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Ischaemia selectivity confers efficacy for suppression of ischaemia-induced arrhythmias in rats

Terrance D. Barrett [a], Eric S. Hayes [b], Sandro L. Yong [a], Alexander B. Zolotoy [c], Shlomo Abraham [d], Michael J.A. Walker [a,*]

[a] *Department of Pharmacology and Therapeutics, Faculty of Medicine, University of British Columbia, 2176 Health Sciences Mall, Medical Block C, Vancouver, BC, Canada V6T 1Z3*

[b] *Institute of Reproduction and Development, Monash University, Level 5, Monash Medical Centre, 246 Clayton Road, Melbourne, Victoria 3168, Australia*

[c] *Nortran Pharmaceuticals Inc., 3650 Wesbrook Mall, Vancouver, BC, Canada V6S 2L2*

[d] *Israel Institute for Biological Research, P.O. Box, 19, Ness-Ziona 70450, Israel*

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Abstract

Eight novel and three reference antiarrhythmics were investigated in anaesthetised rats for antiarrhythmic actions, as well as for effects on the electrocardiogram (ECG) under normal and ''simulated ischaemic'' conditions. In rats subjected to coronary artery occlusion lidocaine, (±)-*trans*-[2-(4-morpholinyl)-cyclohexyl]naphthyl-1-acetate, RSD1000 and (±)-*trans*-[2-(4-morpholinyl)-cyclohexyl]-2-(1-naphthyl)propionate, RSD1030, (Group A) produced dose-related and complete antiarrhythmic protection. Group B compounds, such as (±)-*trans*-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-3, 4-dichlorocinnamamide, RSD995, produced complete antiarrhythmic protection but had aberrant dose–response curves. Group C compounds, such as quinidine and flecainide, failed to give full antiarrhythmic protection and had shallow dose–response curves. The potency of Group A compounds, but not Group B or C compounds, for ECG actions indicative of Na^+ channel blockade (prolongation of PR and QRS intervals) were significantly increased under ''simulated ischaemic'' conditions ([K^+] 10 mM and pH 6.4) in isolated rat hearts. Thus, compounds with ischaemia-selective actions provided superior protection against ischaemia-induced arrhythmias in rats.

Keywords: Antiarrhythmic; Class I; Myocardial ischaemia; Arrhythmia; Proarrhythmia

1. Introduction

A variety of strategies have been adopted in the search for better antiarrhythmics. One strategy centres upon highly selective and potent blockers of specific cardiac ion channels, e.g., Na^+ and delayed rectifier K^+ channels (see The Sicilian Gambit, Anonymous, 1991). Unfortunately, the drugs that have been developed using this strategy are often far from ideal. Examples of such drugs include flecainide in the Cardiac Arrhythmia Suppression Trial (CAST, 1989) for Na^+ channel blockers and D-sotalol in the Survival With ORal D-sotalol trial for K^+ channel blockers (Waldo et al., 1996). Na^+ channel blocking drugs can precipitate the substrate for re-entry arrhythmias (i.e., uni-directional block) and cause the very arrhythmias they were meant to prevent. Drugs that prolong action potential duration, such as the I_{Kr} blockers, are antiarrhythmic by virtue of increasing refractoriness in normal tissue (Singh and Vaughan-Williams, 1970). However, excessive or inappropriate prolongation of action potential duration in normal tissue can cause arrhythmias such as torsades de pointes (Nattel, 1998). Thus, the proarrhythmic actions of such drugs undermine their efficacy.

A second strategy utilises frequency dependent blockade, i.e., ion channel blockade that increases with heart rate (e.g., Campbell, 1983; Hondeghem and Katzung, 1984; Hondeghem and Snyders, 1990). This approach has thus far also been limited in its success, by lack of efficacy, excess toxicity, or both (Guerra et al., 1998). Thus, neither of the first two strategies has been particularly successful, especially in preventing the ventricular tachyarrhythmias causing sudden death.

* Corresponding author. Tel.: +1-604-822-9513; fax: +1-604-822-9578.

E-mail address: rsdaa@unixg.ubc.ca (M.J.A. Walker).

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A third strategy is to develop drugs selective for the pathological processes responsible for the genesis of arrhythmias, in particular, ventricular arrhythmias due to myocardial ischaemia (Bain et al., 1997). Thus, the ischaemic myocardium can be targeted since such tissue is fundamentally responsible for the genesis of ischaemia-induced arrhythmias. If normal tissue is chosen as the target for ion channel blocking antiarrhythmic drugs, then there is always the potential for pro-arrhythmic actions (Shaw and Rudy, 1995).

A pathologically targeted approach has led to the synthesis of a new series of compounds specifically tested for their actions against arrhythmias induced by coronary occlusion in rats (Bain et al., 1997). In such tests, we found that these compounds, plus reference antiarrhythmics, varied greatly in their antiarrhythmic effects. Thus, some compounds provided 100% protection at high doses, dose–response curves with steep slopes and precise ED50 values, while other compounds did not. For the latter compounds, maximum protection was below 40%, the slope of the dose–response curve was low and the coefficient of variation (standard deviation mean^{-1}) for the ED50 estimate was high. The results of this study demonstrate that drugs which act on the ischaemic myocardium provide better antiarrhythmic protection against arrhythmias induced by myocardial ischaemia.

2. Methods

The following studies were performed in male Sprague–Dawley rats weighing 250–350 g. The protocol was approved by the University of British Columbia Animal Care Committee.

2.1. Electrical stimulation studies

Haemodynamic, electrocardiographic (ECG) and electrophysiological variables were measured as previously described (Penz et al., 1992; Walker and Beatch, 1988). Briefly, rats were anaesthetised with pentobarbital (65 mg kg^{-1} i.p.) and a jugular vein and carotid artery cannulated. Tracheotomy was performed and rats were ventilated with oxygen at 60 breaths min^{-1} and 10 ml kg^{-1}. A lead II ECG was recorded from subcutaneous pin electrodes. Mean blood pressure, heart rate and ECG intervals (PR, QRS and QT) were recorded. Measurements were made from a Grass polygraph trace taken at a chart speed of 100 mm^{-1}. All measurements were made 5 min after commencing the infusion.

Electrical stimulation of normal hearts in situ was facilitated by two fine Teflon™-coated silver wires inserted into the apical wall of the left ventricle. Briefly, Teflon™-coated silver wire was passed through a 27-gauge needle and the tip of the wire was bent to form a barb. This barb ensured that the electrode would remain anchored in the left ventricle when the needle was withdrawn. A small incision was made in the skin over the heart to facilitate palpitation of the heart, and the electrode was inserted through the chest wall into the left ventricle. Thoracotomy was not performed. The inter-electrode distance was usually ~2 mm (assessed post mortem).

