# THE ANTIARRHYTHMIC ACTIVITY OF κ-OPIOID AGONIST RSD 939 IS UNRELATED TO κ-AGONISM

by

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# ABSTRACT

Cumulating evidence have indicated that various opioid agonists and antagonists, especially kappa (κ) agonists, can exhibit a variety of cardiovascular and antiarrhythmic actions. Two important questions arise. First, are these cardiovascular and antiarrhythmic actions mediated by the opioid receptors? Second, what is the underlying mechanism of actions if these effects are not mediated by opioid receptors? Previous studies have shown that some of the cardiac and cardiovascular actions of κ agonists are a result of direct actions on cardiac ionic channels and are independent of κ agonism. RSD 939 is structurally related to κ agonists and in binding studies appears to be a potent and selective κ agonist. The present study is an attempt to study the involvement of opioid-receptors in the cardiovascular and antiarrhythmic activity of κ agonists and to determine the underlying mechanism of such activities using RSD 939.

The cardiovascular and antiarrhythmic actions of RSD 939 were investigated in a series of studies. Firstly, the initial profile of acute cardiovascular and toxic actions of RSD 939 were investigated. RSD 939 was given as cumulative i.v. bolus doses to anaesthetised rats whose blood pressure, heart rate and ECG were measured. It was found that at 8 μmole/kg, RSD 939 decreased both blood pressure and heart rate by 25%.

At 5 μmole/kg, it also prolonged the P-R interval and increased RSh of the ECG. However, at a higher dose of 16 μmole/kg, it also produced changes in the Q-T interval.

Since opioid receptors are found in the vagus nerve, in several sympathetic ganglia as well as the heart, opioid peptides can influence the cardiovascular system both centrally and peripherally. Therefore, in order to determine the direct cardiac effects of RSD 939, it is necessary to examine the drug effects in the absence of neuronal and humoral influence on hearts. In isolated rat hearts, over the concentration range 0.1 to 3.0 μM, RSD 939 concentration-dependently prolonged the P-R and QRS intervals of the ECG.

The antiarrhythmic activity of RSD 939 was determined in terms of its ability to prevent both electrically-induced and ischaemia-induced arrhythmias. For electrical stimulation, two silver electrodes were implanted into the rat's left ventricle and the ability of the drug to raise the ventricular fibrillation threshold (VFt) was determined. In the ischaemia model, the left anterior decending coronary artery (LAD) of the rat was ligated. Occlusion of the LAD results in the production of acute myocardial ischaemia and ventricular arrhythmias in a predictable and reproducible manner that mimics conditions found clinically in myocardial ischaemia and infarction. At a dose of 1.5 μmole/kg/min,

RSD 939 significantly increased the threshold voltage needed to induce ventricular fibrillo-flutter. At the same dose, the incidence of ventricular arrhythmias produced by occlusion was also significantly reduced (reduction of arrhythmia score from 7.0 in control groups to 3.2 in RSD 939 group).

Naloxone at a dose which had no cardiovascular or ECG actions, but blocked opioid receptors, was used to differentiate between opioid and nonopioid receptor-mediated actions of RSD 939. In a random and double-blind manner, either control vehicle or 8 μmole/kg(1 μM *in vitro*) naloxone was given to rats or infused into isolated rat hearts. RSD 939 or control vehicle was administered 5 min later. The cardiovascular and antiarrhythmic actions of RSD 939 in naloxone pre-treated preparations and untreated rats were also compared. It was found that the ECG and antiarrhythmic effects of RSD 939 were not antagonized by naloxone. The antiarrhythmic action of naloxone alone was also evaluated and compared in the two groups. Naloxone alone had no effect on any of the ECG variables except for P-R interval which was prolonged slightly. However, naloxone alone reduced the incidence and severity of ischaemia-induced arrhythmia. This action of naloxone was not synergistic with RSD 939 since no difference in antiarrhythmic potency was found between the naloxone pre-treated and nonnaloxone pre-treated

groups. From the above observations, it can be concluded that the cardiac and cardiovascular actions of RSD 939 were not mediated through opioid receptors.

Effects of RSD 939 on the ECG parameters such as P-R, QRS, RSh and Q-T intervals and electrical stimulation parameters such as threshold current (iT), threshold duration (tT), and effective refractory period (ERP) were used to establish the underlying mechanism of actions of its antiarrhythmic activities. RSD 939 dose-dependently prolonged P-R, QRS, RSh, iT, and tT without significant effects on Q-T and ERP until higher doses. Since most sodium channels blockers will increase P-R, QRS, RSh, iT, and tT and most potassium channel blockers will prolong Q-T and ERP, we concluded that RSD 939 mediated its cardiac and cardiovascular effects by direct cardiac sodium and potassium channel blockade.

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| | |
|---|---|
| action potential | AP |
| action potential duration | APD |
| arrhythmia score | AS |
| and | & |
| beta | β |
| blood pressure | BP |
| calcium | $Ca^{2+}$ |
| centimetre | cm |
| degree celcius | $^{0}C$ |
| electrocardiogram | ECG |
| dose of drug producing 25%-or half-maximal response | $ED_{25,50}$ |
| effective refractory period | ERP |
| gram | g |
| hertz | Hz |
| hour(s) | hr |
| intraperitoneally | i.p. |
| intravenous | i.v. |
| kappa | κ |
| kilogram | kg |

| | |
|---|---|
| left anterior descending | LAD |
| less than | < |
| maximum following frequency | MFF |
| micromolar | μM |
| milligram per kilogram | mg/kg |
| milligram per kilogram per min | mg/kg/min |
| millimetre | mm |
| millimetres of mercury | mmHg |
| millisecond(s) | ms |
| minute(s) | min |
| molecular weight | MW |
| mu | μ |
| nanomolar | nM |
| non-spontaneously reverting VT | NSVT |
| non-spontaneously reverting VF | NSVF |
| occluded zone | OZ |
| pacemaker current | $i_f$ |
| percentage | % |
| potassium | $K^+$ |
| premature ventricular contraction | PVC |
| second(s) | s |

| | |
|---|---|
| **sodium** | $Na^+$ |
| **spontaneously reverting VF** | SVF |
| **spontaneously reverting VT** | SVT |
| **standard error of mean** | SEM |
| **threshold current** | iT |
| **threshold duration (threshold pulse width)** | tT |
| **ventricular fibrillation** | VF |
| **ventricular fibrillation threshold** | VFt |
| **ventricular tachycardia** | VT |

# Dedication

**This thesis is dedicated to my beloved parents**

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# 1. Introduction

## 1.1 Myocardial ischaemia and infarction

### 1.1.1 Overview

Myocardial ischaemia occurs when the oxygen supply to a portion of myocardium is insufficient. The underlying cause is coronary arteriosclerosis and/or coronary artery spasm which impairs coronary blood flow and results in insufficient supply of blood to the myocardium. The most common form of myocardial ischaemia is angina pectoris. Angina of effort is always produced by an increased demand on the heart (e.g., by exercise) and is usually due to atherosclerotic narrowing of coronary artery. Variant angina can occur at rest and is associated with coronary artery spasm as a cause of reduced coronary flow. Angina is associated with pain characteristically distributed in the chest, arm and neck. Relief of such pain is obtained by reducing sympathetic drive to the heart with beta-adrenoceptor antagonists, or by dilating coronary vessels with organic nitrites (Poole-Wilson, 1983).

Myocardial infarction is defined as the necrotic and fibrous changes of the myocardial muscle resulting from maintained myocardial

ischaemia. Myocardial infarction is the commonest single cause of death in many parts of the world; death usually results either from mechanical failure of the ventricle or most commonly from ventricular fibrillation (Oliver, 1982). The irreversible cellular damage (infarction) which results from vascular occlusion appears to be triggered by an increase in intracellular calcium concentration resulting from the impairment of two ATP-dependent processes, namely uptake of calcium by the sarcoplasmic reticulum and sodium extrusion from the cell which indirectly control intracellular calcium concentration because of sodium-calcium exchange (Van der Vusse & Reneman, 1985).

### 1.1.2 Electrophysiological changes caused by myocardial ischaemia

Not unexpectedly myocardial ischaemia resulting from occlusion of a coronary artery has a profound effect on the electrophysiological properties of cardiac cells. Changes in resting membrane potential and inward and outward currents during the action potential lead to alterations in conduction, refractoriness, and automaticity, all of which can contribute to the occurrence of ventricular arrhythmias (Janse & Wit, 1989).

Within minutes of experimental coronary artery occlusion, cells within the ischaemic region begin to depolarize, (i.e. resting membrane potential is reduced with a sigmoidal time course (Downar et al., 1977; Kleber, 1983). A major cause of the fall in the resting membrane potential of ischaemic cells is the altered potassium gradient across the cell membrane. This is the result of a net potassium efflux and extracellular potassium accumulation due to lack of blood flow (Hirche et al., 1980). The reasons for ischaemic cells losing potassium are not completely established. In addition to the altered potassium gradient, other mechanisms for depolarization may contribute, such as an increase in intracellular calcium (Isenberg, 1983) and the effects of lipophosphoglycerides (LPG) (Clarkson & Teneick, 1983), produced during early ischaemia, on the cell membrane.

The extracellular fluid composition in ischaemic cardiac muscle has a special composition that results from the lack of blood flow. Not only is extracellular potassium elevated but there is hypoxia, low pH, no substrates, high $P_{CO_2}$ and an accumulation of substances, such as lipophosphoglycerides (LPGs) and catecholamines (Janse & Wit, 1989). Each has an influence on membrane conductances while the various combinations may exert effects that are not predictable from the action of each substance alone. Together they cause changes in the action potential

including a reduction in action potential amplitude, upstroke velocity, and duration. The depressed upstroke, decreased amplitude, and decreased velocity of depolarization of action potentials in partially depolarized membrane potentials are primarily caused by the loss of a fast $Na^+$ current (depressed fast response) and its replacement by a slow inward current (slow response) when the level of resting potential is reduced enough to inactivate the $Na^+$ current (Cardinal et al., 1981).

Due to the reduction in action potential duration, the effective refractory period (ERP) is expected to be abbreviated. However, this is not necessarily so since ischaemic fibers may remain inexcitable even after complete repolarization. In partially depolarized fibers, recovery from inactivation of both fast and slow inward current has been shown to be markedly delayed until many milliseconds after completion of repolarization (Cranefield et al., 1972; Gettes & Reuter, 1974). This "postrepolarization refractoriness" is probably related to depolarization of the resting or maximum diastolic potential of the myocardial cell (Lazzara et al., 1978). In the central ischaemic zone, the phenomenon of postrepolarization refractoriness causes the refractory period to lengthen, whereas in the border zone, refractory periods may become shorter than normal. Inhomogeneity in recovery of excitability is largely caused by inhomogeneities in extracellular $K^+$ (Kodama et al., 1984; Coronel et al.,

1988), which in turn is caused partly by diffusion of $K^+$ from the ischaemic zone towards the normal zone and partly by the difference in coronary collateral perfusion between species. In species with multiple coronary collaterals the ischaemic zone will not be homogeneous and therefore extracelluar $K^+$ may be variable. On the other hand in species without collaterals (e.g. rats), changes in extracellular $K^+$ are uniform.

### 1.1.3 Arrhythmias in the early phase of myocardial ischaemia

In patients with a diagnosis of acute myocardial ischaemia and infarction, approximately one half will experience one or more complications. The type of complication encountered is often related to the patients's age and gender, and to the size and location of the infarction. Conduction disturbances and cardiac arrhythmias are the most common and fatal form of complications in the course of myocardial ischaemia and infarction. More than 85% of myocardial infarction survivors have some evidence of ventricular ectopy. In addition, a significant number of patients have atrial arrrhythmias including either atrial flutter or fibrillation (Lovegrove & Thompson, 1978).

The majority of deaths of cardiac origin are sudden and due to severe tachyarrhythmias. The most frequent of such arrhythmias is

ventricular fibrillation although ventricular tachycardia and asystole can also be a cause of sudden cardiac death (Gordon and Kannel, 1971). The mechanism of arrhythmias following acute infarction, whether enhanced automaticity, or re-entry or both, appears to vary depending on the time after complete occlusion of a coronary artery. Early after the onset of myocardial ischaemia and infarction, ventricular fibrillation may result from re-entrant mechanisms. During the later stages of infarction enhanced automaticity may precipitate ventricular fibrillation (Pogwizd & Corr, 1987).

#### 1.1.3.1 Reentry

Arrhythmias may be generated by recirculating excitation incited by an initiating depolarization. Such arrhythmias, classified as reentrant arrhythmias, are self-sustained but are not self-initiated (Wit and Rosen, 1983). Reentry results from a conduction disturbance whereby the cardiac propagating impulse may not die out after complete complete activation of atria or ventricles but persists to re-excite the tissue after the end of its refractory period. The criteria that must be fulfilled to demonstrate that an arrhythmia is caused by reentry were formulated by Mines as early as 1913 and 1914 and may be summarized as follows: 1)

an area of transient or unidirectional block of conduction must be present; 2) there must be an anatomical or functional "barrier" to conduction such that activity must propagate along alternate pathways around the area of block, activate the tissue beyond the block with delay, and retrogradely traverse the zone of block to reexcite the tissue proximal to the block; 3) interruption of the reentrant pathway must abolish the arrhythmia. Furthermore, the wavelength of the cardiac impulse in the reentrant circuit, where the wavelength is the product of conduction velocity and refractory period, must be shorter than the pathlength of the circuit so that the tissue into which the impulse is reentering has time to recover its excitability. There are difficulties in attributing cardiac arrhythmias to reentry because, with a normal refractory period which is very long, normal conduction of cardiac impulse which is rapid would necessitate quite a long path since the reentrant pathways are reasonably short (Hoffman and Dangman, 1987). As a result of this crucial relationship between pathlength, conduction velocity, and refractory period, it is necessary that conduction velocity must be greatly slowed and refractory period markedly shortened, or both.

Reentry can occur randomly across the heart, as in fibrillation, or be ordered and follow a fixed pathway. It has been suggested in past that fibrillation might represent chaotic reentrant excitation or multiple

continually migrating activation wavefronts (Mines, 1913; Moe et al., 1964). This type of reentry has been termed random reentry (as opposed to stable reentry based on a fixed anatomical path) where the path of excitation continously changes such that individual groups of fibers may be repeatedly excited (Hoffman and Rosen, 1981). Micro-reentry has been used to describe the small circuits such as might occur in the AV node or distal Purkinje fibers. In this case the reentry circuit may be extremely short, perhaps only a few mm (Sasyniuk and Mendez, 1971). The refractory period in the anterograde pathway is also very short allowing excitability to be quickly restored. Microreentry has been determined as a mechanism for ventricular tachycardia whereby small epicardial conduction loops exit into non-refractory subendocardium initiating succeeding beats (Kramer et al., 1985). Alternatively, if the circuit is long enough, there will be sufficient time for the anterograde pathway to recover excitability. This type of longer pathway circuit is referred to as macro-reentry. Reflection is a form of reentry that is produced through reflection in parallel unbranching fibers with depressed segments (Antzelevitch et al., 1980; Jalife & Moe, 1981). If a delayed action potential is caused by the electrotonic depolarization of a blocked impulse and is distal to an inexcitable segment, then reflection occurs (Hoffman and Dangman, 1987). The delayed action potential then

reexcites the tissue proximal to the site of block. These reflected impulses can be modulated by changes in rate and rhythm.

Since acute ischaemia is associated with areas of slow conduction and abbreviated action potential duration and/or prolonged refractory periods, reentry has been implicated for many years as an important cause of ischaemia-induced arrhythmias. Clinically, reentry is the usual cause of paroxysmal supraventricular tachycardia (Wellens et al., 1974). Reentry in the His-Purkinje system is thought to be one cause of coupled ventricular premature depolarizations and ventricular tachycardia, and ventricular tachycardia may degenerate to ventricular fibrillation (Wit, 1972).

#### 1.1.3.2 Abnormal Automaticity

Normally automaticity is found in sinoatrial node (SAN), subsidary atrial fibers, fibers in and around the coronary sinus ostium, cardiac fibers in the tricuspid and mitral valve leaflets, the NH region of the atrioventricular junction, and the His bundle and Purkinje fiber ramifications in the ventricle although, in the latter, automaticity is not normally seen owing to the faster rate of the node (Hoffman and Dangman, 1987; Wit et al., 1974). The faster intrinsic rate of impulse

initiation in the SAN overdrives the spontaneous depolarizations in other regions of the heart, and thus it functions as the primary pacemaker (Rosen, 1988). However, when the rate of firing of the sinus node is slower than the intrinsic rate of the other sites in the specialized conducting system, the region with the higher rate might take over the pacemaker function as a result of overdrive suppression (Rosen, 1988). On the other hand, abnormal automaticity occuring in depolarized ventricular and/or Purkinje fibres is difficult to be suppress by overdrive pacing (Dangman & Hoffman, 1983). Abnormal automaticity simply means that heart tissue other than the sinus node has taken over the pacemaker role of the sinus node (Hoffman and Dangman, 1982; Sasyniuk, 1984), and it has been implicated as the causative agent responsibles for a significant fraction of the arrrhythmias seen 24 hour after myocardial infarction in human subjects (Wellens et al., 1974).

Arrhythmias caused by augmented automaticity in normal specialized conducting tissues are spontaneously generated and therefore do not rely on a prior impulse (Hoffman and Rosen, 1981). One of the currents responsible for the spontaneous diastolic depolarization in SAN is known as the pacemaker current ($i_f$). In the sinus node the pacemaker current has recently been shown to result from the interaction of two currents, the decay of a delayed rectifier $K^+$ current called $i_K$, and the

activation of an inward current carried by $Ca^{2+}$, called $i_{Ca}$ (Shibata and Giles, 1985). Abnormal automaticity may occur as a result of an increasing rate of activation in latent pacemaker tissue, or a shifting in the voltage dependence for activation of $i_f$ toward more positive (depolarized) values thus leading to a reduction in the threshold for generation of the (abnormal) propagated action potential (Hoffman and Dangman, 1987).

#### 1.1.3.3 Triggered Automaticity

Triggered automaticity, by definition is the generation of one or more impulses as a consequence of an exogenous source, and the exogenous source theoretically includes the normal wave of depolarization, particularly the previous impulse (Hoffman and Rosen, 1981). Triggered activity is dependent upon oscillations of membrane potential following an action potential upstroke, i.e. early or delayed afterdepolarizations (Janse and Wit, 1989; Rosen, 1988). Thus it is not self-initiated, but triggered by a prior impulse. Early afterdepolarizations (EADs) are secondary depolarizations that occur before repolarization is complete, i.e. during Phase III repolarization. Early afterdepolarizations are produced when repolarization is interrupted by secondary

depolarizations. Such responses can excite neighboring fibres and be propagated (Rosen, 1988; Wit and Rosen, 1983). Trigered arrhythmias from early afterdepolarizations are more likely to occur during bradycardia, when the action potential duration is prolonged (Hoffman and Dangman, 1987; Brachman et al., 1983). Therefore, early afterdepolarizations have been most closely identified as the cause of torsades de pointes occuring in the setting of the long QT syndrome, and are the proarrhythmic mechanism of some antiarrhythmic drugs (Brachman et al., 1983). Delayed afterdepolarizations (DADs) are secondary depolarization occuring early in diastole after full repolarization has been achieved. After terminal repolarization of an action potential is achieved, membrane potential again transiently depolarizes. If the transient or oscillatory depolarization reaches threshold, a propagating response can occur (Rosen, 1988; Wit and Rosen,1983). Unlike early afterdepolarizations, arrhythmias induced by delayed afterdepolarizations occur more readily when the preceding stimulation rate is rapid and will tend to increase in rate as the preceding drive rate is increased (Monk and Rosen, 1984). Once the delayed afterdepolarizations are large enough to reach threshold, the triggered action potential can initiate a single premature depolarization. That premature depolarization will be followed by a larger than usual delayed

afterdepolarization, and it in turn probably will attain threshold and result in a second impulse (Hoffman and Dangman, 1987). In this way, delayed afterdepolarizations can cause either coupled extrasystoles or runs of tachyarrhythmias.

It is important to recognize that although delayed afterdepolarizations and early afterdepolarizations are routinely classified together as causes of triggered arrhythmias their underlying cellular mechanisms appear to be different. Early afterdepolarizations have been produced in vitro by catecholamines (Hoffman & Cranefield, 1960), acidosis (Coraboeuf et al., 1980), low [$K^+$] (Carmeliet, 1961), low [$Ca^{2+}$] (Sano and Sawanobori, 1972), hypoxia (Trautwein et al., 1954) and numerous drugs (Schmidt, 1960; Dangman & Hoffman, 1981; Strauss et al., 1970; Gough et al., 1988; Levine et al., 1985; El-Sherif et al., 1988; Carlsson et al., 1990). The triggering mechanism of early afterdepolarizations, at least for those arising near the plateau, are thought to be the result of the interaction of surface membrane current systems leading to a time- and voltage-dependent recovery of L-type $Ca^{2+}$ current which can carry depolarizing charge (January et al., 1991). In contrast, the critical mechanism proposed to underlie delayed afterdepolarizations is overloading of cell myoplasm and the sarcoplasmic reticulum with $Ca^{2+}$, such as in reperfusion (Ferrier et al., 1985),

inhibition of $Na^+/K^+$ ATPase by digitalis glycosides, hypokalemia and hypomagnesemia, etc. (Marriott & Conover, 1989). The transient rise in myoplasmic $Ca^{2+}$ also induces a $Ca^{2+}$ sensitive inward current. This $Ca^{2+}$ induced transient inward current ($i_{TI}$) is thought to be the actual mediator of delayed afterdepolarizations (Lederer & Tsien, 1976; Kass et al., 1978; Matsuda et al., 1982).

Abnormal automaticity might not responsible for arrhythmogenesis in acute ischaemia because abnormal automaticity is suppressed by elevated extracellular potassium concentration which occurred subsequent to coronary occlusion (Hoffman & Rosen, 1981; Hirsche et al., 1980). However, triggered activity may play a role in ischaemia induced arrhythmia due to the possible presence of hypoxia (Trautwein et al., 1954), acidosis (Coraboeuf et al., 1953), elevated catecholamines (Brooks et al., 1955), high extracellular potassium concentration, possible myocardial stretch (Pirzada et al., 1976), increased calcium concentration (Clusin et al., 1983), which may contribute to the generation of oscillatory after depolarizations.

### 1.1.4 Pathophysiological progenitors of arrhythmias

Many putative pathophysiological progenitors of arrhythmogenesis

have been proposed. In ischaemia these may include accumulation of catecholamines (Sheridan et al., 1980), lysophosphatides (Corr et al., 1984), thromboxane (Coker et al., 1981), and potassium ( Hill and Gettes, 1980; Hirche et al., 1980). It remains unclear which of these substances, if any, plays the most important role in arrhythmogenesis. Other, as yet unidentified factors almost certainly play a role. Indeed, any substance whose homeostasis is disturbed by ischaemia is a potential arrhythmogen until proven otherwise.

#### 1.1.4.1 Potassium

Potassium was one of the first substances to be suggested to play a role as an endogenous arrhythmogen in myocardial ischaemia. The role of potassium in arrhythmogenesis is supported by evidence that (i) extracellular potassium concentration rises with a time-course similar to the onset of ischaemia-induced arrhythmias (Hill and Gettes, 1980; Hirche et al., 1980) and (ii) regional elevation of extracellular potassium concentration in the absence of ischaemia produces electrophysiological changes liable to precipitate arrhythmias. The latter include slowing of conduction velocity (Schmitt and Erlanger, 1928), a decrease in resting membrane potential (Morena et al., 1980) and a shortening of action

potential duration (Morena et al., 1980), effects similar to those produced by ischaemia itself (Janse et al., 1980; Inoue et al., 1984; Janse, 1986). Furthermore, regional elevation of extracellular potassium in the absence of ischaemia can cause re-entry (Schmidt and Erlanger, 1928), reduce the threshold current necessary to elicit VF (Logic, 1973) and directly precipitate ventricular arrhythmias (Harris et al., 1958; Ettinger et al., 1973; Morena et al., 1980; Pelleg et al., 1989). Thus a role of regional hyperkalemia in arrhythmogenesis seems to be indicated.

