ANTIFIBRILLATORY ACTIONS OF K^+ CHANNEL BLOCKING DRUGS.

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B.Sc.

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

DEPARTMENT OF PHARMACOLOGY & THERAPEUTICS

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

April, 1991

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Vancouver, Canada

Date April 17, 1991

DE-6 (2/88)

ABSTRACT

Class III antiarrhythmic drugs share the common mechanism of widening the cardiac action potential without affecting conduction velocity. This thesis reports on the actions of newly developed putative Class III antiarrhythmic drugs, tedisamil, KC 8851, RP 62719, UK 68798, and risotilide, as well as an ATP-sensitive K^+ channel blocker, glibenclamide. Studies were performed to examine the actions of these drugs in acute myocardial ischaemia and possible mechanisms responsible for these actions. The hypothesis tested was that drug treatment prevented arrhythmias induced by acute myocardial ischaemia. Species dependent actions of these drugs on ECG and blood pressure were examined in rats, guinea pigs, pigs and primates.

The five putative class III drugs listed above were assessed for antiarrhythmic activity in a conscious rat model of myocardial ischaemia. It was found that only tedisamil and KC 8851, which widened the Q-T_C interval of the ECG (by up to 65%), were effective at suppressing fibrillation in this species. None of the drug treatments decreased the incidence of ventricular premature beats. Tedisamil, but not glibenclamide, prevented tachycardias in a rat model of myocardial ischaemia- and reperfusion-induced arrhythmias. In an anaesthetized pig model of acute myocardial ischaemia, tedisamil and UK 68,798 were shown to mildly prolong the Q-T_C interval by less than 20%, but protection against arrhythmias was equivocal.

In further studies, tedisamil and UK 68,798 were compared to each other for effects on ventricular epicardial action potential morphology using intracellular recording *in vivo*, and effects on ventricular effective refractory period using electrical stimulation *in vivo* in both rats and guinea pigs. Tedisamil (4 mg/kg, i.v.) prolonged rat ventricular epicardial action potential duration fourfold *in vivo*, while UK68,798 (up to 1 mg/kg, i.v.) was ineffective in this species. Tedisamil (4 mg/kg, i.v.) widened guinea pig ventricular epicardial potentials by 80%, while UK 68,798 (25 µg/kg, i.v.) increased these by 30%. Action potential widening paralleled increases in ventricular refractoriness to electrical induction of premature beats. It was found that the species selective actions of these drugs was most likely related to differences in selectivity for K^+ channels which contribute to repolarization in myocardium.

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SYMBOLS AND ABBREVIATIONS

action potential	AP
action potential duration	APD
arrhythmia score	A.S.
atrioventricular node	AVN
calcium	Ca^{++}
conductance	g
coronary heart disease	CHD
current	I
effective refractory period	ERP
hour(s)	h
maximum following frequency	MFF
membrane potential	Em
myocardial ischaemia	MI
minute(s)	min
occluded zone	O.Z.
potassium	K^{+}
second(s)	s
slow inward current	I_{si}
sodium	Na^{+}
standard error of the mean	S.E.M.
time constant	τ
torsade de pointes	TdeP
ventricular premature beat	VPB
ventricular tachycardia	VT
ventricular fibrillation	VF
voltage	V

ACKNOWLEDGMENTS

I would like to thank the members of the Department of Pharmacology & Therapeutics, and in particular Drs. Cathy Pang and Bernard MacLeod for their help and encouragement. I am also grateful to the members of my committee for the time and effort spent in review and discussion of this thesis. Michael Pugsley has been an invaluable help and a lot of fun to work with. To my supervisor, Michael Walker, I think the best compliment I can give him is that he was a true educator. I will never forget his spirit and generosity.

To my family and friends I owe a great deal, especially Cindy who has stood by me through both struggles and triumphs. I would also like to thank Dale Hodgins and members of Bad Medicine for keeping me on track.

I am also indebted to the B.C. and Canadian Heart Foundations for their financial support.

1. INTRODUCTION

1.1 Ventricular Fibrillation

1.1.1 Overview

Cardiac arrhythmias can be caused by either abnormal impulse formation or abnormal impulse conduction (Hoffman & Cranefield, 1960). Abnormal impulse formation involves either altered automaticity and/or triggered activity. Conduction abnormalities include unidirectional block, bidirectional block, functional and anatomical reentry, and reflection. The underlying pathological processes responsible for these mechanisms may be ischaemia, electrolyte imbalances, autonomic nervous system dysfunction, cardiomyopathies, anatomical variations e.g., accessory pathways, or drug induced electrophysiological alterations. Differences in mechanism often require different therapeutic approaches.

1.1.2 Myocardial Ischaemia

Cessation of blood flow through the coronary arteries, i.e. myocardial ischaemia, causes rapid changes in the heart's electrical and mechanical activity. Electrophysiological studies have shown that acute ischaemia causes depolarization of the resting membrane potential, reduction in rise rate and height of the AP upstroke, and

brief widening and subsequent abbreviation of the AP plateau (Kardesch *et al.*, 1958; Prinzmetal *et al.*, 1961; Downar *et al.*, 1977; Russel *et al.*, 1979). Conduction velocity increases at first (Holland & Brooks, 1976; Elharrar & Zipes, 1977) but this is soon followed by progressive slowing (Boineau & Cox, 1973; Waldo & Kaiser, 1973; Williams *et al.*, 1974). The refractory period in the ischaemic zone becomes heterogeneous (Elharrar *et al.*, 1977; Han & Moe, 1964; Levites *et al.*, 1975). The edge of the ischaemic zone, or border zone, has been implicated in the genesis of "injury currents" between areas of "out-of-phase depolarization" or "repolarization" (Janse *et al.*, 1980; Nahum *et al.*, 1943; Samson & Scher, 1960). Injury currents have been reinvestigated by numerous investigators searching for the mechanisms of arrhythmogenesis in acute myocardial ischaemia (MI). The role of the metabolic, ionic and neurohumoral events following acute MI has been the focus of intense research.

Initial studies of the role of K^+ ions were carried out by Harris *et al.* (1954) who proposed that the elevation in $[K^+]_o$ secondary to ischaemia was a major arrhythmogen. They were able to show arrhythmias precipitated by K^+ injection in ischaemic hearts (Harris *et al.*, 1958). Subsequent investigations have shown three stage changes in $[K^+]_o$ following ischaemia; initial (30 s to 5-15 min) rapid elevation to up to 15mM, followed by a 10 - 15 minute

plateau and finally a slowly rising phase (Kleber, 1983; Hill & Gettes, 1980). This $[K^+]_o$ accumulation might contribute to the depolarization according to the Goldman-Hodgkin-Katz equation. The equation dictates that high relative permeability to potassium, results in resting membrane potential being largely governed by the log $[K^+]_i/[K^+]_o$ ratio (Goldman, 1943; Hodgkin & Katz, 1949). This depolarization could in turn inactivate voltage dependent sodium channels and thereby slow conduction velocity. However, recent observations by Clusin's group have shown that a greater degree of depolarization and conduction slowing occurs in the first 3 minutes of occlusion than can be accounted for by the accumulation of $[K^+]_o$ alone (Blake *et al.*, 1988). These investigators have pointed to a possible interaction with Ca^{++}, because infusions of Ca^{++}, or pacing, augmented the early ischaemia-induced depolarization. Acidification (Couper *et al.*, 1984) and CO_2 accumulation (Case *et al.*, 1979) occur within seconds of coronary occlusion, and are augmented by pacing. Using the Ca^{++} indicator dye, Indo 1, Lee *et al.* (1988) showed a rapid rise in $[Ca^{++}]$ in response to ischaemia which may stimulate an inward depolarizing current (Colquhoun *et al.*, 1981; Kass *et al.*, 1978). These studies attempt to answer questions generated by clinical experience of myocardial ischaemia induced arrhythmias.

1.1.3 Coronary Heart Disease: Magnitude of the Problem

The mortality rate from coronary heart disease has decreased by 40% in the U.S.A. since 1950 (Feinleib, 1984). this remarkable decline has not occurred in all countries, but Canada has experienced a decline in cardiovascular mortality of 20% in the same period. Despite the decline, 200,000 - 600,000 persons in the U.S.A. and approximately 40,000 Canadians succumb annually to cardiac arrest (Gordon & Kannel, 1971; N.C.H.S. Advance Report, 1981; Goldberg, 1989) which makes CHD the leading cause of death in North America. The term sudden death has been used and abused (i.e. "resuscitated from sudden death") to describe unexpected death occurring within one hour after the onset of signs or symptoms (Rapaport, 1988). The term sudden cardiac death refers to death from any cardiac cause, but it is usually due to atherosclerotic coronary artery disease (Roberts, 1986). Sudden cardiac death can be the result of aortic dissection, ventricular aneurysm and rupture, cardiomyopathy, vascular causes, valvular disease and/or arrhythmias (Roberts, 1990). As antiarrhythmic drugs would only be of benefit to the latter of this incomplete list, further discussion will be limited to sudden arrhythmic cardiac death (SACD). Typical patients suffering an episode of cardiac arrest do not have an acute myocardial infarction associated with an episode of VF (Greene, 1990). While 38% of patients may show leak of intracellular enzymes (e.g., CKMB) into their circulation, only 19% develop Q-waves

associated with transmural infarction (Cobb *et al.*, 1980a; 1980b). In some cases this is probably due to the fact that necrosis takes time to develop after an ischaemic insult (Weisman & Healy, 1987) and ischaemic zones vary in size.

The rhythm seen early in cardiac arrest has been reported as VF in 75%, asystole in 20% and electromechanical dissociation in 5% (Weaver *et al.*, 1986). It must be appreciated that the first rhythm recorded may not be the initiating arrhythmia, VT can of course degenerate into VF and VF further change to asystole. Similarly, there are uncertainties regarding the aetiology of SACD.

1.1.4 Risk Factors

The longitudinal studies of the Framingham population (Kannel & Schatzkin, 1985) have provided most of the information regarding risk factors for sudden cardiac death. The risk of sudden death is apparently greater for males, increases with age, weight, and if underlying coronary disease is present. Interestingly, smoking may not be associated with an increased risk of infarction in women (Dawber, 1980; Doll *et al.*, 1980). The value of risk factors has been questioned, 'correlation does not imply causation' must always be remembered when one looks at epidemiological data. McCormick & Skrabanek (1988) recently reviewed a number of population based risk factor

intervention studies and concluded that CHD was not preventable by altering diet, smoking, blood pressure, exercise levels or body weight. They advocate that the term "risk factor" be replaced by "risk marker" as previously stated by Grundy (1973) in order that the implied causal relation be reduced. Risk factor identification has reached absurd proportions: by 1981, Hopkins and Williams (1981) had found 246. Eventually we all die, half of us from CHD, it seems unlikely that a life untainted by risk factors would escape this destiny.

Sudden cardiac arrhythmic death has different, but in some cases overlapping, risk factors associated with it as compared with CHD. These include a previous myocardial infarction, poor left ventricular function i.e. ejection fraction < 30%, extensive coronary artery disease, high incidence of VPBs, old age, male gender, exercise induced angina pectoris or hypotension and inducibility at electrophysiological study (Greene, 1990). These conditions are, therefore, some of those in which antiarrhythmic drug therapy might be given. Drugs which exacerbate these conditions would not be optimal candidates for therapy. However, drugs that reduce these risk factors may not prevent cardiac mortality, as shown by the CAST study in which encainide and flecainide were associated with increased mortality despite reductions of VPBs and programmed electrical stimulation inducibility (CAST

investigators, 1989). What then can be done? It would seem rational to prevent or ameliorate the arrhythmias themselves which lead to compromised pump function and death. Thus, abolition of VF plus, at the least, slowing of VT, would be desirable properties of any antiarrhythmic. Of the four classes of antiarrhythmics in the Vaughan-Williams (1970, 1984) classification, effective Class III antiarrhythmics would seem the most suitable candidates for slowing reentrant VT and possibly eliminating VF (Bacaner *et al.*, 1986). A selective Class III agent would not impair conduction velocity or contractility as can occur with treatment with gNa^{+} and gCa^{++} blockers (Jaillon & Ferry, 1988). Given that antiarrhythmic drugs are most often given under conditions of impaired pump function, this fact alone makes Class III drugs a more desirable choice.

While sudden cardiac arrhythmic death has been associated with the previously mentioned conditions, what initiates the event? An attractive hypothesis proposed that a sudden occlusive thrombus forms in a coronary artery acutely giving rise to ischaemia (Epstein *et al.*, 1989) and this precipitates the arrhythmias. However, pathological studies indicate that 75% of the cases of sudden cardiac death involve no coronary thrombosis (DiMaio *et al.*, 1990). An interesting corollary of the above hypothesis is that an acute occlusion would be more likely fatal in a previously asymptomatic patient due to a lack of collateral circulation

(Epstein *et al.*, 1989). Human coronary artery collaterals enlarge and mature in response to chronic and severe occlusive disease i.e., greater than 75% narrowing, [as resistance to flow is inversely proportional to radius^4, (Poiseuille, 1842, cf: Shepherd & Vanhoutte, 1979)] (Fulton, 1963; Schaper, 1971; Rentrop *et al.*, 1988; Cohen *et al.*, 1989). While the results of an occluding thrombus would possibly be worse in a patient with mild stenosis (less than 70%), the likelihood of an occluding thrombus developing is much greater in patients with severe stenosis (Moise *et al.*, 1984; Proudfit *et al.*, 1980). In accordance with the above mentioned hypothesis much effort in the last decade has been focused on the phenomenon of silent myocardial ischaemia and its role in sudden arrhythmic death (Gottleib *et al.*, 1986; Amsterdam, 1987). Silent or painless ischaemia is common in coronary artery disease (Cohn, 1986) and occurs often in patients with angina, previous infarction and also in asymptomatic patients (Kannel & Abbot, 1984). Silent ischaemia is generally detected as S-T segment alterations during Holter ambulatory ECG monitoring (Cecchi *et al.*, 1983) or during exercise testing (Amsterdam *et al.*, 1986). This method of ischaemia detection has recently been validated (Tzivoni *et al.*, 1985; Rocco *et al.*, 1986). As with other prognostic indicators, whether or not silent ischaemia plays a causal role or only an association with ventricular arrhythmias is equivocal. The data from a limited number of cases of sudden death in patients

undergoing ambulatory monitoring suggest that most cases of lethal ventricular tachycardia are not associated with ECG evidence of myocardial ischaemia (Meissner *et al.*, 1986; Lereq *et al.*, 1987). However, many isolated instances of individual cases support a role for ischaemia provocation of arrhythmias (Gradman *et al.*, 1977; Savage *et al.*, 1983; Meissner *et al.*, 1986).

Interventions aimed at reversing ischaemia have been developed. Fibrinolytic streptokinase and urokinase as well as tissue type plasminogen activator, t-PA, as well as percutaneous transluminal angioplasty have been used for revascularization in cases of acute coronary thrombus formation and in more chronic stenosis conditions (Lew, 1988). These procedures offer a less invasive approach than coronary artery bypass graft techniques.

1.1.5 Mechanisms of Arrhythmogenesis: Abnormal Automaticity

Ventricular fibrillation in myocardial ischaemia has been reported to be caused by both reentry and abnormal automaticity (Pogwizd & Corr, 1987). Normal automaticity is found in the sinus node and Purkinje fibres, although in the latter automaticity is not normally seen owing to the faster rate of the node. This property is responsible for the sensitivity to overdrive suppression of enhanced

automaticity in Purkinje fibres (Vassalle, 1977). On the other hand, abnormal automaticity occuring in depolarized ventricular and/or Purkinje fibres (Imanishi & Surawicz, 1976) is difficult to suppress by overdrive pacing and is not sensitive to gNa^+ blockers (Cranefield, 1977; Dangman & Hoffman, 1983). It has been suggested that overdrive pacing works by hyperpolarizing the membrane and thus is not effective in severely depressed cells (Hoffman & Rosen, 1981). Abnormal automaticity has been implicated as the causative agent for most tachycardias seen 24 hour after induction of infarction (Wellens *et al.*, 1974). While altered automaticity may occur under any conditions in which a loss of membrane potential is present, triggered activity appears to result from afterdepolarizations (Rosen, 1986). These oscillations are linked to a preceding beat, and can be classified as early, (occurring during Phase III repolarization), or delayed after depolarizations (post phase III) (Rosen *et al.*, 1973; Ferrier, 1973). Early after depolarizations (EAD) have been demonstrated *in vitro* under conditions which either reduce outward, or increase inward, currents. Thus, EADs have been produced *in vitro* by catecholamines (Hoffman & Cranefield, 1960), acidosis (Coraboeuf *et al.*, 1980), low $[K^+]$ (Carmeliet, 1961), low $[Ca^{++}]$ (Sano and Sawanobori, 1972), hypoxia (Trautwein *et al.*, 1954) and numerous drugs (Schmidt, 1960; Dangman & Hoffman, 1981; Strauss *et al.*, 1970; Gough *et al.*, 1988). On the other hand, few reports implicating EADs *in vivo* have

appeared (Levine *et al.*, 1985; El-Sherif *et al.*, 1988; Carlsson *et al.*, 1990). These groups have proposed that U-waves on the surface ECG correspond to EADs, and that the occurrence of torsade de pointes is initiated by EAD (Brachman *et al.*, 1983) (see section 1.3.5). While cesium induced EADs have been reported to be favored by bradycardia (Brachman *et al.*, 1983) delayed after depolarizations (DADs) appear to be augmented by increased rate (Rosen & Reder, 1980). DADs can be produced by $[Ca^{++}]_i$ overload, such as in reperfusion (Ferrier *et al.*, 1985), inhibition of Na^+/K^+ ATPase by digitalis glycosides, hypokalemia and hypomagnesemia, etc. (Marriott & Conover, 1989). Recently, DADs resulting from sympathetic stimulation in conjunction with elevation of $[Ca^{++}]_o$ and strophanthidin treatment, have been directly shown using MAP recordings *in vivo* (Priori *et al.*, 1988). A Ca^{++} overload induced transient inward current may be the actual mediator of DADs (Lederer & Tsien, 1976; Kass *et al.*, 1978; Matsuda *et al.*, 1982). The transient inward current has been reported to be linked to electrogenic Na^+/Ca^{++} exchange (Lipp & Pott, 1988; Arlock & Katzung, 1985; Noble, 1984), while others suggest it is a Ca^{++} induced nonspecific ionic current (Cannell & Lederer, 1986; Colquhoun *et al.*, 1981; Shimoni & Giles, 1987). Whether or not a DAD triggers an extrasystole would depend on both the magnitude of the induced depolarization and the excitability of the local tissue at the DAD's focus.

It might appear unlikely that abnormal automaticity (Hoffman & Rosen, 1981) would be responsible for arrhythmogenesis in acute ischaemia (Janse *et al.*, 1986) because abnormal automaticity is suppressed by elevated extracellular $[K^+]$ (Hoffman & Rosen, 1981; Katzung *et al.*, 1975), while $[K^+]_o$ rises subsequent to coronary occlusion (Hill & Gettes, 1980; Hirsche *et al.*, 1980; Kleber, 1983). However, triggered activity may play a role in ischaemia induced arrhythmias due to the possible presence of hypoxia (Trautwein *et al.*, 1954), acidosis (Coraboeuf *et al.*, 1953), elevated catecholamines (Brooks *et al.*, 1955), high $[K^+]_o$, possible myocardial stretch (Pirzada *et al.*, 1976), increased $[Ca^{++}]$ (Clusin *et al.*, 1983), which may contribute to the generation of oscillatory after depolarizations.

1.1.6 Mechanisms of Arrhythmogenesis: Reentry

Reentry was mentioned over one hundred years ago (McWilliams, 1887, cf: Marriott & Conover, 1989). Proof of its existence in rings of jelly fish subumbrella tissue came 20 years later (Mayer, 1906; 1908, cf: Marriott & Conover, 1989) and the concepts of reciprocating rhythms and beats and their requirements were later demonstrated in heart tissue by Mines (1913 & 1914). The simplest case requirements for reentry are: 1. an available circuit; 2. unequal responsiveness in two segments of the circuit; 3) slow conduction.

Reentry can be random, as in fibrillation, or ordered, and follow a fixed pathway. Micro-reentry has been used to describe the small circuits such as might occur in the AV node or distal Purkinje fibres, while longer pathway circuits are referred to as macro-reentry. Reflection is a form of reentry that is produced through reflection in parallel unbranching fibres with depressed segments (Antzelevitch *et al.*, 1980; Jalife & Moe, 1981).

Since acute ischaemia is associated with areas of slow conduction, short refractory periods and inhomogeneities in refractory periods (see above) reentry has been indicated for many years as the most important cause for ischaemia-induced arrhythmias. Circus movement has been demonstrated using microelectrodes, *in vitro*, when conduction velocity was slowed excessively in Purkinje fibres (Wit *et al.* 1971; 1972; Sasyniuk & Mendez, 1971) or atrial muscle (Allessie *et al.* 1976, 1977). El-Sherif *et al.* (1977) demonstrated reentry in ischaemic myocardium *in vitro*. Epicardial mapping studies in isolated dog or pig hearts implicated injury currents originating in the subendocardium flowing across the border zone of depolarized ischaemic and polarized perfused myocardium as the substrate for reentry in early acute ischaemia (Janse *et al.*, 1980; Janse & Kleber, 1981; Janse *et al.*, 1986). Activation mapping has shown reentry in humans (de Bakker *et al.*, 1988). Three

dimensional activation mapping *in vivo* was used by Pogwizd & Corr (1987) to record reentrant and abnormal automaticity mechanisms participating in the induction and maintenance of arrhythmias following coronary occlusion in the cat. Our own rat studies *in vivo* (Abraham *et al.*, 1989) and *in vitro* (Inoue *et al.*, 1984) have shown a depression of Vmax and shortening of the APD following coronary artery occlusion. This would provide the necessary heterogeneity of refractoriness and conduction slowing for reentrant mechanisms in rats subjected to coronary occlusion. It appears that reentry can be the underlying sustaining mechanism in some cases of ventricular bigeminy, trigeminy or tachycardias as well as supraventricular and nodal arrhythmias.

1.1.7 Animal Models

The clinical experience of arrhythmogenic mechanisms is clouded by uncontrolled variables. Therefore, relationships of causality to lethality are at best equivocal. As a result of this, animal models have been used to systematically investigate the various hypotheses.

Numerous animal models of arrhythmogenesis have been developed (Review see: Winslow, 1984). There are chemically-, electrical stimulation-, mechanically-, and ischaemia/infarction-induced arrhythmia models. Due to the

variability of conditions under which arrhythmias are encountered in patients, there are no single animal models that can be considered as ideal. Recently guidelines have been produced to facilitate interlaboratory comparisons of data (Walker *et al.*, 1988). These Lambeth conventions highlight the need for blind and random protocols and the necessity of gauging the strength of the arrhythmogenic stimulus (e.g. occluded zone size in models utilizing ischaemia). The use of the rat as an assay for antiarrhythmic activity has been recently evaluated (Curtis *et al.*, 1987; Brooks *et al.*, 1989) and found to be generally useful because of size, cost and most importantly consistent coronary artery anatomy (lack of collaterals). There are important species dependent differences in coronary artery anatomy (Johns & Olsen, 1954; Maxwell *et al.*, 1987). Hearts from rats, cats, rabbits, pigs and ferrets have less than 5% collateral vascularization of their coronary arteries, while dog hearts have variable and guinea pig hearts have extensive collateralization. These anatomical differences are important determinants of the extent of ischaemia/infarction produced by permanent coronary artery occlusion. Blood flow in collaterals may prevent infarction of tissue after ligation of a single coronary artery. Since the size of ischaemic zone has been shown to be an important determinant of subsequent arrhythmias, consistently sized ischaemic zones are necessary in order to make comparisons between groups of treated animals (Curtis *et al.*, 1987).

While these studies point to the usefulness of rat models, there are important considerations one must be aware of when testing Class III drugs. As discussed in section (1.2) there are marked species differences in the channels activated in the repolarization phase of the AP. Thus as shown in our studies, blockade of different K^+ channels has species dependent efficacy.

1.2 Potassium Channels

1.2.1 K^+ Channels in Cardiac Tissue: Overview

The understanding of K^+ channels has developed since the introduction of the patch clamp technique (Hamill *et al.*, 1981). The use of single cells has alleviated some of the problems associated with multicellular preparations, such as inadequate voltage clamp control and, of particular interest to K^+ channel electrophysiology, depletion and/or accumulation of K^+ in limited extracellular space (Kline & Morad, 1978). Perhaps as a result of an increased number of investigators in the area, growth in knowledge of K^+ channels has led also to a growth in the number of reputed separately definable K^+ channels. This trend probably will not subside with the introduction of molecular biological techniques to the area. Previous to genetic sequencing studies different K^+ channels were characterized by

qualities such as their current/voltage relationships, time dependent kinetics, as well as responses to pharmacological manipulations. With regard to the latter, the pharmacological tools used in this area have until now been rather limited. The standard drugs, TEA and 4AP, used to define I_{to} and I_K have EC_{50}'s in the mM range. The inorganic ions which are also used to characterize channels are not themselves entirely specific. Despite these possible limitations, recent reviews have given what may be a deceptively clear description of the currently proposed K^+ channels present in cardiac cells (Irisawa, 1987; Cook, 1988; Carmeliet, 1989). There are at least eight separate K^+ channels found in myocardium and their occurrence is both tissue and species dependent. The following list briefly summarizes K^+ channel taxonomy in cardiac tissue:

Those with primarily voltage dependent kinetics; Inward rectifier (I_{K1}), delayed rectifier (I_K), transient outward (I_{to}), "pacemaker" (I_f or I_h), and plateau current (I_{KP}), and those which are primarily chemically dependent; ($I_{K(Ca)}$), ($I_{K(Na)}$), ($I_{K(Ach/Ado)}$) and ($I_{K(ATP)}$) (Carmeliet, 1989).

1.2.2 K^+ Currents Underlying the Cardiac AP.

Originally a single K^+ channel, which only activated, was invoked to explain the cardiac AP (Johnson & Tille, 1964). As apparent from the above, this view was soon

demonstrated to be inadequate. Cardiac cells at rest are selectively permeable to K^+, so that the membrane potential is close to the K^+ equilibrium potential. Wiedemann's (1951) pioneering work, involving resistance measurements in cardiac pacemaking cells, led to the proposal that a slowly decaying outward conductance after repolarization was responsible in part for pacemaker depolarization (Dudel & Trautwein, 1958). Noble's (1962) model incorporated this idea, even before the first description of this delayed K^+ conductance (Hall *et al.*, 1963). Application of voltage clamp techniques to the heart by Deck, Kern & Trautwein (1964) led to a greater complexity, i.e. I_{to} and the reinterpretation of previous currents. The existence of inward currents carried by Ca^{++} was reported by Reuter (1967) after evidence was provided from AP experiments (Niedergerke, 1963) and flux measurements (Winegrad & Shanes, 1962). Ca^{++}-dependent current in the heart has recently been shown to be conducted through two separate channels; the transient (T) and the dihydropyridine sensitive (L) channels (Hess *et al.*, 1984).

With regard to K^+ channels a schism developed over interpretation of pacemaking mechanisms when DiFrancesco & Noble reinterpreted the "pacemaker" current I_{K2} (Noble & Tsien, 1968) as consisting of both the time and $[K^+]_o$ dependent inward rectifier (I_{K1}) and a new current they called, I_f (Brown *et al.*, 1979; DiFrancesco, 1981;

DiFrancesco & Noble, 1982; 1984). This nonspecific, inward and hyperpolarization-activated "funny" current has been rejected as a major contributor to pacemaker depolarization in S.A. Node by other investigators (Noma, Morad & Irisawa, 1983) The gK^+ "decay" hypothesis has again been adopted by the original proponents of I_f although they maintain a modulatory role of I_f especially with regard to adrenaline's positive chronotropic effects (Noble 1984; DiFrancesco, 1985; Irisawa 1987). A detailed description of these and other K^+ channels found in cardiac tissue follows:

1.2.2.1 Inward Rectifier, I_{K1}

The most thoroughly studied K^+ channel which helps maintain the resting potential is the inward rectifier (I_{K1}) originally thought to be time-independent (Hall *et al.*, 1963; McAlister & Noble, 1966; Hutter & Noble, 1960). The channel has rapid activation and inactivation kinetics (Carmeliet, 1982; Sakmann & Trube, 1984) and a characteristic area of negative slope conductance which makes its conductance at plateau potentials almost zero. (Guinea pig: Hume and Uehara, 1985, Sheep: Isenberg, 1976 calf: DiFrancesco *et al.*, 1984, Cat: Tseng *et al.*, 1987, Dog: Shah *et al.*, 1987). I_{KI} is present in atrial (Sackman *et al.*, 1983) and more so in ventricular tissue, but is very sparse in nodal cells, which may explain the lower K^+/Na^+ permeability ratio and thus the low "resting" potential of

nodal tissue (Pelzer & Trautwein, 1987). The high membrane resistance of nodal cells (12-15 KOhms cm^2) allows small changes in membrane current to cause large changes in membrane potential (Bean, 1985; Noma *et al.*, 1984). The single channel conductance of I_{K1} is proportional to the square root of $[K^+]_o$ (Carmeliet, 1982) and thus original studies in multicellular preparations were hampered by accumulation/depletion of K^+ in extracellular compartments (Baumgarten & Isenberg, 1977; Kline & Morad, 1978; Cohen & Kline, 1982). Although the single channel conductance of I_{K1}, in ventricle and atria is similar, in the latter tissue the gating kinetics are much faster (Pelzer and Trautwein, 1987).

The inward rectifier channel can conduct more than one K^+ ion at a time (Shen *et al.*, 1990; Hill *et al.*, 1989) and can be activated by hyperpolarization (Kurachi, 1985). The activation gate of I_{K1} could be a charged particle that moves due to changes in electric field of the membrane or due to the change in local $[K^+]$ ion (subsequent to field changes) at a critical site (Carmeliet 1982; 1989). Changes in $[K^+]_o$ also shifted the voltage range of activation in guinea pig ventricle (Kurachi, 1985). The mechanism of inward rectification was found to be dependent on $[Mg^{++}]_i$ which blocks outward current through the channel at physiological concentrations (Matsuda *et al.*, 1987; Horie *et al.*, 1987; Vandenberg, 1987).

1.2.2.2 Delayed Rectifier, I_K

The delayed rectifier, or time dependent outward current, was first described in Purkinje fibres and called I_{X1} (Noble & Tsien 1969). In ventricle, the current was called I_K (McDonald & Trautwein, 1978). The difference in the reversal potential of I_K between the two tissue types was again found to be related to extracellular cleft K^+ accumulation in multicellular preparations. Thus the term I_K was adopted. Before the current was described, however, the finding of a threshold for all-or-none repolarization (Vassalle, 1966) indicated that repolarization was a consequence of time and voltage dependent changes in membrane conductance. I_K has slow activation kinetics in response to depolarization which can be modulated by protein kinase A (Walsh *et al.*, 1988) and protein kinase C (Tohse *et al.*, 1987). Beta adrenergic stimulation enhances I_K (Bennett *et al.*, 1986). Internal concentrations of Na^+ (Scamps & Carmeliet, 1989) and Ca^{++} (Tohse *et al.*, 1987) also modulated activation, but $[K^+]$ did not. However, the single channel conductance was found to be proportional to the square root of $[K^+]_o$ (Shibasaki, 1987). Its inward rectification properties (Noma and Irisawa, 1976; DiFrancesco *et al.*, 1979; Daytner *et al* 1984) in mammalian tissue have been attributed to fast inactivation (Shibasaki, 1987). Compared to I_K of mammalian cardiocytes, the I_K of

frog atria is simpler, and exhibits a single ohmic I/V relationship (Hume & Giles, 1983). Recently, the classical I_K has been subdivided into a rapidly activating component (I_{Kr}) and a slowly activating component (I_{Ks}) (Sanguinetti & Jurkiewicz, 1990) on the basis of specific block of I_{Kr} by the new class III antiarrhythmic E4031 (see later).

1.2.2.3 Transient Outward Current, I_{to}

Early studies of a transient outward current believed to play a role in action potential repolarization were done using cardiac Purkinje cells from sheep (Peper & Trautwein, 1968; Kenyon & Gibbons, 1979), and cow (Siegelbaum & Tsien, 1980, Siegelbaum *et al.*, 1977). Josephson and colleagues (1984) were the first group to quantitatively describe I_{to} in rat single ventricular myocytes. Canine epicardial Action Potentials have a characteristic notch which has been attributed to I_{to} (Litovsky & Antzelevitch, 1988). The transient outward current of human atrium has been reported to exhibit negative frequency dependence which may be a result of its time and voltage dependence of reactivation (Escande *et al.*, 1985; Shibata *et al.*, 1989). Inactivation of I_{to} develops quickly and decays relatively slowly (Clark *et al.*, 1988; Litovsky & Antzelevitch, 1989; Hiraoka & Kawano, 1986; 1987) thus the current apparently decreases in a frequency dependent manner, i.e. the reverse of the delayed rectifier (Ruiz-Petrich & Leblanc, 1989).

Various authors have pointed to two separate I_{to} currents; one that is voltage dependent and sensitive to 4AP blockade and another that is Ca^{++} dependent (Escande *et al.*, 1987; Benndorf *et al.*, 1987; Tseng & Hoffman, 1989; Coraboeuf and Carmeliet, 1982). Others contend that there is one current that has steady state activation and inactivation curves which are shifted to the right by divalent cations (Agus *et al.*, 1989), similar to depolarizing shift in I_A activation and inactivation gates (Mayer & Sugiyama, 1988). A lack of sensitivity to blockade by Ba^{++} distinguishes I_{to} in cardiac tissue from the I_A current in neurons (Yellen, 1987), otherwise they are very similar. Originally I_{to} was attributed to an inward current carried by Cl^- (Carmeliet, 1961; Dudel *et al.*, 1967; Fozzard & Hiraoka, 1973) on the basis of an apparent sensitivity to $[Cl^-]_o$. This Cl^- sensitivity was later shown to result from the chelation of Ca^{++} by the Cl^- substitutes used (Kenyon & Gibbons, 1979). It is ironic to note that a cAMP dependent background Cl^- conductance with outward rectification (Inward Cl^- movement) has been reported in guinea pig ventricular myocytes, lacking I_{to} (Harvey and Hume, 1989; Harvey *et al.*, 1990). A previous unconfirmed report also described a TTX sensitive Cl^- conductance in rat ventricular myocytes (Pidoplichko & Verkhratsky, 1987). A chloride channel from SR reconstituted into lipid bilayers has been

studied at single channel level (Rousseau, 1989) and from human cardiac SR (Holmberg & Williams, 1989).

Inactivation of I_{to} develops quickly and decays slowly (Clark *et al.*, 1988; Litovsky & Antzelevitch, 1989; Hiraoka & Kawano, 1986; 1987). Thus, it has been argued that repolarization due to I_{to} decreases, whereas I_K increases, with increasing frequency of stimulation, because the former current recovers too slowly while the later current inactivates too slowly (Ruiz-Petrich & Leblanc, 1989).

1.2.2.4 Plateau K^+ Current, I_{KP}

Another K^+ current has been recently described which appears to carry current throughout the duration of the AP (Yue & Marban, 1988). I_{KP} is apparently Ohmic and does not show rectification. Yue and Marban, (1988) suggested that the current which is activated by nicorandil and pinacidil (Kakei *et al.*, 1986; Iijima and Taira, 1987) resembles I_{Kp}. More work needs to be done to confirm the existence of this depolarization activated K^+ channel.

1.2.2.5 "Pacemaker" Current, I_f or I_h

A hyperpolarization activated, inward current carried by Na^+ and K^+, denoted variously as I_f or I_h, has been proposed to contribute to spontaneous ("pacemaker")

depolarization in nodal cells (Brown *et al.*, 1977; 1979; Seyama, 1976; Yanagihara & Irisawa, 1980; DiFrancesco & Ojeda, 1980; DiFrancesco 1981b; 1982b). The kinetics of I_f do not conform to Hodgkin-Huxley type of models (Hart, 1983), but have slow sigmoidal activation and deactivation time courses which can be represented by a complicated model with 3 "closed" and 5 "open" states (DiFrancesco, 1984). This current slowly activates at membrane potentials more negative than -80 mV, with peak current occurring approximately 2 seconds after a voltage step (DiFrancesco, 1984; Earm *et al.*, 1983; Callewaert *et al.*, 1984). These characteristics would tend to negate I_fs contribution to pacemaker depolarization (even in nodal cells which have high input resistance). Pacemaker activity has been recorded in SA node cells even when I_f was fully inhibited (Kreitner, 1981; Brown *et al.*, 1981). However, a modulatory role on pacemaking for I_f is maintained by some investigators (DiFrancesco, 1985; Noble, 1984) due to adrenaline's augmentation of I_f (Brown *et al.*, 1979a), the observation that increased $[Ca^{++}]_i$ increases I_f (Hagiwara & Irisawa, 1989), and its dependence on external $[K^+]$ (DiFrancesco & Ojeda, 1980).

1.2.2.6 Na^+ Activated K^+ Current, $I_{K(Na)}$

This Na^+ activated K^+ channel was first reported in mammalian ventricle by Kameyama *et al.* (1984). For $[Na^+]_i \geq$

20mM this channel has a large single channel conductance of 207pS (Kameyama *et al.*, 1984). It shows outward rectification for $[K^+]_i > [K^+]_o$ (Carmeliet, 1990, Lux, 1990) while the original report showed inward rectification for $[K^+]_i < [K^+]_o$ (Kameyama *et al.*, 1984). $I_{K(Na)}$ is insensitive to $[Ca^{++}]$ and is not voltage dependent at physiologically relevant voltages (Kameyama *et al.*, 1984; Carmeliet, 1990).