Hearts were stimulated using square wave pulses of 1-ms duration at a rate of 7.5 Hz delivered from a Grass SD9 stimulator. The variables measured included: threshold currents for capture (iT — μA) and ventricular fibrillo-flutter (VFT — μA), as well as effective refractory period (ERP — ms). All determinations were made in triplicate.

All responses to electrical stimulation were assessed from the ECG as described below. The value of iT was found by determining the least current required to sustain the basic frequency. ERP was determined at a cycle length of 133 ms (7.5 Hz). Hearts were paced for 10 beats, after which, an extrastimulus was introduced. The coupling interval was systematically increased from the starting value of 20 ms. ERP was defined as the shortest coupling interval which failed to produce a response. VFT was the minimum current required to induce a characteristic VFT pattern on the ECG, plus an accompanying fall in blood pressure, using 50-Hz square wave stimulation (Winslow, 1984). VF was sustained only during electrical stimulation and typically lasted less than 5 s. Drugs were infused in a dose-doubling regime. Blood pressure, heart rate and ECG responses were measured 3 min after commencing infusion while electrical stimulation variables were measured between 3–5 min after commencing the infusion.

We have previously found (Barrett et al., 1995; Beatch et al., 1991; Penz et al., 1992; Pugsley et al., 1992a,b) that such ECG and electrical stimulation studies give functional in vivo estimates of the ion channel (Na^+ and K^+) blocking actions of drugs. The same is also true of ECG measures made in isolated hearts in vitro.

2.2. Isolated heart studies

Effects on local cardiac electrograms were assessed in vitro using a modified Langendorff isolated heart apparatus (Curtis et al., 1986). Rats were anaesthetised with pentobarbital (70 mg kg^{-1} i.p. plus heparin 1000 U i.p.). Hearts were excised and washed with ice cold modified Krebs (see below) before being perfused at a constant pressure of 100 mm Hg and 35°C. After stabilisation for 15 min, hearts were assigned to receive increasing concentrations of test compound, either in normal buffer or in a ''simulated ischaemic'' buffer.

The normal perfusate contained (mM): NaCl 123, KCl 3.4, $MgSO_4 \cdot 7H_2O$ 1.2, PIPES 14.4, glucose 11.1, $CaCl_2 \cdot 2H_2O$ 2.5 titrated with NaOH to pH 7.4. The ''simulated global ischaemic'' perfusate contained (mM): NaCl 117, KCl 10.1, $MgSO_4 \cdot 7H_2O$ 1.2, PIPES 15.3, glucose 11.1, $CaCl_2 \cdot 2H_2O$ 2.5 titrated with NaOH to pH 6.4. The lowered pH and elevated $[K^+]$ was designed to mimic

extracellular conditions found in the ischaemic myocardium in vivo at a time when arrhythmias occurred (Botting et al., 1985).

A local cardiac electrogram was recorded from a pair of silver electrodes placed on the right atrium and left ventricle. Cumulative concentration–response curves were obtained for effects on the PR interval and QRS duration of the local cardiac electrogram, ventricular pressure and heart rate in hearts perfused with normal or ''simulated ischaemic'' buffer. Drugs were administered in a cumulative manner in 0.5 $\log_{10}$ multiples with each concentration applied for 3 min until atrio-ventricular block occurred. All measurements were made 3 min after commencing perfusion with the drug.

2.3. Ischaemia-induced arrhythmias

Regional ischaemia was induced by occlusion of the left anterior descending coronary artery as previously described (Barrett et al., 1995; Paletta et al., 1989). Briefly, rats were anaesthetised with pentobarbital (65 mg kg^{-1}, i.p.) and a jugular vein and carotid artery cannulated. Rats were intubated, via a tracheotomy, and ventilated with oxygen at 60 breaths min^{-1} and 10 ml kg^{-1}. The chest was opened and a polypropylene suture was loosely place around the left anterior descending artery. Thereafter, the chest was closed and the air evacuated from the chest to prevent pneumothorax.

Animals were allowed to recover for 15 min before the material under test was infused continuously starting 5 min prior to occlusion. Monitoring continued for 20 min after occlusion in order to cover the first phase (5–10 min after occlusion) of ischaemia-induced arrhythmias (Curtis et al., 1987).

At the end of 20 min, the heart was excised and the size of the occluded zone was measured by perfusing the heart with cardiogreen dye (0.5 mg ml^{-1}). The occluded zone size was calculated as a percentage of total ventricular mass. Animals were excluded if the occluded zone size was $< 25\%$ or $> 50\%$ of the total ventricular mass. Serum $[K^+]$ was measured before occlusion and animals excluded if the value exceeded 3.5 mM (Saint et al., 1992). All experiments were performed in a blinded fashion using random block design.

2.4. Analysis and statistics

Dose–response curves for blood pressure, heart rate, ECG and local cardiac electrograms (in vitro), as well as electrical stimulation variables, were plotted using best-fit algorithms for individual animals. From these curves, the lowest dose (D) or concentration (C) required to produce a 25% change from the pre-drug value ($D_{25\%}$ or $C_{25\%}$) was interpolated. These values were pooled as appropriate for calculation of the mean and standard error of mean. Statistical significance was tested using analysis of variance (ANOVA) at a significance level of $P < 0.05$ and a Student–Newman–Keuls test for multiple comparisons.

Dose–response curves for antiarrhythmic actions were plotted using an arrhythmia score (AS) since the alternative data, such as quantal incidence of ventricular tachycardia or fibrillation, were less reliable. The Lambeth conventions recommend that such scores can be used to summarise data, but not replace it (Walker et al., 1988). In figures containing antiarrhythmic data, group incidences of ventricular tachycardia and fibrillation are included as an indication of the reliability of the arrhythmia score data. The arrhythmia score used summarises an animal's arrhythmic history as a single, normally (Gaussian) distributed variable (score A from Curtis and Walker, 1988), which takes into account the occurrence, severity and duration of arrhythmias.

The antiarrhythmic response for each dose (AS_{test}) was expressed as the percent protection ($\%P_{test}$) relative to the arrhythmia score in the control group ($AS_{control}$) according to Eq. 1.

$$\%P_{test} = 100 - 100 * (AS_{test}/AS_{control}) \quad (1)$$

By expressing data this way, the effect of the drug is easily seen as an increase in the percent protection. The dose–response data was then fit to a logistic function (Eq. 2) using Slide Write version 2.

$$\%P = \text{maximum} * D^h/(D^h + ED50^h) \quad (2)$$

In Eq. 2, ''D'' is the infused dose in μmol kg^{-1} min^{-1}, ''h'' is the slope factor and ''ED50'' is the dose required to provide 50% of maximum protection. The base line was defined as the occurrence of arrhythmias in controls (i.e., 0% protection, AS = 5.4) while the maximum response was defined as 100% protection (i.e., AS = 0, which corresponds to the complete abolition of arrhythmias).

