

THE ELECTROPHYSIOLOGICAL AND ANTIARRHYTHMIC ACTIONS OF
TEDISAMIL, LIDOCAINE, AND VARIOUS COMBINATIONS OF TEDISAMIL
AND LIDOCAINE MIXTURES AGAINST ISCHAEMIA-INDUCED
ARRHYTHMIAS IN RATS

By

GUILDA SARRAF

B.Sc., The University of British Columbia, 1996

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES
Department of Pharmacology and Therapeutics

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

February 2001

© Guilda Sarraf, 2001

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Pharmacology & Therapeutics
The University of British Columbia
Vancouver, Canada

Date February 10, 2001

ABSTRACT

Sudden cardiac death due to ischaemia-induced ventricular arrhythmias is the leading cause of death worldwide. Today, we have not yet developed a drug that is ideal in suppressing ischaemia-induced arrhythmias. The best drug used clinically is amiodarone. This is an antiarrhythmic with class I, II, III, and IV activities. This multi-antiarrhythmic activity of amiodarone makes this drug pharmacologically complex. However, it also suggests that drug combinations could produce a better antiarrhythmic than single drugs. Researchers have used this hypothesis to study different drug mixtures such as quinidine plus mexiletine. Duff et al. (1986 and 1990), have suggested possible synergistic interaction between quinidine (a class Ia) and mexiletine (a class Ib) drug. However, other researchers, including Nortran Pharmaceuticals (in Vancouver) are trying to develop new antiarrhythmics which have both class Ib and class III activity. Their proposal is based on the prediction of the modulated receptor hypothesis which states that combinations of class Ib and class III drugs could act synergistically to produce a better antiarrhythmic protection than either drug alone. This is based on the possibility that a greater prolongation of the effective refractory period can be produced when an action potential prolonging drug (ie, class III) and an inactive-state sodium channel blocker (ie, class Ib) are mixed. Mixture of class Ib plus class III could provide a better antiarrhythmic than class Ia plus class Ib because a class III drug can produce a greater prolongation of the action potential duration, thus allowing for a greater block of inactive-state sodium channels by class Ib drug.

In the present study, we chose tedisamil (a class III agent) and lidocaine (a class Ib agent) to study the mechanistic interaction between a class III and Ib drug which have been shown by Nortran Pharmaceuticals to have superior antiarrhythmic protection compared to each drug alone.

Rat models were used to induce ischaemia-induced arrhythmias by occlusion of their left main coronary artery. Tedisamil alone, lidocaine alone, and various combinations of tedisamil and lidocaine were studied for their electrophysiological and antiarrhythmic effects.

The results support a possible synergistic interaction between tedisamil and lidocaine. This is because tedisamil produced a leftward shift in the antiarrhythmic dose-response curve of lidocaine alone, and vice-versa. As well, the isobologram showed that the combination of tedisamil and lidocaine produced an ED₅₀ antiarrhythmic isobologram which lay below the line of additivity. Although this was not statistically significant, it still suggests that tedisamil and lidocaine have at least an additive interaction, and possibly a synergistic interaction. Interestingly, 2 μ mole/kg/min tedisamil plus 2 μ mole/kg/min lidocaine produced the best antiarrhythmic protection because the antiarrhythmic ED₅₀ was furthest below the line of additivity in the isobologram. This combination synergistically reduced the arrhythmia score and ventricular tachycardia, as well as completely abolishing ventricular fibrillation.

In summary, this thesis supports the prediction of modulated receptor hypothesis that class Ib plus class III combination can produce a better antiarrhythmic protection than either drug alone.

TABLE OF CONTENTS

	Page
Abstract	ii
List of Tables	vii
List of Figures	viii
List of Abbreviations	x
Acknowledgements	xi
 1 INTRODUCTION	 1
1.1 Myocardial ischaemia and arrhythmia	1
1.1.1 Clinical relevance of myocardial ischaemia	1
1.1.2 Mechanisms of ischaemia-induced arrhythmias	3
1.1.2.1 Overview	3
1.1.2.2 Action potential genesis and conduction in normal myocardium ...	3
1.1.2.3 Ischaemia and electrophysiological changes	6
1.1.2.4 The phases for ventricular arrhythmias in rats subject to coronary occlusion	9
1.1.2.5 Abnormal and triggered automaticity	10
1.1.2.6 Re-entry	13
1.2 Treatment of ischaemia-induced arrhythmias	16
1.2.1 Classification of antiarrhythmic drugs	16
1.2.2 Sodium channel blockers (Class I drugs)	17
1.2.2.1 Sodium channels	17
1.2.2.2 Modulated receptor hypothesis (MRH)	19
1.2.2.3 Subclassification of class I drugs	23
1.2.2.4 Clinical usefulness of class I antiarrhythmics	25
1.2.2.4.1 Antiarrhythmic actions of lidocaine	26
1.2.2.4.2 Proarrhythmic actions of lidocaine	28
1.2.3 Potassium channel blockers (Class III drugs)	29
1.2.3.1 Potassium channels	29
1.2.3.1.1 Transient outward potassium current (I_{to})	30
1.2.3.1.2 Delayed rectifier potassium current (I_K)	31
1.2.3.1.3 ATP-sensitive potassium current ($I_{K(ATP)}$)	31
1.2.3.2 Clinical usefulness of class III antiarrhythmics	33
1.2.3.2.1 Antiarrhythmic actions of tedisamil	35
1.2.3.2.2 Proarrhythmic actions of tedisamil	36
1.3 Antiarrhythmic drug combinations used against ischaemia-induced arrhythmias	38
1.3.1 Clinically used multi-channel blocking antiarrhythmics	40
1.3.1.1 Sotalol	40
1.3.1.2 Amiodarone	41
1.3.1.3 Quinidine	43

1.3.2	Antiarrhythmic therapy using drug combinations vs single agents	40
1.3.2.1	Combination of mexiletine and quinidine	46
1.4	Hypothesis and experimental design	48
2	METHODS	50
2.1	Ischaemia-induced arrhythmias	50
2.1.1	Surgical procedure and experimental design	51
2.1.2	Arrhythmia definitions	53
2.1.3	Inclusion / exclusion criteria	54
2.1.4	ECG and BP measurements	55
2.2	Dose-response curves	57
2.3	Sham occlusions	59
2.4	Isobolograms	59
2.4.1	Terminology	60
2.4.2	Interaction between tedisamil and lidocaine	63
2.5	Drugs	63
2.6	Statistics	64
3	RESULTS	66
3.1	Sham occlusions	66
3.1.1	Overview	66
3.1.2	Antiarrhythmic and proarrhythmic effects of tedisamil	66
3.2	Ischaemia-induced arrhythmias	69
3.2.1	BP, HR, and ECG effects of drugs administered alone and in combination	69
3.2.1.1	Overview	69
3.2.1.2	Lidocaine alone	70
3.2.1.3	Tedisamil alone	70
3.2.1.4	Combinations of lidocaine and tedisamil	74
3.2.1.4.1	Effects on BP	74
3.2.1.4.2	Effects on HR	79
3.2.1.4.3	Effects on PR interval	82
3.2.1.4.4	Effects on QRS duration	82
3.2.1.4.5	Effects on QT interval	85
3.2.2	Antiarrhythmic effect of drugs administered alone and in combination	88
3.2.2.1	Overview	88
3.2.2.2	Lidocaine alone	95
3.2.2.3	Tedisamil alone	95
3.2.2.4	Combinations of lidocaine and tedisamil	95
3.2.2.4.1	Effects on arrhythmia score	100
3.2.2.4.2	Effects on VT incidence	104
3.2.2.4.3	Effects on VF incidence	105
3.2.2.5	Isobolograms	106

4	DISCUSSION	127
4.1	Lidocaine alone	127
4.1.1	Electrophysiological actions of lidocaine alone	127
4.1.2	Antiarrhythmic actions of lidocaine alone	128
4.2	Tedisamil alone	131
4.2.1	Sham occlusions	131
4.2.2	Electrophysiological actions of tedisamil alone	132
4.2.3	Antiarrhythmic actions of tedisamil alone	135
4.3	Combinations of lidocaine and tedisamil	137
4.3.1	Electrophysiological actions of combinations of lidocaine and tedisamil	137
4.3.2	Antiarrhythmic actions of combinations of lidocaine and tedisamil	138
4.3.3	Is the interaction between lidocaine and tedisamil additive, synergistic, or antagonistic?	140
5	CONCLUSION	143
6	REFERENCES	144

LIST OF TABLES

Table		Page
I	The dose-related effects of tedisamil and lidocaine alone on BP, HR, and ECG.	71
II	The effect of various combinations of tedisamil and lidocaine on BP, HR, and ECG.	72
III	BP, HR, and ECG effects of the highest combined doses tested for lidocaine in the presence of a constant dose of tedisamil (Part A) and tedisamil in the presence of a constant dose of lidocaine (Part B) compared to either drug administered alone.	77
IV	The effect of tedisamil and lidocaine on AS and incidence of VT or VF.	94
V	The effect of various combinations of tedisamil and lidocaine on AS and incidence of VT or VF.	101
VI	AS and incidence of VT or VF for the highest combination doses of lidocaine in the presence of constant tedisamil (Part A) and tedisamil in the presence of constant lidocaine (Part B) compared to either drug alone.	108
VII	Antiarrhythmic ED ₅₀ values A) lidocaine in the presence of various doses of tedisamil and B) tedisamil in the presence of various doses of lidocaine.	124

LIST OF FIGURES

Figure		Page
1	A typical rat ECG showing the points used to measure PR, QRS, and QT intervals.	56
2	Construction of an isobologram showing additivism (X), synergy (Y), and antagonism.	62
3	The effect of a bolus dose versus continuous infusion of tedisamil on the QT ₁ interval.	67
4	Mean BP dose-response curves for lidocaine alone, tedisamil alone, or combinations of tedisamil and lidocaine in pentobarbitone-anaesthetized rats.	75
5	HR dose-response curves for lidocaine alone, tedisamil alone, or combinations of tedisamil and lidocaine in pentobarbitone-anaesthetized rats.	80
6	PR dose-response curves for lidocaine alone, tedisamil alone, or combinations of tedisamil and lidocaine in pentobarbitone-anaesthetized rats.	83
7	QRS dose-response curves for lidocaine alone, tedisamil alone, or combinations of tedisamil and lidocaine in pentobarbitone-anaesthetized rats.	86
8	QT ₁ dose-response curves for lidocaine alone, tedisamil alone, or combinations of tedisamil and lidocaine in pentobarbitone-anaesthetized rats.	89
9	QT ₂ dose-response curves for lidocaine alone, tedisamil alone, or combinations of tedisamil and lidocaine in pentobarbitone-anaesthetized rats.	91
10	Normalized antiarrhythmic dose-response curves for lidocaine.	96
11	Normalized antiarrhythmic dose-response curves for tedisamil.	98
12	Normalized antiarrhythmic dose-response curves for lidocaine in the presence of 0.5 $\mu\text{mol/kg/min}$ tedisamil.	110

13	Normalized antiarrhythmic dose-response curves for lidocaine in the presence of 1 $\mu\text{mol/kg/min}$ tedisamil.	112
14	Normalized antiarrhythmic dose-response curves for lidocaine in the presence of 2 $\mu\text{mol/kg/min}$ tedisamil.	114
15	Normalized antiarrhythmic dose-response curves for tedisamil in the presence of 2 $\mu\text{mol/kg/min}$ lidocaine.	116
16	Normalized antiarrhythmic dose-response curves for tedisamil in the presence of 4 $\mu\text{mol/kg/min}$ lidocaine.	118
17	Normalized antiarrhythmic dose-response curves for tedisamil in the presence of 6 $\mu\text{mol/kg/min}$ lidocaine.	120
18	Summary of normalized antiarrhythmic dose-response curves for lidocaine in the presence of varying doses of tedisamil and tedisamil in the presence of varying doses of lidocaine.	122
19	ED ₅₀ isobologram for tedisamil and lidocaine combinations.	125

LIST OF ABBREVIATIONS

AP	action potential
APD	action potential duration
AS	arrhythmia score
AV node	atrioventricular node
BP	blood pressure
CNS	central nervous system
DADs	delayed afterdepolarizations
EADs	early afterdepolarizations
ECG	electrocardiogram
HR	heart rate
MRH	modulated receptor hypothesis
PVB	premature ventricular beat
SA node	sinoatrial node
TdP	Torsade de Pointes
VT	ventricular tachycardia
VF	ventricular fibrillation

ACKNOWLEDGEMENTS

I finally finished my thesis! This is amazing news to all my friends and family. Although it has been a long tough road, I never gave up on finishing my thesis since I have worked so hard for so many nights and days. I am glad that now I can look back at my graduate school experience with fond memories knowing that I have completed that chapter of my life.

I would like to give my greatest thanks to Dr. Walker. He is an outstanding supervisor who always encouraged and supported me throughout my graduate studies. I will never forget all his teachings about life and the joy of science which were extremely inspiring. I would also like to thank my committee members, Dr. Sutter and Dr. Sastry, for their patience and continued support of my thesis.

Special thanks to Dr. Karim who has been a truly supportive friend and excellent teacher since my undergraduate studies at the University of British Columbia. He is a wonderful listener and full of great advice. Thanks for always encouraging me to finish my M.Sc.

I am grateful to Nortran Pharmaceuticals for allowing me to experience the hard work that is required when developing new and better antiarrhythmic drugs.

Terry Barrett was my officemate who helped me out a lot – I sincerely thank him for his friendship and scientific guidance throughout my thesis. Mike Puglsey has been an amazing friend. I thank him for all his supportive emails – I can't believe that he helped me out with my surgical techniques over emails and phone calls. It actually worked! Thanks to Wei who taught me his excellent surgical skills in doing rat experiments. We certainly had good laughs. Dave Dickenson's computer intelligence is very impressive. He developed a data collection program for Nortran. Thank you for teaching me so much about computers and having the patience with all the program crashes that we all went through. I would also like to thank other great friends from the pharmacology department who provided me with wonderful emotional support: Noam Butterfield, Lui Franciosi, Ali Azmudeh, Leah Monds, Ying, Andy Laycock, Maria Walker, and Sandro Yong.

I would also like to thank my friends outside graduate school who were extremely supportive and understanding throughout my thesis. Although I had to sacrifice many hours to work on experiments and write my thesis, these friends stood by me, all the way....special thanks to Stacey, Shelley, Kath, Joanne, Andrew, Ronik, Dave, Sudip, and Regina.

Last, but certainly not least, I owe a huge thanks to my mom, dad, brother, and grandma who have been outstanding emotional support. I really don't know what I would have done without their continuous belief in me. So, thanks to all my friends and family for keeping me focused and helping me get this far!

1 INTRODUCTION

1.1 Myocardial ischaemia and arrhythmia

Myocardial ischaemia can simply be defined as lack of blood flow to a region of the heart (Page et al., 1997). This can lead to an imbalance between cardiac demand and supply of coronary flow. Thus, ischaemic cells convert from aerobic to anaerobic metabolism creating a situation where oxygen, nutrients, and energy are lacking, but waste products such as lactate, carbon dioxide, and protons are accumulating (Hearse et al., 1982). These changes in the ischaemic myocardium can result in arrhythmias (abnormal or irregular heart beats). Patients with ventricular arrhythmias, especially fibrillation, can die of sudden cardiac death.

1.1.1 Clinical relevance of myocardial ischaemia

Sudden cardiac death is a leading cause of death worldwide. In Canada alone, over 40,000 people die of sudden cardiac death each year (Heart Disease and Stroke in Canada, 1997). A widely accepted definition for sudden cardiac death is an unexpected death that occurs within minutes (<1 hour) of symptoms or signs (Rapaport, 1988). The majority of deaths are due to myocardial ischaemia resulting from coronary artery disease (Janse and Wit, 1989). For example, an atherosclerotic plaque or vasospasm of a coronary artery can

narrow the artery and prevent blood flow to a region of the heart, thus leading to myocardial ischaemia (Kannel, 1985). As well, sudden cardiac death is strongly associated with ventricular arrhythmias, particularly ventricular fibrillation (VF) (Janse and Wit, 1989, Janse, 1986, Lown, 1971, and Rapaport, 1988). Numerous clinical findings support a possible link between VF, myocardial ischaemia, and sudden cardiac death (Cobb et al., 1980, reviewed in Janse, 1986; and by Janse and Wit, 1989). Patients receiving long-term ambulatory electrocardiogram (ECG) monitoring often show signs of ischaemia (such as ST segment changes and inverted T waves) just prior to death from fatal arrhythmias (ventricular fibrillation or tachycardia). Fibrillatory death occurs quite suddenly after the onset of ischaemia-related symptoms unless patients are already in the presence of medical care. This very narrow time window before death suggests that VF is most likely caused from myocardial ischaemia rather than infarction (tissue necrosis). Thus, antifibrillatory drugs have a potential in saving many lives by preventing ischaemia-induced arrhythmias that can lead to sudden cardiac death.

Unfortunately, today no drug can adequately prevent ischaemia-induced VF. The only drugs shown to decrease mortality following myocardial infarction are β -receptor blockers (Reiter et al., 1998; Pratt et al., 1982). However, there is no evidence in humans that β -receptor blockers prevent ischaemia-induced VF (Rees et al., 1996). As well, not all patients can use these blockers; for example, β -receptor blockers are contraindicated in obstructive airway disease. Thus,

more research is required to develop better drugs against ischaemia-induced ventricular arrhythmias.

1.1.2 Mechanisms of ischaemia-induced arrhythmias

1.1.2.1 Overview

After coronary occlusion, electrophysiological changes in the ischaemic myocardium can cause arrhythmias (premature ventricular beats (PVB), ventricular tachycardia (VT), and ventricular fibrillation (VF)). These changes include a non-uniform decrease in conduction velocity and increase in effective refractory period (ERP). Possible mechanisms for ischaemia-induced arrhythmias are automaticity and triggered activity (abnormal impulse generation), however, the major mechanism is probably re-entry (abnormal impulse conduction) (Wit et al., 1974, and Page et al., 1997).

1.1.2.2 Action potential genesis and conduction in normal myocardium

In order to better understand the mechanism of arrhythmias arising from the electrophysiological changes in ischaemic myocardium, action potential genesis and conduction in normal myocardium must first be discussed. Under normal conditions, the heart acts as a four-chambered pump and supplies blood to all body organs (see Shih, 1994, for a recent discussion). Interestingly, the

heart can automatically initiate its own beat. Thus, even if the heart is completely removed from the body, it continues to beat as long as the coronary vessels are artificially perfused (Berne et al., 1993). The cells responsible for generating this spontaneous impulse are in the sinoatrial (SA) node known as the natural pacemaker cells. Normally, the high frequency of firing in the SA node suppresses and prevents any other slower automatic cells (such as AV node and Purkinje fibers) from taking over the pacemaker role. Thus, this overdrive suppression by the SA node maintains a pacemaker hierarchy to avoid competition among different automatic sites in the heart (Hoffman et al., 1987). The impulse generated by the SA node travels down the atria to the atrioventricular (AV) node to reach the ventricles via the bundle of His, and Purkinje fibers (recently reviewed by Shih, 1994). The propagation of this impulse occurs due to changes in electrical potential of the cell membrane which eventually lead to the contraction of atria and ventricles. Movement of ions (such as Na^+ , K^+ , and Ca^{2+}) across the membrane can decrease (depolarization) and increase (repolarization) the membrane potential. This change is termed cardiac action potential.

The cardiac action potential can be considered to have five phases (Shih, 1994). The first is the resting membrane potential known as phase 4. In this state, the membrane is highly permeable to K^+ due to the voltage-dependent inward rectifier potassium channels (I_{K1}) which open upon hyperpolarization. I_{K1} is present in the atria and ventricles, but absent in the SA node. This results in a lower resting membrane potential for the SA node (-50 to -60 mV) compared to

other cardiac cells (-60 to -90 mV). The lack of I_{K1} in SA nodal cells is important for the initiation of the pacemaker current. Normally automaticity occurs in nodal cells due to a gradual depolarization during diastole (known as phase 4 diastolic depolarization) which lowers the membrane potential to the threshold for generation of a spontaneous action potential. This pacemaker current (I_f) is due to opening of non-specific cation channels which open upon membrane hyperpolarization. The slow inward pacemaker currents can be generated by I_f as well as a steady background current carried by Na^+ ($I_{\text{Na-B}}$).

Phase 0 of the atrial and ventricular action potentials is due to a depolarization following rapid influx of Na^+ through voltage-gated sodium channels (I_{Na}). However, in the SA and AV nodal cells, this I_{Na} is absent or very small. Thus, the upstroke in nodal cells are due to the slow inward Ca^{2+} current through T-type voltage-gated Ca^{2+} channels ($I_{\text{Ca-T}}$).

Phase 1 (seen in some atrial and ventricular cells) is the early rapid repolarization phase due to inactivation of I_{Na} and activation of a transient outward current (I_{to}). This rapid early repolarization appears as a spike in the cardiac action potential. I_{to} is more prominent in the epicardium compared to the endocardium of the ventricle (Antzelevitch et al., 1991) and varies with different species. Thus, action potential duration and refractoriness can differ depending on tissue and species type.

Phase 2 (seen principally in the ventricles of most species, except rats) is the plateau of the action potential. Most sodium channels recover rapidly from inactivation, but a small number remain open to depolarize the membrane

throughout the plateau phase (Carmeliet, 1993). Thus, this "window current" carried by sodium plus a slow inward current carried by calcium (L-type voltage-gated calcium channels, I_{Ca-L}) depolarize the membrane. However, a concomitant repolarization mediated by I_{Cl} , I_{to} , and $I_{Na-K \text{ pump}}$ keeps the membrane potential stable during the plateau phase. The latter repolarizing current is generated by the sodium-potassium pump which hydrolyzes ATP for the efflux of 3 Na^+ and influx of 2 K^+ . Thus, this pump results in a net outward hyperpolarizing current.

Phase 3 is the final repolarization phase. The time-dependent inactivation of the Ca^{2+} and Na^+ inward currents and the activation of several potassium channels are responsible for this phase (Carmeliet, 1993 and Shih, 1994). Although the presence of different potassium channel subtypes is species-dependent, the main repolarizing current in human myocardium, including nodal cells, is the delayed rectifier potassium channel (I_K). In rat ventricles, I_{to} is the main repolarizing current (Carmeliet, 1993).

The next section will describe ischaemia-induced electrophysiological changes which can disrupt the action potential conduction and lead to arrhythmias.

1.1.2.3 Ischaemia and electrophysiological changes

Acute ischaemia causes severe changes in the myocardium. One of these is the release of K^+ by ischaemic cells (Harris et al., 1954). The lack of

washout by coronary flow during ischaemia results in accumulation of extracellular K^+ . This reduces the resting membrane potential during ischaemic (Janse and Kléber, 1981). In addition, both the reduction in pH (as a result of lactic acid production by anaerobic metabolism) and accumulation of CO_2 enhance depolarization of the resting membrane potential (Orchard et al., 1994 and Yatani et al., 1984). Reduction in resting membrane potential results in voltage-dependent inactivation of fast sodium channels (Janse and Wit, 1989).

Gettes and Reuter (1974) showed that at decreased membrane potentials, the recovery of both the fast and slow inward currents can be dramatically delayed (Janse and Kléber, 1981). Under normal conditions, the time required for recovery of excitability (ie, ERP) is similar to the time required for repolarization, however, under ischaemic conditions with depolarized membrane potential, ERP can be prolonged more than action potential duration (Janse and Kléber, 1981). This increase in ERP observed after complete repolarization is known as post-repolarization refractoriness (Lazzara, 1978). Since recovery of excitability depends on the resting membrane potential, if this is reduced, conduction velocity in the ischaemic myocardium is slowed and conduction block can occur.

Time-dependent recovery can lead to spatial disparity in refractoriness. For example, two neighboring cells in the ischaemic zone can exhibit different degrees of depolarization, thus differences in conduction velocity, action potential duration, and refractoriness (Janse and Kléber, 1981). Ischaemic cells not only

demonstrate a decrease in action potential duration, but also a decrease in action potential amplitude and upstroke velocity (Downar et al., 1977).

The above described electrophysiological changes in the ischaemic tissue occur as a result of a triphasic increase in extracellular K^+ which begins as early as 15 seconds after occlusion (Hill and Gettes, 1980). The mechanism for the accumulation of K^+ is not yet known, but several possibilities exist (Shivkumar et al., 1997; Janse and Wit, 1989). A decrease in the Na^+ - K^+ pump activity (due to a decrease in ATP in ischaemic cells) will result in an accumulation of extracellular K^+ and intracellular Na^+ resulting in depolarization. However, studies have shown the pump still continues to function even after 10-15 minutes of ischaemia, presumably because the cytosolic ATP levels do not decrease significantly during the early phase of ischaemia (Janse and Wit, 1989). For a similar reason, it is unlikely that the K_{ATP} channel has a major role since ATP levels remain relatively unchanged during the early periods of acute ischaemia and therefore, the K_{ATP} channel will probably stay closed.

A more likely possibility is the opening of sodium-dependent potassium channels ($I_{K(Na)}$). Due to increased intracellular Na^+ concentrations in ischaemic cells, more $I_{K(Na)}$ channels can open for K^+ to efflux. In addition, accumulation of intracellular Na^+ could activate the Na^+ - Ca^{2+} exchanger resulting in the efflux of Na^+ in exchange for the influx of Ca^{2+} . This would suggest a membrane depolarization due to calcium rather than sodium influx. Finally, due to the anaerobic production and release of lactate anions in ischaemic cells, potassium

ions could also leave the cells in order to maintain extracellular ionic charge balance.

Whatever the mechanism for the accumulation of extracellular K^+ , studies have found that the rate and magnitude of extracellular K^+ are inhomogeneous within the ischaemic myocardium (Hill and Gettes, 1980; Gettes et al., 1982). For example, extracellular K^+ concentrations were greater and increased more rapidly in cells in the centre of the ischaemic zone compared to the border and non-ischaemic zones. As well, greater K^+ concentrations were found in the subendocardium as opposed to the subepicardium. Thus, the inhomogeneous ionic distributions within the ischaemic, border and non-ischaemic zones lead to a myocardial dispersion of ERP, conduction velocity, and action potential duration (Janse and Wit, 1989). For cells within the centre of the ischaemic zone the potential distribution is more uniform compared to the border zone where the potential gradient is the steepest (Kléber, 1978). This inhomogeneity can then result in the development of injury currents (Janse and Wit, 1989). These currents can result in ventricular arrhythmias due to possible re-entry circuits.

1.1.2.4 The phases for ventricular arrhythmias in rats subject to coronary occlusion

Ischaemia-induced arrhythmias in rats (PVB, VT, and VF) can be divided into two phases (reviewed by Lazzara et al., 1978). The first or early phase occurs within 30 minutes after coronary occlusion and usually ends with VF. The

second or delayed phase occurs 4-8 hours after coronary occlusion and can last for 2-4 days. Unlike the first phase, VF rarely occurs in the second phase. The possibility of two different arrhythmic phases suggests that the mechanism for the arrhythmias is also different (reviewed by Lazzara et al., 1978; Curtis et al., 1987). The most probable cause of ischaemia-induced arrhythmias is re-entry as a result of inhomogeneous conduction and refractoriness in the ischaemic zone. However, abnormal automaticity and triggered automaticity such as early and delayed afterdepolarizations can also occur. The next section will discuss these electrophysiological mechanisms of arrhythmogenesis in more detail.

1.1.2.5 Abnormal and triggered automaticity

Abnormal automaticity is a spontaneous impulse initiation based on phase 4 depolarization in cardiac cells with reduced maximum diastolic potential (Janse and Wit, 1989). This decrease in maximum diastolic potential (to about -60 mV or less can result from a decrease in background potassium conductance (due to a decrease in extracellular K^+ concentration) or an increase in inward current (eg. Na^+ or Ca^{2+}) (Hoffman and Dangman, 1987). Although in normal automaticity, the pacemaker current, I_f , in SA nodal cells is responsible for the slow diastolic depolarization (see section 1.1.2.2), in abnormal automaticity, time- and voltage-dependent changes in potassium and calcium currents are responsible for this depolarization (Hoffman and Dangman, 1987). As well, unlike normal automaticity where SA nodal cells exhibit overdrive suppression, abnormal

automaticity shows very slight or no overdrive suppression. Interestingly, the occurrence of abnormal automaticity during myocardial ischaemia is unlikely because the elevated extracellular K^+ concentrations occurring in ischaemic tissue suppress abnormal automaticity (Katzung and Morgenstern, 1977; Janse and Wit, 1989; Wit et al., 1974). Increases in extracellular K^+ concentrations can shift the pacemaker-like activity to less negative diastolic potentials, decrease maximum phase 4 slope, and decrease membrane resistance, thus preventing the initiation of an abnormal impulse (Katzung and Morgenstern, 1977).

Unlike abnormal automaticity which is an impulse initiated de novo at low membrane potentials, triggered automaticity is a repetitive impulse initiated by a propagated action potential (Damiano et al., 1984; Janse and Wit, 1989). These repetitive impulses (ie, oscillations) in membrane potential which occur after the action potential upstroke are known as afterdepolarizations. If the afterdepolarizations are sufficiently large to reach threshold, the generated action potential is said to be "triggered" by the prior impulse and hence is not automatic (Janse and Wit, 1989; Hoffman and Dangman, 1987). There are two types of afterdepolarizations. Early afterdepolarizations (EADs) occur during phase 3 of an action potential (ie, interrupting the repolarization phase); delayed afterdepolarizations (DADs) occur during phase 4 (ie, after the repolarization phase is complete) (Cranefield, 1977; Hoffman and Dangman, 1987). In vitro studies of isolated cardiac tissue have shown that an increase in inward current (eg. background sodium window current) or a decrease in repolarizing current

(eg. outward potassium current) can prolong the action potential duration resulting in EADs (El-Sherif et al., 1988).

The current changes leading to EADs can occur under conditions including acidosis, low extracellular K^+ concentration, low extracellular Ca^{2+} concentration, hypoxia, and presence of particular drugs (eg. quinidine, tedisamil, aconitine, sotalol, and others which can increase action potential duration) (El-Sherif et al., 1988).

EADs probably only play a minor role in ischaemia-induced arrhythmias perhaps because of their suppression by the elevated K^+ concentrations found in ischaemic tissue. However, they play a major role in drug-induced arrhythmias, particularly torsade de pointes, (a polymorphic VT with a twisting of ECG complexes around the isoelectric line, Davidenko et al., 1989). Drugs which prolong the action potential duration (long QT syndrome) in the presence of bradycardia and hypokalemia can result in torsade de pointes (Davidenko et al., 1989; El-Sherif et al., 1988; Damiano et al., 1984).

Unlike EAD-induced ventricular arrhythmias which are bradycardia dependent, DAD-induced arrhythmias are tachycardia dependent (Damiano et al., 1984). Thus, at rapid heart rates, DADs rather than EADs are the more probable cause of afterdepolarizations. A possible mechanism for DADs is an overload in intracellular calcium which can cause the sarcoplasmic reticulum to continuously release and reuptake calcium (Hoffman and Dangman, 1987). These oscillatory changes in calcium can result in a transient inward

depolarization current (perhaps sodium influx through calcium dependent channels or sodium/calcium exchanger) (Hoffman and Dangman, 1987).

Myocardial ischaemia and inhibition of the Na^+/K^+ pump by digitalis are two conditions which can result in an increase in intracellular Ca^{2+} concentration and thus produce DADs (Coetzee et al., 1987; Hoffman and Rosen, 1981). However, drugs which inhibit influx of Ca^{2+} such as verapamil can prevent DADs (Coetzee et al., 1987). Overall, EADs and DADs can contribute to ischaemia-induced ventricular arrhythmias, however a more likely in vivo mechanism is abnormal impulse conduction such as re-entry.

1.1.2.6 Re-entry

Re-entry as a mechanism for ventricular arrhythmias was first suggested by Mines et al. (1913, 1914). He suggested that re-entering impulses circulate around a "hole" in the heart, either an anatomical barrier or, inexcitable tissue (Hoffman and Dangman, 1987). The minimum conditions required for re-entry to occur are a complete circuit path for the impulse to travel, slow conduction, unequal responsiveness (ie, inhomogeneity in ERP), and a unidirectional block in a region of the heart (Hoffman and Rosen, 1981). Since the combination of the ischaemic and normal myocardium can fulfil these criteria, it is likely that re-entry plays an important role in ischaemia-induced arrhythmias. A re-entry circuit forms when the longer refractory period and slower conduction in the ischaemic tissue causes a unidirectional block of an approaching (anterograde) impulse

(Lazzara and Scherlag, 1988). However, the impulse can still propagate along the normally conducting pathways and then retrogradely enter the damaged, ischaemic zone and thus, re-enter and re-excite the tissue proximal to the area of block. This re-entry can occur only if the propagation of the anterograde pathway is slow enough for the normal tissue proximal to the site of block to recover excitability (Hoffman and Rosen, 1981). Both macro re-entry (long pathway circuits) and micro re-entry (short pathway circuits) can occur within the ischaemic myocardium resulting in the formation of VT and VF, respectively (Janse et al., 1980). VT can occur as a result of ordered re-entry where the circuit propagates around an anatomical block (Hoffman and Rosen, 1981). VF can occur as a result of random re-entry where wavefronts of excitation can fragment leading to multiple chaotic re-entry circuits (Hoffman and Rosen, 1981; Mines 1913 and 1914; Lazzara and Scherlag, 1988). It has been suggested that ischaemia decreases threshold for VF due to an increase in local dispersion of refractoriness leading to fractionation of impulses and re-entry (Han and Moe, 1964).

Allessie et al. (1977) demonstrated that re-entry can also occur when a propagating impulse creates a functionally inactive core to circulate around, and thus, in this case no anatomical fixed barrier is required. This re-entry is known as the "leading circuit concept". In this model, the circuit forms around a thin interface between different tissue zones where absolute and relative refractoriness produce a functional barrier and slow conduction, respectively (Lazzara and Scherlag, 1988).

Re-entry can result not only from circus movement, but also from reflection of an impulse from inexcitable tissue such as occurs in the ischaemic zone (Hoffman and Dangman, 1987). Reflection can occur if the depolarization caused by the blocked impulse causes a greatly delayed action potential in the area distal to the inexcitable tissue. This delayed action potential can then produce a spread of electronic current, and thus, re-excite the normal tissue proximal to the blocked (eg. ischaemic) zone (Hoffman and Dangman, 1987; Lazzara and Scherlag, 1988).

Studies have provided strong evidence that the VT and VF which occur during acute myocardial ischaemia are due to re-entry (Janse et al., 1980; Janse, 1986; Pogwizd et al., 1987). Epicardial mappings using microelectrodes suggest that injury currents flowing from the subendocardium across the border zone of ischaemic (depolarized) to normal (polarized) myocardium, can act to initiate re-entry in acute ischaemia (Janse et al., 1980; Janse 1986).

Antiarrhythmic drugs can break a re-entry circuit and thereby prevent ischaemia-induced ventricular arrhythmias. This can be achieved by either slowing conduction in depressed ischaemic tissue so converting unidirectional to bidirectional block, or prolonging refractoriness in the normal tissue in order to prevent re-entry (Winslow, 1984). Since wavelength of the re-entry circuit is equal to conduction velocity times ERP, increasing ERP will also increase the wavelength travelled by the circuit (Janse, 1992). Thus, prolonging ERP can diminish the number of re-entrant wavelets in random re-entry, hence preventing VF (Janse, 1992).

Ventricular arrhythmias can be treated with varying efficacy using the four different classes of antiarrhythmic drugs (Nattel, 1991). The actions of sodium channel blockers (Class I), potassium channel blocker (Class III), as well as antiarrhythmic drug combinations will be further discussed.

1.2 Treatment of ischaemia-induced arrhythmias

1.2.1 Classification of antiarrhythmic drugs

Historically, Hoffman and Bigger developed an antiarrhythmic classification in the early 1970's which divided drugs into two groups based on their effects on the action potential (AP) (reviewed in Nattel, 1991). Group I drugs decreased myocardial excitability by blocking sodium inward current resulting in a reduction of the V_{max} (the maximum rate of rise of phase 0 of the AP). However, group II drugs either left V_{max} unaffected or increased and did not depress conduction or excitability of the myocardium.

With further research, Singh and Vaughan Williams developed another antiarrhythmic classification which is widely accepted today (Singh and Vaughan Williams, 1970a, 1970b, and 1972). They divided antiarrhythmic drugs into four groups based on their electrophysiological actions on the heart. Class I drugs (eg. lidocaine): sodium channels blockers which also act as local anaesthetics; class II drugs (eg. propranolol): β -adrenergic antagonists which reduce the effects of stimulation of sympathetic nerves; class III drugs (eg. amiodarone and

d-sotalol): mainly block the outward potassium channels responsible for membrane repolarization, hence can increase the action potential duration (APD), and refractoriness (ERP), class IV drugs (eg. verapamil): calcium channel blockers.

One of the problems with the Singh-Vaughan Williams classification is its applicability in clinical settings (Meinertz, 1992). This is due in part because single cells under normal conditions in vitro were used to study drug actions. However, clinically, arrhythmias originate in diseased (eg. ischaemic) myocardium rather than normal cells. Electrophysiological actions of drugs can differ considerably in ischaemic compared to normal cells, thus affecting the drug's classification (Meinertz, 1992). In addition, the Singh-Vaughan Williams classification has been too broad for some drug actions. Therefore, for clarification reasons, class I drugs have been further subgrouped into Ia, Ib, and Ic (Harrison et al, 1981, Campbell, 1983a, and Campbell, 1983b).