#### 1.1.4.2 Calcium

It has been shown that intracellular free calcium $[Ca^{2+}]_i$ is elevated during myocardial ischaemia in isolated perfused rat hearts (Steenbergen et al., 1987b) and in isolated multicellular neonatal rat ventricular myocytes with an arrhythmia profile such as fibrillatory beating activity (Thandroyen et al., 1991). The increase in $[Ca^{2+}]_i$ is a consequence of the release of calcium from intracellular stores and influx of extracellular calcium due to the altered cell permeability to calcium or incomplete sodium-calcium exchange resulted from ischaemia-induced cell injury. The elevation of $[Ca^{2+}]_i$ leads to a number of unfavourable consequences which predispose to development of cardiac arrhythmias. Increase of

$[Ca^{2+}]_i$ can provoke oscillatory depolarization of the cardiac membrane, triggering sustained action potential generation, and extrasystoles (Billman, 1990). $Ca^{2+}$ overload is the proposed mechanism underlying the generation of delayed afterdepolarizations which is possibly the underlying principal cause of triggered arrhythmias. The evidence is as follows. Firstly, administration of BAPTA, a $Ca^{2+}$ chelator which increases cytoplasmic $Ca^{2+}$ buffering capacity, or administration of ryanodine which inhibits calcium release from SR (Sutko et al., 1979), suppresses the generation of delayed afterdepolarizations (Sutko and Kenyon, 1983). Secondly, oscillations in cardiac membrane potential can be abolished by caffeine (Shattock et al., 1991), a drug which enhances the release of calcium from the SR by impairing calcium reuptake by the SR (Lakatta et al., 1985). In addition to the activation of oscillatory inward current flow that precipitates delayed afterdepolarizations, a number of mechanisms may also result in an elevation of $[Ca^{2+}]_i$ leading to arrhythmias. For instance, elevation of $[Ca^{2+}]_i$ impairs intercellular coupling between cells (DeMello, 1982; Pressler et al., 1982) and hence slows conduction and increases the likelihood of re-entry. Furthermore, an increase in $[Ca^{2+}]_i$ can have deleterious metabolic effects which predispose to arrhythmogenesis. For example, high-energy phosphate (ATP) is spent in the sequestration of calcium into the cellular stores such

as SR and mitochondria. Moreover, transient or early elevation of intracellular free calcium will activate a number of calcium-dependent myocardial proteases within the cell so damaging the cytoskeleton and cell plasma membrane (Croall and DeMartino, 1983; Steenbergen et al., 1987a). The development of defects in sarcolemma membrane integrity and early afterdepolarizations leads to an elevation of $[Ca^{2+}]_i$ as a consequence of an influx of calcium from extracellular fluid and hence further exaggerates the genesis of myocardial arrhythmias.

### 1.1.5 Mechanisms of action of antiarrhythmic drugs

Normalization of cardiac rhythm can be achieved if abnormal heterogeneity of excitation, conduction or repolarization caused by ischaemia and infarction is prevented. This can be achieved with drugs that block cardiac ionic channels. A classification of these, and related, drugs was introduced by Vaughan Williams (1970). The definition of drug classes has a strong historical background. Sodium channel blockers were the first drugs to be shown to have antiarrhythmic activity. They were called Class I drugs. Later on, β-adrenoceptor blocking drugs (Class II), potassium channel blockers (Class III) and, finally, calcium channel blockers were developed (Class IV).

Based on the arrhythmogenic mechanisms discussed earlier (abnormal automaticity, triggered activity, and reentry), an agent effective against re-entrant arrhythmias should possess properties which will break a re-entrant circuit. Theoretically, this can be achieved by (1) converting areas of unidirectional block to bidirectional block, (2) prolonging refractoriness in normal myocardium such that the fibers at the sites of origin of the initiating impulses have not recovered their excitability at the time of reentry, and (3) reducing the strength of slowly propagating impulses such that the wavefront dies out before completing the circuit (Winslow, 1984). The antiarrhythmic action of drugs on automatic rhythms could involve an inhibition of $i_f$, the time-dependent, pacemaker inward sodium current, a shifting of the maximum diastolic potential to more negative values, or an increase in APD. These actions together lower the automatic focus firing rate (Davy et al., 1988). Antiarrhythmic action of drugs on triggered activity, on the other hand, could involve suppression of the afterdepolarization by decreasing $Ca^{2+}$ or inward $Na^{+}$ currents (Thale et al., 1987).

Class I antiarrhythmic agents (Class Ia: quinidine, disopyramide, and procainamide; Class Ib: lidocaine, tocainide, and mexiletine; Class Ic: encainide, flecainide, and lorcainide) are sodium channel blockers and thus reduce the fast inward sodium current. This results in reduced

maximum rate of rise (MRD), depressed conduction velocity, and prolongation of the effective refractory period (ERP). Class I agents also reduce spontaneous diastolic depolarization (Campbell, 1983b; Harrison 1985). These agents would therefore be expected to be active against arrhythmias involving reentry and abnormal automaticity. However, sodium channel blockers are almost without effect on abnormal pacemakers in which automaticity is maintained by slow inward current and triggered activity caused by increase in $Ca^{2+}$, as in early and delayed afterdepolarizations.

Class II antiarrhythmic agents (β adrenoceptor blockers) inhibit arrhythmic responses due to endogenous catecholamines (Nattel, 1991). Such adrenergic arrhythmias which occur during physical and mental stress are suppressed most successfully by β adrenoceptor blockers. Long term treatment with β adrenoceptor blockers also tends to prolong both atrial and ventricular action potential duration (Vaughan Williams, 1978). Since re-entrant arrhythmias are favoured by slow conduction and short refractory periods such drugs (which increase refractoriness without slowing conduction) should be of value in treatment of re-entrant arrhythmias.

Class III agents (amiodarone and (±)-sotalol) selectively prolong action potential duration(APD) without slowing conduction velocity.

Prolonging APD delays recovery of voltage dependent $Na^+$ channels, thereby increasing effective refractory periods (Singh & Vaughan Williams, 1970). Class III agents may be of value in preventing re-entrant and abnormal automatic arrhythmias. However, the development of Class III antiarrhythmics has progressed slowly relative to development of Class I agents. This is due in part to their proarrhythmic potential in causing Torsade de Pointes (Dessertenne, 1966).

The use of Class IV agents (verapamil and diltiazem) still remains controversial (Walker and Chia, 1989). It is known that their antiarrhythmic actions depend on their ability to decrease the upstroke velocity of action potentials in the AV node thus slowing AV conduction and increasing the refractory period of the AV node. Furthermore, since pacemaker activity may arise solely from inward calcium currents (Borchard et al., 1989), Class IV agents may be effective in preventing arrhythmias arising from automatic mechanism and re-entrant mechanism involving the AV node as part of the re-entrant circle. Class IV agents are also able to protect the cell against $Ca^{2+}$ overload and thus suppress triggered activity such as afterdepolarization resulted from $Ca^{2+}$ overload.

## 1.2 Experimental arrhythmogenesis

### 1.2.1 Arrhythmia models in general

A large number of models have been designed to produce arrhythmias. These have been reviewed periodically over the last decade or so. For example, Szekeres (1971, 1979) very clearly outlined how arrhythmias may be induced in a variety of species by either electrical stimulation of the heart, administration of arrhythmogenic drugs and chemicals, or pathological damage to the heart via ischaemia or infarction, local cooling, local warming, or mechanical injury. In addition, he listed several methods whereby arrhythmias can be induced by means of electrical stimulation of the central nervous system.

In 1984, Winslow carefully reviewed the methods available for producing arrhythmias and assessing the antiarrhythmic actions of drugs. The methods in her review again involved electrical stimulation, chemical administration, and induction of arrhythmias by pathological means. Curtis and Walker (1988) reviewed in detail all of the models for inducing arrhythmias via myocardial ischaemia and infarction in the rat. The use of rat models has been further summarized by Walker et al. (1991). Except for the last two reviews, most authors specifically

discussed a number of different species in addition to the rat. In any discussion on the production of arrhythmias it is important, for mechanistic and comparative purposes, to be able to classify the resulting arrhythmias.

### 1.2.2 Arrhythmia models in rats

#### 1.2.2.1 Overview

The rat is a common laboratory animal that has been used in many pharmacological, toxicological, biochemical, and pathophysiological studies. It is perhaps the most accepted of all laboratory species and is well understood in terms of anatomy, genetics, physiology, and biochemistry (Carr and Krantz, Jr., 1949; Mitruka, 1976; Ringler and Dabich, 1979; Petty, 1982). There are certain advantages and disadvantages with the use of the rat as an experimental animal. Rats are much smaller than humans; their pharmacokinetics and pharmacodynamics are often quite different (Fox, 1967) and they have a number of peculiarities with respect to biochemistry and physiology (Jorgensen, 1967).

With regard to cardiac electrophysiology, the rat occupies a

somewhat special position in comparison with other laboratory species. This is due to in part to its high heart rate, but more particularly to a more rapid repolarization of cardiac action potentials (Hoffman and Cranefield, 1960). The potassium channels responsible for cardiac potential repolarization vary with species and cardiac tissue type (Carmeliet et al., 1987; Furukawa et al., 1992; Beatch et al., 1990), but are most different in the rat. Thus ventricular action potentials in rats rely predominantly on the transient outward potassium currents ($i_{to}$) for repolarization (Josephson et al., 1984; Dukes and Morad, 1989).

While the rat has distinct differences from man in terms of cardiac anatomy and electrophysiology, its advantages in the study of myocardial ischaemia and arrhythmias may outweigh its disadvantages. The main advantages of rats are that they are small and easy to handle, inexpensive, and can be used in large numbers. In addition, a large variety of human disease states have been modeled in the rat (Petty, 1982). Another notable advantage is the uniform lack of effective coronary collaterals which results in reproducible occluded (ischaemic) zones (Curtis et al., 1987a). This is of prime importance since both ischaemia-induced arrhythmias and infarct size depend upon the extent of collateral anastamoses (Curtis, 1986).

### 1.2.2.2 Chemical model

A variety of arrhythmias can be readily produced in the rat by administration of drugs and chemicals to the whole animal, or to isolated hearts. In many species, cardiac glycosides (e.g., ouabain) are routinely used to induce ventricular arrhythmias but this is not possible in the rat since this species is insensitive to cardiac glycosides (Winslow, 1984).

Some chemicals routinely used to produce arrhythmias (primarily ventricular) in the rat are acontine, calcium, and barium (Vargaftig and Coignet, 1969; Malinow et al., 1953; Ferrara et al., 1990). Normally, these compounds are given intravenously to the whole animal and are not routinely used in isolated hearts. In larger species, such as the cat and dog, such chemical arrhythmogens can be applied locally (e.g., Nakayama et al., 1971; Byrne et al., 1977; Winslow, 1981), but this is not usually done in the rat.

One recently introduced ionic procedure for inducing arrhythmias in isolated hearts is preferential perfusion of different coronary bed with solutions containing an elevated potassium concentration as first performed in isolated rabbit hearts (Curtis, 1989b). Preferential perfusion of a single coronary bed can be achieved by use of an ingenious special perfusion cannula situated within the aorta (Avkiran and Curtis, 1991).

### 1.2.2.3 Electrical induction of arrhythmias

Arrhythmias are induced routinely by electrical stimulation at a variety of cardiac sites such as atria, ventricles, and the atrioventricular node in many species, including humans (Weissberg et al., 1987). The small size of the rat heart does not readily allow for a highly selective placement of electrodes, and, as a result, the site chosen for electrical stimulation in rats is usually the right or left ventricle. Access to these sites can be achieved by opening the chest and exposing the heart. However, it is easy to insert two electrodes, no more than 1-2 mm apart, by a transthoracic route using a 27-gauge needle (Howard and Walker, 1990; Pugsley et al., 1992). Suitable electrodes for this purpose can be readily fashioned from teflon-coated stainless-steel wire.

The types of arrhythmias that can be induced by electrical stimulation include single extrasystoles, tachycardia, and fibrillation. Extrasystoles can be induced by a single extra stimulus added to a chain of stimuli, or interposed during sinus rhythm. Ventricular tachycardia can be induced by stimulating the heart to beat at a rate faster than the sinus rhythm. The rat heart can be driven at rates greater than twice the sinus rate. One variant of this technique is to continuously increase the frequency of stimulation until the heart fails to follow, on a one to one

basis. The frequency of stimulation at which the heart fails to follow is known as the maximum following frequency, an indirect measure of effective refractory period (Martinez and Crampton, 1981; Pugsley et al., 1992).

In order to induce ventricular fibrillation (actually a type of fibrillo-flutter), super threshold square waves are applied at 50 Hz (Marshall et al., 1983). The ease of inducibility of ventricular fibrillation is assessed in terms of the threshold current. Characteristically, such induced ventricular fibrillation has a coarse type of ECG morphology and is best described perhaps as being more of a Torsade de Pointes type of tachycardia rather than true fibrillation. Furthermore, in over 95% of cases this arrhythmia spontaneously reverts to normal sinus rhythm providing that it is not continued for too long. Normally, sinus rhythm reappears on termination of stimulation.

The above electrical stimulation procedures can be readily used in isolated hearts with the stimulating electrodes being placed anywhere within, or upon, the heart. In addition, the atrioventricular node can be ablated thereby freeing the ventricle of interfering impulses originating in the atria.

### 1.2.2.4 Ischaemia and Reperfusion

A common pathological cause of arrhythmia in humans is the occurrence of myocardial ischaemia and infarction, or reperfusion of a previously ischaemic area of myocardium. These events can be readily reproduced in both intact rats and in isolated hearts.

The rat heart, in common with species such as pigs and primates, does not normally have extensive coronary collaterals (Johns and Olson, 1954; Maxwell et al., 1987), i.e. rat coronary arteries are end arteries. Thus when a coronary artery is occluded tissue downstream to the obstruction is rendered uniformly ischaemic. Ischaemic is not absolute since a residual 5% of flow is seen following complete ligation of an artery (Winkler et al., 1984; Maxwell et al., 1987). If occlusion of an artery is maintained for greater than 10 min, irreversible damage occurs and infarction results (Saint et al., 1992). If the occlusion and its resulting ischaemia is permanent, the resulting infarct can occupy an area greater than 80% of the original ischaemic zone. Any period of ischaemia can be terminated by reperfusion but in rat hearts reperfusion will only successfully save all of the ischaemic zone from becoming infarcted if reperfusion is insituted within 15 min of the onset of ischaemia (Saint et al., 1992). Reperfusion is a most powerful stimulus for inducing

arrhythmias (Manning and Hearse, 1984; Curtis and Hearse, 1987). However, the severity and intensiity of such reperfusion arrhythmias depends critically upon the duration of the preceding ischaemic period. The time-dependency effect is quite characteristic in that reperfusion arrhythmias are most severe after 5-10 min ischaemia (Manning and Hearse, 1984; MacLeod et al., 1989). Reperfusion arrhythmias can be induced in both intact and isolated hearts. Methods suitable for intact animals have been described by Manning and Hearse (1984) and MacLeod et al., (1989) and those suitable for intact hearts by Curtis and Hearse (1989) and Lubbe et al. (1978).

A number of methods have been described for producing coronary ligation or occlusion in intact rats, whether anaesthetized (Johns and Olson, 1954; Au et al., 1979; Clarke et al., 1980) or conscious (Johnston et al., 1983; Himori and Akihiro, 1989). In conscious rats, the various responses to occlusion can be recorded for hours and days after occlusion. Responses that have been measured include blood pressure, heart rate, ECG changes such as increases in R-wave height and S-T segment elevation, mortality and ischaemic zone size as well as arrhythmias (Johnston et al., 1983). It has been shown in such preparations that the severity and incidence of arrhythmias following occlusion of a coronary artery is dependent upon the size of the occluded zone (Johnston et al.,

1983; Curtis et al., 1987) and serum potassium concentration (Curtis et al., 1987; Saint et al., 1992). Arrhythmia dependency upon extracellular potassium concentration is also seen in isolated hearts (Curtis, 1989b; Curtis and Hearse, 1989).

In order to obtain consistent results, it is important that factors as occluded zone size and serum potassium concentration are measured. To standardize experimental design in arrhythmia studies, a series of conventions (Lambeth Conventions) were established to improve uniformity and inter-laboratory comparisons (Walker et al., 1988).

## 1.3 Opioid Receptors in the Heart

Although the opioids have been, and continue to be, used primarily as analgesic and general anaesthetics, cumulative evidences has indicated that many opioid agonists and antagonists are also involved in the genesis and prevention of cardiac arrhythmias arising during myocardial ischaemia and infarction. The purpose of this chapter is to discuss our limited current knowledge about the cardiac effects of opioids.

### 1.3.1 Classification of Opioid Receptors

Based on the structural and steric specificity of analgesic action of morphine observed in early behavioural and clinical studies, the existence of specific opioid receptors was suggested (Beckett and Casy, 1954; Portoghese, 1965). The identification of opioid receptor by receptor binding assays (Pert and Snyder, 1973), together with the discovery of enkephalins (Hughes, 1975; Hughes et al., 1975a; Hughes et al., 1975b) and endorphins (Bradbury et al., 1976; Li and Chung, 1976) acting as endogenous ligand for these receptors, it was believed that endogenous opioid peptides produce their effects by interaction with specific receptors. The existence of more than one type of opioid receptors was first postulated by Martin (1967) when he observed that nalorphine had a dual mode of action, antagonizing the analgesic effect of morphine and yet itself possessing analgesic activity. The existence of three types of opioid receptors was suggested based on the differential spectrum of actions produced by different opioids and benzomorphan drugs *in vivo* and the finding that some opioids, but not all, are able to relieve withdrawal symptoms in morphine-dependent dogs (Gilbert and Martin, 1976; Martin, 1976). These were designated as μ-, κ- and σ-receptor for which the prototypical agonists are morphine, ketazocine or

ethylketocyclazocine (EKC) and N-allylnormetazocine (SKF10047), respectively. Activation of each of these receptors by their respective agonists produce distinct pharmacological effects in whole animals. For example, morphine induces analgesia, bradycardia, hypothermia and meiosis, whereas ketazocine induces meiosis, sedation and depression of flexor reflexes. SKF10047 induces mydriasis tachypnoea, tachycardia, and mania. The sigma (σ)-receptor is regarded by some as a non-opioid receptor because other drugs, such as phencyclidine, also act via this receptor. However, it has also been suggested that receptor classification based on the measurements of responses *in vivo* may not be conclusive.

In subsequent *in vitro* studies, employing bioassays with guinea-pig ileum and mouse vas deferens to compare the rank order of potencies of various opioids on guinea-pig ileum and mouse vas deferens, it was found that morphine is more potent on the guinea-pig than the mouse vas deferens whereas met- and leu-enkephalin were more potent on mouse vas deferens than on guinea-pig preparations. Furthermore, naloxone, a non-selective antagonist with preference to μ-receptors, is less potent in antagonizing the actions of other opioids in mouse vas deferens than morphine. The opioid receptors in the guinea-pig, and mouse vas deferens are therefore thought to be μ- and δ-receptors, respectively. The presence of distinct morphine (μ) and enkephalin (δ) binding sites was

confirmed by displacement binding assays with δ- and μ- labelled ligands that were displaced more readily with their respective cold ligands. The discovery of a separate κ-binding site was based on the observation that selective μ- and δ- agonists have low potency in displacing the binding of [$^3$H]EKC (Kosterlitz et al., 1981). Furthermore, responsiveness to δ-, μ- and κ- agonists or ligands can be selectively protected against alkylation by simultaneous incubation with their respective selective ligand whereas the responsiveness to the others is either abolished or significantly reduced. Such selective protection against alkylation provided direct evidence that δ-, μ- and κ-receptors are physically distinct and not interconvertible (Robson and Kosterlitz, 1979; Smith and Simon, 1980; James et al., 1982). To date, it is generally accepted that opioid receptors are classified into at least three main types, namely μ-, κ- and δ- receptors.

### 1.3.2 Pharmacological and Physiological identification of cardiac opioid receptors

Opioid receptors have been shown to be widely distributed in the central nervous system and the periphery (Bloom, 1983; Wamsley, 1983) and are implicated in the regulation of many physiological functions in

addition to analgesia. It was demonstrated in the early 19th century that morphine and other opiate alkaloids possess potent cardiorespiratory effects. The discovery of endogenous opioid peptides and their receptors in the brain nuclei such as nucleus tractus solitarius (De Jong et al., 1983), nucleus ambigus and dorsal vagal nucleus (Laubie et al., 1979; Lang et al., 1982) of the brain stem and hypothalamus, which are important for the modulation of cardiovascular control, implicate endogenous opioid systems in the regulation of cardiovascular function through the central nervous system.

The endogenous opioid system influences the cardiovascular system not only centrally, but also peripherally. Opioid peptides are found in the vagus nerve (Hughes et al., 1977; Lundberg et al., 1979), in several sympathetic ganglia (Schultzberg et al., 1979), and in the heart (Lang et al., 1983; Spampinato and Goldstein, 1983; Weihe et al., 1983, 1985). Adrenal enkephalin has been shown to be involved in cardiovascular regulation. For instance, stimulation of splanchnic nerve in reserpinized dogs causes a naloxone reversible hypotension as a result of enkephalin release from the adrenal gland (Hanbauer et al., 1982). Met-enkephalin produces a naloxone reversible fall in perfusion pressure in isolated cat hindlimb (Moore and Dowling, 1982).

The heart is known to contain opioid peptides such as dynorphin

(Spampinato and Goldstein, 1983), met-enkephalin and leu-enkephalin (Lang et al., 1983). Direct effects of endogenous opioid on the heart were demonstrated by Eiden and Ruth (1982) when they observed that low concentrations of enkephalins are able to antagonize chronotropic responses to noradrenaline on isolated rat atria. Based on the finding that opioids inhibit field-stimulated cardiac noradrenergic responses, but not the effects of exogenous administration of noradrenaline, it was suggested that opioids have effect on the release of noradrenaline from the nerve terminals of guinea-pig atria was suggested (Ledda and Mantelli, 1982). In an attempt to identify the existence of opioid receptors in the ventricular sympathetic nerve terminals, selective opioid agonists, dynorphin and [D-Ala$^2$, D-Leu$^5$]enkephalinamide, were shown to produce a naloxone-reversible potentiation of contraction in field-stimulated isolated guinea-pig ventricular strip (Mantelli et al., 1987), suggesting that the receptors are of κ- and δ- types.

### 1.3.3 Cardiac opioid binding sites

In 1977, using radioligand binding assays with tritiated naloxone and dihydromorphine, as well as non-selective opioid antagonists, as probes, Simantov and his co-workers first demonstrated the existence of

selective opioid binding in crude membranes homogenates prepared from the whole hearts of guinea-pigs and rats. Unfortunately, the extent of saturable opioid binding, relative to the total binding, was very small, 10% for hearts as compared to more than 60% for the brains.

In a subsequent binding study, Burnie (1981) also detected stereospecific opioid binding sites in cardiac papillary muscle from rat right ventricle using titrated diprenorphine, a non-selective opioid antagonist, as a probe. Unfortunately, the study did not provide further details on binding properties.

Subsequent binding studies not only confirmed the existence of opioid binding sites in the heart, but also provided evidence on the type of opioid binding site. However, results were inconsistant. Using indirect binding assays, Krumins et al., (1985) demonstrated that [$^3$H] diprenorphine binding was displaced by DADLE (δ-agonist); ethylketocyclazocine (κ-agonist) and levorphanol (universal opioid agonist), but not by DAGO, (μ-agonist), suggesting that the binding sites were of the δ- and κ- but not μ-type in the right atrium and ventricle of the rat heart. In their subsequent study with competition binding assays to characterize the binding properties of dermorphin, a naturally occuring [D-Ala$^2$]heptapeptide with potent opioid activity and binding selectively for μ-receptors (Westphal et al., 1985), it was found that

[$^3$H]diprenorphine was displaced by increasing concentration of dermorphin in the left atrial membranes of the rat heart, suggesting that the presence of μ-opioid binding sites. In the rat cardiac sarcolemma membrane preparation Ventura et al. (1989) demonstrated the presence of κ- and δ- but not μ- binding sites, in agreement with the finding of Krumins et al. (1985). Further evidence of the properties and distribution of κ-binding sites in the rat heart was provided by Tai et al. (1991) who found that there are substantial specific [$^3$H]U69593 (selective κ-ligand) binding sites in rat heart.

### 1.3.4 Actions of opioids in nerve and cardiac muscle

In general, activation of opioid receptors in nerve cells produced two direct effects-inhibition of neurotransmitter release, and reduction in cell firing. These effects are achieved by affecting ionic conductances.

Activation of μ-receptors increases potassium conductance in preparations such as rat locus coeruleus cells (Yoshimura and North, 1983), mouse dorsal root ganglion cells (Werz and Macdonald, 1983), rat substantia gelatinosa neurones (Yoshimura and North, 1983), guinea-pig myenteric neurones (Morita and North, 1982) and guinea-pig locus coeruleus neurones (Pepper and Henderson, 1980). Study on single-ion

channels suggest a direct interaction between the μ-receptor and potassium channels (Miyake et al., 1989). Opening of potassium channels by μ-agonists can be potentiated by guanosine 5'-[γ-thio]triphosphate (GTPγS), suggesting a G-protein involvement (North et al., 1987).

In a similar manner δ-receptor activation reduces transmitter release by hyperpolarization of the neurones. This involves a shortening of the duration of the calcium action potential resulting from an increase in potassium conductance of the mouse DRG cell membrane (Werz and Macdonald, 1983).

