THE CARDIOVASCULAR AND ANTIARRHYTHMIC ACTIONS OF A SERIES OF KAPPA OPIOID AGONISTS AND RELATED COMPOUNDS

by

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Abstract

The cardiovascular and antiarrhythmic actions of the kappa (κ) opioid receptor agonists are not well characterized. This may be the result of the limited role opioids play in the regulation of cardiovascular function and the fact that pharmaceutical companies concentrate on their analgesic properties. The studies described in this thesis attempt to characterize the cardiovascular and antiarrhythmic properties of a novel series of arylacetamide κ receptor agonists and related compounds. The compounds examined included U-62,066E (spiradoline), U-50,488H, (-)PD129,290 and its inactive enantiomer, (+)PD129,289, (±)PD117,302 and its inactive enantiomer, (+)PD123,497. Studies were conducted to determine whether the κ receptor is involved in the antiarrhythmic actions of these compounds and if it is not, attempt to determine a mechanism by which these compounds may confer antiarrhythmic protection against both electrically-induced and ischaemic arrhythmias. Studies were therefore conducted in rats in the absence and presence of the opioid antagonists, naloxone and Mr2266 or, when possible, with inactive enantiomers of κ receptor agonists.

Six novel compounds sharing the arylacetamide structure were examined for their actions on haemodynamic and ECG actions in intact pentobarbitone-anaesthetised rats. The previously unpublished parts of the thesis focuses on U-62,066E (spiradoline) and also provides results obtained in isolated cardiac myocytes for (±)PD117,302 and its inactive enantiomer, (+)PD123,497. Previously published data contained in the appendices covers the other three compounds. All compounds produced similar actions.

All the compounds examined produced a dose-dependent reduction in heart rate, blood pressure, and prolonged the P-R, QRS duration, and Q-aT intervals with an accompanying increase in RSh (a measure of sodium channel block in the rat).

The effects of the arylacetamides, exemplified by spiradoline, were assessed using a modified Langendorff isolated heart preparation. Spiradoline and related compounds reduced the sinus beating rate and contractility of hearts in a concentration-dependent

manner. ECG effects in isolated hearts included prolongation of the P-R interval and QRS width.

The effects of the compounds on the ability of electrical stimulation to stimulate the heart were examined in pentobarbitone-anaesthetised rats. Spiradoline and related compounds dose-dependently increased the current and duration of stimulus required to stimulate the heart and also increased ventricular fibrillation threshold. All compounds prolonged effective refractory period and reduced maximum following frequency.

In an attempt to determine effectiveness against ischaemic arrhythmias in rats after coronary occlusion a dose of 2.5μmol/kg/min spiradoline was given in the absence and presence of 2.5μmol/kg/min naloxone. Spiradoline reduced the incidence of VT from 100% in controls to 33 and 44% in the absence and presence of naloxone. The incidence of VF was reduced from 100% to 22% and 0% in the absence and presence of naloxone. All chemically related arylacetamides were similarly antiarrhythmic.

In order to delineate a mechanism by which these compounds may be antiarrhythmic their effects on sodium and potassium currents were examined in isolated rat cardiac myocytes. Spiradoline blocked these currents in a concentration-dependent and reversible manner. The EC_{50} for half-maximal sodium current block was 66μM. Spiradoline also produced a hyperpolarizing shift in the voltage-dependence of inactivation of the channel but did not alter activation kinetics. The block produced by spiradoline on sodium channels was both tonic and use-dependent. Additional studies with (±)PD117,302 and its inactive enantiomer, (+)PD123,497 showed that the block produced by these arylacetamides is pH-dependent and that block is consistent with activity at an extracellular site on the sodium channel.

These results indicate that spiradoline and the arylacetamides examined produce antiarrhythmic actions independent of the κ receptor in the rat. Our results demonstrate the sodium channel, and to a lesser extent, potassium channels blocking actions of these compounds.

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α-level	level of significance
ANOVA	analysis of variance
ANS	autonomic nervous system
AP	action potential
APD	action potential duration
AS	arrhythmia score
AVJ	atrio-ventricular junction
AVP	arginine vasopressin
B.P.	blood pressure
CAST	Cardiac Arrhythmia Suppression Trial
°C	degree Celcius
CNS	central nervous system
Da	daltons
ECG	electrocardiogram
ED_{50}	dose of drug producing half-maximal response
EKC	ethylketocyclazocine
EOP	endogenous opioid peptide
ERP	effective refractory period
g	gram
GRH	Guarded Receptor Hypothesis
hr	hour
Hz	hertz
i.p.	intraperitoneally
i.v.	intravenous

kg	kilogram
<	less than
µM	micromolar
mg	milligram
MI	myocardial infarction
min	minute
mL	millilitre
mmHg	millimetres of mercury
mM	millimolar
MRH	Modulated Receptor Hypothesis
msec	millisecond
MW	molecular weight
OZ	occluded zone
pH	hydrogen ion concentration
PVC	premature ventricular contraction
%	percentage
s.e.mean	standard error of the mean
sec	second
SAR	Structure-Activity Relationship
TTX	tetrodotoxin
VF	ventricular fibrillation
VT	ventricular tachycardia

LIST OF APPENDICES

The following papers comprise the latter part of the thesis and are found in the Appendix.

Appendix 6

Penz, W.P., Pugsley, M.K., Hsieh, M.Z. and Walker, M.J.A. A new measure (RSh) for detecting possible sodium channel blockade *in vivo* in rats. J. Pharmacol. Toxicol. Meth. 27(1): 51-58, 1992.

Appendix 7

Hayes, E., Pugsley, M.K., Penz, W.P., Adaikan, G. and Walker, M.J.A. Relationship between Q-aT and RR intervals in rats, guinea pigs, rabbits and primates J. Pharmacol. Toxicol. Meth. 32(4): 201-207, 1994.

Appendix 8

Pugsley, M.K., Penz, W.P. and Walker, M.J.A. Cardiovascular actions of U-50,488H and related kappa agonists. Cardiovasc. Drug Rev. 11(2): 151-164, 1993.

DEDICATION

This thesis is dedicated to my beloved parents, my brother and his family, my sister, grandparents and to my Sharon

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1 Introduction

1.1 Antiarrhythmic Drugs

1.1.1 Brief History of Antiarrhythmic Drugs

The models of ion channel blockade currently used to quantify and qualify the actions of antiarrhythmic drugs in cardiac tissue under ischaemic or normal conditions, are based upon many key observations. Distinctions are based primarily upon differences in electrophysiology in tissues subjected to the two conditions. In addition many studies were conducted in neuronal, rather than cardiac, tissue. However, most findings in neurons have been applied to, and accurately describe, drug actions in the heart.

Hodgkin and Huxley (1952) first examined the electrical properties of the sodium current using the squid giant axon. The results of this work provided the first implicit model for ion channel function whereby the sodium channel may exist in three states: resting (closed), active (open) and closed (inactive). These channel states are a function of voltage and time and are dependent upon membrane potential. Hodgkin and Huxley investigated the kinetics of activation and inactivation of this channel and proposed that these properties were due to "m" and "h" gates, respectively. Weidmann in 1955 found that in the presence of antiarrhythmic drugs (sodium channel blockers) the voltage-function of the maximum rate of depolarization (V_{max}) was shifted to more negative potentials. Weidmann suggested that this was due to drug interaction with the inactivation (h) gate (according to Hodgkin and Huxley formalism) of the sodium channel. These drugs did not alter activation kinetics.

The next observation regarding drug interaction with the sodium channel was made by Johnson and MacKinnon (1957). The action of quinidine on V_{max} was minimal with long diastolic intervals. When the frequency of channel activation increased sodium

channel blockade was enhanced. In 1970 Jensen and Katzung demonstrated that the antiarrhythmic drug diphenylhydantoin (DPH) acted synergistically with elevated external potassium to decrease V_{max} in rabbit atrial preparations. Singh and Vaughan Williams the following year showed, in rabbit atrial and ventricular muscle, a similar action for lidocaine and concluded that this drug may have preferential actions in ischaemic conditions.

The findings of Johnston and MacKinnon in 1957 became important to our understanding of antiarrhythmic drug action in 1973 when Strichartz showed that at an increased stimulation frequency the block produced by quaternary local anaesthetics increased. He also showed that these drugs interact with open sodium channels and that both the drug-free and drug-associated channels obey Hodgkin and Huxley kinetics. Thus, a model of drug interaction with the sodium channel was beginning to emerge which had wide applicability to ion channels. Additional studies by Hondeghem et al. (1974) and Hope et al. (1974) showed that many antiarrhythmic agents (such as quinidine, lidocaine and procainamide) selectively depress electrical activity in hypoxic tissue, and ischaemic myocardium, respectively, as had been suggested by Jensen and Katzung earlier.

Chen et al. (1975) suggested that the selective actions of antiarrhythmic drugs in ischaemic or hypoxic tissue were due to their voltage-dependent properties. They found that in depolarized tissue recovery of channel function from drug block is slow compared to that of normal or highly polarized cells. At the same time Courtney (1975) extended the Strichartz model (1973) to include the interaction of tertiary compounds and sodium channels, and explored the drug-associated channel interaction and its voltage-dependence. It was at this time that Courtney coined the term "use-dependence" to describe the increase in block associated with increased frequency of stimulation.

Hille (1977) and Hondeghem and Katzung (1977) concurrently, but independently, formulated the Modulated Receptor Hypothesis (MRH) in an attempt to describe the effects of local anaesthetics in nerve and cardiac muscle, respectively. The Strichartz-Courtney model could easily account for use-dependent development and recovery from block. It

could not, however, explain the voltage-dependence of recovery described by Chen et al. (1975); the MRH however, could account for this action.

Starmer et al. (1984, 1985) have proposed the Guarded Receptor Hypothesis (GRH) in an attempt to simplify the complex mathematics involved in the global calculation of drug on-rate and off-rate constants (at least 16 in all) for the many states of drug-associated and drug-free channel association. The above outline briefly describes the development of antiarrhythmic drug models that have gained widespread acceptance in describing how antiarrhythmic drugs may effectively suppress supraventricular and ventricular-derived arrhythmias.

1.1.2 Utility of Antiarrhythmics in Ischaemia

With the advent of models describing local anaesthetic and antiarrhythmic drug interaction with the sodium channel, an explosion in the development of antiarrhythmic drugs occurred in the early 1970's. The study of sodium channel antiarrhythmics continued at a high level until 1989 when it was found that in post myocardial infarction (MI) patients, the class I agents, flecainide and encainide, did not prevent, but rather increased mortality (CAST investigators, 1989).

Initial development of antiarrhythmic drugs was fueled by the need for agents which would selectively abolish arrhythmias, possess greater cardiac efficacy and exhibit fewer side-effects. The majority of drugs were local anaesthetics or, according to the Vaughan Williams classification scheme (see below), class I agents, i.e. those which reduce the influx of sodium ions during phase 0 of the action potential (AP).

Sudden cardiac death is responsible for more than 35,000 deaths in Canada each year (Reeder et al., 1993). The majority of such deaths are due to ventricular fibrillation that may or may not preceded by ventricular tachycardia (VT) or premature ventricular contractions (PVC). The Cardiac Arrhythmia Suppression Trial (CAST or CAST-I) was a

large, randomized, multicentre, placebo-controlled study undertaken to examine whether the incidence of cardiac death in patients with asymptomatic or mild ventricular arrhythmias, post-MI, could be reduced with class I antiarrhythmic drugs (CAST investigators, 1989). Clinical trials with flecainide, encainide and later moricizine in CAST-II (CAST-II investigators, 1992) were stopped due to an abnormally high incidence of death in drug treated groups. Additional evidence in human and animal arrhythmia studies shows that class I antiarrhythmic drugs may potentially have proarrhythmic actions (Velebit et al., 1982; El Sherif, 1991; Starmer et al., 1991). Thus the CAST findings merely highlighted clinically what was already known by cardiac pharmacologists and electrophysiologists for many years (Hondeghem, 1987; Tamargo et al., 1992).

Local anesthetics or class I sodium channel blocking antiarrhythmic drugs possess several properties that characterize the actions of this class of drugs. A review of the large number of class I agents developed reveals that most are weak bases i.e., have a pK_a between 7.0-10.0. Thus their protonated:unprotonated ratio varies with pH (Narahashi et al., 1970). In the charged form the compound is hydrophilic and according to Hille (1977) has limited access to the sodium channel through the pore (or hydrophilic pathway). However, in the uncharged form the compound can readily move into the lipid bilayer of the cell, accessing the channel independent of the channel state (Hille, 1977; Hondeghem and Katzung, 1977).

Under ischaemic conditions the acidosis and hyperkalemia (see below for complete discussion) favor the interaction of the charged form of drug with the sodium ion channel. These changes provide the drug with "ischaemic-dependence" and hence a greater degree of sodium channel block is possible compared to that found in the normal myocardium.

Drug asymmetry is also an interesting feature of antiarrhythmic drug actions in ischaemia. The stereochemical properties of drugs result in optically active stereoisomers, which may or may not exhibit similar properties in cardiac tissue. For example the (R)-enantiomer of mexiletine, a class Ib antiarrhythmic agent, is a more potent inhibitor of

sodium currents (Grant, 1990) than its (S)-enantiomer, but is limited in action due to its fast rate of clearance from the body (Igwemezie et al., 1991). The (R)-enantiomer has also been shown to be more effective at reducing ischaemia-induced VF in rats subject to coronary artery occlusion (Igwemezie et al., 1992).

New antiarrhythmic drugs must be developed which show greater selectivity for conditions of ischaemia, possess fewer side effects (i.e., are not proarrhythmic) and which have a greater therapeutic efficacy. The arylacetamide compounds, exemplified by U-62,066E (spiradoline), have properties that favour these desired actions and provide a novel chemical structure which may be explored and antiarrhythmics developed based upon it.

1.1.2.1 Supraventricular Tachyarrhythmias (SVT)

Atrial arrhythmias are defined by the requirement that either the atria or atrio-ventricular junction (AVJ) is involved in initiation, and maintenance of the arrhythmia. Supraventricular arrhythmias involving the AVJ can be subdivided into those requiring conduction in the AV node (AVN) and those mediated by accessory pathways. These types have been extensively reviewed by Waldo and Wit (1993) and Ganz & Friedman (1995) therefore will not be elaborated upon in detail here. Briefly, it should be understood that AVN re-entrant tachycardia is the most common cause of SVT. The majority of SVT's are due to anomalous bands of tissue that conduct abnormal impulses between atria and ventricle. Usually conduction in this accessory pathway occurs alongside conduction in the normal AV system (Ganz and Friedman, 1995). This causes the Wolff-Parkinson-White (WPW) syndrome characterized electrocardiographically as a delta wave accompanied by an ECG with a short P-R interval (Wolff et al., 1930).

Pharmacological intervention is used in order to both relieve symptoms and to prevent stroke which can accompany atrial fibrillation. Treatment includes the use of

intravenous adenosine, an A_1 agonist which rapidly suppresses more than 90% of all SVT arrhythmias (Camm and Garrett, 1991); the calcium antagonists verapamil and diltiazem; the cardiac glycoside digitalis; the ß-antagonists, such as propranolol and nadolol and class I antiarrhythmics such as intravenous (i.v.) procainamide, lidocaine and oral quinidine (the drug of choice for treatment of non-life threatening SVT) (Sokolow and Edgar, 1950; Fenster et al., 1983; Toulboul et al., 1991; Pritchett, 1992). It is suggested that lidocaine may be beneficial since it blocks conduction in accessory pathways but correspondingly may further depress the poor haemodynamic status associated with a rapid ventricular rate occurring during atrial fibrillation (Akhtar et al., 1993). Quinidine is preferred as it can be given both to treat arrhythmias and with continued administration after arrhythmic suppression may reduce the incidence of recurrence (Pritchett, 1992).

Thus many pharmacological treatments exist for the effective reduction of SVT. Radiofrequency catheter ablation is the method of choice by cardiac electrophysiologists for the prevention of atrial arrhythmias (Ganz and Friedman, 1995).

1.1.2.2 Ventricular Arrhythmias

Ventricular arrhythmias resulting from myocardial infarction (MI) are responsible for approximately one-half of the deaths from coronary heart disease in Canada (Reeder et al., 1993). Arrhythmias originating in the ventricle have complicated etiologies and may be the result of many factors. The responsible arrhythmogenic stimuli have been examined in detail and have been the focus of many reviews and therefore will not be elaborated upon in this thesis (see reviews by Botting et al., 1985 and Curtis et al., 1993). Briefly, these stimuli include coronary artery spasm causing angina and its variant forms, platelet thrombosis and embolization, ruptured atheromatous plaques with or without emboli as well as many local ionic (an imbalance of sodium, potassium and calcium) or metabolic substances including phospholipids and eicosanoids. The autonomic nervous system,

especially the sympathetic, may also play an important role in arrhythmogenesis (Botting et al., 1983, 1985). Regardless of which stimuli or factors are involved, the relative importance of each is still uncertain.

While cardiac rhythm disturbances may be the result of a variety of pathophysiological conditions, it is ischaemia of the myocardium which dominates. Myocardial ischaemia or lack of blood flow to ventricular tissue disrupts a balance that exists between myocardial demand for, and coronary delivery of ions oxygen, metabolic substrates and energy (Hearse and Dennis, 1982). Even more damaging is the lack of removal of toxic cellular and metabolic waste products such as protons, carbon dioxide and lactate. The condition of ischaemia produces profound alterations in normal cardiac electrophysiology and cellular metabolism and as a consequence, the establishment of an ectopic focus or perhaps re-entrant patterns of excitation or after-depolarization can arise which precipitate ventricular arrhythmias or fibrillation.

Many models designed specifically to describe the production of arrhythmias have been developed. Models of electrically and chemically-induced arrhythmias induced in a variety of species have been extensively reviewed by Szekeres (1979). As well, Winslow (1984) carefully detailed electrical stimulation, chemical and pathological models of arrhythmia induction while Walker et al. (1991) and Cheung et al. (1993) summarized the use of the rat as a model of arrhythmogenesis.

These models have been essential in the development of drugs for the treatment of ventricular arrhythmias. Effective treatment relies upon the conditions associated with ischaemia and this allows antiarrhythmic drugs to be selective. The required actions of such drugs have been extensively reviewed by Vaughan Williams (1989); they involve rendering infarcted or ischaemic tissue electrically silent, producing greater refractoriness in normal ventricular tissue and reducing or silencing ectopic electrical activity in the border zone between infarcted/ischaemic and normal ventricular tissue. Vaughan Williams determined that blockade of sodium channels met all these criteria. However, more

variability would exist as to the effects of the particular agent or group of agents due to their effects on APD or refractoriness. Drugs such as lidocaine, which do not prolong APD, may not enhance refractoriness in normal tissue, but instead possess marked use-dependent properties which then directs its "ischaemia-selectivity" (Hondeghem et al., 1974; Clarkson et al., 1988; Hondeghem and Snyders, 1990).

Despite the large number of experiments that have been conducted remarkably few sodium channel blocking antiarrhythmic drugs are actually used clinically to suppress arrhythmias. The low number of drugs accentuates the findings from the Cardiac Arrhythmia Suppression Trial (CAST or CAST-I) and CAST-II trial (CAST investigators 1989; CAST-II investigators, 1992). Many previous studies conducted with a variety of sodium channel blocking antiarrhythmic agents suggest that these drugs can effectively suppress arrhythmias but this does not necessarily translate into an improved survival rate after a myocardial infarction (Myerburg et al., 1994). Quinidine, for example, was shown to increase the incidence of mortality as compared with mexiletine in patients with potentially lethal arrhythmias (Morganroth and Goin, 1991).

Lidocaine prevents the occurrence of VF but does not significantly improve survival in post-MI patients (Hine et al., 1989). In 1983 Campbell, after examining many clinical trials and experimental evidence in animals, suggested that lidocaine prophylaxis therapy was effective against ventricular arrhythmias, but pointed out that in humans the risk-benefit ratio of such treatment was not known.

The class II (ß-blocking) antiarrhythmic agents, typified by propranolol and nadolol, are the only drugs that have been consistently shown to produce an increased time to onset of VT (Rosenfeldt et al., 1978), reduction in arrhythmia incidence (Paletta et al., 1989) and to improve survival post MI (Yusuf et al., 1985). In a recent survey of U.S. cardiologists, ß-blockers were the most frequently chosen class of antiarrhythmic drugs used to suppress arrhythmias in newly diagnosed patients (Morganroth et al., 1990).

The outcomes of both CAST-I and CAST-II represented a marked set-back for the development of class I antiarrhythmic drugs. Focus has shifted to the development of selective class III antiarrhythmic agents which prolong refractoriness (see Hondeghem, 1992, 1994; Janse, 1992). Studies with amiodarone and sotalol have shown that these drugs produce an effective reduction in PVC incidence and mortality (Cairns et al., 1991). A recent clinical study which involves amiodarone, is CAMIAT or the Canadian Amiodarone Myocardial Infarction Arrhythmia Trial. The results of this study are currently awaited. However, the recent Electrophysiologic Study versus Electrocardiographic Monitoring (ESVEM) trial compared sotalol to six class I antiarrhythmic drugs and showed that the risk of arrhythmia occurrence was lower with sotalol than the other agents (Mason, 1993). Despite the marked effectiveness of amiodarone and sotalol in clinical trials, the numerous other class III agents under development are "reverse use-dependent" or are essentially less effective at elevated heart rates associated with high frequency arrhythmias such as VT or VF (Hondeghem 1994; Janse, 1992). As well, they may induce bradycardic-dependent arrhythmias such as torsades de pointes (Sasyniuk et al., 1989; Binah and Rosen, 1992).

Thus neither class I or III antiarrhythmic agents currently in use is ideal. The drugs discussed above may be potent, but are not selective, and possess a mixture of pharmacological actions despite their classification as either sodium or potassium channel blockers. Thus it is worthwhile describing the systems of classification in current use.

1.1.3 Classification of Antiarrhythmic Drugs.

Drugs with desired antiarrhythmic qualities in ischaemic tissue should possess little action in normal cardiac tissue including action potentials. To be an effective agent a drug's action must be confined to those action potentials which are involved in the genesis of abnormal cardiac arrhythmias (Singh and Courtney, 1990). As this area of

pharmacology expands it becomes necessary to provide a rational framework in which to interpret the mechanism of action of currently used agents, and perhaps more importantly, newer agents. The schemes by which antiarrhythmic agents have been classified have been controversial (Singh and Hauswirth, 1974) and new propositions (such as the Sicilian Gambit) are no exception to this criticism. It was with the development of an electrophysiological understanding of the cardiac action potential (which will be reviewed below), and its components, that the antiarrhythmic drugs could be reasonably separated into discrete groups based on their electrophysiological actions. These groups, according to Vaughan Williams (1984a, 1984b), were not a categorization but rather a means by which to "describe four putative ways in which abnormal cardiac rhythms can be corrected or prevented". Classification schemes will be discussed briefly below.

1.1.3.1 The Vaughan Williams Classification

Two classification schemes for antiarrhythmic drugs were developed in the early 1970's. The first scheme was developed by Singh and Vaughan Williams in 1970a. They categorized a series of drugs, based upon electrophysiological actions, into four distinct groups. Class I agents were local anaesthetics which reduced the maximum rise rate of depolarization (V_{max}) by reducing sodium currents in heart cells. Class II agents reduced sympathetic nervous system effects on the heart while class III agents, such as sotalol and amiodarone, prolonged action potential duration (APD) and increased refractoriness in cardiac tissue. Subsequently, studies with verapamil provided a fourth class of antiarrhythmic drugs which blocked calcium currents in cardiac tissue (Singh and Vaughan Williams, 1970a, 1972). Hoffman and Bigger (1971) based their scheme on studies with several novel antiarrhythmic agents for that time. They proposed two groups of drugs with distinct properties. Those in group I included agents such as quinidine and procainamide which reduced V_{max} by inhibiting sodium currents in cells and which also prolonged APD.

Those in group II did not reduce V_{max}, or inhibit sodium influx into cells. However group II compounds shortened APD.

The current classification of antiarrhythmic drugs is based primarily upon the Singh and Vaughan Williams categorization, but also is a hybridization of the two schemes. In 1974, Singh and Hauswirth modified the Vaughan Williams scheme by subdividing class I agents into Ia, those drugs which reduce V_{max}, increase APD and which are effective in normal cardiac tissue (e.g., quinidine), and Ib, those agents such as lidocaine which reduce V_{max}, decrease APD and also seem to be most effective in ischaemic or partially depolarized tissue. With the development of potent sodium channel blockers such as flecainide, Harrison (1981) added a third subclassification, class Ic. This group of drugs blocked sodium channels both in normal and ischaemic myocardium.

The possible addition of a fourth subclass to the class I agents which would characterize the electrophysiological actions of the antiarrhythmic agent transcainide has been proposed (Bennett et al., 1987). It blocks sodium channels with little time or voltage-dependence. As well, a fifth class of antiarrhythmic agents which are bradycardic in nature has been identified, and it includes drugs such as alinidine which act to depress pacemaker cell depolarization and increase the threshold potential for spontaneous depolarization in these cells (Kobinger and Lille, 1987).

The inhibition of sodium currents by class I agents increases with frequency of stimulation and results in elevation of electrical thresholds of excitability and depressed cardiac conduction. These properties are consistent for most drugs of this class but properties vary with individual agents. Subclassification of the large number of agents found with these properties was, as noted above, begun. Campbell (1983b) also defined subgroups on the basis of kinetics of drug onset and offset.

1.1.3.2 Subclassification of class I antiarrhythmic agents

The original Singh and Vaughan Williams classification of antiarrhythmic drugs was modified by Harrison in 1981. He attempted to make the classification more useful to both clinicians as well as basic researchers (Harrison, 1985a, 1985b).

Studies with individual agents had shown that rate-dependence was common to all compounds with class I activity (Courtney, 1980; Campbell, 1989). Campbell, using a different set of criteria to Harrison, examined numerous class I agents and suggested that groups of drugs sharing common properties corresponded to the sub-classification described by Harrison (Campbell, 1983a, 1983b). Campbell showed that class I agents fell into three distinct groups based on their rapidity of response to increases in frequency and rate of onset and recovery from rate-dependent block. Drugs with class Ia properties were intermediate in kinetics, class Ib were rapid and class Ic slow (Campbell, 1983b). In addition, the same groups showed differential effects on APD. Class Ia agents prolonged APD, Ib shortened it, and the response to class Ic was variable. Campbell also showed that these same groups altered the effective refractory period (ERP) relative to APD. For class Ia agents there was a moderate increase in ERP relative to APD, while class Ib markedly prolonged ERP relative to APD. The class Ic agents had little effect on ERP-APD (for an in-depth view of these studies and the ERP-APD relationship see Campbell, 1989 and Vaughan Williams, 1984a, 1991).

The implications of subgroup classification included the ability to suggest putative mechanisms for ischaemic and ventricular tissue-dependence of antiarrhythmic drugs (Campbell, 1989). Ischaemia selectivity is displayed predominantly by the class Ib agents. Lidocaine was shown by Hondeghem et al. (1974) to exhibit selectivity. Results from the kinetic studies by Campbell (1983a, 1983b) showed that drug binding occurs during the action potential and unbinding occurs during diastole, in agreement with the suggestion by Hondeghem and Katzung (1977). Lidocaine depresses ischaemic, but not normal myocardium, and is believed to bind to the closed (inactive or I) state of the sodium channel. During depolarization, and in ischaemia-depolarized tissue (see below), this state

of the channel is favoured (Hondeghem and Katzung, 1977) resulting in drug selectivity. This same argument can be used to define the differential selectivity which exists between atrial and ventricular tissue selectivity. The brevity of the atrial action potential dictates that the sodium channel spends little time in the inactive state. This, in turn, reduces the time for drug-inactive channel association and hence less block results during the AP for the suppression of ectopic pacemakers (Campbell, 1989).

Briefly, the other groups within the Vaughan Williams classification scheme include class II (ß-blockers). These act to inhibit sympathetic nervous activity on the heart but do not alter normal cardiac function such as conduction velocity at resting membrane potentials. Class III agents control cardiac arrhythmias by slowing repolarization. This corresponds to an increase in ERP and reduction in arrhythmias (Singh and Courtney, 1990). Many class III antiarrhythmics exist and are very potent blockers of cardiac potassium currents (Hondeghem and Snyders, 1990; Colatsky et al., 1990). The last group of antiarrhythmic agents in this classification scheme are the class IV calcium channel blockers. There are a number of antiarrhythmic agents of diverse chemical nature in this group. However, the common antiarrhythmic mechanism is blockade of the cardiac L-type calcium channel (see review by Walker and Chia, 1989).

1.1.3.3 The Sicilian Gambit

The most recent framework for classifying antiarrhythmic drugs was postulated in the Sicilian Gambit (Task Force of the Working Group on Arrhythmias of the European Society for Cardiology, 1991; reviewed by Rosen et al., 1992, 1995). The premise for this classification scheme was that antiarrhythmic drug effectiveness could be assessed by determining the ability of individual drugs to alter arrhythmogenic mechanisms. This could be accomplished by identifying "vulnerability parameters". These parameters include conduction, phase 4 depolarization, and excitability. This scheme requires that

antiarrhythmic drugs undergo a thorough study of their pharmacological profile for actions on ion channels (and receptors) before an attempt can be made to resolve the mechanism of antiarrhythmic action. The action of a particular drug could then be matched to the mechanism underlying the abnormal cardiac rhythm and be prescribed to treat the patient. According to Katritsis and Camm, (1993, 1994) it is the next logical step following the Vaughan Williams classification scheme. Colatsky (1992) has been quick to criticize this new scheme. He suggests that the Gambit does not offer any new paradigms by which to examine antiarrhythmic drugs, or aid in their development, and criticizes the targeting of specific cellular sites for rational drug choice based on mechanisms which are not well understood. Both Harrison (1992) and Vaughan Williams (1995) also criticized the Sicilian Gambit. They suggest that with this scheme we pretend to know more than we actually do and state that simplicity is best as exemplified by the original Vaughan Williams (1970) classification.

1.2 Mechanisms of Arrhythmogenesis

1.2.1 The Ischaemic Myocardium

Common causes of arrhythmias in humans are myocardial ischaemia, myocardial infarction, or reperfusion of a previously ischaemic myocardium. These conditions can be readily reproduced in both intact and isolated rat hearts.

The rat heart, similar to that of primates and pigs, does not have extensive coronary collaterals (Johns and Olson, 1954; Maxwell et al., 1984), i.e. rat coronary arteries are end-arteries (Winkle et al., 1984). Thus, when a coronary artery is occluded an area is rendered uniformly ischaemic (Schaper, 1971; Schaper et al., 1986). However, ischaemia is not absolute and a residual blood flow of approximately 5% is seen following complete ligation of the artery (Maxwell et al., 1984). If occlusion of an artery persists for more than

ten minutes, irreversible damage occurs and infarction results (Saint et al., 1992). The time-dependency of the onset of arrhythmias after occlusion is quite characteristic for many species. Arrhythmias first occur in conscious chronically prepared rats 5-15 minutes after occlusion (Walker et al., 1991; Johnston et al., 1983). The most common arrhythmias seen include premature ventricular contractions (PVC), ventricular tachycardia (VT) and ventricular fibrillation (VF). A second arrhythmic period occurs after 1-2 hours of occlusion and consists of PVC, VT and VF. Various factors influence the severity and incidence of arrhythmic outcomes after occlusion. These include both the size of the ischaemic zone and the serum potassium concentration (which has an inverse log-linear relationship to arrhythmia score) (Curtis et al., 1986a; Curtis et al., 1987; Podrid, 1990; Saint et al., 1992).

Well-defined electrocardiographic (ECG) changes also occur in the rat model of coronary artery occlusion. The ECG shows an increase in the R-wave height within minutes after occlusion followed by an elevation in the S-T segment (Johnston et al., 1983; Normann et al., 1961). The exact mechanism by which S-T segment elevation occurs is not known (Walker et al., 1991). ECG responses to ischaemia are well-defined in pigs and dogs (for a review in these species the reader is referred to Hirsche et al., 1982; Benzing et al., 1972; Hill and Gettes, 1980; Gettes et al., 1989).

Ischaemia produces changes in the extracellular milieu of the cell. These changes were first reported for potassium by Harris et al. (1954) who showed that coronary occlusion was associated with an increase in extracellular potassium. In addition, Benzing et al. (1972) and Case et al. (1979) showed that the change in potassium is accompanied by changes in pH, O_2 and CO_2 levels within the ischaemic zone. These changes in ion concentrations have become better defined with improved experimental techniques including ion sensitive electrodes, nuclear magnetic resonance (NMR) and voltage-sensitive dyes (Gettes et al., 1989).

The intracellular events which occur as a result of ischaemia include a reduction in pH from 7.2 to 6.0 (Garlick et al., 1979), a slight elevation in sodium from 5 to 20 mM, due

to partial suppression of the sodium/potassium ATPase pump (Wilde and Kleber, 1986), and an increase in calcium (Steenbergen et al., 1987). Yan and Kleber (1992) have recently shown that the pH change is not homogeneous within the ischaemic myocardium due to local variations in accumulation and diffusion of CO_2. This may have implications in the development of arrhythmogenic circuits (see below). Accompanying the intracellular changes are extracellular changes. Within the ischaemic myocardium there is a triphasic increase in potassium which, unless reversed, results in an irreversible loss in membrane integrity (Hill and Gettes, 1980). A reduction in extracellular pH occurs which parallels the changes in extracellular potassium levels. In a similar manner intracellular events are varied and contribute to the heterogeneity within ischaemic tissue (see review by Orchard and Cingolani, 1994; Janse and Opthof, 1995). In addition to the local micro-inhomogeneities of ions which occur within the myocardial extracellular space, Hill and Gettes (1980) have shown in pigs that a disparity exists between the centre and the border zone of the developing ischaemic tissue and still yet between the myocardial subepicardium and subendocardium. These transmural differences have been associated with the wave-like spread of ischaemia from the endocardium to the epicardium (Reimer and Jennings, 1979).

The suggested mechanism producing these changes is based upon biochemical studies of anaerobic metabolism which occurs within cardiac tissue after coronary occlusion (Gettes et al., 1989). Anaerobic metabolism results in glycolysis and a reduction in high energy phosphates which in turn produces lactic acid and reduces pH. The rise in potassium is attributed to the passive movement of potassium with lactate to maintain electrical neutrality across the membrane (Benzing et al., 1972). The lack of blood flow to the ischaemic tissue does not permit a washout of substances within this area thereby contributing to accumulation.

Electrophysiologically, the changes in potassium concentration result in depolarization of the cell membrane according to the Nernst potential. Kodama et al.

(1984) have shown that the changes in pH and CO_2 augment such depolarization. The reduction in resting membrane potential slows the maximum rise rate of depolarization and prolongs recovery of cell excitability by altering sodium channel inactivation (Gettes and Reuter, 1974). Thus, refractoriness is prolonged beyond the effective refractory period (ERP) of the APD (which is shortened itself due to the increase in extracellular potassium) (Yan et al., 1993). The large inhomogeneity which exists at all levels in the ischaemic tissue results in heterogeneous conduction and refractoriness. The development of "injury" currents which flow between non-ischaemic and ischaemic cardiac tissue may precede or generate arrhythmias (Han and Moe, 1964; Janse et al., 1980).

1.2.2 Abnormal Impulse Conduction

Recent advances in cardiac electrophysiological methods have improved our understanding of the mechanisms of cardiac rhythm disturbances. Initially cardiac arrhythmias were classified as being due to an interference with either cardiac impulse generation, conduction or both (Hoffman and Cranefield, 1964; Hoffman, 1981). In 1981 Hoffman and Rosen altered the classification scheme by expanding abnormal impulse generation to include normal/abnormal automaticity and triggered arrhythmias. As well, conduction arrhythmias were expanded to include delayed or blocked impulse propagation and uni-directional conduction block modeled by re-entry. This section will be confined to a brief discussion on the arrhythmic mechanism(s) which are likely due to myocardial ischaemia.

In order to understand how abnormal impulse conduction relates to abnormal automaticity the basis for generation of the action potential and its various phases must be briefly reviewed. Normal automaticity occurs during Phase 4 of the cardiac action potential as a result of a slow diastolic depolarization in (or of) cardiac nodal cells. This inward current is due to the slow opening of i_f, a non-specific cation channel which is activated by

membrane hyperpolarization (DiFrancesco, 1981; Ho et al., 1994). Pacemaker activity in cardiac tissue is hierarchical in that the sinus node dominates and suppresses subsidiary pacemaker sites, reducing the likelihood of the production of ectopic impulses. This is termed overdrive suppression (Vassalle, 1970).

Phase 0 of the action potential is due to the rapid inward movement of sodium ions (I_{Na}) and this causes cell membrane depolarization. Sodium currents account for the rapid upstroke and amplitude characteristics of atrial, ventricular and Purkinje cells. Phase 1 repolarization is thought to be due to inactivation of I_{Na}, and the concomitant activation of a transient outward potassium current (I_{to}) (Coraboeuf and Carmeleit, 1982). The plateau (or Phase 2) of the action potential is due predominantly to calcium current influx via L-type calcium channels at depolarized membrane potentials (Bean, 1985). These "slow inward currents" (i_{si}) are required for excitation-contraction coupling and result in contraction of cardiac muscle (see review by Hess, 1988). However, Coraboeuf et al. (1979) also showed that, in cardiac tissue, not all sodium channels inactivate during depolarization. Thus a sodium "window current" also contributes to the maintenance of the action potential plateau duration. Phase 3 repolarization can be electrophysiologically complex due to the large number of voltage- and ligand-activated potassium channels found in cardiac tissue (Carmeleit, 1993). However, this stage of ventricular repolarization is due predominantly to the opening of delayed rectifier (I_K) potassium channels (McAllister and Noble, 1966). Repolarization of the membrane activates an inward rectifier (I_{K1}) potassium current which ensures that the membrane returns to its resting potential (Carmeleit, 1993). Thus, with the understanding of the components of the action potential and hence normal automaticity, one can discuss abnormal automaticity.

There are many factors that may either suppress normal sinus node pacemaker function or enhance overdrive suppressed pacemaker automaticity and produce an ectopic beat. However, in the ventricle the main factor that results in latent pacemaker activity is damage as a result of stretch, scar formation or ischaemia. Ischaemia results in

depolarization of ventricular tissue, inactivation of sodium channels, and reduction in repolarizing potassium currents due to inactivation of the sodium/potassium ATPase exchanger. This enzyme, under normal conditions, hyperpolarizes the membrane and suppresses ectopic pacemaker sites (Binah and Rosen, 1992).

Abnormal impulse generation can arise from oscillations in the membrane potential and has been characterized as triggered rhythms (Cranefield, 1977; Binah and Rosen, 1992). These triggered rhythms occur in two forms: early or late-afterdepolarizations (EAD or DAD).

1.2.3 Early Afterdepolarizations (EAD)

Early afterdepolarizations interrupt either Phase 2 or 3 repolarization of the action potential. If these afterdepolarizations attain sufficient thresholds they may produce triggered responses and induce single or multiple extrasystoles and even VT (Cranefield, 1977). The EAD is an oscillatory potential which is sensitive to frequency, often occurs at slow stimulation rates (Davidenko et al., 1989); the amplitude of the EAD increases at low rates. High stimulation rates, however, abolish EAD's (Roden and Hoffman, 1986). EAD activity has been shown *in vitro* using many types of isolated cardiac muscle and various cell types including mid-myocardial cells (M-cell) (Antzelevitch and Sicouri, 1994). Induction of EAD activity can be induced by a variety of drugs including class I and III antiarrhythmic agents (Binah and Rosen, 1992; see review by Antzelevitch and Sicouri, 1994), the calcium channel opener, Bay K 8644 (January and Riddle, 1989), and catecholamines (Priori and Corr, 1990). Experimentally, ischaemic conditions also result in EAD-induced triggered activity (El Sherif, 1991).

The ionic basis for EAD development is unclear (January et al., 1991). However, studies suggest the involvement of the slow inward calcium current (i_{si}) of the cardiac L-type calcium channel during the plateau of the action potential. Theoretical modeling

studies conducted by Zeng and Rudy (1995) suggested calcium involvement in sustaining EAD activity as shown experimentally by Marban et al. (1986), January and Riddle (1989), and Priori and Corr (1990). Essentially, i_{si} re-activation acts as a depolarizing charge carrier during the depolarizing phase of the EAD. Prolongation of the plateau phase of the action potential allows for an increased time for calcium channel recovery which enhances the inward current thereby depolarizing the membrane and sustaining the EAD (Zeng and Rudy, 1995). Other proposed mechanisms include a reduction of outward potassium currents resulting in slow repolarization and an increase in sodium window current associated with a prolonged plateau (Coulombe et al., 1984). Ultimately, arrhythmias which result include the long Q-T syndrome and torsades de pointes (Antzelevitch and Sicouri, 1994); however, the genesis and maintenance of these arrhythmias by an EAD mechanism remains unclear.

1.2.4 Delayed Afterdepolarizations (DAD)

Transient depolarizations which occur during Phase 4 of the cardiac action potential are dependent upon the rate of the preceding action potential (Binah and Rosen, 1992). Unlike EAD's the amplitudes of DAD's increase with decreasing cycle lengths (Cranefield, 1977). DAD have been observed under a variety of experimental conditions all of which have a similar end result, i.e. intracellular calcium overload (Tsien and Carpenter, 1978). High intracellular calcium concentrations saturate the sarcoplasmic reticulum sequestration mechanism resulting in calcium oscillations due to calcium-induced calcium release (Binah and Rosen, 1992). The ionic currents which contribute to this mechanism are not known. The DAD is a self-sustaining rhythm and remains either sub-threshold or reaches threshold and initiates a premature response (Ferrier et al., 1973). Ischaemia, digitalis and catecholamines can directly produce DAD by enhancing calcium entry into cells (January and Fozzard, 1988; Antzelevitch and Sicouri, 1994). Thus calcium channel blockers, such

as verapamil, abolish DAD's and studies show that sodium channel blockers including quinidine, lidocaine, amiodarone and the potassium channel activator, pinacidil, may all effectively suppress DAD and DAD-induced triggered activity (Rosen et al., 1974; Rosen and Danilo, 1980; Spinelli et al., 1991; Antzelevitch and Sicouri, 1994). Arrhythmias which result from DAD triggered rhythms *in vitro* include single and multiple PVC's, and tachyarrhythmias (Cranefield, 1977). No direct evidence is available as to the existence of DAD-induced triggered rhythms *in vivo* (Antzelevitch and Sicouri, 1994).

1.2.5 Re-entry

The major cause of ventricular arrhythmias is due to re-entry. Re-entry has been subdivided into either circus-movement excitation or reflection (El Sherif, 1995).

The model for re-entrant circus-movement is based on a scheme developed by Schmitt and Erlanger (1929). A bifurcating Purkinje fibre bundle attached to the ventricle gives rise to different anatomical conduction pathways. Re-entry occurs when antegrade conduction of the impulse is extinguished at a site of uni-directional block. This type of block may arise from ischaemic damage of previously normal conduction pathways. If normal conduction continues in the other branches of the pathway an impulse can retrogradely enter the area of unidirectional block where its conduction is slowed but not extinguished. The impulse can then emerge from this depressed area and, providing that the cells are not refractory, re-excite the tissue proximal to the area of block and generate premature ventricular complexes which can remain as such, or deteriorate into VT or VF (Moe, 1975; Janse and Kleber, 1981; Binah and Rosen, 1992). This results in two forms of re-entry. Ordered re-entry occurs when re-entrant excitation occurs along a pre-existing pathway and usually results in VT (Cranefield et al., 1973). Random re-entry of impulse propagation results when electrophysiological differences exist between areas of cardiac muscle. The development of ischaemia is dynamic therefore the pathway is not constant

for the impulse which circulates. It may fractionate, produce multiple re-entrant circuits, and result in VF (Hoffman and Rosen, 1981). Allessie et al. (1977) showed that random re-entry occurs in the absence of an anatomical pathway and that the propagating impulse produces a central area of inexcitability around which the impulse circulates. This was termed the "leading circle hypothesis".

Many studies show that cardiac tissue types can, under conditions which mimic ischaemia, generate and maintain re-entrant circuits (Sasyniuk and Mendez, 1971; Wit et al., 1972a, 1972b; El Sherif, 1991). Antiarrhythmic drugs can abolish re-entrant arrhythmias by either converting uni- to bi-directional block within the depressed region or prolonging refractoriness (El Sherif, 1991). Which of these is most important is a matter of some debate (Janse, 1992). Since re-entry is only possible if the length (time) of the re-entrant path of the circus wave exceeds the normal cellular refractory period (about 300 milliseconds) antiarrhythmic drugs prevent re-entry by either prolonging refractoriness (as with Class III agents) or slowing conduction (as with Class I agents) (Varro and Surawicz, 1991). Wavelength is the term describing the distance the re-entry impulse travels (mathematically this term is described by conduction velocity multiplied by ERP) and has been suggested as a possible index of differential drug effectiveness (Rensma et al., 1988; Janse, 1992). Spinelli and Hoffman (1989) refute this measure as an index of predictive usefulness because both conduction velocity and ERP are not constant with rate of stimulation or with time in ischaemic tissue. The mechanisms suggested for arrhythmogenesis are complex and all, under ischaemic conditions, may play a significant role (Hoffman, 1981; Binah and Rosen, 1992) . It is most likely that re-entry dominates during VT and VF while the mechanisms for PVC's are less clear.

The following sections discuss basic cardiac electrophysiology of cardiac ion channels. As well, several models of ion channel block by antiarrhythmic drugs are outlined.

1.3 Cardiac Electrophysiology

1.3.1 The Cardiac Action Potential

As discussed above, the action potential (AP) is composed of the upstroke, plateau and repolarization phases. The shape of the AP is governed by ionic current flux via gated channels in the membrane for sodium, calcium and potassium. As well, membrane pumps and exchangers such as for Na/K ATPase and Na/Ca are involved. The properties of the action potential change moderately amongst tissue type. However the fundamentals of action potential generation remain essentially unchanged.

1.3.2 The Sodium Channel

Hodgkin and Huxley (1952) studied sodium conductance in the squid giant axon. They proposed that the voltage-dependent opening and closing of membrane "gates" resulted in a change in membrane permeability to sodium. The permeability change generated the action potential and was responsible for the transmembrane movement of sodium ions. They proposed "m" as an activation gate particle and "h" as an inactivation gate particle which display distinct kinetic properties highly dependent upon changes in membrane potential. Hodgkin and Huxley also postulated that for the conformational transitions of these gates to be voltage-dependent there must be a voltage-sensor, or charge movement, during such transitions. They predicted the existence of the "gating current".

1.3.2.1 Gating Kinetics

Depolarization of the cell membrane opens sodium channels. However this event only occurs after some delay (Armstrong and Bezanilla, 1973). During this short time period (<1msec) charge movement occurs. This delay was described as a series of voltage-dependent, closed-state conformational transitions the macromolecular protein which comprised the sodium channel had to pass through before the channel opened (Hille, 1989). The cause of this delay remained elusive until 1973 when Armstrong and Bezanilla first recorded the "gating current". It was a current of small amplitude (0.13pA) and fast kinetics (80 μsec to reach a maximum) (Armstrong and Bezanilla, 1973; DeFelice, 1993). Thus, the majority of this current flows prior to the opening of the m gate for activation (Hille, 1976).

Molecular studies have revealed characteristics of the sodium channel itself as well as the putative "voltage-sensor" for the gating current. The sodium channel is comprised of approximately 2000 amino acids, containing 4 homologous internal repeats, each of which has 6 putative transmembrane segments (Catterall, 1986). The α subunit contains a particular region, S4, which is composed of a number of positively charged amino acids and has been postulated to be the voltage sensor. Displacement by the change in membrane potential of these amino acids may be responsible for the gating current (Noda et al. 1984, Catterall, 1995). The outward gating charge for sodium is due to the movement of 6 charges across the membrane (Noda et al., 1984). This finding corroborated the proposed charge displacement equivalent to 6 electrons flowing from the extra- to intracellular side of the membrane by Hodgkin and Huxley (1952). However, despite the implication of the S4 region in gating, the mechanism by which activation is initiated is not known.

Until recently local anaesthetics and other drugs were believed to immobilize a fraction of the gating charge when the channel was blocked (Armstrong and Bezanilla, 1973; Bekkers et al., 1984). However, Hanck et al. (1994) suggest that local anaesthetic drug-bound cardiac channels gate with altered kinetics such that all channels continue to

gate but with a reduced voltage dependency. In light of the Modulated Receptor Hypothesis (see below) this may alter our perspectives regarding drug interaction with the sodium channel. Thus drug occupancy may reduce the voltage-dependence of gating by inhibition of voltage-sensitive charge movement rather than by drugs producing a shift in channel states to the favored drug-bound inactive state of the channel (Hanck et al., 1994).

1.3.2.2 Activation Kinetics

Activation of the sodium channel, not unlike gating currents, occurs rapidly and is very steeply dependent upon depolarization (Colatsky, 1980; Hille, 1984). Thus, the rate of activation increases with membrane depolarization (Hodgkin and Huxley, 1952). Activation generally occurs at thresholds between -60 to -70 mV via the voltage-dependent opening of the "m" gate. The change in voltage opens the channel and allows for a rapid increase in sodium permeability (Hille, 1989; Mitsuiye and Noma, 1992). Activation kinetics can be altered by plant alkaloids such as veratridine, scorpion or sea anemone toxins, or by insecticides such as pyrethroids. These kinetics are not altered by most antiarrhythmic or local anaesthetic drugs such as quinidine or lidocaine (Honerjager, 1983; Narahashi, 1992). These agents shift activation to more negative membrane potentials such that at resting potentials the steady-state depolarization is due to a sustained sodium current (Honerjager, 1983). It was originally proposed (for simplicity) that activation was independent of inactivation. However, it was not until Armstrong et al. (1973) perfused the squid giant axon with the enzyme pronase, and showed that inactivation was selectively destroyed and activation was unaltered, that the two processes could be dissociated. Later studies confirmed that activation and inactivation could be separated but that they were not entirely independent (Armstrong et al., 1973; Stimers et al., 1985).

1.3.2.3 Inactivation Kinetics

Ionic conductance of the sodium channel is transient in nature. Prolonged depolarization results in sodium channel inactivation and prevents the influx of sodium into the cell. Thus refractoriness is maintained (Hodgkin and Huxley, 1952). As with activation, the rate of inactivation increases with an increase in the rate of depolarization. Hodgkin and Huxley (1952) postulated that decay of sodium currents to resting values was monoexponential. However, Chiu (1977) found that the rate of inactivation was much better approximated with a bi-exponential function and described two voltage-sensitive components for inactivation: fast and slow. Studies by Khodorov et al. (1976) described the slow component in great detail. Aldrich et al. (1983) used inactivation studies of single channel sodium currents to show that decay was biphasic, and largely coupled to activation. These studies indicate that some fraction of the sodium channels must be open before inactivation proceeds.

The inactivation gate, "h", can be selectively destroyed by the internal application of protease (Armstrong et al., 1973) and chemicals such as the piperazinyl-indole derivative DPI 201-106 (Wang et al., 1990). Veratridine and batrachotoxin, alkaloid toxins, also inhibit inactivation and produce a steady-state depolarization due to enhanced sodium permeability (Brown, 1988; Catterall, 1980, 1986; Honerjager, 1983). At the cellular level this results in a prolongation of the AP and positive inotropism.

Molecular studies have shown that an intracellular linker between domain III and IV of the sodium channel is responsible for fast inactivation kinetics (Stuhmer et al., 1989; Patton et al., 1992; see reviews by Catterall, 1995 and Goldin, 1993). In addition these molecular studies provide evidence for the proposed "ball and chain" model of inactivation whereby this cytoplasmic linker may influence the activation and inactivation coupling process (Moorman et al., 1990). This model suggests that a positively charged cytoplasmic protein particle (the "h" gate using Hodgkin and Huxley formalism) electrostatically interacts with a negatively charged inactivation subunit of the sodium channel (Armstrong and Bezanilla, 1977; Khodorov et al., 1976; Carmeleit, 1987).

Local anaesthetics and antiarrhythmic drugs interact with the inactivation gate (Weidmann, 1955; Courtney, 1975; Hille, 1977; Hondeghem and Katzung, 1977). The inactivation produced by a change in membrane potential and drug block of the channel are interacting processes. These occur as a result of drug binding to a site on or near the "h" gate in a voltage, time and channel state-dependent manner according to the Modulated Receptor Hypothesis (Hille, 1977; Hondeghem and Katzung, 1977). Inactivation is complete with the return of the membrane potential to its pre-depolarizing (resting) level by activation of repolarizing potassium currents.

1.3.3 The Potassium Channels

1.3.3.1 Channel Diversity

Over the last several years interest in the development of drugs which prolong refractoriness, i.e. possess class III antiarrhythmic action, has increased markedly. Several reasons for this resurgence in interest include the negative results of the CAST trial where proarrhythmic tendencies were associated with some class I agents, the effectiveness of sotalol and amiodarone in the clinical setting and the results of long-term studies with amiodarone which suggest that it may, in a manner similar to the β-blockers, decrease post-infarction arrhythmic death (Vaughan Williams, 1982). Repolarization and the configuration of phase 3 of the action potential in cardiac tissue occur as a result of the complex interaction of up to 7 different potassium channels (Colatsky and Follmer, 1989; Carmeleit, 1993). These potassium channels are heterogeneous and differ in gating and permeation properties as well as in susceptibility to modulation by neurotransmitters and intracellular ions such as sodium and calcium (Hume et al., 1990). In essence potassium channels regulate cell function by establishing the resting membrane potential and controlling cell repolarization processes. Individual potassium currents overlap in their

contribution to the total membrane current during the action potential. The relative importance of each may vary under different conditions. During ischaemia changes in cell electrophysiology may alter the degree to which different channels contribute to the action potential (Colatsky et al., 1994).

Important species and regional differences exist in the contribution potassium channels make to repolarization of the cardiac action potential. Studies have shown that electrophysiological distinctions can be made between epi- and endocardial tissue in many species including the dog and rat (Wei et al., 1993). In canine ventricles, epicardial, mid-myocardial (M-cells) and endocardial cells display distinct electrical properties and hence different action potential morphologies (Vaughan Williams, 1985; Sicouri and Antzelevitch, 1991; Antzelevitch et al., 1995). In the rat ventricle at least three cell types have been distinguished based on APD (Watanabe et al., 1983). Wang et al. (1991) have characterized epi- and endo-cardial differences in APD in atrial tissue and suggest that the ionic mechanism for these differences is due to different amplitudes of the transient outward potassium current (i_{to}). Similar differences are also seen in ventricular tissue (Sicouri and Antzelevitch, 1991).

Molecular biologists have recently cloned and characterized voltage-gated potassium currents. All potassium currents have a similar primary amino acid sequence with highly conserved structural regions (Miller, 1991). One region, H5, has been implicated in pore formation as well as being a distinctive intracellular binding site for drug action (Pongs, 1992).

The heterogeneity of potassium channels provides a large potential for the development of diverse compounds with potassium channel blocking properties (Colatsky and Follmer, 1989). The therapeutic potential and benefits of these agents are their ability to increase the time course for repolarization and inhibit SVT and re-entrant ventricular arrhythmias without an associated slowing of intracardiac conduction in an already compromised heart (Katritsis and Camm, 1993). Sotalol, and amiodarone are typical class

III agents which block multiple potassium currents (Singh and Vaughan Williams, 1970a; Colatsky and Follmer, 1989). However, many new agents, at various stages of clinical development, are highly potent and channel selective. These include sematilide, E-4031, and dofetilide (Colatsky and Follmer, 1989; Katritsis and Camm, 1993; Hondeghem, 1994).

Class III agents are effective at maintaining a prolonged APD at low rates of stimulation. However, at high heart rates the effectiveness of these agents is diminished. This "reverse use-dependence" suggests that these drugs have a high affinity for the closed state of the channel (Hondeghem and Snyders, 1990; Colatsky et al., 1994; Hondeghem, 1994). Only amiodarone lacks this effect (Singh, 1983). The resulting bradycardia associated with these agents has been shown to precipitate arrhythmias such as the long Q-T syndrome (Zipes, 1991) and torsades de pointes (Roden, 1994; Katritsis and Camm, 1993).

Of the many potassium channels that exist in cardiac muscle we examined only two in our rat myocytes, i_{to} and the sustained outward delayed rectifier (i_{Ksus}) current. These will be briefly discussed as they contribute predominantly to repolarization of the heart in this species.

1.3.3.2 The Transient Outward Potassium Current

I_{to} is a relatively common current found in a wide variety of species and cell types, except the guinea pig (Campbell et al., 1995). The channel possesses rapid activation and inactivation kinetics (Coraboeuf and Carmeleit, 1982) and is important during early phase I repolarization. It is coupled to both sodium and calcium and is critical for the classic "spike and dome" appearance of the ventricular action potential (Katritsis and Camm, 1993). Channel density and distribution differences amongst cell types results in a variable action potential morphology in various regions of the heart (Antzelevitch et al., 1991). Escande et al. (1987) showed that i_{to} is composed of a large voltage-activated, calcium-independent

component, i_{to1}, and a small calcium-activated component, i_{to2}. The channel activates at membrane potentials more positive than -70mV and inactivates around -10mV (Coraboeuf and Carmeleit, 1982). I_{to} is highly potassium selective, shows little rectification and reaches its peak in 3 msec (Campbell et al., 1993). Inactivation, depending on the species, shows either mono- or bi-exponential rates of decay (Tseng and Hoffman., 1989; Jahnel et al., 1994). In the rat i_{to} is the main repolarizing current and is sensitive to blockade by 4-aminopyridine (4-AP) (Josephson et al., 1984; Castle and Slawsky, 1992). The bradycardic agent, tedisamil (KC8857) is a selective I_{to} blocker that has been used to extensively characterize both the electrophysiology (Dukes and Morad, 1989; Dukes et al., 1990) and involvement of this channel in ischaemic (Beatch et al., 1991; Adaikan et al., 1992) and programmed stimulation-induced arrhythmias (Wallace et al., 1995).

1.3.3.3 The Sustained Outward Delayed-Rectifier Potassium Current

I_{Ksus} is a time-dependent outward current which contributes to the initial phase III repolarization of the action potential. Wang et al. (1993) have extensively characterized this current in atrial cells. However, Escande et al. (1985) described a long lasting outward current (i_{lo}) in human atrial cells and Benz and Kohlhardt (1994) recently described a cardiac outward rectifier ($i_{Koutw.-rect.}$) current in rat myocytes. Whether these are identical current has not been clearly defined however current characteristics are similar. This current is residual in nature and occurs long after i_{to} has inactivated. Interestingly it can be found in cells which lack classical I_K current properties (Wang et al., 1993). Jahnel et al. (1994) suggest it is a third subtype of i_{to} and call it i_{to} steady-state (i_{to-ss}); however, Wang et al. (1993) successfully isolated I_{Ksus} from the i_{to} subtypes and characterized its properties. The channel is slow to inactivate requiring up to 10 sec to complete the process, rapidly activates and is sensitive to tetraethylammonium (TEA) and 4-aminopyridine (4-AP) blockade (Wang et al., 1993).

1.4 Models of Sodium Channel Blockade

The actions of cardiac antiarrhythmic drugs and their interaction with the cardiac sodium channel are based on models developed to describe the action of local anaesthetic drugs in nervous tissue (Grant, 1991). Studies conducted with the marine toxin, tetrodotoxin (TTX), showed that sodium channels were blocked at nanomolar concentrations of this toxin resulting in inhibition of nerve conduction (Narahashi, 1974) while micromolar concentrations are required to inhibit cardiac conduction (Abraham et al., 1989). However, antiarrhythmic drugs show an inverse potency in cardiac and neuronal tissue. Lidocaine inhibits the cardiac sodium channel at concentrations that are 1000 times greater than those which inhibit neuronal sodium channels (Bean et al., 1983). The differences between cardiac and neuronal sodium channels are reflected in single channel studies of gating currents (Kirsch and Brown, 1983). However, despite these differences a large number of similar properties are found regarding kinetics, drug interactions, and channel conducting properties and thus neuronal models have been applied to cardiac channels with success (Grant, 1991).

1.4.1 The Strichartz-Courtney Model

This model was developed by Strichartz in 1973 after conducting studies with several quaternary charged analogs of lidocaine (QX-222 and QX-314) on the frog node of Ranvier. Stimulation in the presence of either drug allowed delineation of two types of block, one that was tonic and the other which was voltage-sensitive. The Strichartz model elaborated upon the Hodgkin and Huxley (1952) model of sodium channel state-dependence. Strichartz assumed that the drug-bound channel complex either opened or closed transitionally following similar states proposed by Hodgkin and Huxley for drug-free channels. Courtney (1975) redefined the model proposed by Strichartz by studying the

voltage-dependent component of block in the presence of the lidocaine derivative GEA 968. In his studies Courtney coined the term "frequency or use-dependence" to describe an increase in the rate of block development seen with increasing rates of stimulation in the presence of the drug. The relevant modifications Courtney made to the model included the fact that drugs could dissociate from the channel when the cells were at rest, i.e., when the resting membrane potential was at rest. He also deduced that drug-bound channel complexes favored the inactive state of the channel which is reflected in a hyperpolarizing shift of the voltage-dependence of inactivation. This action was described by Weidmann (1955) for cocaine; however, Courtney's observations required that only the drug-bound fraction of the sodium channels produced a shift in sodium inactivation. Those channels which were not blocked were not altered.

This model was elaborated upon by Hondeghem and Katzung (1977) and Hille (1977) who independently proposed similar models for the interaction of local anaesthetics with cardiac and neuronal sodium channels, respectively. Courtney's model was based on observations by Chen et al. (1975) who showed that sodium channel recovery was voltage-dependent however could not account for this observation.

The Strichartz-Courtney model could not account for the properties of voltage-dependent sodium channel recovery. This model only described the use-dependent blocking and unblocking of sodium channels with local anaesthetics and antiarrhythmic drugs.

1.4.2 The Modulated Receptor Hypothesis

Hille (1977) proposed a model for local anaesthetic action on nerve based upon the previously described models. He suggested that there was a single specific binding site for local anaesthetics and that drug occupancy (block) alters the inactivation kinetics of the channel as previously shown by Weidmann (1955), Strichartz (1973) and Courtney (1975).

