THE PHARMACOLOGY AND ANTIARRHYTHMIC ACTIONS OF RSD 1000

by

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Abstract

In an attempt to develop a pathologically-specific antiarrhythmic agent, a series of arylacetamide derivatives have been synthesized. One of these compounds, RSD 1000 is very efficacious against arrhythmias produced during myocardial ischaemia and possesses limited toxicity. This thesis provides a preliminary pharmacological profile and investigates the possible mechanisms responsible for the antiarrhythmic actions of RSD 1000.

The pharmacological profile of RSD 1000 was determined by *in vivo* and *in vitro* experiments in rats and mice. Drug effects were monitored using measurements of blood pressure (BP), heart rate (HR) and electrocardiogram (ECG) in pentobarbitone-anaesthetised rats. Additionally, isolated rat hearts were used to investigate the selectivity for "ischaemic" versus normal myocardial tissue. Lethality was tested using both rats and mice. Two models for arrhythmogenesis were used to investigate the antiarrhythmic activity of RSD 1000 in unconscious rats and these were electrically-induced and ischaemia-induced arrhythmias via coronary ligation.

RSD 1000 produced a dose-dependent prolongation of P-R, QRS, RSh and Q-T intervals at higher doses. The estimated effective dose for a 25% change (ED25%) from pre-drug for PR, RSh and Q-T were 20, 12, and $\cong$ 20 µmole/kg/min, respectively. The effective dose range of RSD 1000 on electrically-induced arrhythmias was much lower. RSD 1000 increased the threshold currents for induction of ventricular fibrillo-flutter (VFt) (ED25% = 3.0 µmoles/kg/min), extrasystoles (iT) (ED25% = 4.5 µmoles/kg/min) and effective refractory period (ERP) (ED25% = 4.0 µmoles/kg/min) in a dose-related manner. The effect of RSD 1000 on

ischaemia-induced arrhythmias was tested between 1.0 and 8.0 μmoles/kg/min and an antiarrhythmic ED50% value (or AA50%) of 2.5 μmole/kg/min was determined. The highest dose, 8.0 μmoles/kg/min, provided the greatest protection, since it reduced the incidence of both ventricular tachyarrhythmias and fibrillations from a control value of 95% to 0%. Unlike many antiarrhythmic agents, complete protection against ischaemia-induced arrhythmias with RSD 1000 was conferred with minimal toxicity on the cardiovascular system. Effects on blood pressure and heart rate were minimal. This finding was substantiated with time-effect data from sham-occlusions by infusing with RSD 1000 at 8.0 μmoles/kg/min.

RSD 1000 showed selectivity for conditions simulating myocardial ischaemia in isolated rat hearts. RSD 1000 was approximately 64 times more potent in terms of ECG changes (presumed due to channel blockade) in conditions of low pH (6.4) and high [K+] (10mM) than in normal (pH=7.4; [K+]=4mM) buffer conditions. Studies on morbidity and lethality in both rats and mice indicate that RSD 1000 is well tolerated at doses which provided complete antiarrhythmic protection. The LD50 value in rats and mice were 64 and 67 μmole/kg, respectively.

The same experiments were completed for lidocaine for the purpose of comparing its effects with those of RSD 1000. The results of this study suggest that RSD 1000 is more cardiac selective and produces greater channel blockade in "ischaemia"-simulated conditions than lidocaine. RSD 1000 and its effects on both the sodium and potassium channels suggest that the observed antiarrhythmic effect is the result of a combination of class I and III actions such that the prolongation of the cardiac action potential duration was sufficiently lengthened to provide antiarrhythmic protection while limiting the susceptibility for an arrhythmogenic substrate

by the same action. It is possible that this proposed mechanism of antiarrhythmic activity for RSD 1000 acts independently or in combination with the observed cardiac and/or "ischaemia"-selectivity to contribute to the overall antiarrhythmic action. The results from this study suggests that RSD 1000 possesses strong antiarrhythmic properties and may perhaps be a good candidate for the next generation of antiarrhythmic agents.

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ABBREVIATIONS

AA50%	dose producing a 50% of antiarrhythmic response
ANOVA	analysis of variance
AS	arrhythmia score
AVB	atrio-ventricular block
AVN	atrio-ventricular node
BP	blood pressure
Ca	calcium
CO_2	carbon dioxide
[ ]	concentration
dP/dt	maximum rate of rise of phase 0 of the cardiac action potential
ECG	electrocardiogram
ED25%	dose of a drug producing 25% change from control
ERP	effective refractory period
gNa	sodium current
Hz	hertz
iT	current threshold
i.v.	intravenous
i.p.	intraperitoneal
K	potassium
LAD	left anterior descending
Lidol	lidocaine in "ischaemic"buffer
LidoN	lidocaine in normal buffer
LD50	dose of drug producing 50% lethality
µM	micromolar
µmoles/kg	micromoles/kilogram

µmoles/kg/min	micromoles/kilogram/minute
mg/kg	milligram/kilogram
MFF	maximum following frequency
Na	sodium
O_2	oxygen
OZ	occluded zone
pH	hydrogen ion concentration
PVC	premature ventricular contraction
Q-Tc	Q-T interval corrected for heart rate
RSD	Rhythm Search Development
RSDI	RSD 1000 in "ischaemic" buffer
RSDN	RSD 1000 in normal buffer
SA	sino-atrial node
SEM	standard error of the mean
TTX	tetrodotoxin
tT	duration threshold
Vmax	maximum upstroke velocity of phase 0 of cardiac action potential
VFt	ventricular fibrillation threshold

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My final thanks is to Mike and Leon who took their time to guide me and make me grab the bull by the horns. Thanks!

DEDICATION:

To my grandmother, who raised me since I was born and has never truly realized how well she has done in raising me, until now.

1. - INTRODUCTION

1.1.1. - Cardiac Arrhythmias: An Overview

Cardiac arrhythmias are disturbances of rate, rhythm, or depolarization propagation which may be due to either disorders of impulse formation or impulse conduction (Hondeghem and Mason, 1989). The potential underlying causes of arrhythmias are numerous. They include myocardial ischaemia, heart failure, blood electrolyte imbalances (especially K^+ and H^+), excessive sympathetic nervous system discharge or adrenal medulla adrenaline release (Szekeres, 1981), and drug toxicity (e.g. digitalis) (Hondeghem and Mason, 1989; Hoffman and Dangman, 1987). It is thought that some arrhythmias arise because of heterogeneous transmission through branches of the conduction system with subsequent "reentry" into proximal conduction site (Hondeghem and Mason, 1989; Hoffman and Dangman, 1987). The reentry theory suggests that because of ischaemia or other injury there is a unidirectional block in one bundle of tissue (because of partial depolarization) and a delayed and slowed retrograde conduction. The retrograde impulse from the unblocked limb may penetrate the damaged segment, reenter, and cause a premature contraction (Hondeghem and Mason, 1989; Hoffman and Dangman, 1987). The process may become repetitive and precipitate into arrhythmias (Hondeghem and Mason, 1989; Hoffman and Dangman, 1987).

In addition to disordered conduction, arrhythmias may also arise because of newly developed centers of impulse formation (Hondeghem and Mason, 1989; Hoffman and Dangman, 1987). All cardiac tissue may,

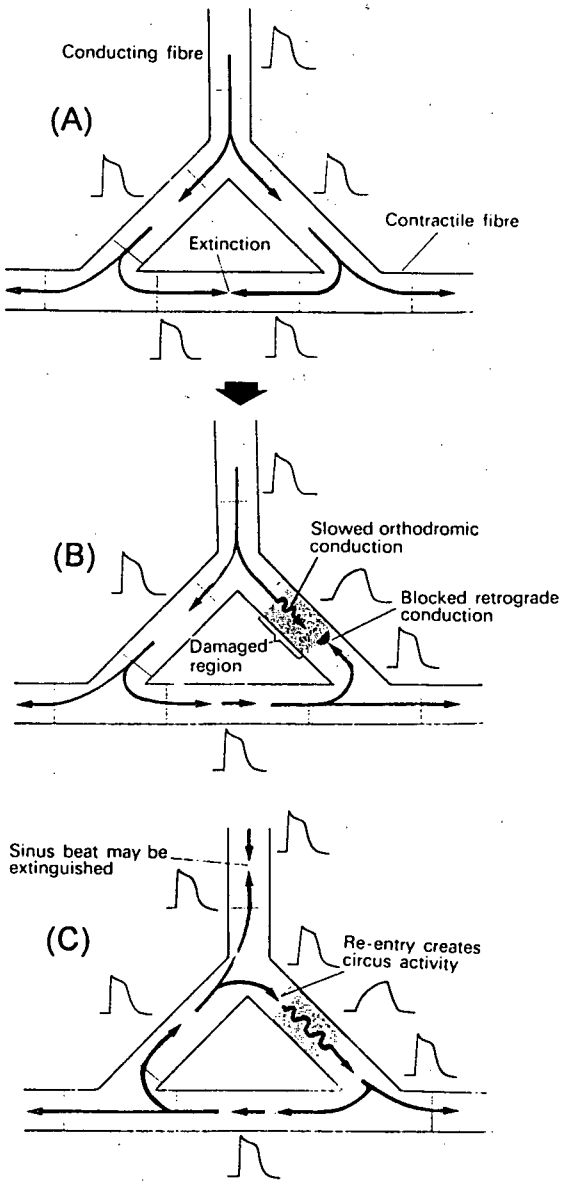


Figure 1: Possible explanation of re-entrant circus movement that is triggered by a premature action potential when there is damage to one of the conducting fibers connected to a chain of contractile fibres. (A) illustrates the normal situation: the action potential travels along both branches at similar speeds and extinguish each other by collision in the contractile fibres, which therefore contract only once in response to each impulse. Diagrams (B) and (C) represent the sequence of events involved in a re-entry circuitin caused by damage in one of the branches. In (B), the contractile fibres are excited by the impulse arriving by one branch while the action potential passes slowly through the damaged region of the other branch. In (C), the slowed action potential regains its full conduction velocity after passing through the damaged region and re-excites the contractile fibres that have now passed out of the refractory period. The sequence is self-perpetuating, giving rise to a circus movement. (Source: Bowman and Rand, 1980).

as a result of ischaemia, injury, or heightened excitability due to ionic imbalance, become a temporary or permanent pacemaker for the heart and thus usurp the pacing role of normal pacemaker. Such sites are called "ectopic foci" or "ectopic pacemaker", a pacemaker at a site other than the SA (or AVN) node. Despite cardiac muscle being a functional syncytium, conduction of excitation is anisotropic; consequently, at any instant, different parts of the heart exhibit different degrees of refractoriness (i.e., repolarization is heterogeneous). The normal cardiac rhythm is dependent on the fact that action potentials generated in the SA node are spread in number by the branching of conducting and contractile fibres and are simultaneously conducted in an orderly sequence throughout the walls of the chambers (Scheidt, 1983). After a single passage, the action potentials, having traveled by specific anisotropic pathways, collide at common points and thereby extinguish each other (Scheidt, 1983). The existence of an ectopic focus would prevent the extinction of the normal cardiac action potential by readily establishing a re-entrant pathway which may dominate and produce continuous asynchronous electrical activity throughout the heart which may be intermittent (premature ventricular contraction) or continuous (ventricular tachycardia or ventricular fibrillation) in nature.

1.1.2. - Ischaemia-Induced Arrhythmias

Sudden cardiac death brought upon by ventricular fibrillation is a major cause of mortality (Pantridge, 1976). Ischaemia-induced arrhythmias are the result of complete and prolonged occlusion of a coronary artery in

which there is an inadequate blood flow, hypoxia and ultimately necrosis of the region of myocardium previously supplied with arterial blood. The main sites of occurrence of such blockade are the anterior descending branch of the left coronary artery, the left circumflex branch, or the main trunk from which these branches arise (Herrick, 1912; Fuster et al., 1992). Following the onset of myocardial ischaemia, accumulation of metabolites may be of primary importance in the genesis of electrophysiological alterations underlying malignant ventricular arrhythmias. These profound electrophysiological alterations induced by ischaemia and their relation to arrhythmogenesis have been reported in detail elsewhere (Bigger, et al., 1977; Corr, et al., 1979; Williams, et al., 1974). A general consensus from these reports suggests that regional intra- and extracellular pH and K^+ in the ischaemic myocardium are the main contributors for arrhythmogenesis (Davies, 1981; Goldstein et al., 1981).

1.1.3. - Regional Intra- and Extracellular pH and K+ in the Ischaemic Myocardium as Contributors to Arrhythmogenesis

Ischaemia represents an imbalance between the myocardial demand for, and the vascular supply of, coronary blood (Carmeliet, 1983). As a consequence, there is a deficit of oxygen, substrates and energy in the myocardial tissues but most importantly, there is an insufficient removal of potentially toxic metabolites such as lactate, carbon dioxide, K^+ and protons (Kléber, 1987).

In the heart cell, protons are continuously generated as a result of ongoing metabolism. In the normal myocardium, intracellular pH is approximately 7.3 and mechanisms exist to extrude the protons which are

generated during cellular metabolism. The ability of cardiac cells to maintain intracellular pH was first observed by Ellis and Thomas (1976). Since then it has been accepted that the Na^+/H^+ exchanger represents the most important mechanism for regulation of intracellular pH. The primary function of the Na^+/H^+ exchanger is to extrude protons against an inwardly directed Na^+ gradient for restoration of normal pH following intracellular acidification. The acidification of extracellular and intracellular fluid which occurs in ischaemic myocardium results from metabolic reactions producing acid equivalents as well as from the accumulation of acid products after cessation of perfusion (Case et al., 1979; Ichihara et al., 1984; Gevers, 1977; Seeley, 1980). The main proton source appears to be anaerobic glycolysis which release protons on hydrolysis with associated formation of lactate and ATP (Williamson, 1966; Gevers, 1977; Seeley, 1980). Along with a decreased pH, there is net intracellular K^+ loss, as evident by a rise in extracellular K^+ associated with myocardial ischaemia (Harris et al., 1954; Hill and Gettes, 1980; Hirche et al., 1980). Early hypoxia increases the conductance of K^+ ions (presumably by interfering with Na^+/K^+ pump (Kléber, 1983)), as indicated by voltage clamp experiments on hypoxic Purkinje fibers (Vleugels et al., 1980) and as a result, there is an increased K^+ efflux in the presence of maintained K^+ influx.

Physiological cellular [H+] and [K+] are maintained at their respective levels during the normal functioning of cardiac tissues. When this balance is upset, as occurs in myocardial ischaemia, regional changes in intra- and extracellular pH and [K+] are likely contributors for arrhythmogenesis.

Intracellular pH is one of the factors determining the rate of anaerobic glycolysis in ischaemic and hypoxic conditions (Rovetto et al., 1975). It is also an important determinant of active tension development (Fabiato and Fabiato, 1978) and is likely to contribute to the genesis of early ventricular arrhythmias (Yan and Kléber, 1992). The arrhythmogenic effect may be mediated through several mechanisms since intracellular pH has been shown to modulate ionic membrane currents (Coraboeuf et al., 1976), Na^+-Ca^{+2} exchange (Philipson et al., 1982), and electrical cell-to-cell coupling (Burt, 1987; Noma and Tsuboi, 1987) in normoxic heart tissue.

The increase of extracellular K^+ associated with ischaemia has been described by several investigators (Hill, et al., 1980; Hirche, et al., 1980; Kléber, et al., 1983; Weiss, et al., 1982). Harris et al. (1954) were the first to observe that the increase in extracellular K^+ was associated with the frequent occurrence of ventricular arrhythmias. In what may be a related finding, clinical studies by Nordrehaug et al. (1983) have shown that an inverse relationship exists between serum [K+] and the incidence of VF in patients suffering a heart attack, i.e., the incidence of VF was five times higher in those patients who had a low serum [K+]. Curtis et al. (1986a) and Saint et al. (1992) have experimentally demonstrated this inverse relationship between serum K^+ concentration and arrhythmias occurring within 4 hr of permanent coronary occlusion in conscious rats i.e., elevated serum K^+ concentration was shown to protect against ischaemic arrhythmias. The accumulation of extracellular K^+ in the ischaemic myocardium causes depolarization (Lee, et al., 1975) and changes in the action potential (Weidmann, et al., 1956) and refractoriness (Gettes, et al.,

1974) of cardiac tissue. These changes are not an effect of elevated extracellular K^+ alone but are more marked during ischaemia when the resting membrane is depolarized (Kodama, et al., 1984; Moréna, et al., 1980). The mechanism responsible for the slowing of impulse propagation associated with the elevation of extracellular K^+ is not yet known. It is likely to be related to acidosis and/or lack of O_2 in the ischaemic myocardium, because the combination of elevated extracellular K^+ concentration, acidosis, and hypoxia closely mimics the action potential changes observed in hypoxia (Moréna, et al., 1980). In view of the preceding arguments, extracellular K^+ as a contributing factor in arrhythmogenesis remains controversial. Nevertheless, the acidotic and hyperkalemic conditions of the ischaemic myocardium provide ideal substrates for the genesis of cardiac arrhythmias.

1.1.4. - Re-entrant Arrhythmias

Arrhythmias as mentioned above, can be due to enhanced automaticity, triggered activity, re-entry, or to various combinations of these mechanisms (Hoffman, 1981). Enhanced automaticity of the type occurring in Purkinje fibers and ventricular muscle is unlikely to occur in ischaemic tissue. This is because abnormal automaticity is suppressed by elevated extracellular K^+ (Hoffman et al., 1981; Katzung et al., 1975) and it is known that extracellular K^+ rises rapidly within the ischaemic myocardium after coronary occlusion (Hill et al., 1980; Hirche et al., 1980; Kléber, 1983). Triggered activity, during which impulses are generated by either early or delayed after-depolarizations (Cranefield, 1977; Rosen et al.,

1981), is a possible mechanism for ectopic impulse formation during acute ischaemia, although absolute proof of its occurrence based on microelectrode recording in ischaemic tissue is lacking (Janse et al., 1982). Re-entrant excitation has been implicated for many years as being the most important cause for ischaemia-induced arrhythmias (Durrer et al., 1971; Boineau and Cox, 1973; Waldo and Kaiser, 1973; El-Sherif et al., 1977a). The strongest evidence for reentry has been the demonstration of continuous electrical activity in extracellular recordings between basic propagated beats and ventricular premature beats (Durrer et al., 1971; Boineau and Cox, 1973; Waldo and Kaiser, 1973; El-Sherif et al., 1977a). Furthermore, recordings of transmembrane potentials and determination of refractory periods of acutely ischaemic cells have shown that the conditions necessary for reentry are present in acute regional myocardial ischaemia (Downar et al., 1977).

Re-entrant activation can occur when the propagating impulse does not die out after complete activation of the heart and may persist to participate in reexciting the heart at the end of the refractory period (Hoffmann and Dangman, 1987). Within the ischaemic myocardium, areas of slow impulse conduction and conduction block in the re-entrant circuit provide the necessary conditions for the occurrence and maintenance of re-entrant activation (Cranefield, 1975; Janse et al., 1986). Unidirectional block enables an excitable pathway to persist, through which a reentering impulse can return to reexcite regions previously excited and no longer refractory (Hoffmann and Dangman, 1987).

1.1.5. - Proposed Models of Re-entrant Arrhythmias in Myocardial Ischaemia

There are three possible models of re-entrant arrhythmias in myocardial ischaemia which can be postulated. In the first model re-entrant arrhythmias occur within the ischaemic zone and the odd reentry circuit escapes to invade the normal myocardium. A second model would involve reentry circuits in which there is a continuous pathway of reentry between the ischaemic and normal myocardium. Re-entrant arrhythmias can also originate at the interface between ischaemic and normal myocardium by virtue of injury currents making a third model of re-entry possible.

The mechanisms for antiarrhythmic protection of the preceding models would include: blockade of the fast inward Na^+ current in both the ischaemic and normal zone; blockade of the fast inward Na^+ current and/or increase in refractoriness of the cardiac action potential in the ischaemic zone; and reduction of the time to spent in the critical period (early after the onset of myocardial ischaemia) for arrhythmias or reduction of injury currents.

Understanding the balance involved in initiating and perpetuating re-entry circuits gives insight into the actions that antiarrhythmic drugs should produce to prevent re-entrant arrhythmia (Brugada, 1987). Re-entry cannot occur when the conduction velocity in the re-entry circuit is so fast, or the refractory period so long, that the circulating impulse is blocked in an area which is refractory for conduction. Re-entry is possible when the revolution time in the re-entry circuit is longer than the refractory period of all structures involved in re-entry (Brugada, 1987). There are several ways

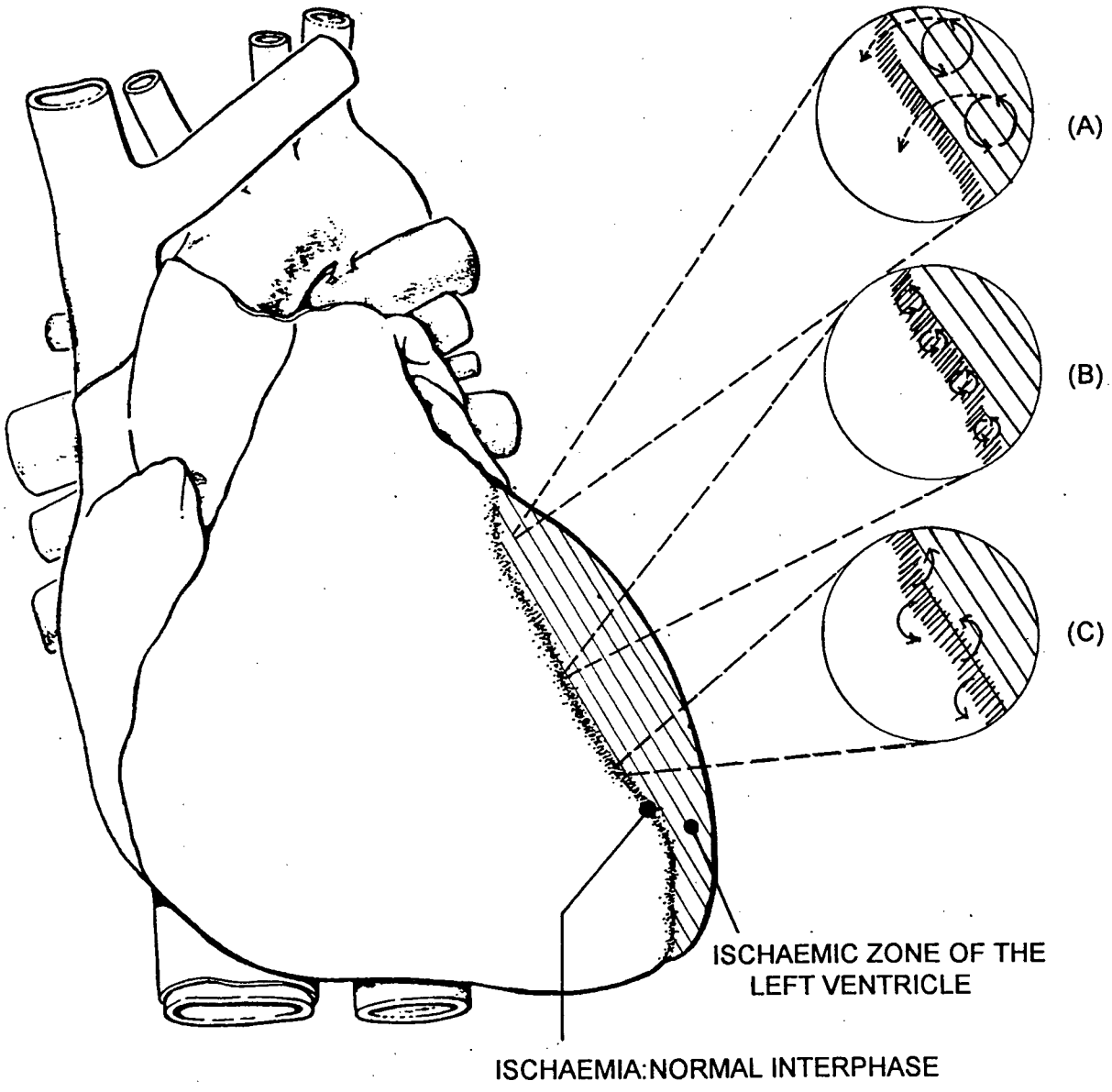


Figure 2: Three possible models of re-entrant arrhythmias. (A): Re-entrant arrhythmias within the ischaemic zone and the odd reentry circuit escapes to invade the normal myocardium. (B): Continuous pathway of reentry between the ischaemic and normal myocardium. (C): Re-entrant arrhythmias originating at the interface between ischaemic and normal myocardium.

of changing a circuit from one which is favourable for re-entry to one which is unfavourable for re-entry. Refractoriness can be prolonged in one of the pathways, or else conduction velocity can be increased or conduction blocked in one of the pathways without prolonging refractoriness (making the cells unresponsive) (Brugada, 1987).