Statistical analyses showed no significant differences between the vehicle controls from different blocks, and therefore, such groups have been combined. The resulting mean arrhythmia score for controls was 5.4 ± 1.7 (mean ± S.D., $n = 75$). This value is not different from our laboratory controls (AS 5.7 ± 1.7, $n > 1000$). In rats used in the present study, 99%, 97% and 83% had premature ventricular beats, ventricular tachycardia and ventricular fibrillation, respectively. The average number of premature ventricular beats, ventricular tachycardia and ventricular fibrillation in animals in which they occurred, was 111 ± 108, 17 ± 23 and 3 ± 3, respectively. The average duration of ventricular tachycardia was 13 ± 23 s while for ventricular fibrillation, the average duration was 89 ± 67 s.

In view of the fact that all algorithms used for curve fitting have limitations, we also used a subjective ranking system for dose–response curves as verification of the pattern recognition we obtained using the logistic algo-

rithm. In a blinded manner, 10 pharmacologist from the Department of Pharmacology and Therapeutics at UBC were asked to rank curves according to their perception of how well data fit the classic sigmoid-shaped dose–response curve.

2.5. Compounds and drugs

The following test compounds were synthesised by Nortran Pharmaceuticals (Vancouver, Canada): (±)-*trans*-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl] benzo[b] thiophene-3-acetamide hydrochloride, RSD944; (±)-*trans*-*N*-methyl-*N*-{2-[1-bis(2-methoxyethylamine)]cyclohexyl]} (3,4-dichlorophenoxy) acetamide hydrochloride, RSD981; (±)-*trans*-*N*-[2-(1-pyrrolidinyl)]-cyclohexyl](3,4-dichlorophenoxy) acetamide hydrochloride, RSD988; (±)-*trans*-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-3,4-dichlorocinnamamide hydrochloride, RSD995; (±)-*trans*-*N*-[2-(1-morpholinyl)-cyclohexyl] benzo[b]thiophene-3-acetamide hydrochloride, RSD996; (±)-*trans*-[2-(4-morpholinyl)-cyclohexyl]naphthyl-1-acetate, RSD1000; (±)-*trans*-[2-(4-methyl)-1-piperazinyl]naphthyl-2-acetate hydrochloride, RSD1015 and (±)-*trans*-[2-(4-morpholinyl)-cyclohexyl]-2-(1-naphthyl)propionate hydrochloride, RSD1030. Quinidine, lidocaine and flecainide were purchased from commercial sources. RSD compounds, quinidine and flecainide were dissolved in a mixture of 22% ethanol and 78% distilled water, whereas lidocaine was dissolved in 10% dimethylsulfoxide, 10% ethanol and 80% distilled water.

3. Results

3.1. Blood pressure and heart rate effects in *vivo*

Infusions of all compounds and drugs reduced blood pressure and heart rate in a dose-related manner, but with varying potencies. All dose–response relationships for indices of Na^+ and K^+ channel blockade in vivo were monotonic and without anomalies. Table 1 summarises the $D_{25\%}$ values for effects on blood pressure and heart rate. RSD944, RSD995 and flecainide were the most potent while RSD981, RSD1000 and RSD1030 were the least.

3.2. Electrophysiological effects in *vivo*

Putative ion channel blocking actions were assessed in terms of changes in the ECG and electrical stimulation variables. All compounds tested increased the PR interval and QRS duration of the ECG, as well as current threshold for induction of an extra systole (iT). Potency values for effects on the PR interval and iT are shown in Table 2. RSD944 and flecainide were the most potent with $D_{25\%}$ values of about 1.0 μmol kg^{-1} min^{-1}. RSD1000 and RSD1030 were the least potent; $D_{25\%}$ values ranged from 14 ± 0.5 to values greater than 40 μmol kg^{-1} min^{-1}. It was not possible to determine a complete potency profile for QRS duration as only 5 out of the 12 compounds tested caused a 25% increase in this variable. All except RSD1030 suppressed the induction of VF (Table 2).

Table 1
Haemodynamic actions of test drugs in vivo
The effects of RSD compounds (by number), quinidine, lidocaine and flecainide on mean arterial blood pressure and heart rate in pentobarbital anaesthetised rats. The lowest dose producing at least a 25% decrease ($D_{25\%}$) in blood pressure and heart rate is given in μmol kg^{-1} min^{-1}, (mean ± S.E.M., $n = 4–6$). Drugs were given as cumulative dose-doubling infusions over different dose-ranges depending on the test drug under investigation. The drug was infused for 5 min and blood pressure and heart rate responses were measured 3 min after commencing the infusion. Blood pressure and heart rate before drug treatment were 130 ± 4 mm Hg (mean ± S.D., range 70–172) and 414 ± 6 beats min^{-1} (range 340–517), respectively.

Test drug RSD#	Blood pressure $D_{25\%}$ (μmol kg^{-1} min^{-1})	Heart rate $D_{25\%}$ (μmol kg^{-1} min^{-1})
944	1.6 ± 0.7	1.0 ± 0.3
981	14.2 ± 7.3	4.2 ± 1.1
988	2.3 ± 0.6	2.5 ± 0.5
995	1.4 ± 0.7	1.4 ± 0.2
996	10.3 ± 4.8	3.6 ± 0.7
1000	2.8 ± 0.8	5.0 ± 1.0
1015	3.2 ± 0.5	4.8 ± 0.8
1030	10.6 ± 3.6	17.5 ± 2.5
Quinidine	1.7 ± 0.7	3.9 ± 0.5
Lidocaine	4.5 ± 2.7	7.5 ± 3.2
Flecainide	1.3 ± 0.3	1.8 ± 0.5

All of the compounds lengthened the QT interval and prolonged the ERP as shown in Table 2. Flecainide and RSD995 were most potent in this regard, 25% 2.4 ± 1.4 and 4.4 ± 1.0 μmol kg^{-1} min^{-1}, respectively. RSD1000 and RSD1030 were the least potent with $D_{25\%}$ values as high as 48 ± 9.2 μmol kg^{-1} min^{-1}. However, all compounds were more potent in their actions on ERP than either the PR interval or QRS duration of the ECG. These $D_{25\%}$ values were very similar to those observed for effects on iT (Table 2).

In general, most of the compounds had more potent Na^+ channel blocking actions when compared with K^+ blocking actions (see Table 2) as assessed in vivo by the means described.