The proposed location of drug action was intracellular (Hille, 1977). Hille also postulated that multiple pathways existed for drug access to these binding sites, thus, unlike previous models it could account for all drug access routes to this binding site. Hondeghem and Katzung (1977) used studies in cardiac muscle to justify the proposal of a similar model for antiarrhythmic drug interaction with cardiac sodium channels. In this cardiac model a series of equations, or global fitting parameters, were developed which defined binding parameters for each state of the channel (rest, open, inactive) and accurately described channel block by quinidine and lidocaine (Hondeghem and Katzung, 1977; Davis et al., 1986). The general model suggests that as sodium channels change states in a voltage-dependent manner local anaesthetic or antiarrhythmic drugs can associate or dissociate from each state (Hondeghem and Bennett, 1989). Thus, each state has a characteristic set of association (k) and dissociation (l) rate constants and binding is modulated by voltage and time (Hondeghem and Katzung, 1977; Hondeghem, 1994). Since the affinity for the binding site is modulated by the state of the channel the proposed model was called the Modulated Receptor Hypothesis (MRH) (Hille, 1977; Hondeghem and Katzung, 1977, 1984; Hondeghem, 1987, 1989; Grant, 1991).

Over the years the model has been widely tested with many drugs in both cardiac and neuronal preparations (Hondeghem and Katzung, 1977; Clarkson et al., 1984, 1988; Hondeghem and Matsubara, 1988; Snyders and Hondeghem, 1990). The only limit to the use of this model was that a number of rate constants were required to be determined simultaneously. Several attempts have been made to simplify this model.

1.4.2 Simplified Versions of the Model

1.4.2.1 Kappa (κ) Repriming Model

Courtney (1983) proposed the kappa repriming model in an attempt to simply describe the quantity of block which develops during the action potential (κ) and the quantity of block which is relieved, due to unblocking, during diastole (λ). In order to calculate both κ and λ the model requires a fixed action potential duration and resting membrane potential during diastole. In this way an accurate determination of rate of block development, steady-state level of block and rate of diastolic recovery of block can be made. The use of the model is limited due to the fact that the APD and resting membrane potential are not fixed under normal conditions and that both κ and λ depend on time which may be highly variable for various drugs and thus may invalidate results.

1.4.2.2 Guarded Receptor Hypothesis

The Guarded Receptor Hypothesis (GRH) was proposed by Starmer et al. (1984, 1985) for the interaction of either local anaesthetic drugs with nerve or antiarrhythmic agents with cardiac sodium channels. This model simplifies binding and adopts the Hodgkin and Huxley model of the sodium channel as it allows for a simple, single-state model (i.e., Closed $\Leftrightarrow$ Open $\Leftrightarrow$ Blocked) on which to base drug action (Starmer et al., 1984). Drugs are theorized to interact with a constant affinity site on the sodium channel. Thus each drug interacts at different rate constants for the open and inactive states of the channel (Starmer, 1987). The drug-bound channels have the same voltage-dependence as drug-free channels but the model requires that only changes between resting, open and inactive states of the channel occur when there is a change in membrane potential, thus the MRH series of global fitting equations simplify into three sets of consecutive first-order equations (Starmer et al., 1984; Grant, 1991). Unlike MRH, drug binding in GRH only occurs in the open channel state. This new model changes the emphasis of drug interaction from the inactivation (h) gate in MRH to the activation gate (m): the activation (m) gate becomes immobilized by the drug-binding site complex and prevents sodium

influx. The frequency and voltage-dependence of the gates, in turn, dicates the voltage and frequency-dependence of the drug with the sodium channel (Grant, 1991).

However despite its simplicity neither the GRH, nor the more complex MRH, can explain block induced by all antiarrhythmic drugs (e.g., transcainide - see Bennett et al., 1987). Antiarrhythmic drug blockade of cardiac sodium channels may be highly dependent upon the structure of the molecule, a factor not incorporated into the models. In addition, experimental evidence has not yet shown that all antiarrhythmic drugs bind to the same site as proposed in the GRH and MRH models.

A novel class of compounds, which are not structurally-related to prototypical antiarrhythmic or local anaesthetic agents discussed above show putative sodium and potassium channel blocking properties. These are the arylacetamide κ opioid receptor agonists, typified by U-62,066E (spiradoline). The properties of these compounds are now discussed.

1.5 Opioid Receptor Heterogeneity

The identification of both agonist and antagonist drugs acting upon the various opioid receptors located in the central and peripheral nervous systems has resulted from the large monetary incentive and market potential for analgesics. Thus, the pharmaceutical industry strives to find less toxic analgesics and in doing so has created many novel classes of agonist and antagonist drugs with potent analgesic properties but which retain many undesired side effects. When this increase in development of drugs is combined with rapidly improving techniques used to distinguish opioid binding sites in tissue, an understanding of the mechanism(s) of opioid analgesia may result. To date, the majority of studies conducted with opioids have been in neuronal tissue in an attempt to elucidate the analgesic mechanism of these agents. The pharmacological profile of action of most opioid agents on the heart and cardiovascular system remains uncharacterized.

1.5.1 Historical Perspective

Opium is an extract derived from the poppy *Papaver somniferum* which for many centuries had been used to produce analgesia and alleviate pain. However, for most of this time the exact mechanism of action by which extracts from this plant alleviated pain was not known. The major alkaloid derived from the plant is morphine. Morphine is a potent analgesic but its use as such is limited by its side effects which include respiratory depression, constipation and physical dependence. The term 'opioid' was coined by Acheson (Martin, 1967) to designate drugs whose action resemble morphine but which may be chemically distinct from its phenanthrene structure. This definition has now been broadened to include antagonists, as well as agonists, which may have a wide spectrum of action on the opioid system (see review by Martin, 1967).

In 1954 Beckett and Casy hypothesized that synthetic analgesic opioid drugs such as morphine and related morphinans 'fit' at a receptor surface stereospecifically. It was through this interaction at the receptor surface that analgesia resulted. However, it was only after studies were conducted in the 1970's using biochemical binding assays that opioid receptor delineation commenced (Fowler and Fraser, 1994). The use of radiolabelled naloxone, which could antagonize morphine or other opioid narcotics such as levorphanol, and the development of stereospecific binding assays for opioid agonists provided for the identification and anatomical localization of the opioid receptors in the brain of mammals (Goldstein et al., 1971; Pert and Snyder, 1973).

In rats it could be shown that electrical stimulation of various brain regions produced analgesia in animals and that naloxone could reverse the effect (Akil et al., 1974). Subsequent to this, the development of bioassays and binding studies provided the necessary methods by which to screen extracts from both the brain and pituitary gland for opioid binding affinity. Hughes et al. (1975) showed that endogenous opioid peptides (EOP) could be isolated from brain extracts and that these peptides possessed morphine-

like properties. These EOP include enkephalins, dynorphins and endorphins and as of 1983 the list had grown to include over 18 chemically distinct peptides derived from mammalian tissue (see reviews by North, 1986; Pasternak, 1993 and Fowler and Fraser, 1994). The effects of these opioid peptides are mediated via specific binding sites or receptors. Many chemically synthesized non-peptide analogues mimic the actions of these peptides.

1.5.2 Classification of Opioid Receptors

The existence of opioid receptors was postulated in the pioneering work of Beckett and Casy (1954). This, in 1965, allowed Portoghese to postulate the existence of separate opioid receptors by correlating analgesic activity to the chemical structure of many opioid compounds.

The first *in vivo* evidence of multiple opioid receptors was obtained by Martin et al. (1976) using congeners of morphine. These workers identified three distinct syndromes produced by these agents in the chronic spinal dog. Thus, it was postulated that these syndromes were due to agonist interaction with three related receptors. The morphine syndrome was mediated by the μ (mu) receptor, the ketocyclazocine syndrome by the κ (kappa) receptor and the SKF 10,047 syndrome by the σ (sigma) receptor (for a complete side effect profile for each syndrome see Martin et al., 1976). It should be noted that studies suggest that the σ receptor is not in fact opioid in nature. Therefore this receptor can no longer be classified as such (Holtzman, 1980; Zukin and Zukin, 1981).

The development of *in vitro* pharmacological bioassays greatly enhanced the elucidation of heterogeneity between and within classes of opioid receptors. The mouse vas deferens and guinea pig ileum preparations were used by Hutchinson et al. (1975) to show that with ketocyclazocine and other related compounds a consistently lower potency ratio for agonism was found in the mouse vas deferens were compared to the guinea pig

ileum. It was later shown by Lord et al. (1977) that there was a higher kappa:mu ratio in guinea pig ileum when compared to the mouse vas deferens. Thus, these tissues appear to have a heterogeneous population of opioid receptors. In addition to having mu (μ) and κ receptor populations the mouse vas deferens was subsequently shown, using endorphins and enkephalins as agonists, to possess another profile of response which was independent of the other opioid receptors. Thus another opioid receptor, the δ (delta) receptor was postulated to exist (Lord et al., 1977; Hutchinson et al., 1980).

Recently, new opioid binding sites have been postulated including the ß-endorphin selective ε (epsilon) receptor (Nock et al., 1990; see review by Pasternak, 1993). However its characteristics have not been clearly defined due to lack of procedures and selective agents with which to label the site. As with other receptor systems in pharmacology many opioid receptor subtypes have been postulated. The subtypes postulated for the μ receptor are the μ_1 and μ_2 receptors which mediate supraspinal and spinal analgesia (Pasternak and Wood, 1986), respectively. The κ receptors are a much more diverse group and many subtypes exist. The analgesic properties of the κ_1 receptor are characterized with κ agonists such as U-50,488H and U-69,593. Use of these compounds has given rise to two binding sites, κ_{1a}, and κ_{1b}.

The pharmacological significance of these binding sites is unknown (Clark et al., 1989). Another κ subtype was postulated after it was shown that U-50,488H-insensitive binding sites could be found in the rat brain (Zukin et al., 1988). The pharmacology of these κ_2 binding sites remains unknown. The most recent U-50,488H-insensitive binding site, κ_3, has been identified using a novel opiate derivative naloxone-benzoylhydrazone (NalBzoH). This binding site is thought to mediate supraspinal analgesia (Clark et al., 1989; Zukin et al., 1988 and Gistrak et al., 1989).

The delta receptor, defined by Kosterlitz as the enkephalin-preferring receptor (Lord et al., 1977), may have two subtypes which are distinguished by the agonists ([D-Pen^2, D-Pen^5]-enkephalin) DPDPE and deltorphin as δ_1 and δ_2, respectively (Mattia et al., 1991).

The pharmacological role of these sites may be related to spinal and supraspinal analgesia, respectively (see Pasternak, 1993). The heterogeneity of opioid receptors is also reflected in the diversity of location and disparity between species (Martin, 1984; Mansour et al., 1988). However, despite this, distribution is consistent with their role in physiological function.

Thus, the present classification of opioid receptors is primarily based upon the pharmacological profiles of numerous opioid drugs as they relate to differences in binding potency ratios in bioassays, in effects of naloxone and other antagonists and also how these drugs affect various physiological actions, be it in the central or peripheral nervous system.

1.5.3 Antiarrhythmic Actions

The first reported effects of opioid compounds on arrhythmias due to myocardial ischaemia were those by Fagbemi et al. (1982). Prior to this it had been observed that opioids were of benefit in endotoxic and haemorrhagic shock states (Holaday and Faden, 1978). Naloxone, when given at doses similar to those used in shock states, reduced the incidence of VT and VF in rats subject to coronary artery occlusion (Fagbemi et al., 1982). It was postulated that EOP such as ß-endorphin might have detrimental electrophysiological effects on the myocardium by 1) directly interacting with opioid binding sites, 2) indirectly by modulating the autonomic nervous system (ANS) or 3) that opioids may act directly on the myocardium and hence alter cardiac action potentials (Fagbemi et al., 1982). Since that time many studies have been conducted using many different models of arrhythmogenesis in many different species.

The direct involvement of the opioid receptor in arrhythmogenesis was examined using the stereoisomers of two different opioid antagonists (Parratt and Sitsapesan, 1986). (-)Mr1452 and (-)WIN 44,441-3, both κ agonists, dose-dependently decreased arrhythmic

incidence of ischaemic arrhythmias suggesting that blockade of κ receptors in the myocardium was antiarrhythmic. Mackenzie et al. (1986) and Sitsapesan and Parratt (1986) showed that naloxone, at doses which inhibit μ and κ receptors and the κ antagonist, Mr2266, reduced ischaemic arrhythmias in rats. The quaternary naloxone derivative, MrZ2593, was also effective against ischaemic arrhythmias. Since it did not cross the blood brain barrier (BBB) it was suggested that the antiarrhythmic actions of opioids were mediated by peripheral opioid receptors. Studies with the μ, κ and δ agonists, diamorphine, U-50,488H and leu-enkephalin, respectively, suggest that the antagonism of μ and κ receptors, but not δ, is important in inhibiting arrhythmias (Sitsapesan and Parratt, 1986).

The majority of published studies suggest the involvement of EOP in ischaemic arrhythmias (Wong et al., 1990; Lin et al., 1991; Lee et al., 1992b). It is thought that EOP are released from cardiac muscle by myocardial ischaemia and that they mediate arrhythmias via their respective opioid receptors (Maslov et al., 1993). Many studies have confirmed the antiarrhythmic actions of naloxone and other opioids against the arrhythmias produced by many methods including chloroform-hypoxia in rats (Wong and Lee, 1985), and ischaemia and reperfusion in isolated rat hearts (Zhan et al., 1985; Lee and Wong, 1987) and in dogs (Huang et al., 1986). In contrast, Rabkin and Roob (1986) found that naloxone potentiated digitalis-induced arrhythmias in guinea pigs and suggested that naloxone inhibition of EOP may provide unopposed parasympathetic activity in the heart (Rabkin and Roob, 1986). Further studies suggest that inhibition of the degradative enzymes for EOP potentiate digitalis arrhythmias (Rabkin and Redston, 1989). Recently, Lee et al. (1992b) have shown that naloxone inhibits ischaemia-induced arrhythmias, hypotension and bradycardia in coronary artery occluded rats. Naloxone also attenuated arrhythmias produced by the κ opioid receptor agonist U-50,488H suggesting that the κ receptor may be involved in the genesis of arrhythmias during ischaemia. These results contrast those published by Pugsley et al. (1992a) and Sitsapesan and Parratt (1989)

which showed that U-50,488H reduced arrhythmic incidence in ischaemia. However, the results of Lee et al. (1991) agree, in part, with the theory that κ opioids may have a dual action in ischaemic arrhythmias. Kaschube and Brasch (1991) and Pugsley et al. (1992b) suggest that the arrhythmogenic actions of κ opioids occur at low doses due to activation of the κ receptor and that antiarrhythmic actions occur at higher doses due to a direct (non-opioid) interaction with the cardiac membrane.

Fagbemi et al. (1983) showed, using the partial opioid agonist, meptazinol, that opioids directly influence the cardiac action potential. Sagy et al. (1987) and Sarne et al. (1988) showed that naloxone exerts a direct local effect (positive inotropism) on isolated rat hearts. Boachie-Ansah et al. (1989) showed that buprenorphine, an opioid having μ-agonist and κ-antagonist properties depressed maximum diastolic depolarization (V_{max}) and increased action potential duration (APD) in sheep Purkinje and rat papillary muscles due to blockade of sodium and delayed-outward potassium channels. The dual action discussed above has been confirmed by Pugsley et al. (1994) using the stereoisomers (-)PD129,290, an active κ agonist, and its inactive enantiomer, (+)PD129,289 and U-50,488H in the absence and presence of naloxone (Pugsley et al., 1992a, 1992b). The antiarrhythmic action of these opioid agonists is due to direct interaction with the sodium and potassium channels of the cardiac membrane. Possible ion channel effects in prolongation in the P-R, QRS width and Q-aT intervals have been observed with many related arylacetamide κ opioid agonists in anaesthetized rats and confirmed in voltage-clamped cardiac myocytes (Pugsley et al., 1992a, 1992b, 1993a, 1994, 1995). In these patch-clamp studies the κ receptor agonists, U-50,488H, (-)PD129,290, (±)PD117,302 and the inactive κ agonists (+)PD129,289 and (+)PD123,497 produced a concentration-dependent block of inward sodium and both transient outward and sustained delayed outward potassium currents in the absence as well as presence of naloxone. Therefore it is suggested that these opioids are antiarrhythmic via blockade of ion channels in myocardial tissue (Pugsley et al., 1995).

1.6 The Kappa (κ) Receptor

The existence of the κ receptor, as outlined above, was originally postulated on the basis of pharmacological studies by Martin in 1976 who characterized sedation, pupil constriction, and depressed flexor response to ethylketocyclazocine (EKC) in the chronic spinal dog as being representative of κ receptor activation. It now appears that responses due to κ receptor activation are very complex and involve a large number of receptor subtypes (Wollemann et al., 1993). In this section of the thesis the biochemistry of the κ receptor system, the arylacetamide agonists which have helped in the description of this receptor system, and the pharmacological actions associated with this opioid receptor type will be discussed.

The pharmacological actions of κ receptors have been characterized by a combination of binding studies and bioassays but the coupling of these receptors to second messenger systems is less well understood. Most studies appear to confirm that μ, κ and δ receptor activation inhibits adenylate cyclase (Haynes, 1988; Childers, 1993). This inhibition is associated with a G protein and requires guanosine triphosphate (GTP) (Hsia et al., 1984). In guinea pig cerebellar membranes both dynorphin-A and U-50,488H produce potent and specific inhibition of adenylate cyclase (Konkoy and Childers, 1989) suggesting that, at least for κ receptors, this may be an important mechanism in cellular function. It is unclear whether this action is associated with μ receptors (Polastron et al., 1990).

The coupling of κ opioid receptors to other systems may be different from that seen in cerebellar membranes. Attali et al. (1989) assessed the effects of opiates on the influx of $^{45}Ca^{2+}$ in rat spinal cord and dorsal root ganglion (DRG) cell co-cultures by an elevation in extracellular potassium or with Bay K 8644, a calcium channel opener. Influx of calcium was dependent upon activation of the L-type calcium channel. U-50,488H and dynorphin-A decreased calcium influx whereas agonists for both μ and δ opioid receptors had no

effect on calcium influx. Further study revealed that this inhibition of L-type calcium channels by κ agonists was mediated by G proteins.

Other studies indicate that there may be additional mechanisms by which κ receptors are coupled to response systems. For example, U-50,488H produced a concentration-dependent increase in phosphatidyl-inositol (PI) turnover in rat hippocampal slices, an effect blocked by naloxone or Mr2266 (Periyasamy and Hoss, 1990).

Molecular biology techniques have resulted in the isolation of complementary DNA (cDNA) sequences for mouse, rat, and guinea pig κ receptors (Nishi et al., 1993; Miniami et al., 1993; Xie et al., 1994, see review by Reisine and Bell, 1993). These κ receptors are structurally similar to the cloned μ (Chen et al., 1993) and δ (Evans et al., 1992) receptors in that they are all members of the highly homologous superfamily of G protein coupled, seven transmembrane domain spanning receptors (Reisine and Bell, 1993). The pharmacological profile of the cloned κ receptor now includes expression in human embryonic kidney cells (Lai et al., 1995) and the human placenta (Mansson et al., 1994). All cells are associated with pertussis toxin sensitive G proteins which mediate inhibition of adenylate cyclase and hence cAMP formation (Avidor-Reiss et al., 1995).

The cloned κ receptor is now used as a probe to screen human and murine genomic libraries for a genetic link to its expression. Yasuda et al. (1994) successfully isolated a clone which contained part of the gene encoding the human κ opioid receptor located on chromosome 8. Recent reports using cloned cDNA from murine κ receptors suggest that the gene for this receptor is on chromosome 1 in this species (Kozak et al., 1994; Giros et al., 1995).

Thus the pharmacological and biochemical profile of κ agonists provided a description of how the effects of κ receptor activation may be mediated and the involvement of second messengers in the translation of the message leading to the eventual response of the cell. The cloning of κ (as well as μ and δ receptors) has

advanced our understanding of the relationship of the κ receptor family to other well characterized pharmacological receptor families (such as the ß-receptors).

1.6.1 Chemical Diversity of κ Agonists

As discussed elsewhere, activation of the κ receptor produces a distinct profile of pharmacological action in addition to analgesia (Martin et al., 1976). At analgesic doses κ agonists such as (±)PD117,302 and U-50,488H produce diuresis and sedation (Von Voightlander et al., 1988; Leighton et al., 1987). They do not however produce the untoward effects of emesis, respiratory depression or constipation associated with μ agonists (Martin, 1984). The pharmacological elucidation of κ opioid receptor activation has resulted in part from synthesis of compounds in the arylacetamide series. This includes drugs such as U-50,488H, U-62,066E (spiradoline), (±)PD117,302, (-)PD129,290 and their inactive κ opioid enantiomers (+)PD123,497 and (+)PD129,289, respectively. These agents will be the primary focus of this section (and this thesis) despite the multitude of other structurally related compounds. Prior to the development of the arylacetamide κ agonists only a limited understanding of κ receptors and their function was known. Researchers were limited by the lack of selectivity of the benzomorphan prototype ligands such as ethylketocyclazocine (EKC) or bremazocine (Kosterlitz et al., 1981; Zukin and Zukin, 1981).

Szmuzkovicz and Von Voightlander (1982) first described the synthesis and analgesic effectiveness of benzamide and benzacetamide structural moieties incorporated into the trans-cyclohexane-1,2-diamine class of antidepressant agents. The benzamide series showed an increased analgesic potency, but retained μ receptor actions. The benzacetamide series, typified by the pyrrolidinyl derivatives, were potent analgesics whose actions were naloxone-reversible but apparently independent of the μ receptor (Szmuzkovicz and Von Voightlander, 1982a; Von Voightlander et al., 1981, 1982a). These

compounds are effective analgesics when given orally. U-50,488H was the first non-peptide benzacetamide compound selective for the κ receptor.

The development of U-50,488H opened the way for the chemical synthesis of numerous arylacetamides. The cyclohexylbenzacetamide group has become interesting because these compounds are analgesics but are structurally dissimilar to morphine (Clark et al., 1988; Hunter et al., 1990). The enantiomer of the cyclohexyl derivative of U-50,488H was the first to be developed and characterized as having high affinity (3nM) for the κ receptor (Lahti et al., 1982; Costello et al., 1991). From this compound a potent κ analgesic analogue, U-62,066E, was developed. Currently it is in Phase I clinical trials (Von Voightlander and Lewis, 1988; P.F. Von Voightlander, Personal Communication).

Further entensive structure-activity relationship (SAR) studies resulted in synthesis of compounds with increased μ to κ selectivity ratio and with optimal affinity for the κ receptor (Clark et al., 1988). SAR studies next involved examination of the N-[(2-aminocyclohexyl)aryloxy]acetamide and N-[(2-aminocyclohexyl)aryl]acetamide series (Clark et al., 1988; Boyle et al., 1990). Sequential examination of electron-donating and electron-withdrawing aromatic substitutions based on U-50,488H was conducted and it was found that the thiophene derivatives increased both κ receptor affinity and selectivity (μ/k ratio) (Clark et al., 1988; Horwell et al., 1990). Thus (±)PD117,302 showed nM affinity for the κ receptor and only μM affinity for the μ receptor (the ratio of EC_{50} values μ/κ was 110). At this same time, (±)PD117,302 was resolved into its enantiomers, (-) PD123,475 which retained κ agonist stereoselectivity and (+)PD123,497 which was essentially inactive at κ receptors (μ/κ = 0.59) (Clark et al., 1988).

Derivatives of (±)PD117,302, containing a benzo[b]thiophene aromatic ring system were synthesized and examined (Halfpenny et al., 1989). Substitution of the cyclohexyl ring of (±)PD117,302 resulted in the development of (-)PD129,290. The analgesic potency for this compound was 25 times that of morphine and 17 times that of U-62,066E (Halfpenny et al., 1989). The high selectivity ratio for the κ receptor (μ/κ = 1520) makes it

one of the most potent κ receptor agonists developed (Halfpenny et al., 1989). The enantiomer, (+)PD129,289, is neither κ selective (μ/κ = 0.38) nor is it an analgesic (Halfpenny et al., 1989).

Thus the novel structure of U-50,488H represented a lead compound which could be extensively altered and resulted in the discovery of many potent κ opioid analgesics lacking the potential for abuse in humans.

1.6.2 Pharmacological actions of arylacetamides

1.6.2.1 Analgesia

The arylacetamides vary in analgesic potency when compared with morphine. U-50,488H produces a species-dependent, equipotent analgesia when compared to morphine using a variety of thermal (hot plate, tail immersion), pressure (tail pinch) and irritation (HCl or acetylcholine abdominal constriction) assays (Von Voigtlander et al. 1981, 1982a, 1982b). However, the analgesic potency of U-50,488H varies markedly with the intensity of the applied nociceptive stimulus (Von Voigtlander et al. 1981, 1988). U-62,066E (spiradoline), (±)PD117,302 and (-)PD129,290 are more potent analgesics compared with U-50,488H and morphine using these same tests (Von Voigtlander and Lewis, 1981; Leighton et al., 1987; Hunter et al., 1990). Selectivity of these agents for κ-receptors is also greater than for μ-receptors. U-50,488H is 53 times more selective for κ than for μ while U-62,066E is 84 times more selective (Lahti et al., 1982; Kunihara et al., 1989). The selectivity of (±)PD117,302 (μ/κ=110) resides in its active κ receptor enantiomer (-)PD123,475 (μ/κ=100, $K_{i\kappa}$=9.6 and the $K_{i\mu}$=1000nM) (Clarke et al., 1988) while its inactive enantiomer (+)PD123,497 has low affinity ($K_{i\kappa}$=1500 and $K_{i\mu}$=880nM) and selectivity (μ/κ=0.59) (Clark et al., 1988; Meecham et al., 1989). Similar enantiomeric potency and selectivity for the κ receptor is found with (-)PD129,290 (Hunter et al., 1990).

The analgesia produced by U-50,488H and its arylacetamide analogues, unlike that of morphine, is not associated with physical dependence nor does it promote self-administration in animals and hence may lack abuse potential in humans (Von Voightlander et al., 1982b; Lahti et al., 1983; Leighton et al., 1987; Kunihara et al., 1989; Hunter et al., 1990). Interestingly, tolerance develops to arylacetamide analgesia which cannot be attributed to enhanced metabolism (Von Voigtlander et al. 1982a; Hunter et al., 1990). Cross-tolerance develops between these compounds and many benzomorphan κ agonists such as bremazocine, or other arylacetamides, but not to morphine (Von Voightlander et al., 1982a; Von Voightlander et al., 1988; Colombo et al., 1991). Unlike morphine and its cogeners, U-50,488H and the arylacetamides do not depress respiration significantly when given at analgesic doses (Martin et al., 1976; Dosaka-Akita et al., 1983; Beecham et al., 1989). At high (supra analgesic) doses, many arylacetamides produce actions in anaesthetized rats which include respiratory depression (Pugsley et al., 1992, 1993a, 1994, 1995) but which are not blocked by naloxone.

Opioid receptor drugs can act at the level of the central (CNS) or peripheral (PNS) nervous systems. Since both high and low CNS centres possess opioid receptors, stimulation can produce a variety of responses including effects on neuroendocrine and cardiovascular systems (Martin, 1984). As well, there are opioid receptors (μ and κ) in the peripheral ANS. Studies by Paton (1957) demonstrated the existence of these peripheral actions. He showed that morphine inhibits autonomic neurotransmitters in guinea pig ileum. The suggested mechanism of such inhibition in the case of morphine and the κ agonists (including U-50,488H, (±)PD117,302, and (-)PD129,290) is blockade of calcium channels pre-synaptically and hence a reduction in the release of neurotransmitters, such as acetylcholine (ACh) in guinea pig myenteric neurons (Cherubini and North, 1985; Xiang et al., 1990; Werling et al., 1988; Mulder et al., 1991; Lambert et al., 1991; Kunihara et al., 1993).

Studies with opioid agonists and antagonists in neuronal tissue suggest that there is a non-opioid receptor mediated action of these compounds on ion channel function. Frazier et al. (1973) first reported that both morphine and naloxone could block squid axon action potentials by inhibiting sodium and potassium currents. Carratu and Motolo-Chieppa (1982) also showed that naloxone blocks sodium currents when applied intracellularly to frog sciatic nerves. The sodium channel blocking actions of U-50,488H and U-69,593 on neuronal tissue have been demonstrated by Alzheimer and ten Bruggencate (1990). Using microelectrode techniques they showed that these κ agonists had local anaesthetic actions in neuronal tissue which could not be reversed by opioid receptor antagonists. Zhu et al. (1992) have developed a series of benzamide U-50,488H analogues which possess anticonvulsant properties and which inhibit sodium currents in mouse neuroblastoma cells. Recent binding studies by Fraser and Fowler (1995) suggest that the κ-receptor binding sites are independent from those sites modulating the actions of local anaesthetic compounds.

Studies have shown that opioid agonists at low concentrations augment voltage-dependent potassium currents and this suggests a means by which these compounds mediate antinociceptive properties (Grundt and Williams, 1993; Moore et al., 1994). At high concentrations these compounds block the current (Moore et al., 1994). Molecular biological studies show that *Xenopus* oocytes express κ opioid specific binding sites (Henry et al., 1995). When cells were co-injected with a cRNA coding for a G-protein linked, inwardly rectifying potassium channel (GIRK1), activation of the κ binding site by low concentrations of U-69,593 resulted in a large potassium current. This increase in current was blocked by norbinaltorphimine. Unlike the sodium or calcium channel block produced by these opioid compounds, potassium channel block may be linked to κ receptor activation by means of an endogenous intermediate or undetermined G-protein (Henry et al., 1995).

1.6.2.2 Systemic Activity

The study of opioids and their involvement in the cardiovascular system is fraught with difficulties. The primary focus of studies with these compounds involves the CNS and ANS. However, in the periphery the locus of opioid neurons and receptors has been defined more clearly for some organ systems (such as in the gastrointestinal tract) compared to others (such as the heart and smooth muscle of the vasculature). The role that opioids, especially κ agonists, play in regulation of these systems is difficult to determine because the highly influential physiological effects are influenced by pharmacological variables such as dose, site and route of administration, receptor specificity and species.

1.6.2.2.1 Diuresis

Opiates have a long history of modulating fluid and electrolyte balance, as demonstrated initially with morphine in dogs (Debodo 1944). The diuretic activity of potent κ agonists such as bremazocine, U-50,488H, U-62,066E, and others, has been the focus of many studies (Huidobro-Toro and Parada, 1985; Leander et al., 1986; Oiso et al., 1988; Yamada et al., 1989, 1990; Bianchi, 1991). All κ agonists studied to date show diuretic properties in many different species, including man (Von Voightlander et al., 1982; 1982a, 1982b; Peters et al., 1987; Rimoy et al., 1991). The diuresis which results can be blocked by opioid antagonists including naloxone and Mr2266 (Huidobro-Toro and Parada, 1985; Yamada et al., 1989).

Leander (1986) and Yamada et al. (1989) showed that U-50,488H and U-62,066E do not produce diuresis in rats which genetically lack arginine vasopressin (AVP). This information led to the suggestion that κ agonists either suppress AVP levels at the level of the neural lobe secretory process, or inhibit the effect of AVP on the kidney (Leander,

1986). In man κ agonists induce water diuresis without any changes in renal blood flow or suppression of AVP levels (Rimoy et al., 1991).

In addition to effects on AVP levels, many studies suggest that the κ agonists influence the serum levels of other circulating hormones. Iyengar et al. (1985, 1986) showed that U-50,488H and EKC elevated plasma corticosterone but decreased plasma thyroid stimulating hormone (TSH) levels in rats. Thus, although many studies show the diuretic actions of κ agonists are likely to be due to a κ opioid receptor effect, clear delineation of the mechanism of action has not been accomplished. This suggests a complex involvement of the opioid system in the regulation of the hypothalamic-pituitary-adrenocortical axis.

1.6.2.2.2 Cardiovascular Actions

The κ agonists and all opioids in general exhibit a variety of complex pharmacological actions on the cardiovascular system (Holaday, 1983). The CNS effects of κ agonists such as analgesia are mediated by opioid receptors (Von Voightlander et al., 1988; Lahti et al., 1982; Leighton et al., 1987; Kunihara et al., 1989) but the actions of these compounds in peripheral tissues including reduction in cardiac contractility and central venous pressure may not be dependent upon opioid receptors and may instead be a direct effect on cardiac muscle and vasculature.

The existence of opioid receptors and their importance for cardiac tissue is uncertain and any involvement in regulation of cardiovascular function, speculative. Hughes et al. (1977) were the first to show that endogenous enkephalins occur in rat and rabbit atria. Much uncertainty exists as to the localization of EOP in the heart despite it being generally agreed that opioid receptors are differentially distributed between atria and ventricles (Holaday, 1983; Lang et al., 1983; Krumins et al., 1985; Weihe et al., 1985; Tai et al., 1991). Studies in the heart are hampered by the lack of appreciable ligand binding

to cardiac membrane fractions. However the most abundant binding for κ agonists, such as U-69,593 and diprenorphine, occurs in the right atrium (Krumins et al., 1985; Tai et al., 1991). It is suggested that rather than globally examining cardiac tissue attention should be given to His-Purkinje and nodal conduction tissue (Holaday, 1983). To date these binding studies have not been performed.

The physiological significance of peripheral κ opioid receptor distribution in vasculature is not fully understood. Peripheral binding of EOP, or the arylacetamides, provides an outline of the direct involvement of the opioid system in regulation of haemodynamic function and cardiac activity in addition to its actions on the cardiovascular control centres found in the brain (Lang et al., 1983).

U-50,488H and other arylacetamides have been examined over a large range of doses producing effects on heart rate (HR) and blood pressure (BP). These actions are dose- and species-dependent. U-50,488H, for example, has a different cardiovascular profile when injected i.v. in rats compared to i.c.v. into the CNS (Feuerstein et al., 1985; Pugsley et al, 1992a; Pugsley et al., 1993b). In anaesthetized dogs U-50,488H produced a dose-related decrease in BP, HR, peak systolic pressure and cardiac contractility over the dose-range 0.08-24 μmol/kg, i.v. Prior administration of 8μmol/kg naloxone abolished such responses (Hall et al., 1988). U-62,066E produced a similar cardiovascular depression in dogs which could be prevented by naloxone (Hall et al., 1988).

Studies conducted in our laboratory in anaesthetized rats showed that U-62,066E, (-)PD129,290, (+)PD129,289, (±)PD117,302 and U-50,488H all dose-dependently decreased BP and HR. In addition, at the highest doses these compounds prolonged the PR, and Q-aT intervals of the ECG (Pugsley et al., 1992a, 1992b; 1993a, 1994, 1995). Neither Mr2266 nor naloxone reduced these cardiovascular actions of the arylacetamides. Studies in rats, using U-50,488H and U-62,066E, report slight depressant actions on HR and BP at low doses similar to those used by Hall et al. (1988) in dogs and cats.

In intact animals the cardiovascular actions produced by κ agonists may be mediated by κ receptor-dependent effects in the CNS. This may be especially true at the supra-analgesic doses used in my studies. Both U-50,488H and U-62,066E, while not noted for actions at μ receptors do suppress respiration, possibly as a result of CNS depression (Clarke et al., 1988; Hall et al., 1988; Pugsley et al., 1992a, 1993b).

To examine the non-opioid pharmacological actions of κ agonists studies were performed in the presence of opioid antagonists, such as naloxone or Mr2266, or by using enantiomers of κ arylacetamides which lack opioid receptor agonist properties. The cardiovascular responses studied in the presence of naloxone or Mr2266 were not influenced by these opioid antagonists, even at doses which block, in addition to μ receptors, κ and δ receptors (Brasch, 1986; Kaschube and Brasch, 1991; Pugsley et al., 1992a, 1993a, 1995). It was concluded that since these responses were not blocked by opioid receptor antagonists they were not mediated by opioid receptors. The inactive enantiomer, (+)PD129,289, produced similar reductions in HR and BP to its enantiomer, the κ agonist (-)PD129,290 (Pugsley et al., 1993a).

A number of studies have shown that opioid drugs can modulate the incidence and severity of arrhythmias (*vide supra*) induced by coronary artery occlusion (see review by Pugsley et al., 1993b). The ECG changes produced by arylacetamides provides a useful indirect method for determining the effects of antiarrhythmic drugs on cardiac ion channels. The ECG changes produced by arylacetamides at high doses are interpreted as indicating ion channel blockade, particularly the sodium channel. Both (-)PD129,290 and its inactive enantiomer (+)PD129,289 produce P-R interval prolongation and QRS widening in rats (Pugsley et al., 1993a) together with an increase in RSh, an index of sodium channel blockade in the rat (Penz et al., 1992). These drugs also produced widening of the Q-aT interval, an index of prolongation of repolarization, indicative of possible potassium channel blockade. Other studies suggest that sodium channel blockade occurs with opioid agonists and antagonists (Sarne et al., 1989, 1991).

In vitro studies conducted in rat hearts complement the *in vivo* studies described above. We examined the effects of U-50,488H and other κ agonists in the Langendorff isolated rat heart and showed these compounds prolonged the P-R interval and QRS duration of the ECG and reduced peak left-ventricular pressure in a concentration-dependent manner. Naloxone (1 μM) did not block these actions (Pugsley et al., 1992a, 1993a, 1995). Such a spectrum of action suggests sodium channel blockade similar to class I antiarrhythmics (Abraham et al., 1989). Few other studies have been reported which examine the effects of κ opioid agonists on isolated heart function and ion channel interaction. The only other report of these effects are by Xia et al. (1994) which show that 1 μM U-50,488H reduced HR and contractility.

The majority of studies involving the non-opioid actions of κ receptor agonists have been conducted in isolated cardiac tissue. These non-opioid actions occur at micromolar (μM) concentrations in the isolated heart, whereas κ agonism usually occurs at nanomolar (nM) concentrations.

Studies using a variety of cardiac isolated muscle preparations have shown that opioid agonists such as morphine and U-50,488H, and antagonists including naloxone and Mr1452, exert similar non-opioid properties on cardiac muscle (see Pugsley et al., 1993b). The chemical diversity and actions of these compounds suggest non-opioid receptor mediated effects (Rashid and Waterfall, 1979; Frame and Argentieri, 1985; Sarne, 1989). In isolated guinea pig and rat atrial and ventricular muscle opiates suppress excitability in cardiac muscle by both increasing the threshold for stimulation and reducing action potential amplitude and the maximum rate of depolarization in a manner similar to local anaesthetics or sodium channel blockade (Alarcon et al., 1993). The results of some studies suggest an interaction of κ opioids with either the potassium or calcium channel found in cardiac muscle. Potassium channel blockade was suggested on the basis of increased action potential duration, especially at the terminal phase of repolarization seen with many opioids (Brasch, 1986; Helgesen and Refsum, 1987; Hicks et al., 1992). The

use of calcium fluorescent techniques for measurement of cardiac myocyte contractility suggests that the negative inotropic actions of κ opioid agonists are due to inhibition of L-type calcium currents (Kasper et al., 1992; Lakatta et al., 1992).

The ion channel or non-opioid actions of the arylacetamides have been examined on currents evoked from isolated cardiac myocytes subjected to patch-clamp. duBell and Lakatta (1991) and Utz and Trautwein (1994) examined the effect of U-50,488H on the slow inward calcium current (I_{Si}). Intracellular application of U-50,488H had no effect on the calcium current (Utz and Trautwein, 1994). U-50,488H inhibited the developed current in a manner indicative of a receptor-independent mechanism at a site accessible from the exterior of the cell. U-50,488H also inhibited a current which had properties similar to the delayed rectifier potassium current. The non-opioid cardiac actions of U-50,488H and other arylacetamides have been extensively characterized. In isolated rat cardiac myocytes U-50,488H, (-)PD129,290, (+)PD123,497, (±)PD117,302 and U-62,066E have all been shown to inhibit both the sodium (I_{Na}) and transient outward (I_{to}) and sustained plateau (I_{Ksus}) potassium currents (Pugsley et al., 1993a, 1994, 1995). Channel block was not reversed by opioid receptor antagonists. The mode of channel block will be elaborated upon later in this thesis, however, blockade is similar to the sodium and potassium channel blockade seen in neuronal tissue (Alzheimer and Ten Bruggencate, 1990).

In contrast to the studies conducted on cardiac tissue, few studies have been performed to determine the effects of κ agonists on isolated vascular tissue. However, non-opioid receptor dependent actions have been observed in several types of vascular tissue. U-50,488H was shown by Altura et al. (1984) to dose-dependently contract the basilar and middle cerebral arteries of dogs. The U-50,488H-induced contractions were not inhibited by naloxone. Illes et al. (1987) showed that U-50,488H and ethylketocyclazocine, at concentrations greater than 3μM, depressed isolated rat tail veins contraction in a manner not inhibited by 10μM naloxone. Verapamil (6μM) has been shown to reduce the inhibitory effect of high concentrations of U-50,488H in abdominal aortic

strips (el Sharkawy et al., 1991) suggesting possible direct interactions of κ agonists with L-type calcium channels on vascular smooth muscle. U-62,066E also produced a concentration-dependent relaxation of pig circumflex coronary arteries attributable to inhibition of voltage-dependent calcium entry into smooth muscle cells (Harasawa et al., 1991). This relaxation was not inhibited by naloxone, even at concentrations of 300μM.

Thus, the sodium, potassium and calcium channel blocking actions of U-50,488H and other arylacetamides have been unequivocally demonstrated in both neuronal (see above) and cardiac tissue. The direct application of this series of compounds in a clinical setting may be limited. However, they provide additional information regarding antiarrhythmic agents.

1.7 Objectives and Outline of Experiments Performed

1.7.1 In this Thesis and in the Appendix

Many studies conducted in rats demonstrate that opioid drugs are antiarrhythmic (Sitsapesan and Parratt, 1989; Pugsley et al., 1992b, 1993, 1995). However, the degree of protection varies with the opioid drug considered. In addition, both antagonists as well as agonists, reduce arrhythmias (Sarne et al., 1991; Pugsley et al., 1992b). The effects of κ receptor agonists and related compounds are not well characterized in the heart and cardiovascular system. The experiments conducted in this thesis with the κ agonist, spiradoline (U-62,066E), (±)PD117,302 and its inactive enantiomer, (+)PD123,497, as well as those described in the Appendix for U-50,488H, (-)PD129,290 and its inactive enantiomer, (+)PD129,289, were designed to answer the following questions:

1. Is the κ receptor involved in arrhythmias produced by coronary artery occlusion in rats?

2. If related arylacetamide κ receptor agonists, such as spiradoline (U-62,066E), and related compounds have antiarrhythmic actions against ischaemic arrhythmias, are these independent of κ receptor agonism?

3. If this independence exists, what then is the putative mechanism by which the arylacetamide κ agonists and related compounds exert their antiarrhythmic effects against ischaemic arrhythmias?

Examination of the cardiovascular profile of spiradoline against both electrical and ischaemic arrhythmias was performed in the absence and presence of naloxone. In

previous studies (those in the Appendix) with U-50,488H, the selective κ antagonist, Mr2266, was used. The use of the inactive enantiomer of κ receptor agonists has also aided in the delineation of κ receptor involvement in arrhythmias. Lastly we characterized the electrophysiological properties of the arylacetamides on sodium and potassium currents evoked in ventricular myocytes.

2 Methods

2.1 Cardiac preparations

Dose-response studies were performed *in vivo* and *in vitro* to ascertain the profile of action of the κ receptor agonist and related arylacetamides on the cardiac and cardiovascular system of the rat.

2.1.1 Intact rat studies

Since there are few studies examining the cardiovascular actions of κ receptor agonists and related compounds (Pugsley et al., 1993b) I examined the haemodynamic, heart rate and ECG effects of these drugs in acutely prepared anaesthetized rats. The rat allows reliable, accurate measurements of cardiovascular function (Schroeder et al., 1981) and is routinely used for drug investigation. As a small animal, it is relatively inexpensive, readily available, and provides reproducible results. An extensive biochemical, physiological and anatomical data base exists for this species. Furthermore, surgical procedures (such as cannulation of blood vessels) are easily performed in the rat for the study of drug actions on organs and/or systems (Curtis et al., 1987). However, like many models it has some drawbacks and as with many models it is unclear as to exactly how closely these models resemble disease in man. However, this does not detract from the usefulness of the rat in the pharmacological characterization of drugs. It is no less valid an animal model than any others which have been used to assess drug actions in the heart and cardiovascular system (Curtis et al., 1987; Walker et al., 1991; Cheung et al., 1993).

2.1.2 Surgical preparation

Male Sprague-Dawley rats (250-350 g) were used in accordance with the guidelines established by the University of British Columbia Animal Care Committee. Rats were anaesthetized with sodium pentobarbital (60 mg/kg, i.p.). All animals had their right jugular vein and left carotid artery cannulated for administration of drugs and blood pressure monitoring, respectively. The electrocardiogram (ECG) was recorded using a unique lead configuration. A needle electrode was placed 0.5 cm from the midline of the trachea at the level of the right clavicle while a second needle electrode was placed 0.5 cm from the midline at the level of the 9^{th} and 10^{th} ribs (Penz et al., 1992). The trachea was cannulated for artificial ventilation at a stroke volume of 10 mL/kg and rate of 60 strokes/min to ensure adequate blood-gas levels (MacLean and Hiley, 1988). Animals were placed in a supine position and body temperature was monitored by rectal thermometer and maintained between 37-38°C with a heating lamp. Blood pressure and ECG were recorded on a Grass polygraph (model 7D) at a bandwidth of 0.1-40 Hz and a chart speed of 100 mm/sec.

2.1.3 Experimental Design

Cumulative *in vivo* dose-response curves for κ receptor agonists and their enantiomers (0.5-32μmol/kg/min, i.v.) were obtained in artificially-ventilated, pentobarbitone anaesthetized rats. All κ receptor arylacetamides were initially solubilized in distilled water as a stock solution and serial dilutions were made in saline vehicle. Drugs were given *in vivo* in one of two ways; either as a bolus with dose-doubling every 5 min, or as infusions with the infusion rate doubled every 5 min. For each case, variables were measured just prior to doubling the dose or rate of infusion. For the infusion studies it was assumed that a pseudo-equilibrium had been achieved by 5 min. Animals (n=5) were randomly assigned to receive either drug or vehicle

control at the end of a 15 min control period. All doses were infused and blood pressure, heart rate and ECG were recorded 5 min later, immediately prior to addition of the next dose.

Accurate analysis of drug-induced changes in the rat ECG presents certain difficulties. Driscoll (1981) exhaustively outlined the many anomalies with the rat ECG. Briefly, the P, QRS and T waves do not share a common baseline and therefore a reference point for determination of the isoelectric line is required. Driscoll (1981) suggests that the point at which the P-R interval terminates and the QRS complex begins is subject to the least variation. We adopted the ECG measures of Budden et al. (1981) which are similar to those of Driscoll (1981) in an attempt to maintain interlaboratory consistency.

Another important factor which influences the rat ECG is electrode position. In all our studies of acutely prepared anaesthetized rats, the positioning of electrodes was as explained previously. There was always some anatomical differences in the position of the rat heart but these were minimized by the method of electrode placement used.

Before starting our studies with arylacetamides we developed a novel ECG measure (RSh) for the detection of possible sodium channel blockade in artificially ventilated, anaesthetized rats. This measure was of value in our studies since it has been shown that κ receptor agonists and related compounds block many different ion channels in both the CNS and systemically (Pugsley et al., 1993b). Conventional measures of sodium channel blockade (QRS complex widening and/or P-R interval prolongation) are limited in their sensitivity in detecting low-dose drug effects. The new measure, termed "RSh" or RS-height, quantifies the height from the peak of the R wave to the bottom of the S wave (Figure 1, Appendix 6). It is more sensitive to sodium channel blockade than conventional measures (Penz et al., 1992). In order to illustrate this, we compared the ECG effects of various Class I sodium channel blockers with other antiarrhythmics. Representative drugs from the three subclasses of Class I, i.e.

quinidine, lidocaine and flecainide, were tested. In each case, changes in RSh occurred before changes in QRS or P-R (see Figure 3a,b,c, Appendix 6). Other antiarrhythmics (Class II, Class III and Class IV) only influenced RSh if they had sodium channel blocking properties and then only at high doses, e.g. propranolol (Class II) and tedisamil (Class III) (Table 3, Appendix 6). Other physiological manoeuvres, such as changing vagal activity, administration of catecholamines, or direct pacing of the right atrium, did not change RSh. Thus, RSh is a useful measure with which to detect possible sodium channel blocking actions of cardiovascular drugs in rats.

The effects of heart rate on the Q-T interval have been examined in several species (Hayes et al., 1994). It is difficult to measure the repolarizing T-wave in a rat ECG since the dominant transient outward current causes a rapid repolarization of the ventricle (Detweiler, 1981; Josephson et al., 1984). To aid analysis we chose to examine the Q-aT interval. This is a more useful measure than that previously used in the investigation of the physiological and pathological factors that underlie Q-T duration and its prolongation (Taran and Szilagyi, 1947) as well as evaluation of drugs such as class III antiarrhythmic drugs, which lengthen refractory periods and action potentials.

In most species the interpretation of Q-T data is complicated with drugs which change heart rate since Q-T depends on rate. It is necessary to correct for this effect. The correction problem has been examined in detail in humans and other experimental animals but no consensus has been reached as to which of the many correction factors is the most useful and appropriate (Browne et al., 1983). We have therefore made a systematic analysis of the effect of variations in heart rate on the Q-T interval in a number of species (rat, guinea pig, rabbit, and primates) (Hayes et al., 1994). In view of the difficulty in determining when the T-wave returns to the isoelectric line, the aforementioned measure, Q-aT, was adopted (Chernoff, 1972). Q-aT is the time from the negative deflection in the Q-wave of the QRS complex to the peak of the T-wave. In rat, there was no correlation between heart rate and the Q-aT (Figure 1c, Appendix

7). As a result, in our studies no correction was made for heart rate effects with respect to Q-aT interval.

2.2 Isolated rat hearts

The isolated perfused heart has many advantages in the study of the actions of drugs on both the mechanical and electrical properties of the heart. The isolated heart was first described by Langendorff in 1895 to be a simple preparation with which to study the actions of drugs. This preparation resolved many of the problems encountered in using preparations involving blood perfusion. In the Langendorff heart, a Krebs-Henseleit solution is used to replace the blood. The isolated heart is also free of both CNS and circulating systemic humoral factors which may alter drug activity (Neely, 1967; Doring and Dehnert, 1988).

Wiggers first critically appraised the Langendorff isolated heart method in 1909. More recently, Broadley (1979) carefully discussed the advantages and limitations of the method. For example, he showed that the perfusate solution had a low oxygen-carrying capacity and that a lack of patency of the aortic valves (and the ease with which they are damaged) allowed perfusion fluid to enter and distend the left ventricle. A perfusion apparatus was developed which reduced or eliminated some of these problems (Curtis et al., 1986b).

2.2.1 Perfusion apparatus

A modified perfusion apparatus for the study of the actions of drugs on the mechanical and electrophysiological behaviour of hearts from small animals such as rats and guinea pigs was developed in this laboratory (Curtis et al., 1986b). Nine chambers (each of a 250 mL capacity) were machined into a plexiglass block and

placed into a bath containing circulating warm water (30-37°C maintained by an external heater). Krebs-Henseleit perfusate from within individually controlled chambers flows via separate silastic tubes to a common manifold and then to the aortic cannula. Since dead-space for each chamber is less than 0.1 mL this allows for a rapid switching of perfusate while an external (5% CO_2 in O_2) gas mixture maintains aortic root pressure between 70-125 mmHg, as required.

Male Sprague-Dawley rats (300-400 g) were killed by a blow to the head, exsanguinated and the heart rapidly removed from the chest cavity. Hearts were perfused with 5 mL of ice-cold Krebs-Henseleit solution to remove remaining blood (Curtis et al., 1986b, Curtis, 1991). Within two minutes of sacrifice, hearts were perfused via an aortic cannula with an oxygenated Krebs-Henseleit solution at 35°C and pH 7.4. The composition (mM) of the Krebs-Henseleit solution was: NaCl, 118; KCl, 4.74; $CaCl_2 \cdot 2H_20$, 2.5; KH_2PO_4, 0.93; $NaHCO_3$, 25; D-Glucose, 10; $MgSO_4 \cdot 7H_20$, 1.2.

The left atrium was removed and a small compliant balloon (volume of approximately 0.5 mL) made of plastic wrapping film (Saran Wrap) was inserted into the left ventricle and adjusted to give an initial left ventricular end-diastolic pressure of 5-10 mmHg. The aortic root of the heart was perfused at a constant pressure of 100 mmHg. Ventricular pressure was measured by a pressure transducer and a Grass Polygraph while the maximum rate of intraventricular pressure development ($+dP/dt_{max}$) was obtained by differentiating left ventricular pressure using a Grass Polygraph differentiator (model 7P20C).

The ECG was recorded from the epicardial surface of the heart with atraumatic, silver-ball electrodes (Curtis et al., 1986b) placed on the right atrium and left-ventricle, i.e., approximating a Lead II configuration. The variability in the rat ECG makes determination of drug effects difficult, since the P-, QRS and T-waves do not share a common baseline (Detweiler, 1981; Driscoll, 1981).

All κ receptor agonists and related compounds were dissolved in distilled water and serial dilutions prepared in Krebs-Henseleit solution. The hearts were perfused with Krebs-Henseleit solution for 15 min prior to perfusion with the drug for a period of 2 min at each concentration. The exposure time was chosen as that during which a steady-state response to drug occurred.

2.3 Electrically-induced arrhythmias

To determine the effectiveness of antiarrhythmic drugs cardiac arrhythmias are usually produced in experimental animal models. Arrhythmias are routinely induced by electrical stimulation at a variety of sites including the atria, ventricles and the atrio-ventricular node. These have been produced in a number of species including rats and humans (Winslow, 1984; Weissberg et al., 1987). The small size of the rat heart does not allow for a highly selective placement of electrodes and as a result usually involves placement in the right or left ventricle. Access to the ventricles involves transthoracic placement of stimulating electrodes. A large variety of stimulation protocols can be used in rat ventricular tissue and can be chosen for the induction of arrhythmias or for indirectly probing the functional status of either sodium or potassium channels. For example, sodium channel availability may be gauged by examining excitability (i-t) curves (Antoni, 1971; Moore and Spear, 1975). While ERP also indirectly reflects sodium channel status it is highly dependent upon the potassium channels which control repolarization (Hoffman and Cranefield, 1960). The influence of drugs on the refractory period is also used for evaluation of antiarrhythmic effectiveness.

The types of arrhythmias induced by electrical stimulation include single extrasystoles, VT and VF (Winslow, 1984). It has been suggested that arrhythmias which result from electrical stimulation represent circus-type re-entrant movement (Antoni, 1971). This method allows for the study of the influence of drugs on

ventricular vulnerability and provides a means to examine drugs with potential antiarrhythmic properties.

2.3.1 Surgical Preparation

Using intact rats, prepared as described in section 2.1.2 of the Methods, electrical stimulation of the left-ventricle was performed using two Teflon-coated silver wire stimulating electrodes which were inserted through the chest wall and implanted into the left-ventricle as described by Walker & Beatch (1988). This placement technique produced an inter-electrode distances of between 1-3 mm. Antoni (1971) showed that the optimal inter-electrode distance for induction of consistent thresholds is between 2-4 mm. Square-wave stimulation was used to determine threshold current (i_t-μA) and pulse-width (t_t-ms) for induction of extrasystoles, ventricular fibrillation threshold (VF_t-μA), maximum following frequency (MFF-Hz) and effective refractory period (ERP-ms) according to Howard and Walker (1990). Although MFF is an approximate inverse measure of ERP we chose to measure both values since it has been shown that there are certain differential sensitivities of each to various drugs (Walker and Beatch , 1988). Control measures were taken 10 min apart until three sets of consistent measures could be obtained sequentially. Usually this required 30 min to accomplish. Measures were repeated in triplicate in the order i_t, t_t, VF_t, ERP and MFF in controls and for each dose of drug (Pugsley et al., 1992a).

2.3.2 Threshold for Capture (i_t)

The threshold for capture (i_t) is the minimum current which is required to capture, or pace the heart, and approximates the rheobase of the i versus t curve for excitability (Vaughan-Williams and Szekeres, 1961). It is a measure of sodium channel

availability (Pugsley et al., 1992a). This threshold is measured for capture of the ventricle at a frequency of 7.5 Hz and occurs when the heart follows a 1 msec square-wave pulse generated by a stimulator (Grass SD9). Capture was detected from the blood pressure and ECG recordings characterized by an increase in signal size, a regular sinus rhythm at a fast rate (slight tachycardia) and a reduction in blood pressure (Howard and Walker, 1988). Usually a range of values (80-100 μA) can be obtained for the rat in controls and up to 1000 μA after drug treatment.

2.3.3 Threshold Pulse Width (t_t)

The threshold pulse width for induction of extrasystoles was determined at 7.5 Hz and at twice i_t and t_t according to Walker and Beatch (1988). This measure approximates the chronaxie of the i versus t curve for ventricular excitability and is also a measure of sodium channel availability (Cheung et al., 1993).

2.3.4 Ventricular Fibrillation Threshold (VF_t)

Ventricular Fibrillation threshold (VF_t) is the minimum current required to produce ventricular fibrillation (Winslow, 1984). A single train of square-wave pulses at twice i_t and t_t, (Walker and Beatch, 1988) was delivered at 50 Hz while the current applied to the myocardium was smoothly increased until fibrillation resulted. This method ensures that the pulse is given during the vulnerable period of late systole, eliciting sustained VF. Once the applied voltage is stopped the rat (as well as the hearts of many other small animals) heart usually spontaneously reverts to normal sinus rhythm (Cheung et al., 1993; Winslow, 1984).

2.3.5 Maximum Following Frequency (MFF)

MFF is an indirect measure of ventricular refractoriness (Antoni, 1971; Pugsley et al., 1992a). It is more of a measure of ventricular functional refractory periods of the action potential, and thus can exhibit a different sensitivity to drugs from ERP. MFF is defined as the frequency at which the ventricle fails to follow on a one-to-one basis with the stimulus (Walker and Beatch, 1988). It is determined by gradually increasing the pacing frequency (at twice i_t and t_t) from 7.5 Hz until the heart fails to follow. Characteristic changes in blood pressure result. The resulting missed beat produces an abrupt decrease, followed by a large increase in blood pressure because of an increased ventricular filling time. This event is thus an index of MFF measurement.

2.3.6 Effective Refractory Period (ERP)

The ERP is determined by pacing the heart at 7.5 Hz at twice the i_t and t_t measures determined previously. An extra-pulse is added at an increased delay during the pacing train such that once the pulse meets or exceeds the absolute refractory period of the action potential an extra-beat is produced in the heart. This extra-beat is detected by an increase in amplitude of the ECG signal and transient compensatory reduction in blood pressure (Antoni, 1971) . Thus ERP is the shortest interval between the added stimulus and the pacing train at which an extrasystole appears.

2.4 Ischaemia-induced arrhythmias

2.4.1 Surgical preparation in acute studies

The surgical procedures used were similar to those employed by Au et al. (1979) and Paletta et al. (1989). In brief, rats were initially anaesthetized with pentobarbitone (60 mg/kg, i.p.) and supplemental doses (6mg/kg) were given i.p. when

necessary to ensure an adequate level of anaesthesia. The trachea was cannulated and all animals artificially ventilated. The left carotid artery was cannulated for measurement of mean arterial blood pressure and withdrawal of blood samples for determination of serum potassium concentrations (Ionetics Potassium Analyzer). The right jugular vein was also cannulated for administration of drugs.

The thoracic cavity was opened and a polyethylene occluder placed loosely around the left anterior descending coronary artery. The chest cavity was closed and body temperature maintained between 35-37°C using a heating lamp.

To obtain the best ECG signal for detection of changes, needle electrodes were placed subcutaneously along the suspected anatomical axis (right atrium to apex) of the heart determined by palpation, according to the method of Penz et al. (1992). The animal was allowed to recover for 30 min prior to drug administration.

2.4.2 Experimental Design

Random and blind experiments were performed (n=9 per group) in which animals received either vehicle, or drug as an i.v. infusion. A control record was taken 15 min before occlusion and 1 min prior to drug administration. Drug or vehicle was infused at a volume of 1.0 mL/hr and traces were taken at 1 min intervals over a period of 5 min, post-infusion. A blood sample (approximately 0.25 mL) was taken for serum potassium analysis. Thereafter, the occluder was pulled so as to produce coronary artery occlusion. ECG, blood pressure, heart rate, arrhythmias and mortality were monitored for 30 min after occlusion.

Arrhythmias were classified as ventricular premature beats (VPB), ventricular tachycardia (VT) or ventricular fibrillation (VF) and the number of each was recorded. The overall arrhythmic history was expressed as an arrhythmia score (A.S.) as

described by Curtis and Walker (1988). At the end of a 30 min period of occlusion and, if the animal survived, a second blood sample was taken.

After death, hearts were removed and perfused by the Langendorff technique (Langendorff, 1895) with Krebs-Henseleit solution to wash out all remaining blood. This was followed by perfusion with saline containing 1 mg/mL indocyanine (Fast green dye, BDH) for 60 sec which revealed the underperfused and occluded zone (zone-at-risk). The occluded zone, which was clearly defined visually as having a distinct border from the non-occluded ventricular tissue was then cut away, blotted, and weighed on an analytical balance. The occluded zone was expressed as a percentage of total ventricular weight.

2.4.3 Pre- and post-occlusion ECG changes

ECG traces were taken 1, 2, 5, 10, 15 and 30 min post-occlusion. Prior to both drug administration and occlusion, the ECG showed a positive ST-segment with respect to the isoelectric baseline. This allowed for signs of drug-mediated changes in the RSh measure, indicative of sodium channel blockade, to be measured in the heart prior to occlusion and examination of changes in the S-T segment, post-occlusion. After ligation of the coronary artery, changes occured in the ECG associated with ischaemia. Initially, there was a rapid increase in the size of the ECG signal, particularly a large increase in the R-wave amplitude. The maximal R-wave was measured as the maximal deflection of the peak of the R-wave from the isoelectric line.

The position of the T-wave of the ECG in rats is not clearly defined and because of this determination of the S-T segment is difficult. However, ST%, i.e. the elevation of the S-wave as a percent of R-wave amplitude, is a consistent measure in the rat (Curtis and Walker, 1986). The S-T segment initially falls to baseline after the onset of occlusion (Johnston et al., 1981; Kane et al., 1981) then elevation immediately follows,

reaches a maximum, and is maintained for the duration of the experiment (Curtis et al., 1986c). Since all measures of S-T segment elevation were not constant with time (Johnston et al., 1983) the maximum S-T segment elevation and the time which this occurred were determined in an attempt to reflect this fact.

2.4.4 Analysis of arrhythmias

The analysis and quantification of the ischaemic arrhythmias produced by occlusion of the coronary artery is complex while the statistical analysis of such arrhythmias is dependent on how the arrhythmia data is categorized and treated. As a result a scoring system was developed with which to summarize the arrhythmic history in a single value- the Arrhythmia Score (Curtis and Walker, 1988).