1.2. - Class I - IV Antiarrhythmics: Their Classification

Presently, the aim in drug therapy for the treatment of cardiac arrhythmias centres on modifying critically impaired conduction, increasing refractoriness and reducing ectopic pacemaker activity. This is accomplished chiefly by selectively blocking the major ionic (Na^+, K^+, Ca^{+2}) channels of cardiac cells. Most antiarrhythmic drugs are agents which selectively block transmembrane ion currents carried by sodium, potassium, and calcium. Vaughan Williams' classification (1970; 1984) of antiarrhythmic drugs is that most frequently used. In this scheme, antiarrhythmic agents are divided into four distinct classes based on their mechanism of action. Class I consists of agents which block the fast inward sodium current (Vaughan Williams, 1984). The subclassification of these agents into *a*, *b*, and *c* groups were originally made based on their effects on the cardiac action potential. Singh and Hauswirth (1974) designated class Ia for drugs whose actions suppress V_{max} and prolong action potential duration and class Ib for drugs which do not change V_{max} and shorten action potential duration. Harrison et al. (1981) added the class Ic for drugs that showed no clear effect on action potential duration with actions similar to those of class Ia. In terms of their kinetics, the

binding and unbinding of the drug to/from the sodium channel is intermediate for class Ia, fast for Ib, and slow for Ic (Campbell et al., 1983). Drugs that reduce adrenergic activity in the heart, the β-blockers, constitute class II. The class III antiarrhythmic agents are comprised of drugs that prolong the effective refractory period (presumably by blockade of K^+-channels; Vaughan Williams, 1984). Class IV consists of calcium channel blockers which block the slow inward calcium current that contributes to the plateau phase of the action potential (Vaughan Williams,1984).

1.2.1. - Therapeutics versus Side-Effects of Antiarrhythmics

A common binding for all antiarrhythmic drugs is their poor ratio of therapeutic versus toxicity and/or side effects. All of the available drugs, particularly those in class I, have serious side-effects which tend to preclude their use in long-term prophylaxis of cardiac arrhythmias (Echt et al., 1991). Lidocaine (Carson, 1986; Bergey, 1982), quinidine (Selzer, 1964) and sotalol (McKibbin, 1984) are some examples of antiarrhythmic agents which produce arrhythmias due to an extension of the same mechanism by which they produce antiarrhythmic activity. This is because the correct functioning of the heart depends on defined routes, rates of conduction and refractoriness, which constitute one orderly sinus beat, and thus, permit extinction of the action potentials after the spread of the impulses (Sheidt, 1983). Therefore, drugs that modify action potentials and refractory periods are capable of inducing arrhythmias as well as abolishing them (Cranefield et al., 1971; Hope et al., 1974; Singer, et al., 1969).

Another example of the fragile balance between the therapeutic benefits of an antiarrhythmic drug and its toxicity is well illustrated with class III antiarrhythmics. The antiarrhythmic effects of class III agents have been attributed to effects on the repolarization process via blockade of one or more potassium channels (Colatsky et al., 1990). However, one major limitation of this antiarrhythmic principle is the occurrence of proarrhythmias, i.e., exacerbation of a pre-existing arrhythmia and/or *de novo* induction of an arrhythmia other than the arrhythmia under treatment (Carlsson et al., 1993; Jackman et al., 1988). Among patients receiving repolarization-delaying (class III) agents, paroxysms of polymorphic ventricular tachyarrhythmia (i.e., torsades de pointes (Jackman et al., 1988; Schwartz, 1985; Surawicz and Knoebel, 1985)), is the most frequently observed form of proarrhythmia.

It is, in part, an irony that the study by Carlsson et al. (1993) showed that proarrhythmias related to delayed repolarization were attenuated by low dose lidocaine. It was found that lidocaine treatment caused a dose-dependent attenuation in the incidence of almokalant-induced torsades de pointes. This is part of the increasing evidence that the "window" for the treatment of arrhythmias is very narrow and is restricted by the same actions which produce other toxicities, e.g. arrhythmogenesis. Clearly, the search for new antiarrhythmic drugs with fewer adverse effects in the normal myocardium is essential for antiarrhythmic therapy.

Many patients continue to be given antiarrhythmic drugs, particularly lidocaine, in an attempt to suppress certain forms of ventricular premature beats (Lie et al., 1974; Bigger et al., 1969; Harrison et al., 1972; Ryden et

al., 1975). Kupersmith et al. (1975) reported that lidocaine prolongs conduction in infarcted but not in the normal tissue of canine models of acute myocardial infarction. Abolition of ventricular reentry can be produced by either causing bi-directional block or by improving conduction in the damaged tissue. Given its antiarrhythmic profile and its limited effect on the normal myocardium, one could argue that lidocaine is an "ideal" agent for preventing re-entrant arrhythmias. This argument is strengthened by its extensive clinical use for prophylaxis of ventricular arrhythmias arising from acute myocardial infarction. However, in one clinical study conducted over a 12-year period (1967-1978) it was suggested that the use of lidocaine to suppress ventricular ectopic activity observed in the first few hours after admission to hospital with acute myocardial infarction was of no significant therapeutic value (Pentecost, et al., 1981). This is further supported by earlier reports which claim that lidocaine had been ineffective in up to 20 per cent of patients (Bigger, et al., 1969; Harrison and Alderman, 1972). In another report, the dose of intravenous lidocaine used in conventional therapy resulted in low blood levels during the first three hours following the start of therapy (Ryden et al., 1975). It should be noted that myocardial infarction significantly influences the metabolism of lidocaine which occurs within the liver and is critically dependent upon hepatic blood flow (Ryden et al., 1975). Many patients admitted to hospital with acute myocardial infarction would not be suitable for high dose lidocaine infusion and would possibly suffer rather than benefit from such treatment (Ryden et al., 1975). Experimental studies in our laboratory (Barret et al., 1994) has shown that despite being selective for

tachyarrhythmias and ischaemia, lidocaine is only able to suppress ischaemia-induced fibrillation at doses which have unacceptable effects on cardiovascular and nervous system. Additionally, low doses of lidocaine have been reported to fail to terminate ventricular arrhythmias complicating acute myocardial infarction in the clinical setting (Alderman et al., 1974; Chopra et al., 1971; Church et al, 1972) In part the failure was due to inadequate plasma concentrations of lidocaine, using the routine dosage of 2mg/min (Bergdahl, et al., 1978). Therefore, we are left with the problem that even our most "effective" antiarrhythmic agent has increasingly been shown to have limited or no therapeutic value.

1.2.2. - Is there an "Ideal" Antiarrhythmic Drug?

The ideal antiarrhythmic drug should consist of a once daily tablet which would act on the specific arrhythmia substrate and selectively remove or suppress it without affecting any other cardiac or extra-cardiac tissues. Reality is much different. At present, only preventative drug treatments instead of a curative treatment are available for arrhythmias. Continuous administration is required to achieve continuous antiarrhythmic action, with all the inherent problems of patient compliance this involves (Echt et al., 1991). Antiarrhythmic drugs do prevent arrhythmias but physicians have to almost arbitrarily select a drug to prevent arrhythmias from a pool of imperfect compounds.

One could argue that the Class III antiarrhythmics may be the "ideal" antiarrhythmic drugs since these agents markedly prolong refractoriness without affecting propagation of electrical impulses in cardiac tissue (Singh

and Nademanee, 1985). In general, Class III agents are clinically used as a second line of defense against life-threatening ventricular arrhythmias that fail to respond to adequate doses of first-line antiarrhythmics (e.g. lidocaine). However, like many other antiarrhythmic agents, clinical and prophylactic use of Class III agents is limited because their effects on action potential prolongation under certain circumstances may constitute the basis for a proarrhythmic effect in terms of the development of the clinical entity of torsade de pointes (Singh, 1989). However, a confounding measure is that there is no relationship between the degree of lengthening of the Q-T interval and the development of torsade de pointes. Similarly, there is no linear relationship between the degree of lengthening of the Q-T interval and antiarrhythmic activity. For example, quinidine produces a modest lengthening of the Q-T interval and has a high risk of producing torsade de pointes, whereas a profound increase in the case of amiodarone is rarely associated with the arrhythmia (Singh and Courtney, 1990). Clearly, the prolongation of the Q-T interval must merely serve as one substrate for the development of antiarrhythmic or proarrhythmic effects. It appears that a given degree of Q-T lengthening in combination with depression of V_{max} may result in the most attractive form of antiarrhythmic treatment since it satisfies the requires needed to prevent or abolish reentry circuits. Therefore, effective antiarrhythmic action is best summarized as having blocking actions on the fast sodium inward current and actions on the action potential duration whereby refractoriness is increased. Quinidine possess both these actions but its extra-cardiac side-effects once again raises the need for better antiarrhythmics. Blockade of

the fast inward sodium current in combination with prolongation of the action potential duration are issues on which this study will be focused.

1.2.3. - Conventional Antiarrhythmics and Ischaemia-Dependency

It was well known that antiarrhythmic agents selectively depress the sodium currents in hypoxic (Hondeghem et al., 1974) and ischaemic tissues (Hope et al., 1974). However, it was the work of Chen, Gettes and Katzung, (1975) which showed that antiarrhythmic selectivity was due to the voltage-dependent action of these drugs. Furthermore, conditions accompanying depolarization such as hypoxia, ischaemia or increased external [K+] increased the effects of the antiarrhythmic agents (Hill and Gettes, 1980). Other researchers (Singh & Vaughan Williams, 1971; Futura, et al., 1982) have reported similar findings for lidocaine in that its intensity of blockade on the fast inward sodium channel of cardiac fibers is strongly dependent on extracellular K^+ concentration.

At the present, it is uncertain as to how changes in pH and extracellular K^+, in the clinical setting, play a part(s) in the mechanisms responsible for the selectivity of class I antiarrhythmics in blocking of sodium currents in normal and ischaemic cardiac tissue.

1.3. - Ischaemic Myocardium and the Na+ Channel

Many Class I antiarrhythmic drugs not only block the cardiac sodium channel but they also block calcium and/or potassium channels. However, Duff et al., (1988) demonstrated that treatment with tetrodotoxin (TTX), a highly selective blocker of the sodium current with virtually no effect on other currents (Hille, 1984a; Narahashi, 1974; Reuter, et al., 1982;

Cachelin, et al., 1983; Bean, 1985; Giles et al., 1988), produced concentration dependent increases in ventricular effective refractory period and conduction time in the infarct zone which were associated with antiarrhythmic activity. Additional evidence has been obtained with TTX such that sodium channels must participate in ischaemic-induced arrhythmias (Abraham, et al., 1989). For these reasons, emphasis on ischaemia-selectivity along with the hypothesis that sodium channel blockade must occur in the ischaemic myocardium to confer antiarrhythmic protection are the factors responsible for the examination of an ischaemia-dependent sodium channel blocker in this study.

1.3.1. - Structural Model of the Na+ Channel

In order to understand the blocking actions of antiarrhythmic drugs, the proposed binding site of the sodium channel must be discussed. The voltage-gated sodium channel is responsible for the inward current during the depolarization phase of an action potential in giant squid axon (Hodgkin and Katz, 1949) and in most excitable cells. Sodium channels expressed in cardiac muscle cells are distinguished by the fact that they are resistant to nanomolar concentrations of tetrodotoxin (Brown et al., 1981), but are more sensitive than neuronal channels to inhibition by lidocaine (Bean et al., 1982). Generally, all voltage-gated sodium channels consists of a highly preserved large subunit, termed α, which comprises the actual pore of the channel. Genes from eel electroplax have been cloned to give the exact amino acid sequence of α subunits (Noda et al., 1984). In addition, these channels consists of four structurally homologous domains (I to IV),

each containing six membrane-spanning α-helical regions (S1-S6) (Noda et al., 1984). In addition, smaller subunits, termed β_1 and β_2 have been identified and are associated with the α subunits. The role of these small subunits is not clear, since functional channels can be constructed from the α-subunit alone (Noda et al., 1986). Beta-subunits may stabilize the structure (Meissner & Catterall, 1986) and modify gating (Auld et al., 1988; Krafte et al., 1988).

S2 and S3 of the α helices have net negative residues and may be the region for ion conduction (Noda et al., 1984). S4 is believed to be the voltage sensor (Catterall, 1986, 1988; Guy et al., 1990), since net positive residues, such as arginine or lysine, repeat at every third position of the transmembrane helical pattern (Noda et al., 1984). Sodium channel activation is thought to be initiated by a conformational change that opens the pore. These positively charged amino acids move in response to changes in membrane potential (Catterall, 1986) causing a conformational change that opens the pore (Armstrong, 1975). Therefore, sodium activation involves the charged residues in the S4 region but the mechanisms by which these regions function are unknown.

The region implicated in sodium channel inactivation is the cytoplasmic linker, of 53 amino acid residues, between domains III and IV (Vassilev et al., 1988; Armstrong and Bezanilla, 1977). Within the highly conserved clusters of positively charged and hydrophobic amino acid residues, inactivation was discovered to correlate with hydrophobicity since conversion of the amino acid residue phenylalanine (F) 1489 to glutamine (Q) was sufficient to prevent fast channel inactivation (West et al., 1992).

Based on these results and those originally proposed by Armstrong and Benzanilla (1977), it is believed that the hydrophobic cluster, with F(1489) as the crucial residue, serves as the inactivating gating loop which enters the intracellular mouth of the transmembrane pore of the sodium channel and initiates inactivation.

1.3.2. - Models of Drug Interactions with the Cardiac Sodium Channel

At present, there is no one model which can accurately describe drug interactions with the cardiac sodium channel. Knowledge of existing models which best describe the drug interactions should be used for predicting antiarrhythmic drug binding. Initial formulations for drug interaction with the sodium channel were based on studies in nerve (Strichartz, 1973). The models developed in nerve have proved useful in the formulation of similar models for cardiac muscle. One such model is the modulated receptor hypothesis for the cardiac sodium channel which explains the effectiveness of local anaesthetics and their relatives in the treatment of cardiac arrhythmias originating in the ventricle (Hondeghem and Katzung, 1977; Courtney, 1980 Hille, 1977). Drug binding is illustrated in terms of state diagrams showing normal gating states and comparing each state to offer different affinities and rates for drug binding reactions (Strichartz, 1973; Courtney, 1975; Hille, 1977; Hondeghem and Katzung, 1977).

The drawback to the application of the modulated receptor model is the large number (16) of rate constants that need to be determined (Grant, 1991). The problem is that it is very difficult and expensive in terms of

computational time to find a unique solution for model parameters when the number of parameters is large (Grant, 1991). Nevertheless, the modulated model is the most widely used to account for the blocking processes of many agents on the sodium channel.

1.3.3. - Antiarrhythmic Actions with the Cardiac Na+ Channel

Current knowledge of the mechanism of action of many antiarrhythmics and their binding sites in cardiac sodium channels is limited. Nerve and skeletal muscle are much more sensitive to local anaesthetics when the drugs are applied inside the cell, indicating a binding site for local anaesthetics on the inside face of those sodium channels (Hille, 1977; Strichartz, 1973). This is also true for lidocaine. The antiarrhythmic effects of lidocaine occur at concentrations 1/100 or less of that required to block the nerve action potential (Ritchie, et al., 1980; Bigger, et al., 1980). Although there is evidence to support the hypothesis that the effect of lidocaine on the fast inward sodium channel (g_{Na}) of cardiac tissue results from an action similar to that demonstrated for nerve (Hondeghem and Katzung, 1977; Grant et al., 1984), there is limited data on the sites of action and active forms of lidocaine on cardiac tissue. To complicate matters, a second external lidocaine binding site on mammalian cardiac cells has been demonstrated by Alpert, et al., (1989).

Antiarrhythmic agents, in general, are somewhat chemically heterogeneous but possess the common chemical property of lipophilic and hydrophilic regions. The cardiac sodium channel consists of a hydrophilic channel transversing the lipid portion of the cell membrane

(Hondeghem and Katzung, 1977). It has been proposed that within the channel, there exists a selectivity filter and the inactivating gate near the outer (Hille, 1975) and inner (Armstrong, 1975) boundaries of the channel molecule, respectively. Quinidine, as well as conventional local anaesthetics, block sodium channels from the inside and have much higher rate constants for association with the non-inactivated channel (Hodenghem and Katzung, 1977). This suggests that the binding site is between the selectivity filter and the inactivation gate.

Access to the channel binding site is a function of the state of the channel and the lipid solubility of the individual drug (Hondeghem and Katzung, 1977). Drug molecules that are less soluble in water, e.g., benzocaine (pKa = 3.5), have hydrophobic access route to the receptor area, i.e., laterally through the membrane (Hodenghem and Katzung, 1977). Conversely, permanently charged molecules, such as quaternary lidocaine derivatives, e.g. QX314, must utilize a hydrophilic access route (Hondeghem and Katzung, 1977). Finally, tertiary compounds may reach the receptor area by either, or both, routes depending on their charge and lipophilicity (Hondeghem and Katzung, 1977). This is the case for quinidine and lidocaine.

1.4. - Frequency-Dependence of Antiarrhythmic Drugs

Frequency dependent drug action is part of the mechanism by which antiarrhythmic drugs provide protection against tachyarrhythmias. Although our most effective antiarrhythmics lack cardiac selectivity, their

potential for providing antiarrhythmic protection is by virtue of their frequency-dependent actions. For example, cardiac action potentials occurring at physiological rates are conducted normally in the presence of lidocaine (i.e., lidocaine has no effect on conduction velocity in normal tissues of the His-Purkinje system or ventricular muscle) but at high heart rates, the intensity of its class I effects are increased (Hondeghem & Katzung, 1977).

The frequency-dependence of antiarrhythmics was first observed by Weidmann (1955) who showed that procainamide HCl and quinidine sulfate led to a decline in V_{max} and eventual inexcitability. Later, Johnson and McKinnon (1957) showed that V_{max} of quinidine-treated ventricular myocardial fibers was the same as control fibers when stimulated infrequently, but declined as stimulation frequency was increased. In summary, some antiarrhythmic drugs (Ib and Ia) at therapeutic concentrations produce little or no block of the sodium channels in the absence of stimulation. With repeated stimulation, block develops in a frequency-dependent manner. Frequency-dependent block would cause strong suppression of beats occurring in quick succession as in a tachyarrhythmia, whereas beats occurring at physiological rates would not be so influenced (Hondeghem and Katzung, 1977). Therefore, these drugs would most effectively block electrical activity when there is a fast tachycardia (many channel activations and inactivations per unit time).

In general, many therapeutically useful antiarrhythmics have a high affinity for activated channels or inactivated channels but very low affinity for rested channels (Hondeghem and Katzung, 1984). Sodium channels in

normal cells that become blocked during normal activation-inactivation cycles will rapidly lose the drug from the receptors during the resting portion of the cycle. Antiarrhythmic drugs differ with respect to their binding and unbinding kinetics, i.e., with respect to their blocking and unblocking kinetics (Hondeghem and Katzung, 1984). If the diastolic interval is long enough to allow complete unbinding, almost all sodium channels recover from block. This explains why certain drugs with fast unbinding time constants exert virtually no sodium channel blocking properties at certain frequencies, i.e., there is no depression of the maximal upstroke velocity of cardiac action potentials nor prolongation of QRS in the ECG (Hondeghem and Katzung, 1984). However, when the diastolic interval becomes brief so that drug unbinding is incomplete, an end-diastolic sodium channel blockade accumulates which becomes apparent in a progressive widening of the QRS complex (Hondeghem and Katzung, 1984).

Frequency dependent drug action can be explained by two assumptions: 1) ion-channel blocking drugs act by binding within the channel pore itself (Hille, 1984b), 2) certain conformational states of the ion-channels are only transiently present throughout the excitatory cycle of cardiac tissue. The latter is further supported by the "modulated receptor" (Hondeghem & Katzung, 1977) and guarded receptor (Starmer, et al., 1984) theories. According to the "modulated receptor theory", the transient nature of the high affinity binding sites is due to a true shift in affinity modulated by the channel state. In contrast, the "guarded receptor theory" explains the transient availability by an access and egress of drug molecules to and from the high affinity binding sites being controlled by the

channel states. Both theories imply that considerable drug binding is restricted to certain phases within an excitation cycle, i.e., the accessibility or availability of the receptor varies depending on the channel conformational state.