1.2.2 Sodium channel blockers (Class I drugs)

1.2.2.1 Sodium channels

The sodium channel is a protein embedded in the cell membrane containing "gates" controlling the passage of sodium ions through the channel pore (see Rosen et al., 1988; and refer to Roden et al., 1997 for details on molecular structure). Studies carried out by Hodgkin and Huxley (1952) in squid

axons led to the development of a model where there are two gates within the inner portion of the sodium channel; the "m" for activation and "h" for inactivation. The opening and closing of these gates led to the suggestion that the sodium channel exists in three states: rested (R), activated (A), and inactivated (I). At negative membrane potentials, during diastole, channels are in the rested (R) state (Hondeghe, 1987, and 1990). These rested channels are closed and do not conduct sodium current, however, they can be opened (activated (A)) by appropriate depolarization. The opening of channels results in a vastly increased permeability to Na^+ ions inward current flow and the action potential upstroke (Rosen et al., 1988). At depolarized membrane potentials, and during the plateau phase, sodium channels convert to the closed-inactivated (I) state. Inactivated channels convert to the closed (resting) state (R) in a voltage and time dependent repolarization (Hondeghe, 1990; Chen et al., 1975). Thus, during a cardiac action potential, sodium channels are mainly in the R state during diastole, the A state during upstroke, and the I state during the plateau phase (or any depolarized tissue such as that found in the ischaemic myocardium) (Hondeghe, 1987).

The interaction of antiarrhythmic drugs with the sodium channels is dependent not only on the channel states described above, but also on channel use, time and voltage (Hondeghe and Katzung, 1977; Chen et al., 1975; Courtney, 1975; Weidmann, 1955). The state-dependent binding of drugs to cardiac sodium channels is described by the modulated receptor hypothesis (Hondeghe and Katzung, 1977).

1.2.2.2 Modulated receptor hypothesis (MRH)

In 1977, the modulated receptor hypothesis (MRH) was proposed by Hondeghem and Katzung for cardiac sodium channels, and independently, by Hille for neuronal sodium channels (Hondeghem and Katzung, 1977; Hille, 1977). The MRH states that a drug's affinity for binding to the sodium channel is modulated by channel state (ie, rested, activated, or inactivated). Different sodium channel blockers have different association and dissociation rate constants for different channel states (Hondeghem and Katzung, 1977; Hondeghem, 1984, 1987). MRH postulates that compared to drug-free channels (R, A, I), drug-bound channels (RD, AD, and ID) do not conduct Na^+ ions and their voltage-dependence shifts to more negative potentials (Hondeghem and Katzung, 1977). Therefore, drug-bound channels tend to accumulate in the inactive state (ie, ID).

Clinically useful antiarrhythmics have a high affinity for activated (open) channels (eg. quinidine) or inactivated channels (eg. amiodarone), or both (eg. lidocaine) (Hondeghem, 1987). However, they have a low affinity for rested channels. Although drugs can unbind from any of the three channel states, they tend to unbind from the R state which predominate during diastole ($\text{RD} \rightarrow \text{R}$), resulting in a low level of block between action potentials. Drugs with high affinity for the activated (open) channels tend to produce increasing block with each action potential upstroke. However, drugs with high affinity for the inactivated

channels tend to produce increasing block throughout the action potential plateau phase which is then observed as further sodium channel block during the next action potential (Hondeghe, 1990).

Tonic block occurs when a drug blocks sodium channels in the absence of stimulation (ie, at rest) (Hondeghe, 1987 and 1990). Although the majority of channels during rest are in the R state, tonic block can also refer to block of (A) open channels. This is because drugs can block the open channels as they become available as the upstroke of the action potential develops and is then recorded as tonic block. As well, at potentials where a fraction of sodium channels are still inactivated, drugs can trap channels in the ID state due to the voltage-dependence of inactivation (Hondeghe, 1990). This form of tonic block is thought to occur in ischaemic myocardium.

In contrast, block which occurs during an action potential, and resolved in a time-dependent fashion, is called phasic block. When channels are repetitively stimulated (eg. during VT), a new steady-state decrease in sodium current occurs after each beat (Courtney, 1975; Hondeghe, 1987 and 1990). This additional block which accumulates with repetitive stimulation is known as use- or frequency-dependent block (Courtney, 1975). Since the channels are used more frequently, there is less time for channels to recover from the block. Thus, channels spend more time in the activated and inactivated states. The rate of binding and unbinding of drugs from the sodium channel is both time and voltage dependent. Thus, recovery from block is also dependent on these two factors. The amount of block at a particular time depends on the concentration of the

drug, its on and off rate constants, and stimulation rate. Since drug unblocking is slow when bound to the inactivated channel state, the time required for the channels to recover from the block is increased (Tamargo et al., 1992; Hondeghem, 1990). The use-dependent unblocking (ie, channel recovery) is just as important as use-dependent blocking of sodium channels (Hondeghem, 1984, 1987, and 1990). The net amount of sodium channel block depends on the balance between the level of block and unblock. Use-dependent block occurs when the period of diastole (recovery period) is not long enough for the channels to recover from the block developed during the preceding action potential (Hondeghem, 1990). Thus, drugs that have fast recovery time constants such as lidocaine (~ 200 msec) will produce use-dependent block only at rapid heart rates (ie, cycle lengths shorter than 600 msec). In other words, lidocaine will not block sodium channels at normal heart rates because 100% of the channels will recover from block by the end of diastole. Hence, all the channels would be available to open for the next action potential (ie, no use-dependent block) (Hondeghem, 1987 and 1990).

The recovery of sodium channel from block is also dependent on extracellular pH, membrane potential (voltage) and the drug itself (eg. lipid solubility and size) (Grant et al., 1995; Hondeghem, 1987). As the drug binding site is suggested to be on the cytoplasmic side of the channel, sodium channel blockers must cross the membrane either by a hydrophilic pathway (ie, enter cell via the open channel pore), or a hydrophobic pathway (ie, via the membrane lipid bilayer) (Hille, 1977; Grant et al., 1995). The latter pathway is faster than the

former. Thus, lipid-soluble drugs with low pK_a (higher concentration in the neutral form at physiological pH), can dissociate faster than lipid-insoluble drugs (Grant et al., 1995; Hondeghem, 1987). As suggested by the MRH, drugs can dissociate from sodium channels at any of the three states (RD, AD, or ID). Since drug-bound channels accumulate in the ID state, unblocking of drugs occur from this state (ie, $ID \rightarrow I$). Lipid-insoluble drugs can be trapped in this ID state unless the drug-bound channel overcomes the inactivation voltage shift so that channel states can change from $ID \rightarrow RD$ (Hondeghem, 1987). Drugs could then dissociate from $RD \rightarrow R$, as well as $AD \rightarrow A$. Since the A state is the only one where channels are open, this could be the main route by which lipid-insoluble drugs can recover (Hondeghem, 1987).

Courtney (1980) showed that faster recovery rates can be achieved with smaller drugs. At physiological pH, most antiarrhythmics (mostly weak bases) are either in the form of cation or neutral (Hondeghem, 1987). However, under ischaemic conditions (acidosis), the drug is mostly in cationic form. This cationic form dissociates more slowly from the sodium channels which further slows recovery of excitability (Grant et al., 1995; Hondeghem, 1987). Both low pH and depolarized potentials present in ischaemic tissue slow recovery of sodium block. This is because most cation-drug-bound channels will be in the inactive state (due to depolarized tissue) from which drugs dissociate slowly. This implies that the antiarrhythmic drugs can selectively produce more block in the ischaemic versus normal myocardium (see section 1.2.2.2.1 for more detail) (Hondeghem, 1987).

Overall, the MRH provides insight on how class I antiarrhythmic drugs act as sodium channel blockers. However, the classification of sodium channel blockers as class I agents is too broad, therefore, these drugs have been further divided into subclasses Ia, Ib, and Ic.

1.2.2.3 Subclassification of class I drugs

In 1974, Singh and Hauswirth decided to incorporate ideas from the Hoffman-Bigger into the Singh-Vaughan Williams classification (discussed in section 1.2.1) by subcategorizing class I drugs into Ia and Ib (reviewed by Nattel, 1991). Class Ia drugs (quinidine, procainamide, and disopyramide) moderately decrease V_{max} (conduction), while increasing APD and ERP. Class Ib drugs (lidocaine, mexiletine, and tocainide) slightly decrease V_{max} , while shortening APD but increasing ERP (Meinertz, 1992; Nattel, 1991). In 1981, Harrison et al. developed a third subclass known as class Ic which greatly depress V_{max} with little effect on APD and ERP.

In 1983, Campbell also divided class I drugs into three subclasses (Ia, b, and c) based on the kinetics of onset of rate-dependent depression of V_{max} . (Campbell, 1983a, 1983b). Campbell's division of class I corresponded exactly to that proposed by Harrison et al. (1981), based on different criteria. All class I drugs in Campbell's experiment produced rate-(or use-)dependent block, however, at all concentrations and rates tested, the drugs differed in the rate at which V_{max} dropped to a new plateau level (Campbell, 1983b). Campbell

divided class I drugs into those with intermediate kinetics as class Ia (eg. quinidine, disopyramide and procainamide), fast kinetics as class Ib (eg. lidocaine, mexiletine, and tocainide), and slow kinetics as class Ic (eg. flecainide, encainide, and lorcainide). Campbell also found that the class I drugs affect the APD: class Ia prolonged, class Ib shortened, and class Ic produced little or varied responses on APD (Campbell, 1983b). Prolongation of the APD by class Ia drugs seems to result from the block of the potassium channels responsible for membrane repolarization (Colatsky, 1982). The shortening of the APD by class Ib drugs is due to block of a tetrodotoxin-sensitive "window" current responsible for maintaining the action potential plateau (Colatsky, 1982).

The class I subgroups also differ with respect to their effects on ERP to APD ratio (Campbell, 1983b). This ratio changes depending on the kinetics of the drugs on the onset of rate-dependent block. Thus, class Ib drugs produce the greatest prolongation of ERP to APD ratio due to their fast kinetics. Class Ia drugs produce limited to moderate prolongation, while class Ic drugs produce only minor effects on the ratio (Campbell, 1983b). The differences in the prolongation of the ERP to APD ratio depends on the ability of drugs to prolong recovery from inactivation. Thus, both APD and speed of onset of rate-dependent block played an important role in Campbell's division of class I antiarrhythmic drugs.

The difference in the kinetics of class I drugs suggests why some sodium channel blockers are more effective against ischaemia-induced ventricular arrhythmias (Campbell, 1983b). For example, class Ib (eg. lidocaine) are the

most ischaemia-selective drugs due to their fast onset and offset of rate-dependent block.

1.2.2.4 Clinical usefulness of class I antiarrhythmics

The Cardiac Arrhythmia Suppression Trial (CAST Investigators, 1989) brought new insights into the usefulness of class I drugs as antiarrhythmics. It provided evidence that class I antiarrhythmics do not reduce mortality in those with PVC's (premature ventricular contractions). Instead of a reduction in mortality, an increase was observed among patients receiving class I drugs for mild ventricular arrhythmias (post myocardial infarction), as compared with their controls. Similar findings have been made with class Ia (eg. procainamide (Kosowsky et al., 1973)), Ib (eg. mexiletine (IMPACT Research Group, 1984)), and Ic (eg. flecainide and encainide (CAST Investigators, 1989)) drugs. Although class I agents can cause proarrhythmia some of which lead to death, they also possess antiarrhythmic effects. For example, lidocaine (class Ib) does not decrease mortality, however, it reduces the incidence of VF in patients with acute myocardial ischaemia (Hine et al., 1989).

Another drawback in the use of class I drugs is their failure to selectively block cardiac rather than neuronal sodium channels (Roden, 1994; Barrett et al., 1995). The block of channels in neurons can cause neuronal (CNS) toxicity such as convulsions. Although class Ib agents are not selective for cardiac sodium channels, they are more selective for the ischaemic rather than normal

myocardium (Barrett et al., 1995). In this way, class Ib agents such as lidocaine can prevent ventricular arrhythmias. Unfortunately, experiments in rats have shown that lidocaine prevents ischaemia-induced arrhythmias only at high doses which also produce convulsions in conscious animals (Barrett et al., 1995). Thus, class I drugs are far from ideal antiarrhythmics for ischaemia-induced arrhythmias.

1.2.2.4.1 Antiarrhythmic actions of lidocaine

Lidocaine is a clinically used antiarrhythmic drug that can provide protection against ischaemia-induced ventricular arrhythmias (Hondeghe, 1984; Barrett et al., 1995). The antiarrhythmic mechanism of this class Ib drug is to selectively block sodium channels in already depressed tissue (eg. ischaemic myocardium) to further depress excitability (Janse, 1992). This can then convert a unidirectional to a bidirectional block, and thus, prevent arrhythmias by breaking the re-entry circuit (Janse, 1992). It is important to note that lidocaine-induced block of sodium channels not only slows conduction, but also prolongs the effective refractory period (ERP) (Janse, 1992; Yin et al., 1997). This is because lidocaine delays recovery from block, so that sodium channels remain inactivated for a longer period of time, thus prolonging ERP (Janse, 1992).

Lidocaine's selectivity for ischaemic (depolarized) rather than normal (polarized) tissue has two main causes (Hondeghe and Katzung, 1977). Firstly, channels in depolarized tissue are predominantly in the inactivated state,

such that drugs with high affinity for inactivated channels produce more block (Hondegghem, 1987). Although lidocaine can block sodium channels in both the activated and inactivated states, it has a higher affinity for inactivated channels. This suggests that open channel blockers such as quinidine (class Ia) would be less effective at depolarized potentials since most channels are in the inactivated state (Hondegghem, 1987).

Secondly, lidocaine has fast kinetics (Davis et al., 1986; Hondegghem and Katzung, 1977). At normal heart rates, lidocaine has little effect because almost all channels recover from block during diastole (Hondegghem and Katzung, 1977). However, at rapid heart rates (eg. VT), use-dependent block occurs since there is not enough time between beats to recover from block (Tamargo et al., 1992). Thus, the faster the heart rate, the more conduction is depressed by lidocaine. During VT, lidocaine can produce a new slowed level of conduction after only two beats (Davis et al., 1986). However, drugs with slower kinetics such as class Ia and Ic, develop a new level of block after ten or more beats (Davis et al., 1986). Another factor which will increase use-dependent block is the low pH in ischaemic tissue (Hondegghem, 1977). This is because acidosis will increase the cationic form of lidocaine which dissociates more slowly from the drug-bound inactivated channel. Thus, both tachycardia and acidosis can increase use-dependent block (Davis et al., 1986). Unlike lidocaine, class Ic drugs (eg. flecainide) have a very slow binding kinetics (Hondegghem, 1987). Thus, class Ic drugs block both normal and depolarized tissue. As a result, they can produce proarrhythmic effects due to excessive depression of cardiac excitability.

Overall, class I antiarrhythmics can prevent arrhythmias, however, in contrast to class Ia and Ic drugs, lidocaine (class Ib) prevents ventricular arrhythmias by selectively blocking sodium channels in ischaemic myocardium (Pallandi and Campbell, 1988).

1.2.2.4.2 Proarrhythmic actions of lidocaine

As mentioned previously, lidocaine has both antiarrhythmic and proarrhythmic actions (Yin et al., 1997). It has been suggested that lidocaine increases the incidence of ventricular fibrillation (VF), thus leading to an increase in mortality and sudden death (Antman and Berlin, 1992; Hondeghem, 1987, Tamargo et al., 1992; Aupetit et al., 1997). This is because lidocaine (similar to other class I agents) can cause further slowing of conduction which leads to areas of inconsistent and non-uniform activation. As a result, lidocaine will precipitate re-entry circuits which lead to VF (Carson et al., 1986; Tamargo et al., 1992; Aupetit et al., 1997).

In summary, lidocaine can selectively block sodium channels in the ischaemic myocardium to prevent ventricular arrhythmias. However, lidocaine does have toxic actions which include: a) induction of VF to increase mortality, and b) induction of convulsions by blocking neuronal sodium channels. Thus, lidocaine must be used cautiously. More research is required to discover drugs

with less dangerous side-effects for use against ischaemia-induced ventricular arrhythmias.

1.2.3 Potassium channel blockers (Class III drugs)

1.2.3.1 Potassium channels

The increase in mortality observed with sodium channel blockers (CAST Investigators, 1989) has led to a growing interest in potassium channel blockers as potentially better antiarrhythmic agents (Colatsky, 1990; Singh, 1998). Potassium channel blockers increase action potential duration and prolong the refractory period; and therefore, are classified as class III antiarrhythmics. Currently, at least eight potassium channel subtypes (see review by Rees et al., 1996). These channels are responsible for myocardial repolarization, as well as maintaining resting membrane potential.

The distribution of potassium channels is species-dependent. In rats, for example, the transient outward (I_{to}) is the major current responsible for ventricular repolarization (Josephson et al., 1984; Akaikan et al., 1992); whereas, in guinea pig ventricles, I_{to} appears to be absent. In common laboratory animals other than rats, such as dogs, guinea pigs, and rabbits, the delayed rectifier (I_K) current plays a more important role in ventricular repolarization (Sanguinetti et al., 1990; Akaikan et al., 1992). The possible class III antiarrhythmic drug, tedisamil, increases action potential duration to a greater degree in rats compared to other

species. This is thought to reflect the greater I_{to} density expressed in rat ventricle (Josephson et al., 1984; Adaikan et al., 1992). Thus, rats are a good model to study the antiarrhythmic actions of tedisamil. Before describing the class III actions of tedisamil, the three potassium channels blocked by this agent will be briefly discussed: transient outward, delayed rectifier (Dukes et al., 1989 and 1990), and ATP-sensitive potassium currents (Faivre et al., 1992).

1.2.3.1.1 Transient outward potassium current (I_{to})

I_{to} is found in many types of mammalian myocardial cells such as rat ventricles (Josephson, et al., 1984), human atria (Escande et al., 1987), and human ventricles (Wettwer et al., 1993). However, there is an absence of I_{to} in guinea pig ventricles (Campbell et al., 1995). I_{to} is referred to as a "transient" current due to its rapid activation and slower, but relatively rapid, inactivation kinetics (Campbell et al., 1995). I_{to} is responsible for the early, phase 1, repolarization. The repolarization of human ventricles consists of this fast phase 1, followed by a longer plateau phase. However, since rat ventricles have a higher density of I_{to} , repolarization occurs much more quickly and the plateau phase is very short (Josephson et al., 1984).

I_{to} is composed of two separate potassium currents: a larger voltage-activated, calcium-independent, and 4-aminopyridine-sensitive current, I_{to1} ; and a smaller calcium-dependent current, I_{to2} (Escande et al., 1987; Campbell et al., 1995). Although both subtypes contribute to myocardial repolarization, I_{to1} is

larger than I_{to2} at slow heart rates. Thus, the relative activation of each subtype is rate-dependent (Campbell et al., 1995). Dukes et al. (1990) showed that tedisamil blocks the calcium independent I_{to1} current.

1.2.3.1.2 Delayed rectifier potassium current (I_K)

I_K is the major time-dependent potassium current responsible for cardiac repolarization in many species such as guinea pigs, rabbits, and humans (Carmeliet, 1992; Rees et al., 1996). As noted earlier, I_K is absent in the rat ventricle. Nobel and Tsien (1969) were the first to study the delayed rectifier current in sheep Purkinje fibers, and found two subtypes: I_{K1} and I_{K2} . Sanguinetti and Jurkiewicz (1990) studied the delayed rectifier current in guinea pig ventricles, and also found two subtypes: I_{Kr} and I_{Ks} . This latter subclassification, which is based on kinetics and drug sensitivity, is still used today. I_{Kr} rapidly activates at a voltage range of -40 and 0 mV, while, I_{Ks} slowly activates at a voltage greater than -10 mV. I_{Kr} is sensitive, while I_{Ks} is insensitive, to blockade by the class III agent, E-4031 (Sanguinetti and Jurkiewicz, 1990; Carmeliet, 1992).

1.2.3.1.3 ATP-sensitive potassium current ($I_{K(ATP)}$)

$I_{K(ATP)}$ is a metabolically regulated potassium current that is sensitive to intracellular levels of ATP (Noma, 1983). In myocardial cells, $I_{K(ATP)}$ is inhibited at

physiological levels of intracellular ATP, but is activated at pathophysiological levels of ATP below 0.2 mM (Noma, 1983). Acute myocardial ischaemia is an example of a pathologic state in which intracellular ATP levels fall, thus potentially opening K_{ATP} channels (Reviewed in: Grover, 1994; Billman, 1994). As discussed earlier (refer to section 1.1.2.3), efflux of K^+ through K_{ATP} channels is only one of several proposed mechanisms by which extracellular K^+ rises during myocardial ischaemia. The regional differences in extracellular K^+ concentration and shortening of action potential duration produce myocardial inhomogeneity in both repolarization and refractory period. Such electrophysiological changes can initiate re-entry arrhythmias and ventricular fibrillation. Hence, K_{ATP} channel blockers such as glibenclamide (a sulphonylurea) may reduce the inhomogeneity of action potential duration and refractory period in ischaemic myocardium and might prevent ventricular arrhythmias (Billman, 1994). On other hand, K_{ATP} channel agonists such as cromakalim can increase the regional myocardial inhomogeneity, leading to ventricular arrhythmias (Grover, 1994; Billman, 1994). Although K_{ATP} channels can be modulated by drugs, whether $I_{K(ATP)}$ is the main current involved in shortening the action potential duration and causing inhomogeneity of refractory period in myocardial ischaemia is controversial (Rees et al., 1996; Wilde, 1997). For example, the block of $I_{K(ATP)}$ by glibenclamide in rat ventricular tissue had no effect on the action potential duration (Wilde et al., 1994). Hence, the usefulness of K_{ATP} channel blockers as antiarrhythmic agents requires more investigation.

1.2.3.2 Clinical usefulness of class III antiarrhythmics

Similar to the CAST trial, the SWORD (Survival with Oral d-sotalol) trial brought new insights into the clinical usefulness of potassium channel blockers (Waldo et al., 1996). The SWORD trial studied d-sotalol (a selective I_K blocker) in post-myocardial infarction patients with left ventricular heart failure. Since about twice as many patients died with d-sotalol treatment compared to placebo, the trial was stopped prematurely. In a related trial, the Danish Trial in Acute Myocardial Infarction of Dofetilide (DIAMOND, 1997), preliminary results showed that dofetilide, a selective I_{Kr} blocker, did not adversely affect the mortality rates.

Although class III agents can have antiarrhythmic actions by prolonging action potential duration, clinical trials such as SWORD show that these agents can also have proarrhythmic actions (Hondeghe et al., 1990). According to Hondeghe and Snyders (1990), the clinical usefulness of class III antiarrhythmics is limited by two main mechanisms. Firstly, many class III agents exhibit reverse use-dependent block of potassium channels. This means that these drugs have fewer effects at fast heart rates and greater effects at slow heart rates.

As a result of reverse use-dependence, the antiarrhythmic effectiveness of class III drugs decreases during tachyarrhythmias, paradoxically when their effects are most needed. Secondly, class III agents can cause excessive prolongation of action potential duration at slow heart rates, in a way that leads to arrhythmias - a proarrhythmic action. These arrhythmias are usually a

polymorphic ventricular tachycardia known as Torsade de Pointes (TdP) (Dessertenne et al., 1966). As mentioned earlier (refer to section 1.1.2.5), drugs that prolong the action potential duration in the presence of bradycardia, can induce EADs leading to TdP. Hence, a good class III antiarrhythmic agent is one that shows use-dependent block, ie, selectively prolongs the action potential duration and effective refractory period at fast heart rates when tachyarrhythmias occur, while producing little or no effect at normal heart rates (Hondeghe, 1992).

Currently, amiodarone is the only clinically used class III agent which prolongs action potentials at fast heart rates with little activity at slow heart rates, thus rarely resulting in TdP (Anderson et al., 1989; Hondeghe, 1990; Reiffel et al., 1998). Although amiodarone is a potent class III antiarrhythmic, it also has class I, II, and IV actions (Reiffel et al., 1998). Thus, amiodarone's therapeutic drawback is its non-selectivity, as well as complex pharmacokinetics, and peripheral toxicities related to the lung, liver, and thyroid gland (Nestico, et al., 1998; Reiffel et al., 1998; Singh, 1998). A second drug used clinically as a class III agent is d,l-sotalol (Singh, 1998). Both d-sotalol and l-sotalol prolong the action potential duration, however, l-sotalol also has β -blocking properties. Thus, sotalol is both a class I and III antiarrhythmic. The problem with sotalol is its reverse use-dependent prolongation of action potential duration which can lead to TdP. Since both amiodarone and sotalol have multiple pharmacological actions, their therapeutic use will be discussed further in section 1.3.1. Although both amiodarone and sotalol are used clinically, more research is required to

develop antiarrhythmics with less toxicity and less proarrhythmic actions. Thus, overall, class III drugs available today are not ideal antiarrhythmics against ischaemia-induced arrhythmias.

1.2.3.2.1 Antiarrhythmic actions of tedisamil

Tedisamil is a class III agent with antiarrhythmic actions against ischaemia-induced ventricular arrhythmias in animal models (Adaikan et al., 1992). As mentioned earlier in section 1.2.3.1, tedisamil blocks (Dukes et al., 1989) the transient outward potassium current (I_{to}) which is the predominant repolarizing current in rat ventricles (Josephson et al., 1984). The block of I_{to} results in prolongation of action potential duration and effective refractory period, thus preventing ventricular arrhythmias (Beatch et al., 1990; Adaikan et al., 1992). Although I_{to} is also present in both human atria and ventricles, tedisamil prolongs the action potential duration more in atria than ventricles (Nemeth et al., 1996). This suggests that tedisamil can be used against supraventricular arrhythmias in humans, however, more clinical trials are required to evaluate tedisamil's therapeutic action against arrhythmias (Singh, 1998).

Although tedisamil selectively blocks potassium channels (I_{to} , I_K , and $I_{K(ATP)}$), it can also block sodium channels at concentrations greater than 20 μ M (Dukes et al., 1989 and 1990). The bradycardia observed with tedisamil, even at low doses, is probably not due to sodium channel block. Instead, tedisamil seems to produce its bradycardiac action by blocking I_{to} in sinoatrial pacemaker

cells, leading to prolongation of the action potential duration and thus a decrease in heart rate (Howard et al., 1989; Adaikan et al., 1992). Since tedisamil can increase the effective refractory period even when the heart is paced at 6 Hz, the prolongation of action potential duration is not solely due to bradycardia (Beatch et al., 1990; Walker et al., 1988). Adaikan et al. (1992) observed antiarrhythmic actions when heart was paced to overcome bradycardia. This is because tedisamil can decrease the time window during which arrhythmias can occur (ie, the non-refractory period). Adaikan et al. (1992) also suggested that tedisamil can produce antiarrhythmic actions in the absence of pacing. If there is no pacing, the bradycardiac actions of the tedisamil will remain, thus the window for arrhythmias would be the same in the presence or absence of tedisamil. In this case, the antiarrhythmic actions of tedisamil are due to its ability to increase the effective refractory period resulting in an increase in the minimal path length for re-entry circuits. Thus, multiple re-entry circuits observed in ventricular fibrillation (VF) would either be abolished or converted to single re-entry circuits, resulting in slow ventricular tachycardia (VT). Overall, tedisamil can act as an antifibrillatory agent by increasing the effective refractory period and extending the minimal path for re-entry circuits.

1.2.3.2.2 Proarrhythmic actions of tedisamil

Although many studies have shown tedisamil's antiarrhythmic actions, none have ever reported any proarrhythmic actions (reviewed by Mitrovic et al.,

1998). However, it is possible for tedisamil to possess proarrhythmic actions due to its reverse use-dependent block of cardiac potassium channels (I_{to} , I_{Kr} , and $I_{K(ATP)}$) (Hondeghe et al., 1990). Thus, similar to other class III agents, tedisamil prolongs the action potential duration and effective refractory period at normal or slow heart rates as opposed to fast heart rates when tachyarrhythmias actually occur. Since the prolonged refractory period is enhanced during bradycardia, tedisamil could precipitate torsade de pointes (TdP) (Hondeghe et al., 1990; Colatsky et al., 1990). Thus, the prolongation of effective refractory period by tedisamil is frequency dependent in humans: prolongation effects are diminished at short cycle lengths (ie, tachycardia), but accentuated at long cycle lengths (ie, bradycardia) (Ohler et al., 1994; Bargheer et al., 1994). One explanation for this reverse frequency-dependent block is that tedisamil binds to potassium channels in the closed-state (Ohler et al., 1994). Interestingly, tedisamil has also been shown to be an open-channel blocker of I_{to} , resulting in a use-dependent block of this current (Wettwer et al., 1998). The reason why Ohler et al (1994) suggest a closed-state channel block by tedisamil is probably because during an action potential repolarization in human myocardium, not only is I_{to} involved, but also I_{Kr} and $I_{K(ATP)}$ (Wettwer et al., 1998). Thus, if they were to study the effects of tedisamil on I_{to} alone, they would probably find that tedisamil is an open-state channel blocker (Wettwer et al., 1998).

Unlike lidocaine, tedisamil is not selective for ischaemic tissue, thus tedisamil prolongs the effective refractory period in the non-ischaemic, normal

myocardium (Adaikan et al., 1992). It is this prolongation in the normal myocardium which could possibly lead to TdP during slow heart rates.

In summary, tedisamil can block cardiac potassium channels to prevent ventricular arrhythmias, however, there are drawbacks to the use of tedisamil as an antiarrhythmic agent. The main reason is the reverse use-dependent block of potassium channels in non-ischaemic, normal myocardium which can lead to proarrhythmia during slow heart rates. However, no one has yet shown any proarrhythmic action by tedisamil. In 1993, Hayes et al., studied tedisamil at toxic doses. They found that at sublethal doses, tedisamil caused proarrhythmia (usually VT or VF), but at lethal doses, tedisamil caused asystole and / or electromechanical dissociation before death. In non-ventilated rats, lethal doses of tedisamil can cause respiratory depression and convulsions (Hayes et al., 1993). Due to tedisamil's dangerous side-effects, more research is required to discover better drugs for use against ischaemia-induced ventricular arrhythmias.

1.3 Antiarrhythmic drug combinations used against ischaemia-induced arrhythmias

Current drug therapy for the prevention of ischaemia-induced arrhythmias is far from ideal. The drugs available today lack efficacy and are toxic. As a result, there is growing interest in treating arrhythmias with drug combinations, instead of single drug therapies or drugs having multiple actions. For example,

Nortran Pharmaceuticals (located in Vancouver, Canada) has recently developed antiarrhythmic drugs with mixed class I and class III actions. As previously mentioned, neither class I, nor class III drugs, provide adequate protection against ischaemia-induced ventricular arrhythmias. However, Nortran has observed that in animal models (rats, rabbits, and primates), the combination of class I and class III drugs can produce good antiarrhythmic protection (Yong et al., 1999).

Based on the modulated receptor hypothesis (refer to section 1.2.2.2), Hondeghem and Katzung (1980 and 1984) predict that administration of a class I and III drug in combination may act synergistically to better prevent arrhythmias compared to either drug administered alone. The suggested mechanism for this synergy was that prolongation of action potential duration by the class III drug will maintain more sodium channels in the inactivate-state which then allows more sodium channel block to occur (Hondeghem and Katzung, 1984). This is because the prolongation of the action potential duration will hold the membrane at a depolarized potential for a longer time, thus allowing for the number of inactive-state sodium channels to increase. A class I agent that has a high affinity for inactive-state sodium channels (ie, class Ib), would bind to more sodium channels during the prolonged action potential duration (Hondeghem, 1987). As a result, combination of class Ib and III drugs would increase the effective refractory period to a greater degree than either drug alone. This is due to the increase in action potential duration (class III) and block of more inactive-state sodium channels (class I); these would both lead to a slower recovery of

sodium channels available for subsequent action potentials. This amplified increase in the effective refractory period could explain why the combination of class Ib and III drugs might better prevent re-entry circuits, and hence the initiation of arrhythmias.

Although use of combinations of class Ib and III is still experimental, there are several clinically available antiarrhythmics which have more than one action: sotalol (class II and III actions), quinidine (class Ia and III actions), and amiodarone (class I, II, III, and IV actions).

1.3.1 Clinically used multi-channel blocking antiarrhythmics

1.3.1.1 Sotalol

dl-Sotalol is an antiarrhythmic agent which blocks β -adrenergic receptors (class II) and increases the effective refractory period by prolonging action potential duration (class III) (Cobbe et al., 1985). It is used clinically for both supraventricular and ventricular tachyarrhythmias (Nestico et al., 1988). Although dl-sotalol is generally well tolerated, it does have some drawbacks. Firstly, sotalol has the typical side-effects associated with β -blockers such as bradycardia and hypotension. This can result in an AV block and other bradycardia-related arrhythmias (Nestico et al., 1988). Secondly, it has been suggested that dl-sotalol loses its class III effects in ischaemic myocardium (Cobbe et al., 1985). This implies that dl-sotalol would not be useful in

ischaemia-induced ventricular arrhythmias. Thirdly, and most importantly, dl-sotalol has proarrhythmic actions (McAlister et al., 1997). This is usually in the form of torsade de pointes due to excessive prolongation of the action potential duration during bradycardia. Thus, dl-sotalol is not only an antiarrhythmic but also a proarrhythmic agent.

Interestingly, in a secondary prevention trial after myocardial infarction, dl-sotalol failed to show a statistically significant decrease in either the incidence of sudden death or total mortality (Julian et al., 1982). However, it was found to significantly lower the risk of reinfarction compared to the placebo group. In another clinical trial, SWORD, the mortality rate in patients treated with d-sotalol was greater than those treated with placebo. The reason is not known, but perhaps it is because d-sotalol has only class III actions and lacks the β -blocking action present in dl-sotalol (McAlister et al., 1997).

Hence, although sotalol is currently used as an antiarrhythmic, it is not ideal due to its undesirable side-effects and limited effectiveness.

1.3.1.2 Amiodarone

Similar to sotalol, amiodarone is currently used to prevent life-threatening ventricular arrhythmias (Singh, 1998). Amiodarone has class Ib, II, III, and IV antiarrhythmic actions (Reiffel et al., 1998). Thus, amiodarone has a very complex pharmacodynamic profile: it can non-specifically block sodium channels, β -adrenergic receptors, potassium channels, and calcium channels. Although

amiodarone has mixed-actions, it is usually classified as a class III due to its ability to potentially increase action potential duration and effective refractory period. However, unlike other class III agents (such as tedisamil and sotalol), chronic administration of amiodarone can increase the action potential duration at both normal and fast heart rates (Hondeghe et al., 1990). Amiodarone rarely causes torsade de pointes (Hondeghe et al., 1990). Interestingly, Mayuga et al., (1992) have shown that amiodarone selectively prolongs myocardial repolarization more in the ischaemic rather than normal tissue. This would result in a decrease in dispersion of repolarization and a decrease in the vulnerability to induce a ventricular arrhythmia.

Several clinical trials have been performed with amiodarone. The most recent were reported in 1997: the European Myocardial Infarct Amiodarone Trial (EMIAT), and the Canadian Amiodarone Myocardial Infarction Arrhythmia Trial (CAMIAT) (Julian et al., 1997; and Cairns et al., 1997; respectively). Both these studies showed that amiodarone produced a statistically significant decrease in arrhythmic deaths and non-fatal arrhythmic event, compared with placebo (McAlister et al., 1997). However, both failed to show any significant decrease in total mortality with the amiodarone treated group. At least, compared to many other antiarrhythmic agents, amiodarone does not increase total mortality. Even though the use of amiodarone is uncertain, it is still used for high risk patients.

One of the major drawbacks of amiodarone is toxic effects (Nattel et al., 1988; Nestico et al., 1988). These include thyroid gland dysfunction, pulmonary toxicity, and gastrointestinal problems. Although amiodarone is currently used as

an antiarrhythmic, it is not ideal. Many patients have been withdrawn from studies due to amiodarone's side-effects (McAlister et al., 1997). A better drug would have a more selective antiarrhythmic action with less toxic effects.

1.3.1.3 Quinidine

Quinidine is a widely used antiarrhythmic agent with class Ia actions (Hondeghe et al., 1990; Hondeghe et al., 1988; Nademanee et al., 1990; Roden et al., 1988). The reason why quinidine is classified as a class Ia is that it not only blocks sodium channels (mainly at fast heart rates), but also prolongs action potential duration (mainly at slow heart rates) (Hondeghe et al., 1980; Roden et al., 1988; Nademanee et al., 1990). Quinidine appears to have a higher affinity for open-state sodium channels as opposed to closed- or inactivated-states (Hondeghe et al., 1984; Tamargo et al., 1992). This is unlike class Ib agents such as lidocaine which block both open- and inactivated-state sodium channels, with a higher affinity for the inactivated-state channels (Hondeghe, 1987; reviewed by Hondeghe and Katzung, 1984). A combination of class Ib plus class III activity might be better against ischaemia-induced ventricular arrhythmias than a class Ia drug alone (Hondeghe et al., 1987). This is because the prolongation of the action potential duration maintains the membrane potential at a depolarized state and increases the number of inactive state sodium channels. Therefore, a class Ib drug (e.g.

lidocaine) would produce a more effective block of sodium channels compared to a class Ia drug (e.g. quinidine) (Hondeghe et al., 1980).

Quinidine has unwanted side-effects. For example, quinidine can cause torsade de pointes (Tamargo et al., 1992). This is similar to the proarrhythmic action of other class III agents mentioned earlier. Selzer et al. (1964), reported that patients treated with quinidine had syncopal episodes, and these are thought to be due to the induction of torsade de pointes. This "quinidine syncope" has been known for years, and is characterized with loss of consciousness, apnea, muscle contractions, and occasionally grand mal seizures (Selzer et al., 1964). Quinidine increases the mortality rate as compared to placebo treated patients (Morganroth, et al., 1991), likely related to its proarrhythmic effects. The mechanism for proarrhythmia (torsade de pointes) may be excessive prolongation of the action potential duration at slow heart rates or perhaps the induction of re-entry circuits due to excessive depression of the myocardium by blocking sodium channels (CAST trial).