The channel may be activated in conditions of Na^+/K^+ ATPase inhibition and thus may contribute to the AP shortening induced by digitalis glycosides (Kameyama *et al.*, 1984; Carmeliet, 1990; Lux 1990). In single channel studies with guinea pig ventricular myocytes (Sanguinetti, 1990), $I_{K(Na)}$ had a high slope conductance of approximately 150pS (compared to I_{K1} = 30pS) with many sub conductance levels at multiples of 1/12 x 150pS or 12-13pS. $I_{K(Na)}$ had a Kd of 66 mM for Na^+ activation in inside out patches and therefore would not be active in normal resting cardiac cells. The open probability increases sigmoidally with logarithmic increase in $[Na^+]_i$. Open probability varies sigmoidally from -200 mV to -80 mV but shows no voltage dependence above -80 mV (Sanguinetti, 1990). Very few (3%) cells had $I_{K(Na)}$ and these had low resting Em (-20 to -10mV), but appeared healthy according to this report.

1.2.2.7 Ca^{++} Activated K^+ Current, $I_{K(Ca)}$

A Ca^{++} activated K^+ current was first reported in Purkinje fibres (Isenberg 1975; 1977; Siegelbaum, 1977) and later in patch clamped bovine Purkinje cells (Callewaert, *et al.*, 1986) and guinea pig atrial myocytes (Baro & Escande, 1989). The channel showed both a linear I/V relationship from +10 to 110mV, and Ca^{++} dependence, exhibiting a rapid activation followed by a slower inactivation (time constant, τ = 30 - 100 ms). In 10.8 mM $[K^+]_o$ the slope conductance was 120pS (Callewaert *et al.*, 1986). This group has designated this channel as different from I_{to} because of its large conductance as compared to I_{to} in sheep Purkinje fibres (18pS) (Coraboeuf & Carmeliet, 1982). This division is tenuous and perhaps not useful as I_{to} has varying conductance in numerous tissues (Review: Pelzer & Trautwein, 1987). The subclassification of an $I_{K(Ca)}$ channel is a seductive endeavor because such a channel would provide an elegant negative feedback mechanism for Ca^{++} entry by shortening AP duration in Ca^{++} overloaded states (Eisner & Vaughan-Jones, 1983).

1.2.2.8 ATP Sensitive K^+ Current, $I_{K(ATP)}$

K^+ channels which were inhibited by normal intracellular levels of ATP were first recorded in rabbit and guinea pig cardiac cells (Noma, 1983; Trube and Hescheler, 1983). When the $[ATP]_i$ falls below 0.2mM in cardiac myocytes these channels are activated (K_i = 100 μM)

(Noma, 1983). Other nucleotide blockers of the cardiac ATP channel include non-hydrolysable analogues of ATP; AMP-PNP, ADP (partial agonist) (Kakei *et al.*, 1986) and GTP (Noma, 1983; Kakei *et al.*, 1985) although in pancreatic cells the latter nucleotide has been reported to have no effects (Cook & Hales, 1984) or to activate the channel (Dunne and Petersen, 1986). As will be discussed later, sulfonylurea drugs inhibit the channel. While almost all of our detailed information about ATP sensitive K^+ channels has been derived from patch clamp studies, early studies of an outward current induced shortening of the AP in conditions of hypoxia (Trautwein *et al.*, 1954) and/or metabolic inhibition (Carmeliet and Boulpaep, 1957) pointed towards the existence of such a channel.

When both sides of the membrane are exposed to equal $[K^+]$ of 150 mM, the channel's K^+ selective unitary conductance in guinea pig cardiac myocytes is 80pS while in $[K^+]_o$ of 5mM it is 20pS (outward at Em $\geq$ -80 mV) (Noma, 1983; Kakei *et al.*, 1985; Trube & Hescheler, 1984; Kakei & Noma, 1984). For large depolarizations the channel shows inward rectification (Noma, 1983; Cook & Hales, 1984; Ashcroft *et al.*, 1984) which results from voltage dependent block of outward K^+ currents by intracellular Mg^{++} (only efficient > 0 mV) and Na^+ cations, and deactivation (Horie *et al.*, 1987). For moderate depolarizations in physiological $[K^+]_o$ outward currents are larger (Findlay,

1987). Typical recordings of the single channel show bursts separated by quiescence which has led to a simplest case model with one open state and one short and one long closed state (Kakei & Noma, 1984; Trube & Hescheler, 1984; Kakei *et al.*, 1985; Rorsman & Trube 1985; Spruce *et al.*, 1987). ATP reduces mean open time and the number of openings per burst (Kakei *et al.*, 1985; Spruce *et al.*, 1987) without affecting single channel current amplitude. Recently the open and "short" closed times have also been found to depend on the electromotive driving force, $V-V_K$, for K^+ (Zilbeter *et al.*, 1988), i.e., with increased $[K^+]_o$ the frequency of closing increases within a burst of openings. In the region of the reversal potential, τ_o was maximal and τ_c was minimal, and open probability decreased with hyperpolarization.

1.2.2.9 Acetylcholine/Adenosine Activated K^+ Current, $K_{ACh/Ado}$

Early indications as to the mechanism of acetylcholine's (ACh) bradycardic effects were derived from observations that ACh increased flux of K^+ from guinea pig atrium and frog and tortoise sinus venosus (Holland *et al.*, 1951; Harris & Hutter, 1956) and decreased membrane resistance in fibres from dog atrium (Trautwein & Dudel, 1958). Later it was shown that $I_{K(Ach)}$ is similar to the inward rectifier I_{K1} (Garnier *et al.*, 1978), but that its gating (shorter open times than I_{K1}) and conductance (13pS

in 5.4 mM $[K^+]_o$ v. 6pS for I_{K1}) properties were different (Sakmann *et al.*, 1983; Soejima and Noma, 1984; Noma & Trautwein 1978; Carmeliet & Mubagwa, 1986; Heidbuchel *et al.*, 1990). Intracellular Mg^{++} is responsible for inward rectification of both channels. Also, $I_{K(Ach)}$ doesn't show sensitivity to Ba^{++} blockade as I_{K1} does. Recently, it was shown that adenosine receptors and ACh receptors couple through a G protein to the same K^+ channels (Belardinelli and Isenberg, 1983; Kurachi *et al.*, 1986; Pfaffinger *et al.*, 1985; Breitwieser and Szabo, 1985; Logothetis *et al.*, 1987; Kirsch *et al.*, 1988). ATP (K_d 9.5 mM) has also been shown to activate an inward rectifying current which was proposed to be conducted through $I_{K(ACh)}$ channels (Friel & Bean, 1988; 1990).

1.2.3 K^+ Channel Distribution in Mammalian Heart

Inward rectifier, I_{K1}: The channel underlying this current has been characterized in ventricular cells from: guinea pig (Sakmann & Trube, 1984; Hume & Uehara, 1985), rat (Payet *et al.*, 1985; Josephson, 1988), rabbit (Kameyama, *et al.*, 1983), cat (Harvey & Ten Eick 1988; Kleiman & Houser, 1989), and dog (Tseng *et al.*, 1987). Similarly ubiquitous in Purkinje fibres I_{K1} has been recorded from canine, sheep and bovine Purkinje cells (Callewaert *et al.*, 1984). I_{K1} has been reported in atrial cells of rabbit (Sakmann *et al.*, 1983; Noma et al., 1984; Giles & Imaizumi, 1988), guinea pig

(Hume and Uehara, 1985), and human (Heidbuchel *et al.*, 1987; 1990). However, others have concluded that I_{K1} was not present in atria of rabbit (Soejima & Noma, 1984), and as discussed earlier, the atrial I_{K1} does not appear to be equivalent to the ventricular I_{K1} (Hume *et al.*, 1990). The reviews of Irisawa (1987) and Pelzer & Trautwein (1987) of currents in cardiac tissue conclude that there are few I_{K1} channels in nodal cells; this agrees with previous reviews (Noble, 1984).

Delayed rectifier, I_K: This current originally called I_{K2} in Purkinje fibres (Hall *et al.*, 1963) was first analyzed in sheep Purkinje fibres by Noble & Tsien (1969) and has been identified in the ventricles of cat (McDonald & Trautwein, 1978) as well as in a number of amphibian atrial preparations (Rougier *et al.*, 1968; Brown & Noble, 1969a & 1969b), SA node (DiFrancesco *et al.*, 1979) and rabbit AV node (Kokubun *et al.*, 1982). The K^+ accumulation/depletion phenomenon seen in voltage clamped multicellular preparations (Baumgarten & Isenberg, 1977; Kline & Morad, 1978) led to a realization of the need for single cell and single channel analyses of I_K. This approach has had its difficulties due to the low single channel conductance (11pS) found in cell attached patches of rabbit nodal cells (Shibasaki, 1987). Whole cell recordings have been made from canine Purkinje cells (Datyner *et al.*, 1984) guinea pig ventricular cells (Matsuura *et al.*, 1987), feline

ventricular cells (Kleiman & Houser, 1989), chick ventricular cells (Clapham & Logothetis, 1988), calf Purkinje (Bennett *et al.*, 1986), rabbit atrial and ventricular cells (Giles & Imaizumi, 1988) rabbit Purkinje cells (Scamps & Carmeliet, 1989) canine ventricular cells (Tseng *et al.*, 1987). I_K appears to be either absent or insignificant in rat ventricle (Josephson *et al.*, 1984; Yatani *et al.*, 1984). Heidbuchel *et al.*, (1990) in an analysis of the K^+ channels in human atrial cells, made no mention of I_K in these cells. Shibata *et al.* (1989) suggested the contribution of I_K to repolarization may be small, i.e., $I_K < 20\% I_{to}$. Pharmacological evidence would suggest that the delayed rectifier is present in human ventricle, because clofilium, an I_K blocker, has class III actions (Arena & Kass, 1988). However, one study of human ventricular cells failed to find I_K although the short (100 ms) test pulses used may have precluded it (Mitchell *et al.*, 1986).

Transient outward, I_{to}: The channel responsible for this current has been reported to be present in Purkinje fibres of cow (Siegelbaum & Tsien, 1980; Kenyon & Sutko, 1987; Callewaert *et al.*, 1986), rabbit, (Carmeliet *et al.*, 1987) sheep, (Deck & Trautwein, 1964; Peper & Trautwein, 1968; Coraboeuf & Carmeliet, 1982; Carmeliet *et al.*, 1987), dog single Purkinje cells (Nakayama & Fozzard, 1988), rabbit atrial and ventricular cells (Hiraoka & Kawano, 1986; 1989;

Imaizumi and Giles 1987; Clark *et al.*, 1988; Giles and Imaizumi, 1988) Crista Terminalis (Giles & van Ginneken, 1985) and AV node (Nakayama and Irisawa, 1985), guinea pig atrial epicardium (Wang & Nattel, 1989). I_{to} has been reported to be present in canine epicardium but not endocardium (Litovsky & Antzelevitch, 1988; 1989). Less anatomically specific studies have shown I_{to} in canine ventricle (Tseng and Hoffman, 1989; Nakayama *et al.*, 1989; Tseng *et al.*, 1987). Human atrium has I_{to} (Escande *et al.*, 1985; Escande *et al.*, 1987; Shibata *et al.*, 1989; Heidbuchel *et al.*, 1990), and it may be in human ventricle (Schoutten *et al.*, 1984). I_{to} has been reported in mouse ventricle (Benndorf *et al.*, 1987), rat ventricle (Josephson *et al.*, 1984) and cat ventricle (McDonald and Trautwein, 1978; McDonald *et al.*, 1984) although this latter finding was questioned by Ten Eick and Robertson, (1983). Frog (Giles & Shibata, 1985) and guinea pig ventricles (Hume & Uehara, 1985; MacDonald *et al.*, 1984) appear not to have an I_{to}. However, I_{to} may be common to most mammalian atrial tissues as even elephant seal atrial muscle has it (Maylie and Morad, 1984).

Plateau, I_{Kp}: This current has only been described in one publication using guinea pig ventricular myocytes (Yue and Marban, 1988) although these authors contend that the current was reported, but not recognized as I_{Kp}, in rat ventricle (Apkon and Nerbonne, 1988) and in chick embryonic

cells (Mazzanti and DeFelice, 1990). It remains to be seen where, and if channels responsible for I_{Kp}, are present in myocardium.

Hyperpolarization-activated, I_f: This current has both supporters and detractors. Its existence in rabbit nodal tissue/cells is supported by a number of studies (Brown *et al.*, 1979a & 1979b; Yanigara & Irisawa, 1980; Noma *et al.*, 1980; Maylie & Morad, 1984; Nathan & Roberts, 1985; van Ginneken & Giles, 1985; DiFrancesco *et al.*, 1986) but, there are negative reports (Kokubun *et al.*, 1982; Nakayama *et al.*, 1984). I_f has been reported to exist in sheep atrium (Earm *et al.*, 1983), cow and sheep Purkinje cells (Callewaert *et al.*, 1984). Studies in human atrial cells have either supported a role for I_f (Heibuchel *et al.*, 1987; Escande *et al.*, 1987) rejected it (Shibata *et al.*, 1989), or not mentioned it at all (Heidbuchel *et al.*, 1990). The latter single channel study may not have been able to see I_f because of the short time course of the voltage steps used.

Sodium dependent, $I_{K(Na)}$: This current has only been reported in a few preparations. It has been shown in guinea pig ventricular myocytes (Kameyama *et al.*, 1984; Sanguinetti, 1990; Carmeliet, 1990; Luk, 1990).

Calcium dependent, $I_{K(Ca)}$:

This current has been suggested to be present in most preparations (Carmeliet, 1989). However, it has also been proposed that a specific Ca^{++} activated K^{+} selective channel has not been found, but that only Ca^{++} modulation of other K^{+} channels occurs (Eisner & Vaughan-Jones, 1983). Despite these reservations, $I_{K(Ca)}$ has been reported in bovine Purkinje fibres/cells (Isenberg, 1975; 1977; Siegelbaum *et al.*, 1977; Callewaert *et al.*, 1986), sheep Purkinje fibres (Coraboeuf & Carmeliet, 1982), guinea pig atrial myocytes (Baro & Escande, 1989), rat T-tubule membrane (Cecchi *et al.*, 1990) and human atria (Escande *et al.*, 1987; Shibata *et al.*, 1989).

Acetylcholine/adenosine activated, $I_{K(ACh/Ado)}$: This current is responsible for the bradycardic effects of acetylcholine (Ach) and adenosine (Ado). The receptors for ACh and Ado are linked to the same G protein which activates this current (Kurachi *et al.* 1986). Thus there is pharmacological evidence for this current in both nodal and atrial tissue, i.e. tissues innervated by the vagus nerve. Since muscarinic receptors are also present in ventricle, this current may also be found there. Patch-clamp/voltage-clamp studies have shown $I_{K(ACh/Ado)}$ in cells from guinea pig atrium (Iijima *et al.*, 1985) AV node (Belardinelli *et al.*, 1983; Sakmann *et al.*, 1983), rabbit atrium (Soejima & Noma, 1984), SA node (Noma *et al.*, 1981; Noma & Trautwein, 1978), AV node (Nishimura *et al.*, 1988), and human atrium

(Escande *et al.*, 1987; Heidbuchel *et al.*, 1987; 1990). $I_{K(ACh/Ado)}$ has also been shown in sheep Purkinje (Carmeliet & Ramon, 1980), and rabbit Purkinje fibres (Mubagwa & Carmeliet, 1983), although its functional significance in this tissue is less clear.

ATP-sensitive, $I_{K(ATP)}$: This current resulting from a channel which responds to metabolic inhibition, or other mechanisms for ATP depletion, was first shown in isolated cells from guinea pig and rabbit atria and ventricle (Noma, 1983; Trube, 1983). Along with numerous confirmations in these tissues the channel has also been shown to exist in rabbit AV node (Kakei & Noma, 1984), rat ventricle (Conrad *et al.*, 1983; Findlay, 1987). An ischaemia-induced outward current in cat ventricle (Vleugels *et al.*, 1980) has been proposed to have been $I_{K(ATP)}$ (Stern *et al.*, 1988). These investigators proposed that $I_{K(ATP)}$ channels were activated in ischaemic cells despite bulk intracellular levels of ATP high enough to suppress the channel, on the premise that compartmentalization of ATP was occurring or that other nucleotides were activating the channel.

1.2.4 K^+ Channel Stimulators

While there are numerous muscarinic receptor and adenosine receptor agonists which would activate $K_{Ach/Ado}$ channels in the heart, these receptor linked channels are

mainly present in supraventricular tissue. The focus of this thesis is on K^+ channels which play putative roles in ventricular arrhythmias. Thus pharmacological activators of $K_{ACh/Ado}$ will not be discussed. Similarily, the activation of the K_{Na} channel by high levels of $[Na^+]_i$ might result from digitalis glycoside treatment, but this has not been shown *in vivo* and Na^+/K^+ ATPase inhibitors will not be discussed here. On the other hand, a newly developed group of vasodilator drugs has recently been shown to activate ATP sensitive K^+ channels in heart, skeletal muscle, pancreatic beta cells and of course vasculature (for review see Escande, 1989; Cole & Leblanc, 1990; Ashcroft, 1988). The prototypes of this new class of drug are nicorandil, (Taira *et al.*, 1979) pinacidil (Arrigoni-Martelli *et al.*, 1980; Bray *et al.*, 1987) and cromakalim (Hamilton *et al.*, 1986; Weir & Weston, 1986). A number of compounds with similar actions is in development, including pinacidil (P1134) analogues P1060, and P1368 (main metabolite inactive), and the nicorandil analogues: SG114; RP49,356; EMD 52,962.

These vasodilator drugs produce an increase in K^+ efflux from cells (Yanagisawa *et al.*, 1979; Yanagisawa & Taira, 1980; Weir & Weston, 1986a & 1986b; Hamilton *et al.*, 1986). This has been shown to shorten the cardiac AP (nicorandil: Yanagisawa & Taira, 1980; Imanishi *et al.*, 1983; cromakalim Nakajuna & Kurachi, 1985; Osterrieder, 1988; Mestre *et al.*, 1988; Scholtysik, 1987) by decreasing

the time constant for activation and increasing the amplitude of outward currents at potentials more positive than -40 mV, in part through removal of inward rectification as shown by Liu *et al.* (1990) with cromakalim. The ATP sensitive K^+ channel shows inward rectification due to both rapid closing upon depolarization and also Mg^{++} block of outward current (Noma, 1983; Cook & Hales, 1984; Horie *et al.*, 1987) similar to I_{K1} (Matsuda *et al.*, 1987). Cromakalim could reduce inward rectification of either I_{K1} or $I_{K(ACh)}$ or both, but since glibenclamide (an $I_{K(ATP)}$ blocker) (Sturgess *et al.*, 1985; Trube *et al.*, 1986; Fossett *et al.*, 1988) reverses cromakalim's effects, these vasodilator drugs are thought to activate $I_{K(ATP)}$ (Escande *et al.*, 1988; Hamilton *et al.*, 1986; Sanguinetti *et al.*, 1988; Osterrieder *et al.*, 1988). It has been shown that cromakalim does not reduce slow inward current, I_{si}, directly, although shortening of the AP would decrease the time available for Ca^{++} entry in a beat (Liu *et al.*, 1988; 1990; Steinberg *et al.*, 1988; Morad & Trautwein, 1968).

1.2.5 K^+ Channel Blockers

There are a number of inorganic ions and a few ammonium compounds that are routinely used to block K^+ channels in cardiac tissue or cells *in vitro*. These ions have, and are being used to dissect out currents for quantitative analysis, but are not generally used *in vivo* because of

their toxicity. For example guinea pig ventricular myocardium has been used to show the effects of Ba^{++} blockade of I_K and I_{K1} on slow response AP (Ehara *et al.*, 1980). Similarly, CsCl has been used to investigate the effects of blockade of I_f and I_{K1} in Purkinje fibres (Isenberg, 1976). Tetraethylammonium, TEA, has been used to observe the effects of reduction of K^+ currents on AP morphology in rat and guinea pig ventricle (Coraboeuf and Vassort, 1968). The 4-aminopyridine sensitive component of I_{to} has been studied in sheep Purkinje fibres (Kenyon & Gibbons, 1979). While useful as tools, these K^+ channel blockers are not cardiac selective, they were actually first used in squid giant axons, and their actions on neural tissue limits their usefulness as antiarrhythmic drugs. The one drug that has been derived from quaternary ammonium compounds (QA) is clofilium, but it is much more potent than the simple "channel plugger" QAs (Arena & Kass, 1988).

1.2.5.1 Sulphonylureas - $I_{K(ATP)}$ Blockers?

Sulphonylurea drugs have been widely used to treat type II diabetes. In the early seventies their use was questioned by findings of the University Group Diabetes Program which suggested tolbutamide treatment increased the risk of cardiovascular death (UGDP, 1970). This sparked interest into their cardiac effects and as reviewed by Levey *et al.* (1974) these hypoglycemic agents were found to have

direct positive inotropic actions. Studies at the time noted cAMP accumulation and suggested phosphodiesterase inhibition as a mechanism of action (Roth *et al.*, 1971). Stimulation of adenylate cyclase was also invoked (Brooker & Fichman, 1971). Others questioned these hypothesis (Brown & Broth al., 1977; Leichter *et al.*, 1981). Kramer *et al.* (1983) observed tolbutamide to increase glycogenolysis and glucose utilization. These early observations can now be explained on the basis of the observation that the sulphonylureas block (Sturgess *et al.*, 1985; Trube *et al.*, 1986; Fosset *et al.*, 1985; Sanguinetti *et al.*, 1988) the ATP sensitive K^+ channel, $I_{K(ATP)}$, (Noma, 1983). Blockade of this channel prevents shortening of the AP in the presence of ATP depletion, a complication which may have been a factor in the original cited work on isolated tissue. Patch clamp studies have shown tolbutamide and glibenclamide to be specific $I_{K(ATP)}$ blockers (Sturgess *et al.*, 1985; Trube *et al.*, 1986; Belles *et al.*, 1987; Ashcroft *et al.*, 1986), with no effects of I_K or Ca^{++} currents (Rorsman & Trube, 1986) $I_{K(Ca)}$ (Trube *et al.*, 1986), or I_{K1} (Belles *et al.*, 1987). Pancreatic beta cell K_{ATP} channels are two orders of magnitude more sensitive to tolbutamide than channels in ventricular myocytes (Trube *et al.*, 1986). Glibenclamide is the most potent sulphonylurea, being 100 x more potent than tolbutamide (Sturgess *et al.*, 1985; Trube *et al.*, 1986).

1.3 Pharmacology of K^+ Channel Blockers

1.3.1 Pharmacology of New Class III's

Since Vaughan-Williams' (1970; 1975) classification of antiarrhythmic drugs, development of class III agents, which prolong refractoriness without slowing conduction velocity, has progressed slowly relative to development of class I, gNa^+ blocking, drugs. This may be related to a perceived proarrhythmic potential of Class III drugs. However, the Cardiac Arrhythmia Suppression Trial, CAST, pointed to a an increased mortality risk associated with use of encainide and flecainide, despite suppression of VPB (CAST investigators, 1989). These results with Class Ic, gNa^+ blocking drugs, may provide a more receptive environment for the introduction of agents with Class III activity (Woosley, 1990) and currently a number of pharmaceutical companies are actively persuing this avenue (Colatsky and Follmer, 1989). New Class III drugs currently in development include: sematilide (Lumma *et al.*, 1987) and CK-3579 (Chi *et al.*, 1990) from Berlex-Schering; E4031 (Katoh *et al.*, 1988; 1990) from Esai; UK68,798 (Gwilt *et al.*, 1989a & 1989b) and UK66,914 (Gwilt, 1988) from Pfizer, (the latter compound has been withdrawn); MDL-11,939 (Koerner & Dage, 1989) from Merril Dow; risotilide (Colatsky *et al.*, 1989) from Wyeth-Ayerst; RP58,866 (Mestre *et al.*, 1989; Escande *et al.*, 1990) and RP62,719 from Rhone Poulenc-Sante, LY190147 from Lilly; MS-551 (Kamiya *et al.*, 1990) from Mitsui. Tedisamil and KC8851 (Beatch *et al.*, 1990) are presently being developed

as antianginal drugs by KaliChemie (Buschmann *et al.*, 1989a). While most of these compounds have been reported to have selective Class III actions, the data supporting these claims is limited. After testing on a broader scale, it will be safer to ascribe selectivity of action to these compounds.

The 4-[methylsulfonylamino] benzamide nucleus of sotalol is the basis of sematilide, CK3579, risotilide, UK68,798, UK66,914, MDL-11,939 and E4031. In sematilide, the N-acetyl group of acecainide has been replaced with a methanesulfonamide group, which is a more stable amide than N-acetyl, because transformation of the latter has been associated with a lupus like toxicity (Lumma *et al.*, 1989). Notable exceptions to this structural similarity are tedisamil and KC8851, which are sparteine derivatives (Kuhl & Buschmann, 1987), RP58,866, which is a benzopyran derivative (Escande *et al.*, 1990), and MS-551, which is a substituted pyrimidinedione (Kamiya *et al.*, 1990).

1.3.2 Class III Drug Mechanisms of Action

The cardiac APD can be prolonged by either, i) blockade of outward K^+ currents which contribute to repolarization, ii) reduction of inactivation or increased conductance of inward Na^+ or Ca^{++} currents, or iii) increased activity of electrogenic Na^+/Ca^{++} exchange. Prolonging APD delays

recovery of voltage dependent Na^+ channels, thus increasing refractory periods. Class III antiarrhythmics that "selectively" block K^+ channels are being developed (Colatsky and Follmer, 1989; 1990).

The exact structural requirements for synthesis of a compound producing specific K^+ channel blockade is not clear at present. The p-aromatic methylsulfonamides listed above have been reported to block the cardiac delayed rectifier K^+ current (risotilide: Follmer *et al.*, 1989; UK66,914: Gwilt *et al.*, 1988; UK68,798: Gwilt *et al.*, 1989; E4031: Sawada, 1989; Follmer and Colatsky, 1990). However, the presence of this group alone does not confer Class III actions (Lumma *et al.*, 1987). Within a group of p-aromatic methylsulfonamides structure activity relationship (SAR) analysis showed that substitutions for the methyl group or removal of the sulfonamide moiety itself renders the compound inactive (Lis *et al.*, 1987). The substitution of an electron withdrawing group in place of an electron-donating group on the aromatic portion of the basic local anaesthetic backbone appears to confer selective Class III actions. (Colatsky & Follmer, 1990). This trend can be seen with the electronegative methylsulfonamide substitution above and with the para-Cl^- or NO_2 in the selective Class III agent clofilium and its tertiary ammonium derivatives (Arena & Kass, 1988).

The sulfonamides familiar to a pharmacologist as the bacteriostatic agents used until the 1940's are different from these Class III drugs in that the amide-N links to the aromatic ring in the antiarrhythmic compounds, whereas the sulfonamide group is linked to a paraamino benzene ring via the sulfur atom in the antibiotics.

Clofilium has been reported to block the delayed rectifier, while its tertiary derivatives, LY97119 and LY97241 also block the inward rectifier in isolated guinea pig ventricular cells (Arena & Kass, 1988). Tedisamil has been shown to block I_{to} and I_K at room temperature in isolated rat and guinea pig ventricular cells, respectively (Dukes *et al.*, 1990).

1.3.3 Cardiovascular Effects of New Class III's

A study with sematilide in isolated canine tissue reported that 3.5 mM widened the APD95 by 20% in Purkinje fibres and 17 mM prolonged the functional refractory period in ventricular muscle by 20% (Lumma *et al.*, 1987). Sematilide (CK-1752A) and CK-3579 have both been reported to have Class III effects in dogs (Chi *et al.*, 1990). *In vivo* there was no effect by either drug on QRS, but P-R was widened after CK-3579 due to beta blockade while Q-T intervals were widened by only 5% (Chi *et al.*, 1990). The refractory period was widened only 5 - 15% by these drugs

whether measured in normal or infarcted tissue and yet 80% protection from mortality following infarction was seen (Chi *et al.*, 1990).

In similar models, E4031 was tested for antiarrhythmic effects (Lynch *et al.*, 1990). In this study, E4031 was without effects on QRS and R-R intervals, while it prolonged $Q\text{-}T_C$ interval by 11%. Similarly ventricular conduction time was unaltered by E4031 treatment but refractory periods were increased by a maximum of 25% in normal, as well as infarcted tissue (Lynch *et al.*, 1990). Again, remarkable protection from mortality (70%) was seen when a second ischaemic insult was applied in the presence of a previous infarct. A second group of investigators found similar selective Class III actions of E4031 in a canine model of programmed electrical stimulation (PES), in 7 day old myocardial infarction (Katoh *et al.*, 1990). In this study effective refractory periods were increased by 18-22% (in both normal and infarcted zones) with plasma levels of 330 ± 150 ng/ml E4031. This study also employed epicardial mapping to suggest that ERP prolongation prevented the induction of reentrant VT.

UK68,798 and UK66,914 have been shown to prolong APD and ERP in dog ventricular and Purkinje fibres and to increase ventricular ERP (VERP) in guinea pig *in vitro* and dog *in vivo* (Gwilt *et al.*, 1988; 1989). These effects were

found to be correlated with widening of the Q-T interval. In addition, both drugs were found to selectively block I_K and not I_{K1} in whole cell patch clamp of guinea pig ventricular cells (Gwilt *et al.*, 1988; 1989). No effects were seen on QRS or P-Q intervals of the surface ECG, or on conduction times. These drugs were remarkably potent with EC_{50} (for Class III effects) values for UK68,798 of 20-50 nM *in vitro* and 10-25 µg/kg *in vivo* (Gwilt *et al.*, 1989a & 1989b; Dalrymple *et al.*, 1989).

Risotilide (WY48,986) has been reported to have selective Class III antiarrhythmic actions in dogs and pigs subjected to coronary artery ligation (Colatsky *et al.*, 1989). As with the drugs reported above, the APD widening and ERP prolonging effects of risotilide were suggested to result from selective I_K blockade (Follmer *et al.*, 1989). Preliminary studies with MDL 11,939 (10 mg/kg) have demonstrated antifibrillatory effects with acute ischaemia or reperfusion in rats and PES in dogs (Koerner & Dage, 1989; 1990). These effects were suggested to result from AP widening seen *in vitro* (Li & Dage, 1989).

RP58,866 has been reported to selectively block I_{K1} (Escande *et al.*, 1989; 1990) in guinea pig myocytes and to prolong APD and refractory periods in guinea pig ventricle and Purkinje fibres (Mestre *et al.*, 1989). These Class III actions were found to protect against ischaemia/reperfusion

induced VF in halothane anaesthetized dogs, and micropigs, at a dose of 0.3 mg/kg (Mestre *et al.*, 1990). As opposed to risotilide, UK68798, sematilide and E4031, RP58866 produced a marked reduction in HR (31%) (Mestre *et al.*, 1990). Unfortunately, the assumption of antiarrhythmic actions of RP58,866, was based on a single dose, open study, with only 5 animals per group in which the occluded zone sizes were not reported (Mestre *et al.*, 1990).

A single abstract has been recently published on the Class III protective effects of MS-551 against arrhythmias induced by PES in 3-5 day old infarcted dogs (Kamiya *et al.*, 1990). In this study, MS-551 was found to increase atrial ERP by 56% and ventricular ERP by only 21% while increasing Q-T interval by 14% at a maximal 10 mg/kg dose. Up to 10mg/kg MS-551 did not have effects on QRS, P-Q intervals, conduction time, blood pressure, aortic flow nor myocardial contractility, while only a slight bradycardia was seen.

Although tedisamil has been tested for its specific bradycardic actions and applications to angina pectoris (Buschmann *et al.*, 1989; Grohs *et al.*, 1989) there has been only one report of its antiarrhythmic actions by a group other than ourselves (Curtis *et al.*, 1990 in press). This group found tedisamil to reduce the duration or "turn-off" VF in isolated rat hearts subjected to ischaemia. Our own work which is presented in this thesis has been published

previously (Walker & Beatch, 1988, Beatch *et al.*, 1988; 1990; 1991).

1.3.4 Clinically Available Class III Drugs

It is ironic that the first class III antiarrhythmic, i.e. amiodarone, can no longer be considered as belonging to any single class in the classification scheme of Vaughan-Williams (1970; 1975). Amiodarone was first shown to be antiarrhythmic by Charlier *et al.* (1969) although it was originally developed as an antianginal drug (Charlier *et al.*, 1962). Initial reports (Singh & Vaughan-Williams, 1970) suggested a selective AP widening effect in rabbit atria and ventricle, with minimal Vmax reduction. Recently, the APD prolonging effects of amiodarone have been shown to result from blockade of both I_K and I_{K1} (Balser *et al.*, 1987; Sato *et al.*, 1987). The historical events leading to the discovery of amiodarone's blocking effects on Na^+ and Ca^{++} channels, interactions with thyroid function and beta adrenoceptors, unusual pharmacokinetics and toxicity have been well chronicled in a number of reviews (Broekhuysen, 1983; Singh, 1983; Nattel & Talajic, 1988; Singh *et al.*, 1989). While the clinical usefulness of amiodarone is apparent, its lack of selectivity of action renders it unsuitable for examinations of specific Class III antiarrhythmic mechanisms in basic research.

The other prototypical Class III antiarrhythmic compound, d,l-sotalol (Singh & Vaughan-Williams, 1970) is a beta blocker with APD widening effects (Schmid & Hanna, 1967; Singh & Vaughan-Williams, 1970; Strauss *et al.*, 1970). While both the dextro- and levo-rotary enantiomers of sotalol have similar APD widening effects and antiarrhythmic effects (Somani & Watson, 1968) d-sotalol is only 7% as potent as a beta blocker (Manley *et al.*, 1986). Sotalol's Class III actions are thought to result from its ability to block I_K and to a lesser extent I_{K1} (Carmeliet, 1985) at concentrations of 10^{-6} - 10^{-4} M in both ventricular muscle and Purkinje fibres. High doses (10^{-4} M) were seen to reduce gNa^+. Although there are species differences in sensitivity to sotalol, typically the APD is widened 15-30% for a plasma concentration of 2 x 10^{-5} M sotalol in man (Edvardsson *et al.*, 1980). The uses and mechanisms of this drug which has been seminal in the design of compounds currently under development (see section 1.3.1) has been recently reviewed (Singh, 1987).

There are a number of drugs which prolong APD as an adjunct to their other actions, such drugs include the adrenergic neuron blocking drug bretylium (Waxman & Wallace, 1972) its analogues bethanidine (Bacaner and Benditt, 1982) and meobentine (Wastilla *et al.*, 1981); the butyrophenone neuroleptic melperone (Arlock *et al.*, 1978; Millar & Vaughan-Williams, 1982) and some tricyclic antidepressants

e.g. amoxapine (Kinugawa *et al.*, 1988). Clofilium, a derivative of bretylium, on the other hand appears to be a relatively specific Class III antiarrhythmic (Steinberg & Molloy, 1979) which blocks I_K (Snyders & Katzung, 1985; Arena & Kass, 1988). Acecainide (N-acetylprocainamide) also has relatively selective Class III actions (Drayer *et al.*, 1974; Jaillon & Winkle, 1979).

The historical absence of selective class III drugs prompted us to assess the antiarrhythmic efficacy of new putatively selective class III compounds (tedisamil, KC8851, UK68,798, RP62,719, and risotilide). We performed experiments designed to determine the mechanism(s) of action of these compounds at equivalent dose levels used to determine antiarrhythmic efficacy.

Section 1.3.5 Torsade de Pointes

Any discussion of antiarrhythmic drugs is incomplete without an examination of their potential for proarrhythmia. A particular form of polymorphic VT which is preceded by a prolonged diastolic interval was first described by Dessertenne (1966) who coined the term, "torsade de pointes" (TdeP). Agents which prolong repolarization have been reported to cause this arrhythmia, which can be suppressed by overdrive pacing and is favoured by hypokalemia. (Review see: Strattmann & Kennedy, 1987; Jackman *et al.*, 1988;

Surawicz, 1987; 1989; Fish & Roden, 1989). Possible induction of this arrhythmia is perhaps the single most important factor influencing the development of Class III antiarrhythmics. The mechanisms underlying TdeP are unclear, but some investigators have pointed to early afterdepolarizations (Brachmann *et al.*, 1983; El-Sherif *et al.*, 1988). Other factors such as bradycardia, hypokalemia and/or hypomagnesemia have also been considered (McKibbin *et al.*, 1984; Surawicz, 1987). One of the difficulties in assessing a drug's potential to cause TdeP is a lack of appropriate animal models for TdeP (Nayebpour *et al.*, 1989).