1.5. - Objectives

The struggle to improve upon prototypical antiarrhythmic Class I agents has not met with much success. Part of the problem is the difficulty in separating the antiarrhythmic activity from the toxic side-effects of the agent since both actions act by virtue of the same mechanism: ion channel blockade. The following is a list of the criteria discussed above which summarizes the possible requirements of an "ideal" antiarrhythmic agent:

1. Limited toxicity.
2. Ischaemia-selectivity (of the cardiac tissue).
3. Frequency dependent channel blockade.

Lidocaine is one agent which possesses two out of the three criteria: ischaemia-selectivity and frequency-dependent actions. Steming from a series of arylacetamides, RSD 1000 was found in our initial studies to selectively act on arrhythmias in the ischaemic myocardium without incurring significant toxicity. To determine which of the following criteria above characterizes the antiarrhythmic properties of **RSD 1000**, a series of comparative studies with lidocaine was made to determine the general pharmacology and antiarrhythmic actions of RSD 1000.

Experiments were performed to determine the pharmacological profile of RSD 1000 in rats and mice. The focus of the pharmacology was on cardiovascular (blood pressure and heart rate) and cardiac (ECG) effects.

The doses required to provide antiarrhythmic protection were compared with those which had effects on the ECG, as a reflection of potential ion current blockade, and upon the responsiveness to electrically-induced arrhythmias of the left ventricle. The latter was compared with the concentrations shown to have similar effects on isolated rat hearts. The relative therapeutic usefulness of RSD 1000 will be assessed by comparing its efficacy against ischaemia-induced arrhythmias to its toxic effects. Whether or not RSD 1000 is an improvement over class I agents in terms of its antiarrhythmic activity and therapeutic ratio, the comparison between RSD 1000 and lidocaine will add to our current knowledge of possible antiarrhythmic mechanisms

2. - METHODS

2.1. - RSD 1000

As one of the products of a search for antiarrhythmic drugs which would selectively provide protection against arrhythmias induced by myocardial ischaemia without causing significant toxicity, RSD 1000 is one of a series of compounds developed by RSD Ltd. Its chemical name, structure and formula are illustrated in Figure 1.

2.2. - Experimental Plan

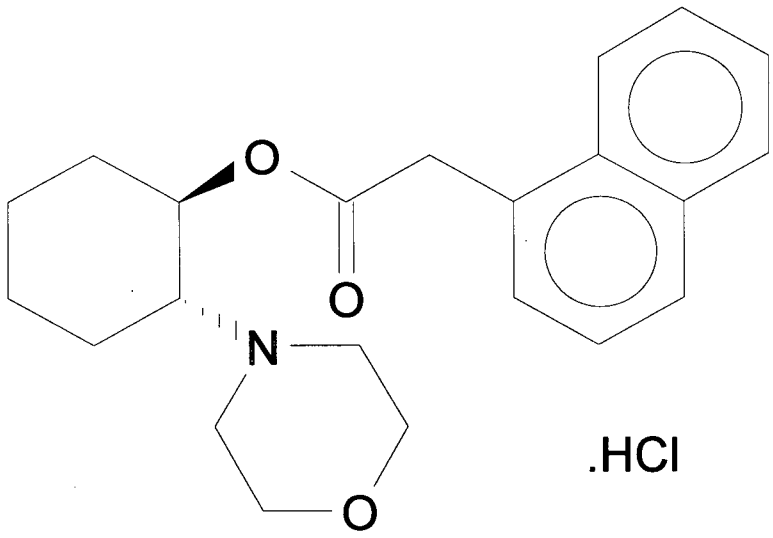
Before investigating the antiarrhythmic actions of RSD 1000, a thorough pharmacological profile was required. The toxicological, haemodynamic and cardiac effects of RSD 1000 was initially investigated in a rat screen. Also, in this screen, the range of effective doses for RSD 1000 was established. From these initial results, appropriate doses for subsequent experiments were made. For comparative purposes, many of the experiments performed on RSD 1000 were repeated for lidocaine (see Section 2.7).

2.3. - General

Male Sprague-Dawley rats weighing between 200 and 300 g were used. Rats were selected at random from a single group and anaesthetised with pentobarbitone (262 µmoles/kg or 65 mg/kg, i.p.). A tracheostomy was performed with a No. 14 (57.2 mm) Jelco teflon catheter for ventilation at 10 ml/kg body weight, 60 cycles per minute (Palmer pump) (MacLean and Hiley, 1988). The right external jugular vein was cannulated

Figure 3 - Chemical Structures of RSD 1000 and Lidocaine

RSD 1000



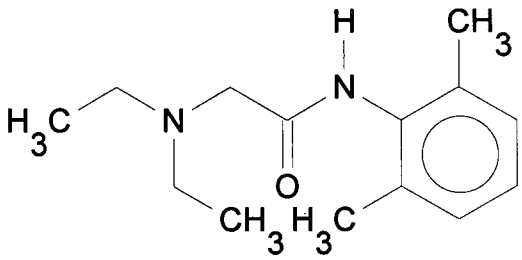
Chemical Formula: $C_{22}H_{27}NO_3 \cdot HCl$

Formula Weight: 389.92gm/mol

Solubility: 22%EtOH:78%dH_2O

Chemical Name: (±)-trans-[2-(4-morpholinyl)cyclohexyl]naphthalene-1-acetate monohydrochloride

Lidocaine



Chemical Formula: $C_{14}H_{22}N_2O$

Formula Weight: 234.33

Solubility: dH_2O

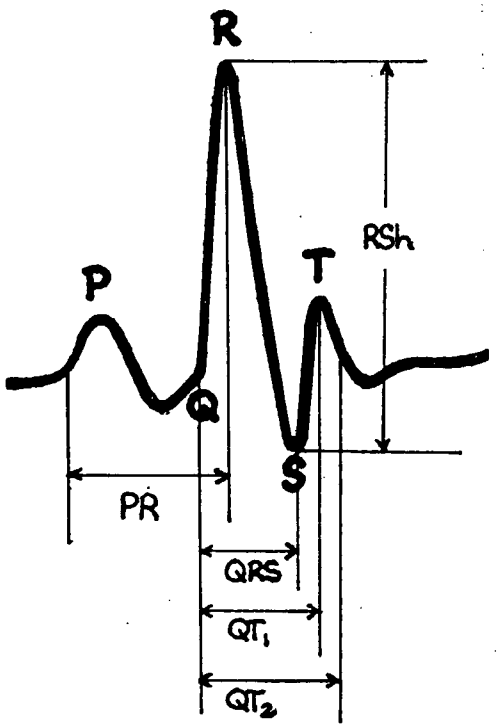
Chemical Name: 2-(Diethylamino)-N-(2,6-dimethylphenyl)acetamide

for intravenous injections, while the left carotid artery was cannulated for recording blood pressure. Prepared rats were allowed to recover for 30 minutes. The preceding surgery was the general protocol for preparing all the rats used in the experiments. Additional surgery associated with electrically-stimulation and occlusion studies will be discussed in later sections.

The electrocardiogram (ECG) (see Fig. B) of the rat was recorded for all the experiments. Permanent ECG leads (10 or 14 cm of insulated stainless steel wire 0.005 in. teflon 316 SS 5T, Medwire Corp., Mt. Vernon, NY) were implanted, through a needle trocar, into the pectoralis muscle overlying the chest, in the forelimb (right atrium to apex, i.e., approximate lead V2 configuration) and in the left hindlimb using a 1.0-cm. exposed tip.

In vitro study of arrhythmias require distinct preparative procedures and these will be discussed in the isolated heart section.

Figure 4: Schematic representation of the rat electrocardiogram.



2.4. - *IN VIVO* STUDIES

2.4.1. - Haemodynamic and Cardiac Effects of RSD 1000 in Rats

The haemodynamic and cardiac effects of RSD 1000 were initially studied in rats with bolus doses given i.v.. Blood pressure, heart rate and ECG were measured and these were expressed as time-effect data. Effects on ECG are indirect measures of ion channel blocking activity, particularly sodium (P-R, QRS and a another, measure of sodium current blockade in the rat, RSh; Penz et al., 1992) and potassium (Q-T interval). The rat has marked differences in ventricular action potential morphology compared with other species. It is characterized by a rapid repolarization phase (presumably i_{to} (transient outward current); Josephson, et al., 1984) instead of the plateau phase seen in guinea pig myocardium and most larger animals (Beatch, et al., 1990). Therefore, the Q-T interval in the rat is poorly defined (Budden et al., 1981) and two measures are made of this interval, i.e., QT_1 and QT_2.

Bolus doses for RSD 1000 and lidocaine were calculated according to rat weight (µmole/kg) and the starting dose was chosen as the dose which produced little or no effect on the variables measured. A series of doubling doses from the initial dose was performed until the animal died from cardiac or respiratory depression (LD50). The dose range for bolus injections of RSD 1000 was tested at 2.0 - 64 µmole/kg. An ED_{25}% is the dose producing a 25% change from control in a variable. Therefore, approximate ED_{25}% values for blood pressure, heart rate ECG were estimated and these were used to calculate the dosing regime for RSD 1000 in the following experiments.

2.4.2. - Lethality and Analgesic Studies in Mice

Lethality: Mice were used to determine the lethality of RSD 1000 in conscious animals and to ascertain possible mechanisms of death. CD-1 mice between 20-25 g were used. Doses were calculated according to mouse weight and all solutions were made up in concentrations of mg/ml. Mice were sufficiently warmed with a heating lamp in order to produce venodilation and allow i.v. injections via the tail veins. Injections were made at the distal end of the tail using a 1 cc syringe and a 27.5 gauge needle with injection volumes $\leq$ 0.2 mls.

Determination of the LD50 was based on the following protocol. The starting concentration was chosen as 50 mg/ml, which was equal to 428 µmole/kg (a dose well above the ED_{25}% for effects observed in the preliminary screen). Initially, two mice are injected with this concentration. If these two mice died, then the LD50 lay below this concentration. A 1:10 dilution was made from the 50 mg/ml and injected into two other mice and, if necessary, additional serial 1:10 dilutions are made and injected until partial or no mortality is present at a given dilution. A total of four mice were injected with the highest minimum, lethal dose while groups of 7 were used to test the diluted concentrations of RSD 1000 in order to construct a dose-response relationship for lethality. Using a statistical computer program (Spearman and Karber), probit (or normal equivalent deviations, or N.E.D.) analysis is used to analyze the graded responses in terms of a regression line. Knowing the minimal maximum lethal dose which produces death in 100% of the mice, reponses at the lower doses will be expressed as a percentage of this maximum. The program then transforms these percentages into probit values. The result is a linear relationship, which is plotted as a function of the dose, with probit of 5 (or N.E.D. = 0) = LD50 dose .

Analgesia: Synthesis of RSD 1000 was based on the parent N-methyl arylacetamide compound, U50 488H, an opioid agonist tested for its antiarrhythmic actions by Pugsley et al. (1992). For this reason, the determination of analgesic actions of RSD 1000 is pertinent to its overall pharmacology. In addition, inferences on the possible causes of death attributed to opioid activity can be made.

RSD 1000 was tested for analgesic actions by pre-treating mice with naloxone (6.1 µmoles/kg or 2.0 mg/kg, i.p.) and using the tail-pinch test (Bianchi, 1952). Mice were selected at random from a single group and divided into five groups (n=5). Treatment groups were as follows: 1) vehicle, 2) RSD 1000 at the LD25 concentration, 3) RSD 1000 at the LD50 concentration, 4) naloxone pre-treated with RSD 1000 at the LD25 concentration and 5) naloxone pre-treated with RSD 1000 at the LD50 concentration.

2.5. - *IN VITRO* STUDIES

2.5.1. - Isolated Rat Hearts

The cardiac actions of RSD 1000 were further studied *in vitro* in isolated rat hearts. The Langendorff rat heart model was first devised by Langendorff in 1895 for studies of the mechanical and electrophysiological properties of the heart. It has many advantages and disadvantages for the investigation of the actions of drugs on both the mechanical (contractile force) and electrical (ECG) activity (see Broadley, 1979). Certain limitations of the Langendorff model, such as the low oxygen-carrying capacity and possible aortic valve damage (Broadley, 1979; Curtis et al., 1986b) have been minimized by the use of a modified perfusion apparatus developed in our laboratory (Curtis et al., 1986b). This apparatus

increases the oxygen-carrying capacity of the perfusate and limits damage to the aortic valves because of improved perfusion.

2.5.1.1. - Perfusion Apparatus

The perfusion apparatus consists of nine chambers (each of 250 ml capacity) connected to the aortic perfusion cannula of the Langendorff perfused heart via separate silastic tubes. The tubes were specially designed to reduce dead-space. The apparatus design enable rapid generation of dose-response data. Perfusates were kept at a constant 37° C by circulating warm water which was heated by an external thermoregulated heater. Oxygen gas (95%O_2:5%CO_2) was used to oxygenate and pressurize (@ 100 ± 5 mmHg) the solution and chamber, respectively. This pressurization was also responsible for driving perfusate through the coronary circulation.

2.5.1.2. - Experimental Preparation

The perfusing solution was formulated in our laboratory to allow maximal oxygen carrying capacity and optimal pH stability. Instead of the Krebs-Henseleit solution used in classical Langendorff model, our PIPES perfusion solution consisted of (in mM) NaCl, 153; KCl, 3.35; $MgSO_4 \cdot 7H_2O$, 1.18; D-Glucose, 11.1; $CaCl_2 \cdot 2H_2O$, 2.52; (Piperazine-N,N'-bis[2-ethanesulfonic acid]), 14.34 and titrated to pH 7.4 with NaOH (Table 1).

Each rat was sacrificed by a blow to the base of the skull and exsanguinated. Next, the heart was rapidly excised from the chest cavity. and immediately retrogradely perfused with 10 ml of ice-cold PIPES buffer solution. Finally, the heart was mounted on the perfusion apparatus via an aortic cannula for perfusion with an oxygenated PIPES buffer solution at 37

Table 1. Ionic Composition of Physiological Salt Solutions used for Cardiac Tissure in Comparison with Rat Serum and Interstitial Fluid.

	Cations				Anions								Gas (%)	
	Na	K	Ca	Mg	Cl	HCO_3	H_2PO_4	SO_4	OH	Urea	Glucose	PIPES	O_2	CO_2
Solution														
Total ion concentration in plasma	152	3.7	2.7	1.06	11	26.5	1.7	0.69		7.0	5.8			
Ionized ion concentration in plasma	150	3.6	1.6	0.76	11	25.7	1.6	0.69		7.0	5.8			
Ionized ion concentration in interstitial fluid	147	3.5	1.5	0.72	11	26.3	1.7	0.73		7.0	5.8			
Krebs-Henseleit buffer	143	5.9	2.5	0	12	24.9	1.2	1.64		0	5.6		95	5
PIPES buffer	153	3.4	2.5	1.18	13	0	0	1.18	6.0	0	11.1	14.3	100	0

The concentrations of anions and cations are given in mM. Note: 2.0 mM sodium pyruvate is added to Krebs-Henseleit solutions. PIPES = Piperazine-N,N'-bis[2-ethanesulfonic acid]. OH^- ions in PIPES buffer solution is from NaOH which is added during titration for a pH = 7.4.

°C and pH 7.4. Within seconds, contractions following normal sinus rhythm resumed in the heart. The left atrium was removed in order to insert a small compliant, non-elastic balloon made of plastic wrapping film ("Saran Wrap") into the left ventricle for measuring ventricular pressure (Curtis et al., 1986). For maximal ventricular contractility, the pressure within the balloon was adjusted to give a left ventricular end-diastolic pressure of 10 mmHg. The aortic root perfusion pressure controlled by the oxygenating gas (95%O_2:5%CO_2) was kept constant at 100 ± 5 mmHg to mimic the normal perfusion pressure of coronary arteries *in vivo*. Both perfusion pressure and ventricular pressure were measured by pressure transducers while the maximal rate of intraventricular pressure development ($+dP/dt_{max}$) and relaxation ($-dP/dt_{max}$) were obtained by differentiating left ventricular pressure using a Grass Polygraph differentiator (model 7P20C). Special atraumatic, silver-ball electrodes were designed for ECG recording from the epicardial surface of the heart (Curtis et al.,1986) using a Grass Polygraph (model 7D) at a bandwidth of 0.1-75 Hz. A length of insulated silver wire (0.003 in., 40 gauge, Teflon Coated Med. Wire Corp., Mount Vernon, NY) passed through the center of a 1-cm circular disk of filter paper (Whatman No. 1); an identical wire was also inserted on the same disk to act as the ground. Adhesion between the filter paper and the moist surface of the heart enabled placement of one of the electrodes on the right atrium (to allow for a large P wave) and another on the left ventricle. Consistent ECG recording was possible with little or no movement of the electrodes.

2.5.1.3. - Experimental Design

Hearts were perfused with PIPES buffer solution alone and measurements of heart rate and ECG made during a period of 15 minutes, or until stable control values were obtained; these pre-drug values were the control values for the experiments. The different concentrations of RSD 1000 were administered cumulatively for a period of 3 minutes at each concentration, at which time, recordings were made at 0.5, 1, 2, and 3 minute intervals. At the end of the drug treatment, a 5 minute wash-out was performed.

Two buffer solutions were used in order to simulate either normal and ischaemic conditions or conditions of ischaemia. The ionic constituents making up the PIPES buffers remained constant varying only the pH and $[K^+]$ for each of the two buffers. In the normal PIPES buffer, pH and $[K^+]$ were maintained at 7.4 and 3.8 mM, respectively, while in the "ischaemia"-simulated PIPES buffer pH and $[K^+]$ were at 6.4 and 10.8 mM, respectively. The starting concentrations of RSD 1000 were chosen to produce minimal changes in any one, or all, of the measured variables. In the normal PIPES buffer, 16, 32, 64 and 128 µM of RSD 1000 were introduced to the perfusate while much lower concentrations of RSD 1000 of 0.25, 0.5, 1.0 and 2.0 µM were used in the PIPES buffer with the higher [H+] and $[K^+]$.

2.5.1.4. - Variables Measured

Heart rate, P-R interval, and QRS complex were measured. However, Q-T was not measured on account of the difficulty in determining the T-wave in isolated rat hearts. During the wash-out period, the on- and off-set kinetics of RSD 1000 was determined as being fast, intermediate or slow by measuring the recovery time of the hearts to their control values.

2.6. - ANTIARRHYTHMIC EFFICACY

2.6.1. - Electrically-Induced Arrhythmias

In order to assess the actions of RSD 1000 on electrical stimulation and the electrical induction of arrhythmias, stimulating electrodes were implanted in the apical region of the left ventricle of intact rat hearts. A program of electrical stimulation allows for the inference of possible effects on myocardial ionic channels such as sodium and/or potassium channels since sodium and potassium channel blocking drugs have clear profiles of action in such tests. It has been well established that drugs which decrease sodium currents increase threshold current & threshold pulse width for capture of single beats (iT-μA & tT-ms) and ventricular fibrillation threshold (VFt-μA) (Vaughan Williams and Szekeres, 1961). In contrast, pure potassium channel blockers do not affect thresholds for capture of single beats, yet suppress VF induction by making the heart refractory to the fractionated wavefront (Winslow, 1984). A pure potassium channel blocker would, therefore, be expected to prolong effective refractory period (ERP-ms) and decrease the maximum following frequency (MFF-Hz) to square wave stimuli (Vaughan-Williams, 1970). In order to establish an index of sodium vs. potassium blocking actions of RSD 1000 changes in iT, tT, VFt, ERP, and MFF (see section 2.6.1.4.) were measured and expressed as dose-response relationships.

2.6.1.1. - Experimental Preparation

The preperative surgery on rats was described earlier. The following methods for electrically-induced arrhythmias have been described elsewhere (Howard and Walker, 1990). A small region of the skin above the level of the heart was removed and the chest palpated to determine the position of the left ventricle. Stimulating electrodes (10cm in length)

consisted of Teflon coated silver wire. From the end of each wire, 1-2 mm segment of insulation was removed and passed through the lumen of a 27 gauge needle. The desheathed tip of the wire was bent back to form a barb. Guided by the needle, the wire was passed through the chest wall and imbedded, with the hooked electrode tip, in the apical region of the left ventricular wall. This process was performed for each of two electrodes implanted and the inter-electrode distance was approximately 1-2mm apart, which was confirmed at the end of the experiment by inspection of the rat heart. Close proximity of both electrodes to each other was important to produce a high voltage and low resistance electrical signal. Stimulation of the left ventricle with square wave pulses was performed using Grass SD9 Stimulators (Howard and Walker, 1990).

2.6.1.2. - Variables Measured

Blood pressure, heart rate, ECG, serum [K^+] and body temperature were recorded from the time of surgical recovery. After 30 minutes and prior to drug infusion, electrical stimulation variables were measured every 2 minutes or until stable consistent values were obtained; the last three consistent set of values were taken as pre-drug values. The average total time from recovery to drug infusion was approximately 45 minutes.

2.6.1.3. - Experimental Design

Rats were selected at random from a single group (n=5). After surgical preparation, they were allowed to recover before being subjected to drug infusion. Infusion of RSD 1000 was performed in an attempt to investigate the changes against electrically-induced arrhythmias at "pseudo"-steady-state plasma serum concentrations. RSD 1000 was tested as cumulative doses starting at 1.0 µmole/kg/min i.v.. Dose-

response curves were constructed for the measured variables. There were two treatment groups: a saline control and RSD 1000 at the starting dose. Each treatment was infused i.v. over 3 minutes and electrical stimulation variables were measured following the 3 minute reading. Drug treatment was performed in a blind and random line manner. Each animal acted as its own control since, prior to drug infusion, control values of electrical stimulation variables were determined. Prior to this experiment, the stability of this preparation as a function of time was examined by performing a time control group. Five individual rats were prepared and infused with saline for a period of 30 minutes at which time the electrical stimulaiton protocol was performed at random.

2.6.1.4. - Experimental Endpoints

Square wave stimulation was used and discrimination of end-points made using an oscilloscope. Each end-point (iT, tT, VFt, ERP, and MFF) was determined in triplicate, 3 minutes after commencing each infusion step. The mean value of three measurements were used. The procedures for the end-points measurement has been described elsewhere (Vaughan Williams and Szekeres, 1961; Winslow, 1983).

2.6.1.4.1. - Threshold Current

Threshold current (iT) is the minimum current required for capture. The heart is captured when it follows the pulses generated by the stimulator and is easily observed. With capture, the following changes in signal were observed:

I. an increase in ECG signal size.

II. a regular rhythm at a fast rate of 7.5 Hz.

III. a slight but sudden drop in blood pressure.

The threshold current was determined at 7.5 Hz, approximately 100 beats/min above the sinus rate, and the signal wave pulse width was 1.0 ms. The threshold current usually fell within the range of 20 to 100 μA.

2.6.1.4.2. - Threshold Pulse Width

Threshold pulse width for capture (tT) was the square wave stimulus at 2 x iT and it was the minimum duration required to capture the heart. It was determined according to the criteria for measuring the threshold current. The average threshold pulse widths were between 0.2 - 0.4 ms.