Overall, quinidine is not an ideal antiarrhythmic agent due to its proarrhythmic actions as well as the lack of affinity for blocking inactive-state sodium channels. This is because most of the sodium channels in an ischaemic tissue are in the inactive-state and if quinidine cannot block these channels, it would not be expected to very effectively protect against ischaemia-induced ventricular arrhythmias. This lack of effectiveness has been shown with quinidine in rats (Barrett et al., 1995).

1.3.2 Antiarrhythmic therapy using drug combinations versus single agents

Since most of the single agents available today are not successful as antiarrhythmics, researchers have studied possible drug combinations. The simultaneous administration of two antiarrhythmics with different mechanisms of action can be more efficacious than either drug administered alone. Using the modulated receptor hypothesis, Hondeghem and Katzung (1980) were the first to predict that the combination of a class Ia (quinidine) and class Ib (lidocaine) could produce a greater depression of conduction velocity of early extrasystoles, compared to either drug alone. Thus, it is more effective to have both a fast-kinetic (e.g. lidocaine) and a slow-kinetic (e.g. quinidine) sodium channel blocker present simultaneously (Hondeghem et al., 1984). The supra-additive (ie, synergistic) reduction in conduction velocity would better prevent re-entry circuits and hence arrhythmias from occurring. Since the 1980's, many other drug combinations have been tested for their ability to prevent arrhythmias. Examples in laboratory animals include lidocaine plus sotalol (Lathrop et al., 1990), and mexiletine plus sotalol (Chézalviel et al., 1993). However, drug combinations have also been studied in clinical experiments; for example, mexiletine plus quinidine (Duff et al., 1983 and 1987). All these studies have shown that drug mixtures are better antiarrhythmics than monotherapy. In order to explain how the presence of two drugs with different mechanisms can provide a better antiarrhythmic protection than either drug alone, Duff's experiments with mexiletine and quinidine will be discussed in more detail.

1.3.2.1 Combination of mexiletine and quinidine

Clinical studies have shown that combination therapy for mexiletine plus quinidine is more effective against ventricular tachycardia than either drug alone (Duff et al., 1983 and 1987). Two possible mechanisms have been proposed for the synergistic action of mexiletine (class Ib) plus quinidine (class Ia) (Hondeghe et al., 1980; Duff et al., 1983; Wang et al., 1993; Duff, 1989; Duff et al., 1990). Firstly, the supra-additive decrease in conduction velocity could be the mechanism by which quinidine (activate-state sodium channel blocker) and mexiletine (activate- and inactivate-state sodium channel blocker) produce their enhanced antiarrhythmic action (Hondeghe et al., 1980). Secondly, enhanced effective refractory period prolongation can be observed when the two drugs are given simultaneously. This is because the increase in action potential duration produced by quinidine can increase the number of inactivate-state sodium channels blocked by mexiletine (Wang et al., 1993). Hence, the prolongation of refractoriness is due to the simultaneous prolongation of the action potential duration and block of inactive-state sodium channels. Overall, the mixture of mexiletine and quinidine is a better antiarrhythmic than monotherapy because of a greater increase in effective refractory period and a greater decrease in conduction velocity in the periinfarct zone (Duff et al., 1990).

Although Duff et al. (1991) have shown that the combination of class Ia and Ib agents is a good mixture against ventricular arrhythmias, even better

antiarrhythmic agents can be developed. There are at least two possible areas for improvement. Firstly, if the antiarrhythmic mechanism of mexiletine plus quinidine is dependent on quinidine's ability to prolong the action potential duration, then a class III agent could produce a greater prolongation of the action potential duration. This would be beneficial because the longer the action potential duration, the greater the opportunity for a class Ib drug to block inactive-state sodium channels. Thus, compared to a class Ia plus Ib combination, the combination of a class III plus Ib could produce a greater prolongation of the effective refractory period leading to a greater antiarrhythmic efficacy (Hondegheem et al., 1987; Tamargo et al., 1992). Secondly, if a class Ia and Ib drugs are given simultaneously, then the block of sodium channels by the class Ib drug can be compromised (Hondegheem et al., 1987). This is because most of the activate-state sodium channels in the upstroke of the action potential will be blocked by class Ia, thereby reducing the effects of class Ib during the plateau phase. The reason is that less drug-free sodium channels will reach the inactive-state if class Ia has already blocked most of the channels in the active-state. Hence, if a sodium channel blocker were to be used in a drug mixture, a class Ib (e.g. lidocaine) would be better than a class Ia (e.g. quinidine). This is mainly because class Ib drugs are more selective for depolarized ischaemic myocardium, and in tissue with prolonged action potential duration. Since both these situations have a greater number of inactive-state sodium channels, a class Ib agent would produce more block compared to a class Ia agent (Hondegheem et al., 1987).

1.4 Hypothesis and experimental design

To date, no drug therapy has proven ideal against ischaemia-induced ventricular arrhythmias. Since the single agents available today are inadequate antiarrhythmics, researchers are investigating drug combinations as a more favorable option.

Based on the modulated receptor hypothesis, Hondeghem et al. (1984), have predicted that the combination of class Ib and III agents could provide better antiarrhythmic protection than either drug alone. Interestingly, neither class I alone nor class III alone are effective antiarrhythmics. Nortran Pharmaceuticals (located in Vancouver, Canada) has developed new agents which have both class Ib and III activity. These mixtures have been shown to produce superior antiarrhythmic protection.

In this present study, lidocaine and tedisamil were chosen to test a proposed mechanism for the observed antiarrhythmic actions of the mixed class Ib and III drugs developed by Nortran Pharmaceuticals.

It was proposed that a combination of lidocaine (class Ib) and tedisamil (class III) drugs would act synergistically to prevent ischaemia-induced arrhythmias more effectively than either drug alone. Electrocardiograms, heart rate, blood pressure, antiarrhythmic dose-response curves (as isobolograms) were used to determine the interaction of lidocaine and tedisamil combinations at various doses.

In addition to the hypothesis stated above, the present study will answer the following questions:

1. Is lidocaine a class Ib antiarrhythmic in rats?
2. Is tedisamil a class III antiarrhythmic in rats?
3. Does the presence of lidocaine influence the actions of tedisamil (and vice versa) on blood pressure, heart rate, ECG parameters?
4. Does the presence of lidocaine potentiate the efficacy and potency of tedisamil (and vice versa) against ischaemia-induced arrhythmias?
5. Is the interaction between lidocaine and tedisamil additive, synergistic, or antagonistic?

2 METHODS

2.1 Ischaemia-induced arrhythmias

Rats were used to study ischaemia-induced arrhythmias. This rodent was chosen for three reasons. Firstly, compared to other animals such as dogs, and guinea-pigs, the coronary arteries in rats are end arteries with rare interarterial anastomoses; therefore, the occlusion of a rat coronary artery should give a consistent ischaemic zone (John and Olson, 1954). The apparent absence of collateral arteries in rat hearts is a major advantage in myocardial occlusion experiments (Curtis et al., 1987) and results in arrhythmic responses to coronary occlusion being more reproducible and predictable especially compared to dogs. The left coronary artery in rat lies under the epicardium, has no true circumflex branch; and provides the main blood supply to the left ventricle (John and Olson, 1954). Due to the presence of circumflex arteries branching from the left coronary artery in most species, the left anterior descending coronary artery is normally occluded. However, due to the absence of circumflex arteries in rats, the main left coronary artery is occluded in this species. Thus, ischaemic myocardial tissue (occluded zone) is easily defined in rats. Secondly, unlike other species such as guinea-pigs, rat ventricles have a large transient outward current responsible for ventricular repolarization (Josephson et al., 1984). Thus, the antiarrhythmic and electrophysiological actions of tedisamil, a transient outward potassium channel

blocker, can be better observed in rats as compared to guinea pigs (Adaikan et al., 1992). Thirdly, rats are cheap and readily available.

2.1.1 Surgical procedure and experimental design

The surgical preparation for coronary occlusion of anaesthetized rats has been previously described (Au et al., 1979; Paletta et al., 1989). In the present study, male Sprague-Dawley rats (190-400 g) were anaesthetized with pentobarbitone (65 mg/kg) administered i.p. The trachea was cannulated for artificial ventilation. The right carotid artery and left jugular vein were cannulated (PE-50 tubing) for BP recording and drug administration, respectively. ECG signals were recorded by placing three subcutaneous electrodes at the apex of the heart, right clavicle, and right abdominal region. Wires were used to connect both ECG and blood pressure transducer to a Honeywell Monitor (Electronics for Medicine). The signals were then connected to a computer with LabView program (version 4.0.1 for Windows 95). All the recordings were first saved on the computer hard drive and then transferred to a compact disc for storage.

After cannulation, rats were artificially ventilated with pure oxygen at a rate of 60 strokes/min and a stroke volume of 10 mL/kg. The region above the heart was located and the skin cut. The muscles in the left thoracic cavity between the 4th and 5th rib were separated. Retractors were used to open the chest. The pericardium was cut and stretched using the same retractors to form a sac. This exposed the heart for the placement of an occluder around the left coronary artery. The

occluders were made of a polypropylene suture (5-0) threaded through a polyethylene (PE-10) tubing (Johnston et al., 1983). The left atrium was lifted using blunt forceps and the needle attached to the occluder was placed around the left coronary artery. Although the positioning was done blindly, it was placed at a downward 30 degree angle from underneath the left atrium toward the left ventricle. The thread was then passed through the occluder to form a loop. Then the chest was closed using suture (4-0).

Before the start of experiment, serum potassium concentration of arterial blood was measured. 0.5 mL samples were placed in a test tube and potassium concentrations measured using a potassium electrode (Accumet; Fisher Scientific; Model 25). Throughout, the temperature of rats was monitored using a rectal thermometer and maintained using a lamp. After surgery, animals were allowed 10 min for recovery. Once BP had stabilized and ECG parameters well defined, the protocol was started. The first stage was a 3 min (predrug) recording of BP and ECG.

An infusion pump (Harvard apparatus, Model 55-2222) was used to continuously infuse drug starting 5 min before and continuing until 15 min after occlusion. Occlusion was performed by pulling the suture through the occluder and secured by melting the exteriorized end. Throughout the experiment, rats were monitored for arrhythmias and haemodynamic changes. Prior to cessation of artificial ventilation at 15 min after occlusion, serum potassium concentration of arterial blood samples (0.5 mL) were measured. This was performed in order to determine whether the drug had effects on serum potassium concentration.

Following cessation of ventilation, drug infusion was continued for another 5 min in order to determine effects on breathing as a possible indication of adverse effects.

After completion of the above protocol, the occluded zone (OZ) was measured (Johnston et al., 1983) by perfusing the heart with saline (Langendorf technique), and cardiac green dye (0.2 - 0.5 g/L) until a clear distinction was made between the normal (green coloured) and occluded (light brown coloured) tissue. Using fine scissors, the ventricles were separated from atria and any blood vessels. The occluded zone was measured as a percent of total ventricular weight.

2.1.2 Arrhythmia definitions

Ischaemia-induced arrhythmias were defined according to the Lambeth Conventions (Walker et al., 1988). Although BP changes may occur with arrhythmias, the ECG was used as the sole basis for defining arrhythmias. A premature ventricular beat (PVB) was defined as the occurrence of a QRS complex with no P wave. Salvoes of less than 4 PVBs were not differentiated from PVBs. Ventricular tachycardia (VT) was defined as 4 or more consecutive PVBs. VT was not defined in relation to rate. VF was defined as morphological instability of the ECG with no distinction between QRS complexes and no possibility of rate determination from the signal. Arrhythmias were classified as being either PVB, VT, or VF.

An arrhythmia score was used to summarize arrhythmias based on the time of occurrence, incidence and severity of arrhythmias (Curtis and Walker, 1988).

This score is Gaussian distributed and therefore allows the use of parametric statistical tests. The following is the scoring system:

0 = 0 - 49 PVBs

1 = 50 - 499 PVBs

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

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

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

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

6 = fatal VF starting at > 15 min after occlusion

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

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

9 = fatal VF starting < 1 min after occlusion

2.1.3 Inclusion / exclusion criteria

In order to ensure consistency between experiments, certain inclusion criteria were used. Rats were included only if predrug mean BP was above 70 mmHg. Secondly, predrug serum potassium concentration had to be in the range of 2.5 to 4.5 mM. Thirdly, no more than 15 PVBs, and no episodes of VT or VF could occur before drug administration. Fourthly, the ECG parameters (P wave, QRS complex, and T wave) should be easily recognized. Fifthly, rectal temperature should be within the range of 33 – 36°C. Finally, the occluded zone must be between 25 –

50% of total ventricular weight. If any of the mentioned criteria were not met, animals were excluded from the study and replaced.

2.1.4 ECG and BP measurements

ECG intervals were measured as the average of five ECG complexes according to Penz et al. (1992) (Figure 1). ECG and BP measures at 2 min before drug infusion (defined as predrug), 30 sec before occlusion (defined as preocclusion), and 15 min after occlusion (defined as postocclusion). HR was calculated from the distance between two consecutive R waves. PR was defined as the interval from the beginning of the P wave to the peak of the R wave along the isoelectric line. QRS was defined as the interval from the beginning of the R wave to the end of the S wave. QT_1 was defined as the interval from the beginning of the R wave to the peak of the T wave. QT_2 was defined as the interval from the beginning of the R wave to a point half-way in the downward phase of the T wave. If an inflection point was observed in the T wave, this was considered to be the peak for QT_2 measurement. QT was not corrected for changes in HR since QT does not vary with HR in rats (Hayes et al., 1994). BP was measured as the average of systolic and diastolic pressures at 30 sec intervals for each stage.

A LabView program (version 4.0.1 for Windows 95) was used for ECG and BP measurements. Briefly, this program automatically places cursors on the P, Q, R, S, T_1 and T_2 positions on each of five ECG complexes. After confirming each of these values (recorded in milliseconds) manually, they were then transferred to an

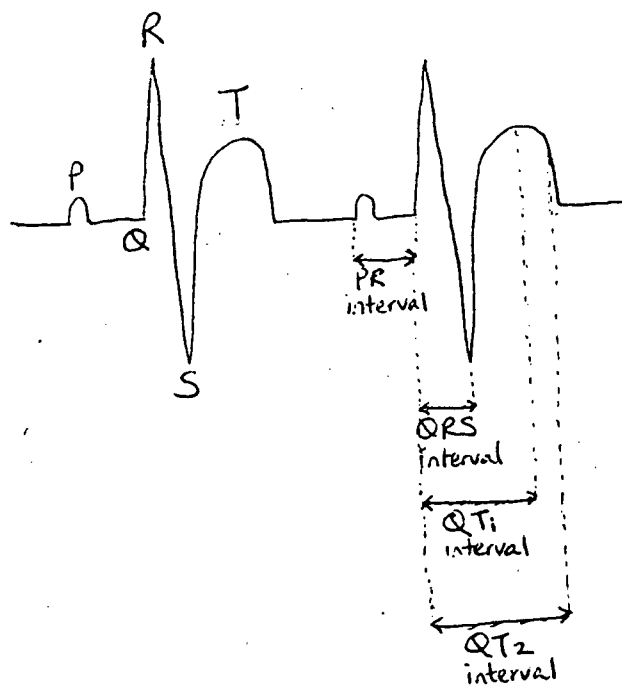


Figure 1 A typical rat ECG showing the points used to measure PR, QRS, and QT intervals. These two normal cycles of ECG are hand-drawn. Please refer to text for more detail.

Excel (Windows 95) template to determine the PR, QRS, QT₁, and QT₂ intervals as well as HR, and BP.

2.2 Dose-response curves

In a double blind and random line design, rats were placed in either vehicle or drug treated groups. A total of fourteen blocks consisting of treatment plus controls were performed with 33% of each block being vehicle treated rats. Antiarrhythmic and electrophysiological dose-response curves were constructed for tedisamil alone, lidocaine alone, tedisamil plus lidocaine at 2, 4, and 6 $\mu\text{mol/kg/min}$, and lidocaine plus tedisamil at 0.5, 1, and 2 $\mu\text{mol/kg/min}$. The vehicle control (total $n=85$) was 10% dimethylsulfoxide (DMSO), 30% ethanol (EtOH), and 60% distilled water (dH_2O). Treated groups were administered one of the following drugs ($n=5$ for each dose in $\mu\text{mol/kg/min}$):

tedisamil alone (0.125, 0.25, 0.5, 1, 2, and 4)

lidocaine alone (2, 4, 6, and 8)

lidocaine 2, plus tedisamil (0.125, 0.25, 0.5, 1, and 2)

lidocaine 4, plus tedisamil (0.063, 0.125, 0.25, 0.5, and 1)

lidocaine 6, plus tedisamil (0.063, 0.125, 0.25, 0.5, and 1)

tedisamil 0.5, plus lidocaine (1, 2, 4, and 6)

tedisamil 1, plus lidocaine (0.5, 1, 2, 4, and 6)

tedisamil 2, plus lidocaine (0.25, 0.5, 1, and 2)

Antiarrhythmic dose-response curves were fitted to a logistic function ($y=x^n/(ED_{50}^n+x^n)$) where x is the dose, n is the slope (Hill coefficient), and ED_{50} is the dose producing 50% of maximal response using SlideWrite (4.0 32 Bit Edition). For each drug, antiarrhythmic dose-response curves were expressed both as dose-dependent changes in arrhythmia score as well as percent antiarrhythmic protection. Scores were converted to percent protection (y) using the equation:

$y=((\text{score}_{\text{control group}} - \text{score}_{\text{treated rat}})/\text{score}_{\text{control group}})100\%$. For tedisamil alone and lidocaine alone, $\text{score}_{\text{control group}}$ was the average antiarrhythmic score obtained for all vehicle treated rats (10% DMSO, 30% EtOH, and 60% dH₂O) in the study ($n=85$), and $\text{score}_{\text{treated rat}}$ was the arrhythmia score for the individual rat treated at a particular dose. For drug combinations, $\text{score}_{\text{control group}}$ was the antiarrhythmic score for the background drug-dose which was kept constant. This score was determined from the antiarrhythmic dose-response curves for that drug alone. For example, the $\text{score}_{\text{control group}}$ for increasing doses of lidocaine in the presence of constant tedisamil at 2 $\mu\text{mol/kg/min}$ was that score obtained from the antiarrhythmic dose-response curve for tedisamil alone at 2 $\mu\text{mol/kg/min}$. The use of either actual arrhythmia scores or normalized arrhythmia scores (expressed as percent changes from control) produced the same ED_{50} for a particular drug. ED_{50} values were used to determine the relative potency of drugs administered alone compared with combinations.

Dose-response curves for electrophysiological effects were constructed for drugs administered either alone, or in combination. Dose-dependent changes in HR, BP, PR, QRS, QT₁ and QT₂ were fitted to a second order polynomial equation

and an ED_{25} defined as the dose producing a 25% change from control was determined for each curve. Control values for drugs administered alone, or in combination, were defined as previously described for the antiarrhythmic dose-response curves.

2.3 Sham occlusions

In our laboratory, bolus administration of tedisamil against ischaemia-induced arrhythmia in rats has previously been studied (Beatch et al., 1991; Adaikan et al., 1992). In order to compare the antiarrhythmic and electrophysiological actions of tedisamil in the present study using continuous infusions, with published data using bolus administration, sham occlusions with tedisamil were investigated in a random and open design. The surgical and experimental procedures were same as those previously described for coronary artery occlusion studies with the following exception: an occluder was loosely placed around the left coronary artery, but the vessel was not occluded. The sham occlusions were performed with tedisamil ($n=3$) at a dose of 0.5 and 2 $\mu\text{mol/kg/min}$ continuous infusion, compared to 4 mg/kg bolus (over a 10 min period).

2.4 Isobolograms

The term isobologram was introduced by Loewe and Muischnek (1926) to describe the interactions between drug combinations. However, this method was

first used by Fraser (1870-1; 1872) to study antagonism. Isoboles are used to determine whether the action of two drugs in combination is additive, synergistic, or antagonistic. Although isoboles have been used for over a century, lack of consistent terminology has led to confusion among researchers (Kodell and Pounds, 1991). For example, Goldin and Mantel (1957) explicitly state the inconsistency in nomenclature by describing seven different concepts for synergy used by investigators. Thus, in order to study drug interaction, a clear definition of each term is crucial.

2.4.1 Terminology

Isobolograms can show whether drug combinations are non-interactive (additive) or interactive (synergistic or antagonistic). As described by Hewlett and Plackett (1959), drug interaction is when one drug can alter the biological actions of another. Additive interactions usually occur when drugs with the same mechanism are combined (Rosow, 1997). Antagonistic interactions usually occur when one drug competitively competes for the same binding site on a receptor as another drug. Synergistic interactions usually occur when drugs of different classes with different mechanisms lead to the same effect. Note that with synergism, the combined dose can produce a larger response than either drug alone.

In order to understand the construction and interpretation of isobolograms used hereafter, the interaction between drugs A and B will be discussed (Berenbaum, 1977). Suppose that the x and y-axes represent doses of drugs A and

B administered alone, respectively. A straight line can be drawn to join the doses of each drug alone producing the same response. As shown in Figure 2, this isobole (*isos*, equal; and *bole*, effect) is referred to as the line of additivity (Naguib et al., 1995). Mathematically, the additivity line is the following equation: $(\text{dose of A} / A_e) + (\text{dose of B} / B_e) = 1$ (Berenbaum, 1977). This means that if A and B are the same drug with different dilutions, then a mixture of A and B in an amount proportional to their relative potencies can maintain the same response (Plummer et al., 1990). For example, if drug A is 10 times more potent than drug B, then 100 mg of drug A would produce the same effect as 50 mg of A plus 500 mg of drug B. Thus, if the above equation for the additivity line is equal to, less than, or greater than 1, then the interaction is denoted as additive, synergistic, or antagonistic, respectively.

Although the most commonly used effect is the dose producing 50% of the maximal response (ED_{50}), due to the least amount of error in obtaining this value, other responses such as ED_{25} and ED_{75} can also be studied (Gessner, 1995). If the isobole for the combination of drugs A and B lies to the left of the additivity line (concave shaped), then the two compounds probably act synergistically. If the isobole for the combination lies to the right of the additivity line (convex shaped), then the two compounds probably act antagonistically. If however, the isobole for the combination lies close to the additivity line, then the interaction is additive. In other words, if the ED_{50} for the combination lies to the left, to the right, or close to the ED_{50} additivity line, the interaction is probably synergistic, antagonistic, or additive, respectively.

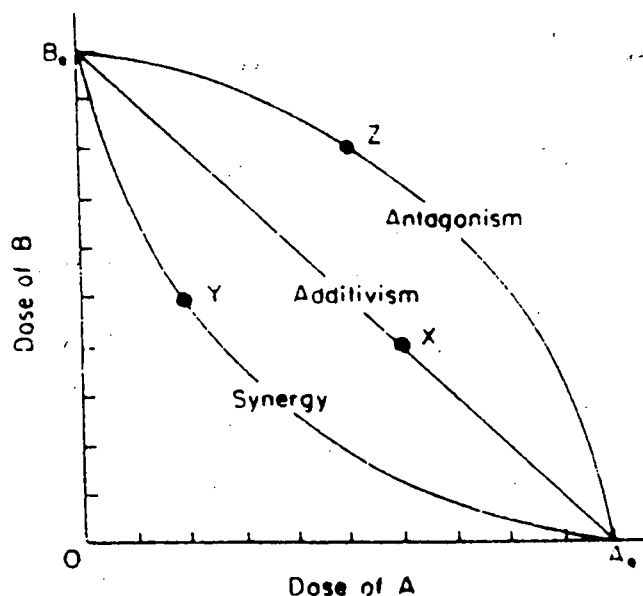


Figure 2 Construction of an isobologram showing additivism (X), synergy (Y), and antagonism (Z). A_e and B_e are doses of drugs A and B which produce the same response. The additivism isobole is a straight line which joins A_e and B_e which is a combination of drugs A and B equaling 1. The synergistic isobole is a combination of the drugs equaling less than 1. The antagonistic isobole is a combination of the drugs equaling greater than 1 (reproduced from Berenbaum, 1977).

2.4.2 Interaction between tedisamil and lidocaine

An ED_{50} isobologram was used to determine the interaction between tedisamil and lidocaine. A straight line of additivity was drawn by joining the antiarrhythmic ED_{50} for tedisamil alone (y-axis) with that for lidocaine alone (x-axis). The antiarrhythmic ED_{50} for the various combinations of tedisamil and lidocaine were also plotted on the isobologram. The ED_{50} s for the mixtures were then fit to an exponential equation. As mentioned previously, if this curve lies to the left, right or close to the line of additivity, then it can be predicted that tedisamil and lidocaine interact synergistically, antagonistically, or additively, respectively.

2.5 Drugs

Tedisamil dihydrochloride (KC8857) was a generous gift from Kali-Chemie Pharma GmbH. Lidocaine was obtained from Sigma Chemical Company, St. Louis, MO. Both drugs were dissolved in vehicle (10% DMSO, 30% ethanol, and 60% dH_2O) before administration to rats.

2.6 Statistics

In order to test whether the means of parameters such as ECG, BP, HR, ED₅₀, and arrhythmia score changed with dose, single factor analysis of variance (ANOVA) was performed (Zar, 1984). A statistically significant F ratio suggested that the means changed dose-dependently since the variance between groups was greater than within a group. Duncan's multiple range test was performed on means that showed a statistical difference after ANOVA. Although several tests are available to determine which means differ from one another, Duncan's multiple range test is a widely used procedure (Duncan, 1955). This is quite a powerful test and thus very popular (Montgomery, 1984). For detecting a statistical difference between groups on incidences of PVB, VT, or VF, a 2X2 Fisher's exact test was used (Zar, 1984). This test is often used for statistics on proportions and is preferable with small cell frequencies. All statistical tests were performed using Sigma Stat version 2.0 with statistical significance set at an α -level of 0.05.

A major drawback in the use of isobolograms is that no recognized statistical tests exist to determine whether drug combinations deviate significantly from the line of additivity (Casseo et al., 1998). The lack of a standard statistical method to account for biological variability has led to wrong conclusions on classification of drug interactions (Könemann et al., 1996). One possible statistic is the joining of the ED₅₀ for each drug alone along with the SEM for each drug (Wessinger, 1986 and Berenbaum, 1989). If the combination ED₅₀s lie outside the confidence limits for the additivity line, then the combination is said to be significantly different from

the line of additivity. However, a problem with this method is that the ED_{50} usually has a large SEM, and so the confidence limits for the line of additivity are also large (Casseo et al., 1998). In view of the above, the interaction between tedisamil and lidocaine was only investigated qualitatively for a synergistic trend with combinations of tedisamil and lidocaine.

3 RESULTS

3.1 Sham occlusions

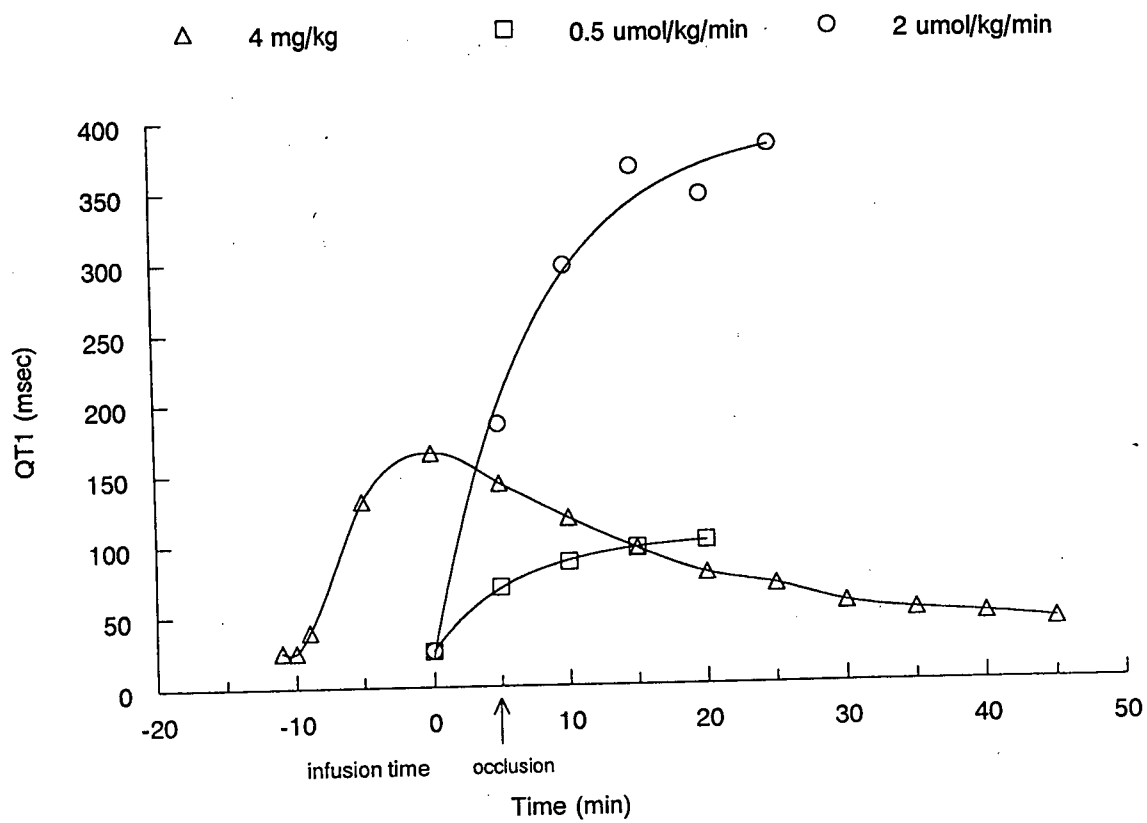
3.1.1 Overview

Beatch et al. (1991) determined the antiarrhythmic and electrophysiological effects of intravenous bolus injection of tedisamil in rats. They found that a 4 mg/kg bolus of tedisamil significantly reduced VF incidence in a coronary artery occlusion model. In the present experiment, tedisamil was administered by continuous infusion rather than bolus to compare tedisamil's effect on the QT interval at a dose equivalent to the 4 mg/kg bolus (Figure 3).

3.1.2 Antiarrhythmic and proarrhythmic effects of tedisamil

Tedisamil dose-dependently increases the QT_1 . This variable was used to compare the two routes of administration in sham occlusion experiments. Ten min after sham occlusion, tedisamil administered either as 4 mg/kg bolus or 0.5 μ mol/kg/min continuous infusion, increased QT_1 interval by approximately 4 fold compared to predrug values (Figure 3). Thus, tedisamil at 0.5 μ mol/kg/min was considered equivalent to 4 mg/kg bolus. Although the effects of these two routes of administration on QT interval were similar, their antiarrhythmic effects were different. Four mg/kg tedisamil significantly decreased VF incidence, while 0.5 μ

Figure 3 The effect of a bolus dose versus continuous infusion of tedisamil on the QT₁ interval. Tedisamil at a bolus dose of 4 mg/kg (Δ) was compared to continuous infusion at 0.5 μ mol/kg/min (\square), and 2 μ mol/kg/min (O). The bolus infusion time was 10 min. Occlusion was performed 5 min after end of bolus or start of continuous infusion. Values are an average of n=3 rats with sham occlusion. Error bars and statistical analysis are not shown due to small sample size.



mol/kg/min tedisamil had no effect on VF incidence (refer to the antiarrhythmic section and Table IV). Interestingly, 4 μ mol/kg/min tedisamil, and not 0.5 μ mol/kg/min, significantly decreased VF incidence.

As well, a high dose of tedisamil at 2 μ mol/kg/min was tested to show that a very large increase in QT₁ interval (about twelve fold) can be obtained (Figure 3). This may be due to the accumulation of the drug with the continuous infusion. Tedisamil at this high dose produced proarrhythmic actions (1 out of 3 rats). The arrhythmias were observed as PVB and one episode of VT in normal, non-ischaemic tissue 20 minutes after sham occlusion.

3.2 Ischaemia-induced arrhythmias

3.2.1 BP, HR, and ECG effects of drugs administered alone and in combination

3.2.1.1 Overview

The study was divided into 14 random and double blind experiments consisting of 1/3 vehicle and 2/3 drug treated rats according to a block design. No statistically significant differences for BP, HR, PR, QRS, QT₁ and QT₂ were detected for the vehicle groups for each experiment. Thus, all vehicle treated rats (n=85) were grouped together for comparison with drug treated rats (n=5 at each treated dose).

The effect of tedisamil alone, lidocaine alone, and various combinations of both drugs on BP, HR, and ECG are shown in Tables I and II. These data were then used to summarize the effects of the highest combined doses of lidocaine in the presence of 0.5, 1, and 2 $\mu\text{mol/kg/min}$ tedisamil and tedisamil in the presence of 2, 4, and 6 $\mu\text{mol/kg/min}$ lidocaine, in order to determine whether drugs administered as a mixture differed significantly from either drug alone (Table III). As well, BP, HR, PR, QRS, QT_1 and QT_2 dose-response curves were constructed to determine whether drugs alone or in combination produced an ED_{25} compared to control (Figures 4 to 9, respectively).

3.2.1.2 Lidocaine alone

Lidocaine produced a dose-dependent decrease in both BP and HR compared to vehicle (Table I). The PR interval was dose-dependently increased. Lidocaine had no significant effect on QRS, QT_1 and QT_2 .

3.2.1.3 Tedisamil alone

Compared to vehicle, tedisamil significantly increased BP only at a dose of 2 $\mu\text{mol/kg/min}$ (vehicle: 111 ± 2 mmHg; tedisamil: 134 ± 4 mmHg). Tedisamil dose-dependently decreased HR (Table I). Tedisamil (≥ 0.5 $\mu\text{mol/kg/min}$) increased PR, QRS, QT_1 , and QT_2 intervals. Compared to vehicle, the highest dose of tedisamil (4 $\mu\text{mol/kg/min}$) increased QT_1 interval by approximately 7 fold

Table I The dose-related effects of tedisamil and lidocaine alone on BP, HR, and ECG.

Ted	Lido	BP (mmHg)	HR (beats/min)	PR (msec)	QRS (msec)	QT1 (msec)	QT2 (msec)
0	0	111 ± 2	359 ± 4	62	16	29	42
0	2	111 ± 6	321 ± 21 #	63 ± 1	18 ± 1	30 ± 1	42 ± 1
0	4	75 ± 9 #	298 ± 29 #	68 ± 2 #	18 ± 1	32 ± 1	47 ± 2
0	6	73 ± 5 #	266 ± 8 #	68 ± 2 #	18 ± 1	29 ± 1	42 ± 2
0	8	75 ± 7 #	245 ± 18 #	69 ± 2 #	17 ± 1	30	43 ± 1
0.125	0	122 ± 5	339 ± 27	66 ± 2	16 ± 2	38 ± 1	56 ± 3
0.25	0	124 ± 3	312 ± 17 #	68 ± 2	20 ± 3	53 ± 3	69 ± 3
0.5	0	130 ± 3	302 ± 13 #	75 ± 1 #	25 ± 4 #	67 ± 9 #	88 ± 10 #
1	0	114 ± 3	237 ± 12 #	70 ± 4 #	16 ± 1 #	91 ± 5 #	124 ± 5 #
2	0	134 ± 4 #	199 ± 16 #	77 ± 2 #	23 ± 3 #	121 ± 20 #	157 ± 21 #
4	0	115 ± 14	108 ± 9 #	74 ± 4 #	21 ± 1 #	201 ± 18 #	259 ± 27 #

The action of tedisamil (Ted) and lidocaine (Lido) administered alone in pentobarbitone-anaesthetized rats are shown. Values are mean ± SEM. At each dose (μmol/kg/min), n=5, except for the vehicle treated rats where n=85. The variables were measured at 5 min after drug infusion. The symbol # denotes a statistically significant difference (p<0.05) from vehicle group.

Table II The effect of various combinations of tedisamil (Ted) and lidocaine (Lido) on BP, HR, and ECG. Values are mean \pm SEM. For all drug combinations, n=5 anaesthetized rats at each dose (μ mol/kg/min). For the vehicle treated rats in the absence of both tedisamil and lidocaine, n=85. The variables were measured at 5 min after beginning drug infusion. Dose-response curves for these data are shown in Figures 4 to 9. The symbol # denotes a difference from vehicle. The symbol * denotes a difference from the experimental control (ie, the constant dose in the mixture). Statistical significance was detected at $p < 0.05$.

