final time point was taken 50 minutes after administration of drug of vehicle control. Values are shown as the mean±SD, n=14, for controls (C) and the mean±SEM, n=7 for treated rabbits. Statistical significance was assessed using ANOVA ($p<0.05$) and Dunnett's test for differences. The asterisk (*) indicates $p<0.05$ from pre-drug value while [#] indicates differences from control at that time point.

Figure 22. MAP duration at 90% repolarisation recorded from the epicardium of pentobarbital anaesthetised rabbits before and after induction of ischaemia; glibenclamide did not prevent ischaemia-induced MAP shortening. Values for controls are expressed as mean $\text{MAPD90}\% \pm \text{SD}$, $n=14$, while for treated rabbits values represent mean $\text{MAPD90}\% \pm \text{SEM}$, $n=7$. $\text{MAPD90}\%$ is shown before ischaemia (Pre-Isch.) and after 5 minutes of ischaemia (+5'-Isch.). Glibenclamide, at doses of 3, 6, 12, and 24 mg/kg, was administered iv, 20 minutes before induction of ischaemia. Statistical significance was determined by ANOVA ($p < 0.05$) followed by a Tukey test for differences. The symbol (*) indicates a statistically significant difference between the $\text{MAPD90}\%$ 5 minutes after induction of ischaemia from the pre-ischaemia value.

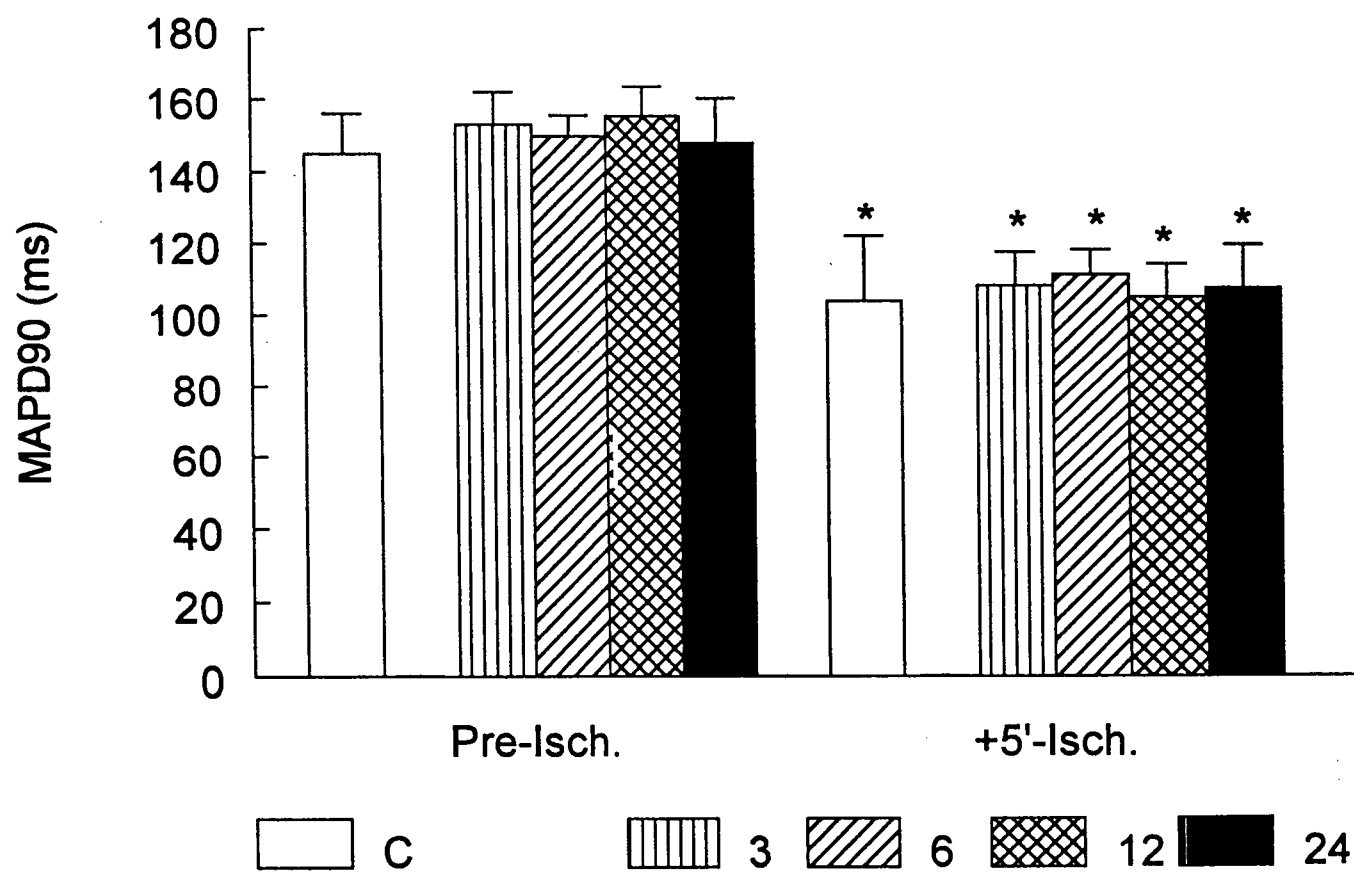
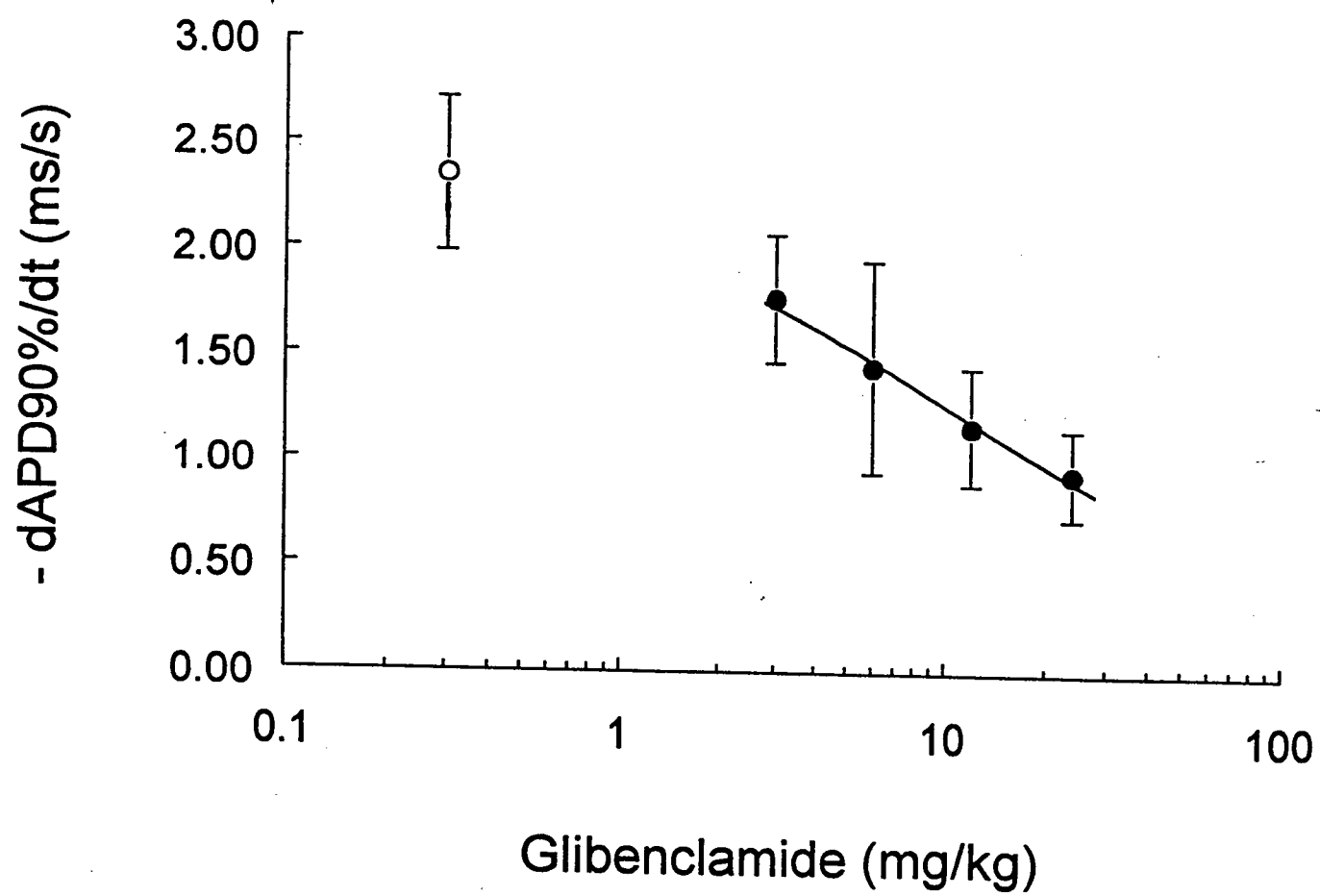


Figure 23. The effect of glibenclamide on the initial rate of MAP shortening ($-dAPD/dt$) caused by ischaemia in pentobarbital anaesthetised rabbits. Data are expressed as the mean \pm SD, $n=14$, for vehicle controls (open circles) and the mean \pm SEM, $n=7$, for glibenclamide treated rabbits (filled circles). The line shows the best fit to the logistic equation $-dAPD/dt = Dose^h / (Dose^h + ED_{50\%}^h)$ where " $-dAPD/dt$ " is the maximum rate of MAP shortening, "Dose" is the dose of glibenclamide (mg/kg), " $ED_{50\%}$ " is the dose producing 50% of the maximum response and " h " is the slope factor. The $ED_{50\%}$ was 13 ± 0.8 mg/kg (mean \pm SEM, $n=28$, $k=4$) and the slope factor was -0.7 ± 0.1 . The curve fit statistic (r) was 0.99 ($p < 0.01$ for slope equal to zero).



occlusion values of 20 ± 0.8 mV (mean \pm SEM, $n=42$) to half this value by approximately 8 minutes. Maximum MAP upstroke velocity decreased to 1.4 ± 0.2 V/s from the pre-ischæmia value of 10 ± 1 V/s within 2 minutes of the start of ischaemia. Maximum MAP upstroke velocity showed no evidence of recovery after the initial decrease. No differences in the occurrence or time distribution of activation alternans were noted.

6.6.3 Antiarrhythmic actions of glibenclamide.

Glibenclamide significantly reduced the incidence of VF compared to controls (Table 6). In vehicle treated rabbits, 8 of 14 had VF compared with 7 of 28 for all glibenclamide treated rabbits combined ($p < 0.05$ versus control). If rabbits suffering severe hypotension were excluded from analysis (e.g., those in the 24 mg/kg glibenclamide group) the result is similar with 5 of 21 having VF ($p < 0.05$ versus control). The antifibrillatory actions of glibenclamide were not clearly dose-related and reductions in VF incidence and arrhythmia score were not significant for any individual dose group (see Table 6). No differences were found between groups in the time of VF occurrence. The average latency after induction of ischaemia to the occurrence of VF was 10.6 ± 1.1 minutes ($n=19$). Glibenclamide had no significant effect on occluded zone size nor blood $[K^+]$ (see Table 6).

6.6.4 Exclusions.

Two animals suffered severe hypotension immediately after the onset of ischaemia and were replaced. In these animals inadvertent occlusion of the septal artery had

occurred resulting in occluded zone sizes larger than the accepted maximum value. One animal was treated with vehicle, the other was treated with 3 mg/kg glibenclamide. Rabbits treated with 24 mg/kg glibenclamide suffered severe hypotension approximately 10 minutes after occlusion which did not allow determination of the arrhythmias score, nor other variables.

6.6.5 Glibenclamide single dose study.

In the single dose study, glibenclamide (0.3 mg/kg iv) had no effect on the initial rate of MAP shortening nor the magnitude of ischaemia-induced MAP shortening 5 minutes after the start of ischaemia (Table 8). At this dose, glibenclamide had no effects on the occurrence of ischaemia-induced arrhythmias and the arrhythmia score was not effected by treatment with 0.3 mg/kg glibenclamide (Table 9).

Treatment with 0.3 mg/kg glibenclamide iv transiently increased mean BP compared to pre-drug values. Mean BP was increased from 89 ± 4 to 97 ± 3 mmHg ($p < 0.05$) by 0.3 mg/kg glibenclamide. Vehicle control had no effect on mean BP (96 ± 4 mmHg before and after injection of vehicle). The effect of glibenclamide on BP was short lived and no difference was detected at the time of coronary artery occlusion.

No differences between the two groups were found in terms of blood gas characteristics, blood $[K^+]$, nor occluded zone size (Table 9). Blood gas characteristics were similar to those observed in anaesthetised rabbits in which a sternal split was not performed (e.g., those used for the glucose lowering assay, see below). Blood pH and

Table 8. The effects of a low dose of glibenclamide on ischaemia-induced MAP shortening in pentobarbital anaesthetised rabbits.

Dose (mg/kg)	Pre-ischaemia (ms)	Maximum (ms)	+5' ischaemia (ms)	-dAPD/dt (ms/s)
Vehicle	169±7	196±8	101±5	0.32±0.07
Glibenclamide 0.3	167±7	201±7	114±8	0.26±0.05

Table 8 shows the effect of a low dose (0.3 mg/kg) of glibenclamide on ischaemia-induced changes in MAP characteristics. The pre-ischaemic MAPD90% (Pre-ischaemia), maximum MAPD90% obtained after induction of ischaemia (Maximum), MAPD90% 5 minutes after induction of ischaemia (+5' ischaemia) and the maximum rate of ischaemia-induced MAP shortening (-dAPD/dt) are shown as the mean±SEM, n=9. Statistical significance was assessed using an unpaired t-test at a significance level of $p<0.05$. No significant differences were found.

Table 9. Antiarrhythmic actions of a low dose of glibenclamide in pentobarbital anaesthetised rabbits.

Dose (mg/kg)	[K ⁺] (mM)	OZ%	AS	PVB (x/n)	VT (x/n)	VF (x/n)
Vehicle control	2.6±0.1	50±1	1.6±0.7	7/9	3/9	2/9
Glibenclamide-0.3	2.3±0.2	47±4	1.8±0.9	8/9	4/9	2/9

Table 9 shows the effects of a low dose of glibenclamide (0.3 mg/kg iv) on ischaemia-induced arrhythmias in pentobarbital anaesthetised rabbits. Data are expressed as the mean±SEM, n=9, except for the arrhythmia incidence data which are expressed as the number of rabbits in each group which had the arrhythmia over the total number in the group. Blood [K⁺] and occluded zone size (OZ%) are also shown. Statistical significance was assessed at a significance level of p<0.05 using an un-paired t-test. Arrhythmia incidence data was tested for statistical significance using Maitland contingency tables. No significant differences were found. The abbreviations are the same as those used in the text.

PCO₂ were within physiological limits while PO₂ exceeded physiological limits since rabbits were ventilated with oxygen. Blood pH was 7.48 ± 0.01 while PCO₂ was 41 ± 2 mmHg (mean \pm SEM, n=18).

Blood glucose concentration was not significantly influenced by glibenclamide treatment as compared to controls ($p > 0.05$). In this study, blood glucose tended to increase at the final time point but this was not significantly different from the pre-drug value. Blood glucose concentrations in controls were 7.7 ± 0.3 mM (mean \pm SEM, n=9) before vehicle administration, 7.2 ± 0.3 mM immediately before the onset of ischaemia (20 minutes after vehicle administration) and 8.0 ± 0.3 mM after 30 minutes of ischaemia. Blood glucose in the treated group was 8.3 ± 0.3 mM before administration of glibenclamide, 7.2 ± 0.3 mM 20 minutes after glibenclamide administration, and 8.6 ± 0.3 mM after 30 minutes of ischaemia (a total of 50 minutes after drug administration).

6.6.6 Core body versus epicardial surface temperature.

Heart and core body temperature were measured during this series of experiments in order to assess the degree of cooling caused by maintaining the chest open during the experiment. No differences due to drug treatment were noted and data are presented as the group mean \pm SEM, n=18. Core body temperature decreased from 37.3 ± 0.2 to 36.7 ± 0.2 °C over 35 minutes ($p < 0.05$). The epicardial surface also cooled over the course of the experiment from 35.9 ± 0.3 to 34.6 ± 0.2 °C ($p < 0.05$). At both time points the epicardial surface was cooler than core body temperature ($p < 0.05$). This difference averaged -1.7 ± 0.1 °C (mean \pm pooled SEM, n=18).