2.6.1.4.3. - Ventricular Fibrillation Threshold

Ventricular fibrillation threshold (VFt) provides the means to measure the "electrical stability" of the heart, and its propensity to fibrillate spontaneously, in a control state and in response to various interventions. An elevated VFt implies that the heart is electrically more stable and less likely to fibrillate; a lower threshold implies the opposite. In this study, ventricular fibrillation threshold was defined as the current necessary to produce a fibrillo-flutter pattern on the ECG and a precipitous drop in blood pressure. Periods of stimulation of approximately 4.0 seconds duration were required for each determination. The end point was determined by increasing the current strength (at 50 Hz and twice the threshold pulse width) until fibrillation occurred. The ECG characteristics were generally a non-sustained fibrillo-flutter accompanied by a precipitous fall in blood pressure.

2.6.1.4.4. - Effective Refractory Period and Maximum Following Frequency

Effective refractory period (ERP) in the ventricle was defined as the shortest interval between two stimuli at which the ventricle responds. It

was determined by the extra-stimulus method. In this method the heart is paced at a frequency of 7.5 Hz, at twice the threshold current and twice the threshold pulse width. A single extra stimulus of the same current strength, and pulse width was applied at a variable delay after the pacing stimuli. The minimum delay at which the extra-stimulus resulted in a extra-systole was taken as the effective refractory period.

Maximum following frequency (MFF) was defined as the frequency at which the heart failed to follow, on a 1:1 basis, when given a steadily increasing frequency of stimulation from a baseline frequency of 5 Hz. It was determined at twice threshold current and pulse width. The frequency of stimulation was rapidly increased until the heart was unable to follow as determined from the blood pressure, which had been reduced by the increasing tachycardia with increasing rate, suddenly showing a large escape beat. The maximum following frequency is reciprocally related to the effective refractory period such that MFF(Hz) = 1000/ERP(ms). However, interventions which increase effective refractory period should not be expected to directly affect maximum following frequency. The difference between these two measures is that ERP is a resonable measure of effective refractory period while MFF is more of a measure of relative refractory period. Both measures exhibit different sensitivities to drugs (Walker and Beatch, 1988) and may falsely interpreted in the presence of elevated extracellular K+. Despite their relationship, both measures are sufficiently different to warrant reporting both.

2.6.1.5. - Data Analysis

Electrical stimulation end-points were examined at baseline and 2 minutes after beginning each infusion. These end-points were compared with control values and baseline values using analysis of variance, (ANOVA) followed by Duncan's multiple range test.

2.6.2. - Myocardial Ischaemia-Induced Arrhythmias

As reviewed by Curtis et al. (1987) and Cheung et al. (1993), a wide range of studies have established the validity of rat models for the study of ischaemia-induced arrhythmias. The antiarrhythmic activity of RSD 1000 was investigated in the model for myocardial ischaemia-induced arrhythmias in the anaesthetised rat. Effects on cardiac arrhythmias and mortality was determined for each infused dose of RSD 1000.

2.6.2.1. - Experimental Preparation

The occluder was first described by Au et al. (1979). Its design and manufacture have been extensively described by Clarke, et al. (1980) and Johnston et al. (1983). In brief, a 5.0 gauge atraumatic polypropylene suture (Ethicon) was threaded through the polyethylene guide such that the needle end of the suture appeared at the flared end of the guide.

The surgical procedures used were a modification of techniques described by Johns and Olson (1954) and Selye et al. (1960), and they were the same as those employed by Au et al. (1979), Johnston et al. (1983) and Paletta et al. (1989). An incision was made through the skin at the base of the sternum, where it was loosened from the underlying muscle mass. Blunt dissection was continued until the 4th to 6th ribs were revealed. The 5th intercostal space was then punctured and enlarged by

blunt dissection at which time four small steel retractors were used to widen the intercostal incision and maintain the opening to the heart. Careful manipulation of the retractors produced a pericardial sling whereby the heart was lifted towards the intercostal opening to reveal the left anterior descending coronary artery.

The procedures for ligation of the left anterior descending (LAD) coronary artery have been described in detail by Johnston et al. (1983) and Curtis et al. (1986a). In the rat, the left anterior descending coronary artey, which lies beneath the epicardium, is predominant and supplies blood supply to the left ventricle (Halpern, 1957). Proper ligation will produce myocardial infarct and induce fatal arrhythmias. The size area of infarct is important since it has been shown that the incidence and severity of arrhythmia correlates with the size of the infarct (i.e., square root of the occluded zone weight; Curtis et al., 1987).

During the occlusion procedure, the left atrial appendage was carefully lifted away from the surface of the heart with a cotton swab (Q-tips). Using a pair of needle forceps, the needle of the polypropylene suture was inserted a few millimeters distal to its origin. Once the suture was secured to the guide tubing, a loose ligature was implanted in the ventricular muscle containing the main trunk of the left coronary artery - a distance of approximately 2.0 mm from the point of entry and exit. This existing snare could be tightened by pulling the suture through the polyethylene guide at its exteriorized end. Occasionally, there was minor bleeding, amounting to less than 1.0 ml of blood, which was collected with a cotton swab. Air from the chest was removed with suction using a 5cc syringe via a small plastic tubing as silk sutures were used to join the sides of the pectoralis musculature together to form a seal. Ventilation was continued and adjusted when needed to reduce the pneumothorax. After

the surgery, rats were left to recover for 30 minutes prior to drug administration. In addition, arterial blood samples (≈ 1.0 ml) were taken before and after occlusion for determination of serum potassium concentrations.

2.6.2.2. - Experimental Design

The doses required to provide antiarrhythmic protection were chosen over a range which had minimal effects on ECG, blood pressure and heart rate. Vehicle or RSD 1000 were administered according to a blind randomized line design. There was one vehicle group with four other groups consisting of 1.0, 2.0, 4.0 and 8.0 µmoles/kg/min RSD 1000. After 5 minutes of infusion, a "pseudo"-steady-state serum concentration, relative to the redistribution, was assumed to have been attained based on the peak versus recovery responses observed in the bolus injections of RSD 1000. The suture was then pulled through the polyethylene guide so as to produce coronary artery occlusion and the traction between suture and guide was made permanent by heat-sealing suture to the guide. ECG, arrhythmias, blood pressure and heart rate were monitored for 15 minutes after occlusion or until the animal died from sudden cardiac death caused by irreversible ventricular fibrillation. Rats were excluded from the study if they met exclusion cirteria published by Curtis (1986a). Generally, if there was (1) a fall in mean blood pressure below 60 mmHg before drug adminstration, (2) an occurrence of arrhythmias for more than 5 minutes pre-drug (i.e., after occlusion surgery), (3) an occluded zone size was below 25%, or above 45%, of the total ventricular weight rats were excluded and immediately repeated, and/or (4) pre-occlusion serum [K+] was not within the range of 2.5-4.5 mM.

2.6.2.3. - Variables Measured

Blood pressure, heart rate, ECG and arrhythmias were recorded from the time of surgical recovery, 5.0 min drug infusion and 15 min after occlusion. These were recorded on a Grass polygraph (model 79D). In addition, a delayed loop ECG monitor (Honeywell, Model PM-2A) was used to facilitate arrhythmia analysis. Determination of serum K^+ concentrations was made using an ion-selective electrode (Ionetics Potassium Analyzer, Ionetics, CA, U.S.A.). Body temperature was measured by a rectal thermometer (YSI model 73A) and maintained at 37.0 ± 0.5°C by means of a heating lamp.

2.6.2.4. - Occluded Zone Determination

At the end of the experiment, surviving rats were sacrificed by an overdose of pentobarbitone (80-160 µmoles/kg or 20-40 mg/kg). All excised hearts (with occluder intact) were perfused (Langendorff technique) with PIPES solution to remove blood in all areas except the ischaemic zone. The perfusion was then switched to a cardiogreen dye solution (1.0 mg/ml in PIPES; Fast green dye. BDH) to differentiate perfused (green) from underperfused (occluded) tissue. The latter was cut out, along with atrial tissue, and weighed to give occluded zone size (OZ) as percentage of total ventricular weight.

2.6.2.5. - Data Analysis: S-T segment and R-wave Amplitude Changes Post-Occlusion

In the absence of drug treatments, coronary artery occlusion produces a rapid increase in ECG signal, characterized by a large increase in R-wave amplitude (Akiyama and Richeson, 1979) and an initial depression of S-T segment (Klébler et al., 1978). R-wave height was measured from the

isoelectric baseline to the peak of the positive deflection and was expressed in mV. Following an initial decrease in the first 2.0 min of coronary occlusion, the S-T segment gradually elevates and is often maintained for the duration of the experiment. The S-T segment elevation was expressed as a percentage of the R-wave amplitude, where the S-T segment is defined as the height of the S wave position above the isoelectric baseline. The isoelectric baseline was defined as the voltage at the foot of the P wave of the preceding beat. Although these effects are produced by occlusion, and presumably are influenced in some way by myocardial ischaemia, the mechanisms which are responsible for its effects are uncertain at this point.

2.6.2.6. - Analysis of Arrhythmias

Ischaemia-induced arrhythmias appear in a biphasic time-dependent manner as early arrhythmias (0-0.5 hr) or late arrhythmias (0.5-4.0 hr) (Wit and Bigger, 1977; Johnston et al., 1981; Kléber, 1987). In these experiments, the antiarrhythmic actions of RSD 1000 were only studied in the early arrhythmia phase since the incidence of arrhythmias is variable in the late phase (Scherlag et al., 1974).

Arrhythmias were analyzed according to guidelines established by the Lambeth Conventions (Curtis and Walker, 1988) as premature ventricular contractions (PVC), ventricular tachycardia (VT) or ventricular fibrillation (VF):

PVCs were defined as extrasystoles with QRS complexes occuring independently of the P wave. They were generally accompanied by a transient drop in aortic blood pressure. Only singlets, doublets and triplets were counted as PVCs. Runs of 4 or more consecutive extrasystoles were recorded as VT. Singlets, doublets, and triplets were not classified as

distinct arrhythmias but were combined together and considered as one of the same arrhythmia.

VT was defined as a run of 4 or more consecutive extrasystoles with distinguishable QRS complexes and were not subclassified according to rate. VT was subdivided into spontaneously reverting VT (SVT), which was reversible (i.e., returned to normal sinus rhythm), and non-spontaneously reverting VT (NSVT) which became irreversible (ceased to return to normal sinus rhythm). A reduction in blood pressure was also seen with VT.

VF was defined as a disordered ECG accompanied by a precipitous fall in blood pressure. As opposed to VT, VF has a chaotic ECG pattern with no identifiable complexes and a blood pressure of less than 10 mmHg. As in VT, any VF which was reversible was defined as spontaneously reverting VF (SVT), and any VF which became irreversible was defined as non-spontaneously reverting VF (NSVF).

The arrhythmia history of each rat was expressed as an arrhythmia score (AS) (Curtis and Walker, 1988). An arrhythmia score, an arbitrary numerical grading of the severity of ventricular arrhythmias, was used to summarize the arrhythmia profile of each animal. There are many possible different scoring systems but the following scoring system was used (Curtis and Walker, 1988):

Scoring System:

0 0-49 PVCs

1 50-499 PVCs

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

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

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

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

6 fatal VF starting at >15 min after occlusion

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

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

9 fatal VF starting <1 min after occlusion

2.7. - COMPARISON OF RSD 1000 WITH LIDOCAINE

For a comparison, the effects of lidocaine were tested in rats using the same experiments performed with RSD 1000. These included bolus and infused regimes, electrical stimulation, isolated rat hearts and coronary artery occlusion. Groups of 9 rats were used for lidocaine in each of the experiments. Drug treatment was performed in a blind and random line design. In the electrical stimulation study, treatment groups of a vehicle control and lidocaine at a starting concentration of 1.0 µmole/kg/min were used. In the occlusion study, drug treatment was performed in a random and blind line design consisting of a vehicle control and five different doses of lidocaine (1.0, 2.0, 4.0, 8.0 and 16 µmle/kg/min) were used. Finally, in the isolated rat heart study, 1.0 to 60 µM and 1.0 to 128 µM were used in the "ischaemia"-simulated and normal buffers, respectively. As with RSD 1000, the starting concentrations for lidocaine were chosen for their minimal affects on heart rate and ECG. The overall data for lidocaine was expressed as dose-response relationships and compared with the relative dose-response relationships of RSD 1000.

3. - RESULTS

3.1 IN VIVO

3.1.1. - Haemodynamic and Cardiac Effects of RSD 1000 in Comparison with Lidocaine: Bolus Doses in Rats

Blood Pressure and Heart Rate Changes: Blood pressure and heart rate responses to bolus doses of RSD 1000 and lidocaine are shown in Figure 5. RSD 1000 decreased blood pressure and heart rate in a dose-related manner with an ED25% value estimated at ≥ 64 µmole/kg. However, initial decreases in mean blood pressure and heart rate for RSD 1000 soon reached a "pseudo" steady state at 16 µmole/kg. Lidocaine lowered blood pressure and heart rate at much lower doses with an estimated ED25% value of 16 µmole/kg. The ED25% was the dose required to produce 25% decrease from its pre-drug value. The ED25% has to be differentiated from the ED25 value which is the dose which reduces or increases a variable 25% of the maximum possible. Except for blood pressure and heart rate, the ED25% is always a lower dose than the ED25 value. This initial data shows that RSD 1000 was much less bradycardic and hypotensive than lidocaine (by at least four times). For RSD 1000, death was due to respiratory failure followed by cardiac output failure 8 minutes after the 64 µmole/kg dose. Similarly, respiratory failure followed by complete cardiac arrest was seen with lidocaine at the onset of the final bolus dose.

Effects on ECG: The effects of RSD 1000 and lidocaine on ECG intervals are shown in Figures 6, 7, and 8 in terms of changes in P-R, QRS, Q-T_1, Q-T_2 intervals and RSh. RSD 1000 had minimal effects on QRS

and Q-T intervals while slightly increasing P-R at 32 µmole/kg. Similarly, lidocaine had no effect on QRS, and only minimal effect on P-R, $Q\text{-}T_1$ and $Q\text{-}T_2$ at 16 µmole/kg. Any increases in P-R, $Q\text{-}T_1$ and $Q\text{-}T_2$ were only detected at higher doses for both agents However, the initial changes seen in the ECG for both drugs were with RSh. Both RSD 1000 and lidocaine increased RSh in a dose-dependent manner with an estimated ED25% values of 12 and 32 µmole/kg, repectively.

Figure 5: - Bolus Injections: Time-Effect Data for Mean Blood Pressure and Heart Rate

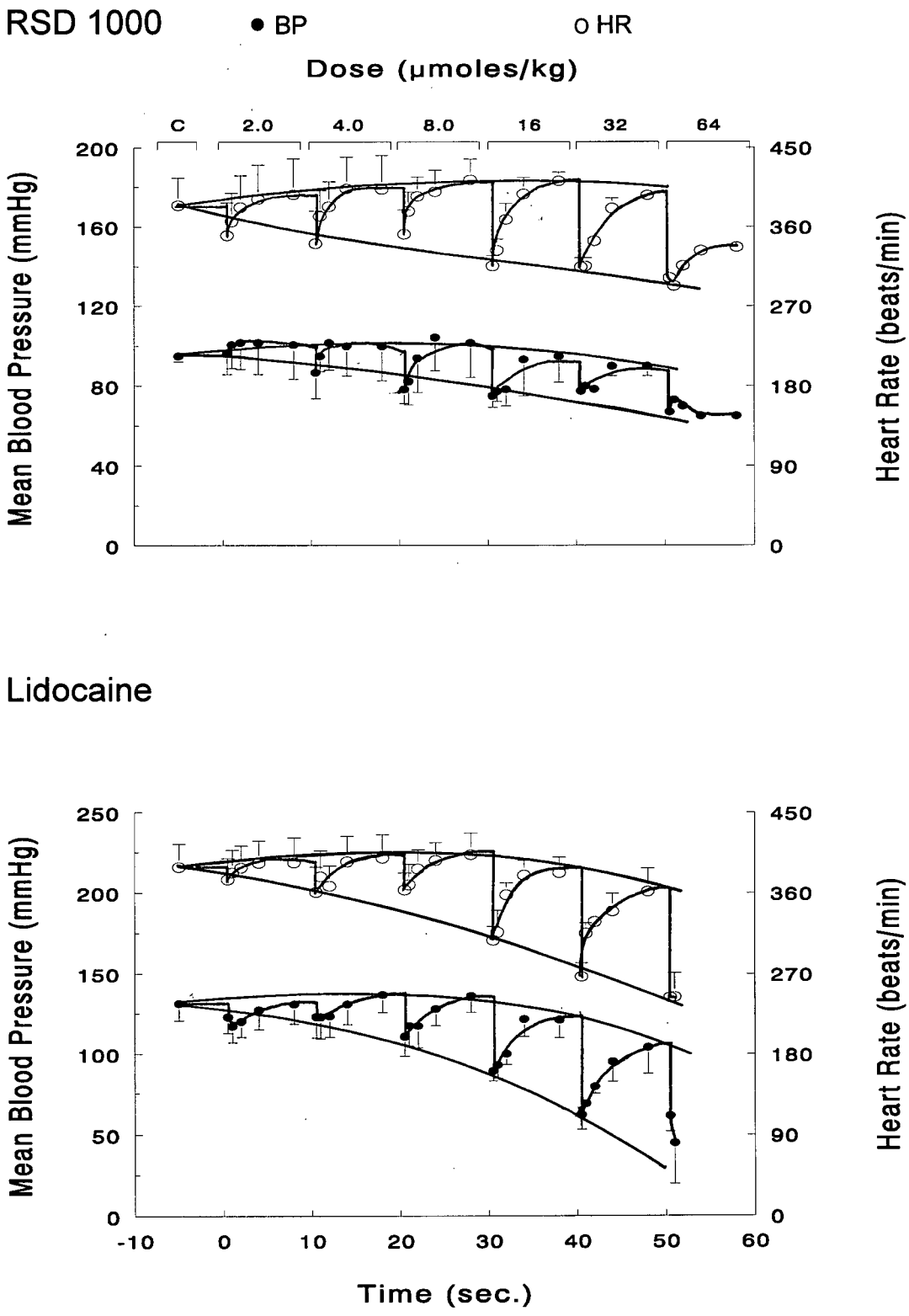


Figure 5: Effects of RSD 1000 on blood pressure and heart rate in comparison with lidocaine. Each point is the mean ± SEM (n=5) for both RSD 1000 and lidocaine and these were plotted as a function of time on the lower abscissa. Readings were taken at 0.5, 1, 2, 4 and 8 minutes followed by the next doubling dose at every 10 minute interval; the time between each interval was 10 minutes. The symbol (•) is for blood pressure and (o) for heart rate. Bolus doses tested for RSD 1000 and lidocaine were 2, 4, 8, 16, 32 and 64 µmole/kg and are indicated on the upper abscissa. Each dose was administered at the beginning of each time interval. Peak effects for blood pressure and heart rate are shown as initial decreases following drug administration while time to recovery for both agents was approximately 8-10 minutes. Both peak and recovery responses were best fitted with curves to illustrate the changes with increasing dose. For lidocaine, both blood pressure and heart rate decreased with increasing dose while similar effects were observed for RSD 1000 in which a "pseudo" steady state was reached at 16 µmole/kg. ED25% values were estimated from the peak response curve. The estimated ED25% value for lidocaine was 16 µmole/kg in lowering both mean blood pressure and heart rate; only an ED25% value for lowering HR was estimatable for RSD 1000. At the final doses, death was due to respiratory failure followed by cardiac output with RSD 1000 while cardiac arrest and respiratory failure were immediate soon after lidocaine administration.

Figure 6 -Bolus Injections: Time-Effect Data for P-R and QRS Intervals

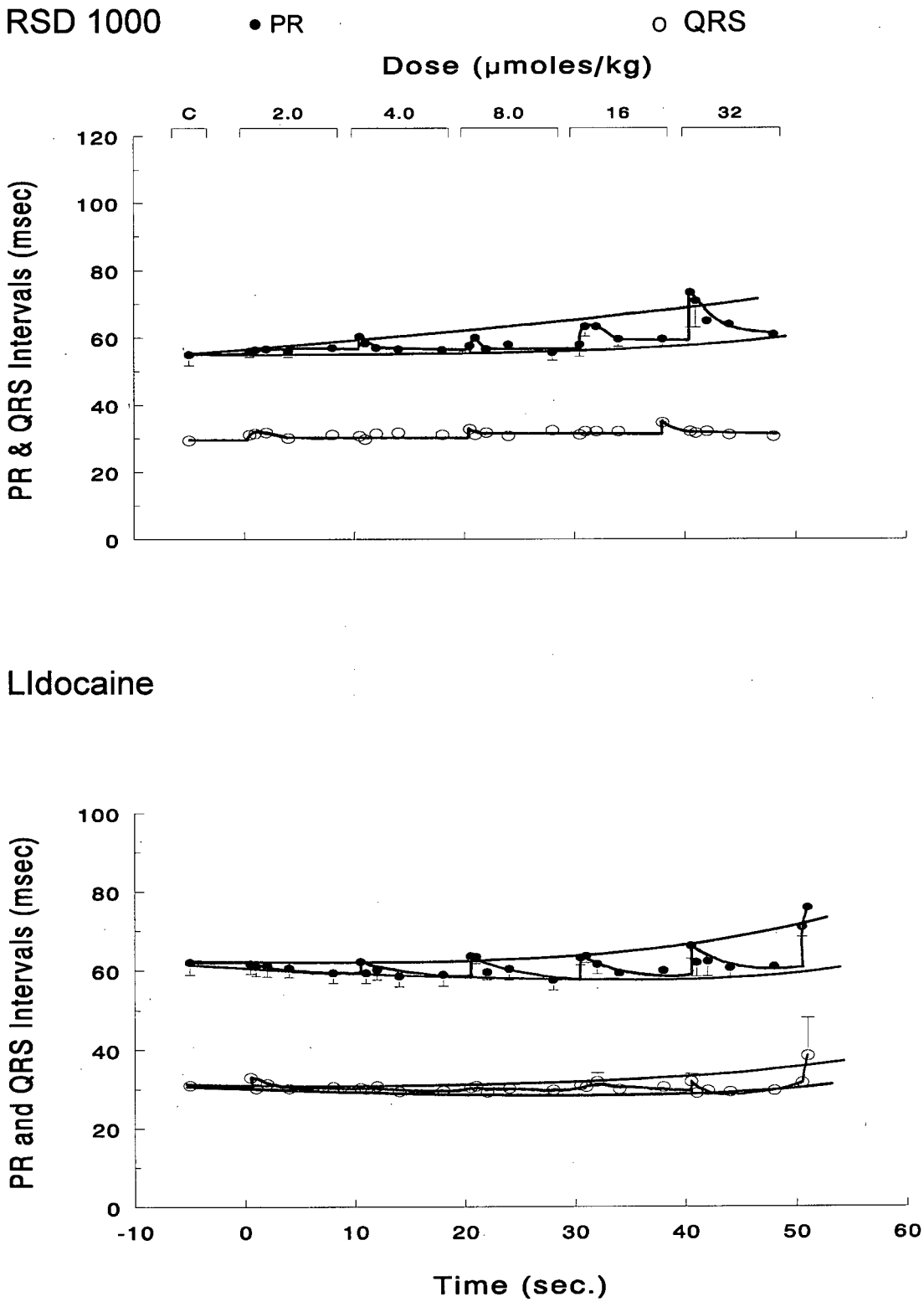


Figure 6: Effects of RSD 1000 on P-R and QRS intervals of the ECG compared with lidocaine. The symbol (•) is for P-R and (o) for QRS. Slight effects on P-R interval with no effect on the QRS interval were observed with each agent with. The estimated ED25% values in increasing P-R interval for RSD 1000 and lidocaine were 32 and 64 μmole/kg, respectively.