Ted	Lido	BP (mmHg)	HR (beats/min)	PR (msec)	QRS (msec)	QT1 (msec)	QT2 (msec)
0	0	111 ± 2	359 ± 4	62	16	29	42
0.5	0	130 ± 3	302 ± 13 #	75 ± 1 #	25 ± 4 #	67 ± 9 #	88 ± 10 #
0.5	1	113 ± 4 *	287 ± 17 #	70 ± 2 #	22 ± 2 #	66 ± 5 #	89 ± 6 #
0.5	2	109 ± 3 *	248 ± 9 # *	74 ± 1 #	21 ± 1 #	69 ± 7 #	98 ± 10 #
0.5	4	117 ± 6	238 ± 10 # *	74 ± 1 #	20 ± 1 #	71 ± 4 #	99 ± 7 #
0.5	6	107 ± 5 *	211 ± 5 # *	75 ± 2 #	20 ± 2 #	71 ± 2 #	96 ± 4 #
1	0	114 ± 3	237 ± 12 #	70 ± 4 #	16 ± 1 #	91 ± 5 #	124 ± 5 #
1	0.5	132 ± 4	232 ± 13 #	70 ± 1 #	24 ± 3 #	84 ± 4 #	117 ± 6 #
1	1	114 ± 6	204 ± 8 #	72 ± 2 #	22 ± 2 #	87 ± 10 #	133 ± 16 #
1	2	115 ± 6	197 ± 12 #	70 ± 3 #	20 ± 1 #	107 ± 20 #	146 ± 18 #
1	4	114 ± 5	205 ± 6 #	76 ± 1 #	26 ± 3 # *	88 ± 5 #	131 ± 6 #
1	6	117 ± 7	167 ± 9 # *	79 ± 2 #	22 ± 2 #	94 ± 4 #	137 ± 8 #
2	0	134 ± 4 #	199 ± 16 #	77 ± 2 #	23 ± 3 #	121 ± 20 #	157 ± 21 #
2	0.25	109 ± 1 *	164 ± 11 #	74 ± 2 #	24 ± 2 #	119 ± 8 #	166 ± 5 #
2	0.5	126 ± 4	171 ± 12 #	75 ± 4 #	25 ± 2 #	154 ± 19 # *	200 ± 17 #
2	1	115 ± 5 *	164 ± 3 #	74 ± 1 #	22 ± 1 #	116 ± 12 #	159 ± 13 #
2	2	126 ± 7	152 ± 13 #	73 ± 3 #	22 ± 2 #	112 ± 8 #	166 ± 14 #
0	2	111 ± 6	321 ± 21 #	63 ± 1	18 ± 1	30 ± 1	42 ± 1
0.125	2	111 ± 9	335 ± 9	70 ± 2 #	17 ± 1	35 ± 2 #	53 ± 3 #
0.25	2	126 ± 4	307 ± 15 #	69 ± 1 #	19 ± 2	56 ± 9 # *	77 ± 12 # *
0.5	2	112 ± 8	267 ± 10 # *	72 ± 1 # *	19 ± 1	64 ± 4 # *	101 ± 5 # *
1	2	113 ± 5	233 ± 5 # *	69 ± 2 #	18 ± 1	99 ± 5 # *	137 ± 5 # *
2	2	87 ± 5 # *	142 ± 7 # *	74 ± 3 # *	26 ± 4 #	105 ± 4 # *	151 ± 6 # *
0	4	75 ± 9 #	298 ± 29 #	68 ± 2 #	18 ± 1	32 ± 1	47 ± 2
0.063	4	107 ± 5 *	303 ± 21 #	72 ± 2 #	19 ± 1	33 ± 2 #	48 ± 2
0.125	4	98 ± 6 *	273 ± 19 #	69 ± 2 #	20 ± 2 #	40 ± 2 #	55 ± 2 #
0.25	4	112 ± 7 *	258 ± 24 #	70 ± 3 #	19 ± 2	58 ± 8 # *	92 ± 13 # *
0.5	4	104 ± 6 *	241 ± 12 #	75 ± 3 #	23 ± 4 #	62 ± 3 # *	87 ± 5 # *
1	4	98 ± 5 *	205 ± 7 # *	73 ± 3 #	18 ± 2	88 ± 6 # *	129 ± 7 # *
0	6	73 ± 5 #	266 ± 8 #	68 ± 2 #	18 ± 1	29 ± 1	42 ± 2
0.063	6	88 ± 5 #	272 ± 9 #	69 ± 1 #	18 ± 1	37 ± 2 #	52 ± 2 #
0.125	6	88 ± 2 #	262 ± 7 #	67 ± 2 #	17 ± 1	39 ± 1 #	56 ± 1 #
0.25	6	97 ± 3 *	249 ± 11 #	75 ± 2 # *	20 ± 2 #	51 ± 3 # *	69 ± 4 # *
0.5	6	106 ± 8 *	222 ± 11 # *	76 ± 3 #	21 ± 3 #	66 ± 5 # *	103 ± 13 # *
1	6	107 ± 7 *	191 ± 6 # *	77 ± 1 # *	21 ± 1 #	80 ± 3 # *	118 ± 8 # *

(vehicle: 42 msec; tedisamil: 201 ± 18 msec). Similar results were observed for effects on QT₂ interval.

3.2.1.4 Combinations of lidocaine and tedisamil

3.2.1.4.1 Effects on BP

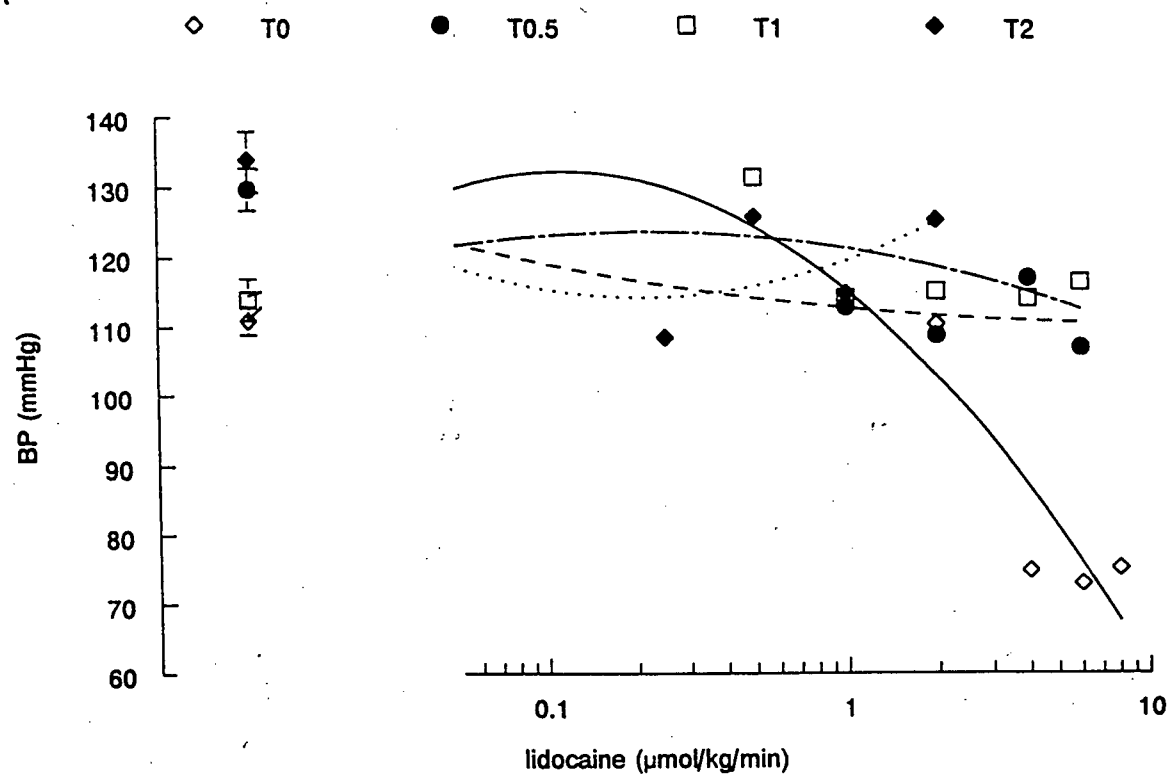
Lidocaine alone dose-dependently decreased BP; however, when combined with 0.5, 1 or 2 $\mu\text{mol/kg/min}$ tedisamil, this response was no longer observed (Table II). Compared to control, lidocaine alone decreased BP with an ED₂₅ of about 3.5 $\mu\text{mol/kg/min}$; however, lidocaine co-infused with tedisamil had no effect on BP (Figure 4). Thus, co-infusion of tedisamil prevented lidocaine's hypotensive effects.

Table II shows that compared to vehicle, tedisamil produced a decrease in BP when co-administered with lidocaine (2 and 6 $\mu\text{mol/kg/min}$). Neither tedisamil alone nor tedisamil plus 2 $\mu\text{mol/kg/min}$ lidocaine produced a 25% change in BP compared to control (Figure 4). However, in combination with higher doses of lidocaine (4 and 6 $\mu\text{mol/kg/min}$), tedisamil's hypotensive effects were reduced. Hence, with increasing doses of lidocaine (2, 4, and 6 $\mu\text{mol/kg/min}$), tedisamil produced greater increases in BP compared to control.

Table III summarizes the effect on BP produced by the highest combined doses of the two drugs compared to tedisamil alone, lidocaine alone, and vehicle.

Figure 4 Mean BP dose-response curves for lidocaine (L) alone, tedisamil (T) alone, or combinations of tedisamil and lidocaine in pentobarbitone-anaesthetized rats. A, BP changes after administration of lidocaine alone (\emptyset), or lidocaine in the presence of tedisamil at 0.5 (\bullet), 1 (\square), and 2 (\blacklozenge) $\mu\text{mol/kg/min}$. B, BP changes after administration of tedisamil alone (Δ), or tedisamil plus lidocaine at 2 (\blacksquare), 4 (\circ), and 6 (\blacktriangle) $\mu\text{mol/kg/min}$. The symbols not on the x-axis correspond to the experimental control of the constant dose in the mixture. For drugs administered alone, the control was vehicle treated rats. All data points are $n=5$ at each dose except for the vehicle ($n=85$). Error bars are only shown for control values (mean \pm SEM) and not for any other points for clarity. Curves are graphical representation of data in Table II in order to determine ED_{25} s (25% change in BP compared to control).

A



B

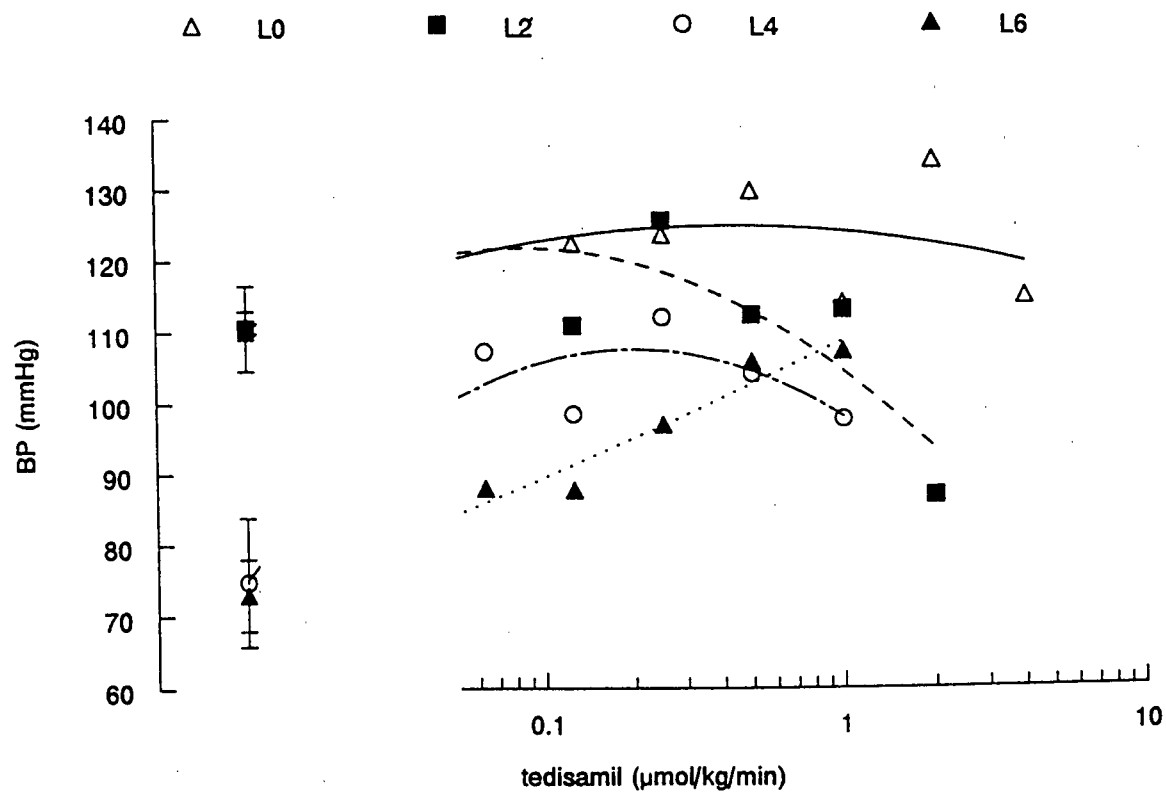


Table III BP, HR, and ECG effects of the highest combined doses tested for lidocaine (L) in the presence of a constant dose of tedisamil (T) (Part A) and tedisamil in the presence of a constant dose of lidocaine (Part B) compared to either drug administered alone. Values (mean \pm SEM) are summarized from Tables I and II. For each dose, n=5 anaesthetized rats except for the vehicle (n=85) in the absence of both tedisamil and lidocaine. The symbol # denotes a difference from vehicle. The symbol * denotes a difference between drugs administered alone compared to combination. Statistical significance was detected at $p < 0.05$.

Drug ($\mu\text{mol/kg/min}$)	BP (mmHg)	HR (beats/min)	PR (msec)	QRS (msec)	QT1 (msec)	QT2 (msec)
Vehicle	111 ± 2	359 ± 4	62	16	29	42

Part A: Lidocaine plus 0.5, 1, and 2 $\mu\text{mol/kg/min}$ tedisamil

T0.5	$130 \pm 3 \#$	$302 \pm 13 \# *$	$75 \pm 1 \#$	$25 \pm 4 \# *$	$67 \pm 9 \#$	$88 \pm 10 \# *$
L6	$73 \pm 5 \# *$	$266 \pm 8 \# *$	$68 \pm 2 \# *$	18 ± 1	$29 \pm 1 *$	$42 \pm 2 *$
T0.5 plus L6	107 ± 5	$211 \pm 5 \#$	$75 \pm 2 \#$	$20 \pm 2 \#$	$71 \pm 2 \#$	$96 \pm 4 \#$
T1	114 ± 3	$237 \pm 12 \# *$	$70 \pm 4 \# *$	$16 \pm 1 *$	$91 \pm 5 \#$	$124 \pm 5 \# *$
L6	$73 \pm 5 \# *$	$266 \pm 8 \# *$	$68 \pm 2 \# *$	$18 \pm 1 *$	$29 \pm 1 *$	$42 \pm 2 *$
T1 plus L6	117 ± 7	$167 \pm 9 \#$	$79 \pm 2 \#$	$22 \pm 2 \#$	$94 \pm 4 \#$	$137 \pm 8 \#$
T2	$134 \pm 4 \#$	$199 \pm 16 \# *$	$77 \pm 2 \#$	$23 \pm 3 \#$	$121 \pm 20 \# *$	$157 \pm 21 \#$
L2	111 ± 6	$321 \pm 21 \# *$	$63 \pm 1 *$	$18 \pm 1 *$	$30 \pm 1 *$	$42 \pm 1 *$
T2 plus L2	126 ± 7	$152 \pm 13 \#$	$73 \pm 3 \#$	$22 \pm 2 \#$	$112 \pm 8 \#$	$166 \pm 14 \#$

Part B: Tedisamil plus 2, 4, and 6 $\mu\text{mol/kg/min}$ lidocaine

T2	$134 \pm 4 \# *$	$199 \pm 16 \# *$	$77 \pm 2 \#$	$23 \pm 3 \#$	$121 \pm 20 \# *$	$157 \pm 21 \#$
L2	$111 \pm 6 *$	$321 \pm 21 \# *$	$63 \pm 1 *$	$18 \pm 1 *$	$30 \pm 1 *$	$42 \pm 1 *$
T2 plus L2	$87 \pm 5 \#$	$142 \pm 7 \#$	$74 \pm 3 \#$	$26 \pm 4 \#$	$105 \pm 4 \#$	$151 \pm 6 \#$
T1	114 ± 3	$237 \pm 12 \#$	$70 \pm 4 \#$	16 ± 1	$91 \pm 5 \# *$	$124 \pm 5 \#$
L4	$75 \pm 9 \# *$	$298 \pm 29 \# *$	$68 \pm 2 \#$	18 ± 1	$32 \pm 1 *$	$47 \pm 2 *$
T1 plus L4	98 ± 5	$205 \pm 7 \#$	$73 \pm 3 \#$	18 ± 2	$88 \pm 6 \#$	$129 \pm 7 \#$
T1	114 ± 3	$237 \pm 12 \# *$	$70 \pm 4 \# *$	$16 \pm 1 *$	$91 \pm 5 \# *$	$124 \pm 5 \#$
L6	$73 \pm 5 \# *$	$266 \pm 8 \# *$	$68 \pm 2 \# *$	$18 \pm 1 *$	$29 \pm 1 *$	$42 \pm 2 *$
T1 plus L6	107 ± 7	$191 \pm 6 \#$	$77 \pm 1 \#$	$21 \pm 1 \#$	$80 \pm 3 \#$	$118 \pm 8 \#$

Tedisamil-lidocaine mixtures had no effect on BP compared to vehicle; however, compared to lidocaine, most combinations increased BP.

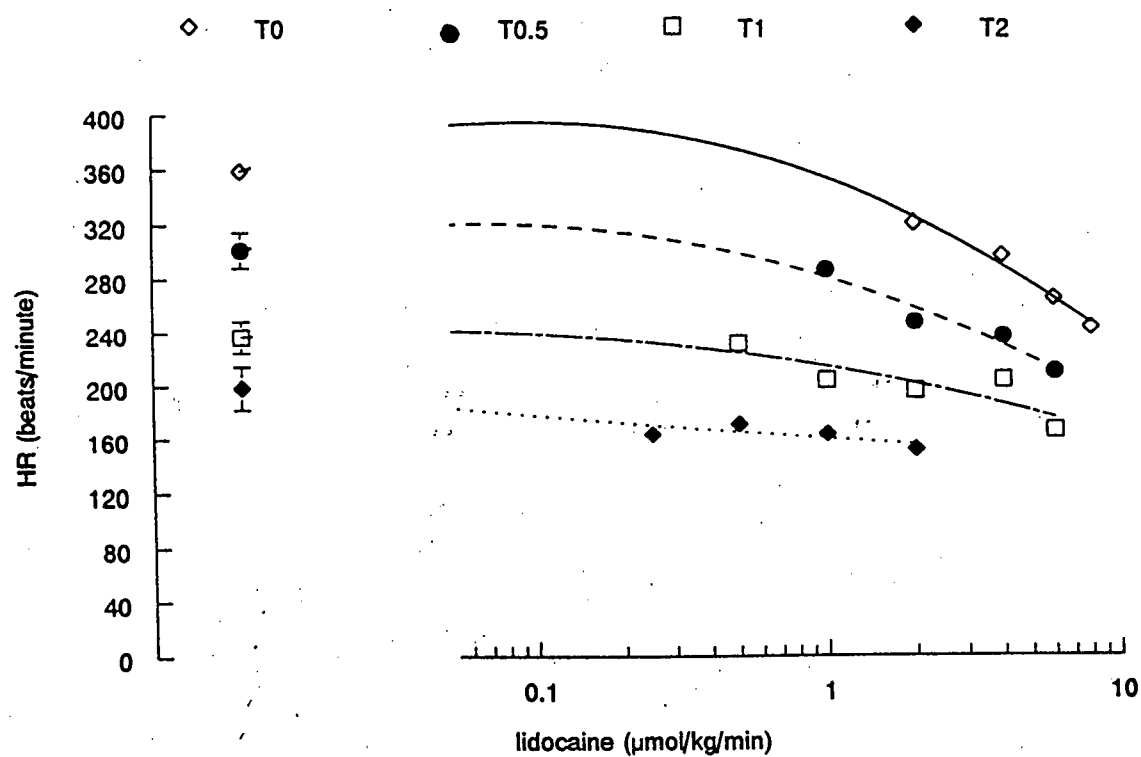
3.2.1.4.2 Effects on HR

All drug combinations produced a dose-dependent decrease in HR compared to vehicle and control (Table II). However, lidocaine plus 2 μ mol/kg/min tedisamil was the only combination to produce no change in HR compared to control. Lidocaine alone, and in the presence of varying doses of tedisamil (0.5 and 1 μ mol/kg/min), decreased HR with similar ED_{25} 's (\sim 5.7, 5, and 5.8 μ mol/kg/min, respectively) (Figure 5). However, lidocaine plus 2 μ mol/kg/min tedisamil failed to reduce HR. Thus, lidocaine's dose-dependent decrease in HR was only affected at the highest dose of tedisamil tested (2 μ mol/kg/min). Tedisamil alone and in the presence of 2, 4, and 6 μ mol/kg/min lidocaine, decreased HR with an ED_{25} of about 0.7, 1, 0.7, and 0.8 μ mol/kg/min, respectively. Since the potencies are similar, the bradycardia produced by tedisamil alone is not further increased with increasing doses of lidocaine. This is despite the fact that lidocaine itself has bradycardiac actions.

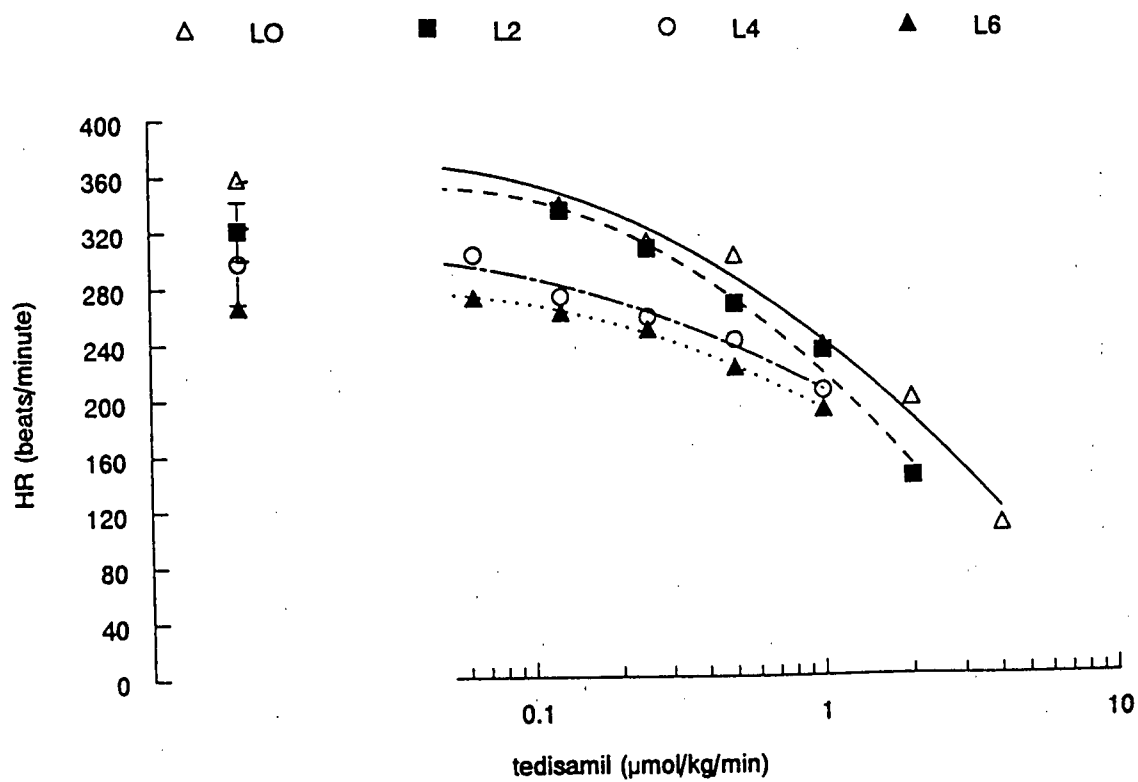
Overall, all the highest combined doses of tedisamil and lidocaine significantly decreased HR compared to vehicle and either drug alone (Table III). The only exception was for 1 μ mol/kg/min tedisamil plus 4 μ mol/kg/min lidocaine: this mixture decreased HR compared to vehicle and 4 μ mol/kg/min lidocaine only.

Figure 5 HR dose-response curves for lidocaine (L) alone, tedisamil (T) alone, or combinations of tedisamil and lidocaine in pentobarbitone-anaesthetized rats. A, HR changes after administration of lidocaine alone (\emptyset), or lidocaine in the presence of tedisamil at 0.5 (\bullet), 1 (\square), and 2 (\blacklozenge) $\mu\text{mol/kg/min}$. B, HR changes after administration of tedisamil alone (Δ), or tedisamil plus lidocaine at 2 (\blacksquare), 4 (\circ), and 6 (\blacktriangle) $\mu\text{mol/kg/min}$. The symbols not on the x-axis correspond to the experimental control of the constant dose in the mixture. For drugs administered alone, the control is vehicle treated rats. All data points are $n=5$ at each dose except for the vehicle ($n=85$). Error bars are only shown for control values (mean \pm SEM) and not for any other points for clarity. Curves are graphical representation of data in Table II in order to determine ED_{25}s (25% change in HR compared to control).

A



B



3.2.1.4.3 Effects on PR interval

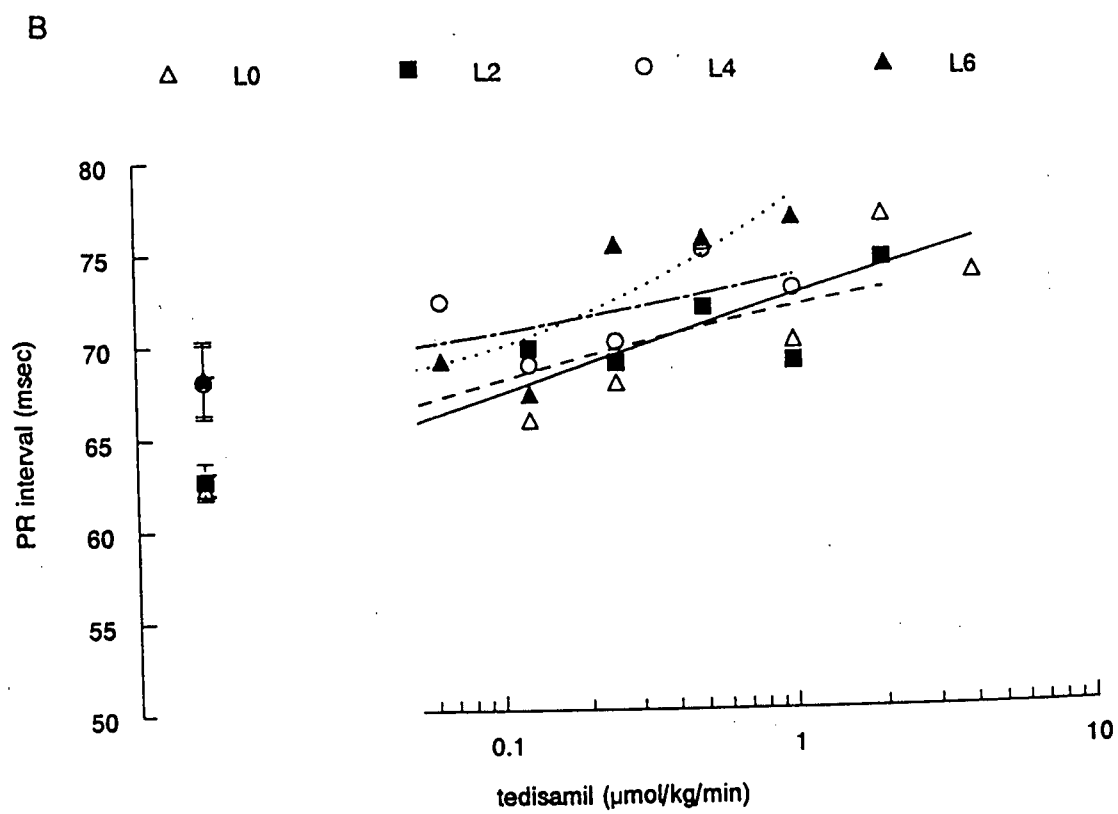
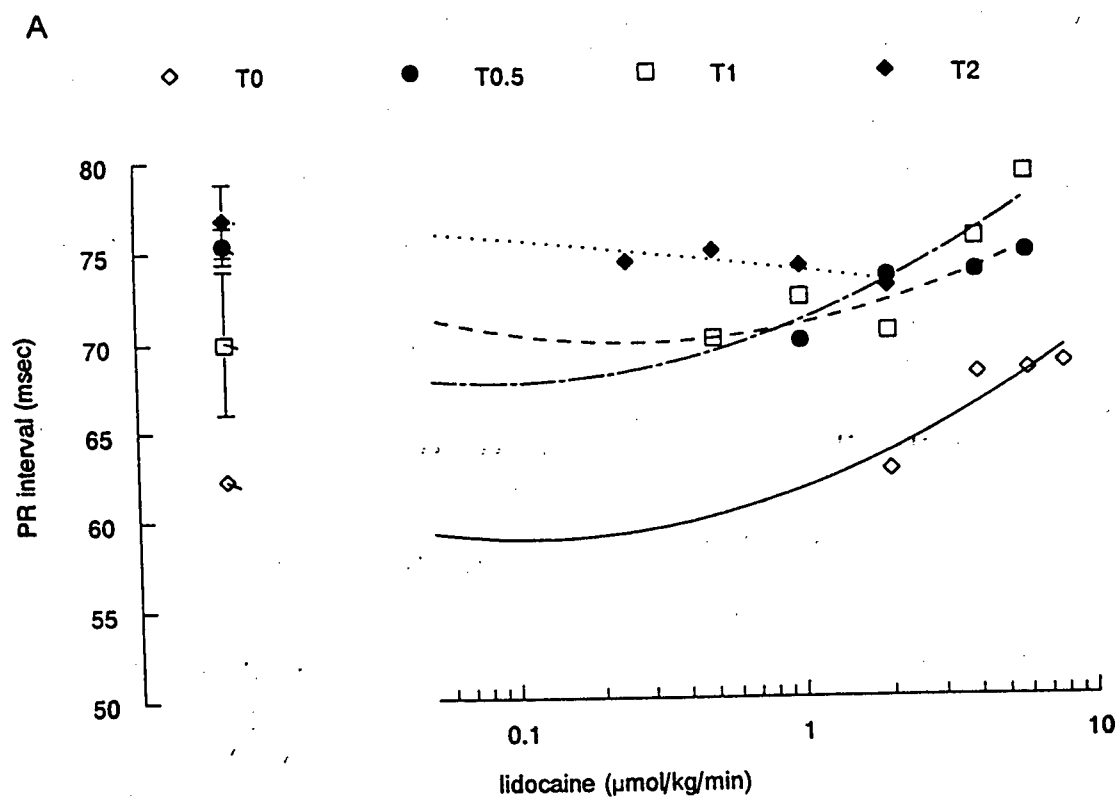
Infusion of tedisamil and lidocaine alone or in combination produced an increase in PR interval compared to vehicle (Tables I and II). However, drugs administered either alone or in combination did not produce a 25% change compared to control. Thus, the co-administration of tedisamil (0.5, 1, and 2 μ mol/kg/min) did not change the effect of lidocaine on PR. Similarly, the co-administration of lidocaine (2, 4, and 6 μ mol/kg/min) did not change the effect of tedisamil on PR (Figure 6).

All the highest doses of tedisamil and lidocaine alone and in combination significantly increased PR compared to vehicle (Table III). One μ mol/kg/min tedisamil plus 6 μ mol/kg/min lidocaine was the only combination to significantly increase PR compared to either drug alone.

3.2.1.4.4 Effects on QRS duration

Lidocaine alone had no effect on QRS duration compared to vehicle (Table I). However, tedisamil alone produced an increase in QRS duration. In the presence of tedisamil (0.5, 1 and 2 μ mol/kg/min), lidocaine dose-dependently increased QRS duration (Table II). As well, in the presence of lidocaine (2, 4, and 6 μ mol/kg/min), tedisamil dose-dependently increased QRS duration. Thus, combination of tedisamil and lidocaine increased QRS, even though lidocaine alone produced no effect.

Figure 6 PR dose-response curves for lidocaine (L) alone, tedisamil (T) alone, or combinations of tedisamil and lidocaine in pentobarbitone-anaesthetized rats. A, PR changes after administration of lidocaine alone (\emptyset), or lidocaine in the presence of tedisamil at 0.5 (\bullet), 1 (\square), and 2 (\blacklozenge) $\mu\text{mol/kg/min}$. B, PR changes after administration of tedisamil alone (Δ), or tedisamil plus lidocaine at 2 (\blacksquare), 4 (\circ), and 6 (\blacktriangle) $\mu\text{mol/kg/min}$. The symbols not on the x-axis correspond to the experimental control of the constant dose in the mixture. For drugs administered alone, the control was vehicle treated rats. All data points are $n=5$ at each dose except for the vehicle ($n=85$). Error bars are only shown for control values (mean \pm SEM) and not for any other points for clarity. Curves are graphical representation of data in Table II in order to determine ED_{25}s (25% change in PR compared to control).



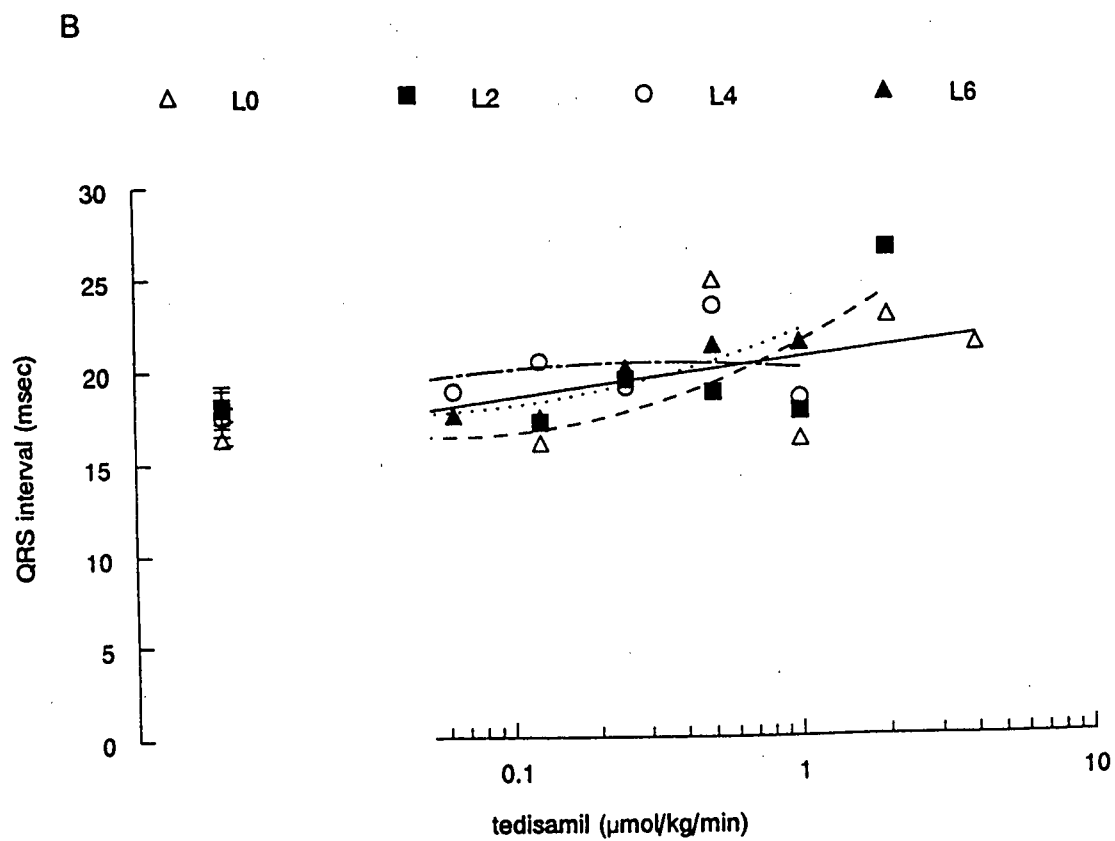
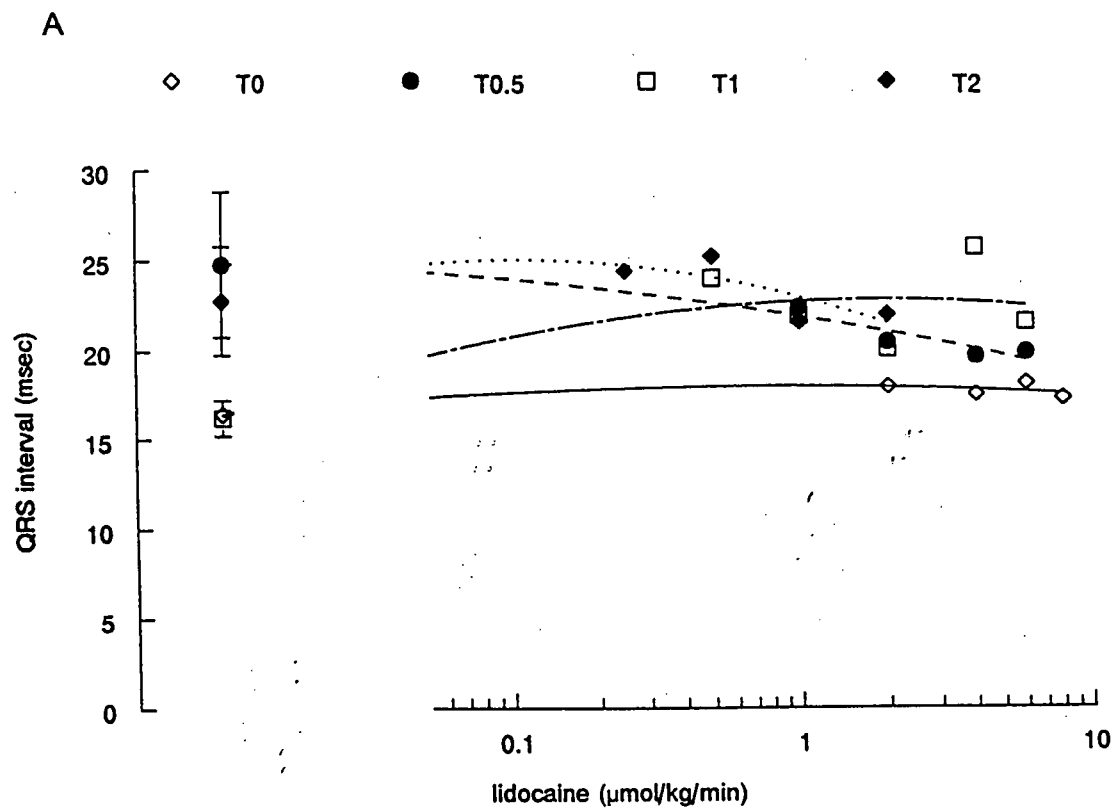
Compared to control, none of the drug combinations produced a change in QRS duration, except for tedisamil at 1 $\mu\text{mol/kg/min}$ plus lidocaine at 4 $\mu\text{mol/kg/min}$ (Table II). Thus, no ED_{25} was determined for either lidocaine alone or in combination with 0.5, 1, and 2 $\mu\text{mol/kg/min}$ tedisamil (Figure 7). In contrast, tedisamil alone increased the QRS duration with an ED_{25} of about 0.5 $\mu\text{mol/kg/min}$. In the presence of increasing doses of lidocaine (2, 4, and 6 $\mu\text{mol/kg/min}$), the potency of tedisamil to prolong QRS duration was increased ($\text{ED}_{25} \sim 0.5$ and 1.6, for tedisamil alone, and tedisamil plus 2 $\mu\text{mol/kg/min}$ lidocaine, respectively). The co-administration of higher doses of lidocaine (4 or 6 $\mu\text{mol/kg/min}$ lidocaine), prevented tedisamil's QRS widening effect. Thus, in the presence of lidocaine, the QRS widening produced by tedisamil alone was reduced.