Kappa-agonists such as dynorphin, tifluadom and U50,488H have no effect on nerves of the rat locus coeruleus (Yoshimura and North, 1983) and guinea-pig submucous plexus (Mihara and North, 1986) which contains μ- and δ- receptors, respectively. On the other hand, κ-agonists shortens action potential duration in guinea-pig myenteric plexus (Cherubini and North, 1985), a tissue with μ- and κ- opioid receptors. Shortening is still observed when the action potential is prolonged by caesium, suggesting an effect mediated via a direct action on calcium conductance, rather than on the potassium channel. Direct reduction of calcium currents in mouse dorsal root ganglion cells by κ-agonists was first postulated by Werz and Macdonald (1983). In a subsequent study, they demonstrated, using voltage clamp techniques, that κ- but not μ- and

δ- agonists reduces calcium currents in these nerve cells (Werz and Macdonald, 1984). On the other hand, recent electrophysiological studies into opioids actions on cultured dorsal-root ganglion cells revealed direct excitatory actions of opioids. Activation of δ-, μ- and κ- opioid receptors can prolong action potential duration when opioids are applied at nanomolar concentrations (Shen and Crain, 1989). Prolongation of action potentials by δ- or μ- and κ-opioid receptor activation is due to a decrease in a voltage-sensitive potassium conductance and an increase in voltage-sensitive calcium conductance of the membrane, respectively (Shen and Crain, 1989; Crain and Shen, 1990). The prolongation of action potentials is not affected by pertussis toxin. However, treatment of the DRG neurones with cholera toxin blocks the excitatory effects of the opioid, suggesting that the involvement of G-protein in the signal transduction of the response (Crain and Shen, 1990).

It has been shown that opioid peptides inhibits the release of norepinephrine (Gaddis and Dixon, 1982; Illes et al., 1985) and acetylcholine (Konishi et al., 1981; Wong-Dusting and Rand, 1987) from sympathetic and parasympathetic nerve endings in various preparations. Presynaptic modulation of neurotransmitter release by opioids has been shown to affect contractility in ventricular tissues (Mantelli et al., 1987). Interaction of opioids with neurotransmitters in the regulation of cardiac

function was reported by Kosterlitz and Taylor (1959) who demonstrated that morphine reduces the cardiac slowing produced by vagal stimulation. The association of cardiac opioid receptor-mediated action with cellular calcium was first demonstrated by Ruth et al. (1983). In an attempt to further clarify mechanism of enkephalin in attenuating noradrenaline-induced positive chronotropic effect in isolated and spontaneously beating rat atria (Eiden and Ruth, 1982), they found that leu-enkephalin produces a naloxone-revesible antagonism of noradrenaline-induced $^{45}Ca^{++}$ uptake in the isolated rat atrial slices. Interestingly, the same authors demonstrated in other studies that noradrenaline-induced positive chronotropy was augmented by leu-enkephalin. In association with this, leu-enkephalin causes a further increase in noradrenaline-activated $^{45}Ca^{++}$ uptake in isolated guinea-pig atria (Ruth et al., 1983). In addition to indirect action of opioids on cardiac functions, opioids have also been shown to act directly on ventricular myocytes, which are devoid of nervous influence (Laurent et al., 1985; Ventura et al., 1991). Therefore, the actions of opioids on cardiac muscle may via the autonomic nervous system or directly on muscle cells.

Although activation of cardiac opioid receptors has been shown to increase $[Ca^{2+}]_i$ (Ventura et al., 1991). Knowledge of the effects of cardiac opioid receptor stimulation on ionic fluxes across sarcolemmal

membrane is scanty. Meptazinol, a opioid partial agonist, has been shown to increase action potential duration by 40% in rat papillary muscle (Fagbemi et al., 1983). The opioid agonists, fentanyl and sufentanil, prolong action potential duration at 50% and 90% repolarization ($APD_{50}$ and $ADP_{90}$) in canine Purkinje fibers, in a non-naloxone-reversible manner, suggesting a direct membrane effect (Pruett et al., 1987; Blair et al., 1986). It has been reported that U50,488H at $10^{-5}$M reduces the slow inward current, a main current that maintains the plateau phase of the action potential in guinea-pig myocytes (duBell and Lakatta, 1991). Whether the effects of the opioids on ionic fluxes in muscle is mediated via opioid receptors have not been tested.

### 1.3.5 Opioids and arrhythmias

In addition to the effects on heart rate and contractility, cardiac opioid receptors have also been implicated in cardiac arrhythmogenesis. This was first demonstrated by Stein (1976) who showed that high doses of morphine can induce cardiac arrhythmias including atrial fibrillation and atrio-ventricular block in conscious rats. Involvement of opioid receptors in arrhythmias arising during myocardial ischaemia were suggested when naloxone, a universal opioid antagonist, was

demonstrated to attenuate arrhythmias in both anaesthetized and conscious rats subjected to coronary artery ligation (Fagbemi et al., 1982). It was then generally believed that attenuation of responses by naloxone was a pathognomonic involvement of opioid receptor. In support of the notion that opioid receptors were involved in ischaemic arrhythmogenesis, it was shown that the opioid antagonists, (-)-naloxone (Lee, 1992), posses antiarrhythmic activity while their pharmacologically inactive structural isomers did not. However, direct actions of naloxone could also contribute to its antiarrhythmic activity. This is supported by the finding that (+)-naloxone, which is 1,000-10,000 less potent as an opioid antagonist (Iijima et al., 1978) is equipotent as an antiarrhythmic agent (Sarne et al., 1988; Sarne et al., 1991).

The use of isolated perfused rat heart preparations makes it possible to determine directly whether cardiac opioid receptors are involved in arrhythmogenesis. Several lines of evidence suggest that activation of cardiac opioid receptors are contributory to the genesis of cardiac arrhythmias arising during myocardial ischaemia and reperfusion. Firstly, naloxone attenuates the incidence of arrhythmias following myocardial ischaemia and reperfusion in the isolated rat heart preparation (Zhan et al., 1985; Lee and Wong, 1986; 1987a). In addition, naltrexone (Liu et al, 1988), another prototypical opioid antagonist, also possesses

antiarrhythmic activity. Isolated rat hearts of chronically morphine-treated rats exhibit less arrhythmias in response to dynorphin$_{1-13}$ (Wong and Lee, 1987), a phenomenon characteristic of receptor mediated event. The hearts of chronically morphine treated rats also exhibit less arrhythmias in response to myocardial ischaemia and reperfusion (Wong and Lee, 1987), suggesting that ischaemia and reperfusion induced arrhythmia may also involve cardiac opioid receptors. Further, a selective κ receptor agonist, U50,488H was found capable of eliciting ventricular arrhythmias in isolated rat hearts (Wong et al, 1990). The same drug has also been found to exacerbate ischaemia induced arrhythmias in rats (Lee et al., 1992).

However, there are also problems which make it difficult to ascribe a pathophysiologically relevant arrhythmogenic role to opioid agonists and an antiarrhythmic role to opioid antagonists. Firstly, there is no convincing evidence that endogenous opioids actually accumulate in the myocardium during ischaemia or reperfusion although endogenous opioid peptides were shown to have released following acute myocardial ischaemia (Oldroyd et al., 1992). Secondly, data on arrhythmogenic and antiarrhythmic effects of opioid agonist and antagonist substances are not consistent. Certain opioid agonists have been reported to reduce rather than increase the incidence and severity of arrhythmias elicited by

myocardial ischaemia (Pugsley et al., 1992a,b & 1993; Fagbemi et al., 1983; Boachie-Ansah et al., 1989). In contrast with Wong et al & Lee et al, U-50,488H was found to be pro-arrhythmic at low doses but was antiarrhythmic at high doses (Pugsley et al., 1992a,b). Thirdly, evidence for protection of arrhythmias by antiopioid agents is inconsistence. Bergey and Beil (1983) found that naloxone at varies doses were ineffective against ischaemia induced arrhythmias in pigs. Consistent with this, Pruett et al (1991) found that naloxone at varies concentrations had no effect on cardiac action potential configuration. Thus the question of whether opioid agonists and antagonists are pro- or anti-arrhythmic still has to be further investigated.

### 1.3.6 κ-opioids and arrhythmias

Although findings from previous studies have implicated the involvement of cardiac opioid receptors in arrhythmogenesis and antiarrhythmic activities during myocardial ischaemia and infarction, the type opioid receptor(s) involved remains to be determined. In an attempt to identify the cardiac opioid receptor subtype(s) involved, Wong et al (1990) has shown that U50,488H, a selective κ-agonist, and MR2266, a selective κ-antagonist, have significant greater arrhythmogenic effects

and antiarrhythmic effects, respectively, than μ- and σ- opioid agonists and antagonists such as DAGO, DPDPE, DADLE, and naloxone. In agreement with the above finding, Sitsapesan and Parratt (1989) found that in the anaesthetized rat MR2266 is the most potent antiarrhytmic agent among the three types of opioid antagonists during ischaemia. Overall, the results of these studies suggest if opioid receptors are involved then the cardiac κ-receptors are the most likely receptor-subtype involved in arrhythmogenesis or antiarrhythmic activities during ischaemia.

### 1.3.7 Mechanism of antiarrhythmic effects mediated by κ agonists

#### 1.3.7.1 κ-opioid receptor mediated antiarrhythmic effects

κ-receptor mediated adenylate cyclase inhibition with the use of dynorphin and U-50,488H have been reported in membranes from rat spinal cord neurons (Attali et al., 1989) and guinea pig cerebellum (Konkoy and Childers, 1989). Adenylate cyclase, which inhibited by the α-(GTP)subunit of the $G_i$ protein, inhibits the formation of cAMP from ATP, and thus disrupts the protein phosphorlation step that controls a

variety of cellular activities. Opioid receptor mediated adenylate cyclase inhibition can lead to attenuation of the cAMP-dependent protein kinase activity that mediated the phosphorylation (activation) of the voltage-dependent calcium channels. Modulation of calcium channels by cAMP has been reported in several studies (Cachilin et al., 1983; Chad et al., 1984; Kostyuk et al., 1981). Recent studies by Attali et al (1988) and Gross et al (1987) also showed that U50,488 and dynorphin decrease both L and N-type calcium currents in dorsal root ganglion cells indirectly via a cAMP-dependent mechanism. In addition, Gross et al (1990) and North et al (1987) have reported the inhibitory effects of dynorphin and U50,488 on calcium conductance are direct effect to the voltage-dependent calcium channels via G-protein. Although these studies were all done in neuronal tissue, it is acceptable to suggest that the second messenger system in the cardiac tissue would function in similar manner. Therefore, the antiarrhythmic activities mediated by κ agonists or antagonists are possibly effects of cardiac calcium channel blockade secondary to receptor binding.

Another possible mechanism for the antiarrhythmic activity mediated by κ agonists and antagonists is the change of intracellular calcium concentration mediated via κ agonists. Recent study by Misawa *et al.* (1990) showed that binding of U50,488 to the κ-receptors in the

guinea pig cerebellum inhibit GTP-stimulated phospholipase C (PLC) activity. PLC is important in the hydrolysis of phosphatidylinositol 4,5-bisphosphate into diacylglycerol and inositol 1,4,5-trisphosphate ($IP_3$) which serve as an important intracellular second messengers for protein kinase C activation and intracellular calcium mobilization. An opposing finding is also reported by Periyasamy and Hoss (1990, 1991) who suggested that U50,488, dynorphin, and ketocyclazocine (at high concentrations) all stimulate phosphoinositol turnover in rat hippocampal slices by stimulating GTP-stimulated phospholipase C activity. $IP_3$ is known to liberate $Ca^{2+}$ from cardiac SR (Nosek et al., 1986; Fabiato, 1990; Kentish et al., 1990). Increase in $IP_3$ can lead to an increase in $[Ca^{2+}]_i$ The increase or decrease in $[Ca^{2+}]_i$ resulted from stimulation of protein kinase C or PLC inhibition may play an important role in the arrhythmogenesis and antiarrhythmic activities of κ agonists and antagonists since $[Ca^{2+}]_i$ levels has been linked with myocardial arrhythmia occurrences as discussed earlier. However, at present, there is no evidence to support these 2 proposed antiarrhythmic mechanisms.

### 1.3.7.2 Cardiac ion channel blockade

Many studies have shown that the antiarrhythmic actions of opioids are independent of the opioid receptors but instead involve cardiac ion channel blockade. Studies in rats by Pugsley et al (1992a,b, 1993) have shown that the antiarrhythmic effects of κ agonists, U50,488H, and PD 129290 were unaltered by naloxone pretreatment suggesting the antiarrhythmic actions are independent of κ and other opioid receptors. In addition to observing whether U50,488H and related compounds have effects on blood pressure and heart rate which are not attenuated by naloxone, ECG observation is a useful indirect method for determining the effects of antiarrhythmic drugs on cardiac ion channels. ECG changes induced by U50,488H can be interpreted as indicating ion channel blockade, particularly for the sodium channel (Class I activity). Thus, U50,488H produced P-R prolongation and QRS widening in rats (Pugsley et al., 1992b), together with elevation of "RSh," an ECG index of sodium channel blockade in this species (Penz et al., 1992). These actions were unaltered by naloxone. As with U50,488H, PD 129290 also showed sodium channel blocking action that are not blocked by naloxone. The evidence for the above events being unrelated to the κ receptors was strengthened by findings with the R,R (+)-enantiomer of PD 129290, PD

129289 (Pugsley et al., 1993). Unlike PD 129290, PD 129289 has very low affinity for κ receptors. Yet, both compounds increased the size of the P-R, QRS and RSh intervals of the ECG. However, it should also be noted that changes in the Q-T interval of the ECG seen at higher doses of these compounds suggest that a degree of potassium channel blockade (Class III activity) also occurred. It is possible that this action which is independent of opioid receptors may also contribute to antiarrhythmic actions.

Studies by Sarne et al (1989 & 1991) using a variety of opioid agonists and antagonists: naloxone and its non-opioid stereoisomer (+)-naloxone; Win 44,441-3 and its stereoisomer Win 44,441-2; levorphanol and its (+)-stereoisomer, dextrorphan, also suggested that the antiarrhythmic effects of these opioid drugs are direct effect on ionic currents in cardiac muscle and are not mediated by agonism or antagonism of the stereospecific opioid receptors. This is in agreement with the previous finding that naloxone suppresses sodium and potassium currents (Carratu and Mitolo-Chieppa, 1982; Brasch, 1986). Further studies by Oldroyd et al (1993) have also shown that naloxone has both a non-opioid-receptor-mediated Class III antiarrhythmic effect on normal myocardium and a Class I effect on ischaemic myocardium since both racemic naloxone (active at opioid receptors) and d-naloxone (inactive)

prolonged action potential duration and effective refractory period in normally perfused rabbit interventricular septa, and enhanced the fall in maximum upstroke velocity of action potential in partially depolarized ventricular myocardium. In addition, buprenorphine, the opioid which possesses both partial μ-agonist and κ-receptor antagonist activity, and meptazinol, a partial opioid agonist, have also been shown to be antiarrhythmic during myocardial ischaemia in the anaesthetised rats, by reducing the action potential height and maximum rate of depolarization of phase zero (MRD) and prolonging the duration of the action potential, effects that resembled the actions of Class I and Class III antiarrhythmic agents (Boachie-Ansah et al., 1989; Fagbemi et al., 1983). These effects, indeed, may not be mediated via specific opioid receptors because the concentrations required to confer protection against ischaemic arrhythmia were much higher than the concentrations required for opioid receptor antagonism.

## 1.4 RSD 939

RSD 939 (Figure 1) was developed by Rhythm Search Development Ltd. in an effort to discover a more effective and less toxic drug for the treatment of cardiac arrhythmias. It has a pKa value of 8.3, the empirical

formula is $C_{19}H_{26}N_2O_2Cl_2 \cdot HCl$, and it has a molecular weight of 421.79 g/mole.

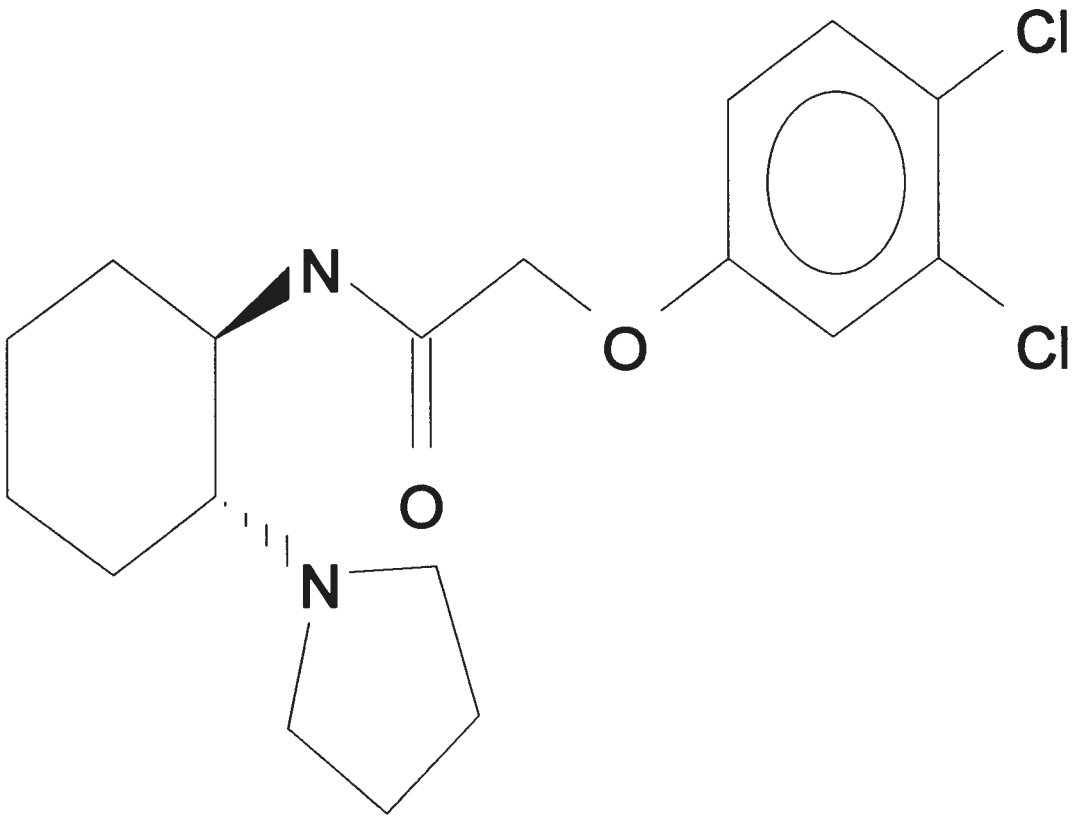


Figure 1. The structure of RSD 939

## 1.5 Aims of studies

In myocardial ischemia or infarction, ventricular fibrillation is responsible for the majority of deaths (approximately 90%). Class I antiarrhythmic agents (sodium channel blockers) have a long history, both in the treatment of a variety of arrhythmias and in their development by modification of basic chemical structures. However, endeavours to improve on prototypical class I agents have not met with success despite the introduction of numerous newer agents. Therefore, there is a continuing need to search for safer and more efficacious drugs (Podrid, 1989). However, the route to new drugs is difficult due to the lack of exact knowledge concerning the mechanisms underlying the genesis of arrhythmias in the clinical population and the relative lack of knowledge regarding pharmacological properties which confer antiarrhythmic activity (Botting et al., 1986).

Recently, accumulating evidence has indicated that various opioid agonists and antagonists, especially κ agonists, exhibit a variety of cardiovascular and antiarrhythmic actions. Two important questions arise. First, are these cardiovascular and antiarrhythmic actions mediated by the opioid receptors? Second, what is the mechanism underlying these actions if these effects are not mediated by opioid receptors? Previous

studies have shown that some of the cardiac and cardiovascular actions of κ agonists are a result of its direct action on cardiac sodium channels and are independent of opioid agonism. RSD 939 is structurally related to κ agonists and it appears to be a potent and selective κ agonist in binding studies (unpublished data by Abraham et al). The present study is an attempt to study the involvement of opioid receptors in the cardiovascular and antiarrhythmic activity possessed by κ agonists and to determine the underlying mechanism of these activities with the use of RSD 939.

# 2 Methods

All animal experiments conducted at U.B.C. were according to guidelines established by the U.B.C. Animal Care Committee.

## 2.1 Experimental plan

The overall goal of the laboratory investigation was to study the involvement of opioid receptors in the cardiovascular and antiarrhythmic activity possessed by κ agonists and to determine the underlying mechanism of these activities with the use of RSD 939. In keeping with this, the following studies were performed with RSD 939 in the presence and absence of naloxone:

1) Assays of antiarrhythmic efficacy against ischaemia-induced arrhythmias in rats.
2) Assays of antiarrhythmic efficacy against electrical induction of fibrilloflutter in rats.
3) Characterization of the pharmacological profile and determination of possible mechanisms of underlying antiarrhythmic efficacy through analysis of effects on ECG,

haemodynamic data, and electrical stimulation parameters in rats and in isolated rat hearts.

## 2.2 Opioid actions

### 2.2.1 Analgesia assays in vivo

Analgesic properties of RSD 939 were measured in mice by the use of the tail pinch method. This involved pinching the tail of the mouse with a guarded arterial clip for a time not greater than 5-10 seconds. The duration of analgesia was not followed, but the fact of its presence or absence within 5 to 15 min of injection. Injection of RSD 939 was from the distal part of the tail via the tail veins using a 1 cc syringe and a 27.5 gauge needle.

Determination of $ED_{50}$, a dose that causes analgesia in 50% of the population studied, was based on the following protocol: Four groups of animals composed of 4 mice per group, were injected with different doses of RSD 939. This gave us 4 dose levels for these groups. Among these 4 groups, at least 2 groups should show partial analgesia. $ED_{50}$ was determined by probit analysis of the dose-analgesia curve in which percentage of animal experiencing analgesia was plotted against log-dose.

## 2.2.2 Binding studies

Opioid receptor binding studies were conducted by Dr. S. Abraham[†]. The experimental procedure used is given below. It is a modification of procedures used by other groups (Millan, 1990; Rothman et al, 1989).

Guinea pig were sacrificed by decapitation and their brains were dissected free of membrane. Brains (excluding cerebella, for μ receptors) or cerebella only (for κ receptors) were homogenized with a polytron homogenizer in 20 volumes of 50 mM Tris HCl (pH 7.5) buffer and centrifuged at 30000 x g for 15 min. The pellet was rehomogenized and centrifuged 2 addition times. Membranes were then suspended in Tris HCl buffer to a tissue concentration of 20 mg wet weight per ml.

Tissue homogenates (0.5ml) were incubated at $25^0$C with [$^3$H]DAGO (0.5 nM) or [$^3$H]U-69593 (0.5 nM) and 9 concentrations of RSD 939 in a total volume of 1 ml Tris HCl buffer. The reaction mixtures were incubated for 90 or 60 minutes, for μ or κ receptors, respectively. All assays were performed in duplicate and two separate determinations were performed for each observation. Nonspecific binding was determined in the presence of 10 μM of morphine or the unlabelled

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[†] Department of Pharmacology, Israel Institute for Biological Research, Ness Ziona, Israel.

ligand U-69593. The reaction was terminated by filtration through glass fiber filters (Whatman GF/B). Radioactivity bound to the filters was measured by lipid scintillation spectrometry.

$IC_{50}$ values were determined by linear regression of the log-probability plots of the displacement curve obtained with each sample.

## 2.3 Cardiovascular assessment

In this study, RSD 939 was given as cumulative i.v. bolus doses to anaesthetized rats whose blood pressure, heart rat and ECG were measured. This study gave an initial profile of the acute cardiovascular and toxic actions of RSD 939, and data from these studies was used to establish the dose range to be used in subsequent isolated heart, electrical stimulation and myocardial ischaemia assays. The involvement of opioid receptor in mediating responses was determined by the administration of naloxone, at a dose which had no cardiovascular or ECG actions but blocked opioid receptors.

### 2.3.1 Surgical preparation

Male Sprague-Dawley rats (Charles River Laboratories, Montreal,

Quebec) weighing 200-300 g were subjected to preparative surgery under a large loading dose of sodium pentobarbitone anaesthesia (65 mg/kg, i.p.). Supplemental doses of diluted pentobarbitone (1/10 dilution) were given i.v. when, and if, necessary to ensure an adequate level of anaesthesia. Body temperature was monitored by a rectal thermometer and maintained between 36-37 °C with a heating lamp.

The right external jugular vein was cannulated for intravenous injections of drugs, while the left carotid artery was cannulated for measurement of mean arterial blood pressure through a calibrated pressure transducer connected to a Grass Polygraph (Model 79D). Animals were placed in a supine position, a tracheotomy performed and a blunt 15 gauge needle was secured in the trachea to prevent obstruction of the airway. However, the animal was not ventilated and was allowed to breathe spontaneously in order to allow the cause of death to be recorded. In order to obtained the best ECG signal in rat, a modified lead II configuration was used. Three ECG needle limb leads were placed subcutaneously along the suspected anatomical axis of the heart (right atrium to apex) as determined by palpation. The superior electrode was placed at the level of the right clavicle, approximately 0.5 cm from the midline of the trachea, and the inferior electrode was placed on the left side of the thorax, approximately 0.5 cm from the midline at the level of

the ninth and tenth ribs (Penz et al., 1992). The ECG records were obtained on a Grass Polygraph using a 7P1F low level preamplifier and associated driver amplifiers at a bandwidth of 0.1-40 Hz. Both blood pressure and ECG measurements were made directly from Grass polygraph records recorded at chart speed of 100 mm $sec^{-1}$.