Arrhythmia appearance is biphasically time-dependent in many species including the rat (Johnston et al., 1981). In the following studies, the severity and incidence of arrhythmias were quantified during the initial occlusion phase (0-30 min post-occlusion).

Arrhythmias were categorized according to guidelines established by the Lambeth conventions (Walker et al., 1988). Ventricular premature beats (VPB) were defined as single QRS complexes which occurred before any identifiable P wave. Doublets (bigeminal) or triplets (trigeminal), variations in the single complex, were not classed as distinct arrhythmias but rather were summed for each group (Curtis and Walker, 1988). Ventricular tachycardia (VT) was defined as 4 or more consecutive VPB's and not subclassified according to rate. VT incidence was classified by characteristic changes in ECG morphology, elevation in heart rate and fall in mean BP.

Ventricular fibrillation (VF) was defined as a chaotic ECG pattern in which no distinguishable QRS complexes could be discerned accompanied by a precipitous fall

in blood pressure to less than 10 mmHg. Animals were not defibrillated and if VF did not spontaneously revert, the animal died.

2.5 Isolated Ventricular Myocytes

2.5.1 Patch-Clamp Apparatus

The patch-clamp was developed in the early 1980's by Neher and Sakmann to record membrane ion channel currents. At that time these currents were undetectable due to the high background noise associated with standard voltage-clamp techniques (Neher and Sakmann, 1976; Hamill et al., 1981). By using a glass micropipette a localized voltage-clamp could be produced and the electrical activity (channels) in an isolated cell or small area of cell membrane (the patch) could be measured (Cahalan and Neher, 1992). The patch-clamp drastically improved recording techniques. Further refinements included the formation of gigaohm seals with cells rather than megaohm seals recorded previously. These new seal resistence's improved current amplitude resolution by reducing background noise, reduced leak currents, and stabilized the current recording (Hamill et al., 1981; Hondeghem et al., 1981).

Several configurations exist in patch-clamp recording. These include the "cell attached patch", "whole-cell recording clamp", "inside-out patch" and "outside-out patch" described in detail by Hamill et al. (1981). For studies conducted in this thesis, only the whole-cell recording mode was used to elicit sodium and potassium currents.

2.5.2 Cell isolation

Freshly isolated adult ventricular myocytes from Wistar rats were used to examine the electrophysiological properties of arylacetamide drug action on ionic

currents. Ventricular myocytes were isolated according to the method of Farmer et al. (1983). Briefly, male Wistar rats (300-400 g) were killed by cervical dislocation followed by exsanguination. The chest was opened, the heart removed and immersed in ice-cold, oxygenated, calcium-free Tyrode solution composed of (mM): NaCl 134; KCl 4; NaH_2PO_4 1.2; $MgCl_2$ 1.2; glucose 11; TES (N-tris-(hydroxymethyl)-methyl-2-aminoethanesulphonic acid) 10, and the solution adjusted to pH 7.4 with 1.0 M NaOH. The heart was then attached, via an aortic cannula, for perfusion with the same calcium-free Tyrode solution warmed to 37°C to facilitate removal of blood from ventricular chambers and coronary vasculature. After a 5 min wash, the heart was subjected to enzymatic dissociation in 25 μM calcium Tyrode solution containing protease (0.1 mg/mL, Sigma Type XIV), collagenase (1 mg/mL, Worthington CLS II), and fetal calf serum (1 μg/mL).

After 20-25 min of perfusion the ventricles were removed in one-third sections. Each section was carefully cut into small pieces in fresh 25 μM calcium-Tyrode solution and triturated to dissociate myocytes. Cell suspensions were then gently centrifuged and washed in a 200 μM calcium-Tyrode solution. Cells were then resuspended in a 1 mM calcium-containing Tyrode solution and 1-2 hr later plated onto glass coverslips. All cells were prepared and stored at room temperature (25-27°C). The cells used in our studies were rod-shaped, clearly striated and quiescent in the 1 mM Ca-Tyrode solution.

2.5.3 Recording Solutions

All experiments were performed at room temperature (25-27°C). Cells were externally perfused with a Tyrode solution of the following composition (mM): NaCl 70; KCl 5.4; $MgCl_2$ 1.0; glucose 10; TES 10; $CaCl_2$ 2.0; $CoCl_2$ 5.0; $CsCl_2$ 5.0; choline Cl 60, the solution adjusted to pH 7.4 with 1.0 M NaOH. The pipette solution used for

recording both sodium and potassium currents contained (in mM): KF 140; TES 10; $MgCl_2$ 1.0; K-EGTA (ethyleneglycol-bis-(ß-amino ethyl ether)N,N,N',N'-tetraacetic acid); $CaCl_2$ 2.0; ATP-disodium 5.0; ATP-Mg 5.0 and pH adjusted to 7.4 with 1.0M KOH. When recording sodium currents, the intracellular potassium was replaced with caesium to inhibit any evoked potassium currents and while recording potassium currents, 20 µM tetrodotoxin was added to the bath solution to inhibit sodium currents which may have been evoked.

2.5.4 Microelectrode Preparation

Patch-clamp electrodes were made from borosilicate glass (A-M Systems, Washington, U.S.A.) with an internal diameter of 1.2 mm and an external diameter of 1.6 mm. Electrodes were prepared using a two-stage vertical puller (Narishige Scientific Instruments, Tokyo, Japan). The microelectrode glass used required a fixed pulling length in order to minimize tip diameter (between 2-4 µm) variability. Pipettes were fire polished using a home-made heating forge. This consisted of a U-shaped platinum filament connected to a variable DC voltage supply. The pipette was mounted on a rotating holder at a speed of 30 r.p.m.. The heating filament was turned on and the pipette tip, under a light microscope (100X magnification), was moved in close proximity to the filament. After 1-2 secs the pipette tip was withdrawn, allowed to cool, and placed in a covered petri dish for subsequent use.

Pipette tips were initially back-filled by applying suction to the pipette while in the filtered recording solution and was followed by normal filling with a needle to approximately one-third the length of the pipette. This ensured removal of all air bubbles and proper immersion of the reference wire (from the head-stage amplifier). The pipette was inserted into a holder on the head-stage amplifier (gain=0.1) which, in turn, was connected to the patch-clamp amplifier (Axopatch 200A, Axon Instruments).

The headstage and pipette could then be moved, in any direction, by a micromanipulator. Only microelectrodes with tip resistances of between 5-10 MΩ were used.

2.5.5 Patching Ventricular Myocytes

Once secure in its holder a small positive pressure was applied to the pipette in order to prevent debris becoming attached to the tip when immersed in the bathing solution. Pipette tip resistance was determined by the application of a 5mV test pulse to the electrode. The pipette offset on the Axopatch amplifier was adjusted to baseline in order to compensate for the developed junction potential (Barry and Lynch, 1991). Although the junction potential can be compensated for on the amplifier, compensation is never absolute. In our studies the liquid junction potential was usually between 5-10 mV at 25°C.

Positioning of the electrode above the cell was performed during visual observation. The pipette tip was slowly lowered onto the cell and once the pipette tip touched the cell, as indicated visually on the oscilloscope by a reduction in the 5mV test pulse (related to formation of a megaohm seal resistance) a brief suction pulse (negative pressure) was applied to the pipette to produce a gigaohm resistance seal and to rupture the cell membrane. The latter was accomplished using a mouth suction tube connected to the pipette holder and confirmed by the disappearance of the test pulse and formation of membrane capacitance currents.

Current flow across the entire cell membrane were recorded 10 min after achieving a whole-cell patch-clamp configuration (Hamill et al., 1981). Current recording was performed using an Axopatch 200A amplifier which allowed for 90% compensation and reduction of both capacitance transients and leak currents from computer-generated voltage commands. Output signals were filtered at 5 kHz,

digitized with a 12-bit A/D converter and recorded on a Total Peripherals 386/250p (Marlboro, MA) computer. Final capacitance and leak compensation was performed at the time of data analysis by subtraction of a 20 mV hyperpolarization pre-pulse current which always preceded the test voltage step.

2.5.6 Patch-Clamp Experiments

Only cells which were quiescent, rod-shaped and which presented clear striations were used for studies. Complete sets of current data (control + drug + recovery) were obtained for each cell studied. Experiments were performed in a small tissue bath mounted on an inverted Nikon microscope. The low volume (0.5-1.0 mL) plexiglass recording bath allowed for rapid exchange (1-2 sec) between control and experimental solution fed from gravity-flow reservoirs. A suction flow ensured that solutions superfused cells at 1-2 mL/min maintaining a constant fluid level. Evoked sodium and potassium currents were obtained only from myocytes which displayed a minimal reduction in current amplitude (less than 5%) during the control period prior to drug application. Adequate voltage control was achieved by both reducing the transmembrane sodium concentration and recording currents at reduced temperatures (25-27°C) according to Brown et al. (1981) and Bennett et al. (1988). With this method, the sodium current could be recorded for between 30-60 min after achieving whole-cell configuration.

2.5.6.1 Sodium Currents

Sodium current dose-response curves were evoked by application of 10 msec depolarizing pulses from a fixed conditioning pre-pulse of -150 mV (which ensured removal of sodium channel inactivation) to a test pulse of 0 mV at an interval of one

pulse every 6 sec. Measurements were most accurate when clear capacitance artifact cancellation and compensation was achieved resulting in a distinct separation between sodium channel activation and the capacitance transient decay.

The voltage-dependence of activation (m_∞) of the peak sodium current was examined using a voltage-step to a variable test potential (between -70 mV and +50 mV) from a fixed conditioning pre-pulse potential of -150 mV. Test potentials were evoked at 1 sec intervals (or greater) to ensure adequate recovery of channel function between pulses. A plot of current amplitude against test pulse potential yields a current-voltage relationship for sodium current. Conductance (G_{Na}) can be calculated using the Hodgkin and Huxley model where $I_{Na}=G_{Na}(V-E_{rev})$. The line approximating this relationship is the Boltzmann equation $I_{Na}=\{G_{max}/[1+\exp((V-V')/k)]\}\times(V-E_{rev})$. In this equation G_{max} is the maximal channel conductance for sodium, V' is the voltage for half-maximal sodium channel activation, k is a slope factor, and E_{rev} is the reversal potential for the sodium channel. This equation allows for an examination of drug effects on channel kinetics of activation.

The steady-state voltage-dependence of inactivation (h_∞) was studied by measuring the effect of various conditioning pre-pulses between -140 mV and -30 mV on the sodium current elicited at a fixed test potential to -20 mV according to Hodgkin and Huxley (1952). The best-line fit for the isolated myocyte data was determined using the Boltzmann equation $I_{Na}=G_{max}/[1+\exp((V-V')/k)]$ for inactivation kinetic values as determined above.

To elucidate use or frequency-dependence of drug action, studies were conducted by examining drug effects on evoked sodium currents at various frequencies. The arylacetamides were initially examined by bath application of a single drug concentration followed by the delivery of a train of depolarizing pulses at various frequencies or by providing a train of depolarizing pulses at a frequency of 10 Hz to cells. Sodium currents were evoked as above except that the trains of depolarizing

pulses were provided by an external Grass stimulator. The stimulator frequencies and durations were regulated by custom computer software developed in the laboratory of Dr. D.A. Saint.

Several studies were conducted to determine the putative site of action of (±)PD117,302 and its inactive enantiomer, (+)PD123,497. Studies on sodium currents involved addition of arylacetamide to the bathing solution or to the pipette solution used to patch the myocyte. In a comprehensive review Horn and Korn (1992) discuss the potential for exchange of material between the cell interior and pipette solution. Since the speed of the resulting "washout" depends upon both pipette resistance and cell size it is nonetheless possible to prevent the rundown of recorded currents by supplementation of the pipette solution with constituents which maintain ionic current function. No significant rundown of current amplitude was seen during patch-clamp recording. Verification of drug perfusion into the cell interior was achieved by intracellular application of lidocaine in several preliminary experiments which quickly blocked the evoked sodium current (data not shown).

Sodium currents were evoked beginning approximately 4 min after achieving whole-cell mode of the patch-clamp configuration. Current amplitude was monitored for an additional 4 min with currents being evoked every 6 sec. Arylacetamides were then added to the bath solution perfusing the cells and currents continuously evoked. Peak current amplitudes were plotted as a function of time.

An additional study was conducted in an attempt to determine the effects of pH on drug blockade of sodium currents. (+)PD123,497 (13 μM) was added to bath solutions at pH=6.4 and pH=7.4. Control currents were obtained following which the pH=7.4 bath solution containing (+)PD123,497 was added. Approximately 2 min later a sustained current block resulted at which time the acidic pH solution containing (+)PD123,497 was exchanged for the pH=7.4 solution. Sodium currents were

continuously recorded for 1.5 min at which time the pH=7.4 bath solution was reapplied to the cell. Peak current amplitude for the responses were plotted as a function of time.

2.5.6.2 Potassium Currents

Studies were conducted to determine the effects of spiradoline on potassium currents in isolated myocytes. Potassium currents were evoked by depolarization to +50 mV from a pre-pulse potential of -150 mV for a duration of 300 msec. The concentration-dependent effects of bath applied arylacetamides on the transient outward (i_{to}) and sustained outward plateau (i_{Ksus}) potassium currents were examined. The potassium current amplitude for i_{to} was measured at the peak, approximately 5 msec after evoking the outward current. A measure of drug effectiveness on this current was obtained by subtraction of the current magnitude 300msec after evoking the outward current from the peak amplitude. At 300 msec the i_{to} current has inactivated and the sustained outward current, i_{Ksus}, remains. Compounds were examined for blocking effects on these currents. Construction of a line tangential to the decay curve of i_{to} approximated drug effect on the rate of decay of this current. We assumed a monoexponential decay of evoked outward potassium current, although i_{to} may be composed of two components (Coraboeuf and Carmeleit, 1982; Campbell et al., 1995).

2.5.7 Drugs

Spiradoline (U-62,066E) (a gift from Dr. P.F. Von Voightlander, The Upjohn Co., Kalamazoo, MI), naloxone (Sigma Chemical Co., St. Louis, MO) and tetrodotoxin (TTX) were initially dissolved in distilled water prior to dissolution in the external bath solution for single cardiac myocytes.

2.6 Statistical Analysis

All *in vivo* studies were performed according to a randomized block experimental design. This block design compensates for heterogeneity in the experiment and controls for variability which may arise from experimental error. Experimental error reflects a combination of both random error and biological variability. The random block design essentially reduces the variability in the system and hence decreases experimental error (Montgomery, 1984). The only randomization that occurs is confined to treatments within blocks. The blocks then represent a restriction on randomization and thus yield a simple statistical model (Li, 1964).

Experiments with the above design lend themselves to Analysis of Variance (ANOVA) particularly as this test demands that treatments are from as uniform an environment as possible (Li, 1964). ANOVA allows one to compare many treatments, thus making it a most useful statistical test (Zar, 1984; Gad and Weil, 1988). Statistical significance was determined at an α-level of 0.05 using the General Linear Model ANOVA (GLM ANOVA) from the NCSS Statistical Package (Hintze, 1981). Values are shown as the mean±s.e.mean for *n* experiments.

A *post hoc* or multiple comparison test was performed to determine which treatment means differed after ANOVA. A large number of tests exist for this purpose; we chose Duncan's multiple comparison test (Duncan, 1955). This test compares groups of continuous and randomly distributed data of equal sample size and is a powerful test for detecting differences between means (Montgomery, 1984). This test was used throughout the *in vivo* experiments performed except in arrhythmia studies. Mainland's contingency tables were used rather than analysis of covariance (ANCOVA) to determine significance between arrhythmic groups. Exclusion criteria for these studies were according to Pugsley et al. (1992b). Arrhythmic PVC incidence was $\log_{10}$ transformed to a normal distribution (Winkle, 1979; Walker et al., 1988).

3 Unpublished Results for other Arylacetamides

3.1 Studies with U-62,066E (Spiradoline)

The chemical structure of spiradoline, (U-62,066E), and the other arylacetamides examined in previously published studies can be found in Figure 1.

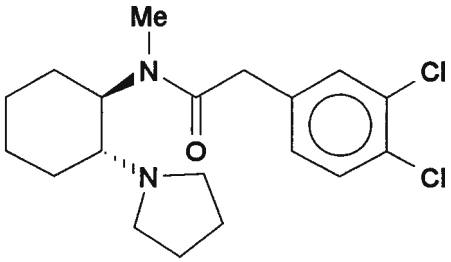
3.1.1 Isolated Heart studies - Contractility and ECG effects

Spiradoline produced concentration-dependent increases in heart rate, P-R interval and QRS duration of the isolated rat heart ECG. Figure 2A shows the concentration-related reduction in heart rate produced by 100 μM spiradoline (n=5 hearts). In the presence of 1 μM naloxone, changes in heart rate with spiradoline paralleled spiradoline alone. Observed ECG changes included a P-R interval prolongation and increased QRS width (Figure 2B). The P-R interval changed from 69±4 to 189±15 msec in hearts exposed to 100 μM spiradoline. Likewise, QRS increased from 29±2 msec to 76±14 msec in hearts exposed to the same concentration of drug. For sake of clarity, naloxone pre-treatment dose-response curves are not shown. In the presence of 1 μM naloxone, spiradoline (100 μM) increased the P-R interval from a control of 55±1 to 170±11 msec. There were no differences between spiradoline effects in the absence and presence of naloxone.

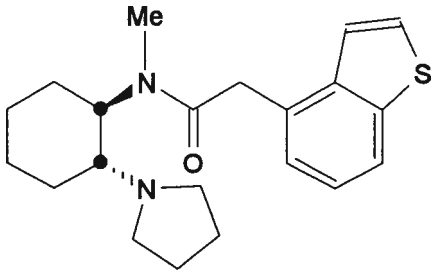
Cardiac contractility was reduced in a concentration-dependent manner by spiradoline. Peak systolic pressure (Figure 3A) and maximum rate of peak systolic pressure development ($+dP/dt_{max}$) and relaxation ($-dP/dt_{max}$) were equally reduced by spiradoline (Figure 3B). The peak systolic pressure was reduced by 54% with 100 μM spiradoline (from a control of 93±5 to 43±11 mmHg). A transient elevation (10%) in end-diastolic pressure occurred at this concentration (data not shown).

Figure 1 Chemical structure of U-50,488H, trans-(±)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]-benzene-acetamide methane sulphonate, (+)PD123,497 (+)-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzo[b]thiophene-4-acetamide monohydrochloride (the inactive κ enantiomer of (±)PD117,302), (±)U-62,066E (spiradoline) 5α,7α,8β-(±)-3,4-dichloro-methyl-N-[7(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl]benzeneacetamide methane-sulfonate), (±)PD117,302 ((±)-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzo[b]thiophene-4-acetamide), (-)PD129,290 (-)[5R-(5α,7α,8ß)]-N-methyl-N-[7-1-pyrrolidinyl)-1-oxaspiro [4.5]dec-8-yl]-4-benzofuran acetamide, (±)PD117,302 (±)-N-methyl-N-[2-(1-pyrrolidinyl) cyclohexyl]benzo[b]thiophene-4-acetamide monohydrochloride and (+)PD129,289 (+)5S-(5 α,7α,8ß)]-N-methyl-N-[7-1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-4-benzofuran acetamide (the inactive enantiomer of (-)PD129,290).

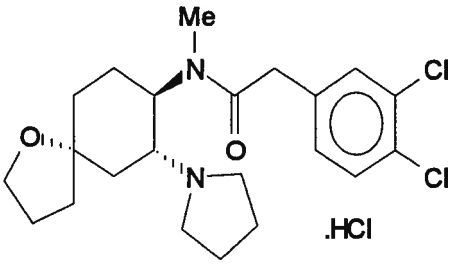
Arylacetamide structures



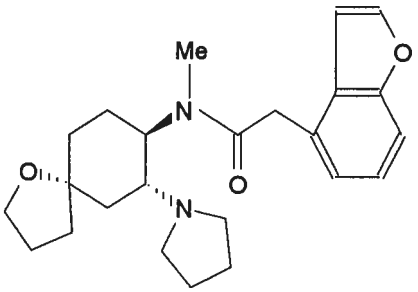
U-50,488H



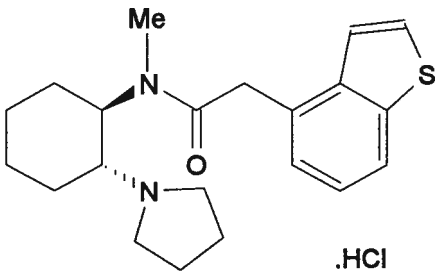
(+) PD123,497



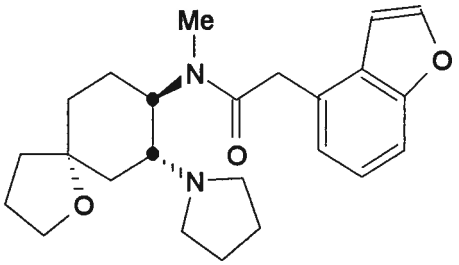
U-62,066E (Spiradoline)



(-) PD129,290



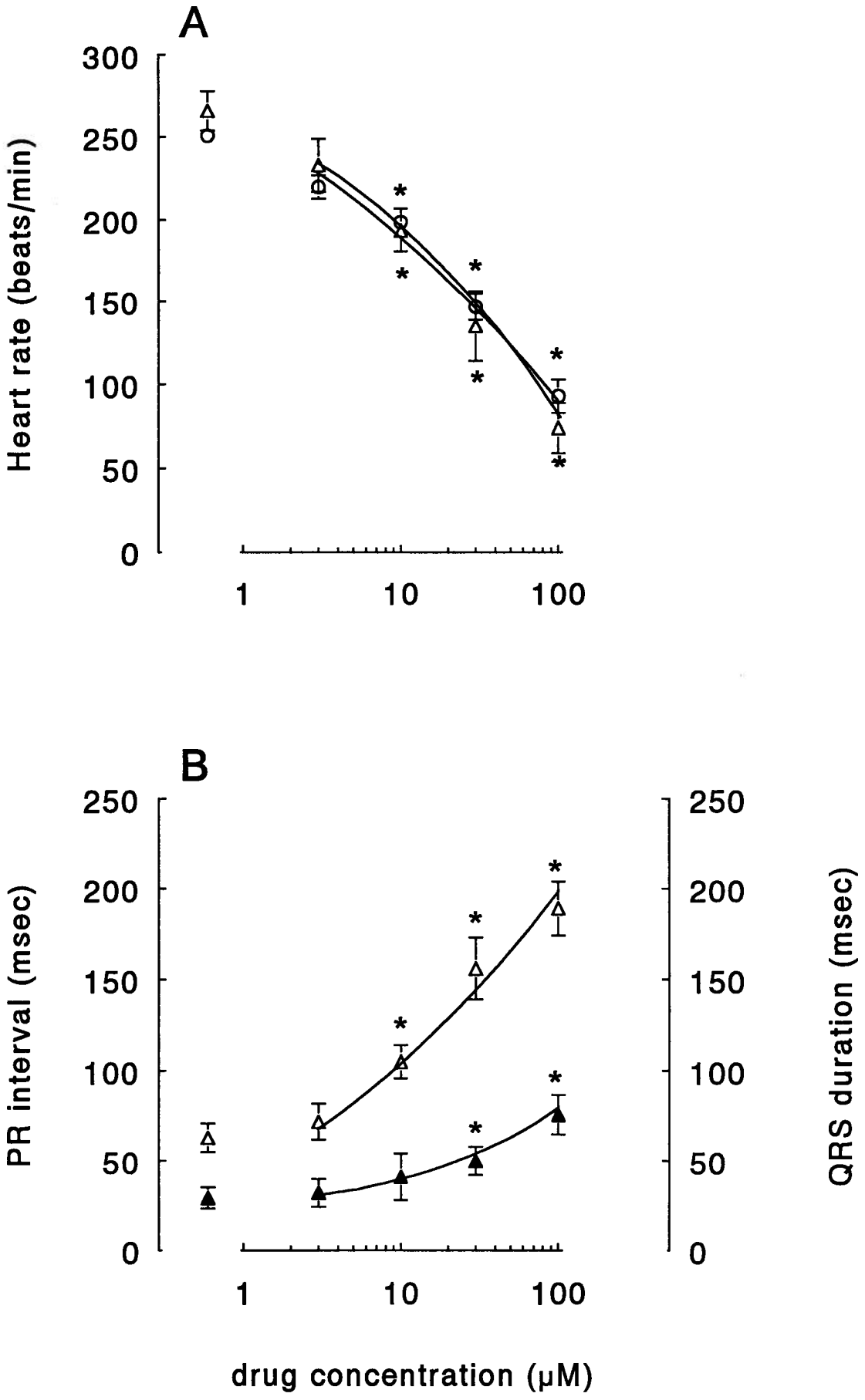
(±) PD117,302



(+)PD129,289

Figure 1

Figure 2 Concentration-dependent effects of spiradoline on heart rate (A) and the ECG (B) in Langendorff-perfused rat hearts Panel A shows the effects of spiradoline on heart rate in the absence (Δ) and presence (O) of 1μM naloxone. Panel B shows the effects of spiradoline alone on P-R interval (Δ) and QRS duration (▲). The effects of spiradoline tested in the presence of 1μM naloxone (data not shown) did not differ significantly from those obtained in the absence of naloxone. Values are shown as mean±s.e.mean for 5 hearts/group. Pre-drug values are shown to the left of the spiradoline concentration axis. Statistically significant difference is shown as * for $p<0.05$, as compared with pre-drug values.


A
Heart rate (beats/min)
300
250
200
150
100
50
0
1
10
100
B
PR interval (msec)
QRS duration (msec)
250
200
150
100
50
0
1
10
100
drug concentration (μM)

The decrease in peak systolic pressure in the presence of 1μM naloxone paralleled the effects of spiradoline alone. The peak systolic pressure in control hearts was reduced from 105±8 to 46±6 mmHg at a concentration of 100 μM spiradoline and in the presence of 1 μM naloxone. The rate of intraventricular pressure development ($+dP/dt_{max}$) and relaxation ($-dP/dt_{max}$) were also reduced by spiradoline (Figure 3B). Data in the presence of naloxone is not shown for sake of clarity. In the presence of 1 μM naloxone, $+dP/dt_{max}$ was reduced from a control of 2250±110 to 1130±95 mmHg/sec by 100 μM spiradoline. The reduction in the rate of relaxation in the presence of naloxone also paralleled the curve describing spiradoline alone. The rate of relaxation was reduced from a control of 1850±225 to 490±45 mmHg/sec with 100 μM spiradoline. Thus, these experiments in isolated hearts suggest that the drug produces a direct depression of cardiac contractility which may be related to ion channel blockade.

3.1.2 Haemodynamic and ECG actions of Spiradoline

Blood pressure and heart rate were stable over the duration of drug infusion in all vehicle (n=5) and 2.5 μmol/kg/min naloxone treated (n=3, data not shown) animals. Spiradoline, in the absence and presence of naloxone, produced a marked dose-dependent reduction in both blood pressure and heart rate (Fig. 4A and B, respectively). At a dose of 32 μmol/kg/min spiradoline reduced blood pressure from 126±7 to 51±4 mmHg and heart rate from 352±14 to 124±7 beats/min. In the presence of naloxone, blood pressure was equally reduced (from 118±6 to 57±7 mmHg); however, heart rate was reduced less than in the absence of naloxone (from 360±19 to 201±17 beats/min).

ECG measures were also influenced in a dose-related manner by spiradoline. The highest dose of spiradoline (32 μmol/kg/min) produced a 36% increase in the P-R interval (from 61±1 to 95±2 msec). In animals treated with naloxone, the highest dose of

Figure 3 Concentration-dependent effects of spiradoline on peak systolic pressure (A) and rates of change of pressure (B) in Langendorff-perfused rat hearts Panel A shows the effects of spiradoline on peak systolic pressure in the absence (Δ) and presence (O) of 1μM naloxone. Panel B shows the effects of spiradoline alone on the maxium rate of rise of ventricular pressure (Δ) and maximum rate of fall of ventricular pressure (▲). The effects of spiradoline tested in the presence of 1μM naloxone (data not shown) did not differ significantly from those obtained in the absence of naloxone. Values are shown as mean±s.e.mean for 5 hearts/group. Pre-drug values are shown to the left of the spiradoline concentration axis. Statistically significant difference is shown as * for $p<0.05$, as compared with pre-drug values.

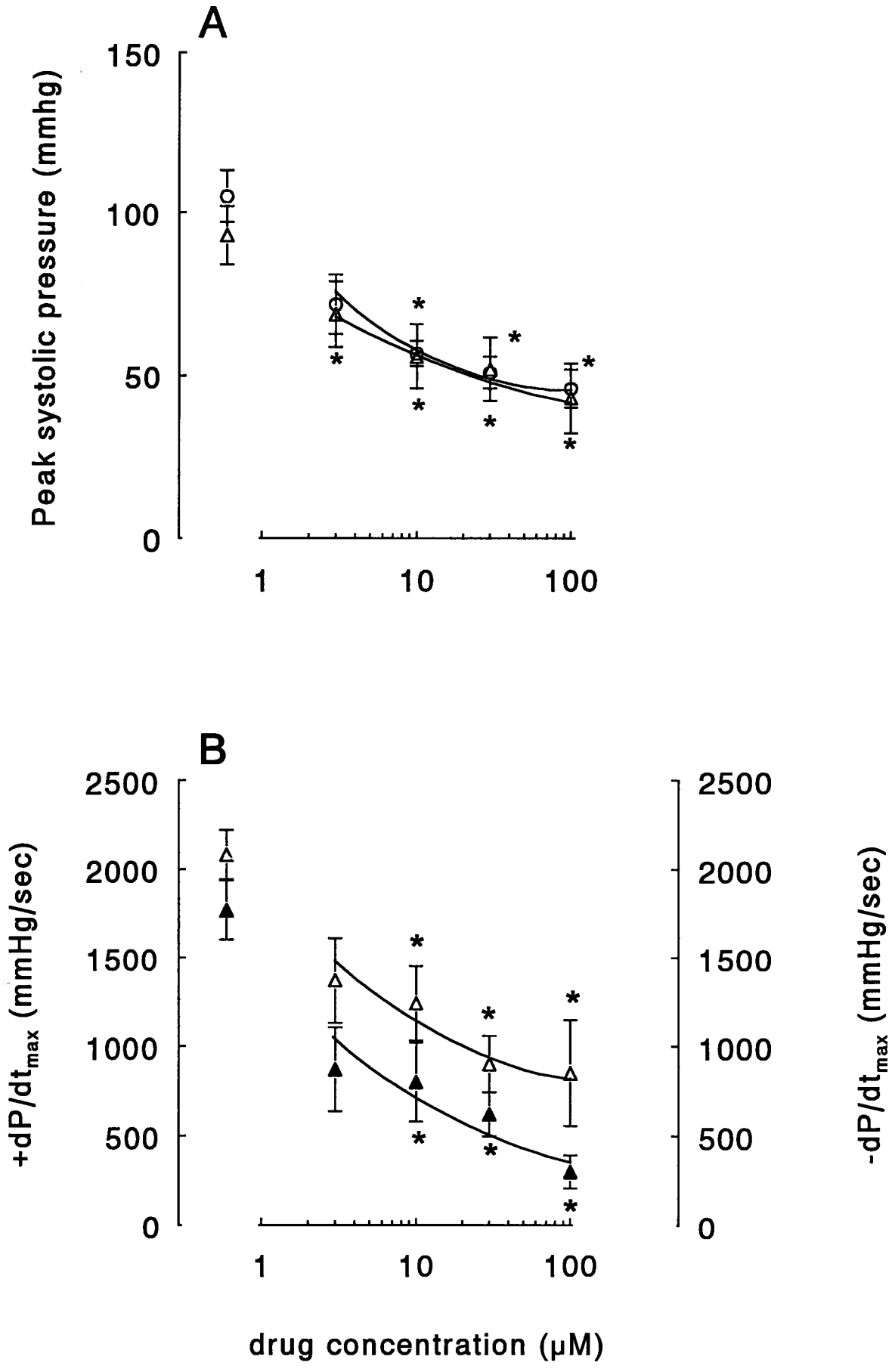
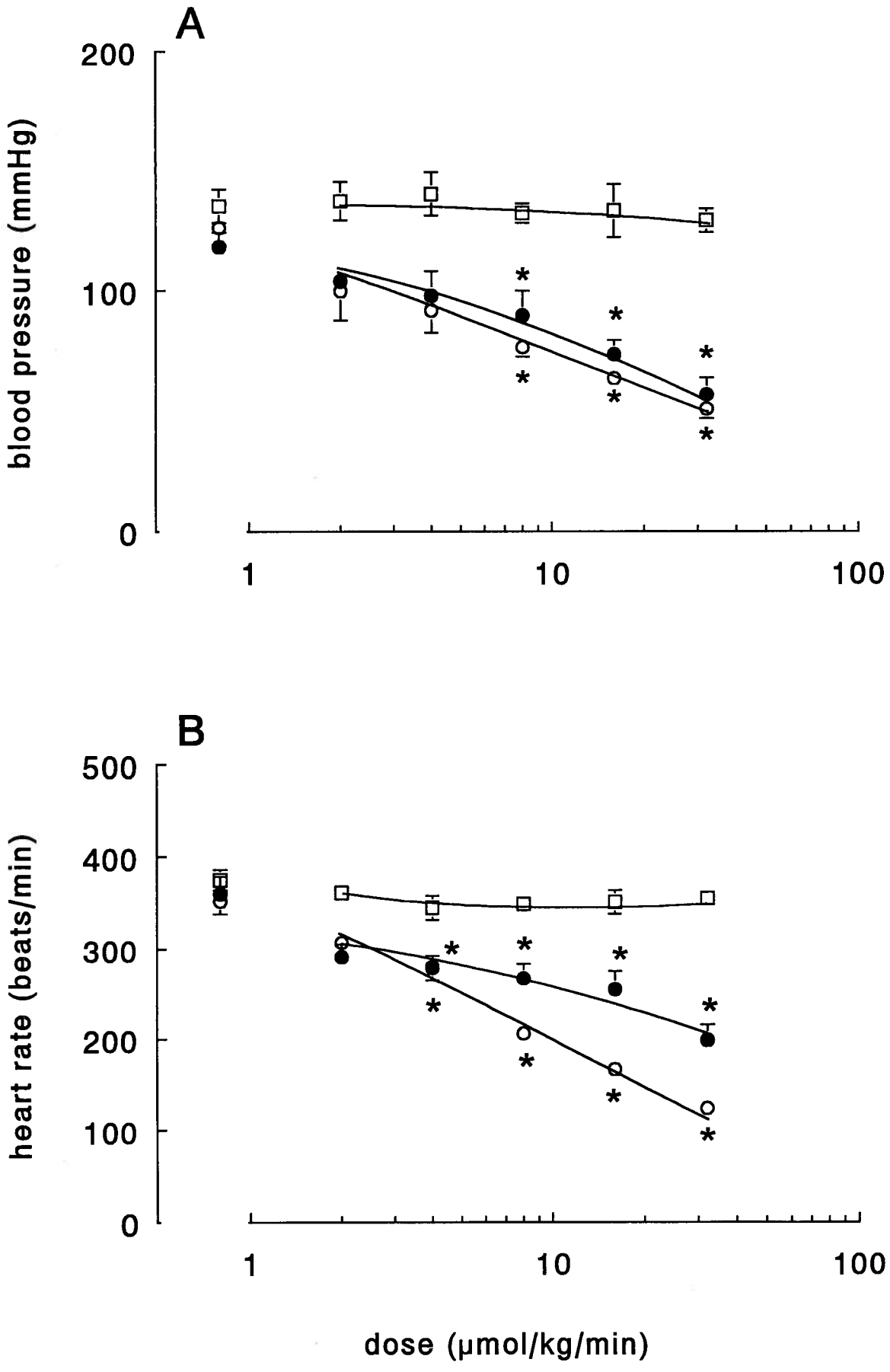

A
Peak systolic pressure (mmhg)
150
100
50
0
1
10
100
B
+dP/dt$_{max}$ (mmHg/sec)
-dP/dt$_{max}$ (mmHg/sec)
2500
2000
1500
1000
500
0
1
10
100
drug concentration (μM)

Figure 4 Dose-related effects of spiradoline on blood pressure (A) and heart rate (B) in pentobarbitone-anaesthetized rats in the absence and presence of 2.5 μmol/kg/min naloxone. Values are mean±s.e.mean for n=5. *Indicates a significant difference from pre-treatment at $p<0.05$. The pre-treatment means for blood pressure were 126±7, 118±6, 135±7 mmHg while those for heart rate were 352±14, 360±19 and 375±11 beats/min for spiradoline (O), spiradoline+naloxone (●) and saline vehicle (□), respectively. Saline control had no significant effect upon either measure.


A
200
100
0
blood pressure (mmHg)
1
10
100
B
500
400
300
200
100
0
heart rate (beats/min)
1
10
100
dose (μmol/kg/min)

spiradoline caused a 35% increase in P-R interval prolongation (from 62±2 to 96±3 msec) (Figure 5A). Spiradoline did not affect the QRS width until the highest doses were administered (Figure 5B). RSh, a novel measure of sodium channel blockade in the rat, was increased in a dose-dependent manner by spiradoline (Figure 6A). The RSh interval, at 32 μmol/kg/min, was increased by 49%, from 0.46±0.03 to 0.91±0.10 mV, while in the presence of naloxone the prolongation of this measure was 60% at this equivalent dose (from 0.48±0.09 to 1.20±0.21 mV). From Figure 6B it can be seen that spiradoline dose-dependently increased the Q-aT interval. The Q-aT measure was increased 34 and 39% by spiradoline in the absence and presence of naloxone, respectively. The vehicle-control did not affect any of the ECG measures over the duration of the experiment.

3.1.3 Electrical Stimulation studies

The patterns of drug action in isolated hearts and in the intact rats indicated that spiradoline may alter both sodium and potassium channel function. In order to determine whether or not these properties of spiradoline may confer antiarrhythmic activity we examined the effectiveness of spiradoline against electrically-induced arrhythmias in the rat. Figure 7A and B shows that spiradoline dose-dependently increased thresholds for capture or induction of extrasystoles (i_t) and ventricular fibrillation (VF_t). The i_t values for spiradoline, in the absence and presence of naloxone, were increased 64 and 66% (or from 80±11 and 95±18 to 220±12 and 247±60 μA), respectively. The dose-dependent changes in VF_t produced by spiradoline were much more marked than those on I_t. VF_t increased from 147±30 to 323±55 μA (54%) in the absence of naloxone and from 124±15 to 525±35 in the presence of naloxone (76%). Changes in t_t, the time to threshold, transiently increased with spiradoline administration but this was not significant ($p>0.05$) (data not shown).

Figure 5 Effects of spiradoline on P-R interval (A) and QRS duration (B) in pentobarbitone-anaesthetized rats in the absence and presence of 2.5 μmol/kg/min naloxone. Values are mean±s.e.mean for n=5. *Indicates a significant difference from pre-treatment at $p<0.05$. The pre-treatment means for the P-R interval were 61±1, 62±2, 57±3.5 msec while those for the QRS duration were 31±1, 30±0.4 and 28±0.8 msec for spiradoline (O), spiradoline+naloxone (●) and saline vehicle (□), respectively. Saline alone had no significant effects upon either measure.

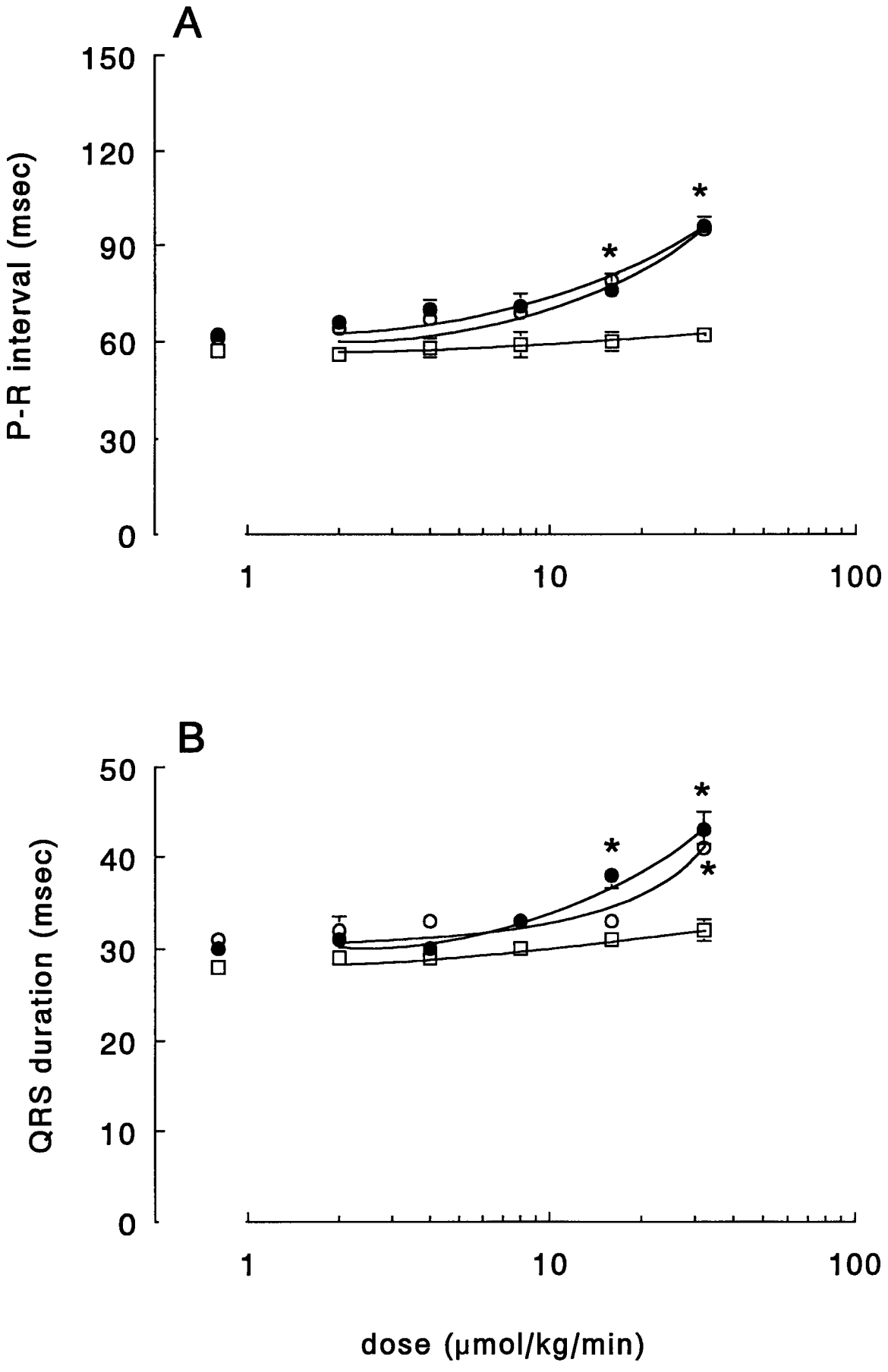

A
150
120
90
60
30
0
P-R interval (msec)
*
*
1
10
100
B
50
40
30
20
10
0
QRS duration (msec)
*
*
*
1
10
100
dose (μmol/kg/min)

Figure 6 Effects spiradoline produced on RSh (a novel index of sodium channel blockade in the rat described in full in Appendix 6) (A) and the Q-aT interval (described in full in Appendix 7) (B) in pentobarbitone-anaesthetized rats. Values are mean±s.e.mean, n=5. *Indicates a significant difference from pre-treatment at $p<0.05$. The pre-treatment values for RSh were 0.48±0.10, 0.46±0.03, 0.54±0.03 mV while those for the Q-aT interval were 35±0.6, 37±1 and 37±1 msec for spiradoline (O), spiradoline+naloxone (●) and saline vehicle (□), respectively. Saline alone produced no significant effects upon either measure.

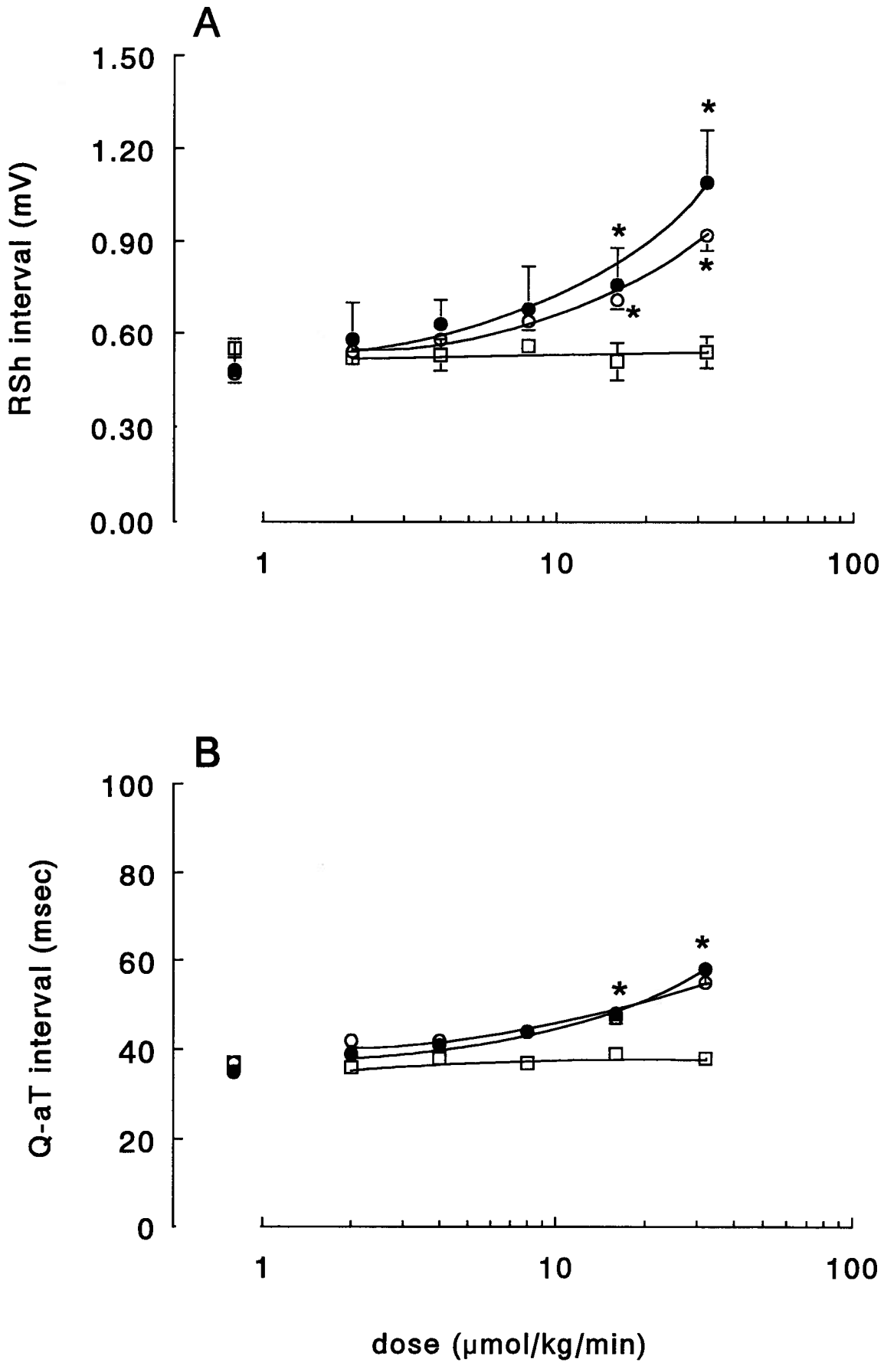

A
1.50
1.20
0.90
0.60
0.30
0.00
RSh interval (mV)
1
10
100
B
100
80
60
40
20
0
Q-aT interval (msec)
1
10
100
dose (μmol/kg/min)

Figure 7 Effects of the saline vehicle (□), and spiradoline in the absence (○) and presence of 2.5 μmol/kg/min naloxone (●) on the threshold current for capture (i_t) (A) and ventricular fibrillation threshold, (VF_t) (B) in pentobarbitone-anaesthetized rats. Saline alone had no significant effect upon either measure. Values (mean±s.e.mean for n=5 animals/group) were measured 3 min after completion of infusion. *Indicates a significant difference from pre-treatment at $p<0.05$.

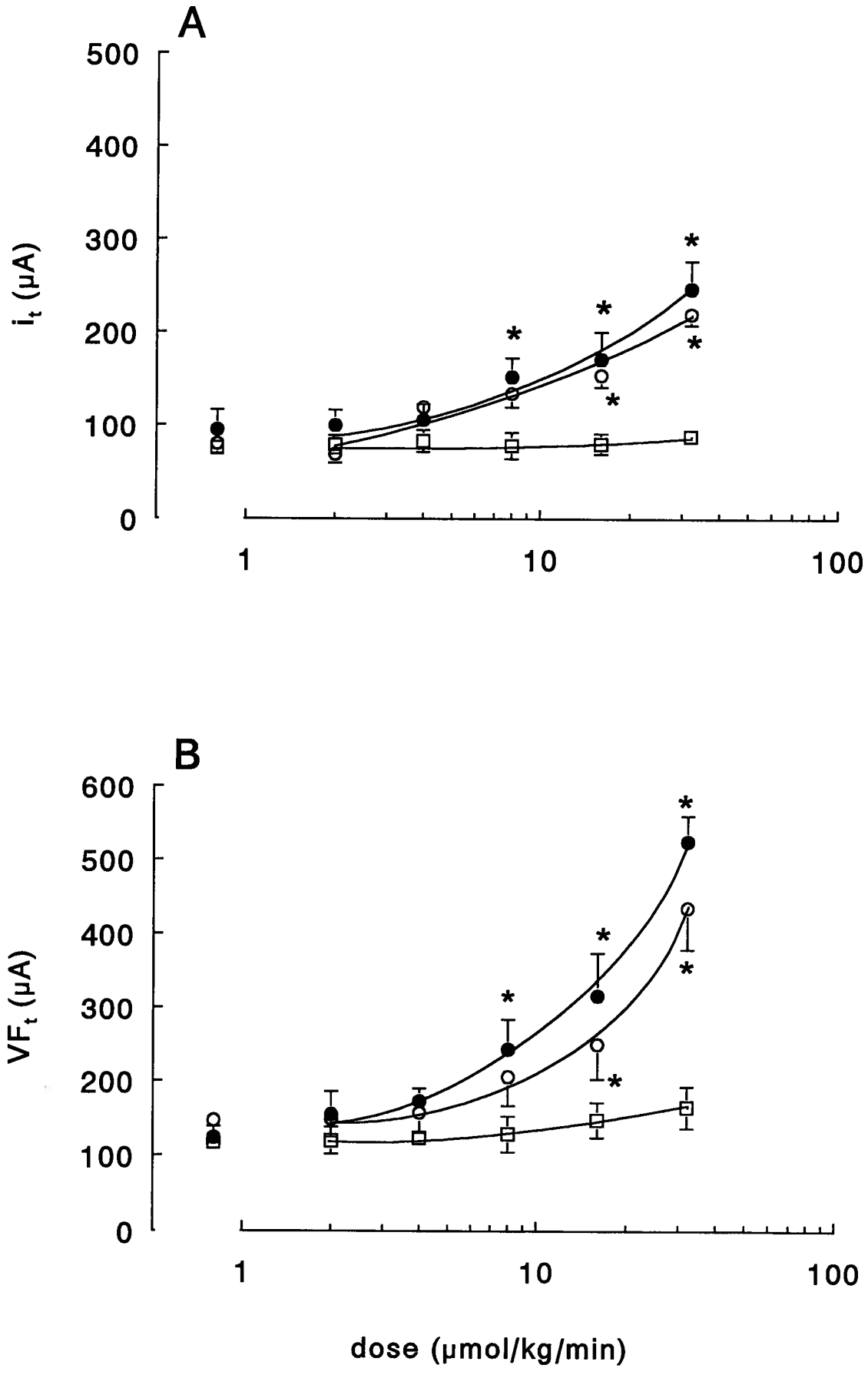
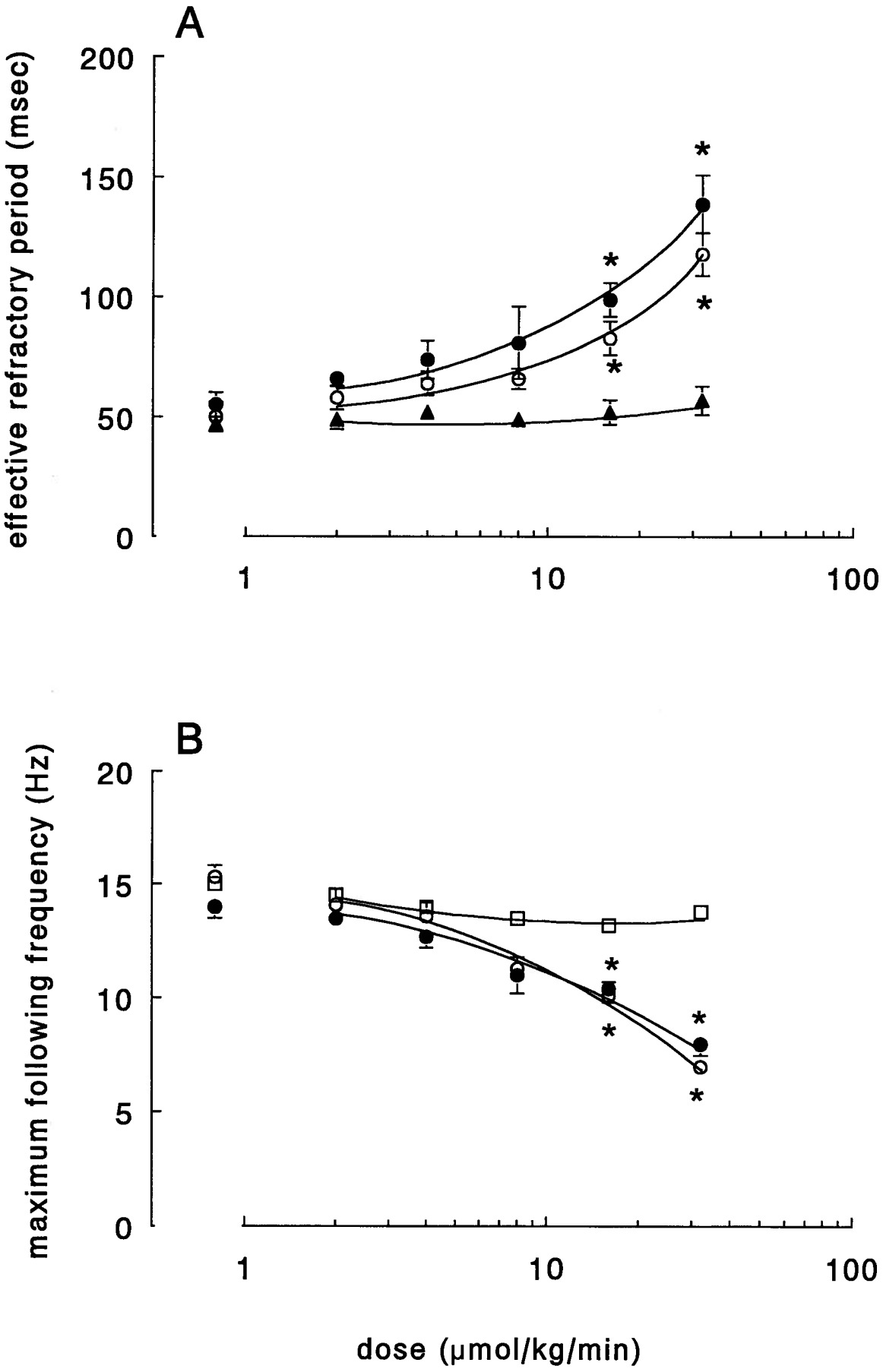

A
500
400
300
200
100
0
i_t (μA)
1
10
100
B
600
500
400
300
200
100
0
VF_t (μA)
1
10
100
dose (μmol/kg/min)

Figure 8 Effects of the saline vehicle (□), and spiradoline in the absence (O) and presence of 2.5 μmol/kg/min naloxone (●) on the effective refractory period (ERP) (A) and maximum following frequency (MFF) (B) in pentobarbitone-anaesthetized rats. For the saline vehicle (□) group, no change resulted over the duration of the experiment. Values are mean±s.e.mean for n=5 animals/group. *Indicates a significant difference from pre-treatment at $p<0.05$.


A
effective refractory period (msec)
200
150
100
50
0
1
10
100
B
maximum following frequency (Hz)
20
15
10
5
0
1
10
100
dose (µmol/kg/min)

The effective refractory period, ERP, a direct measure of refractoriness was dose-dependently prolonged by spiradoline with and without naloxone pre-treatment (Figure 8A). Maximum following frequency (MFF) was significantly decreased by spiradoline (with, or without, naloxone) at doses greater than 8.0 µmol/kg/min (Figure 8B). Spiradoline (32µmol/kg/min) reduced MFF from 15.3±0.5 Hz to 7.0±0.4 Hz in control animals and from 14.1±0.6 to 6.0±0.5 Hz in the presence of naloxone. The vehicle control values for thresholds (i_t and VF_t) and refractoriness (MFF and ERP) were stable over the treatment period.

3.1.4 Coronary Artery Occlusion studies

In the coronary occlusion-arrhythmia study a dose of spiradoline (2.5 µmol/kg/min) was chosen which produced changes in haemodynamic and ECG measures in the absence and presence of naloxone as was seen *in vivo* (Table 1). None of the changes induced by spiradoline were prevented by naloxone pre-treatment.

Spiradoline (2.5 µmol/kg/min) statistically significantly reduced arrhythmias induced by coronary occlusion (Table 2). Both ventricular tachycardia and fibrillation incidence were reduced as exemplified by an arrhythmia score of 2.4±0.4 in the spiradoline treated group compared to 6.3±0.4 in saline controls (n=9 rats/group).

Naloxone had no antiarrhythmic effect when administered alone. However, VF was abolished when naloxone was given with 2.5 µmol/kg/min spiradoline.

The reduction in arrhythmia incidence could not be ascribed to either occlusion zone size (zone-at-risk) or serum potassium levels. Table III shows that there were no significant differences between group occluded zone sizes, hence the arrhythmic insults were assumed to be the same in all groups. Similarly, serum potassium levels were not influenced by drug treatment. The post-occlusion serum potassium levels were only

Table I The cardiovascular and ECG effects of 2.5 µmol/kg/min spiradoline, in the absence and presence of 2.5 µmol/kg/min naloxone in pentobarbitone-anaesthetised rats subject to acute coronary artery occlusion.

Dose (µmol/kg/min)	BP	HR	P-R	QRS	RSh	Q-aT
saline	128±4	383±17	55±1	26±1	0.52±0.04	34±1
naloxone (2.5)	116±6	361±12	58±1	29±1	0.55±0.05	35±2
spiradoline (2.5)	91±6*	298±13*	61±2*	30±2*	0.62±0.05*	38±1
spiradoline(2.5) + naloxone (2.5)	85±4*	289±11*	62±1*	30±1*	0.68±0.03*	40±2*

The effects of spiradoline alone, or in the presence of naloxone (2.5 µmol/kg/min), are expressed as mean±s.e.mean (n=9) for the variable indicated. BP = mean arterial blood pressure in mmHg; HR = heart rate in beats/min, and the P-R, QRS and Q-aT are ECG intervals in msec while RSh is in mV. *Indicates $P<0.05$ for comparison with saline.

Table II Antiarrhythmic effect of spiradoline (2.5 µmol/kg/min) in the absence and presence of 2.5 µmol/kg/min naloxone against coronary artery occlusion-induced arrhythmias in pentobarbitone-anaesthetized rats.

		Group Incidence			
Drug (µmol/kg/min)	log VPB	VT	VF	VT and /or VF	A. S.
Saline	1.9±0.2	9/9	9/9	9/9	6.3±0.4
Naloxone (2.5)	1.8±0.1	8/9	8/9	8/9	5.1±0.5
Spiradoline (2.5)	1.7±0.1	3/9*	2/9*	3/9*	2.4±0.4*
Spiradoline (2.5) + Naloxone (2.5)	1.7±0.3	4/9	0/9*	4/9	2.2±0.4*

The antiarrhythmic actions of spiradoline, either in the absence or presence of naloxone (2.5 µmol/kg/min) are expressed in terms of group incidence of one or more episodes of the major arrhythmias of ventricular tachycardia (VT) or ventricular fibrillation (VF). Ventricular premature beats (VPB) were $\log_{10}$ transformed for normalization. Arrhythmia score (A.S.), which summarizes and grades arrhythmic incidence and severity, was expressed as mean±s.e.mean (n=9). *$P<0.05$ when compared with saline.

slightly elevated (no significant difference between treatment groups) however, this did not play a role in reducing arrhythmia incidence.

The time to S-T segment elevation and R-wave maximum were prolonged after spiradoline treatment, with or without naloxone pre-treatment. Although spiradoline delayed the time for the S-T segment to reach maximum elevation and R-wave maximum, it did not reduce the maxima. Naloxone itself slowed the time to development of an R-wave maximum, indicative of an antiarrhythmic effect. Mortality was abolished in the spiradoline groups regardless of naloxone pre-administration.

Table III The actions of 2.5 µmol/kg/min spiradoline, in the absence and presence of naloxone (2.5 µmol/kg/min), on ECG, mortality and serum potassium changes induced by coronary artery occlusion in pentobarbitone-anaesthetized rats.

	Time to				***Serum K+***			
Dose (µmol/kg/min)	ST-seg. max. (min)	R-wave max. (sec)	S-T max. (%)	R-max (mV)	Pre-drug (mM)	Post-drug (mM)	OZ size (%)	Mortality
Saline	17±3	22±2	81±5	1.3±.3	3.8±0.3	4.4±0.2	38±2	5/9
Nal (2.5)	18±2	30±5*	74±7	1.6±.5	3.6±0.5	4.0±0.2	37±1	3/9
Spir(2.5)	25±2*	48±4*	89±4	1.2±.2	3.7±0.2	4.2±0.3	39±1	0/9
Spir + Nal	24±4*	42±6*	70±9	1.7±.4	3.3±0.4	3.8±0.4	35±2	0/9

The effects of spiradoline (spir) alone, or in the presence of naloxone (nal) (2.5µmol/kg/min), are expressed as mean±s.e.mean for n=9 (except for post-drug serum potassium levels which correspond to the group size remaining at the end of the experiment as indicated in the mortality column) for the variable indicated. *Indicates significant difference from saline at $p<0.05$. S-T max. is the maximum height of the S-T segment expressed as a % of the height of the R-wave.