The highest combined doses of tedisamil and lidocaine, produced an increase in QRS duration compared to vehicle (except for 1 $\mu\text{mol/kg/min}$ tedisamil plus 4 $\mu\text{mol/kg/min}$ tedisamil) (Table III). The only combination to increase QRS duration compared to either drug alone was 1 $\mu\text{mol/kg/min}$ tedisamil plus 6 $\mu\text{mol/kg/min}$ lidocaine.

3.2.1.4.5 Effects on QT interval

The drugs administered either alone or in combination produced similar effects on QT_1 and QT_2 intervals (Figures 8 and 9). As a result of similar drug effects, QT_1 and QT_2 intervals were not differentiated, and therefore referred to

Figure 7 QRS dose-response curves for lidocaine (L) alone, tedisamil (T) alone, or combinations of tedisamil and lidocaine in pentobarbitone-anaesthetized rats. A, QRS changes after administration of lidocaine alone (\emptyset), or lidocaine in the presence of tedisamil at 0.5 (\bullet), 1 (\square), and 2 (\blacklozenge) $\mu\text{mol/kg/min}$. B, QRS changes after administration of tedisamil alone (Δ), or tedisamil plus lidocaine at 2 (\blacksquare), 4 (\circ), and 6 (\blacktriangle) $\mu\text{mol/kg/min}$. The symbols not on the x-axis correspond to the experimental control of the constant dose in the mixture. For drugs administered alone, the control was vehicle treated rats. All data points are $n=5$ at each dose except for the vehicle ($n=85$). Error bars are only shown for control values (mean \pm SEM) and not for any other points for clarity. Curves are graphical representation of data in Table II in order to determine ED_{25} s (25% change in QRS compared to control).



as the QT interval. Compared to vehicle, lidocaine alone, tedisamil alone, and tedisamil-lidocaine combinations produced a dose-dependent increase in QT interval (Tables I and II). The dose-dependent lack of effect of lidocaine was not changed when co-infused with varying doses of tedisamil (0.5, 1, and 2 μ mol/kg/min). Consequently, neither lidocaine alone nor lidocaine plus varying doses of tedisamil allowed estimation of ED_{25} (Figures 8 and 9). Similarly, the dose-dependent QT interval effect of tedisamil alone was not changed when co-infused with varying doses of lidocaine (2, 4, and 6 μ mol/kg/min). Since lidocaine produced no effect on QT interval, the ED_{25} for tedisamil was not affected when combined with varying doses of lidocaine. However, compared to lidocaine alone, tedisamil dose-dependently increased QT in the presence of lidocaine.

Table III summarizes the QT effects of the highest combined doses of tedisamil and lidocaine. All mixtures and tedisamil alone significantly increased QT interval compared to vehicle; however, lidocaine alone produced no effect. For all the mixtures, the QT interval was increased compared to lidocaine alone.

3.2.2 Antiarrhythmic effects of drugs administered alone and in combination

3.2.2.1 Overview

The arrhythmia scores, occluded zone sizes, and post-occlusion serum potassium concentrations were not statistically different among the vehicle treated rats in the 14 random and double blind experiments. As a result, all

Figure 8 QT₁ dose-response curves for lidocaine (L) alone, tedisamil (T) alone, or combinations of tedisamil and lidocaine in pentobarbitone-anaesthetized rats. A, QT₁ changes after administration of lidocaine alone (∅), or lidocaine in the presence of tedisamil at 0.5 (●), 1 (□), and 2 (◆) μmol/kg/min. B, QT₁ changes after administration of tedisamil alone (Δ), or tedisamil plus lidocaine at 2 (■), 4 (○), and 6 (▲) μmol/kg/min. The symbols not on the x-axis correspond to the experimental control of the constant dose in the mixture. For drugs administered alone, the control was vehicle treated rats. All data points are n=5 at each dose except for the vehicle (n=85). Error bars are only shown for control values (mean ± SEM) and not for any other points for clarity. Curves are graphical representation of data in Table II in order to determine ED₂₅s (25% change in QT₁ compared to control).

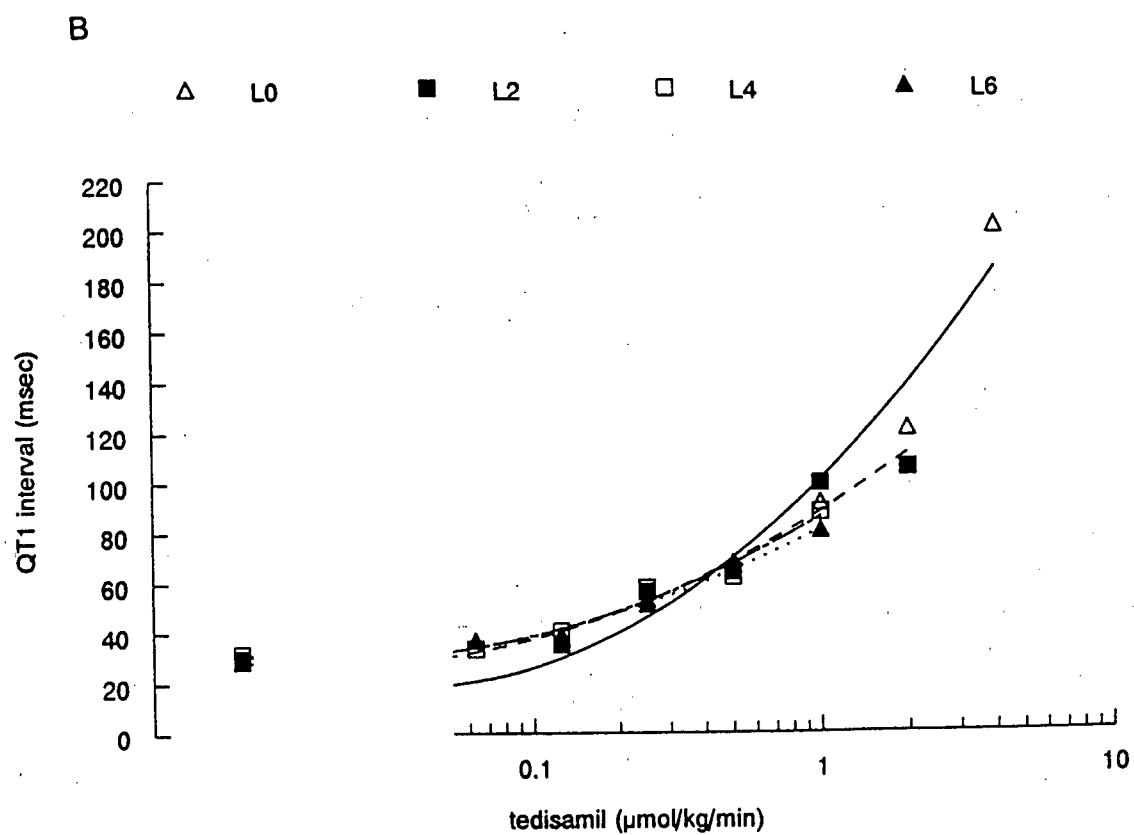
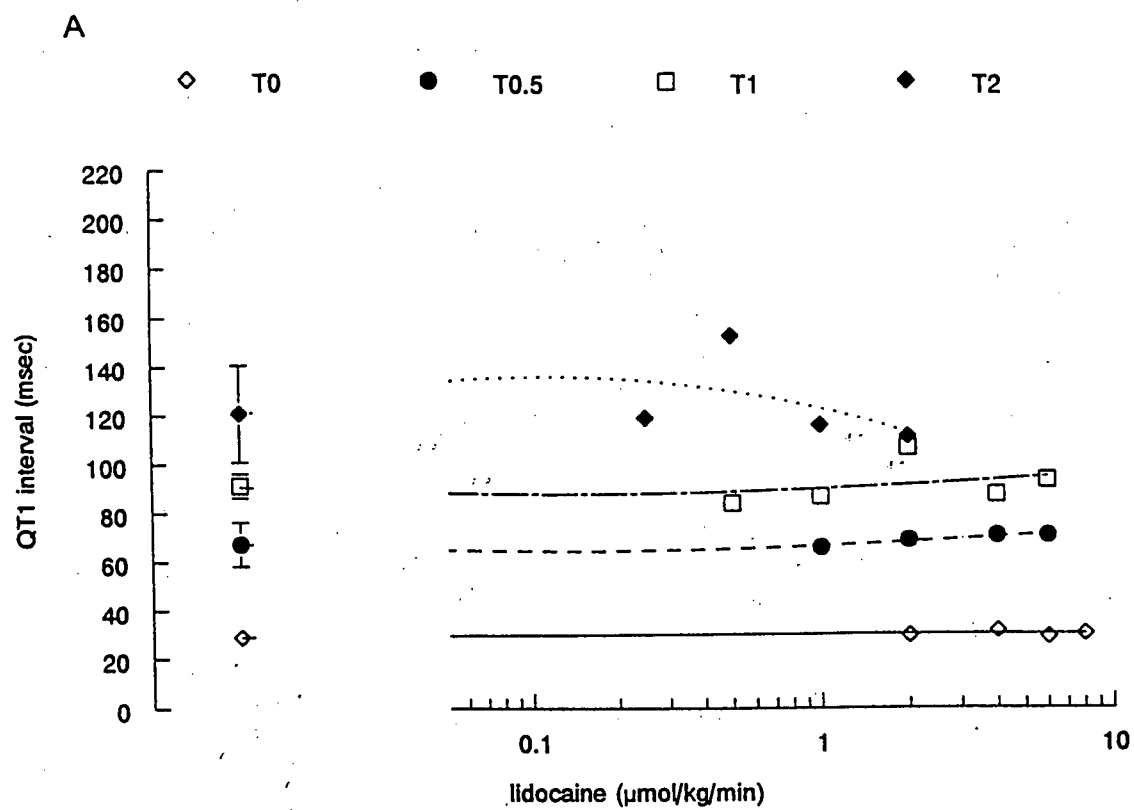
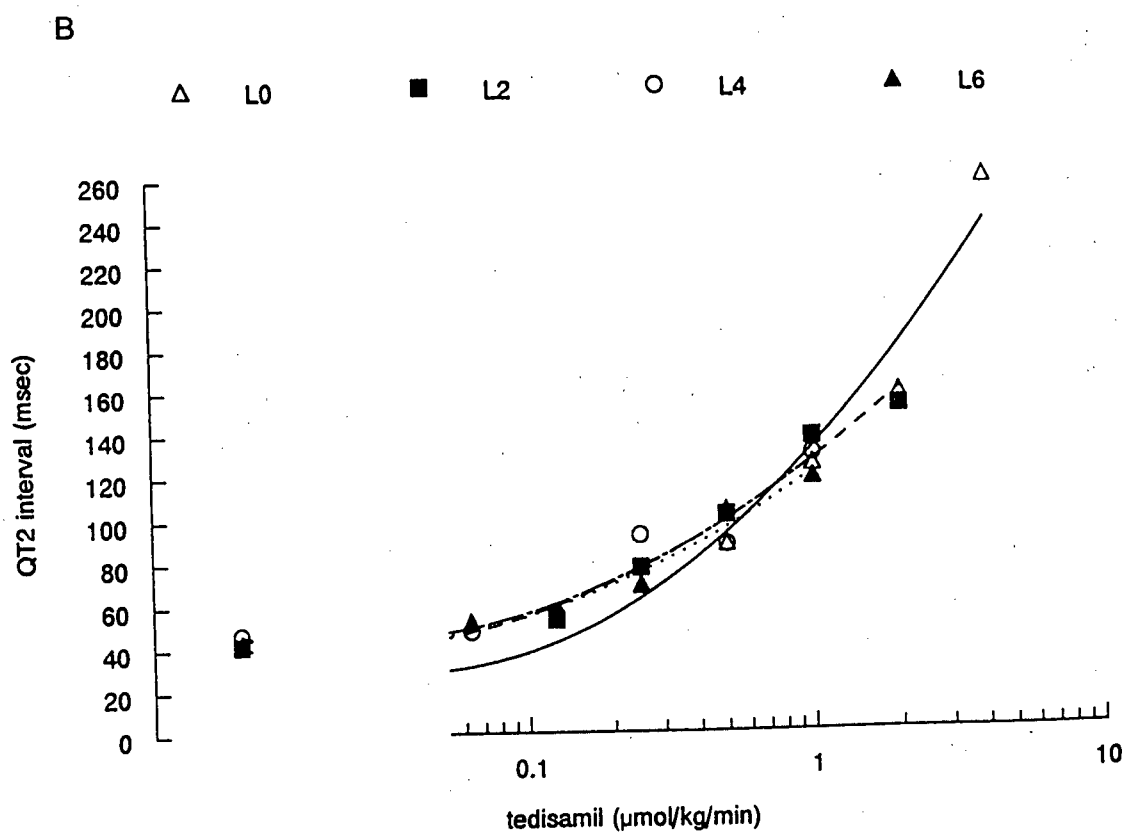
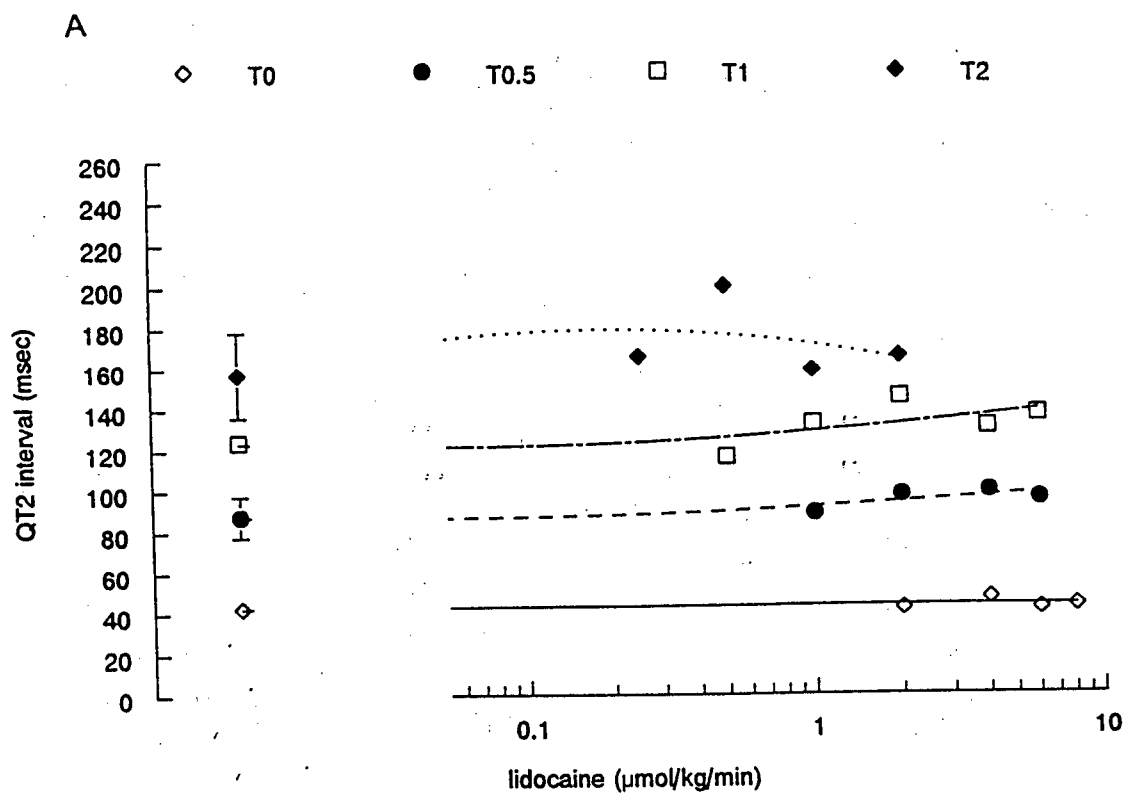


Figure 9 QT₂ dose-response curves for lidocaine (L) alone, tedisamil (T) alone, or combinations of tedisamil and lidocaine in pentobarbitone-anaesthetized rats. A, QT₂ changes after administration of lidocaine alone (♢), or lidocaine in the presence of tedisamil at 0.5 (●), 1 (□), and 2 (◆) μmol/kg/min. B, QT₂ changes after administration of tedisamil alone (Δ), or tedisamil plus lidocaine at 2 (■), 4 (○), and 6 (▲) μmol/kg/min. The symbols not on the x-axis correspond to the experimental control of the constant dose in the mixture. For drugs administered alone, the control was vehicle treated rats. All data points are n=5 at each dose except for the vehicle (n=85). Error bars are only shown for control values (mean ± SEM) and not for any other points for clarity. Curves are graphical representation of data in Table II in order to determine ED₂₅s (25% change in QT₂ compared to control).



vehicle treated (n=85) were grouped together for comparison with drug treated rats (n=5 at each dose).

Neither variability in occluded zone sizes, nor drug effects on post-occlusion potassium concentration played a role in decreasing arrhythmia occurrences. This is because no statistically significant difference was found between the occluded zone size for vehicle treated and drug treated rats (mean \pm SEM: $37 \pm 0.5 \%$ and $37 \pm 0.4 \%$, respectively). Also, the post-occlusion serum potassium was not significantly different between vehicle treated and drug treated rats (mean \pm SEM: 3.7 ± 0.1 mM and 3.5 ± 0.08 mM, respectively). Thus, in all experimental groups, rats received the same arrhythmic insult (occluded zone size) and drugs had no effect on serum potassium concentration. This suggests that any suppression of arrhythmias was due to drug effect.

The effects of tedisamil alone, lidocaine alone and their combinations on arrhythmia score, VT and VF incidences are shown in Tables IV and V. These data were then used to summarize the effects of the highest combined doses of lidocaine in the presence of 0.5, 1, and 2 $\mu\text{mol/kg/min}$ tedisamil and tedisamil in the presence of 2, 4, and 6 $\mu\text{mol/kg/min}$ lidocaine, in order to determine whether drugs administered as a mixture significantly differed from either drug alone (Table VI). The effect of drug combinations on the normalized antiarrhythmic dose-response curves for tedisamil alone and lidocaine alone were investigated (Figures 10 to 18). Finally, ED_{50} isobolograms were used to study the interaction between tedisamil and lidocaine (Figure 19).

Table IV. The effect of tedisamil and lidocaine on AS and incidence of VT or VF.

Ted	Lido	AS	VT incidence	VF incidence
0	0	5.8 ± 0.2	82/85	68/85
0	2	5.6 ± 0.6	5/5	4/5
0	4	4.0 ± 1.3 #	4/5	2/5
0	6	1.6 ± 0.8 #	2/5 #	1/5 #
0	8	1.2 ± 0.5 #	1/5 #	0/5 #
0.125	0	5.6 ± 0.6	5/5	5/5
0.25	0	4.4 ± 0.6	5/5	4/5
0.5	0	4.6 ± 0.4	5/5	4/5
1	0	4.0 ± 0.4	5/5	4/5
2	0	3.6 ± 0.4 #	5/5	2/5
4	0	2.4 ± 1.3 #	3/5 #	0/5 #

The antiarrhythmic effects of tedisamil (Ted) and lidocaine (Lido) alone in pentobarbitone-anaesthetized rats. At each dose ($\mu\text{mol/kg/min}$), $n=5$, except for the vehicle treated rats where $n=85$. AS values are mean \pm SEM. Antiarrhythmic normalized dose response curves using the AS data are shown in Figures 8 and 9 for lidocaine and tedisamil, respectively. The number of animals with VT or VF incidences are shown as a fraction of total sample number. The symbol # denotes a statistically significant difference ($p<0.05$) from vehicle.

3.2.2.2 Lidocaine alone

Lidocaine dose-dependently decreased the arrhythmia score compared to vehicle (Table IV and Figure 10). The highest dose of lidocaine (8 $\mu\text{mol/kg/min}$) produced about 83% protection against arrhythmias compared to vehicle (AS: 1.2 ± 0.5 for lidocaine versus 5.8 ± 0.2 for vehicle).

VT and VF incidences were also decreased dose-dependently by lidocaine (Table IV). Eight $\mu\text{mol/kg/min}$ lidocaine decreased VT incidence to 20% from a control of 96% (82/85). In addition, this dose of lidocaine completely abolished VF.

3.2.2.3 Tedisamil alone

Tedisamil dose-dependently decreased the arrhythmia score compared to vehicle (Table IV and Figure 11). The highest dose of tedisamil (4 $\mu\text{mol/kg/min}$) produced only about 57% protection against arrhythmias compared to vehicle (AS: 2.4 ± 1.3 for tedisamil versus 5.8 ± 0.2 for vehicle).

Compared to vehicle, tedisamil produced no protection against VT (100%; 5/5), except for the highest dose tested (60%; 3/5). At this dose (4 $\mu\text{mol/kg/min}$), tedisamil completely abolished VF.

3.2.2.4 Combinations of lidocaine and tedisamil

Figure 10 Normalized antiarrhythmic dose-response curves for lidocaine. Individual rat data (**+**) and mean values (**+**) are shown for each dose (n=5). Mean vehicle control (n=85) lie to the left \pm its 95% confidence interval for SEM and SD shown in bold and non-bold, respectively. Insertion is the normalized dose-response curve for percent protection against arrhythmias with increasing doses of lidocaine. Data were curve fitted as described in Methods.

Normalized antiarrhythmic dose-response curve for lidocaine

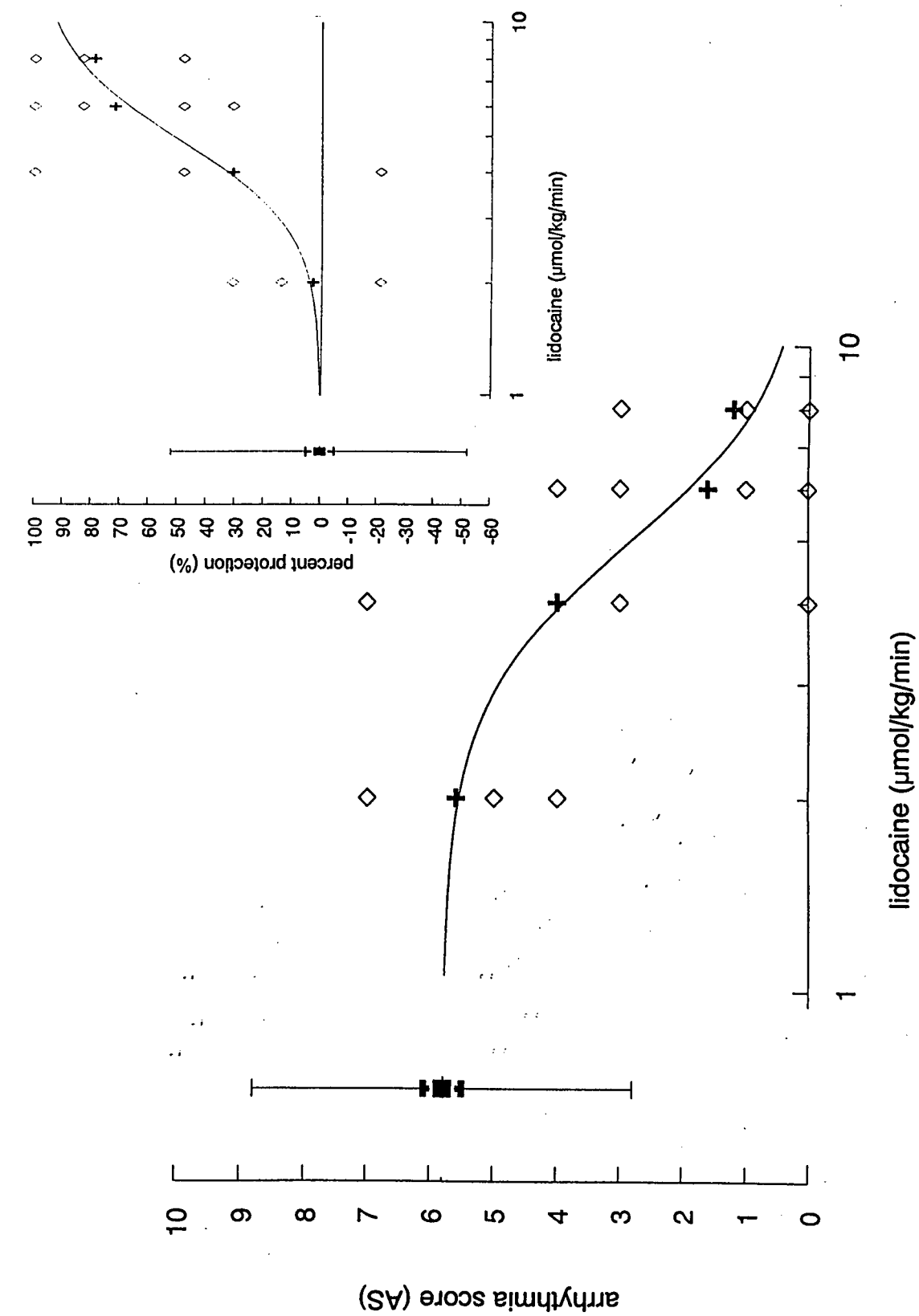
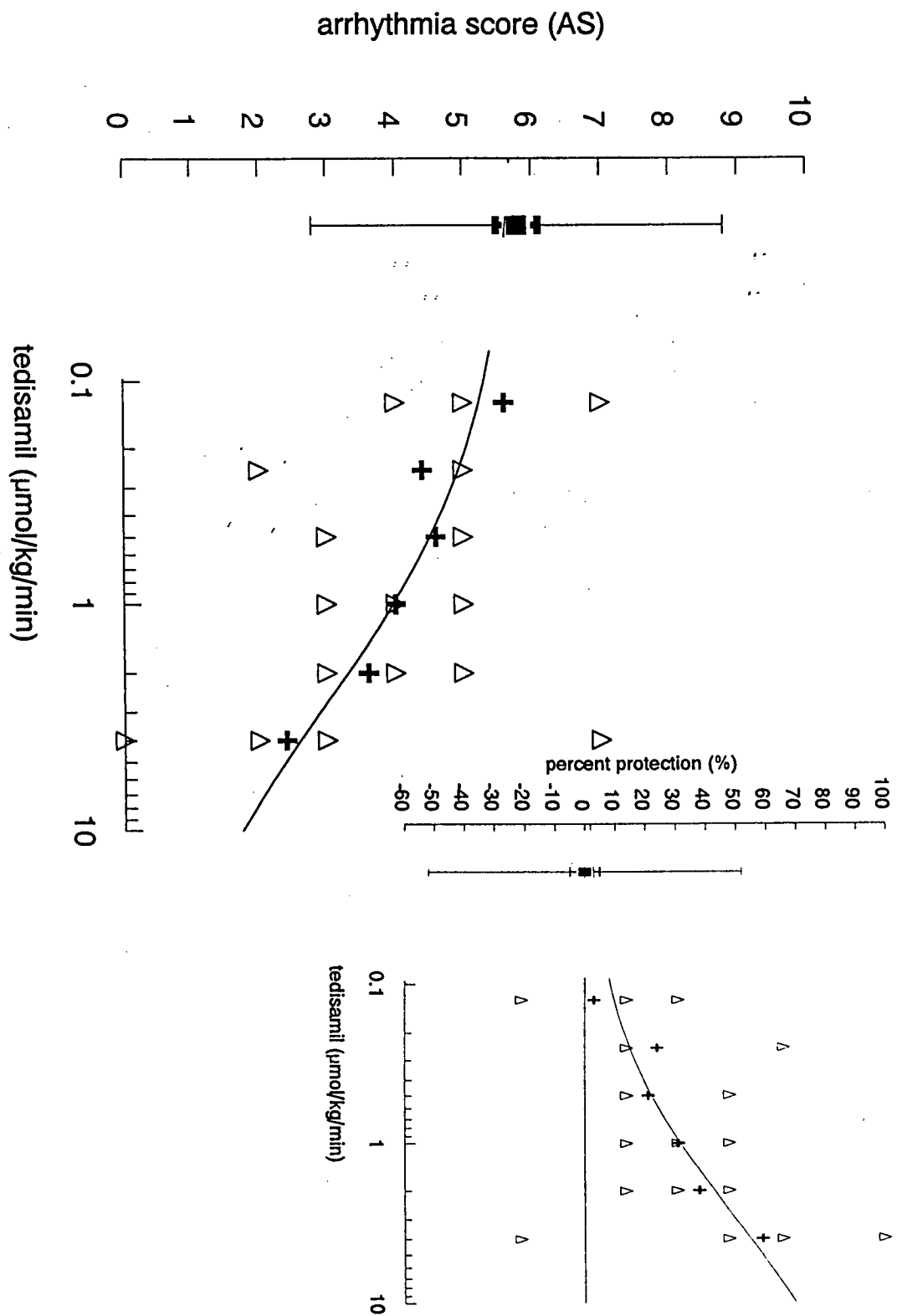


Figure 11 Normalized antiarrhythmic dose-response curves for tedisamil. Individual rat data (Δ) and mean values (+) are shown for each dose (n=5). Mean vehicle control (n=85) lie to the left \pm its 95% confidence interval for SEM and SD shown in bold and non-bold, respectively. Insertion is the normalized dose-response curve for percent protection against arrhythmias with increasing doses of tedisamil. Data were curve fitted as described in Methods.

Normalized antiarrhythmic dose-response curve for tedisamil



3.2.2.4.1 Effects on arrhythmia score

Lidocaine alone and tedisamil alone dose-dependently decreased the arrhythmia score compared to vehicle (Table IV). However, neither lidocaine nor tedisamil produced a 100% protection against ischaemia-induced arrhythmias at the highest doses tested. Thus, various mixtures of the two compounds were studied to determine whether antiarrhythmic efficacy and potency were enhanced compared to either drug alone.

In the presence of increasing doses of tedisamil (0.5, 1, and 2 μ mol/kg/min), lidocaine dose-dependently decreased arrhythmia score compared to both vehicle and control (Table V and Figures 11 to 15). With co-administration of varying doses of lidocaine (2, 4, and 6 μ mol/kg/min), tedisamil dose-dependently decreased the arrhythmia score compared to vehicle (Table V and Figures 16 to 18). However, the only combination to produce a significant decrease in arrhythmia score compared to lidocaine alone was the 2 μ mol/kg/min tedisamil plus 2 μ mol/kg/min lidocaine mixture (Table V and Figure 15). The normalized dose-response curves for tedisamil in the presence of 4 and 6 μ mol/kg/min graphically demonstrates that the mean arrhythmia scores at each dose lay within the 95% confidence interval for the control (Figures 16 and 17). Hence, increasing doses of tedisamil at the latter two constant doses of lidocaine had no statistically significant effect on the arrhythmia score.

Figure 18 summarizes the normalized antiarrhythmic dose-response curves for tedisamil alone, lidocaine alone, and their combinations. In the

Table V The effect of various combinations of tedisamil (Ted) and lidocaine (Lido) on AS and incidence of VT or VF. For all drug combinations, n=5 anaesthetized rats at each dose ($\mu\text{mol/kg/min}$). For the vehicle treated rats in the absence of both tedisamil and lidocaine, n=85. AS values are mean \pm SEM. Antiarrhythmic normalized dose-response curves for the AS data are shown in Figures 12 to 17. The number of animals in which VT or VF occurred are shown as a fraction of total sample size. The symbol # denotes a difference from vehicle treated rats. The symbol * denotes a difference from the experimental control (ie, the constant dose in the mixture). Statistical significance was detected at $p < 0.05$.

Ted	Lido	AS	VT incidence	VF incidence
0	0	5.8 ± 0.2	82/85	68/85
0.5	0	4.6 ± 0.4	5/5	4/5
0.5	1	$4.0 \pm 0.3 \#$	5/5	5/5
0.5	2	$3.8 \pm 0.2 \#$	5/5	1/5 #
0.5	4	$2.8 \pm 0.7 \# *$	4/5	1/5 #
0.5	6	$0.2 \pm 0.2 \# *$	0/5 # *	0/5 # *
1	0	4.0 ± 0.4	5/5	4/5
1	0.5	$4.2 \pm 0.2 \#$	5/5	4/5
1	1	$3.2 \pm 0.6 \#$	4/5	3/5
1	2	$2.8 \pm 0.2 \#$	5/5	2/5
1	4	$2.2 \pm 0.6 \#$	4/5	1/5 #
1	6	$0.6 \pm 0.6 \# *$	1/5 # *	0/5 # *
2	0	$3.6 \pm 0.4 \#$	5/5	2/5
2	0.25	$2.6 \pm 0.2 \#$	5/5	0/5 #
2	0.5	$2.4 \pm 0.6 \#$	4/5	0/5 #
2	1	$1.0 \pm 0.6 \# *$	2/5 #	1/5 #
2	2	$0.8 \pm 0.6 \# *$	1/5 # *	0/5 #
0	2	5.6 ± 0.6	5/5	4/5
0.125	2	$4.4 \pm 0.7 \#$	5/5	5/5
0.25	2	$4.2 \pm 0.4 \#$	5/5	4/5
0.5	2	$3.8 \pm 0.9 \#$	5/5	4/5
1	2	$3.2 \pm 0.7 \#$	4/5	3/5
2	2	$0.2 \pm 0.2 \# *$	0/5 # *	0/5 # *
0	4	$4.0 \pm 1.3 \#$	4/5	2/5
0.063	4	$3.8 \pm 0.8 \#$	4/5	2/5
0.125	4	$2.8 \pm 0.8 \#$	4/5	1/5 #
0.25	4	$2.6 \pm 0.7 \#$	4/5	2/5
0.5	4	$2.0 \pm 0.6 \#$	3/5 #	1/5 #
1	4	$0.6 \pm 0.6 \#$	1/5 #	1/5 #
0	6	$1.6 \pm 0.8 \#$	2/5 #	1/5 #
0.063	6	$2.4 \pm 0.9 \#$	3/5 #	0/5 #
0.125	6	$2.4 \pm 0.6 \#$	3/5 #	0/5 #
0.25	6	$2.6 \pm 0.2 \#$	5/5 #	0/5 #
0.5	6	$0.6 \pm 0.6 \#$	1/5 #	0/5 #
1	6	$0.6 \pm 0.4 \#$	1/5 #	0/5 #

presence of varying doses of tedisamil (0.5, 1, and 2 $\mu\text{mol/kg/min}$), lidocaine's normalized antiarrhythmic dose-response curve was shifted to the left. Similarly, in the presence of varying doses of lidocaine (2, 4, and 6 $\mu\text{mol/kg/min}$), tedisamil's normalized antiarrhythmic dose-response curve was shifted to the left. As shown in Table VII, lidocaine's antiarrhythmic potency was significantly increased by about 7 times when co-infused with 2 $\mu\text{mol/kg/min}$ tedisamil. Similarly, tedisamil's antiarrhythmic potency was greatly (but insignificantly) increased by about 7.5 times when co-infused with 4 $\mu\text{mol/kg/min}$ lidocaine. The antiarrhythmic ED_{50} of tedisamil is compared to tedisamil plus 4 instead of 6 $\mu\text{mol/kg/min}$ lidocaine because this latter combination produced a normalized antiarrhythmic dose-response curve with a very steep slope and an ED_{50} with a very large error. Due to the large error associated with tedisamil plus 6 $\mu\text{mol/kg/min}$ lidocaine, the ED_{50} is probably unreliable. Overall, the increase in potency observed for combinations compared to drugs given alone suggests that tedisamil and lidocaine can interact synergistically.

Table VI shows the effects of the highest combined doses on the arrhythmia score. All combinations significantly decreased the arrhythmia score compared to vehicle. Also, the combinations decreased the arrhythmia score compared to at least one of either tedisamil or lidocaine alone administered at the same dose as that present in the combined doses (Table VI). One of the best mixtures was 2 $\mu\text{mol/kg/min}$ tedisamil plus 2 $\mu\text{mol/kg/min}$ lidocaine. This is because at this mixture dose, the co-infusion of tedisamil with lidocaine significantly decreased the arrhythmia score compared to each drug alone and

vehicle. Interestingly, 2 $\mu\text{mol/kg/min}$ lidocaine alone had no effect on the arrhythmia score, and 2 $\mu\text{mol/kg/min}$ tedisamil alone produced a small, but significant decrease in arrhythmia score. Thus, based on arrhythmia score data, the antiarrhythmic efficacy of combinations was increased compared to either tedisamil or lidocaine alone.