6.6.7 Glucose lowering actions of glibenclamide.

Statistically significant reductions in blood glucose concentration occurred only 110 minutes after drug administration (i.e., after the period relevant to the separate arrhythmia study). Before drug administration the group mean blood glucose concentration was 6.2 ± 0.2 mM (mean \pm SEM, $n=6$). This variable did not change as a function of time in vehicle treated rabbits. In controls, 110 minutes after vehicle administration blood glucose concentration was 6.7 ± 0.3 mM ($n=3$). Treatment with 3.0 mg/kg glibenclamide iv reduced blood glucose concentration to 5.0 ± 0.5 mM ($n=3$, $p < 0.05$ versus time matched control).

Blood gas characteristics and blood $[K^+]$ in these rabbits were grouped together to define the base line characteristics of pentobarbital anaesthetised, artificially ventilated rabbits. No differences were found between groups with respect to blood gas characteristics or $[K^+]$. Group mean blood $[K^+]$ before starting the experiment was 2.8 ± 0.1 mM (mean \pm SEM, $n=6$). Blood pH, PCO_2 and PO_2 values before starting the experiment were 7.48 ± 0.03 , 40 ± 3 mmHg and 430 ± 22 mmHg, respectively.

6.7 Effects of lidocaine, tedisamil and RSD1019 in the anaesthetised rabbit preparation.

6.7.1 Effects of lidocaine, tedisamil and RSD1019 on BP, HR and ECG intervals.

Before induction of ischaemia: lidocaine, tedisamil and RSD1019 had similar bradycardic actions in pentobarbital anaesthetised rabbits. However, these drugs had different effects on BP (Table 10). BP was reduced by lidocaine at the highest dose tested (10 $\mu\text{mol/kg/min}$) while the 0.125 and 0.25 $\mu\text{mol/kg/min}$ doses of tedisamil increased systolic BP. RSD1019 was without effect on BP.

The effects of lidocaine, tedisamil and RSD1019 on ECG intervals were confined to the QT interval (Table 10). At the doses tested no significant changes in PR nor QRS intervals of the ECG were observed. Lidocaine was without effect on raw QTa and QT intervals. However, when the QT prolonging effects of bradycardia were taken into account, the 10 $\mu\text{mol/kg/min}$ dose of lidocaine significantly reduced the QTc interval. Tedisamil and RSD1019 prolonged raw QTa and QT intervals in a dose-related fashion. These effects were independent of the effects of bradycardia and QTac and QTc intervals were also prolonged. The QT widening produced by the highest dose of RSD1019 (8 $\mu\text{mol/kg/min}$) was similar to that produced by the middle dose of tedisamil (0.125 $\mu\text{mol/kg/min}$). The highest dose of tedisamil (0.25 $\mu\text{mol/kg/min}$) producing significantly greater QT widening than the 8 $\mu\text{mol/kg/min}$ dose of RSD1019.

6.7.2 Effect of lidocaine, tedisamil and RSD1019 on ischaemia-induced changes in MAPs.

Lidocaine, tedisamil and RSD1019 had different effects on MAPs before and after induction of ischaemia. The effects of these drugs on MAP duration in normal tissue paralleled their effects on the QT interval of the ECG. Lidocaine had no effect on MAPD90% before or after induction of ischaemia (Figure 24). Tedisamil prolonged

Table 10. Summary of the effects of lidocaine, tedisamil and RSD1019 on BP, HR and ECG intervals on pentobarbital anaesthetised rabbits included in the arrhythmia study.

GROUP PRE-DRUG VALUES		POST DRUG										
		PERCENT CHANGE FROM PRE-DRUG										
		LIDOCAINE			TEDISAMIL			RSD1019				
DOSE ($\mu\text{mol/kg/min}$)	CO / Systolic BP (mmHg) Diastolic BP (mmHg) HR (beats/min) PR (ms) QRS (ms) QTa (ms) QT (ms) QTac (ms/ \sqrt{s}) QTc (ms/ \sqrt{s})	2.5	5	10	0.063	0.125	0.25	2	4	8		

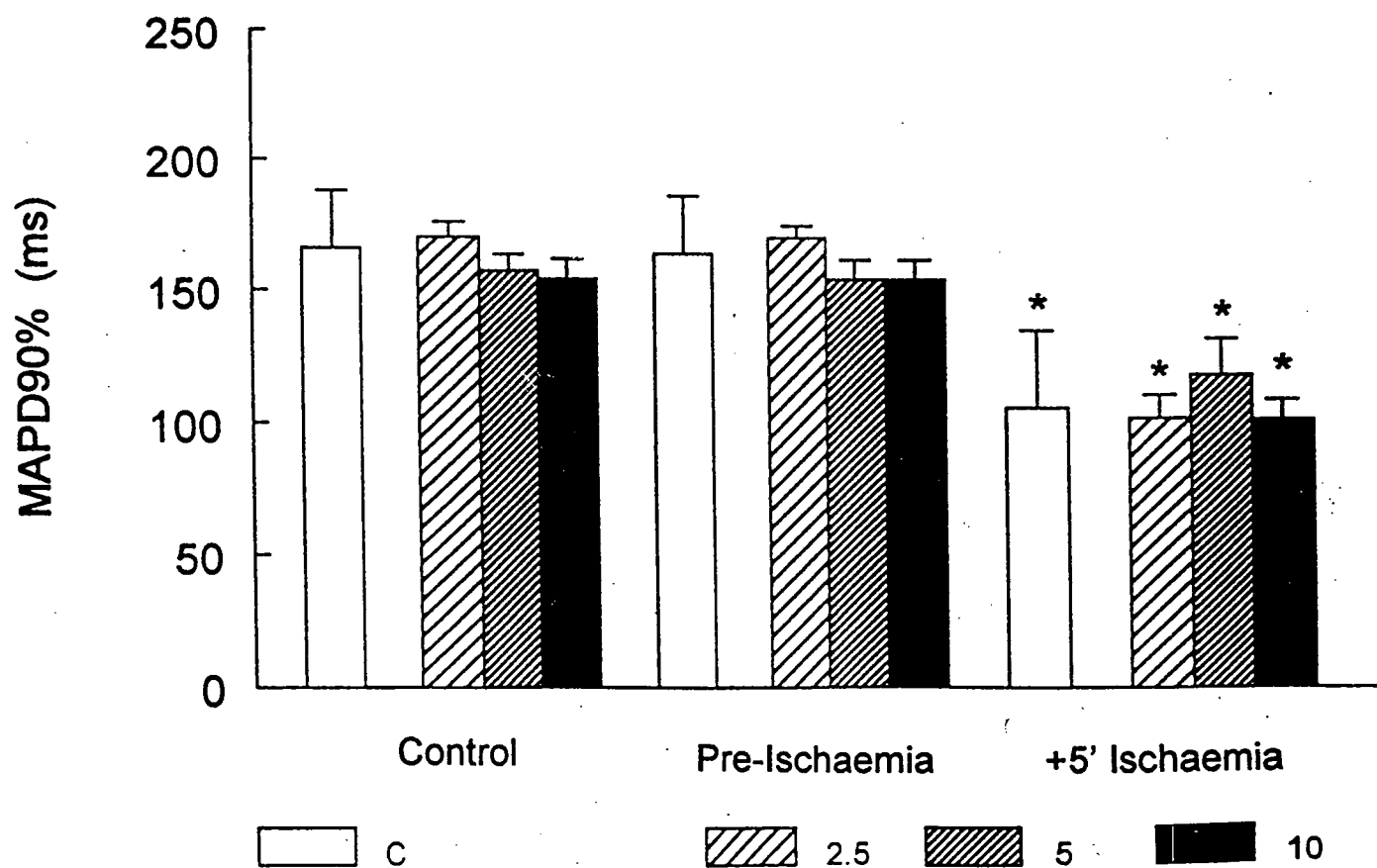
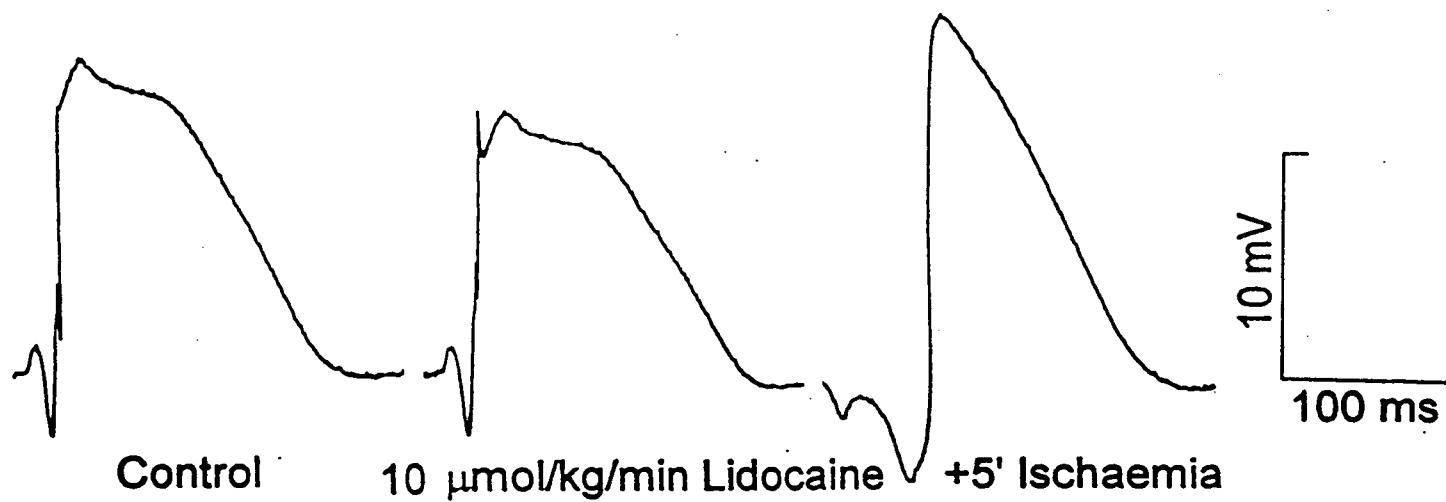
The effects of infused doses of lidocaine, tedisamil and RSD1019 on BP, HR and ECG intervals in pentobarbital anaesthetised rabbits. Doses (in $\mu\text{mol/kg/min}$) of lidocaine, 2.5, 5 and 10, tedisamil, 0.063, 0.125, 0.25, and RSD1019, 2, 4 and 8, or vehicle control (CO) are indicated on top of the column. No differences were found in the pre-drug values (ANOVA, $p>0.05$) and the values represent the population mean \pm SD, $n=105$, for all rabbits included in the study. Post-infusion values represent the mean change \pm SD ($n=42$) for controls or the mean change \pm SEM ($n=7$) from pre-drug values for treated groups. Post-infusion values were measured 5 minutes after commencing infusion. Statistical significant was tested using ANOVA at a significance level of $p<0.05$ followed by Tukey's test for differences. Significant differences from control are indicated with an asterisk (*), differences between the lowest and highest dose are noted with the (#)symbol and differences between the two lower doses and the highest dose are noted with the (&) symbol/

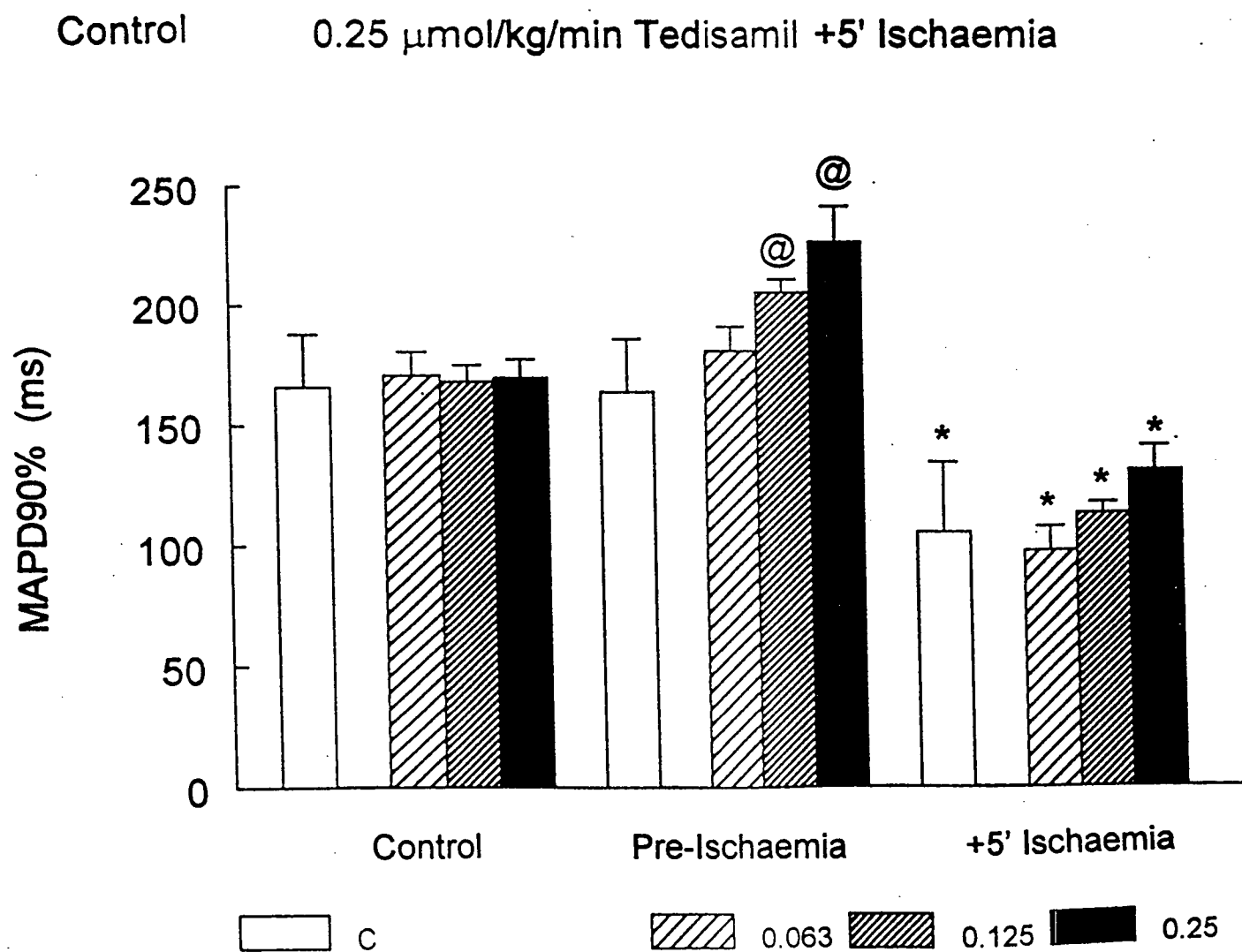
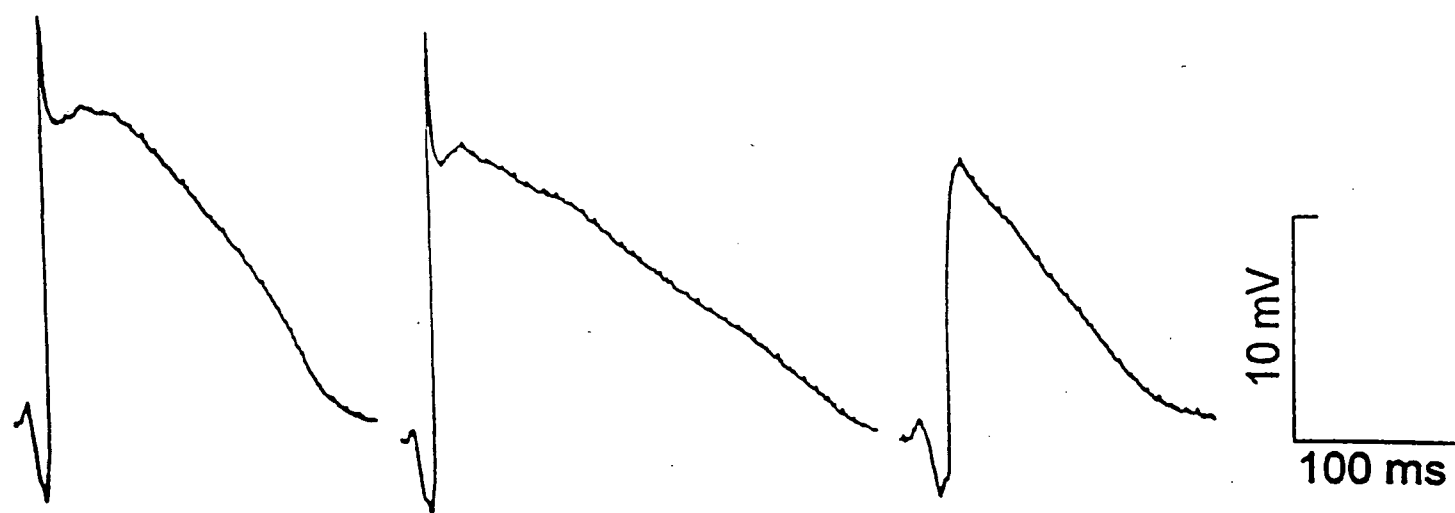
MAPD90% before induction of ischaemia in a dose-related fashion; however, such prolongation was rapidly lost after induction of ischaemia (Figure 25). No significant differences in MAPD90% were found between vehicle control and tedisamil treated rabbits 5 minutes after induction of ischaemia. In contrast, RSD1019 significantly prolonged MAP duration in normal myocardial tissue only at the highest dose tested (8 $\mu\text{mol/kg/min}$). This prolongation was partly maintained after induction of ischaemia (Figure 26). Compared to controls, RSD1019 significantly prolonged MAPD90% 5 minutes after induction of ischaemia. This effect also appeared to be dose-related (see Figure 26). MAP duration at times greater than 5 minutes after the induction of ischaemia were prolonged, however, statistics were not performed on these data for reasons previously discussed. In short, the MAP prolonging actions of RSD1019 were maintained in ischaemic tissue in contrast to the actions of tedisamil.

