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RSD1019 suppresses ischaemia-induced monophasic action potential shortening and arrhythmias in anaesthetized rabbits

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1 The electrophysiological actions of lidocaine, tedisamil and RSD1019 were assessed on normal and ischaemic cardiac tissue using monophasic action potentials (MAPs) recorded from the epicardium of anaesthetized rabbits. Drug effects on ischaemia-induced arrhythmias were assessed simultaneously in the same rabbits.

2 Lidocaine, infused at 2.5, 5 and 10 μmol kg^{-1} min^{-1} i.v., accelerated and worsened the electrophysiological derangement caused by ischaemia, had profibrillatory actions and reduced the time to the occurrence of ventricular fibrillation (VF) relative to controls.

3 Tedisamil, infused at 0.063, 0.125 and 0.25 μmol kg^{-1} min^{-1} i.v., prolonged MAP duration at 90% repolarization ($MAPD_{90\%}$) before induction of ischaemia in a dose-related manner; however, this effect was not maintained 5 min after induction of ischaemia. Tedisamil had no significant antiarrhythmic actions over the dose-range tested.

4 RSD1019, infused at 2, 4 and 8 μmol kg^{-1} min^{-1} i.v., produced a small increase in $MAPD_{90\%}$ before induction of ischaemia and only at the highest dose tested. In contrast to tedisamil, RSD1019 suppressed ischaemia-induced MAP shortening assessed 5 min after induction of ischaemia. This effect was dose-related. RSD1019 completely prevented ischaemia-induced tachyarrhythmias at the mid and highest infusion levels tested.

5 The results of this study illustrate a pathologically targeted approach for preventing ischaemia-induced arrhythmias. Suppression of ischaemia-induced MAP shortening, demonstrated herein for RSD1019, represents a novel antifibrillatory approach.

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Abbreviations: AP, action potential; $I_{Cl(NA)}$, protein kinase A-activated Cl^- current; I_K, delayed rectifier K^+ current; $I_{K(ATP)}$, ATP-dependent K^+ current; I_{Kr}, rapidly activating delayed rectifier potassium current; I_{to}, transient outward K^+ current; MAP, monophasic action potential; $MAPD_{90\%}$, MAP duration at 90% repolarization; VF, ventricular fibrillation; VT, ventricular tachycardia

Introduction

Ventricular arrhythmias associated with myocardial ischaemia are a major cause of morbidity and mortality for which no complete therapeutic solution has been found. Since the downfall of Class I antiarrhythmics, drug development programs have focused on drugs which delay the recovery of excitability by prolonging action potentials (APs) in normal myocardial tissue. A majority of the drugs developed thus far prolong APs by blocking the rapidly activating delayed rectifier potassium current (I_{Kr}) in ventricular myocytes (Sanguinetti & Jurkiewicz, 1990). Despite the development of a number of drugs with this profile, results from clinical trials have been disappointing (Waldo *et al*., 1996; Torp-Pedersen *et al*., 1999). Of the Class III drugs used clinically, only amiodarone has been shown to be effective in man (Connolly, 1999); however, the pharmacology of this drug is complex and the mechanism for its beneficial actions is not known with certainty.

A number of hypotheses have been put forward to explain the lack of efficacy observed with I_{Kr} blockers in clinical trials. The most popular of these relates to the reverse use-dependent actions of such drugs (Hondeghem & Snyders, 1990). Thus their lack of effect on mortality in clinical trials is attributed to the loss of AP prolongation at high heart rates and its accentuation at low heart rates. The increased AP prolongation at low heart rates is not innocuous since it has been linked to the occurrence of torsades de pointes. It is commonly believed that the proarrhythmic actions of such drugs underlie the increased mortality observed in clinical trials (Nattel, 1998).

An alternative hypothesis that might account for the failure of these drugs to reduce mortality is suggested by the observations of Yang & Roden (1996) and Duff *et al*. (1997). These investigators found that dofetilide, a typical I_{Kr} blocker, was less effective under conditions of raised extracellular [K^+], such as those that occur in the ischaemic myocardium ([K^+] 8–12 mM, Coronel *et al*., 1988). As a result, dofetilide is unable to prolong AP duration in the ischaemic myocardium and thereby prevent arrhythmias. This observation might be particularly important since AP shortening is one of the hallmarks of myocardial ischaemia (Janse & Wit, 1989). In general, regional shortening of APs is associated with an increased risk of arrhythmias. Taken together, these observations suggest that preventing AP shortening in the ischaemic myocardium might prevent ischaemia-induced arrhythmias.