3.1.5 Electrophysiological actions on Sodium Currents

3.1.5.1 Concentration-response curves

Spiradoline produced a concentration-dependent reduction in the magnitude of the sodium current (Figure 9A, B) evoked in isolated rat myocytes at concentrations which produced marked effects on isolated hearts (as shown in Figures 2A, B and 3A, B). Figure 9A shows data obtained from an experiment in which sodium currents were evoked

by voltage steps to 0 mV from a pre-pulse potential of -150 mV. These voltage steps were given at 6sec intervals and the currents generated were plotted as a function of time (on a time scale of 10 msec). Spiradoline (15-100 μM) was added to the bath solution and produced a concentration-dependent readily-reversible block of sodium current. These concentration-response experiments were repeated in 4 additional cells and similar dose-response relationships were obtained. Figure 9B, describes the concentration-response data (n=5 cells) for the block of sodium current by spiradoline. An estimate of the half-maximal sodium current block (EC_{50}) from this data (using the best fits of the equation $I_{Na}=1/[1+(K_A/A)]$ was 66 μM. In the presence of 1 μM naloxone there was no change in the concentration-response curve (data not shown).

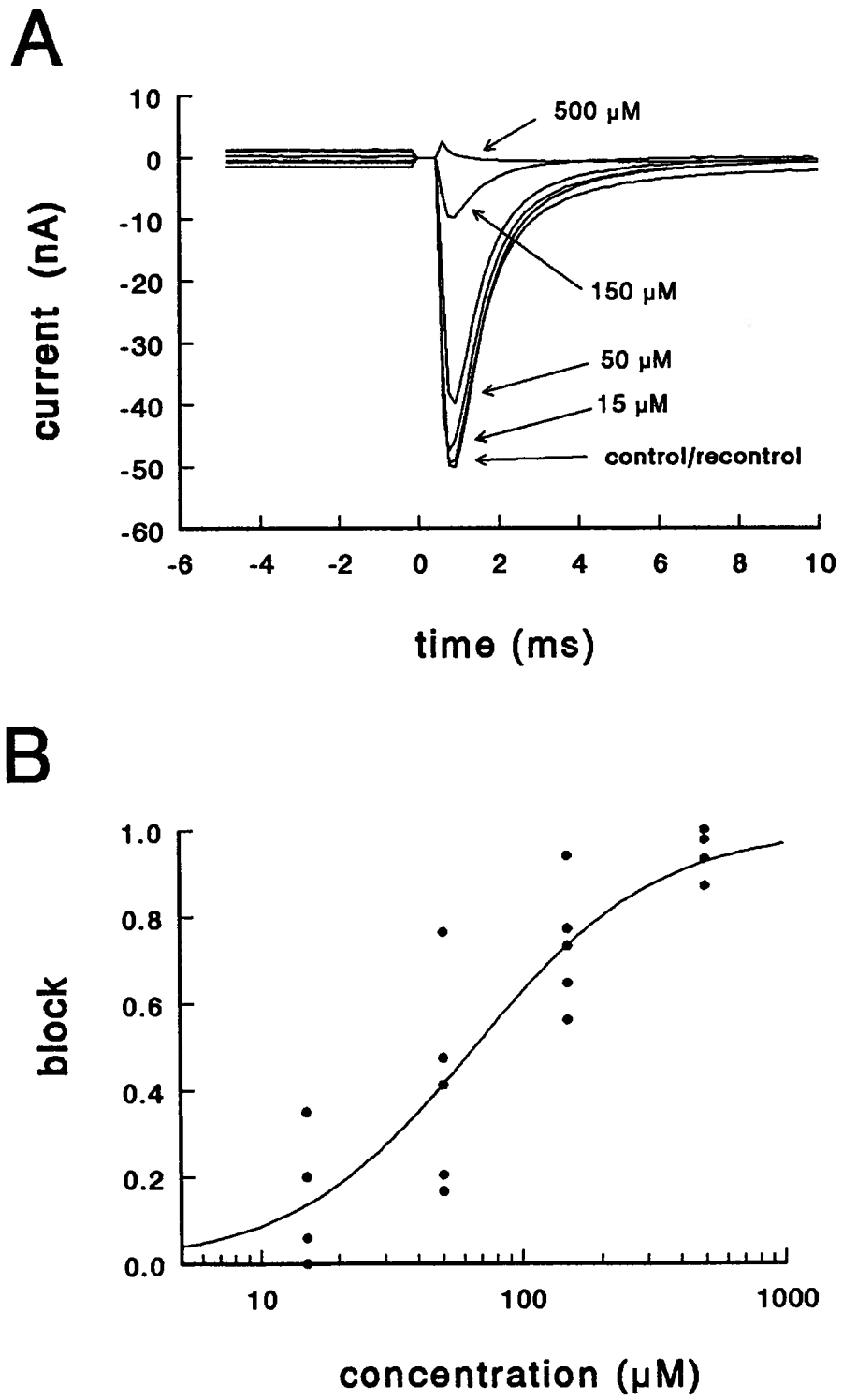
3.1.5.2 Current-Voltage effects

3.1.5.2.1 Activation kinetics

Spiradoline block of sodium currents was examined by determining whether or not it produced a change in the voltage-dependence of activation or inactivation (h_∞) (n=6 cells per study). The effect of 150 μM spiradoline was examined on the voltage-dependence of activation of the sodium current. A voltage-step to various test potentials between -70 mV and +50 mV was given from a fixed pre-pulse potential of -150 mV. The peak current amplitude of I_{Na} is shown in Figure 10A plotted against the pre-pulse potential. To test for the effects of the drug on activation kinetics we defined the peak sodium conductance (G_{Na}) versus the reversal potential (E_{rev}) over the potential range of -70 mV to +60 mV. Conductance (G_{Na}) was calculated using the Hodgkin-Huxley model [$I_{Na}=g_{Na}\times(V-E_{rev})$] and approximated by a Boltzmann equation fit producing the relationship $I_{Na}=\{G_{max}/1+\exp[(V-V')/k]\}\times(V-E_{rev})$ where G_{max} is the maximal channel conductance for sodium, V' is the voltage at which G_{Na} is half-maximal, k is the slope factor, and E_{rev} is the

Figure 9. Effect of spiradoline block on sodium currents in rat cardiac myocytes. Panel A shows sodium currents evoked by a voltage-step from a pre-pulse potential of -150 mV to a potential of 0 mV. The voltage step was delivered at 6 sec intervals and spiradoline was added to the bath solution at the concentrations indicated. The re-control current is indistinguishable from control. Spiradoline was added to the bath solution for 2 min before evoking currents at the concentrations examined. Similar results were obtained in 4 additional cells.

Panel B shows the concentration-response curve for the degree of blockade of the transient sodium current by spiradoline. Sodium currents were evoked as above. The degree of block, $I_{(control)}-I_{(block)}/I_{(control)}$, is shown as a function of $\log_{10}$ concentration of spiradoline. Data from five individual cells is plotted with the line of best fit for the equation $y=1/(1+(K_a/[A]))$ shown.


A
10
0
-10
-20
-30
-40
-50
-60
current (nA)
500 µM
150 µM
50 µM
15 µM
control/recontrol
-6
-4
-2
0
2
4
6
8
10
time (ms)
B
1.0
0.8
0.6
0.4
0.2
0.0
block
10
100
1000
concentration (µM)

reversal potential for sodium. For the rat ventricular myocyte shown in Figure 10A, the peak G_{max} was 1.49 nS, with a V' of -42 mV, a slope factor (k) of 3.0 mV per e-fold change in I_{Na} and a reversal potential (E_{rev}) of 47 mV. After exposure to 150 μM spiradoline the values were 0.22 nS, -42 mV, 3.0 mV per e-fold change in I_{Na} and E_{rev} of 47 mV. Spiradoline only reduced I_{Na} by decreasing G_{max} and did not produce a change in the voltage-dependence of threshold activation of the current.

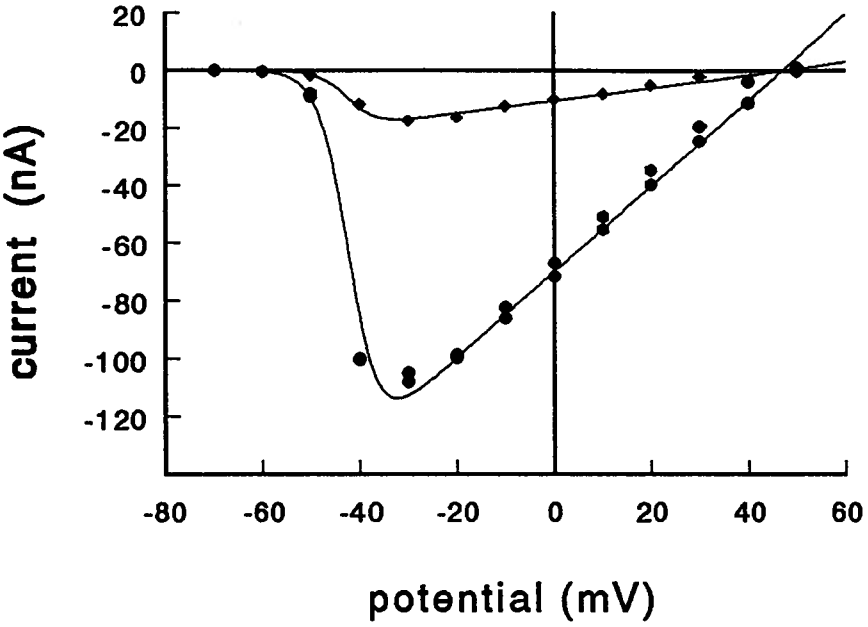
3.1.5.2.2 Inactivation kinetics

The effects of spiradoline on inactivation kinetics were studied by giving a voltage-step to -20 mV from pre-pulse potentials which varied between -140 mV and -30 mV. Spiradoline produced channel blockade by changing the steady-state voltage-dependent inactivation kinetics of the current as shown in Figure 10B. A Boltzmann equation ($I_{Na}=I_{max}/[1+\exp(V-V')/k]$) where I_{Na} is the maximal sodium current, V' is the voltage at which I_{Na} is half-maximal and k is the slope factor (indicating the steepness of the slope or voltage dependence of inactivation) was used to derive a curve for the data obtained under control conditions and during the application of 150 μM spiradoline. In the presence of 150 μM spiradoline a substantial block of the maximum available current was accompanied by a hyperpolarizing shift in the voltage-dependence of I_{Na} by 21±2.9 mV and 24±2.2 mV in the presence of 50 and 150 μM drug concentrations (n=6 cells each, data for 50 μM concentrations not shown). This shift is only revealed when the curve in the presence of spiradoline is scaled to the control/re-control maximum. As well, 150 μM spiradoline increased the slope factor, k , from 7 mV in control to 8.5 mV per e-fold change in I_{Na} after drug exposure. However, from the curves, it can be seen that the maximal current amplitude is greatly reduced with 150 μM spiradoline, even at very negative values of the pre-pulse potential, which indicates that the shift in the voltage-

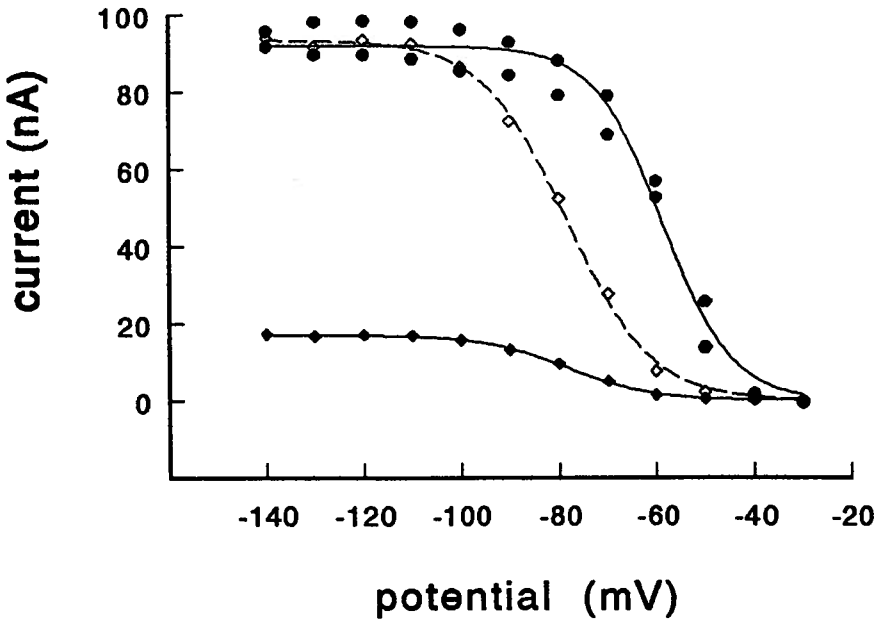
Figure 10 Effect of spiradoline on the current-voltage relationship and inactivation kinetics (h_∞) of sodium currents. In panel A the current-voltage relationship for activation of sodium currents is shown. Currents were evoked by a voltage pulse to potentials of -70 to +50 mV from a potential of -150 mV. Peak current amplitude is plotted against potential for control data (●) and in the presence of 150 μM spiradoline (♦). Conductance (G_{Na}) was calculated from the Boltzmann equation $I_{Na}=\{G_{max}/\{1+\exp[(V-V'/k]\}\times(V-E_{rev})$ where G_{max} is maximal channel conductance for sodium, V' is the voltage at which I_{Na} is half-maximal, k the slope factor, and E_{rev} is reversal potential for sodium. In control and re-control, G_{max} was 1.49 nS, with a V' of -42 mV, a slope factor (k) of 3.0mV per e-fold change in I_{Na} and a reversal potential (E_{rev}) of 47 mV. In the presence of 150μM spiradoline (♦) G_{max} was 0.22 nS, V' was -42 mV, k was 3.0 mV per e-fold change in I_{Na} and E_{rev} was 47 mV. The only difference seen in the cell was a reduction in maximum channel conductance, G_{max}.

In panel B sodium currents were evoked by a voltage step to -20 mV from a pre-pulse potential which varied between -140 and -30 mV. The peak current amplitude is shown plotted against the pre-pulse potential for control and re-control data and in the presence of 150 μM spiradoline. The data is shown by the best-fit line(s) using the Boltzmann equation $y=G_{max}/1+\exp[(V-V')/k)]$ where V is the pre-pulse potential, V' is the half-maximal voltage for inactivation and k is a slope factor. The best fits for the line were obtained for values of G_{max}=0.92 nS, V'=-58.6 mV and k=7.0 mV per e-fold change in I_{Na} for the control solid line curve (●). A curve was obtained in the presence of 150 μM spiradoline (♦). The dotted line curve (◊) shows the data in the presence of spiradoline scaled to the same maximum as control/re-control data. This curve resulted in values of G_{max}=0.17 nS, V'=-78.2 mV and k=8.5 mV per e-fold shift in I_{Na}.

A


20
0
-20
-40
-60
-80
-100
-120
current (nA)
-80
-60
-40
-20
0
20
40
60
potential (mV)

B


100
80
60
40
20
0
current (nA)
-140
-120
-100
-80
-60
-40
-20
potential (mV)

dependence of inactivation may not be the principal means by which current block is produced.

3.1.5.3 Tonic and use-dependent components of spiradoline block of sodium currents.

To determine whether or not spiradoline possesses use-dependent blocking properties, sodium currents were evoked by 10msec voltage-steps at various frequencies. Figure 11 is a record of one sec of evoked sodium current. Spiradoline (50 μM) produced a slowly-developing steady state use-dependent block. Peak current was reduced from 10.2, 10.0 and 10.1 nA (measured as the initial peak control current in panels a, b, c, of Figure 11, respectively) to 6.92, 6.0 and 3.6 nA (measured as the last evoked current in the trace for panels a, b, c, respectively) at (a) 6, (b) 13 and (c) 30 Hz, respectively. The average peak amplitude in (d), control current at 25 Hz, was 10.4±0.2 nA.

Figure 12A depicts the frequency-dependence of block by 150 μM spiradoline. Sodium currents were evoked by a voltage step to 0 mV from a pre-pulse potential of -150 mV. The voltage steps were delivered as frequency trains of 0.7, 3.3, 6.6 and 16 Hz (n=5 cells) with 3 min of recovery time between trains. The bathing solution contained 50 μM spiradoline and cells were exposed to the drug for 2 min before providing pulse trains. The peak current (normalized to the first current evoked) is shown plotted against the time at which it was evoked, for each of the frequencies. In control solution no reduction in peak current was seen at frequencies up to 40 Hz (data not shown, see Figure 11d for 25 Hz representation). The time to development and maximal amount of use-dependent block were increased as the rate of stimulation increased.

Since it has been shown that the rate of block development by antiarrhythmic drugs is strongly concentration-dependent, it was determined whether or not this was true for spiradoline. A plot of peak sodium current amplitude and the number of pulses applied (Figure 12 B) was used to explore the concentration-dependency of rate of

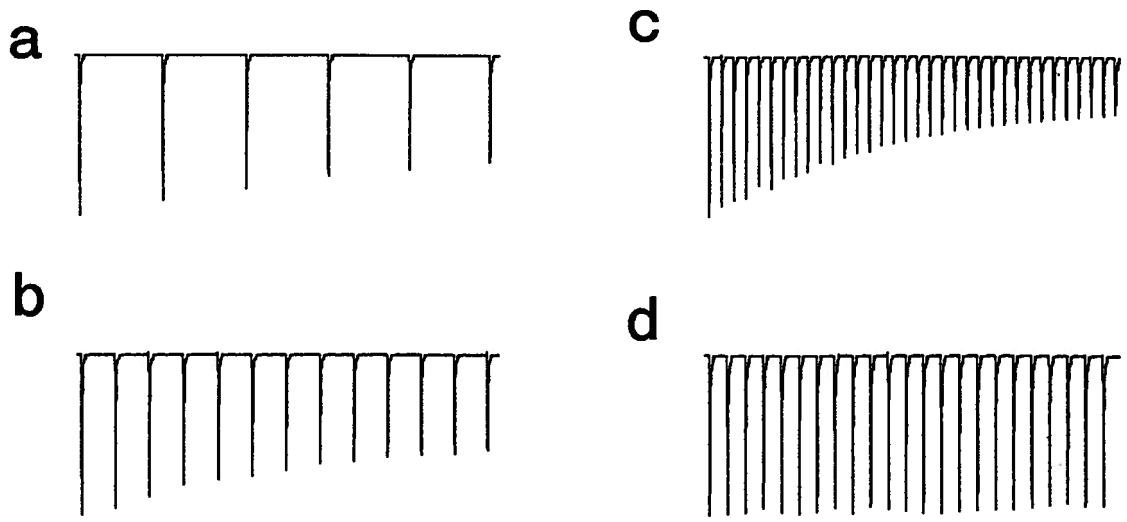


Figure 11 Depicts the frequency-dependent block of the sodium current by spiradoline on a one sec time scale. Sodium currents were evoked by a voltage step to 0 mV for 10 msec from a pre-pulse potential of -150 mV at various frequencies. The traces are representative of a one sec train of such stimuli at (a) 6 Hz, (b) 13 Hz and (c) 30 Hz all in the presence of 50 μM spiradoline. Cells were exposed to the drug for 2 min before delivering frequency pulse trains. For comparison, the one sec train of stimuli shown in trace (d) is the evoked sodium current at 25 Hz in control. Results were obtained in 5 additional cells.

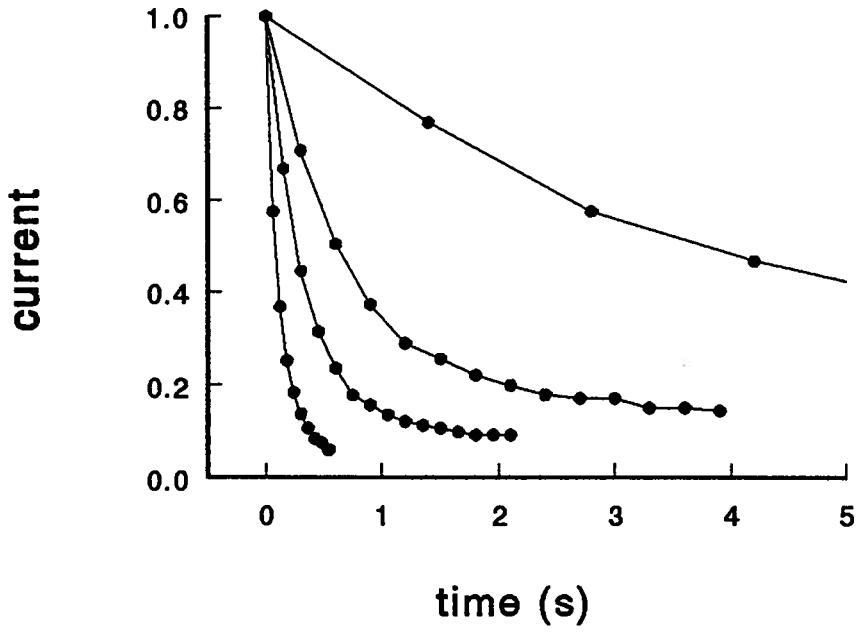
development of use-dependent block and whether a tonic component of drug block exists. A train of depolarizing pulses of 10 msec duration to 0 mV from a pre-pulse potential of -150 mV were given at a stimulation frequency of 10 Hz. In control solutions no noticeable reduction of current with successive pulses was seen (data not shown, see Figure 11d for 25 Hz representation). Spiradoline concentrations were bath applied and trains evoked 2 min after application. Spiradoline block was not fully complete at either of the low concentrations (15 and 50 μM) examined since a steady-state block of current did not result even after 12 pulses (1.0 sec) were evoked, when delivered at 10 Hz. However, fully developed use-dependent block occurred with 150 μM spiradoline after only 7 pulses delivered at this same rate. Although not shown, 500 μM spiradoline blocked all current (Figure 9A) after only 5 pulses.

Although not studied directly several observable effects of current behaviour in the presence of spiradoline suggest that the drug may, in addition to the use-dependent component discussed above, produce a reduction in current by tonic block. The first suggestion of such a block is seen in Figure 10B. Sodium channel block by sspiradoline arises at low rates of stimulation (one sec between evoked current pulses) and at very negative potentials where the channels tend to exist in the resting state (according to Hodgkin and Huxley formalism). The average reduction in peak current was 69±16% in rat cardiac myocytes exposed to 150 μM spiradoline (n=6) over the potential range of -140 to -100 mV (Figure 10B). As well, tonic block can be seen in Figure 12B where spiradoline produced a concentration-dependent reduction of peak current when the amplitude of the second pulse generated is compared to the first (or control pulse number 1) current. Thus, in these experiments, if the amplitude of the second evoked sodium current is compared to the first (control) evoked current, spiradoline cann be seen to reduce the peak current by 2.2, 8.4 and 48% at 15, 50 and 150 μM, respectively, before the onset of use-dependent block.

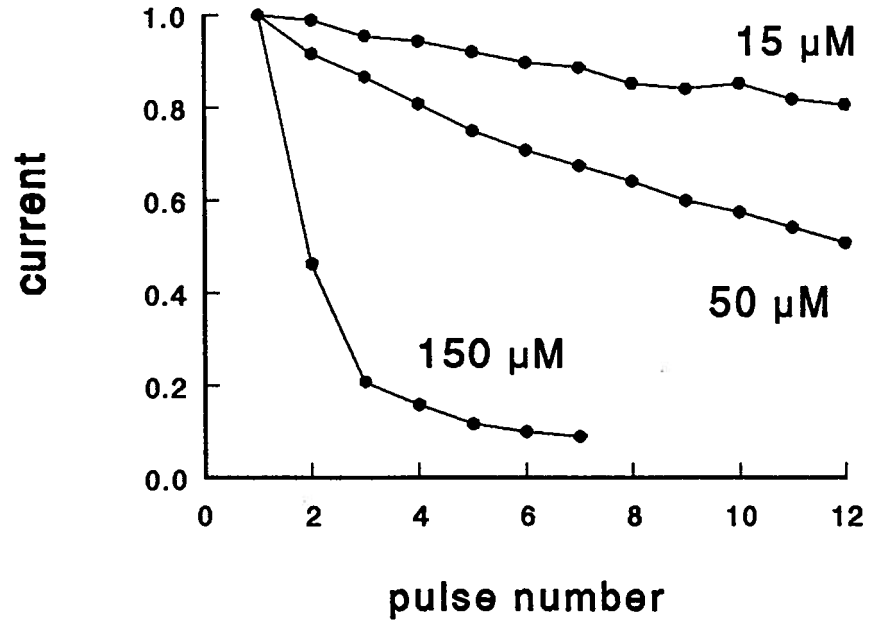
Figure 12 Panel A depicts the frequency-dependence of block by spiradoline. Sodium currents were evoked by a voltage step to 0 mV from a pre-pulse potential of -150 mV at 0.7 Hz, 3.3 Hz, 6.6 Hz and 16 Hz. The bathing solution contained 50 μM spiradoline and cells were exposed to the drug for 2 min before delivering pulses. The peak current (normalized to the first record) is shown plotted against the time at which it was evoked, for each of the frequencies tested. In control solution no reduction in peak current was seen at frequencies up to 40 Hz (data not shown, see Figure 11d for 25 Hz representation).

Panel B shows the relationship between peak sodium current amplitude and the number of pulses applied. A train of depolarizing pulses of 10 msec duration were used. Sodium currents were evoked by depolarizing to 0 mV from a pre-pulse potential of -150 mV at a stimulation frequency of 10 Hz. In control solution no noticeable reduction of current with successive pulses was seen (data not shown, see Figure 11d for 25 Hz representation). Spiradoline concentrations (15-50 μM) were added and trains given 2 min after application.

A


1.0
0.8
0.6
0.4
0.2
0.0
current
0
1
2
3
4
5
time (s)

B


1.0
0.8
0.6
0.4
0.2
0.0
current
15 µM
50 µM
150 µM
0
2
4
6
8
10
12
pulse number

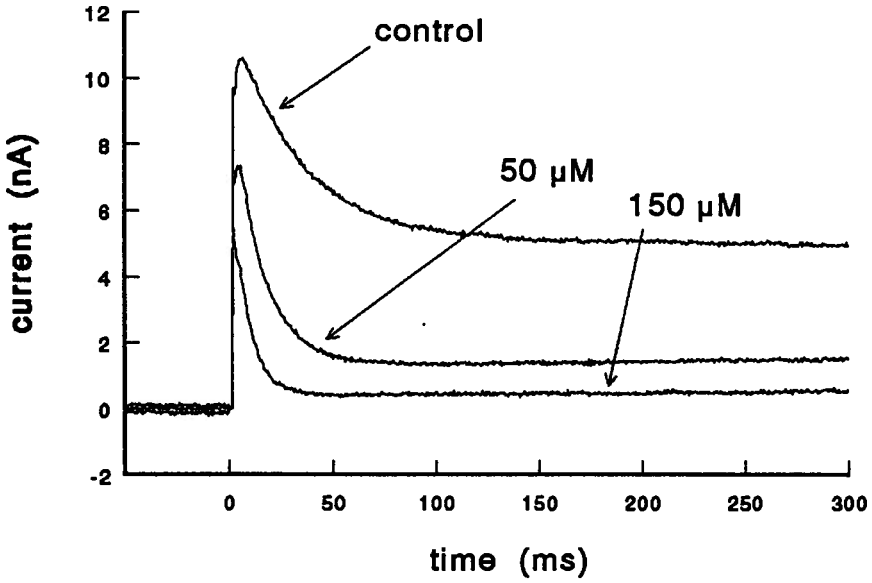
3.1.6 Electrophysiological actions on Potassium currents

3.1.6.1 Transient Outward and Sustained Delayed-Rectifier Potassium Currents

In addition to the above effects of spiradoline on sodium currents we examined the effects on potassium currents in isolated myocytes (Figure 13). The concentration-dependent effect of bath applied spiradoline (50 and 150 μM) was examined on the transient outward (i_{to}) and sustained outward plateau (i_{Ksus}) potassium currents evoked by depolarization to +50 mV from a pre-pulse potential of -150 mV for a duration of 300 msec. The potassium current amplitude was measured at the peak (approximately 5 msec after evoking the outward current). This was obtained by subtraction of the current magnitude remaining at 300 msec from the peak amplitude. After 300 msec complete inactivation of i_{to} occurs at which time the sustained outward current trace remains. Spiradoline produced a concentration-dependent block of i_{Ksus} which was reduced from a control current of 4.9 to 1.5 and 0.5 nA after 50 and 150 μM spiradoline, respectively. The peak component of i_{to} was reduced by spiradoline. In addition, spiradoline increased the rate of decay of i_{to}. Construction of a line tangential to the decay curve of i_{to} allowed for an approximation of the drug effect on the rate of decay of this current. We assumed a monoexponential decay of evoked outward potassium current. In Figure 13 the control rate of decay of i_{to} is approximately 0.115 nA/msec while at a concentration of 50 μM the rate of decay increases three-fold to 0.35 nA/msec and then only marginally increases to 0.38 nA/msec with 150 μM spiradoline. The acceleration of inactivation induced by spiradoline was such that i_{to} inactivation was almost complete within 50 msec as compared with 100 msec in control solution (Figure 13). Similar results were obtained in 4 additional cells.

3.2 Studies with (±)PD117,302 and (+)PD123,497 (not previously reported)

Figure 13. Effects of spiradoline (50 and 150 μM) on depolarization-induced transient outward (i_{to}) and sustained outward plateau (i_{Ksus}) potassium currents. Currents were elicited by depolarization to +50 mV from a pre-pulse potential of -150 mV for 300 msec. The concentrations of spiradoline were applied in the presence of 20 μM TTX and currents evoked 2 min later. The sustained outward current remained after complete inactivation of i_{to}. The potassium current amplitude was measured at the peak and after 300 msec, at which time the sustained outward current trace was essentially flat. Note, however, that this sustained component remains essentially unchanged at the end of the pulse even with prolonged depolarization. The re-control current, after 2 min wash, had a similar time course to control (data not shown for clarity). Similar results were obtained in 4 additional cells.


control
50 µM
150 µM
current (nA)
time (ms)
12
10
8
6
4
2
0
-2
0
50
100
150
200
250
300

3.2.1 Electrophysiological actions on Sodium currents

3.2.1.1 Concentration-response curves

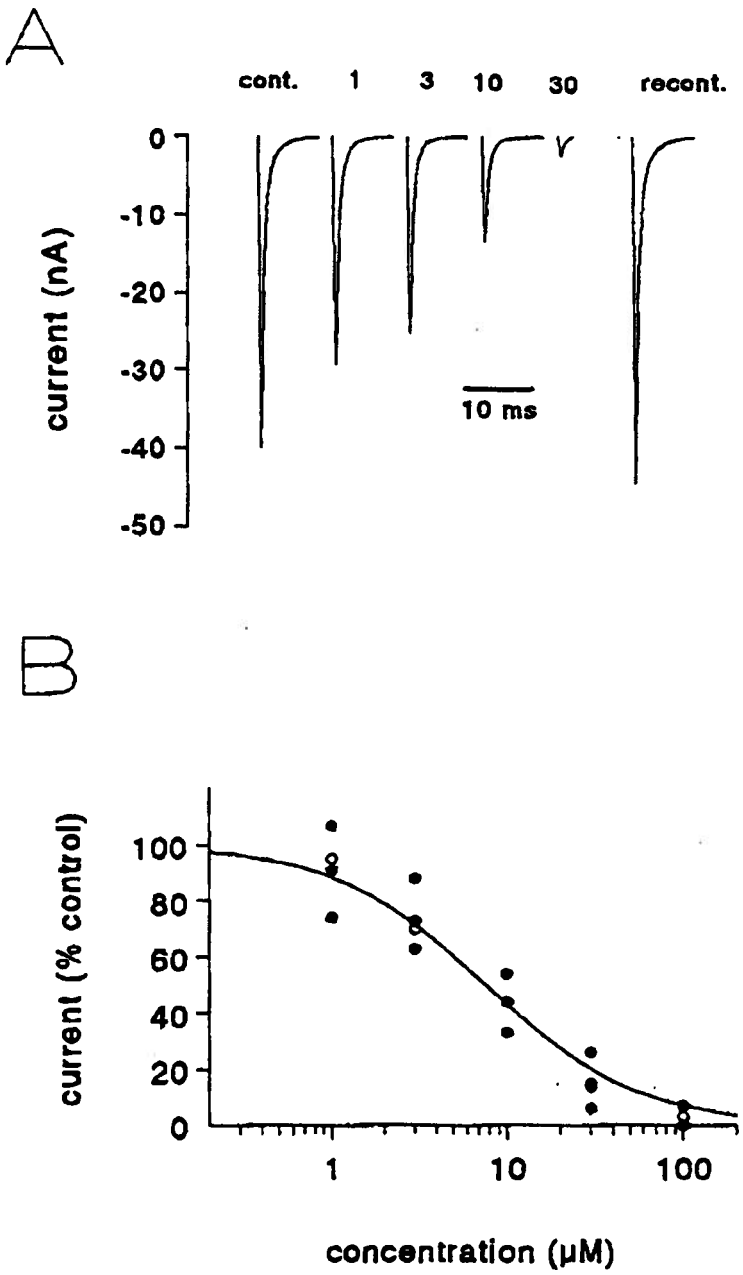
Both the racemic arylacetamide (±)PD117,302 (Figure 14A) and its inactive κ enantiomer (+)PD123,497 (Figure 15A), produced concentration-dependent reductions in the magnitude of sodium currents in isolated rat myocytes. Figure 14A shows data obtained from an experiment in which sodium currents were evoked by a voltage step to 0 mV from a pre-pulse potential of -150 mV. These voltage steps were given at 6 sec intervals. For the evoked sodium currents of 10 msec duration a concentration-response curve was constructed. Either (±)PD117,302 or (+)PD123,497 was applied for 2 min prior to generation of the current over the concentration range of 1-30 μM and 1.3-40 μM, respectively. At the highest concentrations applied both drugs abolished evoked sodium currents. Re-control currents were obtained after 3 min wash. In Figure 14B these concentration-response experiments were repeated in 2 additional cells and a similar dose-response relation was obtained for each cell. In these experiments an additional dose of 100 μM (±)PD117,302 was examined. An estimate of the half-maximal sodium current block (EC_{50}) expressed as % control, was determined to be 8.0μM using the best fit of the equation $1-y=1-1/[1+(K_A/A)]$. For the inactive enantiomer a similar curve resulted in an EC_{50} of 3.0 μM (concentration-response curve not shown). In the presence of 1 μM naloxone there was no change in the concentration-response curve for (±)PD117,302 (Figure 14B).

3.2.1.2 Intracellular vs. extracellular locus of action.

These studies determined the putative site on the sodium channel at which arylacetamide compounds may exert channel blocking actions. In order to examine the

Figure 14 Effect of (±)PD117,302 on sodium currents in rat cardiac myocytes. Panel A shows sodium currents evoked by a voltage-step from a pre-pulse potential of -150 mV to a potential of 0 mV. The voltage step was delivered at 6 sec intervals and (±) PD117,302 was added to the bath solution at the concentrations indicated. The re-control (recont.) current is indistinguishable from the control. (±)PD117,302 was added to the bath solution for 2 min before evoking currents at any of the concentrations examined. Similar results were obtained in 2 additional cells.

Panel B describes the concentration-response curve for the degree of blockade (expressed as a percent control) of the transient sodium current by (±)PD117,302. Sodium currents were evoked as above. The degree of block is shown as a function of the $\log_{10}$ concentration of (±)PD117,302. Data from 3 individual cells is plotted with the line of best fit for the equation $1-y=1-\{1/[1+(K_a/[A])]\}$ shown as a % of control. The estimated K_a from this equation is 8 μM for (±)PD117,302. The effects of 1 μM naloxone (open circles) were also examined in the presence of spiradoline.


A
cont.
1
3
10
30
recont.
0
-10
-20
-30
-40
-50
current (nA)
10 ms
B
current (% control)
100
80
60
40
20
0
1
10
100
concentration (μM)

intracellular actions of these arylacetamides either (±)PD117,302 or (+)PD123,497 were added in the patching pipette at concentrations of 3 μM or 150 μM, respectively.

Figure 15 shows the effects of 30 μM (±)PD117,302 applied intracellularly. (±) PD117,302 showed no significant reduction in sodium currents after 5 min of exposure of drug to the cell interior (as in current a of Figure 15). When a 3 μM concentration of (±) PD117,302 was bath applied the peak sodium current was reduced by 23% (from a control of 45 nA control peak to 34 nA after drug exposure) as is seen in current trace b in Figure 15. Two min washing readily-reversed the block (see current trace c in Figure 15).

Figure 16A shows the bath-applied concentration-dependent block of the sodium current. In Figure 16B the effects of 150 μM (+)PD123,497 were examined intracellularly. Peak sodium currents were evoked every 3 sec beginning 4 min after patch rupture and exposure of the cells interior to the pipette containing (+)PD123,497. After 4 min of exposure only a slight reduction (about 4 nA) in peak current amplitude was recorded. When the same concentration of (+)PD123,497 was added to the perfusate (marked by the horizontal bar) a rapid and readily reversible inhibition of sodium current was observed.

In another experiment we examined the effects of pH on sodium current block (Figure 16C). Current amplitudes in the pH=7.4 bath solution was approximately 48 nA (n=3 cells). In an acidic solution (pH 6.4) the amplitude was reduced to 42 nA (data not shown). When 13 μM (+)PD123,497 was added to the bath solution at pH 7.4 sodium current was reduced by 83% (from a control current of 49 nA to 8 nA after drug application) within 1.5 min. When the same concentration of (+)PD123,497 was added to the bath solution at pH 6.4 some reversal in current block was seen. After 1.5 min at the low pH approximately 22% of the current (or 10 nA) recovered from block. When 13 μM (+)PD123,497 in the pH=7.4 bath solution was immediately re-applied to the cells, the evoked sodium current was blocked to a similar degree as at pH 7.4, previously.

Figure 15 The intracellular effectiveness of (±)PD117,302 block of the sodium current was examined in rat cardiac myocytes. Sodium currents were evoked by a voltage-step to 0 mV from a pre-pulse potential of -150 mV, repeated at 6 sec intervals. (±)PD117,302 was added to the pipette solution at a concentration of 50 μM. Recording was begun 5 min after patch rupture and whole-cell patch-clamp configuration was attained. The 10 msec sample current taken at (a) indicated that relatively little current block was produced by 5.5 min with the intracellular application of (±)PD117,302 (maintained for the duration of the experiment as indicated by the dotted horizontal line). The current trace taken at time (b) indicated the reduction in peak current amplitude after the bath application of 3 μM (±)PD117,302. The time of application is seen as the solid horizontal bar. Current trace (c) is the re-control sodium current after 2.5 min of wash.

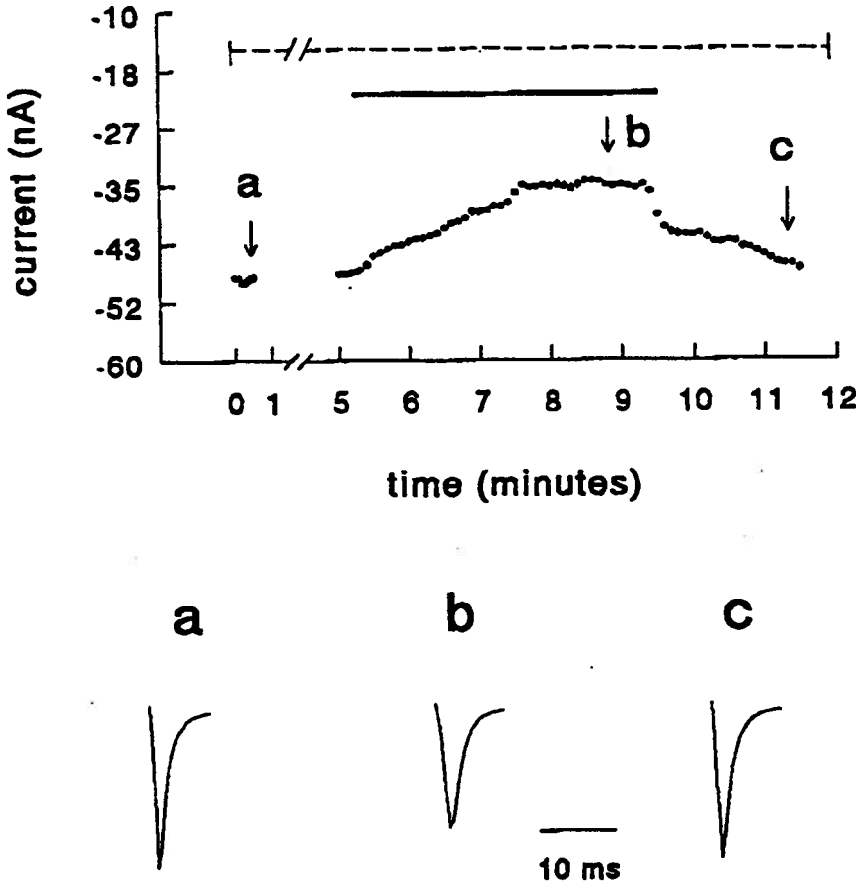
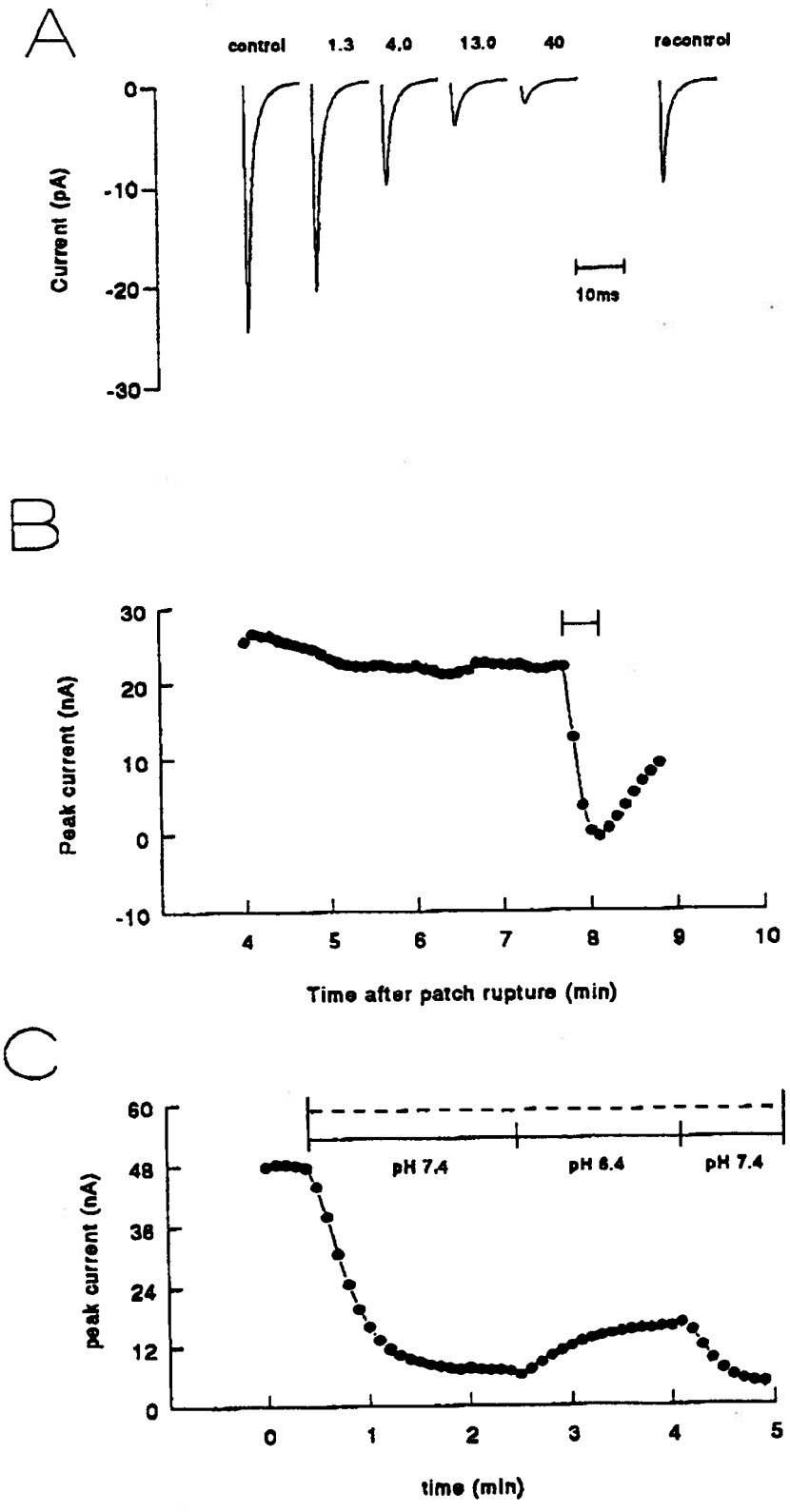

-10
-18
-27
-35
-43
-52
-60
current (nA)
a
b
c
0 1 5 6 7 8 9 10 11 12
time (minutes)
a
b
c
10 ms

Figure 16 Effects of (+)PD123,497 on sodium currents. Panel A shows concentration-dependent block of the sodium current in rat cardiac myocytes by (+)PD123,497 at the concentrations (in μM) indicated. Sodium currents were evoked at 6 sec intervals. (+)PD123,497 was added to the bath solution for 2 min before evoking currents at the concentrations examined. Re-control current shows partial recovery after 2 min wash

Panel B shows the effects of (+)PD123,497 (130μM) on sodium currents when applied to the inside of the cell via the pipette solution. Sodium currents were elicited 4 min after patch rupture. After 4 min 130 μM (+)PD123,497 was applied for 30 sec via the bath perfusate (as indicated by horizontal bar).

In panel C the effects of pH on block of sodium currrent by (+)PD123,497 are shown. A concentration of 13 μM (+)PD123,497 was added to the cell bath solution at a pH=7.4 and pH=6.4 and sodium currents evoked before and after drug in pH 7.4, a pH 6.4, followed by a return to pH 7.4. Time of drug application is shown by the solid horizontal bars.


A
control
1.3
4.0
13.0
40
recontrol
0
-10
-20
-30
Current (pA)
10ms
B
30
20
10
0
-10
Peak current (nA)
4
5
6
7
8
9
10
Time after patch rupture (min)
C
60
48
36
24
12
0
peak current (nA)
pH 7.4
pH 6.4
pH 7.4
0
1
2
3
4
5
time (min)

3.3 Summary of Results obtained in previous studies - manuscripts in Appendices

Studies for this thesis began with a characterization of the pharmacological properties for the arylacetamide, U-50,488H in rat isolated perfused hearts, and in anaesthetized rats at concentrations or doses greater than those required to produce κ-receptor mediated effects. Studies were conducted in the absence and presence of the opioid receptor antagonists naloxone (at either 1 μM or 8 μmol/kg) or Mr2266 (8 μmol/kg). Neither antagonist significantly reduced the cardiovascular actions of U-50,488H *in vivo* or *in vitro*.

U-50,488H dose-dependently reduced blood pressure and heart rate *in vivo* and decreased beating rate and left-ventricular peak systolic pressure *in vitro*. Over a concentration range *in vitro* of 1-30 μM and a dose-range *in vivo* of 0.5-32 μmol/kg, P-R, QRS and Q-aT intervals of the ECG were prolonged. Over the dose-range of 0.5-32 μmol/kg, U-50,488H had a biphasic action on thresholds for induction of VF. Thresholds were reduced at low doses (0.5-4 μmol/kg) but increased at higher doses (8-32 μmol/kg). Naloxone blocked the low dose effects. Both ERP, MFF and threshold pulse width increased with dose (Pugsley et al., 1992a).

The antiarrhythmic actions of a low and high dose of U-50,488H were examined in pentobarbitone-anaesthetized rats subjected to occlusion of the left coronary artery. A high dose (16 μmol/kg) reduced the incidence of VT from 100% in controls to 40% and VF from 67% in controls to 7%. The cardiovascular actions of U-50,488H were not antagonized by naloxone (8 μmol/kg). Naloxone alone may have ($p>0.05$) reduced arrhythmia incidence but to a much lesser extent than U-50,488H. The low dose of U-50,488H (0.2 μmol/kg), in the absence or presence of naloxone, did not affect arrhythmias (Pugsley et al., 1992b).

Another series of experiments examined the actions of U-50,488H on voltage activated sodium and potassium currents in isolated rat cardiac myocytes. U-50,488H

produced a concentration-dependent block of sodium current at an ED_{50} of 15 μM. At higher concentrations, (50 μM) blockade of the plateau potassium current (i_{Ksus}) and an increase in the rate of decay of the transient outward potassium current (i_{to}) was observed. At the EC_{50} for sodium channel blockade, U-50,488H produced a 15 mV hyperpolarizing shift in the inactivation curve (h_∞) for the sodium current. No changes in voltage dependence for activation were seen. U-50,488H had no effect on the voltage dependence of inactivation or activation of the potassium currents studied. The κ receptor antagonist Mr2266 (1 μM) did not influence sodium or potassium currents and did not change the current blocking properties of U-50,488H (Pugsley et al., 1994).

In an attempt to resolve the mechanisms by which κ agonists may be antiarrhythmic we examined the effects of (-)PD129,290, a potent κ agonist and its inactive enantiomer, (+)PD129,289. It was hoped that exploration of the pharmacological and antiarrhythmic profile of the two enantiomers may elucidate putative mechanism(s) by which structurally similar, but pharmacologically different, arylacetamide drugs exert antiarrhythmic actions. (-)PD129,290 and its corresponding (+) enantiomer, PD129,289, were studied in rat isolated hearts and in intact rats for their cardiovascular and antiarrhythmic actions. Using the whole-cell mode of the patch-clamp, the effects of (-) PD129,290 were examined on the sodium channel of cardiac myocytes.

In isolated rat hearts both arylacetamides, at 2-16 μM, dose-dependently reduced peak left ventricle systolic pressure, and heart rate and prolonged the P-R interval and QRS width of the ECG.

In intact rats 1-32 μmol/kg of both enantiomers reduced heart rate and blood pressure. The RSh, P-R, QRS and Q-aT intervals of the ECG were prolonged. In electrical stimulation studies thresholds for capture and VF were dose-dependently increased by both enantiomers. However, (-)PD129,290, the κ agonist, reduced thresholds at low doses (0.5-4 μmol/kg). The reduction in thresholds was blocked by naloxone (8 μmol/kg). Both enantiomers increased ERP. Naloxone did not affect any of the actions of

(-)PD129,290. When examined in rats subject to coronary artery occlusion, both enantiomers (at 2 and 8 μmol/kg) effectively reduced arrhythmia incidence. When (-) PD129,290 was examined in cardiac myocytes, a concentration of 20 μM produced a half-maximal block of the sodium current in the absence and presence of 1 μM naloxone. No effect on the voltage-dependence of activation or inactivation on this current was seen with this compound (Pugsley et al., 1993a).

Our studies show that κ receptor agonists and related compounds possess antiarrhythmic properties in the rat associated with blockade of cardiac sodium and potassium currents. Other studies suggest that drugs with mixed channel blocking properties may have potential therapeutic benefits (Hondeghem and Katzung, 1980) and the arylacetamides may possess these properties. A comparison was made between the κ agonist drugs, (-)PD129,290 and (±)PD117,302 and the inactive enantiomer of (-) PD129,290, (+)PD129,289 by using D_{25} values (potency measures). D_{25} values were estimated from dose-response curves for these compounds over the dose range 0.1-32 μmol/kg/min. D_{25} values for drug effects on blood pressure, heart rate and ECG measures are outlined in Table 1 of Appendix 5. The κ agonist compounds reduced blood pressure at a D_{25} of 0.5 μmol/kg/min while for the inactive enantiomer, (+)PD 129,289, it was 8 μmol/kg/min. In Table 2, Appendix 5, the drugs examined did show some differential effects on sodium compared to potassium channels using the D_{25} value as a measure of current selectivity. At lower doses, the κ agonists affected sodium channels more than potassium channels. Thus the patterns of drug response to ECG, blood pressure, heart rate and electrical stimulation indicate that these drugs are antiarrhythmic by virtue of cardiac ion channel blockade. The measure of drug sensitivity, D_{25}, may be useful as a method by which to quantify and validate the examination of ion channel sensitivity to drug blockade.

Table IV The studies conducted in the appendicised papers have been published in various pharmacology Journals. The Summary Results Table indicates which of the arylacetamide compounds were studied in which papers. For the *in vivo* studies which were conducted the following abbreviations were used: C.V. = cardiovascular studies comprising heart rate, blood pressure and ECG (P-R, QRS, Q-aT and RSh) changes; E.S. = electrical stimulation studies where i_t, t_t, VF_t, ERP and MFF were examined and C.O. = coronary occlusion studies. For the *in vitro* studies, I.H. indicates the studies which were conducted in rat isolated Langendorff hearts for effects on heart rate, contractility and ECG measures. P.C. indicates those studies which were conducted in isolated ventricular myocytes for effects on sodium (Na^+) current amplitudes, kinetics and use-dependence or potassium (K^+) for effects on i_{to} and i_{Ksus} blocking properties. Studies were conducted in the absence and presence of the κ opioid receptor antagonists naloxone or Mr 2266.

Table IV - Summary of Results which can be found in the Appendices

Appendix	Drug	*In vivo*			Figure	Table	Antagonist	*In vitro*			Figure	Antagonist
		C.V.	E.S.	C.O.				I.H.	P.C. Na^+	P.C. K^+		
1	U-50,488H	√			3a,b	1	naloxone Mr 2266	1a,b 2a,b				naloxone
			√		4a,b 5a,b		naloxone					naloxone
2	U-50,488H	√				1	naloxone					
				√		2,3	naloxone					
3	U-50,488H								√		1,2,3, 4,5	Mr 2266
										√	7,8	Mr 2266
4	PD129,289	√			2	1		√			1a,b	
			√		3							
				√		2						
	PD129,290	√			2	1	naloxone	√			1a,b	naloxone
			√		3		naloxone		√		4,5,6	naloxone
				√		2	naloxone					
5	PD117,302	√				1			√		Text	
			√			2						
	PD129,289	√				1						
			√			2						
	PD129,290	√				1			√		Text	
			√			2						

4 Discussion

The major focus of this thesis was to examine the antiarrhythmic actions of a series of arylacetamide κ agonists and related inactive enantiomers. The specific hypothesis being tested was that the antiarrhythmic actions of arylacetamide κ agonists is not related to actions on opioid (particularly κ) receptors. The results clearly indicated that the antiarrhythmic actions of these compounds was independent of actions on opioid receptors but dependent upon channel blocking actions, particularly the block of sodium and potassium channels. In addition to testing this hypothesis these studies also offered insights into a number of related topics. The following discussion will summarize the results of these studies and discuss related topics. This will be accomplished by considering the following questions:-

1) What cardiovascular actions were common to arylacetamide κ agonists and related compounds?

2) Were these cardiovascular actions, particularly those at high doses or concentrations, opioid receptor dependent, or were they dependent on ion channel blockade?

3) What is the nature of the channel blocking actions of the arylacetamide compounds?

4) Do these channel blocking actions account for the antiarrhythmic actions of these compounds?

5) Can any projections be made from these studies regarding the value of arylacetamides in the treatment of arrhythmias and study of ion channel blocking drugs?

The discussion will be concerned with the results obtained with spiradoline, (±) PD117,302 and its inactive enantiomer, (+)PD123,497; all reported for the first time in this

thesis and how they relate to the previously published manuscripts concerning U-50,488H (Pugsley et al., 1992a, 1992b, 1994), (-)PD129,290, and its inactive (+) enantiomer, (+)PD129,289 (Pugsley et al., 1993, 1995).

4.1 Cardiovascular actions of arylacetamides.

All of the compounds tested lowered mean arterial blood pressure, heart rate, widened the P-R, QRS, Q-aT intervals and increased RSh measures of the ECG.

In the foregoing, spiradoline (U-62,066E) was studied for effects on haemodynamic and ECG responses in anaesthetized rats. The Appendix section contains reports of studies with U-50,488H (Appendix 1-3), (-)PD129,290 and its inactive enantiomer, (+)PD129,289 (Appendix 4) and (±)PD117,302 (Appendix 5). The dose-response studies conducted for the arylacetamides provided us with important pharmacological data. It enabled us to select appropriate pharmacological doses for later antiarrhythmic studies and provided toxicological information.

4.1.1 Blood Pressure

The arylacetamides reduced mean arterial blood pressure in rats. Studies conducted in dogs by Hall et al. (1988) showed that U-50,488H and spiradoline dose-dependently reduced blood pressure in this species.

The depressor actions of arylacetamides are consistent with those seen with class Ia antiarrhythmic agents such as quinidine. Quinidine moderately reduces blood pressure as a result of a combined peripheral vasodilation and direct negative inotropic action on the heart (Block and Winkle, 1983; Legrand and Collignon, 1985). Class Ib agents, including lidocaine and mexiletine, are better tolerated and they produce no significant changes in blood pressure at therapeutic doses (Legand and Collignon, 1985). Class Ic agents, such

as flecainide, appear to be also well-tolerated haemodynamically; however, they exert moderate negative inotropic effects and may potentially worsen ventricular function in compromised myocardial tissue such as occurs with congestive heart failure. Several studies suggest that κ agonists depress contraction in rat tail veins (Illes et al., 1987) and porcine coronary circumflex arteries (Harasawa et al., 1991). It is suggested that this action on smooth muscle cells, since it is not inhibited by naloxone, may be due to direct inhibition of voltage-gated channels. The resulting peripheral vasodilation which may occur, in addition to the direct myocardial depression may be the mechanism by which both heart rate and blood pressure are reduced. Further studies are required to explore these actions of the arylacetamides.

4.1.2 Heart Rate

Heart rate was also reduced in a dose-dependent manner by all of the arylacetamides examined. The bradycardia produced by spiradoline (Figure 4B) was similar to that seen with U-50,488H (Figure 3a, Appendix 1). Isolated heart studies suggest that the bradycardia produced by these compounds involves a direct action on the myocardium which may or may not be related to the depression or contractility (see below).

Over a similar dose-range (0.5-32 μmol/kg/min) the arylacetamides produced a dose-dependent decrease in cardiac conduction represented by an increase in the P-R interval and QRS width of the ECG. As well, the Q-aT interval, a measure of refractoriness, was also prolonged *in vivo*. These measures provide a simple, indirect means by which to examine drug action on cardiac ion channels (Cheung et al., 1993).

4.1.3 ECG

In the course of studying the antiarrhythmic actions of many different drugs in the rat a measure of sodium channel blockade (RSh) was developed for this species (Penz et al., 1992). The conventional measures of P-R interval and QRS width prolongation are limited in sensitivity and can be supplemented by RSh. It is the most sensitive measure for sodium channel blockade *in vivo* in the rat (Pugsley et al., 1995). Spiradoline (Figure 6A) as well as (±)PD117,302, (+)PD129,289 and (-)PD129,290 produce changes in RSh at doses approximately 7.5-20 times less than those widening the QRS width and 3 times less than those increasing the P-R interval (See Table 1, Appendix 5).

In addition to producing signs of sodium channel blockade the arylacetamides prolong the Q-aT index which is related to an increase in the refractory period of ventricular cells. Spiradoline dose-dependently prolonged the Q-aT interval (Figure 6B). As well, U-50,488H (Figure 3B, Appendix 1), and both (-)PD129,290 and its inactive enantiomer, (+)PD129,289 prolonged the Q-aT interval (Figure 2D, Appendix 3).

Consistent with the results observed *in vivo*, spiradoline and the other arylacetamides examined in isolated hearts reduced contractility, and heart rate and prolonged the P-R and QRS intervals of the ECG. The isolated hearts provide a means by which to directly assess the effects of drugs on myocardial contractility and electrical activity, free of blood borne constituents and autonomic nervous control (Broadley, 1979). Studies with arylacetamides suggest sodium channel blockade similar to class I antiarrhythmic agents and TTX (Howard et al., 1991; Abraham et al., 1989). Our experiments showed that spiradoline (Figure 2A, B, 3A, B), U-50,488H (Figure 1a, 1b, Appendix 1), (+)PD129,289 and (-)PD129,290 (Figure 1a and 1b, Appendix 4) all produced the described contractility and ECG effects expected of class I antiarrhythmic agents. The P-R interval prolongation which occurs is unlikely to be due to calcium channel blockade in these hearts (Botting et al., 1985).

In conclusion, all arylacetamide compounds produced a similar profile of cardiovascular actions.

4.2 The effects of arylacetamides - opioid receptor dependent mechanisms or ion channel blockade in the heart?

The cardiovascular profile of these compounds was determined in the absence and presence of opioid antagonists, and with inactive κ opioid receptor enantiomers, when available. It is difficult to examine the profile of opioid activity in the cardiovascular system at relevant opioid doses because of the complex interaction between opioid receptors and regulation of hormones such as those of the endocrine system (Holaday, 1983). At the supra-analgesic doses used in these studies these actions may not be important (Pugsley et al., 1992a). The doses of the opioid antagonists used in these studies, to inhibit drug effects on the opioid system, were 50-100 times the pA_2 for antagonism at the κ receptor (Martin, 1984). This ensured examination of non-opioid properties of arylacetamides.

The involvement of κ opioid receptor subtypes, κ_{1a}, κ_{1b} and the U-50,488H-insensitive κ_2 and κ_3 sites, and cardiovascular function is not known. Selective agonists and antagonists for these receptors have yet to be developed. Therefore the involvement of these subtypes in my results will not be discussed.

Hall et al. (1988) examined the cardiovascular actions of spiradoline and U-50,488H in dogs. These compounds produced a dose-related decrease in blood pressure which was abolished by naloxone (8 μmol/kg) pre-treatment and thus could be considered to be due to kappa agonism. In our studies the doses for all arylacetamides were supra-analgesic and furthermore naloxone did not abolish the effects of the active enantiomers (Pugsley et al., 1992a, 1992b, 1993a, 1994). While the arylacetamide compounds used have low affinity for μ receptors, and thus are κ selective, they were administered at approximately 1000 times the minimal dose for κ agonism. As a result spontaneous respiration ceased in animals at only the highest doses of the active κ receptor agonist arylacetamides. Respiratory depression resulted from a non-specific opioid receptor-mediated depression in the CNS (Martin, 1984).

The actions of spiradoline (Figure 5 A,B and 6B), U-50,488H (Figure 3b, Appendix 1) as well as (+)PD129,289 and (-)PD129,290 (Figure 2b, 2c, 2d, Appendix 4) on the P-R, QRS and Q-aT measures were not significantly affected by naloxone pre-treatment at either the 2 or 8μmol/kg/min dose. It is generally accepted that increases in the QRS width of the ECG reflect depression of phase 0 sodium currents and reduced ventricular conduction velocity (see review by Nattel, 1991). Thus, the sodium channel blockade produced by class I agents results in a widened QRS complex, although the degree to which this occurs depends upon heart rate and the subclass of class I being administered (Vaughan-Williams, 1984a; Harrison, 1985). Since all arylacetamides examined widened the QRS complex sodium channel blockade appeared to be a common action of these compounds. P-R interval prolongation can be produced by a variety of different drugs including calcium channel antagonists and class I antiarrhythmics. Prolongation can result from depressed conduction through atrial tissue due to sodium channel blockade or depression of slow inward A-V node calcium currents (Walker and Chia, 1989). Our studies suggest the former, rather than the latter, occurs. This reflects, in part, the fact that in small animal hearts calcium channel blockade does not play the primary role in P-R interval prolongation and rather that sodium channel blockade dominates (Botting et al., 1985).

