PHARMACOLOGICAL AND ANTIARRHYTHMIC PROPERTIES OF QUINACAINOL - A NEW SODIUM CHANNEL BLOCKER?

by

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ABSTRACT

Quinacainol, 1-[2-(1,1-dimethylethyl)-4-quinolyl]-3-(4-piperidyl)-1-propanol is a class I antiarrhythmic agent provisionally subclassified as Ic. Studies were carried out in order to (1) determine the actions of quinacainol in acute myocardial ischæmia, (2) ascertain the mechanism(s) responsible for these actions, and (3) ascertain the appropriateness of its subclassification.

Toxicological, hæmodynamic, and ECG effects in chronically prepared conscious rats were determined following administration of 1, 2, 4, or 8 mg/kg of quinacainol given i.v. over 10 minutes on alternate days. Toxicity referable to the heart was seen at doses of 8 mg/kg and above. In rats given 8 or 16 mgkg, arrhythmias occurred. Quinacainol had no major effects on blood pressure, unlike most class I antiarrhythmics, but lowered heart rate (not statistically significantly) and prolonged P-R interval and QRS duration.

In an attempt to protect against ischæmic arrhythmias, doses of 2 mg/kg and 4 mg/kg were given. The high dose gave the best protection. It reduced the incidence of ventricular tachycardia (VT) from a control value of 80% to 30%, and reduced the incidence of ventricular fibrillation (VF) from a control value of 60% to 10%. An increase in the incidence of premature ventricular contractions was seen at both doses. Blood pressure was not adversely effected although slight bradycardic effects as well as prolongation

of the P-R interval were seen at both doses. Both doses reduced S-T segment and delayed onset of elevation of S-T segment and R-wave which were induced by coronary occlusion.

Sensitivity to electrical stimulation was tested in pentobarbital anæsthetised rats using ventricular electrodes. Doses of 0.5, 1, 2, and 4 mg/kg were given cumulatively as a 10 min infusion every 25 min. Quinacainol did not affect QRS duration or the Q-T_c interval but dose-dependently widened P-R interval when compared to pre-treatment. Quinacainol dose-dependently increased threshold current, threshold duration, and ventricular fibrillation threshold. In addition, quinacainol elevated effective refractory period while decreasing maximum following frequency.

Open-chest rats under pentobarbital anæsthesia were used to record the effects of quinacainol on epicardial intracellular potentials. Recordings were made by conventional microelectrode techniques before and after cumulative doses of 0.5, 1, 2, 4, and 8 mg/kg i.v. Quinacainol dose-dependently reduced phase zero of the action potential (AP) and AP height but did not influence other phases of the AP (with the exception of prolonging repolarization at the highest dose); actions indicative of class Ic.

Effects of quinacainol on isolated rat hearts were assessed using a modified Langendorff heart preparation and were compared with those of tetrodotoxin (TTX). Quinacainol

widened the P-R interval and QRS duration without having major effect on the Q-T_c interval. In addition it slowed the sinus beating rate. Quinacainol was more potent than TTX.

All findings indicated that quinacainol is a potent antiarrhythmic agent with Na^+ channel blocking properties.

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LIST OF ABBREVIATIONS

action potential	AP
action potential duration	APD
action potential duration at 10, 50 or 90% repolarization	$APD_{10,50,90}$
arrhythmia score	AS
blood pressure	BP
current	i
effective dose achieving 50%	ED_{50}
effective refractory period	ERP
electrocardiogram	ECG
hertz	Hz
hour(s)	h
intraperitoneally	i.p.
intravenous	i.v.
left anterior descending	LAD
lethal dose	LD_{50}
log_{10} of the number of PVC's	log_{10} PVC
maximum diastolic potential	MDP
maximum following frequency	MFF
maximum rate of rise of phase 0 of the AP	dV/dt
maximum rate of rise of ventricular pressure	dP/dt
mean	x
milligram per kilogram	mg/kg
millisecond(s)	ms
minute(s)	min

LIST OF ABBREVIATIONS

non-spontaneously reverting VT	NSVT
non-spontaneously reverting VF	NSVF
occluded zone	OZ
pacemaker current	i_f
premature ventricular contraction	PVC
Q-T interval corrected for heart rate	$Q\text{-}T_c$
rate-dependent block	RDB
tetrodotoxin	TTX
second(s)	sec
sino-auricular node	SAN
sodium	Na^+
sodium conductance	$_gNa$
standard error of the mean	s.e.mean
subcutaneous	s.c.
threshold current	i_T
threshold duration (threshold pulse width)	t_T
ventricular fibrillation	VF
ventricular fibrillation threshold	VF_T
ventricular tachycardia	VT

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Dedicated to the memory of my grandfather,

ANDREW COCHRANE HOWARD

1 INTRODUCTION

1.1 The Need for Antiarrhythmic Drugs

Ventricular fibrillation is responsible for the majority of deaths (90%) due to heart attacks (Oliver, 1982). McCormick and Skrabanek (1988) examined the identification of risk factors and the belief that modification of such factors can prevent or reduce the incidence of diseases such as coronary heart disease. Analyses of data from numerous studies such as the WHO, Helsinki, and Framingham studies found that lifestyle interventions (i.e. dietary, smoking, blood pressure, etc.) failed to demonstrate any significant benefit (McCormick and Skrabanek, 1988). Epidemiological studies can identify risk factors as causal agents, but experiment alone provides evidence of cause and effect. Regardless of the above, the need for aggressive antiarrhythmic drug research and development is obvious since sudden death with myocardial ischæmia/infarction is primarily attributable to ventricular fibrillation (Oliver, 1982; Hoffman and Dangman, 1986).

Despite the availability of a large number of antiarrhythmic agents, there is a continuing need for safer and more efficacious drugs (Podrid, 1989). However, the route to new drugs is difficult due to the lack of exact knowledge concerning the mechanisms underlying the genesis of VF in the clinical population and the relative lack of

knowledge regarding pharmacological properties which confer antifibrillatory activity (Botting *et al.*, 1986). A compounding problem in the clinical situation regarding appropriate choice of a particular antiarrhythmic agent is that this decision depends not only on its demonstrable effectiveness against arrhythmias, but also on a knowledge of its pharmacokinetics, hæmodynamic actions, and adverse effects (Muhiddin and Turner, 1985). With respect to the lack of knowledge regarding the pharmacological properties which confer antiarrhythmic actions, it appears that useful drugs might be developed from two routes: (1) agents which prevent the production or action of an essential arrhythmogen or (2) classical antiarrhythmic agents.

The first route has not proven to be successful although it has been suggested on a number of occasions, that essential endogenous arrhythmogens are involved in ischæmic arrhythmogenesis. Possible arrhythmogens include eicosanoids, catecholamines, free radicals, platelet activating factor, etc. (Braquet *et al.*, 1987). Overwhelming evidence for the obligatory involvement of such arrhythmogens has not been provided via studies of blockade of production or action of putative arrhythmogens. In fact, it has been recognized that activation of the ATP-dependent K^+ channel may be sufficient alone to induce the arrhythmogenic changes seen during the early phase of ischæmia (Wilde *et al.*, 1989).

The second route; development of classical anti-arrhythmic agents, has more potential although it is tedious and protracted. It has been consistently reported that, despite the use of a variety of models and species, the classical ion channel blocking antiarrhythmics have antifibrillatory and antiarrhythmic actions against ischæmia-induced arrhythmias (Botting *et al.*, 1986).

1.2 Experimental Approach to Myocardial Ischæmia and Infarction

Sudden cardiac death is considered the foremost challenge of modern cardiology (Bayes de Luna *et al.*, 1988). The most frequent cause is VF although VT and asystole can also result in sudden cardiac death (Cobb *et al.*, 1980; Janse, 1986). The underlying cause of these arrhythmias is atherosclerotic coronary artery disease which narrows or occludes the artery causing cardiac ischæmia and infarction (Davies, 1981; Janse and Wit, 1989). In only a few of those patients resuscitated and reaching hospital alive, are signs of myocardial necrosis found, suggesting that a brief period of ischæmia not even long enough to cause irreversible myocardial damage, can induce fatal arrhythmias (Janse, 1986).

Experimental evidence providing a link between obstruction of a coronary artery, arrhythmias, VF, and sudden death can be traced back to the 19th century (Lazzara

et al., 1978). Erichsen, a pioneer in this field, described a 'slight tremulous motion' after cessation of a regular heart beat following coronary artery occlusion in the dog in 1842 (reviewed in Lazarra *et al.*, 1978). Some 10 years later Porter determined that disturbance of cardiac rhythm was the result of coronary occlusion (reviewed in Lazarra *et al.*, 1978). In 1909, Thomas Lewis demonstrated a correlation between coronary occlusion in experimental animals and the appearance of paroxysmal VT (reviewed in Lazzara *et al.*, 1978). Clinicians observed a multitude of arrhythmias resulting from ischæmia and infarction. Wiggers and coworkers (1941) noted that a region of ischæmia facilitated induction of VF by applying a strong stimulus during the T wave. They concluded that ischæmia lowered the fibrillation threshold (current requirement) and broadened the vulnerable period for induction of VF (Wiggers *et al.*, 1941).

In the early 1950's, Harris and associates (1950) developed a technique to determine the mechanisms underlying disorders of rhythm by directly recording from ischæmic tissues in dogs with occluded coronary arteries. Harris' group drew a number of conclusions. They concluded that ectopic beats were generated near the border between infarcted and normal tissue by automatic foci induced by potassium released from ischæmic cells. They discounted the importance of the interior of the ischæmic zone in the generation of arrhythmias. They also discounted reentry

because they were not able to record continuous activation (i.e. activation potentials detected throughout the interval between 2 beats, during tachycardia). Furthermore, they concluded that ectopic ventricular rhythms occur in 2 phases following coronary occlusion, and that these are separated by a quiescent period of sinus rhythm. The first phase occurs during the first 30 min following occlusion with frequent occurrences of ventricular tachyarrhythmias while the second, or delayed phase, begins 4-8 h post-occlusion and can last for 2-4 days (Harris, 1950). During this time period, ventricular tachyarrhythmias again occur, although episodes of VF are rare. Harris (1950) speculated that the electrophysiological mechanisms involved during the 2 phases were different, as would be concluded in later studies.

The term "acute phase of myocardial ischæmia" refers to events occurring within the first 2-4 h after sudden reduction of blood flow through a coronary artery (Janse and Wit, 1989). In patients resuscitated from VF, only a few subsequently develop a myocardial infarction (Cobb *et al.*, 1980) which suggests that, if myocardial ischæmia is involved, it is transient. Ischæmia need only to exist very briefly (< 1 min) in order to induce arrhythmias (Janse and Wit, 1989). It has also been shown that arrhythmias can result both from transient ischæmia (where S-T segment elevation has already reached maximal levels) or from reperfusion (after the S-T segment changes have returned to normal) (Janse and Wit, 1989).

It is generally accepted that the occurrence of lethal arrhythmias in humans is the result of the interplay between several factors. First, acute ischæmia produces electrophysiological changes thereby creating the setting for reentry circuits within the ischæmic myocardium (Janse and Wit, 1989). Second, the anatomical arrangement of surviving myocardial fibers within a healed infarct provides anatomically defined reentrant circuits (Janse and Wit, 1989). Specific triggers can manifest themselves as arrhythmias in the setting of acute ischæmia or infarction (Szekeres, 1986). PVC's, especially if they occur after a long pause or after increases in heart rate, may be considered a trigger to more severe arrhythmias (Janse and Wit, 1989). Modulating factors include the sympathetic nervous system, electrolyte disturbances (e.g. hyper/hypokalemia), or impaired left ventricular function which may modify a trigger or functional change brought about by acute ischæmia (Szekeres, 1986). The numerous experimental models used to study arrhythmias resulting from ischæmia-infarction vary with respect to the above factors.

1.3 Necessity of Animal Studies and Choice of Species in the Study of Antiarrhythmic Agents

The diverse number of experimental animal models exemplifies the inadequacy of any one particular model to effectively mimic the mechanisms or causes of sudden cardiac

death in man (Davies, 1981) as well as the various types of questions asked.

Models of acute or chronic ischæmia do not necessarily take into account factors which may influence susceptibility for ventricular arrhythmias. Such factors include neuro-humoral influences, emotional responses, anatomical locations of the diseased vessels, regions of infarction, collateral circulation, metabolic derangements, coronary spasm, and unstable coronary artery plaques, i.e. prior coronary or cardiac disease (David *et al.*, 1986; Janse and Wit, 1989; Skereres, 1988).

Experimental methods for inducing myocardial ischæmia and infarction have been developed out of necessity from lack of adequate human data. Unfortunately, experimental preparations do not necessarily mimic those seen in man, due in part to the fact that the underlying mechanisms of clinical arrhythmias are not fully understood (Winslow, 1984). Criteria were described by Curtis *et al.* (1987) for assessing animal models of myocardial ischæmia and infarction. The criteria established that an ideal model would theoretically: (1) completely mimic one or more of the various clinical conditions; (2) respond to drugs in a manner which corresponded with the clinical response exactly; (3) have sufficient precision and accuracy to function as a bioassay; (4) permit a variety of responses to be measured; and (5) be practical in terms of cost, time, and demand (Curtis *et al.*, 1987). As no one model meets all

these requirements, compromises have been made. A number of techniques and models must be used to provide pharmacological and electrophysiological information as to properties which confer antifibrillatory activity in myocardial ischæmia. In order to perform such extensive studies, a readily available, easily used, and cost-efficient species has to be chosen. Such extensive studies in dogs or any other large species make the choice of the rat a logical alternative. While "man is not a rat", discoveries in rats have been proven to have their clinical counterpart. For example, rat studies have shown that blockade of the endogenous systems believed to play a part in ischæmia-induced arrhythmogenesis, does not result in antifibrillatory actions (Beatch *et al.*, 1989). Thus many of the lessons learnt in the rat can be directly transferable to other species.

While the rat has distinct differences from man in terms of cardiac anatomy and electrophysiology, its advantages in the study of myocardial ischæmia and arrhythmias seem to outweigh its disadvantages. A notable advantage is the uniform lack of effective coronary collaterals which results in reproducible occluded (ischæmic) zones (Curtis *et al.*, 1987). This is of prime importance since both ischæmia-induced arrhythmias and infarct size depend upon the extent of collateral anastamoses (Curtis, 1986).

The primary disadvantage of producing ischæmia-induced arrhythmias in rats via coronary artery ligation is shared with all animal species, that is the lack of clarity regarding absolute clinical relevance. As well, some investigators consider the high resting heart rate in the rat (approximately 400 beats/min) to be a disadvantage. However, others have concluded that heart rate does not correlate with ischæmia-induced arrhythmia severity in conscious rats (Johnston *et al.*, 1983). Certain electrophysiological properties of the rat heart complicate analyses. Ventricular APD is brief in rats but it is unclear how, and if, the narrow AP jeopardises rat preparations (Payet *et al.*, 1978; cited in Inoue *et al.*, 1984). The narrow AP may reduce the likelihood of reentry during acute myocardial ischæmia although the fast resting heart rate would be expected to compensate for this (Botting *et al.*, 1986).

It is unavoidable that part of the ventricular muscle and coronary veins must be tied with the artery upon occlusion. However, it has been shown that the tightening of the occluder produces no significant sequelae (e.g. triggering of reflexes or receptor activation contributing to arrhythmogenic effects of ischæmia) unless the artery is also occluded (Hirche *et al.*, 1980).

1.4 Experimental Models used to Test Antiarrhythmic Agents

A number of problems are associated with the treatment of cardiac arrhythmias. Treatment remains largely empirical in nature likely due to the fact that myocardial ischæmia and infarction in man is a disease of multiple aetiology and variable outcome (Breithardt *et al.*, 1989). Numerous models have been developed to help identify the origin and idealistically the actual underlying electrophysiological basis of the various disturbances in cardiac rhythm (Winslow, 1984). A more rational approach to treatment will require characterization of the various types of arrhythmias with respect to the site(s) of origin, underlying electrical abnormalities, and the precise mechanism of action of each antiarrhythmic agent (in both normal and ischæmic areas) (Winslow, 1984). One particular problem with some models of cardiac arrhythmias is that while some deal with model methodology and characterization, few actually consider the model's ability to discriminate among antiarrhythmic agents of different classes or whether all agents in one class are equally effective in a particular model (Brooks *et al.*, 1989). Animal models have primarily attempted to mimic ventricular arrhythmias as these are most often precipitated by acute myocardial infarction and are known to occur in out of hospital patients dying of sudden cardiac death (David *et al.*, 1986; Winslow, 1984).

Numerous methods are available for the induction of arrhythmias in order to assess the effectivenes of putative antiarrhythmic agents or to explore mechanisms associated with the various arrhythmias. Arrhythmogenic stimuli can be divided into 3 groups: mechanical, electrical, and chemical (Winslow, 1984).

One experimental procedure using a mechanical arrhythmogenic stimuli creates a region of acute myocardial ischæmia by the acute occlusion of a major coronary artery (Harris, 1950; Clark *et al.*, 1980; Johnston *et al.*, 1983). This can be done either open-chest under anæsthesia, or closed chest in a chronic conscious model using a previously implanted occluder or catheter technique. A variety of species have been used in this technique including guinea-pig, rat, cat, dog, pig, and baboon (Johnston *et al.*, 1983). Each species has its unique anatomic and electrophysiological characteristics to consider with respect to methodology, interpretation of results and cross-species comparison. Curtis *et al.* (1987) point out that the major source of variability in the extent, severity, and outcome of myocardial ischæmia is coronary artery anatomy (with respect to the extent of collateral vascularization). However, the result of an acute ischæmic event can be compared quantitatively and qualitatively with the effects of treatment upon the ensuing ventricular arrhythmias (Johnston *et al.*, 1983). Drugs can be administered either prophylactically or subsequent to the onset of ischæmia.

Acute myocardial ischæmia in rats (the animal model we have chosen) produces ventricular arrhythmias in a predictable and reproducible manner (Curtis *et al.*, 1987). Rats exhibit 3 phases of arrhythmias in response to coronary occlusion (Curtis *et al.*, 1987). In both conscious and pentobarbitone anæsthetised rats, an early phase of ischæmia-induced arrhythmias begins 4 to 8 min after occlusion, and lasts for 5 to 10 min (Curtis *et al.*,1987). A second phase of severe ventricular arrhythmias starts approximately 1.5 - 2.5 h following occlusion and lasts for several hours (Curtis *et al.*, 1987). A third phase of arrhythmias is present in rats. In at least 90% of animals still alive 24 h post-occlusion, multi-focal PVC's occur (Curtis *et al.*, 1987). VT is rare in rats at 24 h following permanent coronary occlusion (unlike in dogs) (Curtis *et al.*, 1987). Experimental evidence suggests that these different phases of arrhythmias may represent different mechanisms of arrhythmia initiation (David *et al.*, 1986).

Occlusion induced arrhythmias include PVC's, VT, VF, and bradyarrhythmias (Curtis, 1986). Sinus bradycardia and atrial and atrioventricular nodal arrhythmias are much less common than ventricular arrhythmias (Curtis, 1986). Spontaneous reversion of these arrhythmias can occur and is not unique to rats and indeed may be a function of heart size (Curtis *et al.*, 1987).

Numerous variations in a second technique which involves induction of arrhythmias by electrical stimulation

of the heart have arisen since its induction over a century ago (Winslow, 1984). All electrical methods used to induce fibrillation in the heart are dependent upon the basic physiological concept that not all cardiac fibers (even those in close proximity with one another) will repolarize (i.e. recover their excitability) simultaneously (Mines, 1913; Moe *et al.*, 1964; in Winslow, 1984). Thus a vulnerable period is established during the cardiac cycle at the end of systole at which time some fibers will be completely repolarized while others are still refractory (Winslow, 1984). Applying an extra electrical stimulus of sufficient intensity in this time period will induce fibrillation (Winslow, 1984). Allessie *et al.* (1973, 1976) have mapped the spread of activation of a single premature stimulus and measured refractory periods at multiple sites in rabbit atrium. They suggested that the existence of nonuniform recovery of excitability is of primary importance in the genesis of tachyarrhythmias resulting from reentry. If the intensity of the electrical stimulus is further increased, excitation will spread to become disorganized, thereby instigating fibrillation (a measure of the ventricular fibrillation threshold) (Allessie *et al.*, 1976).

In view of the fact that class I and III antiarrhythmic agents are expected to increase the intensity of the electrical stimulus (current) required to evoke fibrillation, this method is often used to ascertain the effectiveness of putative antiarrhythmic agents (Winslow,

1984). The antiarrhythmic is tested against various electrical stimulation variables and its ability to influence arrhythmias induced by electrical stimulation of the heart.

The advantages of the electrical stimulation model include (Winslow, 1984): (1) good reproducability, (2) small cost, (3) duration of drug action can be followed, (4) minimal surgery is required, (5) each animal effectively serves as its own control such that large sample sizes are not required for statistical analysis, (6) electrically induced arrhythmias in animals do have predicted value for man (Horowitz *et al.*, 1980).

Two further experimental models used to test antiarrhythmic agents include chemical agents and reperfusion-induced arrhythmias. A large number of chemical agents, alone or in combination, have been found to induce arrhythmias (Winslow, 1984). It has been found that some chemical agents are species-specific (Brooks *et al.*, 1989; Winslow, 1984) and capable of discriminating among antiarrhythmic agents of different mechanistic classes (Brooks *et al.*, 1989). Chloroform, aconitine, and oubain are the chemical agents most commonly used (Winslow, 1984).

Reperfusion of blood flow to myocardium previously made ischæmic by a period of coronary occlusion, has been associated with arrhythmias and high mortality in animal experiments (Janse and Wit, 1989; Winslow, 1984). In fact, the occurrence of VF may be greater after reperfusion than

after coronary artery ligation (Stephenson *et al.*, 1960; cited in Janse and Wit, 1989). Both *in vivo* and *in vitro* models are used to study reperfusion-induced arrhythmias. There is a relationship between the length of the ischæmic period during the occlusion and the occurrence of reperfusion arrhythmias (reviewed in Janse and Wit, 1989). The incidence of reperfusion-induced VF increases when occlusion periods are lengthened from 5 min to 20 or 30 min but decrease when reperfusion is delayed beyond 30-60 min (Janse and Wit, 1989). Occlusion can be repeated (usually at 30-90 min intervals) so that animals may serve as their own controls for drug assessment (Winslow, 1984).

1.5 Mechanistic Models of Arrhythmogenesis

An arrhythmia is an irregularity in the rhythm of the heart's beating, due to an abnormality of impulse initiation, conduction, or both (Hoffman and Rosen, 1981). This abnormality in the rate, regularity, site of origin of the cardiac impulse, or disturbance in conduction, results in the normal sequence of activation of atria then ventricles being altered (Wit *et al.*, 1974). In considering the mechanisms for cardiac arrhythmias, it is necessary to first identify the abnormalities of cellular electrical function or structure that can induce arrhythmic activity and second, to determine which of these possible mechanisms are actually responsible for specific arrhythmias in the *in*

situ heart (Hoffman and Dangman, 1987). This is a critical point because, if it is possible to make a certain and explicit identification of the cellular electrophysiological mechanism that is involved in the genesis of the arrhythmia, then perhaps it would also be possible that the response of specific arrhythmias to drug intervention would be dependent on the identified arrhythmogenic mechanism.

1.5.1 Abnormalities in Impulse Generation

Certain cardiac cells have the property of automaticity which means that they are capable of spontaneously initiating their own impulses which results from depolarization of fibers during electrical diastole - phase 4 depolarization (Rosen, 1988). This slow spontaneous depolarization during diastole lowers the membrane potential to threshold potential and a spontaneous AP occurs (Vera and Mason, 1981). These fibers are therefore considered automatic (Wit *et al.*, 1974). Normally automatic cells include the pacemaker cells of the SAN, subsidary atrial fibers, fibers in and around the coronary sinus ostium, cardiac fibers in the tricuspid and mitral valve leaflets, the NH region of the atrioventricular junction, and the His bundle and Purkinje fiber ramifications in the ventricle (Hoffman and Dangman, 1987; Wit *et al.*, 1974). The intrinsic rate of impulse initiation in the SAN is faster when compared with the rest of the specialized conducting

system, allowing it to function as the primary pacemaker (Rosen, 1988). However, when the sinus rate is absent, other sites in the specialized conducting system take over the pacemaker function (Rosen, 1988). Thus arrhythmias that are caused by abnormal impulse generation can be regarded as resulting from focal mechanisms (Hoffman and Dangman, 1987). The origin or focus of these arrhythmias is in a single fiber or a small group of well-coupled fibers with ectopic impulses spreading radially from this focus (Hoffman and Dangman, 1987). It is assumed that the rhythms are automatic in the sense that generation of one impulse does not depend on a prior impulse (Hoffman and Rosen, 1981).

Two major classes of arrhythmias caused by abnormalities in impulse generation can be identified; those resulting from truly spontaneous impulse generation or automaticity (and therefore not reliant on a prior impulse) and second, those from triggered activity (i.e. the generation of one or more impulses as a consequence of a prior impulse) (Hoffman and Rosen, 1981).

An important characteristic of automatic pacemakers is their ability to be overdrive suppressed (Rosen, 1988). If a preparation is stimulated at a faster rate than that of the intrinsic pacemaker, the pacemaker rate will be transiently reduced (Rosen, 1988). This is referred to as overdrive suppression (Rosen, 1988). In fibers with high levels of membrane potential, the normal pacemaker mechanism tends to be readily suppressed by overdrive pacing (Rosen,

1988). This is contingent upon the entry of sodium ions during phase 0 of each AP (Rosen, 1988).

Enhanced pacemaker activity in normal specialized conducting tissues can result in automatic arrhythmias (Hoffman and Dangman, 1987). However, many of the properties of normal automatic cells suggest that a significant fraction of the arrhythmias attributed to abnormal impulse generation are not caused by normal automaticity (Hoffman and Dangman, 1987).