Recently, a rabbit model of TdeP has been developed (Carlsson *et al.*, 1990). In this model the $alpha_1$ adrenoceptor agonist methoxamine (15 µg/kg/min i.v.) was infused together with various Class III agents. The authors speculated that the increased incidence of TdeP with concomitant infusions of methoxamine resulted from alpha mediated increases in $[Ca^{++}]_i$, via increased phosphatidylinositol turnover, (Berridge, 1984) and thus greater susceptibility to EADS. However, recent observations of $alpha_1$ adrenoceptor mediated inhibition of I_{to} (Tohse, 1988; Tohse *et al.*, 1990, Ravens *et al.*, 1989) provide an alternate mechanism. The largest time and voltage dependent outward current in rabbit ventricle is I_{to} (Imaizumi & Giles, 1987; Hiraoka & Kawano, 1986). Rabbits also have a greater than average density of myocardial

alpha-adrenoceptors (Mukherjee *et al.*, 1983). Thus, if methoxamine were to inhibit I_{to}, this would cause additional widening of the action potential to that attributed to class III actions. This would explain why 50 x less clofilium was needed to produce TdeP during concomitant methoxamine infusion (Carlsson *et al.*, 1990). Our own rat studies have shown that the proarrhythmic actions of tedisamil depend on an intact autonomic nervous system (Howard *et al.*, 1989). Elevations of intracellular $[Ca^{++}]$ have been reported to augment K^+ currents, such as I_{to} (Tseng & Hoffman, 1989; Hiraoka & Kawano, 1989; Albitz *et al.*, 1988), $I_{K(Ca)}$ (Callewaert *et al.*, 1986), and I_K (Tohse *et al.*, 1987). Thus, if methoxamine in fact did cause a marked increase in $[Ca^{++}]_i$, the effects on K^+ channels would oppose the effects of the Class III agents. Furthermore, the dose of methoxamine used would not appear to have been high enough to disrupt Ca^{++} handling (arterial pressure was minimally affected) to the extent necessary for production of EADS as suggested by Carlsson *et al.*, (1990). Phenylephrine (10^{-7}-10^{-5}M) has been found to inhibit acetylstrophanthidin induction of transient inward current reputed to be responsible for oscillatory after potentials (OAP) in rabbit Purkinje fibres (Ferrier & Carmeliet, 1990). However, these authors did find alpha stimulation to increase OAPs induced by 8mM $[Ca^{++}]_o$. In the absence of $[Ca^{++}]_i$ measurements it is hard to reach a firm conclusion on the mechanism of induction of torsade de pointes by methoxamine/Class III

combination. It would be an interesting area for further research. Since drug treatment may be associated with side effects, other therapies have been developed.

1.4 Non Pharmacological Interventions

In the last 30 years cardiologists have come from a position of recommending bed rest after a cardiac arrest to an aggressive approach to the treatment of rhythm disorders. In 1958, the first pacemaker was implanted for treatment of complete AV block, and for years Mirowski, spurred on by Zoll's demonstration (Zoll *et al.*, 1956) of defibrillation by electric shock in man, struggled to develop a reliable automatic implantable defibrillator (Mirowski *et al.*, 1980). He succeeded in implanting the first one in a patient in 1980 (Mirowski *et al.*, 1980b), although the FDA did not approve these devices until 1985. Presently available devices offer multi-programability, microcircuitry, telemetry, extended battery life, data storage capability and more physiological control of the heart (Dreifus, 1989). Transvenous electrodes are simplifying the surgery necessary for implantation (Campbell, 1990). One of the major limitations to the use of pacemaker and cardioverters is their cost; some $40,000.00 in total in Canada (Eugene Downar, personal communication). These units are apparently effective, with sudden death rates of 1% and total mortality of 4% in one year (Winkle *et al.*, 1989) while the risk for

cardiac death in the year following MI is from 8-15% (Bigger *et al.*, 1984). Perhaps effectiveness has been inflated by counting every non fatal shock as successful defibrillation, as devices have been known to fire inappropriately.

Progress has also been made in suppressing arrhythmias with catheter ablative techniques (Fontaine, 1987). The methods of ablation can be chosen from DC shock fulguration, radio frequency fulguration, cryomodification and laser radiation. A common use of this technique is to ablate accessory pathways responsible for AVN reentrant tachycardia in Wolff-Parkinson-White Syndrome. The obvious benefit of catheter ablation is that a cure is possible for an albeit limited number of arrhythmias. Surgical ablation whether by catheter route or more classical techniques when combined with endocardial/epicardial mapping have been successfully used in patients (Camm & Davies, 1989).

1.5 Rationale

The overall goal underlying studies in the laboratory is to define the pharmacological characteristics required of the ideal antifibrillatory drug in the setting of myocardial ischaemia. In keeping with this, we tested new putative class III drugs for antifibrillatory activity against arrhythmias induced by myocardial ischaemia. In addition we sought, at a whole animal level, to determine the

antiarrhythmic mechanisms involved by means of electrical stimulation protocols and intracellular recording *in vivo*. ECG and haemodynamic data were collected to further characterize the pharmacological profile of each drug. A number of different species were used since the occurrence of different K^+ channels is highly species dependent (Carmeliet, 1977; Pelzer & Trautwein, 1987). For the purposes of the study, Class III antiarrhythmic agents were classified as those drugs which selectively widen the ventricular action potential (Vaughan-Williams, 1970) and as a result increase $Q\text{-}T_C$ interval of the surface ECG and prolong ventricular refractory periods.

1.6 Experimental Plan

In keeping with our rationale, the following studies were performed with a selection of new K^+ channel blockers as well as with reference drugs:

1) Assays of antiarrhythmic efficacy against ischaemia-induced arrhythmias in rats (tedisamil, KC8851, UK68,798, RP62,719, risotilide and glibenclamide) and pigs (tedisamil and UK68,798).

2a) Determination of ECG effects in rats (tedisamil, KC8851, UK68,798, RP62,719, risotilide, quinidine, mexiletine, and propafenone), guinea pigs (tedisamil,

UK68,798), and baboons (tedisamil, UK68,798, RP62,719 and risotilide).

2b) Determination of possible mechanisms of underlying antiarrhythmic efficacy by means of electrical induction of fibrilloflutter in rats, (tedisamil, quinidine, mexiletine, propafenone, UK68,798), and guinea pigs, (tedisamil, UK68,798).

2c) Determination of possible mechanism of action, through analysis of effects on refractory periods assessed by use of electrical stimulation protocols in rats, (tedisamil, quinidine, mexiletine, propafenone, UK68,798), guinea pigs, (tedisamil, UK68,798) and baboons, (tedisamil).

2d) Determination of possible mechanism of action through analysis of effects on ventricular epicardial action potential morphology in rats (tedisamil and UK68,798) and guinea pigs (tedisamil and UK68,798).

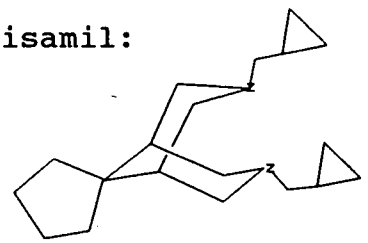
Attempts were made to explain these drug effects on the basis of underlying electrophysiological actions on membrane currents, as reported in the literature.

2 METHODS

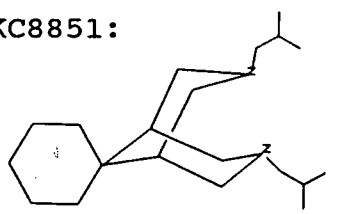
2.1 Pharmacology

The structural formulae of the new compounds used in our studies were: tedisamil, 3,7-di-(cyclopropylmethyl)-9,9-tetramethylene-3,7-diazabicyclo[3,3,1]nonane dihydrochloride; KC8851, 3,7-di(isobutyl)-9,9-pentamethylene-3,7-diazabicyclo [3,3,1]nonane dihydrochloride; risotilide, N-(1-methylethyl)-N-(2-((1-methylethyl)amino)ethyl)-4-((methylsulfonyl)amino)-benzenesulfonamide hydrochloride; UK68,798, 1-(4-methanesulfonamidophenoxy)-2-(N-(4-methanesulfonamidophenethyl)-N-methylamino)ethane; and RP62719 is a benzopyran derivative (complete structural formula not available). The structures of tedisamil and KC8851 are based upon sparteine, while the other compounds are derived from sotalol, as discussed earlier (Section 1.3.1.).

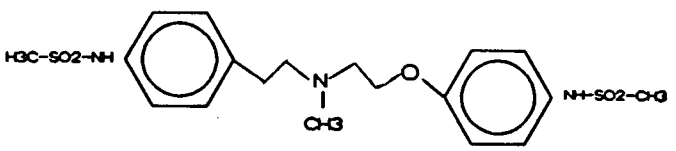
Tedisamil:



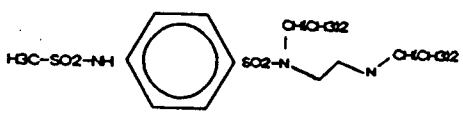
KC8851:



UK68,798:



Risotilide:



2.1.1 Cardiovascular Assessment

Dose response studies were performed to evaluate the blood pressure, heart rate, and ECG responses to the various drugs. In some instances data from these studies was used to establish the dose range to be used in subsequent antiarrhythmic and/or electrophysiological assays.

Section 2:1:1:1 Cardiovascular Assessment in Rats

The toxicity and effective dose range of tedisamil in rats was investigated previously using a continuous infusion protocol (Howard *et al.*, 1989). In this study various autonomic nervous system interventions were used to elucidate the nature of adverse effects associated with high doses of tedisamil. Proarrhythmic effects were also seen at doses above 15 mg/kg. From these studies we decided to perform the remainder of investigations with tedisamil in the 0 - 8 mg/kg range. ECG, HR and BP data were obtained from conscious rats used for coronary artery ligation studies. The insertion of ECG leads and cannulae was done under halothane (1%) anaesthesia one week before the test as previously described (Curtis, 1986). Basically, the abdominal aorta and vena cava were cannulated with free floating cannulae. These cannulae were fashioned from polyethylene tubing (PE10 and PE50) as described originally

by Weeks (Weeks and Jones, 1960; Weeks, 1981) and in replete detail by Curtis (1986). The distal ends of the cannulae were exteriorized at the mid-scapular region, facilitating monitoring of conscious freely moving animals. The ECG leads were made from Teflon coated stainless steel wire and inserted in a V3 configuration as described previously (Johnston *et al.*, 1983; Curtis, 1986). These leads were also exteriorized in the mid scapular region. Blood pressure was recorded on a Grass Polygraph chart recorder (Series VII) utilizing a Statham pressure transducer. The ECG was recorded on the Grass Polygraph and also displayed on an oscilloscope (Honeywell, Model E for M).

All drugs used were infused over 10 minutes via the vena caval cannula. Recordings were measured at a chart speed of 100 mm/sec 4 minutes after the infusion ended. Tedisamil was tested at three separate doses of 1,2 and 4 mg/kg. KC8851 was tested at 4 mg/kg. RP62719 and UK68798 were tested at 1 mg/kg, and finally, risotilide was tested at 5 and 10 mg/kg. These doses were chosen to represent supramaximally effective doses in other species (Gwilt *et al.*, 1989; Colatsky *et al.*, 1989; Cavero personal communication).

2.1.1.2 Cardiovascular Assessment in Guinea Pigs

Guinea pigs were anaesthetized with urethane (1.5 g/kg) and their jugular vein and left carotid artery cannulated with polyethylene tubing (PE50). Blood pressure and ECG (lead II configuration) were recorded on the Grass Polygraph as for rats. Only tedisamil and UK68798 (n = 6) were examined in this species. The doses of tedisamil were 0.5, 0.5, 1, 2, 4 mg/kg i.v. given consecutively every 15 minutes as a bolus, i.e. cumulative doses of 0.5, 1.0, 2.0, 4.0, and 8.0 mg/kg. ECG and blood pressure responses were recorded 10 minutes after dosing. The doses of UK68,798 tested were 10, 10, 20, 40 and 80 µg/kg i.v. given consecutively as above.

2.1.1.3 Cardiovascular Assessment in Pigs

Yorkshire weanling pigs (17 - 25 kg) were used to determine the ECG, BP and left (and right for UK68,798 only) ventricular pressure (plus rate of pressure development, dP/dt) in response to tedisamil and UK68,798. Pigs were anaesthetized with 30 mg/kg i.p. pentobarbitone. A marginal ear vein was cannulated with a 23 gauge (Jelco^{R}, butterfly) needle and additional pentobarbitone was given to achieve surgical anaesthesia, then a 30 mg/kg/hour pentobarbitone infusion was commenced. The femoral vein and artery were then cannulated with a polyethylene tubing (PE 160) and a Swan-Ganz catheter, respectively. The arterial catheter was advanced until it was positioned in the thoracic aorta. A

tracheotomy was performed and a pediatric endotracheal tube was inserted. The pig was then artificially ventilated (Palmer Ventilator Pump) with O_2 at 20 cycles/min, stroke volume 10 ml/kg. A saline infusion (0.5 l/hour) was initiated via the femoral cannula. A midline sternotomy was also performed to permit placement of a loose LAD coronary occluder for subsequent antiarrhythmic assay. Leads I, II, and III were recorded using subcutaneous needle electrodes. In pigs treated with tedisamil, a 14 gauge Jelco^R atraumatic catheter was inserted into the apex of the heart after thoracotomy. The catheter was held in position with a purse string suture (0 silk). In later studies with UK 68,798, the left ventricle was catheterized via the carotid artery with the aid of a J wire locator. The diastolic/systolic pressure difference was taken as evidence of correct placement. In experiments with UK68,798 the right ventricle was also catheterized via the jugular vein - superior vena cava - auricle route. All ECG leads were recorded on a Grass Polygraph (8 channel, Series VII); an oscilloscope (Honeywell Model E for M) was also used for continuous visual assessment of ECG effects. Blood pressure and ventricular pressures were also recorded on the Grass Polygraph. Ventricular pressures were electronically differentiated (dP/dt) and recorded on the chart recorder.

Tedisamil was given in a cumulative manner in doses of 0.5, 0.5, 1, 2, and 4 mg/kg i.v. at 15 minute intervals.

UK68798 was given in consecutive doses of 2.5, 5, 10, 20, 40 and 80 µg/kg i.v. at a dosing interval of 5 minutes.

2.1.1.4 Cardiovascular Assessment in Primates

The effects of tedisamil (and to a lesser extent UK68,798, RP62719, and risotilide) were investigated in male and female baboons (Papio anubis) and male and female pig-tailed monkeys (Macaque fascicularis) at the National University of Singapore. I did not participate directly in the performance of these experiments. These experiments were carried out by my supervisor, Dr. Michael J. A. Walker and Dr. Ganesan Adiakan. However, I participated in planning and data analysis of the experimental records. Since the results of these studies are useful, I feel justified in including some of the relevant data. The data add considerably to the overall pharmacological profile for the new compounds we have investigated.

Preparation of primates

Animals were sedated with ketamine (15 mg/kg, i.m.) and anaesthetized with pentobarbitone (15 mg/kg i.m.) after a 12 h fast. The brachial vein was cannulated and femoral artery catheterized for intravenous access and BP recording respectively. The animals were given extra pentobarbitone as needed in order to maintain a "light" degree of

anaesthesia, and were allowed to recover after completion of each dose response study. A full 12 lead ECG was recorded along with blood pressure, heart rate and respiratory rate. The data reported here were recorded 14 min after each consecutively administered dose; the dosing interval was 15 min. There were two different studies performed in baboons. In the first study, the cumulative doses were 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6 mg/kg tedisamil. Monkeys were also given this dose regimen. In the second study the cumulative doses were 0.5, 1.0, and 2.0 mg/kg, electrical stimulation of the right atrium and ventricle was also performed in this group (see Section 2.2.2.3). In pilot experiments UK68,798 (0-0.4 mg/kg, n=2), RP62,719 (0-0.4 mg/kg, n=2) and risotilide (0-0.3 mg/kg, n=2) were also tested in baboons. The effects on the ECG (QRS, P-R, Q-U, and Q-Uc) were analyzed from recordings made at chart speeds of 25 mm/s.

2.1.2 Drug and Dose Regimens

Tedisamil, KC8851 and risotilide were dissolved in saline in all experiments. UK68798 and RP62719 were dissolved in acidified saline and warmed to promote dissolution. In primate studies, UK68,798 and RP62,719 were dissolved in ethanol and diluted with saline. For the studies in rats the drugs were administered in a double blind randomized design.

2.1.3 Data Acquisition and Analysis

As described above the Grass Polygraph (Series VII) was used throughout (except for primate studies). ECG intervals were measured by hand with a micrometer from recordings made at 100 mm/sec chart speed. In primate studies, a Cardiograph (Model M1700A Page Writer XLi) was used, and traces were recorded at 25 mm/s chart speed, with 3 beats of each lead configuration for each time period printed. ECG intervals were defined as follows:

P-R interval - taken from the foot of the P wave to the upstroke of the R wave.

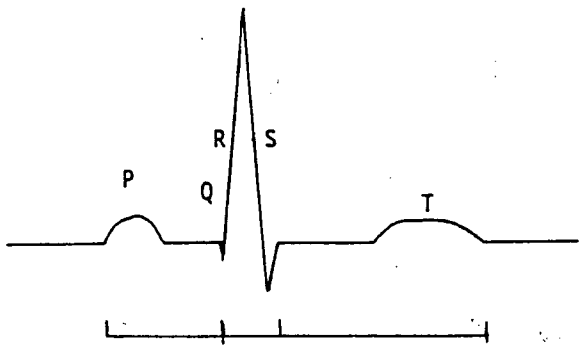
QRS - from the first deflection of the QRS to its return to the isoelectric.

Q-T - from the start of the Q wave to the final return to the isoelectric.

$Q\text{-}T_C = Q\text{-}T/(R\text{-}R)^{1/2}$ from Bazett's formula (Bazett, 1920).

Q-U (where appropriate) from the start of the Q wave to the final repolarization to isoelectric. This was necessary because the shape of the T-wave was often altered by tedisamil treatment. In some cases a U-wave developed, in others the T-wave had a biphasic morphology. Therefore, the interval to the final return to isoelectric, whether from a positive or negative deflection was measured. This was arbitrarily decided, recognizing the previously described

difficulty in accurately determining Q-T intervals (Surawicz, 1987; 1989).



Differences in effects on ECG intervals between treatments were examined by analysis of variance, unweighted means ANOVA, and if significant ($p < 0.05$), were followed by Duncan's multiple range test using NCSS computer packages (Hintze, 1987). As pre-infusion values were not significantly different from each other, comparisons were made between saline and drug treatment post-infusion data in rat studies. For cumulative dosing studies (guinea pigs and baboons) data were compared between pre-drug and dosage level.

2.2 Electrical Stimulation

2.2.1 Introduction

Class III drugs were initially classified (Vaughan-Williams, 1970) as agents which increased APD without additionally decreasing conduction velocity. In order to assess whether drugs increased refractoriness, simple electrical stimulation protocols were carried out *in vivo*.

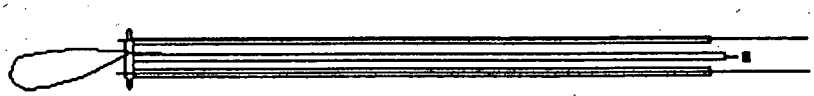
It has been well established that drugs which decrease gNa^+, increase thresholds for capture of single beats and fibrillation threshold (Wiggers & Wegria, 1940). On the other hand, pure Class III drugs might not affect thresholds for capture, yet might suppress VF induction by making the heart refractory to the fractionating wavefront (Review: Winslow, 1984). However, a pure Class III drug would be expected to prolong the effective refractory period (ERP) and decrease the maximum following frequency (MFF) to square wave stimuli (Vaughan-Williams, 1970; 1975). Thus, by testing the drugs for their influence on VF_t, ERP, and MFF we hoped to quickly establish an index of their Class I vs. Class III actions. Simple comparisons could also be made of the effects on electrical stimulation variables with antiarrhythmic effects for a given level of ECG change (e.g. increase in $Q\text{-}T_c$).

2.2.2 Preparation

Initially we attempted to develop a conscious rat model for electrical stimulation of the ventricle (Walker and Beatch, 1988). Basically, rats were anaesthetized with halothane (1%) and free floating cannulae were implanted in the inferior vena cava and abdominal aorta as described previously. The chest was opened and the heart exposed so that a specially designed electrode carriage could be loosely sutured to the anterior apical region of the left

ventricle. The leads from the carriage assembly was routed subcutaneously and exteriorized in the mid scapular region, and the chest closed as in placement of coronary artery occluders (section 2.4.2.1). Animals were given a week to recover from surgery. The electrode carriage consisted of three parallel 11cm lengths of polyethylene tubing (PE10) joined together at one end by melting. A proline suture was inserted in the central tube and stainless steel or silver wire electrodes were inserted in the tubes on either side (see diagram).

Electrode assembly:



Thus, after anchoring the assembly to the heart, gentle traction on the central guide tube could bring the electrodes into apposition with the ventricle. Stimulation protocols are described below.

We used an acutely prepared anaesthetized animal model for all other electrical stimulation experiments. The variables measured were current and pulse width thresholds for capture of the ventricle on a 1:1 basis, maximum following frequency (MFF), Effective refractory period (ERP)

and fibrilloflutter threshold (VF_t). These variables will be described in detail below.

2.2.2.1 Electrical Stimulation in Anaesthetized Rats

Male rats (250 - 400 g) Sprague-Dawley, were anaesthetized with pentobarbitone (45 - 60 mg/kg i.p.) and intubated with a 14 gauge Teflon catheter. The left carotid artery was cannulated with PE50 polythene tubing for blood pressure recording. The right jugular vein was cannulated in the same manner for the purpose of drug administration. The skin overlying the thorax was cut away to facilitate placement of the pacing electrodes. The stimulating electrodes were made from Teflon coated silver wire. A 1-2 mm segment of insulation was removed from the end of the wire and the wire was passed through the lumen of a 27 gauge needle. The desheathed tip of the wire was bent back to form a barb and the needle plunged through the chest wall, and the wire deposited into the left ventricular free wall towards the apex. This process was repeated with a second wire, 2-3 mm from the first one. This technique allowed rapid insertion of stimulating electrodes 2-3 mm apart with minimal trauma to the animal. Stable threshold values, absence of arrhythmias and/or hypotension and post mortem examination confirmed a correct placement of the electrodes. A lead II or III configuration of the ECG was used. The ECG and BP were recorded on a standard Grass Polygraph, and a

delayed loop oscilloscope (Honeywell Model E for M) was used for continuous assessment of the ECG.

2.2.2.2 Electrical Stimulation in Guinea Pigs

Guinea pigs were also used to test for Class III activity because there are species differences in the response to Class III drugs. These species differences are the result of variation in currents underlying action potential repolarization. While the rat has a large transient outward current, this current has been reported to be absent in guinea pigs (Josephson *et al.*, 1984; MacDonald *et al.*, 1984).

Guinea pigs, male Hartley (450 - 600g) were prepared using the same techniques and apparatus as in acutely prepared rats with one exception; urethane (1.5 g/kg i.p.) was used for anaesthesia. This anaesthetic was chosen because it produces a smooth CNS depression in guinea pigs without influencing sympathetic tone.

2.2.2.3 Electrical Stimulation in baboons

Baboons which were prepared for cardiovascular monitoring (study two, above) as described in the previous sections were also subjected to electrical stimulation protocols. These experiments were performed in "recovery"

animals and thus for ventricular stimulation an atraumatic electrode was positioned in the lower right ventricle via the femoral vein, and the right atrium was stimulated with an intra-oesophageal electrode inserted orally. Threshold pulse width and threshold voltage were estimated at fifteen minute intervals and twice these thresholds used to determine MFF. Doses of 0.5, 1.0 and 2.0 mg/kg tedisamil were assessed.

2.2.3 Data Acquisition

For rats and guinea pigs equivalent equipment and protocols were used. A constant current stimulator was used to establish thresholds and estimate refractory periods. Refractory periods were determined by a single extra pulse technique at a basic stimulation frequency of 7.5 Hz (rats) or 6.5 Hz (guinea pigs). All determinations were made using square waves. Each end point (MFF, ERP, VF_t) was determined in triplicate 10 minutes after each dose of drug. The ECG and BP were recorded on a polygraph chart-recorder (Grass, Series VII).

2.2.3.1 Thresholds for capture

The current necessary for capture of the ventricle on a 1:1 basis was determined at the stimulation frequency of 7.5 Hz for rats, and 6.5 Hz for guinea pigs, and a pulse width

of 1 ms. The minimum current necessary to obtain 1:1 capture was taken as the threshold current. The pulse width threshold for capture was determined at the same frequency of stimulation (rats 7.5 Hz, guinea pigs 6.5 Hz) and at twice the current threshold. Average threshold pulse widths were 0.3 ms using this method.

2.2.3.2 Maximum Following Frequency

This variable is a measure of the heart's ability to follow an increasing rate of stimulation on a 1:1 basis. Interventions which increase ventricular refractory periods could be expected to decrease the maximum following frequency (MFF). The MFF is thus another test for class III effectiveness. In normal myocardium, MFF was determined by setting the stimulation current and pulse width at twice their respective threshold values and then smoothly increasing (3 $Hzsec^{-1}$) the stimulation frequency from 6 Hz until the heart failed to follow on a 1:1 basis. This end point was determined by noting the point at which the blood pressure (which steadily decreases at increasing rates of stimulation) suddenly reflected a compensatory pause from a non-captured pulse. The end point was also easily seen (as a sudden missed beat) on the ECG displayed on the oscilloscope. The estimate of effective refractory periods (reciprocal of the MFF) obtained using this method may

reflect underlying drug binding kinetics to sodium channels (Hondeghem & Katzung, 1984).

2.2.3.3 Effective Refractory Period

Another method used to gain an estimate of the effective refractory period in the ventricle was the paired pulse method. In this method the ventricle is stimulated at a baseline frequency (7.5 Hz rats; 6.5 Hz guinea pigs) and a single extra stimulus (of same current strength and pulse width) is added at a variable delay from the entrained stimuli. The minimum delay which resulted in a conducted beat was taken as the effective refractory period. Comparisons between 1/MFF and ERP determined with this method allowed us to ascertain the frequency dependency of refractoriness increase in the upper range of frequencies.

2.2.3.4 Fibrilloflutter Threshold

This threshold is generally accepted as a measure of vulnerability of the ventricle and is normally raised by drugs with class I or gNa^+ reducing actions (Wiggers and Wegria, 1940). The end point was determined by increasing the current strength (at 50 Hz and 0.8 ms duration) until a fibrilloflutter was elicited. The tachycardias were generally non-sustained. The high rate of stimulation was used in order to increase the probability of an R on T type

initiation of the tachyarrhythmia (see sections 2.2.1 & 4.2.1).

2.2.4 Drug and Dose Regimens

Tedisamil was dissolved in saline. UK68798 was dissolved in acidified saline. After establishment of baseline control values rats or guinea pigs were given either 0.5 mg/kg tedisamil or 5 ug/kg UK68798 as a bolus i.v. injection. At 10 minutes after injection, electrical stimulation protocols were initiated and triplicate readings obtained. Fifteen minutes after injection the next dose was given. The cycle was repeated until the maximum dose level was achieved. For tedisamil the doses were 0.5, 0.5, 1, 2, and 4 mg/kg i.v. given consecutively as indicated; for UK68798 the doses were 5, 5, 10, 20, 40, 80 µg/kg i.v. given as above. The exact same protocol was used for rats and guinea pigs. Six animals per group were used for each drug. Primates however received tedisamil 0.5, 0.5, & 1.0 mg/kg, i.v. given every 15 minutes as described above (section 2.1.1.4).

2.2.5 Data Analysis

ECG and blood pressure records were examined at baseline and 10 minutes after each dose for rat and guinea pig studies. ECG intervals were measured manually using the

same conventions as described previously. Electrical stimulation end points determined between 10 and 15 minutes after dosing were compared to baseline values using analysis of variance, ANOVA followed by Duncan's multiple range test (NCSS package, Hintze, 1987). Repeated measures ANOVA was also done to show trends.

2.3 Electrophysiological Analysis

2.3.1 Introduction

In order to examine drug effects on action potential morphology and upstroke velocity, intracellular recordings were made from the ventricular epicardial cell layer in intact rats and guinea pigs. With this technique it was possible to show the effects of the drugs at similar doses used in other *in vivo* studies. An intracellular recording technique with free floating microelectrodes, was used in order to obtain accurate measurements *in vivo*. The flexible tip method we used was modified from the method of Woodbury & Brady, (1956).

2.3.2 *In vivo* preparation

The technique consists of the following:

A rat or guinea pig was anaesthetized with pentobarbitone 45 mg/kg i.p. or urethane 1.5 g/kg i.p., respectively, and intubated with a 14 gauge Teflon Jelco^{R}.

The animal was artificially ventilated with 100% O_2 (60 cycles/minute, 10 ml/kg stroke volume). The right jugular vein and left carotid artery were cannulated with polythene tubing (PE50) as described previously (Section 2.2.2.1). A bipolar oesophageal ECG lead was appropriately placed in the oesophagus or alternatively a surface ECG, lead II configuration, was used. A thermocouple connected to a heating lamp was inserted into the rectum in order to maintain body temperature at 37 ± 1° C. The skin covering the thorax was cut away. The left 4th rib was removed and the over-lying muscles (*pectoralis* and *abdominus rectus*) positioned away from the opening. A pericardial sling was made after retracting the rib cage with specially tooled retractors. A silver/silver chloride reference loop electrode was delicately sutured to the left ventricular surface or, in later experiments, a simple silver wire wick electrode was positioned against the ventricle. The surface of the heart was moistened continuously with Ringer's lactate solution (composition in mM: Na^+ 130; Ca^{++} 1.5; Cl^- 109; K^+ 4; lactate 28). The ECG and BP were recorded on a Grass Polygraph and on video tape (NEC VCR DX1000C) after analogue to digital conversion with a pulse code modulator (initially Sony PCM, later Medical Systems Corp. PCM 4/8). Intracellular electrodes were pulled from fibre filled borate glass (WPI, 1BBL W/FIL 1.0 mm, 4 in), using a pipette electrode puller (Narashige Pa01, set at magnet 7, heat 9), resistance 10-20 MOhms, filled with filtered (0.45 µm,

Millex) 3 M KCl and a coated tungsten wire (0.002 in Am systems Inc.) back inserted down the barrel of the pipette. The tip (1 - 1.5 cm) of the pipette was broken off leaving the wire attached. The wire was bent at right angles 2 cm from the pipette tip and then cut 2 cm distal to this bend. The wire was clipped to a connector which was inserted into the amplifier (WPI instruments Model 750 Dual micro-probe). The connector was positioned using micromanipulators (Narashige #2749), allowing the electrode to be dropped onto the ventricular surface. The intracellular potentials were digitally recorded on video tape after pulse code modulation of the amplified signals. Transmembrane potentials were also differentiated electronically and digitally recorded on video tape. The quality of the impalement was easily monitored by an on-line oscilloscope (Gould Advance OS250B) which displayed both the DC potential and its differential. Stable recordings could often be maintained for 15 minutes using this technique. It was relatively easy to determine the quality of impalement by determining the resting potential (Em), the rise rate (dV/dt), and a "clean" morphology. Unsatisfactory impalements (dV/dt < 100 V/s, Em < -70 mV) were not analyzed. Resting potentials could be ascertained by the voltage shift of the baseline when the electrode passed from the extra cellular space to the intracellular milieu. A continuous spoken record was also made directly on the video tape to log the progress of the experiment.

2.3.3 Drugs and Dose regimens

Tedisamil (dissolved in saline) was given as bolus injections i.v. at doses of 0.5, 0.5, 1, 2, and 4 mg/kg to rats or guinea pigs, n = 4-6 per group. UK68798 dissolved in acidified saline was given as i.v. bolus at doses of 12.5, 12.5, 25, 50, 250, 1000 µg/kg to rats and guinea pigs. The dosing interval was 15 minutes between each dose given consecutively. Multiple impalements were made with the microelectrode throughout the experiment, but when possible, a single cell was held during drug injection and the following 2 minutes. Recordings were made from the lower (apical) half of the left ventricular surface.

2.3.4 Data Analysis

The DC recordings were analyzed from the video tape by feeding the signal back through the A/D converter into a storage oscilloscope (Tektronix type 549). The oscilloscope was calibrated from a 200 V/s sawtooth calibration signal recorded at the beginning of each tape using a saw tooth wave generator. Measurements were made at 10, 25, 50 and 75% repolarization of the AP. The AP height and rise rate were also measured. In addition the slope of the plateau (phase 2) and late repolarization phase (phase 3) were also calculated manually. The digitally recorded ECG & BP

records were played back through the Grass Polygraph allowing fast trace records to be replayed and made at any time. Values were measured at 1, 2, 5, 10 and 15 minutes after each dose.

Data were analyzed by analysis of variance (ANOVA) followed by Duncan's Multiple Range test for means. Repeated measures ANOVA was used to detect trends.

2.4 Myocardial Ischaemia - Induced Arrhythmias:

2.4.1 Introduction

The use of animal models for assessing antiarrhythmic drug efficacy has been a major contributor to the selection of potentially useful drugs. The production of myocardial ischaemia by ligation of one or more coronary arteries in numerous species has been used by numerous investigators. No single species or model of acute ischaemia has proven to be ideal. Recently, guidelines have been published with the aim of standardizing procedures so that interlaboratory comparisons can be made (Walker *et al.*, 1988). These "Lambeth Conventions" were followed in our studies using the rat. Recently the rat has been endorsed as an appropriate model for testing antiarrhythmic drugs by several independent laboratories (Winslow, 1984, Curtis, 1986; Curtis *et al.*, 1987; Brooks *et al.*, 1989). One of the main advantages to the use of the rat is that the species has few collateral arteries in its coronary vasculature (Johns and

Olsen, 1954; Maxwell *et al.*, 1984: 1987) such that consistently sized occluded zones can be produced by ligations of the left anterior descending (LAD) coronary artery. The size of the occluded zone corresponds well to the intensity of the arrhythmogenic stimulus (Austin *et al.*, 1982; Bernauer, 1982; Curtis *et al.*, 1987), thus low inter-animal variability is desirable for multi treatment trials.

As discussed in the Introduction, the rat has fundamental electrophysiological differences from a number of other mammalian species, in that the rat ventricle has negligible I_K and a uniquely large component of transient outward current, I_{to}, which contributes to rapid repolarization of the ventricular AP (Josephson *et al.*, 1984). Thus the rat has a short ventricular AP. This feature renders the rat more sensitive to interventions which inhibit I_{to} and less sensitive to interventions which inhibit I_K or I_{K1} than the guinea pig which relies more heavily on these latter currents for AP repolarization in its ventricle. However, this feature of the rat also makes it ideal to study class III antiarrhythmic mechanisms because its ventricular AP can be widened to such an extent (5 fold) by I_{to} blockade (Dukes *et al.*, 1990; Beatch *et al.*, 1990). Therefore, the degree of widening necessary to produce antiarrhythmic activity in this model can be readily obtained empirically.

2.4.2 Preparation

The preparation commonly used for production of ischaemia consists of simple ligation of the left anterior descending coronary artery (Review: Botting *et al.*, 1986). There are a number of variations to this basic theme. For instance the classic Harris (1950) two stage ligation model in dogs is used to test for arrhythmias arising from ischaemia produced by ligation of a previously partially ligated artery (done to prevent fatal VF). Acutely prepared rats have been widely used to test the effects of drugs on permanent occlusions of the LAD (Johns & Olson, 1954; Selye *et al.*, 1960; Curtis *et al.* 1987). In this model the heart is briefly exteriorized after thoracotomy in order that a silk ligature may be placed around the LAD. After a period of stabilization, the ligature is tied, and the animal monitored. Although there are numerous ways of occluding the artery i.e., glass bead injection (Wilkerson and Downey, 1978), sutures (Johnston *et al.*, 1935), electrically induced thrombogenesis (Salazar, 1961; Patterson *et al.*, 1981), "ameroid cuff" occluders etc. the basic outcome desired is the production of an area of hypoperfusion, or ischaemia, downstream from the occlusion. The sequelae of occlusion have been studied using biochemical, biophysical, pharmacological and electrophysiological techniques, which has resulted in identification of potential mediators of arrhythmias and cardiac dysfunction (Dennis and Moore, 1938; Sobel *et al.*, 1978; Hill and Gettes, 1980; Hirche *et al.*, 1980; Janse *et al.*, 1981; Coker *et al.*, 1981). These

potential mediators have then been used to mimic ischaemia in non-ischaemic tissue in order to determine arrhythmogenic mechanisms or to assay pharmacological interventions (Harris *et al.*, 1958; Ettinger *et al.*, 1973; Ferrier *et al.*, 1985). There is no ideal model for predicting the efficacy of an intervention for transfer to clinical practice (Walker *et al.*, 1988). Recently, a paradigm for examining arrhythmia mechanisms has evolved out of chaos theory (Guevara *et al.*, 1981; Winfree, 1987; Chialvo and Jalife, 1987). These studies suggest that long range predictions of the efficacy of antiarrhythmic interventions may be impossible, if arrhythmia occurrence conforms to the rules governing nonlinear dynamic systems (May, 1976).

2.4.2.1 Conscious rats

The conscious rat model used in these studies was developed in this laboratory from a desire to create a useful drug assay (Johnston *et al.*, 1983). Over the past decade our laboratory has produced a large data base of the effects of interventions on myocardial ischaemia induced arrhythmias using this model. The examination of this model has recently been reviewed (Curtis, *et al.*, 1986). The model as described below was used to evaluate the effects of compounds with reported Class III antiarrythmic actions.