Spiradoline (Figure 6B) dose-dependently prolongs refractoriness, especially at high doses, indicating possible potassium channel blockade. Since the major repolarizing current in the rat is the transient outward potassium current, this is the channel most likely to be affected by these drugs (Josephson et al., 1984; Beatch et al., 1991). In the rat we have shown that, for a similar degree of widening (25%) in the Q-aT interval, the doses required of (+)PD129,289, (±)PD117,302 or (-)PD129,290 are 6-25 times greater than those which produce sodium channel blockade (as assessed by a 25% increase in RSh) (see Table 1, Appendix 5).

Thus the patterns of haemodynamic and ECG changes which occur *in vivo* indicate that spiradoline and the other arylacetamides examined produce their actions by virtue of cardiac sodium and potassium ion channel blockade (Pugsley et al., 1992a, 1993a, 1995). In order to determine whether or not these mixed actions occur by a direct action on the heart we conducted studies in isolated Langendorff perfused hearts.

Spiradoline and the other arylacetamides examined produced a naloxone-resistant depression in cardiac contractility, heart rate, and affected the P-R and QRS intervals of the ECG. As with the results *in vivo,* these measures indicate sodium channel blockade whereby the reduction in contractility may be due to inhibition of the fast sodium current responsible for depolarization.

In addition to transient inward sodium channels it has been postulated, and confirmed, that there are additional cardiac sodium channels in heart tissue (Coraboeuf et al., 1979; Carmeleit, 1993; Fozzard et al., 1985; Saint et al., 1992; Ju et al., 1992, 1995). At concentrations of TTX which do not inhibit the inward sodium current, these sodium channels are blocked. The first is the pacemaker or i_f current which has a small amplitude and activates during hyperpolarization at voltages around -40 and -110 mV corresponding to the diastolic range of cardiac cell depolarizations (DiFrancesco, 1987). It is postulated that this "window current" may be electrogenically produced by membrane pumps or exchangers (Fozzard et al., 1985) and is actually a mixed current, carried by sodium and potassium (DiFrancesco, 1981; Ho et al., 1991). Another type of sodium current is the persistent sodium current which is found in rat ventricle and is also activated over the diastolic range of cell depolarization (Ju et al., 1992; Saint et al., 1992). This current is thought to contribute to the pacemaker current (Ju et al., 1995). This persistent sodium current is blocked by low doses of quinidine and lidocaine and may be involved in arrhythmogenesis (Ju et al., 1992). Therefore, in addition to the sodium current which is blocked by arylacetamide compounds, these other additional sodium currents may also be blocked. This possibility has not been explored.

Honerjager et al. (1986) examined the negative inotropic actions of several class I antiarrhythmic drugs, including TTX, in an attempt to relate this drug action to sodium channel blockade. The study compared drug concentrations which produced half-maximal reductions in V_{max} to those producing the same decrease in contractility. The results suggest that some drugs (quinidine, mexiletine) were more potent sodium channel blockers than negative inotropic agents. Therefore properties inherent to the drug, in addition to sodium channel blockade, are involved in reducing contractility. The arylacetamides reduced contractility at the doses which prolonged P-R interval indicating that negative inotropism occurs over the same dose range that these compounds block sodium channels.

It is difficult to measure the action of arylacetamides on repolarizing potassium currents in rat hearts due to the inherent variability in the Q-aT interval itself and its relationship to the isoelectric line of the ECG (Driscoll, 1981). Thus this measure cannot be used to compare drug effects *in vitro* to *in vivo* responses. The isolated heart results strongly support the suggestion that the actions of spiradoline, U-50,488H and other arylacetamides can be ascribed to effects unrelated to κ agonism. It would be reasonable to suggest that sodium, and to a limited degree at high doses (concentrations), potassium channel blockade occurs with these compounds. This would account for the haemodynamic, contractile and ECG responses observed *in vivo* and *in vitro*.

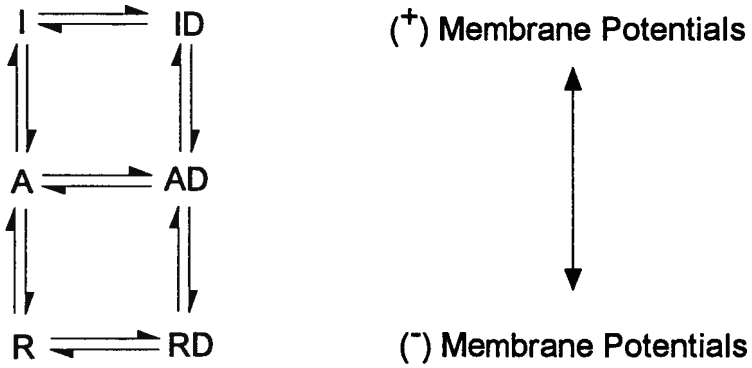
4.3 An electrophysiological basis for the channel blockade seen with arylacetamides in the heart

Electrophysiological studies conducted in isolated cardiac ventricular myocytes showed unequivocally that spiradoline, (-)PD129,290 and U-50,488H blocks sodium and potassium currents and that such an action is not blocked by naloxone or Mr2266. Blockade occurs at concentrations which produce ECG effects in rat isolated hearts and

which are consistent with doses administered to intact rats to produce similar ECG effects. These doses produced patterns of changes in electrical thresholds and refractoriness entirely in keeping with the above.

The arylacetamides produced a concentration-dependent block of sodium currents in single cells. For spiradoline (Figure 9A,B), (±)PD117,302 (Figure 14 A,B), (+)PD123,497 (Figure 15A), U-50,488H (Figure 1 and 2, Appendix 3), and (-)PD129,290 (Figure 4, Appendix 4) the EC_{50} blocking concentrations were 66, 8, 3, 15 and 20μM, respectively. The effects of spiradoline, U-50,488H, and (-)PD129,290 were examined on the current-voltage relationship for sodium currents revealing that these compounds only blocked the channel and did not alter the voltage-dependence of activation (see Figure 10A; Figure 4, Appendix 3 and Figure 4, Appendix 4, respectively). As well the compounds produced, although to varying degrees, a shift in the voltage-dependence of inactivation (h_∞) for the sodium channel. For spiradoline (Figure 10B), U-50,488H (Figure 3, Appendix 3) and (-)PD129,290 (Figure 6, Appendix 4) the relative hyperpolarizing shifts were -20, -15 and -7 mV, respectively, at the approximate EC_{50} concentrations for these compounds. Several of the arylacetamide compounds showed a tonic block component in addition to a frequency or use-dependent block which occurred at frequencies expected to be encountered during VT or VF in the rat. The voltage and frequency-dependent blockade of sodium currents by these arylacetamide compounds is best explained by the Modulated Receptor Hypothesis (Hondeghem and Katzung, 1977, 1984).

When sodium channels are maintained at membrane potentials more negative to -100mV all of the channels, according to the Hodgkin and Huxley (1952) are in the R or rested state. When depolarization occurs, the channels open from the rested (closed) state, however, when the membrane potential becomes more positive than -50mV the sodium channels become inactivated, as in the following schematic:



As discussed earlier, Hille (1977) and Hondeghem and Katzung (1977) formalized the Modulated Receptor Hypothesis shown above where R ⇔ A ⇔ I indicate the drug free channel states, resting, active and inactive, while RD ⇔ AD ⇔ ID indicate drug-associated channel states. The model describes the fraction of channels in each state and can account qualitatively and quantitatively for channel behaviour in the absence and presence of drug (Hondeghem, 1989, 1990). Therefore drug affinity for the binding to the sodium channel is modulated by channel state.

Studies have shown that useful antiarrhythmic drugs with class I properties have a low affinity for resting channels and high affinity for open or inactive channels (Chen et al., 1975; Hondeghem and Katzung, 1977; Hondeghem and Katzung, 1980; Courtney, 1980; Carmeleit et al., 1989; Hondeghem, 1991). During diastole, or the R state of the channel, drugs tend to unbind from the binding site and the level of block is reduced. However, drugs such as lidocaine and quinidine show a differential high affinity (lidocaine>quinidine) for the open state of the channel. This has implications for the tonic and use-dependent blocking properties of each drug. High affinity, open (active) channel state blocking antiarrhythmic drugs would, consequently, have the ability to increase the degree of block attained with each action potential (or evoked sodium current), while high affinity inactive state blockers would continuously develop block throughout the duration of the action potential until a steady-state is reached (Hondeghem, 1990). Since each drug selectively depresses various aspects of the action potential they may have differential effectiveness

against arrhythmias. However, as Hondeghem (1987) has proposed it is irrelevant whether block develops during the upstroke (active) or plateau (inactive) phases of the action potential but rather the amount of block which develops during each evoked action potential be quantified as it is this property which is most important in determining drug effectiveness.

According to this hypothesis, the arylacetamide compounds possess similar actions to drugs such as quinidine or even lidocaine. This is revealed through examination of sodium channel activation/inactivation properties. Studies by Lee et al. (1981) with quinidine and lidocaine in isolated rat myocytes showed that neither of these drugs produced a change in the I-V relationship. The arylacetamides, at concentrations which markedly reduce peak available sodium current, had no effect either.

More interesting results were observed when inactivation kinetics were examined in the presence of the arylacetamides. Drug associated (non-conducting) channels produce shifts in the voltage-dependence of inactivation (Hille, 1984; Hondeghem and Katzung, 1984). Spiradoline and related compounds produced such a shift of the inactivation curve (h_{∞}) for the sodium current in a hyperpolarizing direction indicating that the drug may preferentially bind to the inactive state of the sodium channel (Hille, 1984). Unlike other class I agents, which produce a dose-dependent shift and reduction in peak current at very negative pre-pulse potentials and low rates of stimulation (Bean et al., 1983), spiradoline produced a large block and shift in the inactivation curve which was not dependent upon concentration (since both 50 and 150 μM concentrations produced similar changes in half-maximal inactivation). This action is indicative of a tonic component of block, and a potential affinity for the resting state of the channel.

Estimates of the resting channel affinity for antiarrhythmic drugs are determined for the MRH by using global fitting equations (which consider the rate of kinetics of drug actions simultaneously for all three states of the sodium channel) (Hondeghem and Katzung, 1984). However these estimates are not accurate since the calculated values for

channel state affinities must be obtained from drug effects which occur in the μM concentration range (Hondeghem and Katzung, 1977, 1984). As a result, the values for resting channel affinity for antiarrhythmic drugs are large (K_{dr}>0.1 M) reflecting the inaccuracy of the calculated values (Hondeghem and Katzung, 1984). No attempt was made to characterize the rate constants for arylacetamide interaction using global fitting equations for the various channel states but rather qualitatively describe the observed effects of these compounds.

In a qualitatively similar manner to that seen with quinidine (Lee et al., 1981; Snyders and Hondeghem, 1990) and lidocaine (Bennett et al., 1988) our results showed that the arylacetamide compounds, spiradoline (Figure 10, 11, 12A, B) and U-50,488H (Figure 5a,b,c, Appendix 3) block sodium channels in both a tonic and use-dependent manner. Use-dependence of spiradoline was slow to develop and reminiscent of that exhibited by quinidine (Hondeghem and Matsubara, 1988). Spiradoline (Figure 12A, B) and U-50,488H (Figure 5 a,b,c, Appendix 3), at concentrations producing class Ia effects, produced only a small tonic reduction in sodium current. Since this is only a small fraction of the total block produced at these concentrations, this indicates a low affinity for this state of the channel. If the block were similar to that observed with the higher doses (150 μM) this may lead one to assume that the drug has a high affinity for the resting state of the sodium channel. This form of tonic block, according to Clarkson et al. (1988), may not be an accurate reflection of closed or rest-state drug binding. These studies suggest that with strong depolarizing pulses, and when the drug is at a high concentration, tonic block may result and that this is due to open channel block. Even when a small fraction of channels are inactivated the drug will be trapped in these channels in the ID (inactivated drug-associated) state and produce tonic block (Hondeghem, 1987). Tonic block is composed of two components, an early fast open channel phase following membrane depolarization and a later slow phase which is distinctly associated with sodium channel inactivation (Matsubara et al., 1989; Clarkson et al., 1988). These components in turn depend upon

drug concentration and action potential duration (Courtney, 1975; Mason et al., 1984). With an increase in drug concentration an increase in tonic block results. Both spiradoline (Figure 12A, B) and U-50,488H (Figure 5a, Appendix 3) produce tonic block in a concentration-dependent manner similar to lidocaine (Matsubara et al., 1987) and quinidine (Snyders and Hondeghem, 1990). When drug effects were examined on the cells at several concentrations which span the range of antiarrhythmic doses (low therapeutic concentrations) the increase in tonic block evident with an increase in drug concentration was small at low therapeutic doses, but approached equal blocking activity at high concentrations when compared to the use-dependent block which quickly ensued. The antiarrhythmic doses which were effective against ventricular fibrillation in occlusion studies would approximately be equivalent to 25 μM for spiradoline and 60 μM for U-50,488H in our patch-clamp myocyte studies. If we examine the concentration-response curves (Figures 9A and B) for spiradoline and Figure 2, Appendix 3 for U-50,488H this concentration corresponds to an approximate reduction in sodium current by 20 and 75%, respectively. Thus, at therapeutic doses of the drug tonic block is minimal and hence the drugs would have a low affinity for the resting state of the channels.

The use-dependent block of the cardiac sodium channel consists of an additional reduction in I_{Na} which develops in a frequency-dependent manner with trains of depolarizing pulses (Strichartz, 1977; Courtney et al., 1978). The predicted recovery-time constant ($\tau_{1/2}$) for channel block was calculated using the molecular weight, pK_a and lipophilicity of spiradoline according to an equation developed by Courtney (1980). The predicted $\tau_{1/2}$ for spiradoline was 2.3 sec based on lipophilicity (ethanol:water partition coefficient = 1.3 at pH = 7.4), pK_a = 7.8 and molecular weight (411 Da). The predicted value for spiradoline is similar to a range of values calculated by Courtney for antiarrhythmic agents with slow kinetics (such as procainamide, quinidine and imipramine) using the octanol:water partition coefficient at pH = 7.3 (Courtney, 1980).

The patch-clamp results qualitatively suggest that spiradoline may have a greater affinity for the active (open) or inactive (closed) form of the channel than for rested (closed) channels. Since recovery from block is calculated to be slow (2.3secs for spiradoline and 2.4 sec for U-50,488H) it is predicted that if these compounds interact primarily with either the open or inactive state of the channel, then according to the Modulated Receptor Hypothesis, recovery from the drug-inactivated (ID) channel state will be slow (Hondeghem and Katzung, 1984). Studies for both spiradoline and U-50,488H suggest that these drugs interact with the sodium channel slowly since it takes between 7 and 30 pulses before a steady-state level of block is produced by either compound. Quinidine displays these properties (Hondeghem & Katzung, 1980). *In vivo* confirmation of the patch-clamp results show that, at the high heart rates associated with ventricular fibrillation and tachycardia, spiradoline and U-50,488H effectively reduce these arrhythmias. Thus, the predicted slow kinetics of drug block and low affinity for the resting state of the sodium channel suggest that the antiarrhythmic properties of these drugs may be due to accumulated channel block at high rates. This use-dependent action is an event similar to that seen with quinidine, lidocaine and propafenone (Kohlhardt and Siefert, 1983; Hondeghem & Matsubara, 1988; Snyders and Hondeghem, 1990). These experiments cannot confirm whether open vs. inactive channel block develops with each action potential only that arylacetamide compounds block cardiac ion channels. The accumulation of block occurs at high rates which may be important for antiarrhythmic activity (Hondeghem, 1987).

Hille (1977) postulated that hydrophilic molecules which interact with the binding site on the sodium channel are confined to a hydrophilic pore, formed by the protein comprising the sodium channel, pathway. A second pathway which was suggested was that hydrophobic molecules would diffuse across the lipid bilayer cell membrane to the binding site. It is thought that local anaesthetics and class I antiarrhythmic drugs interact at a site on the cytoplasmic side of the sodium channel in close proximity to the inactivation gate (Hille, 1977; Hondeghem and Katzung, 1977; Courtney, 1980; Hondeghem 1987;

Narahashi et al., 1970). However, studies conducted by Alpert et al. (1989), Baumgarten et al. (1991) and Sheldon and Thakore (1993) suggest that in addition to this intracellular binding site, there may be an extracellular binding site.

Studies with lidocaine and its permanently charged analogue, QX-314, suggest that the charged lidocaine analogue blocks sodium current when applied outside as well as inside in cardiac myocytes (Alpert et al., 1989). The permanently charged quaternary amine, QX-222, was also shown to bind to an external site associated with the cardiac sodium channel (Sheldon and Thakore, 1993). These studies, in addition to highlighting the differences between local anaesthetic and antiarrhythmic drug actions on cardiac and neuronal sodium channels, provide the basis for the putative site of cardiac ion channel blockade by the arylacetamides we examined.

The studies conducted with (±)PD117,302 and its inactive enantiomer (+)PD123,497 suggest that these compounds block the cardiac sodium channel from an extracellular site. Our studies showed that the compounds did not effectively block the evoked sodium current when applied inside the pipette. The low leak current and high seal resistence attained with each whole-cell patch-clamp, would suggest that the drug, when placed in the pipette, could not leak out through this interface. The size of the patch-pipette is of some concern when examining drug effects applied by this route. However, the pore size of our patch-clamp electrodes (approximately 2-4 μm) and resistences of between 5-10 MΩ should provide sufficient intracellular perfusion of the pipette contents with the compounds examined. Thus the observed results are probably not a result of experimental artifacts.

The most convincing evidence of an external site of block by the arylacetamides is the time to onset of and recovery from block when cells were exposed to the drug. The drug perfusion cannula used in our experiments ensured that there was a minimal delay in time to drug exposure (Saint et al., 1991). Figure 15A shows that half-maximal block occured in less than 6 sec, and that this time was not concentration dependent. The

lipophilicity of these arylacetamide compounds is less than that of quinidine (log P=3.6) and imipramine (4.7) (Courtney, 1980) and share similar off-rate kinetics. Patch-clamp studies suggest that these compounds readily interact with the A or I state of the sodium channel using either a hydrophilic pathway to gain access to an intracellular binding site or by simply binding to an external site. It follows that hydrophilic agents gain access to the cell interior when the channel opens during depolarization. However, we propose that the rapid rate of binding which occurs was due to block at an external site, eliminating time-dependent diffusion of the drug to the binding site. The external binding site by which these compounds exert their channel blocking effects is pH-dependent and may be important when we examine changes during ischaemia.

In the acidic conditions associated with ischaemia, arylacetamide compounds with predominantly pK_a values >7.0 become charged and hence are confined to the hydrophilic pathway for their drug channel blocking action. Hondeghem et al. (1974) and Grant et al. (1980) showed that quinidine, procainamide and other class I drugs effectively suppress ischaemic arrhythmias under acidic conditions due to a slowed diastolic recovery from block. Acidic conditions have significant effects of sodium channel properties such as slowing the onset of and reducing peak sodium current amplitude, producing a hyperpolarizing shift in inactivation and slow depolarizing shifts in activation kinetics (Woodhull, 1973 ; Watson and Gold, 1995). Barber et al. (1991) examined the effects of pH changes on amitryptaline and diphenylhydantoin. Their studies suggested two use-dependent binding sites for these drugs, whereby one site was sensitive to external pH and the other was insensitive to external pH. The pH insensitive site was postulated to be located inside the cell since changing the intracellular pH does not change the channel blocking properties of the drug. The pH-sensitive site was located at a more "superficial" site which is accessible to external protons (Barber et al., 1991).

Preliminary studies conducted with (±)PD117,302 and its inactive enantiomer, (+)PD123,497, suggest that a pH-dependent blockade of ion channels occurs and this may

apply to arylacetamides in general. Under acidic conditions (pH = 6.4) there is a reduction in block of the evoked cardiac sodium channel with these compounds. This agrees with the data for the existence of an external binding site (Alpert et al., 1989; Barber et al., 1991). It also agrees well with the proposal by Grant et al. (1980) whereby a reduction in pH (acidification) adversely affects drug hydrophilicity (which is dependent upon the pK_a of the drug) such that when the drug is in the charged form it has a reduced affinity for the extracellular binding site. Drug block could be relieved by such a mechanism. However, it may be that drug block is not relieved under acidic conditions, but only that the availability of sodium channels for block is reduced. Many models have been proposed for the reduction in peak sodium conductance under acidic conditions. The most plausible was postulated by Drouin and Neumcke (1974) and later elaborated upon by Zhang and Siegelbaum (1991). This theory suggests that protons titrate the negative external surface charge of the sodium channel protein reducing the local sodium concentration near the pore entrance and reduce the magnitude of the resulting current. This paradoxically may result in a reduction in channel block or a removal of block by protons.

We do not know whether the proposed binding site is the same extracellular pH-sensitive binding site as discussed by Barber et al. (1991) or for TTX (Narahashi, 1970; Duff et al., 1988). These preliminary studies with arylacetamides do not address this issue, but merely suggest that perhaps these compounds bind to an extracellular locus and may provide the basis for a fundamentally novel mechanism for sodium channel blockade and possibly antiarrhythmic properties.

Spiradoline (Figure 13) and U-50,488H (Figure 6, Appendix 3) reduced at least two repolarizing potassium currents found in the rat heart. The dominant transient outward (i_{to}) current responsible for the early phase of repolarization of the rat action potential (Josephson et al., 1984), and the sustained outward plateau current (Wang et al., 1993) which we ascribe to a delayed rectifier plateau potassium current (i_{Ksus}) are similarly reduced by arylacetamides. Spiradoline (Figure 13) and U-50,488H (Figure 6, Appendix 3)

increased the rate of decay of i_{to} and markedly reduced the sustained plateau component in a concentration-dependent manner. When the actions of U-50,488H were examined in detail with respect to their effects on on i_{to} and i_{Ksus} currents neither the voltage-dependence of activation or inactivation of i_{to} (Figure 7, 8a, Appendix 3) nor voltage-dependence of i_{Ksus} activation (Figure 8b, Appendix 3) was changed. Thus, depression of these potassium currents prolongs refractoriness and in conjunction with the predicted slow rate of drug-bound inactivated (ID) sodium channel recovery should provide antiarrhythmic activity. Snyders and Hondeghem (1990) and Balser et al. (1991) showed that quinidine and amiodarone block transient sodium and potassium currents in myocardial tissue. No other studies have examined the effects of arylacetamide compounds on i_{Ksus} except our laboratory (Pugsley et al., 1993a). However, Roden et al. (1988) examined the voltage-dependence of quinidine on the delayed rectifier current (i_K), of which i_{Ksus} is a member (Wang et al., 1993), and suggest that the i_K channel proceeds from a closed state through two open channel states at which time quinidine associates with the latter open state. The second open state is presumed to occur at membrane potentials greater than +20 mV (Roden et al., 1988). Perhaps the arylacetamides act in a similar manner.

Thus spiradoline is another arylacetamide which possesses both sodium and potassium channel blocking properties in rat cardiac tissue. It is suggested, and has been shown, that the combination of a class Ib and class III antiarrhythmic agent may be of potential therapeutic benefit (Hondeghem & Katzung, 1980; Carlsson et al., 1993). The κ agonists and related compounds may possess the structural properties which confer these actions to ventricular ion channels.

4.4 Are the ion channel blocking actions of the arylacetamides responsible for the antiarrhythmic actions of these compounds?

4.4.1 Electrical Stimulation studies

Both the *in vivo* and *in vitro* results suggest that spiradoline and the arylacetamide compounds depress excitability by virtue of sodium channel blockade and increase refractoriness by blocking potassium channels. However, since prolongation of the P-R interval is not considered a good index of sodium channel blockade, even in the rat, we used increases in QRS width and RSh as additional indices of blockade (Penz et al., 1992; Pugsley et al., 1995). Electrical stimulation studies were also used to examine the antiarrhythmic properties of spiradoline and the other arylacetamides, and to verify and/or delineate ion channel involvement in drug action.

Spiradoline (Figure 7,8), U-50,488H (Figure 4b, Appendix 1), (+)PD 129,289 and (-)PD129,290 (Figure 3a, Appendix 4) and in Table 2 of Appendix 5 for (±)PD117,302 at the relatively high doses and concentrations used in these studies, produced significant actions on electrical stimulation variables. These responses included dose-dependent increases in threshold currents for capture (i_t) and ventricular fibrillation (VF_t) and threshold duration (t_t), responses characteristic of those produced by blockade of sodium channels in rat cardiac muscle and reflecting putative antiarrhythmic actions (Spear et al., 1972; Winslow, 1984; Abraham et al., 1989; Howard et al., 1991).

Electrical stimulation studies take advantage of the properties of inhomogeneity of cardiac muscle repolarization. After a wavefront of excitability occurs in the cardiac muscle there exists a certain degree of heterogeneity of recovery in excitability amongst cardiac cells (Moe et al., 1964; Winslow, 1984). The resulting disparity which occurs amongst cells during this period of ventricular vulnerability occurs at the end of the cardiac cycle (Wiggers and Wegria, 1940). This period corresponds to the phase of the T-wave waveform on the ECG. Therefore, during this period the degree of instability in the ventricle may be such that an extra-stimulus of adequate strength and duration applied at this critical moment may precipitate VF (Han, 1969). This has been termed the "R-on-T" phenomenon since it involves a depolarizing R-S complex appropriately activating a portion of the activatable

ventricle (i.e., cells which are no longer refractory) during the vulnerable period (see review by Moore and Spear, 1975). Thus, electrical stimulation is thought to, via the delivery of high currents (approximately 100 μA), produce VF in this manner (Han, 1969; Sugimato et al., 1967).

Principally, circus movement re-entry is reduced by prolongation of refractoriness. This is because extended refraction of ventricular myocyte action potentials reduces or limits the potential re-entrant loop required to sustain the propagation of the impulse (Adaikan et al., 1992). That portion of the re-entrant loop which is no longer refractory and is therefore susceptible to excitation is known as an excitable gap (Janse 1992; Niwano et al., 1994). If a plot of i_t is made against t_t, the boundary conditions described by the i vs t curve define sodium channel availability, and may also represent a path length for circus movement (McC. Brooks et al., 1951; Antoni, 1971). Thus, any conditions whereby i_t and t_t fall below the defined exponentially-decaying curve provides cause for enhanced ventricular excitability and possible induction of circus movement re-entrant arrhythmias (Antoni, 1971). Blockade of sodium channels by antiarrhythmic drugs, and arylacetamides, produces a shift in the curve by reducing sodium channel availability (through drug-bound channel inactivation) and ventricular excitability such that induction of re-entrant circuits is less probable.

However, it was shown that low doses of U-50,488H (Figure 4a, Appendix 1), (-) PD129,290, (Figure 3c, Appendix 4) and, although not to the same degree, spiradoline (Figure 7) which are associated with selective actions on κ receptors, reduced VF_t (and to a less marked extent, i_t and t_t) in a manner that was blocked by naloxone. Thus, as seen by Brasch with naloxone (Kaschube and Brasch, 1991), the κ agonist arylacetamides appeared to have two actions: a low dose and possible arrhythmogenic effect mediated through opioid receptors, and a higher dose antiarrhythmic effect independent of opioid receptors (Pugsley et al., 1992a, 1993).

It is generally regarded that two possible mechanisms may produce or exacerbate arrhythmias associated with class I antiarrhythmic drug administration or ischaemia (Han, 1969; Hope et al., 1974; Velbeit et al., 1982). The first occurs if conduction is depressed in ventricular tissue without an alteration in refractoriness. This depression in conduction establishes the conditions necessary for potential re-entrant circuit development in the vulnerable period of the myocardium, as discussed above (Binah and Rosen, 1982). The second mechanism by which arrhythmias may occur is due to prolongation of the Q-aT interval or delayed repolarization (Janse, 1992). Development of EADs and DADs, most commonly associated with the torsades de pointes arrhythmia, is characteristic of agents which prolong repolarization, most notably drugs possessing class III properties.

Electrical stimulation studies did not allow us to determine whether one or all of the possible mechanisms of arrhythmogenesis occurs with arylacetamides, however, most probably it is κ receptor mediated. If we compare the doses of class I antiarrhythmic drugs which induce arrhythmias to the potential proarrhythmic doses for the arylacetamides, it is most likely that the class I agent produce arrhythmias at higher than therapeutic doses (Janse, 1992; Nattel, 1991).

ERP (and MFF) was dose-dependently affected by the arylacetamides. This can indicate an action to block sodium channels and/or prolong the duration of the action potential. Similar effects are seen with class Ia antiarrhythmics (Vaughan-Williams, 1984b). MFF is related to ERP by the equation MFF(Hz) = 1000/ERP (msec) (Pugsley et al., 1992a). Although these measures are similar they may be differentially susceptible to antiarrhythmic drug action. ERP is a measure of the effective refractory period while MFF more accurately describes the relative refractory period (RRP) of the ventricle. The manner in which MFF is obtained may be associated with an increase in extracellular potassium which may locally alter drug actions in the vicinity of the depolarized cells. Studies with spiradoline (Figure 8), U-50,488H (Figure 5, Appendix 1) and (-)PD129,290

and its inactive enantiomer (+)PD129,289 (Figure 3b, Appendix 4) suggest that these compounds produce dose-dependent, but differential, actions on these measures.

4.4.2 Ischaemic Arrhythmia Studies

Spiradoline and related arylacetamides demonstrated sodium channel blockade and possible antiarrhythmic properties and these studies showed, for the first time, a novel class of potential antiarrhythmic drugs with an unexplored antiarrhythmic profile. When compared to sodium channel blocking antiarrhythmic drugs, such as quinidine, lidocaine and flecainide, the arylacetamides protect against acute ischaemia-induced arrhythmias at doses which moderately reduce blood pressure and heart rate. In similar rat models quinidine and flecainide produced only moderate antiarrhythmic protection. Lidocaine completely abolished arrhythmias but only at doses which produced convulsions, limiting its usefulness against ischaemia-induced arrhythmias (Barrett et al., in press). Thus, a potentially new class of antiarrhythmic drugs exist with which to explore arrhythmogenesis and antiarrhythmic mechanisms.

The low dose, potentially proarrhythmic actions seen with some of the κ agonists in the electrical stimulation studies did not exacerbate arrhythmias in the ischaemic-arrhythmia model. The low dose, like naloxone, was without effect on the incidence of ventricular arrhythmias.

The proarrhythmic actions of class I antiarrhythmic drug actions are thought to involve at least two components related to pharmacodynamic and pharmacokinetic factors. The pharmacodynamic component of conduction delay relates to a synergism between ischaemic tissue and drug action (Hope et al., 1974). Antiarrhythmic drugs which increase conduction delay in ischaemic tissue may or may not suppress arrhythmias (Cranefield, 1975; Gettes et al., 1982). Antiarrhythmic drugs may suppress conduction and produce arrhythmias by creating an area of myocardium with inconsistent activation, i.e. an area

whereby no uniform conduction can occur (El Harrar et al., 1977; Hoffman et al., 1981). Class I drugs, through perhaps such a mechanism, may facilitate local re-entry and increase ectopic pacemaker site activity (Patterson et al., 1995).

Since coronary artery occlusion can profoundly affect drug distribution in the myocardium, pharmacokinetic consideration may also play a role in causing arrhythmias. Nattel et al. (1981) examined the antiarrhythmic properties of aprindine in dogs subject to coronary artery occlusion. It was found that regional distribution of drug influenced arrhythmia incidence. Although the clinical importance of the cardiovascular actions of κ receptor agonists are likely to be limited, they may be of theoretical interest in view of the proarrhythmic findings with class Ic antiarrhythmic agents in the CAST-I and CAST-II trials (CAST Investigators, 1989; CAST-II Investigators, 1992).

The antiarrhythmic activity of spiradoline (Table 1), U-50,488H (Table 3, Appendix 2), (-)PD129,290 and its (+) enantiomer PD129,289 (Pugsley et al., 1993) could not be ascribed to either an increase in extracellular potassium or occluded zone size. An increase in extracellular potassium occurs in ischaemic tissue shortly after the onset of ischaemia and is responsible for limited depolarization of cells within the ischaemic zone (Harris et al., 1954; Hill and Gettes, 1980). The increase in potassium is most probably due to a decrease in cellular ATP levels which cause the ATP-dependent potassium channels (i_{KATP}) to open and enhance potassium efflux from cells (Wilde and Kleber, 1986). As well, inhibition of the sodium/potassium ATPase membrane exchanger results in an increase in extracellular potassium and intracellular sodium concentrations; consequently, an increase in intracellular calcium usually occurs (Carmeleit, 1984). Lastly, the simple lack of blood flow preventing washout of potassium from the ischaemic area further adds to the elevation in potassium (Janse and Kleber, 1981). However, the exact mechanism by which potassium loss occurs is not defined, only that critical environmental changes occur in the acutely ischaemic myocardium.

In ischaemic tissue, action potential amplitude is reduced as well as the rise rate (V_{max}) of phase 0. This is accompanied by an initial increase, and then decrease, in action potential duration (Downar et al., 1977). Partially depolarized ischaemic fibres show a reduced recovery from excitability compared to normal tissue (post-repolarization refractoriness) (Gettes and Reuter, 1974) These factors, in addition to acidification in the extracellular milieu (Yan and Kleber, 1992), may increase dispersion of refractoriness and precipitate arrhythmias (Janse and Kleber, 1981). The κ receptor agonists and related compounds may be antiarrhythmic by virtue of their ability to differentially suppress excitability and conduction in the ischaemic and non-ischaemic zones in a uniform manner and thereby convert any uni-directional block to bi-directional block preventing re-entrant circuit development.

At higher doses the arylacetamides had potassium channel blocking properties. As suggested by Hondeghem and Katzung (1980), Hondeghem and Snyders (1990) and Carlsson et al. (1993) such blockade may be of therapeutic benefit. Hondeghem and Katzung (1980) examined the antiarrhythmic effectiveness of combinations of drugs such as quinidine and lidocaine and argue that selective depression of abnormal impulse generation could be obtained by combining drugs with slow and fast offset kinetics for sodium channel blockade. Spiradoline and the other arylacetamides may have these desired properties providing a novel structure with which to explore the relationship and interdependence of block between sodium and potassium channels.

In these studies, the anaesthetized rat model of ischaemia was used to assess the onset of ventricular arrhythmias which usually occur within 10 min after the onset of occlusion (Walker et al., 1991). Like many other species a later phase occurs 1-2 hrs after occlusion. Arrhythmias include PVC, VT and VF. In rats VF often spontaneously reverts, which is almost unknown in larger species including man (Janse and Kleber, 1981). Many mechanisms for the induction of VT and VF have been proposed (see review by Binah and Rosen, 1992). This discussion will concentrate upon re-entry first conceptualized by Mines

(1913) as circulating excitation. Many mapping experiments have been conducted which reveal that the movement of excitatory wavefronts are responsible for premature excitation within the heart (Janse and Kleber, 1981). In addition, Harris (1950) and Hoffman (1981) suggested that at the boundary between normal and ischaemic tissue arrhythmogenic "injury currents" occur. This may be due to the variable potential differences which exists between the two tissues.

The abnormal electrical activity and distinct differences in cellular electrophysiology in ischaemic tissue provide an ideal "target" for antiarrhythmic drug development. Consequently, for a drug to effectively suppress the re-entry circuits involving ischaemic tissue it should ideally be activated within the ischaemic zone, i.e. at low pH and high potassium concentrations. Since cells are partially depolarized a useful drug should bind to the channel when it is activated (open block), or inactivated (closed block), and unbind when rested (Hondeghem and Katzung, 1977; Janse, 1992). This would ensure frequency-dependence in a manner similar to class Ib antiarrhythmic drugs. Such frequency-dependence eliminates high-frequency arrhythmias such as VT and VF. In addition, sodium channel inactivation is greatest at the end of the cardiac cycle, maximal block should theoretically occur at this point thereby suppressing ectopic pacemaker activity (Hondeghem, 1990).

When the arylacetamide compounds were examined for antiarrhythmic effectiveness a degree of selective protection against VT and VF was found whereas the incidence of PVC occurrence was not changed. The frequency-dependent actions of the drugs shown in the patch-clamp studies indicate enhanced effectiveness of these compounds at high rates of stimulation. However, in addition to suppressing conduction by blockade of sodium channels, the arylacetamides also prolonged refractoriness. Increased refractoriness can occur by two mechanisms, inhibition of repolarizing potassium currents, and prolongation of sodium channel inactivation (Janse, 1992). As mentioned above, the excitable gap concept of re-entry suggests that drugs which both inhibit sodium channels

and prolong refractoriness may, in addition to abolishing arrhythmias, decrease the possible pro-arrhythmic effects associated with sodium channel blockade alone (Hope et al., 1974; El Harrar et al., 1977). Pro-arrhythmic actions were not seen with the arylacetamides at any of the doses administered. If, according to the long excitable gap theory, excitability and conduction are the requirements by which a portion of the re-entrant loop may be susceptible to arrhythmia induction (Niwano et al., 1994) blockade of sodium channels would selectively suppress conduction along the re-entrant path rendering the tissue inexcitable (Derakhchan et al., 1994). The proposed antiarrhythmic mechanism of the arylacetamides in this study is suggested to be due multiple ion channel block in cardiac ventricular tissue.

In contrast, some studies have shown that excitation may follow rapidly behind refractoriness resulting in a short excitable gap (Janse, 1992). In this case prolongation of repolarization by inhibition of potassium currents may increase refractoriness and abolish arrhythmias (Spinelli and Hoffman, 1989). A large number of repolarizing potassium currents are found in the myocardium (Carmeleit, 1993). It is suggested that the delayed rectifier current, I_K, is an important target for block by antiarrhythmic drugs (Colatsky et al., 1990). In animal species in which I_K plays a dominant, or at least major role in repolarization, block by antiarrhythmic drugs should effectively abolish arrhythmias. However, in the rat model used to conduct the experiments outlined in this thesis, I_K plays only a minor role in contributing to ventricular repolarization.

In the rat, the transient outward potassium current, i_{to}, is the dominant repolarizing potassium current in the ventricle (Josephson et al., 1984). Blockade of this current is antiarrhythmic in the rat and primates (Beatch et al., 1991; Adaikan et al., 1992). Action potentials in atrial tissue are predominantly repolarized by i_{to} currents which, when effectively blocked, abolishes SVT (Wang et al., 1991). A suggested mechanism by which i_{to} blockers effectively abolish high frequency ventricular arrhythmias such as VF has been explained in detail by Adaikan et al. (1992). Essentially these drugs prolong ERP to the

point that the excitatory gap is reduced to the point that the re-entrant arrhythmia is either abolished or the path-length is sufficiently prolonged to sustain only a slow VT. This relates to the concept of wavelength (ERP multiplied by CV) which, as described above, is an index of drug effectiveness against arrhythmias (Rensma et al., 1988). The κ receptor agonists and related compounds may block potassium channels and suppress arrhythmias in rats by a similar manner.

Pure potassium channel blockers, which have been the primary focus of drug company antiarrhythmic drug development for a number of years, possess two inherent problems as antiarrhythmics. Blockade of repolarization can induce arrhythmias due to EAD or DAD-type mechanisms as discussed above (Wit et al., 1972a; Cranefield, 1975). In addition, while sodium channel blockers are characterized by positive frequency-dependence blockers of potassium channels (especially for I_K) exhibit reverse use-dependence (Hondeghem and Snyders, 1990; Hondeghem, 1994). The term reverse- use-dependence refers to the observation that ERP is prolonged at low heart rates with these drugs and reduced at high heart rates (as occurs with VT or VF), thereby limiting antiarrhythmic drug effectiveness. I_{to} blockers, like tedisamil, also appear to be reverse use-dependent (Dukes et al., 1990).

The inherent degree to which compounds such as spiradoline and related arylacetamides block sodium and potassium channels should be explored in detail. The development of analogs of these compounds may result in a class of antiarrhythmic drugs with mixed ion channel blocking properties which could be used to treat various arrhythmias. The properties of antiarrhythmic drug blockade of more than one cardiac ion channel is well known for amiodarone (Singh, 1983) and even for lidocaine (Josephson, 1988). Since our studies show that the predominant ion channel which is blocked by arylacetamides, at doses which abolish or reduce arrhythmias (see RSh vs. Q-aT doses in Table 1, Appendix 5 for comparison), is sodium we could classify these arylacetamide

drugs as class Ia antiarrhythmic drugs according to the Vaughan-Williams classification scheme (Vaughan-Williams, 1984; Pugsley et al., 1992a, 1993).

Spiradoline was examined for effects on ischaemia-induced ECG changes. At a dose of 2.5µmol/kg/min spiradoline prolonged the time to maximum S-T segment elevation and additionally prolonged the time taken to reach the maximum amplitude of the attained R-wave following ischaemia. Arrhythmia studies with TTX and quinacainol, a putative class I antiarrhythmic, show results similar to those with spiradoline (Abraham et al., 1989; Howard et al., 1991). Consistent ischaemic zone sizes between spiradoline and control groups (and the other arylacetamides as well) exclude this as a factor which may contribute to the changes in S-T segment elevation.

It should be noted that despite the differences which exist between rats and humans, ECG responses to occlusion are similar in both species (Walker et al., 1991). Elevation of the S-T segment is the first dramatic change which occurs in the ECG after occlusion (Samson and Scher, 1960; Normann et al., 1961; Walker et al., 1991). This change, an indirect index of the extent of ischaemia occurring in the myocardium, is suggested to be the result of depolarization-induced changes in cardiac cells in the ischaemic zone which include a reduction in action potential amplitude, action potential shortening and delayed activation of these cells (Downer et al., 1977; Kleber et al., 1978; Carmeleit, 1984). A similar delay in onset of S-T segment elevation occurs with other antiarrhythmic agents including the class II ß-blockers and class IV calcium channel blockers (Yusuf et al., 1984; Curtis et al., 1986). As with other class I drugs including quinidine, disopyramide, lidocaine and mexiletine, spiradoline and the related arylacetamides do not reduce maximum S-T segment elevation (Johnston et al., 1983; Igwemezie et al., 1990).

Thus, the ion channel blocking actions seen with the arylacetamides examined can account for the antiarrhythmic actions of these compounds against ischaemic and electrically-induced arrhythmias in the rat.

4.5 Projections regarding the value arylacetamides have in the study and/or treatment of arrhythmias and ion channel blocking drugs.

The arylacetamides were developed as structurally novel agonists for the κ opioid receptor. However, these compounds also possess non-opioid properties in cardiac tissue and may be valuable in the study of mechanisms, or treatment, of cardiac arrhythmia's, or for use as tools in the study of ion channel function. The lack of structural similarity of the arylacetamides to class I and III antiarrhythmic agents offers the opportunity to develop and understand the different structural requirements for drug effectiveness against arrhythmias. These compounds may allow for structure-activity relationship analysis for ischaemia-selectivity ultimately leading to the generation of new antiarrhythmic agents which are superior to both arylacetamide as well as the presently available drugs.

The arylacetamide compounds examined for inhibitory actions on sodium and potassium currents demonstrate a comparable, if not better, potency for ion channel blockade in rat ventricular myocytes when compared to other class I drugs. The basic electronic configurations of these compounds may provide insight into the effectiveness of ion channel blockade and the pharmacophore for recognition and binding on or in ion channels. If arylacetamides can be used to define the geometry of a "pharmacophore" this will provide a model for antiarrhythmic drug development.

5 Summary

Spiradoline, and other arylacetamides, can have both opioid and non-opioid actions on the cardiovascular system. Actions which are dependent upon the opioid receptor are blocked by opioid antagonists such as naloxone or Mr 2266. These antagonists block actions at other types of opioid receptors such as μ, but only at higher drug concentrations. However, the non-opioid actions of the κ agonists and related compounds are not blocked by any opioid antagonist.

The non-opioid cardiovascular actions of κ receptor agonists and related compounds were only seen at high drug concentrations, or doses, 10-1000 times greater than those mediated by the κ opioid receptor. Studies on cardiac tissue presented the clearest evidence of non-opioid actions. Studies in intact and isolated hearts suggested ion channel blocking properties in ventricular myocytes involving sodium, and to a lesser extent potassium, current blockade. These actions were associated with antiarrhythmic effects during electrical stimulation or in the presence of coronary artery occlusion. The sodium channel blocking actions of the arylacetamides are confounded, to some degree, by an opposing opioid-receptor dependent action. In addition to blocking the cardiac sodium and potassium channels, these compounds may have other membrane perturbational effects which may involve reduced calcium influx and potassium release from neuronal tissue.

The clinical relevance of the κ agonist actions on the cardiavascular system are likely to be limited and may be only of theoretical interest. However, recently many suggestions have been made regarding the use of drug combinations in the treatment of clinical arrhythmias. The novel arylacetamide structure may provide important details regarding many aspects of antiarrhythmic drug blockade of cardiac ion channels and allow for the development of antiarrhythmic drugs. Further examination of these compounds may allow for the elucidation of a series of pharmacological structures, devoid of κ-related

properties, but which possess selective sodium and potassium channel blocking properties in cardiac tissue. This would then allow for either an examination of drug combinations as suggested or the development of a novel antiarrhythmic drug which may prove useful in the clinical setting.

6. References

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7 APPENDICES

The following Appendix contains information regarding the actions of several arylacetamide compounds related to U-62,066E (spiradoline), as discussed in the discussion section of this thesis. Permission from the publisher has been obtained for each paper included in the Appendix.

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Hayes, E., Pugsley, M.K., Penz, W.P., Adaikan, G. and Walker, M.J.A. Relationship between Q-aT and RR intervals in rats, guinea pigs, rabbits and primates J. Pharmacol. Toxicol. Meth. 32(4): 201-207, 1994.

APPENDIX 8

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Pugsley, M.K., Penz, W.P. and Walker, M.J.A. Cardiovascular actions of U-50,488H and related kappa agonists. Cardiovasc. Drug Rev. 11(2): 151-164, 1993.

Br. J. Pharmacol. (1992), 105, 521–526 APPENDIX 1

Cardiovascular actions of the κ-agonist, U-50,488H, in the absence and presence of opioid receptor blockade

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1 The cardiovascular actions of U-50,488H, a κ-receptor agonist, were studied in rat isolated perfused hearts, and in anaesthetized rats, over concentrations or doses generally above those required to produce κ-receptor-mediated effects.

2 U-50,488H dose-dependently decreased left-ventricular peak systolic pressure and beating rate *in vitro* and reduced blood pressure and heart rate *in vivo*.

3 Over the concentration range of 1–30 μM *in vitro*, and the dose-range of 0.5–32 μmol kg^{-1} *in vivo*, U-50,488H prolonged the P–R, QRS and Q–T intervals of the ECG.

4 The effects of U-50,488H were not antagonized by an opioid receptor antagonist, naloxone (1 μM or 8 μmol kg^{-1}). Similarly, the opioid receptor antagonist, MR 2266, at 8 μmol kg^{-1} did not significantly reduce the cardiovascular actions of U-50,488H *in vivo*.

5 The actions of U-50,488H on responses to electrical stimulation were also studied. Over the dose range of 0.5–32 μmol kg^{-1}, U-50,488H altered thresholds and effective refractory period. It had a biphasic action on thresholds for induction of ventricular fibrillation. Thresholds were decreased at lower doses (0.5–4 μmol kg^{-1}) but increased at higher doses (8–32 μmol kg^{-1}). The effects of lower doses were blocked by naloxone. Effective refractory period and threshold pulse width only increased with dose.

6 In conclusion, U-50,488H at high concentration, had direct depressant actions on cardiac contractility, electrical excitability and the ECG. These depressant effects were not antagonized by the opioid receptor antagonists, naloxone and MR 2266, and probably do not involve opioid receptors. Furthermore, some of the observed effects were those expected to result from sodium channel blockade.

Keywords: U-50,488H; ECG; electrical stimulation; sodium channel blockade; naloxone; MR 2266; rat hearts

Introduction

U-50,488H, a selective κ-receptor agonist (Lahti *et al.*, 1982), has been shown to induce ventricular arrhythmias in the isolated perfused heart of the rat when given as a bolus of 44 or 132 nmol (Wong *et al.*, 1990). Further, the incidence and severity of ischaemia and reperfusion-induced arrhythmias are reduced by the opioid receptor antagonists naloxone (Sarne *et al.*, 1991) and MR 2266 (Wong *et al.*, 1990). Other studies suggest that κ-receptor blockade may be antiarrhythmic (Sitsapesan & Parratt, 1989) whereas buprenorphine, an agent with mixed actions on μ- and κ-receptors, may exert antiarrhythmic effects directly, independent of opioid receptors (Boachie-Ansah *et al.*, 1989). In view of the possibility that U-50,488H has direct cardiac effects, as well as the fact that the bolus doses used by Wong *et al.* (1990) may have produced transient high concentrations, we decided to extend that study. We used a wide range of steady state concentrations and compared effects *in vitro* with those *in vivo* to determine whether the former actions could be observed in intact animals.

U-50,488H has recently been shown to exert sodium channel blocking actions in neuronal tissue; actions which are not reversed by opioid receptor antagonists (Alzheimer & Ten Bruggencate, 1990). It thus appeared useful to examine U-50,488H for cardiovascular actions which could relate to sodium channel blocking actions in cardiac tissue and to use naloxone to block possible opioid-receptor-mediated effects so revealing direct cardiac effects.

Known sodium channel blockers *in vivo* depress blood pressure and heart rate, prolong P–R and QRS intervals and depress electrical excitability. Concentrations *in vitro* corresponding to effective plasma concentrations *in vivo* depress contractility, lower heart rate and widen P–R and QRS intervals in rat isolated hearts (Walker & Beatch, 1988; Abraham *et al.*, 1989; Howard & Walker, 1990). Such concentrations also depress the rise rate of phase 0 of intracellular action potentials.

We have systematically investigated the actions of U-50,488H in rat isolated hearts and in pentobarbitone-anaesthetized rats. We assessed the effects of U-50,488H on left-ventricular peak systolic pressure, heart rate and the ECG *in vitro*. *In vivo*, blood pressure, heart rate and the ECG effects were examined together with sensitivity to electrical stimulation of the left ventricle. Results suggested that, at concentrations or doses greater than those required to produce agonism at κ-receptors, U-50,488H blocked sodium channels in rat heart.

Methods

Male Sprague-Dawley rats (150–350 g) were used in accordance with the guidelines of the University of British Columbia's Animal Care Committee. Intact rats were anaesthetized with pentobarbitone (60 mg kg^{-1}, i.p.). When required, the trachea was cannulated for artificial ventilation at a stroke volume of 10 ml kg^{-1}, 60 strokes min^{-1}. Body temperature was monitored by rectal thermometer and maintained between 36–37°C with a heating lamp.

Isolated hearts

The procedure described by Curtis *et al.* (1986) was used. Rats were killed and their hearts excised for immediate perfusion with cold Krebs-Henseleit solution. Thereafter they were mounted on a modified Langendorff apparatus (Curtis *et al.*, 1986) and perfused at a constant pressure of 100 mmHg with oxygenated Krebs-Henseleit solution (pH 7.4), and at a temperature of 37°C.

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The left atrium was removed and a compliant balloon inserted into the left ventricle. The end diastolic pressure in the balloon was adjusted to 10 mmHg with approximately 0.5 ml of saline. Pressures within the balloon were monitored on a Grass Polygraph (model 7D) while the maximum rate of intraventricular pressure development ($+dp.dt^{-1}_{max}$) was obtained with a Grass differentiator (model 7P20C).

The ECG was recorded via two atraumatic silver-ball electrodes placed on the epicardial surface of the right atrium and left ventricle, respectively. The ECG was recorded on a Grass Polygraph at a bandwidth of 0.1–40 Hz. All hearts were of similar weight (1.2–1.6 g).

A random block design was used to assign hearts to the following groups: saline, and U-50,488H (at 1, 3, 10 and 30 μM for 10 min each) in the presence or absence of 1 μM naloxone, a concentration at least 100× the pA_2 (Martin, 1983). Hearts were left to stabilize for 10 min before addition of saline vehicle, or naloxone, 10 min prior to dosing with U-50,488H.

In vivo *effects of U-50,488H*

In pentobarbitone-anaesthetized, and artificially ventilated rats ($n = 6$ per group), the left carotid artery and right jugular vein were cannulated. Dose-response curves were constructed for U-50,488H with cumulative doses of 0.5, 1.0, 2.0, 4.0, 8.0, 16.0 and 32.0 μmol kg^{-1} i.v. Each dose was infused over 2 min and the blood pressure, heart rate and ECG were recorded 10 min later, just before addition of the next dose.

In a separate experiment, and according to a random and blind design, either saline, naloxone (8 μmol kg^{-1}) or MR 2266 (8 μmol kg^{-1}) was administered before giving U-50,488H at 16 μmol kg^{-1}. All rats were allowed to stabilize for 10 min before receiving the first injection of saline, or either of the two opioid antagonists. The dose of U-50,488H was chosen from the previous dose-response study as one that produced significant, but not maximal, responses which could be expected to be blocked completely by the high doses of antagonists used. We performed an initial *in vivo* trial study with naloxone and the dose we decided to use was the highest dose without effect on the ECG, blood pressure or heart rate and much higher than the pA_2 (Martin, 1983). This chosen dose could be expected to block effectively any opioid receptor-dependent effects of U-50,488H, even at the highest doses.

Electrical stimulation studies

In pentobarbitone-anaesthetized rats the left-carotid artery and right jugular vein were cannulated. Two Teflon-coated silver wire stimulating-electrodes were inserted through the chest wall and implanted in the left ventricle (Walker & Beatch, 1988). The inter-electrode distance was approximately 1 mm and square wave stimulation was used to determine: threshold current for capture (iT–μA), threshold pulse width for capture (tT–ms), maximum following frequency (MMF–Hz), ventricular fibrillation threshold (VFt–μA) and effective refractory period (ERP–ms) according to previously described methods (Howard & Walker, 1990).

The variables iT and tT approximate to the rheobase and chronaxie, respectively, of the i vs t curve and are a measure of sodium channel availability. Unfortunately, since iT and tT cannot be measured under rigidly controlled conditions they are imperfect measures. 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. Thus while ERP, as measured, is a reasonable measure of effective refractive period, MFF is more a measure of relative refractory period, and ventricular functional refractory period, and thus can exhibit a different sensitivity to drugs from ERP. The process of determination of MFF, namely a steadily increasing frequency of stimulation, can be associated with accumulation of extracellular K^+ thereby adding an extra component to what would otherwise be another measure of ERP. MFF and ERP are not equally sensitive to frequency-dependent sodium channel blockers (Walker & Beatch, 1988). Stimulation studies were performed only *in vivo* since results are more predictable in the intact animal than in isolated hearts. The full profile of U50,488H on the various stimulation variables is given here to allow for comparison with the profiles obtained for other Class 1 anti-arrhythmic drugs (e.g. as in Abraham *et al.*, 1989).

The stimulation variables were determined 5 min after each dose of saline, or U-50,488H, in the absence or presence of a single previous dose (8 μmol kg^{-1}) of naloxone. U-50,488H was given cumulatively at 0.5, 1.0, 2.0, 4.0, 8.0, 16.0 and 32.0 μmol kg^{-1}.

Drugs

The drugs used were: U-50,488H (trans-(±)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]-benzene-acetamide methane sulphonate), naloxone and MR 2266 ((−)-5,9-alpha-diethyl)-2-(3-furylmethyl-2'-hydroxy-6,7-benzomorphan). U-50,488H and MR 2266 were generous gifts from the Upjohn Company and Boehringer Ingelheim (Canada) Ltd., respectively. Naloxone hydrochloride was purchased from DuPont Pharmaceutical Co. All drugs were prepared as stock solutions dissolved in a 0.9% NaCl solution for *in vivo* studies. Drugs were dissolved in Krebs-Henseleit for *in vitro* studies.

Statistical analysis

Statistical analyses were performed with the NCSS statistical package (Hintze, 1987) and values are presented as mean ± s.e.mean. General Linear Model ANOVA was used for balanced studies and multiple comparisons made by Duncan's test. A difference at $P < 0.05$ was considered significant. The effects of U-50,488H are expressed as changes from the pre-U-50,488H value in the absence, or presence, of naloxone. Two types of statistical comparisons were made. With 'raw' data, simple comparisons of means were made. However, in dose-response studies, the mean percentage changes induced by different doses of U50,488H were compared with zero in a manner analogous to that used in *t* tests for differences.

Results

Figure 1 shows the effects of U-50,488H on peak systolic left ventricular pressure and heart rate in terms of changes from the pre-U-50,488H values in isolated hearts. Corresponding changes in ECG intervals recorded from rat isolated perfused hearts are shown in Figure 2.

U-50,488H dose-dependently reduced peak-systolic left ventricular pressure and heart rate, while the maximum rate of intra-ventricular pressure development ($+dp.dt^{-1}_{max}$) was also reduced (data not shown). It also dose-dependently prolonged both P–R interval and QRS duration. All of the above effects were still present in the presence of 1 μM naloxone. The only notable difference between the dose-response curves in the presence or absence of naloxone was for a tendency for higher peak systolic pressure in the presence of naloxone. The effects of naloxone on isolated hearts were not statistically different from those of saline alone.

In intact pentobarbitone-anaesthetized rats, U-50,488H dose-dependently lowered both blood pressure and heart rate (Figure 3a). In addition, it prolonged the P–R and Q–T intervals and widened the QRS complex in a dose-dependent manner (Figure 3b). In both Figure 3a and b changes from pre-drug values are shown. Statistically significant depression of blood pressure and heart rate occurred after 0.5 μmol kg^{-1} whereas larger doses (2–4 μmol kg^{-1}) had to be given before statistically significant changes were seen in P–R interval and QRS width. In the separate experiment summarized in Table 1, blood pressure, heart rate and ECG changes induced by 16 μmol kg^{-1} U-50,488H were not prevented by pretreatment

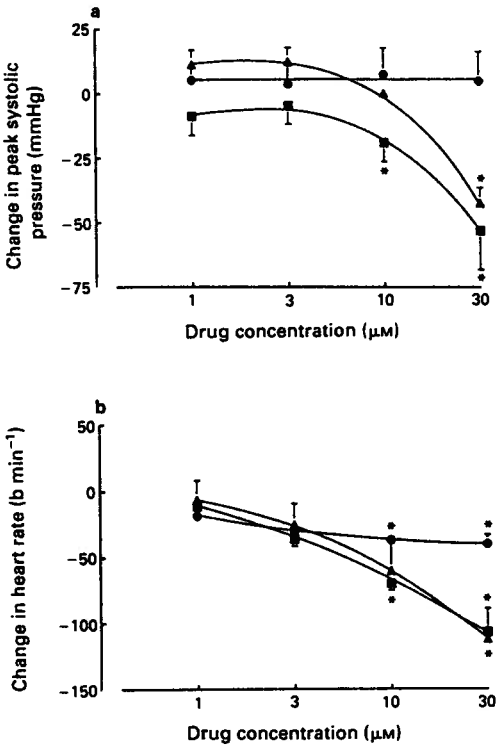

Figure 1 Dose-related effects of U-50,488H on peak systolic ventricular pressure and heart rate in rat isolated perfused hearts: (a) shows changes, from pre-drug values, in peak systolic pressure (mmHg) and (b) heart rate (beats min^{-1}) from pre U-50,488H values. The symbol (●) is for data from control animals treated with saline alone, (■) for U-50,488H treatment, and (▲) for U-50,488H in the presence of naloxone (1.0 μM). Each point is mean with s.e.mean shown by vertical bars, $n = 5$. * $P < 0.05$ for difference from zero change. The pre U-50,488H, or saline, means ranged from 259 ± 11 to 269 ± 12 beats min^{-1} for heart rate and 129 ± 5 to 145 ± 8 mmHg for peak systolic left ventricular pressure.

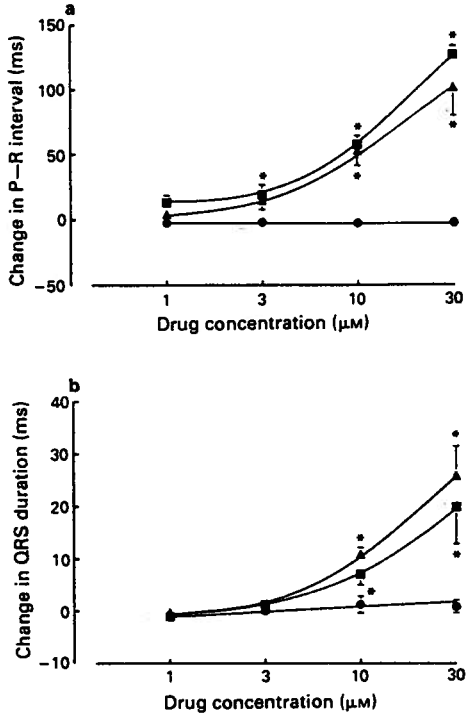

Figure 2 Dose-related effects of U-50,488H on ECG changes in rat isolated perfused hearts: (a) changes, from pre-drug values, in P-R interval (ms) and (b) QRS width (ms). The symbol (●) is for saline control alone, (■) for U-50,488H and (▲) for U-50,488H in the presence of naloxone (1.0 μM). Values are mean with s.e.mean shown by vertical bars, $n = 5$. * $P < 0.05$ for difference from zero change. The pre U-50,488H, or saline, means ranged from 45 ± 2 to 47 ± 3 ms, for P–R, and 33 ± 1 to 35 ± 1 ms for QRS.

with high doses of either MR 2266 or naloxone. The antagonist drugs alone had limited effects; naloxone did not change any of the variables whereas MR 2266 induced slight bradycardia and P–R prolongation (Table 1).