### 2.3.2 Experimental Design

In vivo dose-response curves were constructed for the effects of RSD 939 at a cumulative dose of 1.0, 2.0, 4.0, 8.0, 16.0 μmole $kg^{-1}$ i.v. or until death occured in pentobarbitone anaesthetized rats (n = 5). In a separate group of animal (n = 5), the effects of naloxone on the cardiovascular and ECG effects produced by RSD 939 were also determined.

In this study, rats were selected at random from a single group. After surgical preparation, they were allowed 30 min to recover before drug administration. In a random and double-blind manner rats were given an initial injection of either saline or 8 μmole $kg^{-1}$ naloxone, a dose which is much higher than the $pA_2$ (Martin, 1983) but shown to have no cardiovascular or ECG actions (Pugsley et al., 1992). This dose could be expected to effectively block any opioid receptor-dependent effects of

RSD 939, even when given at the highest doses. The injection of vehicle, or the first dose of RSD 939 was randomly and blindly given 5 min later. This resulted in four groups of animals (n = 5 each group). Group I was naloxone pretreated rats tested with RSD 939. Group II was naloxone pretreated rats tested with vehicle. Group III and IV were saline pretreated rats tested with RSD 939 and vehicle, respectively. All doses were injected i.v. over 30 sec and blood pressure, heart rate and ECG were recorded 0.5, 1, 2, 4, and 8 min after and immediately prior to addition of the next cumulative dose. The vehicle was 22% ethyl alcohol and 78% distilled water. The maximun volume of vehicle or drug given to the rat was 1.0 ml / 100g body weight. The cause of death in each animal was recorded as being due either to arrhythmias (very uncommon), an irreversible decline in blood pressure, or respiratory failure.

### 2.3.3 Data Analysis

Mean blood pressure, heart rate, and ECG variables were recorded using a Grass Polygraph as described above. The ECG variables were measured manually with a micrometer from recordings made at a chart speed of 100 mm/sec (see Figure 2). The mean blood pressure was taken as an approximate average of systolic and diastolic pressures. Heart rate

was calculated by dividing the R-R distance between two beats in mm into 6000 mm/min (recording was made at 100 mm/sec = 6000 mm/min) to get the number of beats per minute. ECG intervals were defined as follows:

P-R interval - measured from the beginning of the P wave to a line drawn from the peak of the R wave to the isoelectric line following the curvature of the paper.

QRS complex - measured from the beginning of the R wave to the end of the S wave allowing for curvature of the paper.

$QT_1$ interval - measured from the beginning of the R wave to the peak of the T wave (i.e. to a line drawn down from the peak of the T wave to the isoelectric line following the curvature of the paper).

$QT_2$ interval - measured from the beginning of the R wave to the point of inflection of the downstroke of the T wave (again following the curvature of the paper).

RSh - measured from the peak of the R wave to the base of the S wave, again following the curvature of the trace. This new measure, termed "RSh" or RS-height, is a more sensitive measure of sodium channel blockade than conventional measures such as QRS complex widening and P-R interval prolongation (Penz et al., 1992). Penz et al, using various Class I sodium channel blockers, have shown that changes

in RSh occurred before changes in QRS or P-R.

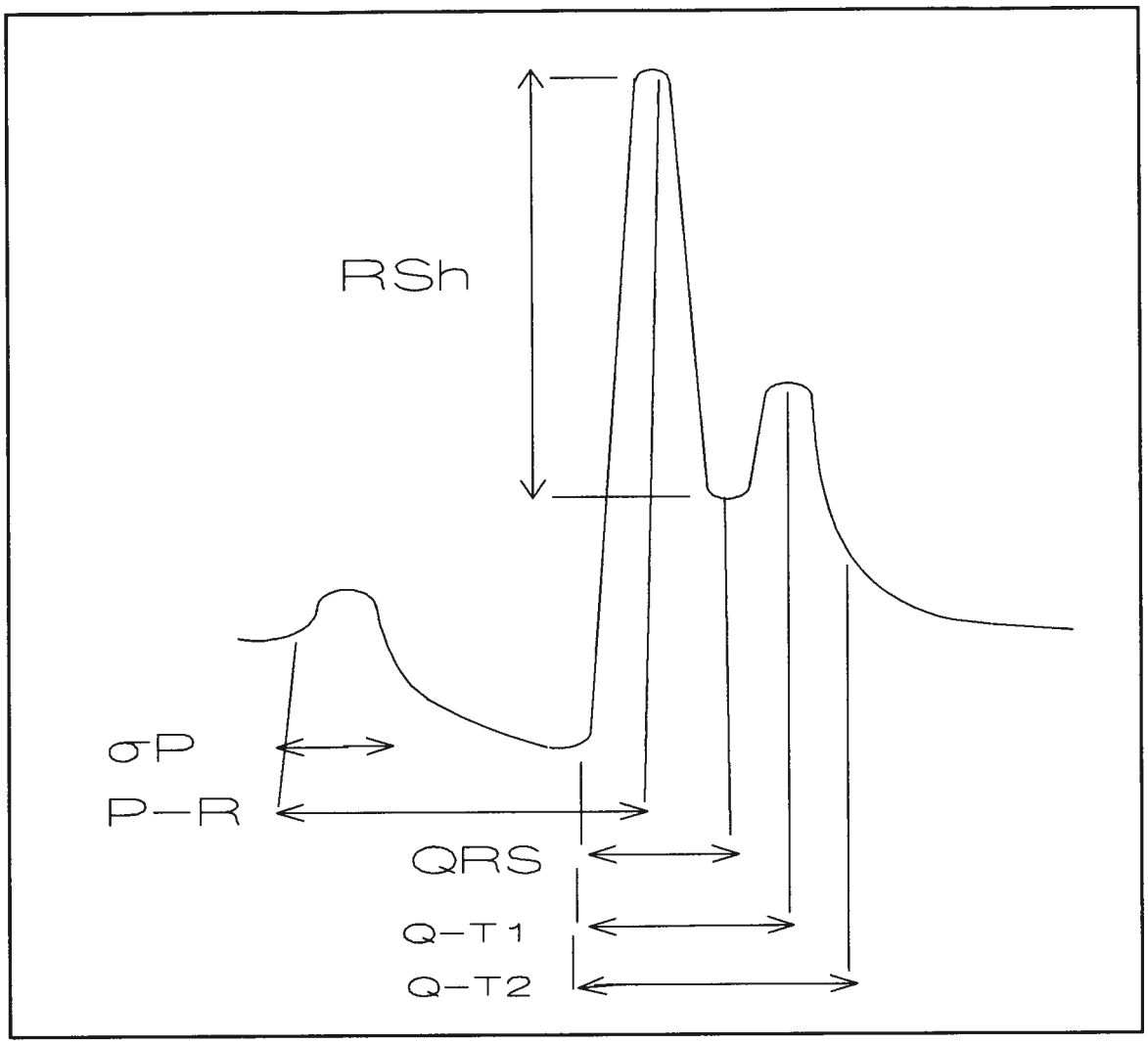


**Figure 2: A typical ECG from the rat. Also shown are the variabless which are measured.**

Differences in effects of blood pressure, heart rate, and ECG intervals between vehicle-control and drug treatment groups were examined by unweighted means ANOVA, and if the sources of variance significant ($p < 0.05$), were followed by Duncan's multiple range test using NCSS computer statistical packages (Hintze, 1987). Since this is a cumulative dose study, the above two statistical tests were also used to test all doses of RSD 939 to determine if drug effects on the variables measured were statistically significant. Thus all data were also compared between pre-drug and dosage level.

## 2.4 Isolated rat hearts

Opioid receptors are found in the vagus nerve, in several sympathetic ganglia as well as the heart, thus opioid peptides can influences the cardiovascular system not only centrally but also peripherally. Since myocardial tissue was the tissue in which antiarrhythmic actions of RSD 939 were expressed, it was of interest to evaluate the cardiac pharmacology of RSD 939 in the absence of neuronal and humoral innervationorganism so conventional studies were performed on unpaced Langendorff perfused rat hearts. The Langendorff rat heart model was first devised by Langendorff in 1895. It has many advantages

for the investigation of the actions of drugs on both the mechanical (contractile force) and electrical (ECG) activity. Yet we also perceived a number of deficiencies of this model. In the Langendorff heart, a filtered Krebs-Henseleit solution is used to replace the blood. This results in gradual failure with edema. In addition, only the ventricles are filled with solution. Measurements of coronary flow by simply collecting the outflow is also subject to considerable error. However, the use of five hearts for each determination tended to reduce such sources of variance and allowed sufficient accuracy for observing pharmacological effects on the myocardium which might explain any antiarrhythmic actions of RSD 939. Other limitations to the use of this method such as the low oxygen-carrying capacity of the perfusate and the ease of damage of the aortic valves which allowed perfusion fluid to enter and distend the left ventricle have been minimized by the use of a modified perfusion apparatus developed by our laboratory (Curtis et al., 1986a).

### 2.4.1 Perfusion apparatus

Experiments were performed using the modified perfusion apparatus designed and constructed in our laboratory to study the actions of drugs on the mechanical and electrophysiological behavior of hearts

from small animals (e.g., rat, guinea pig) hearts (Curtis et al., 1986a). This 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 was designed to allow a low volume dead-space for rapid switching between different solutions in order to permit the rapid generation of dose-response data. Perfusates were kept constant at 37 $^{0}$C by circulating warm water heated by an external thermoregulated heater. 100% $O_2$ was used to oxygenate each solution and to pressurize the chambers (at 100 mmHg) to drive perfusate through the coronary circulation.

### 2.4.2 Preparation

The perfusing solution was formulated in our laboratory to allow maximal oxygen carrying capacity and optimal pH stability. It consisted of (in mM) NaCl, 1.23; KCl, 3.35; $MgSO_4 \cdot 7H_2O$, 1.18; D-Glucose, 11.1; $CaCl_2 \cdot 2H_2O$, 2.52; PIPES (Piperazine-N,N'-bis[2-ethanesulfonic acid]), 14.34 and NaOH titrated to pH 7.4 (Table 9). This newly formulated buffer together with our modified apparatus improved the oxygen delivery to the heart because this system reduces loss of oxygen to the

**Table 9.** **Ionic composition of physiological salt solutions used for cardiac tissue in comparison with rat serum and interstitial fluid**

| | Cations | | | | Anions | | | | | | | | Gas (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Solution | *Na* | *K* | *Ca* | *Mg* | *Cl* | $HCO_3$ | $H_2PO_4$ | $SO_4$ | *OH* | *Urea* | *Glucose* | *PIPES* | $O_2$ | $CO_2$ |
| Total ion concentration in plasma | 152 | 3.7 | 2.7 | 1.06 | 114 | 26.5 | 1.7 | 0.69 | | 7.0 | 5.8 | | | |
| Ionized ion concentration in plasma | 150 | 3.6 | 1.6 | 0.76 | 112 | 25.7 | 1.6 | 0.69 | | 7.0 | 5.8 | | | |
| Ionized ion concentration in interstitial fluid | 147 | 3.5 | 1.5 | 0.72 | 115 | 26.3 | 1.7 | 0.73 | | 7.0 | 5.8 | | | |
| Krebs-Henseleit buffer | 143 | 5.9 | 2.5 | 0 | 128 | 24.9 | 1.18 | 1.64 | | 0 | 5.6 | | 95 | 5 |
| PIPES buffer | 153 | 3.4 | 2.5 | 1.18 | 131 | 0 | 0 | 1.18 | 6.0 | 0 | 11.1 | 14.3 | 100 | 0 |

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

dead space between the reservoir and the heart, and because the apparatus oxygenates preheated buffer, rather than heating pre-oxygenated buffer.

Male Sprague-Dawley rats weighing 300 to 400 g were sacrificed by a blow to the base of the skull and exsanguinated before hearts were rapidly excised from the chest cavity. Hearts were immediately retrogradely perfused with 10 ml of ice-cold PIPES buffer solution and then mounted on the perfusion apparatus via an aortic cannula. Hearts immediately were perfused with an oxygenated PIPES buffer solution at 37 °C and pH 7.4. Within seconds, the heart began beating in sinus rhythm. The left atrium was then removed in order to insert a small compliant, but non-elastic balloon made of plastic wrapping film ("Saran Wrap") into the left ventricle for ventricular pressure measurements (Curtis et al., 1986a). For maximal ventricular contractility, the pressure within the balloon was adjusted to give an left ventricular end-diastolic pressure of 10 mmHg. The aortic root perfusion pressure controlled by the oxygenating gas (100% $O_2$) was kept constant at 100 mmHg to mimic the normal perfusion pressure of the coronary arteries in vivo. Both perfusion pressure and ventricular pressure were measured by pressure transducers while the contractility or the maximal rate of intraventricular pressure development ($+dp/dt_{max}$) and maximal rate of intraventricular 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, 1986) using a Grass Polygraph (model 7D) at a bandwidth of 0.1-40 Hz. Electrodes were placed in an approximately Lead II configuration thus one electrode was placed on the right atrium to allow recording of a large P wave, and the second on the left ventricle. Measurements of mean coronary flow perfusate was done by collecting effluent at one minute intervals in a graduated cylinder.

### 2.4.3 Experimental design and data analysis

To assess direct effects of RSD 939 on rat cardiac tissue in vitro dose-response curves were constructed for the effects of RSD 939 at a cumulative dose of 0.1, 0.3, 1.0, and 3.0 μM on isolated rat hearts (n = 5). In a separate group of hearts (n = 5), the effects of naloxone on the contractility and ECG effects produced by RSD 939 was also determined.

For 15 minutes, hearts were perfused with PIPES buffer solution alone and measurements of heart rate, contractility, and ECG were taken every minute for a minimum of 15 minutes, or until stable control values were obtained. For the dose-response study, RSD 939 at concentrations of 0.1 to 3.0 μM were administered cummulatively for a period of 3

minutes at each concentration. Recordings were made at 0.5, 1, 2, and 3 minutes interval. A 5 minutes wash period then followed. Experiments with naloxone were performed by adding naloxone to the perfusate for a period of 5 minutes before the co-administration of RSD 939 and naloxone. Two sets of control experiments were done. The first one used only the vehicle for RSD 939. The second one used both vehicle and naloxone. The pre-drug period also act as the control values for the experiments.

Heart rate, systolic ventricular pressure, contractility ($+dp/dt_{max}$ and $-dp/dt_{max}$), P-R interval, and QRS complex were measured. However, Q-T was not measured as a result of the difficulty in determining the T-wave in isolated rat hearts. Statistical analysis were performed as in the previous studies.

## 2.5 Electrical stimulaltion

Electrical stimulation was designed to further define the electrophysiological actions of RSD 939 in intacts rats. It involved cumulative i.v. infusions of RSD 939 to anaesthetised rats, with implanted left ventricular electrodes, in order to test for responsiveness to electrical stimulation. Electrical stimulation adds further information regarding

effects on blood pressure, heart rate, ECG and toxicity. A program of electrical stimulation allows assessment 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 test. 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) (Wiggers & Wegria, 1940). On the other hand, pure potassium channel blockers might not affect thresholds for capture yet suppress VF induction by making the heart refractory to the fractionating wavefront (Winslow, 1984). However, a pure potassium channel blocker would be expected to prolong the effective refractory period (ERP-ms) and decrease the maximum following frequency (MFF-Hz) to square wave stimuli (Vaughan-Williams, 1970; 1975). Thus, by testing the drugs for their influence on iT, tT, VFt, ERP, and MFF, we hoped to establish an index of their sodium vs. potassium blocking actions. In addition, electrical stimulation was also useful in the assessment of the potency and efficacy of RSD 939 in protecting against electrically-induced arrhythmias.

### 2.5.1 Experimental preparation

Male Sprague Dawley rats weighing 250 to 350 g were prepared in a manner similar to that used for cardiovascular assessment studies, with one exception. A tracheotomy was performed and rats were artificially ventilated using room air with a Palmer small animal respirator at a stroke volume of 10 ml/kg and rate of 60 strokes/min to ensure appropricate blood-gas levels (MacLean and Hiley, 1988).

The skin above the the level of the heart was removed and palpation performed to determine the position of the left ventricle. Stimulating electrodes were made from Teflon coated silver wire by removing 1-2 mm segment of insulation from the end of the wire which was passed through the lumen of a 27 gauge needle. The desheathed tip of the wire was bent back to form a barb. The needle were passed into the thoracic cavity and the electrode lodged in the left ventricular apical free wall. This process was repeated for a second electrode 1-2 mm apart from the first one. The positioning of the electrodes were confirmed at the end of the experiment by dissection. This technique allowed rapid insertion of stimulating electrodes 1-2 mm apart with minimal trauma. Stimulation of the left ventricle with square wave pulses was with Grass SD9 Stimulators (Howard and Walker, 1990). Subcutaneous ECG electrodes in a Lead II

configuration were used. The ECG and BP were recorded on a Grass Polygraph (model 79D), and a delayed loop oscilloscope (Honeywell Model E for M) was used for continuous assessment of the ECG.

### 2.5.2 Experimental design

In vivo dose-response curves were constructed for the effects of RSD 939 at infusions of 1.0, 2.0, 4.0, 8.0, 16.0 μmole/kg/min i.v. (n = 5). In a separate group of animal (n = 5), the effects of naloxone on responses to RSD 939 were determined.

In this study, rats were selected at random from a single group. After surgical preparation, they were allowed 30 min to recover before drug administration. In a random and double-blind manner rats were given an initial injection of either saline or 8 μmole $kg^{-1}$ naloxone. The infusion of vehicle, or the first dose of RSD 939 was randomly and blindly given 5 min later. This resulted in four groups of animals (n = 5 each group). Group I was naloxone pretreated rats tested with RSD 939. Group II was naloxone pretreated rats tested with vehicle. Group III and IV were saline pretreated rats tested with RSD 939 and vehicle, respectively. Each dose was infused i.v. over 3 minutes and electrical stimulation variables were measured in triplicate after 2 minutes of

infusion. Each animal also acted as its own control since prior to drug infusion, control values of electrical stimulation variables were determined every five minutes until stable control values were obtained. The last set of values was taken as pre-drug values.

Prior to determination of electrical stimulation variables and at the end of the experiment, a 1 ml sample of blood was withdrawn from the carotid artery line and the initial and final [$K^+$] was determined (Ionetics Potassium Analyzer).

### 2.5.3 Experimental end-points

Square waves stimulation was used and discrimination of the end-points were made on an oscilloscope. Each end-point (iT, tT, VFt, ERP, and MFF) was determined in triplicate 2 minutes after commencing each infusion step. The mean value of three measurements were used. The procedures for the end-points measurement has been described by Curtis et al. (1984, 1986).

#### 2.5.3.1 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 were observed:

i) an increase in 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 a pulse width of 1 ms. Theshold current usually falls within the range of 20 to 100 μA.

#### 2.5.3.2 Threshold pulse width

Threshold pulse width for capture (tT) was the minimum duration to capture the heart. It was determined according to the criteria for measuring the threshold current and at twice current threshold. Average threshold pulse widths were 0.3 ms.

#### 2.5.3.3 Ventricular fibrillation threshold

Ventricular fibrillation threshold was defined as the current necessary to produce fibrillation and a precipitous drop in blood pressure.

Periods of stimulation of approximately 4 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 characteristic were generally non-sustained fibrillo-flutter. Therefore, fibrillation threshold measurements allowed determination of the effectiveness of RSD 939 against a non-damaging type of arrhythmia.

#### 2.5.3.4 Effective refractory period and maximum following frequency

Effective refractory period (ERP) in the ventricle was defined as the shortest interval between two stimuli to which the ventricle responds. It was determined by the extra-stimulus method. In this method the heart is paced at a baseline frequency of 7.5 Hz, twice the threshold current and twice the threshold pulse width. A single extra stimulus of the same frequency, current strength, and pulse width was applied at a variable delay after the pacing stimuli. The minimum delay at which an 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, a steadily increasing frequency of stimulation from a baseline of 5 Hz. It was determined by 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. In addition, there was a missing beat on the ECG. The maximum following freqency is reciprocally related to the effective refractory period and interventions which increase effective refractory period could be expected to decrease the maximum following frequency.

### 2.5.4 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 (NCSS package, Hintze, 1987).

## 2.6 Myocardial ischaemia-induced arrhythmias

This set of experiments involved estimation of the antiarrhythmic activity of RSD 939 in an anaesthetized rat model of coronary occlusion. Occlusion was made of the coronary artery in the presence of an infusion of RSD 939. The dependency of opioid receptors on the antiarrhythmic activity of RSD 939 was determined with the use of naloxone.

### 2.6.1 Experimental preparation

Male Sprague Dawley rats (250-350 g) were subjected to preparative surgery similar to those in electrical stimulation studies but with a left coronary artery occluder implanted. The occluder, manufactured from polyethylene, was first described by Au et al. (1979) and Johnston et al. (1983). Its design and manufacture have been extensively described by Curtis et al. (1986). 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 procedure used was a modification of techniques described by John and Olsen (1954), and they were the same as those

employed by Au et al. (1979) and Paletta et al. (1989). After tracheotomy and cannulation of carotid artery and jugular vein, an incision was made through the skin at the base of the sternum, using blunt scissors. The skin was loosened from the underlying muscle mass using blunt dissection to the base of the neck. The skin was then cut from the base of the sternum to the neck and peeled back, revealing the chest musculature. Artificial respiration was applied at this time using 100% oxygen to ensure adequate oxygenation. Fine pointed scissors were used to make a 1 cm skin incision over the 4th to 6th ribs on the left thorax and this was enlarged by blunt dissection. The forceps were then inserted under the pectoralis muscle, which was gently separated from the underlying rectus abdominus, exposing the Intercostal muscles beneath. The 5th or 6th intercostal space was then punctured and this incision was enlarged by blunt dissection. If the heart was exposed unfavourably for placement of the occluder, then a second intercostal incision was made. This was necessary in less than 5% of preparations. Retractors were used to widen the intercostal incision and hold the chest wall back, and blunt forceps were used to open the pericardium. By inserting the retractors' tips through the small pericardial tear, such that the pericardium was included with the retracted tissue, the heart was lifted toward the intercostal incision and a pericardial cradle that facilitated subsequent surgery was

created.

The procedures for ligation of the left coronary artery has been described in detail by Johnston et al. (1983) and Curtis et al. (1986). The left anterior descending coronary artey (LAD) is predominant and supplies the left ventricle. There is no true circumflex artery in the rat. The coronary arteries lie beneath the epicardium and sometimes can be seen at operation in the intact beating heart as tiny red streaks beneath the surface of the heart. In our experiments, the LAD was located using the highly visible coronary veins as landmarks. In the rat the main left coronary artery can be ligated at a point just beneath the left auricular appendage. Occasionally, branching will have already begun under the left atria. In this situation it is necessary to ligate several branches at the same time in order to obtain a good-sized area of infarct. A good-sized area of infarct is important since it has been shown that the incidence and severity of arrhythmia correlates with the size of the infarct (Johnston et al., 1983, 1983a).

In the experiments, the left atria was lifted with wetted cotton stick (Q-tips). The needle of the polypropylene suture of the occluder, held in straight hemostats, was looped under the LAD. The suture was then sewn through the flared end of the guide tubing and was cut off and melted down to form a small ball. A loose occluder was thus implanted in the

ventricular muscle, with the needle entered and left the myocardium approximately 2 mm either side of the artery, to ensure that the artery was occluded. Occasionally, there was a minor bleeding, amounting to less than 1 ml of blood. Any bleeding was stopped by allowing the blood to clot and the thoracic cavity was cleared of excess blood. The chest was closed with silk sutures and negative pressure was applied inserting a length of PE90 polythene tubing as the chest was closed to prevent pneumothorax.

ECG and blood pressure recordings were made as in previous cardiovascular assessment and electrical stimulation studies.