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The objective of the present study was to define the electrophysiological actions of RSD1019 on ischaemic and normal cardiac tissue *in vivo* and to correlate these effects to suppression of arrhythmias. Tedisamil was included in the study as a representative Class III antiarrhythmic drug which might be expected to prolong APs in ischaemic cardiac tissue. Tedisamil might be expected to have such an effect *via* blockade of the transient outward K^+ current (I_{to}; Dukes & Morad 1989), delayed rectifier K^+ current (Dukes & Morad, 1989), ATP-dependent K^+ current ($I_{K(ATP)}$, Bray & Quast, 1992), or the protein kinase A-activated Cl^- current ($I_{CI(NA)}$, Faivre *et al*., 1998). Aside from the well known role of $I_{K(ATP)}$ in mediating ischaemia-induced AP shortening, I_{to} (Lukas & Antzelevitch, 1993), and $I_{CI(NA)}$ (Ruiz-Petrich *et al*., 1996) have also been implicated in the process. The molecular mechanism(s) whereby RSD1019 might have electrophysiological actions on ischaemic tissue have not been investigated in detail; however, RSD1019 has been demonstrated to block the molecular correlate of Ito (Kv 4.2) as well as the ultrarapid delayed rectifier current (Kv1.5; Bain *et al*., 1997). Lidocaine was also included in the study as a standard ischaemia-selective drug (Cardinal *et al*., 1981). Preliminary reports of this work have appeared in abstract form (Barrett *et al*., 1997a; 1998) and as a dissertation (Barrett, 1999).

Methods

The following studies were approved by the University of British Columbia's animal care committee according to internationally accepted guidelines. The method has been previously described (Barrett *et al*., 1997b).

General preparation

Male rabbits (2–3.5 kg) were used. They were kept in house for at least 1 week before the day of the experiment. During this time they had access to standard rabbit chow and water *ad libitum*. On the day of the experiment a marginal ear vein was cannulated and the rabbit was given 10 ml kg^{-1} of 5% dextran-70 in saline before inducing anaesthesia with 50 mg kg^{-1} pentobarbital i.v. Additional pentobarbital was given as required to maintain anaesthesia. After the corneal reflex was abolished, a tracheotomy was performed and the animal was ventilated with 100% oxygen at 25 strokes min^{-1} and a stroke volume of $\sim$10 ml kg^{-1}. Arterial blood pH and P_{CO2} were monitored using an AVO OPTI I blood gas analyser and kept within physiological limits. All rabbits were hyperoxaemic since they were ventilated with oxygen. The jugular vein and left carotid artery were cannulated for injection of drugs and measurement of blood pressure, respectively. A lead II ECG was recorded using subcutaneous pin electrodes. Blood pressure and the ECG were displayed simultaneously on a Grass polygraph (model 79D). ECG intervals were measured from paper traces taken at a chart speed of 100 mm s^{-1}. Body temperature was monitored *via* a rectal thermometer and maintained between 36–40°C using a heating lamp.

A mid-line sternal opening was made and a pericardial cradle formed by suturing the pericardium to the chest wall. Two sutures (5-0 polypropylene) were placed in the heart for traction purposes enabling an unobstructed view of the coronary arteries to be attained. The first of these sutures was placed near the apex of the left ventricle and was used to suture the monophasic action potential (MAP) electrode to the epicardium. Thus the MAP electrode was positioned over the area to be made ischaemic by coronary artery occlusion. The second suture was passed through the margin of the left atrium. Coronary artery occlusion was carried out at two sites in order to obtain an occluded zone size that was consistently associated with the occurrence of ischaemia-induced arrhythmias (Barrett *et al*., 1997b). Bellemin-Baurreau and co-workers (1994) description of the rabbit coronary vasculature was used as a guide. The first snare was placed just distal to the left anterior descending artery where the vasculature branches to form two small arteries in the rabbit. The second snare was placed around the left ventricular branch of the coronary artery as close to its origin as possible (i.e., near the base of the heart). Coronary artery occlusion was produced by simultaneously tightening both snares.

MAP recording

MAPs were recorded from the epicardium of the left ventricle as previously described (Barrett *et al*., 1997b; 1998). A Philbrick instrumentation amplifier (model P65AU) configured in the differential mode was used to amplify signals. A gain of 100 times was used to facilitate analyses. No filters were used to condition the signal. MAPs were displayed on a Tektronics oscilloscope (model R5013N) and recorded digitally (OT2831G-Data Translations A/D board) on a personal computer (486, Trison PC). Global Lab software was used to record MAPs and analysis of MAP recordings was carried out using custom designed software (Dickenson *et al*., 1997). A sampling rate of 5 kHz was used. MAP recordings were archived to video tape (NEC video recorder) after conversion by a pulse code modulator (Medical Systems Corporation PCM-4/8 CO).