Figure 7 - Bolus Injections: Time-Effect Data for RSh

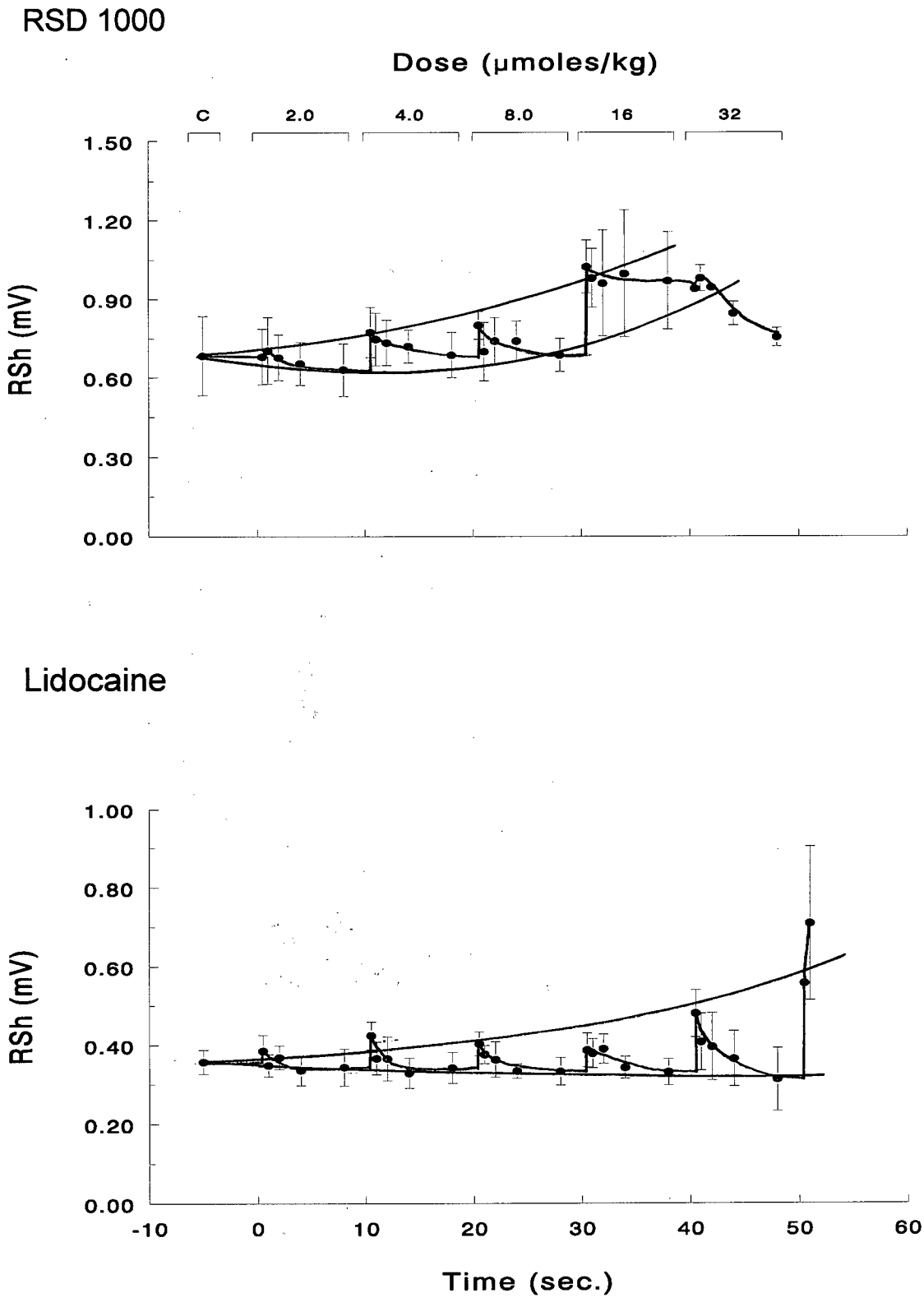


Figure 7: Effects of RSD 1000 on RSh in comparison with lidocaine. RSD 1000 increased peak effects on RSh with increasing dose. For the initial three doses, values returned to baseline after 4 minutes, but at 16 µmole/kg there was a much greater increase in the peak effects. In a dose-related manner, lidocaine also increased peak effects of RSh but following each dose, values returned to baseline after 4 minutes. The estimated ED25% value for both RSD 1000 and lidocaine in increasing RSh interval were 12 and 19 µmole/kg.

Figure 8 - Bolus Injections: Time-Effect Data for Q-T_1 & Q-T_2 Intervals

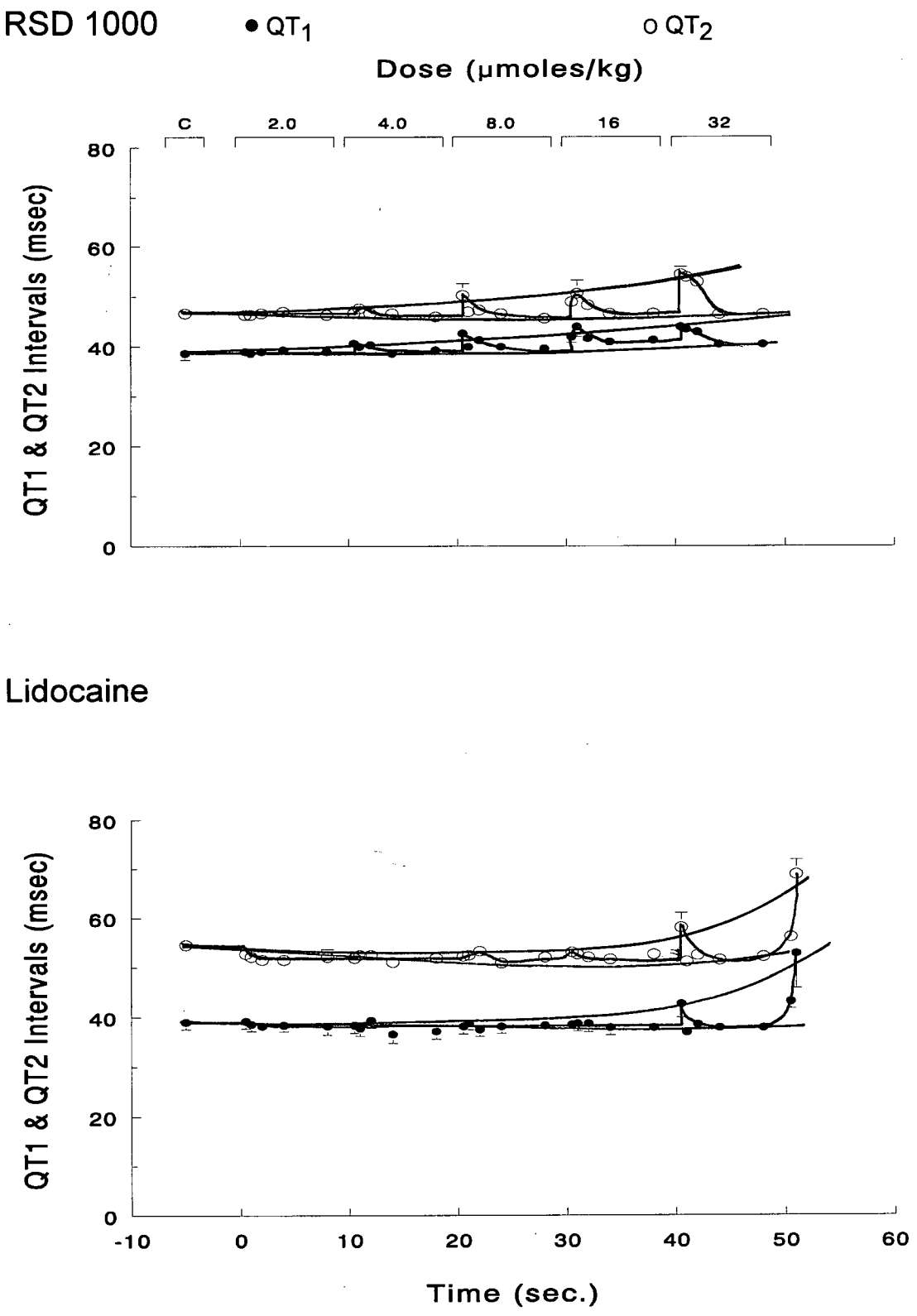


Figure 8: Effects of RSD 1000 on Q-T_1 and Q-T_2 intervals in comparison with lidocaine. The symbol (•) is for Q-T_1 and (o) for Q-T_2. Increases in peak effects for Q-T_1 and Q-T_2 were initially observed at 8.0 µmole/kg for RSD 1000. In contrast, initial effects on Q-T interval with lidocaine were seen at 32 µmole/kg. ED25% values for RSD 1000 and lidocaine were not estimatable.

3.1.2. - Haemodynamic and Cardiac Effects of RSD 1000 in Comparison with Lidocaine: Infused Doses in Rats

Blood Pressure and Heart Rate Changes: Figure 9 illustrates the infused dose-response effectiveness of RSD 1000 on blood pressure and heart rate in comparison with lidocaine. RSD 1000 and lidocaine lowered blood pressure and heart rate with increasing dose. In comparison to bolus injections, cumulative infusion doses of both agents also showed that RSD 1000 is less vaso-depressant than lidocaine over the same dose range. The ED25% values for lowering blood pressure and heart rate were 9.0 and 8.0 µmoles/kg/min, respectively, for RSD 1000 and 4.0 and 8.0 µmoles/kg/min, respectively, for lidocaine. In both cases, animals were ventilated throughout the experiment and death was due to atrio-ventricular block (AVB) followed by cardiac arrest at the onset of 64 and 32 µmoles/kg/min for RSD 1000 and lidocaine, respectively.

Effects on ECG: Similar dose-response curves were constructed for the effects of RSD 1000 on the ECG variables of P-R, QRS, RSh and Q-T intervals (Figures 10, 11 & 12). RSD 1000 produced a slight increase in P-R with an estimated ED25% value of 20 µmoles/kg/min while slight or no effects on QRS interval were observed. Q-T widening was evident at doses greater than 10 µmoles/kg/min with an ED25% value of 30 and 15 µmoles/kg/min for $Q\text{-}T_1$ and $Q\text{-}T_2$, respectively. In contrast, lidocaine had minimal or no effects on P-R, QRS and Q-T intervals, as evidence by the absence of estimated ED25% values for these variables and changes, if any, were only seen at the highest doses tested. Both RSD 1000 and lidocaine increased RSh with equal potency at an ED25% value of 12 µmoles/kg/min.

Figure 9: Infused Doses: Dose-Response Curves for Mean Blood pressure and Heart Rate

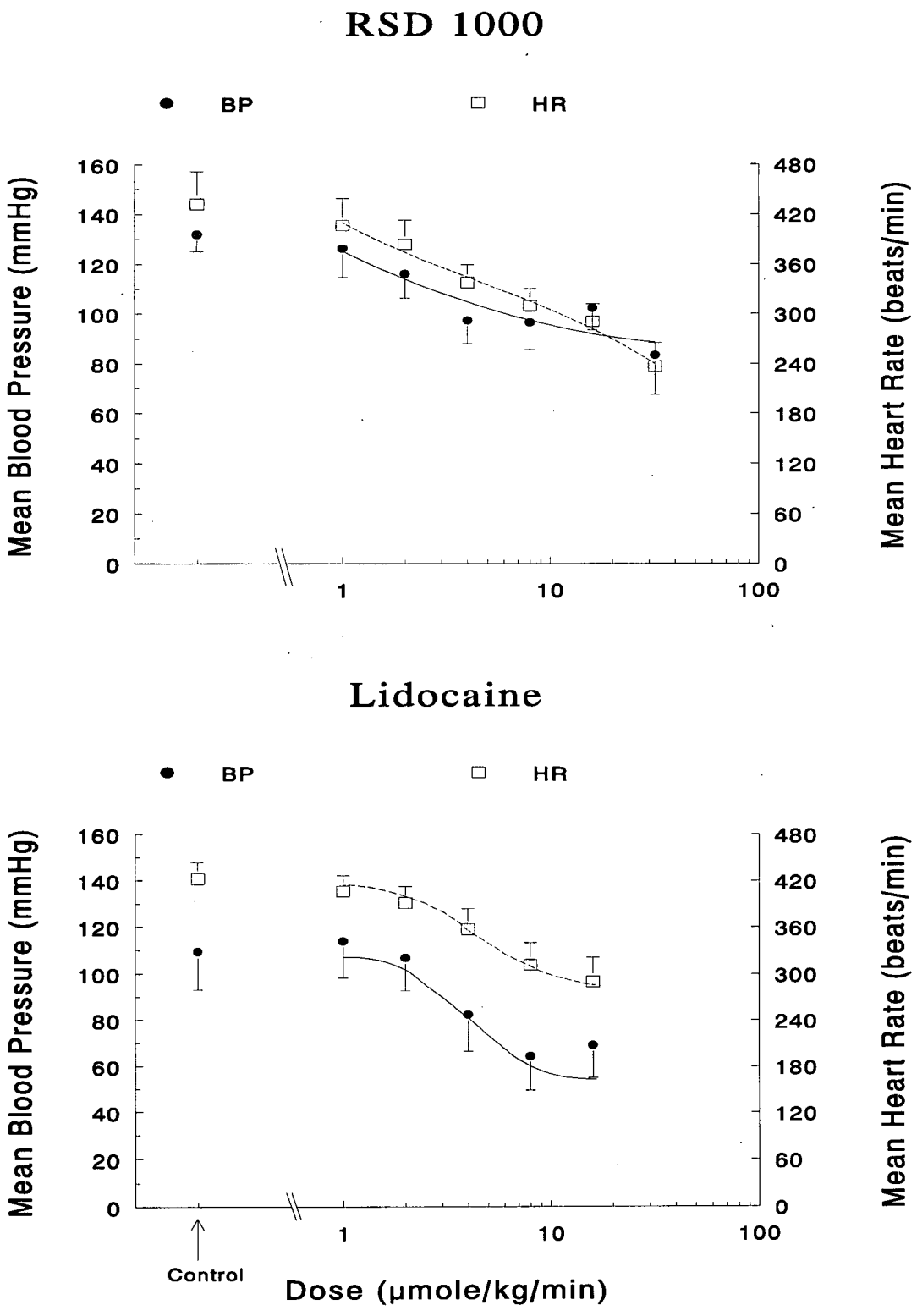


Figure 9: Effects of RSD 1000 on mean blood pressure and heart rate in comparison with lidocaine. Each point is the mean ± SEM (n=5) for both RSD 1000 and lidocaine. Measurements were made after 3 minutes of infusion at each infusion level and values are changes from pre-drug values. RSD 1000 and lidocaine were tested at infused doses of 1.0 to 32 µmoles/kg/min. The predrug values were not significantly different for the two agents; the overall mean blood pressure was 120 ± 7mmHg, and corresponding heart rate was 420 bpm. The symbol (●) is for blood pressure and (□) for heart rate. Both agents decreased blood pressure and heart rate in a dose-dependent manner. The ED25% values for lowering blood pressure and heart rate are 9.0 and 8.0 µmoles/kg/min, respectively, for RSD 1000 and 4.0 and 8.0 µmoles/kg/min, respectively, for lidocaine.

Figure 10: Infused Doses: Dose-Response Curves for PR & QRS Intervals

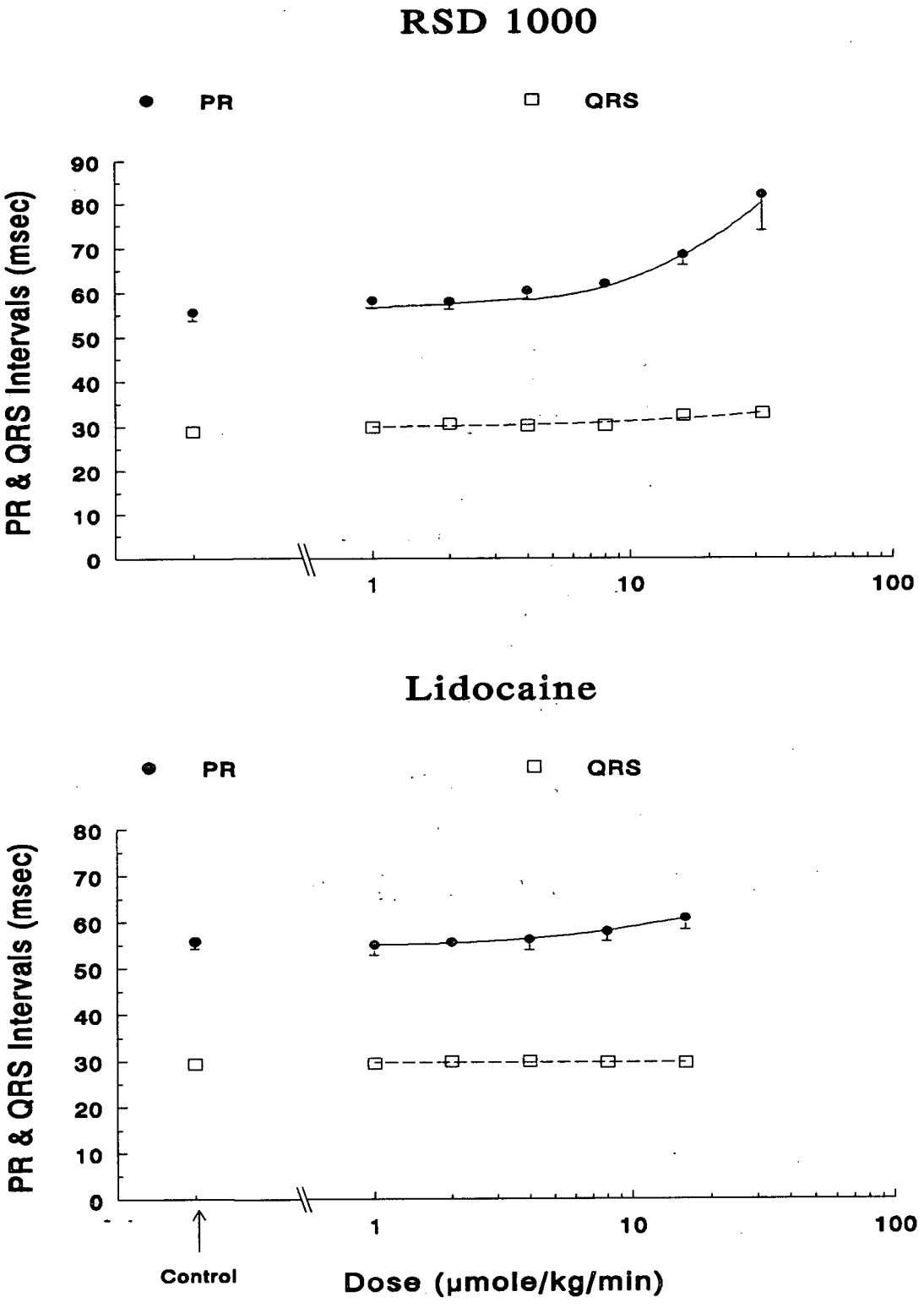


Figure 10: Effects of RSD 1000 on P-R and QRS intervals in comparison with lidocaine. Effects on P-R with RSD 1000 began at doses greater than 8 μmoles/kg/min while no changes in QRS width was detected. In contrast, lidocaine had minimal or no effects on P-R and QRS over the same dose range. The estimated ED25% value for RSD 1000 for changes in the P-R interval was approximately 20 μmoles/kg/min.

Figure 11: Infused Doses: Dose-Response Curve for RSh

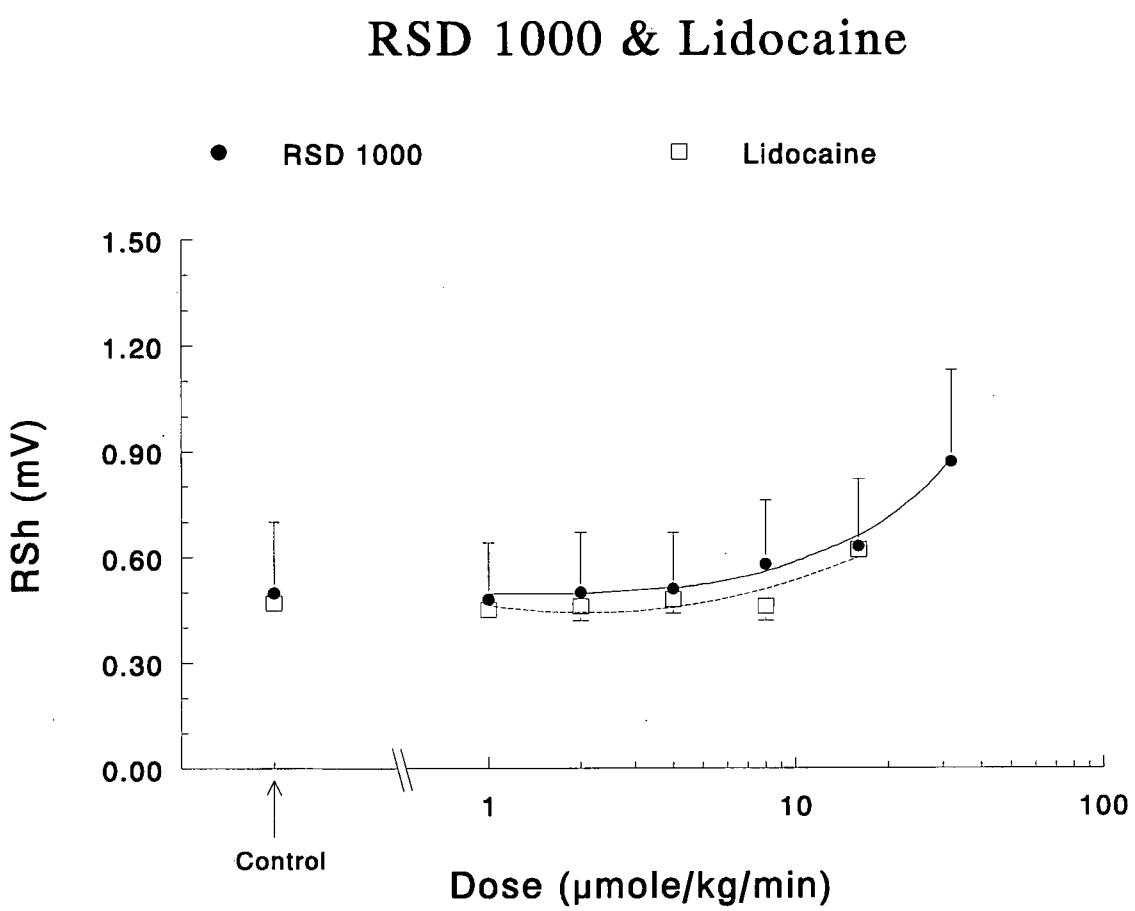


Figure 11: Effects of RSD 1000 on RSh in comparison with lidocaine. RSD 1000 dose-dependently increased RSh, while lidocaine produced no effects. The estimated ED25% values for RSD 1000 and lidocaine at increasing RSh was 12 and 19 µmoles/kg/min, respectively.