Abnormal automaticity can be the occurrence of phase 4 depolarization (i.e. spontaneous impulse initiation), at levels of transmembrane potential considerably less negative than the normal maximum diastolic potential or normal resting potential of the fibers involved (Hoffman and Rosen, 1981; Janse and Wit, 1989). If there is a decrease in background potassium conductance or an increase in inward Na^+ current, the resting transmembrane potential is reduced to -60 mV or less, initiating a spontaneous impulse (Hoffman and Dangman, 1987). When the transmembrane potential is reduced, slow diastolic depolarization seems not to result from the pacemaker current, i_f but from time- and voltage-dependent changes in K^+ and Ca^{2+} currents that occur at the plateau potential (Hoffman and Dangman, 1987). Unlike normal automatic impulses, single premature impulses do not perturb rhythms caused by abnormal automatic impulses (Hoffman and Dangman, 1987). In addition, the marked overdrive suppression that can occur in normal automatic

cells is reduced or even lost in abnormal automatic cells (Hoffman and Dangman, 1987; Rosen, 1988). This may reflect the fact that the Na^+ channels are largely inactivated by the low maximum diastolic potential (Hodgkin and Huxley, 1952; Hoffman and Dangman, 1987).

A third proposed arrhythmogenic mechanism is triggered activity. It is repetitive impulse formation initiated by a propagated or automatic AP (Janse and Wit, 1989). Triggered activity is dependent on oscillations in membrane potential that follow the AP upstroke, i.e. afterdepolarizations (Janse and Wit, 1989), and may be either early or delayed (Rosen, 1988). When such an oscillation occurs during repolarization it is called an early afterdepolarization; when it occurs after the membrane has repolarized to its maximum diastolic potential (Rosen, 1988), or nearly so, it is called a delayed afterdepolarization (Janse and Wit, 1989). When afterdepolarizations are large enough to reach threshold, the resultant AP, or repetitive AP's, is said to be "triggered" and may result in atrial or ventricular arrhythmias (Janse and Wit, 1989). Impulses resulting from afterdepolarizations are by definition triggered by a prior impulse and thus are not automatic (Hoffman and Dangman, 1987). Arrhythmias induced by delayed afterdepolarizations occur more readily when the preceding stimulation rate is rapid and will tend to increase in rate as the preceding drive rate is increased (Monk and Rosen, 1984). It has been suggested that delayed afterdepolarizations reflect

oscillations of calcium-loaded sarcoplasmic reticulum (Lazzara and Scherlag, 1988).

In summary, the mechanisms involving arrhythmias caused by abnormal impulse generation may be either automatic or triggered: the automatic rhythms can be due to either normal or abnormal automaticity, and triggered rhythms by either early or delayed afterdepolarizations.

1.5.2 Abnormalities in Impulse Conduction

Abnormalities in impulse conduction may be the result of complete failure of propagation or due to unidirectional block and reentry of an impulse (Rosen, 1988). The term reentry is used in the sense that during the arrhythmia there is continuous propagation of the impulse (Rosen, 1988).

Evidence is accumulating in support of reentry as a mechanism for generation of arrhythmias by ischæmic tissues in animal models (Lazzara and Scherlag, 1988). Certain conditions are necessary for the initiation and maintenance of reentry and include: (1) unidirectional block of the impulse in a region(s) of the heart; (2) stable propagation at a sufficiently low velocity; (3) delayed excitation of the tissue just distal to the blocked site, and (4) sufficent repolarization of the tissue proximal to the site of block such that the sodium channels can be opened when

the impulse that is propagating around the barrier enters the region of initial block (Sasyniuk and Mendez, 1971; Spach and Dolber, 1985). Thus conduction time over the alternative route must be longer than the refractory period of the path to be reentered (Sasyniuk and Mendez, 1971). Difficulty arises when consideration is given to the long refractory periods (particularly in the ventricles) and brings about the question whether propagation over an alternative route in ventricular tissue could be slow enough to allow tissue with extended refractory periods to be reexcited (Sasyniuk and Mendez, 1971).

Modifications have been made regarding these conditions and the mechanisms that cause the slow conduction and conduction block. Allesie *et al.* (1977) demonstrated in atrial tissue that stable reentry circuits need not form around a hole or fixed barrier but can form around a thin interface between regions of tissue that are alternately refractory and receptive during a single circuit. In Allesie's model, absolute refractoriness serves as the functional barrier to the circulating impulse while relative refractoriness meets the requirements of slow conduction (Lazzara and Scherlag, 1988).

It has been suggested in past that fibrillation might represent chaotic reentrant excitation or multiple continually migrating activation wavefronts (Mines, 1913; Moe *et al.*, 1964). This type of reentry has been termed random reentry (as opposed to stable reentry based on a

fixed anatomical path as discussed above) where the path of excitation continuously changes such that individual groups of fibers may be repeatedly excited (Hoffman and Rosen, 1981). Myocardial ischæmia can produce all of the conditions necessary for reentry to occur: holes (scars or fixed barriers), slow conduction, and abnormal refractoriness, either abbreviated or prolonged (Lazzara and Scherlag, 1988). Consequently, strong support is given to the important role reentry may have as a mechanism for arrhythmias in ischæmia.

Mapping has been used extensively to study reentrant circuits and to offer modifications to the conditions for reentry. Kramer *et al.* (1985) used a computerized system capable of detecting, storing and assessing information from 232 sites in dog hearts after permanent or transient coronary occlusion. They determined that intramural reentry (microreentry) was a mechanism for VT whereby small epicardial conduction loops exited into non-refractory subendocardium initiating succeeding beats. Kramer *et al.* (1985) also determined that the site of conduction delay neccessary for reentry was the thin surviving epicardial tissue rim, i.e. a fixed hole or barrier was not needed for a reentry circuit (Lazzara and Scherlag, 1988). Furthermore, the "preferred pathways" of exit into the subendocardium occurred at the "border zone" of the infarct and were of variable configuration (Kramer *et al.*, 1985).

Reentry can also result from reflection of an impulse from an inexcitable segment (Antzeleritch *et al.*, 1985; Cranfield, 1975; cited in Hoffman and Dangman, 1987). If a delayed AP is caused by the electrotonic depolarization of a blocked impulse and is distal to an inexcitable segment, then reflection occurs (Hoffman and Dangman, 1987). The delayed AP then reexcites the tissue proximal to the site of block (Hoffman and Dangman, 1987). These reflected impulses can be modulated by changes in rate and rhythm (Hoffman and Dangman, 1987).

Spach and Dolber (1985) proposed that the spread of excitation in cardiac muscle occurs by a previously unrecognized type of propagation that is discontinuous in nature (proceeding in steps) due to recurrent discontinuities of axial resistivity that affect the kinetics of the fast Na^+ channels (i.e. membrane currents). They proposed that this propagation in cardiac muscle plays a primary role in cardiac conduction disturbances leading to reentry. This hypothesis does not meet the requisites for reentry (detailed earlier) due to spatial nonuniformity of refractory periods (Spach and Dolber, 1985). Following the direction of Spach and co-workers it has been suggested that the alignment of fibers and directional differences in the density of tight junctions may determine the loci of block and the configuration of reentrant circuits (Lazzara and Scherlag, 1988).

1.5.3 Electrophysiological Consequences of Myocardial Ischæmia

Acute ischæmia causes myocardial cells to release K^+ (Hirche *et al.*, 1980). This increase in extracellular K^+ is accompanied by a decrease in the resting membrane potential. This causes a decrease in AP amplitude, maximum rate of depolarization, and APD (Janse and Wit, 1989). A combination of factors produce these effects and include increased extracellular K^+, acidosis, and the combined lack of oxygen and substrate in the ischæmic tissue (Janse and Wit, 1989). Both fast and slow currents are equally depressed by ischæmia (Cardinal *et al.*, 1981). Due to the reduction in APD, ERP is also expected to be abbreviated (Lazzara *et al.*, 1978). However this is not the case. ERP is in fact prolonged in acutely ischæmic cells since it continues beyond full repolarization (Lazzara *et al.*, 1978). This is known as post-repolarization-refractoriness (Lazzara *et al.*, 1978). The above electrophysiological changes occur in different ischæmic cells to different degrees (Janse *et al.*, 1979). Thus delayed conduction and conduction block; necessary for reentry and subsequent reentrant arrhythmias, are present during acute ischæmia (Botting *et al.*, 1986).

1.6 Mode of Action of Antiarrhythmic Agents

While the cardiac electrophysiological actions of antiarrhythmic agents have been extensively described and indeed are the basis for the Vaughan Williams classification system, the mechanisms of the antiarrhythmic actions of these drugs is far less defined (Davy *et al.*, 1988). Antiarrhythmic agents can affect the arrhythmogenic substrate (whatever its electrophysiological origin), or the initiating events such as ectopic beats or changes in the sinus cycle length (Davy *et al.*, 1988). Both diseased cells and normal cells are involved, and can be profoundly affected by such drugs (Davy *et al.*, 1988). Consequently, the choice of an antiarrhythmic agent often remains empirical. An empirical basis of therapy first may result in failure but, more importantly, the arrhythmia being treated may be aggravated or a new arrhythmia generated (Zipes, 1987).

Mechanisms of antiarrhythmic actions have been demonstrated *in vitro* and *in vivo* both experimentally and clinically. Based on the arrhythmogenic mechanisms discussed earlier (automaticity, triggered activity, and reentry), the antiarrhythmic action of drugs on automatic rhythms could involve an inhibition of i_f, the pacemaker current, a shifting of the maximum diastolic potential to more negative values, or an increase in APD, these actions

altogether lowering the automatic focus firing rate (Davy *et al.*, 1988).

Triggered rhythms, as opposed to automatic rhythms, are more commonly responsible for clinical arrhythmias (Davy *et al.*, 1988). Antiarrhythmic action on triggered activity could involve suppression of the afterdepolarization (Davy *et al.*, 1988). Antiarrhythmic agents can decrease Ca^{2+} or Na^{+} inward currents (Thale *et al.*, 1987). Finally, early afterdepolarizations can be effectively suppressed by decreasing the basic cycle length while delayed afterdepolarizations can be suppressed by increasing it (Davy *et al.*, 1988).

Antiarrhythmic action of drugs on reentrant rhythms could include an increase in refractoriness in one part of the circuit or secondly, a conduction block changing a unidirectional block, into a bidirectional block (Hoffman, 1985). It is important to realize that the underlying mechanisms are unknown for some arrhythmias and that the same drug can act in different ways.

1.6.1 Classification System for Antiarrhythmic Agents

The classification of antiarrhythmic agents was initially based on drug effects on AP morphology (Vaughan Williams, 1970). This classification has become the most widely accepted. Antiarrhythmic agents are grouped into class I, those that block sodium channels; class II, those

agents which block sympathetic activity - the β blockers; class III, potassium channel blockers; and class IV, calcium channel blockers (Singh and Vaughan Williams, 1972; Vaughan Williams, 1970, 1984a). Vaughan Williams emphasized that his classification system was not so much a categorization of drugs according to chemical structures or physical properties (as exemplified by the diversity of agents in any given class), but merely described four ways in which abnormal cardiac rhythms could be prevented or corrected (Vaughan Williams, 1984a).

Some basic problems arose when attempts were made to transfer Vaughan Williams classification to the clinic. First, the Vaughan Williams classification of antiarrhythmic agents was based on observations made on the cardiac AP under normal conditions (Brugada, 1990). Extrapolation of such information to the clinical selection of an antiarrhythmic agent has proven difficult (Zipes, 1987). The same antiarrhythmic agent can have different effects on the same cell depending upon a multitude of factors such as the presence or absence of ischæmia, electrolyte disturbances, drug concentration at the target site, rate of stimulation, etc. (Brugada, 1990; David *et al.*, 1986; Szekeres, 1986). The behaviour of normal and abnormal cells in the presence of a drug depends not only on the intrinsic characteristics of the cells but also upon the autonomic balance (Brugada, 1990). Arrhythmias seen clinically

involve areas (as opposed to single cells) of myocardium and relatively long activation pathways (Brugada, 1990).

Further analysis within each class both experimentally and clinically has resulted in further clarification and subclassification of the Vaughan Williams classification system perhaps giving it more clinical relevance. The clinical differences between various drugs with class I action has necessitated their subdivision (Harrison *et al.*, 1980).

1.6.2 Class I Agents

1.6.2.1 The Sodium Channel

Attempts to elucidate the nature and properties of the cardiac sodium channel are relevant to hypotheses concerning the action of class I antiarrhythmic agents on these channels. Ion channels are molecules that form pores in the membrane to allow ion flow (Hille, 1984; Jan and Jan, 1989). The electric potential across the cell membrane is determined largely by ion channels opening and closing (Jan and Jan, 1989). Channel specificity to a particular ion species is conferred by a "selectivity filter" that resides in the outer portion of the channel (Rosen and Spinelli, 1988). Voltage or channel gates are structures controlling the passage of ions so that ions do not enter the channel *ad libitum* until the concentration and charge gradients are

balanced (as this would not maintain the transmembrane potential) (Rosen and Spinelli, 1988). These gates are on the inner portion of the channel and depending on whether they are in an open or closed position, passage of ions will be permitted or prevented (Rosen and Spinelli, 1988). From Hodgkin and Huxley's (1952) classic studies on squid axon, these gates were designated m and h. Hodgkin and Huxley (1952) introduced the concept of channel gates in an attempt to mathematically model the time and voltage dependent stages of the inward Na^+ current and depolarization. In the closed state of the channel, the m gate is closed and the h gate open (Sheldon *et al.*, 1989). In the open state, the m gate opens in response to an electrical stimulus (voltage sensitive) and Na^+ ions enter causing the AP upstroke (Rosen and Spinelli, 1988). As the cell depolarizes, the voltage-sensitive h gate closes, inactivating the channel (Rosen and Spinelli, 1988). Both gates are time-dependent such that during repolarization, the m gate closes and the h gate opens (Rosen and Spinelli, 1988; Sheldon *et al.*, 1989). These voltage sensors or gates act independently of the selectivity filter (Rosen and Spinelli, 1988).

Electrophysiological studies have examined the movements of charges intrinsic to the cation channels (known as the gating current) as predicted by Hodgkin and Huxley (1952) (Jan and Jan, 1989). These studies have lead to the prediction of a string of positive charges acting as a voltage sensor which may pair with neighbouring negative

charges in the cell membrane (Armstrong, 1981). Subsequent studies (reviewed by Hille, 1984) indicated that components intrinsic to these channels function as the voltage sensor. The differences between the inactivated state and the closed state is clearly demonstrated by measuring the movement of charges intrinsic to the channel (the gating current) under various conditions which affect inactivation of Na^+ channels (Jan and Jan, 1989). Inactivation immobilizes the gating charges in the open state (Armstrong, 1981), implying that a channel has to be open before it can be inactivated (Jan and Jan, 1989). Na^+ channels inactivate more readily when they are open, although it is possible that some channels may be inactivated before reaching the open state (Jan and Jan, 1989).

The sodium channel is composed of 1820 amino acid residues and contains four homologous internal domains, each of which has six putative transmembrane segments (Stuhmer *et al.*, 1989) of 19 or more predominantly hydrophobic residues (Jan and Jan, 1989). One of these segments, S4, contains several arginine or lysine residues at every third position interposed with mostly nonpolar residues (Stuhmer *et al.*, 1989). This S4 sequence has been postulated to form a transmembrane helix, so that the positively charged residues reside within the membrane and function as the voltage sensors discussed above (Catterall, 1986). In the S4 hypothesis, the four S4 sequences may correspond to the gating particles responsible for channel activation and the

steep voltage-dependence as proposed by Hodgkin and Huxley (Jan and Jan, 1989; Stuhmer *et al.*, 1989). From measurements of gating charge, it has been estimated that four to six charges within the channel molecule move from one side of the membrane to the other as the channel opens (Jan and Jan, 1989). The S4 model accounts for this if depolarization causes each of the S4 sequences to move by roughly one helical turn (Jan and Jan, 1989).

Stuhmer *et al.* (1989) identified functional regions of the Na^+ channels by investigating the effects of site directed mutations on rat sodium channels. They provided evidence that the positive charges in segment S4 were involved in the voltage-sensing mechanism for activation of the channel, and that the region between repeats III and IV were important for channel inactivation.

Na^+ channels in squid giant axon are said to exist in 3 states, as are Na^+ channels of Purkinje cells: (1) closed, at potentials near the resting potential, but available to be opened by depolarization; (2) open, selectively permitting passage of Na^+ ions; and (3) closed, and not available to be opened, i.e. inactivated (Fozzard *et al.*, 1985). After the cell membrane depolarizes, Na^+ permeability markedly increases and then after 1 msec decreases to the base-line level (Catterall, 1987). This biphasic behaviour represents activation (opening of Na^+ channels) and inactivation (closing of Na^+ channels) (Catterall, 1987). These events can be described as

separate voltage-dependent processes that change their rates instantly as a function of voltage (Hodgkin and Huxley, 1952). Both resting and inactivated states are non-conducting (Catterall, 1987). However, evidence has been accumulating over the years that the cardiac Na^+ currents are not accurately described by the original Hodgkin-Huxley formulations. Armstrong (1981) and others (reviewed in Fozzard *et al.*, 1985) have shown that activation occurs with a lag which is thought to reflect the intermediate state that the channel must pass through prior to opening. Inactivation also occurs with a lag, thus suggesting that channels must open before they can inactivate (Armstrong, 1981). Inactivation and recovery from inactivation often occurs with more than one time constant (Chiu, 1977) suggesting that the channel may have more than one inactivated state (Fozzard *et al.*, 1985). Meves and Nagy (1989) discussed multiple conductance levels in the sodium channel of mouse neuroblastoma cells. They pointed out that these subconductance states were rare events and that it did not seem likely that they contributed significantly to the macroscopic inward Na^+ current responsible for the rising phase of the AP (Meves and Nagy, 1989). However, the subconductance states may help explain function as related to structure of the sodium channel (Meves and Nagy, 1989). In this regard, the four repeated homology units of the sodium channel undergo conformational changes during depolarization before an "ion permeability pathway" is

formed (Meves and Nagy, 1989). Meves and Nagy (1989) speculated that the subconductance levels of the sodium channel corresponded to the activation of each homology unit in turn.

The delay in the onset of inactivation could be very important in heart muscle since the current involved in generating the conducted AP upstroke requires 100-200 μsec to develop (Fozzard *et al.*, 1985). A very slow phase of recovery from inactivation in heart muscle is influenced by the duration of depolarization or the presence of antiarrhythmic agents (Cohen *et al.*, 1981).

Based on recent experimental results, variations to the Hodgkin-Huxley model have been made. Figure 1 is an illustration of one such modification. In a time- and voltage-dependent manner, Na^+ channels pass between the closed (C), open (O), and inactivated (I) states (Hondeghem, 1984). Time and voltage dependent rate constants or probability functions can describe these transitions (Hondeghem, 1984). The closed state of the channel is most prevalent in the resting membrane (Hondeghem, 1984). Upon depolarization of the membrane, the channel may first go to the open state and then the inactivated state (or go there directly) then returning to the closed state before any further channel opening (Fozzard *et al.*, 1985).

The development of the patch clamp technique by Neher and colleagues (Hamill *et al.*, 1981) has allowed the study of single membrane channels. Data obtained from patch clamp

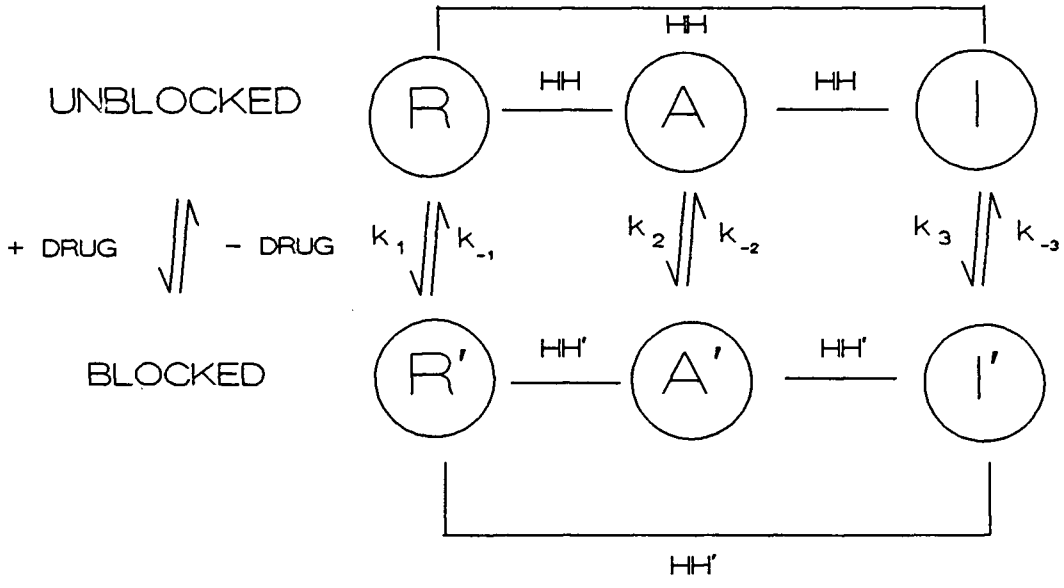


Figure 1. Diagram illustrating the mechanism of action of antiarrhythmic drugs in a sodium channel undergoing transitions between three states. R = resting (closed), A = activated (open), and I = inactivated (closed) drug-free fractions of the population of sodium channels. R', A', and I' are the respective drug-associated fractions. The transition between the states follows Hodgkin and Huxley first-order kinetics with voltage-dependent rate constants (HH). HH' are the same rate constants shifted on the voltage axis for the drug-associated channels. k_1, k_2, and k_3 are the association rate constants for the antiarrhythmic drug and k_{-1}, k_{-2}, and k_{-3} are the dissociation rate constants. (From Hondeghem and Katzung, 1977).

analysis of cardiac Na^+ channels has allowed the following conclusions to be drawn (Fozzard *et al*, 1985): (1) Na^+ channel density is 2-10/μm^2. Whether channel distribution is random or localized is not yet known (Almers *et al.*, 1983; cited in Fozzard *et al.*, 1985); (2) Under certain defined conditions, a single Na^+ channel has a conductance of approximately 15 pS, which is about 10^7 ions/sec per channel. With an open time of 1 msec, a single channel event is recorded by the movement of about 10 000 Na^+ ions; (3) The Na^+ current changes its magnitude upon depolarization which increases probability of opening and by a somewhat longer mean open time (Rosen and Spinelli, 1988). Studies have provided data suggesting that there may be two populations of Na^+ channels, based on channel currents, kinetics, and/or response to TTX (Ten Eik *et al.*, 1984; cited in Fozzard *et al.*, 1985).

1.6.2.2 Subclassification of Class I Agents

Despite the complexity of the classification of antiarrhythmic agents based on the classical ion channel blocking properties, such agents have consistently demonstrated antifibrillatory and antiarrhythmic actions against ischæmia-induced arrhythmias. The class I agents have a long history, both in their use in the treatment of a variety of arrhythmias, and in their development by modification of basic chemical structures (Courtney, 1988).

However, endeavours to improve on prototypical class I agents have not met with much success despite the introduction of numerous new agents (Schlepper, 1989).

Agents differ in their mode of action on Na^+ channels and in other properties (Borchard *et al.*, 1989) such that subclassification within this group has simplified matters and provided additional relevant information for experimental and clinical purposes.

In reviewing the complex electrophysiological actions of various new class I antiarrhythmic agents, Vaughan Williams (1984b) proposed a subclassification of this group of drugs. Vaughan Williams (1984b) suggested placing lidocaine and flecainide in one class, and quinidine, procainamide and disopyramide in another sub-class on the basis of their effects on APD. In 1979, Harrison *et al.* proposed a further division (i.e. the introduction of the Ic subclassification) of class I into subgroups 1a, 1b, and 1c (Harrison *et al.*, 1979). Harrison (1985) subclassified class I agents primarily on the different effects of clinical concentrations of these drugs, on conduction in the specialized conducting tissue, and on ventricular refractoriness and repolarization. According to Harrison (1985); class 1a agents widen the QRS duration, slow conduction at high concentrations, prolong the Q-T interval and widen the AP. Prototypical 1a agents include quinidine, procainamide, and disopyramide. Class 1b agents demonstrate limited (if any) effect on QRS duration and conduction while

shortening the Q-T interval and APD and elevating the fibrillation threshold (Harrison, 1985). Lidocaine, mexiletine, tocainide, and ethmozine are all 1b agents. Class 1c agents widen the QRS complex, slow conduction at low concentrations and have little effect on repolarization and duration of the AP. Class 1c agents produce small changes in refractoriness, e.g. flecainide, encainide, lorcainide, propafenone, and indecainide (Harrison, 1985). These characteristic electrophysiologic differences can also be seen clinically (Milne *et al.*, 1984).

Criticism could be made against Harrison's subclassification not only for compounds with multiple electrophysiologic actions such as amiodarone, but also for drugs such as quinidine and procainamide, which prolong repolarization of the AP at higher concentrations by mechanisms unrelated to sodium channel blockade (Harrison, 1985). Since all class 1 agents block fast sodium channels, it has been postulated that the subclassification is not a fundamental one but one based on pharmacodynamics in that the only difference between the subclasses is in the concentration necessary to block Na^+ channels (Harrison, 1985).