Table 1 The effects of U-50,488H at a dose of 16 μmol kg^{-1} on blood pressure, heart rate and ECG in pentobarbitone-anaesthetized rats in the absence or presence of the opioid antagonists, naloxone and MR 2266 (both at 8 μmol kg^{-1})

Treatment	*Pretreatment*	*Post-treatment*	*Post U-50,488H*	%
		BP (mmHg)		
Saline	132 ± 4	136 ± 6	107 ± 5†	(−20 ± 6)†
Naloxone	137 ± 6	143 ± 5	120 ± 8†	(−16 ± 7)†
MR 2266	136 ± 7	142 ± 9	118 ± 11†	(−18 ± 4)†
		HR (beats min^{-1})		
Saline	365 ± 13	364 ± 6	261 ± 14†	(−25 ± 2)†
Naloxone	405 ± 20	403 ± 25	323 ± 24†	(−20 ± 3)†
MR 2266	389 ± 1	337 ± 20*	271 ± 12†	(−22 ± 3)†
		P–R (ms)		
Saline	49 ± 1	48 ± 1	58 ± 2†	(+18 ± 4)†
Naloxone	47 ± 2	48 ± 2	57 ± 2†	(+20 ± 3)†
MR 2266	48 ± 1*	54 ± 2*	58 ± 2†	(+8 ± 3)†
		QRS (ms)		
Vehicle	29.6 ± 0.7	30.0 ± 0.7	32.8 ± 0.4†	(+10 ± 2)†
Naloxone	28.6 ± 0.4	29.4 ± 0.2	31.8 ± 0.2†	(+8 ± 2)†
MR 2266	30.0 ± 0.8	31.1 ± 0.5	33.8 ± 0.2†	(+9 ± 2)†

Values are mean ± s.e.mean, $n = 5$. BP = mean blood pressure; HR = heart rate. Values in parentheses are the percentage changes between post-treatment and post U-50,488H values. After administration of U-50,488H all values were statistically significantly (†) different ($P < 0.05$) from the pre-U-50,488H, i.e. post-saline, naloxone or MR 2266 treatment values. * $P < 0.05$ for means after treatment with either saline, naloxone or MR 2266 versus pretreatment means.

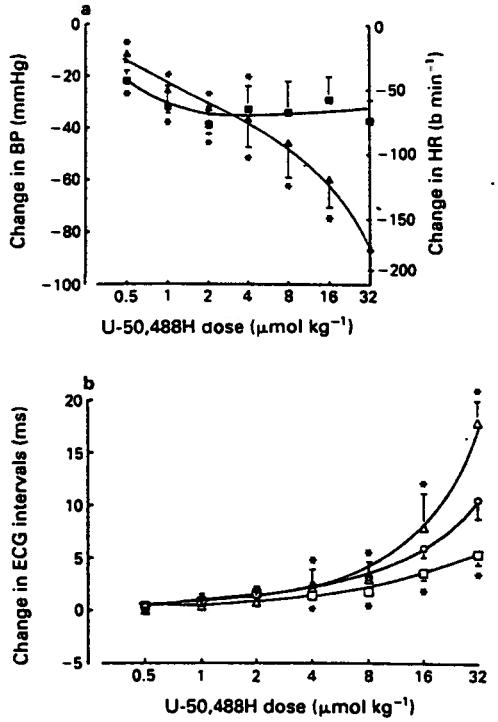

Figure 3 Dose-related effects of U-50,488H on blood pressure, heart rate and the ECG in pentobarbitone-anaesthetized rats: (a) changes, from pre-drug values, in blood pressure (mmHg) and heart rate (beats min^{-1}) while (b) shows the corresponding changes in P–R, QRS and Q–T (ms). The symbol (■) is for blood pressure, (▲) for heart rate, (△) for P–R interval, (□) for QRS and (○) for Q–T. Values are mean with s.e.mean shown by vertical bars, $n = 5$–10. Significant difference from zero change at $^*P < 0.05$. The pretreatment means were 123 ± 8 mmHg for blood pressure, 381 ± 26 for heart rate, 47 ± 1 ms for P–R, 29 ± 1 for QRS and 42 ± 1 for Q–T.

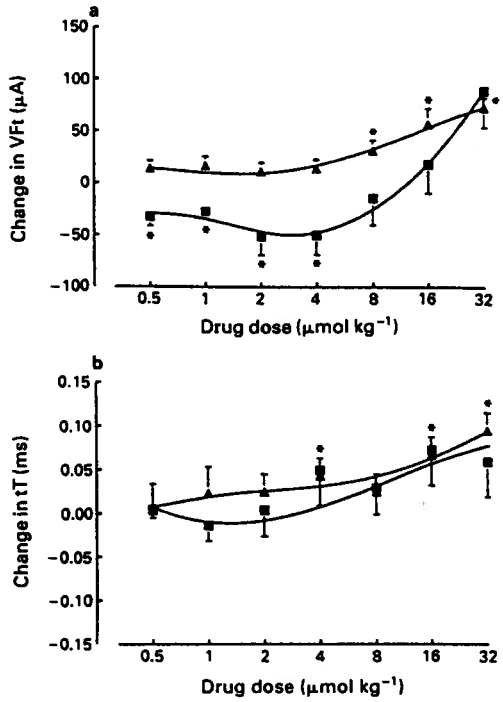

Figure 4 Effects of U-50,488H on ventricular fibrillation threshold (VFt) (a) and threshold pulse width (tT) (b) in pentobarbitone-anaesthetized rats subject to electrical stimulation. The symbol (■) indicates changes from pre-drug values with U-50,488H alone, or in the presence of naloxone pretreatment (▲). Saline control values are not shown but are indicated in the text. Values are mean with s.e.mean shown by vertical bars, $n = 5$–12. $^*P < 0.05$ for difference from zero change. Control (pre-drug) values ranged from 208 ± 15 to $265 \pm 25\,\mu$A for VFt and 53 ± 3 to 54 ± 4 ms for tT.

Figures 4 and 5 summarize the effects of U-50,488H on responses to electrical stimulation in intact rats expressed as changes from values just prior to administration of U-50,488H. Data for saline control rats showed no changes with time over the experimental period. Thus values at the beginning and end of the experimental period in the saline-treated group were 240 ± 25 and $294 \pm 36\,\mu$A for VFt, 0.32 ± 0.04 and 0.27 ± 0.03 ms for tT, 53 ± 3 and 48 ± 4 ms for ERP and 13.9 ± 0.6 and 14.7 ± 0.7 Hz for MFF, respectively.

The dose-dependent changes in VFt produced by U-50,488H (Figure 4) were biphasic in nature. Reductions occurred over the dose range of 0.5–4 μmol kg^{-1} while VFt increased with dose after attaining a minimum value at 4 μmol kg^{-1}. The initial phase of reduced values was greatly attenuated by pretreatment with naloxone. In Figure 4 it can be seen that tT was prolonged in a dose-related manner with only a suggestion of an initial fall and this was not seen after naloxone pretreatment. Similar findings were made with iT. In the absence of naloxone, iT initially fell by $-30 \pm 10\,\mu$A ($P < 0.05$) after a total dose of 4 μmol kg^{-1} and then increased with dose to reach a value of $+79 \pm 34\,\mu$A ($P < 0.05$). With naloxone pretreatment no significant initial fall was seen and iT rose in a monotonic manner with increasing dose.

ERP lengthened dose-dependently at all dose levels (Figure 5a) in a monotonic dose-dependent manner and this was not influenced by naloxone pretreatment. In keeping with the prolongation of ERP, the closely related but different variable, MFF, was dose-dependently reduced. This change was accentuated rather than reduced by pretreatment with 8 μmol kg^{-1} naloxone.

Naloxone, *in vivo*, had no statistically significant effects on electrical stimulation. Thus differences before and after naloxone were $+19 \pm 12\,\mu$A for iT, $+32 \pm 18\,\mu$A for VFt, -0.01 ± 0.03 ms for tT, -0.1 ± 0.8 Hz for MFF and $+2 \pm 4$ ms for ERP. None of these changes was statistically significant.

Discussion

The present study revealed some interesting actions of U-50,488H on rat hearts. These actions occurred at doses and concentrations above those required for κ-agonism and for the most part were not abolished by naloxone. In isolated hearts a naloxone-resistant depression of contractility, rate and the ECG were seen. Similar depression *in vivo* would account at least in part, for reductions in blood pressure and heart rate seen in intact rats.

In both isolated and intact hearts, QRS width and P–R interval were prolonged by U-50,488H in a manner unaffected by naloxone. It is generally accepted that Class I antiarrhythmics, i.e. myocardial sodium channel blockers, can produce increases in QRS width. Prolongation of the P–R interval can be produced by a variety of different drugs including Class I antiarrhythmics.

U-50,488H, at the relatively high doses and concentrations used in this study produced significant actions on responses to electrical stimulation. These included increases in iT, tT and VFt. These responses were characteristic of those produced by blockade of sodium channels in rat heart (Abraham *et al.*, 1989). However, it was noticeable that lower concentrations of U-50,488H, i.e. those most likely to be associated with selective effects on opioid receptors, reduced VFt in a manner that was attenuated by naloxone. The same pattern, but to a less marked extent, was seen with iT and tT. Thus, especially in

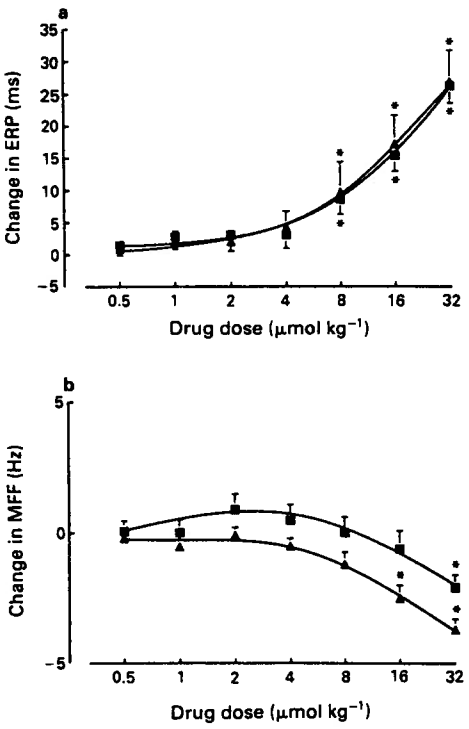

Figure 5 Effects of U-50,488H on effective refractory period (ms) (a) and maximum following frequency (Hz) (b) in pentobarbitone-anaesthetized rats subject to electrical stimulation. The symbol (■) indicates changes from pre-drug values with U-50,488H alone, or in the presence of naloxone pretreatment (▲). Saline control values are not shown but are indicated in the text. Values are mean with s.e.mean shown by vertical bars, $n = 5$–12. * $P < 0.05$ for difference from zero change. Control values ranged from 53 ± 3 to 58 ± 3 ms for ERP and 13.4 ± 0.4 to 15.3 ± 1.0 Hz for MFF.

the case of VFt, U-50,488H appeared to have two actions; a lower dose and possibly arrhythmogenic effect mediated through opioid receptors, and a higher dose and possible anti-arrhythmic effect independent of opioid receptors.

ERP was increased and MFF decreased in a dose-dependent manner by U-50,488H. These are characteristic actions of drugs which block sodium channels and/or prolong action potential duration. U-50,488H prolonged Q–T in the manner typical of a Class Ia antiarrhythmic drug.

In summary the profile of action of U-50,488H, especially at higher doses and concentrations, was consistent with sodium channel blockade. Depression of blood pressure, or contractility, and heart rate, prolongation of P–R and QRS, elevation of thresholds for induction of ventricular fibrillation or capture, are all produced by sodium channel blockade as has been illustrated previously with tetrodotoxin (Abraham *et al.*, 1989). Prolongation of ERP can be expected to occur with Class Ia sodium channel blockers and Class III anti-arrhythmics. It should be noted that for the majority of the measured variables there was a clear dose-response relationship. Even in the case of VFt, where such a dose-response relationship was not so obvious, because of the initial fall, blockade with naloxone clearly revealed the same underlying trend as seen with other variables. In concert with this, preliminary studies (unpublished observations) in rat isolated cardiac cells show that U-50,488H blocks sodium currents in these cells at concentrations found effective in rat isolated hearts.

The relationship between the above findings and potential arrhythmogenic or antiarrhythmic actions of U-50,488H are complex. Thus reductions in VFt seen at lower doses can be interpreted as being arrhythmogenic whereas sodium channel blockade can be either antiarrhythmic or arrhythmogenic in a manner that depends both upon dose and prevailing conditions. Thus U-50,488H may be biphasic in action: arrhythmogenic or pro-arrhythmic, at lower doses, and antiarrhythmic at higher doses. Such actions agree with previous findings in another study in which U-50,488H induced arrhythmias in rat isolated hearts when given in bolus doses (Wong *et al.*, 1990). They also are in agreement with the suggestion that opioid agonists and antagonists have anti-arrhythmic actions independent of opioid receptors (Sarne *et al.*, 1991).

At lower doses the possible arrhythmogenic action of U-50,488H on VFt was blocked by naloxone, indicating an opioid receptor-mediated effect, a finding in agreement with the suggestions of Parratt & Sitsapesan, (1986) and Wong & Lee (1987). Thus the present study helps explain discrepancies as to the effects of opioid agonists and antagonists on arrhythmias (see Sarne *et al.*, 1990).

In a separate study (unpublished observations), we found that U-50,488H, given at a single dose of 8 μmol kg^{-1} had antiarrhythmic actions against arrhythmias induced by occlusion of a coronary artery in anaesthetized rats. These antiarrhythmic actions were not blocked by pretreatment with naloxone. In the present study the same dose, albeit given cumulatively rather than as a single dose, gave incomplete indications of sodium channel blockade. Thus P–R and QRS were both increased by this dose. On the other hand Q–T was increased as was ERP, findings associated with Class Ia actions and/or potassium channel blockade. The 8 μmol kg^{-1} (cumulative) dose was associated with an increased VFT in presence of naloxone but not in its absence, an indication, perhaps, of the presence of sodium blockade masked by an equal but opposite action mediated via κ-agonism. It remains to be tested whether with lower doses, where κ-agonism would be expected to predominate, proarrhythmic actions occur with coronary occlusion in the rat.

In conclusion, the present study provides evidence that U-50,488H, a κ-receptor agonist, can have complex cardiovascular actions. Some of the effects may be mediated via opioid receptors while others, especially at higher doses, are independent of opioid receptors and may involve sodium channel blockade. The exact relationship between the findings in this study and effects on arrhythmias have still to be fully explained.

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APPENDIX 2

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Antiarrhythmic effects of U-50,488H in rats subject to coronary artery occlusion

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The antiarrhythmic actions of low and high doses of U-50,488H, a selective κ-receptor agonist, were examined in pentobarbitone-anaesthetized rats subjected to occlusion of the left anterior descending coronary artery. At a high dose (16 μmol/kg) U-50,488H reduced blood pressure, heart rate and prolonged the P-R and QRS intervals of the electrocardiogram. This dose reduced the incidence of ventricular arrhythmias produced by occlusion. The blood pressure, heart rate, ECG and antiarrhythmic actions of a high dose of U-50,488H were not antagonized by 8 μmol/kg naloxone, a dose which had no cardiovascular or ECG actions. Naloxone alone reduced arrhythmia incidence but to a lesser extent than U-50,488H. A low dose (0.2 μmol/kg) of U50,488H in the absence or presence of naloxone had no effect on arrhythmias. Thus, U-50,488H had antiarrhythmic actions at a high dose which were independent of opioid receptors.

Antiarrhythmic activity; Ischaemic arrhythmias; U-50,488H; Naloxone

1. Introduction

In a previous study, we found that U-50,488H, a specific κ-receptor agonist (Lahti et al., 1982), prolonged both the P-R interval and QRS duration in vitro and in vivo and that these actions could not be blocked by naloxone (Pugsley et al., in press). Doses producing the above actions increased the resistance of the heart to electrical stimulation in a manner expected of a sodium channel blocking drug. In unpublished observations in isolated rat heart cells U-50,488H was found to block sodium-dependent currents. In the above study, U-50,488H also increased refractory periods. Such results suggested that U-50,488H may block sodium channels and have antiarrhythmic properties. At doses below 1 μmol/kg none of the above actions were seen whereas doses below 1 μmol/kg are capable of stimulating κ-opioid receptors.

Both opioid agonists and antagonists have been reported to change the incidence of arrhythmias induced by myocardial ischaemia in the rat. For example, Frame and Argentieri (1985) reported that high concentrations of naloxone have properties similar to Class I antiarrhythmics. However, Pruett et al. (1991) showed that concentrations of naloxone, between 2–10 μM, had no effect on action potential morphology.

We have examined the antiarrhythmic actions of U-50,488H and naloxone on ischaemia-induced arrhythmias. In order to assess the antiarrhythmic efficacy of U-50,488H, we investigated its actions at doses of 0.2 and 16 μmol/kg on the incidence of cardiac arrhythmias in pentobarbitone-anaesthetized rats subject to coronary artery occlusion. Our results indicate that U-50,488H, at the dose of 16 μmol/kg, has antiarrhythmic actions but not at a lower dose of 0.2 μmol/kg. The latter dose would be expected to only have actions on κ-receptors and had previously been shown to be pro-arrhythmic in a model similar to that used in this study (Lin et al., 1991).

2. Materials and methods

Male Sprague-Dawley rats (Charles River Laboratories, Montreal, Quebec) weighing 150–200 g were used for all experiments. All experiments were conducted according to guidelines established by the U.B.C. Animal Care Committee.

2.1. Experimental protocol

Rats were subjected to preparative surgery under pentobarbitone anaesthesia and then assigned to one

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of five groups in a random block design. Groups 1–5 contained 15 rats whereas groups 6 and 7 contained 10 rats. The groups were: (1) saline vehicle; (2) atenolol (30 μmol/kg); (3) U-50,488H (16 μmol/kg); (4) naloxone (8 μmol/kg); (5) naloxone (8 μmol/kg) plus U-50,488H (16 μmol/kg); (6) U-50,488H (0.2 μmol/kg); (7) naloxone (8 μmol/kg) plus U-50,488H (0.2 μmol/kg).

Experimental design and analyses were performed according to the Lambeth Conventions (Walker et al., 1988).

2.2. *Surgical techniques and coronary artery occlusion study*

The surgical procedures used were similar to those previously employed by Au et al. (1979) and Paletta et al. (1989). In brief, rats were initially anaesthetized with pentobarbitone (60 mg/kg i.p.) and supplemental doses (1/10 dilution) were given i.v. when necessary to ensure an adequate level of anaesthesia. The trachea was cannulated and all animals were artificially ventilated at a stroke volume of 10 ml/kg and at a rate of 60 strokes/min, a regimen which keeps blood gases at normal levels in the rat (MacLean and Hiley, 1988). The left carotid artery was cannulated for measurement of mean arterial blood pressure, and withdrawal of blood samples for determination of serum K^+ concentrations (Ionetics Potassium Analyzer). The right jugular vein was also cannulated for injection of drugs.

The thoracic cavity was opened and a polyethylene occluder loosely placed around the left anterior descending coronary artery. The chest cavity was closed and the rat allowed to recover for 30 min. Body temperature was maintained between 35–37°C.

The ECG was recorded by insertion of ECG electrodes placed along the anatomical axis of the heart determined by palpation. The superior electrode was placed at the level of the right clavicle, approximately 0.5 cm from the midline, and the inferior electrode was placed on the left side of the thorax, 0.5 cm from the midline and at the level of the ninth rib.

In a random and double-blind manner rats were given an initial injection of either saline (groups 1, 2, 3 and 6) or 8 μmol/kg naloxone (groups 4, 5 and 7). A second injection of saline vehicle, or appropriate drug, was given 5 min later. Blood pressure and ECG were recorded 5 min after injection and a blood sample (approximately 1.0 ml) taken 5 min after the second injection. Thereafter the occluder was pulled so as to produce coronary artery occlusion.

ECG, arrhythmias, blood pressure, heart rate and mortality were monitored for 30 min after occlusion. Arrhythmias were recorded as ventricular premature beats (VPB), ventricular tachycardia (VT) and ventricular fibrillation (VF) and summarized as an arrhythmia score (AS) described by Curtis and Walker (1988). An animal's AS summarizes its post occlusion arrhythmic history in one single value. At the end of the 30 min period, if the animal survived, a second blood sample was taken.

After death, hearts were removed and perfused by the Langendorff technique with Krebs-Henseleit solution to wash out all remaining blood. This was followed by cardiogreen dye (1 mg/ml) perfusion which revealed the underperfused occluded zone (zone-at-risk).

2.3. *Exclusion criteria*

Throughout the experiment the following criteria were used to retain rats in the study: (1) mean arterial blood pressure of the animal did not fall below 25 mm Hg for more than 5 min; (2) pre-occlusion ECG had a recognizable P-wave, QRS complex and T-wave, was in normal sinus rhythm, and the height of the S-T segment was lower than the R-wave; (3) pre-occlusion arrhythmias were no more than 15 VPB and no VT or VF; (4) pre-occlusion serum potassium concentrations were within the range of 2.9–3.9 mM; (5) occlusion was associated with increases in R-wave height and 'S-T' segment elevation. The 'S-T' segment height was measured before and after occlusion and changes induced by occlusion were expressed as a percentage of R-wave size (Johnston et al., 1983); (6) the size of the occluded zone (zone-at-risk) was in the range of 25–50% of total left-ventricular weight.

Any rat failing to meet these criteria was immediately excluded from the study and replaced with another rat.

2.4. *Drug treatment*

U-50,488H (The Upjohn Co, Kalamazoo, MI) was given at either 0.2 or 16 μmol/kg. The latter dose has previously been shown to reduce blood pressure and heart rate while prolonging P-R interval and QRS duration in anaesthetized rats (Pugsley et al., in press). The former dose had no effects on blood pressure, heart rate or the ECG.

Naloxone (Du Pont Pharmaceutical Co., Scarborough, Ontario) was given at 8 μmol/kg; a dose which antagonizes the actions of κ-agonists in vivo (Sarne et al., 1991).

The dose of atenolol (Sigma Chemical Co., St. Louis, MO), 30 μmol/kg, was chosen to reduce blood pressure and heart rate (Paletta et al., 1989) to levels similar to those observed with 16 μmol/kg U-50,488H.

In a subsiduary experiment dose–response (blood pressure) curves to noradrenaline were obtained before and after the administration of 16 μmol/kg U-50,488H. In trials involving three anaesthetized rats no evidence of α-receptor blockade was observed.

TABLE 1

The blood pressure, heart rate and ECG effects of U-50,488H, atenolol, and naloxone in pentobarbitone-anaesthetized rats before coronary artery occlusion. Values are means ± S.E.M. with n = 15 animals/group, except in the last two groups where n = 10. U-50 = U-50,488H (16 and 0.2 μmol/kg) and Nal = naloxone (8 μmol/kg). Atenolol was given at 30 μmol/kg.

Group	BP (mm Hg)	HR (bpm)	P-R (ms)	QRS (ms)	Q-T (ms)
Saline	103 ± 5	394 ± 11	53 ± 1	31 ± 0.4	42 ± 1
Atenolol	82 ± 4 [a]	346 ± 10 [a]	57 ± 1 [a]	32 ± 0.2	41 ± 1
Nal	102 ± 4	396 ± 12	57 ± 1 [a]	31 ± 0.3	41 ± 1
U-50 (16)	79 ± 6 [a]	252 ± 8 [a]	69 ± 2 [a]	35 ± 1.0 [a]	46 ± 1 [a]
U-50 (16) + Nal	82 ± 6 [a]	285 ± 9 [a]	63 ± 2 [a]	34 ± 0.5 [a]	44 ± 1 [a]
U-50 (0.2)	93 ± 6	369 ± 11	56 ± 1	33 ± 1.6	40 ± 1
U-50 (0.2) + Nal	112 ± 5	398 ± 11	57 ± 1	31 ± 1.0	39 ± 1

[a] Indicates significant difference from saline at $P < 0.05$.

TABLE 2

Table showing the antiarrhythmic properties of U-50,488H compared to naloxone and atenolol on arrhythmia incidence during coronary artery occlusion in pentobarbitone-anaesthetized rats. Values are expressed as the percent of animals experiencing particular arrhythmias or means ± S.E.M. for AS. U-50 = U-50,488H (16 and 0.2 μmol/kg); Nal = naloxone (8 μmol/kg); VT = ventricular tachycardia and VF = ventricular fibrillation.

Group	Incidence of VT and VF (%)			AS
	VT	VF	VT and/or VF	
Saline	100	67	100	5.1 ± 0.6
Atenolol	93	40	93	4.0 ± 0.5
Nal	87	20 [a]	87	3.0 ± 0.2 [a]
U-50 (16)	40 [a]	7 [a]	40 [a]	1.5 ± 0.4 [a]
U-50 (16) + Nal	40 [a]	13 [a]	40 [a]	1.7 ± 0.5 [a]
U-50 (0.2)	100	60	100	4.7 ± 0.5
U-50 (0.2) + Nal	100	60	100	4.5 ± 0.6

[a] Indicates significant difference from saline at $P < 0.05$ for N = 15 animals/group except for the last two grops where n = 10.

2.5. *Statistical analysis*

Values are mean ± S.E.M. Statistical significance was determined using the NCSS statistical package (Hintze, 1987). The general linear model analysis of variance was used and multiple comparisons were made using Duncan's test. Arrhythmia incidence was assessed by Mainlands contingency tables (Mainland et al., 1956) and the VPB number was $\log_{10}$ transformed before analysis.

3. Results

The in vivo actions of the high dose of U-50,488H were similar to those found previously (Pugsley et al., in press) in that a dose of 16 μmol/kg, i.v., significantly decreased both blood pressure and heart rate. This same dose also prolonged both P-R and QT intervals in addition to QRS duration (table 1). These effects were not antagonized by naloxone at a dose of 8 μmol/kg. A much smaller (0.2 μmol/kg) dose produced none of the effects of the high dose.

Naloxone alone had no effect on any of the variables except for P-R interval which was prolonged from 53 ± 1 ms to 57 ± 1 ms ($P < 0.05$). Atenolol decreased blood pressure to the same degree as U-50,488H; however, it did not reduce heart rate to the level seen after 16 μmol/kg. Atenolol prolonged the P-R interval to the same extent as naloxone, yet had no effects on any of the other ECG variables (table 1). Naloxone did not antagonize any of the blood pressure, heart rate and ECG effects of U-50,488H.

TABLE 3

Table showing the occluded zone (OZ) Size and serum K^+ levels in pentobarbitone-anaesthetized rats subject to coronary artery occlusion. N = 10–15 as indicated except in the post-occlusion serum potassium groups where n was reduced after occlusion (n value in parentheses). Values are means ± S.E.M.

Group	N	OZ (%)	Serum K^+ levels (mM)	
			Pre-occlusion	Post-occlusion
Saline	15	32.5 ± 0.8	3.4 ± 0.1	3.6 ± 0.2 (6)
Atenolol	15	34.6 ± 1.5	3.4 ± 0.1	3.7 ± 0.1 (10)
Naloxone	15	30.0 ± 1.0	3.3 ± 0.1	3.6 ± 0.1 (13)
U-50 (16)	15	31.2 ± 1.0	3.3 ± 0.1	4.0 ± 0.2 (14)
U-50 (16) + Nal	15	30.7 ± 1.4	3.4 ± 0.1	3.9 ± 0.1 (14)
U-50 (0.2)	10	32.0 ± 1.2	3.5 ± 0.1	3.7 ± 0.2 (7)
U-50 (0.2) + Nal	10	33.6 ± 1.0	3.3 ± 0.1	3.6 ± 0.4 (7)

With respect to the incidence of arrhythmias induced by coronary artery occlusion, it can be seen from table 2 that atenolol at a dose of 30 μmol/kg did not reduce the severity nor incidence of arrhythmias. Naloxone alone, however, reduced the incidence of VF as exemplified by the reduction in AS, but did not reduce the incidence of VT. This action of naloxone was not seen when it was given in combination with 0.2 μmol/kg U-50,488H.

On the other hand, the high dose of U-50,488H statistically significantly reduced both the incidence and severity of all arrhythmias. This was most noticeable with reduction in VF. The incidences of VT and VF were not different between U-50,488H alone or when naloxone was given prior to U-50,488H. On the otherhand 0.2 μmol/kg failed to influence arrhythmias in the absence or presence of naloxone.

The arrhythmic insult to all groups were assumed to be the same since the size of the occluded zones (zone-at-risk) did not differ between groups. In a similar manner serum potassium concentrations before and after occlusion were the same in all groups, as is shown in table 3.

4. Discussion

In this study, we examined the effects of low and high doses of U-50,488H, a κ-receptor agonist on blood pressure, heart rate and incidence of arrhythmias in pentobarbitone-anaesthetized rats subjected to occlusion of the left anterior descending coronary artery. The high dose of U-50,488H decreased both blood pressure and heart rate and prolonged the P-R and QRS intervals (table 1) confirming our findings in a previous study of U-50,488H's actions on blood pressure and the ECG (Pugsley et al., in press). The high dose, but not the low dose, also reduced the incidence of severe ventricular arrhythmias; a finding which is in agreement with previous findings which showed that U-50,488H over the dose range of 16–32 μmol/kg increased resistance to the electrical induction of VT (Pugsley et al., in press).

In neither this or the other study were the actions of U-50,488H antagonized by naloxone when the latter was given at a dose which has been shown to antagonize the actions of κ-agonists (Sarne et al., 1991). We suggest that such non-opioid actions of U-50,488H are the result of sodium channel blockade. U-50,488H has previously been shown to have such an ion channel blocking action in neuronal tissue by Alzheimer and Ten Bruggencate (1990).

The evidence for a direct antiarrhythmic action of U-50,488H against ischemia-induced arrhythmias includes the fact that such actions cannot be ascribed to opioid receptor actions, or to changes in blood pressure or heart rate. The indirect evidence for sodium channel blocking actions is strong in that U-50,488H prolonged P-R interval and QRS width and had the actions expected of a sodium channel blocker in electrical stimulation studies (Pugsley et al., in press) as well as blocking sodium currents in isolated rat heart cells (unpublished observations).

There was a slight, but not statistically significant elevation of serum K^+ concentrations associated with the administration of U-50,488H (table 3) but this was not enough to protect against ischaemia-induced arrhythmias. Previous studies have shown the protective effects of raised serum potassium concentrations in this model (Curtis et al., 1985). Similarly atenolol had little action on ischemia-induced arrhythmias which is in accord with previous studies (Paletta et al., 1989).

While naloxone did not antagonize the actions of U-50,488H on the ECG or arrhythmias, it did show some antiarrhythmic actions of its own. Previous studies have suggested a complex relationship between opioid receptors and arrhythmias in anaesthetised rats subjected to coronary artery occlusion (Fagbemi et al., 1982; Sarne et al., 1991).

In conclusion, the present study provides evidence that U-50,488H possesses antiarrhythmic activity against ischaemia-induced arrhythmias. We suggest here that this antiarrhythmic action is dependent upon sodium channel blocking actions and is possibly similar to Class Ia antiarrhythmics. These actions occurred at a high dose with respect to actions on the κ-opioid receptor but not with respect to antiarrhythmic doses of sodium channel blocking antiarrhythmics. The antiarrhythmic actions described here may not have any particular relevance to the clinical use of U-50,488H as a κ-receptor agonist but to ancillary pharmacological actions of this compound which can be seen at high doses.

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APPENDIX 3

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Electrophysiological and antiarrhythmic actions of the κ agonist PD 129290, and its R,R (+)-enantiomer, PD 129289

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1 The S,S (−)-enantiomer PD 129290, a κ agonist, and its corresponding inactive R,R (+)-enantiomer (PD 129289) were studied in rat isolated hearts and in intact rats for cardiovascular and antiarrhythmic actions. The electrophysiological actions of PD 129290 were also studied in rat isolated cardiac myocytes using voltage clamp.

2 Ventricular pressure, heart rate and ECG were studied in isolated hearts while blood pressure, heart rate and ECG were studied in pentobarbitone-anaesthetized rats. In the latter, responses to electrical stimulation and coronary occlusion were also investigated.

3 In isolated hearts both enantiomers, over the concentration range 2–16 μM, dose-dependently reduced systolic ventricular pressure and heart rate while prolonging the P–R and QRS intervals of the ECG.

4 At doses of 1–32 μmol kg^{-1} both enantiomers reduced blood pressure and heart rate in anaesthetized rats. In addition, both enantiomers increased the size of the RSh and increased P–R, QRS, and Q–T intervals of the ECG. The thresholds for premature beats and ventricular fibrillation were dose-dependently increased by PD 129289. At lower doses PD 129290 decreased thresholds. These decreases were blocked by naloxone to reveal underlying increases similar to those seen with PD 129289. Both enantiomers increased refractory periods.

5 Naloxone (8 μmol kg^{-1}) did not alter any of the actions of PD 129290, except to abolish the initial decreases in thresholds in intact rats seen with lower doses of PD 129290.

6 Both enantiomers (2 and 8 μmol kg^{-1}) equally reduced arrhythmias in anaesthetized rats subject to occlusion of a coronary artery.

7 In rat isolated cardiac myocytes 20 μM PD 129290, in the presence and absence of naloxone decreased the amplitude of the transient sodium current by about 50% without affecting the voltage-dependence of activation or inactivation of this current.

8 The antiarrhythmic actions of both enantiomers appear to primarily result from their Class I (sodium channel blockade) properties which are independent of κ agonism.

Keywords: Opioid; antiarrhythmic; sodium channel; κ agonist

Introduction

A range of different studies in rats have demonstrated that a wide spectrum of opioid drugs reduce arrhythmias induced by occlusion of a coronary artery (e.g. Sitsapesan & Parratt, 1989; Pugsley *et al.*, 1992b). The degree of antiarrhythmic protection varied with the opioid drug studied and there was no simple correlation between opioid type (agonist or antagonist) and antiarrhythmic activity. After examining a series of agonist and antagonist opioid drugs, Sarne *et al.* (1991) came to the provisional conclusion that sodium channel blockade was responsible for observed antiarrhythmic actions.

We recently studied the antiarrhythmic actions of the κ agonist, U-50,488H in rats and were able to relate its antiarrhythmic actions to sodium channel blockade (Pugsley *et al.*, 1992a,b) and not to κ agonism. PD 129290, an arylbenzacetamide similar in chemical structure to U50,488H, is a newly developed κ agonist (Hunter *et al.*, 1990). PD 129290 is an S,S (−)-enantiomer whereas PD 129289 is the corresponding R,R (+)-enantiomer which has less than 1/1,000 of the κ receptor binding activity of PD 129290. Its affinity for μ receptors is 0.5 that of the S,S-enantiomer (Halfpenny *et al.*, 1990). In view of the large difference in κ agonist activity between the two enantiomers it was apparent that a comparison of the pharmacological and antiarrhythmic profiles of PD 129289 and PD 129290 might help resolve the mechanisms by which such structurally similar, but pharmacologically different, drugs exert an antiarrhythmic action.

We have examined the actions of PD 129289 and PD 129290 in isolated hearts and in intact rats. The latter were subjected either to coronary occlusion, or to electrical stimulation of the left ventricle. The present studies are analogous to those recently completed with U50,488H (Pugsley *et al.*, 1992a,b); similar doses and concentrations were used in both studies. In the U50,488H studies possible opioid receptor-dependent (κ and μ) effects *in vivo* and *in vitro* were abolished with naloxone. In the present study we used naloxone for the same purpose and in addition used the enantiomer PD 129289 which is inactive at κ opioid receptors. We have also examined the inhibitory effects of PD 129290 on sodium currents recorded in isolated cardiac myocytes by use of the whole-cell variant of the patch clamp technique.

Methods

General

Male Sprague-Dawley rats (Charles River Laboratories, Montreal, Quebec) weighing 150–200 g were used for all experiments conducted at UBC. The isolated cardiac myocyte experiments conducted at Canberra used the available male Wistar (200–300 g) strain rats. All experiments conducted at U.B.C. were according to guidelines enforced by the U.B.C.

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Animal Care Committee while those performed in Australia were according to the guidelines used by the Australian National University.

Intact rats were anaesthetized with pentobarbitone (60 mg kg^{-1}, i.p.) and the trachea cannulated for artificial ventilation at a stroke volume of 10 ml kg^{-1}, 60 × min^{-1}. Body temperature was monitored by a rectal thermometer and maintained between 36–37°C with a heating lamp.

Isolated hearts

Hearts were mounted on a modified Langendorff apparatus (Curtis *et al.*, 1986) and perfused at a pressure of 100 mmHg with Krebs-Henseleit solution (pH 7.4), bubbled with 5% CO_2 and O_2 and maintained at a temperature of 35°C. A compliant saline-filled balloon was inserted into the left ventricle and inflated with approximately 0.5 ml saline to a diastolic pressure of 10 mmHg. The developed pressure in the balloon was recorded together with the maximum rate of intraventricular pressure development ($+dp.dt_{max}^{-1}$) by means of a Grass differentiator, model 7P20C. ECGs were recorded (band width of 0.1–40 Hz) from silver-ball electrodes placed on the epicardial surfaces of the right atrium and left ventricle.

Hearts weighing between 1.2 and 1.6 g were, according to a random design, perfused for 5 min at each concentration with saline or either of PD 129289 or PD 129290 at 1, 2, 4, 8 and 16 μM.

Intact rats

Dose-response curves were constructed for the effects of PD 129289 and PD 129290 (cumulative doses of 0.5, 1.0, 2.0, 4.0, 8.0, 16.0 and 32.0 μmol kg^{-1}, i.v.) in pentobarbitone-anaesthetized and artificially ventilated rats ($n = 6$ per group). A carotid artery and jugular vein were cannulated for recording blood pressure and administering drugs, respectively. Drug was given over a 2 min period and recordings made 5 min later, i.e. just before the next dose. In a separate experiment naloxone (8 μmol kg^{-1}) was administered just before dosing with PD 129290. This dose of naloxone was the largest which could be given without influencing ECG, blood pressure or heart rate.

P–R, QRS and Q–T intervals of the ECG were measured. Since the Q–T interval in rats does not change with rate (unpublished observations), it was not corrected for heart rate. A new ECG measure, RSh (mV), was used as a reflection of possible sodium channel blockade (Penz *et al.*, 1992). RSh is the difference in mV between the peak of the R-wave and the trough of the S-wave.

In intact rats, electrical stimulation of the left ventricle was performed via two Teflon-coated silver wire stimulating electrodes inserted through the chest wall and implanted in the left ventricle (Walker & Beatch, 1988); the inter-electrode distance was approximately 1 mm. Square wave stimulation was used to determine the threshold current (i_T–μA) and pulse width (t_T–ms) for induction of extrasystoles, threshold currents for induction of ventricular fibrillation (VF_T–μA), effective refractory period (ERP–ms) and maximum following frequency (MFF–Hz) (Howard & Walker, 1990). Although MFF is related to ERP there are differences in their sensitivity to drugs (Walker & Beatch, 1988) and so both measures were made.

Coronary occlusion studies

The surgical procedures used were those of Au *et al.* (1979) and Paletta *et al.* (1989). In brief, rats were anaesthetized with pentobarbitone and artificially ventilated as described above. A carotid artery cannula was used to record blood pressure and to withdraw blood samples for determination of serum K^+ concentrations (Ionetics Potassium Analyzer). A polyethylene occluder was used to occlude the main left coronary artery in animals whose chest cavity was loosely closed after the occluder had been implanted. Rats were allowed to recover for 30 min after surgical preparation.

All rats were given an initial injection of saline except for one group which received 8 μmol kg^{-1} naloxone. In a random and double-blind manner a second injection of saline, or enantiomer was given 5 min later. Blood pressure and ECG were recorded 5 min after injection and a blood sample (0.25 ml) taken. The occluder was then tightened so as to produce occlusion.

ECG, arrhythmias, blood pressure, heart rate and mortality were monitored for 30 min after occlusion. Arrhythmias, consisting of ventricular premature beats (VPB), ventricular tachycardia (VT) and ventricular fibrillation (VF) were recorded and summarized as an arrhythmia score (AS) according to the system of Curtis & Walker (1988). After 30 min, a second blood sample was taken in surviving animals. Hearts were then removed and perfused via the Langendorff technique with cardiogreen dye (1 mg ml^{-1}) to reveal the underperfused occluded zone (zone-at-risk). The treatment groups were a saline control, PD 129290 (2 or 8 μmol kg^{-1}), PD 129289 (2 or 8 μmol kg^{-1}) and PD 129290 (8 μmol kg^{-1}) after pretreatment with naloxone 8 μmol kg^{-1}. Experimental design and analyses were performed according to the Lambeth Conventions (Walker *et al.*, 1986) with replacement of lost animals to maintain balance between groups. Rats were only included in the study providing that: (1) Mean arterial blood pressure did not fall below 25 mmHg for more than 5 min before and after occlusion. (2) Pre-occlusion ECG had well-defined P, QRS and T waves, was in normal sinus rhythm, and the S–T segment was below the R-wave peak. (3) Arrhythmias before occlusion were less than 15 VPB and no VT or VF occurred. (4) Serum potassium was between 2.9 and 3.9 mM. (5) Occlusion produced an increase in R-wave and elevation of the S–T segment (Johnston *et al.*, 1983). (6) Occluded zone size was 25–50% of total left-ventricular weight.

Statistical analyses

Statistical analyses were performed by use of the NCSS statistical package (Hintze, 1987); values are given as mean ± s.e.mean (n = group size). A general linear model ANOVA was used and multiple comparisons were made by Duncan's test. The statistically significant differences in group incidences of ventricular tachycardia (VT) or ventricular fibrillation (VF) were determined by reference to Mainland's contingency tables (Mainland *et al.*, 1956). The number of VPB was $\log_{10}$ transformed before parametric statistical analyses.

Electrophysiological studies of PD 129290 in isolated myocardial cells

In view of a limited supply of the R,R-enantiomer (PD 129289) it was only possible to perform electrophysiological studies on isolated cell using the S,S-enantiomer, PD 129290. Single cardiac myocytes were prepared by enzymic dissociation of rat isolated hearts; the method has been described in detail elsewhere (Saint *et al.*, 1992). Isolated cells were allowed to settle onto a glass slide coated with poly-l-lysine in a chamber of 1 ml volume which was perfused at 1 ml min^{-1}.

Evoked currents were recorded by conventional whole-cell patch clamp techniques. The bathing solution (temperature 34°C) had the following composition in mM: NaCl 130, KCl 5.4, $MgCl_2$ 1.0, $CaCl_2$ 2.0, $CoCl_2$ 5.0, CsCl 5.0, tetraethyl sulphonic acid 10.0, NaOH 5.0, glucose 10.0 with the pH adjusted to pH 7.4. The composition of the electrode solution in mM was: CsF 50.0, NaF 70.0, K-EGTA 20.0, $CaCl_2$ 2.0, tetraethyl sulphonic acid 10.0, ATP-Na_2 5.0, ATP-Mg 5.0, with the pH adjusted to 7.4 with KOH. Microelectrodes were fabricated from borosilicate glass and typically had a resis-

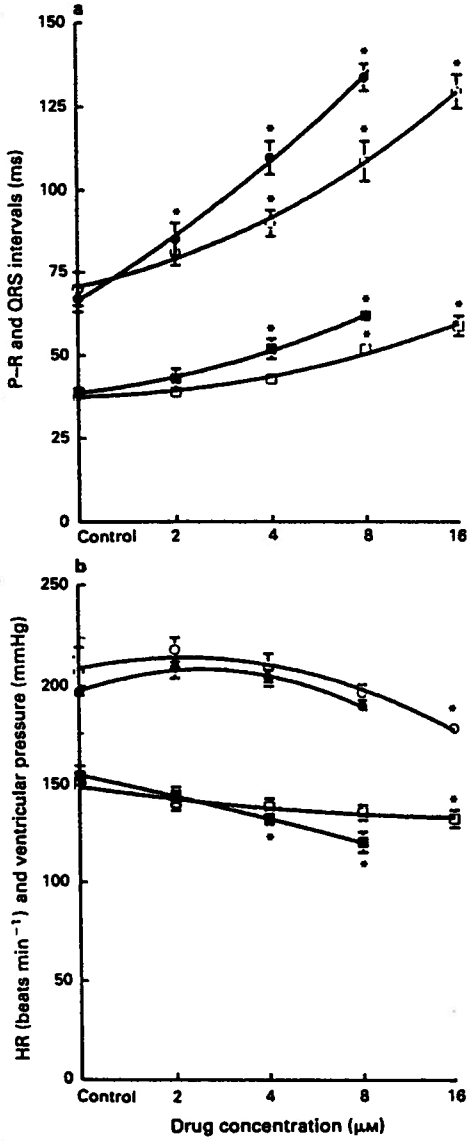

Figure 1 Concentration-response effects of PD 129289 and PD 129290 on ventricular pressure, heart rate and the ECG of rat isolated hearts. Effects of different concentrations of the two enantiomers, PD 129289 (filled symbols) and PD 129290 (open symbols) are shown as changes in ventricular pressure (□), heart rate (○) (b) and the ECG intervals of QRS (□) and P–R (○) (a) from control values after 5 min exposure to different concentrations of drug. Values are mean and vertical lines s.e.mean for $n = 5$. Control values were 154 ± 5 mmHg for ventricular pressure, 197 ± 3 beats min^{-1} for heart rate and 39 ± 2 ms for QRS and 69 ± 4 ms for P–R. *Indicates $P < 0.05$ for statistically significant differences from control.

tance of 10 MΩ. Cells were voltage clamped by use of an Axopatch 1D amplifier. Currents were filtered at 5 KHz, digitized at 10 KHz and stored in a microcomputer which also generated the voltage pulse protocol. Leakage and series resistance compensation was achieved by using the amplifier controls. Voltage pulses were given at 2 s intervals. PD 129290 was applied by adding aliquots of the stock solution of drug to the solution bathing the cells. Naloxone (1 μM) was used to block opioid receptors.

Drugs

PD 129289 (CAM 569; [5R-(5α,7α,8β)]-N-methyl-N[7-(1-pyrrolidinyl)-1-oxaspiro [4.5]dec-8-yl]-4-benzofuranacetamide HCl) and PD 129290 (CAM 570 = CI-977), the corresponding S,S-enantiomer, were generously supplied by Dr J. Hughes, Parke-Davis Research Institute, Cambridge. Naloxone was from DuPont. All drugs were made up in 0.9% NaCl solution for *in vivo* studies and in appropriate bathing solutions for *in vitro* studies.

Results

Isolated heart studies

Both PD 129289 and 129290 had dose-related effects on the ECG recorded from rat isolated perfused hearts. Figure 1 shows that both enantiomers prolonged P–R and QRS intervals although PD 129289 was somewhat more potent (dose-ratio approximately 2.0) than PD 129290; 8 μM of PD 129289 produced an approximate doubling of P–R and a lesser effect on QRS. Heart rate was reduced by PD 129290 but the change was only statistically significant for the highest concentration (Figure 1b). Dose-related bradycardia was not seen with PD 129289. Both enantiomers tended to reduce ventricular pressure (Figure 1b) and $+dp.dt^{-1}_{max}$ (data not shown). For example with PD 129289 the control peak ventricular systolic pressure of 154 ± 5 mmHg was reduced to 120 ± 5 after 8 μM ($P < 0.05$); similar changes occurred with PD 129290.

The experiments in isolated hearts demonstrated that both enantiomers had direct actions on the ECG. ECG indices were more sensitive to the drugs than either systolic ventricular pressure or heart rate.

Effects in intact rats

Blood presure, heart rate and ECG In saline treated intact rats, blood pressure and heart rate were stable over the measurement period whereas both enantiomers produced falls in blood pressure and heart rate. The fall in blood pressure was dose related for PD 129289. With the initial dose of 0.5 μmol kg^{-1} the change in pressure was − 5 ± 10 mmHg from the pre-drug value of 115 ± 8 mmHg. The fall in pressure increased with dose and was − 35 ± 7 mmHg after 32 μmol kg^{-1}. With PD 129290 the fall after 0.5 μmol kg^{-1} was − 25 ± 6 from a control of 121 ± 5 and − 27 ± 3 from 110 ± 6 mmHg in the presence of naloxone pretreatment. Falls after 32 μmol kg^{-1} were − 34 ± 6 and − 33 ± 7 mmHg in the presence and absence of naloxone, respectively. Between the 0.5 and 32 μmol kg^{-1} doses, falls in blood pressure were not obviously dose-related for PD 129290.

Heart rate in intact rats showed dose-related bradycardia with increasing doses of either enantiomer in the presence or absence of naloxone. A fall in heart rate was seen with PD 129289 after a cumulative dose of 2 μmol kg^{-1} and had fallen 98 ± 17 beats min^{-1} (predrug value 355 ± 27) after 32 μmol kg^{-1}. For PD 129290 in the absence of naloxone the same pattern was seen. The heart rate fell 83 ± 13 from a control of 406 ± 20 beats min^{-1} after 32 μmol kg^{-1}. In the presence of naloxone pretreatment the fall was 32 ± 12 from a control of 391 ± 18 beats min^{-1}.

ECG measures were influenced in a dose-related manner by both compounds (Figure 2). Saline was without effect in that the ECG did not change significantly with time over the experimental period. To an approximately equal extent PD 129289 and PD 129290 (in the presence and absence of naloxone) produced dose-related increases in RSh. For both enantiomers a dose between 1 and 2 μmol kg^{-1} produced a 10% increase in RSh with PD 129289 perhaps being more potent (potency ratio < 2.0).

In terms of effects on QRS duration, PD 129289 (the R,R

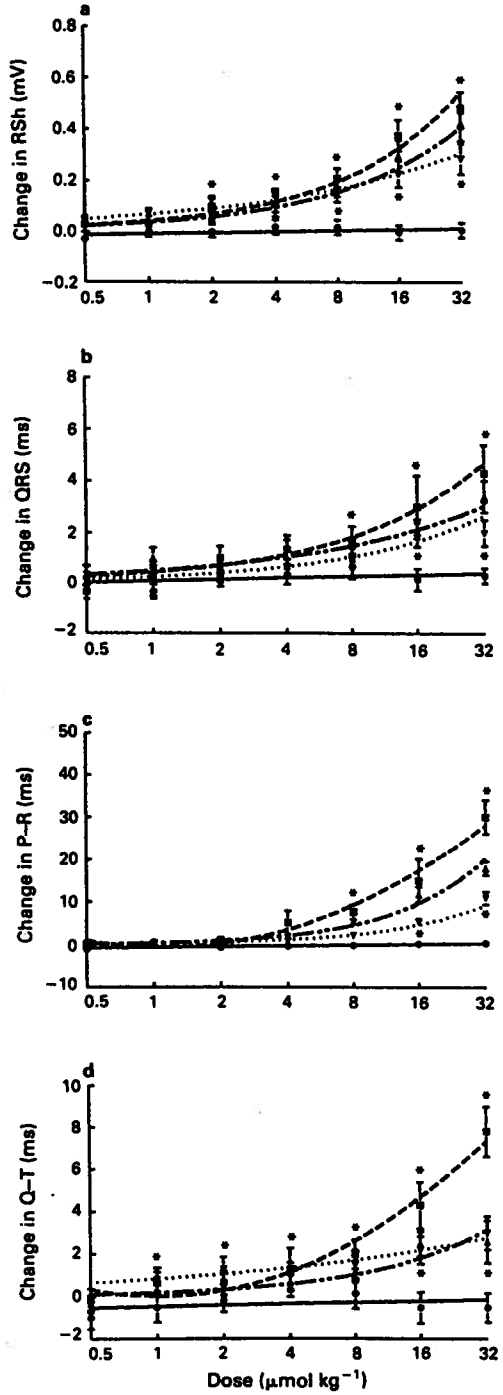

Figure 2 Dose-response curves for the effects of PD 129289 and PD129290 on the ECG of anaesthetized rats. The effects of the two enantiomers in the absence of naloxone, and PD 129290 in the presence of naloxone, are shown as changes in (a) RSh, (b) QRS, (c) P–R and (d) Q–T from control values induced 5 min after administration of a bolus of drug at 1, 2, 4, 8, 16, or 32 μmol kg^{-1}. Values are mean and vertical lines s.e.mean ($n = 8$) for (●) saline, (■) PD 129289, (▲) PD 129290 and (▼) PD 129290 in the presence of naloxone 8 μmol kg^{-1}. Control values were 0.49 ± 0.08 mV for RSh, 28 ± 1 ms for QRS, 53 ± 1 ms for P–R and 38 ± 1 for Q–T. *Indicates $P < 0.05$ for statistically significant differences from control.

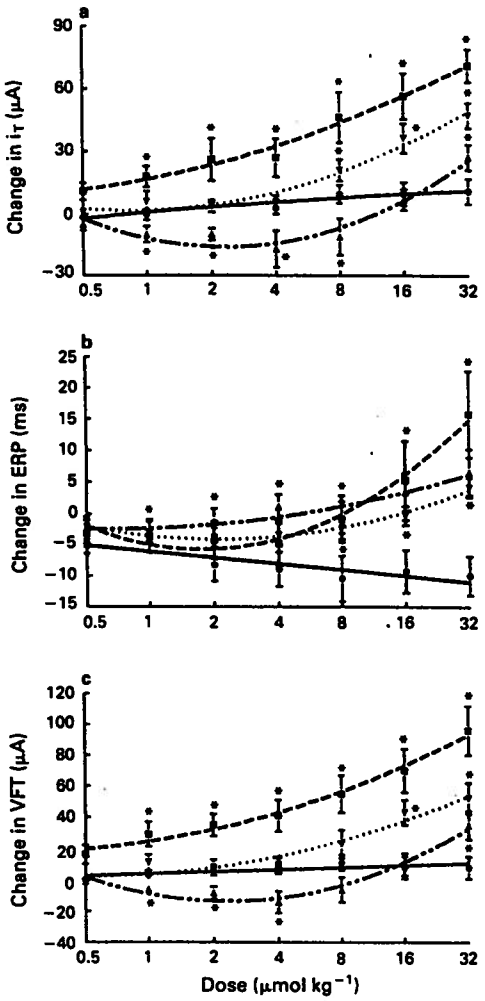

Figure 3 Dose-response curves for the effects of PD 129289 and PD129290 on sensitivity to ventricular electrical stimulation in anaesthetized rats. The effects of the two enantiomers in the absence of naloxone, and PD 129290 in the presence of naloxone, are shown as changes in (a) threshold current (i_T), (b) effective refractory period (ERP) and (c) threshold current for ventricular fibrillation (VF_T) occurring 3 min after administration of either compound at cumulative doses of 1, 2, 4, 8, 16, or 32 μmol kg^{-1}. Values are mean and vertical lines s.e.mean ($n = 6$) for (●) saline, (■) PD 129289, (▲) PD 129290 and (▼) PD 129290 in the presence of naloxone 8 μmol kg^{-1}. Control values were 51 ± 3 μA for i_T, 89 ± 6 μA for VF_T and 54 ± 2 ms for ERP. *Indicates $P < 0.05$ for statistically significant differences from control.

(+)-enantiomer) was slightly more potent than PD 129290 in the presence or absence of naloxone. The dose producing a 10% increase in QRS lay between 16 and 32 μmol kg^{-1}. A similar pattern was seen with P–R interval in that PD 129289 was again more potent (potency ratio < 2.0) than PD 129290 in the presence or absence of naloxone. ED_{10} values for both enantiomers were between 8 and 16 μmol kg^{-1}.

The ECG measurement least influenced by either drug was the Q–T interval. This interval was statistically significantly increased only after the higher doses, such that the approximate ED_{10} was 16 μmol kg^{-1} for PD 129289 and > 32 μmol kg^{-1} for PD 129290, with or without naloxone.

Table 1 Cardiovascular and ECG effects of 2 and 8 μmol kg^{-1} doses of PD 129290 and PD 129289 – antiarrhythmic study

Dose (μmol kg^{-1})	BP	HR	ECG P–R	ECG QRS	ECG Q–T
Saline	105 ± 6	377 ± 12	53 ± 2	29 ± 0.5	38 ± 0.8
PD 129290 2	88 ± 4*	356 ± 16	53 ± 2	31 ± 1.0	37 ± 1.0
PD 129290 8	88 ± 5*	331 ± 11*	62 ± 2*	31 ± 0.3	41 ± 0.4
PD 129290 8 (+N)	76 ± 5*	298 ± 9*	57 ± 2*	33 ± 0.8*	41 ± 1.0
PD 129289 2	71 ± 2*	318 ± 14*	55 ± 1	32 ± 1.0	41 ± 0.6
PD 129289 8	77 ± 6*	338 ± 17*	65 ± 2*	31 ± 0.6	41 ± 0.9

The effects of PD 129290 alone, or in the presence of naloxone (8 μmol kg^{-1}, + N), and PD 129289 are expressed as mean ± s.e.mean ($n = 9$) for the variable indicated. BP = mean blood pressure in mmHg; HR = heart rate in beat min^{-1}; P–R, QRS and Q–T are ECG intervals in ms.
*$P < 0.05$ for comparison with saline.

Table 2 Antiarrhythmic effects of 2 and 8 μmol kg^{-1} doses of PD 129290 and PD 129289 against coronary artery occlusion-induced arrhythmias

Drug and dose (μmol kg^{-1})	Group Incidence VT	VF	VT and/or VF	Arrhythmia score
Saline	8/8	7/8	8/8	5.0 ± 0.6
PD 129290 2	3/8	3/8	3/8	1.1 ± 0.6*
PD 129290 8	4/8	0/8*	4/8	1.5 ± 0.6*
PD 129290 8 (+N)	2/8*	0/8*	2/8*	0.8 ± 0.4*
PD 129289 2	6/8	3/8	6/8	3.0 ± 0.7
PD 129289 8	4/8	0/8*	0/8*	1.3 ± 0.6*
Naloxone 8	6/8	3/8	6/8	3.4 ± 0.2

The antiarrhythmic actions of PD 129290, alone or in the presence of naloxone (8 μmol kg^{-1}), as well as PD 129289, are expressed in terms of the group incidence of one or more episodes of the major arrhythmias of ventricular tachycardia (VT) or ventricular fibrillation (VF), $\log_{10}$ of ventricular premature beats (VPB) and arrhythmia score expressed as mean ± s.e.mean ($n = 9$).
*$P < 0.05$ for comparison with saline.

Effects of treatment on electrical stimulation Figure 3 illustrates the effects of the compounds on sensitivity to electrical stimulation. The control values for thresholds for capture (i_T) and VF (VF_T) were constant over the treatment period whereas those for ERP tended to fall throughout the treatment period. In a clearly dose-related manner PD 129289 increased thresholds and effective refractory periods. PD 129290 in the absence of naloxone initially tended to decrease thresholds (i_T and VF_T) with minima occurring between 2 and 4 μmol kg^{-1}. Thereafter, thresholds increased with increasing doses until finally they rose above control values in the range of 16–32 μmol kg^{-1}. The minimum values with a cumulative dose of 4 μmol kg^{-1} PD 129290 was − 21% from control for i_T and − 14% for VF_T. On the other hand, in the presence of naloxone pretreatment, the i_T and VF_T dose-response curves for PD 129290 were similar in shape to those for PD 129289 and showed no tendency to decrease below control levels. Interestingly in the presence of naloxone the potency of PD 129290 was less than PD 129289 (potency ratio approximately 4.0). Changes in t_T (data not shown) were less consistent.

ERP, and its related variable MFF (not shown), were not markedly influenced by low doses of either drug. The saline control showed a reduction with time for ERP and this was accompanied by the expected increase in MFF. Lower doses of both enantiomers did not have any significant effects on MFF and ERP when compared with saline. At higher doses there were dose-related increases in ERP and corresponding falls in MFF. Notably, responses to PD 129290, in the presence and absence of naloxone, were not markedly different from those for PD 129289.

Antiarrhthmic study In the coronary occlusion-arrhythmia study both 2 and 8 μmol kg^{-1} doses had the ECG and cardiovascular effects (Table 1) expected from the foregoing study. The lower dose (2 μmol kg^{-1}) minimally changed QRS and Q–T intervals. The most noticeable effect of the low dose was on the P–R interval. The large dose (8 μmol kg^{-1}) produced its expected effects on blood pressure, heart rate and ECG. None of the changes induced by the (−)-enantiomer (PD 129290) were reversed by naloxone pretreatment.

Both doses of PD 129289 and PD 129290 statistically significantly reduced arrhythmias induced by coronary occlusion (Table 2). The high dose (8 μmol kg^{-1}) was more antiarrhythmic than the low dose (2 μmol kg^{-1}). Naloxone had no effect on the antiarrhythmic effects of 8 μmol kg^{-1} PD 129290 although, on its own, naloxone appeared to reduce arrhythmias but not to a statistically significant extent.

The antiarrhythmic activity of either enantiomer could not be ascribed to changes in either occluded zone size or serum potassium concentrations. Group mean occluded zone size varied from a group minimum of 30.0 ± 1.2 (% total ventricular weight) to a maximum of 32.5 ± 1.6% (not statistically significant). Similarly, serum potassium concentrations were not influenced by the treatments. Group mean serum potassium concentrations varied from a low of 3.2 ± 0.2 mM to a high of 3.9 ± 0.4 mM and were not related to treatment.

Whole cell voltage clamp studies

Concentrations of PD 129290 (10 and 20 μM) which produced marked effects on isolated hearts (Figure 1) also

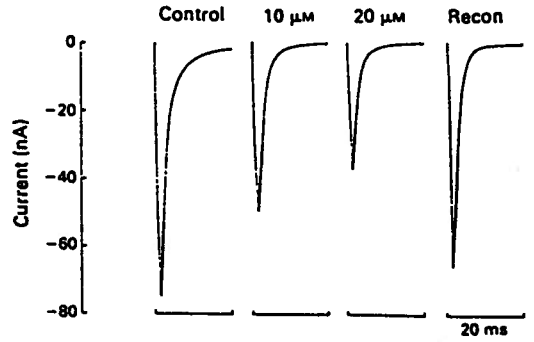

Figure 4 Sodium currents and the effect of PD 129290. Sodium currents were evoked in a single cardiac myocyte by a depolarizing step to − 40 mV from a holding potential of − 140 mV. The current under control conditions had a peak amplitude of 75 nA. This was reduced to 48 nA by 10 μM and to 38 nA by 20 μM PD 129290. These effects could be reversed by wash-out as shown by the re-control (Recon) current obtained 4 min after returning to the control solution. Some decrease in the control sodium current is seen during the course of the experiment, a finding typical of whole-cell records.

reduced sodium currents recorded in whole cell voltage clamp studies (Figure 4). These concentrations are 100–1,000 times greater than those producing κ agonism. The higher concentration (20 μM) of PD 129290 reduced current amplitude by about 50%, without perceptible changes in kinetics. These effects were readily reversible and could not be blocked by naloxone (data not shown).

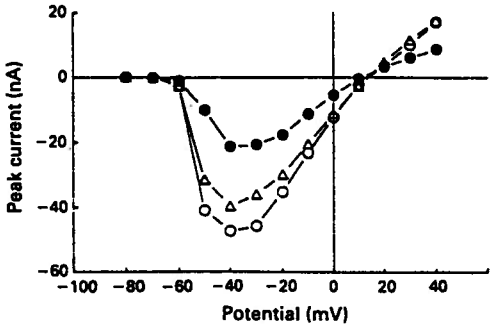

Figure 5 Current-voltage relation for the sodium current and the effect of PD 129290. Peak sodium current evoked by depolarizing steps to various voltages from a holding potential of −140 mV is shown plotted as a current-voltage relationship. Maximal sodium current of 50 nA was evoked by a step to −40 mV in control solution (○). The maximal current in the presence of 20 μM PD 129290 was also evoked at −40 mV, but had a magnitude of only 22 nA (●). The voltage at which the current reversed was not appreciably changed in the presence of PD 129290. It was 14 mV for both curves. E_{NA} was calculated as 14 mV for the solutions used. Recontrol data, after about 4 min of recovery in control solution are shown by the open triangles.