Figure 12: Infused Doses: Dose-Response Curves for QT_1 & QT_2 Intervals

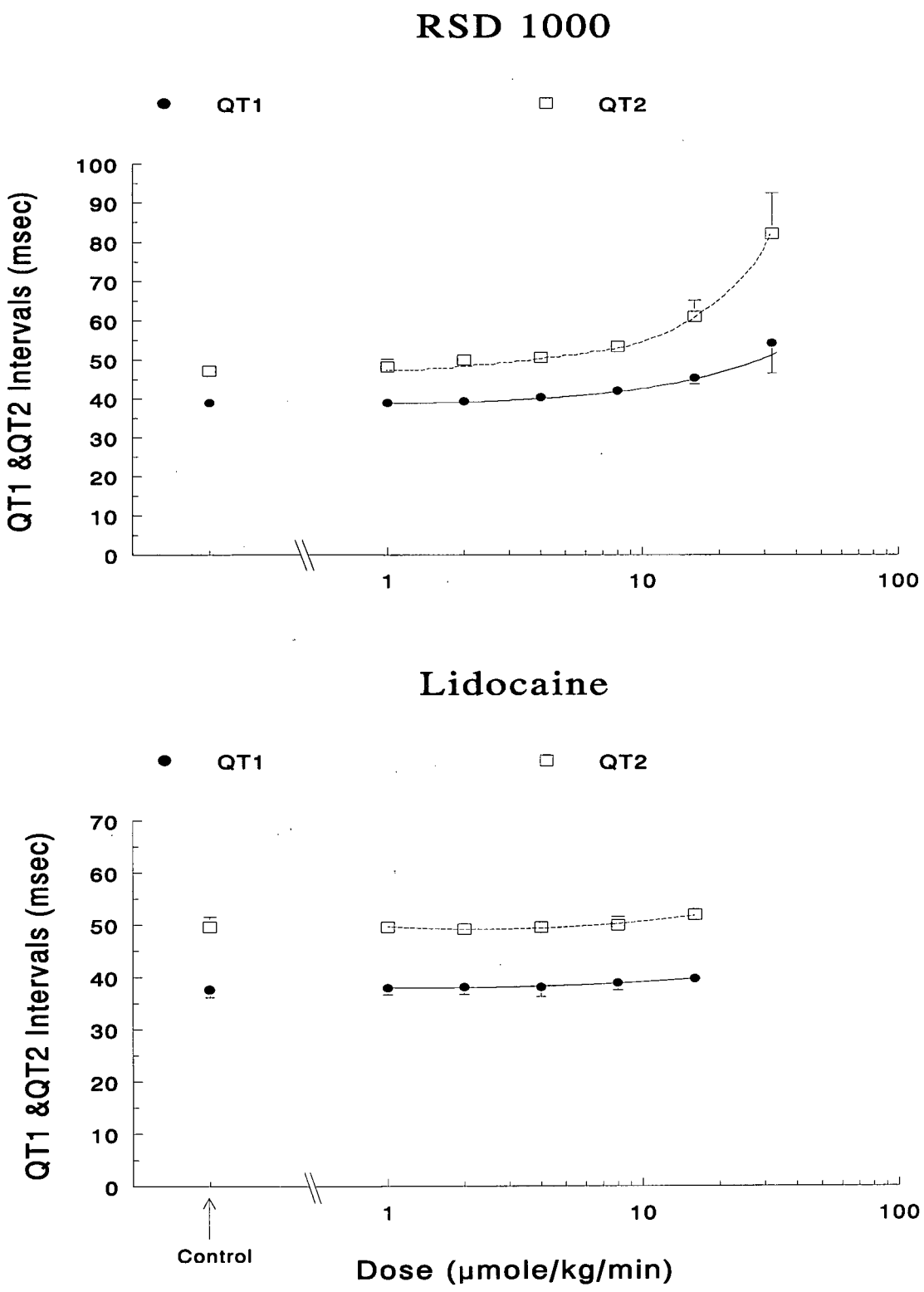


Figure 12: Effects of RSD 1000 on Q-T interval ($Q\text{-}T_1$ & $Q\text{-}T_2$) in comparison with lidocaine. $Q\text{-}T_1$ was measured from the start of the QRS complex to the peak of the T-wave. Similarly, $Q\text{-}T_2$ was measured from the start of the QRS complex to the midpoint of the downstroke of the T-wave. Q-T prolongation was most marked at doses greater than 10.0 µmoles/kg/min with RSD 1000. However, lidocaine had no effects on Q-T interval. The estimated ED25% value for RSD 1000 for changes in $Q\text{-}T_1$ and $Q\text{-}T_2$ was 30 and 15 µmoles/kg/min, respectively.

3.2. - RSD 1000: Lethality and Analgesic Studies in Mice:

Table 1 shows the doses of RSD 1000 tested for lethality in CD-1 mice. The following doses of RSD 1000 were tested i.v.: 171, 85, 42.7 and 17.1 µmoles/kg. The dose required to produce death in 50% of the mice, was estimated at 67 µmoles/kg using the computer program (Spearman and Karber) in a compatible PC (486-66MHz). The minimal lethal dose used was 171 µmoles/kg, in which all the mice died from respiratory failure. A 1/10 dilution was made from the above dose and all the mice survived with minimal analgesic effects. As the dose was increased to 42.5 and 85 µmoles/kg, those which did survive showed similar signs of tolerance to painful stimulus via the tail pinch method. Evidence of convulsive behaviour coupled with laboured breathing were also observed at the higher doses while lethargy as most common physical response at the lower doses. The mice which did survive the 85 µmoles/kg dose were lethargic for a period of 10-15 minutes, while those surviving the 42.5 µmoles/kg dose showed similar responses lasting less than 10 minutes.

In the naloxone-pretreated mice, the analgesic effects were investigated and the results showed a negative response to analgesia with RSD 1000. The absence of reported deaths at the 85 µmoles/kg dose, but, with deaths reported at double the dose, suggests that more than one mechanism was responsible for producing lethality with RSD 1000.

Table 2 - RSD 1000: LD50 Studies in Mice with Naloxone

LD50 Study:

RSD 1000 (μmoles/kg, i.v.)	n	Analgesia	Death
17.1	7	Y in 2/7	N in 0/7
42.5	7	Y in 3/7	Y in 1/7 (resp.)
85	7	Y in 3/7	Y in 5/7 (resp.)
171	4	--	Y in 4/4 (resp.)

Analgesia Study with Naloxone:

RSD 1000 (μmoles/kg, i.v.)	n	Analgesia	Death
85	5	Y in 4/5	Y in 3/5 (resp.)
171	5	--	Y in 5/5(resp.)
85 w/ naloxone	5	N in 5/5	0/5
171 w/ naloxone	5	N in 1/5	Y in 4/5 (resp.)

Table 2 - Lethality studies of RSD 1000 with pre-treatment of naloxone. CD-1 mice were used and i.v. injections were made in the tail vein. Mice were pre-treated with naloxone (6.1 μmoles/kg or 2.0 mg/kg, i.p.) 2 minutes prior to the administration of RSD 1000. The tail-pinch method was used to assess analgesic effects of RSD 1000. Lethargy was the most common physical response following the administration of RSD 1000. The estimated LD50 was 67 μmoles/kg and death was primarly due to repiratory failure. In the presence of naloxone, there was no mortality of mice at the 85 μmoles/kg dose. Mortality was not abolished, however, at the 171 μmoles/kg dose, suggesting that other mechanisms with RSD 1000 were involved in producing lethality.

3.3 - *IN VITRO*

3.3.1. - Effects on Isolated Rat Heart

The *in vitro* effects of RSD 1000 on heart rate (Fig.13A), P-R interval (Fig.13B) and QRS interval (Fig.13C) in isolated rat hearts are presented in comparison with lidocaine. Since the starting concentration for RSD 1000 in the normal buffer was relatively high, a limited supply of the drug prevented further investigation at lower concentrations. The concentrations used in the "ischaemia"-simulation buffer were dilutions made from the stock concentration used in the normal buffer. The data was expressed as a percent change from control, i.e., a percent decrease for HR and a percent increase for both PR and QRS. The concentrations for lidocaine which were chose show the relative concentration range in which the changes produced by RSD 1000 were comparable to those produced by lidocaine.

In normal buffer, RSD 1000 produced a dose-dependent decrease in heart rate and an increase in P-R and QRS intervals followed by episodes of AVB at 128 μM. Decreases in heart rate and increases in both P-R and QRS intervals of the ECG with lidocaine were also produced in a dose-dependent manner followed by the production of AVB at 512 μM.

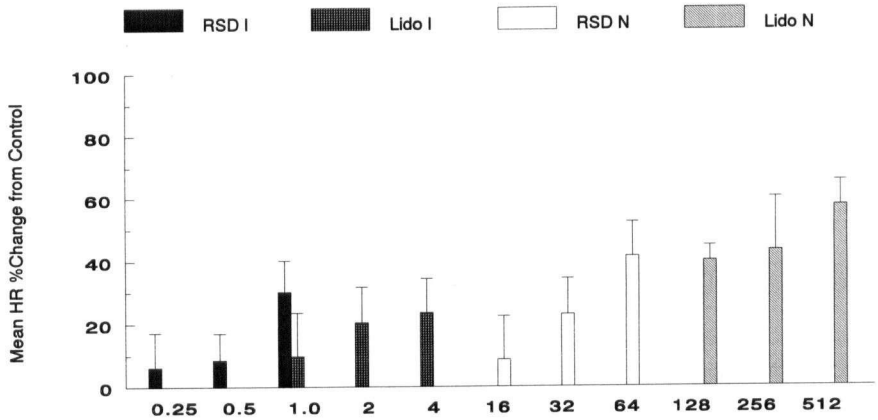
In the "ischaemia"-simulation buffer, the concentrations of RSD 1000 used to produce effects on heart rate, P-R and QRS were much lower than lidocaine. The starting concentration was 0.25 μM which was approximately 64 times more effective than the concentration used in the normal buffer. Concentrations of RSD 1000 were increased until AVB in 4/5 heart was produced at 2.0 μM. As in the normal buffer, RSD 1000 lowered heart rate and increased P-R and QRS intervals with increasing concentration. However, its effects on QRS prolongation were much greater in the "ischaemic" than in the normal buffer.

Lidocaine was also activated by conditions of high [H+] and [K+] indicated by dose-dependent effects on HR, PR and QRS which were also produced at concentrations 100-fold lower than in the normal buffer. Despite the similar 100-fold shift in concentration for both agents between the normal and "ischaemia"-simulated buffers, RSD 1000 was the more potent of the two by at least 8-10 times. This potency difference was clearly illustrated by the production of AVB in the normal buffer with RSD 1000 at 64 µM compared with 512 µM for lidocaine. One other observable difference between RSD 1000 and lidocaine was the difference in their on-set and off-set times for producing effects in the isolated hearts. The time to drug effects and the recovery from those effects during washout was longer for RSD 1000 (about 4-5 minutes) than those observed for lidocaine (0.5-1minutes). These results suggest that there may be difference in the lipophilicity and/or binding between these two agents.

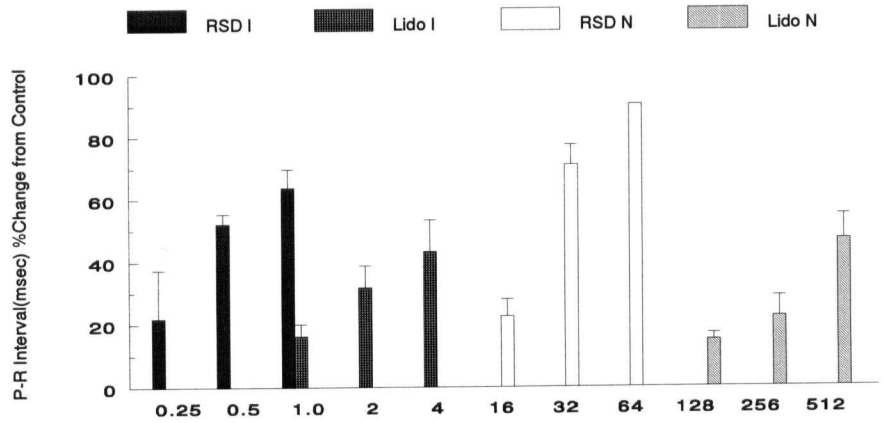
The decrease (as in heart rate) and increase (as in P-R and QRS intervals) in baselines control values for the measured variables in the "ischaemia"-simulated buffer are the result of the low pH and high [K+] concentration, both of which are known to depress cardiac excitability (Curtis et al., 1986; Fabiato and Fabiato, 1978; Yan and Kléber, 1992; Couper, et al., 1984; Gasser and Vaughan-Jones, 1990). In the normal buffer, changes in systolic ventricular pressure and contractility were no different from control values. Since acidotic and hyperkalemic conditions affect the active tension of the heart and, as a result, produces a large variance when measuring systolic pressure and contractility, a comparison between these measures in normal and "ischaemic" buffers was not made.

Figure 13: Effects on Heart Rate, P-R and QRS Intervals in Isolated Rat Hearts

A.



B.



C.

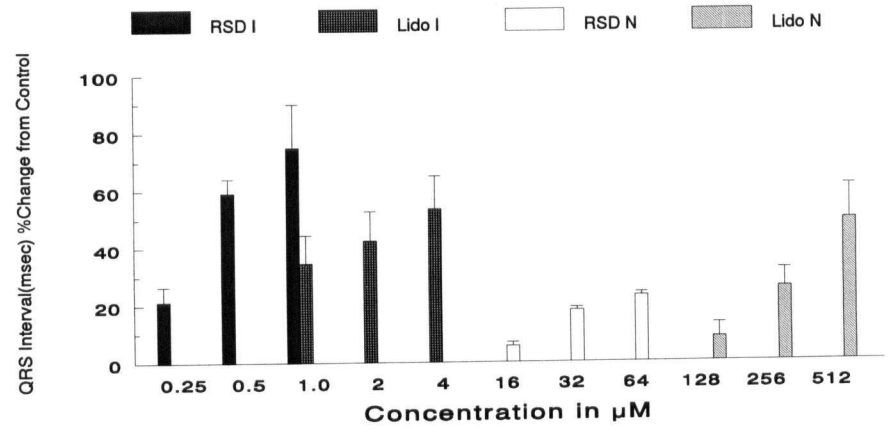


Figure 13: RSD 1000 and lidocaine and their effects on heart rate, P-R and QRS intervals in the presence of "ischaemia"-simulation (pH=6.4; [K+]=10mM) and normal buffer (pH=7.4; [K+]=4mM). I and N denote "ischaemia"-simulated and normal buffer, respectively. Each value (n=5) is expressed as a percent change from control values. Control values for *heart rate* (HR) for RSD 1000 and lidocaine were RSDI=198±21 and RSDN=376±12beats/min and LidoI=190±24 and LidoN=287±8 beats/min, respectively. The control values for *P-R interval* were RSDI=97±13 and RSDN=59±5msec for RSD 1000 and LidoI=72±2 and LidoN=64±5msec for lidocaine. The control values for *QRS interval* were RSDI=36±3 and RSDN=29±0.8msec for RSD1000 and LidoI=32±2 and LidoN=31±2msec for lidocaine. In the normal buffer, both RSD 1000 and lidocaine decreased heart rate and increased P-R and QRS intervals in a dose-dependent manner. At concentrations greater than 64 μM for RSD 1000, 4/5 hearts in the normal buffer experienced AVB. Similarly, at 512 μM for lidocaine, AVB was produced in all the hearts after 30 seconds of drug administration. The effects of both agents were potentiated by low pH and high [K+] but with RSD 1000 being more potent than lidocaine. Increases in P-R and QRS intervals were greatest with RSD 1000 in both buffers. AVB in the "ischaemic" buffer was produced at 2 μM for RSD 1000 and at 32 μM for lidocaine (data not shown).

3.4. - ANTIARRHYTHMIC EFFICACY

3.4.1. - RSD 1000 and its Effects Against Electrically-Induced Arrhythmias

RSD 1000 was tested against arrhythmias induced by electrical stimulation of the left ventricle over the same dose range which produced its cardiodepressant effects to assess and compare its frequency dependence with lidocaine. Figure 14, 15 & 16 showed that RSD 1000 increased the threshold currents for extrasystoles (iT-Fig.14), induction of ventricular fibrillo-flutter (VFt-Fig.15), and effective refractory period (ERP-Fig.16) in a dose-related manner. In the same figures, lidocaine also produced comparable effects on iT, ERP and VFt suggesting that both RSD 1000 and lidocaine were equally effective against electrically-induced arrhythmias. The approximate ED25% values for elevations of iT, ERP and VFt were 4.5, 3.0 and 4.0 µmoles/kg/min, respectively, for RSD 1000 and 8.0, 6.0 and 5.0 µmoles/kg/min, respectively, for lidocaine indicating that RSD 1000 provided antiarrhythmic protection against electrically-induced arrhythmias at doses marginally lower than lidocaine and at doses which produce minimal ECG and cardiovascular effects. These results show that elevations in these thresholds by RSD 1000 and lidocaine were dependent on dose (and frequency for VFt only).

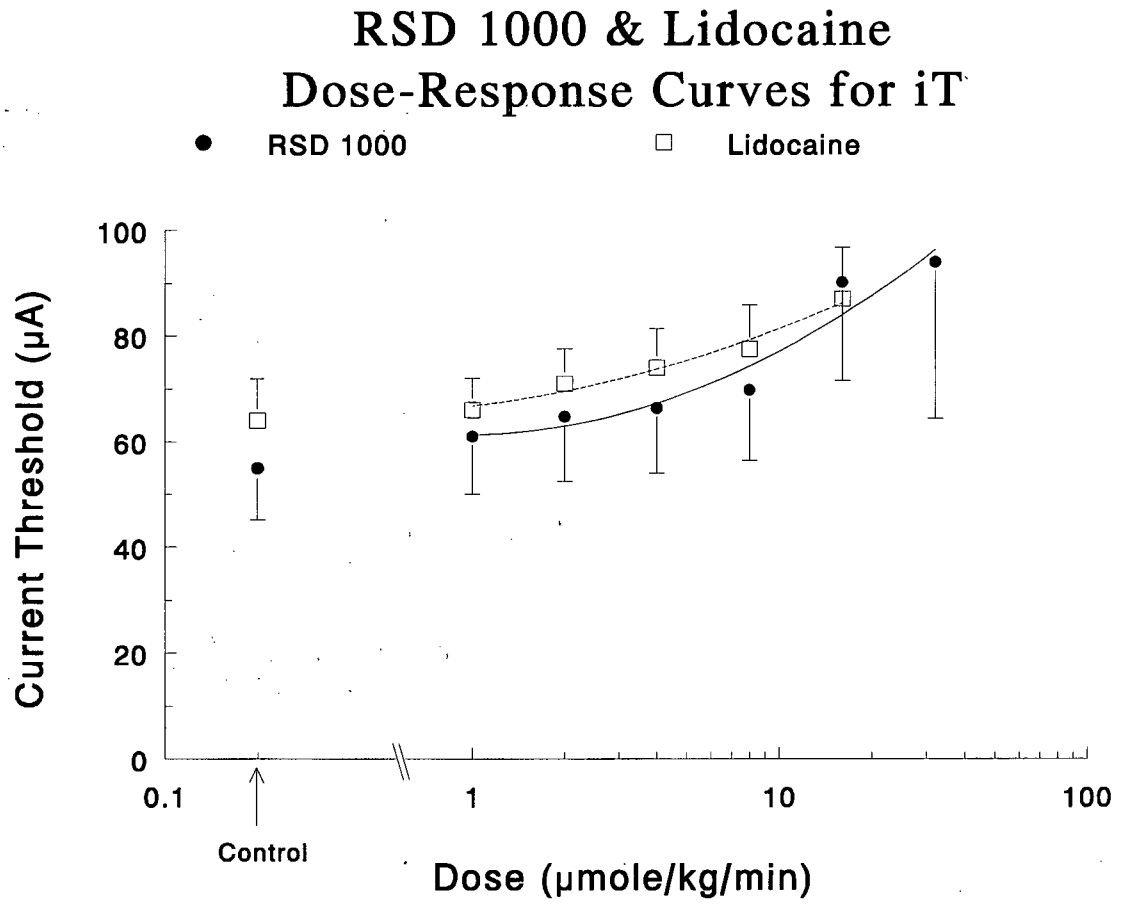


Figure 14: The effects of RSD 1000 and lidocaine on iT. Each point is the mean ± SEM (n=5) for both RSD 1000 (•) and lidocaine (□). Results are presented as changes from the pre-drug value (RSD 1000 = 55 ± 9 µA; lidocaine = 64 ± 4 µA) induced by infusion of RSD 1000 or lidocaine from 1.0 to 32.0 µmoles/kg/min. Both agents increased the current threshold in a dose-related manner. The ED25% values for RSD 1000 and lidocaine were 4.5 and 8.0 µmole/kg/min, respectively. Note, the absence of values for lidocaine at 32µmole/kg/min is due to death occurring prior to measuring electrical stimulation end-ponts.