In 1983, Campbell subdivided class 1 agents based on their kinetics of onset and rate dependent depression of the maximum rate of depolarization (V_{max} or MRD) *in vitro* (Campbell, 1983a, 1983b). Campbell's laboratory investigated 3 new class I agents: encainide, flecainide,

and lorcainide. These agents all markedly depressed conduction in the His-Purkinje system and ventricle in vivo, not surprisingly based on their effects on V_{max} *in vitro* (Campbell, 1983b). At therapeutic concentrations these agents did not prolong refractoriness in the atrium and ventricle to the extent expected (Campbell, 1983b). In addition, and common to other class I agents, increased frequency of stimulation progressively enhanced the depression of V_{max} (i.e. rate-dependent block) (Campbell, 1983b). However, the rate at which V_{max} was depressed following a sudden increase in frequency was much slower than reported for other class I agents in current clinical use (Campbell, 1983b). Campbell (1983b) studied nine class I antiarrhythmic agents and found that they fell into 3 well-demarcated subgroups based on onset kinetics of rate dependent block (RDB). While all agents produced comparable amounts of RDB, it was possible to separate them into 1b, 1a, and 1c subgroups based on the marked differences observed in the speed at which V_{max} fell to the new plateau level with these differences persisting at all concentrations and driving rates studied (Campbell, 1983b). 1b agents (lidocaine, tocainide, and mexiletine) demonstrated fast onset kinetics; 1a (quinidine, disopyramide, and procainamide), intermediate; and 1c (encainide, flecainide, and lorcainide), slow onset kinetics (Campbell, 1983b).

A major factor in determining the ability of a class I drug to prolong the ERP relative to the APD is the speed with which the drug can further depress V_{max} in response to a sudden increase in stimulation frequency (i.e. the speed of onset of RDB) (Campbell, 1983b). Reasons for these kinetic differences among class I drugs involves a number of aspects. The depression of V_{max} alone is insufficient to block conduction of a propagated AP *in vitro* at a basic diastolic interval of 1000 ms (Campbell, 1983b). An additional factor must result in further depression of the number of Na^+ channels available at 300 ms (in addition to the depression already present at the time of the preceding basic drive beat), such that an AP will not propagate and the tissue would be, by definition, refractory (Campbell, 1983b). This factor may be the ability of a given drug to further depress V_{max} in response to a step increase in rate which persists for one interstimulus interval (Campbell, 1983b).

When the kinetic differences between subgroups are taken into account, then drugs with the fastest onset of RDB (1b agents) will produce the greatest increases in ERP-APD_{90} (Campbell, 1983b). Agents with fast kinetics have relatively greater effects on refractoriness than on APD thus conferring selectivity of Ib agents for high frequency arrhythmias (Nattel and Zeng, 1984; Varro *et al.*, 1985). Thus the differences between drugs in their onset kinetics at concentrations producing equal depression of V_{max}, and

their ability to prolong ERP relative to APD, can be seen to be a direct consequence of their differing abilities to prolong recovery from inactivation (Campbell, 1983b). The class Ic agents may then be considered more potent since their longer offset kinetics will produce a steadily accumulating depression of V_{max} without significant interference from diastolic recovery (Varro *et al.*, 1985). However, they are also more toxic since they can exert their effects at normal sinus rhythm resulting in adverse side effects such as myocardial depression (Schlepper, 1989). Class Ib agents on the other hand, can be given at concentrations that have little effect on V_{max} and hence conduction velocity at normal heart rates, but selectively depress conduction of premature AP's (Pallandi and Campbell, 1988; Varro *et al.*, 1985). Class Ia agents are less able to respond to sudden changes of rate and thus depress conduction of normal beats at concentrations that depress premature beats (Pallandi and Campbell, 1988). This is seen to an even greater extent with Ic agents (Pallandi and Campbell, 1988).

Experimental studies on cardiac and nerve tissue that focus on molecular mechanisms of action of class I agents have provided further insight on the kinetic differences of class I agents (Campbell, 1983b). It has been established that the primary action of class I drugs is to prolong recovery from inactivation, possibly by binding directly to the inactivation gating mechanism (Hondeghem and Katzung,

1977; Courtney, 1980). In addition, before "large-scale" drug binding can occur, it appears that the channels must be in the inactivated state (Campbell, 1983b). Therefore, repetitive stimulation enhances the binding of class I drugs to the sodium channel by producing repetitive inactivation and is known as the rate-dependent effect (Campbell, 1983b). Kodama's group (1986, 1987) have shown channel state selectivity of blockade. They determined that Ia agents block the Na^+ channel mainly during the upstroke of the AP (i.e. activated channel block) while Ib agents do so mainly during the plateau phase (inactivated channel block) (Kodama and Toyama, 1988).

The importance of physico-chemical properties in determining the kinetics of the effects of the class I agents on maximum rate of depolarization have been examined (Campbell, 1983c). Properties taken into account include molecular weight, lipophilicity, and the spatial dimensions of the molecule. Numerous studies of structure-activity relationships of class I agents have been published (Courtney, 1980). These various groups found that the degree to which class I agents could produce resting block (i.e. depress V_{max} in unstimulated myocardium) correlated well with the agent's lipophilicity (from logP, the partition coefficient, and pKa). Poor correlations have been found with some other physico-chemical properties such as molecular weight (Campbell, 1983c). However as molecular weight increased, the affinity for the receptor and the

persistence of drug action also increased (Campbell, 1983c). This is normally attributed to greater Van Der Waal's (and other) forces between drug and binding site (Campbell, 1983c). The rapidity of onset of rate dependent block correlated with molecular weight; agents with lower molecular weights being faster (Campbell, 1983c). The time constant of recovery from RDB on cessation of stimulation also correlated well with molecular weight (Campbell, 1983c), this correlation being improved by taking lipophilicity (log P and pKa) into account (Campbell, 1983c). Permanently charged molecules were associated with very slow recovery regardless of molecular weight (Campbell, 1983c).

Voigt et al. (1988) demonstrated that the binding of class I antiarrhythmics to phosphatidylcholine membranes was determined mainly by their lipophilicity irrespective of pKa. Based on previous concepts and Campbell's (1983c) data on the physico-chemical properties of class I agents, Campbell proposed a model of the sodium channel and its interaction with class I antiarrhythmic agents. Campbell (1983c) felt that the class I agents act on a site, possibly within, or functionally associated with the Na^+ channel, this site being accessible from the cytosol or hydrophobic membrane, but not directly from the extracellular side of the membrane. Furthermore, depolarization greatly enhances drug binding to the binding site (Campbell, 1983c). If the depolarization is rapid and repetitive, then the increased

binding is manifest as RDB, but if a steady depolarization is applied, it will appear as an increase in resting block (Campbell, 1983c). These are essentially two different aspects of a single voltage-dependent process (Campbell, 1983c). Destruction of the inactivation gating process by some technique which leaves the activation intact largely eliminates this voltage dependence, thus strongly suggesting that the binding site is the inactivation gate or a similar structure (Cahalan, 1980; Shapiro and Almers, 1980; cited in Campbell, 1983c). Depolarization presumably changes the inactivation gate which exposes the receptor site to the cytosol so that binding is facilitated (Campbell, 1983b). Reactivation in response to repolarization is only possible if the drug dissociates from the binding site (Campbell, 1983b; Courtney, 1987).

Another important factor in determining the kinetics of offset of RDB of various class I agents aside from ionic charge (permanently charged agents have very long offset kinetics regardless of molecular weight), and molecular weight is stereospecificity (Hill *et al.*, 1988). The orientations of aromatic and amine groups on class I agents are important in drug binding to the cardiac Na^+ channel (Hill *et al.*, 1988).

Structural hypotheses have been proposed for class I agents with respect to their widely differing abilities to block myocardial Na^+ channels during individual AP's and their associated repriming kinetics. The size-solubility

hypothesis just discussed provides support for smaller antiarrhythmic drugs with good lipid distribution capabilities (e.g. Ib agents) exhibiting rapid repriming kinetics (Courtney, 1990). While the hypothesis seemed to successfully predict the unblocking kinetics, it was not clear from a group of drugs tested by Courtney (1983), whether a high lipid solubility would speed up recovery kinetics for a larger drug, or whether just a low lipid solubility would slow recovery kinetics for a smaller drug. Courtney (1983) suggested that hydrophilic drugs but not lipophilic drugs show modulation of their size-dependent kinetics. Furthermore, the more tightly bound waters of hydration could be effectively adding to the size of such hydrophilic drug molecules (Courtney, 1983).

With the introduction of new drugs, predictions based on a quantitative structure-activity relation proposed by Courtney (1987) have been in error. Such was the case with a putative class I agent BW A256C which has an unusually high pKa (11.2) (Donoghue *et al.*, 1987). As a result of this failure to make correct predictions regarding new drugs, Courtney (1990) looked at agents structurally and three-dimensionally. He found that the end-on view of the molecule, provided a better explanation for the size dependence of repriming kinetics than molecular weight thus offering a modification to the size-solubility hypothesis. Courtney (1990) described a quantitative model for recovery time which coupled proton exchange rates with a drug-size

dependent process related to recovery from Na^+ channel inactivation. Thus drugs having a wider span at their aromatic recovery kinetics from inactivation (Courtney, 1990).

Clinically, the main interest of subdivision of class I agents, based on purely objective electrophysiological measurements *in vitro*, is that the quantitative disparities in such characteristics as frequency-(or rate) dependency, voltage-dependency, and time-dependency to the channels may account for observed differences in the clinical profiles of class I agents (Vaughan Williams, 1984b). Subgrouping experimentally then may possibly correlate with that determined clinically (Vaughan Williams, 1984a).

1.7 Quinacainol

Quinacainol (1-[2-(1,1-dimethylethyl)-4-quinolyl]-3-(4-piperidyl)-1-propanol) (Figure 2) was developed by Rhone Poulenc Santé in an effort to discover a more effective and less toxic drug for the treatment of cardiac arrhythmias. A preliminary report produced by Rhone Poulenc Santé (PK 10139, 1984) summarized a number of pharmacological studies with quinacainol. These studies assessed quinacainol's pharmacokinetics, its effects in conscious and anaesthetised animals, its toxicological properties, and its clinical effectiveness.

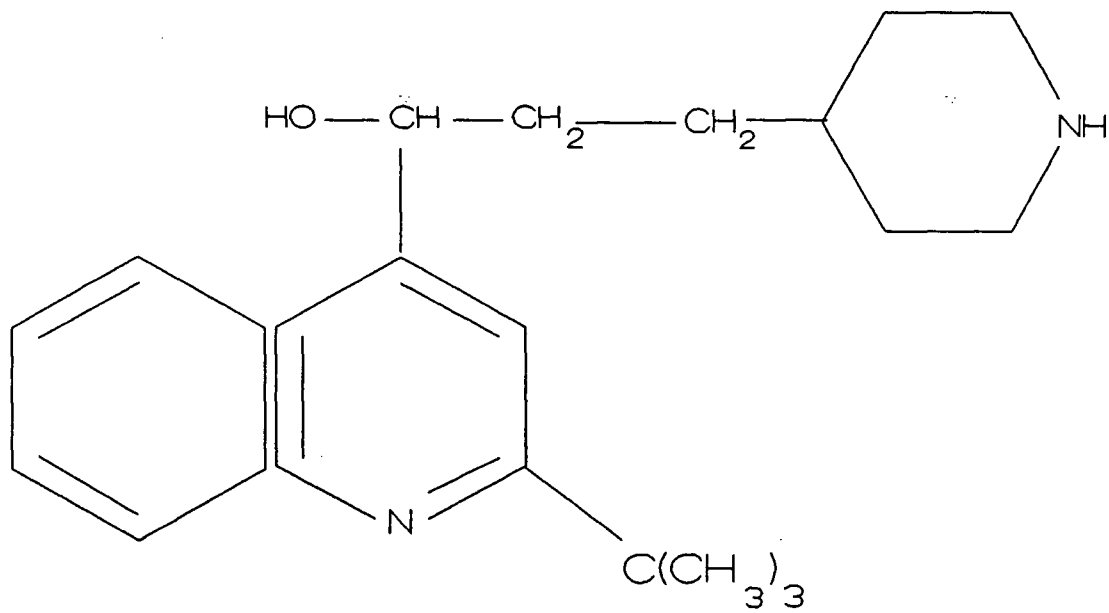


Figure 2. The structure of quinacainol, (1-[2-(1,1-dimethylethyl)-4-quinolyl]-3-(4-piperidyl)-1-propanol). It has a pKa of 10.8, the empirical formula is $C_{21}H_{30}N_{20}$, and it has a molecular weight of 326.4 g/mole.

Pharmacokinetic results showed that the urinary and fæcal routes were equally important for elimination; unchanged quinacainol was the largest urinary and biliary component with only small amounts of conjugated derivatives found. At least 4 metabolites were found in plasma and urine although their structures still need to be determined.

In conscious and anæsthetised dogs as well as in anæsthetised rat and rabbit, quinacainol was well tolerated. Quinacainol's effects on P-R interval and QRS duration were seen from the lowest doses up to approximately twice the 100% active dose. Quinacainol did not compromise blood pressure but reduced contractility slightly.

The antiarrhythmic activity of quinacainol was assessed in mice, rats, or dogs on arrhythmias induced by various methods. Quinacainol was potent and lacked deleterious side effects in comparison with other class I antiarrhythmic agents. The electrophysiological actions of quinacainol were assessed in a number of different models. Concentrations of quinacainol above 10^{-6} M decreased amplitude and dV/dt in sheep Purkinje fibres, a depression of plateau phase (60% repolarization), shortening of the APD and a slight decrease in RMP. Quinidine was tested in a similar manner for comparative purposes and its effects were completely reversed while partial effects of quinacainol could still be seen 1 h later. Similar effects were seen in sheep ventricular fibers and canine atrial fibers for

quinidine and quinacainol although both produced a slight increase in APD in these preparations.

The acute toxic effects of quinacainol (LD_{50}) were similar to other class I (Ia) agents. Quinacainol was devoid of effects on the CNS and autonomic nervous system.

1.8 Objectives

Historically, class I agents have proven to have antiarrhythmic and antifibrillatory properties against ischæmia-induced arrhythmias. Recent endeavours to improve upon prototypical agents have not met with much success. Drugs have been produced with the appropriate 1b profile, fast kinetics of blockade, etc., but these advantages have not translated into ideal drugs (Impact Research Group, 1984). Class Ib agents penetrate the brain and produce CNS-related side-effects (Davy *et al.*, 1988). In an analogous manner, there have been developments in class Ic agents, but recently newer class Ic agents have come under a cloud of suspicion as a result of the much discussed CAST trial (1989). Clinical studies with class Ic agents have been terminated and research on this subgroup has been reduced (Woosley, 1990). A recent meta-analysis by Hine *et al.* (1989) supported the conclusion that the entire class of class I antiarrhythmic agents cause increased mortality.

In view of such difficulties, it is not surprising that attention has been directed to developing better

antiarrhythmics from other classes. Most notable are the class III antiarrhythmic agents. Interestingly, many of the newer drugs that have been developed as class III antiarrhythmics were developed by chemical manipulations which change sodium channel blockers into potassium channel blockers (Lumma *et al.*, 1990). However, the question remains as to whether the ideal antifibrillatory drug can be developed from class III agents. Studies within our laboratory have shown that anti-fibrillatory actions occur only after massive increases in APD (Beatch *et al.*, 1990). This brings us back then to the class I agents. Perhaps a real possibility exists of developing a class I antiarrhythmic agent which affords excellent protection against arrhythmias within the setting of myocardial ischæmia without incurring significant cardiovascular toxicity while demonstrating selectivity for the ischæmic myocardium. The most recently marketed class Ic agents (quinacainol's putative subclassification) have been noted to have potent effects in the treatment of ventricular arrhythmias but are limited by their cardiac and non-cardiac toxic effects (Kreeger and Hammill, 1987; Nathan *et al.*, 1985).

Our experiments were designed to reveal the underlying electrophysiological actions of quinacainol and the appropriateness of its putative antiarrhythmic classification, i.e. Ic (PK 10139, 1984). The electrophysiological studies were chosen to show how these

actions may be transferred to effects on responses to electrical stimulation. The relative therapeutic usefulness of quinacainol was assessed by comparing its efficacy against ischæmia-induced arrhythmias to its toxic effects. Efficacy against electrically-induced arrhythmias could be compared with efficacy against ischæmia-induced arrhythmias.

2 METHODS

2.1 Hæmodynamic Studies in Conscious Rats

The dose-response relationship is essential in studying drug actions. This experiment was designed to study the hæmodynamic effects of quinacainol, underlying toxic effects, and to ascertain therapeutic ratios and ED_{50}'s in the rat. As all studies were performed in the same species under only two conditions, conscious or pentobarbital anæsthetised, extrapolation with respect to the doses used in the various studies was possible due to similarity of conditions in different models.

2.1.1 Preparation

Blood pressure and drug infusion cannulae for permanent implantation were modeled after Weeks (1981) and their manufacture has been described elsewhere (Johnston *et al.*, 1981, 1983; Curtis, 1986; Igwemezie, 1990).

Following induction of anæsthesia with 5% halothane in oxygen delivered by a vapourizer to a glass chamber, male Sprague Dawley rats (245-340 g) were intubated with a 14 gauge human intravenous catheter with the aid of a modified paediatric laryngoscope. The intubation tube was secured and the anæsthetic maintained with 1-1.5% halothane throughout the remainder of the surgery. A midline

laparotomy was performed and the inferior vena cava and the abdominal aorta cannulated for drug administration and blood pressure recording, respectively. These vessels were used (as opposed to carotid, jugular, or femoral blood vessels) because they are large, easily accessible, and do not tend to become obstructed by blood or muscle tissue (Curtis, 1986). The cannulae were pushed through the back muscles and routed subcutaneously using a trocar to the mid-scapular region and exteriorised. The abdomen was dusted with Cicatrin^{R} antibiotic powder and the abdominal muscle was closed using a continuous suture (4-0 silk). The local anæsthetic, Marcaine^{R} was liberally applied prior to suturing the skin with discontinuous sutures. The cannulae were heat-sealed following injection of 0.5-1.0 ml of saline to ensure patency.

ECG leads (prepared from teflon coated stainless steel wire, 0.0001 cm diameter, with the insulation removed from either end) were next placed in a lead II configuration using a 21 guage needle. These were twisted together and secured to the skin. A chest lead was inserted through the pectoralis major muscle and exteriorised in the mid-scapular region and secured to the skin.

The anæsthetic was discontinued. Animals were left until it was evident the anæsthesia was wearing off at which time the tracheal tube was removed. Animals were placed in separate cages in a temperature and light-controlled animal

room, given rat chow and water *ad libitum* and allowed to recover from surgery for 5 to 7 days.

2.1.2 Experimental Design

Animals (n=6) received a randomly chosen dose (or vehicle) every 48 h over a period of 10 days. Using a blind, random, cross-over design 1, 2, 4, or 8 mg/kg i.v. doses of quinacainol or vehicle alone, were each given as an infusion over 10 min. Dosing was performed on alternate days as a precautionary measure because quinacainol produces at least 4 metabolites which disappear within 36 h (PK 10139, 1984). In some animals a final dose of 16 mg/kg was given. The drug was dissolved in 26% ethanol in saline. This vehicle acted as the control. The vehicle for the 16 mg/kg dose of quinacainol was 40% ethanol in saline. Rats were brought into the laboratory 1 h prior to beginning the experiment. The aortic cannula was connected to a Grass Polygraph (model 79D) and kept open by attaching a leak pump in series with the line according to Weeks' method (1981). ECG leads were also connected to the Grass Polygraph. Animals were allowed to stabilise for 30 min prior to starting the experiment. Blood pressure and ECG were recorded during this time and for 20 minutes following dosing. In addition to recording cardiovascular responses to quinacainol, toxic effects were also noted throughout the experiment and during post-mortem analysis of internal

organs. Driscoll (1981) stressed that the use or the non-use of anæsthesia (e.g. telemetry, restraint, etc.) is perhaps the most important factor having a direct bearing on toxicological testing as it pertains to the rat ECG.

2.1.3 Data Analysis

The P, QRS, and T deflections in the rat ECG usually do not share a common baseline; any given wave may terminate at a level different from that of its origin (Driscoll, 1981). In order to measure the magnitudes of the various deflections it is necessary to choose a reference point for determination of the isoelectric baseline (Driscoll, 1981). The best reference point is that point at which the P-R interval terminates and the QRS complex begins (Driscoll, 1981). This point is influenced the least by other electrical events which may occur during the cardiac cycle (Beinfield and Lehr, 1968; cited in Driscoll, 1981). As is common with most leads of the rat ECG, the Q wave is absent in most cases. This peculiarity, along with the lack of a definite S-T segment and the occurrence of an asymmetric T wave has been discussed extensively in the literature (reviewed in Driscoll, 1981) and has been widely studied. A composite of a normal rat ECG is given in Figure 3 which notes the points taken to measure ECG variables. The Q-T interval was measured from the Q wave to the mid-peak of the T wave as the T wave was often ill-defined with respect to

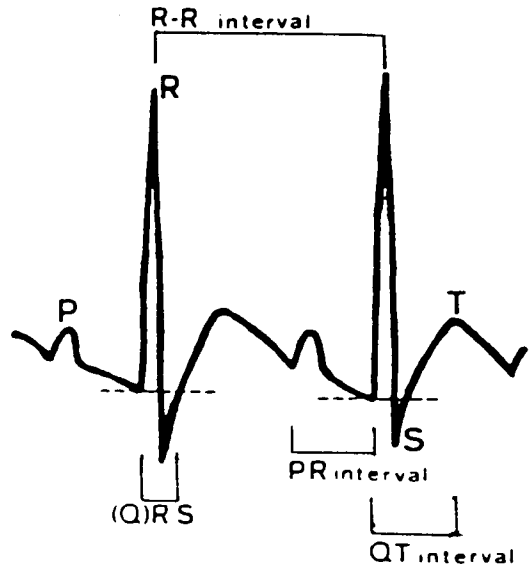


Figure 3. A composite drawing of a typical ECG trace from the rat. Two normal cycles are shown. Dashed lines represent isoelectric baselines for each. Also shown are the points used for measurement of the intervals indicated. (Reproduced from Driscoll, 1981). The Q-T interval measurement was modified; instead of using the end-point of the T wave (Driscoll, 1981), the mid-point was used as shown above.

an obvious upstroke or downstroke. The Q-T interval was corrected for heart rate (QT_c), QT_c = Q-T interval (sec)/$\sqrt{}$R-R interval (sec) due to the consistent differences in the heart rate seen between anæsthetised and non-anæsthetised rats (Driscoll, 1981).

In all experiments with quinacainol the peak of the systolic wave was measured and the lowest point of the diastolic was measured. For the hæmodynamic study mean blood pressure was calculated from the following formula: Mean BP = systolic BP + 1/3(systolic BP - diastolic BP). For all other experiments the average of systolic and diastolic pressures was taken to be the mean blood pressure.

2.2 Ischæmia-induced Arrhythmias

2.2.1 Experimental Preparation

Male Sprague Dawley rats (270-350 g) were prepared as described in 2.1.1 except for the additional surgical preparation needed for implantation of the occluder. Cannulae were implanted first followed by an occluder.

We routinely study the effects of myocardial ischæmia in chronic, conscious rats and consequently have designed a snare device which can be implanted and left intact for up to 7 days. The occluder, manufactured from polyethylene, was first described by Au *et al.* (1979) and Johnston *et al.*, (1983). Its design and manufacture have been extensively

described elsewhere (Curtis, 1986; Igewemezie, 1990). A 5.0 gauge atraumatic polypropylene suture (Ethicon) was threaded through the polyethylene guide such that the needle end of the suture appeared at the flared end of the guide.

The implantation of the occluder has also been described in detail elsewhere (Johnston *et al.*, 1983; Curtis, 1986). Briefly, an occlusion site was chosen to produce an ischæmic zone 35-40% of total ventricular mass. The polypropylene suture of the occluder was looped under the left anterior descending coronary artery. This was done blindly since the vessel could not be seen with the naked eye, thus we relied upon knowledge of the anatomy of the rat heart and when visible, we were able to use the coronary veins as landmarks (see Figure 4). A wide loop (approximately 4 mm) was made in the ventricular muscle to ensure that the artery was occluded. Minor bleeding was apparent in less than 15% of cases. Any bleeding was stopped by allowing the blood to clot and the thoracic cavity was cleared of excess blood. After implantation of the coronary artery occluder the lungs were hyperinflated during closure of the chest by blocking the exhalation tube for 3 consecutive breaths in order to avoid a pneumothorax.