Measurement of the MAP foot to peak (MAP f-p) interval before and after induction of ischaemia revealed differences in drug action on ischaemic myocardial tissue. None of the drugs tested, nor vehicle control, had effects on this interval before induction of ischaemia. MAP f-p interval increased progressively after induction of ischaemia in vehicle controls. Lidocaine accelerated and exacerbated the ischaemia-induced increase in MAP f-p interval. Five minutes after induction of ischaemia, lidocaine significantly prolonged the MAP f-p interval as compared to control (Figure 27). Tedisamil and RSD1019 had no effect relative to vehicle control (Figure 27). Data gathered at times greater than 5 minutes after the onset of ischaemia supported this differences between

Figures 24-26. The effect of lidocaine, tedisamil and RSD1019, respectively, on MAPD90% recorded from the epicardium of pentobarbital anaesthetised rabbits before and after induction of ischaemia. The top panel shows original records which illustrate the effect of each drug on MAPs. The lower panel summarises the group mean data. The open bars show mean MAPD90% \pm SD, n=42, for control rabbits before vehicle treatment (Pre-drug), before occlusion and after 5 minutes of vehicle infusion (Pre-Ischaemia), as well as the value 5 minutes after occlusion (+5' Ischaemia). For treated groups, the hatched and filled bars represent MAPD90% \pm SEM, n=7.

The following drugs and doses (in μ mol/kg/min) were tested: lidocaine, 2.5, 5 and 10, Figure 24; tedisamil, 0.063, 0.125 and 0.25; Figure 25; and RSD1019, 2, 4 and, 8, Figure 26. Each treatment was infused continuously starting 5 minutes before coronary artery occlusion and maintained for the duration of the experiment. Statistical significance was determined by ANOVA ($p < 0.05$) followed by a Tukey test for differences. The asterisk (*) indicates statistical significance between the +5'-ischaemia value and the respective pre-drug and pre-ischaemia values as well as versus vehicle control. The symbol (@) indicates a difference between the pre-drug and pre-occlusion values while the symbol (#) indicates a difference between groups at that time point. No difference was found between the pre-occlusion and the +5 minute ischaemia value of MAPD90% for the 8 μ mol/kg/min dose of RSD1019 (Figure 26).





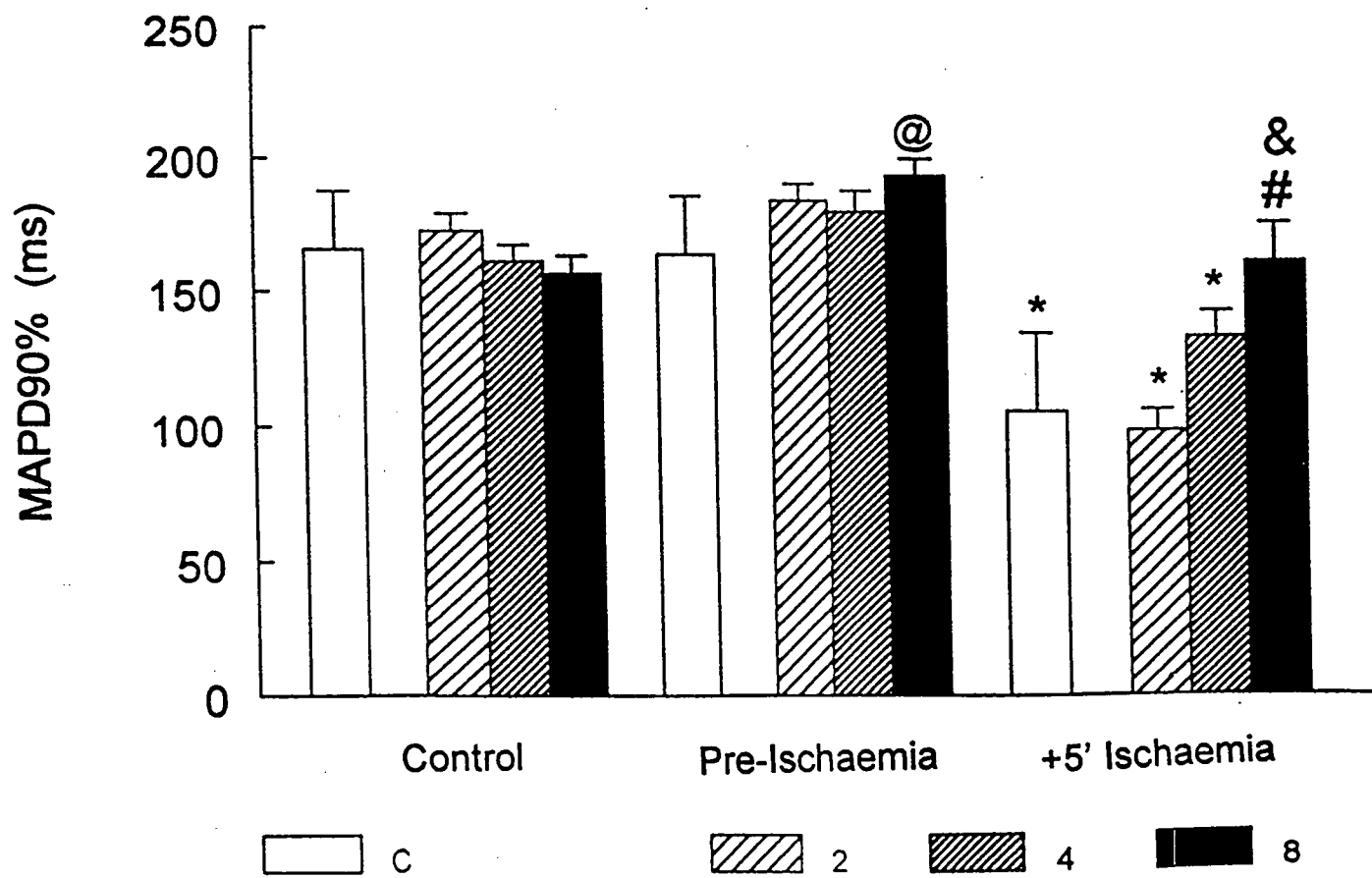
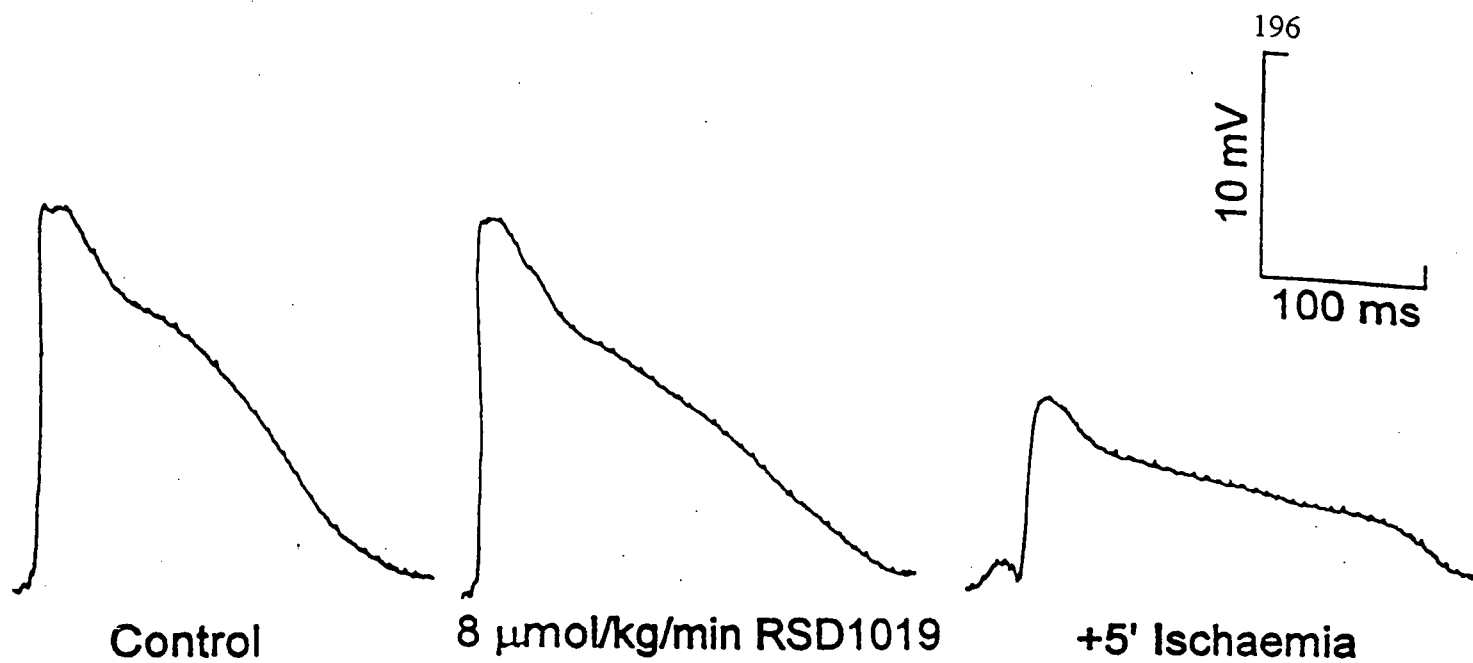
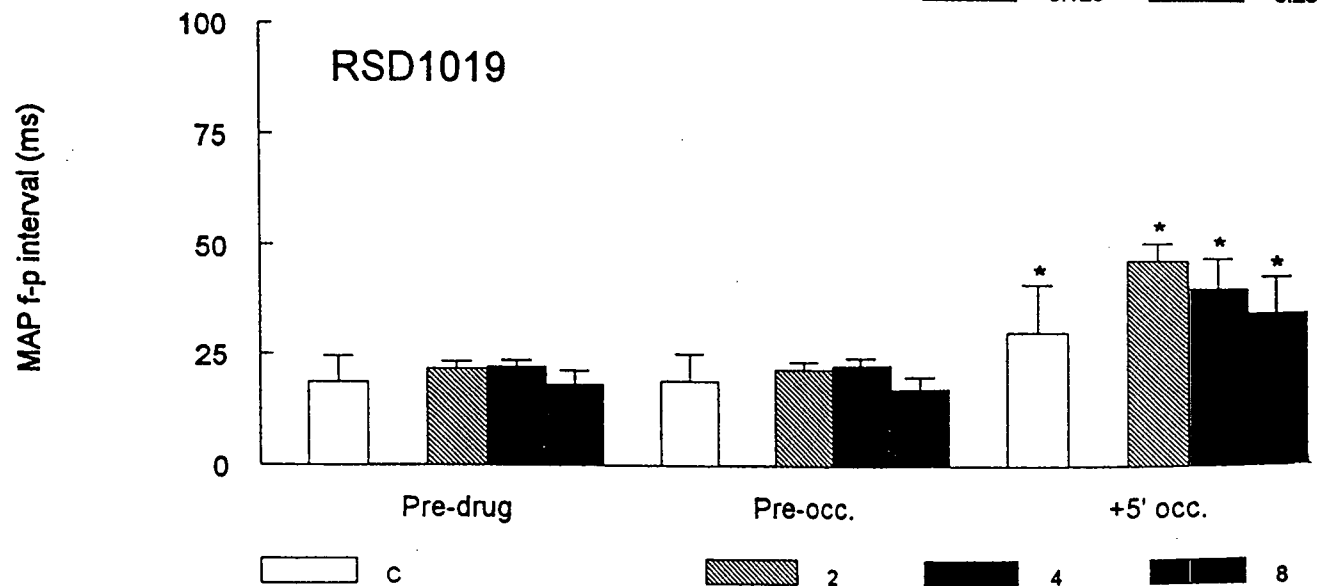
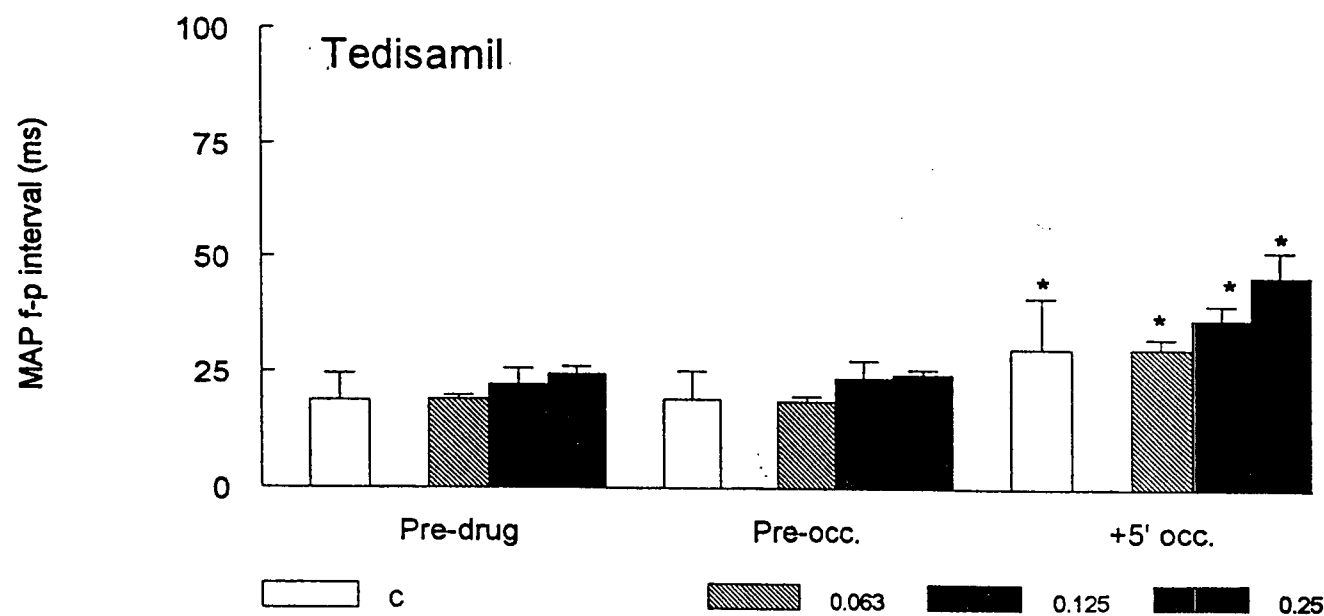
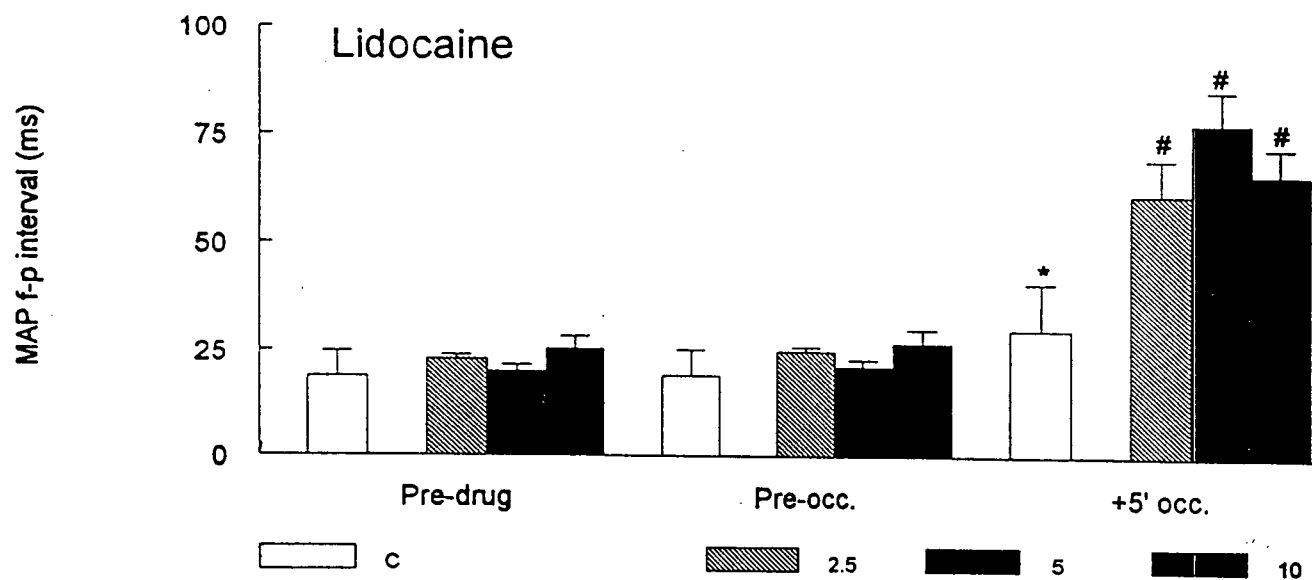


Figure 27. The effects of lidocaine, tedisamil and RSD1019 on the MAP f-p interval before and after induction of ischaemia. The open bars show the mean MAP f-p interval \pm SD, n=42, for control rabbits before vehicle treatment (Pre-drug), before occlusion and after 5 minutes of vehicle infusion (Pre-Isch.), as well as the value 5 minutes after occlusion (+5' Isch.). For treated groups, the hatched and filled bars represent the mean \pm SEM, n=7. The following drugs and doses (μ mol/kg/min) were tested: A) lidocaine, 2.5, 5 and 10, top panel; B) tedisamil, 0.063, 0.125 and 0.25, middle panel; and C) RSD1019, 2, 4 and, 8, bottom panel. The dose of each drug is also indicated next to the bar with shading that corresponds to the MAP f-p intervals for each group. Each treatment was infused continuously starting 5 minutes before coronary artery occlusion and maintained for the duration of the experiment. Statistical significance was determined by ANOVA ($p < 0.05$) followed by a Tukey test for differences. The asterisk (*) indicates statistical significance between the +5'-ischaemia value and the respective pre-drug and pre-ischaemia values, as well as versus vehicle control. The symbol (#) indicates a difference between lidocaine treated groups and all other groups 5 minutes after occlusion.