### 2.6.2 Experimental design

The antiarrhythmic actions of RSD 939 at a high dose of 1.5 μmole/kg/min i.v., a dose chosen from the previous dose-response studies as one that produced significant, but not maximal ECG, heart rate, and blood pressure changes, and at a low dose of 0.5 μmole/kg/min, a dose which had minimal effects on ECG, heart rate and blood pressure but noticeable effect on RSh, were examined in pentobarbitone anaesthetized rats subjected to occlusion of the left anterior descending

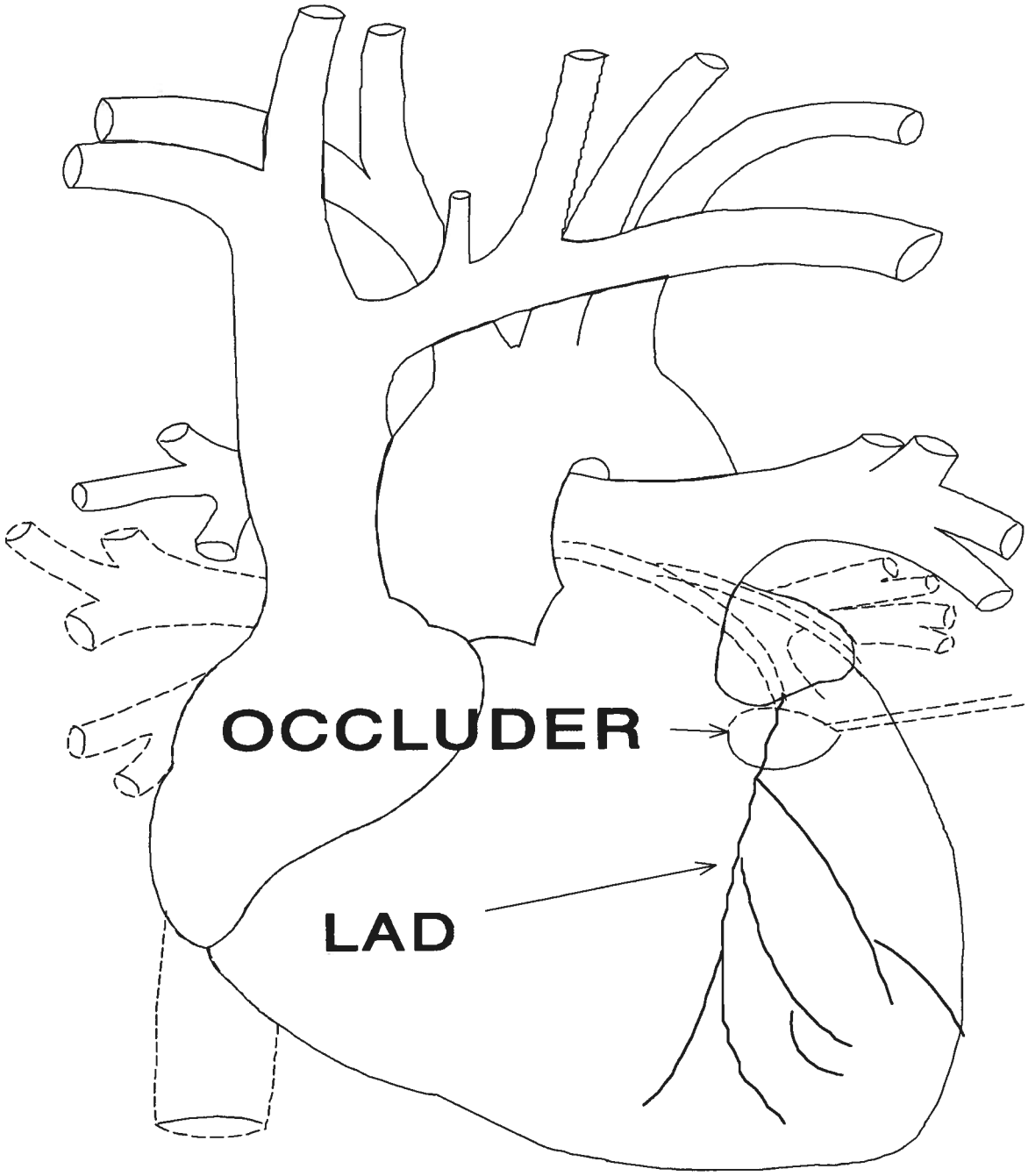


Figure 21. Diagram of the rat heart showing approximate placement of the occluder around the left anterior descending coronary artery (LAD).

coronary artery. In a separate group of animal, the effects of naloxone on the ischaemia induced arrhythmias reduced by RSD 939 was also determined.

The animal was allowed to recover for 30 minutes prior to drug administration. A blood sample of approximately 1 ml was drawn to measure serum potassium levels prior to drug administration and at the end of the experiment if the animal survived. Drug were administered in a double blind randomized design as described earlier in cardiovascular assessment studies and electrical stimulation studies.

In a random and double-blind manner rats were given an initial injection of either saline or 8 μmole $kg^{-1}$ naloxone. The infusion of vehicle, or RSD 939 was randomly and blindly given 5 min later. This resulted in four groups of animals (n = 5 each group). Group I was naloxone pretreated rats tested with RSD 939. Group II was naloxone pretreated rats tested with vehicle. Group III and IV were saline pretreated rats tested with RSD 939 and vehicle, respectively. Blood pressure and ECG were recorded 5 minutes after begining of infusion. Thereafter the occluder was pulled so as to produce coronary artery occlusion. ECG, arrhythmias, blood pressure, heart rate, and mortality were monitored for 30 minutes after occlusion, and recordings at fast paper speed (100 mm/sec) were taken every minutes for the first 5

minutes and every 5 minutes thereafter. Arrhythmias during the monitoring period were diagnosed from the oscilloscope screen and noted directly on the chart for analysis, as described below. The criteria for exclusion have been detailed by Curtis (1986). In the event of exclusion of a rat by these criteria the treatment was immediately repeated in another rat before continuation.

All rats surviving 30 minutes were sacrificed by an overdose of pentobarbitone. After death, the size of the occluded zone was measured by perfusing the hearts by the Langendorff technique with PIPES solution. Blood quickly washed out of all areas except the infarct. This was followed by perfusion with PIPES solution containing 1 mg/ml indocyanine (Fast green dye. BDH) for 60 sec. The perfused tissue stained dark green and the ischaemic area (occluded zone) remain red. The occluded zone was then cut out and weighed as the percent of the whole ventricular weight.

### 2.6.3 Data analysis

#### 2.6.3.1 S-T segment and R-wave amplitude changes post-occlusion

Coronary occlusion produces a rapid increase in ECG signal, characterized by a large increase in R-wave amplitude and an initial depression of S-T segment. The increase in R-wave amplitude gradually returns towards baseline (pre-occlusion) with time (Johnston et al., 1981). R-wave height was measured from the isoelectric baseline to the peak of the positive deflection and was expressed in mV. Follows an initial decrease, the S-T segment elevates and is 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 were produced by occlusion, and presumably myocardial ischaemia, they were not the primary concern of the present study.

### 2.6.3.2 Analysis of arrhythmia

Ischaemia-induced arrhythmias appear in a biphasic time-dependent manner corresponding to early arrhythmias (0-0.5 hr) and late arrhythmias (0.5-4.0 hr) (Johnston et al., 1981). In these experiments, the antiarrhythmic actions of RSD 939 were only studied in the early arrhythmia phase.

Arrhythmias were analyzed according to the guidelines established by the Lambeth conventions (Walker et al., 1988) and Curtis (1986) as premature ventricular contractions (PVC), ventricular tachycardia (VT) or ventricular fibrillation (VF). The arrhythmia history of each rat was expressed as an arrhythmia score (AS) (Curtis and Walker, 1988).

PVC 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 (bigemini) 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 arrhythmia but rather were pooled implying that they were one and the same arrhythmia.

VT was defined as a run of 4 or more consecutive extrasystoles with a clearly distinguishable QRS and were not subclassified according

to rate. VT was subdivided into spontaneously reverting VT (SVT), lasting less than 10 sec, and non-spontaneously reverting VT (NSVT), which lasted more than 10 sec or was irreversible. A drop 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 QRS complex and a blood pressure of less than 10 mmHg. As in VT, any VF lasted less than 10 sec was defined as spontaneously reverting VF (SVT), and any VF lasted more than 10 sec or irreversible was defined as non-spontaneously reverting VF (NSVF).

An arrhythmia score, an arbitrary numerical grading of the severity of ventricular arrhythmias with time post-occlusion, was used to summarize the arrhythmia profile of each animal. There are many possible different scoring systems (Curtis and Walker, 1988) but the following scoring system was used.

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

# 3 Results

## 3.1 Opioid effects

**Table 10.** Binding studies

| | μ opioid receptor | κ opioid receptor |
|---|---|---|
| $IC_{50}$ | 1.0 μM | 0.006 μM |

**Table 11.** Analgesia assays

| | Tail Pinch |
|---|---|
| $ED_{50}$ | 1.5 μmole/kg |

In the above two assays, it was found that RSD 939 binded 1000 times more selective to κ receptor, and it produced analgesia at an $ED_{50}$ of 1.5 μmole/kg (Table 10 & 11).

## 3.2 Haemodynamic effects of RSD 939 in vivo

In intact pentobarbitone anaesthetized rats, dose-response curves (Figure 3 & Figure 4) and $ED_{25}$ values (Table 1) for effects of RSD 939 on blood pressure and heart rate in the presence and absence of naloxone pre-treatment were obtained. RSD 939 dose-dependently lowered blood pressure and heart rate in a statistically significant manner after 1 to 2 μmole/kg as compared with the vehicle control. The $ED_{25}$, for 25 % changes from pre-treatment values, for both blood pressure and heart rate were 8.0 μmole/kg. All tested animals died from an irreversible decline in blood pressure after 32 μmole/kg. Effects of RSD 939 on blood pressure and heart were reduced by pre-treatment with naloxone and significant depression of blood pressure and heart rate was not observed until after 4 to 8 μmole/kg in the naloxone pre-treated group. In this group, $ED_{25}$ for blood pressure and heart rate were 12.0 μmole/kg and 15.0 μmole/kg, respectively. In the saline vehicle control group, both blood pressure and heart rate were stable over the measurement period. Naloxone alone produced no statistical significant haemodynamic effects.

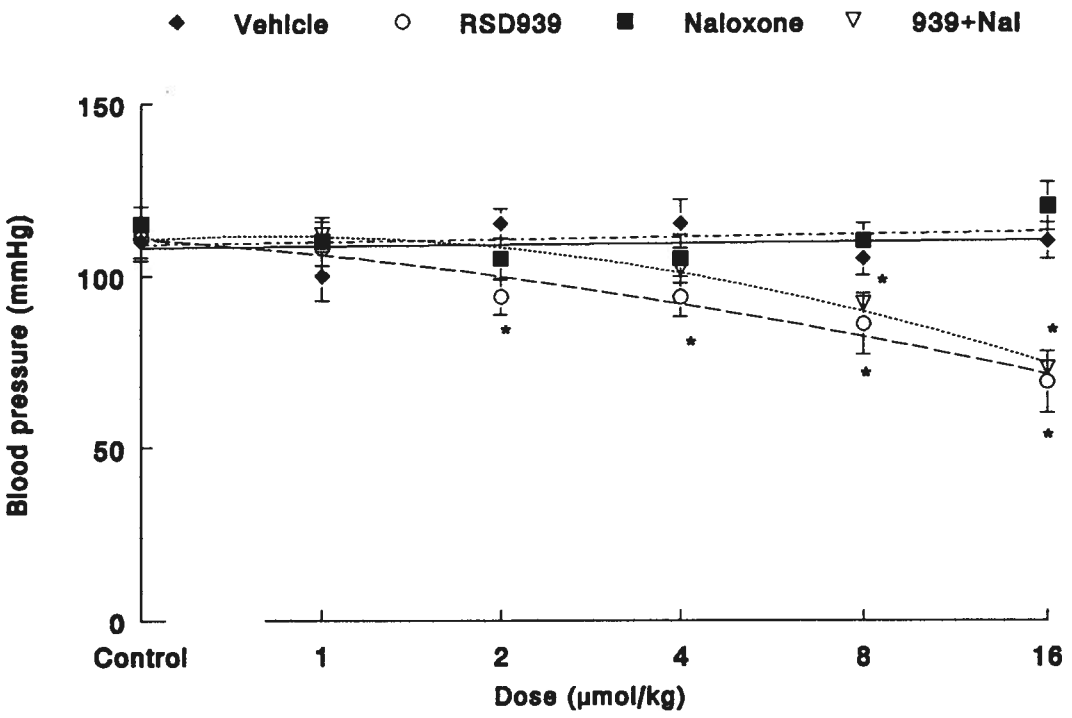


Figure 3. Dose-response effects of RSD 939 in the presence and absence of naloxone pre-treatment, effects of vehicle control, and effects of naloxone on mean arterial blood pressure in pentobarbital anaesthetized rats. Intravenous doses were given every 8 minutes. Measurements were made 0.5, 1.0, 2.0, 4.0, and 8.0 minutes after drug administration, and the peak effects of these measurements used. The groups indicated are: ◆ = saline pre-treated vehicle control; ○ = cumulative doses of RSD 939; ■ = naloxone pre-treated vehicle control (8 µmole/kg); ▽ = cumulative doses of RSD 939 with naloxone pre-treatment. Controls are the pre-drug values or the post-naloxone treatment values for the naloxone pretreated groups. * indicates $p<0.05$ as compared to the vehicle control group. † indicates significant difference between RSD 939 in the presence and absence of naloxone pre-treatment at $p<0.05$ (ANOVA and Duncan's range test). All values are expressed as mean ± S.E.M. with n = 5 per group.

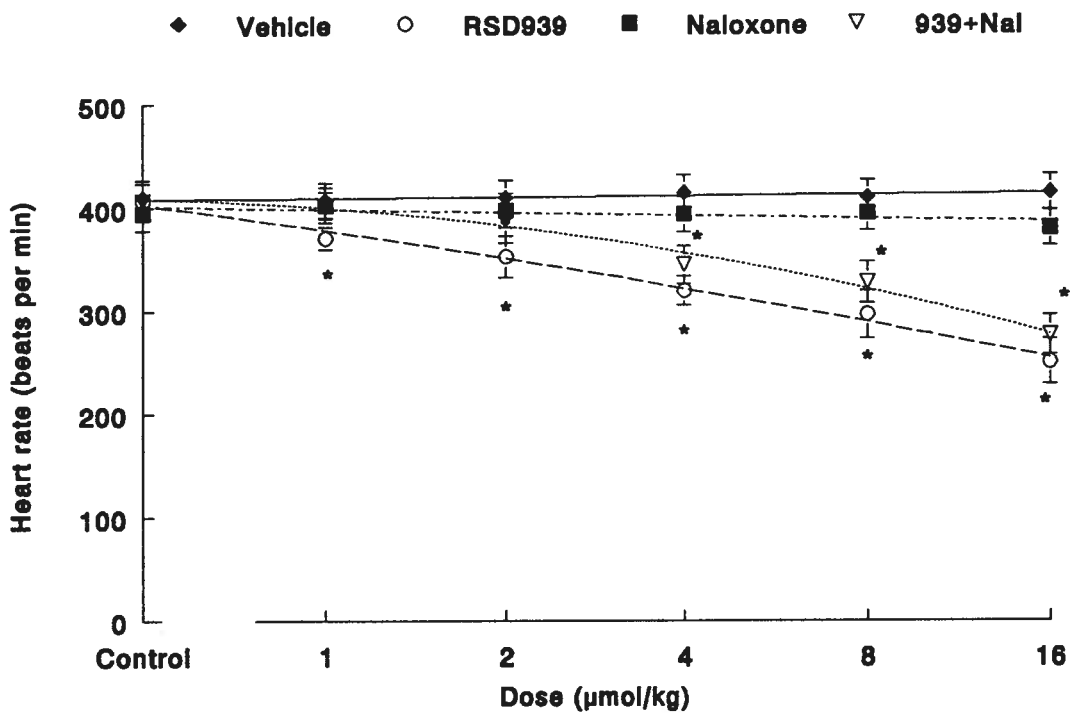


Figure 4. Dose-response effects of RSD 939 in the presence and absence of naloxone pre-treatment, effects of vehicle control, and effects of naloxone on heart rate in pentobarbital anaesthetized rats. Intravenous doses were given every 8 minutes. Measurements were made 0.5, 1.0, 2.0, 4.0, and 8.0 minutes after drug administration, and the peak effects of these measurements used. The groups indicated are: ◆ = saline pre-treated vehicle control; ○ = cumulative doses of RSD 939; ■ = naloxone pre-treated vehicle control (8 µmole/kg); ▽ = cumulative doses of RSD 939 with naloxone pre-treatment. Controls are the pre-drug values or the post-naloxone treatment values for the naloxone pre-treated groups. * indicates $p<0.05$ as compared to the vehicle control group. † indicates significant difference between RSD 939 in the presence and absence of naloxone pre-treatment at $p<0.05$ (ANOVA and Duncan's range test). All values are expressed as mean ± S.E.M. with n = 5 per group.

Table 1. Potencies of RSD 939 in the presence and absence of naloxone pre-treatment with respect to haemodynamic responses in vivo.

| Group | Blood Pressure | Heart Rate |
|---|---|---|
| | $ED_{25}$ (μmole/kg) | |
| RSD 939 | 7.7 | 8.0 |
| RSD 939 + Nal | 12.0 | 15.0 |

The potencies of RSD 939 in the presence and absence of naloxone pre-treatment with respect to blood pressure (BP), and heart rate (HR) in pentobarbital anaesthetized rats. Nal = naloxone (8μmole/kg) pre-treatment. Values are expressed as the dose required to produce a 25% change from the pre-drug values, i.e. $ED_{25}$.

## 3.3 ECG effects of RSD 939 in vivo

ECG measures in intact rats were also influenced in a dose-related manner by RSD 939 both in the presence and absence of naloxone pre-treatment (Figure 5 to Figure 9). No statistical significant difference was found between the RSD 939 group and the naloxone pre-treated RSD 939 group. The P-R, QRS, and RSh measure from the ECG were significantly increased at doses of 1 μmole/kg or 2 μmole/kg. Although significant prolongation of QRS interval occurred at 2 μmole/kg, RSD 939 was not as potent in increasing the QRS duration as compared with P-R interval and RSh. The $ED_{25}$ value for QRS interval was 26.0 μmole/kg, 5 times higher than the $ED_{25}$ values for P-R interval (4.6) and RSh (6) (Table 2). The ECG measurements least influenced by RSD 939 were QT1 and QT2. These intervals were not statistically significantly increased until 4 μmole/kg. $ED_{25}$ values for QT2 and QT1 were 11.0 and 16.0 μmole/kg, respectively. Vehicle and naloxone pre-treatment alone had no statistically significant effects on the ECG measures except for a slight increase in P-R interval after naloxone.

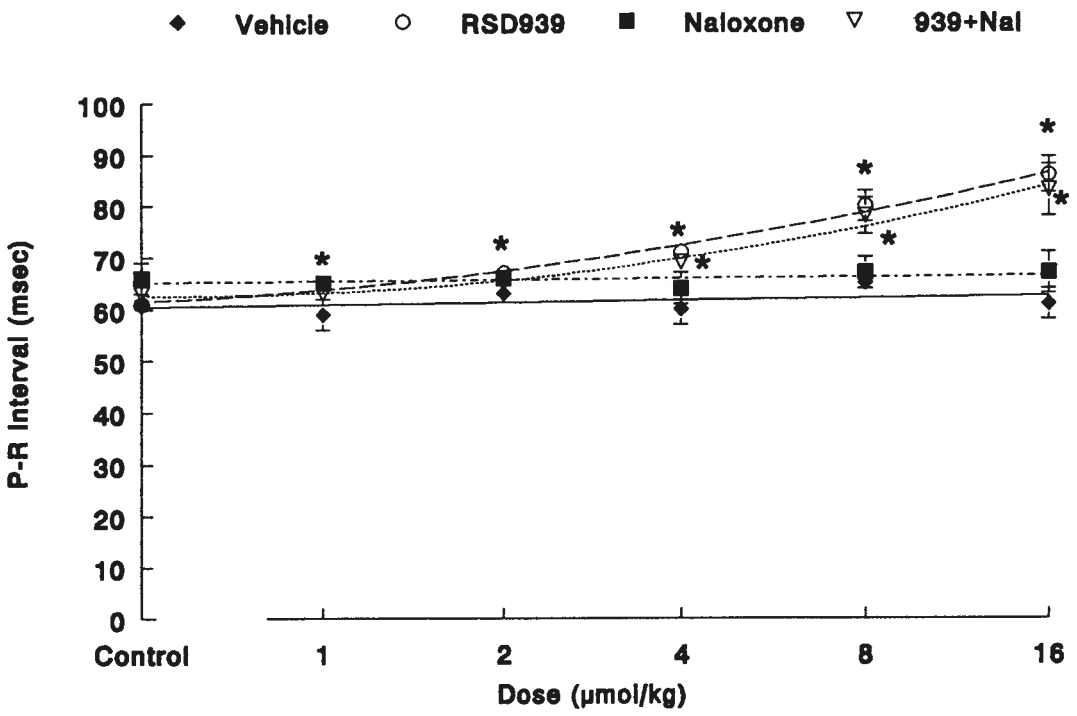


Figure 5. Dose-response effects of RSD 939 in the presence and absence of naloxone pre-treatment, effects of vehicle control, and effects of naloxone on P-R interval of ECG in pentobarbital anaesthetized rats. Intravenous doses were given every 8 minutes. Measurements were made 0.5, 1.0, 2.0, 4.0, and 8.0 minutes after drug administration, and the peak effects of these measurements used. The groups indicated are: ◆ = saline pre-treated vehicle control; o = cumulative doses of RSD 939; ■ = naloxone pre-treated vehicle control (8 µmole/kg); ▽ = cumulative doses of RSD 939 with naloxone pre-treatment. Controls are the pre-drug values or the post-naloxone treatment values for the naloxone pre-treated groups. * indicates $p<0.05$ as compared to the vehicle control group. † indicates significant difference between RSD 939 in the presence and absence of naloxone pre-treatment at $p<0.05$ (ANOVA and Duncan's range test). All values are expressed as mean ± S.E.M. with n = 5 per group.

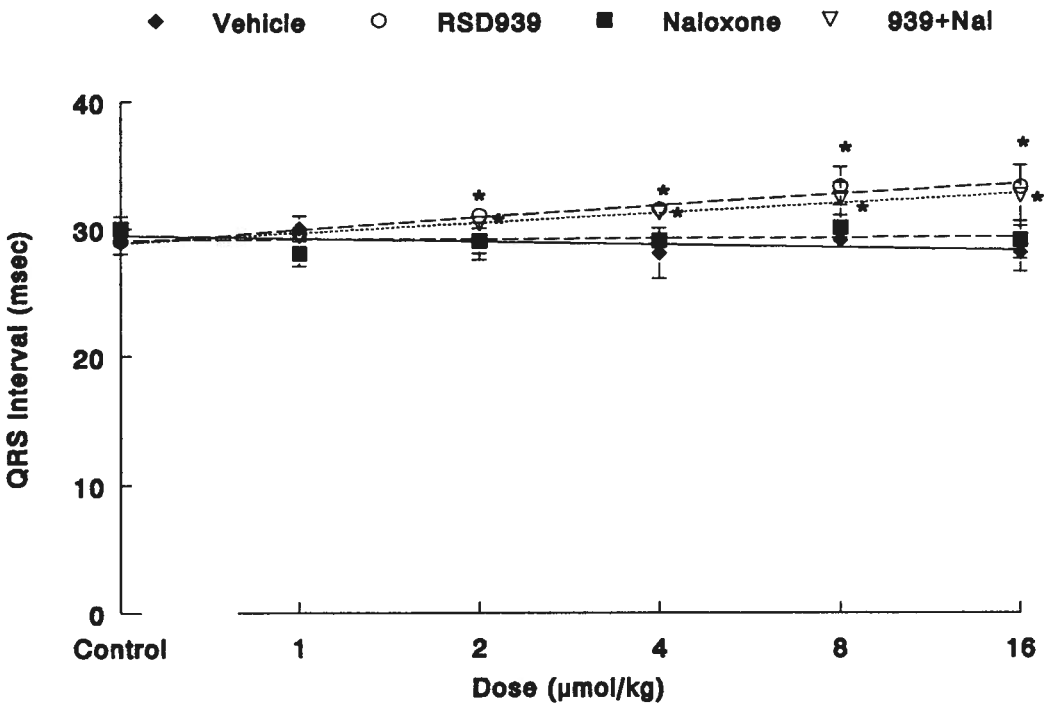


Figure 6. Dose-response effects of RSD 939 in the presence and absence of naloxone pre-treatment, effects of vehicle control, and effects of naloxone on QRS interval of ECG in pentobarbital anaesthetized rats. Intravenous doses were given every 8 minutes. Measurements were made 0.5, 1.0, 2.0, 4.0, and 8.0 minutes after drug administration, and the peak effects of these measurements used. The groups indicated are: ◆ = saline pre-treated vehicle control; ○ = cumulative doses of RSD 939; ■ = naloxone pre-treated vehicle control (8 µmole/kg); ▽ = cumulative doses of RSD 939 with naloxone pre-treatment. Controls are the pre-drug values or the post-naloxone treatment values for the naloxone pre-treated groups. * indicates $p<0.05$ as compared to the vehicle control group. † indicates significant difference between RSD 939 in the presence and absence of naloxone pre-treatment at $p<0.05$ (ANOVA and Duncan's range test). All values are expressed as mean ± S.E.M. with n = 5 per group.