3.2.2.4.2 Effects on VT incidence

Lidocaine alone dose-dependently decreased VT incidence compared to vehicle (Table IV). However, tedisamil decreased VT incidence only at the highest dose (4 $\mu\text{mol/kg/min}$). In the presence of varying doses of tedisamil (0.5, 1, and 2 $\mu\text{mol/kg/min}$), only the highest doses of lidocaine significantly decreased VT incidence compared to vehicle and control (Table V). In the presence of 2 and 4 $\mu\text{mol/kg/min}$ lidocaine, only the highest doses of tedisamil significantly decreased VT incidence compared to vehicle. Note that all doses of tedisamil combined with 6 $\mu\text{mol/kg/min}$ lidocaine decreased VT incidence compared to vehicle. In the presence of varying doses of lidocaine, 2 $\mu\text{mol/kg/min}$ tedisamil plus 2 $\mu\text{mol/kg/min}$ lidocaine was the only mixture to produce a decrease in VT compared to control.

As shown in Table VI, all highest combined doses of tedisamil and lidocaine decreased VT incidence (0/5 or 1/5) compared to vehicle (82/85). Also, the combinations decreased the VT incidence compared to at least one of either tedisamil or lidocaine alone, administered at the same dose as that present in the

combined doses (Table VI). The best combination was 2 $\mu\text{mol/kg/min}$ tedisamil plus 2 $\mu\text{mol/kg/min}$ lidocaine. This is because neither tedisamil alone (5/5) nor lidocaine alone (5/5) produced an effect on VT incidence compared to vehicle (82/85); however, their combination significantly decreased VT incidence (0/5 or 1/5) compared to each drug alone. Thus, when co-infused, tedisamil and lidocaine can act synergistically to decrease VT incidence compared to vehicle and each drug alone.

3.2.2.4.3 Effects on VF incidence

Lidocaine alone dose-dependently decreased VF incidence compared to vehicle (Table IV). However, tedisamil alone completely abolished VF incidence only at the highest dose tested (4 $\mu\text{mol/kg/min}$). In the presence of a constant dose of tedisamil (0.5, 1 and 2 $\mu\text{mol/kg/min}$), lidocaine dose-dependently decreased VF incidence compared to vehicle (Table V). Only two combinations, 0.5 $\mu\text{mol/kg/min}$ tedisamil plus 6 $\mu\text{mol/kg/min}$ lidocaine and 1 $\mu\text{mol/kg/min}$ tedisamil plus 6 $\mu\text{mol/kg/min}$ lidocaine, produced a significant decrease in VF incidence compared to their respective controls (ie, doses of the drug alone). In the presence of a constant dose of lidocaine (2, 4, and 6 $\mu\text{mol/kg/min}$), tedisamil decreased VF incidence dose-dependently compared to vehicle. Two $\mu\text{mol/kg/min}$ tedisamil plus 2 $\mu\text{mol/kg/min}$ lidocaine was the only combination to completely abolish VF compared to 2 $\mu\text{mol/kg/min}$ lidocaine alone.

As shown in Table VI, all the highest combined doses completely abolished VF (0/5), except for 1 $\mu\text{mol/kg/min}$ tedisamil plus 4 $\mu\text{mol/kg/min}$ lidocaine which still did produce a significant decrease in VF incidence (1/5) compared to vehicle (68/85). However, the best combination was 2 $\mu\text{mol/kg/min}$ tedisamil plus 2 $\mu\text{mol/kg/min}$ lidocaine. This is because neither tedisamil alone (2/5) nor lidocaine alone (4/5) decreased VF incidence significantly compared to vehicle (68/85); however, their combination completely prevented VF. Thus, co-administration of tedisamil and lidocaine can act synergistically to decrease VF incidence.

3.2.2.5 Isobolograms

The antiarrhythmic ED_{50} s for drugs administered alone and in combination were used to construct an ED_{50} isobologram (see section 3.2.2.4.1 and Table VII). The ED_{50} isobole for tedisamil and lidocaine mixtures lies below the additivity line (Figure 19). Thus, tedisamil and lidocaine can act synergistically to increase the antiarrhythmic potency compared to either drug alone. Note that the isobole for the mixtures does not lie significantly outside the confidence interval for the additivity line. However, as discussed in the methods section, the statistics for using isobolograms to study drug interactions have not yet been established. Thus, the possibility that tedisamil and lidocaine act synergistically cannot be excluded.

Interestingly, the only two ED_{50} s which lie furthest below the additivity line were tedisamil in the presence of 2 $\mu\text{mol/kg/min}$ lidocaine and lidocaine in the presence of 2 $\mu\text{mol/kg/min}$ tedisamil. As discussed earlier, 2 $\mu\text{mol/kg/min}$ tedisamil plus 2 $\mu\text{mol/kg/min}$ lidocaine (the highest combination doses; Table VI) was the best mixtures studied. This combination synergistically reduced both the arrhythmia score and VT incidence, and completely abolish VF.

The ED_{50} for tedisamil plus 6 $\mu\text{mol/kg/min}$ lidocaine lies above the additivity line. Thus, this high dose of lidocaine in combination with tedisamil suggests a possible antagonistic effect between the two drugs. However, as mentioned earlier, this combination produced a steep normalized antiarrhythmic dose-response curve and an ED_{50} with a very large error. Hence, this mixture's ED_{50} is probably unreliable.

Table VI AS and incidence of VT or VF for the highest combination doses of lidocaine (L) in the presence of constant tedisamil (Part A) and tedisamil (T) in the presence of constant lidocaine (Part B) compared to either drug alone. For each dose, n=5 anaesthetized rats except for the vehicle (n=85). AS values are mean \pm SEM. The number of animals with VT or VF incidences are shown as a fraction. The symbol # denotes a statistically significant difference from vehicle. The symbol * denotes a statistically significant difference between drugs administered alone compared with combination. Statistical significance was detected at $p < 0.05$.

Drug ($\mu\text{mol/kg/min}$)	AS	VT incidence	VF incidence
vehicle	5.8 ± 0.2	82/85	68/85

Part A: Lidocaine plus 0.5, 1, and 2 $\mu\text{mol/kg/min}$ tedisamil

T0.5	$4.6 \pm 0.4 *$	5/5 *	4/5 *
L6	$1.6 \pm 0.8 \#$	2/5 #	1/5 #
T0.5 plus L6	$0.2 \pm 0.2 \#$	0/5 #	0/5 #

T1	$4.0 \pm 0.4 \# *$	5/5 *	4/5 *
L6	$1.6 \pm 0.8 \#$	2/5 #	1/5 #
T1 plus L6	$0.6 \pm 0.6 \#$	1/5 #	0/5 #

T2	$3.6 \pm 0.4 \# *$	5/5 *	2/5
L2	$5.6 \pm 0.6 *$	5/5 *	4/5 *
T2 plus L2	$0.8 \pm 0.6 \#$	1/5 #	0/5 #

Part B: Tedisamil plus 2, 4, and 6 $\mu\text{mol/kg/min}$ lidocaine

T2	$3.6 \pm 0.4 \# *$	5/5 *	2/5
L2	$5.6 \pm 0.6 *$	5/5 *	4/5 *
T2 plus L2	$0.2 \pm 0.2 \#$	0/5 #	0/5 #

T1	$4.0 \pm 0.4 \# *$	5/5 *	4/5
L4	$4.0 \pm 1.3 \# *$	4/5	2/5
T1 plus L4	$0.6 \pm 0.6 \#$	1/5 #	1/5 #

T1	$4.0 \pm 0.4 \# *$	5/5 *	4/5 *
L6	$1.6 \pm 0.8 \#$	2/5 #	1/5 #
T1 plus L6	$0.6 \pm 0.4 \#$	1/5 #	0/5 #

Figure 12 Normalized antiarrhythmic dose-response curves for lidocaine in the presence of 0.5 $\mu\text{mol/kg/min}$ tedisamil. Individual rat data (∇) and mean values (+) are shown for each dose ($n=5$). Mean vehicle (\blacksquare ; $n=85$) with 95% confidence interval for SEM and mean control (\bullet ; tedisamil at 0.5 $\mu\text{mol/kg/min}$; $n=5$) with 95% confidence interval lie to the left. Insertion is the normalized dose-response curve for percent protection against arrhythmias with increasing doses of lidocaine in the presence of 0.5 $\mu\text{mol/kg/min}$ tedisamil. Data were curve fitted as described in Methods.

Normalized antiarrhythmic dose-response curve for lidocaine
in the presence of 0.5 $\mu\text{mol/kg/min}$ tedisamil

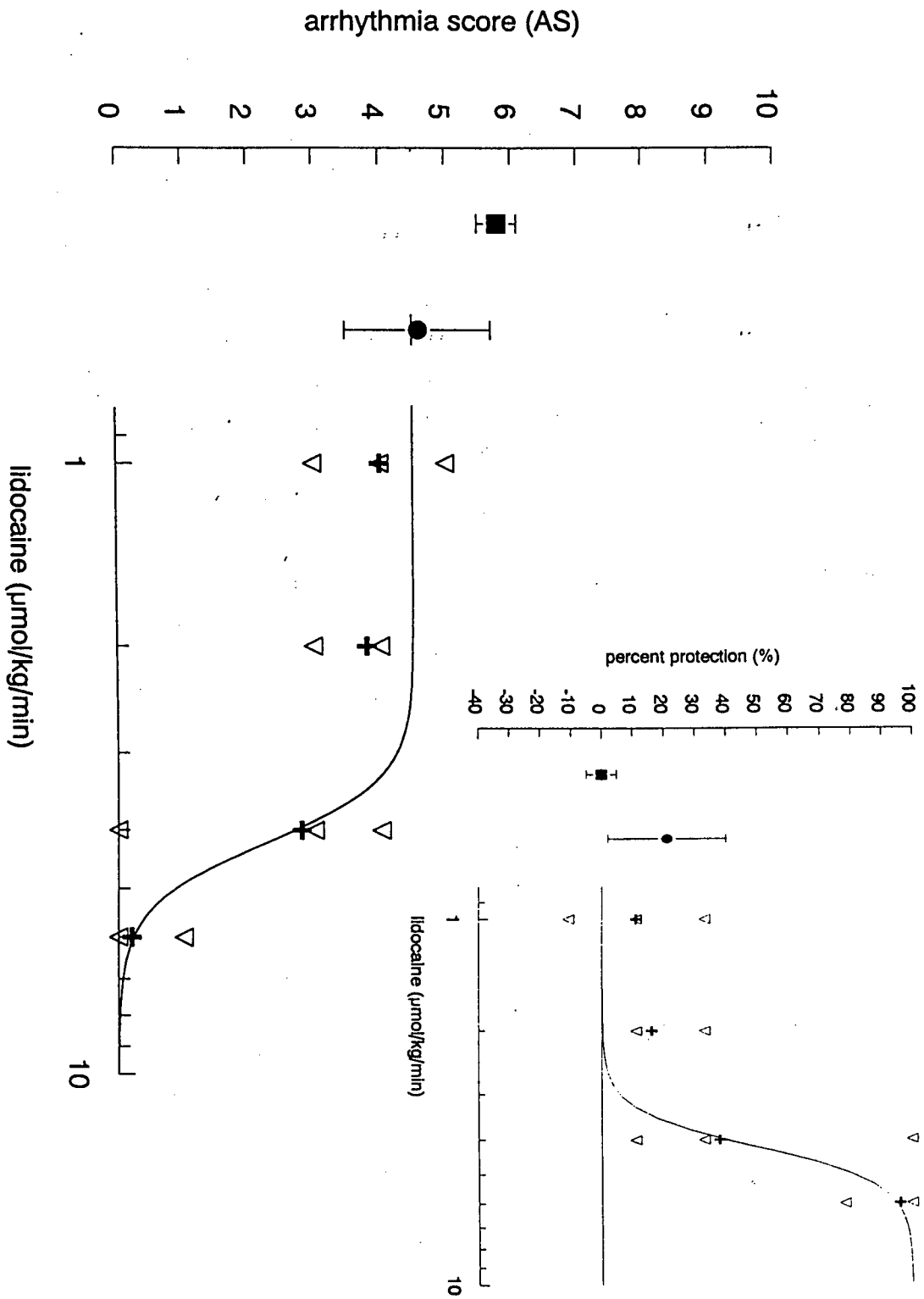


Figure 13 Normalized antiarrhythmic dose-response curves for lidocaine in the presence of 1 $\mu\text{mol/kg/min}$ tedisamil. Individual rat data (∇) and mean values (+) are shown for each dose ($n=5$). Mean vehicle (\blacksquare ; $n=85$) with 95% confidence interval for SEM and mean control (\bullet ; tedisamil at 1 $\mu\text{mol/kg/min}$; $n=5$) with 95% confidence interval lie to the left. Insertion is the normalized dose-response curve for percent protection against arrhythmias with increasing doses of lidocaine in the presence of 1 $\mu\text{mol/kg/min}$ tedisamil. Data were curve fitted as described in Methods.

Normalized antiarrhythmic dose-response curve for lidocaine in the presence of 1 $\mu\text{mol/kg/min}$ tedisamil

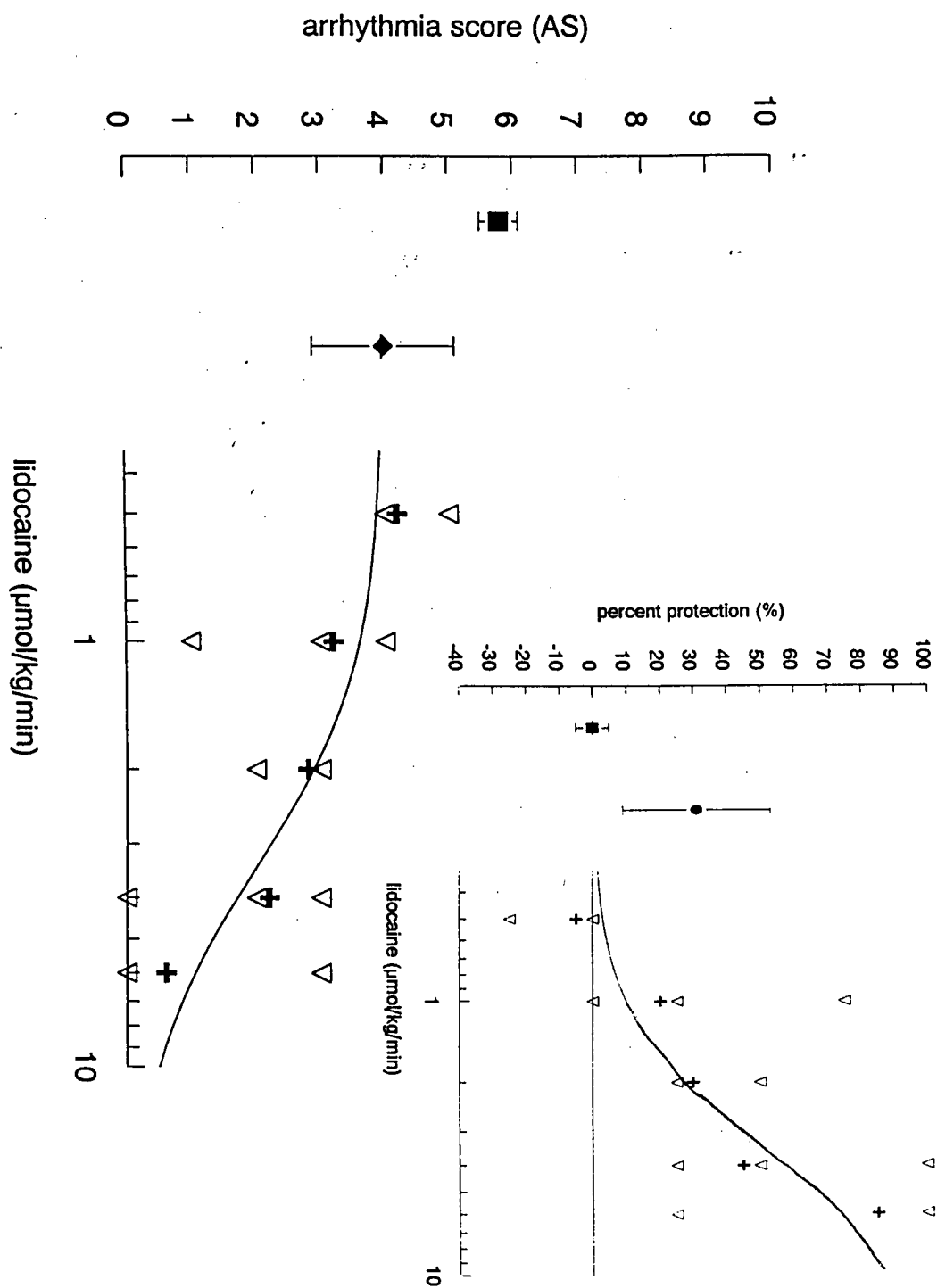


Figure 14 Normalized antiarrhythmic dose-response curves for lidocaine in the presence of 2 $\mu\text{mol/kg/min}$ tedisamil. Individual rat data (∇) and mean values (+) are shown for each dose ($n=5$). Mean vehicle (\blacksquare ; $n=85$) with 95% confidence interval for SEM and mean control (\bullet ; tedisamil at 2 $\mu\text{mol/kg/min}$; $n=5$) with 95% confidence interval lie to the left. Insertion is the normalized dose-response curve for percent protection against arrhythmias with increasing doses of lidocaine in the presence of 2 $\mu\text{mol/kg/min}$ tedisamil. Data were curve fitted as described in Methods.

Normalized antiarrhythmic dose-response curve for lidocaine in the presence of 2 $\mu\text{mol/kg/min}$ tedisamil

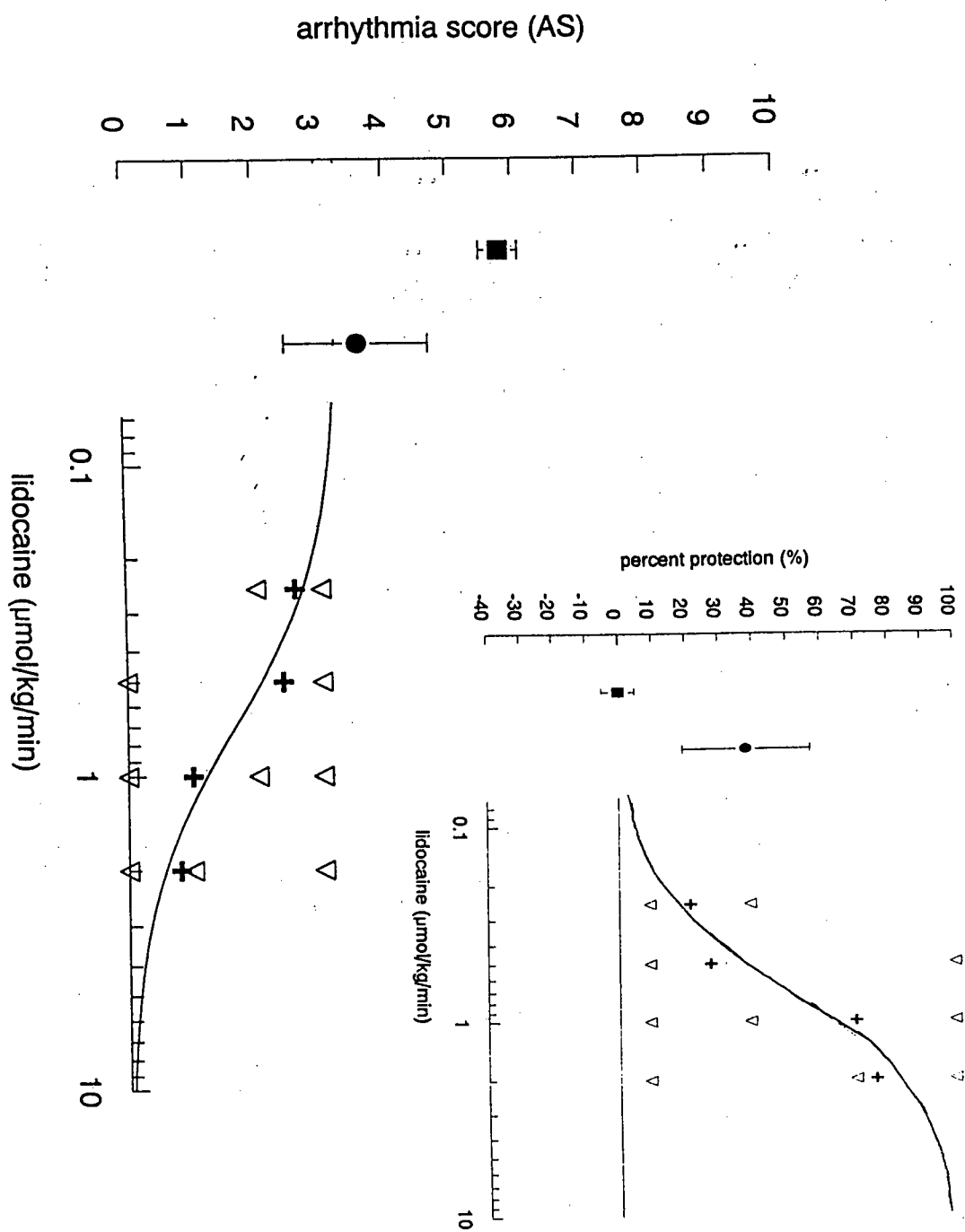


Figure 15 Normalized antiarrhythmic dose-response curves for tedisamil in the presence of 2 $\mu\text{mol/kg/min}$ lidocaine. Individual rat data (∇) and mean values (+) are shown for each dose ($n=5$). Mean vehicle (\blacksquare ; $n=85$) with 95% confidence interval for SEM and mean control (\bullet ; tedisamil at 1 $\mu\text{mol/kg/min}$; $n=5$) with 95% confidence interval lie to the left. Insertion is the normalized dose-response curve for percent protection against arrhythmias with increasing doses of tedisamil in the presence of 2 $\mu\text{mol/kg/min}$ lidocaine. Data were curve fitted as described in Methods.

Normalized antiarrhythmic dose-response curve for tedisamil
in the presence of 2 $\mu\text{mol/kg/min}$ lidocaine

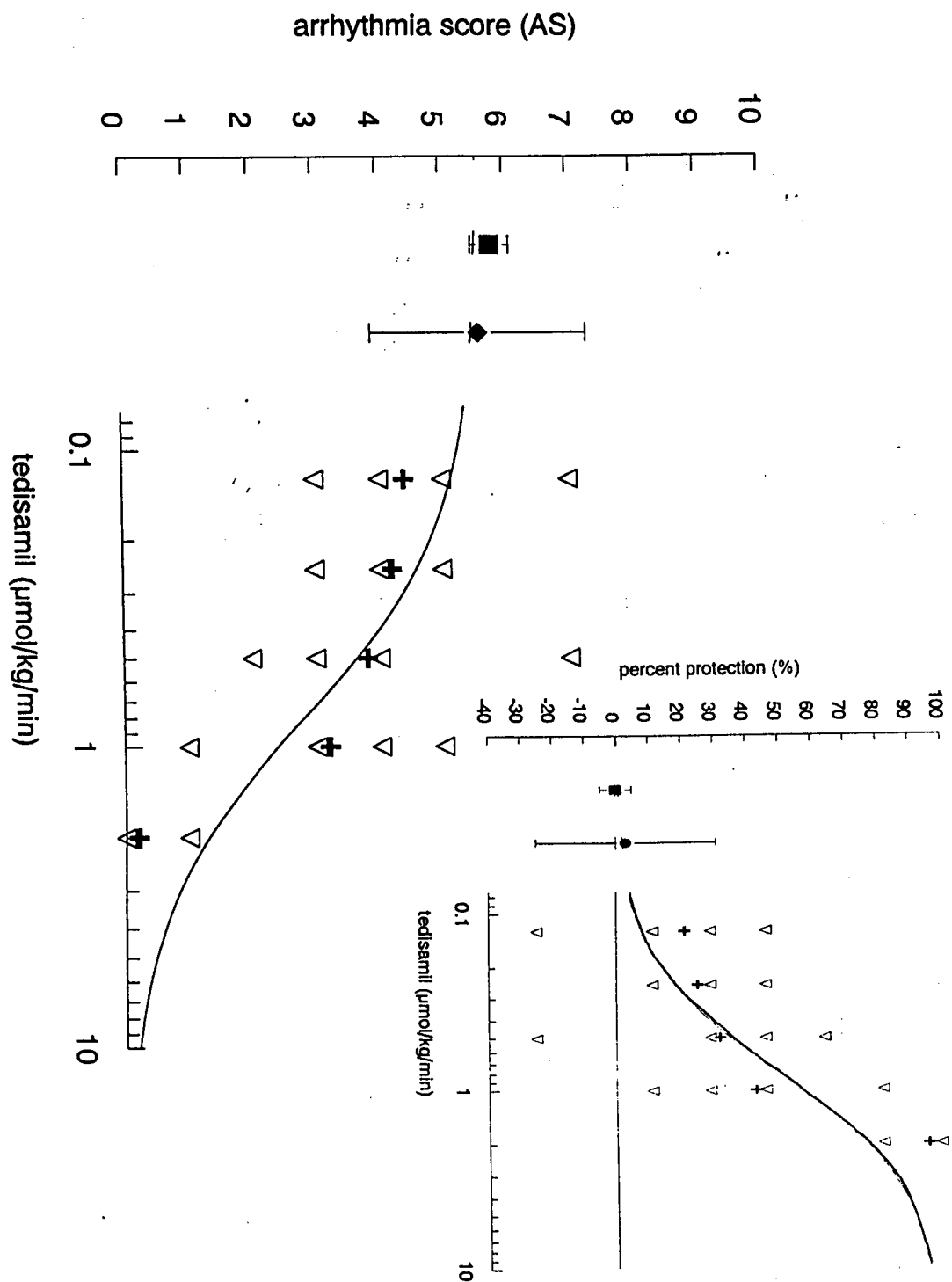


Figure 16 Normalized antiarrhythmic dose-response curves for tedisamil in the presence of 4 $\mu\text{mol/kg/min}$ lidocaine. Individual rat data (∇) and mean values ($+$) are shown for each dose ($n=5$). Mean vehicle (\blacksquare ; $n=85$) with 95% confidence interval for SEM and mean control (\bullet ; tedisamil at 4 $\mu\text{mol/kg/min}$; $n=5$) with 95% confidence interval lie to the left. Insertion is the normalized dose-response curve for percent protection against arrhythmias with increasing doses of tedisamil in the presence of 4 $\mu\text{mol/kg/min}$ lidocaine. Data were curve fitted as described in Methods.

Normalized antiarrhythmic dose-response curve for tedisamil
in the presence of 4 $\mu\text{mol/kg/min}$ lidocaine

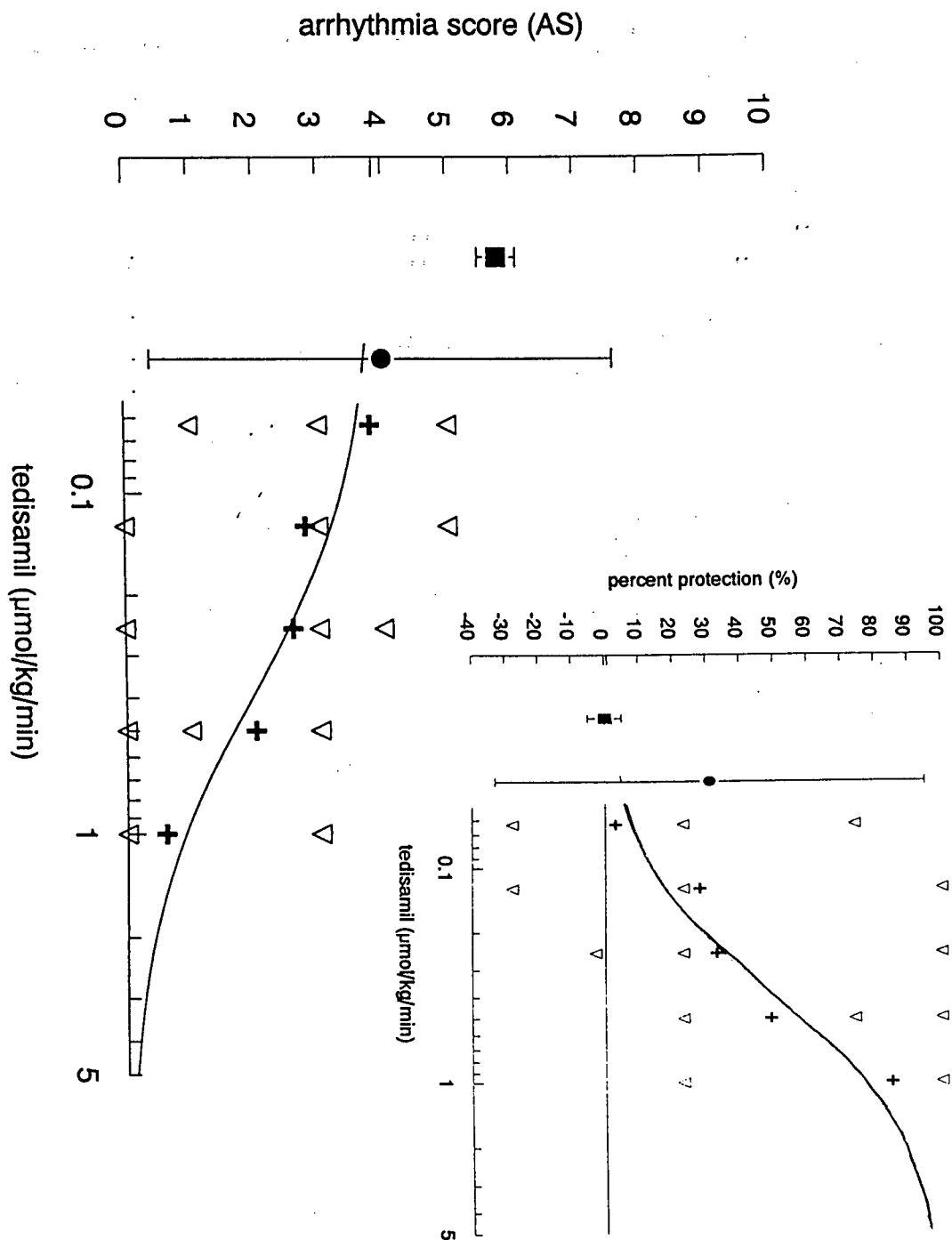


Figure 17 Normalized antiarrhythmic dose-response curves for tedisamil in the presence of 6 $\mu\text{mol/kg/min}$ lidocaine. Individual rat data (∇) and mean values (+) are shown for each dose ($n=5$). Mean vehicle (\blacksquare ; $n=85$) with 95% confidence interval for SEM and mean control (\bullet ; tedisamil at 6 $\mu\text{mol/kg/min}$; $n=5$) with 95% confidence interval lie to the left. Insertion is the normalized dose-response curve for percent protection against arrhythmias with increasing doses of tedisamil in the presence of 6 $\mu\text{mol/kg/min}$ lidocaine. Data were curve fitted as described in Methods.

Normalized antiarrhythmic dose-response curve for tedisamil in the presence of 6 $\mu\text{mol/kg/min}$ lidocaine

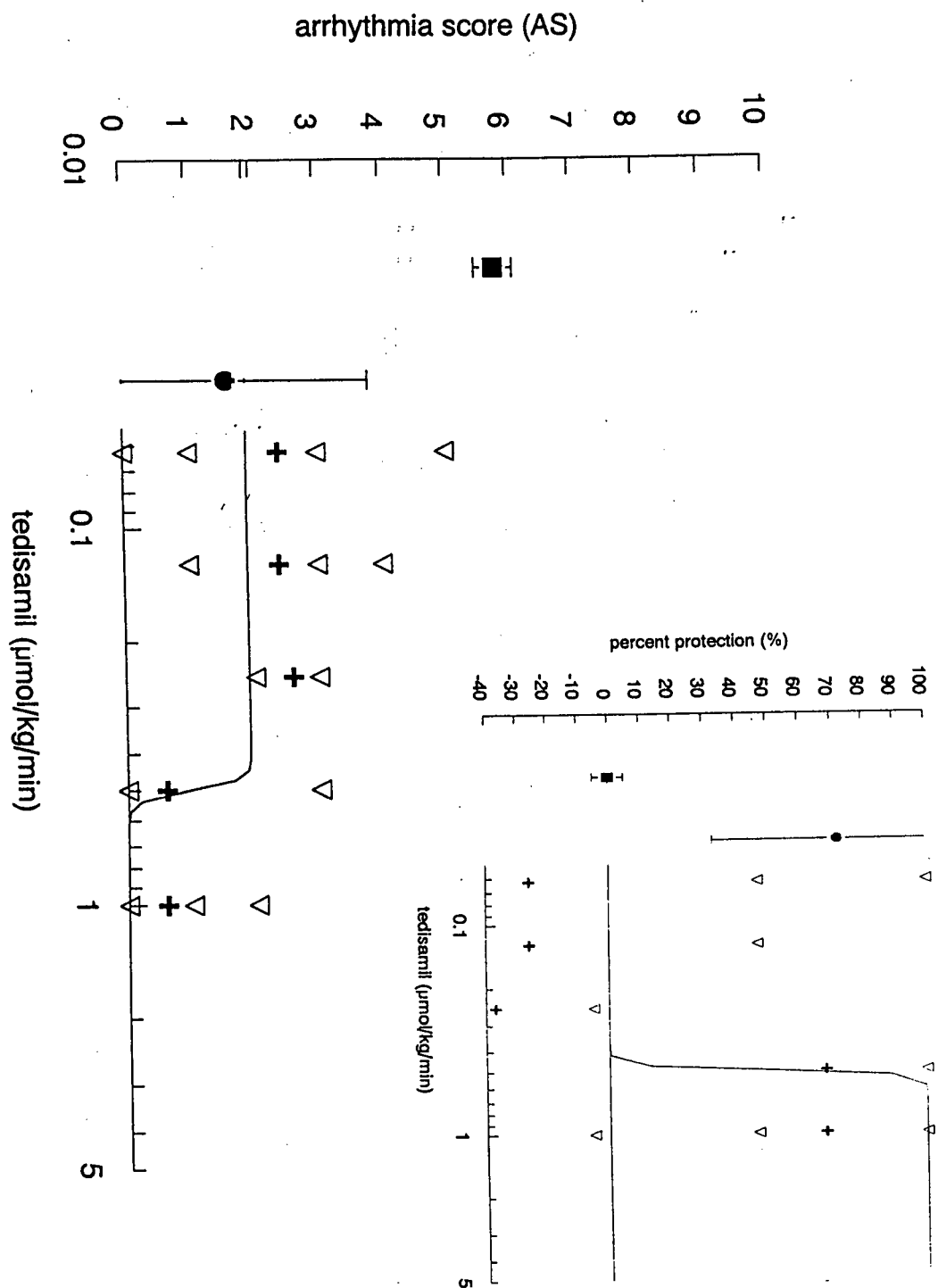
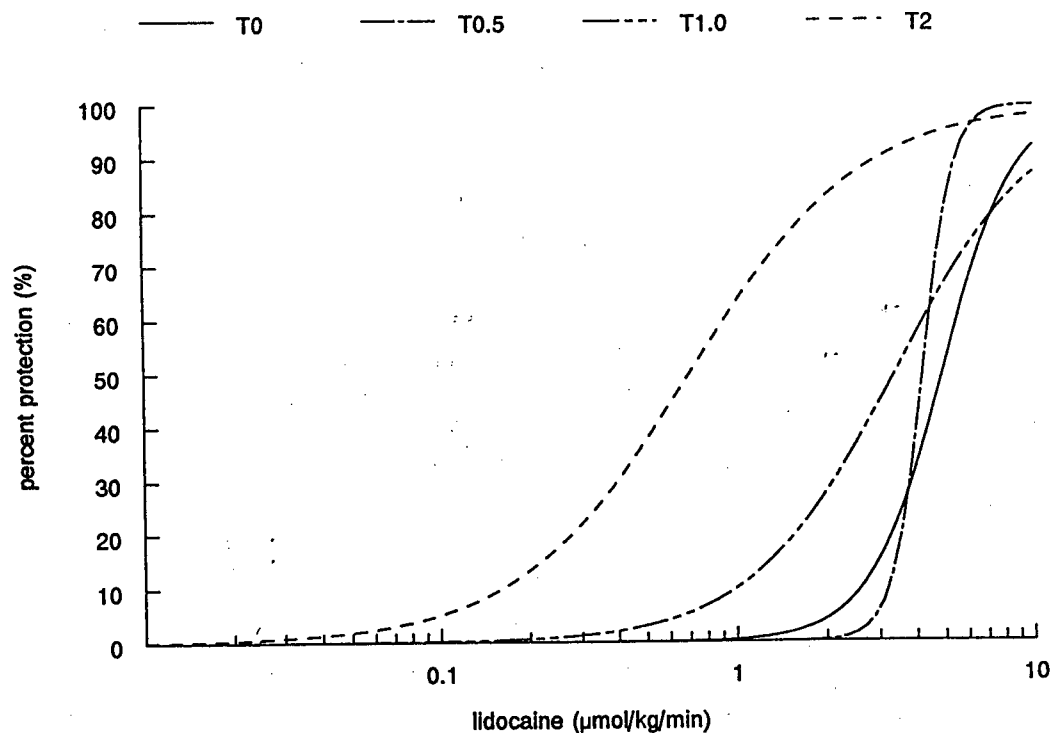


Figure 18 Summary of normalized antiarrhythmic dose-response curves for lidocaine (L) in the presence of varying doses of tedisamil (0, 0.5, 1 and 2 μ mol/kg/min) (top graph) and tedisamil (T) in the presence of varying doses of lidocaine (2, 4, and 6 μ mol/kg/min) (bottom graph). All the dose-response curves for drug alone or in combination are extracted from Figures 8 to 15 in order to illustrate the leftward shift of the dose-response curves for lidocaine co-administered with tedisamil and tedisamil co-administered with lidocaine. Values \pm SEM are not shown for clarity.