Following completion of surgery, the rats were placed in individual rat cages and given tap water *ad libitum*. The rats remained in the laboratory until the anæsthesia had worn off. They were placed in a separate room used only to house rats and given rat chow and water *ad libitum*. The

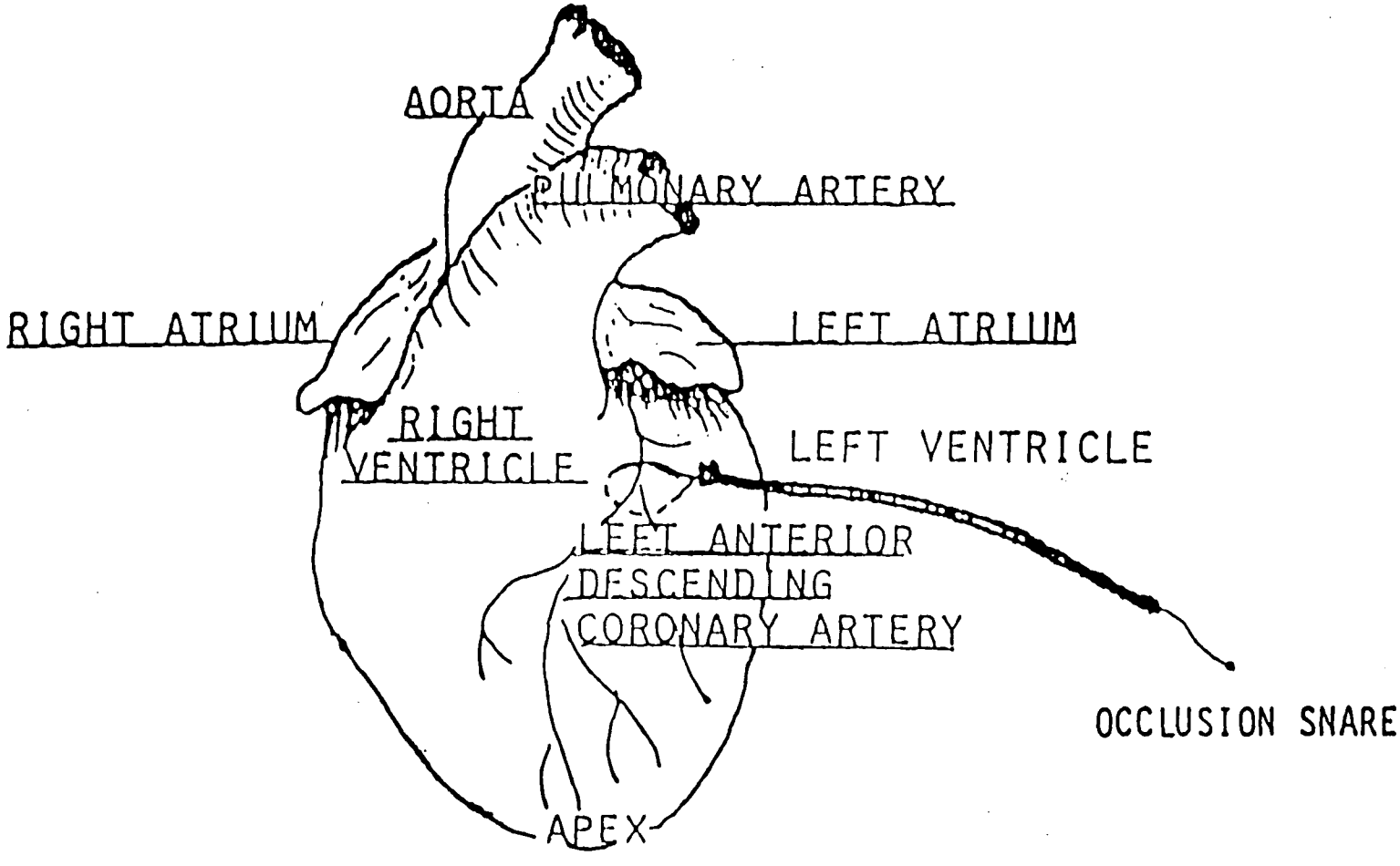


Figure 4. Diagram of the rat heart showing approximate placement of the occluder around the left anterior descending coronary artery (reproduced from Igwemezie, 1990).

entire surgical procedure took 40-60 min to complete from induction of anæsthesia to recovery.

2.2.2 Experimental Design

Six to eight days following surgery, rats were brought into the laboratory in their home cages 2 h before ligation and allowed free access to food and water. ECG and cannulae were connected. Blood samples (1 ml) were taken to measure serum potassium levels (Ionetics Potassium Analyzer) prior to drug infusion and 4 h post-occlusion in those animals surviving to this time. Recordings at fast paper speed (100 mm/sec) were taken 15 min and 5 min prior to drug infusion once calibration was completed. These served as control periods for data analyses. The basic design was a 3x10 randomized block design with replacement of excluded animals. Our criteria for exclusion were based on the Lambeth Conventions (1988) and have been detailed by Curtis (1986). In a double-blind and random manner, appropriate solutions were given by i.v. infusion over a 10 min period in a total volume of 2 ml or less. Thereafter, a further 5 min elapsed before occlusion. Occlusion was performed as described by Curtis (1986). Usually, occlusion causes a sudden change in the ECG but this could not be used as a criterion for occlusion since it was possible that drug treatment could mask or at least delay the ECG changes caused by occlusion. Rats were monitored continuously for 4

h post-occlusion or until death. Recordings at fast paper speed were taken every min for the first 10 min, every 5 min for the following 20 min, then every 15 min. All rats surviving 4 h were sacrificed by concussion and exsanguination and occluded zone determined. The size of the occluded zones in the hearts were also determined in animals dying prior to 4 h. The heart was excised taking care not to cut through the occluder and the occluded zone was determined as follows. The heart was perfused via the aorta according to Langendorff (1895) with 0.9% saline followed by saline containing indocyanine (Fast Green dye, BDH) 0.5 g/l. Approximately 30-60 s was necessary to allow the dye to pass through the coronary circulation to stain the perfused tissue a dark green colour and leave the ischæmic area (occluded zone) an opaque colour. The occluded zone was cut away from the normal or non-occluded ventricular tissue and each was weighed after blotting to remove excess perfusate; the occluded zone could be expressed as a percent of ventricular weight.

Arrhythmias, arrhythmias score (discussed below), and mortality were also recorded along with the blood pressure and ECG. All responses (to drug and coronary occlusion) were monitored with the aid of permanent recordings of the ECG. For classification and quantification of arrhythmias during the experiment, a delayed loop oscilliscope (Honeywell type E for M) with a 4 sec delay and a 4 sec real time display was used.

2.2.3 ECG Changes Pre and Post Occlusion

The ECG was recorded prior to drug infusion, following infusion and 1 min prior to occlusion so that the full electrophysiological effects of the drug could be assessed. Characteristic ECG changes produced by occlusion include an immediate and rapid rise in the height of the R wave and a slower but consistent elevation of S-T segment. A Q wave occurs in the chest lead approximately 2 h following occlusion. A significant Q wave has been defined as an initial downward deflection from the isoelectric potential approximately 10% of the R wave amplitude (Curtis, 1986). The time at which this was noted was recorded.

R wave amplitude was easily measured (mV) from the isolectric to the peak of the R wave. Maximum R wave amplitude was also recorded as was the time at which this occurred.

Before occlusion the S wave is negative to the isoelectric potential so that values of S-T segment elevation are negative as well. All negative values were assigned a value of zero. The elevation of the S-T segment was expressed as a percent of R wave amplitude. Maximum value of the S-T segment elevation were also determined.

2.2.4 Ischæmia-induced Arrhythmias Commonly Seen with Occlusion

Variations exist with respect to defining the various arrhythmias seen with occlusion of the LAD coronary artery. A varying amount of subjectivity is inevitable in determining what arrhythmias are seen. However, blind and random designs result in random distribution of the inconsistencies in defining the arrhythmias. The following definitions were used in the diagnosis of arrhythmias following occlusion of the LAD in rats.

A premature ventricular contraction (PVC) was defined as a premature QRS complex occurring independent of a P wave, normally accompanied by a transient drop in blood pressure. Singlets, doublets (bigeminy), and triplets were counted as PVC's while longer runs (4 or more) were recorded as ventricular tachycardia. This pooling of singlets, doublets and triplets implies that they were one and the same arrhythmia. However, these arrhythmias were associated only for convenience in analysis. In fact, the incidence of bigeminy and triplets is variable and lower than the incidence of PVC's. However the study group size would have to be enlarged to investigate the drug effects on each arrhythmia just mentioned. In the absence of clear evidence of a common electrophysiological mechanism amongst PVC's, doublets, and triplets and knowledge of that point in which

a run of PVC's should actually be considered a short run of VT, we arbitrarily defined PVC and VT.

VT was defined as 4 or more ventricular ectopic beats (PVC's) in succession. No restriction was made on associated rate. VT was subdivided based upon duration, into spontaneously reverting VT (SVT), which lasted less than 10 s, and non-spontaneously reverting VT (NSVT) in which, after 10 s, cardioversion was attempted by flicking the chest with a forefinger to attempt to convert the heart back to sinus rhythm.

VF was defined as chaotic disorder in the ECG (i.e. no identifiable QRS complex) accompanied by a precipitous fall in BP (essentially, zero cardiac output). As in VT, defibrillation was attempted in all rats experiencing greater than 10 s of VF, and could therefore be differentiated into spontaneously reverting VF (SVF, <10 s and not requiring defibrillation), and non-spontaneously reverting VF (NSVF, >10 s, requiring defibrillation). VT sometimes degenerated into VF; both episodes were counted.

Figure 5 illustrates a typical ECG pattern induced by coronary occlusion and examples of the arrhythmias discussed above.

2.2.5 Rationale and Methods of Defibrillation

Manual defibrillation was attempted to convert all episodes of VT or VF lasting longer than 10 s to avoid

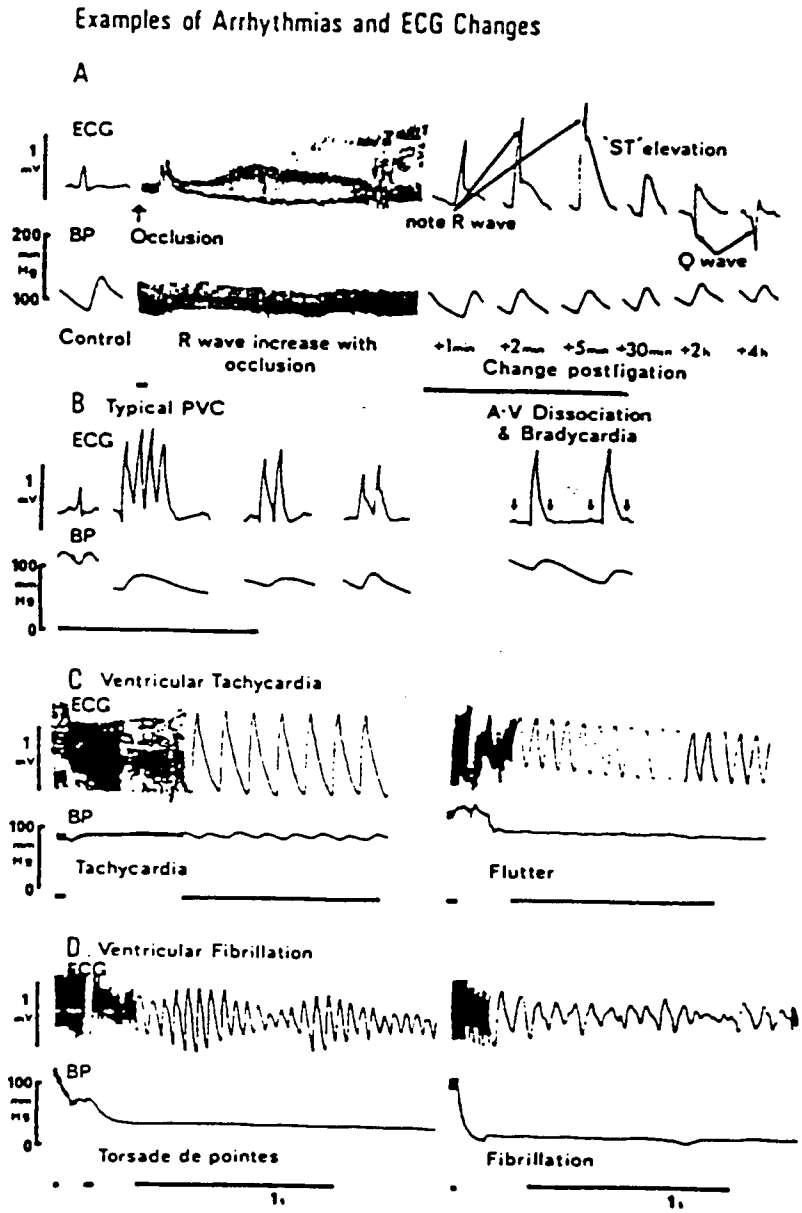


Figure 5. Typical ECG pattern induced by ligation of the left anterior descending (LAD) coronary artery and appearance of arrhythmias. Panel A shows ECG and blood pressure traces with R-wave increase, S-T segment changes, and Q-wave appearance. Typical arrhythmias of PVC and A-V dissociation (bradycardia) are shown in panel B, VT in C, and VF in D. The horizontal bars indicate 1 s at various chart speeds. (Reproduced from Johnston et al, 1983).

excessive censoring due to death following VF. It is expected that death due to VF will be higher in control groups when testing antifibrillatory drugs, such that the group size may become so small as to have to forego meaningful comparisons. Also, the experiment was set up to examine the effects of antifibrillatory drugs over time. Much information concerning the time course and interrelationships of variables is lost as animals die during the course of the study. For all these reasons, defibrillation was attempted in all episodes of any VT or VF lasting longer than 10 s. Defibrillation was accomplished by lifting the rat and flicking the chest directly over the heart with a fore-finger until sinus rhythm returned. Normally, less than 10 flicks were required; however, in those rats where "thump-version" was not successful, the chest was compressed with the thumb and 2 forefingers to defibrillate. Reversion was diagnosed by a sudden increase in aortic BP and return to sinus rhythm. VF normally produced convulsions within a few seconds of its initiation, and syncope quickly followed. This was not apparent with episodes of VT.

2.2.6 Scoring System for Arrhythmia Analysis

In order to facilitate analysis of arrhythmias, particularly with myocardial ischæmia, a number of arrhythmia scores have been developed (Curtis and Walker,

1988). It is possible to design many arrhythmia scores that will show changes in severity of arrhythmias when more conventional analyses show only non-statistically significant trends (Curtis and Walker, 1988). The Lambeth Conventions (1988) recommends a cautionary use of arrhythmia scores. Arrhythmia scores should be considered only in relation to dose-response data and in conjunction with the raw arrhythmia data in order to avoid false claims for a drug's effectiveness (Curtis and Walker, 1988). Curtis and Walker (1988) examined seven scoring systems. The following scoring system was used.

Scoring system for quantification of arrhythmias:

0 = 0-49 PVCs

1 = 50-499 PVCs

2 = >499 PVCs and/or 1 episode of spontaneously reverting VT or VF

3 = >1 episode of VT or VF or both (< 60 s total combined duration)

4 = VT or VF or both (60-119 s total combined duration)

5 = VT or VF or both (>119 s total combined duration)

6 = fatal VF starting at >15 min after occlusion

7 = fatal VF starting at between 4 min and 14 min 59 s after occlusion

8 = fatal VF starting at between 1 min and 3 min 59 s after occlusion

9 = fatal VF starting <1 min after occlusion

2.3 Electrically-Induced Arrhythmias

2.3.1 Preparation

Male Sprague Dawley rats (250-350 g) were anæsthetised with sodium pentobarbital (50 mg/kg i.p.). The left carotid artery and right jugular vein were cannulated for blood pressure monitoring and drug infusion, respectively. A tracheotomy was performed and all rats were allowed to breathe spontaneously throughout the experiment. Using a 27 gauge needle, teflon coated silver wire electrodes (with exposed ends) were passed through the thoracic cavity and lodged 2-5 mm apart in the upper left ventricle. This was confirmed by dissection at the end of the experiment. Subcutaneous ECG electrodes in a Lead II configuration were used and all 4 limbs were grounded to reduce noise.

2.3.2 Experimental Design

Blood K^+ concentrations were measured on 1 ml arterial blood samples prior to determination of electrical stimulation variables and at the end of the experiment.

Quinacainol was administered as a solution in acidified (HCl, pH 2.7), distilled water. Doses of 0.5, 1, 2, and 4 mg/kg were each given over a 10 min infusion period in a

cumulative manner with addition of a new dose every 25 min. The same regimen was used with control animals but equivalent volumes of vehicle were substituted for drug doses. Thus there were 2 groups, a control group (n=6) given vehicle alone, and a quinacainol treated group (n=6). Each animal acted as its own control. Each group was tested randomly with all experimental components blinded to the experimenter. Stimulation of the left ventricle with square wave pulses was accomplished using a Grass Stimulator (see also Howard and Walker, 1990a).

Prior to drug infusion, three sets of control values of electrical stimulation variables were determined five min apart. The last set of values was taken as the control. All measurements, plus ECG, heart rate and blood pressure were made 10 min after the end of infusion.

2.3.3 Experimental Endpoints

End-points were determined using a delayed loop oscilloscope (Honeywell Type E for M) and square wave pulses were used to determine the following electrical stimulation variables: threshold current (i_T), threshold duration (t_T), maximum following frequency (MFF), ventricular fibrillation threshold (VF_T), and effective refractory period (ERP). Each variable was measured 3 times and a mean value obtained. The procedure for the measurements has been described elsewhere (Curtis *et al.*, 1984, 1986).

Threshold current for capture was determined at 1 ms duration and 7.5 Hz by uniformly increasing current applied until capture. The threshold pulse width was determined at 7.5 Hz and twice the threshold current.

MFF was determined at twice the current and pulse width thresholds. MFF was taken at that point when the heart failed to follow, on a 1:1 basis, a steadily increasing frequency of stimulation from 7 to 20 Hz. This was readily seen as a sudden upward spike in the blood pressure after an initial sustained drop.

VF_T was determined at 50 Hz and twice threshold duration. The frequency was chosen to ensure that a pulse was delivered during the vulnerable period, i.e. the terminal portion of the Q-T interval in the ECG. The maximum current which elicited sustained fibrillation with a precipitous fall in blood pressure was taken as threshold.

ERP was determined by the extra-stimulus method. The heart was paced at 7.5 Hz and a single extra stimulus was applied at varying intervals behind the pacing stimuli. The shortest interval between the pacing stimuli and the extra-stimulus in which an extra-systole was obtained was taken as the ERP.

2.4 **Epicardial Intracellular Recordings** *in vivo*

Sodium channel blockade in normal ventricular tissue can be assumed from effects on the ECG, e.g., widening of

the QRS complex or the P-R interval suggests inhibition of cardiac Na^+ channels. The technique described below offered two advantages: it demonstrated quinacainol's effects on the cardiac AP *in vivo*, and second, it allowed us to (sub)classify quinacainol on the basis of its electrophysiological actions.

2.4.1 Preparation

Male Spraque Dawley rats (320-390g) were anæsthetised with pentobarbital (50 mg/kg i.p.) (n=6). The right jugular vein and left carotid artery were cannulated for drug administration and blood pressure recording respectively. The left jugular vein was cannulated for administration of saline and pentobarbital as needed during the experiment since the drug infusion line in the right jugular vein could not be disturbed once drug infusion had started. The rat was mounted on a stainless steel plate raised to a 15° angle to help maintain venous return. Under artificial ventilation with room air, a thoracotomy was performed by cutting through the fourth and fifth ribs to expose the heart. The upper epicardial surface of the left ventricle was immobilized by suturing it to a silver/silver chloride metal loop using a sterile absorbable surgical suture (5-0 Dexon Plus; Davis & Geck). The loop also served as a reference electrode. For ECG recording, an electrode was inserted down the oesophagus and a needle electrode inserted

into the chest wall with the loop still acting as the reference electrode.

Microelectrodes (20 megaohm resistance) were prepared using glass tubing (1BBL w/Fil 1.0 MM, 4 inches; World Precision Instruments, Inc.) and a Narishige (Pa-01) electrode puller. The microelectrodes were filled with 3 M KCl that had been filtered through a 0.45 μm Filter unit (Millex-HA). Coated tungsten wire (0.003 inches, A-M Systems, Inc.) was inserted into the electrode until it reached the end of the tip. This end was then carefully snapped off and attached to the electrode holder. Angling the tungsten wire at 90° buffered the microelectrode against the beating of the heart and assisted in keeping the microelectrode within the cell.

The microelectrodes were connected to a WPI preamplifier. The output was passed through a differentiator and the signals recorded on a video tape after pulse code modulation (Sony PCM). An oscilliscope was used to monitor cell penetration and to determine dV/dt_{max}, while ensuring a good record had been acquired.

2.4.2 Experimental Design

In a 30 min control period prior to drug infusion, APs were recorded using a multi-sampling technique. Penetration within a cell was considered good if the AP could be held for 20 sec or longer and V_{max} was adequate.

Quinacainol, (acidified, distilled water, pH 2.7) was given in a cumulative manner at doses of 0.5, 1, 2,4, and 8 mg/kg. Each dose was infused over a 3-4 minute period using an i.v. infusion pump (Harvard Apparatus) with the next dose beginning 10 min after the end of the previous infusion. A multiple impalement technique (Inoue *et al.*, 1982; Abraham *et al.*, 1989) was used for the 10 min period between doses.

2.4.3 Experimental Endpoints and Data Analysis

Using a storage oscilliscope (Tetronix), acceptable APs were examined at two time periods, within the first minutes following infusion (0-3 min and no greater than 5 min post-infusion), and during the last 5 min of the 10 min period between doses. From the AP, the following parameters were measured: AP height, the maximum value of dV/dt for the rising phase of the action potential (dV/dt_{max}) and AP duration at 10, 50, and 90% of repolarization. For each rat preparation, values obtained within the time frames mentioned were averaged and the averages for all rats were measured to give mean and s.e. mean values with n equal to the number of rats tested (n=6).

2.5 Effects of Quinacainol in Isolated Rat Hearts

Investigation of the mechanical (contractile force) and electrical (ECG) activity of the isolated mammalian heart

was first devised by Langendorff in 1895. The primary advantage to using isolated organ preparations is that they are without nervous and humoral regulation as well as substrate supply and modification of blood content by the intact organism.

2.5.1 Perfusion Apparatus

A perfusion apparatus for mechanical and electrophysiological studies in small animals such as rat, guinea pig, and rabbit hearts was designed and constructed in our laboratory (Curtis *et al.*, 1986). The apparatus consists of nine individual perfusion chambers connected to the aortic perfusion cannula of the Langendorff perfused heart. The multiple chambers allow for various drug concentrations or solutions. The design of our apparatus allows for rapid switching of up to nine different pre-heated perfusates and involves a small dead space (less than 0.1 ml) while aortic route perfusion pressure may be easily varied from 0 to 200 mmHg.

2.5.2 Preparation

While the use of whole blood or solution containing hæmoglobin may limit hypoxia, multiple complications are associated with their use (e.g. foaming, clotting erythrocyte aggregation, etc.) (Doring and Dehnert, 1988).

Thus we chose to use a modified Kreb's-Henseleit solution. The composition (mM) of the Kreb's solution was: NaCl, 118; KCl, 4.74; $CaCl_2$-$2H_2O$, 2.5; KH_2PO_4, 0.93; $NaHCO_3$, 25; Glucose, 10; $MgSO_4$-$7H_2O$, 1.2.

Male Sprague Dawley rats (290-400g) (n=7) were sacrificed by concussion and exsanguination and the heart was immediately excised. The heart was retrogradely perfused with cold Kreb's solution using a 50 cc syringe to remove blood. The heart was then transferred to the perfusion apparatus and tied to the cannula. Within seconds of inititating perfusion, the heart began beating in sinus rhythm. The left atrium was then cut off in order to insert a balloon (Curtis, 1986) for ventricular pressure measurements and the diastolic pressure within the balloon was adjusted to maintain left ventricular end diastolic pressure of 10 (±5) mmHg. Special atraumatic electrodes were designed for ECG recording (Curtis, 1986). One electrode was placed on the right atrium to allow the recording of a large P wave, and the second on the left ventricle. Using a Grass polygraph for ECG recording, a differentiator was connected to the channel measuring left ventricular pressure to convert it to dP/dt, a measure of contractility. Perfusion pressure was kept constant at 115 mmHg (±10 mmHg). Measurement of mean coronary flow was done by collecting the perfusate draining out of the right atrium. Shifting of the electrodes was fairly common and care was taken to ensure they remained in approximately the

same position. Some difficulty was seen in determining the beginning and end of the T-wave so that the apex of the T wave was used for all measurements (refer to Figure 3).

2.5.3 Experimental Design

To assess the direct effects of quinacainol in rat cardiac tissue, isolated rat hearts were perfused with varying concentrations of quinacainol. Quinacainol was compared with tetrodotoxin (TTX), an agent whose action is a highly specific blocking action on sodium channels (Fuhrman, 1986).

The heart was allowed to equilibrate using the modified Kreb's solution as the perfusate for a minimum of 10 min. Each heart (n=7) was first exposed to the following concentrations of TTX (M): $3x10^{-7}$, $1x10^{-6}$, $3x10^{-6}$, $1x10^{-5}$. Each concentration of TTX perfused the heart for 2 min. A 10 min wash period then followed. Hearts were exposed to TTX first due to its fast onset and offset of effects (Fuhrman, 1986). After 2 min of perfusing the heart with modified Kreb's, most hearts had reverted back to pre-drug values. At the end of the 10 minute wash period the following concentrations (M) of quinacainol were tested: $3x10^{-7}$, $1x10^{-6}$, $3x10^{-6}$, $1x10^{-5}$. Hearts were perfused with each concentration of quinacainol for 10 min. The wash period acted as the control values for quinacainol to

determine drug-induced changes whereas for TTX, control values were taken during the equilibration period.

2.6 Statistical Analyses

The General Linear Model (all ANOVA models from Hintze, NCSS Statistical Package, 1981) was used to compare treatment means with vehicle in the hæmodynamic study in conscious rats as cell frequencies were balanced. Original data values in the vehicle control group and the quinacainol treated group were separately tested in the electrical stimulation study (GLM ANOVA) to determine if treatment (vehicle or drug) and time were statistically significant.

An unweighted means analysis (UWM ANOVA) was used because of missing cells to discover significant sources of variance ($p<0.05$) between treatment as compared to control (pre-drug) values and to determine if treatment effects varied with time for the epicardial intracellular potential study.

UWM ANOVA ($p<0.05$) was also used to test all concentrations of quinacainol and TTX in the isolated heart experiment to determine if drug effects on the variables measured were statistically significant. As hearts were perfused with each concentration of TTX and quinacainol over a given time period, time-dependent effects were tested for in an analogous manner to treatment effects. All values

were compared to pre-treatment values for the given treatment (TTX or quinacainol).

In the experiment testing quinacainol's actions against ischæmia-induced arrhythmias, a control group (receiving drug vehicle) was compared with two treated groups. The variables compared were both Gaussian-distributed and binomially-distributed (Johnston *et al.*, 1983). In accordance with these types of distributed data, an ANOVA (UWM model) was carried out for Gaussian-distributed variables, while Mainland's contingency tables of minimum contrasts were used for chi-squared testing (Mainland *et al.*, 1956) for binomially-distributed variables.