3.3. Potentiating actions of ''simulated ischaemic'' perfusate in isolated rat hearts

As an assessment of the relative selectivity of drug effects for normal vs. ''simulated ischaemic'' conditions, drug-induced changes in the ECG were recorded from isolated rat hearts perfused with normal or ''simulated ischaemic'' perfusate. Table 3 summarises the $C_{25\%}$ values under these conditions. In normal perfusate, RSD944 and RSD995 were the most potent with $C_{25\%}$ values ranging from 0.9 ± 0.06 to 6.7 ± 0.8 μM. RSD1000 and RSD1030

Table 2
Potency for ECG and electrical stimulation effects in vivo
The influence of RSD compounds (by number), as well as of quinidine, lidocaine and flecainide on ECG and electrophysiological properties of the rat heart in vivo. The lowest dose producing at least a 25% increase ($D_{25\%}$) in the ECG or electrophysiological variable indicated is given in μmol kg^{-1} min^{-1}, (mean ± S.E.M., $n = 4–6$). If the compound did not cause a 25% change in the variable in question, then the maximum dose tested is given. Drugs were given as cumulative dose-doubling infusions over different dose-ranges depending on the test drug under investigation. The drug was infused for 5 min and ECG responses measured 3 min after commencing infusion while responses to electrical stimulation were measured between 3–5 min after commencing infusion. Pre-drug values for PR, QRS and QT intervals of the ECG were 56 ± 1 ms (mean ± S.D., range 42–68 ms), 29 ± 0.4 ms (range 23–32 ms) and 37 ± 1 ms (range 27–42 ms), respectively. While for iT, ERP and VFT, the pre-drug values were 105 ± 8 μA (range 37–260 μA), 40 ± 2 ms (range 20–60 ms) and 221 ± 20 μA (range 50–540 μA), respectively.

Test drug RSD#	PR $D_{25\%}$ (μmol kg^{-1} min^{-1})	QRS $D_{25\%}$ (μmol kg^{-1} min^{-1})	QT $D_{25\%}$ (μmol kg^{-1} min^{-1})	IT $D_{25\%}$ (μmol kg^{-1} min^{-1})	ERP $D_{25\%}$ (μmol kg^{-1} min^{-1})	VFT $D_{25\%}$ (μmol kg^{-1} min^{-1})
944	1.0 ± 0.4	> 8.0	> 8.0	0.9 ± 0.3	0.6 ± 0.1	0.6 ± 0.1
981	20.0 ± 5.4	36.0 ± 16.2	16.8 ± 3.2	2.8 ± 0.7	2.7 ± 0.8	2.3 ± 0.6
988	5.0 ± 1.0	> 8.0	7.0 ± 1.0	4.0 ± 1.4	2.3 ± 0.6	1.5 ± 0.3
995	3.0 ± 0.6	4.0 ± 1.4	4.4 ± 1.0	1.6 ± 0.4	1.8 ± 0.2	1.1 ± 0.3
996	11.3 ± 2.4	28.9 ± 3.3	8.0 ± 0.9	3.0 ± 0.7	2.3 ± 0.3	1.5 ± 0.1
1000	18.0 ± 5.0	> 32.0	20.0 ± 4.0	14.0 ± 5.0	4.4 ± 1.0	9.2 ± 5.8
1015	9.6 ± 1.6	> 16.0	10.0 ± 2.0	5.0 ± 1.0	4.5 ± 1.3	4.5 ± 1.3
1030	> 40.0	> 40.0	> 40.0	15.0 ± 2.9	11.3 ± 3.1	> 25.0
Quinidine	9.5 ± 2.4	20.0 ± 8.1	5.3 ± 1.0	2.4 ± 0.6	1.1 ± 0.2	1.4 ± 0.3
Lidocaine	> 16.0	> 16.0	> 16.0	11.0 ± 1.1	6.6 ± 1.5	5.4 ± 2.0
Flecainide	1.8 ± 0.2	4.5 ± 0.2	2.5 ± 1.4	1.2 ± 0.2	2.0 ± 0.4	1.4 ± 0.4

were the least potent with $C_{25\%}$ values from 19 ± 1.8 to 233 ± 38 μM.

In ''simulated ischaemic'' buffer, RSD944 and RSD995 retained potency ($C_{25\%}$ values of 0.5 ± 0.08 and 1.5 ± 0.3 μM, respectively), whereas for RSD1000 and RSD1030, potency was dramatically increased. For the latter compounds, $C_{25\%}$ values ranged from 0.3 ± 0.1 to 0.4 ± 0.1 μM, respectively. This relative selectivity for conditions of ''simulated ischaemia'' is expressed in Table 3 as a ratio of the potency for effects on the PR interval and QRS duration (''simulated ischaemic'' buffer $C_{25\%}$/normal buffer $C_{25\%}$; $C_{25\%I}/C_{25\%N}$). Ischaemic/normal (I/N) ratios close to unity indicate poor ischaemic tissue selectivity, whereas ratios much less than unity indicate selectivity for ischaemic tissue. Good selectivity was seen with RSD1000 and RSD1030, whereas it was much less with quinidine, flecainide, RSD944 and RSD995.

3.4. Dose-related effects on ischaemia-induced arrhythmias

Antiarrhythmic protection against ischaemia-induced arrhythmias was dependent on both the drug and dose administered. Three different types of dose–response

Table 3
Drug effects on ECG intervals in hearts perfused with normal or ''simulated ischaemic'' buffer in vitro
The effect of RSD compounds (by number), quinidine, lidocaine and flecainide on the PR interval and QRS duration of the rat hearts perfused with normal or ''simulated ischaemic'' buffer in vitro. The concentration (in μM) required to produce a 25% increase ($C_{25\%}$) in the PR interval and QRS duration in each of the buffers is given (mean ± S.E.M., $n = 3–6$). I/N ratios were calculated from average $C_{25\%}$ values for the PR interval in normal and ''simulated ischaemic'' buffer. Changes in ECG intervals were measured 3 min after commencing perfusion with the drug. Pre-drug values for PR interval and the QRS duration in hearts perfused with normal buffer were 63 ± 3 ms (mean ± S.D.) and 32 ± 3 ms, while in hearts perfused with ''simulated ischaemic'' buffer, these values were 76 ± 3 and 40 ± 5 ms, respectively.