Stable MAP recordings were obtained. The following inclusion criteria were used to ensure acceptable records: (1) a flat base line, (2) amplitude greater than 15 mV before starting the experiment, (3) a clear sharp rising phase, (4) a clearly distinguishable plateau phase, and (5) stable morphology. The location of the MAP electrode was confirmed *post mortem*. This ensured that MAPs were recorded from the ischaemic myocardium after coronary artery occlusion was performed. The electrode was never closer than 5 mm to the normal/ischaemic tissue interface. The stability of MAP recordings was assessed by visual inspection for 3–5 min before starting the experiment.

Experimental protocol

The experiment was started 15 min after completing surgical preparation. During this time MAP recording was started. Each animal was randomly assigned to receive one of the following treatments (infusions in μmol kg^{-1} min^{-1}): tedisamil 0.063, 0.125 or 0.25; lidocaine 2.5, 5 or 10; or RSD1019 2, 4 or 8; or vehicle control (10% DMSO, 20% ethanol, 70% distilled water; 1.2 ml total volume). Infusion commenced 5 min before coronary artery occlusion and was continued thereafter (total of 35 min). Drug and drug plus ischaemia-induced changes in MAPs (see later), ECG variables and the occurrence of arrhythmias were monitored for 30 min of occlusion. This time is sufficient to capture both of the early phases of ischaemia-induced arrhythmias (Barrett *et al*., 1997b). Arrhythmias were defined according to the Lambeth conventions (Walker *et al*., 1988). After completing the experiment, the occluded zone size was measured and the position of the MAP electrode confirmed.

Analysis of ischaemia-induced changes in MAP morphology

In addition to analysis of conventional MAP variables (e.g., MAP duration at 90% repolarization), ischaemia-induced changes in MAP morphology were also assessed. These changes in morphology included 'activation alternans' and 'multiple wave forms.' An activation alternan was defined as a repetitive change in morphology which occurred on every other beat (Figure 1). For example, the AP amplitude of the 1st beat was larger than that of the 2nd beat with the 3rd beat having an AP amplitude similar to that of the 1st beat and the 4th beat being similar to the 2nd. The term 'multiple wave forms' was used to describe the occurrence of more complicated patterns than activation alternans in which a repetitive sequence was not apparent (Figure 1). The occurrence of changes in MAP morphology were noted for each min after occlusion.

Measurement of occluded zone size

The size of the occluded zone (zone at risk) was measured by the dye exclusion technique previously described (Barrett *et al*., 1997b). The heart was excised at the end of the experiment and