In all cases, if treatment constituted a significant source of variation as revealed by F test values, Duncan's multiple range test and Newman Keul's tests were used to compare means. If any discrepancy existed Duncan's results were accepted. ANOVA results were checked by making further comparisons between vehicle control group and treatment group using two-sample t-tests where appropriate.

3 RESULTS

3.1 Effects of Quinacainol on Blood Pressure, Ventricular Pressure, and Heart Rate

The effects of quinacainol on blood pressure and heart rate were examined *in vivo* in conscious and anæsthetised animals and on ventricular pressure and heart rate *in vitro*. In conscious animals quinacainol showed more dramatic effects in the presence of coronary artery occlusion. In the dose-response study in conscious rats, quinacainol produced a slight depression in blood pressure and a slight elevation in heart rate with this trend reversing at the highest dose (8 mg/kg) (Table 1) when expressing the effect as change from pre-treatment values for the indicated dose. None of these effects achieved statistical significance. Significant effects of quinacainol on hæmodynamics were seen after coronary artery occlusion. Treatment did not have marked actions on blood pressure and only occlusion-dependent effects were seen 4 h following occlusion for all groups (Figure 6a). The effects of quinacainol on heart rate were seen much earlier with significant bradycardia noted 15 min post-occlusion as compared to vehicle control group (Figure 6b).

Quinacainol's hæmodynamic effects were more predominant in anæsthetised animals than in conscious rats.

Table 1. Blood pressure and heart rate effects of quinacainol in conscious rats.

Time and Dose	Change in BP (mmHg)	Change in Heart Rate (beats/min)
vehicle		
+5 min	-4±8	23±13
+20 min	-13±6	34±19
1.0 mg/kg		
+5 min	-5±3	11±7
+20 min	-7±4	23±15
2.0 mg/kg		
+5 min	-9±6	31±24
+20 min	-12±9	21±16
4.0 mg/kg		
+5 min	-11±5	6±17
+20 min	-14±6	5±18
8.0 mg/kg		
+5 min	-3±7	-10±20
+20 min	6±4	-24±29

Rats (n=6) were given quinacainol, 1, 2, 4, or 8 mg/kg i.v. as an infusion over 10 min. Doses were administered random and blind from a randomized block design with doses being administered on alternate days. Values are given for that dose, time following end of drug infusion, and variable indicated (n=6). Results are presented as changes from pre-treatment values (for that dose). All variables are mean ± s.e. mean. No significant differences were found with respect to time or dose (GLM ANOVA).

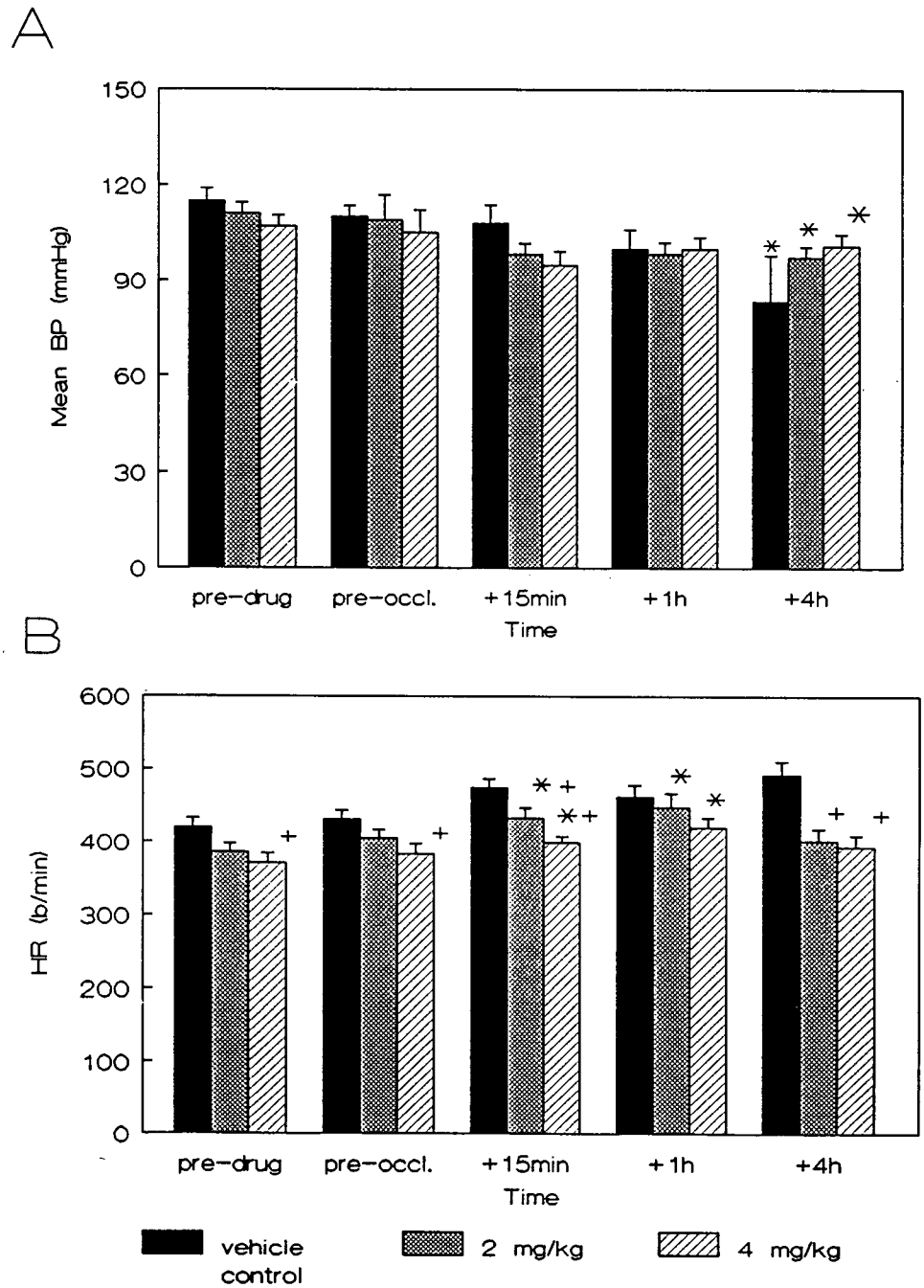


Figure 6. Blood pressure and heart rate effects of quinacainol before and after coronary artery occlusion. Each bar graph represents mean ± s.e.mean, n=10. Time periods are -15 min (pre-drug) and -1 min pre-occlusion (indicated in the graphs as pre-occl.) and +15 min, 1 h, and 4 h after occlusion. Blood pressure effects are shown in part A and effects on heart rate in part B. * Indicates $p<0.05$ versus pre-occlusion values (i.e. occlusion-dependent effects). + Indicates $p<0.05$ versus vehicle control group.

In the rats used in the electrical stimulation study, the vehicle alone had no major effect on blood pressure or heart rate while quinacainol produced a dose-dependent decrease in blood pressure (statistically significant only for the highest dose) and produced a statistically significant dose-dependent decrease in heart rate for doses of 1, 2, and 4 mg/kg (Figure 7a and 7b). As in conscious animals, quinacainol produced a greater effect on heart rate than on blood pressure.

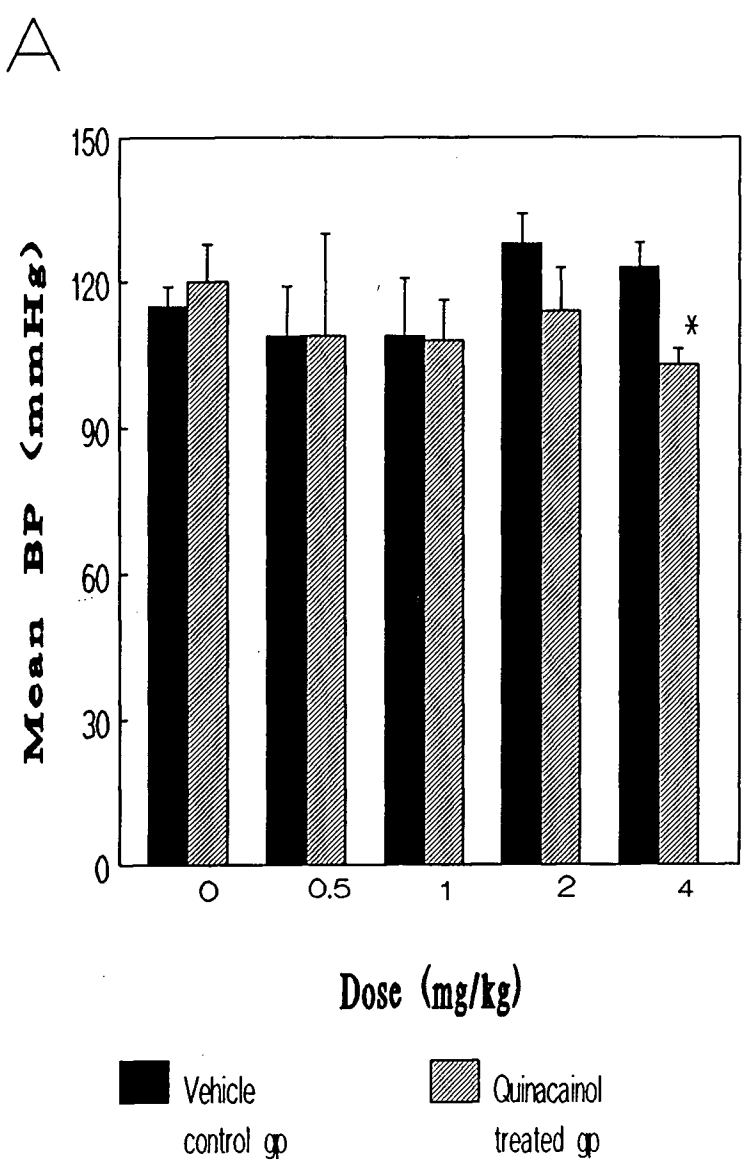
In the anaesthetised rats (n=7) used to investigate the effects of quinacainol on epicardial transmembrane potentials, a dose-dependent decrease in heart rate and blood pressure was also seen (Table 2). The control and drug treatment period each occupied approximately 90 min. Since control readings did not vary over the 90 min control period, they are presented as a single value in the table. The effects of treatment did not vary with time.

Bradycardic effects of quinacainol were also seen *in vitro* and were compared to TTX (Table 3). Quinacainol reduced ventricular pressure and coronary flow though not statistically significantly (Table 3).

3.2 ECG effects of Quinacainol

Quinacainol had marked effects on the ECG. In conscious animals it prolonged both the P-R interval and QRS duration (Figure 8,9 and 10) dose-dependently while the

Figure 7. Effects of quinacainol and vehicle control on blood pressure (A) and heart rate (B) in pentobarbital anæsthetised rats. All results are presented as absolute values, each value is mean ± s.e.mean, n=6. * Indicates $p<0.05$ from vehicle control. No significant differences were seen when comparing groups to their own pre-treatment or pre-drug values.


A
150
120
90
60
30
0
Mean BP (mmHg)
0
0.5
1
2
4
*
Dose (mg/kg)
Vehicle control gp
Quinacainol treated gp

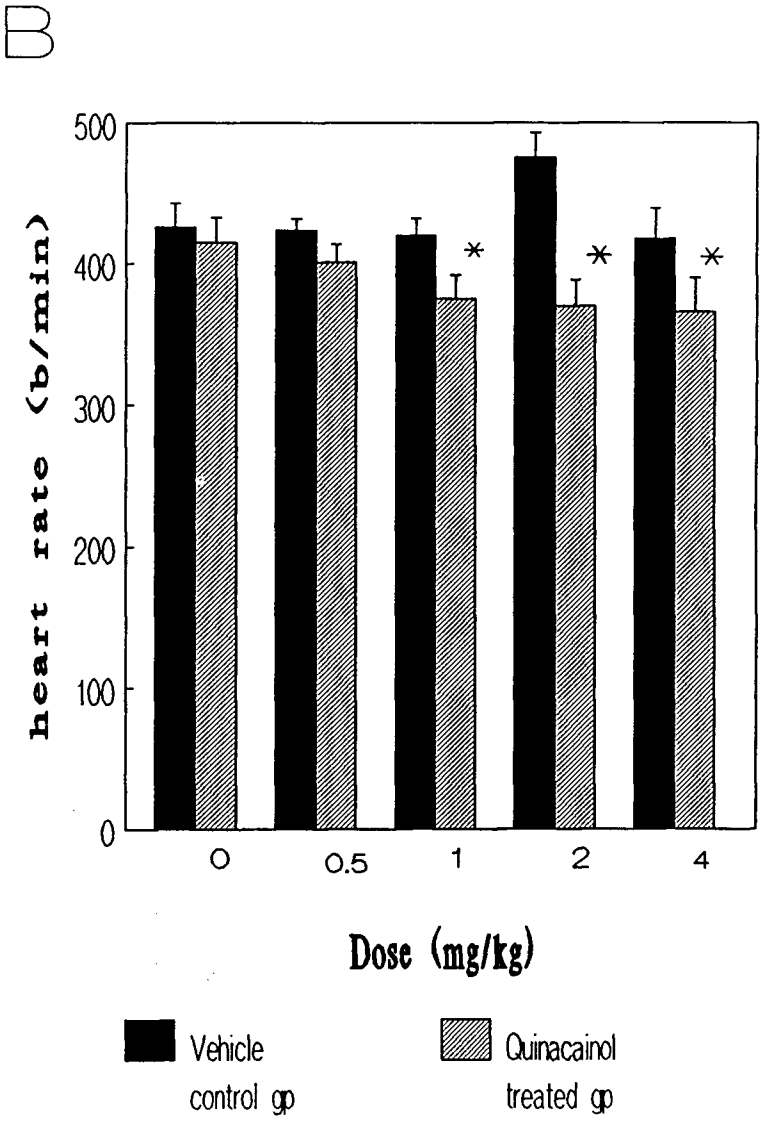

B
500
400
300
200
100
0
heart rate (b/min)
0
0.5
1
2
4
*
*
*
Dose (mg/kg)
Vehicle control gp
Quinacainol treated gp

Table 2. Blood pressure and heart rate effects of quinacainol during epicardial intracellular potential recordings in vivo.

Dose (mg/kg)	Mean BP (mmHg)	Heart Rate (beats/min)
control	116±8	433±11
0.5	112±9	407±18
1.0	105±10	393±19*
2.0	110±9	387±11*
4.0	95±15*	365±21*
8.0	69±2*	317$^{\phi}$

The effects of quinacainol on blood pressure and heart rate are shown as recorded during epicardial intracellular potential recordings. * Indicates $p<0.01$ from control values. Treatment was determined to be a significant source of variance. As time was not a significant source of variance all values represent x±s.e.mean for the averaged values in the second time period (i.e. 5-10 min post-infusion). $^{\phi}$ Indicates that data was insufficient to perform statistical tests or record s.e.mean as heart rates were recorded for only 2 animals at this dose.

Table 3. Hæmodynamic effects of tetrodotoxin (TTX) and quinacainol in vitro.

Dose	Heart Rate (beats/min)	Ventricular Pressure (mmHg)	Coronary Flow (ml/min)
a) Tetrodotoxin			
control	226±7.5	134±17	10±1.3
$3x10^{-7}$	228±7.9	135±18	9.5±2.5
$1x10^{-6}$	211±9.5	151±16	11±1.3
$3x10^{-6}$	193±11*	150±17	9.3±1
$1x10^{-5}$	200±18	150±17	8.9±2
b) Quinacainol			
control	179±6.8+	109±16	8.0±1
$3x10^{-7}$	191±6.6+	105±18	7.2±0.5
$1x10^{-6}$	185±12	97±19	6.6±0.6
$3x10^{-6}$	175±6.8	89±12	6.2±0.7
$1x10^{-5}$	163±15*+	86±19	6.8±0.7

All values (x ± s.e. mean) were recorded at the end of 2 min of perfusion with TTX, and 10 min perfusion with quinacainol. * Indicates $p<0.05$ from control values. + Indicates $p<0.05$ as compared to respective doses of TTX (Unweighted Means ANOVA).

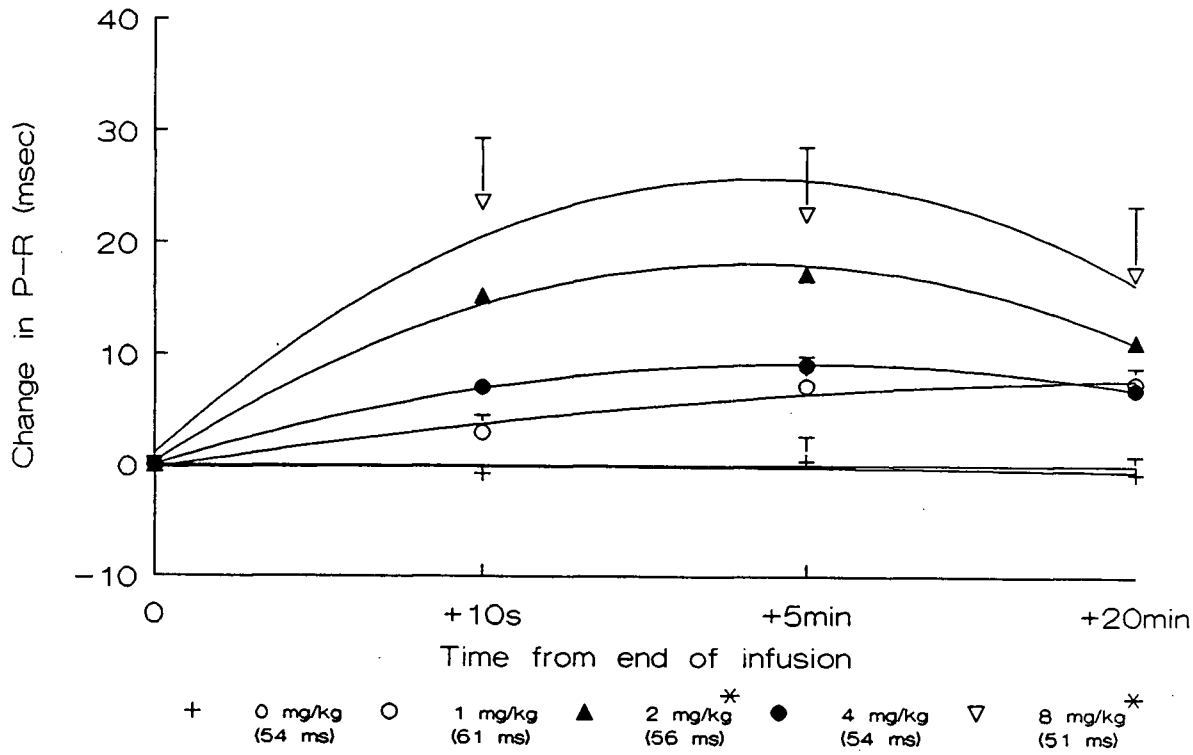


Figure 8. Effects of quinacainol and vehicle on P-R interval in the conscious rat. Values are expressed as change in msec (mean ± s.e.mean) from pre-treatment values. The values indicated at 10 s are those measured immediately following end of infusion, and those indicated at 5 min and 20 min are from that time following end of infusion. * indicates that treatment was a significant source of variance as compared to vehicle (0 mg/kg) ($p<0.001$). The effect of a given dose did not change with time. Pre-treatment values are indicated in brackets above. Some s.e.mean values are omitted for clarity.

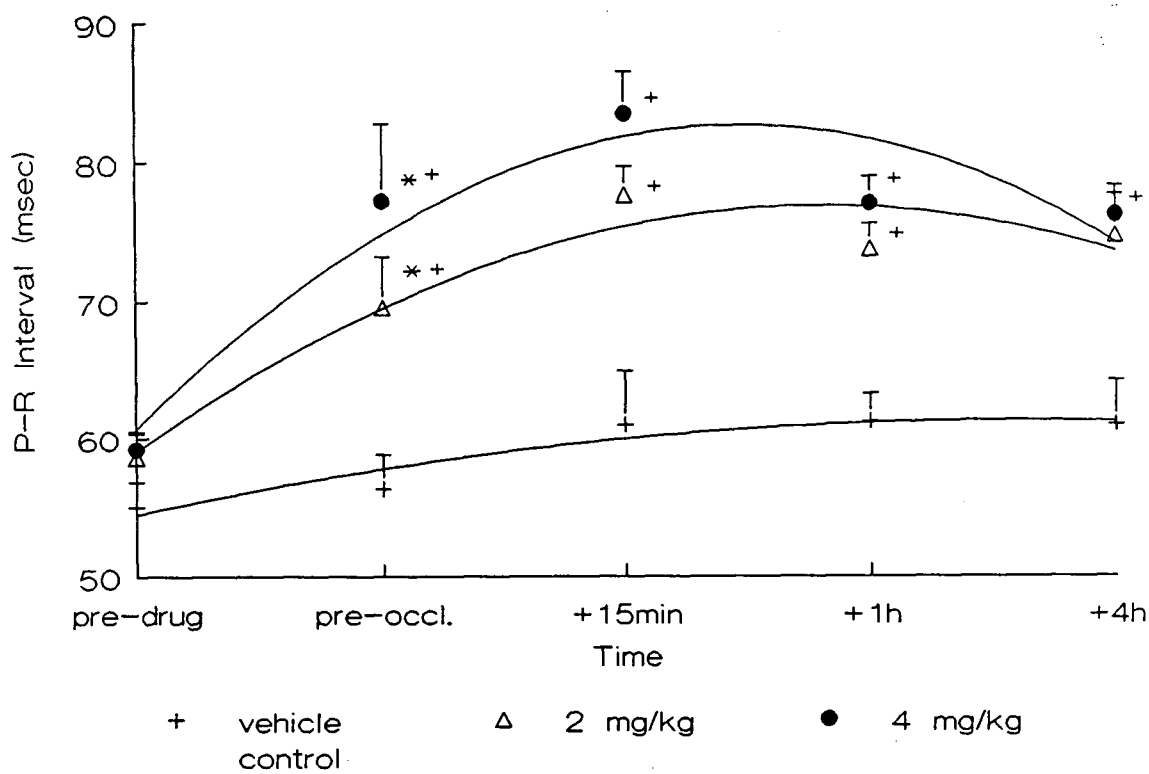


Figure 9. Effects of quinacainol and vehicle control on P-R interval before and after occlusion. The abscissa indicates time (min and h) with respect to ligation of the coronary artery. Results are expressed as x± s.e.mean, n=10. * Indicates $p<0.001$ versus pre-drug values. + Indicates significant differences ($p<0.05$) between treatment groups and vehicle control group at that time period indicated on the abscissa.

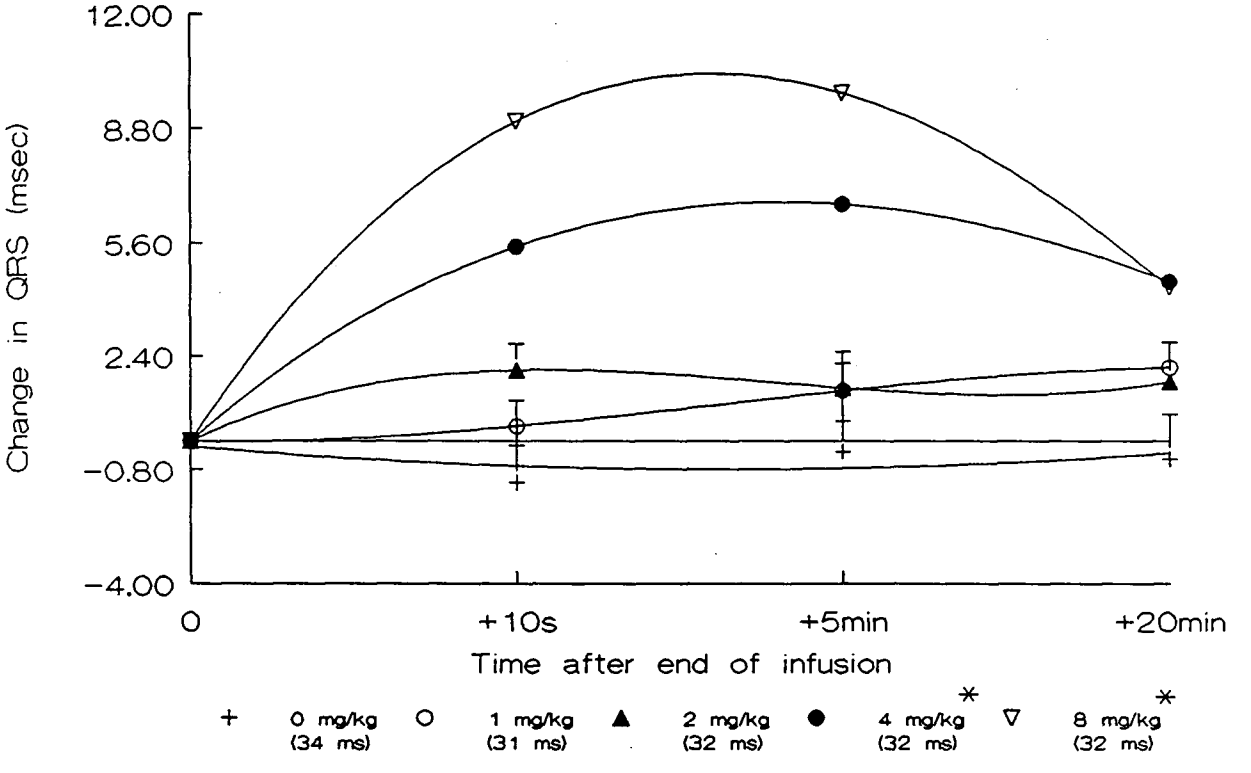


Figure 10. The effects of quinacainol and vehicle on QRS duration in the conscious rat. Values are expressed as change from pre-treatment values (x±s.e. mean). Bracketed values indicate the mean of control values prior to indicated treatment. Values indicated at 10 s were recorded immediately following end of infusion. Compared to vehicle, 4 mg/kg and 8 mg/kg were a significant source of variance ($p<0.01$). Treatment effects did not vary significantly with time.

vehicle was without effect. The Q-T interval, corrected for heart rate ($Q\text{-}T_c$), was also prolonged dose-dependently although not as markedly (Figure 11). It was interesting to note that ECG effects were most marked 5 min post-infusion in the dose-response study and began to wane slightly 20 min post-infusion.