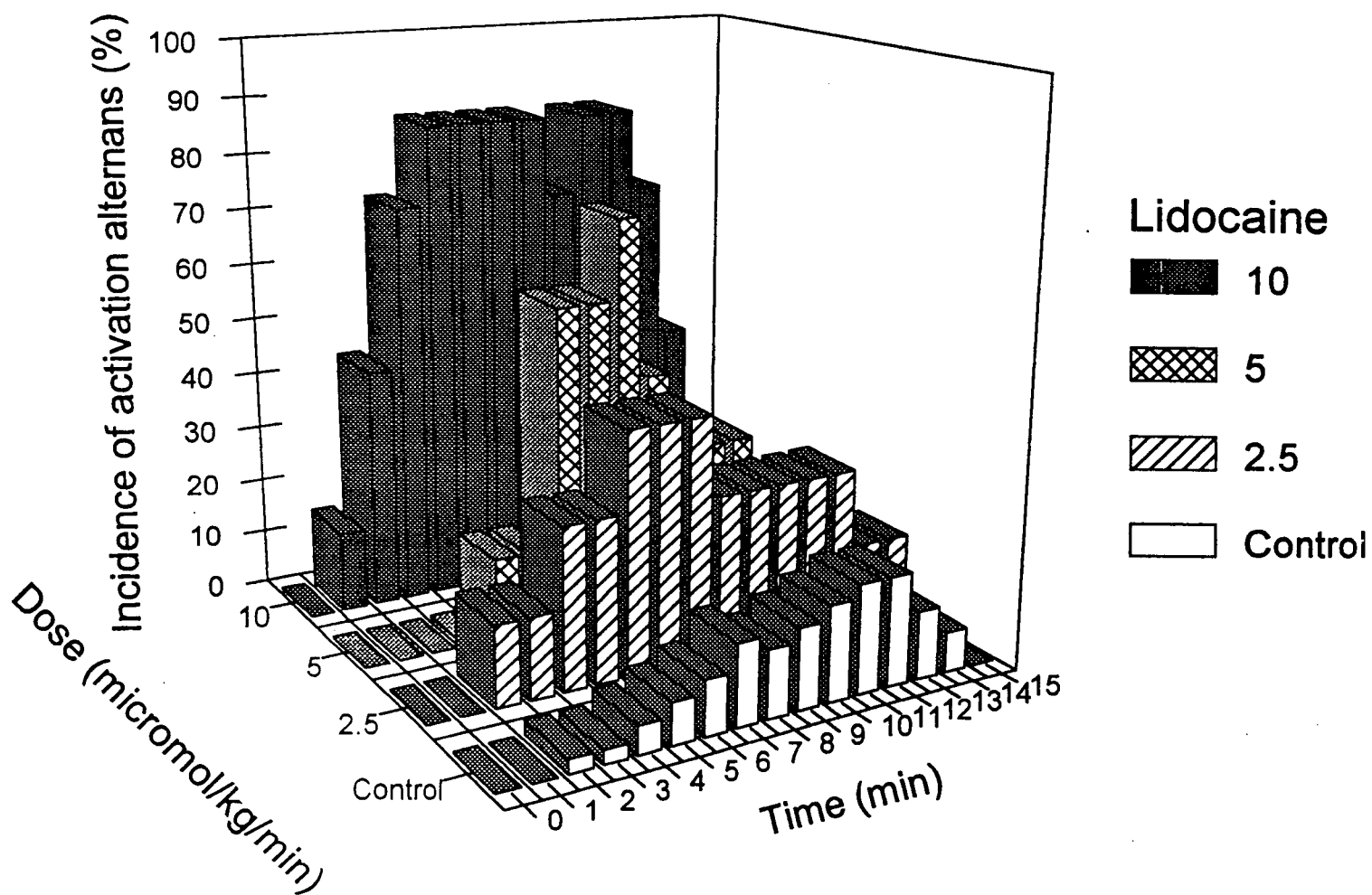
drugs. These data were not subjected to statistics due to a potential censoring bias (see methods and later).

6.7.3 Effect of lidocaine, tedisamil and RSD1019 on activation alternans.

Time dependent changes in MAP morphology were observed after induction of ischaemia. These changes included the occurrence of alternans in MAP duration, activation alternans and multiple MAP morphologies. Departures from smooth phase 3 repolarisation (see Figure 18, 30 seconds after induction of ischaemia), defined as EADs by some, were observed in most rabbits after induction of ischaemia. These abnormalities in MAP morphology were short lived and were never observed after the first minute of ischaemia. In vehicle controls the occurrence of activation alternans increased with time after induction of ischaemia. Activation alternans were first observed 2 minutes after the induction of ischaemia and peaked after 10 minutes (Figure 28). Fifteen minutes after induction of ischaemia, and times thereafter, MAPs were no longer recognisable as such. Multiple MAP wave forms were rarely observed in vehicle controls. When they were observed they occurred transiently between activation alternans and MAPs becoming unrecognisable as such.

Lidocaine increased the occurrence of activation alternans and reduced the latency to their occurrence (Figure 28). Activation alternans quickly become more complex such that multiple MAP wave forms were commonly observed in lidocaine treated rabbits (see Figure 19). Multiple wave forms were observed in all lidocaine treated rabbits while they were only observed in 2 of 42 control rabbits ($p < 0.05$). Tedisamil and RSD1019 had no

Figure 28. The temporal distribution of activation alternans in MAP recordings and the effect of lidocaine on this distribution. The bars represent the percentage of recordings in which activation alternans were observed in that minute. The dose of lidocaine (in $\mu\text{mol/kg/min}$) is indicated next to the bar with the shading that corresponds to the temporal distribution of activation alternans for each group. Each treatment was infused continuously starting 5 minutes before coronary artery occlusion and maintained for the duration of the experiment. The time to the first occurrence of activation alternans in the control group was 8.0 ± 1.0 minutes (mean \pm SEM, $n=12$) while in the 2.5, 5 and 10 $\mu\text{mol/kg/min}$ lidocaine groups it was 6.0 ± 0.6 ($n=5$), 5.2 ± 1.0 ($n=5$) and 2.3 ± 0.4 ($n=7$) minutes, respectively. The median time after occlusion to the occurrence of activation alternans in MAPs recorded from vehicle control rabbits was 7 minutes; in rabbits treated with 2.5, 5 and 10 $\mu\text{mol/kg/min}$ lidocaine this value was reduced to 6, 6 and 2 minutes, respectively.



effect on the occurrence nor the time distribution of activation alternans and multiple MAP wave forms were not observed.

6.7.4 Effect of lidocaine, tedisamil and RSD1019 on the rate of ischaemia-induced MAP shortening.

Tedisamil, at a dose of 0.125 $\mu\text{mol/kg/min}$, significantly increased the initial rate of ischaemia-induced MAP shortening (Table 11). This effect was not dose-related. The effects of RSD1019 on this variable were difficult to assess as the data are statistically censored; ischaemia did not cause MAP shortening in all rabbits treated with RSD1019. The available data suggest that RSD1019 did not influence the initial rate of ischaemia-induced MAP shortening but rather prevented MAP shortening after this initial change. Lidocaine had no effect on the rate of ischaemia-induced MAP shortening.

6.7.5 Effect of lidocaine, tedisamil and RSD1019 on ischaemia-induced arrhythmias.

Ischaemia-induced arrhythmias were differentially influenced by lidocaine, tedisamil and RSD1019 (Table 12). Lidocaine increased the occurrence VT and VF and reduced the latency to the occurrence of VF (Table 12; Figure 29). Neither of these effects appeared to be dose-related over the dose range tested. The proarrhythmic effects of lidocaine were seen as a significant increase in the arrhythmia score. Tedisamil increased the occurrence of VT at the lowest dose tested (0.063 $\mu\text{mol/kg/min}$) but was without significant antiarrhythmic effects at any dose. The proarrhythmic actions of the low dose of tedisamil occurred within the first 2 minutes of ischaemia. In control rabbits,

Table 11. The effect of lidocaine, tedisamil and RSD1019 on the maximum rate of ischaemia-induced MAP shortening.

	-dAPD/dt (ms/s)
Control	5.7±4.1
<hr/>	
Drug-dose (μmol/kg/min)	
Lidocaine-2.5	4.0±1.2
Lidocaine -5	3.1±1.4
Lidocaine -10	6.0±1.0
Tedisamil-0.063	8.4±1.9
Tedisamil -0.125	11.4±2.5*
Tedisamil -0.25	6.5±2.1
RSD1019-2	4.0±1.4
RSD1019-4	4.2±1.3
RSD1019-8	3.8±0.8

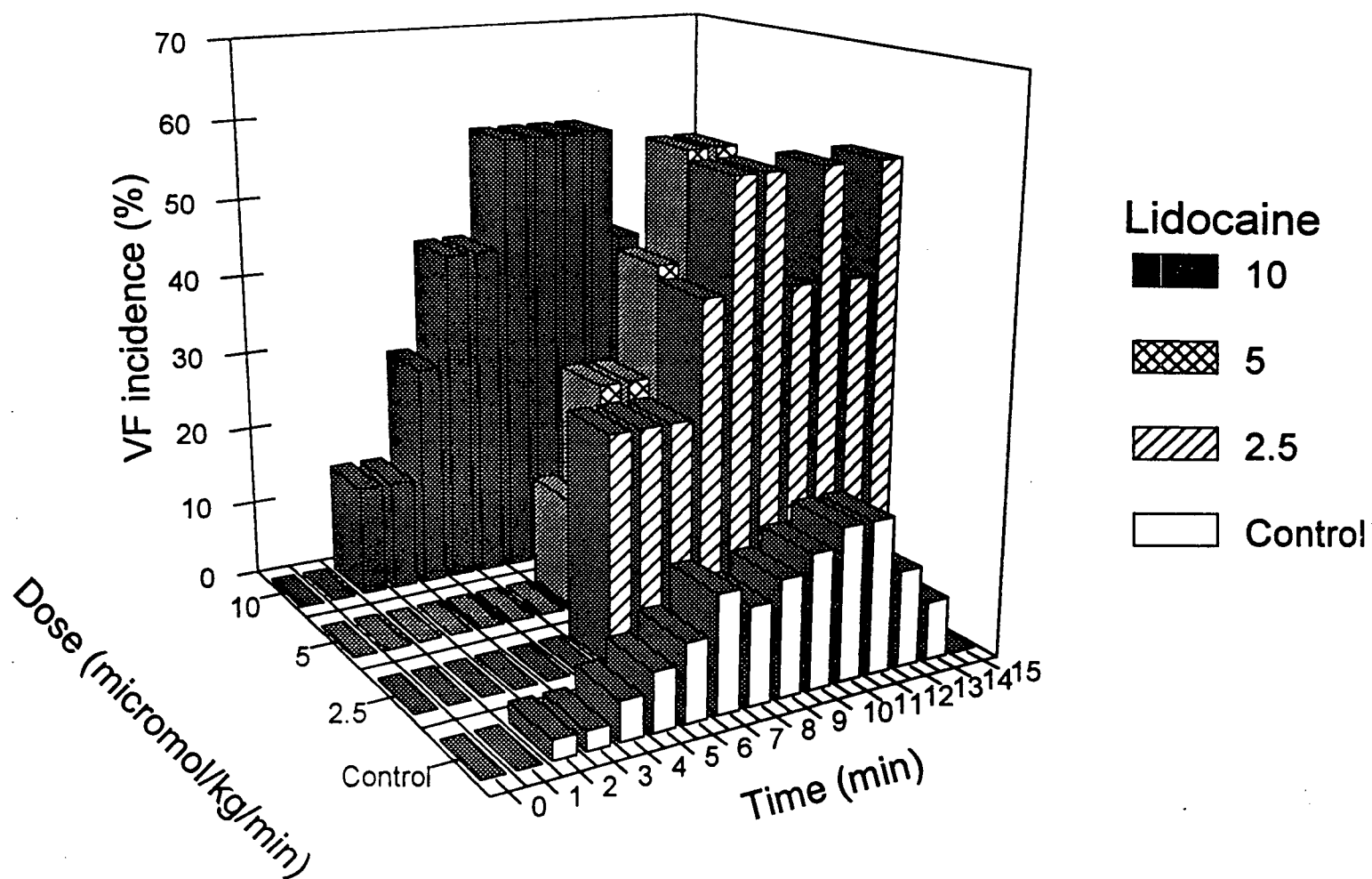
Table 11 shows the effects of infused doses of lidocaine, tedisamil and RSD1019 on the maximum rate of ischaemia-induced MAP shortening in pentobarbital anaesthetised rabbits. The value for vehicle controls is the mean±SD, n=39, while values for treated groups are the mean±SEM, n=7. Statistical significance was tested using ANOVA at a significance level of $p<0.05$ followed by Dunnett's test for differences. Significant differences from control are indicated with an asterisk (*).

Table 12. Summary of the effects of lidocaine, tedisamil and RSD1019 on ischaemia-induced arrhythmias in pentobarbital anaesthetised rabbits.

ISCHAEMIA-INDUCED ARRHYTHMIAS							
Drug-Dose	[K ⁺]	OZ%	AS	VPB	VT	VF	Latency to VF
Vehicle control	2.5±0.5	50±7	2.6±2.1	39/42	24/42	10/42	14.5±1.4
Lidocaine, 2.5	3.1±0.3	53±2	5.5±0.7*	7/7	6/7*	7/7*	8.4±1*
Lidocaine, 5	2.4±0.2	44±3	4.0±1.0	5/7	6/7*	5/7*	9.3±1.7*
Lidocaine, 10	2.7±0.2	51±2	5.4±1.0*	7/7	6/7*	6/7*	9.1±1.9*
Tedisamil, 0.063	2.4±0.1	50±2	4.4±1.0	7/7	6/7*	3/7	10.6±5.2
Tedisamil, 0.125	2.4±0.3	48±3	1.7±0.7	7/7	1/7	3/7	14.9±1.8
Tedisamil, 0.25	2.2±0.2	46±1	1.8±0.7	6/7	2/7	4/7	12.6±3.4
RSD1019, 2	2.6±0.3	52±1	1.9±0.6	7/7	4/7	1/7	15.3
RSD1019, 4	2.8±0.3	48±3	0.2±0.2*	4/7	0/7	0/7	/
RSD1019, 8	2.4±0.2	52±1	0.0±0.0*	6/7	0/7	0/7	/

Table 12 summarises the antiarrhythmic actions of lidocaine, tedisamil and RSD1019 in pentobarbital anaesthetised rabbits. Data for blood [K⁺] (mM), occluded zone size (OZ%) and arrhythmia score (AS) are shown as the mean±SD, n=42 for controls or mean±SEM, n=7 for treated groups. The arrhythmia score data was analysed using ANOVA followed by Dunnett's test for differences. Arrhythmia incidence data are shown as the number of rabbits having the arrhythmia over the total number in the group. The latency to the occurrence of VF, in minutes, is expressed as the mean±SEM for the number of rabbits in which the arrhythmia occurred. Arrhythmia occurrence data was analysed using Fisher's exact test. The symbol (*) indicates a statistically significant difference from vehicle control at p<0.05.