Basically, as described previously (Johnston *et al.*, 1983, Curtis, 1986), male Sprague-Dawley rats (250 - 450 g) were anaesthetized with halothane (1%), intubated with a Teflon 14 gauge Jelco^{R} catheter and ventilated on 100% O_2, (10 ml/kg, 60 cycles/min) to keep blood gases in the normal range (MacLean & Hiley, 1988). Needle electrodes were used to obtain a lead II ECG which was displayed on an oscilloscope (Honeywell, Model E for M) for the purposes of monitoring the animal during surgery. Surgical instruments were cleaned and disinfected with A35 detergent and then soaked in 70% ethanol. After midline laparotomy, free floating cannulae, made from polyethylene tubing (Weeks & Jones, 1960; Weeks, 1981; Curtis, 1986) were inserted in the abdominal aorta and inferior vena cava in close proximity to the renal artery branches. The distal ends of the cannulae were exteriorized in the mid-scapular region with the aid of a trocar. The cannulae were flushed with saline and the ends heat sealed. The abdomen was closed with silk sutures. In some cases the blood pressure line was connected to the transducer and a recording was made on the Grass Polygraph during surgery. The chest was then opened at the fourth intercostal space, a pericardial sling was made, and a loose coronary occluder placed around the LAD. The chest was closed with a silk purse string suture, a Teflon coated stainless steel electrode wire implanted over the third intercostal space (approximate V3 configuration), and both the occluder guide and ECG lead exteriorized at the mid-

scapular region. The chest was closed with silk sutures and negative pressure applied to prevent pneumothorax. All wound sites were infiltrated with Marcaine^{R} and sprinkled with Cicatrin^{R}. Reference stainless steel wire ECG leads were implanted subcutaneously on the left and right forelimbs and left hindlimb, connected together and exteriorized at the mid-scapular region. The animal was then allowed to recover after removal of the intubation tube. Total surgical preparation took about 40 minutes in most cases. The animal was allowed to recover for one week before use. During this time they had access to food (Purina Rat Chow) and water *ad libitum*. On the day of the experiment the conscious animal was weighed and its cannulae and leads connected to the Grass polygraph, oscilloscope and drug infusion pump as appropriate. The animal was allowed to stabilize for at least 15 minutes before a ten minute drug infusion was initiated. A blood sample (0.2 ml) was drawn just prior to infusion and serum [K^{+}] was measured with K^{+} sensitive electrodes (Ionetics Instruments). The LAD ligature was tightened 5 minutes after the infusion was complete. Drug treatments were administered in a double blind randomized design as described later. Responses to ligation were monitored for a four hour period before disconnecting the animal. A repeat drug dose was given as a 30 minute infusion, commencing 1.5 hours after occlusion in order to compensate for metabolism and excretion of the drugs. Arrhythmias during the monitoring period were

diagnosed from the oscilloscope screen and noted directly on the chart recording for later analysis, as described below. Twenty-four hours after occlusion the rat was reconnected and monitored for a further 30 minutes. In the case of tedisamil, fourteen of these rats with one day old infarcts were further analyzed (section 2.4.2.2.). After this monitoring period (or as soon as the animal died), the animal was sacrificed and the heart removed and retrogradely perfused (Langendorff mode) in order to assess the occluded zone (O.Z.), size by cardiac green dye exclusion. The O.Z. was defined as weight of non-perfused ventricle / weight of perfused ventricle x 100%. The animals were examined post mortem for any obvious signs of pathology e.g. scarring and adhesion of the myocardium, infarction of the kidneys, pulmonary oedema etc. A rigid set of exclusion criteria as outlined by Curtis (Curtis, 1986) was adhered to. In the event of exclusion of a rat by these criteria the treatment was immediately repeated in another rat before continuation. This conscious rat model of myocardial ischaemia was used to test the efficacy of tedisamil, KC8851, UK68798, RP62719 and risotilide.

2.4.2.1.1 Infarcted Rats

The proarrhythmic potential of tedisamil was explored in rats with previous myocardial infarction. These studies were carried out in 14 rats with a one day old infarction, 5

rats with a one week old infarction and 5 rats with a one month old infarction. The rats were prepared as for ischaemia-induced arrhythmias in conscious rats (section 2.4.2.1). It was initially hoped to assay the antiarrhythmic activity of tedisamil against infarction-induced arrhythmias, however the low incidence of arrhythmias in the animals with one week and one month old infarcts precluded such investigations. Rats were monitored for a 30 min stabilization period, during which arrhythmia incidence (VPBs) was tabulated, then bolus injections of tedisamil were given (1.0, 2.0, 4.0 mg/kg) at 15 minute intervals. Increases or decreases in VPB or other arrhythmias were recorded along with the BP and ECG in these conscious chronically infarcted rats.

2.4.2.2 Acutely Prepared Anaesthetized Rats

Acutely prepared, pentobarbitone (45 mg/kg) anaesthetized, rats were used to detect the antiarrhythmic efficacy of tedisamil and glibenclamide against arrhythmias induced by a 10 minute ischaemic period followed by reperfusion. These investigations were part of a larger study reported previously (Beatch *et al.*, 1989). The rats were cannulated for drug administration and blood pressure recording as in electrical stimulation studies (section 2.2.2.1). The rats were intubated with a 14 gauge Teflon catheter and artificially ventilated with room air. The

occluder was loosely placed around the LAD coronary artery as for chronically prepared animals. An ECG (lead II) was recorded along with BP on a Grass Polygraph. The rat was allowed to stabilize for 15 min before a 10 min drug infusion commenced. Just prior to drug infusion a blood sample (0.2 ml) was drawn to measure serum $[K^+]$. The LAD was occluded 5 min post infusion. Alternatively, because glibenclamide was insoluble in polar solvents, a slurry of glibenclamide in water was administered *per os* 30 min before occlusion. After 10 min of occlusion, the occluder was loosened and reperfusion effected. Ten min after reperfusion the heart was excised and retrogradely perfused (Langendorff mode) with saline to verify reperfusion and to measure the occluded zone after retightening the occluder. Eleven rats were occluded after glibenclamide (10 mg/kg) treatment and 7 reperfused, 5 treated with tedisamil (4mg/kg) and all 5 reperfused. There were seven control rats.

2.4.2.3 Anaesthetized Pigs

The antiarrhythmic efficacy of tedisamil and UK68,798 was investigated in acutely prepared anaesthetized pigs. The preparation of the pigs is described above (section 2.1.1.3.). The primary aim of the pig studies was to obtain dose/response relationships of the cardiovascular and ECG effects of tedisamil (n=6) and UK68,798 (n=5). After

carrying out the cumulative dose regimen, we decided to ligate the LAD in these preparations and compare the antiarrhythmic effects at the maximal dose with historical control pig data. The number of pigs used in these studies, the use of historical controls, the open design and the lack of antiarrhythmic efficacy dose/response determinations were recognized and thus antiarrhythmic efficacy could not be determined with any degree of scientific certainty. In short, these were pilot studies.

2.4.3 Data Analysis

With any scientific endeavor, care must be taken in both the design and analysis of experiments. The Lambeth Conventions (Walker *et al.*, 1988) were compiled in an attempt to approach these problems in a systematic manner. In accordance with these recommendations, the antiarrhythmic assays in conscious rats were carried out in a double blind and random design, with predetermined statistical analysis and exclusion criteria (Curtis, 1986; Igwemezie, 1990). While a number of the pilot studies were not as rigidly controlled, we did attempt to obtain dose/response curves such that the variance due to drug treatment would become apparent. The analysis we used in these experiments is described below.

2.4.3.1 ECG changes

The analysis of standard ECG intervals (QRS, P-R, Q-T) has been previously defined in Section 2.1.4, however the effects of ischaemia on the R-wave height, S-T segment elevation and Q-wave appearance were examined in addition to these standard variables. Numerous investigators have shown that acute ischaemia and infarction have dramatic and reproducible effects on the morphology of the ECG (Review: Holland and Brooks, 1977). Drug treatments have also been shown to influence these sequelae of ischaemia (Clusin *et al.*, 1984). Ca^{++} antagonists delay the onset of S-T segment elevation in our model (Johnston *et al.*, 1983; Curtis *et al.*, 1984; Walker and Beatch, 1990) while Abraham *et al* (1989) have shown that TTX reduced both R-wave elevation and S-T segment elevation in chronically prepared anaesthetized rats. The analysis of these ECG changes has been described explicitly in previous publications (Curtis, 1986) and the following description is only a brief synopsis.

The R-wave height was measured from the isoelectric baseline to the peak of the positive deflection and expressed in mV. The S-T segment elevation was expressed as % of R-wave height, where the S-T segment was defined as the height (in mV) above isoelectric where the negative deflection of the S-wave was interrupted in its return to isoelectric. This second peak after the QRS was not confused with a second peak seen with splitting of the QRS

secondary to conduction blockade by measuring peaks at least 30 msec after the QRS initiation. Q-wave onset was measured as the time after occlusion when a negative deflection just prior to the R-wave greater than 10% of R-Wave height was seen.

2.4.3.2 Arrhythmia Analysis

Occlusion of the LAD in rats and pigs resulted in the occurrence of ventricular arrhythmias which were equivalent to the premature beats (VPB), bigeminy, tachycardia (VT) and fibrillation (VF) seen in the clinical setting. The size of the O.Z. was determined by dye (Cardiac green) exclusion in hearts retrogradely perfused at the end of the monitoring period. Arrhythmias were analyzed as described by Curtis (1986) i.e. VT was defined as four or more consecutive extrasystoles with a clearly distinguishable QRS, as opposed to VF which has a chaotic appearance.

Arrhythmias were divided into time periods corresponding to early arrhythmias (0-0.5 hr in rats, 0-1hr in pigs) and late arrhythmias (0.5-4hr rats) in accordance with the biphasic occurrence with time of ischaemia-induced arrhythmias (Wit and Bigger, 1977). The mean ± SEM $\log_{10}$ number of VPBs per time period was determined for each group. The mean ± SEM of $\log_{10}$ number and duration of VT and VF episodes also were counted for each group in each time period. Log_{10} transformation normalized these data for

subsequent parametric statistical analysis by one way ANOVA (Johnston *et al.*, 1983). The number of animals per group which had at least one episode of VT and VF also was recorded. These nonparametric data were analyzed by the Chi-square test with the aid of Mainland's contingency tables (Mainland *et al.*, 1956). Arrhythmia score (Johnston *et al* 1983) was used to summarize the arrhythmia profile for each animal. The use of arrhythmia scores has recently been evaluated (Curtis & Walker, 1988).

3 RESULTS

3.1 Pharmacology

3.1.1 Species Dependent Effects on the Cardiovascular System.

The effects of tedisamil have been reported to vary quantitatively with species (Buschmann *et al.*, 1989). Similar species dependent effects have been reported with amiodarone, d-sotalol and clofilium (Kopia *et al.*, 1985). Since little was known about the new drugs used in these studies, we initially sought to demonstrate whether or not they had class III effects on the ECG and BP. Data from these experiments could then be compared to more detailed assessments of class III actions, i.e., effects on refractoriness and AP morphology. Finally, antiarrhythmic efficacy was determined and interpreted according to the pharmacological profile obtained for each drug.

3.1.1.1 Effects on HR, BP & ECG in Rats

Tedisamil reduced heart rate significantly in all species tested. Comparison of Table 1 with Tables 3, 4, and 6 shows that the bradycardic actions of tedisamil (and KC8851) were greatest in those species with the highest resting heart rate (rats, guinea pigs). In conscious rats a dose-dependent bradycardia was seen with the 4 mg/kg dose producing a 34% drop in heart rate 4 minutes after infusion.

Table 1. Haemodynamic Effects of K^+ Channel Blockers in Conscious Rats.

Group	HEART RATE (b/min) predrug	HEART RATE (b/min) postdrug	BLOOD PRESSURE (mmHg) predrug	BLOOD PRESSURE (mmHg) postdrug
C1	403±16	418±15	120± 7	121± 6
T1	420±12	305± 7*	114± 3	124± 6
T2	395± 7	263± 6*	121± 3	145± 6*
T4	382±17	252±10*	119± 4	145± 5*
C2	436±16	417± 2	111± 4	111± 4
KC	416±13	260±11*	110± 4	122± 5
UK	397±11	376± 7	102± 4	105± 4
RP	376±11	382±12	105± 3	109± 3
R5	398±16	387±16	104± 4	105± 3
R10	420±12	414±14	104± 3	106± 5

The effects of treatment upon heart rate and mean arterial blood pressure in conscious rats are shown. Predrug values were recorded immediately prior to initiation of a 10 min infusion. Postdrug values were recorded 4 min after the infusion was complete. The symbol * indicates $p < 0.05$ versus predrug, by ANOVA and Duncan's range test (all predrug values were N.S. versus appropriate control group). The data are from two separate studies, each with their own control, and are shown here for sake of comparison. The groups in the first study are C1 = control, T1 = tedisamil (1 mg/kg), T2 = tedisamil (2 mg/kg), T4 = tedisamil (4 mg/kg). The groups in the second study are C2 = control, KC = KC8851 (4 mg/kg), UK = UK68,798 (1 mg/kg), RP = RP62,719 (1 mg/kg), R5 = risotilide (5 mg/kg), R10 = risotilide (10 mg/kg). n = 9 per group.

Similarly, 4mg/kg KC8851 produced a 37.5% drop in heart rate at this time period. On the other hand, risotilide 5 mg or 10 mg/kg, UK68798 (1mg/kg) and RP62719 (1 mg/kg) did not reduce heart rate significantly compared to control (Table 1).

Effects on BP in conscious rats: At doses of 2 & 4 mg/kg tedisamil and the 4 mg/kg dose of KC8851 blood pressure was elevated by 20% ($p < 0.05$) and 11% respectively, 4 minutes after infusion. The 1 mg/kg dose of tedisamil, as well as the doses of UK68798, risotilide, and RP62719, did not alter blood pressure in conscious rats. The elevation in blood pressure was maintained for the first hour after occlusion for only the 2mg/kg dose of tedisamil; all other treatments did not attenuate the decrease in blood pressure produced by coronary artery occlusion in conscious rats (section 3.4.3, Figure 17).

Effects on the ECG in conscious rats: None of the drug treatments (tedisamil, KC8851, UK68798, risotilide nor RP62719) prolonged the QRS duration when measured 4 minutes post infusion. Tedisamil (2 & 4 mg/kg) and KC8851 (4 mg/kg) infusions prolonged the P-R interval ($p<0.05$) when compared to predrug values. All doses of tedisamil and KC8851 markedly increased the Q-T_C interval while no significant effects were seen with UK68798, RP62719 nor risotilide in conscious rats (Table 2).

Table 2. ECG Effects of K^+ Channel Blockers in Conscious Rats.

Group	Q-Tc		P-R		QRS	
	pre	post	pre	post	pre	post
C1	215±10	210±10	41±1	42±1	22±1	22±1
T1	220±5	280±9*	38±1	44±1	22±1	23±1
T2	205±5	320±7**	38±2	47±1*	23±1	23±1
T4	195±5	330±9*	44±2	53±2*	22±1	23±1
C2	217±6	217±10	42±1	42±1	23±1	23±1
KC	211±5	332±10*	45±1	50±2*	23±1	25±1
UK	205±5	213±8	41±2	43±1	22±1	22±1
RP	197±7	202±6	46±1	47±1	23±1	22±1
R5	204±9	200±10	41±1	44±1	22±1	23±1
R10	203±4	206±4	45±2	47±2	24±1	24±1

The effects of treatment upon the ECG in conscious rats are shown. Values shown are in ms. Predrug values were recorded immediately prior to initiation of a 10 min infusion. Postdrug values were recorded 4 min after the infusion was complete. The symbol * indicates $p < 0.05$; ** indicates $p < 0.01$ versus predrug, by ANOVA and Duncan's range test (all predrug values were N.S. versus appropriate control group). The data are from two separate studies, each with their own control, and are shown here for sake of comparison. The groups in the first study are C1 = control, T1 = tedisamil (1 mg/kg), T2 = tedisamil (2 mg/kg), T4 = tedisamil (4 mg/kg). The groups in the second study are C2 = control, KC = KC8851 (4 mg/kg), UK = UK68,798 (1 mg/kg), RP = RP62,719 (1 mg/kg), R5 = risotilide (5 mg/kg), R10 = risotilide (10 mg/kg). n = 9 per group.

3.1.1.2 Effects in Anaesthetized Guinea Pigs

In acutely prepared anaesthetized guinea pigs, tedisamil produced a dose dependent bradycardia and widening of the Q-T_C. The bradycardic effect as well as the Q-T interval lengthening effects reached statistical significance after the second dose (i.e., cumulative 2 mg/kg i.v.) while the Q-T_C interval widening reached statistical significance after a total of 4 mg/kg i.v. On the other hand there were no effects on the QRS duration, and P-R intervals in this dose range (1 - 8 mg/kg). UK68,798 in the dose range of 10 - 160 µg/kg produced no significant effects on any ECG interval (Table 3).

Tedisimal and UK68,798 did not affect blood pressure in acutely prepared guinea pigs. The 8 mg/kg tedisamil dose when given rapidly occasionally precipitated VPB and VT. Also, arrhythmias which were characterized by alternating sinus brady/tachycardia i.e. sick sinus were seen at this dose.

3.1.1.3 Effects in Pigs

Tedisamil was least efficacious with regard to Q-T_C widening in pigs compared with other species at a dose producing a 25% reduction in HR (Buschmann *et al.*, 1989). However, it did cause a dose dependent bradycardia and Q-T_C

Table 3. Cardiovascular Effects of tedisamil and UK68,798 in Anaesthetized Guinea Pigs.

Dose	Q-Tc (ms)	P-R (ms)	QRS (ms)	HR b/min	BP (mmHg)
pre.	270±10	49±2	31±2	300±20	55±4
T1	280±10	52±1	30±2	220±15	65±5
T2	300±10	52±1	29±2	200±20*	65±8
T4	315±15*	52±/	29±2	190±20**	63±9
T8	330±/	54±/	27±/	160±/	65±/
pre.	260±10	57±4	31±2	300±15	42±5
UK10	265±15	54±3	31±2	260±20	42±5
UK20	265±15	54±3	31±2	265±20	43±4
UK40	260±15	53±3	32±2	270±20	41±4
UK80	260±15	54±3	31±2	260±20	39±5
UK160	265±10	53±3	31±2	270±20	39±6

The effects of treatment upon ECG intervals, heart rate and mean arterial blood pressure in anaesthetized guinea pigs are shown. Predrug values were recorded immediately prior to initiation of a cumulative dosing regimen. Doses were given at 15 min intervals and values were recorded 10 min after each dose. The data are from two separate studies, and are shown here for sake of comparison. The treatments indicated are, pre = predrug control, T1 = tedisamil (1 mg/kg), T2 = tedisamil (2 mg/kg), T4 = tedisamil (4 mg/kg), T8 = tedisamil (8 mg/kg), / indicates n = < 4 for particular value. The treatments in the second study are pre = predrug control, UK10 = UK68,798 (10 mg/kg), UK20 = UK68,798 (20 mg/kg), UK40 = UK68,798 (40 mg/kg), UK80 = UK68,798 (80 mg/kg), UK160 = UK68,798 (160 mg/kg), n = 6 throughout. All doses indicated are cumulative amounts given. The symbol * indicates $p < 0.05$; ** indicates $p < 0.01$ versus predrug, by ANOVA and Duncan's range test.

widening. On the other hand, P-R, QRS were not affected. Blood pressure was elevated dose dependently, up to a cumulative dose of 8 mg/kg. However, LVEDP was not affected. Unfortunately, the catheter inserted into the apex did not faithfully record LVP. Since peak systolic pressure was dampened using this technique, LVP and dP/dt data were excluded from analysis. This problem was rectified in studies with UK68,798 by catheterizing the ventricle via the carotid artery. UK68,798, (0 - 167.5 µg/kg) was without effects on HR, BP, LVP, RVP, dp/dt or any ECG intervals except Q-T_C interval which rose by 21% after the 20 µg/kg dose (37.5 µg/kg cumulative). (Tables 4 & 5).

3.1.1.4 Effects in Primates

Baboons and macaque monkeys responded similarly to tedisamil with dose dependent elevations in diastolic and systolic BP, reductions in HR and prolongations of the Q-U_C interval. The bradycardic effect maximized at 1 mg/kg, but the Q-U continued to widen. In baboons and monkeys the P-R interval and QRS duration also tended to widen at doses above 0.5 mg/kg (Tables 6 & 7). In baboons (n=2, for each drug), UK68,798 (50 µg/kg), risotilide (0.3 mg/kg) and RP62,719(0.4 mg/kg) also tended to increase the Q-U_C interval, by a maximum of 30%, without ancillary effects on HR, BP or other ECG intervals (data not shown).

Table 4. Cardiovascular Actions of Tedisamil and UK68,798 in Pigs.

	Haemodynamics (mm/Hg)				
Dose	MAP	LVP	LVEDP	dP/dt	RVP
pre	93±1	/	1.5±2	/	/
T1	97±4	/	1.3±1	/	/
T2	103±4	/	1.8±2	/	/
T4	106±6	/	6.0±4	/	/
T8	(112±5	/	4.5±2	/	/)
pre	86±7	119± 9	3±1	2800±300	27±4
UK2.5	85±6	118± 9	3±1	2700±300	27±4
UK7.5	89±8	116± 9	2±1	2700±300	28±4
UK20	87±7	118±10	1±1	2800±300	28±4
UK40	88±9	112±12	0±1	2600±300	28±4
UK80	90±10	112±13	0±1	2700±300	27±4
UK160	93±10	111± 9	0±1	2700±300	28±5

The effects of treatment upon haemodynamics in anaesthetized pigs are shown. Predrug values were recorded prior to initiation of a cumulative dosing regimen. Doses were given at 15 min intervals for tedisamil and 5 min intervals for UK68,798. Values were recorded 5 min after each dose. The data are from separate studies, shown here for comparison. The cumulative doses indicated are, pre = predrug control, T1 = tedisamil (1 mg/kg), T2 = tedisamil (2 mg/kg), T4 = tedisamil (4 mg/kg), T8 = tedisamil (8 mg/kg), UK2.5 = UK68,798 (2.5 μg/kg), UK7.5 = UK68,798 (7.5 μg/kg), UK20 = UK68,798 (17.5 μg/kg), UK40 = UK68,798 (37.5 μg/kg), UK80 = UK68,798 (77.5 μg/kg), UK160 = UK68,798 (157.5 μg/kg). The symbol * indicates $p < 0.05$ versus predrug, by ANOVA and Duncan's multiple range test. T8 values obtained between episodes of arrhythmias, therefore are approximate. Mean ± S.E.M.

Table 5. Effects on the ECG of Tedisamil and UK68798 in Pigs

	ECG Intervals (ms)			HR (b/min)
Dose	QRS	P-R	Q-Tc	
pre	46±1	96±4	345± 4	125±9
T1	47±1	98±4	361± 6	104±4
T2	46±1	98±5	369±14	101±3
T4	47±1	95±6	391±18*	104±3
T8	/	/	/	sinus arrhythmias
pre	39±2	84±5	306± 9	141±11
UK2.5	40±3	82±5	332±13	140±10
UK7.5	41±3	82±5	333±14	139±11
UK20	41±2	83±5	341±17*	132±13
UK40	41±2	83±5	354±17*	128±12
UK80	41±2	84±4	346±17*	127±12
UK160	42±2	84±5	340±30	132±14

The effects of treatment upon ECG intervals and heart rate in anaesthetized pigs are shown. Predrug values were recorded prior to initiation of a cumulative dosing regimen. Doses were given at 15 min intervals for tedisamil and 5 min intervals for UK68,798. Values were recorded 5 min after each dose. The data are from separate studies, shown here for comparison. The cumulative doses indicated are, pre = predrug control, T1 = tedisamil (1 mg/kg), T2 = tedisamil (2 mg/kg), T4 = tedisamil (4 mg/kg), T8 = tedisamil (8 mg/kg), UK2.5 = UK68,798 (2.5 μg/kg), UK7.5 = UK68,798 (7.5 μg/kg), UK20 = UK68,798 (17.5 μg/kg), UK40 = UK68,798 (37.5 μg/kg), UK80 = UK68,798 (77.5 μg/kg), UK160 = UK68,798 (157.5 μg/kg). The symbol * indicates $p < 0.05$ versus predrug, by ANOVA and Duncan's multiple range test. Mean ± S.E.M.

Table 6. BP and HR Effects of Tedisamil in Primates.

Dose (mg/kg)	Blood pressure (BP) Systolic (mmHg)	Diastolic	Heart Rate (HR) (beats/min)
	BABOONS		
	Study (i) (n = 3-5)		
Pre-drug	159±15	99±4	101±6
0.05	+6±5	+5±3	-6±3
0.10	+7±2	+3±2	-7±4
0.2	+13±2	+3±2	-20±11
0.4	+18±6	+4±3	-20±7
0.8	+20±11	+19±13	-29 /
1.6	+25±3	+19 /	-30 /
	Study (ii) (n = 4-5).		
Pre-drug	160±5	117±4	108±3
0.5	+9±2	+8±1	-16±3
1.0	+10±8	+6±3	-27±5
2.0	+10±8	+5±3	-27±5
	MONKEYS (n = 5)		
Pre-drug	104±5	68±5	144±6
0.05	+2±2	+5±2	-14±2
0.1	+5±5	+5±2	-22±4
0.2	+8±6	+9±6	-32±8
0.4	+13±7	+15±8	-43±12
0.8	+22±3	+17±3	-52±9
1.6	+31±4	+20±5	-59±10

The bradycardic and hypertensive responses to tedisamil are shown. Two different studies (i and ii) were performed in baboons. In study (i) the cumulative doses were 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 mg/kg while in study (ii) the cumulative doses were 0.5, 1.0 and 2.0 mg/kg i.v. Values = Mean ± S.E.M. where $n > 3$; otherwise, only Mean is given. In the pre-drug period actual values are given, all others are changes from pre-drug. The symbol (-) indicates a decrease from pre-drug whereas (+) indicates an increase. Statistical analyses for trends (correlation) showed increasing changes in variables with increasing doses which were statistically significant at $p < 0.01$ (diastolic pressures) to $p < 0.001$ (heart rate) in both species.

Table 7. ECG Effects of Tedisamil in Primates.

Dose	P-R (ms)	QRS (ms)	Q-U (ms)	$Q\text{-}U_C$ (ms)
		BABOONS		
		Study (i) (n = 3-5).		
Pre-drug	116±7	34.4±0.9	332±18	413±19
0.05	+2.8±2.7	+2.2±1.8	+25±9	+20±8
0.1	-2.3±2.9	+3.4±1.7	+53±13	+57±10
0.2	+4.0±2.7	+4.5±1.8	+86±21	+65±20
0.4	+3.8±3.1	+5.3±1.1	+152±37	+108±17
0.8	-1.7±1.2	+3.5±1.5	+165±41	+133±42
		Study (ii) (n = 4-5).		
Pre-drug	135±7	34±2	302±19	401±16
0.5	+2±3	+0.8±0.8	+99± 5	+89±20
1.0	+7±1	+1.4±2.3	+158±47	+118±25
2.0	+11±1	+2.8±1.5	+263±75	+181±15
		MONKEYS (n = 5)		
Pre-drug	95±9	23± 2	262± 9	413±19
0.05	+8±4	-0.3±1.0	+36±11	+15±14
0.1	+9±4	+0.3±0.8	+56±10	+42±14
0.2	+9±5	+1.0±2.2	+86±27	+52±23
0.4	+13±6	+2.0±2.2	+121±28	+100±36
0.8	+15±7	+1.8±1.7	+173±70	+109±52
1.6	+22±3	+3.3±1.7	+271±77	+152±72

In order to assess the effects of tedisamil on the ECG, the P-R, QRS and Q-U intervals were measured. The Q-U interval was corrected for rate ($Q\text{-}U_C$) by Bazett's, square root of R-R correction. Values = Mean ± S.E.M. where $n > 3$ otherwise only Mean is given. In the pre-drug period actual values are given, all others are changes from pre-drug. The symbol (-) indicates a decrease from pre-drug whereas (+) indicates an increase. Time had no consistent effect. Statistical analyses of trends showed that variables with increasing dose were statistically significant at $p < 0.05$ for P-R changes, and $p < 0.001$ for Q-U and Q-Uc.

3.2 Electrical Stimulation Studies

3.2.1 Tedisamil vs. Class Is in Rats

In an initial attempt to develop a reusable conscious rat model for electrical stimulation a method using an electrode carriage was tried. The intention was to use a crossover design, so eliminating between rat variability. This method was successful in that the electrodes could be reversibly positioned against the ventricle. However in order to have stable threshold values, the animal had to be anaesthetized. This latter requirement nullified the benefit accrued by the time-consuming and intricate preparative surgery. However, this method was used to compare tedisamil with representatives of the three Class I subclasses; quinidine (1a); mexiletine (1b) and propafenone (Ic). Later electrical stimulation studies were performed in acutely prepared pentobarbitone anaesthetized animals.

The effects of tedisamil were different from those of the Class I drugs. Tedisamil prolonged ERP and decreased MFF markedly but did not elevate VF_t. However, after 4 mg/kg tedisamil, the characteristic fibrilloflutter could not be induced by electrical stimulation. Instead, a VT at a maximum heart rate of 7 Hz was all that could be achieved by this "burst pacing" method (50 Hz, 0.8 ms). Class I drugs increased VF_t in a dose dependent fashion with propafenone having the lowest EC_{50} for this effect.

Propafenone also had the greatest potency in prolonging QRS duration which is consistent with its Class 1c classification. The class 1 drugs widened P-R intervals with apparent EC_{50}s of 8 mg/kg. Tedisamil widened the QRS duration and Q-T_C interval significantly in this preparation with only minimal effects on the P-R interval. Tedisamil was eight times more potent than quinidine at widening the Q-T_C interval (Figures 1 & 2). These results have been published previously (Walker and Beatch, 1988).

3.2.2 Tedisamil vs. UK68,798 in Rats

After abandoning attempts at developing a conscious reusable model of electrical stimulation, we tested tedisamil and UK68798 in acutely prepared, pentobarbitone (45 mg/kg i.p.) anaesthetized rats. Results with tedisamil were the same as those in the chronically prepared halothane anaesthetized model (Figure 3), and were less time consuming and less invasive. In acutely prepared animals, there was of course no risk of a surgically induced infarct myocardial adhesion influencing the results. However, in a few cases if the stab electrodes damaged the ventricle, which caused an unsteady baseline of threshold values, results from such animals were discarded.

As seen in the previous study (3.2.1) tedisamil prolonged the ERP and rendered the heart refractory to VF

Figure 1 shows the responses to electrical stimulation of halothane anaesthetized chronically prepared rats treated with tedisamil, quinidine, mexiletine, or propafenone in a cumulative dosing regimen. Values shown are mean ± S.E.M. (n = 6) for: change in threshold voltage for induction of fibrillation, (VFt); decrease in maximum following frequency, (MFF); and increase in effective refractory period, (ERP). Curves were fit (second order polynomial) by Slidewrite software. Determinations were made 10-15 min after each dose. Dosing interval was 15 min.

Figure 1. Electrical Stimulation: Tedisamil vs. Class I Drugs.

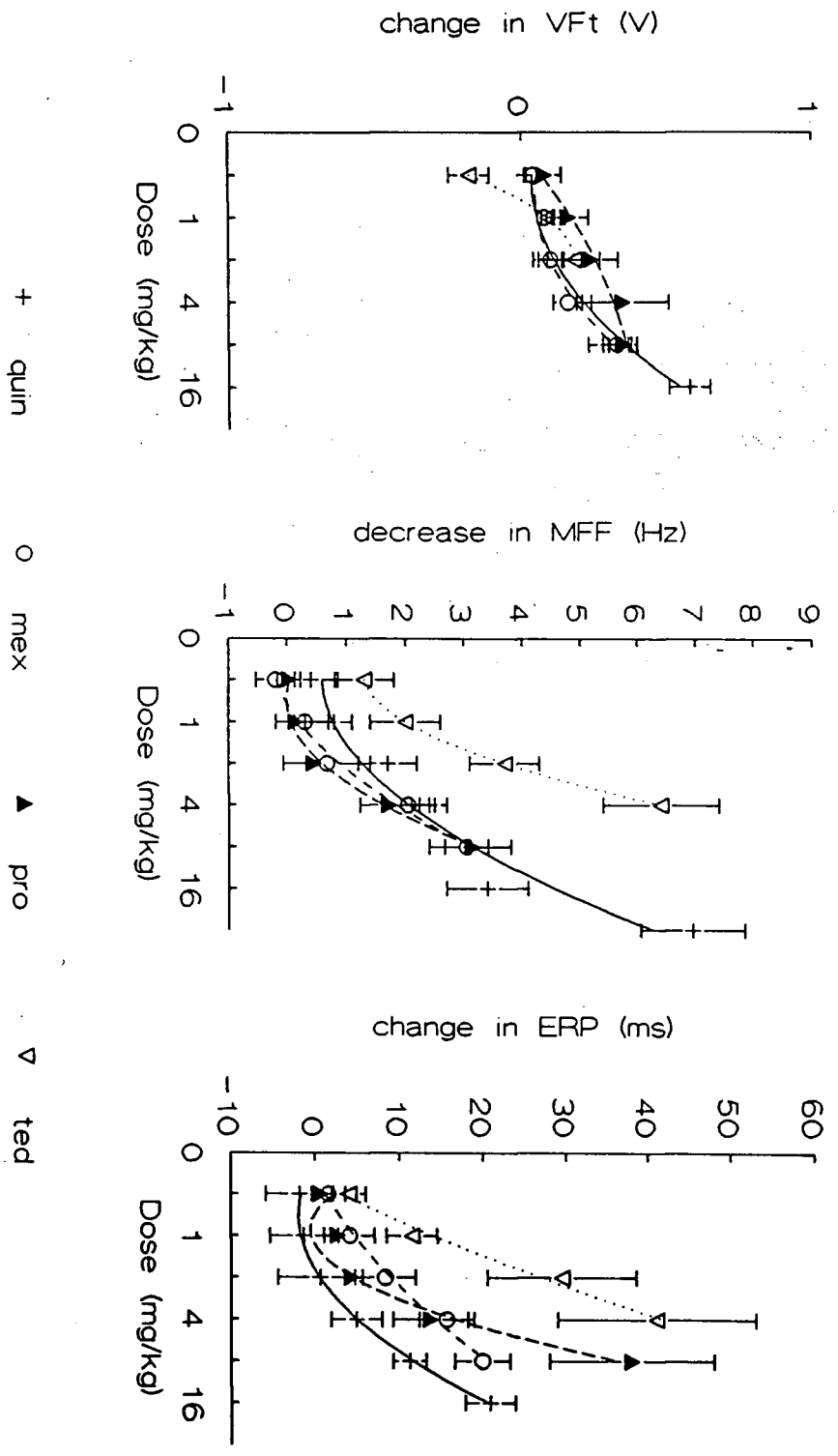


Figure 2 shows the ECG responses to drug treatment in halothane anaesthetized chronically prepared rats treated with tedisamil, quinidine, mexiletine, or propafenone in a cumulative dosing regimen. Values shown are mean ± S.E.M. (n = 6) for: change in QRS duration; P-R interval; and Q-T_C interval (x1000). Curves were fit (second order polynomial) by Slidewrite software. Measurements were made 10 min after drug infusion. Increasing doses were given at 15 min intervals.

Figure 2. ECG Effects of Tedisamil & Class I Drugs.