In anæsthetised animals quinacainol demonstrated similar ECG effects compared to those seen in conscious animals. In the electrical stimulation study coincident with the lowering of heart rate, the P-R interval was prolonged (Figure 12) as seen in conscious animals although little effect was seen on the QRS duration or $Q\text{-}T_c$ interval (Table 4). The vehicle alone had no major effects on the ECG. The P-R prolongation was significant statistically ($p<0.05$) for all doses when compared to vehicle control group infusions (Figure 12).

In vitro, quinacainol widened the P-R interval and QRS duration (Figures 13 and 14) without having a major effect on the $Q\text{-}T_c$ interval (Figure 15).

The major acute toxic effects of quinacainol in conscious rats occurring within 1 h of administration can be seen in Table 5. Proarrhythmic effects occurred at doses of 8 mg/kg and above.

3.3 Coronary Artery Occlusion-Induced Arrhythmias in Conscious Rats

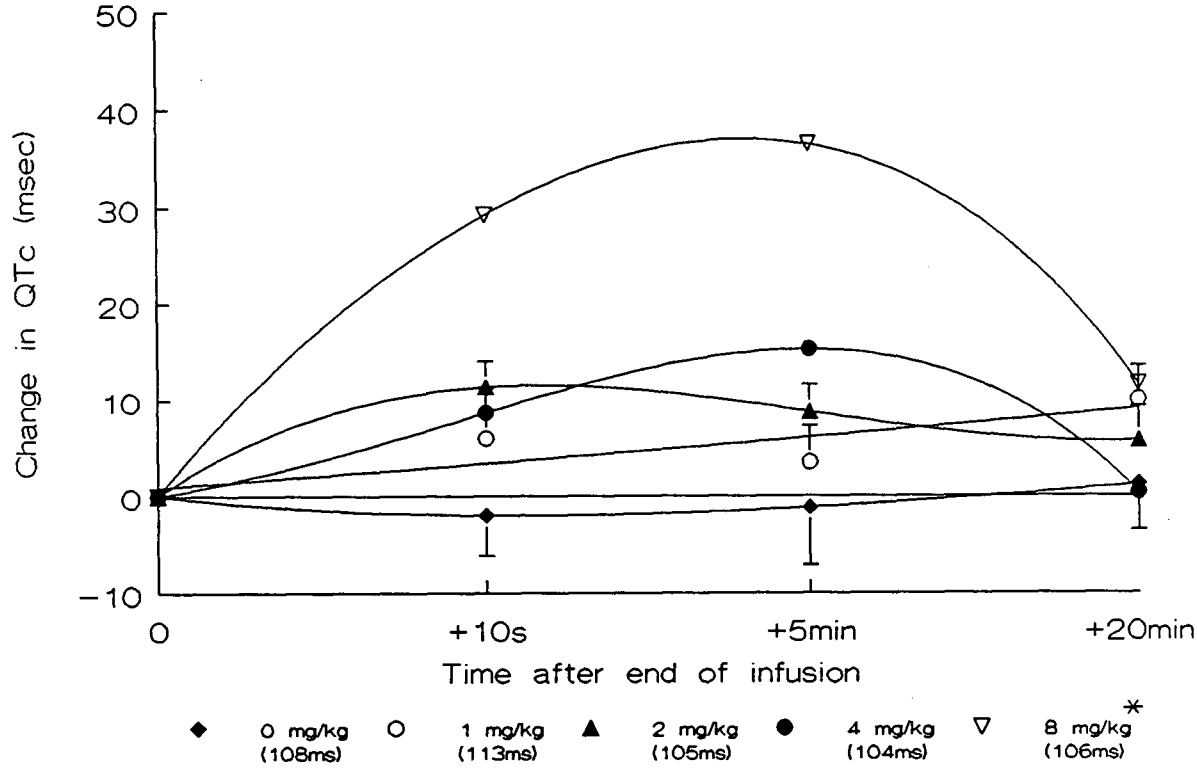


Figure 11. The effects of quinacainol and vehicle on Q-T interval corrected for heart rate ($Q\text{-}T_c$) in the conscious rat. Values are expressed as change from pre-treatment values (x±s.e. mean). Bracketed values indicate the mean of control values prior to indicated treatment. Values indicated at 10s were recorded immediately following end of infusion. Compared to vehicle (indicated as 0 mg/kg above), the highest dose was a significant source of variance ($p<0.002$). Effects of treatment did not vary significantly with time.

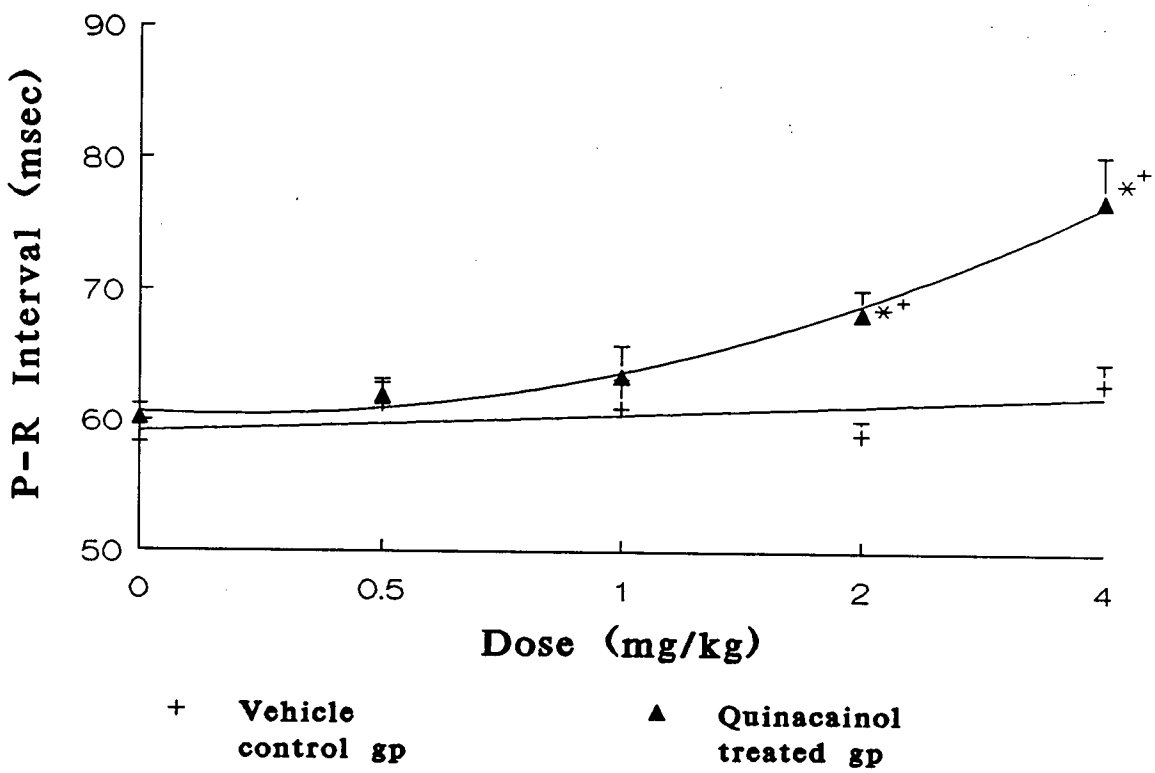


Figure 12. Effects of quinacainol and vehicle control on P-R interval in pentobarbital anæsthetised rats. Drug treatment with quinacainol was a significant source of variance (* $p<0.001$) as compared to its own control values. + Indicates $p<0.05$ as compared to the vehicle control group for the indicated dose of quinacainol. All values are expressed as x±s.e.mean.

Table 4. ECG effects of quinacainol as compared to vehicle control in pentobarbital anæsthetised rats.

Dose	QRS Duration (msec)	Q-Tc Interval (msec)
a) vehicle control group		
pre-treatment	32±1.9	113±8.4
infusion 1	32±1.4	111±6.8
infusion 2	32±1.2	107±6.0
infusion 3	32±1.4	114±8.7
infusion 4	32±1.8	111±9.4
b) quinacainol treated group		
pre-treatment	32±0.9	107±5
0.5 mg/kg	33±1.1	110±2
1.0 mg/kg	33±1.3	106±5
2.0 mg/kg	33±1.0	107±3
4.0 mg/kg	33±0.7	113±3

All variables are mean ± s.e.mean, n=6. Pre-treatment values are those obtained prior to infusion of specified dose of quinacainol or equivalent volume for body weight of vehicle control (indicated as infusion 1, 2, 3, or 4). * Indicates $p<0.05$ from pre-treatment values. + Indicates $p<0.05$ as compared to appropriate infusions in the vehicle control group.

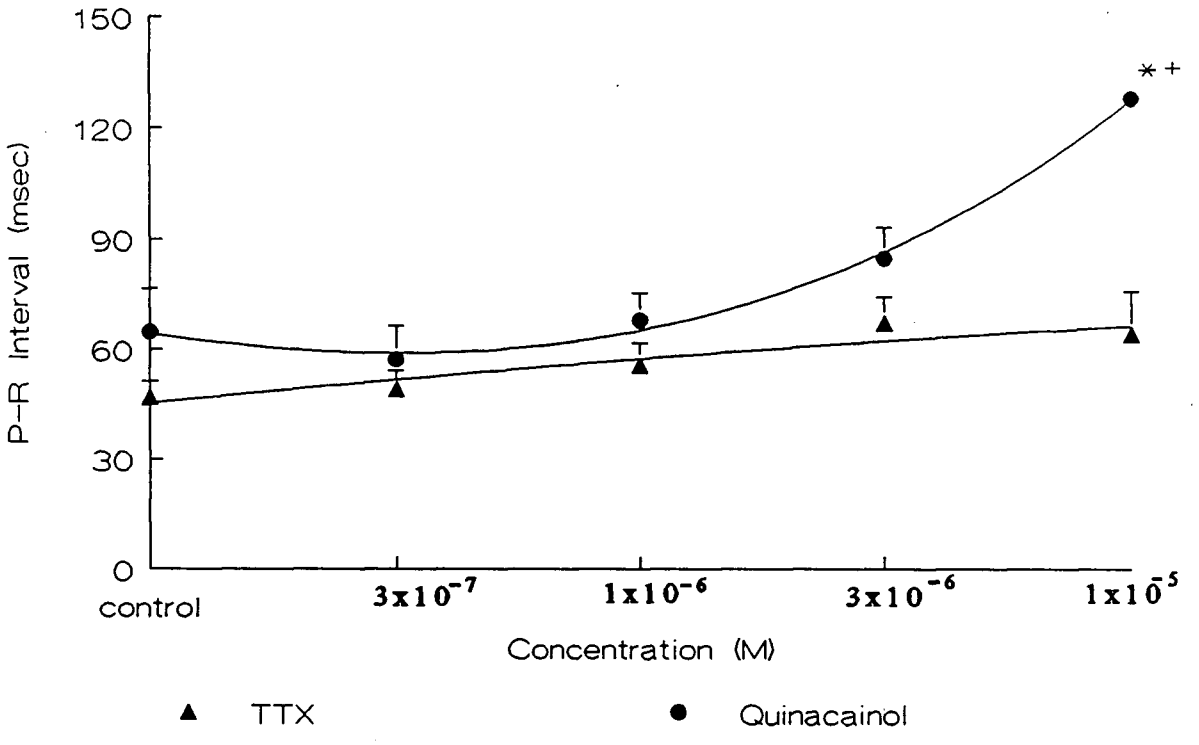


Figure 13. In vitro effects of tetrodotoxin (TTX) and quinacainol on the P-R interval. * Indicates $p<0.001$ as compared to each drug's own control values. + Indicates $p<0.05$ as compared to respective doses of TTX (Unweighted Means ANOVA).

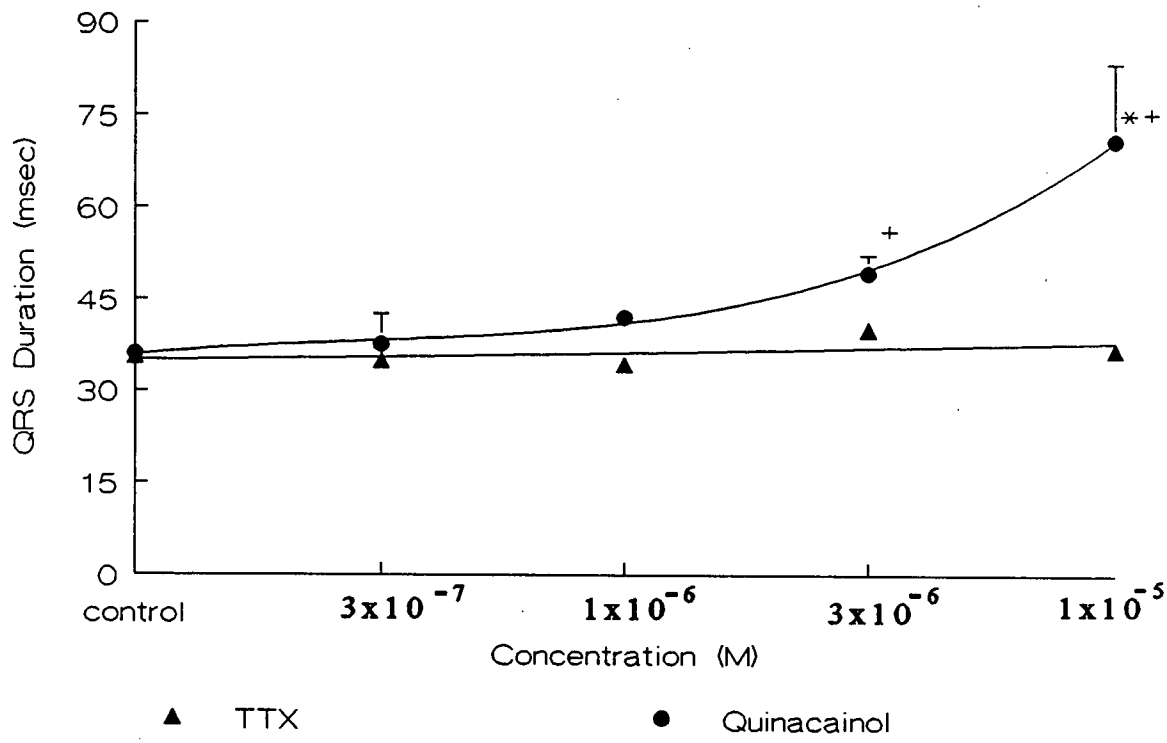


Figure 14. In vitro effects of tetrodotoxin (TTX) and quinacainol on the QRS duration. * Indicates $p<0.001$ as compared to each drug's own control values. + Indicates $p<0.05$ as compared to respective doses of TTX (Unweighted Means ANOVA).

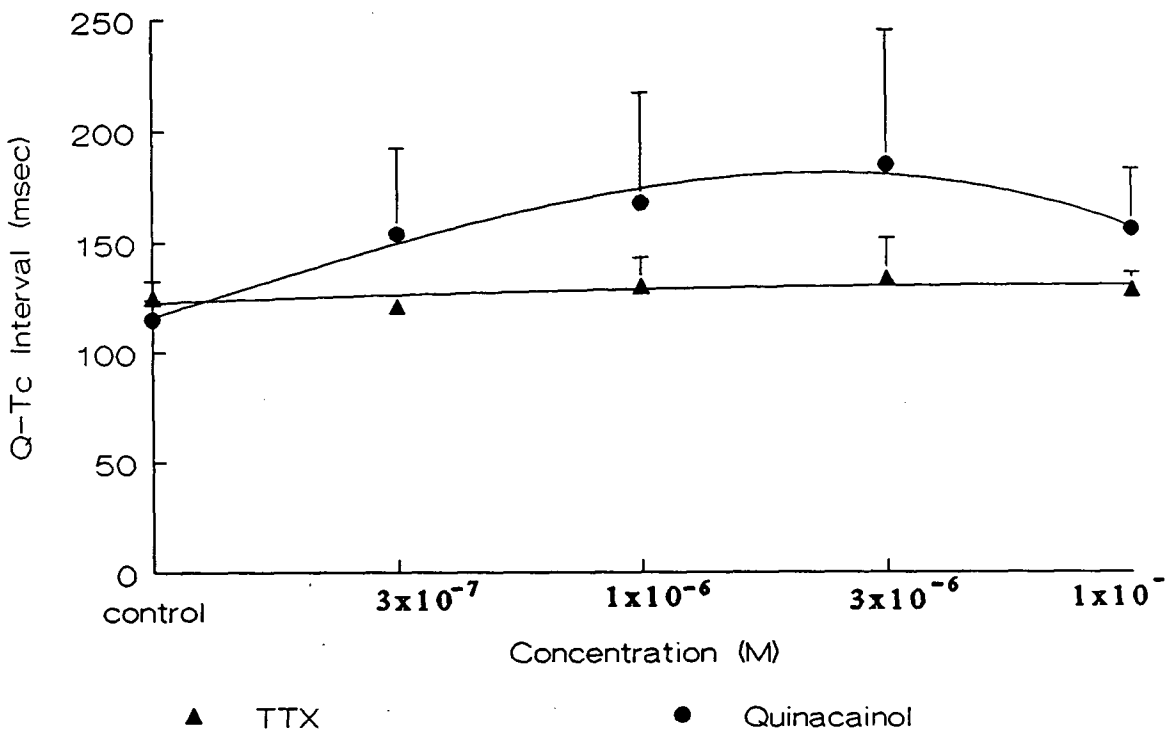


Figure 15. In vitro effects of tetrodotoxin (TTX) and quinacainol on the Q-T_c interval. No significant effects were seen when comparing treatment and vehicle to their respective control values nor when comparing quinacainol to TTX (Unweighted Means ANOVA).

Table 5. Major acute toxic effects of quinacainol in conscious rats.

Toxic Effect	Dose (mg/kg)					
	Vehicle	1.0	2.0	4.0	8.0	16
Mortality	0	0	0	0	0	1
Arrhythmias	0	0	0	1	3	4
Convulsions	0	0	0	0	0	0

Results given for vehicle and doses of 1, 2, 4, and 8 mg/kg are based on a sample size of 6 while n=4 for 16 mg/kg. Excessive grooming and piloerection were noted during infusion.

3.3.1 Occluded Zone and Mortality

To ensure equality of arrhythmic stimulus between groups, the OZ size (as a percentage of total ventricular weight) was determined for all groups and exclusion criteria (Curtis, 1986) were followed throughout the experiment. No statistically significant differences were seen. The OZs for control, 2 mg/kg, and 4 mg/kg were: 41±4, 33±3 , and 41±6% respectively.

Dose-related effects of quinacainol on mortality were not seen, but quinacainol reduced mortality due to VF but not that due to non-arrhythmic causes (Table 6). The high mortality rate in the control group was due to the high incidence of NSVF.

3.3.2 Occlusion-Induced ECG Effects

Interesting effects of quinacainol treatment on ECG changes produced by coronary occlusion (in regard to S-T segment and R-wave changes) were seen. Quinacainol prolonged the time to maximum S-T segment elevation and R wave amplitude increases, and reduced the extent of S-T segment elevation (expressed as %R wave amplitude (ST%) in the manner used by Bernauer (1982; sited in Curtis, 1986)). These results are summarized in Table 7 and Figure 16. Maximum R-wave values obtained following occlusion were similar in all groups.

Table 6. Mortality following coronary occlusion from arrhythmic and non-arrhythmic causes in vehicle control and quinacainol treated conscious rats.

Dose	Arrhythmic Cause	Non-Arrhythmic Cause	Total
a) 0-0.5 h post-occlusion			
control	5/10	1/10	6/10
2 mg/kg	0/10*	0/10	0/10*
4 mg/kg	0/10*	0/10	0/10*
b) 0.5-4 h post-occlusion			
control	1/4	1/4	2/4
2mg/kg	0/10	2/10	2/10*
4 mg/kg	0/10	1/10	1/10*

All variables are expressed as incidence (out of n). Incidence of arrhythmias was separated into two time periods (0-0.5 h and 0.5-4 h) following occlusion due to the bimodal distribution of arrhythmias following occlusions. * Indicates a significant source of variance as compared to controls (as determined by chi^2 analysis).

Table 7. ECG changes produced by coronary artery occlusion.

Dose	Max %S-T (% of R)	Time of Max %S-T (min)	Max R (mV)	Time of Max R (min)	Time of Q-Wave (min)
control	73±8	8±1.4	0.7±0.1	2.3±0.5	/
2 mg/kg	52±6*	20.5±4*	0.7±0.1	11.7±5.8	98±25
4 mg/kg	43±6*	26.3±2.6*	0.7±0.1	12.2±6.3*	93±24

Time (in min) is the time required for the R-wave and S-T changes to reach their maxima while it indicates the appearance of a Q-wave. It was not possible to determine a value for the control group as indicated by /. All variables are expressed as mean ± s.e.mean. * Indicates $p<0.05$ versus control.

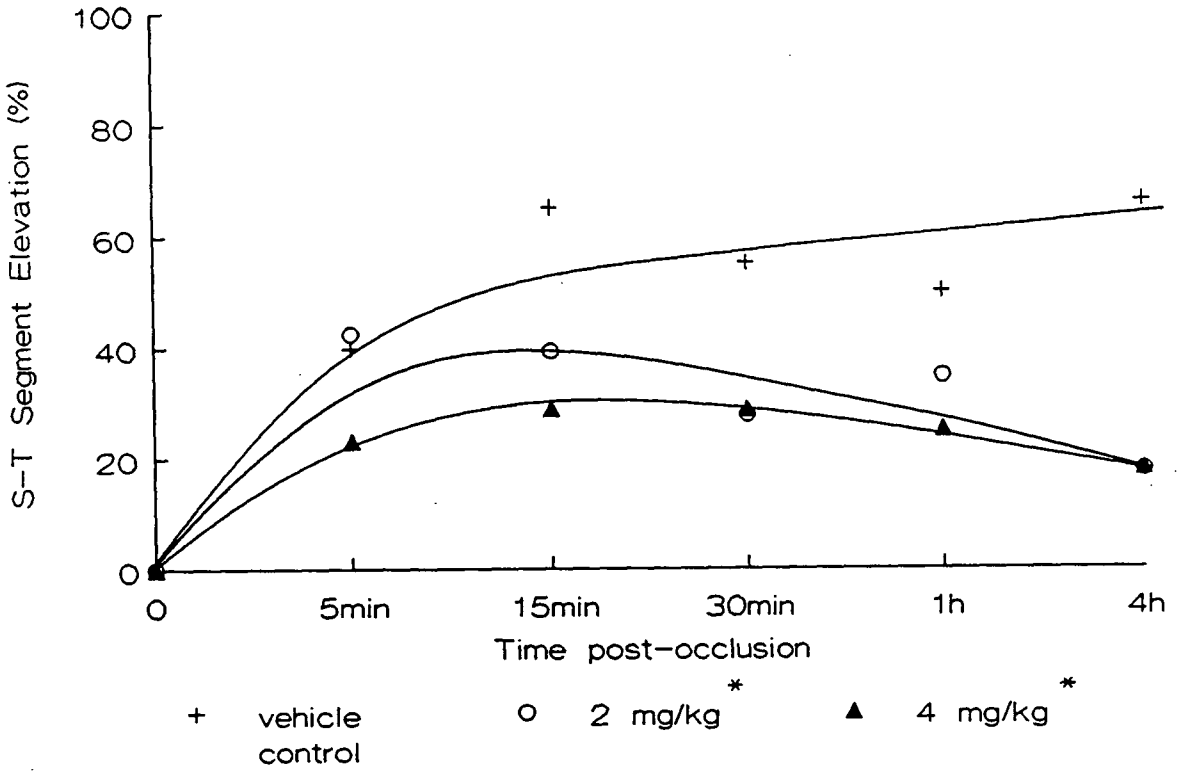


Figure 16. Effects of quinacainol and vehicle control over time on S-T segment elevation (expressed as a % of R wave amplitude) following occlusion. The abscissa indicates the time (in min) following coronary occlusion. Results are expressed as x±s.e.mean, n=10. * Indicates $p<0.05$ versus vehicle control group. + Indicates significant differences ($p<0.05$) between treatment groups and vehicle control group at that time period indicated on the abscissa.

3.3.3 Arrhythmias

Extensive analysis within our laboratory of the arrhythmias following occlusion of the LAD in conscious rats showed a bi-modal distribution with time (Johnston *et al.*, 1983). Within the 4 h time period in which we test anti-arrhythmic activity in our conscious ischæmia model, peak frequency of arrhythmias occurs within the first 15 min and 2 h following occlusion, with a quiescent period between these periods (Table 8) (Curtis, 1986).

Arrhythmia incidence in the various groups is shown in Table 8 for the two time periods, 0-0.5 h and 0.5-4 h, after occlusion; arrhythmia scores are also given. Both doses of quinacainol statistically significantly reduced arrhythmia score in the first time period 0-0.5h after occlusion while quinacainol's protective effects over the remaining time period, 0.5-4 h, were less.

With regard to various types of arrhythmias, the incidence of PVC's was similar in all groups. The $\log_{10}$ number of PVC was slightly higher in the high dose of 4 mg/kg quinacainol treated group but not to a statistically significant degree. Quinacainol dose-dependently reduced the incidence of VT and VF over the 4 h following occlusion. This was statistically significant for VT and VF in the 0-0.5 h period and for VF in the 0.5-4 h period.

Table 8. Antiarrhythmic effects of quinacainol against ischæmia-induced arrhythmias in conscious rats in early (0-0.5 h) and late (0.5-4 h) periods following coronary artery occlusion.