Test drug RSD#	Normal buffer pH = 7.4, [K] = 3.4 mM		''Ischaemic buffer'' pH = 6.4, [K] = 10.1 mM		I/N ratio PR	I/N ratio QRS
	PR $C_{25\%}$ (μM)	QRS $C_{25\%}$ (μM)	PR $C_{25\%}$ (μM)	QRS $C_{25\%}$ (μM)	$C_{25\%I}/C_{25\%N}$	$C_{25\%N}/C_{25\%I}$
944	0.9 ± 0.06	2.2 ± 2.1	0.5 ± 0.08	1.5 ± 0.3	0.63	0.68
981	16.0 ± 2.8	28.0 ± 2.0	0.3 ± 0.05	0.9 ± 0.3	0.02	0.03
988	9.3 ± 2.0	16.0 ± 0	2.0 ± 0	5.3 ± 0.8	0.21	0.33
995	1.8 ± 0.6	6.7 ± 0.8	1.0 ± 0.3	1.2 ± 0.2	0.54	0.18
996	2.7 ± 0.6	4.3 ± 0.7	0.7 ± 0.7	0.7 ± 0.7	0.26	0.16
1000	19.3 ± 1.8	48.0 ± 2.9	0.3 ± 0.02	0.4 ± 0.05	0.02	0.008
1015	3.3 ± 0.5	3.3 ± 0.5	1.2 ± 0.1	2.2 ± 0.2	0.35	0.67
1030	53.0 ± 13.0	233.0 ± 38	0.3 ± 0.1	0.3 ± 0.1	0.006	0.001
Quinidine	12.8 ± 2.3	65.0 ± 8.8	8.4 ± 1.5	5.4 ± 1.3	0.65	0.08
Lidocaine	95.0 ± 18.0	75.0 ± 19	14 ± 2.0	11.0 ± 1.6	0.15	0.15
Flecainide	4.4 ± 0.4	12.0 ± 1.1	1.8 ± 0.3	2.0 ± 0.2	0.41	0.17

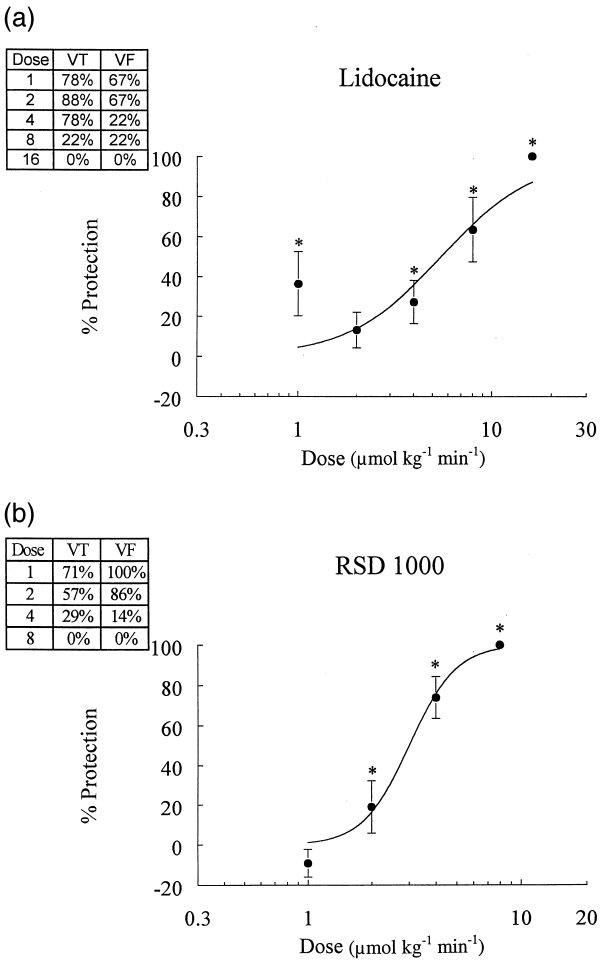

Fig. 1. Dose–response curves for (a) lidocaine and (b) RSD1000-induced suppression of ischaemia-induced arrhythmias in pentobarbital anaesthetised rats. Drugs were infused starting 5 min before the coronary artery occlusion and continued for 25 min. Each point represents the mean arrhythmia score ± S.E.M., $n = 7–9$. The line represents the best fit to a logistics function as described in the text. The ED50 and slope of the antiarrhythmic dose–response curves for lidocaine and RSD1000 were 5.8 ± 5.2 and 3.0 ± 0.3 μmol kg^{-1} min^{-1}, and 1.8 ± 0.6 and 3.9 ± 1.2, respectively. The curve–fit coefficients (r) were 0.66 and 0.83, respectively. An asterisk ''*'' indicates a significant difference from control ($P < 0.05$).

curves, as assessed from the arrhythmia score, were observed and classified as being sigmoidal, aberrant or incomplete. Effects on the incidence of high frequency arrhythmias, ventricular tachycardia and fibrillation, substantiated the dose–response curve analyses in which the arrhythmia score was used (see Figs. 1–3).

In the case of sigmoidal curves, the arrhythmia score, as well as the incidence of ventricular tachycardia and fibrillation, were clearly reduced in a dose-dependent manner. Additionally, both types of arrhythmias were completely abolished at the highest dose(s) tested (e.g., lidocaine, RSD1000 and RSD1030; Group A). In other cases, the arrhythmia score and the incidence of ventricular tachycardia and fibrillation appeared to increase in the middle portion of the dose range with a complete, or near com-