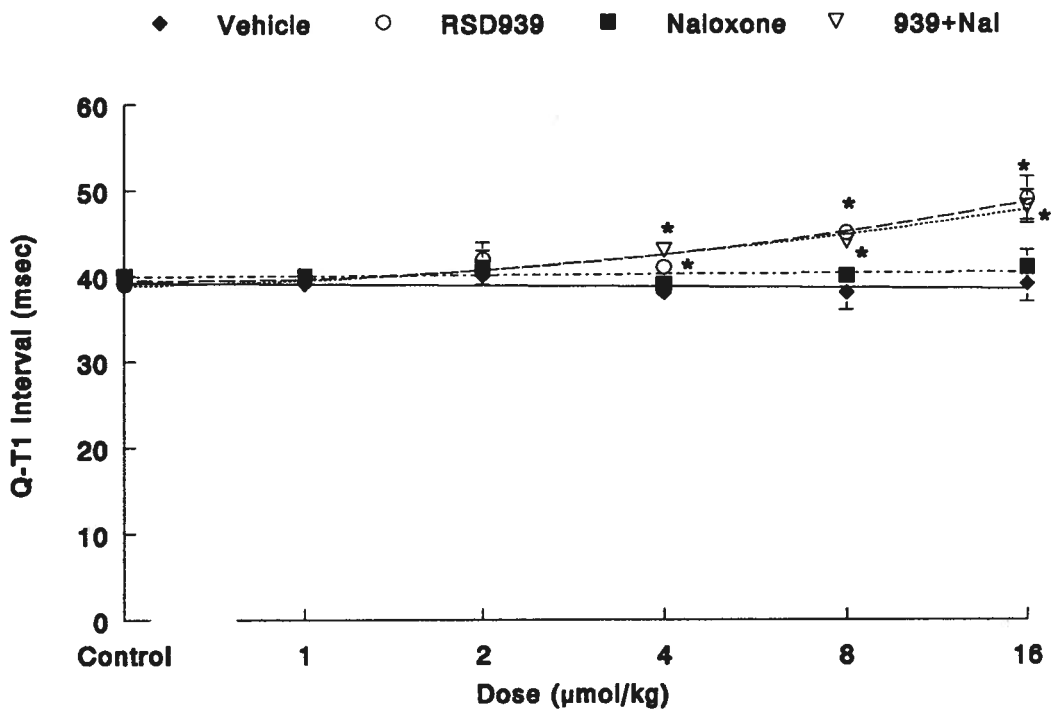


Figure 7. Dose-response effects of RSD 939 in the presence and absence of naloxone pre-treatment, effects of vehicle control, and effects of naloxone on Q-T1 interval of ECG in pentobarbital anaesthetized rats. Intravenous doses were given every 8 minutes. Measurements were made 0.5, 1.0, 2.0, 4.0, and 8.0 minutes after drug administration, and the peak effects of these measurements used. The groups indicated are: ◆ = saline pre-treated vehicle control; ○ = cumulative doses of RSD 939; ■ = naloxone pre-treated vehicle control (8 µmole/kg); ▽ = cumulative doses of RSD 939 with naloxone pre-treatment. Controls are the pre-drug values or the post-naloxone treatment values for the naloxone pre-treated groups. * indicates $p<0.05$ as compared to the vehicle control group. † indicates significant difference between RSD 939 in the presence and absence of naloxone pre-treatment at $p<0.05$ (ANOVA and Duncan's range test). All values are expressed as mean ± S.E.M. with n = 5 per group.

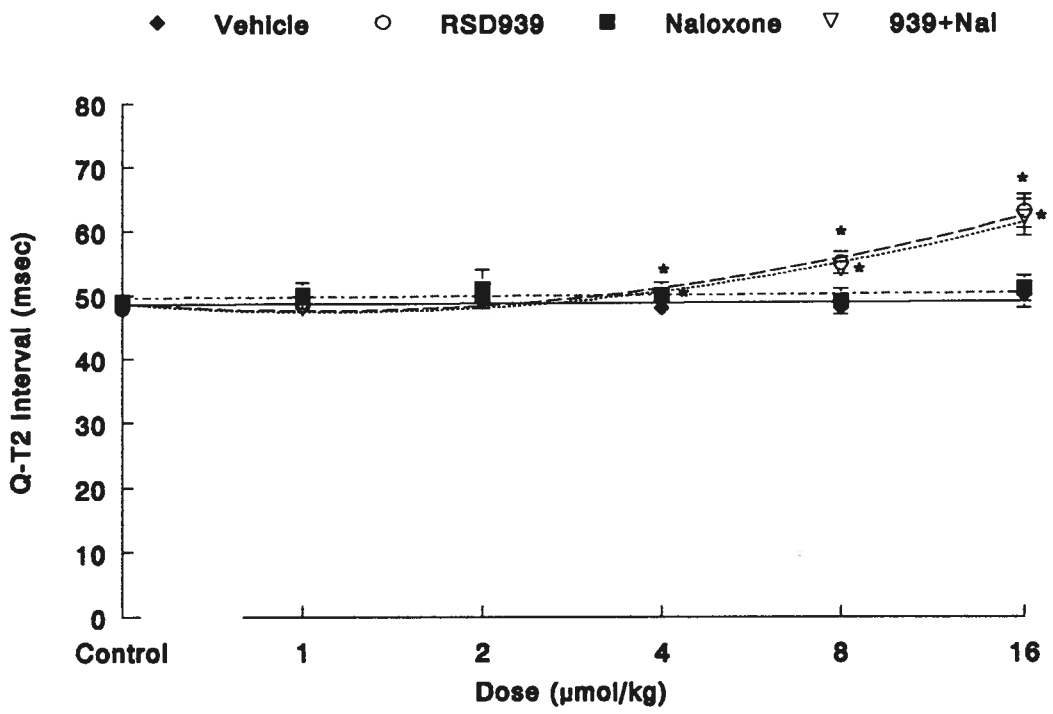


Figure 8. Dose-response effects of RSD 939 in the presence and absence of naloxone pre-treatment, effects of vehicle control, and effects of naloxone on Q-T2 interval of ECG in pentobarbital anaesthetized rats. Intravenous doses were given every 8 minutes. Measurements were made 0.5, 1.0, 2.0, 4.0, and 8.0 minutes after drug administration, and the peak effects of these measurements used. The groups indicated are: ◆ = saline pre-treated vehicle control; o = cumulative doses of RSD 939; ■ = naloxone pre-treated vehicle control (8 µmole/kg); ▽ = cumulative doses of RSD 939 with naloxone pre-treatment. Controls are the pre-drug values or the post-naloxone treatment values for the naloxone pre-treated groups. * indicates $p<0.05$ as compared to the vehicle control group. † indicates significant difference between RSD 939 in the presence and absence of naloxone pre-treatment at $p<0.05$ (ANOVA and Duncan's range test). All values are expressed as mean ± S.E.M. with n = 5 per group.

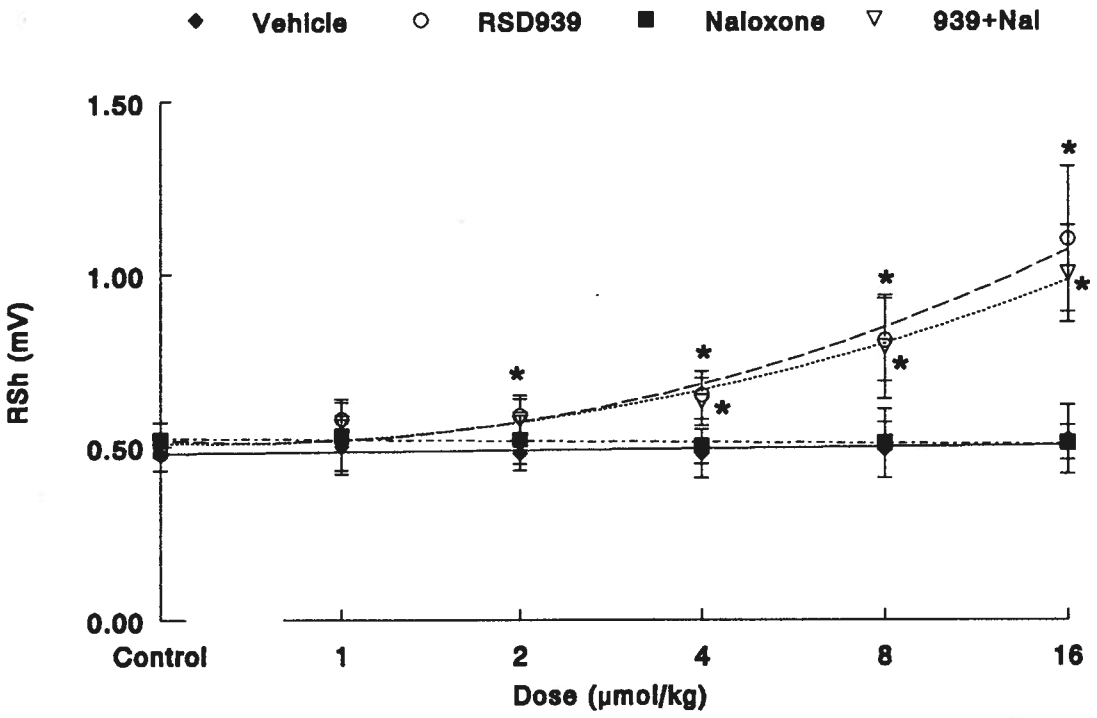


Figure 9. Dose-response effects of RSD 939 in the presence and absence of naloxone pre-treatment, effects of vehicle control, and effects of naloxone on RSh of ECG in pentobarbital anaesthetized rats. Intravenous doses were given every 8 minutes. Measurements were made 0.5, 1.0, 2.0, 4.0, and 8.0 minutes after drug administration, and the peak effects of these measurements used. The groups indicated are: ◆ = saline pre-treated vehicle control; ○ = cumulative doses of RSD 939; ■ = naloxone pre-treated vehicle control (8 µmole/kg); ▽ = cumulative doses of RSD 939 with naloxone pre-treatment. Controls are the pre-drug values or the post-naloxone treatment values for the naloxone pre-treated groups. * indicates $p<0.05$ as compared to the vehicle control group. † indicates significant difference between RSD 939 in the presence and absence of naloxone pre-treatment at $p<0.05$ (ANOVA and Duncan's range test). All values are expressed as mean ± S.E.M. with n = 5 per group.

Table 2. Potencies of RSD 939 in the presence and absence of naloxone pre-treatment with respect to ECG responses in vivo.

| Group | P-R | QRS | Q-T1 | Q-T2 | RSh |
|---|---|---|---|---|---|
| | | $ED_{25}$ (μmole/kg) | | | |
| RSD 939 | 4.6 | 26.0# | 16.0 | 11.0 | 6.0 |
| RSD 939 + Nal | 7.0 | 28.0# | 17.0# | 11.0 | 8.5 |

The potencies of RSD 939 in the presence and absence of naloxone pre-treatment with respect to P-R interval, QRS interval, Q-T1 interval, Q-T2 interval, and RSh in pentobarbital anaesthetized rats. Nal = naloxone (8μmole/kg) pre-treatment. Values are expressed as the effective dose necessary to produce 25% change from the pre-drug values, $ED_{25}$. # indicates the values of QRS interval and Q-T1 interval are extrapolated from the extended portion of the dose-response curve.

## 3.4 In vitro effects of RSD 939

Figure 15,16,17, and 20 shows the effects of RSD 939, in the presence and absence of naloxone, on heart rate, peak systolic left ventricular pressure, and contractility in isolated perfused rat hearts. Corresponding changes in ECG intervals recorded from isolated perfused rat hearts are shown in Figure 18 and Figure 19.

In contrast with the haemodynamic effects observed with RSD 939 in vivo, concentration-related bradycardia and reduction in ventricular pressure were not seen in isolated perfused rat hearts. RSD 939 produced no significant changes in heart rate, peak systolic left ventricular pressure, maximum rate of intraventricular pressure development ($+dp/dt_{max}$), and maximal rate of intraventricular relaxation ($-dp/dt_{max}$) at the tested dose range of 0.1 to 3 μM in vitro. $ED_{25}$ values could not therefore be estimateabled (Table 5). The only notable difference in the dose-response curves were a tendency for higher peak systolic pressure, $+dp/dt_{max}$, and $-dp/dt_{max}$, and a slight decrease in heart rate in the presence of RSD 939.

RSD 939 concentration-dependently prolonged both P-R interval and QRS duration. The P-R and QRS prolongation were statistically significant at 1 μM when compared to vehicle control. As with effects

observed in intact rats, RSD 939 had a much lesser effect on QRS. The potency of RSD 939 on QRS duration was 10 times lower than its potency on P-R interval. The $ED_{25}$ values for P-R interval and QRS duration were found to be 1.0 μM and 10.0 μM, respectively (Table 6).

All of the above effects were still present in the presence of 1.0 μM naloxone. No significant difference on the above measures were found between the naloxone pre-treated RSD 939 group and RSD 939 group. The effects of naloxone on isolated hearts were not statistically different from those of vehicle control. The slight increase in P-R interval after naloxone treatment observed in intact rats were also seen in isolated perfused rat hearts though to a lesser degree.

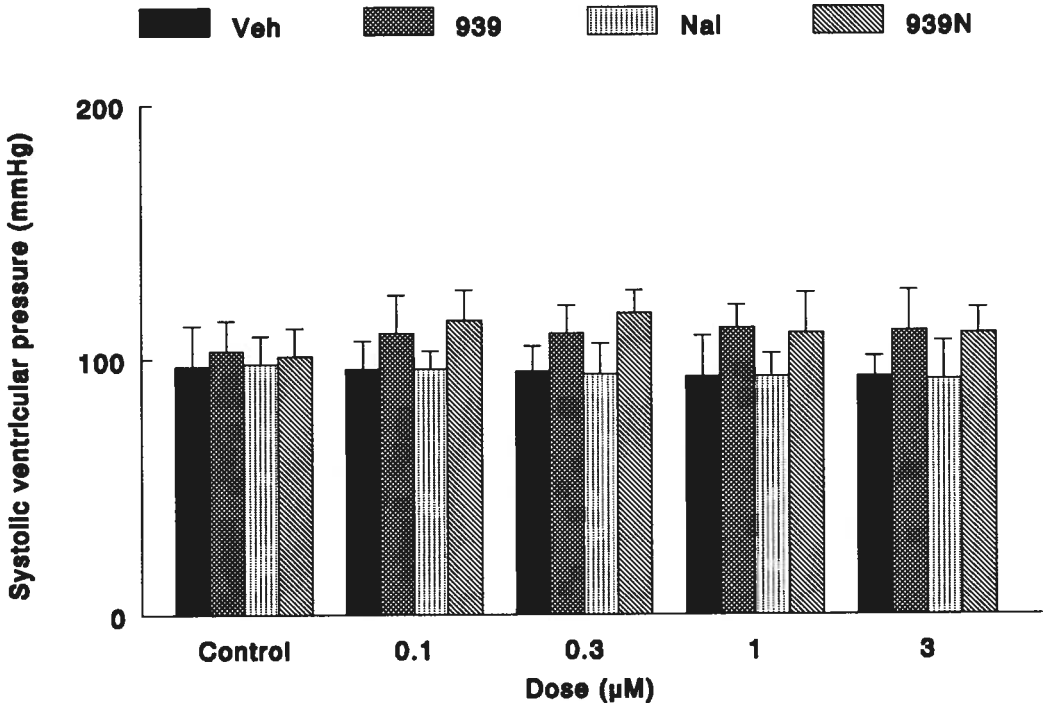


Figure 15. Concentration-response effects of RSD 939 in the presence and absence of naloxone pre-treatment, effects of vehicle control, and effects of naloxone on systolic ventricular pressure in isolated rat hearts. Hearts were perfused with PIPES buffer solution containing either vehicle, RSD 939, naloxone, or RSD 939 plus naloxone. Each dose of drug was infused for 3 minutes, and the steady state values at 3 minutes used in the analysis. The groups indicated are: Veh = vehicle control; 939 = cumulative concentrations of RSD 939; Nal = naloxone (1.0 μM); 939N = cumulative concentrations of RSD 939 with naloxone. Controls are the pre-drug values. * indicates $p<0.05$ as compared to the vehicle control group. † indicates significant difference between RSD 939 in the presence and absence of naloxone pre-treatment at $p<0.05$ (ANOVA and Duncan's range test). All values are expressed as mean ± S.E.M. with n = 5 per group.

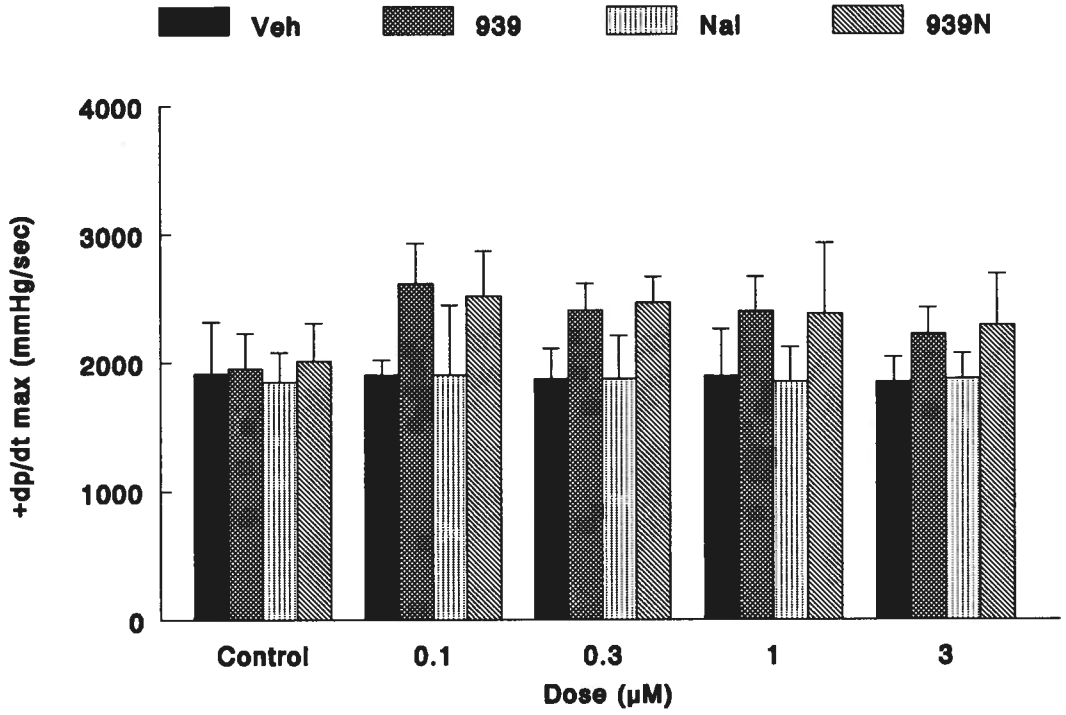


Figure 16. Concentration-response effects of RSD 939 in the presence and absence of naloxone pre-treatment, effects of vehicle control, and effects of naloxone on a measure of contractility, the maximal rate of intraventricular pressure development ($+dp/dt_{max}$) in isolated rat hearts. Hearts were perfused with PIPES buffer solution containing either vehicle, RSD 939, naloxone, or RSD 939 plus naloxone. Each dose of drug was infused for 3 minutes, and the steady state values at 3 minutes used in the analysis. The groups indicated are: Veh = vehicle control; 939 = cumulative concentrations of RSD 939; Nal = naloxone (1.0 μM); 939N = cumulative concentrations of RSD 939 with naloxone. Controls are the pre-drug values. * indicates $p<0.05$ as compared to the vehicle control group. † indicates significant difference between RSD 939 in the presence and absence of naloxone pre-treatment at $p<0.05$ (ANOVA and Duncan's range test). All values are expressed as mean ± S.E.M. with n = 5 per group.

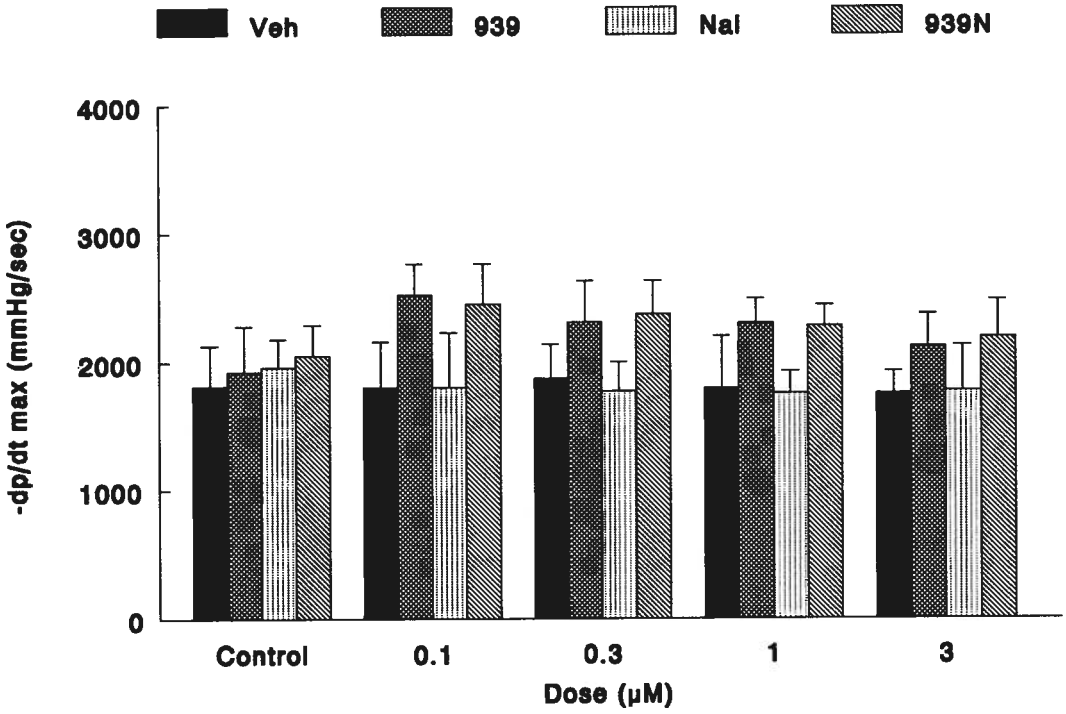


Figure 17. Concentration-response effects of RSD 939 in the presence and absence of naloxone pre-treatment, effects of vehicle control, and effects of naloxone on a measure of contractility, the maximal rate of intraventricular relaxation ($-dp/dt_{max}$) in isolated rat hearts. Hearts were perfused with PIPES buffer solution containing either vehicle, RSD 939, naloxone, or RSD 939 plus naloxone. Each dose of drug was infused for 3 minutes, and the steady state values at 3 minutes used in the analysis. The groups indicated are: Veh = vehicle control; 939 = cumulative concentrations of RSD 939; Nal = naloxone (1.0 μM); 939N = cumulative concentrations of RSD 939 with naloxone. Controls are the pre-drug values. * indicates $p<0.05$ as compared to the vehicle control group. † indicates significant difference between RSD 939 in the presence and absence of naloxone pre-treatment at $p<0.05$ (ANOVA and Duncan's range test). All values are expressed as mean ± S.E.M. with n = 5 per group.

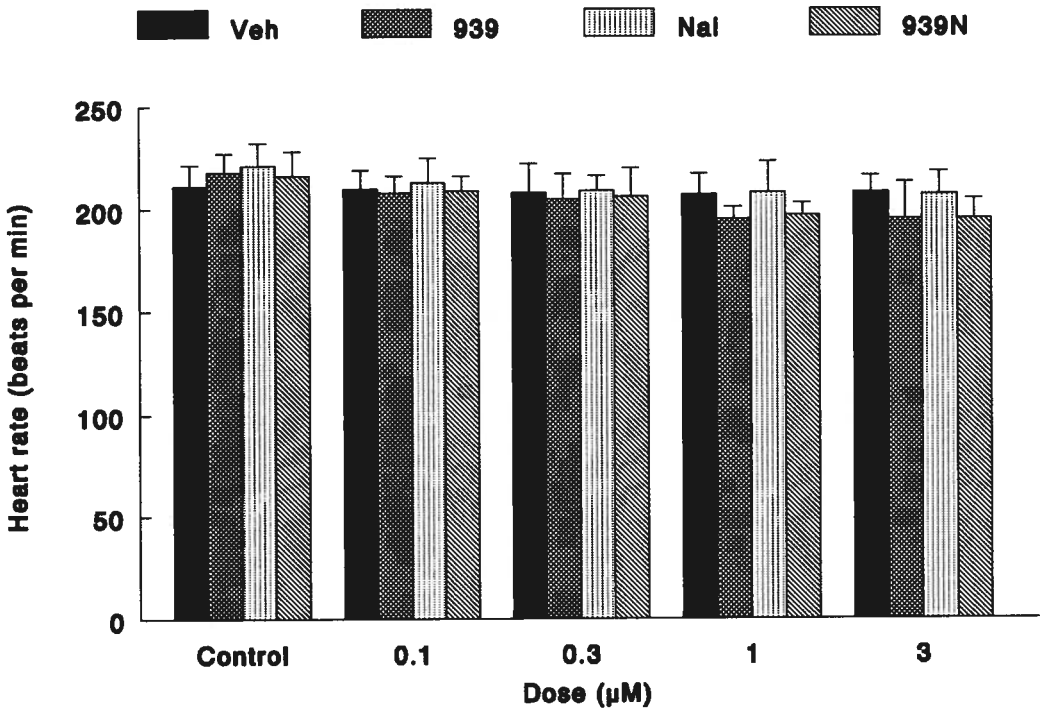


Figure 18. Concentration-response effects of RSD 939 in the presence and absence of naloxone pre-treatment, effects of vehicle control, and effects of naloxone on heart rate in isolated rat hearts. Hearts were perfused with PIPES buffer solution containing either vehicle, RSD 939, naloxone, or RSD 939 plus naloxone. Each dose of drug was infused for 3 minutes, and the steady state values at 3 minutes used in the analysis. The groups indicated are: Veh = vehicle control; 939 = cumulative concentrations of RSD 939; Nal = naloxone (1.0 μM); 939N = cumulative concentrations of RSD 939 with naloxone. Controls are the pre-drug values. * indicates $p<0.05$ as compared to the vehicle control group. † indicates significant difference between RSD 939 in the presence and absence of naloxone pre-treatment at $p<0.05$ (ANOVA and Duncan's range test). All values are expressed as mean ± S.E.M. with n = 5 per group.