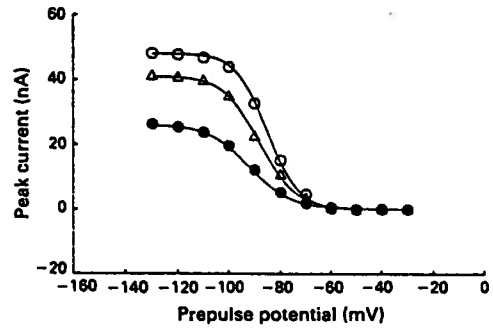

Figure 6 Inactivation curves for the sodium current and the effect of PD 129290. Sodium currents were evoked by a depolarizing step to −40 mV from prepulses of various potentials. The magnitude of the sodium current is shown plotted against prepulse potential. The inactivation curve so produced was fitted by the Boltzman equation, shown by the line and of the form $y = I_{max}/1 + (e^{((v-v')/k)})$ where v is the prepulse potential, v' is the voltage at which 50% inactivation occurs and k is a slope factor. The best fits to the data were obtained with values of max = 48 nA, v' = −85 mV and k = 6 for the control curve (○), and max = 26 nA, v' = −92 mV and k = 8 for the curve obtained in the presence of 20 μM PD 129290 (●). Recontrol data obtained after 4 min of recovery are shown by the triangles.

Figure 5 shows the effect of 20 μM PD 129290 on the current-voltage relationship for the sodium current. Sodium current amplitude was maximum at −40 mV in control solution and the current reversed at 14 mV. Neither of these values was appreciably altered by the application of 20 μM PD 129290, although maximum sodium current was reduced by about 60%. PD 129290 (20 μM) did not affect the voltage-dependence of inactivation of the sodium current, as shown in Figure 6. Thus, although 20 μM PD 129290 caused large decrease in maximum current amplitude, this was not due to a shift in the voltage-dependence of either the current-voltage relation or inactivation. Thus, in rat isolated heart cells PD 129290 added to the bathing solution was capable of blocking sodium currents.

Discussion

Overall, the actions of the S,S (−)-enantiomer (PD 129290) in these studies were remarkably similar to those of the arylbenzacetamide κ agonist, U50,488H, which we have tested previously under similar conditions (Pugsley *et al.*, 1992a,b). Thus, both U50,488H and PD 129290 (κ agonists) prolonged the P–R and QRS intervals of the ECG in isolated and P–R, QRS, RSh and Q–T in intact hearts. In addition they lowered blood pressure and heart rate and increased threshold currents in the presence of naloxone. Both drugs had lesser effects on the other stimulation variables measured and both were antiarrhythmic against occlusion-induced arrhythmias.

Notably, as was the case with U50,488H, the only unequivocal effect of naloxone pretreatment on the actions of PD 129290 was to antagonize the decrease in threshold currents seen at low doses. Such evidence suggests that most of the antiarrhythmic and other actions of U50,488H and PD 129290 can be ascribed to effects unrelated to κ agonism.

The evidence for the above events being unrelated to the κ receptor is strengthened by findings with PD 129289, the R,R (+)-enantiomer of PD 129290, which has very low affinity for κ receptors. Measurement of the binding constants for PD 129289 and PD 129290 on κ receptors indicate at least a 1,000 fold selectivity for PD 129290 (the S,S (−)-enantiomer). Thus, it is reasonable to assume that the effects of PD 129290 on RSh, P–R, QRS and Q–T intervals, which were not blocked by naloxone and are similar to those of PD 129289, are probably independent of κ receptors. Similarly, increases in thresholds seen with all doses of PD 129289 and with PD 129290 at higher doses (or at lower doses in the presence of naloxone) may also be independent of κ receptors. Furthermore, PD 129289 was as equally effective as PD 129290 in its antiarrhythmic actions.

Although PD 129289 has very little activity at the κ receptor it has appreciable actions on μ receptors. Thus PD 129289 has almost the same μ receptor binding as PD 129290, and PD 129289 is effective in the hot plate test for analgesia, a test believed to depend upon μ recptors. The ED_{50} for PD 129290 in this test was 2.5 mg kg^{-1} (approx 6 μmol kg^{-1}), i.e. within the dose range tested in this study.

Table 3 Potencies of PD 129290 and PD 129289 with respect to blood pressure (BP), heart rate (HR), and ECG intervals, sensitivity to electrical stimulation (ES) and antiarrhythmic actions

				ECG			*ES*		*Antiarrhythmic*		
Drug	*BP*	*HR*	*P–R*	*QRS*	*Q–T*	i_T	VF_T	*ERP*	*VT*	*VF*	*AS*
PD 129289	16	8	8	>32	>32	8	0.5	3	<2	<2	<2
PD 129290	0.5	0.5	16	>32	>32	32	4	6	2	2	2
PD 129290 (N)	16	32	32	>32	>32	8	4	8	8	<8	<8

In the above table estimates for ED_{25} values (μmol kg^{-1}) have been extrapolated from the data given in the Tables and Figures. PD 129290 (N) refers to PD 129290 in the presence of naloxone. For abbreviations used see text in Methods section.

However naloxone is even more effective in blocking μ receptors than κ receptors (Rees & Hunter, 1990) thus μ effects cannot account for the action of PD 129290 after naloxone treatment.

With respect to the mechanisms underlying the antiarrhythmic actions of the two enantiomers, Table 3 lists the doses of PD 129289 and PD 129290 (in the presence or absence of naloxone) required to produce 25% changes in blood pressure, ECG variables, electrical stimulation variables and antiarrhythmic effects. From this Table it is apparent that marked antiarrhythmic effects occurred at doses which produced limited but consistent changes in the other variables.

In our studies with U50,488H (Pugsley *et al.*, 1992a,b) we suggested that the antiarrhythmic, ECG and most of the electrical stimulation effects of U50,488H were due to sodium channel blockade. In view of the similar actions of PD 129289 and PD 129290 (not blocked by naloxone) it would be reasonable to suggest that sodium channel blockade occurs with all three drugs and that such an action accounts for their antiarrhythmic actions. For PD 129290 we have direct proof that the compound blocks sodium currents in rat isolated cardiac myocytes and that such an action is not blocked by naloxone. Such blockade occurs at concentrations similar to those which produce ECG effects in rat isolated hearts and are consistent with the doses administered to intact rats to produce similar ECG effects. 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. However, it should be noted that changes in the Q–T interval of the ECG seen at higher doses suggest that a limited degree of potassium channel blockade may occur with these compounds. It is possible that this action may also contribute to antiarrhythmic actions.

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APPENDIX 4

ELSEVIER

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An electrophysiological basis for the antiarrhythmic actions of the κ-opioid receptor agonist U-50,488H

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Abstract

This study examined the actions of the selective κ-opioid receptor agonist, U-50,488H, on voltage activated Na^+ and K^+ currents in isolated rat cardiac myocytes. U-50,488H produced a concentration-dependent block of the transient Na^+ current with an ED_{50} of about 15 μM, and, at higher concentrations (40–50 μM), a block of the plateau K^+ current and an increase in the rate of decay of the transient K^+ current. In addition U-50,488H produced a hyperpolarising shift in the inactivation curve for the transient Na^+ current without altering the voltage dependence for activation and without an effect on the voltage dependence of inactivation or activation of K^+ currents. The block of Na^+ currents by U-50,488H showed pronounced use dependence. The κ-opioid receptor antagonist MR2266 did not itself produce any change in the Na^+ or K^+ currents and did not change the channel blocking properties of U-50,488H. Thus, since the antiarrhythmic actions of U-50,488H are not blocked by MR2266 or naloxone, the effects of U-50,488H to block Na^+ and K^+ currents are the most likely reasons for its antiarrhythmic actions, rather than an action at κ-opioid receptors.

Key words: Cardiac electrophysiology: Ionic current; Cardiac myocyte, isolated; κ-Opioid receptor agonist; U-50,488H

1. Introduction

The cardiovascular actions of opioid receptor ligands, especially κ-opioid receptor agonists, are complex and may involve direct actions on blood vessels and the heart as well as actions mediated through opioid receptors located in both the central and peripheral nervous system (Martin, 1984; Seth et al., 1991; Pugsley et al., 1992b, c). Because of this complexity, the involvement of opioid agonists and antagonists in arrhythmogenesis is obscure. A number of studies suggest the involvement of opioid agonists and antagonists in fatal ventricular arrhythmias resulting from myocardial ischaemia and reperfusion (Parratt and Sitsapesan, 1986; Sarne et al., 1991) and it has been shown that some opioid agonists, such as U-50,488H and even partial agonists, such as meptazinol, have antiarrhythmic actions in rats subject to coronary artery occlusion (Fagbemi et al., 1983; Pugsley et al., 1992a, c). In this context, recent studies by Tai et al. (1991) have shown that saturable, reversible and stereospecific binding sites for κ-opioid agonists exist on the heart.

It has been suggested that the non-opioid properties of κ-opioid receptor and other opioid agonists and antagonists result from blockade of Na^+ channels, as shown with U-50,488H by Alzheimer and Ten Bruggencate (1990) in neuronal cells and recently by Zhu and Im (1992) with the anticonvulsant κ-opioid receptor agonist U-54494A in neuroblastoma cells. Antiarrhythmic studies also suggest that the non-opioid effects of these drugs on the heart result from Na^+ channel blockade (Sarne et al., 1991; Pugsley et al., 1992c; Zhu et al., 1992).

In this study we have used the whole-cell configuration of the patch clamp technique to examine the

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electrophysiological actions of U-50,488H on the transient Na^+ current and on the transient outward (I_{to}) and plateau (I_k) K^+ currents of isolated rat cardiac myocytes. The effects of U-50,488H on Na^+ and K^+ channel activation and inactivation curves, as well as its actions in the presence of the κ-opioid receptor antagonist MR2266, were examined. The use-dependence of block of Na^+ currents by U-50,488H was also investigated.

2. Materials and methods

2.1. Isolated ventricular myocytes

Ventricular myocytes were isolated according to the method of Farmer et al. (1983). Briefly, male Wistar rats (300–400 g) were killed by cervical dislocation followed by exsanguination. The chest was opened, the heart removed and immersed in ice-cold, oxygenated, Ca^{2+}-free Tyrode's solution containing (in mM): NaCl 134; KCl 4; NaH_2PO_4 1.2; $MgCl_2$ 1.2; glucose 11; TES (*N*-tris-(hydroxymethyl)-methyl-2-aminoethanesulphonic acid) 10, the solution adjusted to pH 7.4 with 1.0 M NaOH. The heart was then perfused, via an aortic cannula, with the same Ca^{2+}-free Tyrode's solution warmed to 37°C to facilitate removal of blood from ventricular chambers and coronary vasculature. After 5 min of wash the heart was subjected to enzymatic dissociation in 25 μM Ca^{2+}-Tyrode's solution containing protease (0.1 mg/ml, Sigma Type XIV), collagenase (1 mg/ml, Worthington CLS II), and fetal calf serum (1 μg/ml).

Approximately 20–25 min later the ventricles were removed in one-third sections. Each section was carefully cut into small pieces in fresh 25 μM Ca^{2+}-Tyrode's solution and triturated to dissociate myocytes. Cell suspensions were then gently centrifuged and washed in a 200 μM Ca^{2+}-Tyrode's solution. Cells were then resuspended in a Tyrode's solution containing 1 mM Ca^{2+} and 1–2 h later plated onto glass coverslips. Cells were stored at room temperature (21–23°C).

2.2. Recording solutions and electrodes

2.2.1. Recording solutions

All experiments were performed at room temperature (21–23°C). Cells were externally perfused with a Tyrode's solution of the following composition (mM): NaCl 70; KCl 5.4; $MgCl_2$ 1.0; glucose 10; TES 10; $CaCl_2$ 2.0; $CoCl_2$ 5.0; $CsCl_2$ 5.0; choline-Cl 60, pH adjusted to 7.4 with 1.0 M NaOH. The pipette solution used to allow for the simultaneous recording of both Na^+ and K^+ currents contained (in mM): KF 140; TES 10; $MgCl_2$ 1.0; K-EGTA (ethyleneglycol-bis-(β-amino ethyl ether) *N,N.N',N'*-tetraacetic acid) 10; $CaCl_2$ 2.0; ATP-disodium 5.0; ATP-Mg 5.0 and pH adjusted to 7.4 with 1.0 M KOH. For recording of K^+ currents 20 μM tetrodotoxin was added to the bath solution; for recording Na^+ currents the intracellular K^+ was replaced with Cs^+.

2.2.2. Microelectrodes

Patch clamp electrodes were fabricated from thin wall borosilicate glass using a two-stage pipette puller (Narishige Scientific Instrument Co., Tokyo, Japan). Typical electrode resistance, when containing the pipette solution, was between 3 and 5 MOhm. Electrodes were fire polished immediately before use.

2.3. Data acquisition and analysis

Myocyte currents were typically recorded 5–10 min after achieving whole cell patch clamp configuration (Hamill et al., 1981). Current recording was performed using an Axopatch 200 A amplifier (Axon Instruments, Foster City, CA, USA) which allowed for compensation and reduction of both capacitance transients and leak currents. Voltage command pulses were generated by a computer using software written for the purpose. Output signals were digitized with a 12-bit analogue-to-digital converter and recorded on the hard disk of the computer. Final capacitance and leak compensation was performed at the time of data analysis by subtraction of the current produced by a 20 mV hyperpolarizing voltage pulse current which always preceded the test voltage step.

2.4. Drugs

U-50,488H (a gift from Dr. P.F. Von Voightlander, The Upjohn Co., Kalamazoo, MI, USA) and tetrodotoxin (Sigma Chemical Co., St. Louis, MO, USA) were initially solubilized in distilled water prior to addition to the external bath solution. MR2266 (Boeringer Mannheim, Germany) was initially solubilized in dimethyl sulfoxide (DMSO) and added to the bath solution to a final concentration of 1 μM. At this concentration, DMSO alone did not have any actions on either Na^+ or K^+ currents in control experiments. All drugs were bath applied via superperfusion of cells at a flow rate of 1–2 ml/min.

3. Results

3.1. Effects of U-50,488H on membrane currents in isolated myocytes

Preliminary experiments were performed under conditions where both Na^+ and K^+ currents were recorded. The membrane potential of the cell was

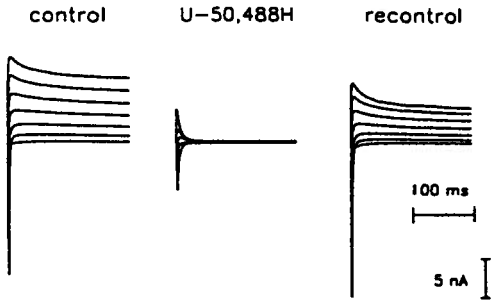

Fig. 1. Effect of U-50,488H on Na^+ and K^+ currents recorded simultaneously. Voltage steps lasting 200 ms were given from a fixed pre-pulse potential of −150 mV. These voltage steps were to potentials of −60, −40, −20, 0, 20, 40 and 60 mV. The currents evoked by these voltage steps in control solution are shown in the left panel. A repetition of the voltage protocol in the presence of 50 μM U-50,488H yielded the currents shown in the centre panel. The currents obtained 5 min after return to control solution are shown in the right panel. All currents are plotted on the same scale.

stepped from a potential of −150 mV to voltages between −60 and +60 mV. This protocol produced progressive activation of Na^+ and then K^+ currents (both transient and plateau), as shown in Fig. 1, which shows superimposed current records. A repeat of this procedure in the presence of 50 μM U-50,488H revealed profound changes in the currents (Fig. 1, centre panel). A large block of the Na^+ current was seen (downward current in the figure), together with a block of the plateau K^+ current and a greatly accelerated rate of decay of the transient K^+ current (upward current in the figure). These effects were readily reversible, as shown by the recontrol traces in Fig. 1, which were obtained about 5 min after return to control solution. Subsequent experiments were designed to examine the effect of U-50,488H on the individual currents more carefully.

3.2. Concentration dependence of the effects of U-50,488H on the transient Na^+ current

Fig. 2 shows the dose-response relationship for the effect of U-50,488H to block the transient Na^+ current. The concentration of U-50,488H which reduced the peak Na^+ current by 50% (i.e., the ED_{50}) was estimated as 15 μM. It should be noted that depolarising pulses to evoke the Na^+ current were given at 6 s intervals during the collection of this data. Even at this low pulse rate there is some degree of use-dependent block of the Na^+ current by U-50,488H, especially at higher drug concentrations, which will distort the estimate of potency slightly (see section 3.4).

3.3. Effects of U-50,488H on the activation and inactivation curves of the Na^+ current

Na^+ currents were evoked by a voltage step to −40 mV from various conditioning pre-pulse potentials between −140 mV and −70 mV. A plot of peak current amplitude against pre-pulse potential showed voltage dependent inactivation of the Na^+ current, as shown in Fig. 3. In the presence of 15 μM U-50,488H the voltage dependence of this inactivation was shifted to the left (towards more hyperpolarised potentials). For the cell shown in Fig. 3 the shift was approximately 15 mV in the presence of 15 μM U-50,488H. In another 4 cells examined the mean shift induced by 15 μM U-

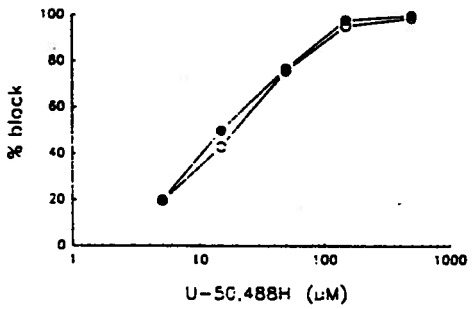

Fig. 2. The concentration dependence of the block of the Na^+ current by U-50,488H. Na^+ currents were evoked by a voltage step to −40 mV from a potential of −150 mV. The voltage steps were given at 6 s intervals. The maximal peak current amplitude was measured in control solution and in solutions with different concentrations of U-50,488H. The peak current amplitude is shown plotted against the concentration of U-50,488H on a logarithmic scale (filled circles). These data points are the mean value from 4 cells. The open circles represent the data obtained from a different cell in the presence of 1 μM MR2266.

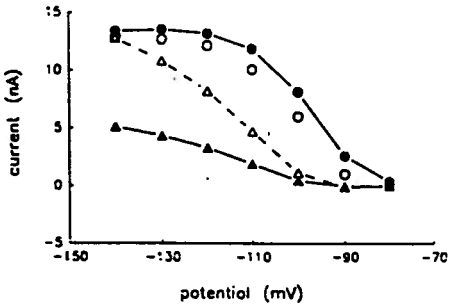

Fig. 3. The effect of U-50,488H on the inactivation of the transient Na^+ current. A voltage step to a fixed test pulse potential of −40 mV was preceded by a conditioning pre-pulse to various potentials between −140 mV and −80 mV. The peak amplitude of the Na^+ current evoked by the test pulse is shown plotted against the pre-pulse potential (filled circles). Data obtained in the same cell in the presence of 15 μM U-50,488H is shown by the filled triangles. When the data in the presence of U-50,488H is scaled to the same maximum as the control data the points shown as open triangles are obtained. The data shows a shift in the hyperpolarising direction of about 15 mV in the presence of U-50,488H. Open circles depict recontrol data.

50,488H was 16 mV (range 15–20 mV). A higher concentration of U-50,488H (50 μM) was tried in two cells, and gave shifts in the curve of 25 mV and 30 mV, respectively.

The effect of U-50,488H on the voltage dependence of activation of the Na^+ current was also examined. Currents were evoked by a voltage step to a variable test potential from a fixed conditioning pre-pulse potential of −150 mV. Fig. 4 shows a plot of the current amplitude evoked in one cell plotted against test pulse potential, to give a current-voltage relation for the Na^+ current. This current-voltage relation was not changed, other than by a scaling of the maximum current attained, in the presence of 15 μM U-50,488H. Hence the voltage dependence of the current activation was unaffected, although a marked block of the current amplitude was recorded. This result was confirmed in 5 other cells.

3.4. Use dependence of the block of Na^+ currents by U-50,488H

The block of Na^+ currents by U-50,488H showed pronounced use dependence at the concentrations used. Fig. 5, panel A, shows the effect of increasing concentrations of U-50,488H upon the Na^+ currents evoked by a train of stimuli delivered at 20 Hz. In the absence of the drug there was no diminution in the size of the Na^+ current during the train at this frequency. In the presence of 10 μM U-50,488H, however. an obvious attenuation of the current with successive pulses was evident, although little block of the first current in the train was seen. This use dependence of

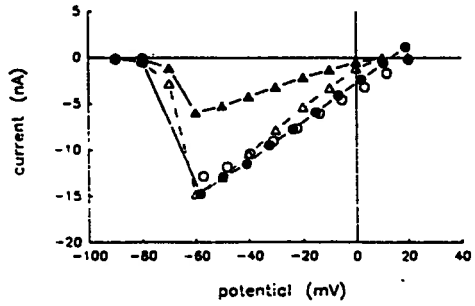

Fig. 4. The effect of U-50,488H on the current-voltage relation for the Na^+ current. Na^+ currents were evoked by a voltage step to potentials between −90 mV and +20 mV from a fixed pre-pulse potential of −150 mV. The peak amplitude of the current is shown plotted against potential for control data (filled circles), in the presence of 15 μM U-50,488H (filled triangles) and recontrol (open circles). When the data in the presence of U-50,488H is scaled to the same maximum current as control the data points shown by the open triangles are obtained.

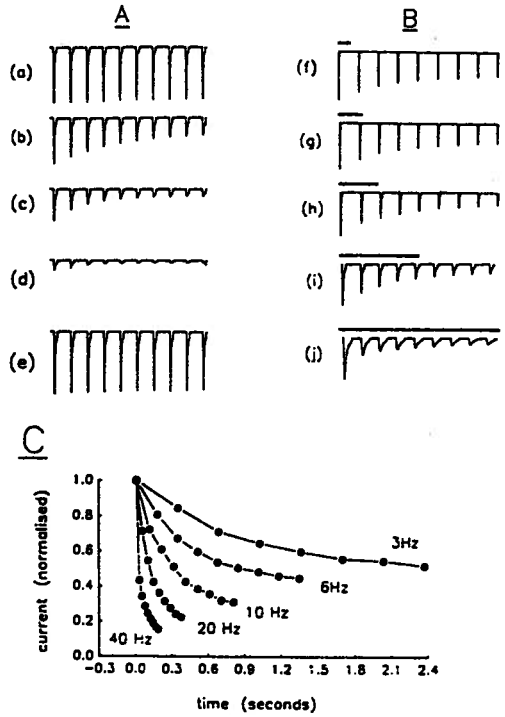

Fig. 5. The use dependence of block of Na^+ currents. Panel A shows Na^+ currents evoked by trains of depolarising pulses to −30 mV lasting 10 ms from a holding potential of −130 mV, stimuli being delivered at 20 Hz for 1 s. A gap of 1 min was allowed between trains during which no stimuli were given. The top trace (a) shows the currents evoked under control conditions and below it are shown the currents evoked in the presence of increasing concentrations of U-50,488H: (b) = 10 μM. (c) = 30 μM and (d) = 100 μM. The bottom trace (e) shows re-control. Panel B shows the effect of increasing the frequency of stimulation in the presence of U-50.488H. Pulses were delivered as above. at various frequencies. The first nine currents for each train are shown, with the horizontal axis scaled appropriately for clarity. The frequencies of stimulation were 3 Hz (f). 6 Hz (g), 10 Hz (h), 20 Hz (i), and 40 Hz (j). U-50,488H was present throughout the experiment at a concentration of 10 μM. The horizontal bar above each current trace is 200 ms long in each case. With an identical stimulation train, there was no attenuation of the Na^+ current at 40 Hz in the absence of the drug (data not shown). Panel C shows the magnitudes of each current evoked, normalised to the size of the first current. plotted against the time at which it was evoked, for each of the five trains.

the block was more pronounced at higher concentrations, and these higher concentrations also produced a greater degree of tonic block. Similarly, at a fixed concentration of 10 μM, trains of increasing frequencies produced a more pronounced use-dependent block. as shown in Fig. 5. panel B. Panel C shows this data plotted as the normalised current amplitude for each pulse against the time at which it was evoked for each of the 5 trains.

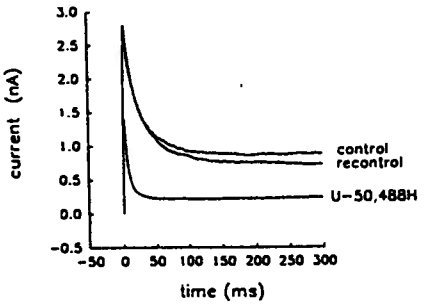

Fig. 6. The effect of U-50,488H on K^+ currents. Records of K^+ currents obtained from a myocyte in response to a voltage step to +50 mV from a potential of −120 mV. 20 μM tetrodotoxin was present in the bath throughout the experiment. The currents were obtained under control conditions (control), in the presence of 50 μM U-50,488H (U-50,488H) and recontrol conditions (recontrol).

3.5. The block of Na^+ current is not affected by opioid antagonists

U-50,488H is an agonist at κ-opioid receptors (Von Voightlander and Lewis, 1982), and κ-opioid receptors have been shown to be present in the heart (Tai et al., 1991). We therefore investigated whether κ-opioid receptors play any role in the block of the Na^+ current by U-50,488H. The dose-response relation for block of the Na^+ current by U-50,488H (Fig. 2) was repeated in several cells in the presence of 1 μM MR2266, a selective blocker of κ-opioid receptors (Wong et al., 1990). Typical data is shown plotted in Fig. 2. 1 μM MR2266 is approximately a 100-fold greater concentration than the K_D for antagonism of cerebral κ-opioid receptors (Clark et al., 1989), and so any effect mediated by action at κ-opioid receptors should be completely abolished. However, the dose-response relation for U-50,488H block of Na^+ currents was completely unchanged in the presence of this concentration of MR2266. Hence it appears that κ-opioid receptors play no role in the observed block of Na^+ currents by U-50,488H.

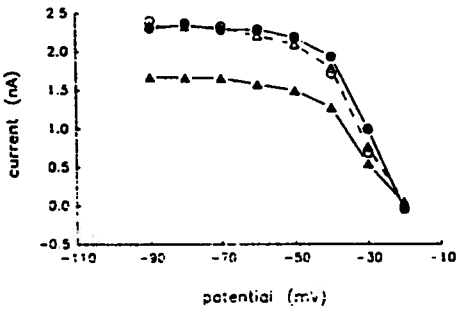

Fig. 7. Effect of U-50,488H on the inactivation of the transient K^+ current. K^+ currents were evoked in the presence of 20 μM tetrodotoxin by a voltage step to +50 mV from a pre-potential which varied between −120 mV and −20 mV. The magnitude of the transient component of the current was measured (by subtracting the current amplitude at 300 ms from the peak amplitude) and is shown plotted against the pre-pulse potential (filled circles). Data obtained in the same cell in the presence of 50 μM U-50,488H is shown by the filled triangles. When this data is scaled to the same maximum as control the points shown as open triangles were obtained. There was no shift in the voltage dependence of inactivation of the transient K^+ current.

3.6. The effect of U-50,488H on K^+ currents

The effect of U-50,488H was also investigated on K^+ currents recorded in isolated myocytes. The pre-

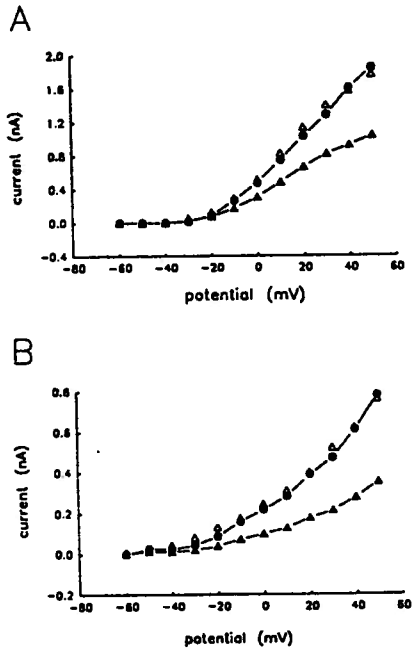

Fig. 8. Effect of U-50,488H on the current-voltage relation of K^+ currents. K^+ currents were evoked by a test pulse lasting 300 ms to potentials between −90 mV and +30 mV from a fixed pre-pulse potential of −120 mV in the presence of 20 μM tetrodotoxin. The transient component of the current was measured by subtraction of the amplitude of the current at the end of the trace from the peak current. The amplitude of this transient component of the current is shown plotted against the test pulse potential in panel A (filled circles). These data points show the mean of control and recontrol (after washout of U-50,488H). In the presence of 50 μM U-50,488H the current was blocked by about one third, as shown by the filled triangles. Scaling the data in the presence of U-50,488H to the same maximum as control revealed no shift in the voltage dependence of the activation (open triangles). Panel B shows the amplitude of the plateau current (current at the end of a 300 ms pulse) plotted against test pulse potential for mean of control and recontrol (filled circles). 50 μM U-50,488H (filled triangles) and scaled U-50,488H (open triangles). At a concentration of 50 μM U-50,488H blocked this component of the current by about 55%. No shift in voltage dependence for activation of the plateau current was seen in the presence of U-50,488H.

dominant effects of U-50,488H were to cause a block of the plateau K^+ current (which we ascribe to I_k) and to greatly accelerate the decay of the transient component of the current (Fig. 6). Both of these effects were seen at concentrations of U-50,488H of the same order as those which blocked the Na^+ current. Although 50 μM U-50,488H caused a greatly accelerated decay of the transient current, no shift in the voltage dependence of inactivation of this current was apparent (Fig. 7). In addition, U-50,488H did not affect the voltage dependence of activation of either the transient or plateau components of the K^+ current, as shown in Fig. 8. Although U-50,488H greatly accelerated the decay of I_{to}, the block of the peak amplitude of I_{to} was much less pronounced than the block of I_k.

Several experiments were also performed on K^+ currents in the presence of 1 μM MR2266; no obvious difference in the effects of U-50,488H could be seen (data not shown).

4. Discussion

U-50,488H has been shown to be antiarrhythmic in animal models of both electrical- and ischaemia-induced arrhythmias (Pugsley et al., 1992b, c). It has also been shown to be an agonist at κ-opioid receptors (Von Voightlander and Lewis, 1982). Since it has been shown that there are κ-opioid receptors in the heart (Tai et al., 1991) it seems possible that the antiarrhythmic actions of U-50,488H are due to its action at κ-opioid receptors.

However, several studies refute this hypothesis. It has been shown that activation of opioid receptors is arrhythmogenic (Wong et al., 1990), and that block of these receptors reduces the incidence of arrhythmias (Sitsapesan and Parratt, 1989). Indeed, concentrations of U-50,488H which are selective for κ-opioid receptors have been shown to reduce the thresholds for electrical induction of ventricular arrhythmias in rats (Pugsley et al., 1992b). In addition, the antiarryhthmic actions of PD129290, a κ-opioid receptor agonist closely related to U-50,488H, have been shown to be independent of κ-opioid receptor activation, since the antiarrhythmic actions of this agent were not stereospecific (Pugsley et al., 1993). These authors demonstrated that PD129290 blocks sodium currents in cardiac myocytes, and suggested that this may be the basis of its antiarrhythmic actions. Since the antiarrhythmic effects of U-50,488H are not blocked by naloxone (Pugsley et al., 1992c), this data strongly suggested that the antiarrhythmic actions of U-50,488H may not be mediated via an action at κ-opioid receptors, but rather by a channel blocking action.

We have shown in this study that U-50,488H is a potent blocker of the transient Na^+ current in cardiac myocytes and that it affects the inactivation of the channel, producing a hyperpolarising shift in the voltage dependence of inactivation. The block of Na^+ currents showed an obvious use dependence at moderate concentrations of U-50,488H, and at frequencies of stimulation comparable to those expected to be encountered during fibrillation (about 20 Hz). This use dependence of block may greatly enhance the potency of U-50,488H as an antarrhythmic agent.

In addition to effects on Na^+ currents, U-50,488H produces a block of I_k and a greatly increased rate of decay of the transient K^+ current at concentrations similar to those that block Na^+ currents. None of the electrophysiological effects of U-50,488H are affected by high concentrations of MR2266, and thus these actions of U-50,488H are not dependent on κ-opioid receptor activation.

The combined effect of U-50,488H on Na^+ and K^+ currents makes the Vaughan-Williams classification of the antiarrhythmic actions of this drug somewhat difficult. Nevertheless, it seems reasonable to propose that the antiarrhythmic effects of U-50,488H are due to its direct electrophysiological effects on Na^+ and K^+ currents. Within the constraints of the Vaughan-Williams classification scheme of antiarrhythmic drugs U-50,488H has a spectrum of action which is similar to that of quinidine on cardiac myocytes (Ju et al., 1992) and this would tentatively define U-50,488H as a class I antiarrhythmic agent.

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APPENDIX 5

Proc. West. Pharmacol. Soc. 38: 25-27 (1995)

Do Related Kappa Agonists Produce Similar Effects on Cardiac Ion Channels?

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We have shown, using in vivo and in vitro techniques, that several kappa opioid receptor agonists possess antiarrhythmic properties in the rat with associated blockade of cardiac Na^+ and K^+ currents [1-4]. Other studies, using similar rat models, suggest that the basis for antiarrhythmic activity of opioids is interaction(s) with cardiac ion channel(s) [5]. The use of opioid antagonists, such as naloxone and MR 2266, confirm the independence of opioid receptors from antiarrhythmic activity [1-3].

Hondeghem and Katzung [6] suggest that there may be potential therapeutic benefits in using combinations of antiarrhythmic agents, and have examined combinations of drugs such as quinidine and lidocaine. They argue that selective depression of abnormal impulse generation should be obtainable by combining any 'slow' drug with any 'fast' drug, and have modeled such drug combinations. Other studies suggest similar beneficial effects of combined drug therapy [7,8]. Kappa agonists may have such actions. In this study we compared the actions of two structurally-related kappa receptor agonists and the inactive enantiomer of one agonist in an attempt to determine whether these drugs possess preferential cardiac ion channel blockade.

METHODS: Male Sprague-Dawley rats (UBC Animal Care Centre) weighing between 200-300 g were used for all whole animal studies conducted at UBC while available male Wistar rats (200-300g) were used for isolated cell studies conducted in Canberra. All studies were conducted according to guidelines established by the Animal Care Committees of UBC and ANU.

Intact rats were prepared according to Penz et al. [9]. Dose-response curves for electrical stimulation of the left ventricle were established using two Teflon-coated silver wire stimulating electrodes. Square-wave stimulation was used to determine: threshold current, i_T, in μA for induction of extrasystoles; ventricular fibrillation threshold, VF_t, in μA; maximum following frequency, MFF, in Hz; and effective refractory period, ERP, in ms [10]. Cumulative doses of drug (0.1-32 μmol/kg/min iv) were infused, each dose over a 5 min period, and recordings were made at the end of an infusion just prior to the elevation of the infusion rate to the next level. Sodium channel blockade was assessed by measuring the depression of RSh; the height in mV from the peak of the R-wave to the trough of the S-wave in the EKG [9].

Ventricular myocytes were isolated according to a method described by Saint et al. [11]. Cells were prepared, plated onto glass coverslips and stored at room temperature (25-27°C) until used. Cells were externally perfused with a Tyrodes solution while the pipette solution allowed for the recording of Na^+ currents generated by a voltage step to -40mV from a holding potential of -140 mV. Microelectrodes (1-3 MΩ) were prepared from thin-wall borosilicate glass and currents were recorded 5-10 min after achievinq whole-cell patch-clamp configuration [12] using an Axopatch 200A amplifier. Records were digitized and capacitance and leak compensation performed during data analysis by subtraction of a 20 mV hyperpolarization pre-pulse current.

Drugs studied were: (i) PD 117302, (±)-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzo[b]thiophene-4-acetamide; (ii) PD 129290, (-)[5R-(5α,7α,8ß)]-N-methyl-N-[7-1-pyrrolidinyl)-1-oxaspiro [4.5]dec-8-yl]-4-benzofuran acetamide; and (iii) PD 129289, (+)5S-(5α,7α,8ß)]-N-methyl-N-[7-1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-4-benzofuran acetamide. Drugs were solubilized in distilled water prior to iv injection or dissolved in the external Krebs or bath solution of isolated hearts and single cardiac myocytes, respectively, to reach the final desired concentrations. Drug responses were determined as D_{25}, the dose producing a 25% change in given response. This measure allows a determination of differential actions on cardiac ion channels.

RESULTS: From Table 1 it can be seen that both kappa agonist drugs, PD 129290 and PD 117302, reduce blood pressure at a D_{25} of 0.5 μmol/kg/min. PD 129289, the inactive kappa enantiomer of PD 129290, also produced the same reduction in blood pressure but its D_{25} was 16 times that of the other drugs. All drugs had varying D_{25} doses for heart rate reduction. PD 117302 produced evidence of Na^+ channel blockade at lower D_{25} values than either of the other two drugs. This can be seen with RSh, a sensitive index of Na^+ channel blockade, which was prolonged at a D_{25} dose of 1.0 μmol/kg/min. In comparison, both PD 129290 and its enantiomer produced similar Na^+ channel blocking effects on RSh at similar doses to each other. However, these D_{25} doses were 6 and 4.5 times, respectively, greater than the D_{25} values for PD 117302. PD 117302 consistently prolonged the P-R and QRS intervals at lower D_{25}'s compared with PD 129290 and its enantiomer. The D_{25} for PD 1129290 and PD 129289 for QRS prolongation were estimated to be >120 μmol/kg/min. These D_{25} doses were estimated by extrapolating the dose-response curves constructed for the drugs over the dose range 0.1-32 μmol/kg/min fourfold

Table 1. D_{25} drug doses of kappa agonists in intact rats - effects on heart rate, blood pressure, and EKG measures

Drug	Heart Rate (beats/min)	Blood Pressure (mm Hg)	P-R Interval (ms)	QRS Interval (ms)	RSh (mV)	Q-aT (ms)
PD 117302	5.5	0.50	3.0	7.5	1.0	6.0
PD 129290	>120	0.50	20	>120	6.0	>120
PD 129289	30	8.0	10	>120	4.5	>120

Values are expressed as D_{25} doses (μmol/kg/min iv) in intact animals for 6 determinations per measure; the infused drug dose which produced a 25% change from control. The control values were: heart rate 360 ± 12 beats/min; blood pressure 115 ± 6 mm/Hg; P-R interval 52 ± 1 ms; QRS interval 30 ± 1 ms; and RSh 0.59 ± 0.07 mV for RSh.

Table 2. Effects of D_{25} drug doses on thresholds for electrical stimulation

Drug	i_T (μA)	VF_i (μA)	ERP (ms)	MFF (Hz)
PD 117302	0.45	0.35	0.45	0.75
PD 129290	2.5	3.0	40	>120
PD 129289	0.60	1.0	20	>120

Values are expressed as D_{25} doses (μmol/kg/min iv) in intact animals for 6 determinations per measure. The control values were for: induction of extrasystoles (i_T) 50 ± 1 μA; threshold of fibrillation (VF_i) 85 ±5 μA; effective refractory period (ERP) 55 ± 1 ms; and maximum following frequency (MFF) 11 ± 1 Hz.

above the maximum limit of the dose range (i.e., for these studies 32 μmol/kg/min was our upper limit, so 120 μmol/kg/min was chosen to be the maximum dose of extrapolation). The Q-aT interval, a measure of cardiac refractoriness, was prolonged at doses 6 to 25 times greater than those producing Na^+ channel blockade.

Electrical stimulation measures showed a differential effect of the drugs. PD 117302 had lower D_{25} values than either of the other drugs examined. It showed initial Na^+ channel blockade at 0.35 μmol/kg/min with prolongation in i_T at 0.45 μmol/kg/min. PD 129290 and PD 129289 produced Na^+ blockade at relatively similar doses. PD 129290 prolonged i_T and VF_i at 2.5 and 3.0 μmol/kg/min while these effects for PD 129289 were seen at at 0.60 and 1.0 μmol/kg/min. All drugs prolonged the measures of refractoriness (ERP and MFF) at doses greater than those blocking Na^+ channels. MFF was insensitive to changes in dose for PD 129290 and PD 129289 until >120 μmol/kg/min.

Isolated rat cardiac myocyte concentration-response curves for block of Na^+ currents were 2 and 10 μM for PD 117302 and PD 129290, respectively.

DISCUSSION: The overall effect of the high doses of kappa receptor agonists PD 117302 and PD 129290 and its inactive enantiomer, PD 129289, is to produce dose-dependent signs of mixed Na^+ and K^+ blockade. We have shown previously that this occurs with U-50,488H [4], and the literature contains data for many opioid drugs showing many effects on ion channels in various tissues [13]. The in vivo measures which we use to examine drug effects on ion channels, such as changes in the EKG, provide an easy means by which to screen a drug for its effects on the heart. The drugs examined in this study did show some differential effects on Na^+ versus K^+ channel effects using the D_{25} value as a measure of current selectivity. At lower doses, the kappa agonists affected Na^+ channels more than K^+ channels. The D_{25} values for RSh, a measure of Na^+ channel blockade in the rat, are

significantly lower than those seen for Q-aT, an index of K^+ channel function.

The patterns of drug response to electrical stimulation indicate that these drugs are antiarrhythmic by virtue of cardiac ion channel blockade [1,3]. Our measure of drug sensitivity, D_{25}, should be further assessed using several other antiarrhythmic opioid compounds with similar structure as well as their inactive enantiomers. In this way we could quantitate and validate this method of examining ion channel sensitivity to drug blockade. Further quantification may provide details on drugs which uniquely block both Na^+ and K^+ channels, and allow for examination of drug combinations which may prove useful in the clinical setting.

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APPENDIX 6

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A New ECG Measure (RSh) for Detecting Possible Sodium Channel Blockade in Vivo in Rats

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A new electrocardiographic (ECG) measure for detecting possible sodium channel blocking actions of drugs in anaesthetized rats is described. The conventional measures for sodium channel blockers are increased QRS width and/or P–R prolongation, however, these are limited in their sensitivity. This new measure, RSh, is the height from the peak of the R wave to the bottom of the S wave; it is more sensitive to known sodium channel blocking agents than conventional measures. This was shown by comparing the ECG effects of sodium channel blocking class I antiarrhythmic drugs from the three subclasses lidocaine (Ia), quinidine (Ib), and flecainide (Ic). In each case, RSh increased before changes could be detected in QRS or P–R. With tetrodotoxin and quinacainol, a new class I agent, changes in RSh correlated directly with previously reported changes in dV/dt_{max} of intracellular potentials recorded in vivo from epicardial cells. Representatives from antiarrhythmic classes II, III, and IV were also tested and only changed RSh when they had known sodium channel blocking properties at high doses. Other physiological maneuvers for altering heart rate, such as changing vagal activity, administration of catecholamines, or direct right atrial pacing, did not alter RSh. Thus RSh is a useful in vivo measure for the detection of possible class I antiarrhythmic actions. It has the advantages of being sensitive, selective, easy to measure, and involving minimal preparation.

Keywords: ECG variable; RSh segment; Sodium channel blockers; Anesthetized rats

Introduction

In assessing the electrophysiological actions of a variety of drugs, electrocardiographic (ECG) recordings are often made in conscious or anesthetized rats. The ECG of the rat is more difficult to analyze than those of other small species, such as the guinea pig, because of the relative lack of definition of various important inflection points such as those for the S and T waves. However, because the rat is commonly used in cardiac drug toxicity studies, it is important to be able to make precise and accurate estimates of changes in ECG patterns (Detweiller, 1981).

The rat is a readily available and inexpensive species often used to investigate antiarrhythmic drugs (Winslow, 1984). ECG changes can be used to gain clues for whether a drug is blocking ion channels for sodium, calcium, or potassium ions. For example, most drugs that produce significant degrees of sodium channel blockade can be expected to widen the QRS complex and increase the P–R interval (Vaughan Williams, 1984). The latter interval is also increased by calcium channel blockers and drugs that block beta adrenoceptors in the atrio–ventricular (A–V) node (Angus et al., 1982; Paletta et al., 1989). On the other hand, drugs such as tedisamil that block potassium channels in rat ventricles prolong the Q–T interval (Beatch et al., 1991). We have routinely used such measures in helping to assess the mechanisms of action of various classes of antiarrhythmics (Walker and Chia, 1989; Howard and Walker, 1990).

During the course of studies with drugs known to produce sodium channel blockade in the rat, and while trying to measure QRS intervals, which is difficult in this species because of poor definition of inflection points, we became aware that such drugs appeared to increase the height between the R and the S wave in a

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dose-related manner. We have therefore examined three representative class I antiarrhythmics, plus control drugs. for actions on the S wave to determine whether it was possible to devise an alternative measure to QRS widening or P–R prolongation, and an in vivo indicator of sodium channel blockade.

Materials and Methods

Male Sprague–Dawley rats weighing between 200 and 300 g were used throughout this study. They were anesthetized with pentobarbitone (50 mg/kg i.p.) with additional anesthetic given when, and if, necessary. The right external jugular vein was cannulated for intravenous injections, while the left carotid artery was cannulated for recording blood pressure on a Grass Polygraph (Model 79D).

In order to obtain the best ECG signal for detection of S-wave changes, needle electrodes were placed along the suspected anatomical axis (right atrium to apex) as determined by palpation. The superior electrode was placed at the level of the right clavicle about 0.5 cm from the midline, while the inferior electrode was placed on the left side of the thorax, 0.5 cm from the midline and at the level of the ninth rib. Permanent ECG records were obtained on a Grass Polygraph using a 7P1F low-level preamplifier and associated driver amplifiers. The band width (at −3 dB) was a nominal DC-40Hz. The ECG was also monitored on an E for M/Honeywell ECG monitor, model PM-2A with a band width of 0.05–200 Hz. ECG measurements were made directly from Grass polygraph records recorded at 100 mm/sec, or were measured directly from the memory trace of the monitor. Both methods of measurements gave the same findings in terms of ECG responses to drugs.

Rats were selected at random from a single group. They were anesthetized with pentobarbitone and surgically prepared. Thereafter, 30 min was allowed for recovery time before commencing drug administration. In the study of class I antiarrhythmics, three drugs plus vehicle were studied in a random and blind manner. Either vehicle, quinidine (class Ia), lidocaine (class Ib), or flecainide (class Ic) were given in a cumulative manner with a doubling of dose (administered i.v.) every 15 min. The first dose was always 1.0 mg/kg, and doses were doubled until death occurred or a maximum dose of 16 mg/kg for the last dose was given (32 mg/kg cumulative). All doses were given as an infusion over 1–2 min. The initial diluent was 14% ethanol plus 2% dimethylsulfoxide (DMSO) in distilled water, and all drugs were dissolved at a concentration of 10 mg/mL and at a temperature of 45°–50°C. Subsequent dilution to 1 mg/mL was made with 0.9% saline. The vehicle, time, and volume controls had no effects.

In order to show that S-wave changes were selective for sodium channel blockers, the following drugs were given: atenolol, propranolol, and tedisamil (at 1 and 10 mg/kg) and verapamil (at 0.4 and 4 mg/kg). Doses were given as an i.v. infusion over 1–2 min, whereas longer periods of infusion (10 min) were required for verapamil. Lower doses of drugs were chosen as those producing their maximal characteristic pharmacological action. It was expected that the higher dose of some of the drugs might have, in addition to supramaximal actions with respect to their antiarrhythmic classification, ancillary pharmacological actions such as sodium channel blockade. Specifically, this was anticipated with propranolol (Smith, 1982) and tedisamil (Beatch et al., 1991).

Blood pressure, heart rate, and ECG measurements were taken 5 and 15 min after completing a dose. The first period was assumed to be within the distribution phase for the drug, whereas the second period was chosen to be within the drug metabolism phase. For all the drugs used in this study except lidocaine, the 15-min measurements were used for data analysis. Due to the rapid redistribution and metabolism of lidocaine (Nattel et al., 1987), by 15 min postadministration, drug actions had returned nearly to predrug levels. Hence, for lidocaine the measurements taken at 5 min were used.

Because sodium channel blockers are bradycardic, it was necessary to ascertain whether changes in heart rate altered the RSh measure. A group of animals were subjected to vagal stimulation, direct right atrial stimulation, and i.v. administration of adrenaline. The vagus nerve was isolated in the neck region and decentralized before being stimulated at twice threshold current (100 μA) and pulse width (0.2 msec) with variable frequencies (5–20 Hz) to produce different degrees of bradycardia. Higher frequencies of vagal stimulation were found to product AV blockade, and therefore, were not used. Tachycardia was elicited by direct stimulation of the right atrium by means of a bipolar electrode inserted via the right external jugular vein. After ascertaining that stimulation did not change the configuration of the P wave and QRS complex, the atrium was stimulated at twice threshold current (150 μA) and pulse width (0.3 msec) from 7 to 10 Hz. Another method used to elicit tachycardia was i.v. bolus administration of adrenaline at doses of 0.01, 0.1, and 1.0 μg/kg. By these procedures, the heart rate was controlled between the range of 280–600 beats per minute (bpm).

ECG measurements were made according to the diagram in Figure 1. These measurements were similar to those described by others (Budden et al., 1981) except for the two new variables, σP and RSh. The σP measurement was taken as a measure of the time taken for the depolarization wave to cross the atria (e.g., the

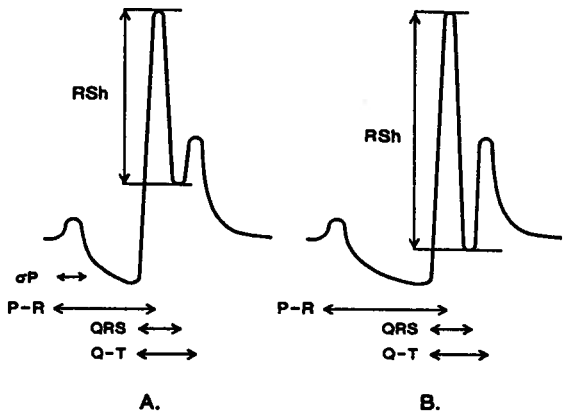

Figure 1. Illustration of the various measurements made from the rat ECG. The figure illustrates an idealized ECG from the rat recorded according to the lead configuration described in the text. (A) is a control ECG and (B) is after administration of a class I sodium channel blocking antiarrhythmic.

span of the P wave). The P wave is well defined, unlike the QRS where the S wave is slurred owing to the early repolarization seen in the rat. It was supposed that the P wave might be prolonged by sodium channel blocking drugs. The RSh magnitude was taken as a measure of the extent of S-wave depression (the height between the peak of the R and S wave). In pilot experiments, RSh was found to be the best measure of S-wave changes. The Q-Tc measure used in this experiment was the Q–T interval corrected for heart rate (Bazett, 1920).

Correlation of RSh with Other Indices of Sodium Channel Blockade

In previous studies we determined doses of tetrodotoxin (Abraham et al., 1989) and quinacainol (Howard et al., 1990) which depress dV/dt_{max} in intracellular potentials recorded from the epicardium of intact open-chest anesthetized rats. In those studies, depression of dV/dt_{max} correlated with other indices of sodium channel blockade including sensitivity to electrical stimulation and prolongation of P–R and/or QRS intervals of the ECG. We determined the effects of tetrodotoxin in a previously described anesthetized and artifically ventilated rat model (Abraham et al., 1989) at low and high doses for effects on RSh. The low dose (10 μg/kg) was chosen to produce only neuronal sodium channel blockade, whereas the high dose (50 μg/kg) also produced some cardiac sodium channel blockade (50% reduction in dV/dt_{max}). The three doses (0.5, 1, and 2 mg/kg) of quinacainol tested for effects on RSh produced a 10%–50% reduction in dV/dt_{max}. Tetrodotoxin was dissolved in saline and quinacainol in acidified saline. Correlations between RSh and dV/dt_{max} were investigated by plotting reductions in dV/dt_{max} against RSh.

Statistical Analyses

Statistical analyses were based on ANOVA followed by Duncan's test for differences of means using an NCSS statistical package (Hintze, 1987). The significance for trends were determined for dose–response relationships. In order to demonstrate the relationships between dose and drug effects, standard cumulative dose–response curves were constructed. In addition, isodose curves were used to analyze the relationships between the different measures. This involved plotting the values for the two measures under consideration, on *X* and *Y* axes, where each point represented measurements made at the same dose level.

Results

Blood Pressure and Heart Rate Changes

Blood pressure and heart rate responses to cumulative doses of the representative class I drugs are shown in Figure 2 (A, B). The analogous changes induced by the control drugs are shown in Table 1.

All of the class I drugs lowered blood pressure and heart rate in a dose-related manner with the potency order (greatest to least) of quinidine, lidocaine, and flecainide for a fall in blood pressure and flecainide, lidocaine and quinidine for a fall in heart rate.

With respect to blood pressure and heart rate responses to the control drugs (Table 1), atenolol and propranolol produced the expected decreases in both blood pressure and heart rate. Tedisamil also produced its characteristic decrease in heart rate accompanied with an increase in blood pressure. Verapamil elicited a significant decrease in blood pressure with a negligible effect on heart rate.

ECG Effects

The effects of the class I antiarrhythmics on ECG intervals are shown in Figure 3 (A–C) in terms of changes in P–R and Q–T intervals, QRS width, and RSh. The control (predrug) values from which the changes were calculated are given in Table 2. Although not shown, σp was not changed in any consistent manner by these drugs, and so data for this variable is not given. Figure 3 clearly shows that, relative to the other ECG measures, changes were first seen in RSh and

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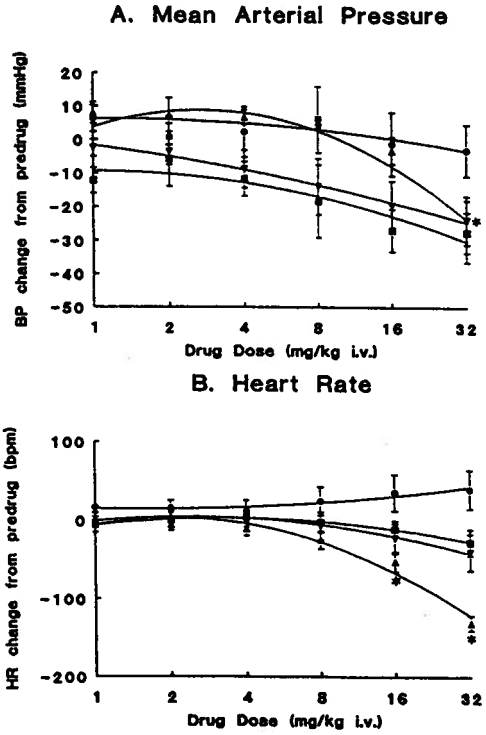


Figure 2. Blood pressure (A) and heart rate (B) changes with quinidine, lidocaine, and flecainide. Each point is the mean ± SEM (n = 6) for the three drugs and a vehicle control. The predrug values were not significantly different for the different groups; the overall mean blood pressure was 120 ± 5 mmHg, and corresponding heart rate was 385 ± 24 bpm. The symbol (●) is for vehicle, (■) quinidine, (▼) lidocaine, and (▲) flecainide. *$p < 0.05$ versus no change.

Table 1. Changes in Blood Pressure and Heart Rate Produced by the Control Drugs

Group	Dose mg/kg	Blood Pressure mmHg	Heart Rate Beat/min
Atenolol	Predrug	120 ± 4	433 ± 10
	1	116 ± 5	377 ± 23
	10	102 ± 4*	349 ± 24*
Propranolol	Predrug	119 ± 4	413 ± 20
	1	105 ± 5	343 ± 34
	10	99 ± 5*	333 ± 26*
Verapamil	Predrug	118 ± 3	435 ± 8
	0.4	111 ± 5	447 ± 10
	4.0	81 ± 5*	409 ± 27
Tedisamil	Predrug	118 ± 1	379 ± 12
	1	131 ± 1*	361 ± 5
	10	134 ± 5*	177 ± 21*

Note: All values are mean ± SEM (n = 4) for changes 15 min after completion of drug administration.

* Indicates $p < 0.05$ versus predrug values.

drug dose was increased. At equal doses, the different drugs produced different degrees of response, and if potency could be expressed in terms of a dose producing a 10% change from predrug, then for RSh changes, lidocaine [Figure 3(A)] was the least potent, flecainide [Figure 3(C)] the most potent, and quinidine [Figure 3(B)] occupied an intermediate position. The efficacies of the drugs could not be assessed in the absence of maximal responses.

In addition to the above measures, the height of the R wave was also measured, but normalization of RSh for changes in R-wave height (e.g., RSh/R) did not improve the measure of RSh. This was because the R wave did not change in height until very high doses were given (data not shown). The only drugs to significantly change Q–T were quinidine and, to a lesser extent, flecainide.

The representative drugs from antiarrhythmic classes II–IV had very different profiles of activity on the ECG compared with the class I antiarrhythmics (Table 3). Thus verapamil (class IV) caused marked P–R widening with no effect on the other ECG measurements. Propranolol (primarily class II) increased the P–R interval, did not decrease Q–T (but reduced Q–Tc), and had no statistically significant effect on both RSh and QRS width. Tedisamil (primarily class III) at the high dose increased all three intervals in addition to increasing RSh. Finally, atenolol had effects on the ECG similar to those of propranolol. During the course of the experiments, changes in RSh were seen shortly after completion of dosing with propranolol (e.g., during the pharmacokinetic redistribution phase).

Influence of Heart Rate

Changes in heart rate had little effect on RSh as summarized in Table 4. Increasing or decreasing heart rate by a number of different techniques had no consistent effect on RSh. Interestingly, the Q–T interval was also unaltered by changes in heart rate (Table 4). Changes observed in Q–Tc due to changes in heart rate were, therefore, due solely to changes in the R–R interval and not to a direct action on ventricular depolarization and repolarization.

Correlation of RSh with Indices of Sodium Channel Blockade

By using doses of tetrodotoxin and quinacainol with known effects on dV/dt_{max} of intracellular potentials recorded in vivo from the epicardium of rats, we attempted to correlate changes in RSh with changes in dV/dt_{max}. As shown in Figure 4, a good correlation

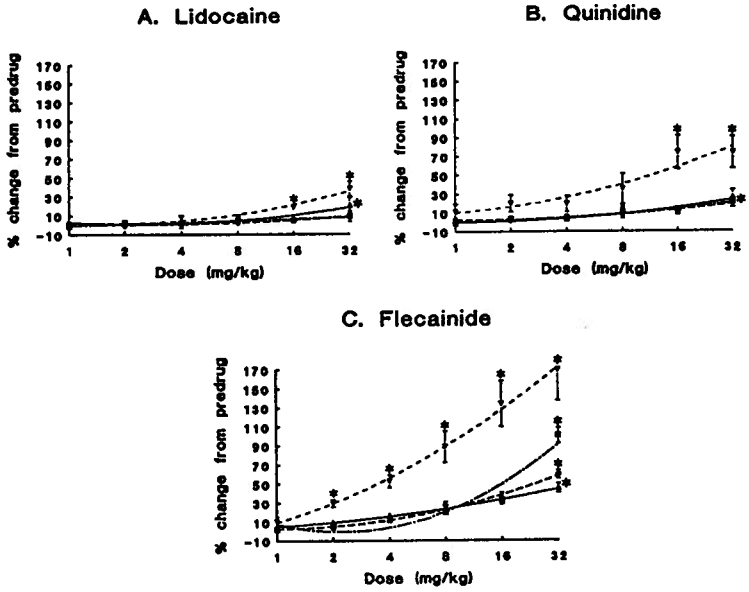


Figure 3. ECG changes induced by quinidine (A), lidocaine (B), and flecainide (C). The symbol (●) is for P–R, (■) for QRS, (▲) for Q–T, and (▼) for RSh. Each point is the mean ± SEM (n = 6) for changes from the control values given in Table 2. *$p < 0.05$ versus no change.

Table 2. Predrug Control Values for ECG Variables for the Three Drug-Treated Groups and Vehicle Control

Group	P–R (msec)	QRS (msec)	Q–T (msec)	Q–Tc (msec)	RSh (mV)
Vehicle	56.2 ± 1.8	30.8 ± 0.4	40 ± 1	99 ± 4	0.40 ± 0.06
Quinidine	54.8 ± 1.8	30.0 ± 0.4	41 ± 1	101 ± 2	0.39 ± 0.04
Lidocaine	56.8 ± 2.2	30.2 ± 0.3	39 ± 1	100 ± 1	0.40 ± 0.04
Flecainide	57.1 ± 2.0	29.2 ± 0.4	39 ± 1	94 ± 1	0.45 ± 0.06

Note: All values are mean ± SEM (n = 6).

Table 3. Changes in ECG Measurements Produced by Control Drugs

Group	Dose mg/kg	P–R (msec)	QRS (msec)	Q–T (msec)	Q–Tc (msec)	RSh (mV)
A	Predrug	56 ± 1	31.3 ± 0.2	43 ± 1	114 ± 2	0.31 ± 0.06
	1	58 ± 1	31.5 ± 0.3	42 ± 1	105 ± 3	0.28 ± 0.05
	10	58 ± 2	31.8 ± 0.4	42 ± 1	103 ± 3[a]	0.29 ± 0.05
P	Predrug	56 ± 1	33.5 ± 0.6	42 ± 1	111 ± 2	0.39 ± 0.06
	1	57 ± 1	34.0 ± 0.8	43 ± 1	103 ± 4	0.39 ± 0.05
	10	63 ± 2[a]	34.3 ± 0.6	44 ± 1	102 ± 7	0.43 ± 0.06
V	Predrug	53 ± 1	33.3 ± 0.4	43 ± 1	115 ± 3	0.29 ± 0.02
	0.04	55 ± 1	33.0 ± 0.8	45 ± 1	121 ± 4	0.29 ± 0.02
	4.0	63 ± 3[a]	34.0 ± 0.6	45 ± 1	116 ± 5	0.27 ± 0.03
T	Predrug	57 ± 1	31.3 ± 0.4	44 ± 1	110 ± 3	0.32 ± 0.04

Note: All values are mean ± SEM (n = 4) for changes 15 min after completion of administration of the particular drug given as a fast infusion.

Abbreviations: A, atenolol; P, propanolol; V, verapamil; T, tedisamil.

[a] Indicates $p < 0.05$ versus predrug values.