Figure 29. The effect of lidocaine on the temporal distribution of ischaemia-induced VF in pentobarbital anaesthetised rabbits. The bars represent the percentage of rabbits which had VF in that minute. Lidocaine, at doses of 2.5, 5 or 10 $\mu\text{mol/kg/min}$, or vehicle control was infused continuously starting 5 minutes before the induction of ischaemia and continued throughout. The dose of lidocaine is also indicated next to the bar with the shading that corresponds to the temporal distribution of VF in that group.



10 of 42 had VT and none had VF within this time period. Over the same time period VT occurred in 4 of 7 rabbits treated with 0.063 $\mu\text{mol/kg/min}$ tedisamil ($p < 0.08$, NS) and 1 of 7 had VF. RSD1019 completely prevented the occurrence of VT and VF at doses of 4 and 8 $\mu\text{mol/kg/min}$. These antiarrhythmic effects were reflected as a significant reduction in the arrhythmia score. The latency to the occurrence of VF (in the rabbit in which it occurred) was similar to that observed in vehicle controls.

6.7.6 Exclusions.

Rabbits not meeting the inclusion criteria were excluded and replaced. All exclusion were made on the basis of severe hypotension after coronary artery occlusion. In all, 12 animals were excluded from the following groups: 2, 1 and 3 rabbits from the 2.5, 5 and 10 $\mu\text{mol/kg/min}$ lidocaine groups, respectively; 1 in the 0.25 $\mu\text{mol/kg/min}$ tedisamil group; 1, 1 and 3 from the 2, 4, and 8 $\mu\text{mol/kg/min}$ RSD1019 groups, respectively. More lidocaine and RSD1019 treated rabbits were replaced than control or tedisamil treated animals.

6.7.7 Sham occlusion experiments.

Infusion of the highest doses of lidocaine, tedisamil and RSD1019 tested in the occlusion studies had similar effects on sham occluded rabbits (Table 13). HR was reduced by all three drugs. Tedisamil increased BP, RSD1019 had no effect and lidocaine reduced BP. Tedisamil and RSD1019 increased the QT interval while lidocaine had no effect. Blood $[\text{K}^+]$ was similar between groups before and after the experiment.

Table 13. Summary of the effects of infused doses of lidocaine, tedisamil and RSD1019 in pentobarbital anaesthetised rabbits; sham occlusion study.

	Control		Lidocaine		Tedisamil		RSD1019	
	Pre-drug	+5'	+35'	10 $\mu\text{mol/kg/min}$ +5'	+35'	0.25 $\mu\text{mol/kg/min}$ +5'	+35'	8 $\mu\text{mol/kg/min}$ +5'
BP sys	92 \pm 9	3 \pm 3	4 \pm 6	-19 \pm 7*	-10 \pm 8	10 \pm 7	11 \pm 6	2 \pm 2
BP dia	76 \pm 8	2 \pm 3	4 \pm 5	-26 \pm 9*	-39 \pm 16*	14 \pm 7	11 \pm 5	6 \pm 3
HR	221 \pm 18	2 \pm 1	2 \pm 2	-6 \pm 1	-9 \pm 1	-7 \pm 8	-21 \pm 13	-13 \pm 3
PR	83 \pm 9	0 \pm 5	0 \pm 6	11 \pm 3	-6 \pm 2	7 \pm 4	8 \pm 3	-1 \pm 3
QRS	38 \pm 5	4 \pm 4	4 \pm 2	1 \pm 3	9 \pm 3	8 \pm 2	8 \pm 3	5 \pm 3
QTa	126 \pm 34	-4 \pm 3	-3 \pm 4	8 \pm 5	13 \pm 8	20 \pm 3*	19 \pm 2*	11 \pm 7
QTac	241 \pm 63	-3 \pm 3	-2 \pm 4	5 \pm 5	9 \pm 8	17 \pm 5	11 \pm 6	6 \pm 7
QT	168 \pm 32	-4 \pm 2	0 \pm 1	5 \pm 3	3 \pm 4	21 \pm 3*	24 \pm 2	18 \pm 6*
QTc	321 \pm 59	-3 \pm 2	1 \pm 2	2 \pm 3	-1 \pm 5	18 \pm 4*	17 \pm 5	13 \pm 6*
Vmax	18 \pm 4	19 \pm 1	20 \pm 2	18 \pm 1	21 \pm 2	20 \pm 1	22 \pm 2	18 \pm 2
APA	19 \pm 5	18 \pm 2	9 \pm 2	18 \pm 2	12 \pm 1	13 \pm 1	7 \pm 1	19 \pm 3
APD90	159 \pm 13	163 \pm 5	157 \pm 6	153 \pm 3	156 \pm 6	206 \pm 16*	240 \pm 20*#	206 \pm 5*
MAP F-P	14 \pm 6	11 \pm 2	8 \pm 2	14 \pm 5	12 \pm 5	10 \pm 2	9 \pm 2	11 \pm 1
[K ⁺]	2.5 \pm 0.4	NM	2.4 \pm 0.1	NM	2.1 \pm 0.3	NM	2.1 \pm 0.1	NM
								2.2 \pm 0.2

Table 13 shows the effects lidocaine, tedisamil and RSD1019 on BP, HR, ECG intervals and MAP variables in pentobarbital anaesthetised rabbits. The drug and doses are indicated at top of the column as are the times when variables were measured (5 and 30 minutes after commencing infusion). The abbreviations are the same as those used in Tables 10. No differences were found in the pre-drug values (ANOVA, $p>0.05$) and pre-drug values are presented as the mean \pm SD ($n=20$). Values for each group are expressed as the mean percent change \pm SEM ($n=5$) while the MAP data is presented as the mean \pm SEM. Statistical significant was tested using ANOVA at a significance level of $p<0.05$ followed by Tukey's test for differences. Significant differences from control are indicated with an asterisk (*) while differences between the 5 and 35 minute time points are noted with the (#) symbol.

MAP amplitude, maximum upstroke velocity and foot to peak interval did not differ significantly between groups at any of the time points measured. MAP f-p interval did not change in a time dependent fashion (Table 13 & Figure 27). RSD1019 and tedisamil produced greater MAPD90% widening after 35 minutes of infusion as compared with 5 minutes. However, the extent of MAP widening produced by RSD1019 and tedisamil was similar at both time points. Judged from the pharmacological effects, it appears that all three drugs were accumulating at the highest infusion rates.

Tedisamil had proarrhythmic actions in the sham occlusion experiments. One of 5 rabbits infused with 0.25 $\mu\text{mol/kg/min}$ tedisamil had VT while 4 had PVBs. Arrhythmias were not observed in the lidocaine or RSD1019 treated rabbits. Two of 5 rabbits infused with 10 $\mu\text{mol/kg/min}$ lidocaine suffered severe hypotension and death. In these cases death occurred between 15 and 20 minutes after starting infusion.

6.8 Summary.

Despite prolonging MAP duration and the QT interval of the ECG, tedisamil failed to prevent ischaemia-induced arrhythmias. In fact, tedisamil had proarrhythmic actions in the occlusion and sham occlusion experiments. Lidocaine had proarrhythmic actions after the induction of ischaemia and reduced the latency to VF. These proarrhythmic actions were associated with the following changes in MAPs recorded from ischaemic tissue: 1) the latency to the occurrence of activation alternans was reduced and their occurrence increased, 2) activation alternans degenerated into multiple MAP wave forms, and 3) ischaemia-induced increases in the MAP f-p interval were accelerated and increased.

RSD1019 effectively prevented ischaemia-induced arrhythmias in anaesthetised rabbits. MAP duration in normal tissue was prolonged, however, RSD1019 also suppressed ischaemia-induced MAP shortening. After 5 minutes of ischaemia 8 $\mu\text{mol/kg/min}$ RSD1019 prolonged MAP duration relative to controls. The ischaemia-selective MAP prolonging effects of RSD1019 were unique among the drugs tested and may be related to its ability to suppress ischaemia-induced arrhythmias. Selective AP prolongation in ischaemia tissue represents a novel approach to suppression of arrhythmias.

7.0 Discussion.

7.1 Ischaemia-induced electrophysiological changes and arrhythmias in anaesthetised rabbits.

The following sections will discuss the characteristics of the anaesthetised rabbit as used for the study of ischaemia-induced arrhythmias. This will be followed by a detailed discussion of the drug studies performed in this preparation. A specific discussion of the use of MAPs as a tool to study the electrophysiological effects of myocardial ischaemia and the action of drug thereon will be presented. This will include a discussion of the measurement of drug effects under conditions of ischaemia and the interpretation of such effects. Following the discussion of studies performed in rabbits, the mechanism(s), advantage(s) and pitfall(s) of ischaemia-selectivity as an antiarrhythmic principle will be discussed. Finally general conclusions will be drawn.

7.1.1 Use of anaesthetised rabbits for studying ischaemia-induced arrhythmias.

Since Cooker's original description (1989) of ischaemia-induced arrhythmias in pentobarbital anaesthetised rabbits, a number of groups have used this preparation for various purposes (Bril *et al.*, 1991, 1994; Chakrabarty *et al.*, 1991; Holbrook & Cooker 1989, 1991; Burton *et al.*, 1992; Farkas *et al.*, 1996). The reported incidence of VF varied widely; ranging from 27 to 71% ($50 \pm 15\%$, mean \pm SD). The large variability between studies highlights the need for a randomised, blind experimental design and the absolute requirement for concurrent controls. Cooker's original paper noted that spontaneous reversion of VF to sinus rhythm did not occur in rabbits not treated with antiarrhythmic drugs. However, spontaneous reversion of VF has been reported by others (Bril *et al.*, 1991, 1994) and was commonly observed in the present study. Subsequent studies by Cooker's group showed that spontaneous reversion from VF did occur in control rabbits (Holbrook & Cooker 1989, 1991).

Ischaemia-induced arrhythmias are known to occur in distinct phases (Kaplinsky *et al.*, 1979; Russel *et al.*, 1984; Curtis *et al.*, 1987; Smith *et al.*, 1995). In this regard the anaesthetised rabbit preparation is no different. Cooker (1989) reported that VF occurrence peaked between 8 and 12 minutes after the onset of ischaemia and was reduced thereafter. This suggests that a single arrhythmic phase occurs in the rabbit, although this was not explicitly stated. Others find a similar time profile for the occurrence of VF (Bril *et al.*, 1991, 1994). VPBs could occur any time during the ischaemic period but tended to peak between 2-3, and 8-12, minutes after the onset of ischaemia. Thus my studies paralleled those in the literature in terms of the time of

arrhythmia occurrence. The occurrence of VF peaked between 8 and 12 minutes and waned thereafter. However, analysis of the time of occurrence of PVB and VT suggests that two arrhythmic phases occurred. A very early phase (0-3 minutes) followed by a second phase (8-15 minutes). Data from other investigators also show evidence of 2 arrhythmia phases after coronary artery occlusion in rabbits (see Bril *et al.*, 1994, p. 314).

The bi-model distribution of arrhythmias after the onset of ischaemia in rabbits is similar to that found in other species. In the conscious rat preparation, two arrhythmia phases are observed (Curtis, 1986; Curtis *et al.*, 1987). The first between 0-2 minutes and the second between 7-12 minutes (Curtis *et al.*, 1987). The first phase typically consists of PVBs and VT; VF rarely occurred during this phase (Curtis, 1986). In dogs, the first phase occurs between 2-4 minutes after the onset of ischaemia with the second occurring between 15-25 minutes (Kaplinsky *et al.*, 1979; Russel *et al.*, 1984). In pigs, the first phase occurs between 2-10 minutes, the second occurs between 20-40 minutes (Dilly & Lab, 1988).

A number of factors are known to influence the occurrence of ischaemia-induced arrhythmias including occluded zone size, blood $[K^+]$ and the anaesthetic used. Despite its known electrophysiological actions on the heart, pentobarbital is commonly employed as an anaesthetic (Nattel *et al.*, 1990). In rabbits, no difference between anaesthetic agents, at least those tested, has been found (Chakrabarty *et al.*, 1991). These authors also noted that pentobarbital gave a consistent heart rate throughout occlusion studies. This was considered an advantage for the present studies.

In a series of untreated rabbits, occluded zone size was found to correlate in a linear fashion with the arrhythmia score. Large occluded zone sizes were associated with higher arrhythmia scores (i.e., an increase in the severity, incidence and duration of arrhythmias). In the conscious rat preparation, the arrhythmia score correlated better with the square root of the occluded zone size than with the raw data (Curtis *et al.*, 1987). This observation suggests that the surface area between the ischaemic and normal tissue is a major determinant of the incidence of ischaemia-induced arrhythmias. In the anaesthetised rabbit preparation, this nuance was not observed and these data neither support nor refute Curtis and co-workers (1987) observation.

Blood $[K^+]$ is known to influence ischaemia-induced arrhythmias with hypokalaemia being associated with an increased incidence and severity of arrhythmias (Curtis & Hearse, 1989; Curtis, 1991; Saint *et al.*, 1992). Under pentobarbital anaesthesia and artificial ventilation, rabbits in the present study were hypokalaemic (blood $[K^+]$ =2.6 mM versus 4.0 mM in the conscious state). This effect might be mediated by the increased sympathetic tone found in acutely prepared, pentobarbital anaesthetised animals (Lazzara *et al.*, 1978; also see Paletta *et al.*, 1989). Adrenaline is known to reduce blood $[K^+]$ by stimulating β_2 adrenoceptors on skeletal muscle (Hohnloser *et al.*, 1986; Kaltofen *et al.*, 1990). The effects of hypokalaemia do not have a major bearing on the interpretations of these studies as such effects were evenly distributed throughout all groups. A resting hypokalaemia, hitherto unreported, is a consistent feature in pentobarbital anaesthetised rabbits (Barrett & Walker, 1997, 1998; SJ Cooker, personal communication, 1998).

Interestingly, *in vitro* preparations used to study arrhythmias in the rabbit typically use a lower than normal $[K^+]$ (e.g., 2.5-3.0 mM; e.g., Curtis, 1991; Fagbemi *et al.*, 1993; Rees & Curtis, 1993a,c; Chi *et al.*, 1996). Thus, even *in vitro* a $[K^+]$ between 2.5-3.5 mM is a consistent feature of preparations used to study ischaemia-induced arrhythmias in rabbits.

In short, the characteristics of the pentobarbital anaesthetised rabbit preparation used for the studies presented are comparable to those published in the literature. Ischaemia-induced arrhythmias in the rabbit occur in two phases and VF incidence varies greatly between studies. Ischaemia-induced VF in rabbits commonly spontaneously reverts to sinus rhythm but may also be sustained (and fatal). Blood $[K^+]$ is lower than expected in the pentobarbital anaesthetised rabbit. The reason for this is not clear but it appears to be a consistent feature of pentobarbital anaesthetised rabbits.

7.2 Use of MAPs to study the electrophysiological changes caused by ischaemia.

MAPs have been extensively used to study repolarisation processes in experimental and clinical investigations (see reviews by Franz, 1991; Mohabir *et al.*, 1991; Zipes, 1991; Xie & January, 1993; Yuan *et al.*, 1994). Lab and Woollard (1978) first used MAPs to study the electrophysiological consequences of ischaemia. Since then, many other groups have used the technique for both experimental (e.g., Lab & Woollard, 1978; Franz *et al.*, 1986) and clinical studies (see Yuan *et al.*, 1994). Intracellular recordings have been used as the "gold standard" to validate MAPs. Under conditions of ischaemia, MAPs have been shown to faithfully parallel intracellular recordings (Hoffman *et al.*, 1959; Franz *et*

al., 1986; Ino *et al.*, 1988). Automation of MAP analysis by computer software, such as MAPPER© (Dickenson *et al.*, 1997), allows time dependent changes in the electrophysiological behaviour of myocardial tissue to be easily followed.

The above properties make MAP recordings suitable for assessing the time-dependent electrophysiological changes caused by ischaemia. Despite their utility, relatively few studies have employed this recording technique to assess the effect of drugs on ischaemic myocardial tissue (e.g., Smallwood *et al.*, 1990; Timour *et al.*, 1991; Aupetit *et al.*, 1995, 1997).