Dose	Antiarrhythmic Effects			
	AS	Log_{10} PVC	VT	VF
a) 0-0.5 h post-occlusion				
control	4.8±1.0	1.2±0.1	8/10	6/10
2 mg/kg	2.5±0.5^{+}	1.8±0.2	8/10	4/10
4 mg/kg	1.5±0.5^{+}	1.8±0.4	3/10*	1/10*
b) 0.5-4 h post-occlusion				
control	2.0±0.2	1.7±0.4	2/4	2/4
2 mg/kg	1.9±0.4	1.7±0.4	4/10	3/10
4 mg/kg	2.4±0.5	2.0±0.5	3/10	1/10*

AS=arrhythmia score. Calculations to determine AS were discussed in the methods and all values represent mean ± s.e.mean. PVC's are expressed as log_{10} PVC number. For VT and VF, values are incidence (out of n). * Indicates $p<0.05$ versus control (vehicle) while $^{+}$ indicates $p<0.01$ versus control.

Serum K^+ levels did not increase statistically significantly 4 h post-occlusion as compared to pre-occlusion values for quinacainol-treated group.

In summary quinacainol provided marked protection against ischæmia-induced arrhythmias and was particularly antifibrillatory at 4 mg/kg. The dose-dependent effect of quinacainol in reducing the frequency of arrhythmias is exemplified not only by the arrhythmia scores but also by the decrease in the incidence of serious arrhythmias (VT and VF). Higher doses of 8 mg/kg were tested in a separate study and it was found to potentiate rather than reduce arrhythmias. This pro-arrhythmic effect took the form of precipitating arrhythmias within 1 min of occlusion. This was different from the groups tested with 2 and 4 mg/kg, where arrhythmias only occurred at least 5 min after occlusion.

3.4 Electrically-induced Arrhythmias in Anæsthetised Rats

Time was a confounding variable in the electrical stimulation studies necessitating the presence of a time control, i.e. the vehicle control group. For this reason, and the sake of continuity in data analysis of all variables (hæmodynamics, ECG, and electrical stimulation), vehicle data were not pooled.

The vehicle did not influence responsiveness to electrical stimulation whereas quinacainol dose-dependently increased i_T, t_T, and VF_T (Figures 17a, 17b, and 18). In addition, quinacainol lengthened the effective refractory period while reducing the maximum following frequency (Figure 19b and 19a).

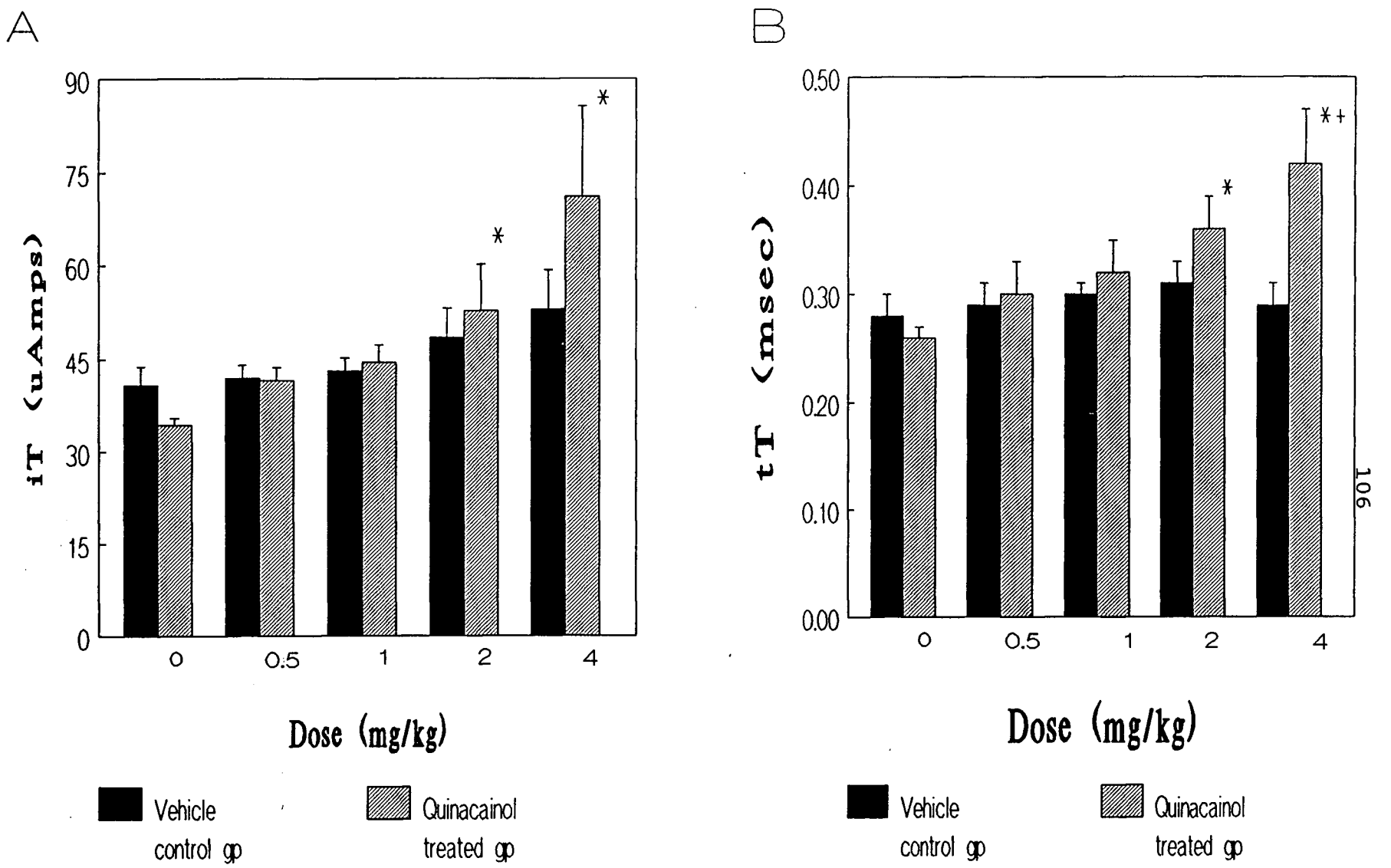
In summary, quinacainol produced a reduction in excitability to electrical stimulation. This was associated with antiarrhythmic effects against electrically-induced ventricular fibrillation.

Serum K^+ levels increased from a control value of 3.73±0.2 to 4.14±0.15 at the end of the experiment for the vehicle control group. For the quinacainol treated group, serum K^+ levels increased from a control value of 3.65±0.19 to 3.92±0.2 at the end of the experiment. No statistically significant differences were seen when comparing control values with post experiment values within each group or comparing control values or post experiment values between groups.

3.5 Effects of Quinacainol on Epicardial Action Potentials in the Anæsthetised Rat

It can be seen that quinacainol markedly and dose-dependently decreased the rise rate of the AP (phase 0), dV/dt_{max} (Figure 20a). The height of the AP was also dose-dependently depressed (Figure 20b) while the resting

Figure 17. Effects of quinacainol as compared to vehicle control on threshold current, i_T (A) and threshold duration, t_T (B) for induction of VT in pentobarbital anæsthetised rats subjected to electrical stimulation of the left ventricle. As time was not a significant source of variance, the indicated values were measured 10 min after drug or vehicle administration and expressed as mean $\pm$ s.e.mean. Treatment was a statistically significant source of variance when compared to pre-drug values (0 mg/kg on the abscissa), * indicating $p<0.05$. + Indicates a significant difference ($p<0.05$) between the vehicle control group and quinacainol treated group at the dose indicated.


A
90
75
60
45
30
15
0
iT (uAmps)
*
*
0
0.5
1
2
4
Dose (mg/kg)
Vehicle control gp
Quinacainol treated gp
B
0.50
0.40
0.30
0.20
0.10
0.00
tT (msec)
*+
*
0
0.5
1
2
4
Dose (mg/kg)
Vehicle control gp
Quinacainol treated gp

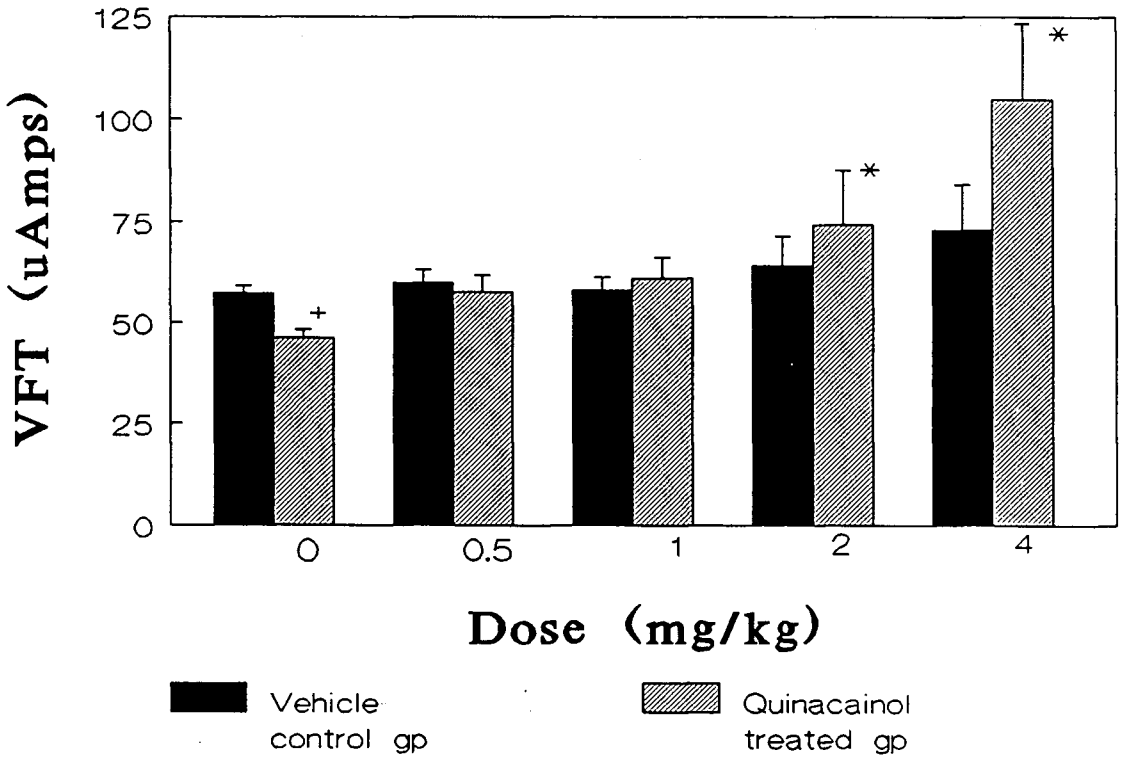
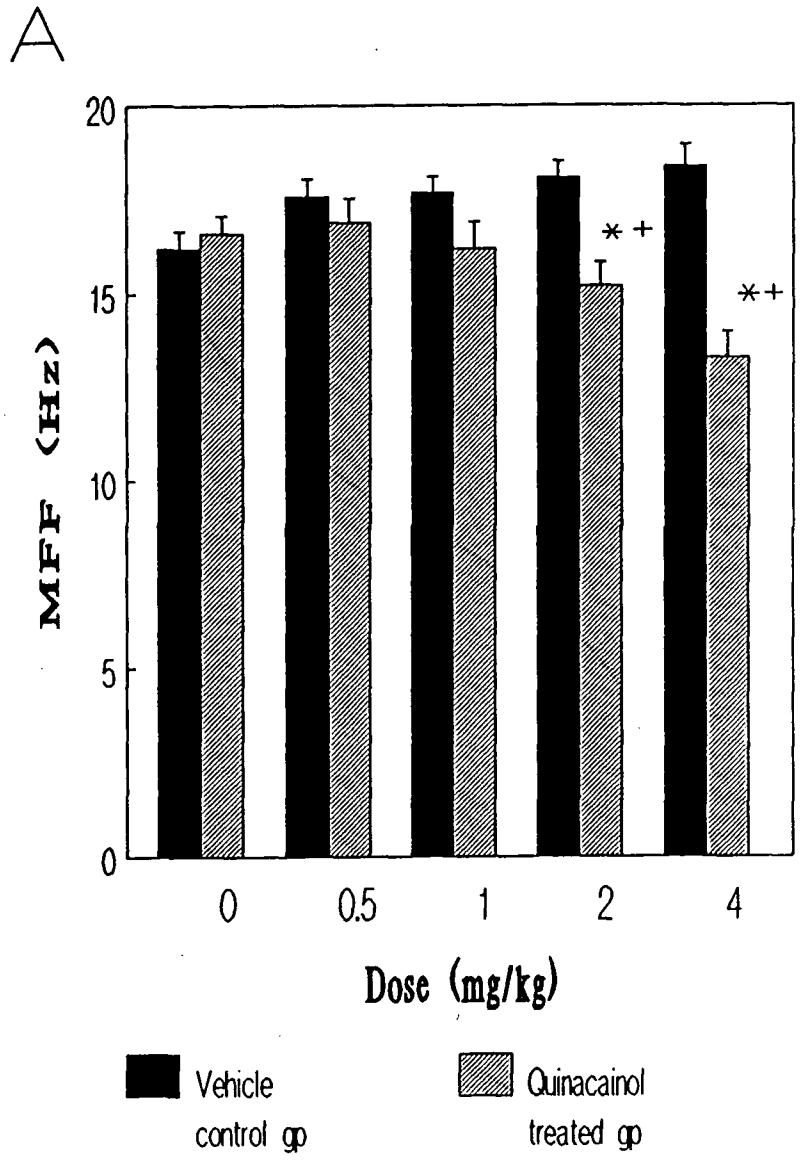
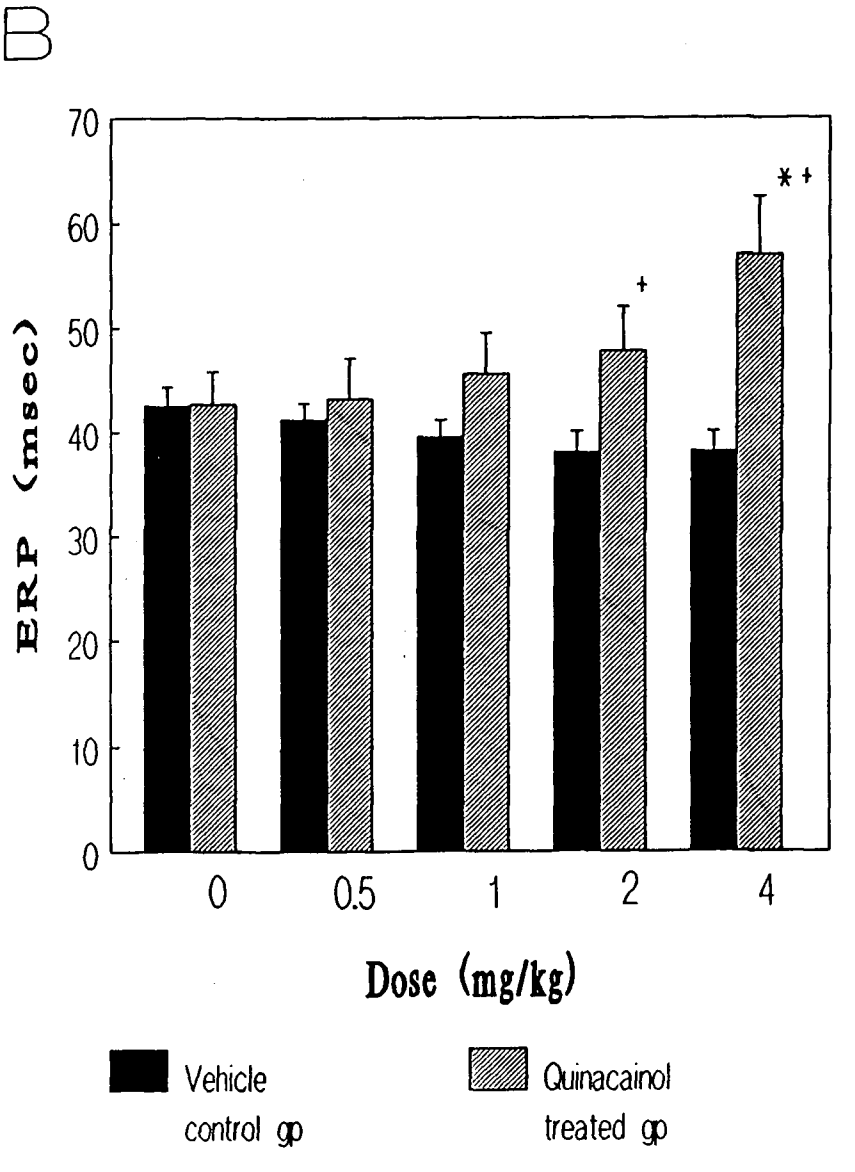


Figure 18. Comparison of quinacainol versus vehicle control on the induction of ventricular fibrillation threshold (VF_t) in pentobarbital anæsthetised rats in vivo by stimulation of the left ventricle. * Indicates $p<0.05$ from pre-drug values. + $p<0.05$ as compared to vehicle control.

Figure 19. Effects of quinacainol as compared to vehicle control on maximum following frequency, MFF (A) and effective refractory period, ERP (B) as determined by the extra stimulus method. Indicated values were measured 10 min after end of drug or vehicle administration and expressed as mean ± s.e.mean. Time was not a significant source of variance while treatment was ($p<0.05$). * $p<0.05$ as compared to pre-drug values (0 mg/kg). + $p<0.05$ as compared to vehicle control.


A
MFF (Hz)
20
15
10
5
0
0
0.5
1
2
4
* +
* +
Dose (mg/kg)
Vehicle control gp
Quinacainol treated gp


B
ERP (msec)
70
60
50
40
30
20
10
0
0
0.5
1
2
4
* +
+
Dose (mg/kg)
Vehicle control gp
Quinacainol treated gp

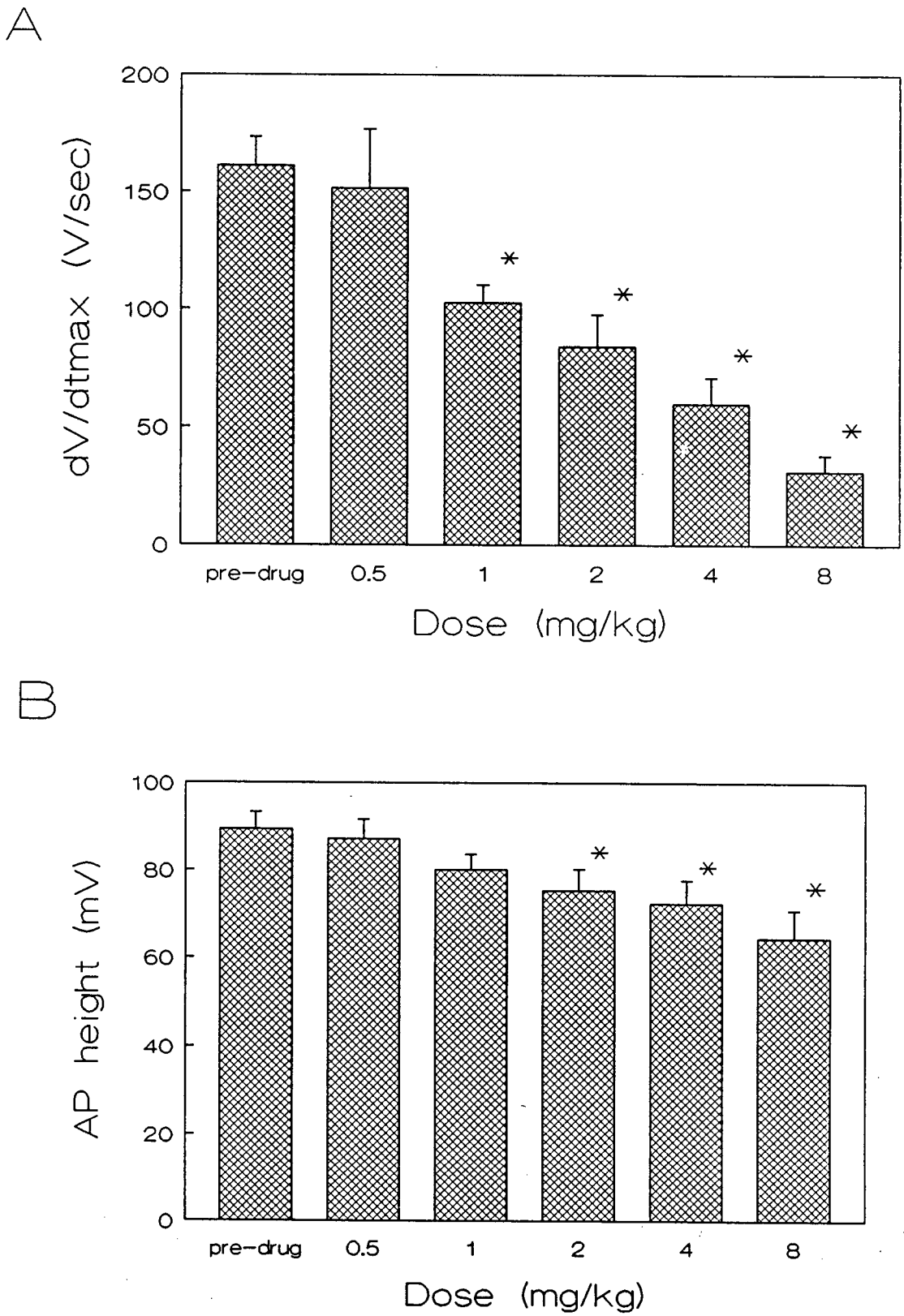


Figure 20. Effects of quinacainol on phase 0 of the action potential. Intracellular recordings of the epicardial action potential show that the maximum rise rate of the action potential (dV/dt_{max}) (A) and action potential height (B) are dose-dependently decreased in the presence of cumulative doses of quinacainol. * Indicates $p<0.001$ from pre-drug values.

membrane potential was not significantly affected although a slight depression was seen at increasing doses (Figure 21). AP duration was measured at 10, 50, and 90% repolarization (Figure 22). Preferential effects on different phases of repolarization were never observed with the highest dose of 8 mg/kg significantly ($p<0.001$) prolonging repolarization at all times. An obvious trend of delayed repolarization (for $APD_{10,50,\ 90}$) with increasing doses could be seen although this was significant for all phases only at the highest dose (8 mg/kg).

3.6 Isolated Perfused Rat Hearts

In order to assess the direct effects of quinacainol in cardiac tissue, isolated rat hearts were perfused with varying concentrations of quinacainol (modified Langendorff apparatus, Curtis *et al.*, 1986) and compared to that of the classical sodium channel blocker TTX.

Figure 23 depicts the effects of quinacainol and TTX on contractility as measured by dP/dt. TTX did not appear to depress contractility while a slight dose-dependent cardiodepressant effect was seen with quinacainol. This may have been associated with it's bradycardic actions.

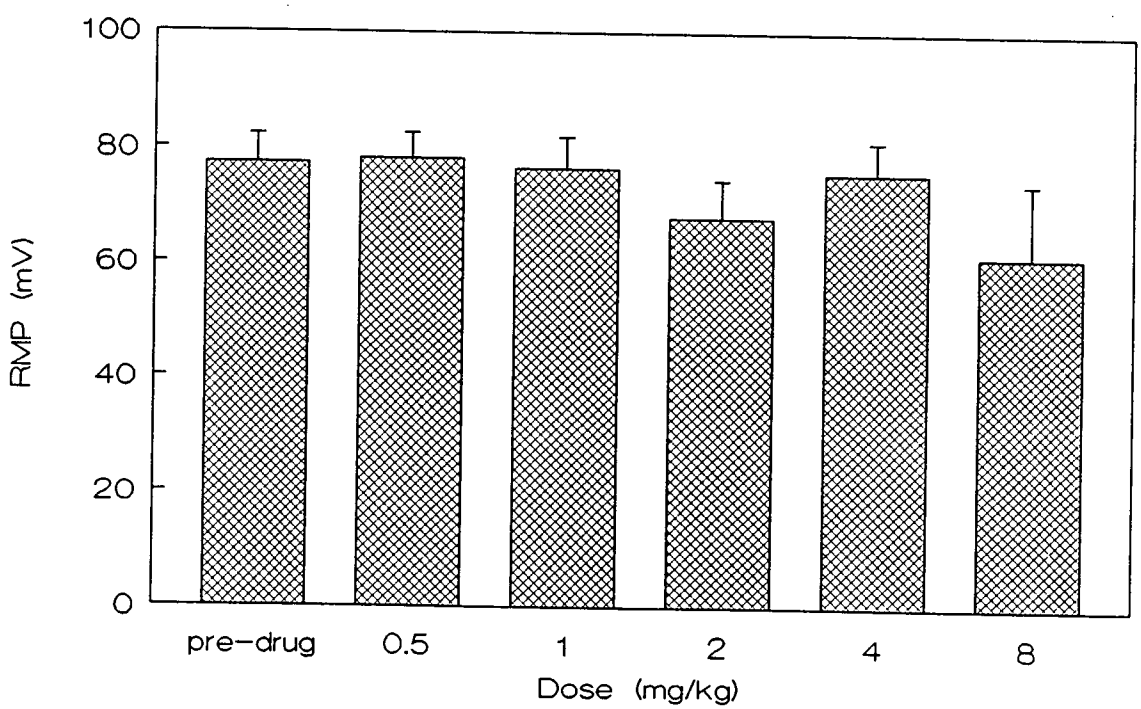


Figure 21. Effects of quinacainol on the resting membrane potential (RMP).