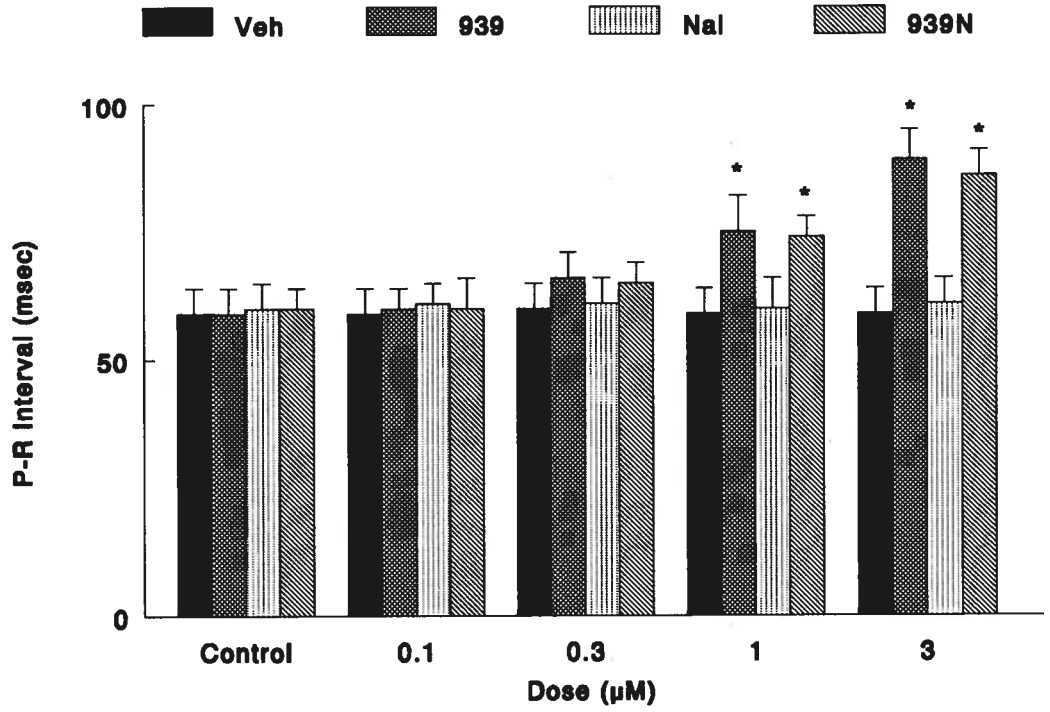


Figure 19. Concentration-response effects of RSD 939 in the presence and absence of naloxone pre-treatment, effects of vehicle control, and effects of naloxone on P-R interval of ECG in isolated rat hearts. Hearts were perfused with PIPES buffer solution containing either vehicle, RSD 939, naloxone, or RSD 939 plus naloxone. Each dose of drug was infused for 3 minutes, and the steady state values at 3 minutes used in the analysis. The groups indicated are: Veh = vehicle control; 939 = cumulative concentrations of RSD 939; Nal = naloxone (1.0 μM); 939N = cumulative concentrations of RSD 939 with naloxone. Controls are the pre-drug values. * indicates $p<0.05$ as compared to the vehicle control group. † indicates significant difference between RSD 939 in the presence and absence of naloxone pre-treatment at $p<0.05$ (ANOVA and Duncan's range test). All values are expressed as mean ± S.E.M. with n = 5 per group.

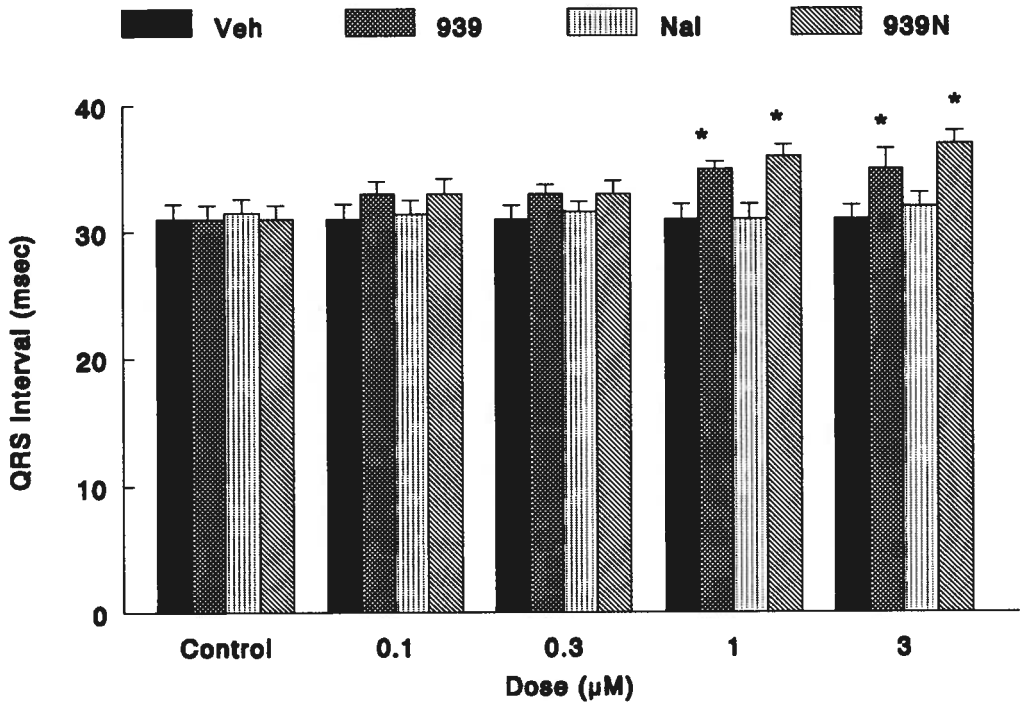


Figure 20. Concentration-response effects of RSD 939 in the presence and absence of naloxone pre-treatment, effects of vehicle control, and effects of naloxone on QRS interval of ECG in isolated rat hearts. Hearts were perfused with PIPES buffer solution containing either vehicle, RSD 939, naloxone, or RSD 939 plus naloxone. Each dose of drug was infused for 3 minutes, and the steady state values at 3 minutes were in the analysis. The groups indicated are: Veh = vehicle control; 939 = cumulative concentrations of RSD 939; Nal = naloxone (1.0 μM); 939N = cumulative concentrations of RSD 939 with naloxone. Controls are the pre-drug values. * indicates $p<0.05$ as compared to the vehicle control group. † indicates significant difference between RSD 939 in the presence and absence of naloxone pre-treatment at $p<0.05$ (ANOVA and Duncan's range test). All values are expressed as mean ± S.E.M. with n = 5 per group.

Table 5. Potencies of RSD 939 in the presence and absence of naloxone pre-treatment with respect to cardiac functions in vitro.

| Group | Heart Rate | Systolic Ventricular Pressure | $+dp/dt_{max}$ | $-dp/dt_{max}$ |
|---|---|---|---|---|
| | | $ED_{25}$ (μM) | | |
| RSD 939 | >3‡ | >3‡ | >3‡ | >3‡ |
| RSD 939 + Nal | >3‡ | >3‡ | >3‡ | >3‡ |

The potencies of RSD 939 in the presence and absence of naloxone pre-treatment with respect to heart rate, systolic ventricular pressure, contractility ($+dp/dt_{max}$ & $-dp/dt_{max}$) in isolated rat hearts. Nal = naloxone (1.0 μM). Values are expressed as the effective dose necessary to produce 25% change from the pre-drug values, $ED_{25}$. ‡ indicates $ED_{25}$ are non-estimateable at the tested dose range (changes from pre-drug values are less than 25% at the highest tested dose).

Table 6. Potencies of RSD 939 in the presence and absence of naloxone pre-treatment with respect to ECG responses in vitro.

| Group | P-R | QRS |
|---|---|---|
| | $ED_{25}$ (μM) | |
| RSD 939 | 1.0 | 10.0# |
| RSD 939 + Nal | 1.5 | 8.0# |

The potencies of RSD 939 in the presence and absence of naloxone pre-treatment with respect to P-R interval, and QRS interval in isolated rat hearts. Nal = naloxone (1.0 μM). Values are expressed as the effective dose necessary to produce 25% change from the pre-drug values, $ED_{25}$. # indicates the values of QRS interval are extrapolated from the extended portion of the dose-response curve.

## 3.5 Effects of electrical stimulation in vivo

Figure 10 to Figure 14 illustrates the effects of RSD 939 in the presence and absence of naloxone pre-treatment on sensitivity to electrical stimulation in intact rats. In a clearly dose-related manner RSD 939 increased threshold current (iT), threshold pulse width (tT), and ventricular fibrillation threshold (VFt). In addition RSD 939 dose-dependently lengthened the effective refractory period (ERP) while reducing the closely related but different variable, maximum following frequency (MFF). Statistically significant difference on iT, VFt, MFF, and ERP occurred at doses of 2 μmole/kg/min and at a dose of 4 μmole/kg/min for tT when compared to vehicle control. However, after 4 μmole/kg/min RSD 939, the characteristic ventricular fibrillo-flutter could not be induced by electrical stimulation despite the use of the maximum current of 1000 μA produced by the stimulator. Instead a ventricular tachycardia was all that could be achieved by this "burst pacing" method (50 Hz, 1.0 ms). The potency of RSD 939 on ERP ($ED_{25}$ = 2.3 μmole/kg/min) was slight lower than its potencies on iT ($ED_{25}$ = 2.0 μmole/kg/min) and VFt ($ED_{25}$ = 1.5 μmole/kg/min) (Table 4). The potency of RSD 939 on tT ($ED_{25}$ = 4.6 μmole/kg/min) was much lower

than iT, suggesting that RSD 939 was less potent in preventing extrasystoles induced by stimulus with long pulse width. ERP and MFF are reciprocally related such that it might be expected that MFF = 1000/ERP. However, the potency of RSD 939 on MFF was much lower than on ERP. The $ED_{25}$ for MFF was found to be 3.6 μmole/kg/min as compared to of 2.3 μmole/kg/min for ERP.

Naloxone had no statistically significant effects on electrical stimulation, although iT and VFt showed a slight increase after naloxone treatment. Naloxone pre-treatment did not influence the changes induced by RSD 939. However, the dose-dependent reduction in MFF was accentuated by pre-treatment with naloxone though the responses were not markedly different from the RSD 939 group. Data for vehicle control rats showed no changes with time over the experimental period.

Serum $K^+$ levels increased from a control values of 3.4 ± 0.1 to 3.6 ± 0.2 and 3.3 ± 0.1 to 3.6 ± 0.1 at the end of the experimental period for the vehicle control and the naloxone pre-treated control group, respectively (Table 3). For the RSD 939 treated group, and the naloxone pre-treated RSD 939 group, serum $K^+$ increased from a control value of 3.3 ± 0.1 to 3.9 ± 0.2 and 3.4 ± 0.1 to 3.9 ± 0.1, respectively, by the end of the experimental period. The changes of $K^+$ prior to and after experiment for all 4 groups were statistically significant but no

statistically significant differences were seen for comparison between groups.

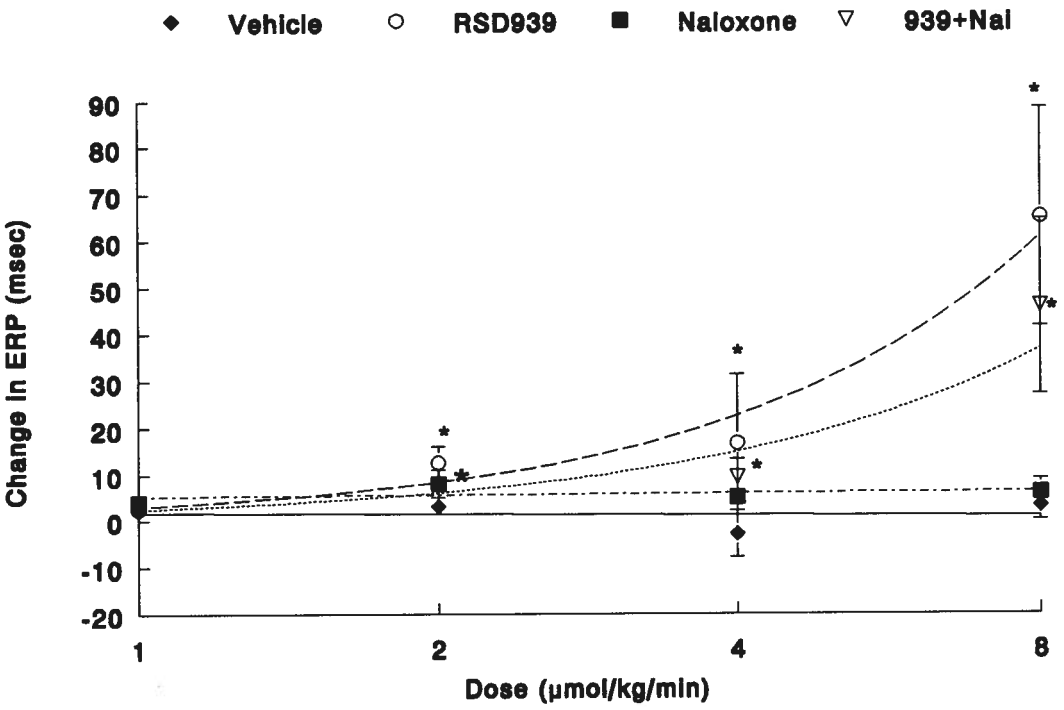


Figure 10. Dose-response effects of RSD 939 in the presence and absence of naloxone pre-treatment, effects of vehicle control, and effects of naloxone on effective refractory period, ERP as determined by the extra stimulus method in pentobarbital anaesthetized rats subjected to electrical stimulation of the left ventricle, are shown as changes from pre-drug values. Each dose of drug or vehicle were infused for 3 minutes. Measurements of the electrical stimulation variables were made triplicately at 2 minutes of each infusion. The average of the 3 measurements were used. The groups indicated are: ◆ = saline pre-treated vehicle control; o = cumulative doses of RSD 939; ■ = naloxone pre-treated vehicle control (8 µmole/kg); ▽ = cumulative doses of RSD 939 with naloxone pre-treatment. * indicates $p<0.05$ as compared to the vehicle control group. † indicates significant difference between RSD 939 in the presence and absence of naloxone pre-treatment at $p<0.05$ (ANOVA and Duncan's range test). All values are expressed as mean ± S.E.M. with n = 5 per group.

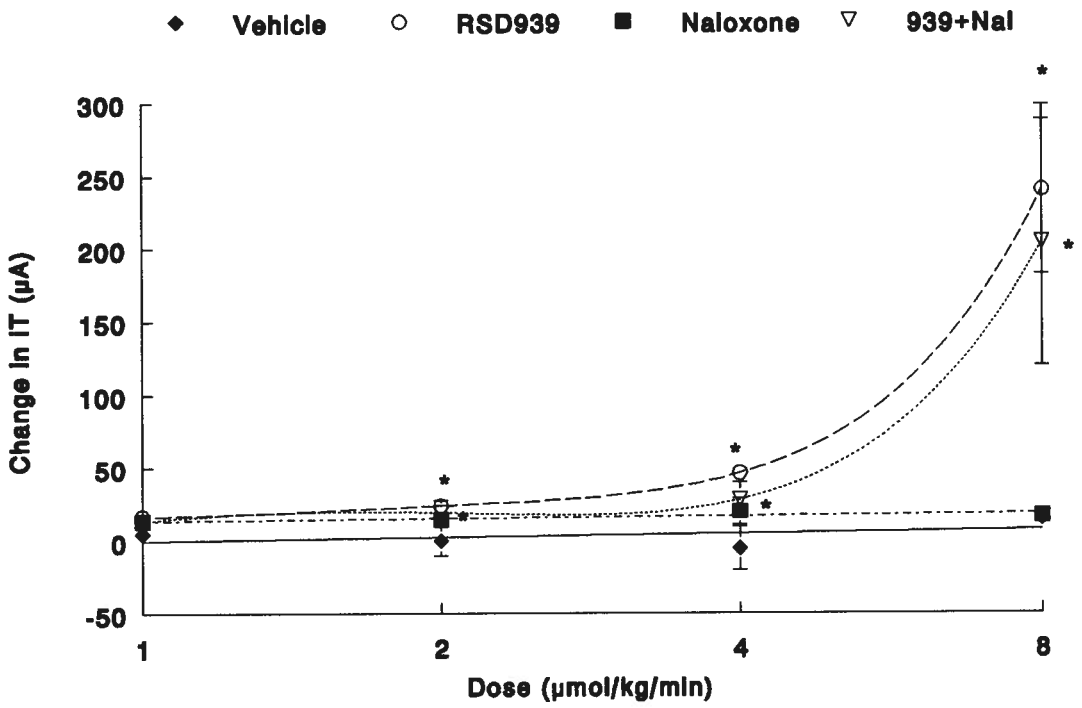


Figure 11. Dose-response effects of RSD 939 in the presence and absence of naloxone pre-treatment, effects of vehicle control, and effects of naloxone on threshold current, iT in pentobarbital anaesthetized rats subjected to electrical stimulation of the left ventricle, are shown as changes from pre-drug values. Each dose of drug or vehicle were infused for 3 minutes. Measurements of the electrical stimulation variables were made triplicately at 2 minutes of each infusion. The average of the 3 measurements were used. The groups indicated are: ◆ = saline pre-treated vehicle control; o = cumulative doses of RSD 939; ■ = naloxone pre-treated vehicle control (8 μmole/kg); ▽ = cumulative doses of RSD 939 with naloxone pre-treatment. * indicates $p<0.05$ as compared to the vehicle control group. † indicates significant difference between RSD 939 in the presence and absence of naloxone pre-treatment at $p<0.05$ (ANOVA and Duncan's range test). All values are expressed as mean ± S.E.M. with n = 5 per group.

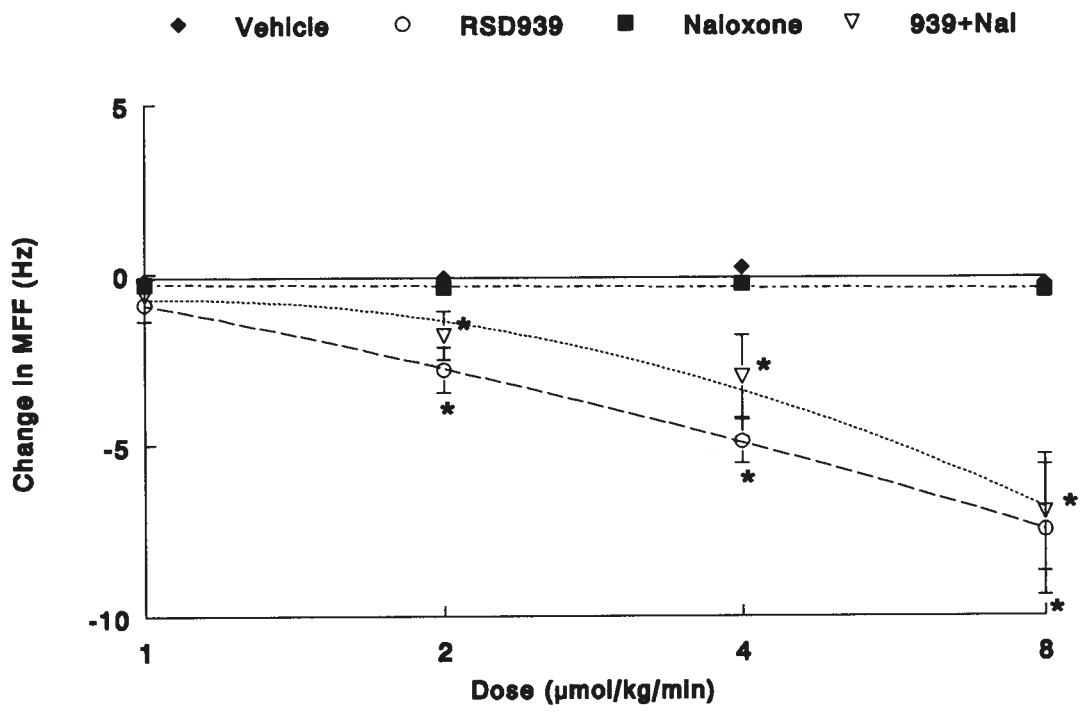


Figure 12. Dose-response effects of RSD 939 in the presence and absence of naloxone pre-treatment, effects of vehicle control, and effects of naloxone on maximum following frequency, MFF in pentobarbital anaesthetized rats subjected to electrical stimulation of the left ventricle, are shown as changes from pre-drug values. Each dose of drug or vehicle were infused for 3 minutes. Measurements of the electrical stimulation variables were made triplicately at 2 minutes of each infusion. The average of the 3 measurements were used. The groups indicated are: ◆ = saline pre-treated vehicle control; o = cumulative doses of RSD 939; ■ = naloxone pre-treated vehicle control (8 µmole/kg); ▽ = cumulative doses of RSD 939 with naloxone pre-treatment. * indicates $p<0.05$ as compared to the vehicle control group. † indicates significant difference between RSD 939 in the presence and absence of naloxone pre-treatment at $p<0.05$ (ANOVA and Duncan's range test). All values are expressed as mean ± S.E.M. with n = 5 per group.

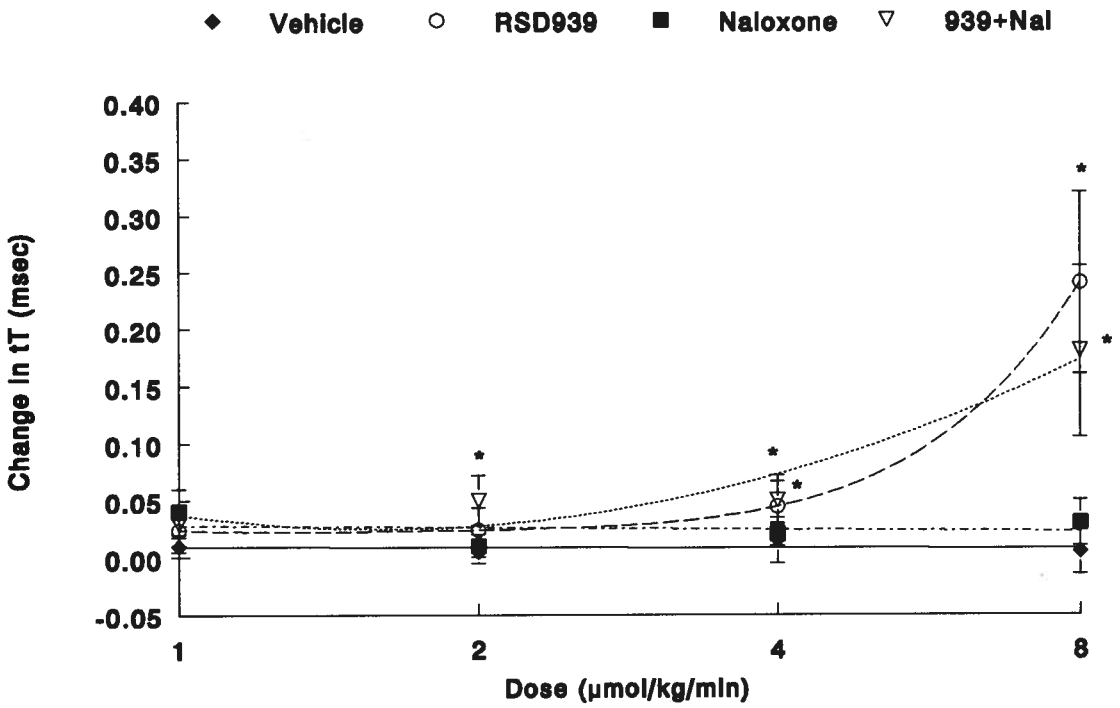


Figure 13. Dose-response effects of RSD 939 in the presence and absence of naloxone pre-treatment, effects of vehicle control, and effects of naloxone on threshold duration, tT in pentobarbital anaesthetized rats subjected to electrical stimulation of the left ventricle, are shown as changes from pre-drug values. Each dose of drug or vehicle were infused for 3 minutes. Measurements of the electrical stimulation variables were made triplicately at 2 minutes of each infusion. The average of the 3 measurements were used. The groups indicated are: ◆ = saline pre-treated vehicle control; o = cumulative doses of RSD 939; ■ = naloxone pre-treated vehicle control (8 µmole/kg); ▽ = cumulative doses of RSD 939 with naloxone pre-treatment. * indicates $p<0.05$ as compared to the vehicle control group. † indicates significant difference between RSD 939 in the presence and absence of naloxone pre-treatment at $p<0.05$ (ANOVA and Duncan's range test). All values are expressed as mean ± S.E.M. with n = 5 per group.