7.3 Actions of glibenclamide in the anaesthetised rabbit preparation.

7.3.1 Effect of glibenclamide on blood glucose concentration.

The glucose lowering actions of glibenclamide observed in this study were both dose- and time-dependent. In the occlusion studies, the expected glucose lowering actions of glibenclamide appear to have been masked by a time dependent increase in blood glucose associated with the experimental model (i.e., present also in the control group). Paradoxically, treatment with glibenclamide appeared to increase the blood glucose concentration. The observation that basal and glucose-stimulated insulin secretion is reduced by anaesthesia (Halter & Pflug, 1980) might explain why the glucose lowering response expected was blunted; however, this observation cannot account for the apparent *reversal* of glibenclamide's action. The reason for this reversal is not clear. As a result of the time dependent increase in blood glucose, only the 24 mg/kg dose of glibenclamide

reduced blood glucose 50 minutes after administration relative to control. This result must be interpreted cautiously as these rabbits suffered severe hypotension and are therefore not directly comparable to the other groups. The relevance of the apparent lack of glibenclamide's glucose lowering action to its antiarrhythmic actions is likely to be negligible, since significant decreases in blood glucose concentration required a time to manifest that was longer than the observation period for the arrhythmia study.

The mechanism of the time dependent increase in blood glucose concentration is not clear. Anaesthesia is known to reduce insulin secretion (Halter & Pflug, 1980) and cause hyperglycaemia (Fahmy & Battit, 1975; Halter & Pflug, 1980). It is also noteworthy that sympathetic tone is increased in pentobarbital anaesthetised, open chested preparations (Lazzara *et al.*, 1978). Beta-adrenoceptors stimulation increases blood glucose concentration via stimulation of glycolysis (see Hoffman & Lefkowitz, 1996). In support of the latter suggestion is the observation that blood glucose concentration did not increase as a function of time in rabbits in which a sternal split was not performed (i.e., rabbits in the hypoglycaemia assay). These rabbits can be expected to have less sympathetic activation due to the lesser degree of surgical stimulus. Blood glucose also did not increase in the separate study with 0.3 mg/kg glibenclamide. The reason for these differences is not clear.

7.3.2 Ischaemia selective Class III antiarrhythmic actions of glibenclamide.

The influence of glibenclamide on ischaemia-induced changes in the electrophysiology of myocardial tissues *in vivo*, as measured with MAPs, were not

marked. Glibenclamide (3-24 mg/kg iv), had no effect on the maximum upstroke velocity, nor the amplitude of MAPs, before or after occlusion but caused a dose-related decrease in the rate at which ischaemia caused shortening of epicardial MAPs. This finding can be viewed as a limited ischaemia-selective Class III antiarrhythmic action. Thus, the electrophysiological actions of glibenclamide are attributable to $I_{K(ATP)}$ block, since $I_{K(ATP)}$ is only activated under conditions of ischaemia (Wilde & Janse, 1994). Nevertheless, this action of glibenclamide was rapidly lost during the following minutes.

Others have noted that modulation of $I_{K(ATP)}$ influences the rate of ischaemia-induced MAP shortening (Moritani *et al.*, 1994; Miyoshi *et al.*, 1996). During short episodes of ischaemia (5 minutes) 5-hydroxydecanoate suppressed, while nicroandil increased, ischaemia-induced MAP shortening (Miyoshi *et al.*, 1996). Ischaemia caused epicardial MAPs to shorten more than endocardial MAPs. Epicardial MAPs were also more sensitive to $I_{K(ATP)}$ modulation. These observations support the notion that $I_{K(ATP)}$ participates in the early stages of ischaemia-induced AP shortening.

The results of this study do not agree with the findings reported by Smallwood and co-workers (1990). They reported that 3 mg/kg glibenclamide iv prevented ischaemia-induced shortening of endocardial MAPs 5 minutes after the onset of ischaemia in rabbits. There are two major differences between the Smallwood *et al.* (1990) study and the present one. The former study employed multiple episodes of ischaemia and recordings were made from the endocardium. While MAP duration returned to base line values before subsequent episodes of ischaemia in the Smallwood *et al.* (1990) study the effects of episode(s) of ischaemia on electrophysiological responses to subsequent episodes of

ischaemia cannot be discounted. The "protective" effects of ischaemic preconditioning reduce biochemical, electrophysiological and arrhythmic responses to subsequent episodes of ischaemia (see Yao & Gross, 1994). Differences in the recording site are unlikely to explain the opposite results obtained as the epicardium is more sensitive to the electrophysiological effects of ischaemia (Taggart *et al.*, 1988; Moritani *et al.*, 1994; Miyoshi *et al.*, 1996) while Smallwood *et al.* (1990) reported that glibenclamide prevented ischaemia-induced MAP shortening in the endocardium.

Most of the evidence supporting the view that $I_{K(ATP)}$ blockers have ischaemia-selective AP prolonging effects has been obtained *in vitro*, and may not accurately reflect actions *in vivo*. Despite the reported effectiveness of $I_{K(ATP)}$ blockers in preventing AP shortening caused by hypoxia or metabolic poisoning (e.g., Fosset *et al.*, 1988; Gasser & Vaughan-Jones, 1990; Cole *et al.*, 1991; Furukawa *et al.*, 1991; Nakaya *et al.*, 1991; MacKenzie *et al.*, 1993; Tweedie *et al.*, 1993), the effect is transient and incomplete even *in vitro* (Cole *et al.*, 1991; Ruiz-Petrich *et al.*, 1992; Findlay, 1993; Moritani *et al.*, 1994). Venkatesh *et al.* (1991) have shown that the $I_{K(ATP)}$ blocking actions of sulfonylureas are sensitive to the conditions used to simulate ischaemia, whereby conditions that more closely resemble *in vivo* conditions diminished the effectiveness of the drugs. Findlay (1993) reported that sulfonylureas were effective blockers of $I_{K(ATP)}$ induced by moderate metabolic inhibition but blockade was reduced as the degree of metabolic inhibition increased. Surprisingly, sulphonylurea drugs have been reported to **activate** $I_{K(ATP)}$ expressed in xenopus oocytes during metabolic stress (Guillemare *et al.*, 1995). The

blocking actions of other $I_{K(ATP)}$ blockers, such as tedisamil, were unaffected under these conditions.

In summary, the AP prolonging effects of $I_{K(ATP)}$ blockers in the myocardium under conditions of metabolic stress, hypoxia, or ischaemia are modest and dependent on a number of factors. These factors may include, but are not limited to, the severity of the metabolic challenge and the method used to produce it (e.g., ischaemia versus hypoxia).

7.3.3 Antiarrhythmic actions of glibenclamide.

Glibenclamide tended to reduce the arrhythmia score and the incidence of VF. This was statistically significant when all the glibenclamide treated groups were combined and compared to control. Other whole animal studies, using species other than rabbit, show that low doses of glibenclamide (doses used in man for the treatment of diabetes) have no effect on the occurrence of VF (Chi *et al.*, 1989). With higher doses, such as the those used in this study and by Billman *et al.* (1993), antifibrillatory actions of glibenclamide can be detected. *In vitro* studies yield conflicting results, with both positive (Wolleben *et al.*, 1989; Kantor *et al.*, 1990; Ballagi-Pordány *et al.*, 1990; Gwilt *et al.*, 1992; D'Alonzo *et al.*, 1994; Bellemin-Baurreau *et al.*, 1994) and negative reports (Ballagi-Pordány *et al.*, 1990; Adams *et al.*, 1990; Rees & Curtis, 1995) in the literature (see overview by Rees & Curtis, 1996). The results cannot be easily explained on the basis of the concentration used (see review by Rees & Curtis, 1996). It seems reasonable to conclude from such data that high doses / concentrations of glibenclamide can have weak antifibrillatory actions.

7.3.4 Mechanism for the antiarrhythmic actions of glibenclamide.

The mechanism(s) by which sulphonylurea $I_{K(ATP)}$ blockers produce their antiarrhythmic actions has not been clearly demonstrated. $I_{K(ATP)}$ blockers have been shown to slow the accumulation of extracellular K^+ during ischaemia (see Kantor *et al.*, 1990; see introduction). Slowing the accumulation of extracellular K^+ can be expected to delay but not reduce the occurrence of arrhythmias. VF occurred at similar times in vehicle control and glibenclamide treated rabbits, suggesting that this mechanism did not contribute importantly to the antifibrillatory actions observed. Various other studies also suggest that the antiarrhythmic actions of $I_{K(ATP)}$ blockers may not be mediated by $I_{K(ATP)}$ blockade in the heart. Rees and Curtis (1995) found that the antiarrhythmic effects of co-administration of the $I_{K(ATP)}$ channel opener RP49356 and glibenclamide effectively prevented ischaemia-induced VF while either drug alone was without effect. These data are clearly inconsistent with the antiarrhythmic effects of glibenclamide being mediated by $I_{K(ATP)}$ blockade. Glibenclamide has also been reported to have antiarrhythmic effects against glycoside induced arrhythmias in rabbits (Pogátga *et al.*, 1985), a situation where $I_{K(ATP)}$ is not expected to be activated (Wilde & Janse, 1994). Furthermore, the antiarrhythmic actions in ischaemia of first and second generation sulphonylurea $I_{K(ATP)}$ blockers are divergent (Pogátga *et al.*, 1988; Ballagi-Pordány *et al.*, 1990). These data, taken together, suggest that any antiarrhythmic effects of these drugs are not related to $I_{K(ATP)}$ blockade in the heart and may relate to their other pharmacological actions.

7.3.5 Actions of glibenclamide not related to blockade of $I_{K(ATP)}$.

Glibenclamide has a number of actions on the cardiovascular system unrelated to blockade of $I_{K(ATP)}$ which might be relevant to its use as a tool to study myocardial ischaemia (for a review see Schotborgh & Wilde, 1997). Typically, these effects occur at concentrations greater than those required to block $I_{K(ATP)}$ in the pancreas but are similar to the concentrations which effect the cardiovascular system (compare Zunckler *et al.*, 1988 to Findlay, 1992). Glibenclamide has been reported to block the cAMP-activated Cl^- current in cardiac myocytes (Tominaga *et al.*, 1995) and block voltage gated K^+ and Ca^{++} channels in cultured cells (Reeve *et al.*, 1992). Glibenclamide has also been shown to reduce intracellular Ca^{++} stores in rabbit bronchiole smooth muscle (Chopra *et al.*, 1992). Cook (1987) demonstrated that glibenclamide inhibited palmitoyl-transferase, a key enzyme in metabolism of fatty acids in cardiac muscle. As previously discussed, Yan *et al.* (1993), and more recently Shivkumar *et al.* (1997), showed that the efflux of K^+ from cardiac muscle and AP shortening could be dissociated from $I_{K(ATP)}$ blockade. Thus, the alterations in energy metabolism and blockade of various ion channels (other than K_{ATP}) in the heart might mediate the modest antiarrhythmic actions of glibenclamide.

7.3.6 Summary of the action of glibenclamide in the anaesthetised rabbit preparation.

Glibenclamide failed to elicit a maintained effect on MAP duration in ischaemic myocardial tissue at the time when arrhythmias occurred. The antiarrhythmic actions of glibenclamide were found to be modest. The relevance of the scant ischaemia-selective

AP prolonging effects of glibenclamide to its observed antiarrhythmic actions is tenuous at best.

7.4 Comparison of the actions of lidocaine, tedisamil and RSD1019 in the anaesthetised rabbit preparation.

Comparison of the antiarrhythmic and electrophysiological actions of lidocaine, tedisamil and RSD1019 in the anaesthetised rabbit preparation illustrates the potential benefits, as well as the risks, of ischaemia-selectivity as an antiarrhythmic approach. The actions of lidocaine in ischaemic myocardial tissue clearly lead to an undesirable end, while the lack of selectivity shown by tedisamil parallels its effect on ischaemia-induced arrhythmias. In contrast to the actions of both drugs, RSD1019's action in ischaemic tissue was associated with antiarrhythmic actions. While it is not possible to definitively prove that the antiarrhythmic actions of RSD1019 were related to its actions on ischaemic tissue (depending on your philosophical view point, see Popper 1972) these studies provide evidence that they are.

Lidocaine had profibrillatory actions in the rabbit which were associated with marked electrophysiological derangement in ischaemic myocardial tissue. Ischaemia-induced increases in the MAP f-p interval were accelerated and increased by lidocaine. The fact that this effect was not observed before induction of ischaemia, or in sham occlusion experiments, illustrates lidocaine's ischaemia-selectivity. Analysis of MAP morphology after induction of ischaemia shows that lidocaine exacerbated electrophysiological derangement. The occurrence of activation alternans in ischaemic

tissue was increased and the latency to their occurrence was reduced. Moreover, changes in MAP morphology were worsened to such an extent that MAP morphology changed on a beat to beat basis; lidocaine caused multiple MAP morphologies in ischaemic tissue. While the interpretation of the electrophysiological significance of multiple MAP morphologies is complicated (see later), an increase in their occurrence clearly represents an increase in the heterogeneity of the electrophysiological properties of the ischaemic myocardium. Thus, lidocaine's ischaemia-selective conduction slowing actions were associated with an increase in the incidence of VF and a decrease in the latency to VF.

The profibrillatory actions of lidocaine after induction of myocardial ischaemia have also been reported in the porcine heart *in vivo* (Carson *et al.*, 1986). Lidocaine/ischaemia-induced VF typically occurred very rapidly after induction of ischaemia (1-2 minutes). Concurrent epicardial activation mapping shows that lidocaine selectively slowed conduction in the ischaemic regions of the heart. Carson *et al.* (1986) did not, however, attribute the profibrillatory actions of lidocaine to ischaemia-selective conduction slowing for two reasons. First, conduction slowing was minimal during sinus beats. Second, similar conduction delays were observed with a longer duration of ischaemia in untreated pigs. In these pigs VF did not occur. Carson and colleagues comment is in fact very telling and suggests that the degree of conduction slowing in the ischaemic myocardium is not itself profibrillatory but rather some other factor is responsible. MAP recordings from the present study suggest that this other factor might be increased heterogeneity of the electrophysiological properties of the ischaemic myocardium. As the actions of lidocaine are rate- and voltage-dependent (see review by

Hondeghem & Katzung, 1984), beat to beat differences in AP morphology (i.e., AP amplitude & duration) can be expected to give rise to beat to beat variations in the degree of Na⁺ channel block produced. Once initiated, positive feedback is an inherent property of this mechanism.

Lidocaine increases the heterogeneity of conduction velocity and refractoriness in the ischaemic myocardium by producing different degrees of block and recovery from block on a beat to beat basis. The degree of Na⁺ channel block and recovery from block will depend on heart rate as well as AP duration and amplitude. These AP characteristics will in turn vary, depending on the degree of Na⁺ channel block. Example electrograms presented by Carson *et al.* (1986) show signs of increased heterogeneity in the ischaemic myocardium. This variability is particularly evident in the records which show the occurrence of VT and VT preceding VF (i.e., at higher heart rates Carson *et al.*, 1986, Figure 8). The properties of ischaemia-selective conduction slowing, and its relationship to pro- versus antiarrhythmic actions of such drugs, will be discussed later in the thesis. Other investigators have reported the profibrillatory actions of lidocaine in combination with regional myocardial ischaemia (Bergey *et al.*, 1982; Aupetit *et al.*, 1995, 1997; Yin *et al.*, 1997).

A number of other investigators have demonstrated proarrhythmic and antiarrhythmic actions for lidocaine after myocardial infarction (El-Sherif *et al.*, 1977; Fazekas *et al.*, 1994; Patterson *et al.*, 1982). These studies generally attribute the effects on arrhythmias to the drug's action on conduction and refractoriness in or near infarcted tissue.

While it is possible that the proarrhythmic actions of lidocaine are clinically significant it seems unlikely to be so. In the clinical situation, lidocaine is always given after the onset of ischaemia and/or infarction. Thus, the first arrhythmic phase caused by ischaemia has already passed. The work of Bergey and co-workers (1982) supports this idea. They found that lidocaine was proarrhythmic when given before coronary artery occlusion, but had no effect when given after occlusion.

Lidocaine shortened the QT interval of the ECG compared to controls only when it was corrected for heart rate. While lidocaine is known to shorten AP duration *in vitro* (e.g., Colatsky, 1982), *in vivo* this effect is counteracted by the drug's bradycardic actions. Bradycardia tends to prolong AP duration and therefore the QT interval of the ECG (Bazett, 1920).