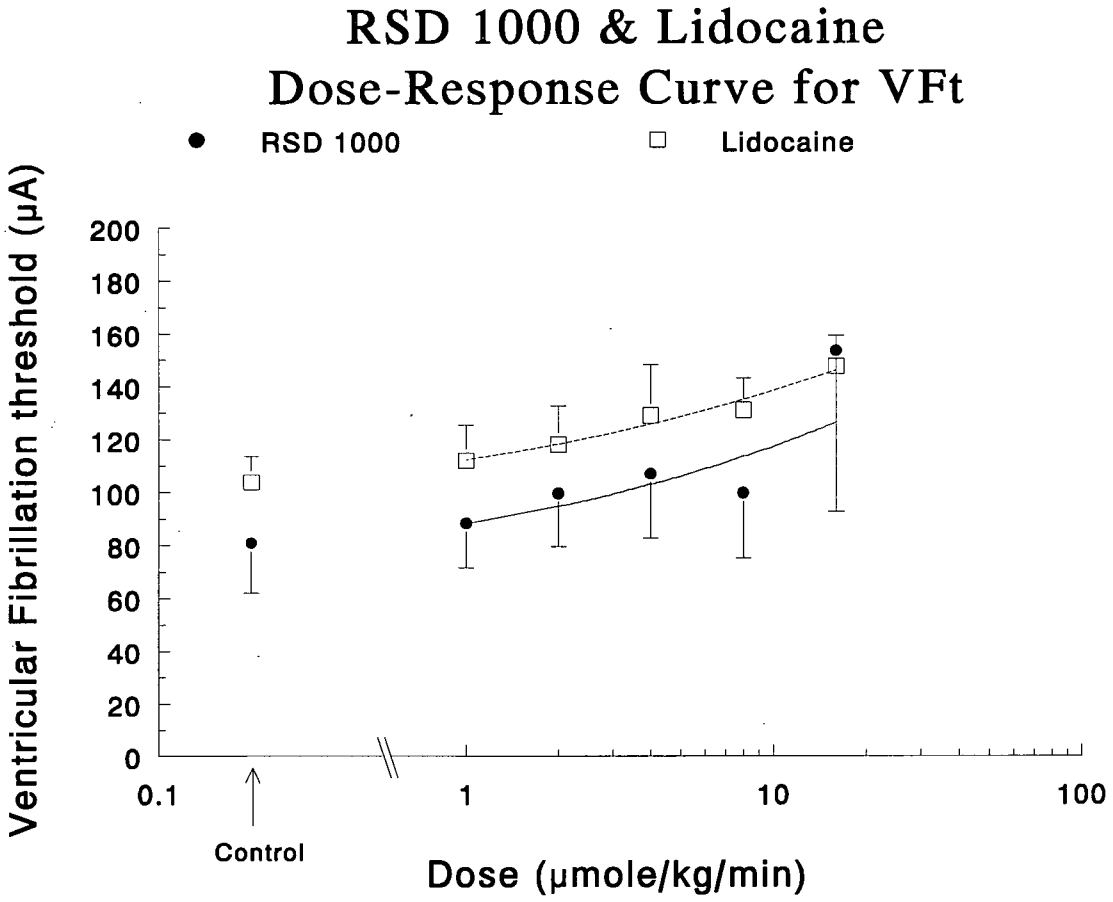


Figure 15: RSD 1000 and its effects on VFt in comparison with lidocaine. Each point is the mean ± SEM (n=5) for both RSD 1000 and lidocaine. Results are presented as changes from the pre-drug value (RSD 1000 = 81 ± 9 µA; lidocaine = 104 ± 4 µA) induced by infusion of RSD 1000 or lidocaine from 1.0 to 32.0 µmoles/kg/min. Both agents showed the same effectiveness at increasing the ventricular fibrillation threshold with increasing dose. The ED25% values for RSD 1000 and lidocaine were 4.0 and 5.0 µmole/kg/min, respectively.

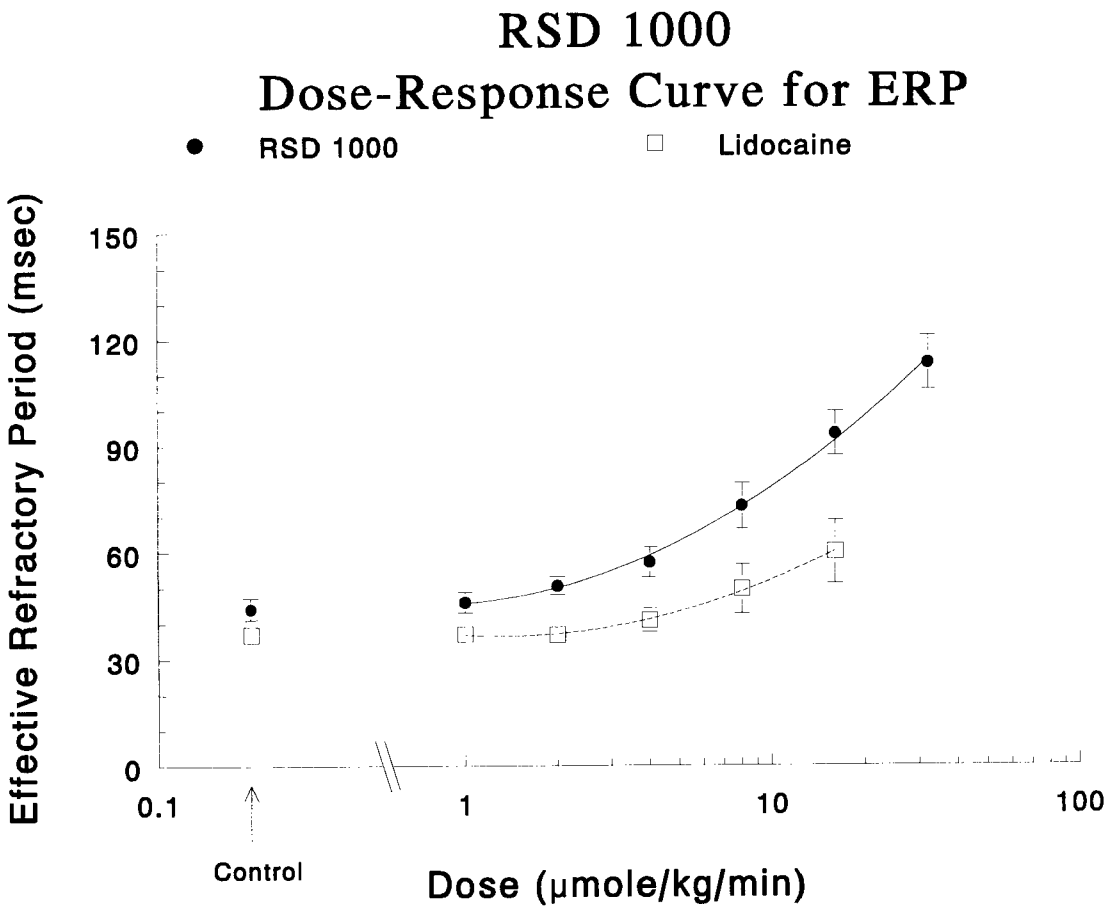


Figure 16: A comparison between RSD 1000 and lidocaine and their effects on ERP. Each value is the mean ± SEM (n=5) for both RSD 1000 and lidocaine. Results are presented as changes from the pre-drug value (RSD 1000 = 44 ± 3 msec; lidocaine = 37 ± 2 msec) induced by infusion of RSD 1000 or lidocaine from 1.0 to 64.0 µmoles/kg/min. Both agents increased the effective refractory period in a dose-related manner, but RSD 1000 was 2 times more effective in prolonging ERP than lidocaine. The ED25% values for RSD 1000 and lidocaine were 3.0 and 6.0 µmole/kg/min, respectively.

3.4.2. - RSD 1000 and its Effects on Ischaemia-Induced Arrhythmias

RSD 1000 provided dose-related protection against arrhythmias induced by coronary artery occlusion in anaesthetized rats as illustrated in Figure 17. Table 3 is a summary of antiarrhythmic actions for each dose of RSD 1000 and lidocaine. The number of premature ventricular contractions (PVC) was expressed as a $\log_{10}$ transform, since this variable is Gaussian distributed whereas the untransformed variable is not (Johnston et al., 1983). The transformed PVC data allows its expression as mean(±SEM). Arrhythmias were expressed in terms of group mean arrhythmia scores (±SEM) for groups of 7 rats.

Increase in the R-wave amplitude and inital depression of the S-T segement following coronary artery occlusion were present in all the rats. The occurrence of the majority of arrhythmias were observed between 6-8 minutes post-occlusion. In the vehicle controls and groups treated with the lower doses, episodes of tachyarrhythmias followed by irreversible ventricular fibrillation were most common. Mortality was observed as an episode of irreversible VF, degenerating into fine VF along with a blood pressure of approximately 15 mmHg. Serum potassium concentrations and occluded zone sizes in all the groups were within acceptable limits.

The ED_{50} value for RSD 1000 was 2.5 µmoles/kg/min with the greatest antiarrhythmic effect at 8.0 µmoles/kg/min. Similar antiarrhythmic protection was produced with lidocaine with a significant reduction in the incidence of arrhythmias at 16 µmoles/kg/min. The estimated ED_{50} for lidocaine was 4.0 µmoles/kg/min. The antiarrhythmic actions of RSD 1000 followed a dose-related relationship, whereas lidocaine exhibited inconsistent antiarrhythmic responses with each dose. Although both agents appear to be equipotent in their antiarrhythmic actions, the

differences in their therapeutic indices (see Table 4) show that RSD 1000 has a safety margin that is 3-4 times greater than lidocaine for lowering blood pressure. Except for the fact that lidocaine produced little effect on P-R and Q-T intervals, the remaining therapeutic indices are comparable and reflects the many similarities found in the earlier studies between both agents. Therefore, RSD 1000 is identical to lidocaine in terms of antiarrhythmic activity and ECG effects, except for the fact that RSD 1000 appears to be less hypotensive than lidocaine.

Figure 17: - Antiarrhythmic Dose-Response Curves for RSD 1000 and Lidocaine

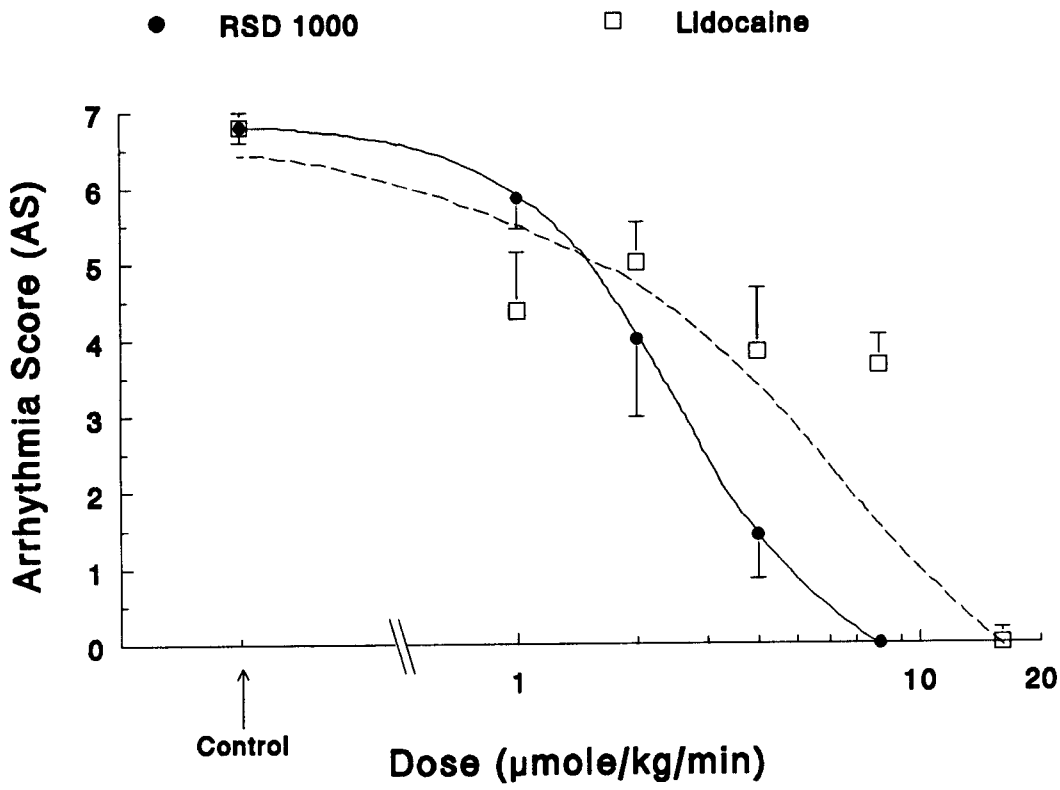


Figure 17: Antiarrhythmic efficacy of RSD 1000 and lidocaine in terms of their effects on arrhythmia score (AS). RSD 1000 and lidocaine were tested at 1.0, 2.0, 4.0 & 8.0 µmoles/kg/min with an additional dose at 16 µmoles/kg/min for lidocaine. Each value is expressed as the mean AS (±SEM) of the group with n=7. The estimated ED_{50} values for RSD 1000 and lidocaine were 2.5 and 4.0 µmoles/kg/min, respectively. An AS = 0 implied few or no arrhythmias, and RSD 1000 was two times more potent than lidocaine in this respect.

Table 3: Arrhythmia incidence and score.

GROUP	log_{10}PVC	VT	VF	MORTALITY	AS
CONTROL	1.9±0.1	19/21	20/21	20/21	6.8±0.3
RSD 1000					
1.0 µmoles/kg/min	1.5±0.2	5/7	7/7	6/7	5.8±0.4
2.0 µmoles/kg/min	1.6±0.1	4/7*	6/7	6/7	4.0±1.0
4.0 µmoles/kg/min	1.8±0.1	2/7*	1/7*	1/7*	1.4±0.3*
8.0 µmoles/kg/min	0.5±0.2	0/7*	0/7*	0/7*	0*
Lidocaine					
1.0 µmoles/kg/min	2.2±0.1	5/7	4/7*	4/7*	4.3±0.8
2.0 µmoles/kg/min	2.0±0.3	6/7	4/7*	5/7	5.0±0.5
4.0 µmoles/kg/min	2.1±0.2	5/7	3/7*	3/7*	3.8±0.8
8.0 µmoles/kg/min	1.0±0.3	1/7*	1/7*	2/7*	3.7±0.4
16 µmoles/kg/min	NE	0/7*	0/7*	1/7*	0*

Table 3: A summary illustrating the antiarrhythmic activity of RSD 1000 and lidocaine. The arrhythmia history of each rat was expressed as an arrhythmia score and is reported as the mean for the group (mean ± SEM) (7/group). The number of animals in the group having each type of arrhythmias was also recorded. The types of arrhythmias were expressed as PVC (premature ventricular contraction), VT (ventricular tachycardia) and VF (ventricular fibrillation). NE indicates no estimate was made since no PVCs were not recorded. Mortality was defined by an episode of irreversible VF, which degenerated to a fine VF, together with a BP of approximately 15 mmHg. The symbol * indicates statistical significance at $p < 0.05$ for difference from control.

Table 4 - Therapeutic Indices:

	AA50%(µmoles/kg/min)
RSD 1000	2.5
Lidocaine	4.0

	RSD 1000		Lidocaine	
	ED25% (µmoles/kg/min)	T.I. = AA50/ED25%	ED25% (µmoles/kg/min)	T.I. = AA50/ED25%
BP	9.0↓	0.27	4.0↓	1.0
HR	8.0↓	0.32	8.0↓	0.5
PR	20↑	0.13	NE	--
QRS	NE	--	NE	--
RSh	12↑	0.21	19↑	0.21
QT_1	30↑	0.08	NE	--
QT_2	15↑	0.16	NE	--
iT	4.5↑	0.55	8.0↑	0.5
VFt	4.0↑	0.63	5.0↑	0.8
ERP	3.0↑	0.83	6.0↑	0.66

Table 4: Therapeutic index was defined as the ratio of the 50% effective doses for antiarrhythmic protection and the ED25% value for blood pressure, heart rate, ECG and electrical stimulation end-points (i.e., TI = AA50%/ED25%).

3.4.2.1. - Haemodynamic Effects in the Absence of Coronary Occlusion: Time-Effect Data

To further investigate the effects of RSD 1000 on haemodynamic and ECG responses in rats subjected to coronary artery occlusion surgery, but in the absence of occlusion of the LAD, hence, a sham operation was performed. Five rats were prepared and infused with 8.0 μmoles/kg/min RSD 1000 for a total of the 20 minutes required for the occlusion study. The results of this study are illustrated in Figures 18-21 as time-effect curves showing that RSD 1000 had little or no effect on haemodynamic and ECG responses, independent of coronary occlusion, during the course of the experiment. Figures 20 and 21 show that the greatest changes in ECG were on Q-T and RSh intervals, respectively. Evidence of Q-T widening and RSh increase, in previous studies over the dose range of 8.0-32 μmoles/kg/min are consistent with the sham-occlusion data. These results adds to the evidence that channel blocking actions of RSD 1000 is a mixture of both sodium and potassium. Although changes in P-R and QRS intervals were minimal in this and previous experiments, the sensitivity of the RSh as an indirect measure of sodium blockade suggests that the blockade of sodium channels is less potent at equivalent doses for potassium channel blockade. Clearly, the results show that RSD 1000 possesses activity in both the sodium and potassium channels with a greater potency for blockade on potassium channels.

Figure 18: - RSD 1000: Sham Occlusion - Time-Effect of Mean Blood Pressure and Heart Rate

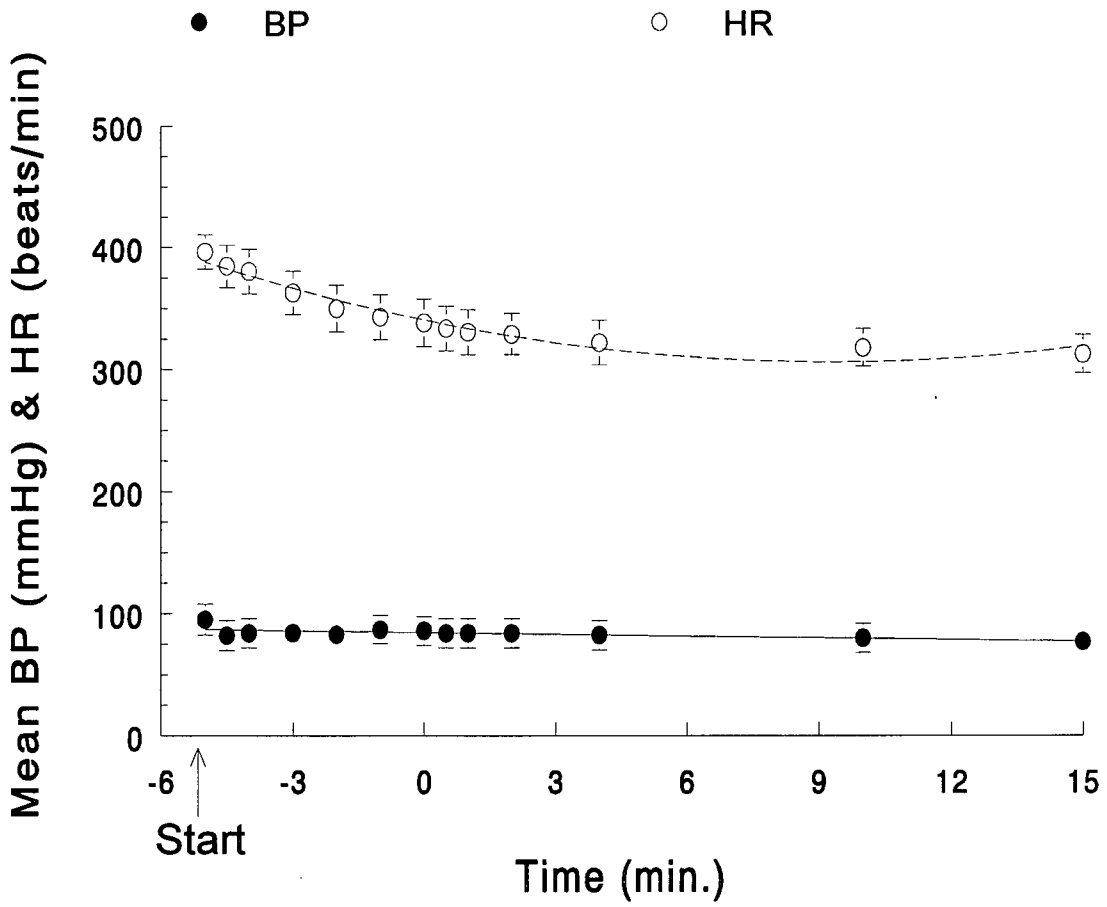


Figure 18: RSD 1000 and its effects on mean blood pressure and heart rate during sham occlusion. Following occlusion surgery, rats were infused with 8.0 µmoles/kg/min RSD 1000 for 20 minutes (-5 to + 15 minutes). There was little change from control suggesting that RSD 1000 has limited vasodepressant effects at this dose. Initially, RSD 1000 produced bradycardia which eventually reached a steady state between 3 to 6 minutes and was maintained for the remaining period of the experiment.