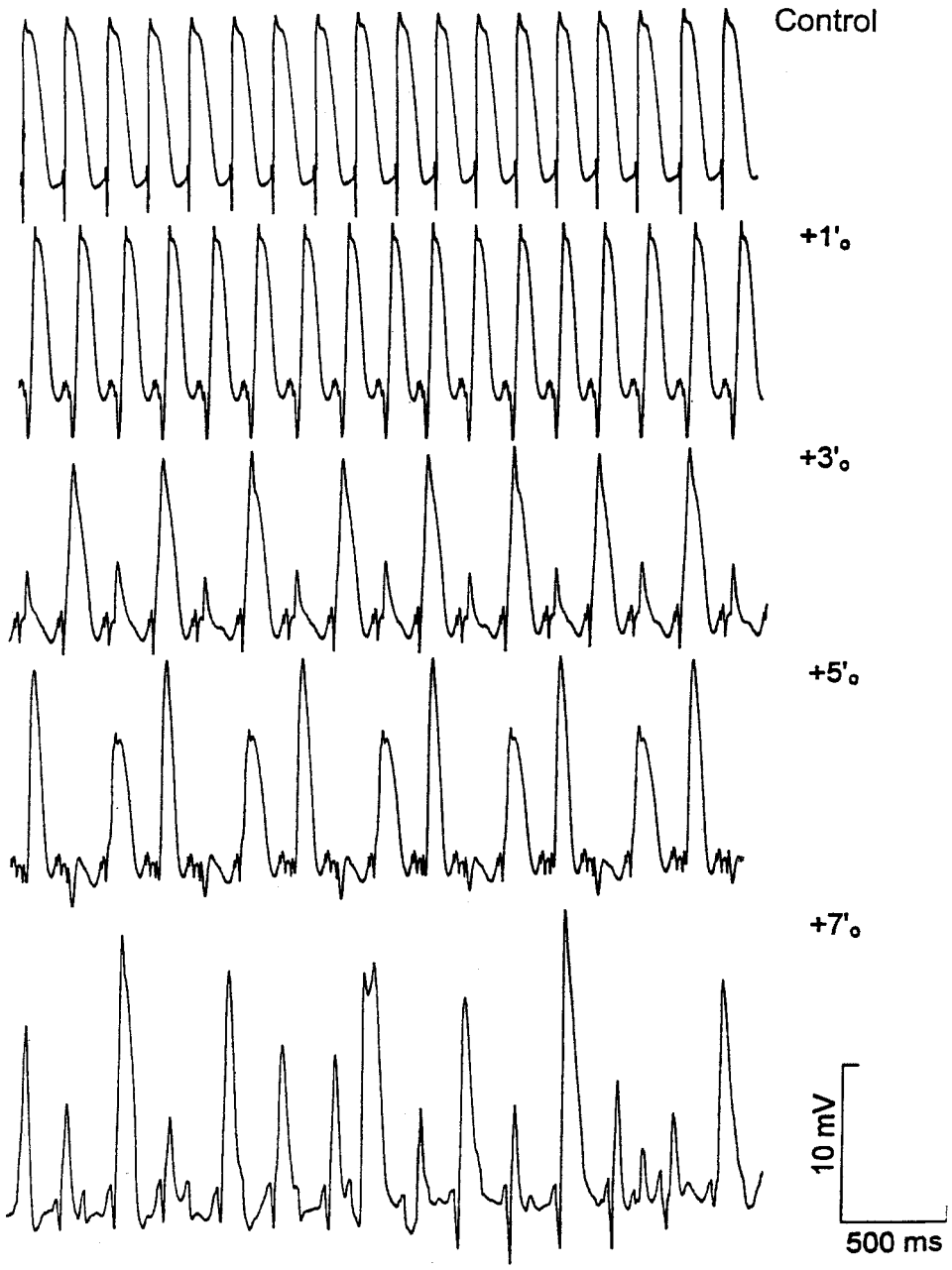

Figure 1 Typical activation alternans and multiple wave forms in MAPs recorded from ischaemic myocardial tissue. Recordings are labelled according to the time after coronary artery occlusion, except for the first recording labelled 'Control' which was taken before induction of ischaemia. All traces were taken during normal sinus rhythm except for the final trace. The final trace was taken during VF. The trace at 3 min shows typical activation alternans while the traces at 5 and 7 min shows multiple wave forms. The trace at 5 min after occlusion is somewhat misleading in that the pattern of multiple wave forms was more regular than was typically observed. When multiple wave forms occurred, MAP morphology could change on a beat to beat basis and repetitive sequences were not commonly observed. This rabbit was treated with 5 μmol kg^{-1} min^{-1} lidocaine i.v. starting 5 min before induction of ischaemia.

perfused by the Langendorff technique with 0.9% saline followed by a saline solution containing dye (0.5 mg ml^{-1} of Cardiogreen™, indocyanne green). The perfusion pressure was 100 mmHg. The occluded zone size was defined as the mass of ventricular tissue not dyed green over the total mass of the heart (expressed as a percentage).

Drugs

Lidocaine was purchased from Sigma. Tedisamil was provided by Kali-Chemi (Hannover, Germany). RSD1019, (±)-trans-[2-(4-morpholinyl)cyclohexyl](4-bromophenyl) acetate monohydrochloride, was a gift from Nortran Pharmaceuticals (Vancouver, Canada).

Analysis and statistics

The effects of treatment on blood pressure, heart rate, ECG intervals and MAP variables were summarized as the mean ± s.e.mean, $n=42$ for controls and $n=7$ for each treated group. Before drug treatment no differences were found between groups. This allowed pre-drug data to be pooled and presented as the mean ± s.e.mean for all of the rabbits included in the study ($n=105$). Arrhythmia data was summarized using an arrhythmia score adapted from score A described by Curtis & Walker (1988).

Statistical significance was tested using analysis of variance followed by Tukey's or Dunnett's test for differences (as considered appropriate). Fisher's exact test was used to test the significance of arrhythmia occurrence data. A probability value of $P<0.05$ was taken as being statistically significant and all statistical tests were two tailed.