Tedisamil effectively prolonged AP duration in normal tissue but had no significant effect on ischaemia-induced arrhythmias in this species. Its antifibrillatory actions have been reported in rabbits (Chi *et al.*, 1996) and other species (Beatch *et al.*, 1991; Adaikin *et al.*, 1992; Wallace *et al.*, 1995). The doses / concentrations producing antifibrillatory actions were higher and resulted in greater AP widening than that seen in the present study. In the rat, antifibrillatory doses of tedisamil increased the QT interval by 200 to 600%! In comparison, the highest dose of tedisamil tested in rabbits (0.25 $\mu\text{mol/kg/min}$) prolonged MAPD90% and the QT interval of the ECG by less than 30%.

Chi *et al.* (1996) demonstrated tedisamil's antifibrillatory actions in isolated rabbit hearts at concentrations that produced similar prolongation of AP duration to that observed in the present study. This might be explained by the observation that this group

did not consider the bradycardic actions of tedisamil. The antiarrhythmic actions of AP prolongation can also be described in terms of a reduction in the duration of the vulnerable period during the cardiac cycle. Prolonging the cardiac cycle (i.e., bradycardia) increases the duration of the vulnerable period. Thus, tedisamil's bradycardic actions counter-act the potential antiarrhythmic actions conferred by AP prolongation. In the preparation used by Chi *et al.* (1996), arrhythmias were induced by activation of $I_{K(ATP)}$ (by a combination of pinacidil and hypoxia) and are not directly comparable to ischaemia-induced arrhythmias. Therefore it is not surprising that arrhythmias are more readily prevented by $I_{K(ATP)}$ blockade (Fagbemi *et al.*, 1993; Friedrichs *et al.*, 1994). Tedisamil can be expected to have antiarrhythmic effects in this model via blockade of $I_{K(ATP)}$ (Bray & Quast, 1991, 1992; Kreye *et al.*, 1992; Guillemare *et al.*, 1994), as well as prolongation of AP duration produced by I_K and I_{to} block (Dukes & Morad, 1989; Duke *et al.*, 1990).

In the present studies, the AP prolonging effects of tedisamil were not maintained in the ischaemic zone after induction of myocardial ischaemia. These results are generally in agreement with those reported in the literature. AP prolonging produced in normal tissue is not maintained after induction of ischaemia. The AP prolonging effects of sotalol were lost under conditions of "experimental ischaemia" (Culling *et al.*, 1984; Cobbe *et al.*, 1985a,b). A number of other studies using a selection of I_{Kr} blockers suggest that this is a class effect (MacKenzie *et al.*, 1993; Tweedie *et al.*, 1993; Baskin & Lynch, 1994). The elegant study of Duff *et al.* (1997) demonstrated that the loss of effectiveness of I_{Kr} blockers under conditions of ischaemia is mediated by K^+ -induced antagonism of the binding of these drugs to the ion channels which underlay I_{Kr} . The non-selective K^+

channel blocker, 4-aminopyridine, has been investigated by Antzelevitch's group (Lukas & Antzelevitch, 1993; DiDiego & Antzelevitch, 1994) and shown to prolong AP duration *in vitro* under "simulated ischaemic" conditions. The antiarrhythmic actions of this compound have not been investigated, likely because it is too toxic to use *in vivo*. *In vitro* investigation using various K^+ channel blockers, including Ba^{++} , Cs^+ , tetraethylammonium, 4-aminopyridine and glibenclamide, suggested that AP shortening caused by hypoxia is partly, but not completely, prevented by these compounds (Ruiz-Petrich *et al.*, 1992).

Tedisamil is a very potent blocker of $I_{K(ATP)}$ ($EC_{50\%}$ for $I_{K(ATP)} \sim 50$ nM vs $EC_{50\%}$ for $I_{to} \sim 3$ μ M, Bray & Quast, 1991, 1992; Kreye *et al.*, 1992; Guillemare *et al.*, 1994; Dukes *et al.*, 1990; Dukes & Morad, 1989). Differences in its pharmacology under certain conditions suggests that it acts at a different site than sulphonylurea drugs, such as glibenclamide (see Bray & Quast, 1992; Guillemare *et al.*, 1995). These results may be taken as further support for the lack of effect of $I_{K(ATP)}$ blockade on AP shortening in the early stages of myocardial ischaemia.

Interpretation of the lack of antiarrhythmic effectiveness of tedisamil in the anaesthetised rabbit preparation is complicated by its proarrhythmic actions. In sham-occlusion experiments, 0.25 μ mol/kg/min tedisamil caused PVBs in 60% of rabbits and VT in 20%. Proarrhythmic actions of tedisamil have been reported in rats (Beatch *et al.*, 1991) and primates (Adaikin *et al.*, 1992). While the AP prolonging effects of tedisamil might have prevented some arrhythmias caused by ischaemia, the drug also precipitated arrhythmias. As it is not possible to identify the mechanism(s) of arrhythmogenesis in these studies, it could be argued that the arrhythmias precipitated by tedisamil are not

ischaemia-induced arrhythmias *per se*. Tedisamil may therefore be more effective against ischaemia-induced arrhythmias than evident from these studies. However, the issue at hand is drug action in the ischaemic myocardium and its relationship to ischaemia-induced arrhythmias. The lack of effect of tedisamil on the electrophysiological properties of ischaemic tissue and its net lack of effect on arrhythmias induced by ischaemia is the major finding. Moreover, in a bigger picture, a drug that prevents arrhythmias by one mechanism, and precipitates arrhythmias by another, will have limited utility for preventing cardiac arrhythmias and SCD. Indeed, this has been observed clinically (e.g., CAST, 1989; Morganroth & Goin, 1991; Waldo *et al.*, 1996; see Nattel, 1998 for a review).

The proarrhythmic actions of tedisamil in sham-occlusion studies highlight the proarrhythmic potential common to drugs which prolong AP duration in normal tissue. Drugs which prolong AP duration, particularly Class Ia and III antiarrhythmic drugs, have been associated with the occurrence of the potentially fatal cardiac arrhythmia torsade de pointes (see review by Levine *et al.*, 1989; Bayés de Luna *et al.*, 1989; Morganroth & Goin, 1991).

Torsade de point is an ill defined phenomenon (see Dessertenne *et al.*, 1966; Curtis, 1991; Clayton *et al.*, 1993) which is commonly thought of as an arrhythmia but may in fact be better described as a syndrome (Curtis, 1991). Regardless, it is typically described as a cardiac arrhythmia with a characteristic undulating ECG morphology that is associated with long QT intervals, hypokalaemia, hypomagnesaemia, bradycardia and may have a "short-long-short" initiation sequence (Dessertenne *et al.*, 1966; Morganroth, 1993).

The mechanism(s) of torsade de pointes has been the subject of much investigation (for a review see Weissenburger *et al.*, 1983; Sasyniuk *et al.*, 1989; Tan *et al.*, 1995). Generally, two mechanisms are considered possible candidates. The first is EADs leading to triggered activity (Lazzara *et al.*, 1980; El-Sherif *et al.*, 1989; Brachmann *et al.*, 1983). The second is dispersion of AP duration and electrotonic reflection (Brugada & Wellens, 1985; Habbab & El-Sherif, 1990). A combination of the two mechanisms has also been considered (Brugada & Wellens, 1985; Habbab & El-Sherif, 1990; Antzelevitch *et al.*, 1995). EADs are easily produced *in vitro* by Class III drugs but have never been observed in intracellular recordings *in vivo*. On the other hand, reflection has not been shown to occur *in vivo* either. While the mechanism(s) underlying torsade de pointes remains enigmatic, the association between Class III drugs and torsade de pointes is strong (Nattel, 1998). Fear of precipitating torsade de pointes is likely to halt further development of Class III drugs, at least as we now know them (see Hondeghem & Snyders, 1990; Hondeghem, 1994; Colatsky, 1995).

RSD1019 prolonged AP duration before and after induction of ischaemia in the anaesthetised rabbit preparation and prevented ischaemia-induced arrhythmias. The QT interval of the ECG was prolonged in parallel with effects on AP duration. The antiarrhythmic actions of RSD1019 cannot be attributed to its AP prolonging effects in normal tissue since tedisamil prolonged AP duration to a similar or greater extent but did not prevent arrhythmias. While the effects of RSD1019 were not absolutely *selective* for the conditions of myocardial ischaemia, they were maintained under such conditions unlike the actions of other drugs. Thus, RSD1019's AP prolonging effects in ischaemic tissue

represent a unique antiarrhythmic mechanism. The discussion of ischaemia-selective Class III antiarrhythmic actions will be expanded upon later.

In contrast to lidocaine, RSD1019 had no effect on MAP f-p interval after the induction of ischaemia. This is consistent with the results of the "simulated ischaemia" assay carried out in isolated rat hearts. RSD1019 was less potent and less "ischaemia-selective" than lidocaine. Studies in rabbits provide no direct evidence for RSD1019's ischaemia-selective Na⁺ channel blocking actions. Despite this, the ischaemia-selective Na⁺ channel blocking actions might contribute to the antiarrhythmic actions observed. A number of lines of evidence support this notion. Firstly, the actions of drugs on Na⁺ channels are not known to vary with species. Secondly, the modulated receptor hypothesis (Hondegheem & Katzung, 1984) suggests that AP prolongation, and therefore time at positive potentials, can be expected to potentiate the actions of drugs which bind to the inactivated state of Na⁺ channels. Note that this assumes that RSD1019 blocks Na⁺ channels by binding to the inactivated state. While there is no direct evidence for this, the drug's profile was similar to that of lidocaine, which is known to bind to the inactivated state (Chen *et al.*, 1975; Bean *et al.*, 1983). In this regard the acidotic and K⁺-depolarised myocardium may be particularly sensitive to this interaction (see introduction and Grant *et al.*, 1982; Hondegheem & Katzung, 1984; Wendt *et al.*, 1993).

7.5 Potential species dependent antiarrhythmic actions of RSD1019.

In contrast to lidocaine, RSD1019 exhibited similar antiarrhythmic actions in rats and rabbits. Further studies in anaesthetised primates showed that 4 µmol/kg/min

RSD1019 selectively increased VFT after induction of ischaemia but was without effect before ischaemia (Bain *et al.*, 1997). The antiarrhythmic actions of RSD1019 were observed in a number of preparations and occur at the same dose in both species. An interesting facet of the ischaemia-selective approach to antiarrhythmic drug development is that it may overcome the problem of species dependence which is common to antiarrhythmic drugs (particularly K^+ channel blockers, see later).

7.6 Use of MAPs to investigate the electrophysiological actions of drugs on ischaemic myocardial tissue.

One of the reasons that ischaemic myocardial tissue has not been the targeted in the development of antiarrhythmic drugs is the lack of appropriate techniques to measure its electrophysiological characteristics, and drug effects thereon. As described previously, MAPs might provide a useful tool for such investigations. Despite this, relatively few studies have used MAPs to study the effect of drugs on ischaemic myocardial tissue. The following discusses the use of MAPs under conditions of myocardial ischaemia, ischaemia-induced changes in MAP recordings and drug effects thereon.

7.6.1 Interpretation of the MAP f-p interval.

MAP recordings in combination with ECG recordings can be used to measure activation times and therefore may be used as a surrogate for conduction velocity (e.g., Kurz *et al.*, 1993 also see reviews by Franz, 1991; Yuan *et al.*, 1994). However, another measure of conduction velocity might be attained from MAP recordings. Measurement of

the interval from the first deflection seen in MAP recordings to the intrinsic deflection, or the peak, could be useful as a surrogate measure of conduction velocity. The small biphasic deflection seen at the beginning of a MAP recording is thought to be the remnant of the intracardiac QRS complex (Franz, 1991; Yuan *et al.*, 1994). This complex may serve as a marker for activation of the heart in the same way the Q wave of the surface ECG is taken to represent the onset of ventricular activation. The intrinsic deflection of the MAP represents activation of the cells directly beneath the electrode (see Franz, 1991; Yuan *et al.*, 1994). As the intrinsic deflection is not clearly seen in all recordings the peak of the MAP might serve equally as well. In short, measuring the interval between the remnant of the intracardiac QRS complex and the peak of the MAP may be useful as a surrogate measure of conduction velocity. This interval was defined as the MAP foot to peak (MAP f-p) interval.

To test the hypothesis that the MAP f-p interval can be used as a surrogate for conduction velocity this interval was measured during the following experimental manipulations; coronary artery occlusion with and without drug treatment, as well as sham occlusion, with and without drug treatment. Under conditions where no changes in conduction velocity are expected (e.g., sham occlusion and sham occlusion experiments with drugs that do not slow conduction) no change in MAP f-p interval was seen. These data demonstrate that MAP f-p interval was stable over time. Induction of myocardial ischaemia is known to slow conduction. As expected, MAP f-p intervals increased in a time-dependent fashion after the onset of ischaemia. Further evidence can be inferred from drug effects on MAP f-p interval after induction of ischaemia. Tedisamil had no

effect on MAP foot to peak interval before or after induction of ischaemia. This is consistent with the observation that this drug only blocks Na^+ channels at high concentrations (Dukes & Morad, 1989; Dukes *et al.*, 1990; Beatch *et al.*, 1991; Wallace *et al.*, 1995; Chi *et al.*, 1996). Lidocaine, on the other hand, is known to reduce conduction velocity preferentially in ischaemic tissue. As predicted from the ischaemia-selectivity hypothesis (Hondeghe *et al.*, 1974), lidocaine had no effect on MAP f-p interval before induction of ischaemia but potentiated ischaemia-induced increases in this measure. These data support the hypothesis that MAP f-p interval can be used as a surrogate for conduction velocity. One important piece of experimental evidence is missing from these data set. These studies do not include a drug that slows conduction in normal myocardial tissue. Evidence that a drug which is known to slow conduction (e.g., flecainide) increased the MAP f-p interval would further strengthen this hypothesis.

Another interpretation of the MAP f-p interval is related to the synchronicity of depolarisation beneath the electrode. It is clear that MAP electrodes record potentials from a number of cells (see Franz, 1991; Yuan *et al.*, 1994). Activation of all of the cells in the electrode's field of view at the same time can be expected to produce a uniform, rapid MAP upstroke. However, if differences in the time of activation of cells beneath the electrode occur, a reduction in the MAP upstroke can be expected. The maximum rate of depolarisation of the MAP (V_{max}) and the MAP f-p interval can be expected to be reduced by manipulations that reduce the synchronicity of activation of myocardial cells beneath the electrode. One such manipulation is myocardial ischaemia. Myocardial ischaemia is known to reduce conduction velocity and increase the dispersion of activation

times (e.g., Kutz *et al.*, 1993). Thus, an increase in the MAP f-p interval might be indicative of an increase in the dispersion of activation and not simply a slowing of conduction. Such increases in the dispersion of activation times have been implicated in the development of re-entrant arrhythmias (see introduction).

7.6.2 Activation alternans and multiple wave forms in MAPs recorded from ischaemic myocardial tissue.

The term "alternans" has been used to describe mechanical and electrical phenomena associated with the heart. Mechanical alternans, such as those occurring in BP, will not be discussed here. Electrical alternans originating from the heart can be observed by a number of techniques including the surface ECG (see Hellerstein & Liebow, 1950; Nearing *et al.*, 1991; Verrier & Nearing, 1994; Rosenbaum *et al.*, 1994), unipolar electrograms (Carson *et al.*, 1986), MAP recordings (Lab & Wollard, 1978; Nakashima *et al.*, 1978; Hashimoto *et al.*, 1984; Dilly & Lab, 1987; Lee *et al.*, 1988; Taggart *et al.*, 1988) and intracellular recordings (Downar *et al.*, 1977; Kléber *et al.*, 1978; Russel *et al.*, 1979; Janse, 1980; Penny & Sheridan, 1983). In terms of changes in AP morphology, electrical alternans can be divided into two groups depending on when the alternation occurs. Alternans which effect phase 0 of the AP, and consequently all phases thereafter, have been defined herein as activation alternans since they occur during the activation of the AP. Alternans which occur exclusively during phase III of the AP are defined as repolarisation alternans. Dilly & Lab (1988) have further sub-divided repolarisation

alternans into those occurring on even or odd numbered beats after some initiating event (e.g., a PVB).

A brief cautionary note is required before discussing electrical alternans; observer bias is always present. In these studies, MAPs were recorded from a single site. Lee and co-workers (1988) have shown that alternans in AP duration can occur out of phase at sites very close together (5 mm) and that alternans may be observed at one site but not at another. This makes it nearly impossible to link the occurrence of alternans to the occurrence of arrhythmias with certainty. Activation alternans were observed in rabbits which did not have VF and were not observed in rabbits which had VF.