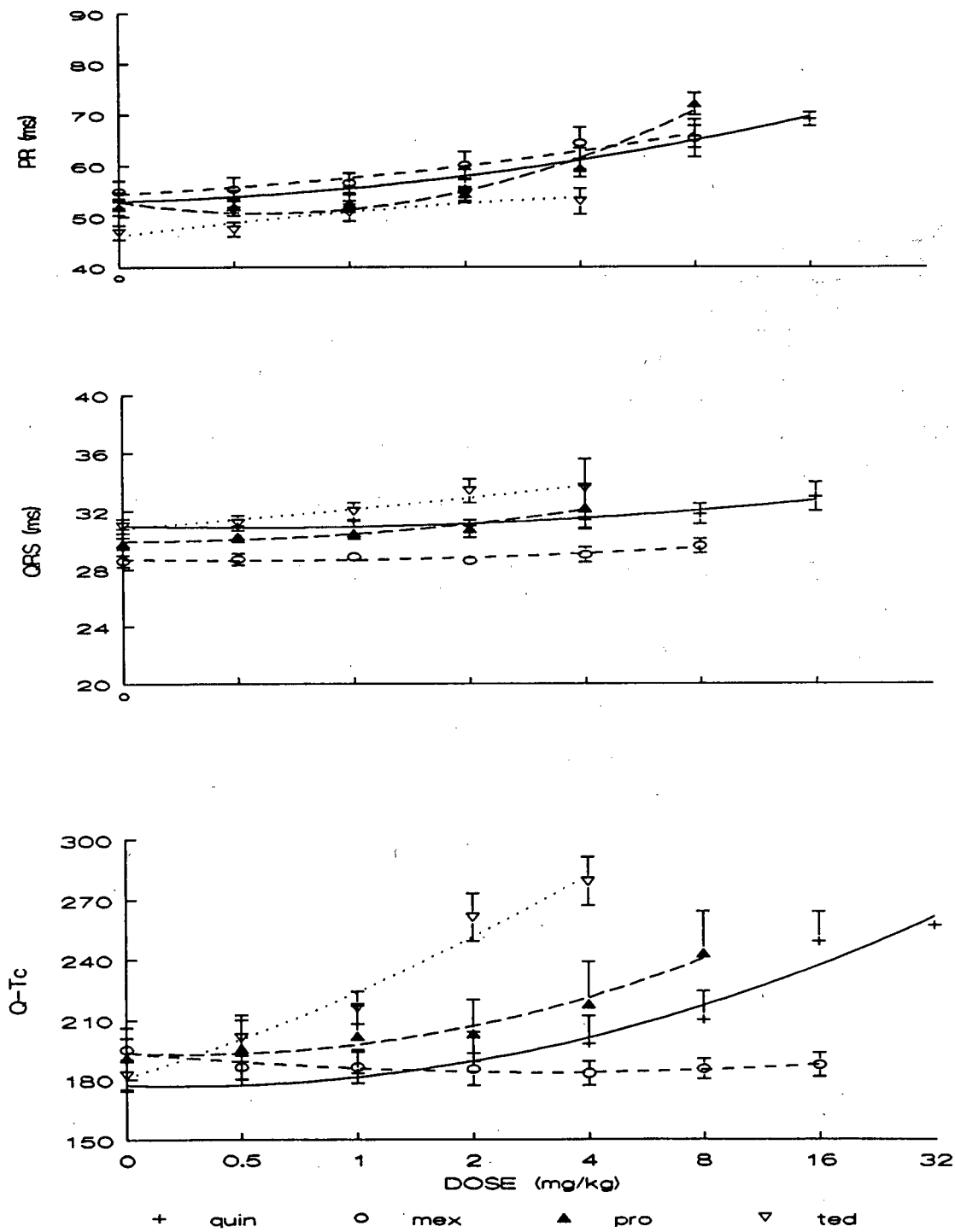
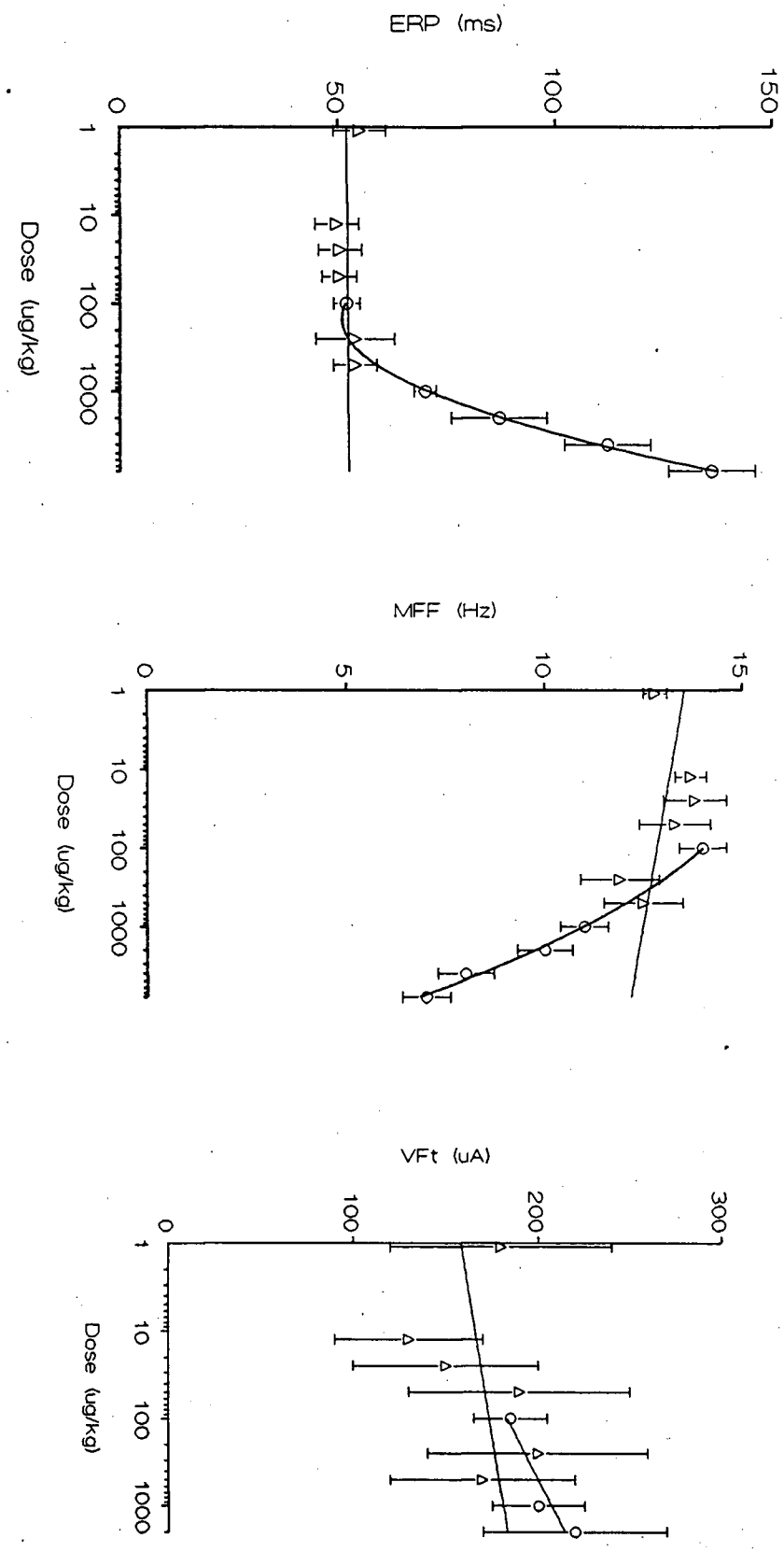


Figure 3 shows the response to electrical stimulation in pentobarbitone anaesthetized acutely prepared rats, treated with tedisamil or UK68,798. Values shown are mean ± S.E.M. for: ventricular effective refractory period, ERP; maximum following frequency, MFF; and ventricular fibrillation threshold, VF_t. Curves were fit (second order polynomial) by Slidewrite software. Determinations were made 10-15 min after each dose. Dosing interval was 15 min.

Figure 3. Electrical Stimulation Studies in Rats: Tedisamil vs. Uk68,798.



induction. UK68,798 had no significant effects on either MFF, ERP, or VF_t (Figure 3). UK68798 also had no effects on the ECG intervals of QRS, P-R, Q-T_c or R-R while tedisamil again prolonged the Q-T_c interval and slowed HR. In this study the P-R and QRS intervals were not prolonged significantly by tedisamil treatment (Figure 4).

3.2.3 Tedisamil *vs.* UK68,798 in Guinea Pigs

UK68,798 tended to be more potent, but much less efficacious, than tedisamil in prolonging Q-T_c intervals and ERP in guinea pigs. However, these trends did not reach significance (unweighted means ANOVA), nor did any of the other ECG effects or VF_t. Tedisamil did however dose dependently widen the R-R, QT_c and ERP ($p < 0.05$) while not affecting QRS, P-R nor VF_t (Table 3 and Figure 5).

3.2.4 Tedisamil in Baboons

In electrical stimulation studies in baboons, tedisamil had no significant effects on stimulation threshold for capture (of a 25% increase in rate train), but tedisamil did decrease MFF by a maximum of 26% after 1.0 mg/kg i.v. In contrast, when the right atrium was stimulated via the oesophagus, the ventricular MFF was not significantly decreased by tedisamil, which suggested that A.V. node refractoriness was not affected by tedisamil treatment (Figure 6).

Figure 4 shows the ECG responses to tedisamil or UK68,798 treatment in the same pentobarbitone anaesthetized acutely prepared rats as shown in figure 3. Values shown are mean ± S.E.M. for: heart rate, HR; QRS duration; P-R interval; and Q-T_C interval. Curves were fit (second order polynomial) by Slidewrite software. Measurements were made 10 min after drug infusion. Increasing doses were given at 15 min intervals.

Figure 4. ECG Effects of Tedisamil & UK68,798 in Anaesthetized Rats.

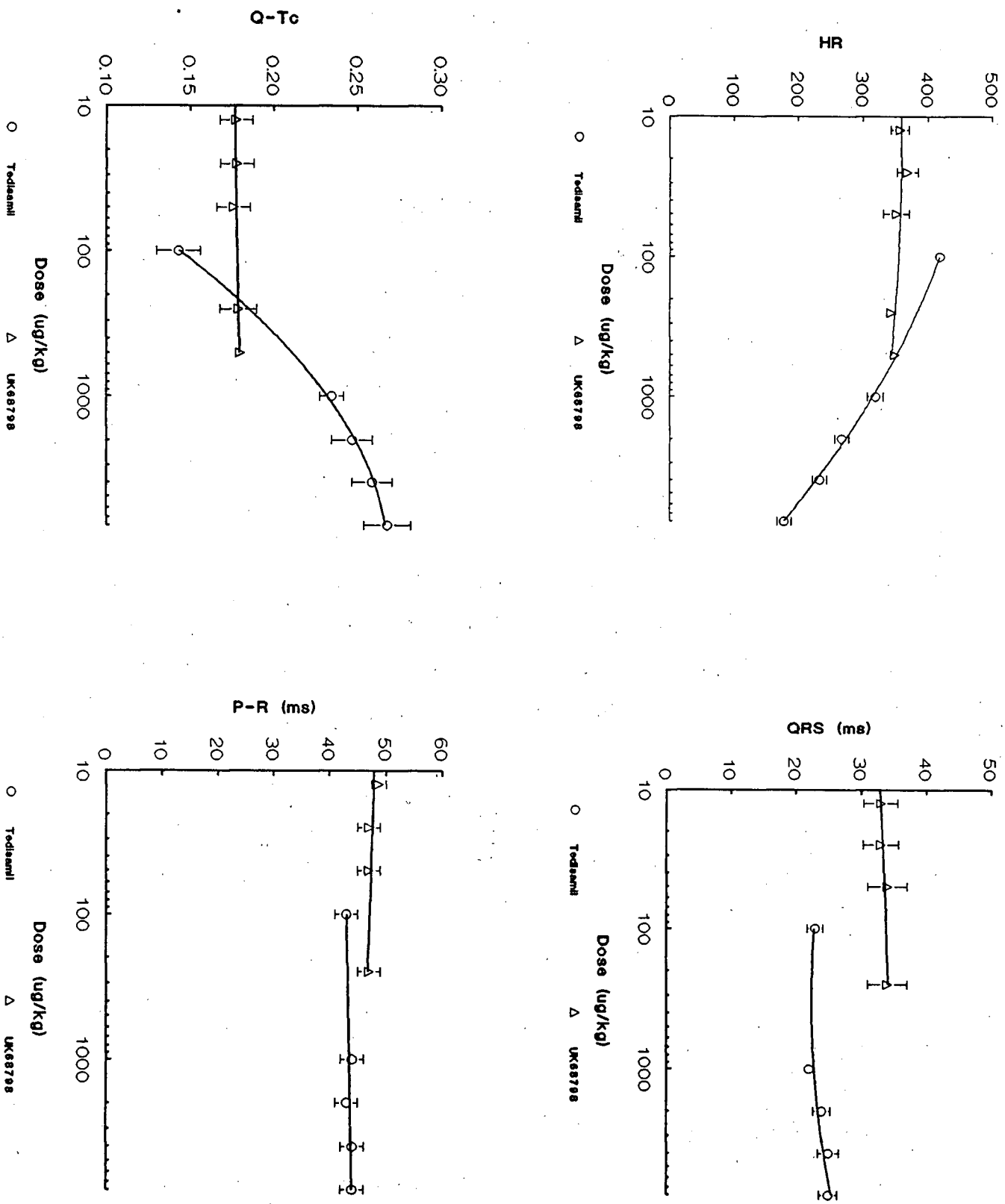


Figure 5 shows the response to electrical stimulation in urethane anaesthetized acutely prepared guinea pigs, treated with tedisamil or UK68,798. Values shown are mean ± S.E.M. for: ventricular effective refractory period, ERP; maximum following frequency, MFF; and ventricular fibrillation threshold, VF_t. Curves were fit (second order polynomial) by Slidewrite software. Determinations were made 10-15 min after each dose. Dosing interval was 15 min.

Figure 5. Electrical Stimulation Studies in Guinea Pigs: Tedisamil vs. UK68,798.

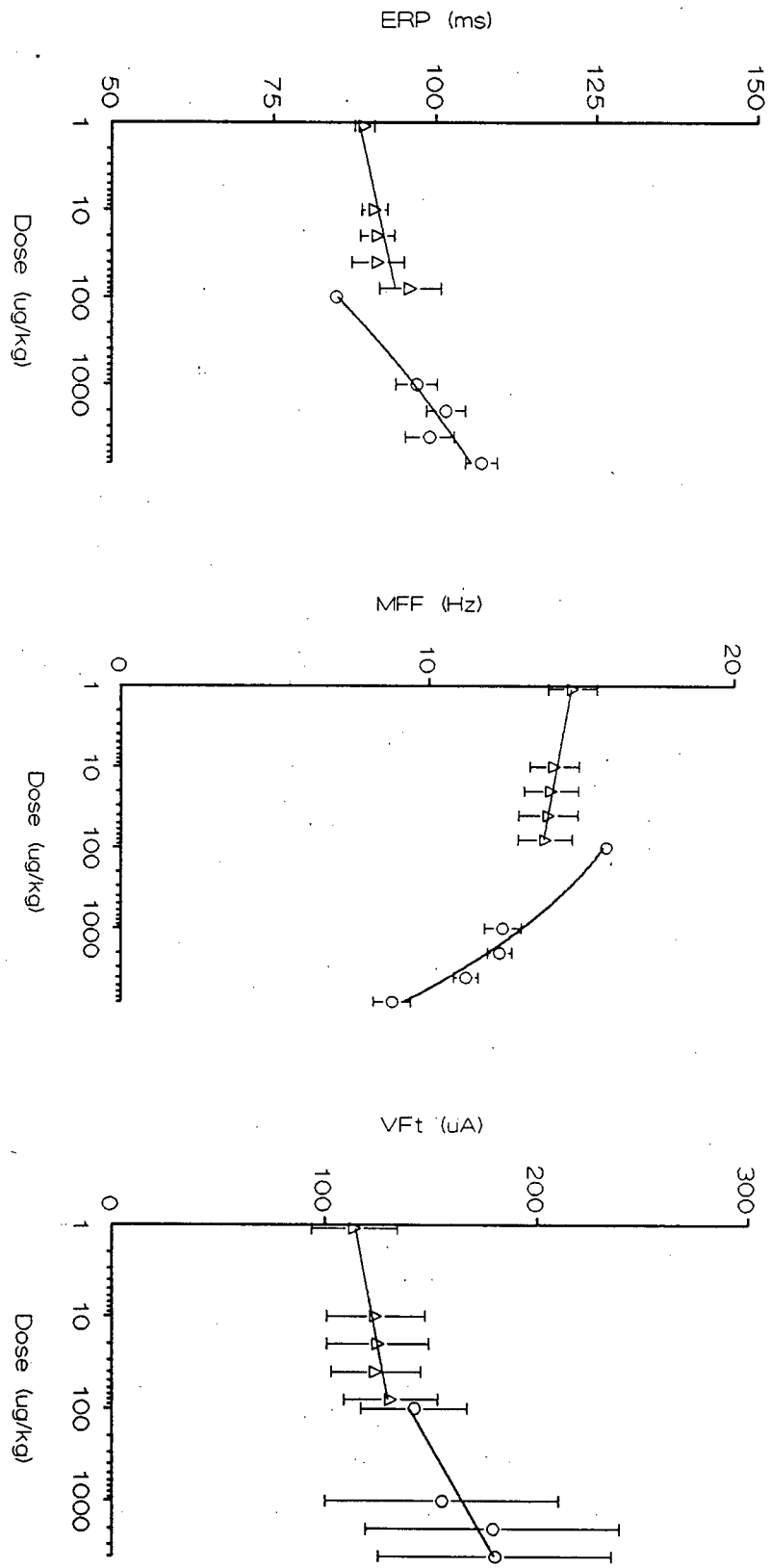
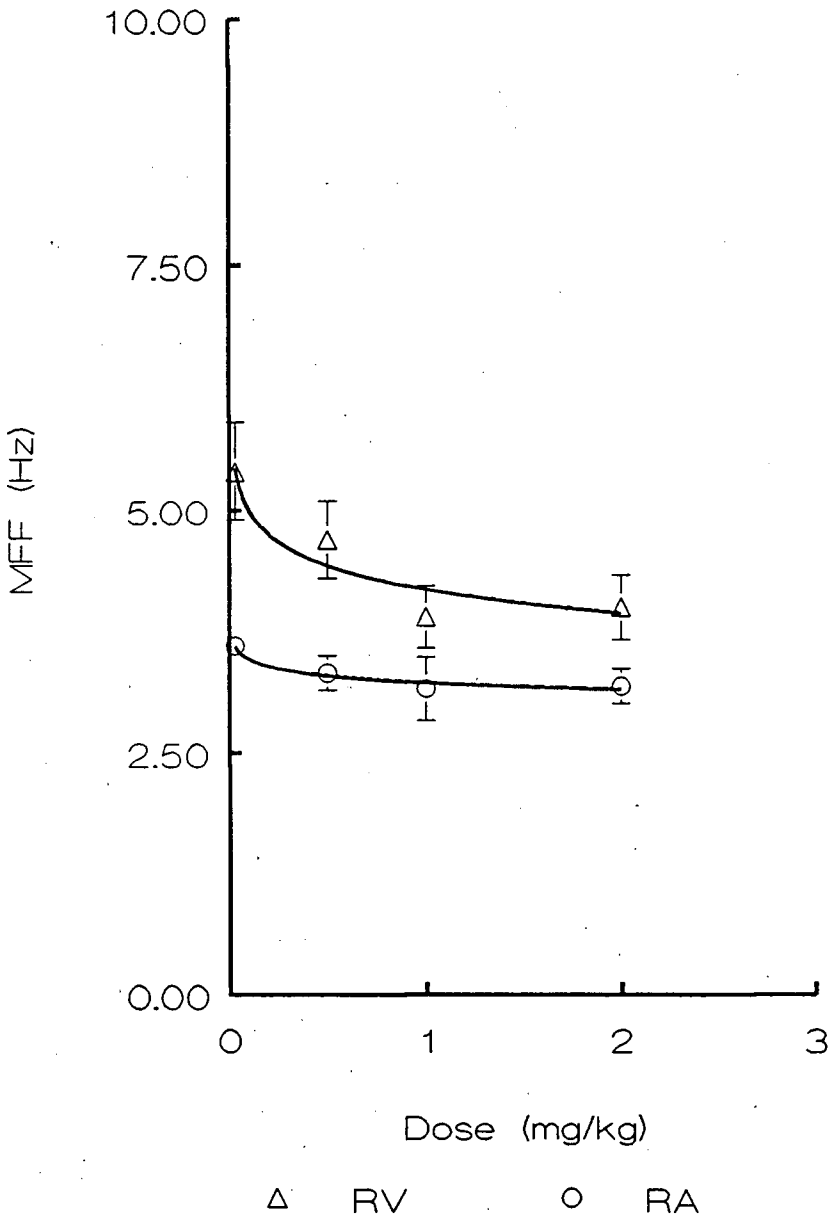


Figure 6. Effects of Tedisamil on MFF in Baboons.



The effects of tedisamil on ventricular responses to electrical stimulation of the right atrium (RA) and right ventricle (RV) in anaesthetized baboons are shown. Cumulative doses of 0.5, 1.0, and 2.0 mg/kg were given. Means and S.E.M. are shown.

3.2.5 Summary

In non-ischaemic hearts *in vivo*, tedisamil prolonged ventricular refractoriness in rats, guinea pigs and baboons. The increase in refractory periods were not a result of bradycardia as MFF was lowered by tedisamil and ERP was determined by the extra stimulus method at a fixed rate. When compared with class I drugs, tedisamil had a different profile of action on electrical stimulation. Tedisamil was without effects on the threshold for electrical induction of VF, but in rats tedisamil ($\geq$4 mg/kg) treatment prevented the electrical induction of VF.

UK68,798 was far less efficacious than tedisamil and did not produce any significant effects on MFF, ERP and VF_t nor on any of the ECG intervals in anaesthetized rats or guinea pigs.

3.3 Intracellular Recording Studies *In Vivo*.

These investigations were carried out as an effort to explore the mechanism of action of tedisamil and UK68798 in electrophysiological terms. Doses were similar to those used in other studies. In order to allow for comparisons between studies, a similar degree of $Q\text{-}T_c$ interval prolongation was assumed to indicate similar plasma concentrations for each drug.

3.3.1 Tedisamil in Rats

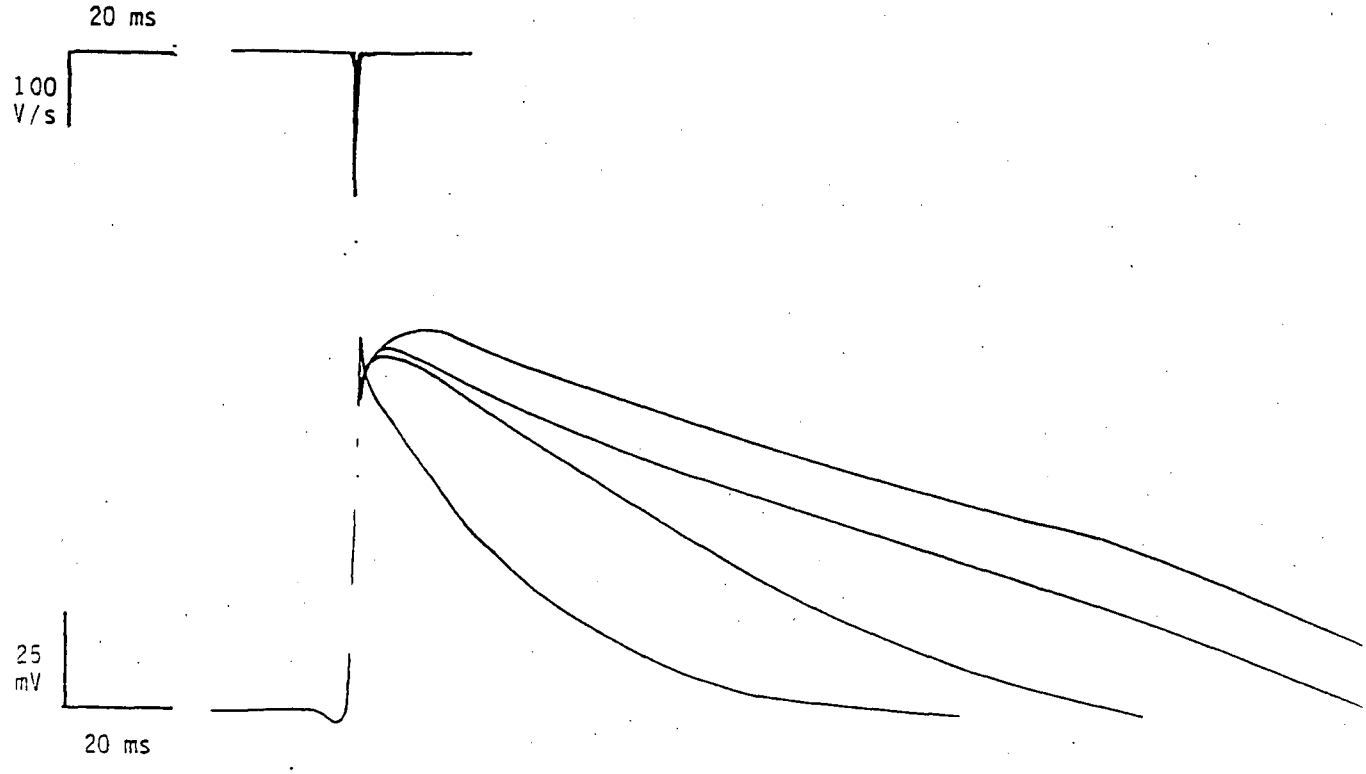
Tedisamil dose-dependently widened rat action potentials (AP). The early phase of rapid repolarization was delayed markedly, such that the normally narrow rat epicardial AP appeared similar to that seen in larger mammalian species. The later stage of repolarization (phase 3) was not appreciably slowed. The slope of the early repolarization phase was reduced 5 fold by 4 mg/kg tedisamil. As a result of this decreased rate of repolarization the APD at 10, 25, 50, and 75%, repolarization was prolonged 5 fold after 8 mg/kg cumulative. Concomitant to these highly significant APD effects, tedisamil slightly elevated AP height and only decreased AP rise rate at one dose level (Figure 7).

3.3.2. Tedisamil in Guinea Pigs

In guinea pigs tedisamil also tended to widen the APD although only by a maximum of 80%. This effect was substantially reduced by bolus injections of adrenaline (6.7 µg/kg and 13 µg/kg) which restored HR to pre-tedisamil levels. There were no consistent trends in either AP height or rise rate after tedisamil treatment. Both the plateau (phase 2) and late repolarization (Phase 3) stages of repolarization were decreased by 50% at 4 mg/kg cumulative tedisamil (Figure 8).

Figure 7 shows the effects of tedisamil treatment on ventricular epicardial AP morphology in acutely prepared pentobarbitone anaesthetized rats. Composite representative AP are drawn and mean ± S.E.M. values (n=6) are given for: action potential height, AP; maximum rise rate, +dV/dt; action potential duration at 10, 25, 50 and 75% repolarization, APD10 etc.; and repolarization rate of the "plateau", -dV/dtp. Values obtained 10 min after dosing are shown. Cumulative doses were given at 15 min intervals. *$p < 0.05$, **$p < 0.01$ from control.

Figure 7. Electrophysiological Effects of Tedisamil in Rats.

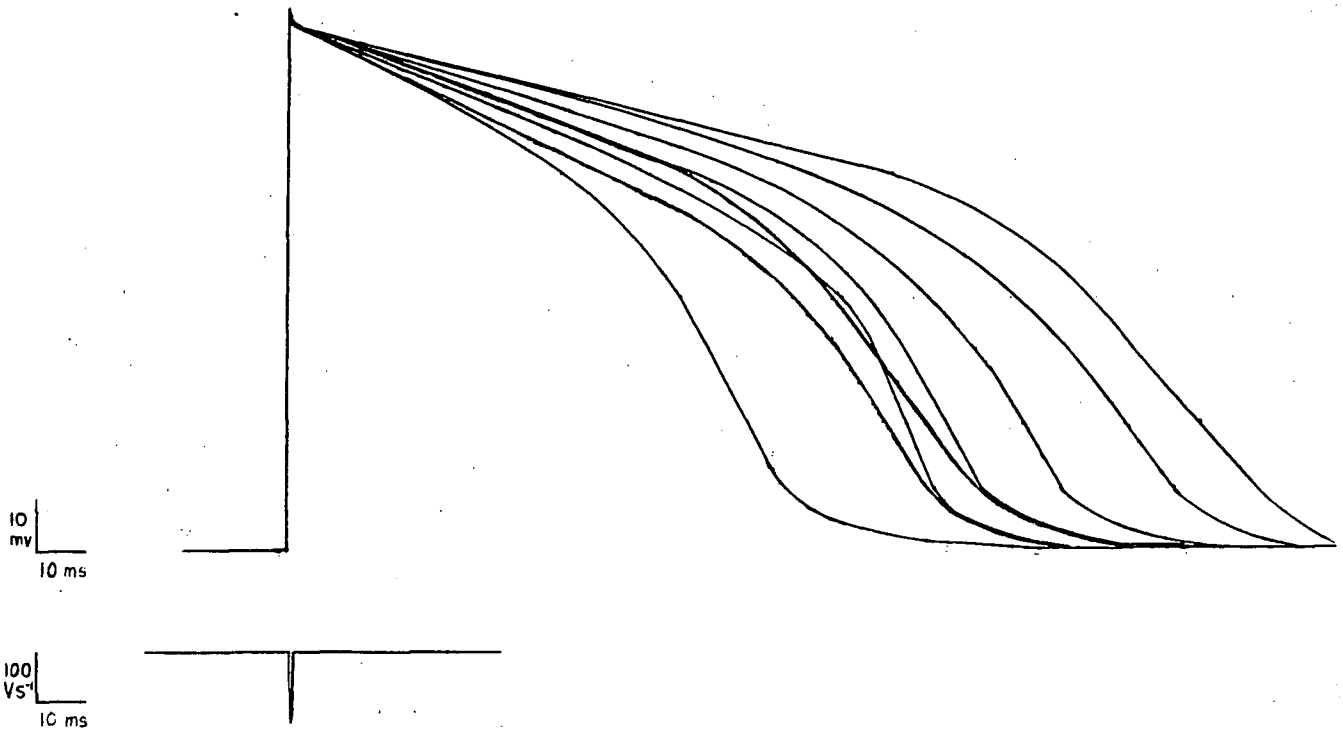


TEDISAMIL in Rats

Dose mg/kg	AP mV	+dV/dt V/s	APD10 ms	APD25 ms	APD50 ms	APD75 ms	-dV/dtp V/s
con	97±3	183±6	5±1	10±1	19±1	45±3	2.7±0.2
0.5	103±3	173±8	8±1	19±2*	41±3**	81±5**	1.0±0.3*
1.0	105±2	175±7	11±1**	26±2**	58±6**	95±4**	0.9±0.2*
2.0	110±2	170±15	17±1**	37±1**	69±2**	114±3**	0.7±0.2**
4.0	102±4	115±18*	24±4**	53±8**	100±14**	162±22**	0.5±0.2**
8.0	107	165	29	67	125	195	0.5

Figure 8 shows the effects of tedisamil treatment on ventricular epicardial AP morphology in acutely prepared urethane anaesthetized guinea pigs. The effects of adrenaline on tedisamil induced changes are also shown. Composite representative AP are drawn and mean ± S.E.M. values are given for: action potential height, AP; maximum rise rate, dV/dt; action potential duration at 25, 50 and 75% repolarization, APD25 etc.; repolarization rate of the "plateau", -dV/dtp; and phase 3 repolarization rate, -dV/dt. Values obtained 5 min after dosing are shown. Cumulative doses were given at 15 min intervals (n=4). *$p < 0.05$, **$p < 0.01$ from control.

Figure 8. Electrophysiological Effects of Tedisamil in Guinea Pigs.



TEDISAMIL in Guinea pigs

Dose mg/kg	AP mV	dV/dt V/s	APD25 ms	APD50 ms	APD75 ms	-dV/dt*p* V/s	-dV/dt V/s
con	105±6	140±20	54±3	78±2	91±2	0.52±.05	2.6±.3
0.5	110±7	160±20	72±6	109±9	122±6	0.37±.04**	1.9±.1
1.0	105±7	145±25	81±4*	115±5*	130±11*	0.36±.03**	1.9±.3**
2.0	100±7	135±20	93±8**	126±13**	146±11*	0.31±.05**	1.6±.2**
4.0	107±7	140±30	105±8**	144±11**	165±13**	0.28±.04**	1.3±.2**
8.0	110	130	115	155	180	0.26	1.1
Adren ug/kg							
6.7	106±4	140±20	80±7	108±15	126±18	0.42±.04	1.6±.2
13	100	160	60	100	118	0.40	1.5

3.3.2.1 Effects of Vagal Stimulation

The vagus nerve was stimulated in rats and guinea pigs in order to study the direct effects of bradycardia on ventricular AP. The results of these experiments showed that guinea pigs APD widening was rate dependent *in vivo* while rats showed no such sensitivity. This study demonstrated that the bradycardic effect of tedisamil was not a factor in its ability to widen the APD in rats, but that bradycardia produced in guinea pigs would have had a major contribution to tedisamil's action in the species (Figure 9).

3.3.3 UK68798 in Rats *In Vivo*

In rats UK68798 was without effects on rise rate, AP height, and APD_{25}. Slight prolongation of APD_{50} and APD_{75} also did not reach statistical significance (by ANOVA). It was potent (apparent EC_{50} 25µg/kg) despite its low efficacy in this species. The early phase of repolarization, which is rapid in the rat, was not affected by UK68798 treatment, while slight slowing of the (Phase 3) final repolarization was seen (Figure 10). Comparison of figures 7 & 10 also show the slight variability in morphology (predrug) between individual action potentials recorded from rat epicardium.

Figure 9 shows the effects of vagal stimulation on ventricular epicardial action potential duration, APD, in guinea pigs (top) and rats (bottom). Animals were prepared as for treatment with tedisamil or UK68,798, except their left vagal nerve was stimulated in order to slow the heart rate. Each point represents a single recording of APD vs. R-R interval. Curves were fit (second order polynomial) by Slidewrite software.

Figure 9. Effects of HR on APD in G. Pigs and Rats.

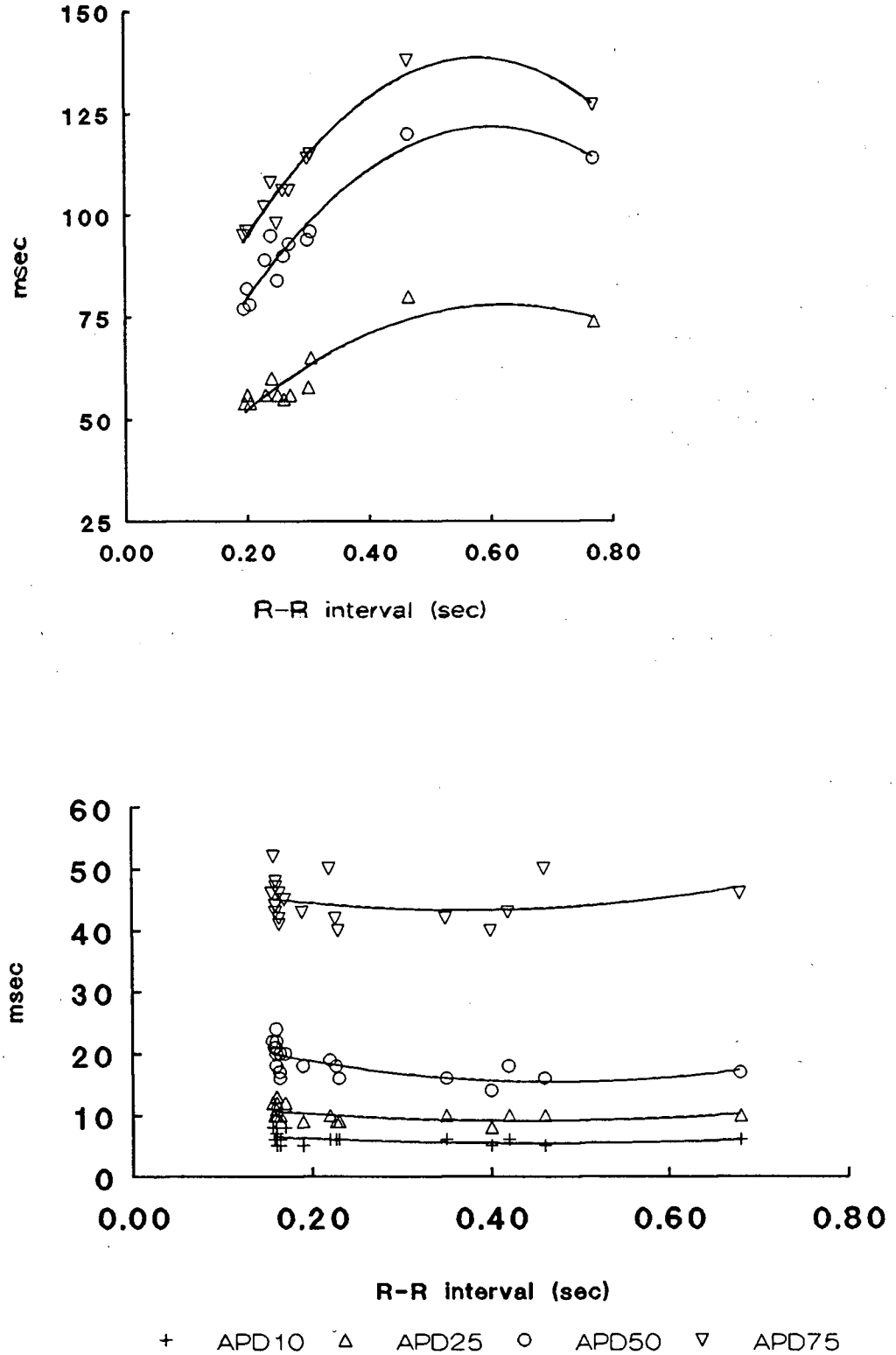
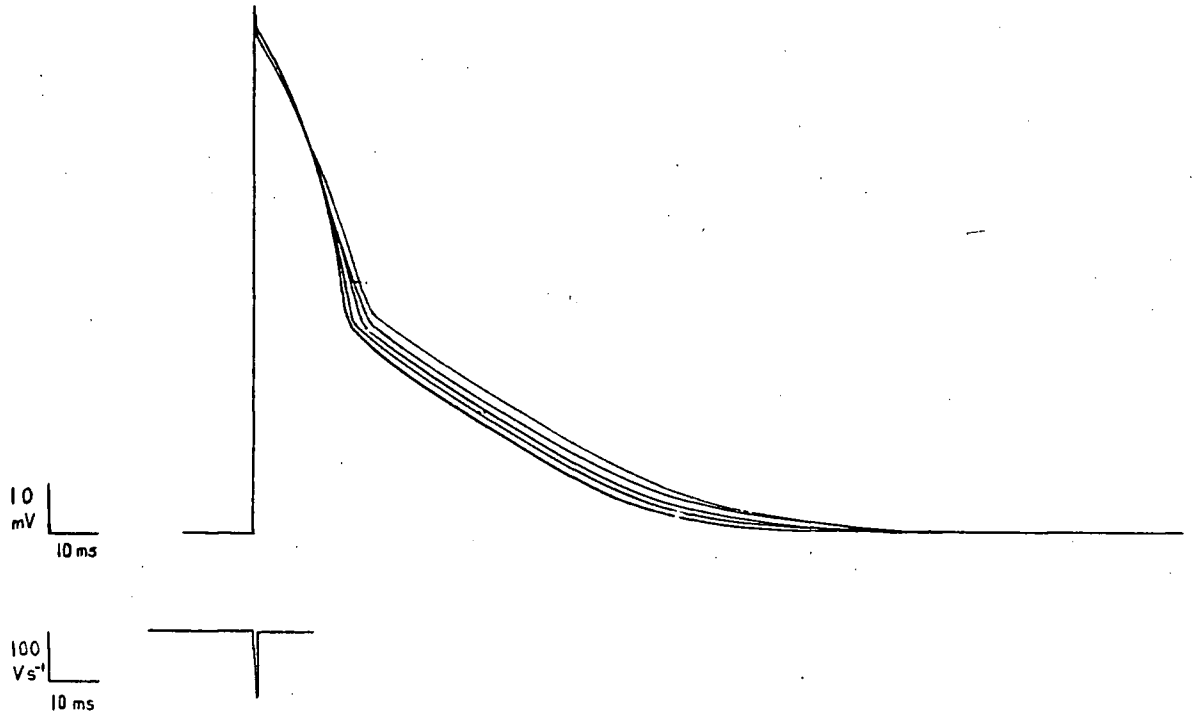


Figure 10 shows the effects of UK68,798 treatment on ventricular epicardial AP morphology in acutely prepared pentobarbitone anaesthetized rats. Composite representative AP are redrawn and mean ± S.E.M. values are given for: action potential height, AP; maximum rise rate, +dV/dt; action potential duration at 25, 50 and 75% repolarization, APD25 etc.; repolarization rate of the "plateau", -dV/dtp; and phase 3 repolarization rate, -dV/dt. Values obtained 10 min after dosing are shown. Cumulative doses were given at 15 min intervals (n=3). *$p < 0.05$ from control.