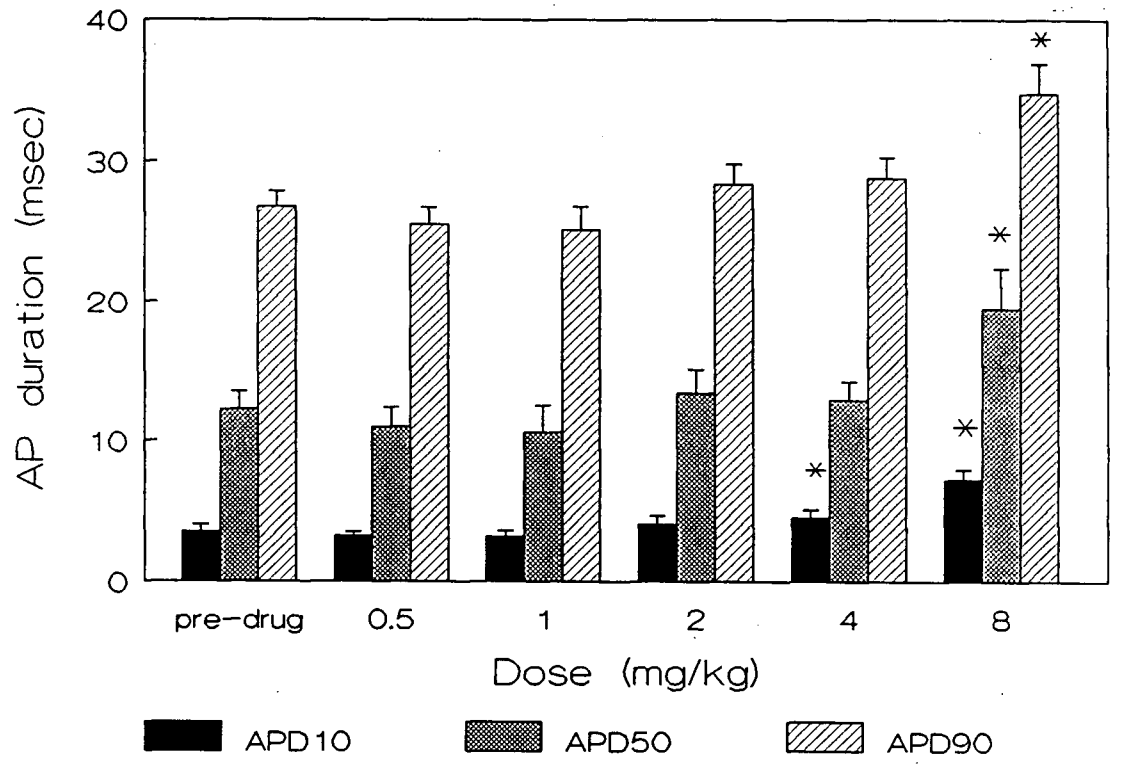


Figure 22. Effects of quinacainol on different phases of repolarization of the epicardial intracellular action potential, i.e. APD_{10} = AP duration following 10% repolarization. * Indicates $p<0.001$ as compared to pre-drug values (Unweighted Means ANOVA).

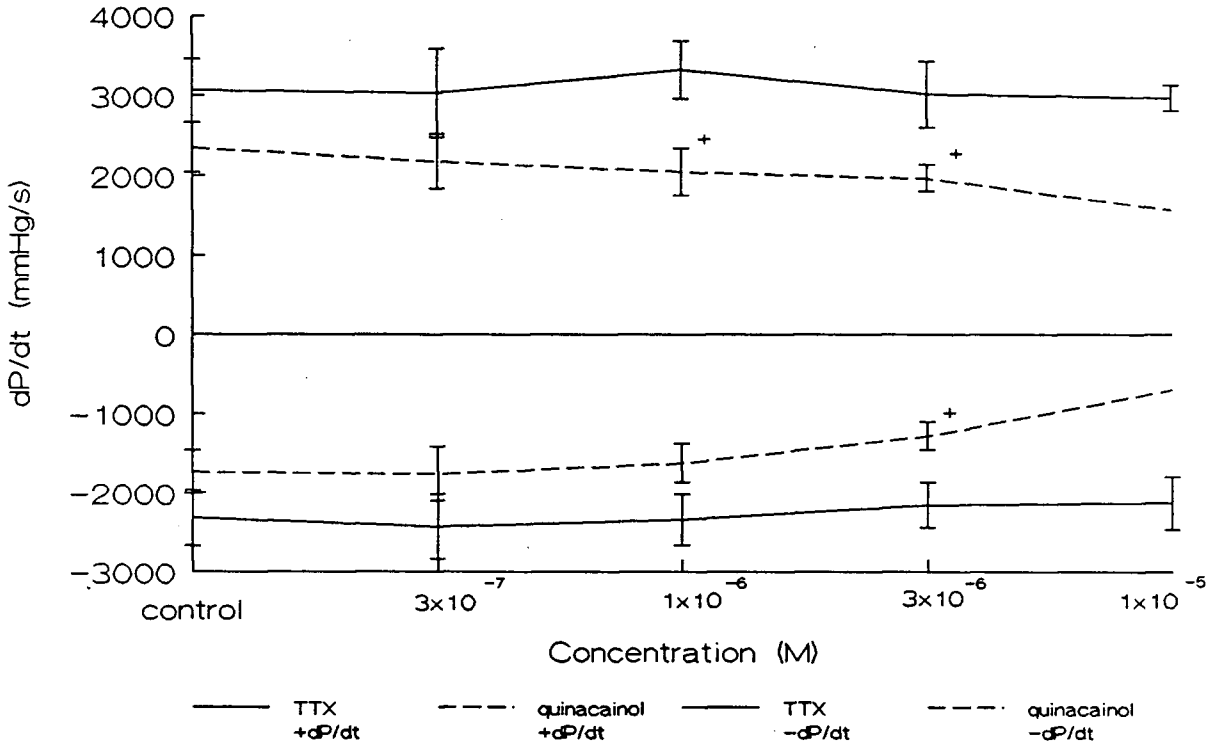


Figure 23. The effects of tetrodotoxin (TTX) and quinacainol on a measure of contractility (dP/dt) in the isolated rat heart. Quinacainol had greater effects on contractility than TTX. Treatment was not a significant source of variance when comparing quinacainol and TTX to their own control values. Significant differences were detected when comparing respective doses of quinacainol with TTX ($^{+}$ indicates $p<0.05$) (Unweighted Means ANOVA). No error bars are indicated for $1x10^{-5}$ M in the quinacainol treated group as 6 of 7 hearts were not in sinus rhythm but rather experienced either atrioventricular block or PVC's.

4 DISCUSSION

4.1 Hæmodynamic and ECG Effects of Quinacainol

The results of the hæmodynamic dose-response study enabled the selection of appropriate doses for further experiments. The ED_{50} for BP effects was estimated to be 4 mg/kg from this experiment.

Significant toxicity was only encountered at doses of 8 mg/kg and above and was referable to the heart, i.e. spontaneously reverting tachyarrhythmias. When compared with the vehicle, quinacainol had no major effects on BP unlike many class I antiarrhythmic agents (Legrand and Collignon, 1985; Honerjager *et al.*, 1986). Quinacainol's major effects were to lower HR and prolong the P-R interval and QRS duration of the ECG.

With regard to the toxicity of class I agents and their deleterious hæmodynamic effects, the most appropriate choice of an agent must take into account cardiovascular depression (Legrand and Collignon, 1985). Legrand and Collignon (1985) noted that while class Ia agents have minor hæmodynamic effects and class Ib drugs are exceptionally well tolerated, the cardiodepressant effects of class Ic agents have potentially adverse effects especially in patients with left ventricular dysfunction. There is controversy however, over the association between Na^+ channel blockade and negative inotropy. Honerjager *et al.*

(1986) stated that sodium channel block invariably has a moderate negative inotropic influence, as demonstrated with TTX, although this may not be seen with Ib agents due to their fast offset kinetics. Cowan and Vaughan Williams (1981) and Courtney (1984) found no association between the electrophysiological effects of class I agents and negative inotropy.

4.2 Antiarrhythmic Actions of Quinacainol against Ischæmia-induced Arrhythmias

Quinacainol demonstrated a rather attractive profile as compared to other sodium channel blockers tested for antiarrhythmic efficacy. It gave protection against acute ischæmia-induced arrhythmias without inducing convulsions, significantly depressing blood pressure nor reducing heart rate. However, as indicated by electrophysiological actions, antiarrhythmic doses caused detectable depression of sodium current in non-ischæmic myocardium. This action in normal myocardium is typical of class Ic agents and, to a lesser extent, Ia agents (Pallandi and Campbell, 1988). At higher doses (8 mg/kg and above), the drug precipitated arrhythmias shortly after occlusion of the coronary artery; a finding reminiscent of the pro-arrhythmic findings with other class Ic agents (CAST Investigators, 1989; Horowitz, 1990; Levine *et al.*, 1989; Morganroth and Horowitz, 1984).

Quinacainol had interesting effects on ischæmia-induced ECG changes. Both doses (2 mg/kg and 4 mg/kg) reduced S-T segment elevation and prolonged the time to maximum S-T segment elevation and maximum R-wave amplitude. This finding is similar to that seen in a previous study done in our laboratory with TTX (Abraham *et al.*, 1989). In each of these studies (TTX and quinacainol), the decreased extent of S-T segment elevation could not be attributed to significant differences in ischæmic zone size. Greater blockade of sodium channels in ischæmic tissue could explain the unexpected effects of quinacainol on ischæmia-induced ECG changes as has been postulated previously by Abraham *et al.* (1989) after finding similar effects with TTX. In control groups (given vehicle), coronary occlusion produces time-dependent elevations of R-wave and S-T segment (Johnston *et al.*, 1981, 1983; Curtis *et al.*, 1987), indirect indices of the extent and severity of myocardial ischæmia (Janse, 1979; Janse, 1986). Several agents delay the onset of S-T segment elevation, including the adrenergic β blocker, propranolol (Berdeaux *et al.*, 1978). Other agents previously studied in our laboratory produced similar results, most notably, the calcium channel blockers felodipine (Curtis *et al.*, 1985), anipamil (Curtis *et al.*, 1986b), and verapamil (Curtis and Walker, 1986). However, while these agents produce similar delayed effects on the rate of development of S-T elevation, they did not reduce the maximum S-T segment elevation (Curtis *et al.*, 1987).

In isolated hearts, a dose-dependent cardiodepressant effect was seen with quinacainol at concentrations which prolonged the P-R interval and QRS duration (Howard and Walker, 1990b). The depressed contractility seen *in vitro* with quinacainol may have been associated with its bradycardic actions. However, that quinacainol reduces contractility (and minimally at that), seems an unlikely explanation of the ECG results following occlusion inasmuch as calcium channel blockers reduce contractility but do not reduce maximum S-T segment elevation (as reviewed by Curtis *et al.*, 1987; Curtis and Walker, 1986). In addition, reduction of maximum S-T segment elevation has not been seen with any other class I antiarrhythmic agent used in our laboratory which include quinidine (Ia), disopyramide (Ia), lidocaine (Ib) (Johnston *et al.*, 1983), and mexiletine (Ib) (Igwemezie, 1990).

Factors altering myocardial oxygen requirements cannot be excluded as possible contributors to quinacainol's actions on the ischæmic ECG. The primary determinants of myocardial oxygen demand are contractility and heart rate. Both doses of quinacainol produced similar effects on heart rate, and both doses of quinacainol significantly decreased S-T segment elevation, suggesting effects on heart rate (tendency to bradycardia) may be a factor.

The above observations suggest that quinacainol demonstrated some degree of selectivity on partially depolarized tissue under acidic conditions as occurs in

ischæmic tissue although blockade of ventricular sodium channels in normal tissue was also evident. Quinacainol did not demonstrate the degree of selectivity for the ischæmic myocardium that would have been desired of an ideal antiarrhythmic agent.

4.3 Actions of Quinacainol Against Electrically-induced Arrhythmias in the Anæsthetised Rat

Results from the ECG and electrical stimulation study suggested that quinacainol produced sodium channel blockade as demonstrated by the prolongation in the P-R interval without changing the Q-T_c interval. Q-T_c interval prolongation suggested class Ia actions. However, P-R interval widening cannot be considered conclusive evidence of sodium channel blockade since prolongation of the P-R interval in rats is seen with both sodium and calcium channel blockers (Botting *et al.*, 1986). In this study the QRS complex was not markedly widened as might be expected from a clas Ic compound.

In addition to ECG evidence, the effects of quinacainol on i_T, t_T, and VF_T were consistent with sodium channel blockade (Beatch *et al.*, 1988; Hodess *et al.*, 1979; Marshall *et al.*, 1983; Yoon *et al.*, 1974). Quinacainol was also very effective in prolonging the ERP and in reducing the MFF. These actions are consistent with class Ia and Ib agents although the latter produce few ECG signs of sodium channel

blockade at normal sinus beating rates due to their high frequency dependency (Campbell, 1983b; Courtney, 1987).

In the above experiment, characterization of quinacainol was established at 7.5 Hz stimulation frequency and therefore did not take into consideration the marked frequency dependent properties of class I agents (Courtney, 1980). Quinacainol seemed free of toxicity in that it did not produce signs of cardiodepression or CNS toxicity at doses which had marked effects in reducing excitability to electrical stimulation (Howard and Walker, 1990a).

4.4 Actions of Quinacainol on Epicardial Intracellular Recordings

The predominant electrophysiological action of class I antiarrhythmic agents is to block cardiac Na^+ channels (Sheldon *et al.*, 1989). This reduces the rate of depolarization of the AP and slows impulse propagation (Sheldon *et al.*, 1989). Quinacainol had no effect on the resting membrane potential although it had major inhibitory effects on phase 0 of the AP. It clearly demonstrated class Ic actions in that it reduced the maximum rise rate of the AP and decreased AP height without influencing other phases at doses producing equivalent ECG effects in the other experimental preparations. These observations provided evidence for selective ventricular Na^+ channel blockade (Sheldon *et al.*, 1989).

The use of maximal upstroke velocity (V_{max}) as an estimate of available Na^+ conductance is a controversial subject (Grant *et al.*, 1984; Cohen *et al.*, 1984). In general, a curvilinear relationship describes the dependence of V_{max} on Na^+ conductance, rising more sharply at low Na^+ conductance and approaching a maximum value at high Na^+ conductance (Catterall, 1987). Measurements in excitable cardiac cells typically span this nonlinear range such that measurements of V_{max} are only a semi-quantitative measure of Na^+ channel availability (Catterall, 1987). Much of the non-linearity could be attributed to activation kinetics of the Na^+ channel (Cohen *et al.*, 1984).

In the intracellular study, quinacainol had neither class III nor class IV properties since the plateau duration (class IV) and shape (class III) in the rat ventricle were relatively unaffected. There also was no evidence to suggest that quinacainol possessed class II activity.

Quinacainol prolonged APD only at the highest dose tested. This suggested that quinacainol may be acting as a class Ia agent at higher doses. A lack of effect on APD_{90} has been previously described for other class I agents, notably flecainide (Campbell, 1983a) and BW A256C a putative class I agent (Allan *et al.*, 1986). However, these agents also showed lack of effect on ERP (in guinea-pig ventricle) while quinacainol demonstrated a dose-dependent prolongation of ERP. Class I agents such as lidocaine (Ib) have been shown to change both APD_{90} and ERP (Varro *et al.*, 1985).

Such varied effects led in part to Harrison et al's (1981) and Campbell's (1983b) subclassification schemes based on effects on APD and rate-dependent effects (onset-offset kinetics), respectively. Based on the combined results obtained with quinacainol, i.e. lack of effect on all phases of the AP except phase 0 and AP height, and a dose-dependent increase in ERP, quinacainol demonstrated Ic actions with respect to its lack of effects on APD but Ib actions with respect to its lengthening of the ERP independent of changes in APD.

Class I agents alter repolarization in a variety of ways. Lidocaine induces such effects on the sodium "window" current while quinidine affects the delayed rectifier (K^+ channel blockade) (Colatsky, 1982). The effects of encainide (a Ic agent) are attenuated with increasing frequency (Ebharrar and Zipes, 1982; cited in Nattel and Zeng, 1984).

Studies concerning the frequency-dependent action of quinacainol are needed to provide additional evidence of its subclassification. Such a study would test its frequency-dependency and differential effects on APD might play a role in determining the overall effects of the drug on the refractory period. Nattel (1987) inferred from his data that the use-dependence of class I effects on conduction *in vivo* were quantitatively predictable from the interval dependence of effects on V_{max} *in vitro*.

Thus the cellular electrophysiological profile obtained is not strictly consistent with any of the three current subclasses within class I. This anomaly may indicate shortcomings in the present drug classification scheme and may not be indicative of a "novel" antiarrhythmic profile. Similar conclusions can also be drawn from other studies which tested putative class I antiarrhythmic compounds (e.g. Colatsky *et al.*, 1987).

4.5 Effects of Quinacainol *in vitro*

Quinacainol's possible site(s) of action are still imprecisely determined. Quinacainol could have produced its effects in normal myocardium, ischæmic myocardium, or the interface between the two; the border zone. We must rely on indirect evidence in this regard due to the difficulty in obtaining direct evidence of drug effects on ischæmic tissue (Abraham *et al.*, 1989; Inoue *et al.*, 1982). Theoretically, quinacainol could act by protecting the normal myocardium from invasion by arrhythmic impulses arising in the ischæmic zone, or it could act in ischæmic tissue to directly suppress arrhythmogenesis. Patch clamp studies would provide stronger evidence on the voltage-dependency of quinacainol. We know that ischæmia is associated with a 15 to 20 mV depolarization (Inoue *et al.*, 1982), therefore, if blockade of Na+ channels in ventricular cells by quinacainol is dependent on steady-state depolarization, blockade of

sodium channels by quinacainol would be more pronounced in ischæmic tissue. Greater blockade of sodium channels in ischæmic tissue may help explain the unexpected effect of quinacainol on ischæmia-induced ECG changes.

By determining quinacainol's actions in isolated ventricular tissue, an opportunity was provided for the direct assessment of quinacainol's effects and to compare these effects with TTX, an agent whose only known action is blockade of sodium channels. This experiment showed that quinacainol had all of the expected effects of a class Ic agent since it widened the P-R interval and QRS duration without having a major effect on the Q-T_c interval. Quinacainol was more potent than TTX, the classic sodium channel blocker, on all ECG variables measured. Blockade of other channels may also prolong the P-R interval. In the rat heart both sodium and calcium channels participate in A-V conduction such that prolongation of the P-R interval is a useful measure of sodium and calcium channel blockade (Botting *et al.*, 1985). The greater effect of quinacainol compared with TTX on the P-R interval, suggested that quinacainol may also have blocked calcium channels to produce its effect. In any event, calcium channel blockade is not involved in P-R interval prolongation to the same extent as Na^+ channel blockade in smaller species (Botting *et al.*, 1986).

As a result of low doses of TTX (10^{-7} M) shortening the plateau of the cardiac Purkinje fiber AP without affecting

the upstroke velocity, Coraboeuf *et al.* (1979) suggested two cardiac sodium currents - one responsible for the AP upstroke and a second responding to TTX. Thus there could be two populations of cardiac Na^+ channels (Fozzard *et al.*, 1985). TTX-sensitive Na^+ channels could inactivate with time constants of several hundred msec, their sensitivity to TTX being supported by studies in rabbit Purkinje strands demonstrating a small, slowly inactivating component of Na^+ current (Carmeliet, 1984). In addition, some TTX-sensitive Na^+ channels could be voltage and time-independent providing a background inward current important to the resting potential as well as the plateau (Fozzard *et al.*, 1985). These slowly decaying or steady Na^+ currents are very small, their size being in the range of currents that could be generated by Na^+ dependent membrane transport processes but are not gated Na^+ channels (Fozzard *et al.*, 1985). These possibilities include the electrogenic Na^+-K^+ pump and the Na^+-Ca^{2+} exchange system, and possibly the Na^+-H^+ or Na-K^+ transport systems if they are electrogenic (Fozzard *et al.*, 1985). The existence of 2 different cardiac sodium channels may also help explain the different effects observed with quinacainol and TTX (both of which block sodium channels) on the variables measured.

In relation to the hæmodynamic effects of quinacainol, it is well known that negative inotropism is a common side effect of treatment with class I agents (Hoffmeister *et al.*, 1987; Honerjager *et al.*, 1986). Honerjager *et al.* (1986)

compared the negative inotropic effects of TTX and 7 class I antiarrhythmics in relation to Na^+ channel blockade. With the exception of 2 agents (sparteine and AR-LH31) all the drugs produced a larger negative inotropic effect than TTX at concentrations equieffective in reducing V_{max} , suggesting that blockade of Na^+ channels can account for only part of their negative inotropic effect. Honerjager (1986) also found TTX to be significantly more potent in reducing V_{max} than in reducing force of contraction as compared to various class I agents which exerted stronger negative inotropic effects.

One further possibility in regard to the differences seen on contractility between TTX and quinacainol may be that not high enough concentrations of TTX were used to validly compare its effects to quinacainol's. Winslow *et al* (1983), in a study using Langendorff isolated perfused rat hearts, found "antiarrhythmic concentrations" (0.16-1.57 μM) of TTX to reduce contractility up to approximately 48%.

The experimental data from isolated hearts supports two conclusions, first, that quinacainol alone can produce the effects seen in *in vivo* experiments without need for conversion to active metabolites. Secondly, quinacainol exerts effects on the normal myocardium and need not rely on the conditions produced by ischæmia to potentiate its effects.

5 Summary

The studies showed that doses of quinacainol, which in normal ventricular myocardium prolonged the P-R interval, increased electrical stimulation thresholds, and reduced dV/dt_{max} and AP height, possessed antiarrhythmic activity against ischæmia-induced arrhythmias. The latter occurred without compromising hæmodynamic status.

If effects on the ECG, electrical stimulation, and intracellular potentials share a common mechanism of sodium channel blockade (sodium current inhibition) then it must be assumed that the antiarrhythmic action of quinacainol correlates with blockade of ventricular gNa in normal myocardium. All studies were performed in one species under one of two conditions, either conscious or pentobarbital anæsthetised. The similarity of conditions in the different experimental models allowed for ready extrapolation with respect to the doses used in the various studies.

At antiarrhythmic doses, toxic effects referable to either the CNS or cardiovascular system were not seen in marked contrast to other class I agents that we, and others, have tested under similar conditions. The antiarrhythmic efficacy of mexiletine and lidocaine (Ib agents) are severely limited by the occurrence of convulsions at comparatively low doses (Igwemezie *et al.*, 1990) while with drugs such as quinidine and procainamide (Ia agents), the major toxicity is cardiovascular depression (negative

inotropism) and peripheral vasodilation which is not helpful in patients with congestive heart failure (Legrand and Collignon, 1985). Prototypical class Ic agents such as flecainide and encainide also have specific and dangerous toxicities in that proarrhythmic effects may occur in the setting of myocardial ischæmia and infarction (CAST Investigators 1989a, 1989b).

With regard to quinacainol's subclassification, Table 9 presents the pooled results from all experimental studies that are used for definitive purposes in subclassification of class I compounds. Using the characteristics given to the various subclassification definitions and the results obtained with the various experimental protocols used here it can be ascertained that quinacainol demonstrated Ic actions at lower doses and Ia actions at higher doses. Higher doses were not necessary for antiarrhythmic effects.

In conclusion, at doses producing equivalent prolongation of the P-R interval of the ECG, a consistent series of findings were noted with quinacainol. These consisted of: (1) a selective depression of AP height and of dV/dt_{max} of phase 0 of the AP; (2) moderate increase in QRS duration without effects on the $Q\text{-}T_c$ interval; (3) reduction in excitability to electrical stimulation, and (4) lack of cardiovascular depressant effects (i.e. no significant effects on blood pressure, heart rate, or contractility).

All of the above effects were associated with protection against electrically and ischæmia-induced

Table 9. Established and Experimental Effects of Class I Agents.

Class	Hmodynamic and ECG Effects BP	HR	P-R	QRS	Q-T	Effects on Kinetics & AP ERP	MFF	iT	VFT	MRR*	APD
(a) Established Effects (as reviewed in the literature)											
Ia	↓	↓	↕	↑↑	↑↑	↑↑	↓↓	↑	↑	↓	↑
Ib	↔	↔	↔	↔	↔	↑↑↑	↓↓↓	↑	↑↑	↓	↓↔
Ic	↓↓	↓↓	↑	↑↑↑	↔	↑	↓	↑	↑	↓	↔
(b) Experimental Effects of Quinacainol											
(i) Hmodynamic study in conscious rats	↓	↓	↑↑↑	↑↑	↑	/	/	/	/	/	/
(ii) Antiarrhythmic study	↓	↓↓	↑↑↑	/	/	/	/	/	/	/	/
(iii) Electrical Stimulation study	↓	↓↓	↑↑↑	↔	↔	↑	↓↓	↑	↑	/	/
(iv) Epicardial intracellular potential study	↓	↓↓	/	/	/	/	/	/	/	↓	↑
(v) Isolated Heart study	/	↓	↑	↑	↔	/	/	/	/	/	/

Experimental arrows indicate trend for all doses tested. * Indicates that drug effects are dependent on frequency. Based on Vaughan Williams (1984b) classification system which subdivides class I compounds based on their effects on the APD, quinacainol exerts Ic actions at low doses and Ia actions at higher doses. In consideration of frequency-dependency, quinacainol was very effective in prolonging ERP and reducing MFF. These actions are consistent with Ia and Ib agents although the ECG effects at normal sinus rhythm seemed to preclude Ib agents. The ECG effects indicate that quinacainol is a Ic agent since it widened the P-R interval and QRS duration without having a major effect on the Q-T_c interval.

arrhythmias. Unfortunately, the extensive analyses performed still did not provide conclusive evidence with regard to quinacainol's subclassification.

"A drug neatly pigeon-holed by the pharmacologist as the most potent of its group for some allegedly desirable property, may have disastrous side-effects. Precise analysis of individual pharmacological actions is, however, necessary if a proper scientific basis is to be found for a rationale of treatment." (Vaughan Williams, 1970).

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