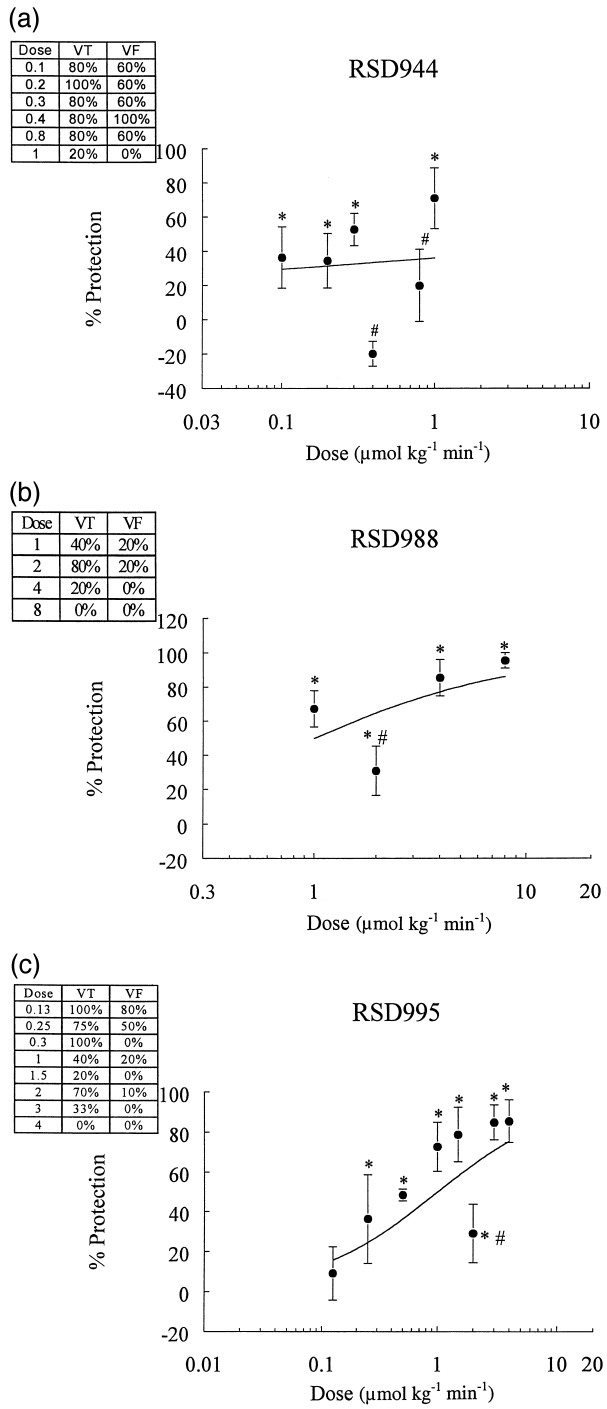

Fig. 2. Dose–response curves for (a) RSD944, (b) RSD988 and (c) RSD995-induced suppression of ischaemia-induced arrhythmias in pentobarbital anaesthetised rats. Drugs were infused starting 5 min before the coronary artery occlusion and continued for 25 min. Each point represents the mean arrhythmia score ± S.E.M., $n = 7–9$. The line represents the best fit to a logistics function as described in the text. The ED50s for the three compounds were 79 ± 14, 1.0 ± 0.6 and 0.8 ± 0.3 μmol kg^{-1} min^{-1}, for RSD944, RSD988 and RSD995, respectively. The slope factors for the same series were 0.1 ± 0.4, 1.8 ± 0.5 and 0.7 ± 0.3. Curve–fit coefficients (r) were 0.05, 0.39 and 0.41, respectively. An asterisk ''*'' indicates a significant difference from control ($P < 0.05$) while ''#'' indicates a significant difference from the next highest and lowest dose.

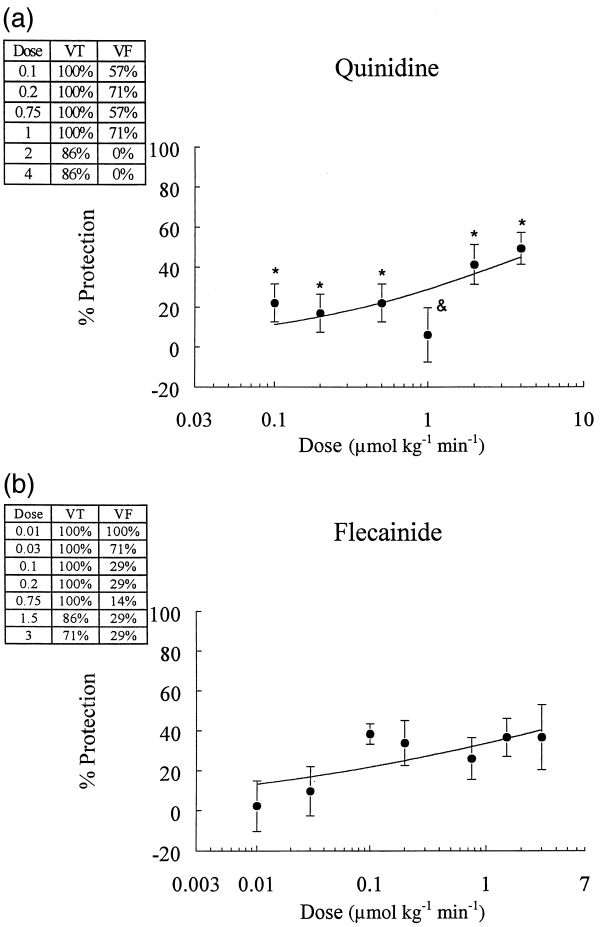

Fig. 3. Dose–response curves for (a) quinidine and (b) flecainide-induced suppression of ischaemia-induced arrhythmias in pentobarbital anaesthetised rats. Drugs were infused starting 5 min before coronary artery occlusion and continued for 25 min. Each point represents the mean arrhythmia score ± S.E.M., $n = 7$. The line represents the best fit to a logistics function as described in the text. The ED50 and slope of the antiarrhythmic dose–response curves for quinidine and flecainide were 5.8 ± 4.0 and 13.3 ± 24 μmol kg^{-1} min^{-1}, and 0.5 ± 0.2 and 0.26 ± 0.13, respectively. The curve–fit coefficients (r) were 0.41 and 0.51, for quinidine and flecainide, respectively. An asterisk ''*'' indicates a significant difference from control ($P < 0.05$) and ''&'' indicates a significant difference from the next highest dose (quinidine).

plete, reduction of one or both types of arrhythmias at the highest doses tested (e.g., RSD981, RSD988 and RSD995; Group B).

For some drugs, the incidence of ventricular fibrillation, but not ventricular tachycardia, was reduced in a dose-dependent fashion such that the dose–response curve for antiarrhythmic protection appeared shallow (e.g., RSD944, quinidine and flecainide; Group C). Despite being given at the highest tolerated dose, flecainide failed to abolish ventricular fibrillation. The goodness of fit statistics and parameter estimates for dose–response curves are summarised in Table 4.

Dose–response curves were considered sigmoidal if they had the following characteristics: (1) good fits to the logistic function ($r^2 > 0.65$); (2) high slope coefficient ($h > 1.0$); and (3) produced 100% protection against ischaemia-induced arrhythmias. RSD1000, RSD1030 and lidocaine produced this type of curve (Group A). Fig. 1a–b shows the antiarrhythmic dose–response curves for lidocaine and RSD1000.

Table 4
Curve fit variables for antiarrhythmic dose–response curves
Curve–fit estimates for antiarrhythmic dose–response curves of RSD compounds (by number), quinidine, lidocaine and flecainide. ED50, slope (h) and curve–fit coefficients (r^2) were derived from logistic fits to mean data. Values for the ED50 and the slope are given as the mean ± S.E.M. Each drug was infused, at dose as described in Figs. 1–3, for 5 min before coronary artery occlusion and infusion was continued for 25 min. The subjective rank order is described in the text. Curve–fit estimates for quinidine, lidocaine and flecainide were adapted from Barrett et al. (1995).