Figure 10. Electrophysiological Effects of UK68,798 in Rats.

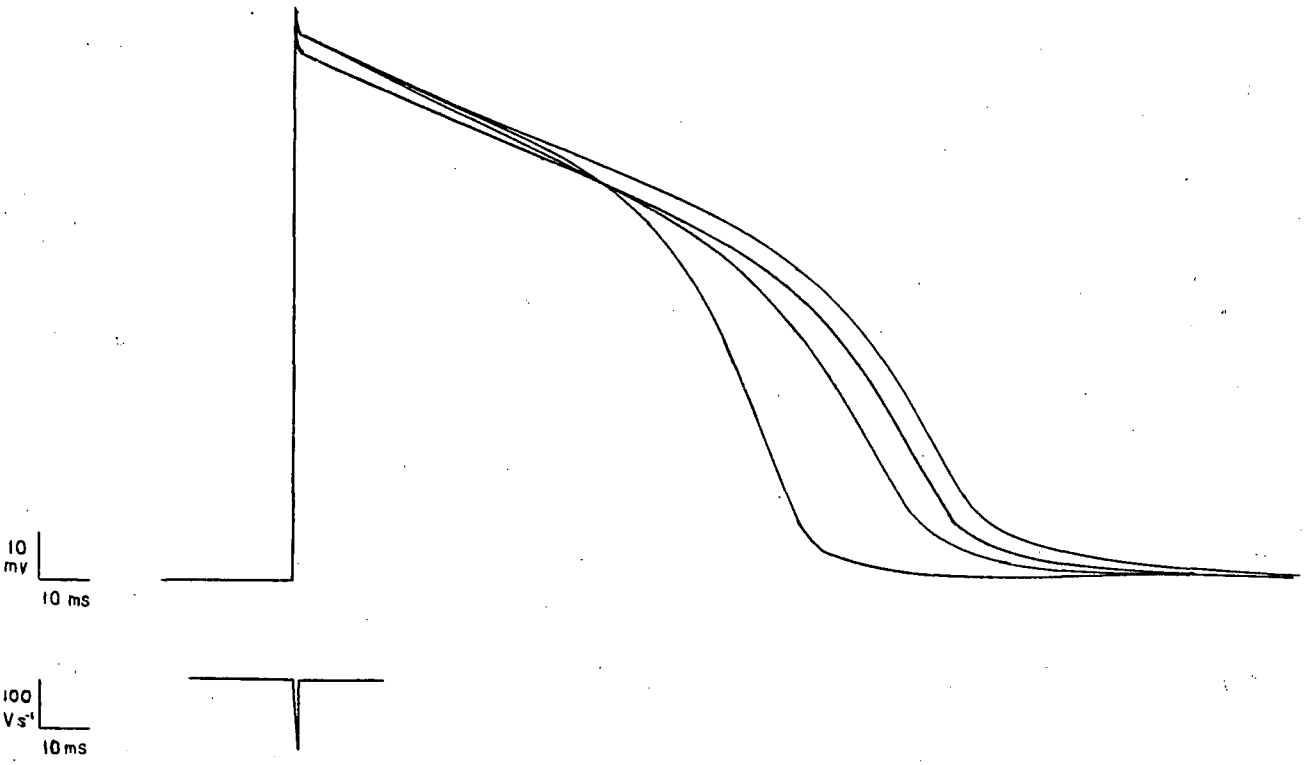


UK 68,798 in Rats

Dose ug/kg	AP mV	dV/dt V/s	APD25 ms	APD50 ms	APD75 ms	-dV/dtp V/s	-dV/dt V/s
con	105±5	130±20	10±2	18±2	43±3	3.0±.2	0.66±.03
12.5	105±8	125±20	10±1	19±2	45±3	3.0±.2	0.68±.04
25	104±7	140±20	9±3	21±6	46±3	2.9±.3	0.64±.04
50	102±6	125±25	10±3	21±5	50±4	2.9±.2	0.60±.05
100	106±9	130±20	10±2	23±3	53±4	2.8±.3	0.60±.03
1000*	106	140	10	22	53	2.8	0.60

Figure 11 shows the effects of UK68,798 treatment on ventricular epicardial AP morphology in acutely prepared urethane anaesthetized guinea pigs. Composite representative AP are redrawn and mean ± S.E.M. values (as in figure 10) obtained 10 min after dosing are shown. Cumulative doses were given at 15 min intervals, (n=6). *$p < 0.05$ from control.

Figure 11. Electrophysiological Effects of UK68,798 in Guinea Pigs.



UK 68,798 in Guinea pigs

Dose ug/kg	AP mV	dV/dt V/s	APD50 ms	APD75 ms	-dV/dtp V/s	-dV/dt V/s
con	110±4	140±10	84±2	95±2	0.47±.04	2.3±.2
12.5	116±7	140±20	98±9	117±6	0.45±.04	1.9±.1
25	114±5	145±15	106±8*	125±9*	0.44±.05	1.8±.1
50	109±7	150±20	103±5	122±9	0.42±.03	1.7±.2
100	110±6	140±20	103±5	121±7	0.42±.04	1.7±.1

3.3.4. UK68798 in Guinea Pigs *In Vivo*

In guinea pigs, as in rats, UK68798 did not affect AP height, nor rise rate. UK68798 widened APD_{75} by 32% maximally at 25 µg/kg. Its main effects were on the later stage of repolarization, slowing phase 3 repolarization rate by 23% while not altering the rate of repolarization of the plateau (Phase 2). The remarkable potency (despite limited efficacy) seen with UK68798 has not been seen with previously available class III drugs (Figure 11).

3.3.5 Summary

The Class III actions of tedisamil were clearly demonstrated in rats and guinea pigs, although in the latter species APD widening could have been partly due to bradycardia. This was evidenced by the partial reversal of APD widening by adrenaline boluses in this species and the well known (Anderson and Johnson, 1976; Payet *et al.*, 1981) frequency dependence of APD in this species. However, marked frequency dependence appears not to be operative in rat epicardium at physiological rates, thus the APD widening seen in this species could not be simply discarded as a secondary response to bradycardia. Class I actions of tedisamil appeared to be of little consequence - i.e. minimal dV/dt effects.

Rabbit SA node experiments suggest that the bradycardia produced by tedisamil is mainly due to its effects on Phase (3) repolarization (Oexle *et al.*, 1987). We also noted a lack of effect on pacemaker potential in similar studies (unpublished observations).

The new class III drug, UK68798, was remarkably potent with an EC_{50} for APD prolongation of 10 - 25 µg/kg. This drug also appeared to be completely free of Class I activity (no effects on dV/dt). However, the APD widening produced by UK68798 was seen as an attenuation of the phase 3 repolarization rate, such that the later stages, i.e. APD_{75}, were more affected than the earlier stages in APD_{25}. The implications for effects on refractoriness will be discussed below.

3.4 Myocardial Ischaemia - Induced Arrhythmias

3.4.1 Overview

In light of the profound effects of tedisamil on APD, ERP and Q-Tc interval in rats, it was seen as an ideal candidate to test the efficacy of Class III antiarrhythmic interventions. It was possible to relate the degree of Q-Tc widening necessary (by implication APD widening) to confer antiarrhythmic activity in the setting of acute M.I. in this species. The congener, KC8851, was expected to behave in a

similar manner to tedisamil, while other newly developed Class III drugs, risotilide, UK68798, and RP62719 were expected to confer less protection because of their lack of effects on the Q-T_C interval in rats. Unfortunately, guinea pigs cannot be used for acute regional myocardial ischaemia studies due to a large number of coronary artery collaterals in this species. However, pigs, which lack extensive collaterals, were chosen as an appropriate additional species in which to test the antiarrhythmic potential of tedisamil and UK68798. Antiarrhythmic efficacy had been reported for UK68798 in dogs (Gwilt *et al.*, 1989) and for risotilide in pigs (Colatsky *et al.*, 1989).

In rats, tedisamil was tested in a dose response study, while maximally effective doses, as assessed from ECG analyses, were used for the other drugs. These studies utilized double blind randomized treatment regimens.

3.4.2. Tedisamil in Conscious Rats

In the first series of experiments, tedisamil at 1, 2 and 4 mg/kg was tested against the arrhythmias occurring in the first four hours following occlusion. An additional group of chronically prepared rats was also given 2 mg/kg tedisamil and their ventricles paced (acutely prepared stab electrodes) at 6.5Hz during the 4 - 10 minute post occlusion period; a highly vulnerable period for early arrhythmias.

An extra saline control group was prepared as a control for pentobarbitone anaesthesia, and electrode insertion, in the animals.

Tedisamil dose dependently reduced VF but not VT nor VPB's in conscious rats subjected to coronary occlusion. In the electrically stimulated (paced) rats both VF and VT occurrence were significantly reduced. A.S. was reduced dose dependently by tedisamil, indicating that the incidence and severity of the arrhythmias were reduced by tedisamil treatment (Figure 12 & Table 8).

Serum [K^+] (3.6 ± 0.2 mM) and O.Z size (33 ± 2% to 37 ± 2%) were equivalent in all the groups. Despite the equivalence in O.Z. sizes, the tedisamil treated groups appeared to have a greater degree of S-T segment elevation (Figure 13). The initial ECG effects of tedisamil infusion in the absence of ischaemia can be seen in section 3.1. The tedisamil induced bradycardia was maintained during the first 30 minutes following occlusion, however the BP elevation seen after infusion was lost immediately following occlusion (Figure 14).

Table 8. Arrhythmias Following Occlusion in Conscious Rats: Effects of Tedisamil.

	VT (0-1/2h)		VF (0-1/2h)	
Dose	l #	l dur	l #	l dur
C	0.5±0.1	0.9±0.2	0.2±0.1	1.7±0.2
T1	0.6±0.2	0.9±0.4	0.3±0.2	1.8±0.3
T2	0.5±0.2	0.7±0.3	0.2± /	1.3± /
T4	0.7± /	0.9± /	0.5± /	1.4± /
T2S	0.7± /	1.0± /	0.6± /	1.3± /

	Log_{10} VPB		Arrhythmia Score	
Dose	0-0.5 h	0.5-4 h	0-0.5 h	0.5-4 h
C	1.5±0.1	2.0±0.1	4.1±0.8	2.6±0.7
T1	1.8±0.2	2.5±0.2	3.7±0.9	2.6±0.6
T2	1.6±0.2	2.1±0.4	2.4±0.4*	1.3±0.4*
T4	1.4±0.2	2.1±0.3	1.2±0.5*	1.9±0.6
C2	1.3±0.2		3.8±0.4	
T2S	1.2±0.2		1.0±0.4*	

The effects of treatments on the arrhythmias induced by coronary artery occlusion in conscious rats are shown. Treatments were C = conscious control, T1 = tedisamil (1 mg/kg), T2 = tedisamil (2 mg/kg), T4 = tedisamil (4 mg/kg), C2 = control for ventricular "paced" rats, T2S = tedisamil (2 mg/kg) in "paced" rats, see Methods. The columns shown are l # = log_{10} number, l dur = log_{10} duration of ventricular tachyarrhythmias (counted only in those animals which experienced them) Log_{10} VPB = log_{10} ventricular premature beats in the time periods indicated, A.S. = arrhythmia score. Values are expressed as Mean ± S.E.M. S.E.M. not shown (/) for $n < 5$. * indicates $p < 0.05$ versus control, by ANOVA followed by Duncan's range test.

Figure 12 shows the effects of tedisamil on the incidence of VT and VF during the 0-1/2 h and 1/2-4 h periods following occlusion in chronically prepared rats. The groups are indicated by: C = controls, T1 = 1 mg/kg, T2 = 2 mg/kg, and T4 = 4 mg/kg tedisamil, CPb = pentobarbitone anaesthetized controls, T2S = 2 mg/kg tedisamil in "paced" rats (0-1/2 h, only). * $p < 0.05$.

Figure 12. Effects of Tedisamil on Arrhythmias.

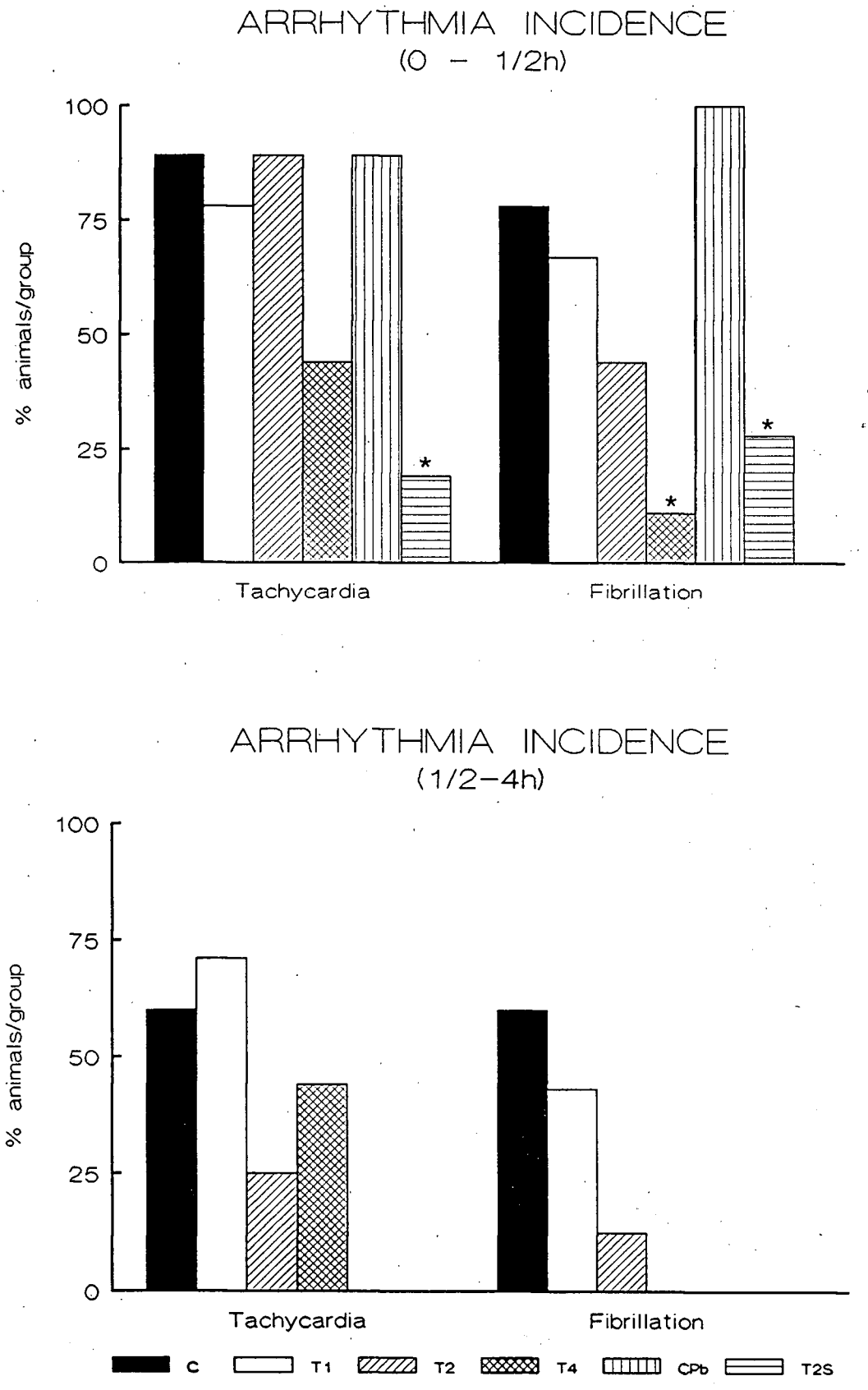


Figure 13 shows the effects of tedisamil (1, 2, and 4 mg/kg) on ST segment elevation (as % R wave amplitude) in the first hour following occlusion. Each point represents mean ± S.E.M. at the time period indicated. Curves were fit by Slidewrite software. *$p < 0.05$.

Figure 13. Effects of Tedisamil on ST Segment Elevation.

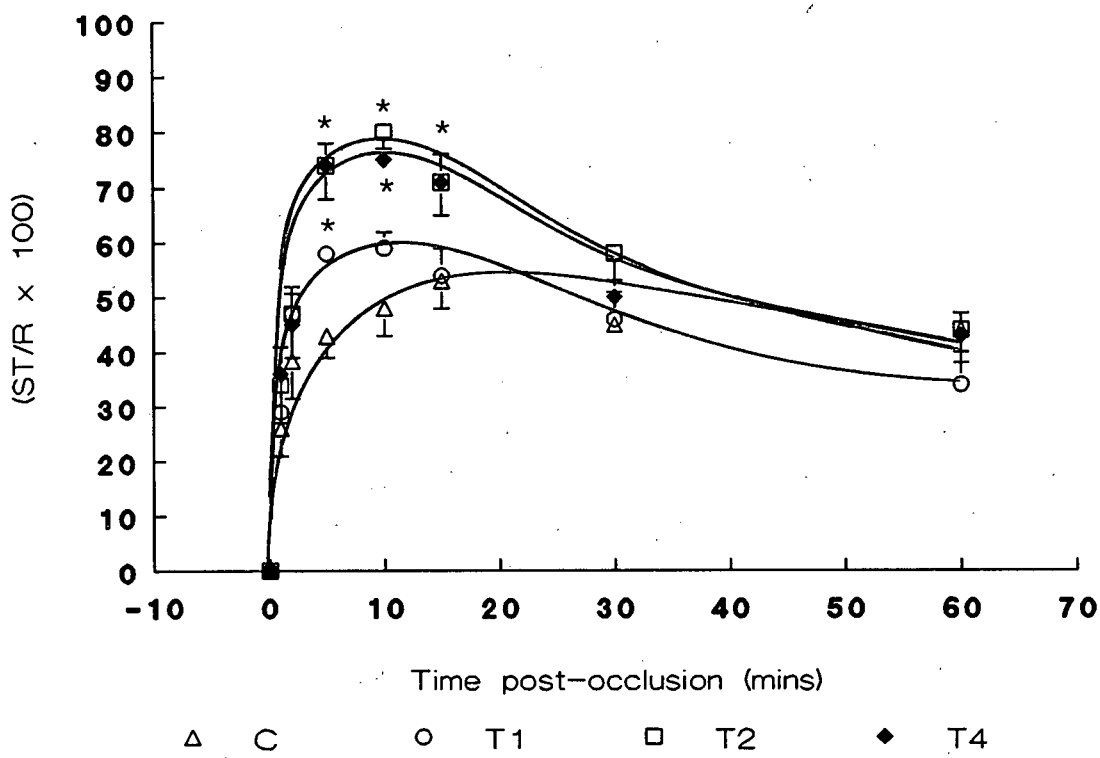
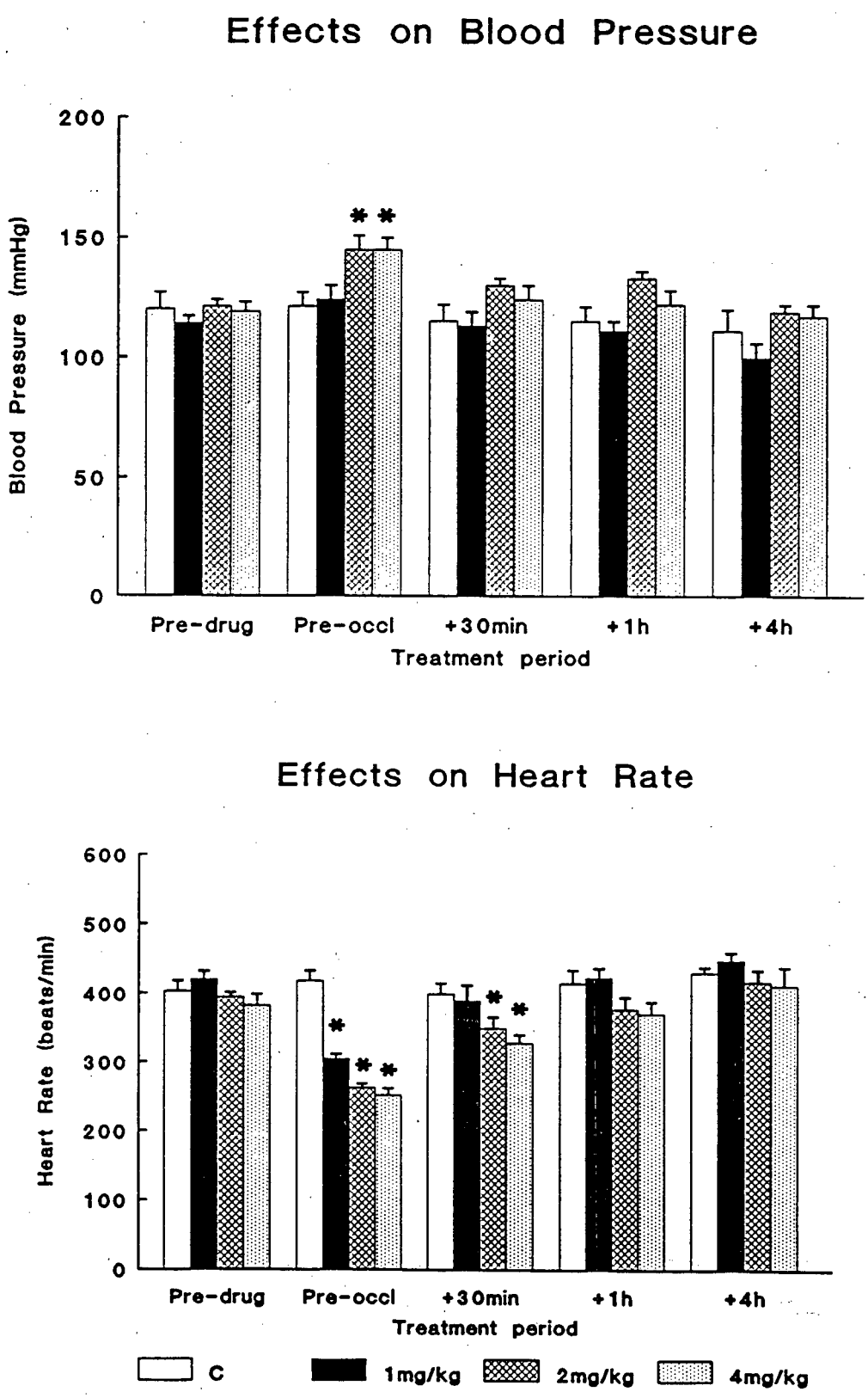


Figure 14 shows the effects of tedisamil on blood pressure and heart rate before and after occlusion in conscious rats. Means ± S.E.M. shown for n=9, * $p < 0.05$.

Figure 14. Effects of Tedisamil on BP and HR.



3.4.2.1. Tedisamil in Infarcted Rats

Administration of tedisamil to rats with one-day, one-week, or one-month old infarcts did not affect blood pressure, but slowed heart rate to the same extent as in non-infarcted rats. For example, control values for heart rate were 440 ± 12, 410 ± 15 and 330 ± 30 beats/min in one-day, one-week and one-month infarcted rats respectively. At 4 mg/kg tedisamil, heart rate in the three groups fell to 259 ± 9, 245 ± 16 and 225 ± 18 beats/min, respectively. As in the acute ischaemia study, ST segment elevation was seen in one-day infarcted rats after tedisamil treatment.

In one-day infarcted rats, tedisamil (1-4 mg/kg) suppressed VPBs in 8/14 rats. The control rate of Log_{10}VPB incidence was 2.0 ± 0.2 over 15 min, in these rats. In rats with older infarcts, VPB incidence was too low to test for antiarrhythmic effects. Proarrhythmic effects (increase in VPB, bigemini or alternating brady/tachycardia) occurred in 8/14 one-day infarcted rats at a median cumulative dose of 7 mg/kg. Proarrhythmic effects were seen in 1/5 one-week infarcted rats after cumulative doses of 1 and 4 mg/kg tedisamil. Similarly arrhythmias were provoked in 1/5 of the one-month infarcted rats after 8 mg/kg tedisamil. Respi ratory toxicity (asphyxia) appeared to be the cause of death in animals dying after doses ranging from 4-16 mg/kg.

3.4.3 Other K^+ Channel Blockers in Rats.

The initial success with tedisamil prompted us to seek out and test a number of other investigational compounds with reported Class III activity. Thus, we tested the analogue of tedisamil, KC8851, as well as the sotalol and N-acetylprocainamide derivatives, UK68,798, risotilide and RP62719 in the conscious rat model of coronary artery occlusion.

These compounds were not effective in prolonging Q-T_C interval in the rat except for KC8851 (section 3.1.1.1). Only KC8851 was effective in preventing ischaemia-induced arrhythmias despite being tested at maximally effective doses (with regard to Q-T_C widening). In the first 30 minutes following occlusion, none of the treatments reduced VT or VF incidence relative to control, although the control incidence was somewhat lower than previous studies have shown (Figure 15). However, when the durations of VT and VF were analyzed, KC8851 reduced VF duration relative to control. Neither VT duration nor Log_{10} VPB number were reduced by any of the treatments. There was no difference in the A.S. for the 0-30 minute time period in any of the groups (Table 9). When arrhythmias were analyzed in the 1/2 - 4 hour post occlusion time period, KC8851 treatment prevented VF occurrence. All other treatments were ineffective in reducing VF incidence (Figure 15). None of

the treatments altered VT incidence nor VPB number. In animals in which VT and/or VF occurred, drug treatment did not shorten the duration of these arrhythmias (Table 9). When arrhythmias over the full 0-4 hour monitoring period were analyzed again only KC8851 reduced $\log_{10}$ # (0.1 ± 0.1) and $\log_{10}$ duration (1.1 ± 0.2) of VF relative to control (0.6 ± 0.2) and (2.1 ± 0.2) respectively. Also, only KC8851 reduced the $\log_{10}$ duration of VT and VF combined (1.3 ± 0.2) versus control (2.2 ± 0.2).

There were no differences in O.Z. size between any of the groups (range 31 ± 4 to 38 ± 2). Similarly the ST segment elevation was similar in all the groups, except for the KC8851 group, in which the ST segment elevation was greater (maximum 81%) than control (maximum 50%) as seen previously with tedisamil (Figure 16). In addition, as seen with tedisamil, the second KC8851 infusion, 1.5 hours post occlusion, produced an increased ST segment elevation.

The blood pressure fall induced by occlusion was not attenuated by any of the drug treatments (Figure 17). In fact, over the first 15 minutes following occlusion risotilide (10 mg/kg) treatment exacerbated hypotension ($p \leq 0.05$), the lower dose (5 mg/kg) also lowered BP relative to control at the 2 minute and 5 minute post occlusion periods (data not shown).

Table 9. Arrhythmias Following Occlusion in Conscious Rats: Effects of K+ Channel Blockers.

	VT (0-4h)		VF (0-4h)	
Dose	l #	l dur	l #	l dur
C	0.9±0.2	1.3±0.2	0.5±0.2	2.0±0.2
KC	0.5±0.3	1.1±0.4	0.1±0.1	1.1±0.2*
UK	0.7±0.1	1.0±0.2	0.7±0.2	2.0±0.2
RP	0.8±0.2	1.3±0.3	0.5±0.2	1.9±0.3
R5	0.7±0.3	1.3±0.3	0.3±0.2	2.0±0.2
R10	0.8±0.2	1.3±0.2	0.5±0.2	1.9±0.2

	Log_{10} VPB		Arrhythmia Score	
Dose	0-0.5 h	0.5-4 h	0-0.5 h	0.5-4 h
C	1.4±0.1	2.3±0.2	2.8±0.9	4.6±0.7
KC	1.5±0.2	2.2±0.2	2.0±1.0	1.4±0.4*
UK	1.3±0.1	2.3±0.2	2.3±0.9	3.7±0.4
RP	1.3±0.3	2.5±0.2	2.8±1.0	4.0±0.5
R5	1.3±0.2	1.7±0.3	3.6±1.2	3.2±0.8
R10	1.7±0.2	2.4±0.2	3.6±0.8	3.6±0.7

The effects of treatments on the arrhythmias induced by coronary artery occlusion in conscious rats are shown. Treatments were C = control, KC = KC 8851 (4m/kg), UK = UK68,798 (1 mg/kg), RP = RP62,719 (1 mg/kg), R5 = risotilide (5 mg/kg), R10 = risotilide (10 mg/kg). The columns shown are l # = log_{10} number, l dur = log_{10} duration of ventricular tachyarrhythmias, Log_{10} VPB = log_{10} ventricular premature beats in the time periods indicated, A.S. = arrhythmia score. Values are expressed as Mean ± S.E.M. * indicates $p < 0.05$ versus control, by ANOVA followed by Duncan's range test.

Figure 15 shows the effects of putative class III antiarrhythmic drugs on VT and VF incidence over the 0-1/2 h and 1/2-4 h time periods following occlusion in conscious rats. The groups indicated are: C = controls; KC = 4 mg/kg KC8851; UK = 1 mg/kg UK68,798; RP = 1 mg/kg RP62,719; R5 = 5 mg/kg risotilide; R10 = 10 mg/kg risotilide. *$p < 0.05$.

Figure 15. Effects of K^+ Channel Blockers on Arrhythmias.

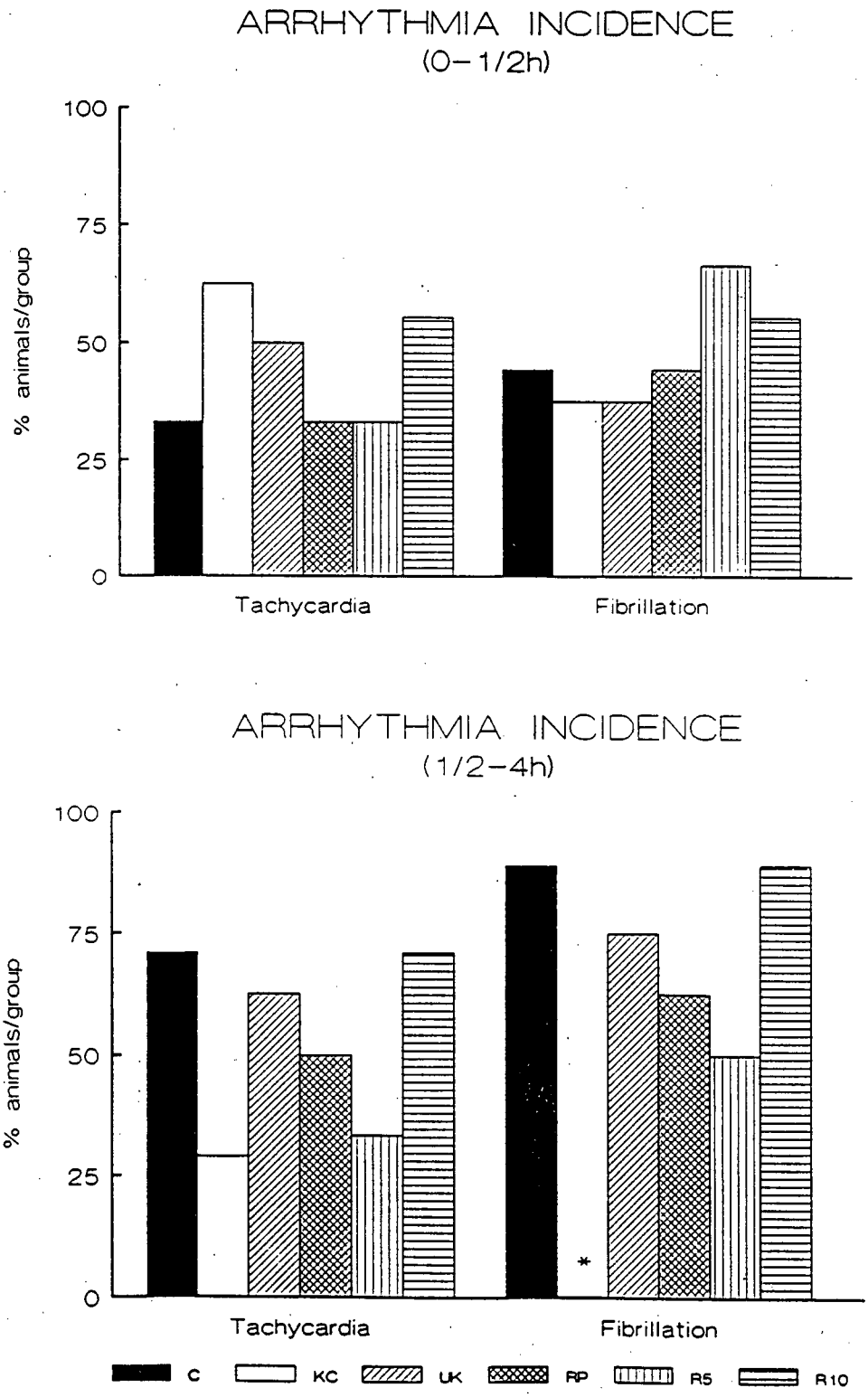


Figure 16 shows the effects of putative class III antiarrhythmic drugs on ST segment elevation (as % R wave amplitude) in the first hour following occlusion in conscious rats. The groups indicated are: C = controls; KC = 4 mg/kg KC8851; UK = 1 mg/kg UK68,798; RP = 1 mg/kg RP62,719; R5 = 5 mg/kg risotilide; R10 = 10 mg/kg risotilide. Each point represents mean at the time period indicated. Curves were fit by Slidewrite software. $^{*}p < 0.05$ versus control.

Figure 16. Effects of K^+ Channel Blockers on ST Segment Elevation.

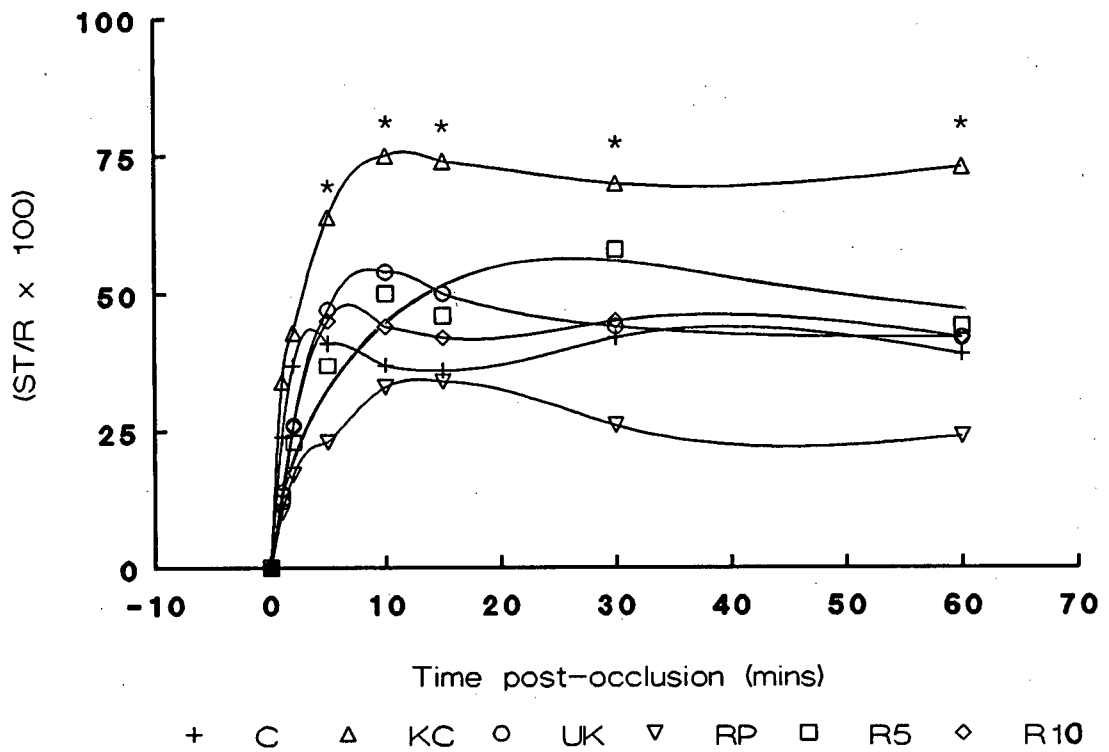
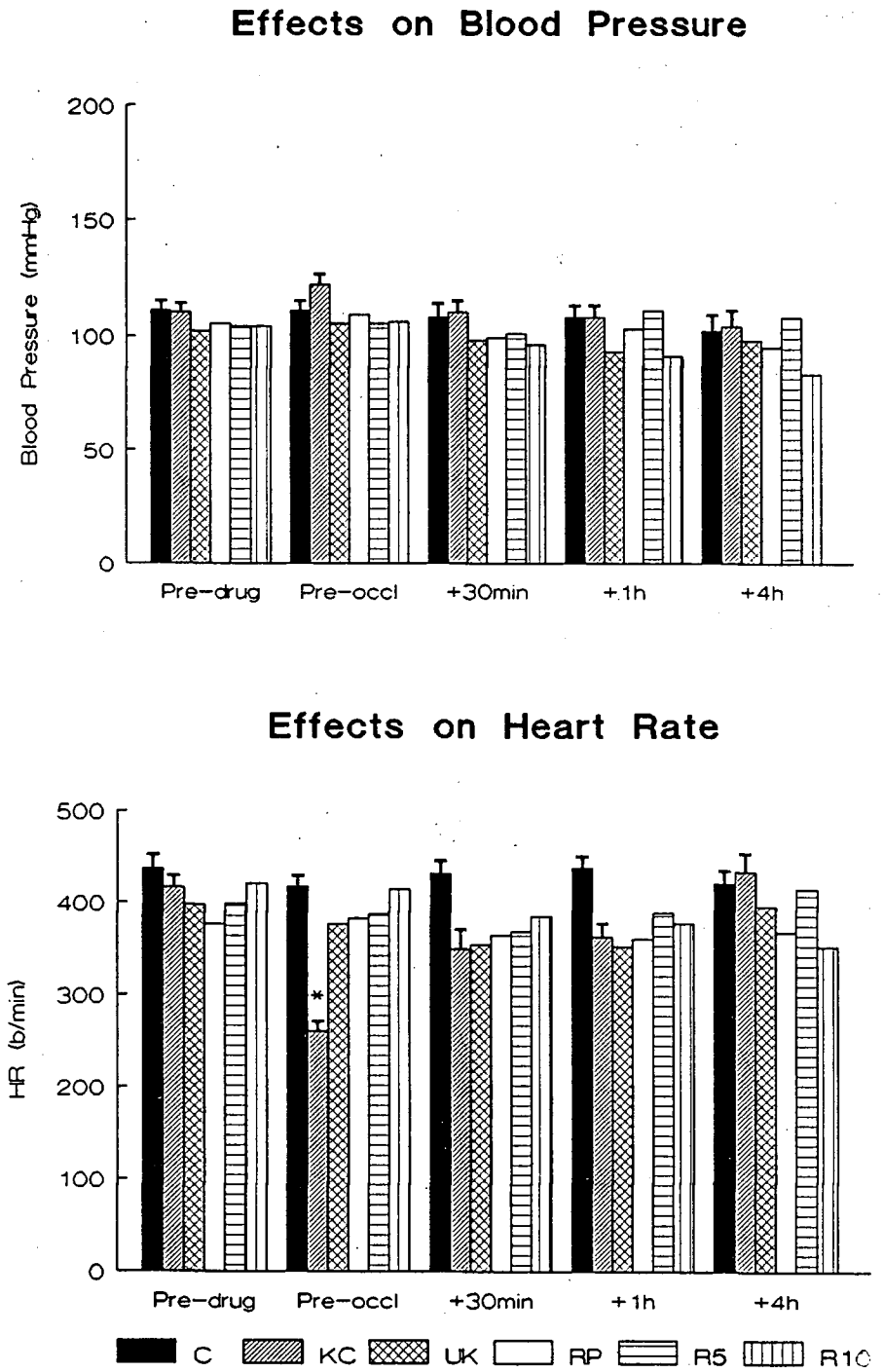


Figure 17 shows the effects of putative class III antiarrhythmic drugs on blood pressure and heart rate before and after occlusion in conscious rats. The groups indicated are: C = controls; KC = 4 mg/kg KC8851; UK = 1 mg/kg UK68,798; RP = 1 mg/kg RP62,719; R5 = 5 mg/kg risotilide; R10 = 10 mg/kg risotilide. Means ± S.E.M. shown for n=9, *$p < 0.05$.