Normalized antiarrhythmic dose-response curves for lidocaine with varying doses of tedisamil



Normalized antiarrhythmic dose-response curves for tedisamil with varying doses of lidocaine

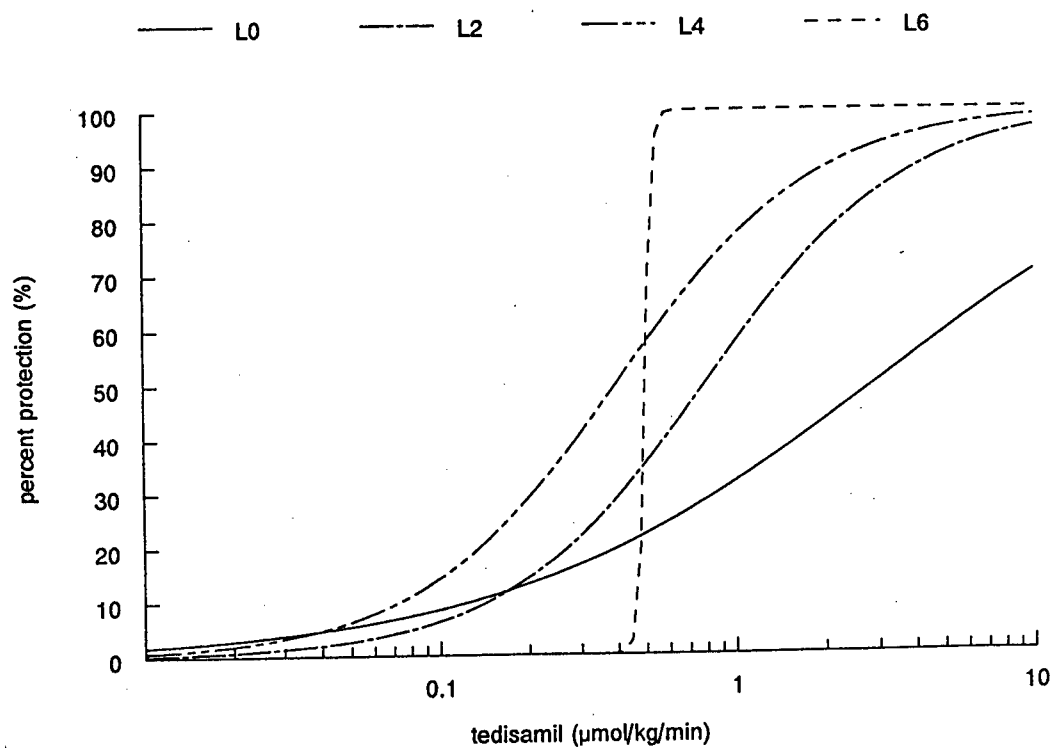


Table VII. Antiarrhythmic ED₅₀ values A) lidocaine in the presence of various doses of tedisamil and B) tedisamil in the presence of various doses of lidocaine.

Part A:

Drug ($\mu\text{mol/kg/min}$)	ED ₅₀ ($\mu\text{mol/kg/min}$)	Ratio (ED ₅₀ alone/ED ₅₀ combination)
Lidocaine alone	4.9 \pm 0.6	
Lidocaine plus 0.5 tedisamil	4.2 \pm 0.2 *	1.2
Lidocaine plus 1 tedisamil	3.4 \pm 0.6 *	1.4
Lidocaine plus 2 tedisamil	0.7 \pm 0.2 #	7

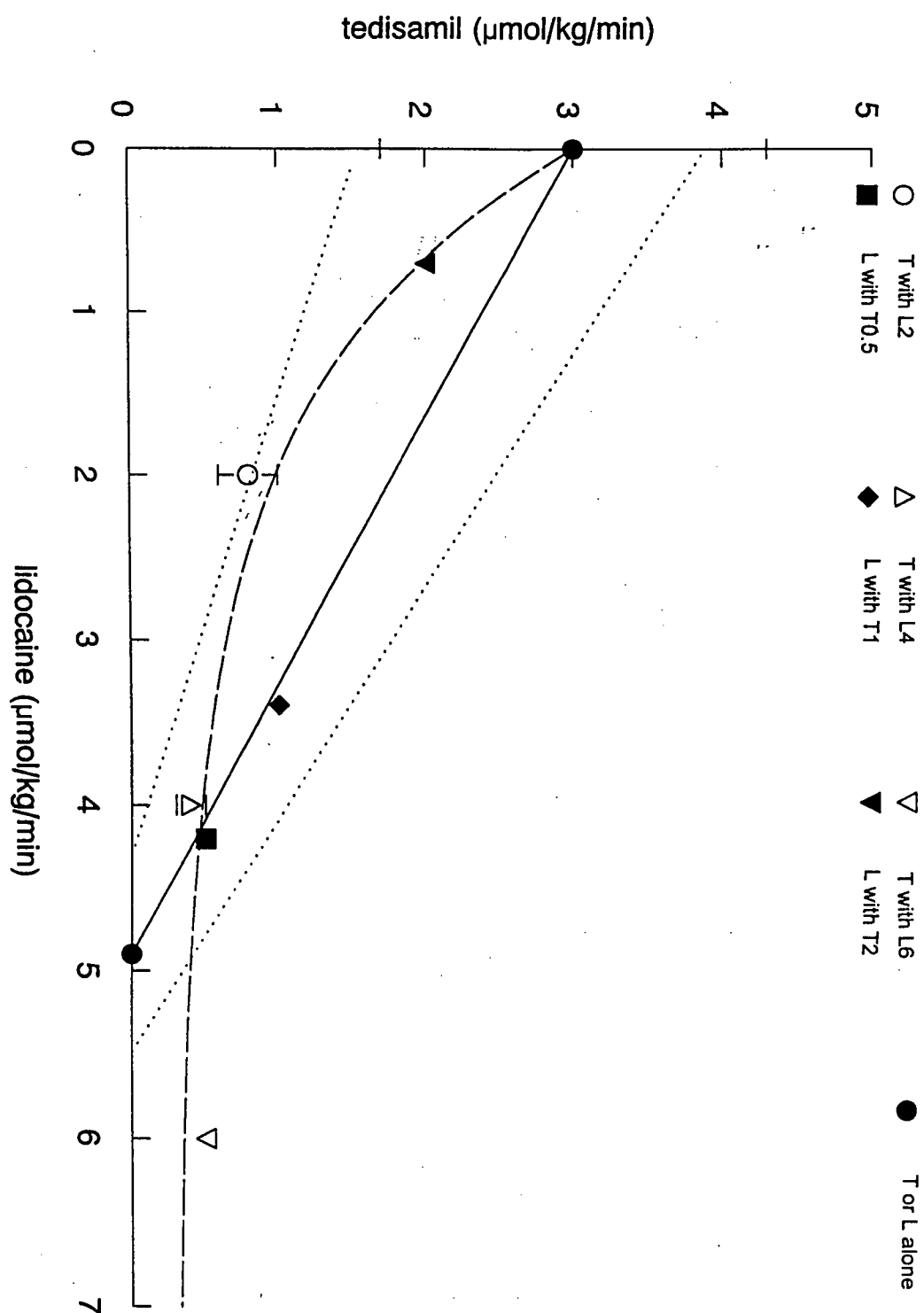
Part B:

Drug ($\mu\text{mol/kg/min}$)	ED ₅₀ ($\mu\text{mol/kg/min}$)	Ratio (ED ₅₀ alone/ED ₅₀ combination)
Tedisamil alone	3.0 \pm 1.3	
Tedisamil plus 2 lidocaine	0.8 \pm 0.2	3.8
Tedisamil plus 4 lidocaine	0.4 \pm 0.1	7.5
Tedisamil plus 6 lidocaine	0.5 \pm /	6

The effect of tedisamil on lidocaine's antiarrhythmic ED₅₀ (Part A) and the effect of lidocaine on tedisamil's antiarrhythmic ED₅₀ (Part B) are shown. For all drugs, the ED₅₀ \pm SEM were determined from their antiarrhythmic normalized dose-response curves (refer to Figures 10 to 17). Note that the SEM for tedisamil plus lidocaine at 6 $\mu\text{mol/kg/min}$ was very large due to a steep slope of the normalized dose-response curve (/). The symbol # denotes a difference between drugs administered alone and in combination. The symbol * denotes a difference between combination drugs and lidocaine plus tedisamil at 2 $\mu\text{mol/kg/min}$.

Figure 19 ED_{50} isobologram for tedisamil (T) and lidocaine (L) combinations. The solid line represents the line of additivity joining the ED_{50} for tedisamil and lidocaine alone with the SEM boundary (.....) for each drug. The combination isobole (dashed line) was constructed as described in Methods. Data points represent $ED_{50} \pm SEM$ for either drug in the presence of various doses of the other as determined from their normalized antiarrhythmic dose-response curves. The following drug mixtures were used: lidocaine with tedisamil at 0.5 (■), 1 (◆), or 2 (▼) $\mu\text{mol/kg/min}$; tedisamil with lidocaine at 2 (○), 4 (Δ), or 6 (▽) $\mu\text{mol/kg/min}$.

ED50 isobologram for tedisamil and lidocaine mixtures



4 DISCUSSION

The questions posed previously in the introduction section will now be discussed in more detail. Initially, the electrophysiological and antiarrhythmic actions of lidocaine alone and tedisamil alone will be discussed. Then, lidocaine and tedisamil mixtures will be studied to determine whether the presence of one drug affects the actions of the other. Finally, an isobologram will be used to classify the interaction between lidocaine and tedisamil as additive, synergistic, or antagonistic.

4.1 Lidocaine alone

4.1.1 Electrophysiological actions of lidocaine alone

Compared to vehicle control, lidocaine produced a significant fall in both blood pressure and heart rate (Table 1). These results support those obtained previously in our laboratory (Barrett et al., 1995). The mechanism for the fall in blood pressure has not yet been determined. Some researchers have suggested that the hypotension is due to a decrease in cardiac contractility (Hammermeister et al., 1972). However, Honerjäger et al. (1986) showed that the amount of sodium channel blockade by class I drugs did not correlate with the negative inotropism. Thus, the hypotension produced by lidocaine is probably due to both a direct myocardial depression as well as a direct vasodilatory effect (Barrett et

al., 1995). Similarly, the bradycardia observed with lidocaine is probably due to a direct action on the myocardium which might be related to the decrease in myocardial contractility.

Lidocaine prolonged the PR interval of the ECG, but had no effect on the QRS and QT intervals. Both sodium and calcium channels play a role in AV conduction and hence the duration of the PR interval. Thus, blockade of either sodium or calcium channels in the AV node can prolong the PR interval. However, in rats and other animals, AV conduction is predominately due to sodium rather than calcium channels (Botting et al., 1985). Hence, lidocaine's prolongation of the PR interval of the ECG is probably due to blockade of sodium channels. The QRS interval of the ECG is a measure of the intraventricular conduction generated by the opening of voltage-gated sodium channels. Although lidocaine has been shown to prolong the QRS interval (Barrett et al., 1995), in the present experiment, lidocaine had no effect on the QRS interval. As well, lidocaine produced no change in the QT interval of the ECG which reflects changes in the action potential duration. This is in accordance with the classification of lidocaine as a class Ib agent. This is because the fast kinetics of class Ib agents prolong the effective refractory period by blocking sodium channels, but have little no effect on the action potential duration (Meinertz, 1992).

4.1.2 Antiarrhythmic actions of lidocaine alone

Lidocaine's antiarrhythmic actions are supported by the dose-dependent decrease in arrhythmia score, ventricular tachycardia (VT) incidence, and ventricular fibrillation (VF) incidence (Table IV). VF was completely abolished with 8 $\mu\text{mol/kg/min}$ lidocaine. These results are similar to those obtained previously in our laboratory (Barrett et al., 1995). Cardinal et al. (1981) also found that in isolated porcine heart, high concentration (5 $\mu\text{mol/ml}$) of lidocaine prevented VF, but not VT. It has been proposed that lidocaine can decrease the incidence of large re-entrant circuits (diameter 1 - 2 cm), thus decreasing the incidence of VT (Cardinal et al., 1981; Janse et al., 1980). However, lidocaine can completely prevent the fractionation of circuits into multiple wavelets and small diameter (0.5 cm) circuits, thus preventing VF.

The antiarrhythmic actions of lidocaine and other class Ib agents is to selectively block sodium channels in the ischaemic myocardium, resulting in the prolongation of the effective refractory period (Janse, 1992). This will then break any re-entry circuits by converting a unidirectional block to a bidirectional block. The reason why lidocaine selectively blocks sodium channels in the ischaemic myocardium was explained by the modulated receptor hypothesis (Hondeghe and Katzung, 1977; Hondeghe, 1984 and 1987). This hypothesis suggests that lidocaine has a high affinity for open and inactivated sodium channels, but a low affinity for resting sodium channels. Since the majority of the sodium channels in the ischaemic (depolarized and acidic) myocardium are inactivated, lidocaine preferentially blocks these channels (Davis et al., 1986; Ye et al., 1993). As a result, lidocaine has little or no effect on the normal myocardium, and normal

heart rates. Lidocaine's fast kinetics of binding and unbinding of channels allows it to selectively block inactivated sodium channels of the ischaemic tissue only at fast heart rates. This is referred to as use- or frequency-dependent block (Courtney, 1975). Several researchers' experiments support the modulated receptor hypothesis (Sanchez-Chapula et al., 1983; Bean et al., 1983; Matsubara et al., 1987; Campbell et al., 1990). They found that increasing the duration of depolarization conditioning clamp pulse, increases lidocaine-induced depression of the sodium current. This suggests that lidocaine preferentially binds more to the inactivated-state sodium channels. Thus, these studies imply that lidocaine mainly binds to sodium channels during the plateau phase of the action potential and that lidocaine's depression of conduction velocity depends on the duration of the action potential (Sanchez-Chapula et al., 1983).

Although no proarrhythmic actions were observed in the present experiment, lidocaine can induce arrhythmias (Thale et al., 1987; Yin et al., 1997; Aupetit et al., 1997). The proarrhythmic actions of lidocaine probably occur when the depression of conduction in the ischaemic myocardium is greater than the prolongation of the effective refractory period (Yin et al., 1997). This means that for lidocaine to act as an antiarrhythmic and break the re-entry circuits, it must prolong the effective refractory period more than slowing the conduction velocity, so that wavelength of refractoriness exceeds that of the re-entrant circuit length (Yin et al., 1997).

Other than its proarrhythmic actions, lidocaine's use can be limited by its CNS toxicity. Barrett et al. (1995) found that 8 $\mu\text{mol/kg/min}$ lidocaine produced

convulsions in 1 out of 3 conscious rats, and that 16 $\mu\text{mol/kg/min}$ lidocaine produced convulsions in all 3 conscious rats. This was not observed in the present study because the rats were under anaesthesia. A possible explanation for the convulsions is that lidocaine is a small, lipid-soluble drug which can easily pass the blood brain barrier and enter the CNS (Igwemezie et al., 1992). Also, the action potential firing in the CNS has a high frequency, and so lidocaine can produce a frequency-dependent block of sodium channels on neuronal cells.

Overall, lidocaine is a class Ib drug with antiarrhythmic actions against ischaemia-induced arrhythmias. However, the lidocaine dose which completely abolishes VF (8 $\mu\text{mol/kg/min}$), also produces convulsions. Thus, lidocaine's toxic effects can limit its clinical use.

4.2 Tedisamil alone

4.2.1 Sham occlusions

As mentioned earlier in the results section (refer to 3.1), Beatch et al. (1991) have studied the effects of bolus injections of tedisamil in rats. In order to compare the results obtained from the present study with those from Beatch et al. (1991), sham occlusions were performed with both bolus and continuous intravenous injections of tedisamil. It was found that 0.5 $\mu\text{mol/kg/min}$ continuous administration is equivalent to 4 mg/kg bolus administration of tedisamil. This decision was based on the observation that both doses of tedisamil produced a

four fold increase in QT₁ interval of the ECG (Figure 3). Although similar effects were seen with the QT₁ interval prolongation, the two routes of administration did not produce the same antiarrhythmic effects: 4 mg/kg tedisamil decreased VF incidence, but 0.5 µmol/kg/min tedisamil had no effect (Table IV). There are several possibilities for this discrepancy in antiarrhythmic activity. Firstly, the rats in the sham occlusions were anaesthetized, while those in Beatch et al.'s (1991) experiment were conscious. This is only a hypothesis because there is no published evidence that rat preparations can affect tedisamil's antiarrhythmic action. Secondly, perhaps the QT₁ interval is not a good indicator for quantifying the amount of drug in rats. If so, the comparison made here between bolus and continuous administration of tedisamil is not valid. Measuring the actual serum concentrations of the drug would probably be a better indicator. Thirdly, the sham occlusions were only a pilot experiment with n = 3. Thus, no statistical analysis was performed on the results. In order to make better conclusions from the sham occlusion results, the sample number of the experiment must be increased, as well, it must be determined whether the QT₁ interval is an adequate indicator of the amount of drug in rats.

4.2.2 Electrophysiological actions of tedisamil alone

Tedisamil tended to increase the blood pressure, however 2 µmol/kg/min tedisamil was the only dose that produced a statistically significant increase (Table 1). This is similar to that reported by Brill et al. (1993) who found that

tedisamil does not increase blood pressure in pentobarbitone-anaesthetized rats. Interestingly, tedisamil has been shown to increase blood pressure in conscious rats (Beatch et al., 1991), as well as in patients with coronary artery disease (Thormann et al., 1993). It is hypothesized that the increase in vascular tone is due to a direct inhibition of potassium channels in the vascular smooth muscle (Mitrovic et al., 1998; Hermann et al., 1998). Tedisamil can non-competitively block a large conductance calcium-dependent, cromakalim-sensitive potassium channel (Volker et al., 1992; Bray et al., 1991). The inhibition of this potassium channel will lead to an increase in calcium influx in the smooth muscle cells, thus increasing vascular resistance. Indirect support for this hypothesis is the vasorelaxant effects of potassium channel openers (Escande et al., 1993; Anderson, 1992).

Tedisamil dose-dependently decreased heart rate (Table 1). Other researchers have also observed tedisamil's bradycardiac actions (Howard et al., 1989; Beatch et al., 1990; Dukes et al., 1989). Since I_{to} potassium current is responsible for the repolarization of sinoatrial node (Irasawa, 1987), tedisamil's inhibition of this current will prolong the action potential duration in the pacemaker cells, and thus decrease heart rate (Oexle et al., 1987; Dukes et al., 1989 and 1990).

Both the PR and QRS intervals of the ECG were dose-dependently increased by tedisamil (Table 1). This implies that tedisamil can block sodium channels in the AV node to prolong the PR interval (Botting et al., 1985), and in the ventricular myocardium to prolong the QRS interval. Other researchers have

also reported tedisamil's ability to block sodium channels, however this was only seen at high doses (Dukes et al., 1989; Beatch et al., 1991; Adaikan et al., 1992; Bril et al., 1993). Adaikan et al. (1992) found that QRS interval was prolonged only at doses of tedisamil higher than 4 mg/kg. Similarly, in the present study, tedisamil prolonged the QRS interval starting at a dose of 0.5 $\mu\text{mol/kg/min}$ which was shown to be equivalent to 4 mg/kg tedisamil (refer to the sham occlusion experiments, section 4.2.1). Thus, tedisamil can be classified as a class I agent due to its ability to block sodium channels, resulting in an increase in PR and QRS intervals of the ECG. However, tedisamil can also be classified as a class III agent due to its ability to block I_{to} potassium channels in rats, resulting in an increase in the QT interval of the ECG (Table 1). Hence, in the present study, tedisamil has a combination of class I and class III action. The PR and QRS prolongations were observed at the same dose range (0.5 to 4 $\mu\text{mol/kg/min}$) as that for the QT prolongation (Table 1). This suggests that tedisamil blocks sodium and potassium channels at the same dose range. This observation is in contrast with other researchers which have shown that tedisamil's effect on the QT interval starts at a lower dose compared to its effect on the PR and QRS intervals (Howard et al., 1989; Bril et al., 1993). The reason for this discrepancy is not known. It may be due to the route of administration of tedisamil: this study used continuous intravenous tedisamil, while others have used bolus tedisamil.

If tedisamil has both class I and III action, can it be classified as a class Ia drug such as quinidine? The answer is no. The main reason is that tedisamil's prolongation of the QT interval was much greater than that of either PR or QRS

intervals. For example, 4 $\mu\text{mol/kg/min}$ tedisamil prolonged QT_1 by 6.9 fold, but prolonged both PR and QRS intervals by only 1.2 fold. As well, the ED_{25} for QT prolongation was at a low dose of about 0.2 $\mu\text{mol/kg/min}$ (Figure 8). However, the ED_{25} for QRS prolongation was at a dose of about 0.5 $\mu\text{mol/kg/min}$, which is about 2.5 fold larger than the dose for QT prolongation (Figure 7). Tedisamil did not even produce an ED_{25} for the PR interval, because it did not produce a large enough increase in the PR interval (Figure 6). Thus, the present study showed that tedisamil is very potent at increasing the QT interval compared to the PR and QRS intervals. Interestingly, Barrett et al. (1995) showed that quinidine (a class Ia agent with both class I and III action) prolonged the QT, PR, and QRS intervals all at the same dose of 10 $\mu\text{mol/kg/min}$. This suggests that quinidine is equipotent in blocking either potassium or sodium channels.

The present study showed that tedisamil is not a pure class III agent. However, tedisamil should not be classified as a class Ia agent because it is much more potent in prolonging the QT interval compared to class Ia agents such as quinidine. Tedisamil's effectiveness in prolonging the QT more than the PR and QRS intervals, means that it will also prolong the action potential duration to a much greater degree compared to class Ia agents.

4.2.3 Antiarrhythmic actions of tedisamil alone

Tedisamil was not a very effective antiarrhythmic drug because even at the highest dose tested (4 $\mu\text{mol/kg/min}$), it only produced a 57% protection

against ischaemia-induced arrhythmias (Table IV and Figure 11). In addition, tedisamil had absolutely no effect on VT or VF, except at the highest dose of 4 $\mu\text{mol/kg/min}$. At this dose, VF was completely abolished. Similarly, Beatch et al. (1991) found that the highest dose of tedisamil (4 mg/kg) significantly decreased VF. As mentioned earlier in the introduction section (refer to 1.2.3.2.1), tedisamil provides antiarrhythmic protection by blocking I_{to} potassium channels which results in prolongation of the action potential duration and effective refractory period (Adaikan et al., 1992). Since tedisamil can increase the action potential duration at any heart rate, tedisamil's antiarrhythmic effects are not related to its ability to produce bradycardia (Beatch et al., 1990; Walker et al., 1988). Adaikan et al. (1992) proposed that tedisamil can produce antiarrhythmic action independent of whether the heart is paced or non-paced. With the paced hearts, tedisamil can decrease the window for the occurrence of arrhythmias, while with the non-paced hearts, tedisamil can prolong the effective refractory period. This increase in the effective refractory period can increase the minimal path length for the re-entrant pathway. This may explain why tedisamil can convert VF to VT (Adaikan et al., 1992). If tedisamil produces a large enough increase in the effective refractory period, then multiple re-entrant circuits of VF will be abolished, but single re-entrant circuits of VT will still continue.

Although the present study showed that tedisamil possesses antiarrhythmic action, its clinical use is limited due to the possibility of proarrhythmia. This is because tedisamil, similar to other class III agents, blocks potassium channels in a reverse-use dependent manner (Hondeghe et al.,

1990). This means that tedisamil produces a greater prolongation of the effective refractory period during bradycardia as opposed to tachycardia. The combination of prolonged effective refractory period plus decreased heart rate could lead to torsade de pointes (Hondeghe et al., 1990; Colatsky et al., 1990). Thus, even though 4 $\mu\text{mol/kg/min}$ tedisamil was able to abolish VF, tedisamil is not an ideal antiarrhythmic.

Hence, since tedisamil abolished VF only at a very high dose and can possibly cause proarrhythmia, it is not an ideal antiarrhythmic agent.

4.3 Combinations of lidocaine and tedisamil

4.3.1 Electrophysiological actions of combinations of lidocaine and tedisamil

Overall, the electrophysiological actions of combinations of lidocaine and tedisamil were additive. For example, the hypotensive action of lidocaine alone counteracted the hypertensive action of tedisamil alone, thus the combination of the two drugs produced no change in BP compared to vehicle (Table II). Similarly, the effects of tedisamil and lidocaine mixtures on HR, PR, QRS, and QT intervals were additive (Table II).

If one antiarrhythmic potentiates the antiarrhythmic actions of a second drug without having potentiating effects on BP, HR, and ECG parameters, then one can improve the therapeutic ratio of the first drug by adding the second. Interestingly, combinations of tedisamil and lidocaine seem to increase the

therapeutic ratio compared to either drug alone. This is because tedisamil increased the antiarrhythmic potency of lidocaine (Table VII), without potentiating the effects of lidocaine on BP, HR, and ECG parameters. Similarly, lidocaine increased the antiarrhythmic potency of tedisamil (Table VII), without potentiating the effects of tedisamil on BP, HR, and ECG parameters. This improvement in the therapeutic ratio is a very important finding when searching for better antiarrhythmic drugs.

4.3.2 Antiarrhythmic actions of combinations of lidocaine and tedisamil

Combinations of lidocaine and tedisamil produced a better antiarrhythmic compared to either drug alone. Firstly, the co-infusion of lidocaine and tedisamil produced a leftward shift in the antiarrhythmic dose-response curve compared to either drug alone (Figure 18). In the presence of 2 $\mu\text{mol/kg/min}$ tedisamil, lidocaine's antiarrhythmic potency was increased by about 7 times (Table VII). Similarly, in the presence of 4 $\mu\text{mol/kg/min}$ lidocaine, tedisamil's antiarrhythmic potency was increased by about 7.5 times (Table VII). Secondly, combination of the highest doses of tedisamil and lidocaine significantly decreased VT incidence to either 1/5 or 0/5 which was less than administration of either drug alone (Table VI). Thirdly, combination of the highest doses of tedisamil and lidocaine completely abolished VF, except for 1 $\mu\text{mol/kg/min}$ tedisamil plus 4 $\mu\text{mol/kg/min}$ lidocaine which decreased VF incidence to 1/5 (Table VI). All these results suggest a possible synergistic interaction between lidocaine and tedisamil.

The best combination that supports synergy between tedisamil and lidocaine is the mixture of 2 $\mu\text{mol/kg/min}$ tedisamil plus 2 $\mu\text{mol/kg/min}$ lidocaine. This is because co-infusion of 2 $\mu\text{mol/kg/min}$ tedisamil plus 2 $\mu\text{mol/kg/min}$ lidocaine produced a statistically significant decrease in both the arrhythmia score and VT incidence compared to administration of either drug alone (Table VI). As well, this mixture completely abolished VF (Table VI).

The possible synergy between a class III agent (eg. tedisamil) and a class Ib agent (eg. lidocaine) was predicted by the modulated receptor hypothesis (Hondegheem and Katzung, 1980 and 1984; for more detail refer to results section 1.3.2). The hypothesis would support the possible mechanism whereby tedisamil prolongs the action potential duration allowing for a greater number of inactive-state sodium channels to be blocked by lidocaine. Thus resulting in a greater prolongation of the effective refractory period with co-infusion of lidocaine and tedisamil compared to either drug alone. This could explain why the combination of tedisamil and lidocaine produced a better protection against ischaemia-induced arrhythmias compared to either drug alone. This hypothesis has been supported by several researchers for different drug mixtures such as quinidine plus lidocaine (Duff et al., 1983, 1987, 1991; Wang et al., 1993), and mexiletine plus sotalol (Chéزالviel et al., 1993).

Duff et al. (1989, 1990) have suggested that the mechanism for the antiarrhythmic action of quinidine and lidocaine mixtures is the synergistic increase in the effective refractory period in the periinfacted tissue. However, data from our laboratory (not yet published) shows that tedisamil and lidocaine

combinations act synergistically to increase the effective refractory period in the normal myocardium. It was shown that tedisamil produced a dose-dependent increase in the effective refractory period and that the co-administration of 2 $\mu\text{mol/kg/min}$ lidocaine, produced a leftward shift in tedisamil's dose-response curve. Interestingly, 2 $\mu\text{mol/kg/min}$ lidocaine alone had no effect on the effective refractory period. This finding suggests that tedisamil and lidocaine mixtures can produce better antiarrhythmic protection than either drug alone, by interacting synergistically to increase the effective refractory period in normal myocardium.

4.3.3 Is the interaction between lidocaine and tedisamil additive, synergistic, or antagonistic?

As mentioned above in section 4.3.2, several findings support a possible synergistic interaction between tedisamil and lidocaine. For example, the antiarrhythmic potency of each drug alone was increased by several folds when administered in combination. As well, the electrical stimulation study (not yet published) showed that 2 $\mu\text{mol/kg/min}$ lidocaine was able to produce a leftward shift in tedisamil's dose-dependent increase in the effective refractory period in the normal myocardium. However, we will now discuss what the isobologram showed as a possible interaction between tedisamil and lidocaine (Figure 19).

The isobologram was constructed by drawing a straight line between the ED_{50} for antiarrhythmic dose-response curve of tedisamil and lidocaine alone (Figure 19). This is referred to as the line of additivity (please refer to the

methods section for more details on isobologram construction). If the ED_{50} of drug combinations lie at the line, above, or below, they are referred to as additive, antagonistic, or synergistic interactions, respectively. The isobologram for the antiarrhythmic action of tedisamil and lidocaine mixtures was below the line of additivity, thus suggesting a possible synergistic interaction between the two drugs. However, when analyzing each of the ED_{50} 's for the various combinations of tedisamil and lidocaine, it seems that lidocaine potentiates tedisamil but tedisamil does not potentiate lidocaine and that it is only additive to lidocaine. This is because the ED_{50} for 2 $\mu\text{mol/kg/min}$ lidocaine plus tedisamil is the furthest one below the line of additivity.

The interaction between lidocaine and tedisamil can be explained by using the equation that the re-entry path length is conduction velocity \times refractory period (Janse 1992). The possible additive interaction of tedisamil when co-infused with lidocaine may be that tedisamil only increases the refractory period prolonging action of lidocaine. Thus, in the above equation, the re-entry path length is increased due to an additive increase in the refractory period, without any change in the conduction velocity. In contrast, lidocaine may potentiate the antiarrhythmic action of tedisamil synergistically by adding rate dependent reduction of conduction velocity to the refractory prolonging action of tedisamil.

Thus, the antiarrhythmic interaction of tedisamil and lidocaine are at least additive, and perhaps synergistic. Interestingly, the isobologram supports the finding that 2 $\mu\text{mol/kg/min}$ tedisamil plus 2 $\mu\text{mol/kg/min}$ lidocaine is the best

antiarrhythmic combination in this study since the ED_{50} is the furthest below the line of additivity.

5 CONCLUSION

The results of this study support the hypothesis that combinations of tedisamil and lidocaine can produce better protection against ischaemia-induced arrhythmias than either drug alone. As suggested by the modulated receptor hypothesis, the mechanism for the improved antiarrhythmic protection is probably due to a greater prolongation of the effective refractory period produced by combinations of class III agent tedisamil and class Ib agent lidocaine, compared to either drug alone. This is an important finding because it suggests that newer antiarrhythmics can be developed by combining class III with class Ib activity. One example of such a drug used clinically today is amiodarone. Although amiodarone can prolong the action potential duration (class III action) and block inactive-state sodium channels (class Ib action), it also has drawbacks of low specificity (can act as class I, II, III, and IV) and some unwanted side-effects such as thyroid and lung disease. Thus, further studies are required to develop newer antiarrhythmics with more specificity and less side-effects compared to amiodarone. This is why Nortran Pharmaceuticals is developing new agents with both class Ib and III activity. Interestingly, this thesis suggests that the mechanism for the superior antiarrhythmic protection produced by the mixture of class Ib and III drugs is due to their possible synergistic interaction.

6 REFERENCES

- Adaikan, G., Beatch, G.N., Lee, T.L., Ratnam, S.S., and Walker, M.J.A. Antiarrhythmic actions of tedisamil: studies in rats and primates. *Cardiovascular Drugs and Therapy*. 6: 345-352, 1992.
- Allessie, M.A., Bonke, F.I.M., and Schopman, J.G. Circus movement in rabbit atrial muscle as a mechanism of tachycardia, III. The "leading circle" concept: a new model of circus movement in cardiac tissue without the involvement of an anatomical obstacle. *Circulation Research*. 41: 9-18, 1977.
- Anderson, K.P., Walker, R., Dustman, T., Lux, R.L., Ershler, P.R., Kates, R.E., and Urie, P.M. Rate-related electrophysiologic effects of long-term administration of amiodarone on canine ventricular myocardium in vivo. *Circulation*. 79: 948-958, 1989.
- Anderson, K.E. Clinical pharmacology of potassium channel openers. *Pharmacology and Toxicology*. 70: 244-254, 1992.
- Antman, E.M., and Berlin, J.A. Declining incidence of ventricular fibrillation in myocardial infarction: implications for the prophylactic use of lignocaine. *Circulation*. 86: 764-773, 1992.
- Antzelevitch, C., Sicouri, S., Litovsky, S.H., Lukas, A., Krishnan, S.C., Diego, J.M.D., Gintant, G.A., and Liu, D. Heterogeneity within the ventricular wall. Electrophysiology and pharmacology of epicardial, endocardial, and M cells. *Circulation Research*. 69: 1427-1449, 1991.
- Au, T.L.S., Collins, G.A., Harvie, C.J., and Walker, M.J.A. The actions of prostaglandins I₂ and E₂ on arrhythmias produced by coronary occlusion in the rat and dog. *Prostaglandins*. 18: 707-720, 1979.
- Aupetit, J., Loufoua-Moundanga, J., Faucon, G., and Timour, Q. Ischaemia-induced loss or reversal of the effects of the class I antiarrhythmic drugs on vulnerability to fibrillation. *British Journal of Pharmacology*. 120: 523-529, 1997.
- Bargheer, K., Bode, F., Klein, H.U., Trappe, H.J., Franz, M.R., and Lichtlen, P.R. Prolongation of monophasic action potential duration and the refractory period in the human heart by tedisamil, a new potassium-blocking agent. *European Heart Journal*. 15: 1409-1414, 1994.
- Barrett, T.D., Hayes, E.S., and Walker, M.J.A. Lack of selectivity for ventricular and ischaemic tissue limits the antiarrhythmic actions of lidocaine, quinidine and flecainide against ischaemia-induced arrhythmias. *European Journal of Pharmacology*. 285: 229-238, 1995.

Bean, B.P., Cohen, C.J., and Tsien, R.W. Lidocaine block of cardiac sodium channels. *The Journal of General Physiology*. 81: 613-642, 1983.

Beatch, G.N., MacLeod, B.A., Abraham, S., and Walker, M.J.A. The in vivo electrophysiological actions of the new potassium channel blockers, tedisamil and UK 68,798. *Proceedings of the Western Pharmacological Society*. 33: 5-8, 1990.

Beatch, G.N., Abraham, S., MacLeod, B.A., Yoshida, N.R., and Walker, M.J.A. Antiarrhythmic properties of tedisamil (KC8857), a putative transient outward K⁺ current blocker. *British Journal of Pharmacology*. 102: 13-18, 1991.

Berenbaum, M.C. What is synergy? *Pharmacological Reviews*. 41: 93-141, 1989.

Berenbaum, M.C. Synergy, additivism and antagonism in immunosuppression, a critical review. *Clinical and Experimental Immunology*. 28: 1-18, 1977.

Bern, R.M., and Levy, M.N. Electrical activity of the heart. In *Physiology*, third edition. Mosby-Year Book, Inc., U.S.A. pp.364-396, 1993.

Billman, G.E. Role of ATP sensitive potassium channel in extracellular potassium accumulation and cardiac arrhythmias during myocardial ischaemia. *Cardiovascular Research*. 28: 762-769, 1994.

Botting, J.H., Curtis, M.J., and Walker, M.J.A. Arrhythmias associated with myocardial infarction. *Molecular Aspects of Medicine*. 8: 307-422, 1985.

Bray, K., and Quast, U. Tedisamil (KC 8857) differentially inhibits the ⁸⁶Rb efflux-stimulating and vasorelaxant properties of cromakalim. *European Journal of Pharmacology*. 200: 163-165, 1991.

Bril, A., Landais, L., and Gout, B. Actions and interactions of E-4031 and tedisamil on reperfusion-induced arrhythmias and QT interval in rats in vivo. *Cardiovascular Drugs and Therapy*. 7: 233-240, 1993.

Cairns, J.A., Connolly, S.J., Roberts, R., Gent, M., for the Canadian Amiodarone Myocardial Infarction Arrhythmia Trial Investigators. Randomised trial of outcome after myocardial infarction in patients with frequent or repetitive ventricular premature depolarisations: CAMIAT. *Lancet*. 349: 675-682, 1997.