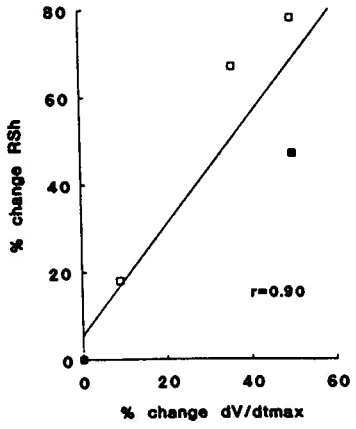
Table 4. The Effect of Changes in Heart Rate on ECG Variables in the Anesthetized Rat (n = 5)

Condition	Stim Freq (Hz)	HR (bpm)	RSh (mV)	Q–T (msec)	Q–Tc (msec)
Control	0	366 ± 24	0.52 ± 0.06	41.8 ± 0.2	103 ± 3
Atrial Pacing	7.0	436 ± 7[a]	0.50 ± 0.06	42.0 ± 0.3	113 ± 1
	7.5	456 ± 3[a]	0.51 ± 0.06	41.8 ± 0.2	115 ± 1[a]
	8.0	481 ± 1[a]	0.51 ± 0.06	41.6 ± 0.4	118 ± 1[a]
	8.5	513 ± 3[a]	0.51 ± 0.06	41.6 ± 0.5	118 ± 1[a]
	9.0	540 ± 2[a]	0.49 ± 0.07	42.2 ± 0.5	127 ± 2[a]
	9.5	565 ± 1[a]	0.50 ± 0.09	42.3 ± 0.5	130 ± 2[a]
	10.0	601 ± 3[a]	0.51 ± 0.09	42.8 ± 0.4	135 ± 2[a]
Control	0	372 ± 25	0.51 ± 0.07	41.8 ± 0.2	104 ± 3
Vagal Stimulation	5	349 ± 22	0.51 ± 0.07	42.0 ± 0.0	101 ± 3
	10	317 ± 22[a]	0.51 ± 0.07	42.0 ± 0.0	96 ± 3
	15	292 ± 19[a]	0.51 ± 0.08	41.4 ± 0.4	91 ± 3[a]
	20	282 ± 18[a]	0.50 ± 0.07	42.0 ± 0.2	90 ± 3[a]
Condition	Dose	HR (bpm)	RSh (mV)	Q–T (msec)	Q–Tc (msec)
Control	0	339 ± 19	0.50 ± 0.04	42.4 ± 0.2	101 ± 2
Adrenaline	0.01	380 ± 17	0.49 ± 0.03	41.9 ± 0.3	105 ± 2
	0.1	397 ± 13	0.50 ± 0.03	42.3 ± 0.5	109 ± 1[a]
	1.0	417 ± 14[a]	0.51 ± 0.04	42.7 ± 0.4	113 ± 1[a]

[a] Indicates $p < 0.05$ versus control values.

was obtained with both quinacainol (three doses) and tetrodotoxin for a percent increase in RSh *versus* a percent fall in dV/dt_{max}. In the case of tetrodotoxin, the low dose (10 μg/kg) produced no changes in dV/dt_{max}, whereas the high dose (50 μg/kg) produced a marked lowering.

Figure 4. Correlation between changes in RSh and dV/dt_{max} of phase 0 of rat epicardial actions potentials. Three doses of quinacainol (open symbols) and tetrodotoxin (filled symbols) were assessed for effects on RSh and compared with effects on dV/dt_{max} determined previously (Abraham et al., 1989 and Howard and Walker, 1990).



Effects of Vehicles

In all of the tests with the various vehicles, there was no effect on any of the measured responses in terms of changes in blood pressure, heart rate, and ECG intervals.

Discussion

The purpose of this study was to evaluate a new and sensitive measure for detecting possible drug-induced sodium channel blockade in anesthetized rats. In general, all of the drugs tested had their expected pharmacological actions on blood pressure, heart rate, and ECG. This is important because this study involved the measurement and comparison of a number of cardiovascular parameters that are used as indirect in vivo indicators of sodium channel blockade.

Thus class I antiarrhythmics lowered blood pressure and heart rate and widened the P–R interval and QRS width, although to varying degrees. The representative drugs from the three subclasses of class I antiarrhythmics produced the expected findings in that flecainide and quinidine produced significant ECG changes, whereas lidocaine was less effective. This was as expected as class Ic antiarrhythmics (e.g., flecainide) produced marked sodium channel blockade at normal sinus beating rats, class Ia (e.g., quinidine) less so, and class Ib (e.g., lidocaine) least of all (see review by Vaughan Williams, 1991). The relative lack of efficacy

of lidocaine at 15 min postadministration presumably also reflects its rapid redistribution and metabolism. Within the context of the varying efficacies mentioned above, the class I antiarrhythmics produced the expected QRS and P–R widening (e.g., flecainide > quinidine > lidocaine). It is important to note, however, that changes in RSh were seen at lower doses than changes in the P–R interval and QRS width, demonstrating the greater sensitivity of the RSh measure.

In terms of the blood pressure and heart rate effects of class I antiarrhythmics, we observed what could be called an *inverse potency order* for the two variables. Hence, at equal doses, quinidine produced greater effects than lidocaine on blood pressure, and lidocaine greater than flecainide. On the other hand, flecainide had greater bradycardic effects than quinidine and lidocaine. This occurrence was presumably the result of an interaction between direct sodium channel blockade (favoring bradycardia) and a tendency for reflex tachycardia in response to falls in blood pressure. Although the direct cardiac action of the class I agents would be expected to decrease heart rate, the parallel decrease in blood pressure would tend to elevate heart rate. Such interactions would explain the potency order for the decrease in blood pressure (quinidine > lidocaine > flecainide) being opposite to that seen for decreases in heart rate (flecainide > lidocaine > quinidine).

The other antiarrhythmics also produced their expected blood pressure, heart rate, and ECG responses. Thus beta blockers reduced blood pressure and heart rate and prolonged the P–R interval. Tedisamil was bradycardic and elevated blood pressure, whereas verapamil reduced blood pressure and prolonged P–R interval. Despite these actions, none of these drugs significantly changed RSh except for tedisamil at its high dose, a dose at which it also produced a statistically significant increase in QRS width. These responses to tedisamil were not unexpected as tedisamil has been suggested to possess sodium channel blocking properties at high doses (Beatch et al., 1991). Propranolol, although having no effect on QRS, appeared to also increase RSh at the high dose (not statistically significant). This too may be explained by propranolol's ancillary sodium channel blocking properties (Smith, 1982). Hence, considering the cardiovascular responses to all the antiarrhythmic agents used in this study, RSh changes appear to be selective for drugs with sodium channel blocking characteristics and occur independently of heart rate and blood pressure changes.

In a separate experiment in which heart rate was experimentally increased or decreased, there was no effect on RSh. Thus RSh changes that were induced selectively by class I antiarrhythmics were independent of changes in heart rate. Interestingly, changes in heart rate were not accompanied by changes in Q–T interval. This relative independent of Q–T interval changes from heart rate in rats implies that the Q–Tc correction for heart rate is not appropriate in rats.

Widening of the P–R interval can be produced by both sodium and calcium channel blockade in the atrio–ventricular node as well as by beta adrenoceptor blockage (Vaughan Williams, 1984; Angus et al., 1982; Paletta et al., 1989). Classically, QRS widening is only produced by sodium channel blocking agents. Sodium channel blockade causes depression of the rise rate of phase 0 of the action potential (dV/dt_{max}) as well as excitability. Although conduction velocity was originally believed to be proportional to dV/dt_{max}, it relates better to excitability (Peon et al., 1978). It is difficult to differentiate effects on excitability from those on dV/dt_{max} as both depend to some extent on sodium channel availability. The speed of impulse conduction depends upon current densities flowing through quiescent tissue and the ease with which that tissue is activated, i.e., excitability (see discussion by Arnsdorf, 1990). Usually, depression of dV/dt_{max} by sodium channel blockade is associated with reductions in excitability and conduction velocity. The latter is manifested in the ECG by both increases in P–R and QRS intervals due to slowed A–V and ventricular conduction, respectively. Unfortunately, the QRS width is difficult to measure with precision, particularly in the rat where the S wave can meld imperceptibly into the T wave making definition of the QRS difficult. Further, although the rat P–R interval is responsive to sodium channel blockade, it is also sensitive to other drugs such as calcium channel and beta blockers. These problems with the QRS and P–R measurements indicate there is a need for alternative ECG measures of sodium channel blockade and RSh seems to be an attractive alternative.

Two lines of evidence support our conclusion that RSh changes appear to be due to sodium channel blockade. First, on a general level, RSh changes occur in unison with other classical ECG changes that result from administration of sodium channel blockers (i.e., P–R and QRS). Second, on an electrophysiological level, as shown in the experiments conducted with quinacainol and tetrodotoxin, RSh changes correlated nicely with changes in dV/dt_{max} which is a fairly direct measure of sodium channel blockade.

The question as to why sodium channel blockade should cause depression of the S wave and thereby the useful measure of RSh, is not easily answered. In order to detect RSh it is important that the lead configuration described in the Materials and Methods section is used. It may be that this particular configuration has a relationship to the electrical axis in such a manner that small changes in conduction velocity cause an axis shift

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resulting in S-wave depression. Although not tested in other species, it is also possible that RSh changes result from a modification of the underlying ionic currents responsible for the unusual shape of the action potentials in rat ventricles. If early repolarization in rat ventricle masks the expected S wave, then slowing of repolarization might be responsible for the lowering of the S wave. However, this was not so because a dose of tedisamil that produced marked Q–T increases did not change RSh.

It is important to appreciate that the RSh measure is seen with both the Grass Polygraph low-fidelity recording system and with a high-fidelity recorder. It is, however easier to measure directly off the Grass trace without correction for the nonlinearity of the trace.

In conclusion, an alternative in vivo measure for sodium channel blocking properties of drugs is needed. RSh is an attractive choice because drug classes known to affect commonly accepted indices of sodium channel blockade (e.g., P–R and QRS intervals), such as the beta blockers and calcium channel blockers, have been shown to not affect the RSh measure. Further, RSh seems to be a more sensitive measure than P–R and QRS intervals because it responded at lower doses of the sodium channel blockers. Finally, this new measure appears to be unaffected by changes in heart rate. Hence, due to its sensitivity and selectivity, the RSh measure has potential for use as an effective in vivo assay for sodium channel blocking drugs.

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APPENDIX 7

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Relationship Between QaT and RR Intervals in Rats, Guinea Pigs, Rabbits, and Primates

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The ECG is routinely used in many species to monitor effects of drugs. While it is relatively easy to measure both PR and QRS, measurement of QT is complicated by the fact that this interval can change with heart rate. In order to compensate for variations in QT due to variations in heart rate, various correction factors have been used, including those of Bazett and Hodges. Such corrections were devised for humans and may have limited applicability in other species. We have systematically varied heart rate in anesthetized rats, guinea pigs, rabbits, and primates using procedures such as vagal stimulation, direct atrial stimulation, injection of cold saline and drugs, including anesthetics, and measured the resulting QT (as QaT and related measures). Over a wide range of heart rates we tested various formulas for their value in correcting for the variation in QT interval associated with changes in heart rate. In rats the "QT" interval did not change appreciably with heart rate. In the other species QaT intervals varied in the expected manner with heart rate in that they decreased with tachycardia and increased with bradycardia. Various formulas were tested for their utility in correcting measures of the QaT interval (QaTc) for changes in heart rate in guinea pigs, rabbits, and primates. In species other than rats, there was little difference between the various formulas in their ability to increase the precision of QaTc and the normality of its distribution, although the best correction is that derived from the regression (either linear, square root, or polynomial) equation relating RR and QaT.

Key Words: QaT correction; Vagal and atrial stimulation; Heart rate; Small animals

Introduction

The QT interval varies with heart rate in many species (Kisch, 1953). Generally such changes involve widening of the QT at low heart rates and shortening at high heart rates. These changes in rate can be usefully corrected in humans using formulas such as those due to Bazett (1920), Fredericia (1920), Hodges et al. (1983), and others. (See Simonson et al., 1962, for a complete list of additional formulas.) While these corrections are useful in humans (Ashman, 1942), their utility may be limited in other species (Browne et al., 1983). In nonprimate species, various procedures have been suggested for correcting rate-dependent changes in QT interval while it is often assumed that the formulas for humans apply equally well to primates. Carlsson et al. (1993) have corrected (i.e., normalized) the QT in rabbits on the basis of a linear regression relating QT interval and heart rate as found in their own series of rabbits. QT correction (QTc) has been attempted in a similar manner for other common laboratory species such as the dog (Van de Water et al., 1989), the rat (Bienfeld and Lehr, 1968), and primates (Adaikan et al., 1992).

Measurements of the ECG and QT interval are useful for a number of purposes, and therefore the most precise and normally distributed (Gaussian) derivative of QT is to be preferred. The QT interval is used in the investigation of the physiological and pathological factors that underlie QT duration and its prolongation (Taran and Szilagyi, 1947) as well as evaluation of drugs, such as class III antiarrhythmics, which lengthen ventricular ac-

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tion potentials and increase refractory periods. In order to assess the actions of various class III antiarrhythmics in various species, we have made use of various measures of the QT interval as an indicator of drug effects on ventricular action potential duration in various laboratory species, i.e., rat, guinea pig, rabbit, and primates, and for cross-species comparison we sought the most precise measure of QT. Interpretation of such QT data is complicated by the fact that some class III drugs lower heart rate and therefore require correction, and so we have made a systematic analysis of the effect of variations in heart rate on measures of QT interval in these species. In the following study, anesthetized rats, guinea pigs, rabbits, and primates were subjected to various procedures chosen for their ability to slow or increase heart rate.

Materials and Methods

Approval for experiments with rats, rabbits, and guinea pigs was obtained from the Animal Care Committee of the University of British Columbia. Standard laboratory animals were used for this part of the study. Experiments on primates were performed at the Department of Obstetrics and Gynaecology, National University of Singapore, Singapore.

General Surgery

Male Sprague-Dawley rats weighing between 250–300 g, male Hartley guinea pigs weighing between 500–800 g, and male New Zealand white rabbits weighing between 1.5–2.5 kg were used in the studies. Two different anesthetic regimens were used; pentobarbitone was used to anesthetize both rats (60 mg/kg i.p.) and rabbits (30 mg/kg i.v.), while guinea pigs were anesthetized with urethane (1 g/kg i.p.). Additional anesthetic was given when necessary. The anesthetic used in these species were those conventionally used in our laboratory.

Primates were anesthetised with either halothane (0.5–1.0%) or pentobarbitone (10–20 mg/kg i.v.) after being initially tranquilized with ketamine (50 mg/kg i.m.). The pentobarbitone anesthesia was used to induce high heart rates whereas halothane anesthesia was used for lower heart rates. The primates were baboons (*Papio anubis*) or macaque monkeys (*Macaque fascicularis*). Blood pressure was recorded from a transcutaneous cannula placed in the femoral artery.

In the small species the left carotid artery was cannulated for recording blood pressure on a Grass polygraph (Model 79D) while the right external jugular vein was cannulated for drug administration. The ECG was recorded using a Lead II type of configuration along the anatomical axis of the heart as determined by palpation. (For a complete description, see Penz et al., 1992). ECGs were recorded on Grass polygraph chart paper at a standard chart speed of 100 mm/sec and on a Honeywell E for M storage oscilloscope. Measurements of intervals were made on the Grass polygraph chart recorder and from the memory trace of the monitor. Both measurements were compared and did not differ significantly in terms of ECG intervals.

Heart rate was altered by various procedures including vagal stimulation, direct atrial stimulation and administration of a bolus of cold saline or treatment with various drugs and anaesthetics.

Vagal Stimulation

Vagal stimulation was accomplished by isolating both the right branch of the vagal nerve as well as the accompanying ascending cervical sympathetic nerves. The nerves were separated by blunt dissection and cut at the level of the submandibular gland. The cardiac end of the vagus nerve was stimulated with bipolar electrodes using square wave stimulation at twice threshold current (it), pulse width of 0.5 msec, and at suitable frequencies (25–60 Hz) to obtain a heart rate which varied between 60 beats/min and sinus rhythm. In some animals the resting heart rate was reduced by metoprolol (1.0 mg/kg i.v.) administration. It was assumed that vagal stimulation of the right branch of the nerve did not result in the significant release of acetylcholine in the ventricles. During vagal stimulation an attempt was made to ensure that atrioventricular (AV) conduction was still intact and that the heart rate was not of an AV nodal origin.

Atrial Stimulation

For atrial stimulation studies, a specially constructed stimulating electrode was prepared using polyethylene (PE) tubing. Two teflon-coated silver wires were inserted into either PE50 (rats and guinea pigs) or PE90 (rabbits) tubing. In order to ensure that electrodes were positioned equidistant apart, a second PE tubing was inserted between the silver wires. For rats and guinea pigs the second tubing was PE10, while for rabbits it was PE50. All tubing was then filled with polyethylene glycol (PEG_{400}) to prevent blood from entering the electrode and to electrically insulate the wires. The Teflon coating of the wires was removed from the stimulating end of the electrode to expose bare metal to allow for pacing of the atria once the electrode was threaded through the right jugular vein into the right atria. The suitability of the electrode position was determined by ensuring that a minimum threshold current at 8 Hz and 1 msec gave a tachycardia with an ECG recording close to that obtained with the animal in sinus

rhythm. Tachycardia was induced over the range of 5.5–9.0 Hz.

Cold Saline and Drug Administration

In addition to electrical pacing, changes in heart rate were also induced by the administration of ice-cold saline and drugs including the beta adrenoceptor agonist adrenaline or isopropylnoradrenaline (0.1–10.0 μg/kg i.v.). Ice-cold saline, at various volumes (1.0–5.0 ml), was injected into the jugular vein, and the resulting changes in heart rate monitored.

Q-T Interval Determination

In view of the difficulty that was sometimes experienced in determining when the T wave returned to the isoelectric line, an alternative measure of QT (QaT) was used for species other than rat. QaT is measured to the peak of the T wave, which could be more clearly defined in this study. Calculations of the QaT interval were made directly from the surface of paper charts. In cases where a clear positive or negative T wave was seen, the QT interval was the time between the negative peak of the Q wave and the peak (negative or positive) of the T wave. In some circumstances when heart rate changed, there were alterations in the shape of the T wave. When this occurred, an attempt was made to use the part of T-wave configuration that best related to the T wave seen in the normal condition, that is, sinus rate. In the cases where changing heart rate produced a change in the configuration of the T wave, a note was made of the change in such configuration. The use of QaT was suggested originally by Lepeschkin (1955), and its value substantiated by Beck and Marriott (1959) and more recently by Nierenberg and Ransil (1979). Chernoff (1972) has discussed QaT in relation to QT in detail.

In the case of rat, it is difficult to detect a T wave that corresponds exactly with the T wave seen in other species (Beinfeld and Lehr, 1968; Driscoll, 1981). In this species therefore, T-wave calculations were made on the basis of the repolarization wave that followed the QRS complex. In previous studies we have considered the difficulties associated with measurement of QT and as a result measure a surrogate of QT ("QT"). This measurement is taken from the Q wave to the first major inflection point on the repolarization phase. Exact details of this measurement is illustrated in Figure 1 of Penz et al. (1992). The RR interval was the mean of 7 beats.

In individual animals, QaT estimates were made over a range of heart rates, and the regression between QaT and heart rate was plotted graphically. If the same regression line occurred between different individuals of the same species, the individual regressions were accumulated to give an overall regression for the species. These regressions were used to determine a regression formula for each species. The effects of using such derived formulae to correct QaT intervals to a common RR of 250 msec were compared with the effects of the other common correction factors.

The utility of the different correction factors was tested by correcting all rates to a basic 240 beats/min and examining the distribution of QaTc intervals for closeness to normality (Gaussian) in terms of heteroscedascity by means of D'Agostino's test and calculation of kurtosis and skewness. In addition, the coefficient of variation (mean/standard deviation) was calculated as an index of precision.

Results

Figures 1(A–C) show the relationships between uncorrected QaT as the dependent variable and heart rate (RR) as the independent variable in individual rabbits, guinea pigs, and rats, regardless of the technique used to alter heart rate. As can be seen in Figure 1, the relationships between heart rate and QaT were similar for the different individuals of the same species, except for rabbit 2 (Figure 1-A), which was statistically significantly different from the other animals ($p < 0.05$ for difference for the parameters of its regression line from the group). In the case of rats (Figure 1-C), QaT did not change with rate. However, with both rabbits and guinea pigs, there was a clear positive regression between RR and QaT.

In order to determine whether the method of changing heart rate had an effect on QaT intervals independent of rate, data for all members of a particular species were accumulated and plotted in Figures 2 and 3. In Figure 2 the data points obtained by the different techniques for changing heart rate show that the relationship between RR and QaT for the rabbit (a) and the guinea pig (b) were the same regardless of the procedures used to change heart rate. In the case of primates (Figure 3), values for monkeys and baboons initially were plotted individually, but in view of the fact that the data were not statistically different, they were grouped together for calculation of the regression lines of best fit.

An attempt was made to determine the line of best fit for the data using linear, square root, and polynomial (power 2) functions, and the resulting best fit lines are indicated in Figures 2 and 3. In the case of rabbit data, the regression coefficients were 0.97, 0.90, and 0.97, respectively, for the three types of regression. In the case of guinea pig data, the corresponding coefficients were 0.84, 0.84, and 0.85. For the accumulated primates, the values were 0.75, 0.75, and 0.75.

In view of the fact that a good regression relationship between RR and QaT was obtained for the guinea pig,

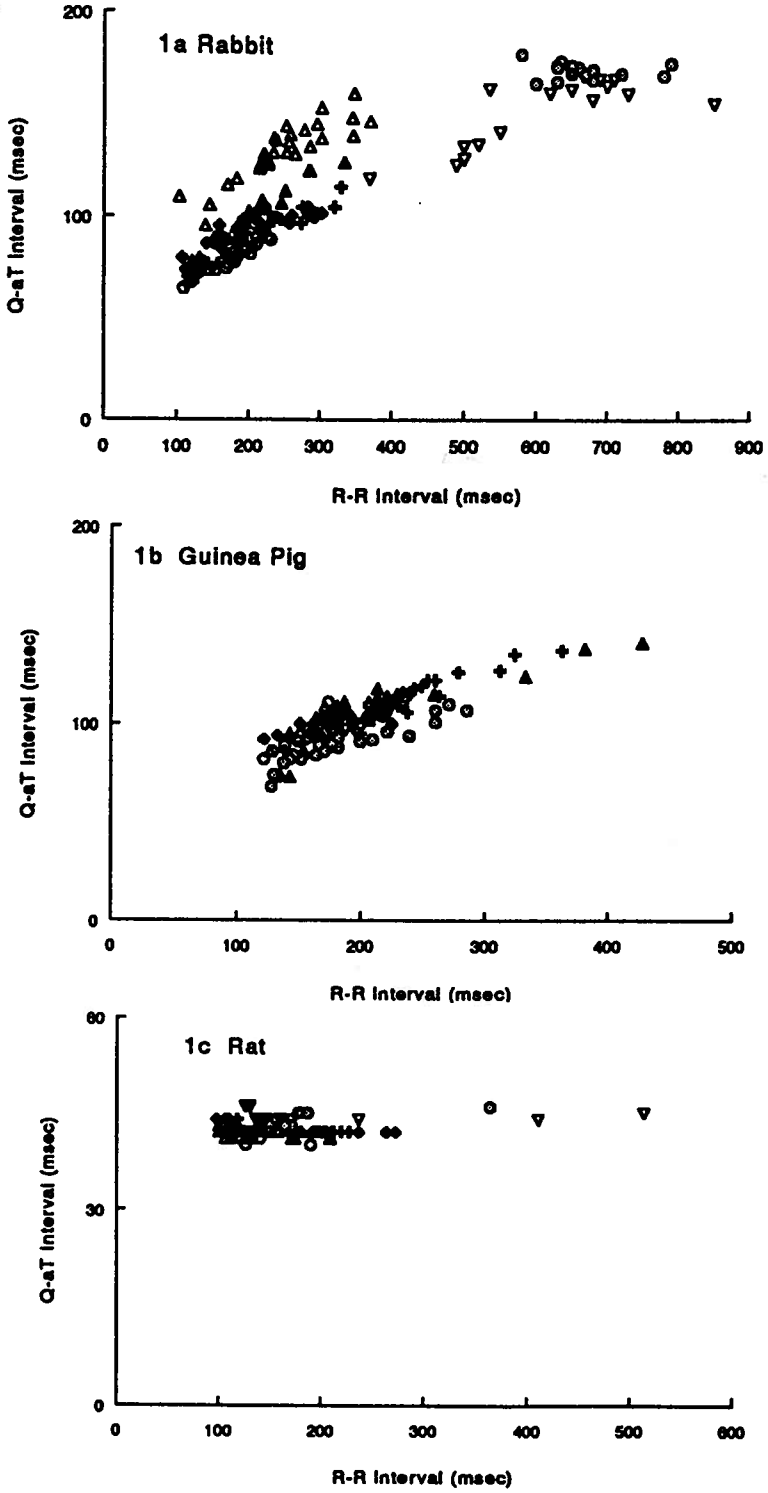

Figure 1 (A–C). Relationship between heart rate (as RR interval in msec) and QaT interval duration (in msec) in individual rabbits (a), guinea pigs (b), and rats (c). Data points are shown for individual members of the three species anesthetised and prepared as indicated in the Methods section. Data points for individual animals are indicated by the following symbols: animal 1 (◆), animal 2 (△), animal 3 (○), animal 4 (+), animal 5 (▲), animal 6 (●), animal 7 (▽). From 8–10 data points were obtained for each animal over a range of RR intervals using the variety of procedures for manipulating heart rate described above.

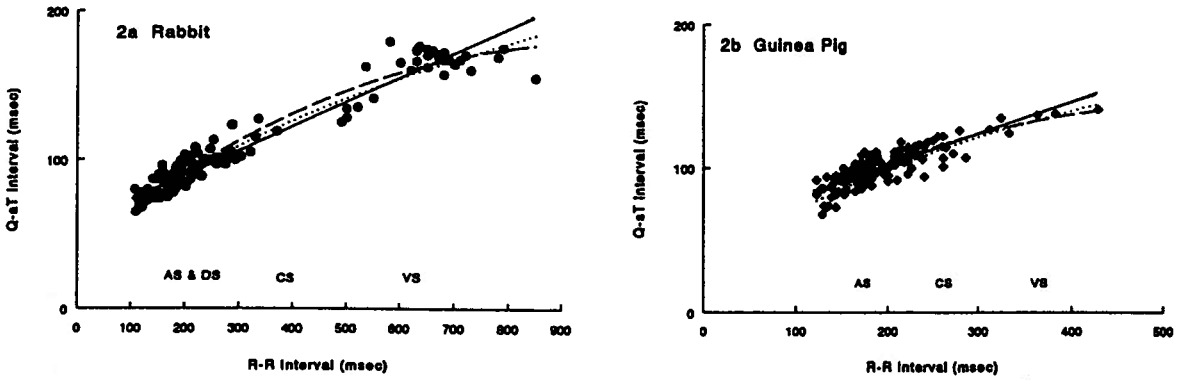

Figure 2. Regression between heart rate (as RR interval in msec) and QaT interval duration for rabbits (a) and guinea pigs (b). Data in Figure 1 for rabbits and guinea pigs are replotted and the lines of best fit shown for the linear relationship $QaT = a_L + b_L * RR + a$ (solid line), square-root relationship $QaT = b_s * RR^{1/2}$ (dotted line), and the polynomial relationship $QaT = a_p + b_p * RR + c_p * RR^2$ (dashed line), where QaT is the QaT interval in msec and RR in msec. No regression lines or data are shown for the rat because in this species there was no relationship between QaT and RR (Figure 1c). As discussed in the text, rabbit 2 was excluded from Figure 2a since it was a statistically significant outlier and therefore can be excluded on statistical grounds according to the well-accepted Chauvenet's criteria. The range of RR values over which various techniques were used to change heart rate are shown in the figure and denoted by VS for vagal stimulation, AS for direct atrial stimulation, CS for cold saline, and DS for drug stimulation.

rabbit, and primates—whereas there was no such relationship for the rat—an attempt was made to determine the most useful correction formulas for normalizing QaT for changes in heart rate in guinea pigs, rabbits, and primates (Table 1). The underlying premise for this aspect of the study was that the best correction of QaT would result in data that was normally (Gaussian) distributed, had the least heteroscedascity (by skewness and kurtosis), and had the lowest coefficient of variation (mean/standard deviation). The results of such corrections are summarized in Table 2. This table compares the correction using the above regression formulas with a number of standard correction formulas that have been used in human and other species. Examination of Table 2 shows that the best correction utilized the linear regression equations found in Table 1 for the rabbit and guinea pig, but not those for the rat because there was no regression between QaT and heart rate in rats.

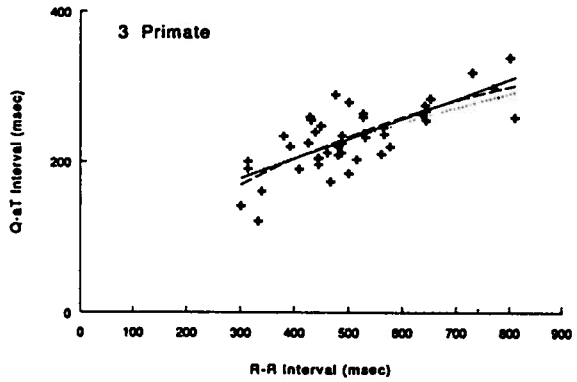

Figure 3. Regression relationship between heart rate (as RR interval in msec) and QaT interval duration for primates. Values are accumulated data for both baboons and monkeys because no significant difference between the two species was found. As in Figure 2a and b, the lines of best fit are shown for linear (solid line), square root (dotted line), and polynomial (dashed line) formulas using the same equations as those found in the Figure 2 legend.

Discussion

Variations of QT interval with heart rate in humans and other species has been noted many times. A complete explanation, in terms of the underlying ionic currents, is not available, but current hypotheses center on the various potassium channels responsible for repolarization (Hume and Uehara, 1985; Carmeliet, 1993). It is recognized that, at shorter diastolic intervals, a portion of the repolarizing channels, such as those responsible

Table 1. Fitting Parameters for the Equations Used in Calculation of Lines of Best Fit According to the Linear and Nonlinear Formulas

	Linear		Square root	Polynomial		
Species	b_L	a_L	b_s	a_p	b_p	c_p
G Pig	0.21	58.9	7.0	41.4	0.38	−0.00034
Rabbits	0.16	59.0	6.3	41.3	0.28	−0.00014
Primate	0.27	98.7	10.3	40.6	0.49	−0.00020

The equations used for the above fitting parameters were Linear: $QaT = a_L + b_L * RR$; Square root: $QaT = b_s * \sqrt{RR}$; Polynomial: $QaT = a_p + b_p * RR + c_p * RR^2$.

The fitting parameters in the above table are indicated by a, b, and c with the appropriate subscript for the different functions.

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Table 2. Statistical Analysis of Effects of Various Correction Formulas on the QaT Interval

	QaT	Linear	SqRt	Poly	Bazett	Hodges	Driscoll
Rabbit							
Mean	108.9	99.0	102.0	102.4	102.7	113.7	6.5
SD	34.3	8.9	8.2	7.9	7.6	17.8	0.5
C.V.(%)	49.0	8.9	8.0	7.7	7.4	16.0	7.4
K	−0.66	2.6	−0.23	−0.09	0.23	0.92	0.23
S	0.90	−0.26	−0.23	−0.08	0.23	0.92	0.23
P for Fit	<0.01	<0.01	>0.20	<0.20	0.1–0.2	<0.01	0.1–0.2
Guinea Pig							
Mean	101.3	112.4	113.8	116.8	115.2	118.6	7.3
SD	13.1	7.1	6.9	7.0	8.2	8.2	0.52
C.V.(%)	13	6.3	6.1	6.0	7.1	6.9	7.1
K	0.83	−0.35	−0.40	−0.50	−0.37	0.54	−0.37
S	0.32	−0.47	−0.38	−0.44	−0.25	0.10	−0.25
Fit	0.02–.05	>0.20	>0.20	>0.20	>0.20	>0.20	>0.20
Primate							
Mean	223.8	163.7	165.7	149.0	164.4	202.6	10.4
SD	44.2	29.7	29.8	29.4	22.0	38.5	1.39
C.V.(%)	19	18	18	20	13	19	13
K	0.44	−0.27	−0.35	−0.56	0.16	0.30	0.16
S	−0.14	−0.13	−0.06	0.08	−0.27	−0.08	−0.27
Fit	>0.20	>0.20	>0.20	>0.20	>0.20	>0.20	>0.20

The above table shows the results of the following formulas used to correct the QaT interval for changes in heart rate in rabbits, guinea pigs, and primates. For the linear correction the equation used was $QaTc = QaT - b_L * (RR-250)$, while for the square root (SqRt) correction the equation used was $QaTc = QaT + b_s * (\sqrt{250} - \sqrt{RR})$. Finally, the equation $QaTc = QaT - b_p * (RR-250) - c_p * ((250)^2-(RR)^2)$ was used as the polynomial (Poly) correction. For each corrected mean, the standard deviation (SD) and coefficient of variation (CV) were calculated. As well, the shape of the distribution of values for each species or kurtosis (K) and heteroscedasticity (S) can be seen, and the level of significance for the fit (P for fit) completes the table.

for iK_r, would still remain activated from the previous action potential and thereby shorten the action potential (Hauswirth et al., 1972; Boyett and Fedida, 1984). In addition, over time, changes in rate can be expected to change the intracellular concentration of sodium and calcium ions (Nierenberg and Ransil, 1979) as well as extracellular potassium (Kunze, 1977), and, thus, influence repolarization. Such changes will, less directly, result in corresponding changes in the repolarization currents. The importance of such processes varies with species, and, thus, rate dependent changes in QT could be expected to vary with species. The lack of iK_r and predominant i_{to} in the rat (Josephson et al., 1984) may account completely for the lack of any major effect on rate on "QT" in this species.

It is necessary to recognize that changes in QT are relatively poor indices in how repolarization might be influenced by heart rate. The QT interval reflects, probably in a manner proportional to the number of each type of cell present in the ventricle, repolarization processes in the different types of cells found in ventricles. Thus the repolarization process and its sensitivity to rate could probably differ between ventricular and Purkinje cells, between endocardial and epicardial cells, and between Spike and Dome cells, etc. Unfortunately the possible differential effects of heart rate changes on the shape and configuration of the QT interval has not been systematically investigated. Regardless of such complications, the QT interval probably still represents a useful approximation of action potential duration in the ventricle and therefore is worth measuring.

If the QT is to be measured, there is a need to correct for any concomitant changes in heart rate. This problem has been studied many times for humans and even for common laboratory species but without any consensus as to the most useful and appropriate correction factor. It is, however, apparent that there cannot be any one correction formula that applies equally well to all species.

In the case of the rat, there appeared to be little variation in QT, as measured in this study, and heart rate. Other investigators have previously investigated this problem in rats and found some variability in QT with rate (Beinfeld and Lehr, 1956).

In the guinea pig, a positive relationship between heart rate and QaT was apparent and best corrected by formulas derived from various regression equations. Corrections according to the procedures of Bazett, Driscoll, and Hodges were also beneficial in correcting QaT for heart rate in this species.

In the rabbit, the various regression-corrected QaT distributions are much better than the uncorrected data. Corrections proposed by Bazett and Driscoll were of some value, whereas the correction proposed by Hodges

appeared to be of lesser use. In primates, the regression corrected QaT distributions were not a great improvement over the uncorrected data.

In conclusion, it appears that no one correction formula has marked advantage over other correction formulas for any species.

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APPENDIX 8

Cardiovascular Drug Reviews
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Cardiovascular Actions of U50,488H and Related Kappa Agonists

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Key Words: Cardiovascular actions—Kappa agonist—Opioid—U50,488H.

INTRODUCTION

Both agonist and antagonist drugs acting upon the various types of opioid receptors located in central and peripheral nervous systems are under development in the pharmaceutical industry. One of the most important of the opioid receptors is the kappa receptor as its activation results in the alleviation of pain without producing either abuse liability or many of the other adverse effects of opioids; this is a valuable attribute for a new analgesic drug (66,77). Pharmaceutical companies have invested much in the development of new synthetic opioids (kappa agonists) in the hope of producing better analgesics.

In view of the supposition that opioid receptors play a relatively limited role in cardiovascular regulation, kappa agonists might be expected to have only limited actions on the cardiovascular system. However, recent experiments (described in this paper) with the kappa agonist U50,488H, and to a lesser extent with other kappa agonists, have suggested that these compounds have significant actions on the cardiovascular system. Some of these actions appear to be mediated via activation of opioid receptors located in the central and/or peripheral nervous systems, whereas others are independent of such receptors and appear to be due to direct actions on the heart or blood vessels.

This review will concentrate upon the cardiovascular actions of kappa agonists, as exemplified by U50,488H. In the first part of the review we will consider the general pharmacological actions of U50,488H and related kappa agonists with a similar chemical structure. The second part of the review will consider in detail their actions on the intact cardiovascular system, isolated hearts, and blood vessels.

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GENERAL PHARMACOLOGY OF U50,488H AND RELATED KAPPA AGONISTS

U50,488H was one of the first compounds synthesized as part of a series of kappa receptor agonists which chemically are cyclohexyl derivatives of benzeneacetamide (73). U50,488H has the chemical structure *trans*-(±)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]-benzene-acetamide methane sulfonate (Fig. 1). In general, compounds in the cyclohexylbenzeneacetamide series have potential value both as clinical analgesics and as tools for exploring the functional significance of kappa receptors in the central nervous system (CNS) and elsewhere (43,79,80).

The present classification of opioid receptors is based on the pharmacological profiles of different opioid drugs (12,49,66) as they relate to various physiological actions, differential effects of naloxone antagonism, different potency ratios in bioassays, and differences in binding profiles to putative opioid receptors. The individual opioid receptors themselves appear to be separate glycoprotein entities with molecular sizes which range between 55–65 kDa (30,72).

Molecular Mechanisms

Recent studies have suggested that mu (μ), delta (δ), and kappa (κ) opioid receptors are linked negatively to adenylate cyclase (30). These receptors are associated with a specific pertussis sensitive G-protein.

Experiments using selective kappa agonists, such as U50,488H and d-pro-dynorphin indicate that adenyl cyclase activity can be inhibited by these compounds. This action is G-protein–dependent and can be inhibited by naloxone (41). The coupling of the kappa receptor to adenylate cyclase may not be direct but a direct link is highly probable for the μ receptor (61).

Differences exist in the coupling of kappa opioid receptors to other effector systems. Evidence for this comes from studies by Attali et al. (4). These authors assessed the effects of opiates on $^{45}Ca^{2+}$ influx induced in co-cultures of rat spinal cord and dorsal root ganglia by either Bay K 8644 or elevated extracellular potassium. Influx under these

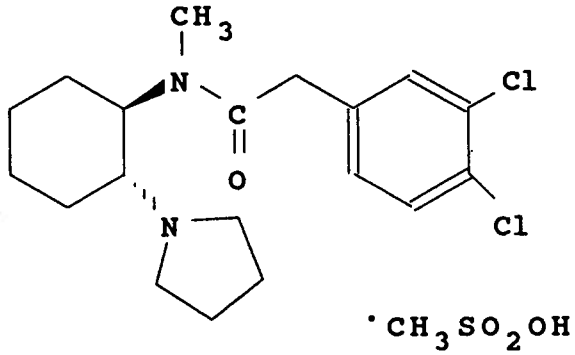

FIG. 1. The chemical structure of the κ-receptor agonist U50,488H (*trans*-(±)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolodinyl)cyclohexyl]-benzene-acetamide methane sulfonate).

conditions depends upon activation of L-type calcium channels. Agonists for both μ and δ opioid receptors had no effect on the calcium influx. U50,488H and dynorphin, on the other hand, decreased calcium influx. Further investigation by these authors showed that kappa receptor activation, acting via a G-protein inhibited the opening of L-type calcium channels. Although kappa agonist-mediated inhibition of the channel is blocked by naloxone, U50,488H at a concentration of 100 μ*M*, does not competitively or allosterically inhibit specific binding of calcium channel blockers.

There are indications that there may be more than one mechanism by which kappa receptors are coupled to other systems. For example, U50,488H produces a dependent increase in inositol phosphate in hippocampal slices which is blocked by naloxone (57). On the other hand, the same workers (58) also showed that U50,488H inhibits carbachol-stimulated PI turnover (IC_{50} = 33 μ*M*) without influencing responses to other agonists. The above studies offer partial explanations of how the effects of kappa receptor activation are mediated as well as the possible involvement of second messengers.

Analgesic Actions

In terms of analgesic actions, U50,488H is equipotent to morphine in thermal, pressure, and irritation analgesia assays in some species (77) although the analgesic potency of U50,488H varies markedly with the intensity of the nociceptive stimulus applied (77). Furthermore, analgesia produced by U50,488H is not associated with physical dependence, neither is U50,488H self-administered, although tolerance does develop to its analgesic actions. This tolerance cannot be attributed to enhanced metabolism, nor does cross-tolerance to morphine occur (77). In addition, U50,488H also produces sedation and muscle weakness in rats and monkeys (75).

Other Actions on Neuronal Tissue

When opioid receptor drugs act on nervous tissue they can act at the level of the central or peripheral nervous systems. Both higher and lower centers in the CNS have opioid receptors, stimulation of which can produce a variety of responses in different body systems including the cardiovascular system (50). In addition, there are also μ and kappa opioid receptors in the peripheral autonomic nervous system (ANS). This is exemplified by a classical peripheral action of morphine, i.e., inhibition of the release of autonomic transmitters as was first shown in guinea pig ileum (55). This inhibitory action was central to the discovery of endogenous opioids in that inhibition of transmitter (norepinephrine) release from a peripheral tissue was the test system used by Hughes et al. (34) to detect enkephalins.

As a result of activation of kappa receptors, U50,488H alters both calcium action potential generation and the release of acetylcholine from presynaptic nerve terminals of guinea pig myenteric neurons (13). It has been suggested that the kappa receptor is linked with N-type calcium channels in such a way that activation of the receptor directly reduces calcium influx into the nerve terminal thereby reducing transmitter release (84,85). In a similar manner many kappa agonists (including U50,488H, PD117302, and U69593) have recently been shown to inhibit dopamine release from neuronal tissue (52,82).

A related action is inhibition of K^+-induced histamine release from cortical slices. Evoked histamine release was dose-dependently inhibited by the kappa agonists ketocy-

clazocine, dynorphin A (1–13), PD 117302, spiradoline, U50,488H, and U69,593 (24). This action was prevented by the kappa antagonist MR 2266 and nor-binaltorphiimine. It was suggested that inhibition of cerebral histamine release may account for the sedation produced by kappa agonists.

Antagonism at Kappa Receptors

In the investigation of the possible complex actions of opioid drugs on the cardiovascular system, naloxone is a particularly useful tool for differentiating between opioid and nonopioid receptor-mediated actions. All opioid receptor-dependent actions are easily blocked by opioid antagonists, including those involving kappa agonists. However, the potency of naloxone in antagonizing morphine is greater than that for blocking kappa receptor-mediated responses produced by U50,488H (43). Recently selective kappa antagonists such as MR2266 have become available; they have been used to selectively block kappa receptor actions (63,83).

When studying the pharmacological actions of U50,488H and related kappa agonists it is important to realize that such drugs may have at least three distinct types of pharmacological action. The most notable of these are due to actions on the kappa receptor. Such kappa receptor-mediated actions generally occur at lower doses and are easily blocked by naloxone. Another type of action seen at higher doses may involve actions on other types of opioid receptors, such as the μ and δ types. These opioid receptor-dependent actions are also blocked by naloxone. A third type of action, seen at the highest doses, does not involve opioid receptors and is characteristically not blocked by naloxone. In discussing the pharmacological actions of kappa agonists it is, therefore, important to differentiate between low and high dose effects, and actions blocked by naloxone.

Kappa Agonists Similar to U50,488H

A variety of other kappa agonists (cyclohexylbenzacetamide derivatives), or compounds with similar chemical structures, have been synthesized (16,17,25). These include U62066 (spiradoline), an analog of U50,488H in which a spirotetrahydrofuranyl substituent is placed at the C4 position of the cyclohexane ring (81), and the Parke-Davis compounds, PD 117302 and CI-977 (also known as CAM 570 and PD 129290, respectively) (14,16,36,37).

The kappa- and μ-opioid binding affinities of the kappa-selective ligand U62066 (spiradoline) and its optical enantiomers, (+) R,R U63639 and (−) S,S U63640, have been compared with those of the structurally related ligand PD 117302 (CAM 20) (51) and its respective isomers, (+) R,R PD 123497 and (−) S,S PD 123475. Both the racemic compounds and the (−) S,S-enantiomer display high affinity and selectivity for kappa receptors (51). Interestingly, the (+) R,R-enantiomer (PD 123497) has only 1/1,000th the affinity of the S,S-enantiomer for kappa receptors, but has approximately equal affinity for μ receptors. A similar pairing of enantiomers, also synthesized by Parke-Davis, exists between the (+) R,R-enantiomer PD 129289 and the (−) S,S-enantiomer PD 129290. The (−) S,S-enantiomer has by far the greatest affinity for the kappa receptor (25).

The pharmacological actions of U50,488H in comparison with other kappa agonists are summarized in Table 1. The table also indicates actions which are not blocked by naloxone and therefore can be classified as being non-opioid in nature. The chemical names of drugs mentioned in this article are listed in Table 3.

TABLE 1. *Pharmacological actions of kappa agonists*

Kappa agonist	Kappa receptor dependent actions				Ref.
	Analgesia	Sedation	Diuresis	Arrhythmias	
U50,488H	+	+	+	+	A
ICI-204448	+	+	+	?	B
Spiradoline	+	+	+	?	C
EKC	+	+	+	?	D
Bremazocine	+	+	+	?	E
CI-977	+	+	+	?	F

Actions not blocked by naloxone (kappa receptor independent)

	Arrhythmias	Channel blocking actions			
	Anti	Na^+	Ca^{2+}	K^+	Ref.
U50,488H	+	+	+	+	G
Spiradoline	+	?	+	–	I
Ethylketocyclazocine	?	?	+	–	J
Bremazocine	?	?	+	–	K
CI-977	+	+	–	–	L

+ indicates a well established pharmacological action; ? indicates insufficient data available to make a definitive statement; — indicates no data available. EKC, ethylketocyclazocine; Pro, proarrhythmic; Anti, antiarrhythmic.

A. refs. 3,4,10,26,39,43,44,60,63,76,77,78
B. ref. 71
C. refs. 17,25–27,32,42,59,60,81
D. refs. 15,31,35,86
E. refs. 7,9,23,74,79
F. refs. 36,37
G. refs. 3,4,7,18,63,76,84
I. ref. 27
J. ref. 26
K. refs. 7,9
L. ref. 65

CARDIOVASCULAR ACTIONS OF U50,488H AND RELATED KAPPA AGONISTS

Actions Dependent on Kappa and Other Opioid Receptors

Kappa agonists and opioids in general can exhibit a variety of cardiovascular actions. For example, morphine, the prototypical opiate, is an important drug in the management of congestive heart failure. The exact mechanisms by which morphine produces beneficial effects in congestive heart failure are not known, although some of the effects undoubtedly depend on its interaction with opioid receptors (28). The CNS effects of morphine such as calming and anxiety reducing, are mediated by opioid receptors (48) but effects on the peripheral circulation which result in reduced central venous pressure may not depend on opioid receptors.

More specifically for kappa receptors, U50,488H tested over a range of doses produces effects on blood pressure and heart rate which are species- and dose-dependent. Cardio-

vascular responses to U50,488H intravenously injected differ from those following direct injection into the CNS (70). Over the dose range 0.01–3.0 mg/kg (i.v.), U50,488H produced dose-related reductions in mean arterial pressure, left ventricular systolic pressure, left ventricular *dP/dt*, and heart rate in anesthetized dogs. Such responses were completely prevented by the prior administration of 1 mg/kg naloxone (26). Spiradoline (U62066), a structurally related kappa agonist, produced similar cardiovascular depression in dogs which was also prevented by pretreatment with naloxone (26). The actions of spiradoline on blood pressure and heart rate depend upon dose, species, and level of anesthesia; spiradoline can raise or lower blood pressure and heart rate dependent upon the test situation (26).

In our own studies in pentobarbital-anesthetized rats, U50,488H (0.5–32 μ*M*/kg i.v.) dose-dependently reduced heart rate and blood pressure. In addition, at the higher end of the dose range it prolonged the P-R and Q-T intervals of the ECG and widened the QRS complex in a dose-dependent manner (63). Neither naloxone nor the kappa receptor selective antagonist MR 2266 reduced the cardiovascular actions of higher doses of U50,488H on heart rate, blood pressure, and ECG. Most studies in rats using U50,488H or other kappa agonists report similar depressant actions on blood pressure and heart rate, particularly at higher doses. However, such actions are reported as being blocked by naloxone in cats and dogs (26).

While diuresis is not always considered a cardiovascular response it is produced by kappa agonists in a variety of species. Potent kappa agonists such as U50,488H (6,44) and bremazocine produce dose-dependent diuresis in rats, as does ethylketocyclazocine (35). The diuresis produced by kappa agonists can be blocked by kappa antagonists such as MR2266 and WIN 44,441 (35). Oiso et al. (53) showed that U50,488H does not induce diuresis in rats which genetically lack arginine vasopressin (AVP). This led to the suggestion that the diuresis produced by kappa agonists may involve suppression of AVP release. However, in humans, kappa agonists induce diuresis by a mechanism which does not involve suppression of vasopressin or changes in renal blood flow (67). In addition to a possible action on vasopressin release, the kappa agonists U50,488H, ethylketocyclazocine, trifluadom, and MR 2034 influence the release of other hypothalamic hormones. Thus they elevate plasma corticosterone and reduce thyroid stimulating hormone levels in animals (39,40).

Some of the cardiovascular responses produced by kappa agonists in intact animals are mediated partially by receptor-dependent effects in the CNS (11). This is particularly true for supraanalgesic doses. For example, while U50,488H is not noted for its actions on μ receptors, high doses are capable of inhibiting respiration and depressing the central nervous system (80).

In order to test for pharmacological actions not involving opioid receptors, it is necessary to perform studies in the presence of opioid receptor antagonists, such as naloxone, or to use enantiomers which lack activity at kappa receptors. For example, in our own studies of U50,488H cardiovascular responses observed in intact anesthetized rats were not influenced by naloxone (63). Since these cardiovascular depressant actions are not blocked by naloxone they do not depend upon opioid receptors. Interestingly, the R,R-enantiomer (PD 129289) of the kappa agonist Cl-977 (PD 129290) lacks kappa agonist actions but produces similar naloxone-resistant falls in blood pressure and heart rate (unpublished observations).

Effects on Arrhythmias

A variety of studies (Table 2) have shown that opioid drugs can influence the severity and incidence of arrhythmias induced by myocardial ischemia and infarction. Our own studies in rats showed that at a high dose (16 μ*M*/kg) U50,488H reduced arrhythmias induced by myocardial ischemia, an action not blocked by naloxone (64). In an unpublished study in which the two enantiomers PD 129289 and PD 129290 were similarly tested, antiarrhythmic activity was seen against ischemia-induced arrhythmias at high doses (2 and 8 μ*M*/kg i.v.) of both enantiomers. These antiarrhythmic effects were unaltered by naloxone pretreatment.

Actions Independent of Kappa 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 U50,448H at high doses can be interpreted as indicating ion channel blockade, particularly for the sodium channel. Thus, U50,488H produced P-R prolongation and QRS widening in rats (63), together with elevation of "RSh," an ECG index of sodium channel blockade in this species (56). In addition to signs of sodium channel blockade, there was also limited widening of the Q-T interval, a possible indication of potassium channel blockade.

In the above study, responses to electrical stimulation (63) in the presence of U50,488H were only partially consistent with sodium channel blockade. Subtle differences in responses to U50,488H occurred in the presence and absence of naloxone pretreatment. In the presence of naloxone U50,488H dose-dependently, and in a monotonic manner, increased the threshold currents for induction of extrasystoles and ventricular fibrillation. However, in the absence of naloxone a paradoxical initial decrease, rather than increase, in thresholds was seen at lower doses. Such decreases may suggest an increased sensitivity to electrical stimulation, i.e., a potential proarrhythmic action mediated by opioid receptors.

TABLE 2. *Opioid receptor agonists and antagonists which have antiarrhythmic actions*

Drug*	Reference(s)
Buprenorphine	5
Dextrorphan	69
Levorphanol	69
Meptazinol	21
Morphine	69
U-69593	3
(−)WIN 44,441-3	54,69
(+)WIN 44,441-2	69
MR-2266	47,83
(−)MR-1452	54
(+)Naloxone	8,69
Naloxone	20,22,33,45,47,62,63,68,69

* Antiarrhythmic actions were against arrhythmias induced by coronary occlusion. All drugs are in the racemic form unless otherwise indicated.

TABLE 3. *Full chemical names and CAS numbers of compounds mentioned in this article*

Drug	CAS	Chemical name
Bremazocine	756B4-07-0	(±)-6-ethyl-1,2,3,4,5,6-hexahydro-3-[(1-hydroxycyclopropyl)methyl-11,11-dimethyl-2,6-methano-3-benzazocin-8-ol hydrochloride
Ethylketocyclazocine	58640-84-9	(2α,6α,11S$^+$)-(±)-3-cyclopropylmethyl)-6-ethyl-3,4,5,6-tetrahydro-8-hydroxy-11-2,6-methano-3-benzazocin-1(2H)-one
ICI 204448	40994-80-7	R,S-[3-[1-[[(3,4-dichlorophenyl)acetyl]methylamino]-2-(1-pyrrolidinyl)ethyl]phenoxy]-acetic acid hydrochloride
MR 1452	72656-86-1	1-(N-furylmethyl)-α-norme-azocine methane-sulfonate
MR 2034	57236-85-8	(−)-(1R,5R,9R,2″S)-5,9-dimethyl-2′-hydroxy-2-(tetrahydrofurfuryl)-6,7-benzomorphan
MR 2266	56649-86-1	(−)-5,9α-diethyl-2-(3-furylmethyl)-2′-hydroxy-6,7-benzomorphan
PD 117302	111728-01-9	(±)-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzo[b]thiophene-4-acetamide monohydrochloride
PD 129289	None	(+)-[5S-(5α,7α,8β)]-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]-dec-8-yl]-4-benzofuran acetamide monohydrochloride
PD 129290	107431-28-7	(−)-[5R-(5α,7α,8β)]-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]-dec-8-yl]-4-benzofuran acetamide monohydrochloride
Spiradoline	87151-85-7	(5α,7α,8β)-(±)-3,4-dichloro-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-4-benzeneacetamide methane sulfonate
Tifluadom	83386-35-0	N-[[5-(2-fluorophenyl)-2,3-dihydro-1-methyl-1H-1,4-benzodiazepin-2-yl]methyl]-3-thiophenecarboxamide
U50488H	67198-13-4	*trans*-(±)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]-benzene-acetamide methane sulfonate
U69593	69744-75-1	(5α,7α,8β)-(+)-3,4-dichloro-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-4-benzeneacetamide methane sulfonate
WIN 44441	69924-29-4	(2α,6α,11s)-(−)-cyclopentyl-D-(1,2,3,4,5,6-hexahydro-3,6,11-trimethyl-2,6-methano-3-benzazocin-11-yl)-3-pentanone methane sulfonate

In an unpublished study using R,R-(PD 129290) and S,S-(PD 129289) enantiomers analogous observations were made in that the R,R-enantiomer, which lacks affinity for kappa receptors, only increased thresholds whereas the kappa agonist (S,S-enantiomer) decreased thresholds at lower doses, but increased them at higher doses. Thus the pattern for the kappa agonist S,S-enantiomer was identical to that for U50,488H. As with U50,488H the initial decrease in threshold seen with the S,S-enantiomer was blocked by naloxone. Also in a manner analogous to that seen with U50,488H, the increases in thresholds produced by the R,R-enantiomer (PD 129289) and higher doses of S,S-enantiomer (PD 129290) were not blocked by naloxone. Thus it appears that both enantiomers may have sodium channel blocking action at high doses and that such actions are not blocked by naloxone. The initial decreases in thresholds seen only with kappa agonist enantiomers (which could be blocked by naloxone) appear to be opioid-receptor dependent and still have to be explained.

Some studies have suggested that sodium channel blockade occurs with a variety of opioid agonists and antagonists (69). U50,488H and related kappa agonists have also been implicated as having sodium channel blocking actions. Evidence for this comes from studies on nervous tissue conducted by the Upjohn group (87).

In vitro studies in isolated rat hearts showed that U50,488H prolonged the P-R and QRS intervals of the ECG and reduced peak systolic left-ventricular pressure, as well as the maximum rate of intraventricular pressure development ($+dP/dt$), in a concentration-dependent manner. Naloxone (1 μM) did not block these actions of U50,488H which

were seen only at concentrations greater than 1.0 μ*M* (63), i.e., above those producing kappa agonism. Such a spectrum of action in isolated hearts is similar to that which occurs with known sodium channel blockers (1). Where chemically related kappa agonists have been examined in isolated hearts, similar actions have been observed (unpublished observations).

Previous studies in isolated rat hearts have suggested that U50,488H has a dose-dependent arrhythmogenic action (46,83). This finding is in accord with other studies (see references in Table 2) which suggest that opioid receptors may play a role in arrhythmogenesis induced by coronary artery occlusion in rats. Thus, opioid antagonists prevent arrhythmias whereas endogenous opioids such as β-endorphin, enkephalins, and specific kappa agonists may initiate arrhythmias.

Evidence for the arrhythmogenic actions is equivocal. MacKenzie et al. (47) have shown that, while naloxone and MR2266 both reduce the incidence and severity of arrhythmias in rats subjected to coronary artery occlusion, U50,488H (0.1–1.0 mg/kg) was without effect. In our own studies into the action of U50,488H on arrhythmias, antiarrhythmic actions were seen in anesthetized rats subjected to coronary occlusion (64) but these only occurred at high doses. Similar antiarrhythmic actions have been found with the R,R- (PD 129289) and S,S- (PD 129290) enantiomers. Such antiarrhythmic actions were not prevented by prior administration of naloxone.

Few studies (see below) have been performed to determine the effects of kappa agonists on isolated vascular tissue. However, if these agonists at high concentrations are sodium channel blockers, it might be expected that such high concentrations would produce a pattern of responses in isolated vascular tissue similar to that produced by high concentrations of conventional sodium channel blockers. There is no reason to suppose that kappa agonists, as exemplified by U50,488H, have unexpected actions on vascular tissue due to activation of kappa receptors.

MECHANISMS INVOLVED IN THE NONOPIOID ACTIONS OF KAPPA AGONISTS

As discussed previously, nonopioid actions on isolated cardiac tissue have been demonstrated for U50,488H and related kappa agonists. These actions characteristically occur at micromolar (μ*M*) concentrations in contrast with kappa receptor agonism which usually occurs at nanomolar (n*M*) concentrations.

Nonopioid receptor-dependent actions have been seen in vascular tissue. Altura et al. (2) tested a series of kappa agonists on the basilar and middle cerebral arteries of dogs. U50,488H and MR 2034 dose-dependently contracted both types of arteries and these contractions were not blocked by naloxone. However, ethylketocyclazocine and bremazocine relaxed the strips. In isolated rat tail veins, Illes et al. (38) showed that U50,488H and ethylketocyclazocine depressed stimulation-induced contraction while naloxone (10 μ*M*) did not prevent such actions. In abdominal aortic strips, contractions induced by either 1 μ*M* norepinephrine or 10 μ*M* prostaglandin $F_{2\alpha}$ were potentiated by low concentrations (0.1–10 μ*M*) of U50,488H, but were depressed by high concentrations (50 and 100 μ*M*) (19). Verapamil (6 μ*M*) reduced the inhibitory effect of high concentrations of U50,488H. Such a finding raises the possibility that opioid receptor agonists may exert direct actions on vascular smooth muscle via direct interaction with L-type calcium channels.

Spiradoline, a U50,488H analog, has inhibitory actions on agonist-induced contractions of strips of circumflex coronary artery. Naloxone (300 μM) did not reverse this inhibitory action of spiradoline whether contractions were induced by elevated K^+ (15–25 mM) or by prostaglandin $F_{2\alpha}$ (1–3 μM). The inhibitory response was, in part, attributable to interference with Ca^{2+} entry (27).

In isolated guinea pig cardiac myocytes subjected to patch-clamp, duBell et al. (18) showed that at 10 μM U50,488H reduced steady-state twitch contractions by 78%, and slow inward calcium current (I_{si}) by 44%. In addition, U50,488H inhibited a repolarizing current which had properties similar to delayed-rectifier potassium currents. U50,488H also produced a concentration-dependent negative inotropism in field-stimulated rat left ventricular myocytes and enhanced myofilament responsiveness to calcium (76).

The sodium channel blocking actions of U50,488H and U69,593 on neuronal tissue have been demonstrated by Alzheimer and Ten Bruggencate (3). Using microelectrode techniques they showed that U50,488H and U69,593 had local anesthetic actions in neuronal tissue which were not reversed by opioid receptor antagonists. Their evidence is the clearest yet presented; it unequivocally demonstrates the sodium channel blocking actions of U50,488H.

CONCLUSIONS

Kappa agonists, such as U50,488H can have both opioid and nonopioid actions on the cardiovascular system. The actions which depend on kappa receptor activation are easily blocked by opioid antagonists. Actions on other types of opioid receptors (especially on μ receptors) are also blocked by antagonists but such actions are seen only at higher concentrations of these drugs. Nonopioid actions are not blocked by any of the opioid antagonists.

Nonopioid receptor actions which include cardiovascular effects are only seen at concentrations, or doses, 10–1000 times those which activate kappa receptors. The clearest nonopioid actions are those involving cardiac tissue. They appear to involve sodium (and possibly to a lesser degree potassium) channel blockade. Such actions are associated with antiarrhythmic effects in the presence of coronary artery occlusion or electrical stimulation. The blocking actions of kappa agonists on sodium channels are confounded to some extent by an opposing opioid receptor-dependent action. In addition to the possible blockade of sodium and potassium channels, kappa agonists may have other membrane effects which result in reduced calcium fluxes and release of potassium from neuronal tissue.

The clinical importance of the cardiovascular actions of kappa agonists are likely to be limited, although they may be of theoretical interest, while the clinical usefulness of the diuretic actions of kappa agonists have yet to be fully assessed. The cardiovascular depressant effects of kappa agonists (both opioid and nonopioid) may have clinical significance if these compounds are used in situations such as myocardial infarction where the cardiovascular system is impaired.

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