Figure 17. Effects of K^+ Channel Blockers on BP & HR.



The bradycardia produced by KC8851 was maintained until 30 minute post occlusion. The heart rate was also decreased relative to control in the first 5 minutes following occlusion by 5 mg/kg risotilide ($p < 0.05$) (data not shown). However, by 30 min post occlusion there were no significant differences in HR between control and any treatment (Figure 17).

3.4.4 K^+ Channel Blockers in Acutely Prepared Rats

Acutely prepared rats were used in a pilot study to assess whether tedisamil (4mg/kg) and glibenclamide (10 mg/kg) gave protection against reperfusion induced arrhythmias. Rats were subjected to 10 minute ischaemia followed by reperfusion. The results of this study have been published in detail previously (Beatch *et al.*, 1989). In this study tedisamil (4 mg/kg) and glibenclamide (10 mg/kg) were given before occlusion and compared to a number of interventions which reduced the influence of free radicals and various eicosanoids. These latter treatments were ineffective and are not relevant to the focus of this thesis and therefore will not be discussed. It was of interest to note whether the putative ATP sensitive K^+ channel blocker, glibenclamide, could offer protection in this model given the numerous speculations and few studies in the literature (Kantor *et al.*, 1990; Bekheit *et al.*, 1990).

In this study tedisamil (4 mg/kg, n = 5) eliminated VT & VF in the 10 minute ischaemia period and VF in the reperfusion period. The small group size and short duration of ischaemia may have biased these results. Glibenclamide (10 mg/kg, n = 11 occlusion; n = 7 reperfusion) had no protective effect on either arrhythmias in either time period, and actually increased the duration of the VF seen in the 10 minute occlusion period ($p < 0.05$) (Figure 18 & Table 10). Neither drug significantly decreased the occurrence of VPB. Serum [K^+] (3.6 ± 0.2 to 4.1 ± 0.2) and O.Z. (33 ± 1 to 36 ± 2) were equivalent in the groups. Tedisamil caused bradycardia (190 ± 25 b/min) vs. control saline (380 ± 30 b/min) and increased maximum ST segment elevation (86 ± 5%) relative to control (45 ± 8%) ($p < 0.05$).

Table 10. Antiarrhythmic Effects of Glibenclamide and Tedisamil.

Arrhythmias Following Occlusion in Anaesthetized Rats

	VT		VF	
Dose	l #	l dur	l #	l dur
C	0.9±0.1	1.5±0.1	0.2±0.1	1.3±0.3
T	n/a	n/a	n/a	n/a
G	0.9±0.1	1.5±0.2	0.2±0.1	1.9±0.1*

Arrhythmias Following Reperfusion in Anaesthetized Rats

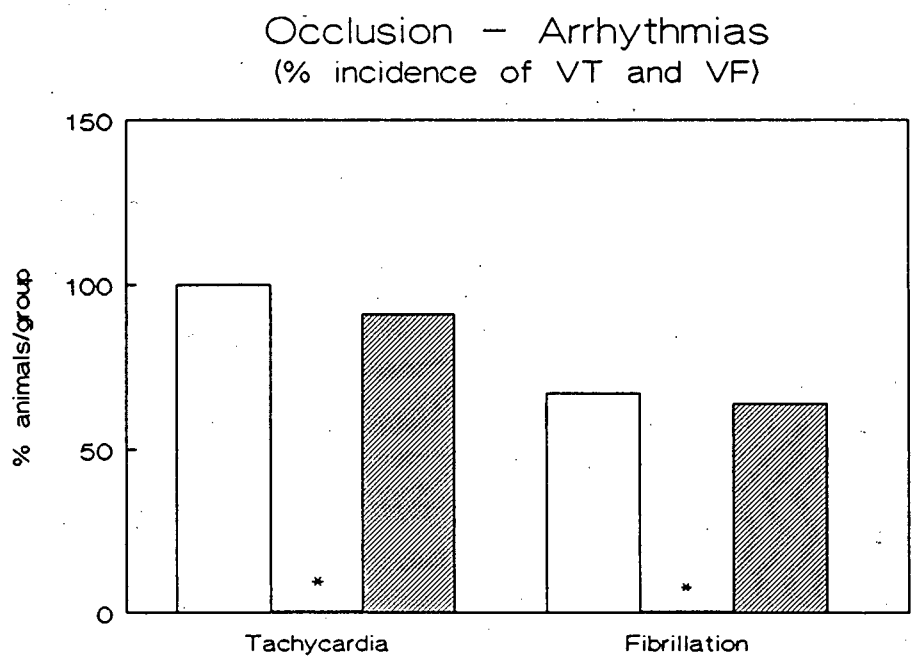
	VT		VF	
Dose	l #	l dur	l #	l dur
C	0.6±0.2	1.5±0.2	0.2±0.1	1.8±0.5
T	0.3± /	1.3± /	n/a	n/a
G	0.7±0.2	1.4±0.2	0.4±0.2	1.5±0.4

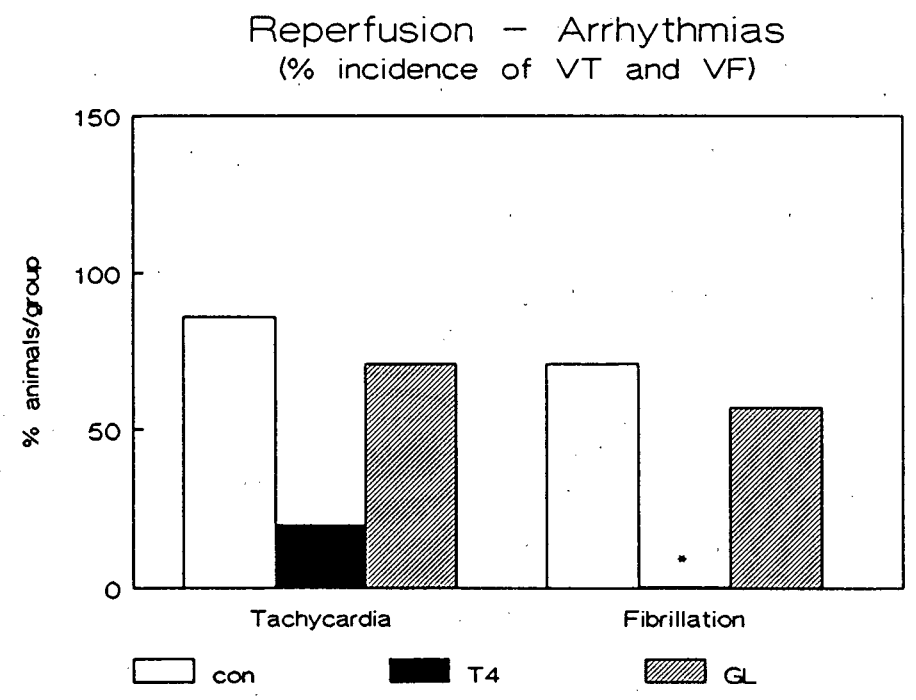
	Log_{10} VPB		Time to VT (s)	
Dose	Occlusion	Reperfus.	Occlusion	Reperfus.
C	1.9±0.1	1.8±0.2	330±30	10
T	1.3±0.3	1.3±0.1	n/a	10
G	1.5±0.2	1.5±0.1	320±30	7

The effects of treatments on the arrhythmias induced by 10 min coronary artery occlusion followed by reperfusion in anaesthetized rats are shown. Treatments were C = control, T = tedisamil (4m/kg), G = glibenclamide (10 mg/kg). The columns shown are l # = log_{10} number, l dur = log_{10} duration of ventricular tachyarrhythmias, Log_{10} VPB = log_{10} ventricular premature beats in the time periods indicated. Values are expressed as Mean ± S.E.M. * indicates $p < 0.05$ versus control, by ANOVA followed by Duncan's range test.

Figure 18 shows the effects of tedisamil and glibenclamide on incidence of VT and VF during 10 min of ischaemia and followed by reperfusion in pentobarbitone anaesthetized acutely prepared rats. Con, indicates saline control (empty bars); T4, indicates tedisamil, 4 mg/kg (solid bars); GL, indicates glibenclamide, 10 mg/kg (hashed bars); * indicates $p < 0.05$ versus control.

Figure 18. Effects of Glibenclamide and Tedisamil on Arrhythmia Incidence.





3.4.5 K^+ Channel Blockers in Acutely Prepared Pigs

Tedisimal (8 mg/kg) and UK68798 (167.5 ug/kg) were tested against arrhythmias produced by acute myocardial ischaemia in anaesthetized pigs. The studies were an adjunct to the cardiovascular assessment of the drugs in this species and were conducted in an open design. Only 5-6 animals per group were used and the arrhythmia incidence was compared to historical controls conducted in our laboratory.

Neither drug decreased $\log_{10}$ VPB incidence in the first hour following occlusion. Likewise, neither tedisamil (4/6) nor UK68,798 (4/5) decreased VT incidence relative to control (11/16). The incidence of VF was low in control pigs (3/16) as compared to rats and as such detecting an antifibrillatory effect would require a large sample size. There were no episodes of VF in the tedisamil treated group while VT degenerated into VF in two pigs treated with UK68,798. No primary VF was seen in the UK68,798 group. The groups had similar O.Z. size. (Table 11).

Table 11. Arrhythmias Following Occlusion (0-1h) in Pigs

Dose	Log_{10} VPB	VT	VF	O.Z.
C	1.8±0.1	11/16	3/16	33±2
T	2.4±0.5	4/6	0/6	26±2
UK	2.5±0.2	4/5	2/5[1]	29±2

The effects of treatments on the arrhythmias induced by coronary artery occlusion in anaesthetized pigs are shown. Treatments were C = control, T = tedisamil (8 mg/kg), UK = UK68,798 (157.5 μg/kg). The columns shown are Log_{10} VPB = log_{10} ventricular premature beats, O.Z. = occluded zone size as percent ventricular weight, (Mean ± S.E.M.), VT = VT incidence per group, VF = VF incidence per group. [1]VF initiated by VT.

3.4.6 Summary: Ischaemia induced Arrhythmias

Tedisamil and KC8851 demonstrated dose dependent antifibrillatory activity in rats subjected to myocardial ischaemia and in rats further subjected to reperfusion. An additional protection against VT was also seen in rats whose hearts were stimulated such that the tedisamil induced bradycardia was reversed. Tedisamil otherwise did not affect the incidence of VT nor VPBs in the rat. Similar results were seen in pigs subjected to acute myocardial ischaemia, however, an unequivocal demonstration of antifibrillatory effect in pigs would have required a larger sample size. UK68798, RP62719 and risotilide were not antiarrhythmic in the rat at all. This was consistent with their lack of effects on the ECG in this species. UK68798, was not protective in pigs, despite a small but significant widening of the Q-T_C interval in this species. Again, due to the small sample size used, an antifibrillatory effect of UK68798 would not have been detectable in this study. However, it would appear safer to speculate that no protection was afforded against VPB or VT incidence by this drug in the pig model.

4 DISCUSSION

4.1 Pharmacology

The first section of the discussion deals with the cardiovascular effects of class III drugs. Initially, drug effects on heart rate and blood pressure will be examined. Species dependent actions of the drugs on the ECG will be discussed in relation to possible electrophysiological actions. Possible proarrhythmic actions associated with these drug effects will be discussed. Finally, the ECG effects of tedisamil, KC8851, UK68798, RP62719 and risotilide will be compared to other class III drugs.

In subsequent sections, mechanisms of drug effects will be interpreted through analysis of responses to electrical stimulation and intracellular recording studies. Antiarrhythmic effectiveness will be compared for the drugs, and evaluated in relation to other class III drugs. Finally, general conclusions will be discussed.

4.1.1 Class III Drug Effects on Heart Rate and Blood Pressure.

The most prominent effect of tedisamil administration in all species was dose dependent bradycardia. At a 4mg/kg dose, the reduction in heart rate ranged from 17% in pigs to 34% in conscious rats. The order of sensitivity to the

bradycardic effects (% reduction in HR) of tedisamil was guinea pig = rat = rabbit > cat > baboon > pig (Buschmann *et al.* 1989). The absolute reduction in heart rate (among the different species) was dependent on the initial heart rate. This suggests that tedisamil alters a current which normally controls cycle length in sinus nodal cells. Autonomic blockade in cat (our unpublished observations) and in rat (Howard *et al.*, 1989) had no effect on the bradycardic action of tedisamil. Tedisamil did not affect pacemaker depolarization rate or threshold potential in isolated sinus node and apparently prolongation of the AP in pacemaker tissue contributed to the bradycardia (our unpublished observations). An earlier study reported a reduction in pacemaker depolarization rate, which was insufficient to account for the bradycardia seen (Oexle *et al.*, 1987).

KC8851, tedisamil's analogue, had similar potency and efficacy to tedisamil in rats (Table 1). The other putative Class III agents, UK68,798, RP62,719, and risotilide had slight or no effects on heart rate when tested in rats, pigs and guinea pigs and baboons. This would suggest that these drugs had minimal effects on the currents contributing to pacemaker rate in sinus nodal cells. Since these drugs have been reported to selectively block I_K (see section 1.4.1), it would appear that I_K is either not present, or makes only a minor contribution to pacemaker rate in nodal cells (Irisawa, 1987). These results suggest that tedisamil's

bradycardic effects result from its inhibition of I_{to} and are not related to its additional suppression of I_K (Dukes *et al.*, 1990).

The bradycardic action of tedisamil is the basis for its potential use as an antianginal (personal communication, Ulrich Kuhl; Grohs *et al.*, 1989). Although, this application might be of questionable benefit, studies have shown tedisamil to reduce O_2 demand via its bradycardic action in dogs (Grohs *et al.*, 1989). Bradycardia does not reduce ischaemia-induced arrhythmias in the conscious rat model (Johnston *et al.*, 1983; Abraham *et al.*, 1989).

In all species tested, tedisamil treatment tended to increase BP, which was possibly secondary to bradycardia and AP widening (Carmeliet, 1977; Beatch *et al.*, 1988), which in itself might augment contractility. Alternatively, tedisamil may have caused vasoconstriction. Slight elevation of BP (not statistically significant) was seen in rats treated with KC8851. The hypertensive response to tedisamil was lost in infarcted rats. None of the other Class III drugs nor glibenclamide altered BP. This suggests that I_K blockers at the doses used did not cause vasoconstriction nor did they augment contractile force. The apparent lack of effects on BP of these new compounds, or possible mild hypertensive effects of tedisamil, would make Class III drugs a more desirable choice for patients

with an infarction, than Class I and IV drugs which may depress contractility if given in sufficient dosage (Jaillon & Ferry, 1988). As infarction already lowers ejection fraction, clearly drugs without negative inotropic effects are desirable in this population of patients. However, in hypertensive patients, tedisamil's BP elevating effects might be undesirable.

4.1.2 Overview of ECG Analysis

Class III drugs, as discussed previously, delay repolarization of the cardiac AP. Since the T-wave of the surface ECG reflects the repolarization phase in the ventricle (Einthoven, 1912; Katz, 1928), Class III drugs should widen the Q-T_c interval of the ECG. For drugs with selective Class III actions no other effects on QRS or P-R intervals should be seen due to a lack of effect on conduction velocity in atrial, nodal, or ventricular tissue. Typically Class I drugs widen QRS and P-R intervals if given in doses sufficient to slow conduction velocity. Class II drugs , beta blockers, can widen P-R intervals *in vivo* if AV nodal conduction has been enhanced by a significant degree of sympathetic tone before administration, as the P-R interval reflects the conduction time through the AV node. Class IV drugs, Ca^{++} antagonists, can widen P-R intervals if given in sufficient doses to inhibit I_{si}. *In vivo* the dihydropyridines (e.g. nifedipine) are less potent in this

regard than phenylalkylamines (e.g. verapamil) or benzothiazepines (e.g. diltiazem). Thus as a first approximation, a drug which selectively widens the $Q\text{-}T_C$ interval in a dose dependent manner may be characterized as having Class III effects. However, not all drugs which widen the $Q\text{-}T_C$ interval can be considered primarily as Class III antiarrhythmics. For example, prolonged beta-blocker therapy has been reported to prolong APD (Raine & Vaughan-Williams, 1981) possibly through an alpha-receptor mediated action (independent of direct APD prolongation as with d-sotalol). $Q\text{-}T_C$ prolongation has also been encountered with neuroleptics, tricyclic antidepressants, organophosphate insecticides, erythromycin and numerous non-cardiac drugs (Fish & Roden, 1989). Bradycardia produced by specific bradycardic agents or as a side effect or by activation of reflexes also widens the Q-T interval. Due to this effect of bradycardia, numerous formulae have been devised to normalize the Q-T interval for changes in HR, the most widely accepted formula is that of Bazett (1920), which is $Q\text{-}T/R\text{-}R^{-1/2}$. This formula has been criticized however as it tends to overcorrect at fast rates and undercorrect at slower rates. Antiarrhythmic drugs with mixed electrophysiological actions, for example, the Class Ia antiarrhythmics, quinidine, disopyramide and procainamide block both gNa^+ and gK^+ and therefore widen both QRS duration and $Q\text{-}T_C$ intervals if given in sufficient dosage.

There are also pathological conditions in which long $Q\text{-}T_C$ intervals are observed in the drug free state. The long Q-T syndrome occurs in two major conditions, one in which congenital deafness is present and one without this affliction (Jackman *et al.*, 1988). There has been an increased risk of TdeP associated with long Q-T syndrome (Jackman *et al.*, 1988). This apparent relationship as well as reports of TdeP associated with Class III drugs has added an air of caution to the introduction of Class III drugs as a therapy for arrhythmias.

4.1.2.1 Effects on ECG intervals

In rats, only tedisamil and KC8851 widened the $Q\text{-}T_C$ interval, while the putative I_K blockers were without effects in this species (Table 2). These results further support the observation that the major K^+ current contributing to repolarization in rat ventricle is I_{to} (Josephson *et al.*, 1984) and either I_K is not present or contributes little to the repolarization process in rat ventricle. None of the putative K^+ channel blocking drugs we tested in the rat prolonged the duration of the QRS interval suggesting that at the doses used no signs of gNa^+ blocking activity were manifest. Tedisamil has been shown to decrease gNa^+ at room temperature *in vitro* at concentrations > 50µM (Dukes & Morad, 1989; Dukes *et al.*, 1990). Interestingly, this gNa^+ blockade was effective from

the external surface in a manner similar to TTX (Kao, 1966; 1986). This property allowed for rapid reversibility upon washout (Dukes & Morad, 1989) and may be related to the proarrhythmic effects of tedisamil seen after bolus administration, as gNa^+ channel blockade-induced conduction slowing, coupled to possible heterogeneous refractoriness may precipitate reentry (Mines, 1913; 1914). While no thorough studies on the tissue selectivity of tedisamil have been performed, the species dependent effects of tedisamil hint at a tissue selectivity as well. One abstract has appeared which shows tissue selectivity of tedisamil in isolated rabbit SA node and guinea pig hearts and papillary muscle (Oexle *et al.*, 1987). Guinea pig ventricle lacks I_{to}, but effects on I_k would be seen in this tissue (Giles & Imaizumi, 1988).

None of the drugs tested had effects on the P-R interval in rats except for tedisamil and KC8851, which both widened P-R to a similar extent. This effect was unlikely due to Ca^{++} antagonism, as these drugs tended to increase blood pressure and not impair contractility. In addition, Dukes *et al* (1989) have shown tedisamil (0-100μM) does not impair gCa^{++} in isolated rat myocytes. Alternatively, P-R interval is sensitive to gNa^+ blocking drugs and bradycardia (Driscoll *et al.*, 1981). Classically the central nodal cells of the AVN do not use Na^+ currents for depolarization (Hoffman & Cranefield, 1960). However, border cells might

be more sensitive to the gNa^+ blocking effects of tedisamil than ventricular muscle, owing to voltage dependent inactivation of a large proportion of Na^+ channels in the AVN border cells. Further reduction of their small component of gNa^+ by tedisamil would be equivalent to an increase in the apparent number of central nodal cells. Thus, with a greater area of Ca^{++} dependent slowly conducting tissue to traverse, the wave of excitation might take longer to reach the ventricles. The influence of the atrial tissue surrounding the sinus node has recently been studied (Kirchof *et al.*, 1987).

In primates, pigs and guinea pigs UK68,798 was remarkably potent (apparent EC_{50} 10 µg/kg) but minimally efficacious in widening $Q\text{-}T_C$ or $Q\text{-}U_C$ interval. UK68798 widened the $Q\text{-}U_C$ interval maximally by 20% in primates and pigs and only 9% in guinea pigs. The larger species have slower resting heart rates and longer APD than guinea pigs, which would allow the delayed rectifier, I_K, to have a larger role in controlling APD, thus blockade of I_K would possibly have more pronounced effects in larger species. RP62719 and risotilide had similar effects to UK68,798 in primates, which suggests a similar mechanism of action. While most efficacious in rats, tedisamil was more efficacious at $Q\text{-}T_C$ or $Q\text{-}U_C$ widening in baboons than in guinea pigs, and least effective in pigs. The implication from these primate studies is that humans might be sensitive

to the Q-T_c widening effects of tedisamil. Also the initial resting heart rate did not govern sensitivity to tedisamil as for the putative I_K blockers, suggesting that the Q-Tc widening effects of tedisamil were secondary to its I_{to} blocking effects. The pig's relative lack of sensitivity to tedisamil suggest that ventricular repolarization is not markedly dependent on I_{to} or I_K in this species. The currents underlying the pig ventricular action potential may be inferred from the morphology of AP recorded from the ventricular subepicardium. These studies (Downar *et al.*, 1977) showed a broad plateau AP without the "spike and dome" appearance which suggests I_{to} is not a major contributor to AP repolarization in pig ventricular subepicardium. In a recent report using transmembrane recording of baboon myocardium it was shown that epicardial cells had a distinct notch after phase 0 and a shorter duration AP than endocardial cells (Dangman *et al.*, 1988). These observations might be explained by a tissue dependent distribution of the channels responsible for I_{to}, as in canine ventricle (Litovsky & Antzelevitch, 1988), and may partly explain tedisamil's tendency to generate U waves in the ECG of baboons.

None of the putative I_K blockers prolonged QRS or P-R intervals in any species tested. Tedisamil however tended to widen the QRS and P-R intervals in baboons with minimal effects in pigs and guinea pigs. These effects in baboons

most likely resulted from the Na^+ channel blocking actions of tedisamil as discussed above.

4.1.2.2 Proarrhythmic Effects

An interesting observation, which was especially prominent in pigs (although least sensitive to tedisamil's bradycardic action), was the tendency towards a "sick sinus" syndrome or alternating sinus bradycardia/tachycardia after high doses of tedisamil. As the course of membrane potential changes is a dynamic process in pacemaker cells, alterations in the repolarization phase could have influences over the entire cycle. The possibility exists that a bradycardia dependent early after depolarization triggered a tachyarrhythmia which self terminated by "overdrive suppression." Alternatively, since sinus node cells have slow conduction a leading circle micro reentry (Allessie *et al.*, 1977) could have been initiated if there was a dispersion of refractoriness in the nodal tissue.

Another anecdotal observation, made during studies with primates, was that tedisamil induced ventricular arrhythmias which could be converted to sinus rhythm by UK68,798, RP62,719 or risotilide. It is possible to speculate that the latter drugs reduced the dispersion of refractoriness caused by tedisamil, thereby terminating a reentrant VT. Since activation mapping was not done and the observations

are anecdotal, no firm conclusions can be drawn. It is interesting to note in this regard that amiodarone has been used successfully to treat patients with previous history of drug induced torsade de pointes (Mattioni *et al.*, 1989).

None of the Class III drugs which have been reported to selectively block I_K (UK68,798, RP62,719, risotilide) caused arrhythmias at the doses used. Tedisamil, on the other hand, with mixed electrophysiological effects (Dukes *et al.*, 1990) and multiple ECG effects did trigger episodes of VPB and VT. It therefore appears that some proarrhythmic effects seen with previous Class III and Ia agents may be related to their mixed electrophysiological effects. Selective AP widening may be necessary but insufficient to provoke arrhythmias in normal myocardium without concomitant hypokalemia, hypomagnesemia, or other abnormalities. This is not entirely surprising as the heart already has AP of differing durations depending upon the tissue, i.e. Purkinje fibres have the longest APD, yet normally the heart functions in a remarkably coordinated manner.

As noted above, TdeP has been associated with hypomagnesemia (Loeb *et al.*, 1968) and hypokalemia (Tamura *et al.*, 1967). Since hypomagnesemia may be associated with a loss of the inward rectification properties, and inward conductance through I_{K1} is dependent on $[K^+]_o^{1/2}$, both of these conditions impair the membrane potential stabilizing function of I_{K1}. In addition Na^+/K^+ ATPase is also

inhibited by these conditions. Thus, the net result might be a loss of control over membrane polarization and subsequent voltage-mediated inactivation of Na^+ channels. Quinidine has been associated with TdeP (Selzer & Wray, 1964). These two separate observations suggest that a suppression of conduction velocity is an additional component that may be necessary to reveal the proarrhythmic effects of drugs which prolong APD.

The other factor which may have been important in the proarrhythmic potential of tedisamil was its bradycardic effects. Several recent studies suggest that torsade de pointes may be due to bradycardia-dependent early after depolarizations (EADs) and triggered activity (Brachman *et al.*, 1983; Damiano & Rosen, 1984; Roden & Hoffman, 1985; Levine *et al.*, 1985; El-Sherif *et al.*, 1988). The latter two studies referred to CsCl and anthopleurin-A induced polymorphic VT *in vivo*, respectively which may not be related to the tedisamil induced arrhythmias. The first two references refer to cesium chloride and the third to quinidine-induced EADs *in vitro*. Clearly these studies are even further removed from our studies. In rats, up to 4 mg/kg tedisamil or KC8851 was not proarrhythmic. Previous studies (Howard *et al.*, 1989) showed the proarrhythmic effects of tedisamil occurred at doses of $\geq$ 15 mg/kg in pentobarbitone anaesthetized rats. At this dose level significant QRS and P-R prolongation was seen. This study also showed that the proarrhythmic effects of tedisamil were

eliminated when the autonomic nervous system was blocked. The proarrhythmic effects of tedisamil occurred at lower doses (4-7 mg/kg) in infarcted rats (Section 3.4.2.1). Numerous studies have shown the arrhythmic potential of damaged tissue (review: Hoffman & Dangman, 1987). Whether tedisamil caused arrhythmias by the same mechanism in both normal and damaged hearts is a moot point (although deserving of further research), the fact that tedisamil was proarrhythmic at doses showing antiarrhythmic properties against acute ischaemia suggests the drug may have significant proarrhythmic potential at therapeutic levels in post-infarcted hearts. As the majority of patients receiving antiarrhythmic treatment have had a previous infarction, the usefulness of tedisamil as an antiarrhythmic drug may be severely limited, if patients show the same sensitivity as rats. Clearly, more studies in primates with infarcted hearts should be carried out before consideration is given to treat human subjects with previous myocardial infarction. Although, not directly shown in our studies, the proarrhythmic potential of tedisamil would suggest proarrhythmic effects may result from combination therapy using one of the more "selective" Class III agents (UK68798, RP62719, risotilide) and a Class I antiarrhythmic, although Class I and III combination has been proposed to be a beneficial endeavor, theoretically, (Hondeghem & Snyders, 1990) and with Class Ia drugs.

4.1.2.3 Summary

In conclusion, analysis of the ECG showed tedisamil and KC8851 exert mixed actions which are species dependent and different from the other putative Class III agents tested. Tedisamil and KC8851 widened Q-T_C in a manner consistent with dose dependent blockade of myocardial K^+ channels (Dukes et al., 1990). Instances of P-R interval prolongation may have resulted from Na^+ channel blockade. UK68798 selectively widened Q-T_C intervals in pigs, consistent with I_K blockade (Gwilt *et al.*, 1991). However, Q-T_C widening was not seen in guinea pigs, which suggests UK68798 may be ineffective at higher HR. UK68798, RP62719 and risotilide had no ECG effects in rats, at supramaximal doses, which suggests they have selective electrophysiological actions, as rats are sensitive to gNa^+ and gCa^{++} blocking drugs. These results should now be considered in the context of other research with Class III drugs.

4.1.3 Comparison with Other Class III Drugs

4.1.3.1 Amiodarone

As mentioned in the introduction, amiodarone is a drug with complex actions. Not only does amiodarone have complex pharmacokinetics, multiple side effects unrelated to its

electrophysiological profile, e.g. corneal deposits, thyroid dysfunction and lung fibrosis (Singh *et al.*, 1989), but its antiarrhythmic actions fit all four of Vaughan-Williams original classes (Class I: Singh & Vaughan-Williams, 1970; Mason *et al.*, 1983; Class II: Charlier *et al.* 1968; Polster & Broekhuysen, 1976; Class IV: Gloor *et al.*, 1983; Class III: Singh & Vaughan-Williams, 1970). Also the tissue levels do not correspond well with electrophysiological actions. Thus amiodarone would be a poor choice for a reference standard Class III drug. However, the effectiveness of amiodarone makes it an agent of last resort for life-threatening ventricular arrhythmias despite its toxicity, unclear mechanism of action and bizarre pharmacokinetics (Lazzara, 1989).

4.1.3.2 In Search of a Reference Standard

Thus, what is an appropriate reference Class III drug? According to Vaughan-Williams' classification scheme, the ideal compound would have homogeneous AP widening effects in the absence of any effects on conduction velocity (Vaughan-Williams, 1970), or other actions unrelated to AP prolongation. Clearly there are no "Class III" drugs in clinical use which fit this category. Bretylium is an adrenergic neuron blocking agent (Boura & Green, 1959). Its progeny, bethanidine, alters gNa^{+} and gCa^{++} in addition to blocking gK^{+} (Bkaily *et al.*, 1988), and meobentine has been

shown to block gNa^+ (Wang *et al.*, 1977), d,l-Sotalol has beta blocking activity although d-Sotalol is 1/50 times as potent as its enantiomer in this regard (Kato *et al.*, 1986). Acecainide appears to be selectively Class III, but in chronic therapy it may be deacetylated *in vivo* and thus block gNa^+ as well (Lumma *et al.*, 1987). Certain neuroleptic drugs have been reported to have Class III actions, e.g. melperone (Arlock *et al.*, 1978) and amperozide (Hoglund *et al.*, 1986), but, clearly these, and the numerous other drugs with coincidental Class III properties, can not be considered as ideal reference drugs. The quaternary ammonium derivative, clofilium, has been suggested to interact with alpha adrenoceptors and possibly Ca^{++} channels (Arena & Kass, 1988). Similarly, cesium chloride, TEA or 4-AP have profiles of action *in vivo* that are markedly different from an ideal (cardioselective) Class III drug. It would be folly to use one of the other newly developed drugs as a reference Class III, as sufficient data are not available to make such a claim.

Thus, one is left to choose the lesser of evils. In this regard, the effects of acecainide (on acute administration) d-sotalol and clofilium appear to be the most selective Class III drugs for which there are sufficient data for purposes of comparison. It must be recognized that using a reference standard even if ideal, will only assist in identifying drugs with similar

properties (Brugada, 1987; 1990) and that drugs with potentially beneficial, but unrelated actions may be missed. It goes without saying that if the reference standard is falsely chosen to begin with, the likelihood of arriving at an ideal drug is greatly diminished. The development of histamine H_2 receptor antagonists demonstrated the value of an appropriate bioassay (here, gastric acid secretion), versus screening for compounds with *in vitro* binding properties of a false reference standard (Black *et al.*, 1972). The only way to test whether the third class of antiarrythmic activity is indeed antiarrhythmic is to assay compounds which fit the proposed definition. Thus, if a drug possesses selective AP widening effects, and all other actions of the drug are accounted for, and it is found to be protective against arrhythmias, then it can be assumed (with a defined probability) that Class III actions confer antiarrhythmic protection in the model used. However, it may only be proved that Class III actions are not antiarrhythmic, if under the same rigidly controlled conditions no protection is seen when the requirements of the definition are fulfilled. In order to obtain proof of a causal relationship there is a necessity for rigidly controlled conditions. Thus rarely in biological science can proof be attained. Dose response (D/R) curves aid the identification of drug induced effects, but are themselves limited by co-existence of dose dependent additional actions acting as covariants. Thus, even with D/R curves,

mechanistic certainty is equivocal. It is rare in pharmacology to have a complete D/R curve for each of the effects of a drug. Further complications arise when non-sigmoidal D/R curves are obtained, e.g. serum $[K^+]$ vs. arrhythmia incidence.

With all of these conditions in mind then, the ideal Class III drug would theoretically have predictable effects on the ECG. A Class III drug should not affect QRS duration nor P-R interval since these reflect conduction velocity in ventricles and AVN, respectively. By definition, a Class III drug should prolong the $Q\text{-}T_c$ interval since this reflects the repolarization time of the ventricle (Einthoven, 1912; Katz, 1928). A homogeneous prolongation of the plateau of the AP, as occurs with hypocalcemia or hypothermia widens the S-T segment, where as a dispersion of repolarization times, or alterations in phase 3 repolarization, result in $Q\text{-}T_c$ widening secondary to T- wave broadening or slurring. Both mechanisms may operate simultaneously. Thus, it is important to distinguish between Q-T intervals measured from the Q wave to the initial foot of the T ($Q\text{-}T_i$), the apex of the T ($Q\text{-}T_a$) or end of the T wave ($Q\text{-}T_e$). Widening of the Q-T interval can also result from QRS prolongation (Review see: Surawicz, 1987; Jackman *et al.*, 1988). While it is relatively easy to distinguish Q-T widening caused by the latter mechanism, detection of the first two mechanisms by ECG analysis is

very challenging and requires multiple ECG leads. The presence of U waves further complicates analysis. U waves were initially interpreted as regions of myocardium with delayed repolarization relative to the main mass of the ventricles (Einthoven, 1912). U waves were later ascribed to afterpotentials (Nahum, 1939) and recently this idea has received support from el-Sherif and Lazzara's groups (Lazzara *et al.*, 1978). As heart rate has been reported to alter Q-T interval, as discussed previously, measurements should be made at a constant heart rate. The underlying mechanism responsible for AP widening (or dispersion) cannot be arrived at by analysis of Q-T intervals, since it can occur either as a result of an increase of inward currents or a decrease in outward currents. As discussed in the introduction, numerous drugs, toxins, adrenergic agents, electrolyte imbalances, or metabolic conditions can in turn influence these currents.