Test drug RSD#	ED50 (μmol kg^{-1} min^{-1})	Curve–fit slope (h)	Curve fit coefficient (r^2)	Subjective rank order (mean)
944	79 ± 14	0.1 ± 0.4	0.05	9
981	1.8 ± 0.6	1.1 ± 0.4	0.63	6
988	1.0 ± 0.6	1.8 ± 0.5	0.39	7
995	0.8 ± 0.3	0.7 ± 0.3	0.41	6
996	3.7 ± 0.4	4.3 ± 1.5	0.88	1
1000	3.0 ± 0.3	3.9 ± 1.2	0.83	4
1015	1.3 ± 0.7	0.7 ± 0.5	0.28	8
1030	2.7 ± 0.2	2.1 ± 0.3	0.98	2
Quinidine	5.8 ± 4.0	0.5 ± 0.2	0.41	10
Lidocaine	5.8 ± 5.2	1.8 ± 0.6	0.66	3
Flecainide	13.3 ± 24	0.3 ± 0.1	0.51	11

Aberrant dose–response curves had the following characteristics: (1) poor fits to the logistics function ($r^2 < 0.65$); (2) low slope coefficients ($h < 1.0$); and (3) did not always provide 100% antiarrhythmic protection. This type of curve was often characterised by a prominent notch in the mid or upper portion of the curve. RSD944, RSD988 and RSD995 (Group B) were examples of compounds producing this type of antiarrhythmic dose–response curve (Fig. 2a–c). It is important to note that RSD995 produced a statistically significant ($P < 0.05$) increase in the occurrence of ventricular tachycardia at 2 μmol kg^{-1} min^{-1} (vs. 1.5 and 3 μmol kg^{-1} min^{-1}).

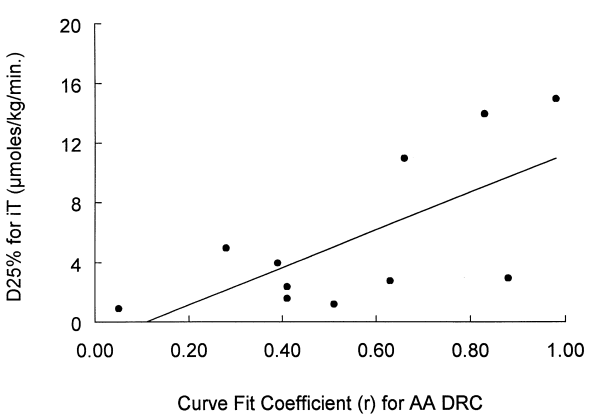

Fig. 4. Correlation between threshold current for capture (iT) and the curve–fit coefficient for antiarrhythmic dose–response curves ($r^2 = 0.68$, $P < 0.01$).

The third type of dose–response curve was incomplete and may be a variant of the aberrant dose–response curve. The dose–response curves produced by RSD1015, quinidine and flecainide are examples of this (Group C). They are characterised by: (1) very poor fits to a logistic function ($r^2 < 0.50$); (2) very shallow slopes ($h < 0.75$); and (3) failure to provide complete antiarrhythmic protection. Fig. 3a–b shows such curves for quinidine and flecainide.

3.5. Correlation of functional electrophysiological effects with antiarrhythmic dose–response curves for ischaemia-induced arrhythmias

$D_{25\%}$ values for effects on threshold for capture (iT) in normal hearts correlated with the curve–fit correlation coefficient (Fig. 4; $r^2 = 0.51$) for the antiarrhythmic dose–response relationships. Compounds that were potent on iT in the normal myocardium (low $D_{25\%}$ for iT) gave poor fits (low curve–fit coefficients). In contrast, compounds with high curve–fit coefficients and slopes (good curves) had low potency for iT. Thus, $D_{25\%}$ values for iT with RSD1000 and RSD1030 were significantly greater than $D_{25\%}$ values for RSD944 and flecainide ($P < 0.005$; Table 2).

Significant correlations were also obtained for $D_{25\%}$ values for ERP and QT and curve–fit coefficients. However, unlike iT, ERP and QT were not powerful predictors of antiarrhythmic activity. The slope factor for the antiarrhythmic dose–response curves correlated poorly with $D_{25\%}$ values for both ERP and QT. The ED50 estimate for antiarrhythmic actions did not correlate well with $D_{25\%}$ values for drug effects on iT ($r^2 = -0.32$, $n = 12$), ERP ($r^2 = -0.34$) or QT ($r^2 = -0.17$) in the normal myocardium.

A significant negative correlation was found between curve fit coefficients for dose–response curves for antiarrhythmic protection against ischaemia-induced arrhythmias and the I/N ratio. Thus, the best fit coefficients were found with those compounds which had the lowest I/N ratio (Fig. 5; $P < 0.05$). The slope factor was also significantly correlated inversely with the I/N ratio ($P < 0.01$). The major finding was that the quality of the dose–response curves for antiarrhythmic protection against ischaemia-induced arrhythmias correlated very significantly with I/N ratios.

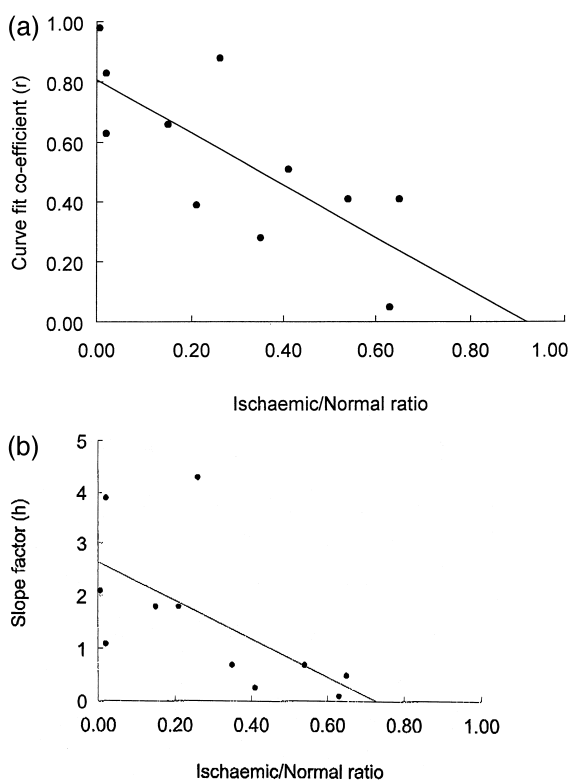

Fig. 5. Correlation between (a) I/N ratio and curve–fit coefficient for antiarrhythmic dose–response curves (r, panel a, $r^2 = 0.76$, $P < 0.01$) and (b) the slope factor (h, panel b, $r^2 = 0.61$, $P < 0.01$).