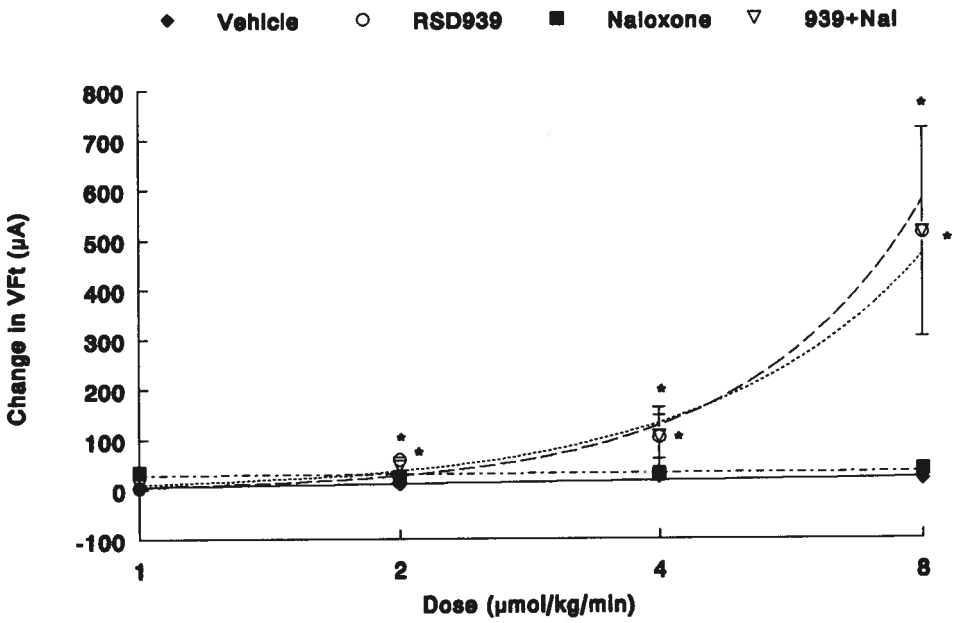


Figure 14. Dose-response effects of RSD 939 in the presence and absence of naloxone pre-treatment, effects of vehicle control, and effects of naloxone on the induction of ventricular fibrillation threshold, VFt in pentobarbital anaesthetized rats subjected to electrical stimulation of the left ventricle, are shown as changes from pre-drug values. Each dose of drug or vehicle were infused for 3 minutes. Measurements of the electrical stimulation variables were made triplicately at 2 minutes of each infusion. The average of the 3 measurements were used. The groups indicated are: ◆ = saline pre-treated vehicle control; ○ = cumulative doses of RSD 939; ■ = naloxone pre-treated vehicle control (8 μmole/kg); ▽ = cumulative doses of RSD 939 with naloxone pre-treatment. * indicates $p<0.05$ as compared to the vehicle control group. † indicates significant difference between RSD 939 in the presence and absence of naloxone pre-treatment at $p<0.05$ (ANOVA and Duncan's range test). All values are expressed as mean ± S.E.M. with n = 5 per group.

At 8 μmole/kg/min RSD 939 both in the presence and absence of naloxone, only ventricular tachycardia can be induced with the maximum current output of 1000 μA in all the rats.

Table 3. Serum $K^+$ levels in pentobarbital anaesthetized rats subjected to electrical stimulation.

| Group | Serum $K^+$ levels (mM) | |
|---|---|---|
| | Pre-electrical stimulation | Post-electrical stimulation |
| Vehicle control | 3.3 ± 0.2 | 3.7 ± 0.1* |
| Naloxone | 3.2 ± 0.2 | 3.8 ± 0.3* |
| RSD 939 | 3.3 ± 0.1 | 3.9 ± 0.2* |
| RSD 939 + Nal | 3.4 ± 0.2 | 3.9 ± 0.2* |

Values are expressed as means ± S.E.M.. Nal = naloxone at 8μmole/kg. * indicates significant difference between pre- and post-electrical stimulation values. † indicates $p<0.05$ versus vehicle control.

Table 4. Potencies of RSD 939 in the presence and absence of naloxone pre-treatment with respect to sensitivity to electrical stimulation in vivo.

| Group | iT | tT | VFt | ERP | MFF |
|---|---|---|---|---|---|
| | | | $ED_{25}$ (μmole/kg/min) | | |
| RSD 939 | 2.0 | 4.6 | 1.5 | 2.3 | 3.6 |
| RSD 939 + Nal | 1.8 | 5.0 | 1.5 | 2.5 | 3.0 |

The potencies of RSD 939 in the presence and absence of naloxone pre-treatment with respect to threshold current (iT), threshold pulse width (tT), ventricular fibrillation threshold (VFt), effective refractory period (ERP), and maximum following frequency (MFF) in pentobarbital anaesthetized rats. Nal = naloxone (8μmole/kg). Values are expressed as the effective dose necessary to produce 25% change from the pre-drug values, $ED_{25}$.

## 3.6 Antiarrhythmic actions of RSD 939

In the antiarrhythmic study for ischaemic arrhythmias, the two infusion doses of RSD 939 used were chosen on the basis of dose-response effects seen with RSD 939 in the electrical stimulation study (data not shown). The higher dose (1.5 μmole/kg/min) of RSD 939 had significant, but sub-maximal effects on heart rate, blood pressure, and ECG. The lower dose (0.5 μmole/kg/min) minimally changed heart rate, blood pressure and ECG but had noticeable effect on RSh. Both doses of RSD 939 statistically significantly reduced the incidence of arrhythmias induced by coronary artery occlusion (Table 7). The high dose (1.25 μmole/kg/min) was more antiarrhythmic than the low dose (0.5 μmole/kg/min). The high dose of RSD 939 reduced the incidence of NSVT by 40%, the incidence of NSVF by 60%, and mortality by 100% when compared with the vehicle control group. The low dose reduced the incidence of NSVF by 40%, mortality by 60%, but did not reduce the incidence of NSVT. Although both doses statistically significantly reduced AS, the reduction in AS by the high dose RSD 939 was much higher than with the low dose. The AS of high dose RSD 939 was 3.2 ± 0.2 while the AS of low dose was 5.4 ± 0.2 which indicated low dose RSD 939 almost failed to influence arrhythmias.

Naloxone alone also reduced the incidence of NSVF as exemplified by the reduction in AS, but did not reduce the incidence of NSVT. The incidence of NSVT and NSVF were not different between high dose RSD 939 alone or when naloxone was given prior to RSD 939. Naloxone appeared to have no effect on the antiarrhythmic effects of high dose RSD 939 since no statistical significant difference were found. On the other hand, while low dose RSD 939 alone had limited effects on arrhythmias, when combined with naloxone it reduced arrhythmias to a statistically significant extent. The AS of low dose RSD 939 was reduced to 4.0 ± 0.2 when combined with naloxone pre-treatment. This reduction was most likely resulted from naloxone treatment rather than from RSD 939 treatment.

The antiarrhythmic activity of RSD 939 and naloxone could not be ascribed to changes in either the size of the occluded zone (zone-at-risk) or serum potassium concentrations. The group mean occluded zone size did not differ statistically significantly between groups (Table 8). In a similar manner serum potassium concentrations before and after occlusion were also not differ statistically significantly between groups although they were significantly increased after occlusion in the high dose RSD 939 treatment group.

Statistical test was not performed to determine the significant difference in the group incidences of NSVT or NSVF because the 1-tailed $chi^2$ test referenced by Mainland's contingency tables (Mainland et al., 1956) required the minimum group size to be 9 in order to be able to reveal a 50% reduction in NSVT and NSVF.

Table 7. Antiarrhythmic properties of RSD 939 in the presence and absence of naloxone pre-treatment against ischaemia-induced arrhythmias in pentobarbital anaesthetized rats in the early (0-0.5 hr) period following coronary artery occlusion.

| Group | Incidence of VT and VF (%) | | | | | Mortality (%) | AS |
|---|---|---|---|---|---|---|---|
| | SVT | SVF | NSVT | NSVF | NSVT and/or NSVF | | |
| Vehicle | 100 | 0 | 100 | 100 | 100 | 100 | 7.0 ± 0 |
| Nal | 100 | 0 | 100 | 40 | 100 | 0 | 3.4 ± 0.2* |
| RSD939 (H) | 100 | 20 | 60 | 40 | 60 | 0 | 3.2 ± 0.2* |
| RSD939 (H) + Nal | 100 | 0 | 80 | 20 | 80 | 0 | 3.0 ± 0.3* |
| RSD939 (L) | 100 | 0 | 100 | 60 | 100 | 40 | 5.4 ± 0.2*† |
| RSD939 (L) + Nal | 100 | 20 | 100 | 40 | 100 | 0 | 4.0 ± 0.2*† |

The antiarrhythmic actions of 1.5 μmole/kg/min (H) and 0.5μmole/kg/min (L) RSD 939, alone or in the presence of naloxone pre-treatment, as well as naloxone, are expressed in terms of the percent of animals experiencing one or more episodes of the particular arrhythmias. Nal = naloxone (8 μmole/kg). NSVT = non-spontaneously reverting ventricular tachycardia. NSVF = non-spontaneously reverting ventricular fibrillation. SVT = spontaneously reverting ventricular tachycardia. SVF = spontaneously reverting ventricular fibrillation. AS = arrhythmia score. Calculations to determine AS were discussed in the methods and all values represent mean ± S.E.M.. % = % animal per group (n=5). * indicates significant difference from vehicle control at $p<0.05$. † indicates $p<0.05$ between RSD 939 and RSD 939 + Naloxone.

Table 8. Occluded zone size and serum $K^+$ levels in pentobarbital anaesthetized rats subjected to coronary artery occlusion.

| Group | OZ (%) | Serum $K^+$ levels (mM) | |
|---|---|---|---|
| | | Pre-occlusion | Post-occlusion |
| Vehicle | 37.5 ± 1.5 | 3.9 ± 0.2 | 4.0 ± 0.2 |
| Nal | 36.0 ± 1.0 | 3.8 ± 0.1 | 4.1 ± 0.2 |
| RSD 939 (H) | 37.0 ± 1.0 | 3.8 ± 0.1 | 4.4 ± 0.2* |
| RSD939 (H) + Nal | 38.0 ± 1.5 | 3.8 ± 0.1 | 4.2 ± 0.2* |
| RSD 939 (L) | 38.0 ± 1.5 | 3.9 ± 0.2 | 4.1 ± 0.2 |
| RSD939 (L) + Nal | 37.0 ± 1.0 | 3.8 ± 0.2 | 4.2 ± 0.2 |

RSD 939 (H) = 1.5 μmole/kg/min. RSD 939 (L) = 0.5 μmole/kg/min. Values are means ± S.E.M.. Nal = naloxone (8 μmole/kg/min). OZ = weight % of the occluded zone in ventricle. * indicates significant difference between pre- and post-occlusion serum $[K^+]$ values. † indicates $p<0.05$ versus vehicle control for serum $[K^+]$ levels or OZ.

# 4. Discussions

## 4.1 Haemodynamic effects of RSD 939 in vivo and in vitro

It has been proposed that opiate mechanisms play a role in central and/or peripheral cardiovascular regulation (Holaday, 1983). κ agonists, ethylketocyclazocine (EKC), U50,488H, and spiradoline, have shown to induce decreases in blood pressure and heart rate via either autonomic nervous system regulation or direct peripheral actions at κ sites located on the heart (Laurent and Schmitt, 1983; Wu and Martin, 1983; Hall et al., 1988). These effects were mediated by κ receptors and were completely reversed by the opioid receptor antagonist naloxone. In our studies, RSD 939 (over a range of doses from 1 to 16 μmole/kg i.v.) dose-dependently reduced blood pressure and heart rate in pentobarbitone anaesthetized rats. However, these effects could only be partially antagonized by naloxone. Naloxone reduced the actions of low doses RSD 939 on heart rate and blood pressure but not at higher doses. These actions were remarkably similar to those of high doses of U50,488, PD 129289, and PD 129290, which our laboratory has tested previously under similar conditions (Pugsley et al., 1992a,b & 1993). In contrast, over the concentration range 0.1 to 3.0 μM, RSD 939 produced no significant

changes on heart rate, systolic ventricular pressure, and dp/dt in isolated rat heart studies, although a noticeable decrease in heart rate and an increase in systolic ventricular pressure as well as dp/dt were observed at higher doses. These responses were not prevented by naloxone. From such results, it is reasonable to suggest that at least part of the effects of RSD 939 on heart rate are mediated by receptor-dependent effects in the central nervous systme (vagal system and/or sympathetic nervous system) since there is a significant reduction in the effects on heart rate in the isolated heart preparations. The fall in blood pressure could have been due partly to a possible decrease in cardiac output which may result from the bradycardia. Our results also suggest that the haemodynamic effects of RSD 939, particularly at higher doses, are not mediated via κ opioid receptors since the effects were naloxone-resistant. In addition, the reduction in heart rate seen with isolated heart preparations may not be related to the direct peripheral actions of RSD 939 on κ opioid receptors located in the heart since the effects were insensitive to naloxone. The influence of opioid agonists and antagonists on cardiac muscle contractile force has been examined in a variety of preparations with a variety of results, including positive, negative, and biphasic (both positive and negative) inotropic effects (Rendig et al., 1980; Laurent et al., 1985; Goldberg & Padget., 1969; Strauer, 1972). The increase in systolic

ventricular pressure and contractility with RSD 939 in isolated heart is not related to opioid receptor but may be the results of increased $Ca^{2+}$ loading subsequent to an increase in action potential duration as discussed later.

## 4.2 Antiarrhythmic actions of RSD 939

Opioid agonists and antagonists had been shown to be effective against a variety of experimental arrhythmias in isolated heart and intact animal studies (Alzheimer et al., 1990; Boachie-Ansah et al., 1989; Brasch, 1986; Frame et al., 1985; Fagbemi et al., 1982 & 1983; Helgesen et al., 1987; Huang et al., 1986; Lee et al., 1986; MacKenzie et al., 1986; Parratt et al., 1986; Pruett et al., 1991; Pugsley et al., 1992a,b & 1993; Sarne et al., 1988 & 1991; Wong et al., 1990). In this study, we found that low dose (0.5 μmole/kg/min) RSD 939 offered only very minimum protection against arrhythmias induced by myocardial ischaemia but at a high dose (1.5 μmole/kg/min), RSD 939 reduced the incidence of arrhythmias significantly. At the same dose, RSD 939 also increased resistance to electrical induction of ventricular fibrillo-flutter. A much higher dose of 16 μmole/kg/min, RSD 939 completely prevented the electrical induction of ventricular fibrillo-flutter as only ventricular

tachycardia could be induced despite the use of maximum current of 1000 μA. These results suggested the antiarrhythmic actions of RSD 939 could be dose-related. However, the antiarrhythmic activities against ischaemia-induced arrhythmia at this dose range were not investigated because of marked depression effect on blood pressure. In neither of the previous studies was the actions of RSD 939 antagonized by naloxone given at a dose which has been shown to antagonize the actions of κ agonists (Sarne et al., 1991). This finding is in agreement with our previous studies in which κ agonists, U50,488H and PD 129290 were shown to have antiarrhythmic activities unrelated to opioid receptors (Pugsley et al., 1992a,b & 1993). We thus suggest that such non-opioid actions of RSD 939, like those of U50,488H and PD 129290 are also the result of cardiac ion channel blockade.

While naloxone did not antagonize the antiarrhythmic effects of RSD 939, naloxone itself possessed slight antiarrhythmic properties since the incidence of ischaemia-induced arrhythmias were significantly reduced by pre-treatment with 8 μmole/kg naloxone. The mechanism underlying the antiarrhythmic action of naloxone has been the subject of several investigations with many proposing that its antiarrhythmic action is unrelated to opioid receptors (Sarne et al., 1988 & 1991; Brasch, 1986; Oldroyd et al., 1993). In our study, naloxone might have been

responsible for the increase in antiarrhythmic effectiveness of low dose RSD 939. However, naloxone appeared to have no synergistic effect with the high dose RSD 939 since no difference in arrhythmia score was found between the naloxone treated and untreated group.

There was a statistically significant elevation of serum $K^+$ concentrations post-occlusion associated with administration of RSD 939 (Table 8), particularly at the high dose. Elevations in serum $K^+$ are known to occur following acute surgery, and this may contribute to the results of this study. Elevation of serum $K^+$ is associated with a fall in the incidence of VF in patients with acute myocardial infarction (Nordrehaug and von der Lippe, 1983; Solomon, 1984) and experimentally in rats (Saint et al., 1992). In isolated perfused hearts, elevation of the $K^+$ concentration of the perfusate drastically reduces arrhythmias induced by coronary occlusion (Lubbe et al., 1978; Daugherty et al., 1981). The mechanism by which hyperkalaemia protects against ischaemia-induced arrhythmias might be due, at least in part to the elevation of the threshold for electrical excitation resulted from high serum $K^+$. Thus the normal myocardium would be protected from invasion by aberrant impulses emanating from ischaemic tissue. In our study, the serum $K^+$ levels with high dose RSD 939 were 4.4 ± 0.2 mM post-occlusion. According to the results obtained by Curtis et al (1985),

the incidence of VF was reduced by 23% in groups of rats whose serum $K^+$ were in the range of 4.0 - 4.9 mM. However, in our study, we have been able to induce VF in 100% of the animals in the control group, which has a serum $K^+$ of 4.0 ± 0.2 mM post-occlusion. The serum $K^+$ in control group was not significantly different from the RSD 939 group. Therefore, we believed that the slight increase in serum $K^+$ was not enough to protect against ischaemia-induced arrhythmias.

According to previous studies in our laboratory, arrhythmias (incidence and duration) depend on the size of the ischaemic (occluded) zone (OZ) such that arrhythmia score (AS) is linearly correlated with square root of the OZ (Johnston et al., 1983a). This implies that it is not directly the amount of ischaemic tissue, but the presence of both normal and ischaemic tissue which is necessary for arrhythmogenesis since the site of arrhythmogenesis is the interface area between the ischaemic and normal tissue (Brofman et al., 1956; Beck, 1958; Janse et al., 1979 &1980). Therefore, OZ size should be measured in all rats in order to verify that a proper occlusion has taken place. From our past experiences, the optimal OZ size for arrhythmogenesis in rats should be in the range of 25-45% (Curtis, 1984; Johnston et al., 1983a). Since the OZ size in our study average at 37% and the variance for OZ size is small, antiarrhythmic activity could not be ascribed to changes in OZ in

this study.

## 4.3 Non-opioid actions of RSD 939

A variety of electrophysiological studies have shown that opioid agonists and antagonists have actions independent of opioid receptors and can produce electrophysiologic changes consistent with those seen with Class I antiarrhythmic agents (Blari et al., 1986; Pruett et al., 1987; Oldroyd et al., 1993; Carratu and Mitolo-Chieppa, 1982; Brasch, 1986; Boachie-Ansah et al., 1989; Fagbemi et al., 1983; Alzheimer and Bruggencate, 1990; Pugsley et al., 1993). In additional to electrophysiological studies, ECG observation and responses to electrical stimulation are also useful in determining the effects of antiarrhythmic drugs on cardiac ion channels. The ECG effects of the 4 classes of antiarrhythmic agents have been summarized by Botting et al (1986) and Penz et al (1992). Class Ia $Na^+$ channel blockers slow conduction velocity at high concentrations thus widen the QRS duration, prolong P-R intervals, and increase RSh. They also widen the AP thus prolonged the Q-T intervals. Class Ib agents demonstrate limited (if any) effect on P-R interval, QRS duration, RSH, and conduction while shortening the Q-T interval and APD. Class Ic agents slow conduction at low concentrations

thus prolong P-R interval, increase RSh, and widen QRS complex, but have little effect on repolarization and APD. Class II drugs, β-blockers, can widen P-R intervals in vivo if AV nodal conduction has been enhanced by a significant degree of sympathetic tone before administration, as the P-R interval reflects the conduction time through the AV node. Class III drugs, $K^+$ channel blockers, delay repolarization of the cardiac action potential. Since the T-wave of the surface ECG reflects the repolarization phase in the ventricle (Einthoven, 1912; Katz, 1928), selective Class III drugs should widen the Q-T intervals of the ECG with no other effects on QRS or P-R intervals due to a lack of effect on conduction velocity in atrial, nodal, or ventricular tissue. Class IV drugs, $Ca^{2+}$ channel blockers, can widen P-R intervals if given in sufficient doses to inhibit the slow inward current, $i_{si}$. In intact hearts, RSD 939 prolonged the P-R interval and elevated the RSh at low doses while Q-T interval and QRS duration were not affected until higher doses. This is in agreement with the results obtained from isolated hearts where the drug was shown to be more potent in prolonging the P-R interval than QRS duration. These evidences suggested that RSD 939 acted directly on cardiac tissue and might have Class I actions at lower doses. However, the effects on Q-T intervals at higher doses could either be achieved by APD prolongation (Class III) or was accompanied by conduction slowing

(Class Ia). The ECG effects also slightly resemble those of Class Ib compounds which have limited action on QRS duration at normal sinus beating rates due to their high frequency dependency (Campbell, 1983b; Courtney, 1987). Since P-R interval widening is seen with both $Na^+$ and $Ca^{2+}$ channel blockers, we can not rule out the possibility of calcium channel blockade. However, by studying the responses to electrical stimulation, it is proved that sodium channel blockade is most likely the cause of P-R interval prolongation.

In addition to the ECG evidence, the effects of RSD 939 on iT, tT, and VFt were consistent with sodium channel blockade. It has been well established that $Na^+$ channel blockers increase iT, tT, and VFt in response to electrical stimulation (Wiggers & Wegria, 1940; Beatch et al., 1988; Hodess et al., 1979; Marshall et al., 1983; Yoon et al., 1974). On the other hand, unlike Class I drugs which dose dependently elevated iT, tT, and VFt, $K^+$ channel blockers might not affect iT and tT but might render the heart completely resistant to VF at a high enough dose. This resulted when the refractoriness was prolonged to such an extent that multiple fractionations of induced reentrant wave fronts were not possible (Sugimoto et al., 1989; Winslow, 1984). At low doses, VFt was gradually increased dose-dependently. Yet, at 8 μmole/kg/min, RSD 939 suddenly and completely suppressed the induction of ventricular fibrillo-flutter,

thus again suggested the involvement of potassium channel blockade at such high dose.

The recovery of excitability after a preceding impulse is determined mainly by the availability of sodium channels, which are voltage dependent and thus AP widening can prolong refractoriness. Thus prolongation of ERP can be expected to occur with Class Ia sodium channel blockers and Class III antiarrhythmics (Vaughan-Williams, 1970 & 1975). In the present study with RSD 939, ERP was increased as was Q-T interval, findings associated with Class Ia and/or potassium channel blockade. ERP and MFF are related such that it might be expected that MFF(Hz) = 1000/ERP(ms). However, although the two are similar they are sufficiently different to warrant reporting both. ERP, as measured, is a reasonable measure of effective refractory period. MFF is more a measure of relative refractory period, and ventricular functional refractory period, and thus can exhibit a different sensitivity of drugs from ERP. The process of determination of MFF, namely a steadily increasing frequency of stimulation, can be associated with accumulation of extracellular $K^+$ (see Table 3) thereby adding an extra component to what would otherwise be another measure of ERP. Thus MFF and ERP are not equally sensitive to frequency-dependent sodium or potassium channel blockers (Walker & Beatch, 1988). Comparisons between 1/MFF

and ERP allow us to ascertain the frequency dependency of refractoriness increase with RSD 939. In our study, RSD 939 treatments did not change the ratio of 1/MFF to ERP values, thus rule out the possibility of frequency dependent sodium channel blockade (Class Ib).

The ECG effects and the responses to electrical stimulation seen with RSD 939 were unrelated to κ opioid receptors since these actions occurred at doses and concentrations above those required for κ-agonism, and naloxone did not abolish nor reduce any of these effects statistically significantly. However, the dose of naloxone used have caused a minimum degree of sodium channel blockade as indicated by the slight increase in RSh, P-R interval, iT, and tT.

# 5 Conclusion

In conclusion, the present study provides evidence that RSD 939, a potent and selective κ agonist, possess antiarrhythmic activity against ischaemia-induced and electrically-induced arrhythmias. We suggest that these antiarrhythmic actions are independent of κ opioid receptors but are dependent upon sodium and/or potassium channel blocking actions. These results are similar to those previously obtained with U50,488, PD 129289, and PD 129290 (Pugsley et al., 1992a,b &1993). Sodium channel blockade explains the observed P-R prolongation, QRS widening and the increase in threshold currents, all of which occurred at doses which conferred antiarrhythmic actions. Potassium channel blockade, on the other hand, explains the changes in Q-T, MFF, ERP, and the sudden increase in VFt seen at higher doses. It is most likely that both actions may have contributed to the antiarrhythmic actions.

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