Campbell, T.J., and Hemsworth, P.D. Selective depression of maximum rate of depolarization of guinea-pig ventricular action potentials by amiodarone and lignocaine in simulated ischaemia: comparison with encainide. *Clinical and Experimental Pharmacology and Physiology*. 17: 135-145, 1990.

Campbell, D.L., Rasmusson, R.L., Comer, M.B., and Strauss, H.C. The cardiac calcium-independent transient outward potassium current: kinetics, molecular properties, and role in ventricular repolarization. In: Zipes, D.P., and Jalife, J. (eds) Cardiac electrophysiology: from cell to bedside. Chapter 10. W.B. Saunders Ltd., U.S.A., 1990.

Campbell, T.J. Resting and rate-dependent depression of maximum rate of depolarization (V_{max}) in guinea-pig ventricular action potentials by mexiletine, disopyramide and encainide. *Journal of Cardiovascular Pharmacology*. 5: 291-296, 1983a.

Campbell, T.J. Kinetics of onset of rate-dependent effects of Class I antiarrhythmic drugs are important in determining their effects on refractoriness in guinea-pig ventricle, and provide a theoretical basis for their subclassification. *Cardiovascular Research*. 17: 344-352, 1983b.

The Cardiac Arrhythmias Suppression Trial (CAST) Investigators. Increased mortality due to encainide and flecainide in a randomized trial of arrhythmia suppression after myocardial infarction. *New England Journal of Medicine*. 321: 406-412, 1989.

Cardinal, R., Janse, M.J., Eeden, I.V., Werner, G., D'Almoncourt, C.N., and Durrer, D. The effects of lidocaine on intracellular and extracellular potentials, activation, and ventricular arrhythmias during acute regional ischaemia in the isolated porcine heart. *Circulation Research*. 49: 792-806, 1981.

Carmeliet, E. Mechanisms and control of repolarization. *European Heart Journal*. 14 (Supplement H): 3-13, 1993.

Carmeliet, E. Voltage- and time-dependent block of the delayed K^+ current in cardiac myocytes by dofetilide. *The Journal of Pharmacology and Therapeutics*. 262: 809-817, 1992.

Carson, D.L., Cardinal, R., Savard, P., Vasseur, C., Nattel, S., Lambert, C., and Nadeau, R. Relationship between an arrhythmogenic action of lidocaine and its effects on excitation patterns in acutely ischaemic porcine myocardium. *Journal of Cardiovascular Pharmacology*. 8: 126-136, 1986.

Cassee, F.R., Groten, J.P., van Bladeren, P.J., and Feron, V.J. Toxicological evaluation and risk assessment of chemical mixtures. *Critical Reviews in Toxicology*. 28: 73-101, 1998.

Chen, C.M., Gettes, L.S., and Katzung, B.G. Effect of lidocaine and quinidine on steady state characteristics and recovery kinetics of $(dV/dt)_{max}$ in guinea pig ventricular myocardium. *Circulation Research*. 37: 20-29, 1975.

Chéزالviel, F., Weissenburger, J., Guhenec, C., Jagueux, M., Davy, J.M., Vernhet, L., Simandoux, V., Poirier, J.M., and Cheymol, G. Antiarrhythmic effect of a sotalol-mexiletine combination on induced ventricular tachycardia in dogs. *Journal of Cardiovascular Pharmacology*. 21: 212-220, 1993.

Chi, L., Park, J.L., Friedrichs, G.S., Banglawala, Y.A., Perez, M.A., Tanhehco, E.J., and Lucchesi, B.R. Effects of tedisamil (KC-8857) on cardiac electrophysiology and ventricular fibrillations in the rabbit isolated heart. *British Journal of Pharmacology*. 117: 1261-1269, 1996.

Cobb, L.A., Werner, J.A., and Trobaugh, G.B. Sudden cardiac death. I. A decade's experience with out-of-hospital resuscitation. *Modern Concepts of Cardiovascular Disease*. 49: 31-36, 1980.

Cobbe, S.M., Manley, B.S., and Alexopoulos, D. The influence of acute myocardial ischaemia on the class III antiarrhythmic action of sotalol. *Cardiovascular Research*. 19: 661-667, 1985.

Coetzee, W.A., Opie, L.H., and Saman, S. Proposed role of energy supply in the genesis of delayed afterdepolarizations-implications for ischemic or reperfusion arrhythmias. *Journal of Molecular and Cellular Cardiology*. 19: 13-21, 1987.

Colatsky, T.J., Follmer, C.H., and Starmer, C.F. Channel specificity in antiarrhythmic drug action. Mechanism of potassium channel block and its role in suppressing and aggravating cardiac arrhythmias. *Circulation*. 82: 2235-2242, 1990.

Colatsky, T.J. Mechanisms of action of lidocaine and quinidine on action potential duration in rabbit cardiac purkinje fibers. An effect on steady state sodium currents? *Circulation Research*. 50: 17-27, 1982.

Cooper, D.K.C. Eelectrocardiographic studies in the rat in physiological and pathological states. *Cardiovascular Research*. 3: 419-425, 1969.

Courtney, K.R. Mechanism of frequency-dependent inhibition of sodium currents in frog myelinated nerve by the lidocaine derivative GEA 968. *The Journal of Pharmacology and Experimental Therapeutics*. 195: 225-236, 1975.

Courtney, K.R. Interval-dependent effects of small antiarrhythmic drugs on excitability of guinea-pig myocardium. *Journal of Molecular and Cellular Cardiology*. 12: 1273-1286, 1980.

Cranefield, P.F. Action potentials, afterpotentials, and arrhythmias. *Circulation Research*. 41: 415-423, 1977.

Curtis, M.J., and Walker, M.J.A. Quantification of arrhythmias using scoring systems: an examination of seven scores in an in vivo model of regional myocardial ischaemia. *Cardiovascular Research*. 22: 656-665, 1988.

Curtis, M.J., MacLeod, B.A., and Walker, M.J.A. Antiarrhythmic actions of verapamil against ischaemic arrhythmias in the rat. *British Journal of Pharmacology*. 83: 373-385, 1984.

Curtis, M.J., MacLeod, B.A., and Walker, M.J.A. Models for the study of arrhythmias in myocardial ischaemia and infarction : the use of the rat. *Journal of Molecular and Cellular Cardiology*. 19: 399-419, 1987.

Damiano, B.P., and Rosen, M.R. Effects of pacing on triggered activity induced by early afterdepolarizations. *Circulation*. 69: 1013-1025, 1984.

Davidenko, J.M., Cohen, L., Goodrow, R., and Antzelevitch, C. Quinidine-induced action potential prolongation, early afterdepolarizations, and triggered activity in canine purkinje fibers: effects of stimulation rate, potassium, and magnesium. *Circulation*. 79: 674-686, 1989.

Davis, J. Matsubara, T., Scheinman, M.M., Katzung, B., and Hondeghem, L.H. Use-dependent effects of lidocaine on conduction in canine myocardium: application of the modulated receptor hypothesis in vivo. *Circulation*. 74: 205-214, 1986.

Dessertenne, F., Fabiato, A., and Coumel, P. Un chapitre nouveau d'électrocardiographie Les variations progressive de l'amplitude de l'électrocardiogramme. *Acta. Cardiol. Angéol. Int.* 15:241-256, 1996.

DIAMOND (Danish Trial in Acute Myocardial Infarction on Dofetilide. Preliminary results presented at the European Congress of Cardiology. Stockholm, August, 1997.

The DIAMOND study Group, Denmark. Dofetilide in patients with left ventricular dysfunction and either heart failure or acute myocardial infarction: rationale, design, and patient characteristics of the DIAMOND studies. *Clinical Cardiology*. 20: 704-710, 1997.

Duff, H.J., Roden, D., Primm, R.K., Oates, J.A., and Woosley, R.L. Mexiletine in the treatment of resistant ventricular arrhythmias: enhancement of efficacy and reduction of dose-related side effects by combination with quinidine. *Circulation*. 67: 1124-1128, 1983.

Duff, H.J., Mitchell, L.B., Manyari, D., and Wyse, D.G. Mexiletine-quinidine combination: electrophysiologic correlates of a favorable antiarrhythmic interaction in humans. *Journal of American College of Cardiology*. 10: 1149-1156, 1987.

Duff, H.J. Mexiletine-quinidine combination: enhanced antiarrhythmic and electrophysiologic activity in the dog. *The Journal of Pharmacology and Therapeutics*. 249: 617-622, 1989.

Duff, H.J., Rahmberg, M., and Sheldon, R.S. Role of quinidine in the mexiletine-quinidine interaction: electrophysiologic correlates of enhanced antiarrhythmic efficacy. *Journal of Cardiovascular Pharmacology*. 16: 685-692, 1990.

Duff, H.J., Mitchell, L.B., Wyse, D.G., Gillis, A.M., and Sheldon, R.S. Mexiletine / quinidine combination therapy: electrophysiologic correlates of anti-arrhythmic efficacy. *Clinical Investigation Medicine*. 14: 476-483, 1991.

Dukes, I.D., and Morad, M. Tedisamil inactivates the transient outward K^+ current in rat ventricular myocytes. *American Journal of Physiology*. 257: H1746-H1749, 1989.

Dukes, I.D., Cleemann, L., and Morad, M. Tedisamil blocks the transient and delayed rectifier K^+ currents in mammalian cardiac and glial cells. *The Journal of Pharmacology and Experimental Therapeutics*. 254: 560-569, 1990.

Duncan, D.B. Multiple range and multiple F tests. *Biometrics*. 11: 1-42, 1955.

El-Sherif, N., Zeiler, R.H., Craelius, W., Gough, W.B., and Henkin, R. QTU prolongation and polymorphic ventricular tachyarrhythmias due to bradycardia-dependent early afterdepolarizations, afterdepolarizations and ventricular arrhythmias. *Circulation Research*. 63: 286-305, 1988.

Escande, D., Coulombe, A., Faivre, J.F., Deroubaix, E., and Coraboeuf, E. Two types of transient outward currents in adult human atrial cells. *American Journal of Physiology*. 252: H142-H148, 1987.

Escande, D., and Henry, P. Potassium channels as pharmacological targets in cardiovascular medicine. *European Heart Journal*. 14 (Supplement B): 2-9, 1993.

Faivre, J.F., and Bril, A. Tedisamil inhibition of ATP-sensitive K^+ channels in adult rat ventricular cells. *Journal of Molecular and Cellular Cardiology*. 24 (Supplement VI): S40, 1992.

Fraser, T.R. An experimental research on the antagonism between the actions of physostigma and atropia. *Proc. Roy. Soc. Edinb.* 7: 506, 1870-1.

Fraser, T.R. The antagonism between the actions of active substances. *British Medical Journal*. ii: 485, 1872.

Gessner, P.K. Isobolographic analysis of interactions: an update on applications and utility. *Toxicology*. 105: 161-179, 1995.

Gettes, L.S., Hill, J.L., Saito, T., and Kagiya, Y. Factors related to vulnerability to arrhythmias in acute myocardial infarction. *American Heart Journal*. 103: 667-672, 1982.

Gettes, L.S., and Reuter, H. Slow recovery from inactivation of inward currents in mammalian myocardial fibers. *Journal of Physiology*. 240: 703-724, 1974.

Goldin, A. and Mantel, N. The employment of combinations of drugs in the chemotherapy of neoplasia: a review. *Cancer Research*. 17: 635-654, 1957.

Grant, A.O., Whalley, D.W., and Wendt, D.J. Pharmacology of the cardiac sodium channel. In: Zipes, D.P., and Jalife, J. (eds). Cardiac electrophysiology: from cell to bedside, second edition. W.B. Saunders Company, 1995.

Grover, G.J. Protective effects of ATP-sensitive potassium-channel openers in experimental myocardial ischaemia. *Journal of Cardiovascular Pharmacology*. 24 (Supplement 4): S18-S27, 1994.

Hammermeister, K.E., Boerth, R.C., Warbasse, J.R. The comparative inotropic effects of six clinically used antiarrhythmic agents. *American Heart Journal*. 84: 643-652, 1972.

Han, J., and Moe, G.K. Nonuniform recovery of excitability in ventricular muscle. *Circulation Research*. XIV: 44-60, 1964.

Harris, A.S., Bisteni, A., Russell, R.A., Brigham, J.C., and Firestone, J.E. Excitatory factors in ventricular tachycardia resulting from myocardial ischaemia: Potassium a major excitant. *Science*. 199: 200-203, 1954.

Harrison, D.C., Winkle, R.A., Sami, M., and Mason, J.W. Encainide: a new and potent antiarrhythmic agent. In: Harrison, D.C. (ed) Cardiac arrhythmias: a decade of progress. Boston, G.K. Hall, 315-330, 1981.

Hayes, E., Pugsley, M.K., Penz, W.P., Adaikan, G., and Walker, M.J.A. Relationship between QT and RR intervals in rats, guinea pigs, rabbits, and primates. *Journal of Pharmacological Methods*. 32: 201-207, 1994.

Hayes, E., Courtice, I.D., Abraham, S., and Walker, M.J.A. Acute toxicity of tedisamil, a new potassium channel blocking drug. *Pharmacology and Toxicology*. 73: 257-261, 1993.

Hearse D.J., and Dennis, S.C. Myocardial ischaemia and metabolic changes associated with the genesis of ventricular arrhythmias. In *Early arrhythmias*

resulting from myocardial ischaemia (edited by Parratt, J.R.), Macmillan Press Ltd, London, Great Britain, pp.15-35, 1982.

Heart Disease and Stroke in Canada. Laboratory Centre for Disease Control. Statistics Canada, 1997.

Hermann, H., Ohler, A., Just, H., and Hasenfuss. Cardiac and hemodynamic effects of the sinus node inhibitor tedisamil dihydrochloride in patients with congestive heart failure due to dilated cardiomyopathy. *Journal of Cardiovascular Pharmacology*. 32: 969-974, 1998.

Hewlett, P.S., and Plackett, R.L. A unified theory for quantal responses to mixtures of drugs: non-interactive action. *Biometrics*. 15: 591-610, 1959.

Hill, J.L., and Gettes, L.S. Effect of acute coronary artery occlusion on local myocardial extracellular K^+ activity in swine. *Circulation*. 61: 768-778, 1980.

Hille, B. Local anaesthetics: Hydrophilic and hydrophobic pathways for the drug-receptor reaction. *Journal of Gen. Physiology*. 69: 497-515, 1977.

Hine, L.K., Laird, N., Hewitt, P., and Chalmers, T.C. Meta-analytic evidence against prophylactic use of lidocaine in acute myocardial infarction, *Archives of Internal Medicine*. 149: 2694-2698, 1989.

Hodgkin, A.L., and Huxley, A.F. A quantitative description of membrane current and its application to conduction and excitation in nerve. *Journal of Physiology*. 117: 500-544, 1952.

Hoffman, B.F., and Bigger, Jr., J.T. Antiarrhythmic drugs. In: DiPalma, J.R. (Ed.) *Drugs Pharmacology in Medicine*. McGraw-Hill Book Co., New York, 1971.

Hoffman, B.F., and Dangman, K.H. Mechanisms for cardiac arrhythmias. *Experientia*. 43: 1049-1056, 1987.

Hoffman, B.F., and Rosen, M.R. Cellular mechanisms for cardiac arrhythmias. *Circulation Research*. 49: 1-15, 1981.

Hondeghem, L.M. Antiarrhythmic agents: modulated receptor applications. *Circulation*. 75: 514-520, 1987.

Hondeghem, L.M. Molecular interactions of antiarrhythmic agents with their receptor sites. In: Zipes, D.P., and Jalife, J. (eds) *Cardiac electrophysiology: from cell to bedside*. Chapter 90. W.B. Saunders Ltd., U.S.A., 1990.

Hondeghem, L.M. Development of class III antiarrhythmic agents. *Journal of Cardiovascular Pharmacology*. 20 (Supplement 2): S17-S22, 1992.

Hondeghem, L.M., and Katzung, B.G. Time- and voltage-dependent interactions of antiarrhythmic drugs with cardiac sodium channels. *Biochemical and Biophysical Acta*. 472: 474-98, 1977.

Hondeghem, L.M., and Katzung, B.G. Test of a model of antiarrhythmic drug action: effects of quinidine and lidocaine on myocardial conduction. *Circulation*. 61: 1217-1226, 1980.

Hondeghem, L.M., and Katzung, B.G. Antiarrhythmic agents: the modulated receptor mechanism of action of sodium and calcium channel-blocking drugs. *Annual Reviews Pharmacology and Toxicology*. 24: 387-423, 1984.

Hondeghem, L.M., and Matsubara, T. Quinidine blocks cardiac sodium channels during opening and slow inactivation in guinea-pig papillary muscle. *British Journal of Pharmacology*. 93: 311-318, 1988.

Hondeghem, L.M., and Snyders, D.J. Class III antiarrhythmic agents have a lot of potential but a long way to go: reduced effectiveness and dangers of reverse use dependence. *Circulation*. 81: 686-690, 1990.

Honerjäger, P., Loibl, E., Steidl, I., Schönsteiner, G., and Ulm, K. Negative inotropic effects of tetrodotoxin and seven class I antiarrhythmic drugs in relation to sodium channel blockade. *Naunyn-Schmiedeberg's Archives of Pharmacology*. 332: 184-195, 1986.

Howard, P.G., Abraham, S., Courtice, I.D., and Walker, M.J. Further studies into the ECG effects of tedisamil (KC 8857) in rats. *Proceedings of Western Pharmacological Society*. 32: 183, 1989.

Igwemezie, L.N., Beatch, G.N., McErlane, K.M., and Walker, M.J.A. Mexiletine's antifibrillatory actions are limited by the occurrence of convulsions in conscious animals. *European Journal of Pharmacology*. 210: 271-277, 1992.

IMPACT Research Group. International mexiletine and placebo antiarrhythmic coronary trial: I. Report on arrhythmia and other findings. *Journal of the American College of Cardiology*. 4: 1148-1163, 1984.

Irasawa, H. Membrane currents in cardiac pacemaker tissue. *Experientia*. 43: 1131-1135, 1987.

Janse, M.J. Electrophysiological effects of myocardial ischaemia. Relationship with early ventricular arrhythmias. *European Heart Journal*. 7 (Supplement A): 35-43, 1986.

Janse, M.J. To prolong refractoriness or to delay conduction (or both)? *European Heart Journal*. 13 (Supplement F): 14-18, 1992.

Janse, M.J., and Kléber, A.G. Electrophysiological changes and ventricular arrhythmias in the early phase of regional myocardial ischaemia. *Circulation Research*. 49: 1069-1081, 1981.

Janse, M.J., van Capelle, F.J.L., Morsink, H., Kléber, A.G., Wilms-Schopman, F., Cardinal, R., D'Almoncourt, C.N., and Durrer, D. Flow of "injury" current and patterns of excitation during early ventricular arrhythmias in acute regional myocardial ischaemia in isolated porcine and canine hearts, evidence for two different arrhythmogenic mechanisms. *Circulation Research*. 47: 151-165, 1980.

Janse, M.J. and Wit, A.L. Electrophysiological mechanisms of ventricular arrhythmias resulting from myocardial ischaemia and infarction. *Physiological Reviews*. 69: 1049-1169, 1989.

Johns, T.N.P., and Olson, B.J. Experimental myocardial infarction. *Annals of Surgery*. 140: 675-682, 1954.

Johnston, K.M., MacLeod, B.A., and Walker, M.J.A. Responses to ligation of a coronary artery in conscious rats and the actions of antiarrhythmics. *Canadian Journal of Physiology and Pharmacology*. 61: 1340-1353, 1983.

Josephson, I.R., Sanchez-Chapula, J., and Brown, A.M. Early outward current in rat single ventricular cells. *Circulation Research*. 54: 157-162, 1984.

Julian, D.G., Camm, A.J., Frangin, G., Janse, M.J., Munoz, A., Schwartz, P.J., and Simon, P., for the European Myocardial Infarct Amiodarone Trial Investigators. Randomised trial of effect of amiodarone on mortality in patients with left-ventricular dysfunction after recent myocardial infarction: EMIAT. *Lancet*. 349: 667-674, 1997.

Julian, D.G., Prescott, R.J., Jackson, F.S., and Szekely, P. Controlled trial of sotalol for one year after myocardial infarction. *Lancet*. 1: 1142-1147, 1982.

Kannel, W. and Schatzkin, A. Sudden death: lessons from subsets in population studies. *JACC*. 5: 141B-149B, 1985.

Katzung, B.G., and Morgenstern, J.A. Effects of extracellular potassium on ventricular automaticity and evidence for a pacemaker current in mammalian ventricular myocardium. *Circulation Research*. 40: 105-111, 1977.

Kléber, A.G., Janse, M.J., Van Capelle, F.J.L., and Durrer, D. Mechanism and time course of S-T and T-Q segment changes during acute regional myocardial

ischemia in the pig heart determined by extracellular and intracellular recordings. *Circulation Research*. 42: 603-613, 1978.

Kodell, R.L., and Pounds, J.G. Assessing the Toxicity of Mixtures of Chemicals. In *Statistics in Toxicology*. Edited by D. Krewski and C. Franklin. pp. 559-591. Gordon and Breach, New York, 1991.

Könemann, W.H., and Pieters, M.N. Confusion of concepts in mixture toxicology. *Food and Chemical Toxicology*. 34: 1025-1031, 1996.

Kosowsky, B.D., Taylor, J., Lown, B., and Ritchie, R.F. Long-term use of procaine amide following acute myocardial infarction. *Circulation*. 47: 1204-1210, 1973.

Kreye, V.A.W., Pfrunder, D., and Theiss, U. Effects of the potassium channel blocker tedisamil on ^{86}Rb efflux induced by cromakalim, high potassium and noradrenaline, and on mechanical tension in rabbit isolated vascular smooth muscle. *Naunyn-Schmiedeberg's Archives of Pharmacology*. 345: 238-243, 1992.

Lathrop, D.A., and Varró, A. The combined electrophysiological effects of lignocaine and sotalol in canine isolated cardiac Purkinje fibres are rate-dependent. *British Journal of Pharmacology*. 99: 124-130, 1990.

Lazzara, R., El-Sherif, N., Hope, R.R., and Scherlag, B.J. Ventricular arrhythmias and electrophysiological consequences of myocardial ischemia and infarction. *Circulation Research*. 42: 740-749, 1978.

Lazzara, R., and Scherlag, B.J. Generation of arrhythmias in myocardial ischaemia and infarction. *American Journal of Cardiology*. 61: 20A-26A, 1988.

Loewe, S., and Muischnek, H. Über kombinationswirkungen. I. Mitteilung: Hiltsmittel der fragstellung. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 114: 313-326, 1926.

Lown, B., and Wolf, M. Approaches to sudden death from coronary heart disease. *Circulation*. XLIV: 130-142, 1971.

Matsubara, T., Clarkson, C., and Hondeghem, L. Lidocaine blocks open and inactivated cardiac sodium channels. *Naunyn-Schmiedeberg's Archives of Pharmacology*. 336: 224-231, 1987.

Mayuga, R.D., and Singer, D.H. Effects of intravenous amiodarone on electrical dispersion in normal and ischaemic tissues and on arrhythmia inducibility: monophasic action potential studies. *Cardiovascular Research*. 26: 571-579, 1992.

McAlister, F.A., and Teo, K.K. Antiarrhythmic therapies for the prevention of sudden cardiac death. *Drugs*. 54: 235-252, 1997.

Meinertz, T. Clinical implications of new insight into mechanism of antiarrhythmic drug action. *Journal of Cardiovascular Pharmacology*. 20 (Supplement 2): S23-S27, 1992.

Mines, G. R. On dynamic equilibrium in the heart. *Journal of Physiology*. 46: 350-383, 1913.

Mines, G.R. On circulating excitations in heart muscles and their possible relation to tachycardia and fibrillation. *Trans. R. Soc. Can., Section IV*: 43-52, 1914.

Mitrovic, V., Oehm, E., Thormann, J., Pitschner, H., and Haberbosch, W. Comparison of the potassium channel blocker tedisamil with the beta-adrenoceptor blocker esmolol and the calcium antagonist gallopamil in patients with coronary artery disease. *Clinical Cardiology*. 21: 492-502, 1998.

Montgomery, D.C. Design and analysis of experiments. Third edition. John Wiley & Sons, New York, 75-77, 1991.

Morganroth, J., and Goin, J.E. Quinidine-related mortality in the short-to-medium-term treatment of ventricular arrhythmias. *Circulation*. 84: 1977-1983, 1991.

Nademanee, K., Stevenson, W.G., Weiss, J.N., Frame, V.B., Antimisiaris, M.G., Suithichaiyakul, T., and Pruitt, C.M. Frequency-dependent effects of quinidine on the ventricular action potential and QRS duration in humans. *Circulation*. 81: 790-796, 1990.

Naguib, M., Samarkandi, A.H., Bakhamees, H.S., Magboul, M.A., and El-Bakry, A.K. Comparative potency of steroidal neuromuscular blocking drugs and isobolographic analysis of the interaction between rocuronium and other aminosteroids. *British Journal of Anaesthesia*. 75: 37-42, 1995.

Nattel, S. Antiarrhythmic Drug Classifications, A critical appraisal of their history, present status, and clinical relevance. *Drugs*. 41: 672-701, 1991.

Nattel, S., and Talajic, M. Recent advances in understanding the pharmacology of amiodarone. *Drugs*. 36: 121-131, 1988.

Nemeth, M., Virag, L., Hala, O., Varro, A., Kovacs, G., Thormahlen, D., and Papp, J.G. The cellular electrophysiological effects of tedisamil in human atrial and ventricular fibers. *Cardiovascular research*. 31: 246-248, 1996.

Nestico, P.F., Morganroth, J., and Horowitz, L.N. New antiarrhythmic drugs. *Drugs*. 35: 286-319, 1988.

Nobel, D., and Tsien, R.W. Outward membrane currents activated in the plateau range of potentials in cardiac purkinje fibers. *Journal of Physiology*. 200: 205-231, 1969.

Noma, A. ATP-regulated K⁺ channels in cardiac muscle. *Nature*. 305: 147-149, 1983.

Normann, S.J., Priest, R.E., and Benditt, E.P. Electrocardiogram in the normal rat and its alteration with experimental coronary occlusion. *Circulation Research*, 9: 282-287, 1961.

Oexle, B., Weirich, J., and Antoni, H. Electrophysiological profile of KC 8857, a new bradycardiac agent. *Journal of Molecular and Cellular Cardiology*. 19 (Supplement III): 195, 1988.

Ohler, A., and Ravens, U. Effects of E-4031, almokalant and tedisamil on postrest action potential duration of human papillary muscles. *The Journal of Pharmacology and Experimental Therapeutics*. 270: 460-465, 1994.

Orchard, C.H., and Cingolani, H.E. Acidosis and arrhythmias in cardiac muscle. *Cardiovascular Research*. 28: 1312-1319, 1994.

Page, C.P., Curtis, M.J., Sutter, M.C., Walker, M.J.A., and Hoffman, B.B. *Integrated Pharmacology*. p154-178, Mosby International, UK, 1997.

Pallandi, R.T., and Campbell, T.J. Selective depression of conduction of premature action potentials in canine Purkinje fibers by class Ib antiarrhythmic drugs: comparison with Ia and Ic drugs. *Cardiovascular Research*. 22: 171-178, 1988.

Paletta, M.J., Abraham, S., Beatch, G.N., and Walker, M.J.A. Mechanisms underlying the antiarrhythmic properties of β -adrenoceptor blockade against ischaemia-induced arrhythmias in acutely prepared rats. *British Journal of Pharmacology*. 98: 87-94, 1989.

Penz, W., Pugsley, M., Hsieh, M.Z., and Walker, M.J.A. A new ECG measure (RSh) for detecting possible sodium channel blockade in vivo in rats. *Journal of Pharmacological Methods*. 27: 51-58, 1992.

Plummer, J.L., and Short, T.G. Statistical modeling of the effects of drug combinations. *Journal of Pharmacological Methods*. 23: 297-309, 1990.

Pogwizd, S.M., and Corr, P.B. Reentrant and nonreentrant mechanisms contribute to arrhythmogenesis during early myocardial ischemia: results using three-dimensional mapping. *Circulation Research*. 61: 352-371, 1987.

Pratt, E., and Lichstein, E. Ventricular antiarrhythmic effects of beta adrenergic blocker drugs: a review of mechanism and clinical studies. *Journal of Clinical Pharmacology*. 22: 335-347, 1982.

Rapaport, E. Sudden cardiac death. *The American Journal of Cardiology*. 62: 31-61, 1988.

Rees, S., and Curtis, M.J. Which cardiac potassium channel subtype is the preferable target for suppression of ventricular arrhythmias? *Pharmacology and Therapeutics*. 69: 199-217, 1996.

Reiffel, J.A., Reiter, M.J., and Blitzer, M. Antiarrhythmic drugs and devices for the management of ventricular tachyarrhythmia in ischemic heart disease. *American Journal of Cardiology*. 82: 31 I-40 I, 1998.

Reiter, M.J., Reiffel, J.A. The importance of beta blockade in the therapy of serious ventricular arrhythmias. *American Journal of Cardiology*. 82: 9 I-19 I, 1998.

Roden, D.M., Bennett, P.B., Snyders, D.J., Balser, J.R., and Hondeghem, L.M. Quinidine delays Ik activation in guinea pig ventricular myocytes. *Circulation Research*. 62: 1055-1058, 1988.

Roden, D.M. Risks and benefits of antiarrhythmic therapy. *The New England Journal of Medicine*. 331: 785-791, 1994.

Roden, D.M., and George, Jr., A.L. Structure and function of cardiac sodium and potassium channels. *American Journal of Physiology*. 273 (Heart Circulation Physiology 42): H511-H525, 1997.

Rosen, M.R., and Spinelli, W. Some recent concepts concerning the mechanism of action of antiarrhythmic drugs. *PACE*. 11: 1485-1498, 1988.

Rosow, C.E. Anesthetic drug interaction: an overview. *Journal of Clinical Anesthesia*. 9: 27S-32S, 1997.

Sanchez-Chapula, J., Tsuda, Y., and Josephson, I.R. Voltage- and use-dependent effects of lidocaine on sodium current in rat single ventricular cells. *Circulation Research*. 52: 557-565, 1983.

Sanguinetti, M.C., and Jurkiewicz, N.K. Two components of cardiac delayed rectifier K^+ current: Differential sensitivity to block by class III antiarrhythmic agents. *The Journal of General Physiology*. 96: 195-215, 1990.

Selzer, A., and Wray, H.W. Quinidine syncope: Paroxysmal ventricular fibrillation occurring during treatment of chronic atrial arrhythmias. *Circulation*. XXX: 17-26, 1964.

Shih, H. Anatomy of the action potential in the heart. *Texas Heart Institute Journal*.. 21: 30-41, 1994.

Shivkumar, K., Deutsch, N.A., Lamp, S.T., Khuu, K., Goldhaber, J.I., and Weiss, J.N. Mechanism of hypoxic K loss in rabbit ventricle. *Journal of clinical investigation*. 100: 1782-1788, 1997.

Singh, B.N., and Hauswirth, O. Comparative mechanisms of antiarrhythmic drugs. *American Heart Journal*. 87: 367-382, 1974.

Singh, B.N., and Vaughan Williams, E.M. The effect of amiodarone, a new anti-anginal drug on cardiac muscle. *British Journal of Pharmacology*. 39: 657-667, 1970a.

Singh, B.N., and Vaughan Williams, E.M. A third class of antiarrhythmic action. Effects on atrial and ventricular intracellular potentials, and other pharmacological actions on cardiac muscle, of MJ 1999 and AH 3474. *British Journal of Pharmacology*. 39: 675-687, 1970b.

Singh, B.N., and Vaughan Williams, E.M. A fourth class of antidysrhythmic action? Effect of verapamil on ouabain toxicity, on atrial and ventricular intracellular potentials, and on other features of cardiac function. *Cardiovascular Research*. 6: 109-119, 1972.

Singh, B.N. Antiarrhythmic drugs: a reorientation in light of recent developments in the control of disorders of rhythm. *The American Journal of Cardiology*. 81: 3D-13D, 1998.

Tamargo, J., Valenzuela, C., and Delpon, E. New insights into the pharmacology of sodium channel blockers. *European Heart Journal*. 13 (Supplement F): 2-13, 1992.

Thale, J., Haverkamp, W., Hindricks, G., and Gulker, H. Comparative investigations on the antiarrhythmic and electrophysiologic effects of class I-IV antiarrhythmic agents following acute coronary artery occlusion. *European Heart Journal*. 8 (Supplement G): 91-98, 1987.

Thormann, J., Mitrovic, V., Riedel, H., Neuzner, J., Strasser, R., Behavar, H., and Schlepper, M. Tedisamil (KC 8857) is a new specific bradycardic drug: does it also influence myocardial contractility? Analysis by the conductance (volume) technique in coronary artery disease. *American Heart Journal*. 125: 1233-1246, 1993.

Tsuchihashi, K., and Curtis, M.J. Influence of tedisamil on the initiation and maintenance of ventricular fibrillation: chemical defibrillation by I_{to} blockade? *Journal of Cardiovascular Pharmacology*. 18: 445-456, 1991.

Waldo, A.L., Camm, A.J., deRuyter, H., Friedmann, P.L., MacNeil, D.J., Pauls, J.F., Pitt, B., Pratt, C.M., Schwartz, P.J., and Veltri, E.P. The SWORD investigators: Effect of d-sotalol on mortality in patients with left ventricular dysfunction after recent and remote myocardial infarction. *Lancet*: 348: 7-12, 1996.

Walker, M.J.A., and Beatch, G.N. Electrically induced arrhythmias in the rat. *Proceedings of the Western Pharmacology Society*. 31: 167-170, 1988.

Walker, M.J.A., Curtis, M.J., Hearse, D.J., Campbell, R.W.F., Janse, M.J., Yellon, D.M., Cobbe, S.M., Coker, S.J., Harness, J.B., Harron, D.W.G., Higgins, A.J., Julian, D.G., Lab, M.J., Manning, A.S., Northover, B.J., Parratt, J.R., Riemersma, R.A., Riva, E., Russell, D.C., Sheridan, D.J., Winslow, E., and Woodward, B. The Lambeth Conventions: guidelines for the study of arrhythmias in ischaemia, infarction, and reperfusion. *Cardiovascular Research*. 22: 447-452, 1988.

Wang, L., Chiamvimonvat, N., and Duff, H.J. Interaction between selected sodium and potassium channel blockers in guinea pig papillary muscle. *The Journal of Pharmacology and Experimental Therapeutics*. 264: 1056-1062, 1993.

Weidmann, S. Effects of calcium ions and local anaesthetics on electrical properties of Purkinje fibers. *Journal of Physiology*. 129: 568-582, 1955.

Wessinger, W.D. Approaches to the study of drug interactions in behavioral pharmacology. *Neuroscience & Biobehavioral Reviews*. 10: 103-113, 1986.

Wettwer, E., Amos, G., Gath, J., Zerkowski, H.R., Reidmister, J.C., and Ravens, U. Transient outward current in human and rat ventricular myocytes. *Cardiovascular Research*. 27: 1662-1669, 1993.

Wettwer, E., Himmel, H.M., Amos, G.J., Li, Q., Metzger, F., and Ravens, U. Mechanism of block by tedisamil of transient outward current in human ventricular subepicardial myocytes. *British Journal of Pharmacology*. 125: 659-666, 1998.

Wilde, A.A.M., and Janse, M.J. Electrophysiological effects of ATP sensitive potassium channel modulation: implications for arrhythmogenesis. *Cardiovascular Research*. 28: 16-24, 1994.

Wilde, A.A.M. ATP and the role of $I_{K(ATP)}$ during myocardial ischaemia: controversies revive. *Cardiovascular Research*. 35: 181-183, 1997.

Winslow, E. Methods for the detection and assessment of antiarrhythmic activity. *Pharmacology and Therapeutics*. 24: 401-433, 1984.

Wit A.L., Rosen, M.R., and Hoffman, B.F. Electrophysiology and pharmacology of cardiac arrhythmias. II. Relationship of normal and abnormal electrical activity of cardiac fibers to the genesis of arrhythmias. *American Heart Journal*. 88: 515-524, 1974.

Yatani, A., Brown, A.M., and Akaike, N. Effect of extracellular pH on sodium current in isolated, single rat ventricular cells. *Journal of Membrane Biology*. 78: 163-168, 1984.

Ye, V.Z., Wyse, K.R., and Campbell, T.J. Lidocaine shows greater selective depression of depolarized and acidotic myocardium than propafenone: possible implications for proarrhythmia. *Journal of Cardiovascular Pharmacology*. 21: 47-55, 1993.

Yong, S.L., Xu, R., McLarnon, J.G., Zolotoy, A.B., Beatch, G.N., and Walker, M.J.A. RSD 1000: a novel antiarrhythmic agent with increased potency under acidic and high-potassium conditions. *Journal of Pharmacology and Experimental Therapeutics*. 289: 236-244, 1999.

Yin, H., El-Sherif, N., Caref, E.B., Ndrepepa, g., Levin, R., Isber, N., Stergiopolus, K., Assadi, M.A., Gough, W..B., and Restivo, M. Actions of lidocaine on reentrant ventricular rhythms in the subacute myocardial infarction period in dogs. *American Journal of Physiology*. 272: H299-H309, 1997.

Zar, J.H. Biostatistical analysis. Second edition. Prentice-Hall, Inc., Englewood Cliffs, New Jersey, chapters 6, 11 and 12, 1984.

Zhi, J. and Levy, G. Isobolographic assessment of the convulsant interaction between theophylline and caffeine or pentylenetetrazol in rats. *Journal of Pharmaceutical Sciences*. 79: 678-681, 1990.