The underlying electrophysiological mechanism of the Q-T interval prolongation effects of d-sotalol, clofilium and acecainide (Carmeliet, 1985; Snyders & Katzung, 1985) have been investigated and shown to result from blockade of K^+ channels. Thus, the ECG effects of these drugs might appear different from the toxins, aconitine, ATX-II, and AP-A which all lengthen Q-T and produce U waves via delayed repolarization secondary to increases in inward Na^+ currents

(Matsuda *et al.*, 1959; Peper & Trautwein, 1967; Platou *et al.*, 1986; el-Sherif *et al.*, 1988).

4.1.3.3 Clofilium

Clofilium (1-100μM), a bretylium analogue lacking sympatholytic actions, "selectively" blocks I_K in guinea pig myocytes (Snyders & Katzung, 1985; Arena & Kass, 1988) and produces Q-T widening in the absence of QRS or P-R prolongation (Kopia *et al.*, 1985; Steinberg *et al.*, 1979). Theoretically, I_K blockade produces widening of the AP without changes in the rate of phase 3 repolarization in ventricular tissue (Arena *et al.*, 1990) thus S-T segment widening without T-wave widening on the surface ECG should result unless there is a tissue selective (e.g., ventricle vs. Purkinje) sensitivity. The Q-T widening effects of clofilium in dogs were not measured so carefully in the report by Kopia *et al.*, (1985). However, clofilium did have a proarrhythmic tendency in these and other studies (Carlsson *et al.*, 1990). In man, clofilium (60 - 300 μg/kg) prolonged Q-T interval without affecting AH or HV intervals (Greene *et al.*, 1983). In the rabbit studies of Carlsson *et al.* (1990) clofilium's proarrhythmic effects were potentiated by methoxamine and/or propranolol. In the two conscious rabbits used, Q-T widening of 100% was seen with 0.36 and 0.80 μmol/kg which led to VT. In anaesthetized rabbits, VPB were seen at 1.8 ± 1.3 μmol/kg clofilium and VT

at 6 µmol/kg although no Q-T prolongation value was reported. Our results, in rabbits, showed that tedisamil (which blocks both I_{to} and I_K) produced less Q-T_C widening (unpublished observations) than clofilium in Carlsson *et al.*'s study. The studies of Arena and Kass (1988) predicted, and our results in other species all showed, less Q-T_C widening with specific I_K blockers than with tedisamil. The marked Q-T prolongation with clofilium may result from its completely different structure (quaternary amine) which allows it to block both the "slow" and "fast" components of I_K (Arena & Kass, 1988; Sanguinetti & Jurkiewicz, 1990), relative to other (methylsulfonamide) I_K blockers, which appear only to block the less prominent "rapid" component (Colatsky *et al.*, 1990). Tedisamil appears to block both the "fast" and isoproterenol-sensitive "slow" components of I_K in guinea pig ventricular myocytes (Dukes *et al.*, 1990).

4.1.3.4 D-Sotalol

D-sotalol blocks both I_{K1} and I_K at 10^{-6} - 10^{-4}M, it blocks gNa^+ at 10^{-4}M (Carmeliet, 1985) and has < 1/50th the beta blocking potency of l-sotalol (Kato *et al.*, 1986). Nattel and colleagues (1989) have shown that the EC_{50} (plasma concentrations) for beta blocking effects of racemic sotalol (0.8 mg/l) was ten fold lower than the EC_{50} for Class III increases in refractoriness (6.8mg/l) in anaesthetized dogs. Apparently, both enantiomers have the

same potency for APD widening (Carmeliet, 1985). These studies suggest a narrow margin of Class III selectivity for d-sotalol, i.e. a potency ratio of for Class II:Class III effects of only 5 fold.

Kato's study showed equipotent APD widening at 10^{-4}M for d- and l-sotalol, at 10^{-5}M no beta blocking action was seen for d-sotalol (Kato *et al.*, 1986). Gomoll & Bartek (1986) using dogs showed d-sotalol had 1/12 - 1/14 and l-sotalol 1.6 - 3.2 X the potency of the racemate as beta-antagonists. However, at plasma concentrations giving equi effective beta blockade, racemic sotalol and its enantiomers had similar potency in prolonging VERP. These studies indicated that doses of d-sotalol producing maximal APD widening would be associated with significant beta blockade. Recently, Reid *et al.* (1990) showed further evidence for an interaction of d-sotalol with beta receptors at concentrations producing delayed repolarization. In this study d-sotalol's EC_{50} for APD widening was 13 µmol/l, while the K_A for beta-adrenoceptors was 4 µmol/l in binding studies. Theoretically, d-sotalol would be expected to widen the Q-T_C interval (by Class III effects) and the P-R and R-R intervals (by Class II effects) without altering QRS duration. This has been shown experimentally, in anaesthetized dogs. D-sotalol (2mg/kg) produced 15% widening of the Q-T_C interval without increasing QRS duration (Feld *et al.*, 1986). Clinical findings gave

similar results (McComb *et al.*, 1987; Sahar *et al.*, 1989). Results with tedisamil on the ECG, at first glance appeared similar to d-sotalol, however no beta receptor affinity has been shown for tedisamil (Kalichemie Internal Report) and autonomic blockade does not prevent tedisamil-induced P-R widening (Howard *et al.*, 1989).

In rats, d-sotalol (2mg/kg) has been shown to have minimal effects on contractility (Hoffmeister & Siepel, 1988; Tande & Refsum, 1988). In a clinical study, d-sotalol (1.5 to 2.75 mg/kg, i.v.) lowered mean BP by 13 ± 9% (Sahar *et al.*, 1989). This minor effect on BP may have been related to beta blockade. The Class III effects of sotalol have been reported to be reduced by ischaemia (Culling *et al.*, 1984; Cobbe *et al.*, 1985); if the patients had such abnormalities the haemodynamic response to d-sotalol may have been more dependent on beta blockade.

As discussed previously, racemic sotalol has been reported to be associated with torsade de pointes (Laakso *et al.*, 1981; McKibbin *et al.*, 1984). So far, no clinical demonstrations of a link between d-sotalol and TdeP have been reported. Sotalol and acecainide have been shown to induce EADS *in vitro* (Strauss *et al.*, 1970; Singh & Vaughan-Williams, 1970; Dangman & Hoffman, 1981).

4.1.3.5 Acecainide

Acecainide (30 mg/kg) widened Q-T_C interval 16% without affecting QRS in anaesthetized dogs (Feld *et al.*, 1988). In humans, acecainide, at plasma concentrations of up to 1.8 x 10^{-4}M, has been reported to have no effects on QRS or P-R intervals (Winkle *et al.*, 1981; Jaillon *et al.*, 1981; Sung *et al.*, 1983), while it increased Q-T_C interval dose dependently by a maximum of 22%. These selective Q-T_C widening effects are similar to our results with UK68,798, RP62,719 and risotilide when given to guinea pigs, pigs and baboons. However, despite similar efficacy, UK68798 appears to be 1000 fold more potent than acecainide.

In anaesthetized dogs, acecainide (12 mg/kg and 60 mg/kg i.v.) was found to have positive inotropic actions and produced a +12%, and +33% increase in force, respectively. However, these actions may have been related to autonomic as well as direct effects (Letora & King, 1986; Letora *et al.*, 1986). The result of the direct and indirect actions of the high dose was a reduction in heart rate and blood pressure, while 12 mg/kg had no chronotropic or hypotensive effects. In the human studies referred to above only Jaillon *et al.* (1981) reported haemodynamic effects, a slight decrease in BP was noted at plasma concentrations above 15 µg/ml. Previous studies in humans showed acecainide to lack myocardial depressant actions (Elson *et al.*, 1975; Atkinson *et al.*, 1977). Similar to these

studies, UK68798, tedisamil, and risotilide lack hypotensive effects (Gwilt *et al.*, 1989, 1991; Dalrymple *et al.*, 1989; Grohs *et al.*, 1989; Colatsky *et al.*, 1989).

4.1.4 Summary: Analysis of Surface ECG Intervals

QRS, P-R and Q-T_C intervals, give useful information on a drug's effects on ventricular and AVN conduction and ventricular repolarization. Tissue dependent effects on the atria and Purkinje or endocardium versus epicardium are not so readily seen with analysis of the standard ECG. Analysis of AH, HV intervals etc. gives further clues to drug effects on some of these tissues. More detailed studies can be done with epicardial mapping and programmed electrical stimulation.

Characteristic Class III electrocardiographic actions i.e. Q-T_C widening, can be shown for drugs which block I_{K1} and I_K in most mammalian species including man. However, these drugs appear to be without effects in rats. Tedisamil which produced Q-T_C widening in a species dependent manner, was most effective in rats. As rats have an abbreviated ventricular AP plateau which may be related to a large I_{to} component (Josephson *et al.*, 1984), tedisamil might widen Q-T_C intervals in rats through blockade of this current (Dukes *et al.*, 1990). Tedisamil and KC8851 appear to be unique in relation to both clinically available Class III drugs and

those under development (see introduction). UK68798 appears to have similar efficacy, but up to 1000 fold greater potency than clinically available class III drugs. UK68798, RP62719, and risotilide had selective Q-T_C widening effects in primates, which were of similar magnitude to those reported for acecainide and d-sotalol in man.

4.2 Electrical Stimulation

4.2.1 Overview

There are many methods of measuring VF threshold in animal experiments, such as single pulse stimulation, and sequential R on T methods. Essentially all that is needed is a correctly timed stimulus of sufficient strength. The vulnerable period results from inhomogeneity of recovery of excitability following normal excitation (Mines, 1913; Moe *et al.*, 1964), and the use of multiple shocks simply increases the probability of applying an appropriately timed stimulus (Han, 1969). Wiggers and Wegria (1940) suggested that the current strength needed to elicit VF, could be used to estimate the sensitivity of the ventricle, and thus determine the influence of antiarrhythmic drugs on VF threshold. Recently, it has been shown that VF threshold is dependent on the method of stimulation (Review: Sugimoto *et al.*, 1989). These observations are based on proposed stages of development of VF from local excitation, through

ventricular excitation, repetitive excitation and then disorganization into VF. These authors showed that drug effects on VF thresholds were dependent upon which stage the drug worked. The methods we used are similar to those used by Marshall *et al.* (1983) which detects Class I and III effects on VF_t.

Electrical stimulation techniques have also been used to measure refractory periods *in vitro* and *in vivo* (see Hoffman & Cranefield, 1960). The recovery of excitability after a preceding impulse is determined mainly by the availability of Na^+ channels, which are voltage dependent and thus action potential widening can prolong refractoriness.

4.2.2 Tedisamil versus Class I Drugs

In rats tedisamil prolonged ventricular refractoriness more potently than the representative Class I subclasses. Tedisamil's effects on MFF and ERP generally paralleled its marked (50% increase) $Q\text{-}T_c$ widening effects. Treatments did not change the ratio of 1/MFF to ERP values, suggesting that frequency dependent effects were not apparent at rates above 7Hz. Unlike the Class I drugs, which dose dependently elevated VF_t, tedisamil, at a high enough dose, ($\geq$ 4mg/kg) rendered the heart completely resistant to VF. This suggests that in the small rat heart, refractoriness was

prolonged to such an extent that multiple fractionations of induced reentrant wave fronts were not possible (Sugimoto *et al.*, 1989). Propafenone most closely resembled tedisamil (except in the latter effect) which supports Wu and Hoffman's (1987) observations that effects of APD alone, *in vitro* do not necessarily predict antiarrhythmic efficacy in a given model. Using the same reentry model Wu *et al.* (1989) showed Class I drugs could be distinguished from Class III drugs because the former prolong cycle length of the reentry more than Class III's.

4.2.3 Tedisamil versus UK68,798

Tedisamil had similar effects on refractoriness and stimulation thresholds in rats anaesthetized with either pentobarbitone or halothane (above). On the other hand, UK68,798 was without effects on any electrical stimulation parameters. This matched the lack of effects of UK68798 on the rat ECG. UK68,798 and UK66,914 have been shown to increase VERP in a frequency dependent manner in guinea pig *in vitro* and dogs *in vivo* which is consistent with its Q-T widening effects in these species (Dalrymple *et al.*, 1989, Gwilt *et al.*, 1989a & 1989b). UK68,798 and UK66,914 also elevated VF_t in their studies, a finding not seen with UK68,798 in rats. In dogs UK68,798 was reported to increase the incidence of spontaneous reversion to sinus rhythm after

VF induction (Gwilt, 1989) by a 50Hz train of stimuli in a manner similar to that used here.

In guinea pigs we found UK68,798 to only have minor non-statistically significant effects on refractoriness and VF_t. The difference between our results and those *in vitro* (see above) may have been due to the high (stimulated & resting) heart rate of guinea pigs *in vivo*. At 5Hz stimulation *in vitro*, minimal VERP prolongation was seen (Gwilt *et al.*, 1989).

Guinea pigs have no I_{to} in their ventricle (Hume & Uehara,1985), thus tedisamil's effects on I_K more effectively increased ERP in guinea pigs than UK68,798 (Section 3.2.3). ERP was determined at a fixed stimulation rate of 6.5 Hz, so tedisamil-induced bradycardia was not a factor. It is also unlikely that tedisamil's gNa^+ blocking properties contributed to the ERP prolongation, because the measurements made at 10 minutes after dosing showed no effects on QRS, P-R or VF_t. It is possible that tedisamil produced more "complete" block than UK68,798 of I_K, as tedisamil has been shown to block the both the "fast" and isoproterenol-induced "slow" components of I_K (Dukes *et al.*, 1990). However, UK68,798 appears to only block the "fast" component of I_K (Gwilt *et al.*, 1989).

4.2.4 Electrical Stimulation Studies in Primates

In primates, tedisamil was less effective at reducing MFF than in rats although it was more effective than in guinea pigs. This paralleled the intermediate effects on Q-T_C in this species (Figure 7). Tedisamil appears to have been more potent in primates than in rats with the maximal response occurring at 1 mg/kg.

In primates, the index of excitability, threshold for capture, was not affected by tedisamil which suggests gNa^+ blockade was not appreciable at the doses used. Despite the bradycardia and slight P-R prolongation produced by tedisamil, AVN refractoriness was not significantly increased by tedisamil (only a slight decrease in ventricular response to atrial pacing). This may reflect a negative frequency dependency of tedisamil in the AVN. It might be worthwhile to determine ERP by the extra stimulus method at a constant rate and compare it to the MFF values obtained in this study. The lower resting MFF obtained by atrial pacing could have reflected longer ERP values in nodal tissue. In this regard the MFF obtained by atrial pacing corresponded to the minimum MFF obtained from ventricular pacing, although direct comparisons can not be made because of the difference in the strength of the stimulus and mode of capture of the ventricle. These observations assume "supernormal" excitability did not influence MFF determination (Childers *et al.*, 1968).

Another simpler reason why tedisamil did not significantly decrease AVN refractoriness is that recovery of excitability may occur after repolarization is complete in nodal cells (Hoffman & Cranefield, 1960) and therefore might depend on factors affecting availability of Ca^{++} channels, which tedisamil does not block directly (Dukes & Morad, 1989).

Section 4.2.5 Effects of Other Class III Drugs on ERP.

In humans, acecainide has been shown to increase atrial and ventricular ERP but not AVN ERP (Sung *et al.*, 1983). However, d-sotalol did increase AVN ERP as well as VERP in patients, at doses producing P-R widening, which suggests a beta blockade contribution (Sahar *et al.*, 1989).

Most studies of Class III drugs include data showing drug effects on refractoriness. This is not surprising as ERP prolongation is generally believed to be the mechanism of action of Class III drugs (Vaughan-Williams, 1970; 1975). Thus, the three "reference" compounds, d-sotalol, acecainide and clofilium have all been shown to increase ventricular refractory periods *in vitro* and *in vivo* (Feld *et al.*, 1986; Strauss *et al.*, 1970; Singh & Vaughan-Williams, 1970; Sahar *et al.*, 1989; Singh *et al.*, 1986; Feld *et al.*, 1988; Harron & Brogden 1990; Sung *et al.*, 1983; Jaillon *et al.*, 1981; Wu & Hoffman, 1987; Wu *et al.*, 1989; Euler & Scanlon, 1988). In studies using programmed electrical stimulation,

prolongation of ventricular ERP was generally associated with an increase in cycle length of induced tachycardias, which often resulted in termination of the arrhythmias. In one model of reentry, ERP prolongation was effective whether it was achieved by APD prolongation (Class III, e.g. acecainide) or was accompanied by conduction showing (Class Ia, e.g. procainamide), although in this study a long path length (relative to ERP) was available for the reentrant impulse (Wu & Hoffman, 1987).

The similar efficacy of UK68,798 in dogs compared to d-sotalol has also been seen with other new Class III agents such as E4031 (Lynch *et al.*, 1990, +23% VERP; Katoh *et al.*, 1990, +18% VERP), MS-551 (Kamiya *et al.*, 1990, +20% VERP), RP58,866 (Mestre *et al.*, 1989, +12% VERP), sematilide (Chi *et al.*, 1990, +15% VERP) and risotilide (Colatsky *et al.*, 1989, +23% VERP). The remarkable potency of UK68,798 is shared by E4031, MS-551 and RP58,866 which have these effects at ug/kg doses. A general feature of all these studies was a greater prolongation of atrial than ventricular refractory periods. The ventricular ERP was maximally prolonged no more than 25% by any of the drugs (see above). RP58,866 which has been reported to "selectively" block I_{K1} (Escande *et al.*, 1989; 1990) produced the least (12%) increase in VERP (Mestre *et al.*, 1989). It thus appears that drugs reported to "selectively" block K^+ channels in myocardium have a maximal effectiveness

at prolonging VERP limited to <25%, at least in dogs and guinea pigs. Due to the different K^+ currents contributing to repolarization of the AP in rats, UK68,798 (and by implication, numerous I_K blockers) was ineffective at prolonging ERP while tedisamil was more effective. Under resting conditions the rat VERP is much shorter than other species, as a result of an abbreviated plateau (see below). However, the increase in rat VERP induced by tedisamil makes this species and drug combination ideally suited for testing the effectiveness of Class III mechanisms. Detailed analysis of reentry and abnormal automaticity models should be possible, and the results compared to models using aconitine, ATX-II, A-PA in other species. While direct applicability to human cardiac arrhythmia mechanisms is never possible with animal models, theoretical hypothesis can be tested over a broader range of ERP than with the other "K^+ blocker" drugs.

4.3 Action Potential Morphology

4.3.1 Frequency Dependence of APD

Since APD width influences ERP, and rats and guinea pigs have morphologically different ventricular epicardial AP (Weidmann, 1956) we used intracellular recording techniques to elucidate tedisamil and UK68,798's disparate effects on AP morphology in rats and guinea pigs.

Tedisamil's marked APD widening effects in rats was consistent with blockade of I_{to} in the ventricle (Dukes & Morad, 1989; Josephson *et al.*, 1984). It has also been reported that frequency dependent APD prolongation occurs at slow rates of stimulation *in vitro* (Carmeliet, 1977, Payet *et al.*, 1981). However, the rat ventricular action potential has an early phase of repolarization (up until APD_{50}) controlled by both decay of I_{si} and increasing I_{to} and a late phase attributed to inward current from either electrogenic Na^+/Ca^{++} exchange (Mitchell *et al.*, 1984a & 1984b; Noble, 1987) or a $[Ca^{++}]_i$ activated inward current (Colquhoun *et al.*, 1981) These two phases respond in opposite directions to frequency of stimulation changes; at slow rates (0.2Hz) APD_{25} shortens and APD_{75} lengthens, while the opposite effects occur at higher rates (5Hz). These effects have been attributed to changes in amplitude and inactivation time constant of I_{si} (Payet *et al.*, 1981).

Alternatively, rate dependent prolongation of APD25 and APD50 may be mediated by inactivation of I_{to}, since I_{to} of cardiac muscle has slow kinetics of recovery from inactivation (Josephson *et al.*, 1984; Coraboeuf & Carmeliet, 1982; Hiraoka & Kawano, 1989). It must be borne in mind, that in rat ventricles the frequency dependent changes in APD_{25} and APD_{75} were only a decrease from 8.3 to 5.2 ms and an increase from 42.7 to 59.1 ms respectively for a decrease in stimulation rate from 5Hz to 0.2 Hz *in vitro* (Nobe *et*

al., 1990). We observed very little change in rat epicardial APD in response to vagal stimulation, (Figure 9). More thorough studies of tedisamil's effects on APD at different stimulation rates *in vitro* still need to be done.

4.3.2 Effects of Tedisamil on AP Morphology.

Our observations indicate that bradycardia produced by tedisamil was not sufficient to account for tedisamil's marked AP widening in rats. Indeed bradycardia would have been expected to shorten the early repolarization phase. However, blockade of I_{to} by 4-AP in rat ventricle does not produce the same degree of AP widening *in vitro* as occurred in our studies (Josephson *et al.*, 1984; Nobe *et al.*, 1990). Although under different conditions from our *in vivo* experiments, a clue to the difference between tedisamil's action and 4-AP can be gained from the *in vitro* studies of Dukes & Morad (1989) and Dukes *et al.* (1990) in which 4-AP reduced peak I_{to} current while tedisamil increased the rate of inactivation of I_{to}. Dukes and Morad were further able to show that the most sensitive measure of tedisamil's action was a reduction of K^+ efflux. By speeding inactivation of I_{to}, a decrease in permeability to K^+ would result at a time when inward currents were activated. Thus, membrane potential would reflect the Na^+ and then Ca^{++} equilibrium potential to a greater extent, due to their increased relative permeability to permeability of K^+. Thus

"window" current (Colatsky, 1982), background inward current, and I_{si} may have had a greater contribution to membrane potential by virtue of the increased relative permeability of Na^+ and Ca^{++} vs K^+ without an antecedent increase in flux of Na^+ or Ca^{++}. This was reflected in the rounding off of the spike in our studies and in gross qualitative terms, tedisamil's transmogrification of rat APs into guinea pig AP morphologies. Additional Ca^{++} flux may have occurred secondary to the prolonged depolarization and contributed to the increased contractile force reported for tedisamil (Grohs *et al.*, 1988). Without a knowledge of the Ca^{++} transient concentration these observations are mere speculations. It is interesting to speculate that tedisamil might have converted rat hearts to "positive staircase" hearts (Bowditch 1871, cf: Sheperd & van Houtte, 1979).

Tedisamil's effects in guinea pig ventricle were most likely unrelated to blockade of I_{to} in the ventricle, because I_{to} is negligible in this tissue (MacDonald *et al.*, 1984). However, I_{to} has been reported in guinea pig atria (Wang & Nattel, 1989) and nodal tissue (Irisawa, 1987). Blockade of I_{to} in the node may be responsible for bradycardia and bradycardia-dependent widening of the ventricular AP (Anderson & Johnson, 1976; Boyett & Jewell, 1980). Adrenaline only partially reversed the APD prolongation, despite normalizing the heart rate, similarly tedisamil prolonged ERP in "paced" guinea pigs. As

discussed earlier, adrenaline enhances I_K, but tedisamil appears to block this effect (Dukes *et al.*, 1990). We have noted that vagal stimulation widens guinea pig ventricular epicardial potentials (Figure 9) but has only minimal effects on rat ventricular epicardial potentials *in vivo*, in agreement with the work cited above (Anderson & Johnson, 1976; Boyett & Jewell, 1980). It is unlikely that K_{ACh} receptors in the ventricle were activated by vagal stimulation because the vagus does not innervate the ventricle, and ACh is rapidly hydrolyzed in plasma by acetylcholinesterase. Indeed, activation of $I_{K(ACh)}$ would have shortened the ventricular APD, which is the opposite to what we saw. The degree of widening for a similar degree of bradycardia was greater for tedisamil than for vagal stimulation. Thus, additional factors must have contributed to tedisamil's APD prolongation besides bradycardia. The simplest explanation would be a blockade of the delayed rectifier (I_K) as has been reported for tedisamil (Dukes *et al.*, 1990). The onset of the delayed rectifier (on top of a maintained component of I_{si}) contributes to initiation of AP repolarization in guinea pig ventricle (MacDonald & Trautwein, 1978; Lee & Tsien, 1982; Hume & Uehara, 1985). Tedisamil has been shown to have no effect (Dukes *et al.*, 1990) on isoproterenol induced Cl^- current, I_{Cl}, (Harvey & Hume, 1989) thus the shortening of the APD after adrenaline could have been due to reversal of the bradycardia (nodal tissue action) and induction of ventricular I_{Cl}.

Tedisamil produced greater effects on APD than on Q-Tc, however their is no *a priori* reason why the degree of epicardial APD prolongation should equal the extent of Q-T lengthening of the surface ECG.

4.3.3 Effects of UK68,798 on AP Morphology.

UK68,798 was without effects on rat ventricular epicardial potentials, consistent with its lack of effects on ECG or electrical stimulation in this species. These findings are consistent with both a lack of I_K in rat ventricle (Josephson *et al.*, 1984) and the selectivity of UK68,798 for I_K (Gwilt *et al.*, 1989;1991).

I_{si} is an important membrane potential generator in the early phase of rat ventricular AP, but has less of a role in the later phase which depends on $[Na^+]_o$ and $[Ca^{++}]_i$ and might be due to electrogenic Na^+/Ca^{++} exchange (Schouten and ter Kerrs, 1985; Mitchell *et al.*, 1984a & 1984b) and ryanodine-sensitive release of Ca^{++} from internal stores (Mitchell *et al.*, 1987). In guinea pig ventricular cells the release of Ca^{++} from internal stores is less important than in rats. Attempts at simultaneous AP recording and patch clamping have been done on chick embryonic ventricular cells, and have shown agreement between microscopic and macroscopic current recording techniques (Mazzanti &

Defelice, 1988). Studies comparing rat and guinea pig ventricular cells have not been done yet, with this technique.

In guinea pigs, UK68,798 produced similar effects to its effects in dog ventricle *in vitro* (Gwilt *et al.*, 1989; 1991). In both species the minor prolongation of plateau can be most easily interpreted as resulting from its blockade of I_K (Gwilt *et al.*, 1989b; 1991). The lack of effects of UK68,798 on rise rate and height of the AP suggest that Na^+ channels were not blocked by the drug, which is consistent with its lack of effect on conduction time in guinea pig papillary muscle *in vitro* (Gwilt *et al.*, 1989a; 1991). This is also consistent with its lack of effects on QRS and P-R intervals. Thus, UK68,798 appears to be a most potent and selective Class III drug, although its efficacy is not remarkable. UK68,798 was more effective (+80% widening) in Purkinje fibres which have 30% wider AP than ventricular muscle in control conditions (Gwilt *et al.*, 1989; 1991; Myerburg, 1971).

4.3.4 Comparison to Other Class III Drugs

The APD prolonging effects of sotalol, at concentrations of up to 10^{-3}M, have been studied in rabbit atrial and canine Purkinje fibres and ventricular muscle (Strauss *et al.*, 1970) as well as cat papillary muscle

(Singh & Vaughan-Williams, 1970). High concentrations, greater than 1.6 x 10^{-4}M, were associated with reductions in rise rate, unlike with UK68,798. As with UK68,798 in guinea pigs, sotalol produces more marked widening of dog Purkinje fibres than ventricular tissue. However, up to 5 x 10^{-4}M sotalol did not prolong rabbit atrial APD (Strauss *et al.*, 1970). This tissue has a large I_{to} component (Giles & Imaizumi, 1988) and resembles rat ventricle more than guinea pig ventricle. Although canine Purkinje fibres have been reported to have a large I_{to} current, sotalol and UK68,798 were more effective in this tissue than ventricle *in vitro*. It may be that Purkinje fibres have greater dependence on I_K for *final* repolarization than ventricular muscle. As, work generating ventricular cells would be expected to have greater efficiency of Ca^{++} handling than Purkinje fibres, and $[Ca^{++}]_i$ has marked effects on APD and contraction, it may be expected that ventricular muscle APD has a greater dependence on Ca^{++} handling relative to I_K. The Purkinje fibre "gating function" (Myerburg, 1971) might be subserved by a slower decay of inward current and repolarization may therefore depend to a greater extent on I_K activation, which by virtue of its slow macroscopic activation kinetics would be expected to contribute more to repolarization in cases of longer APD. This argument is basically the inverse of the situation in rat ventricle, where I_K has little effect and the short late phase is dependent on rapid Ca^{++} handling (Mitchell *et al.*, 1984a & 1984b; Schouten and ter Kerrs,

1985). The prevalence of EADs in Purkinje fibres also suggests poorer Ca^{++} handling in this tissue. *A priori*, it would be less critical to control $[Ca^{++}]_i$ as efficiently as in working muscle.

In vitro d-sotalol would not be expected to differ markedly from d,l-sotalol as both enantiomers have similar potency for APD prolongation (Carmeliet, 1985) and sympathetic influences are irrelevant. A report suggesting d-sotalol (10 - 1000 µmol/l) reduced I_{to} more than I_{K1} in isolated sheep cardiac Purkinje fibres has recently appeared (Berger *et al.*, 1989). Similarly, APD widening has been shown using intracellular recording with acecainide (Dangman & Hoffman, 1981) and clofilium (Steinberg *et al.*, 1981).

4.4 Myocardial Ischaemia

4.4.1 Studies In Rats

Our results in conscious rats support the concept that drugs which can significantly prolong APD, VERP and Q-T_C intervals can suppress ischaemia-induced arrhythmias. However, a marked degree (400% and 100% respectively) of APD and VERP prolongation was needed to suppress fibrillation in this species. The small sized rat heart was still able to support fibrillo-flutter despite increases of VERP of 65% produced by 2mg/kg tedisamil. This suggests that Class III

antiarrhythmic drugs may only be useful for microreentry of path length limited in size by anatomical substrates. In other words, a degree of protection might be afforded by increasing ERP such that the product of ERP and conduction velocity gives a longer path-length than can be supported by the anatomical substrate. A simple calculation illustrates this point; if one assumes a reentrant circuit with a conduction velocity of 0.6 m/s and an ERP of 100 ms (e.g. after tedisamil), then the minimum path-length would be 6 cm as compared with 3 cm for an ERP of 50ms (control conditions). Although tedisamil and KC8851 had antifibrillatory actions in the rat, this was not accompanied by an ability to suppress VPB. Selective antifibrillatory action has been described for the K^+ channel blockers bretylium, bethanidine and meobentine (Bacaner *et al.*, 1986). Tedisamil's and KC8851's S-T segment elevating properties may have resulted from possible greater prolongation of APD in normal vs. ischaemic tissue (Janse, 1986).

The lack of effectiveness of UK68,798, RP62,719 and risotilide in rats supports the concept that their antiarrhythmic actions in other species relates to their ability to prolong ERP in those species and not to some nonelectrophysiological effect. Our own results in pigs suggest the effectiveness of UK68,798 may not be as high as originally proposed (Gwilt *et al.*, 1989). More studies are

needed. UK68,798 may suppress specific types of arrhythmias, such as reentrant tachycardias involving fixed path-lengths in Purkinje fibres. Studies with risotilide in pigs have suggested remarkable protection in an acute ischaemia model (Colatsky *et al.*, 1989). The related compounds E-4031 and sematilide have also been shown to be effective in canine models of acute ischaemia in the presence of infarction (Lynch *et al.*, 1990; Chi *et al.*, 1990). The use of dogs for ischaemia-induced arrhythmias has been questioned due to variability in collateral artery anatomy as discussed previously (Section 1.1.7). Earlier studies with clofilium in a canine model of sudden death found this compound to be ineffective despite elevations of VF_t and prolongations of VERP in non-ischaemic heart (Kopia *et al.*, 1985). Cobbe's group has documented a loss of class III drug induced ERP prolongation in ischaemic conditions (Cobbe *et al.*, 1985, Cobbe, 1988). On the other hand, Gough and El-Sherif (1989) have pointed to an increased proarrhythmic tendency at antiarrhythmic doses with clofilium, d-sotalol and bretylium in "ischaemic" Purkinje fibres surviving infarction. The marked increase in APD in el-Sherif's study was opposite to that seen by Cobbe's group, which may have been related to el-Sherif's experimental conditions (0.25 Hz stimulation rate, superfused fibres chosen from survivors of infarction) whereas Cobbe's model is an arterially perfused

interventricular septum stimulated at > 1 Hz (Cobbe *et al.*, 1985).

Studies in rat ischaemia and reperfusion models have shown protective effects for sotalol (Lamontagne *et al.*, 1989), amiodarone and desethylamiodarone (Riva & Hearse, 1989, Varro *et al.*, 1989). As these drugs are not selective and the rats were prepared acutely, a false positive protective role for Class III antiarrhythmics in rats might have been seen. Similarly Brooks *et al.*, (1989) used acutely prepared anaesthetized rats and found protection against ischaemia induced arrhythmias with melperone but not with sotalol. In reperfusion studies *in vitro* and *in vivo* bretylium, clofilium and melperone were moderately to strongly protective, but sotalol was less effective (Brooks *et al.*, 1989). The broad sensitivity claimed by these authors for rodent models, should be accepted with caution as our results indicate that the new "specific" class III drugs may not be effective in rats. The response of acutely prepared anaesthetized rats to ischaemia is determined by many factors including serum $[K^+]$ which may be changed by recent surgery and therefore must be measured (Curtis *et al.*, 1987, Paletta *et al.*, 1988). Further the only "selective" Class III used was clofilium and it was more effective *in vitro* than *in vivo*. Curtis & Hearse (1989) have shown reperfusion induced arrhythmias to be less

sensitive to $[K^+]$ than ischaemia induced arrhythmias *in vitro*.

4.4.2 Reperfusion-Induced Arrhythmias

A note should be made regarding glibenclamide's lack of effectiveness against ischaemia/reperfusion induced arrhythmias in the rat. We found no protection despite reported antiarrhythmic effects in globally ischaemic rat hearts by Opie's group (Kantor *et al.*, 1990). We did not carry out an in depth analysis of the possible time dependency of glibenclamide's actions, and thus we cannot completely dismiss a role for glibenclamide in the treatment of ischaemia/reperfusion. However, prevention of ischaemia-induced AP shortening by glibenclamide mediated $I_{K(ATP)}$ blockade, might be less effective in rats than in species with "broad plateau" AP. Although $I_{K(ATP)}$ blockade might delay loss of intracellular K^+, eventually the cells would die if ischaemia were maintained. Opie's group further speculated that K_{ATP} channels are activated after such brief durations of ischaemia despite maintained levels of [ATP] in the cytosol, by invoking compartmentalization of ATP or the influence of other nucleotides (Stern *et al.*, 1988; Opie & Clusin, 1990). This was complete speculation. Furthermore, prevention of the AP shortening which occurs in ischaemia may be deleterious by promoting Ca^{++} entry (Cole & Leblanc, 1990). An alternate hypothesis might explain a protective

effect of $I_{K(ATP)}$ blockade, by permitting more rapid necrosis via Ca^{++} overload, the ischaemic cells could be rendered electrically silent. The benefits of such a treatment are obviously outweighed by the potential worsening of the contractile failure. Another group has reported prevention of K^{+} loss with ischaemia by glibenclamide treatment in dogs (Bekheit *et al.*, 1990).

4.4.3 Our Studies in Pigs

Although none of the pigs (n = 6) used in our study fibrillated in response to ischaemia after tedisamil treatment (8mg/kg), the group size was too small to conclusively demonstrate an antifibrillatory effect. The incidence of VF in historical controls was only 3/16. Similarly, UK68,798 was not shown to protect against ischaemia-induced fibrillation in the same model. A similar degree of Q-T_C prolongation was produced by either tedisamil or UK68,798 which may have been due to I_K blockade. There was no VF seen after tedisamil and the VF which occurred in the UK68,798 treated pigs was initiated by VT. These results indicated that although free of toxic effects, UK68,798 had limited efficacy. While tedisamil has less selective actions, its usefulness will need further studies.

4.5 General Conclusions

4.5.1 Species Dependent Actions

The putative class III antiarrhythmic drugs we tested exerted species dependent actions on the ECG. These differences were most likely related to species dependent K^+ channel distribution. Tedisamil was markedly effective in prolonging APD, ERP, and Q-T_C interval in rats, which can be most easily explained by tedisamil's I_{to} blocking actions, as this current contributes to the rapid repolarization of rat ventricle AP (Josephson *et al.*, 1984; Dukes *et al.*, 1990). The lack of effectiveness of UK68,798, RP62,719, and risotilide at prolonging Q-T_C interval or APD (UK68,798) in rats can be explained by the relative unimportance of I_K in determining APD in rats (Josephson *et al.*, 1984; Dukes *et al.*, 1990). However, it was evident from the experiments with UK68,798 that I_K contributes to AP repolarization in guinea pigs, pigs, and primates. These studies highlight the necessity of assessing the pharmacological profile of a class III drug in the same species in which one intends to assay the drug for antiarrhythmic activity.

4.5.2 Antiarrhythmic Actions

Antifibrillatory action was evident at doses of tedisamil which prolonged Q-Tc interval and ERP twofold and APD fourfold in rats. This protection can not be attributed to the tedisamil-induced bradycardia, as heart rate

reduction has been shown not to protect against arrhythmias in this model (Curtis *et al.*, 1987, Abraham *et al.*, 1989). Thus, class III action can protect against fibrillation, provided a sufficient degree of APD prolongation (and ERP increase) is achieved. The amount of APD prolongation necessary, suggests that the other putative class III drugs might not be effective antifibrillatory agents in other species, although these experiments still need to be done fully. Our preliminary results in pigs suggest this lack of efficacy for UK68,798. It appears from our results and the results of others, that drugs which have been reported to block I_K may only maximally prolong $Q\text{-}T_c$ interval by up to 25%. Tedisamil, with additional I_{to} blocking properties, had greater efficacy in primates which suggests that it might be possible to design an effective class III agent for use in man.

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