Figure 19: - RSD 1000: Sham Occlusion - Time-Effect on P-R and QRS Intervals

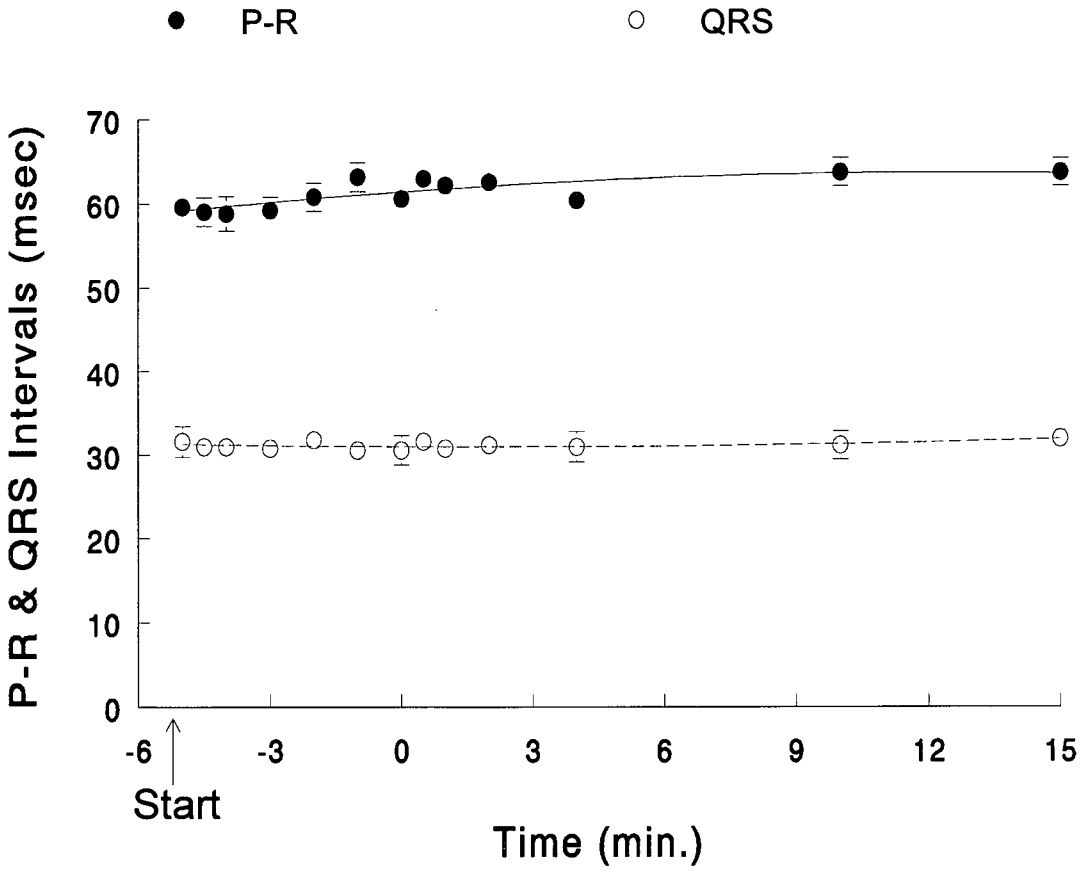


Figure 19: RSD 1000 and its effects on P-R and QRS intervals during sham occlusion. Following occlusion surgery, rats were infused with 8.0 µmoles/kg/min RSD 1000 for 20 minutes (-5 to + 15 minutes). There was a slight increase in P-R in the first minutes of infusion but no further P-R widening was observed for the remainder of the experiment. The absence of P-R widening indirectly suggests that at this dose RSD 1000 has limited effects on sodium channel blockade. No observable effects on the QRS were produced adding to the evidence that there are limited effects on conduction and indirect effects on sodium channels in the presence of RSD 1000 at 8.0 µmoles/kg/min.

Figure 20: - RSD 1000: Sham Occlusion - Time-Effect on Q-T1 and Q-T2 Intervals

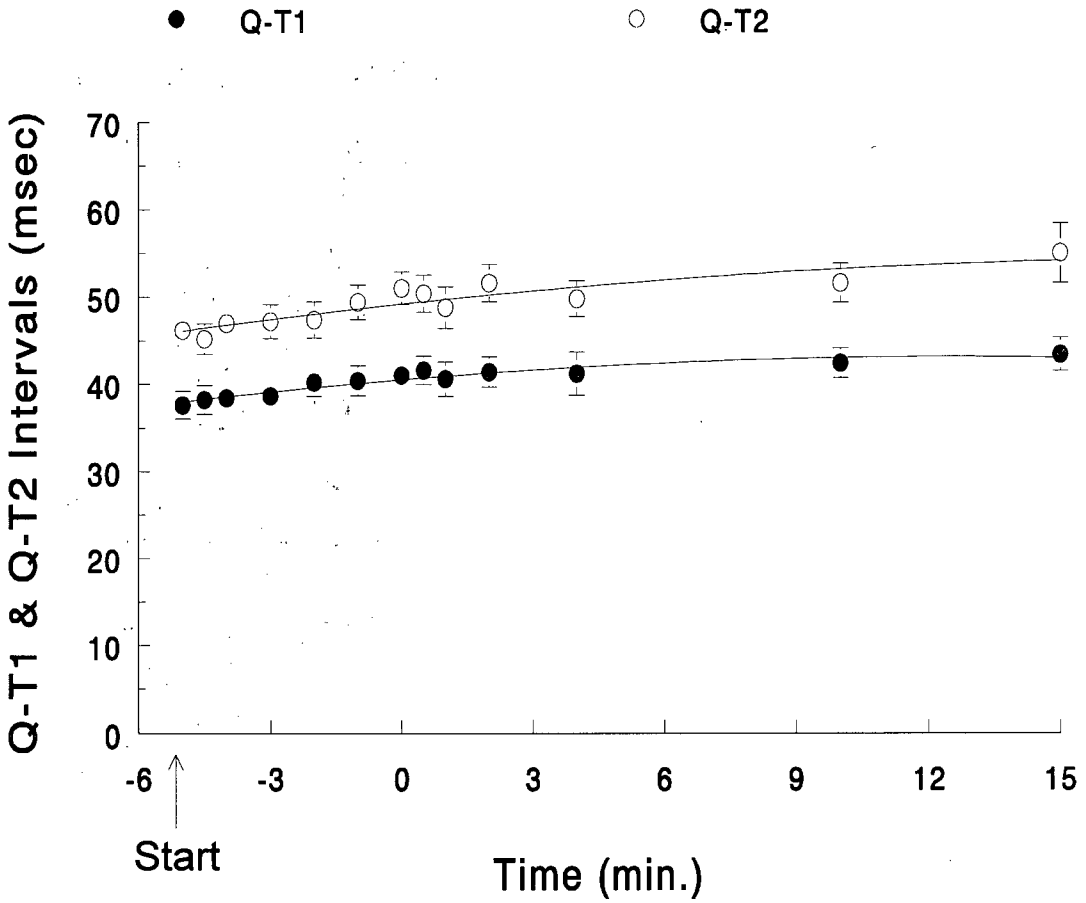


Figure 20: RSD 1000 and its effects on QT_1 and QT_2. Unlike P-R and QRS intervals, QT prolongation slightly increased with time.

Figure 21 - RSD 1000: Sham Occlusion - Time-Effect on RSh

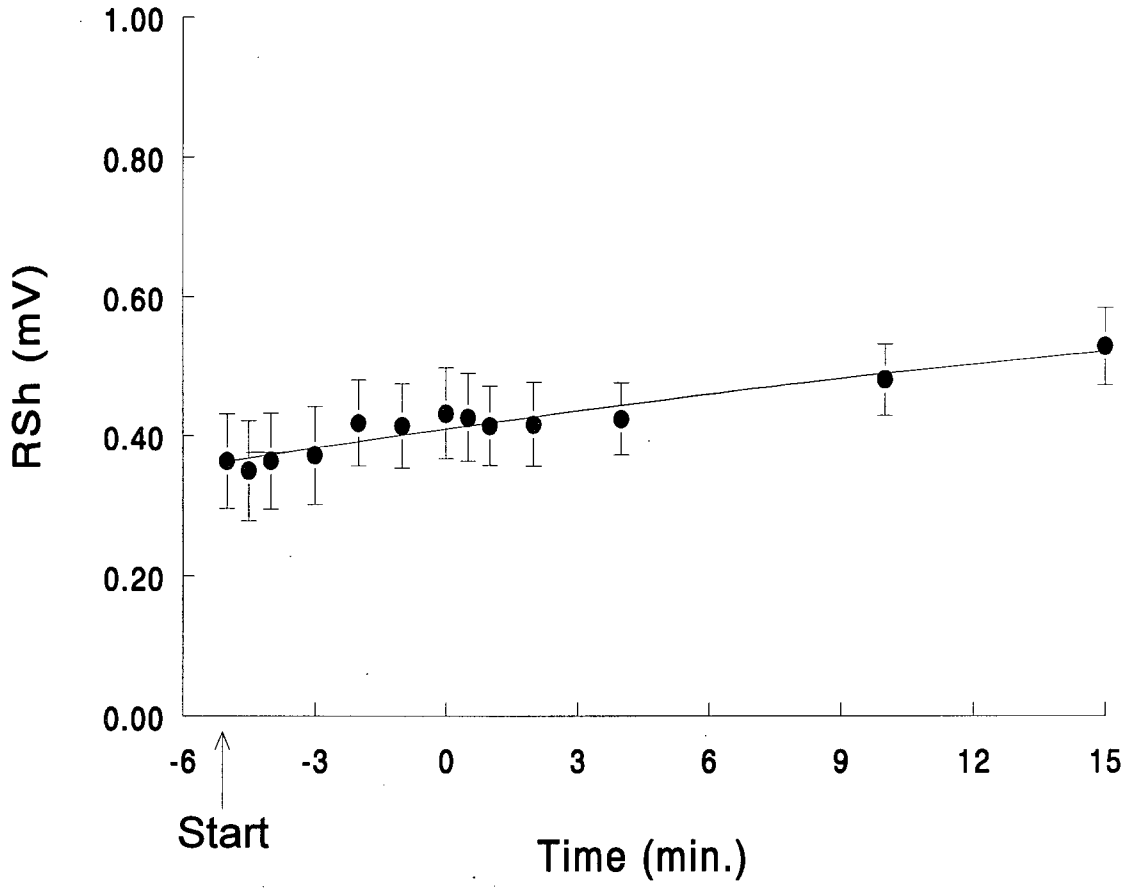


Figure 21: Effects on RSh interval during sham occlusion. Following occlusion surgery, rats were infused with 8.0 µmoles/kg/min RSD 1000 for 20 minutes (-5 to + 15 minutes). Changes in RSh increased with time.

4. - DISCUSSION

The purpose of this study was to characterize the pharmacology and evaluate the antiarrhythmic activity of RSD 1000. Conventional class I antiarrhythmics have been shown experimentally and clinically to provide antiarrhythmic protection at doses which also produce cardiovascular and CNS side-effects (Roden, 1994; Hondeghem, 1991; Feldman et al., 1989; Lie et al., 1974). Therefore, the need to investigate other antiarrhythmic agents was recognized and this study focussed on the investigation of RSD 1000, which was compared to lidocaine, in an attempt to establish the effectiveness of RSD 1000 as a new antiarrhythmic drug.

4.1.- Antiarrhythmic Effects and Cardiac Selectivity of RSD 1000

RSD 1000 has shown to have equal if not improved antiarrhythmic protection against arrhythmias when compared with lidocaine. The ED50 value for antiarrhythmic activity of RSD 1000 and lidocaine were 2.5 and 4.0 µmoles/kg/min, respectively, indicating that RSD 1000 was at least 1.5 times more potent in protecting against ischaemia-induced arrhythmias, a modest but not significant improvement over lidocaine. The results show that the lack of hypotensive actions at doses which provided protection against ischaemia-induced arrhythmias may contribute to the full antiarrhythmic expression of RSD 1000.

One of the problems with lidocaine and other conventional antiarrhythmics is their lack of selectivity for the cardiac tissue. At doses which showed minimal cardiac effects in the rat, lidocaine began to depress the cardiovascular system. At higher doses, 16 µmoles/kg/min of lidocaine provided complete protection from ischaemia-induced arrhythmias in the

rats but at the expense of causing two deaths and critically compromising blood pressure and heart rate. In contrast, complete antiarrhythmic protection with RSD 1000 was conferred at 8.0 μmoles/kg/min with no death compared to the 64 μmoles/kg/min dose which was required to produce respiratory failure and cardiac death. CNS toxicity has been shown in conscious animals with lidocaine (Barret et al., 1994; Feldman et al., 1989) but we did not observe this with RSD 1000 at the same high doses. Although tests in conscious animals have not been formally conducted, preliminary data on conscious rats have shown that at an infusion dose of 8.0 μmoles/kg/min over 20 minutes, the only observable symptoms were moderate lethargy and analgesia. Unlike lidocaine whose therapeutic indices for blood pressure and heart rate lowering are equal to or close to unity, the therapeutic index for RSD 1000 in lowering blood pressure showed that a separation between the antiarrhythmic effects of RSD 1000 and its effects on the circulatory system. These results, particularly with the sham occlusion, are evidence which support the observed results that RSD 1000 was less toxic than lidocaine.

4.2. - Lethality Studies of RSD 1000

The LD50 values determined for the mouse and rat suggest that the toxicological action of RSD 1000 is not species-dependent. In the mice, naloxone was able to abolish one component, presumably μ-receptor activation, which was responsible for the observed toxic side-effects with RSD 1000. The lack of protection from naloxone at higher doses of RSD 1000 suggest that there are other undetermined mechanisms involved in producing the toxic side-effects observed with RSD 1000. Given that the rats were artificially ventilated in the electrical stimulation study, sodium

channel blockade on the phrenic nerves to the diaphragm and/or directly on the diaphragm and intercostal muscles may be one possible mode of action for the respiratory depression in the non-ventilated rats. Effects on the respiratory center in the brain should not be rule out.

4.3. - Time-Effect of RSD 1000

Data from the sham occlusion in rats provided additional information in regards to the cumulative effects of RSD 1000 with time. Infusion at 8.0 µmoles/kg/min showed that there was an initial cumulative effect indicated by a decrease in heart rate with a slight lowering of blood pressure in the first 5 minutes of infusion. Ten minutes after the start of infusion, a steady state level for both measures was reached. Pharmacokinetic data on RSD 1000 using liver microsomes extracts from the rat estimated the $t_{1/2}$ to be approximately 8 minutes (courtesy of Walker and Wall). Ideally, steady state would, therefore, be attained after five half-lives or 40 minutes. However, a "pseudo" steady state would only be reached during the time period of our experiments. Since RSD 1000 is metabolized in the liver microsomes, one obvious explanation for the absence of observed drug accumulation in our experiments may have been that RSD 1000 underwent first-pass metabolism and the apparent steady state was a redistribution phase.

The absence of observed cumulative effects with increasing time at 8.0 µmoles/kg/min suggest that there may be a distinct separation between the antiarrhythmic and cardiovascular effects for RSD 1000. As a prophylactic antiarrhythmic agent, this is a positive and possible advantage over lidocaine and other class I agents, particularly the Ic's since CAST identified a new risk of antiarrhythmic therapy: increased mortality during

long-term treatment (Echt et al., 1991). Future considerations to warrant the use RSD 1000 as a prophylactic agent must be carefully examined with further pharmacokinetic and toxicological data.

4.4. - Frequency and/or Ischaemia Dependence of RSD 1000

Frequency Dependent Effects: The remaining attributes which were originally proposed in characterizing the "ideal" antiarrhythmic agent are frequency and ischaemia dependence. In this study, frequency-dependent sodium channel blockade with RSD 1000 was alluded to in our models. The VFt measure served as an indirect measure of frequency-dependent actions and the frequency of stimulation was only at 7.5 and 50 Hz. Proper frequency dependent studies would involved studying VFt at varying frequencies (0 -100Hz+). Traditionally, frequency dependent investigations have been conducted in single channel recordings of the cardiac muscle (Weidmann, 1955; Johnson and McKinnon, 1957; Heistracher, 1971; Courtney, 1975; Hondeghem and Katzung, 1977, Colatsky, 1982; Grant et al., 1982) with a greater degree of accuracy than the model used in this study. The purpose of studying the frequency dependent actions of RSD 1000 in our rat model was to efficiently gather as much pharmacological and electropharmacological information using one model. The fact that RSD 1000 showed increases in threshold for VFt, which were comparable to those shown by lidocaine, suggests that the sodium channel blocking actions of RSD 1000 may have a frequency-dependent component. Future investigations in single channel recordings should provide more information on the kinetics of RSD 1000.

Ischaemia Dependence: In Vitro studies in isolated rat hearts have shown that the effects of both RSD 1000 and lidocaine were potentiated by conditions of low pH and high [K+]. In fact, RSD 1000 was more potent than lidocaine at decreasing heart rate and increasing P-R and QRS intervals in the "ischaemic" conditions. Unfortunately, the isolated heart preparation was not identical to events or conditions resulting from coronary artery occlusion and myocardial ischaemia in a physiological setting. Ischaemia, by definition is a situation where blood supply to an organ or body part is decreased and anoxia is one of the consequences. Using oxygenated buffer solution in this model was critical, however, in maintaining the normal functioning of the hearts. Since high [H+] and [K+] are also present during normal myocardial ischaemia, this warranted their use in approximating ischaemic conditions.

An accurate index of ischaemia-dependency was also not possible in this model. The acidotic and hyperkalemic conditions of the buffer in the isolated hearts only accounted for a "global ischaemic" effect contrary to an ischaemic effect in a localized region of the heart. To solve this problem, the electrical activity in both the ischaemic zone and the normal myocardium of the same model, in the presence of drug, should be made to detect the relative changes and determine ischaemia-selectivity for the drug in question. Thus, any interpretation of the actions of RSD 1000 in the isolated rat hearts should be made for the entire heart.

In the case of lidocaine, electrophysiological (El-Sherif et al., 1977b; Kupersmith et al., 1979) and clinical evidence (Hine et al., 1989), have shown that lidocaine is selective for arrhythmias in the ischaemic myocardial tissue. This ischaemia-selective component for lidocaine may have been reflected in the in this study by the 100-fold lower concentrations

used in the "ischaemia"-simulated buffer in the isolated rat hearts. Given that RSD 1000 showed similar effects under the same conditions as lidocaine, it may be safe to assume that RSD 1000 may have properties characteristic of ischaemia-dependency.

It is easier to report the effects of RSD 1000 in conditions simulating ischaemia, but a more difficult task to explain the observed potency of RSD 1000 compared to lidocaine in the isolated rat hearts. The slow onset and offset times for RSD 1000 in both buffers indicate a slow binding and unbinding process. As a consequence, this cumulative effect of RSD 1000 may account for AVB which was produced at doses much lower than lidocaine in both buffers. Although direct evidence of intracellular and/or extracellular drug actions was not available with the isolated rat heart preparation, the slow on and off kinetics of RSD 1000 may suggest an intracellular drug effect. Hille (1977) first suggested that there are two access routes to the sodium channel receptor: a hydrophilic and a hydrophobic pathway. Therefore, one would expect the cationic form of a drug to preferentially access the sodium channel receptor through the hydrophilic pathway whereas the neutral species will preferentially interact through the lipophilic pathway. Lipophilic agents such as amiodarone and bupivacaine, rarely interact with open sodium channels and mainly interact with inactivated channels presumably through the lipophilic pathway (Hondeghem, 1989). In light of this, RSD 1000 may act in a similar fashion. The observed slow onset and offset times of RSD 1000 could be attributed to lipophilic properties of the drug. The effect of RSD 1000 acting by the lipophilic route to gain access to the sodium channel receptor and binding to the inactivated state would be amplified in depolarized tissue (e.g., high $[K+]_o$). These considerations are speculative in an attempt to account for

only the observed potency of RSD 1000 in the "ischaemia"-simulated buffer. Until such time the proper electropharmacology is made to positively resolve the issue of intracellular or extracellular binding, the fact remains that RSD 1000 is activated by low pH and high [K+] in isolated rat hearts.

4.5. - Possible Mechanisms for Antiarrhythmic Activity of RSD 1000

The results have shown that RSD 1000 and lidocaine are very similar in their actions. Both agents were found to be equipotent in their antiarrhythmic and sodium channel actions. However, in the same results, there were three distinct evidence of RSD 1000 which distinguishes it from lidocaine as, perhaps, being a better antiarrhythmic agent: 1) therapeutic index, 2) potency and 3) potassium channel actions. The therapeutic index and potency of RSD 1000 have been mentioned above and for the remainder of this section its effects on the potassium channel will be discussed to elucidate the possibility of a combination of class I and III actions for antiarrhythmic activity.

In the present study, both sodium and potassium channel activity was evident with RSD 1000. However, prolongation of the Q-T interval and elevation in threshold for ERP by RSD 1000 relative to those produced by lidocaine showed that RSD 1000 may possess a greater selectivity for potassium than sodium channels. Lengthening of action potential duration can also be achieved by increasing inward currents via depression of the maximum rate of depolarization (e.g. quinidine; Szekeres and Vaughan Williams, 1962). The results have shown that RSD 1000 was equally effective with lidocaine in producing sodium channel blockade. But the fact that the ED25% values for RSD 1000 on its effects on the Q-T interval and ERP were less than those for lidocaine suggests there may be a

preferential activity on potassium channels with RSD 1000. It is possible that this additional potassium channel activity may be responsible for, or contribute to, the antiarrhythmic activity of RSD 1000. Radioligand binding with RSD 1000 in the presence of [3H]batrachatoxin or TTX should resolve this issue of channel specificity.

Current information on class III agents indicate that potassium channel blockade demonstrates a greater efficacy than conventional class I agents in preventing ischaemia- or electrically-induced arrhythmias, while producing less cardiac and haemodynamic depression than other antiarrhythmic drug classes (Kou, et al., 1987; Lynch, et al., 1985; Anderson, 1990). Delayed repolarization with agents demonstrating class III effects may increase the transarcolemmal Ca^{2+} current, and as a consequence augment the release of Ca^{2+} from the sarcoplasmic reticulum, thus increasing the free intracellular Ca^{2+} concentration in the vicinity of the contractile elements (Reiter, 1988; Carlsson, et al., 1991). However, measurements of systolic ventricular pressure and (+/-)dP/dt in isolated rat hearts in the normal buffer were unchanged suggesting that there were no apparent effects on myocardial contractility. Therefore, it is unlikely that RSD 1000 is a "pure" channel blocker.

There are at least five known types of potassium channels responsible for the inward currents during the plateau phase of the cardiac action potential in ventricular cell membranes (see review by Singh and Courtney, 1990). In many cardiac preparations (human, canine, rabbit and rat) transient outward current (I_{to}) is often larger than and more rapidly activated than the delayed rectifier current (I_K). Experimentally, others have found that the degree of "Q-Tc" (i.e. Q-T) widening in the rat is consistent with the blockade of I_{to}.(Adaikan et al., 1992; Beatch et al., 1991; Dukes et

al., 1990). Presumably, the observed action potential widening by RSD 1000 in the rat may be also by blockade of the transient outward current.

Perhaps the mechanisms responsible for the antiarrhythmic actions of RSD 1000 in the rat can be best explained by its actions on both the sodium and potassium channels. A correct combination of class I and III effects may exist which modifies the cardiac action potential, presumably by increasing refractoriness just enough to provide antiarrhythmic protection without leading to a profound lengthening of the Q-T interval and risk the development of torsade de pointes. In association with this "balance" of sodium and potassium channel blockade, full expression of this effect may be possible because of the minimal side-effects observed with RSD 1000.

RSD 1000 was shown to possess a greater antiarrhythmic efficacy with fewer side-effects than lidocaine in the experimental arrhythmia models considered to be most representative of the clinical situation. Of the three criteria initially proposed in characterizing the "ideal" antiarrhythmic agent, minimal hypotensive actions in the rats was the single attribute of RSD 1000 which had been clearly demonstrated in this study. Although work on RSD 1000 remains at an early stage, it is with hope that the pharmacology and antiarrhythmic actions of RSD 1000 presented in this study has helped to extend the knowledge for the mechanisms required for antiarrhythmic activity. Clearly, several issues regarding the validity of ischaemia-dependence, frequency-dependence of channel blockade, internal or external binding, and relative selectivity for sodium, potassium or any other channel remains to be formally tested. Until then, RSD 1000 may be a good candidate in the pursuit of developing the next generation of antiarrhythmic agents.

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