4. Discussion

Overall, the results of this study showed that compounds with low I/N ratios provided the best antiarrhythmic protection. Compounds with low I/N ratios lacked potency in terms of effects on ECG and electrical stimulation variables in normal tissue. Thus, compounds that were the least potent in preventing electrically induced arrhythmias were the best against ischaemia-induced arrhythmias. Compounds that produced poor dose–response curves did so, not because they lacked potency for electrophysiological effects on normal tissue, but because their potency was not increased in ischaemic tissue.

Compounds with low potency for effects in normal tissue and greater potency under conditions of ''simulated ischaemia'' completely prevented ischaemia-induced arrhythmias (i.e., lidocaine, RSD1000 and RSD1030). Compounds equally potent in normal and ''simulated ischaemic'' tissue (e.g., RSD944, RSD988, RSD995, RSD1015, quinidine and flecainide) rarely produced complete antiarrhythmic protection and the antiarrhythmic dose–response curves were poorly defined. Differential ischaemia-selectivity between lidocaine and flecainide has been previously reported (Campbell and Hemsworth, 1990; Campbell et al., 1991).

Two mechanisms can be postulated as causing the increased potency in ''simulated global ischaemia.'' The first is that ''simulated ischaemia'' increases the potency of the compound for binding to ion channels. All compounds tested in the present study are weak bases. If it is assumed that the protonated species is responsible for ion channel blockade, then at lower pH, the concentration of the active species would be increased, and hence, increased potency seen (lower $C_{25\%}$). Increased potency for

Na^+ channel block has recently been demonstrated for RSD1000 in isolated rat cardiac myocytes (Yong et al., 1999). Another mechanism that may contribute to the potentiation of drug action under ''simulated ischaemic'' conditions is that the compound may have different affinities for different states of the Na^+ channel. An increase in extracellular $[K^+]$ in the ischaemic myocardium results in voltage-dependent inactivation of Na^+ channels. Due to the increased availability of binding sites, the actions of compounds that bind to inactivated state Na^+ channels are increased (Hondeghem and Katzung, 1984; Grant et al., 1980, 1982). A selectivity for inactivated Na^+ channels and potentiation of drug effects by ''simulated ischaemic'' conditions has been shown for lidocaine (Hondeghem and Katzung, 1984; Campbell and Hemsworth, 1990; Campbell et al., 1991).

Reduction of excitability and conversion from uni- to bi-directional block is thought to be one mechanism by which Class I antiarrhythmic drugs prevent arrhythmias (Janse, 1992). Cardinal et al. (1981) have shown that the slowing of conduction and conversion from uni- to bi-directional block with lidocaine occurs more commonly in ischaemic tissue (also see Carson et al., 1986). As ischaemia progresses, uni-directional block will ultimately be converted to bi-directional block (unless the tissue is reperfused), and anything that accelerates this process would render ischaemic tissue electrically silent more quickly and thereby result in antiarrhythmic effects (Hondeghem and Cotner, 1978; Hondeghem et al., 1974; Davis et al., 1985).

With respect to the failure of some drugs to provide good antiarrhythmic protection, irregularities in antiarrhythmic dose–response curves might be produced by the proarrhythmic actions of such drugs in normal tissue and antiarrhythmic effect in ischaemic tissue. A drug with moderate ischaemia-selectivity might be expected to produce such effects for the following reasons. At low doses, the ischaemia-selective actions would predominate and antiarrhythmic activity would be seen, i.e., there would be a decreased participation of the ischaemic zone in arrhythmogenesis. At intermediate doses, proarrhythmic actions due to the slowing of conduction in normal tissue would compete with the antiarrhythmic actions, i.e., some re-entry circuits would be abolished while others created (alternatively stated, the window for re-entry is increased; Hondeghem and Cotner, 1978). At high doses, the ischaemia-selective action of the drug effectively removes the substrate for ischaemia-induced arrhythmias by rendering the ischaemic tissue electrically silent and hence, antiarrhythmic effects once more predominate.

RSD944, RSD988 and RSD995 all fit the above profile and their rank order for ischaemia-selectivity is reflected in their antiarrhythmic dose–response curves. RSD944 was the least selective for ''simulated ischaemic'' conditions and had only limited antiarrhythmic actions. On the other hand, RSD988 was relatively more ''ischaemia''-selective than RSD944 and provided greater antiarrhythmic protection.

A drug with little selectivity for ischaemic tissue would be expected to produce an incomplete antiarrhythmic dose–response curve by generating as many re-entry circuits as it prevented. Ultimately, the maximum antiarrhythmic response produced by such a drug would be limited by cardiovascular, or alternatively, by CNS toxicity. Doses that rendered the ischaemic myocardium electrically silent would have adverse effects on the normal myocardium. Quinidine, flecainide and RSD1015 are examples of such drugs.

Computer simulations of antiarrhythmic dose–response curves based on theoretical summations of anti- and proarrhythmic responses show that various types of dose–response curves can be produced. Sigmoidal curves were observed when the proarrhythmic ED50 was higher than the antiarrhythmic ED50. Aberrant curves were observed when the potencies for the two effects were similar. Furthermore, the slope of the antiarrhythmic dose–response curve was directly related to the extent of overlap between the anti- and proarrhythmic dose–response curves (unpublished observation).

The results of the present study are in agreement with the above model and suggest that ischaemia-selectivity determines the degree of separation between the anti- and proarrhythmic dose–response curves. As the proarrhythmic dose–response curve encroaches on the antiarrhythmic dose–response curve, the resultant curve becomes progressively more shallow. A drug with a large separation between its pro- and antiarrhythmic responses (i.e., one that is highly ischaemia-selective) would be expected to produce a steep and sigmoidal dose–response curve. In the present study, the I/N ratio for Na^+ channel block correlated negatively with both the slope ($r = -0.57$) and curve–fit coefficient ($r = -0.78$) of the dose–response curve (Fig. 5). Thus, it appears that compounds which effectively slow conduction in ischaemic myocardium, but which lack such actions in the normal myocardium, have a greater separation between anti- and proarrhythmic responses.

Many of the drugs tested prolonged the QT interval of the ECG. While the mechanism for this effect was not the focus of these studies, it is likely that these compounds block the transient outward current (I_{to}) in the rat ventricle (Beatch et al., 1991).

In summary, ischaemia-selective ion channel blocking actions are associated with sigmodal dose–response relationships for antiarrhythmic actions in a series of standard and novel antiarrhythmic drugs. Drugs that were potent for producing electrophysiological effects in normal myocardial tissue produced shallow and aberrant antiarrhythmic dose–response curves. The results of the present study demonstrate that ion channel blockade in the ischaemic myocardium provides superior protection against ischaemia-induced arrhythmias in rats.

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