While alternans in AP duration were observed in these experiments, they were confined to the early stages of the ischaemia (i.e., the first 3 minutes). These alternans were similar to those observed by Lee *et al.* (1988) in isolated rabbit hearts. AP prolongation produced by tedisamil tended to exacerbate the occurrence of these alternans, however, none of the drugs tested had statistically significant effects. The increased incidence of VT at low doses of tedisamil might be related to exacerbation of repolarisation alternans.

There are a number of mechanisms whereby changes in ionic currents could give rise to alternans. Lee and co-workers (1988) suggested that alternans in AP duration are due to changes in Ca^{++} currents. Changes in Ca^{++} current with prolonged periods of ischaemia may also give rise to activation alternans. The studies by Lee *et al.* (1998) showed a direct correlation between Ca^{++} fluxes across the membrane and alternans in AP duration. Shorter APs had small Ca^{++} fluxes and vice versa. They also noted that

alternans could be abolished by verapamil. Other investigators confirm this observation (Janse, 1980; Hashimoto *et al.*, 1983).

Activation alternans have been observed by others under conditions of myocardial ischaemia and may be fundamentally related to ischaemia-induced arrhythmias (Nakashima *et al.*, 1978; Russel *et al.*, 1979; Dilly & Lab, 1988; Abe *et al.*, 1989; Rosenbaum *et al.*, 1994). Arrhythmias and activation alternans occurred at the same time after induction of ischaemia, and administration of lidocaine increased their occurrence. Moreover, lidocaine reduced the latency to the occurrence of both VF and activation alternans in parallel. These data supports a role of alternans in ischaemic arrhythmogenesis.

The ionic mechanism(s) that underlay ischaemia-induced activation alternans is not clear but a number of suggestions have been made. Alternans are likely to be due to changes in AP morphology and not due to changes in excitability or conduction velocity (Carson *et al.*, 1986). It is important to note that activation alternans have also been observed in intracellular recordings under conditions of myocardial ischaemia and cannot therefore be an artefact related to the use of MAPs (Downar *et al.*, 1977; Kléber *et al.*, 1978; Russel *et al.*, 1979; Janse, 1980; Penny & Sheridan, 1983).

While alterations in Ca^{++} currents likely play a role in the genesis of activation alternans, it is unlikely that they are the only ionic current responsible. It is likely that the fast inward Na^{+} current is also involved. The simple observation that AP amplitude changed on a beat-to-beat basis supports this hypothesis. Experimental evidence for this is provided by the observation that lidocaine increases the occurrence of activation alternans. By retarding the recovery of Na^{+} channels to the activatable state, an effect that is

dependent on membrane voltage and therefore on AP duration and amplitude, lidocaine allows activation alternans to persist once started.

Unlike lidocaine, RSD1019 did not influence the occurrence of activation alternans in ischaemic tissue and multiple wave forms were not observed. Perhaps the AP prolonging effects of RSD1019 prevented activation alternans from degenerating into multiple wave forms. Alternatively, the ischaemia-selective Na^+ channel blocking actions of RSD1019 were insufficient to delay recovery of Na^+ channels to the activatable state and therefore insufficient to cause activation alternans to degenerate into multiple wave forms. This question cannot be answered from the present studies. As expected from its pharmacology, tedisamil had no effect on the occurrence of activation alternans.

7.7 Effects of lidocaine, tedisamil and RSD1019 on BP and HR.

Zetebadine, a specific bradycardic agent, has been reported to have limited antifibrillatory actions in anaesthetised rabbits (Bril *et al.*, 1994). Reversal of the bradycardia by atrial pacing reversed the antifibrillatory actions of this drug. Reductions in heart rate required to produced antifibrillatory effects with zetebadine were much greater than those seen in the present study. A reduction of HR by 90 beats/minute reduced the incidence of ischaemia-induced VF by approximately 50%. The maximum reduction in HR observed in the present study was 30 beats/min (see Tables 10 & 13). Irrespective of the possible antiischaemic effects produced by bradycardia, the drugs tested had very different actions on ischaemia-induced arrhythmias, while effects on HR were similar. The interaction between bradycardia and AP prolongation has already been

discussed (see section discussing the antifibrillatory actions of tedisamil). The same argument applies to the AP prolonging effects of RSD1019.

8.0 General discussion and conclusions

8.1 Vulnerable window hypothesis for Class I antiarrhythmic action.

Changes in excitability of the myocardium after induction of ischaemia range from minimal effects early on to complete suppression late in ischaemia as it progresses to infarction. These two extremes are separated by a time window within which tissue is critically suppressed such that unidirectional block can occur. Unidirectional block is one of the fundamental requirements for re-entry and therefore of ischaemic arrhythmogenesis (see introduction). The time between these two extremes is therefore the vulnerable window for arrhythmogenesis (Hondeghe & Cotner, 1978). Evidence for the existence of a vulnerable window is illustrated in the present studies by the observation that the degeneration of MAP recordings corresponded in time to the major phase of arrhythmias. Moreover, the simple observation that ischaemia-induced arrhythmias occur in phases also speaks to the existence of a vulnerable window (Battle *et al.*, 1974; Corbalan *et al.*, 1976; Menken *et al.*, 1979; Curtis *et al.*, 1987; Bergey *et al.*, 1982).

Drugs which suppress excitability in ischaemic tissue accelerate the occurrence of both critical depression of excitability and inexcitability (Hondeghe & Cotner, 1978). Class I antiarrhythmics can be expected to shorten the vulnerable window by accelerating ischaemia-induced suppression of excitability; however, they cannot prevent the vulnerable

window's occurrence. This hypothesis predicts that Class I drugs have antiarrhythmic actions by virtue of their effects on ischaemic myocardial tissue. It also predicts that the latency to the occurrence of arrhythmias, when they do occur, will be reduced by drugs which suppress excitability in ischaemic tissue. Carson and co-worker's (1986) demonstrated that lidocaine can have proarrhythmic actions and cause a time shift in the occurrence of arrhythmias. The present study also shows that lidocaine significantly reduces the latency to the occurrence of VF after induction of ischaemia. Others have shown that lidocaine increases the vulnerability to VF after induction of ischaemia (Bergey *et al.*, 1982; Aupetit *et al.*, 1995, 1997). These data suggest that lidocaine shifted the vulnerable window for arrhythmogenesis forward in time in a manner which is consistent with the predictions of Hondeghem and Cotner (1978). However, all such data must be viewed cautiously as it is inherently subject to censoring, i.e. was the vulnerable window shifted in animals in which VF did not occur?

The vulnerable window hypothesis can also explain why Class I drugs which lack selectivity for the conditions of ischaemia have only marginal efficacy for suppressing ischaemia-induced arrhythmias. While drugs such as quinidine and flecainide accelerate the process of ischaemia which renders myocardial tissue inexcitable, they also reduce excitability in normal tissue. To be effective against ischaemia-induced arrhythmias, such drugs would have to produce similar suppression of excitability in the ischaemic myocardium to that produced by lidocaine. In order to do this, very high doses would be required. In the rat occlusion experiments, flecainide and quinidine's cardiovascular toxicity did not permit such high doses to be administered. Indeed, at the maximum doses

that could be tested in this preparation, quinidine and flecainide had only marginal antiarrhythmic efficacy. However, even in the theoretical case of a drug which had no other effects but to equally suppress excitability in ischaemic and normal tissue, such a drug would not be expected to *completely* prevent ischaemia-induced arrhythmias. At doses required to render ischaemic tissue inexcitable, normal tissue would also be markedly suppressed. This would interfere with the normal processes of excitation and excitation-contraction coupling and would compromise the heart's function as a pump. In short, the vulnerable window hypothesis illustrates why Class I antiarrhythmic drugs **must** exhibit ischaemia-selectivity if they are to suppress ischaemia-induced arrhythmias.

While the action of Class I drugs in ischaemic tissue may shorten the duration of the vulnerable window, what determines whether the proarrhythmic or antiarrhythmic responses occur? The answer is not known. The vulnerable window hypothesis (Hondeghe & Cotner, 1978) says nothing about the probability of arrhythmia occurrence, although Hondeghe (1987) suggested that Class I drugs should increase the risk of arrhythmias. The induction of multiple wave forms by lidocaine in the present study suggests that one factor might be the heterogeneity in the suppression of excitability. In the rabbit, MAP recordings showed that lidocaine induced greater electrophysiological derangement in ischaemic tissue than that observed in control hearts. No such data were available in the rat; however, the correspondence between the antifibrillatory actions of lidocaine and the complete suppression of ST segment elevation suggest that excitability of the ischaemic tissue was uniformly suppressed.

One factor that might contribute to the differences in the effects of lidocaine on ischaemia-induced arrhythmias in the present study is AP morphology. In absolute terms, rabbits have greater dispersion in ventricular AP duration than rats. Dispersion in AP duration is greater in the rabbit due to differences in the distribution of repolarising K^+ currents (e.g., I_{Kr} , I_{Ks} and I_{to} ; Antzelevitch *et al.*, 1991). These currents are known to be differentially effected by increases in extracellular $[K^+]$ (e.g., Sicouri & Antzelevitch, 1995; Salata *et al.*, 1996; Sanguinetti & Juriewicz, 1990), an effect that can be expected to increase the regional dispersion of AP duration under ischaemic conditions. This, coupled with the time- and voltage-dependence of lidocaine-induced block of Na^+ currents, may set the stage for increased heterogeneity in excitability, refractoriness and therefore lead to proarrhythmia. This mechanism cannot completely account for the proarrhythmic actions observed, as antiarrhythmic actions have been observed in rabbits (e.g., Wolk *et al.*, 1998). An alternative hypothesis is that the relatively low blood $[K^+]$ (~ 2.7 mM) observed in the rabbit preparation increases the arrhythmogenicity of the heart and unveiled the shift in the vulnerable window. Low $[K^+]$ is known to increase the incidence and severity of arrhythmias (Curtis & Hearse, 1989; Curtis, 1991; Saint *et al.*, 1992).

8.1.1 Other considerations on the proarrhythmic potential of Class I antiarrhythmic drugs.

Examining the wave length of re-entry illustrates another mechanism whereby Class I antiarrhythmics may have proarrhythmic actions. As previously noted, the wave length of a re-entry circuit is the product of conduction velocity and refractory period. Under normal conditions re-entry does not occur because this product is too large to

allow re-entry (i.e., the tissue is refractory for too long and/or conduction is too fast). A reduction in conduction velocity brings the system closer to a state where re-entry can occur. In the case where conduction is near sufficiently depressed, further slowing of conduction will allow refractory tissue more time to regain excitability and allow a re-entry pathway to occur that would otherwise not have occurred. The differences between lidocaine and flecainide on ischaemia-induced arrhythmias in the rat coronary artery occlusion studies, and their respective effects on conduction velocity in normal myocardial tissue, stand out when viewed in this light. Flecainide potently suppressed conduction in normal tissue while lidocaine had no such effect; lidocaine provided complete antiarrhythmic protection while flecainide did not.

8.2 Ischaemia-selective Class III antiarrhythmic actions.

The antiarrhythmic effects of selective AP prolongation in ischaemic tissue are not known and can only be speculated upon. Myocardial ischaemia causes AP shortening in all species, an effect that is clearly associated with the occurrence of arrhythmias. Prolonging APs, or even preventing AP shortening, in ischaemic tissue might prevent arrhythmias caused by ischaemia. It is clear that increasing AP duration in normal tissue can prevent arrhythmias (e.g., Singh & Vaughan-Williams, 1972; Beatch *et al.*, 1991; Anderson *et al.*, 1994). Selectively prolonging AP duration in ischaemic tissue may offer distinct advantages over a similar effect in normal tissue.

Ischaemic arrhythmogenesis results from a complex interplay of electrophysiological changes. These changes are time dependent and alterations in their

time dependence could influence the occurrence of arrhythmias. Preventing AP shortening in ischaemic tissue without other effects can be expected to prevent arrhythmias. This follows directly from the wave length of re-entry model previously discussed (see Beatch *et al.*, 1991; Janse, 1992; Adaikin *et al.*, 1992). However, myocardial ischaemia reduces conduction velocity until inexcitability results; a situation which is truly arrhythmic (i.e., without rhythm)! An ischaemia-selective Class III drug can be expected to shorten the vulnerable window for arrhythmogenesis. A longer AP duration in ischaemic tissue requires that conduction be that much slower in order to allow re-entrant arrhythmias to occur. This reduces the probability that arrhythmias will occur. In short, prolonging AP duration in ischaemic tissue will delay the onset of, but not extend, the vulnerable window for arrhythmogenesis. Data from this study support this notion.

While evidence for the antiarrhythmic efficacy of ischaemia-selective Class III actions is scarce, there is some evidence from related studies that supports this hypothesis. Lucchesi's group has shown that drugs which can be expected to prevent AP shortening under "simulated ischaemic" conditions prevent VF (Fagbemi *et al.*, 1993; Friedrichs *et al.*, 1994; Chi *et al.*, 1996). The conditions used to simulate ischaemia in this preparation are of major importance when interpreting the results. In their *in vitro* preparation, ischaemia was simulated by using a combination of hypoxia and the $I_{K(ATP)}$ activator pinacidil. Under these conditions, $I_{K(ATP)}$ blockers such as tedisamil, 5-hydroxydeconoate and glibenclamide can be expected to prevent AP shortening. $I_{K(ATP)}$ blockers have been shown to effectively prevent AP shortening caused by organic $I_{K(ATP)}$ activators (Fosset *et al.*, 1988; Sanguinetti *et al.*, 1988). In short, the antifibrillatory actions of tedisamil, 5-

hydroxydeconoate and glibenclamide in this preparation can be attributed to blockade of $I_{K(ATP)}$ and resulting suppression of AP shortening caused by "simulated ischaemia."

Addition of ischaemia-selective Na^+ channel blockade to ischaemia-selective Class III antiarrhythmic actions might be very effective for suppressing ischaemia-induced arrhythmias. As discussed, ischaemia-selective Na^+ channel blockers accelerate the occurrence of both critical suppression of conduction and inexcitability. The combination of the two ischaemia-selective actions might narrow the vulnerable window maximally.

The pitfall of drugs which prolong AP duration in normal tissue is their proarrhythmic actions (e.g., Waldo *et al.*, 1996; see Nattel, 1998 for a review). Such proarrhythmic actions are thought to result from either EADs or dispersion of AP duration with subsequent reflection (see Introduction). In this regard, ischaemia-selective AP prolongation may offer real advantages. Ischaemia-induced increases in extracellular $[K^+]$ suppress EADs (Coronel *et al.*, 1988; Davidenko *et al.*, 1989) and prolongation of APs that were previously shortened will reduce the dispersion of refractoriness in the heart. In either case, confining AP prolongation to ischaemic tissue can be expected to reduce the proarrhythmic actions of such drugs.

Prolonging AP duration in ischaemic tissue is not without its own pitfalls. Prolonging AP duration can be expected to increase Ca^{++} influx. In the case of transient or lesser degrees of ischaemia, this may lead to worsening of the consequences of ischaemia in terms of ATP consumption and ultimately result in cell death. Moreover, Ca^{++} -induced DADs and resulting triggered activity might be exacerbated by prolonging AP duration in ischaemic tissue. If this is an important mechanism for arrhythmogenesis in

the ischaemic myocardium, ischaemia-selective AP prolongation may well be proarrhythmic!

9.0 Conclusions.

This thesis explored the relationship between ischaemia-selective drug actions and antiarrhythmic efficacy. The studies clearly demonstrated that drug action in the ischaemic myocardium can influence the occurrence of ischaemia-induced arrhythmias. Ischaemia-selective drug actions can be anti- or proarrhythmic, as demonstrated for lidocaine, in rats and rabbits, respectively. In contrast to lidocaine, the actions of RSD1019 in ischaemic tissue were associated with antiarrhythmic actions in both species. Unlike other drugs reputed to prolong AP duration selectively in ischaemic tissue (e.g., glibenclamide), RSD1019 effectively prolonged AP duration after induction of ischaemia. Thus, the ischaemia-selective actions of RSD1019 were associated with suppression of ischaemia-induced arrhythmias. Selective prolongation of AP duration in ischaemic myocardial tissue represents a novel approach to the problem of preventing of ischaemia-induced arrhythmias. One of the reasons that ischaemic myocardial tissue has not been a target for antiarrhythmic drug development is the fact that there are relatively few techniques available to study the electrophysiological actions of drugs on such tissue. These studies highlight the use of monophasic action potential (MAP) recordings as a technique to assess the effects of drugs on ischaemic myocardial tissue. Real advances in antiarrhythmic pharmacology might be made by classifying antiarrhythmic drugs on the

basis of their effects under the pathological conditions under which they act. In the future, RSD1019 may serve as a prototype drug for evaluation and development of an ischaemia-selective approach. If successful, such a drug would prevent VF and SCD in patients at risk and have a significant impact on the human condition.

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