Results

Drug effects on blood pressure, heart rate and ECG intervals

Before induction of ischaemia, lidocaine, tedisamil and RSD1019 had similar bradycardic actions; however, these drugs had different effects on blood pressure. The combined group pre-drug heart rate was 217 ± 3 beats min^{-1} (mean ± s.e.mean, $n=105$). The highest dose of lidocaine reduced heart rate by 14 ± 5% ($n=7$, $P<0.05$). The middle and highest dose of tedisamil (0.125 and 0.25 μmol kg^{-1} min^{-1}) reduced heart rate by 15 ± 2% and 27 ± 5% (both $P<0.05$), respectively. RSD1019 reduced heart rate by 15 ± 3% and 20 ± 3% (both $P<0.05$) at the middle and high dose tested (4 and 8 μmol kg^{-1} min^{-1}).

The group pre-drug arterial blood pressure was 92 ± 2/73 ± 2 mmHg ($n=105$). The highest dose of lidocaine (10 μmol kg^{-1} min^{-1}) reduced mean arterial pressure by 16 ± 4% ($P<0.05$) from the pre-drug value. Tedisamil increased mean arterial pressure by 12 ± 2% and 13 ± 3% (both $P<0.05$) from the pre-drug value at the middle and highest doses tested. Diastolic blood pressure was not effected by tedisamil and the net result was an increase in pulse pressure. RSD1019 had no effect on blood pressure.

The effects of lidocaine, tedisamil and RSD1019 on ECG intervals were confined to effects on the QT interval (Table 1). The PR and QRS intervals were 81 ± 1 ms ($n=105$) and 39 ± 1 ms, respectively, before drug treatment. Neither variable was effected drug treatment. Lidocaine was without effect on QTα and QT intervals. However, when the QT prolonging effects of bradycardia were taken into account by means of Bazett's QTc correction formula, the 10 μmol kg^{-1} min^{-1} dose of lidocaine significantly reduced the interval. Tedisamil and RSD1019 prolonged QTα and QT intervals in a dose-related fashion. These effects were independent of bradycardia since QTc intervals were also prolonged. QT widening produced by the highest dose of RSD1019 (8 μmol kg^{-1} min^{-1}) was similar to that produced by the middle dose of tedisamil (0.125 μmol kg^{-1} min^{-1}). The highest dose of tedisamil (0.25 μmol kg^{-1} min^{-1}) produced significantly greater QT and QTc widening than 8 μmol kg^{-1} min^{-1} dose of RSD1019 ($P<0.05$).

Drug effects on ischaemia-induced changes in MAPs

Lidocaine, tedisamil and RSD1019 had different effects on MAPs before and after induction of ischaemia. The effects of these drugs on MAP duration in normal tissue paralleled their

Table 1 The effects of lidocaine, tedisamil and RSD1019 on QT intervals in anaesthetized rabbits

	*QT*α (ms)	*QT* (ms)	*QT* α*c* (ms)	*QTc* (ms)
Pre-drug value	141 ± 4	177 ± 4	266 ± 7	333 ± 6
Drug, dose (μmol kg^{-1} min^{-1})	*Per cent change from the pre-drug value*			
Control	4 ± 2	3 ± 2	6 ± 3	5 ± 4
Lidocaine, 2.5	3 ± 3	−1 ± 5	1 ± 5	7 ± 10
Lidocaine, 5	2 ± 2	6 ± 5	−2 ± 4	6 ± 8
Lidocaine, 10	5 ± 2	−2 ± 4	3 ± 2	−12 ± 9*#
Tedisamil, 0.063	6 ± 3	7 ± 2	7 ± 7	9 ± 6
Tedisamil, 0.125	15 ± 4	20 ± 5*	15 ± 6	22 ± 8*
Tedisamil, 0.25	35 ± 5*†	53 ± 5*†	34 ± 6*†	60 ± 5*†
RSD1019, 2	18 ± 4*	8 ± 2	29 ± 7*	10 ± 2
RSD1019, 4	14 ± 4*	19 ± 4*	18 ± 7	25 ± 7*#
RSD1019, 8	19 ± 6*	21 ± 4*	23 ± 9*	26 ± 5*#

The effect of infusions of lidocaine, tedisamil and RSD1019 on the QT interval of the ECG in pentobarbital anaesthetized rabbits. The QTα interval is measured from the Q wave to the peak of the T wave, while the QT interval is measured from the Q wave to the end of the T wave. QTc denotes the QT interval corrected for heat rate using Bazett's formula ($QTc = QT/\sqrt{RR}$). Infusions, in μmol kg^{-1} min^{-1}, are indicated in the left column. No differences were found between pre-drug values (ANOVA, $P>0.05$) and therefore data are summarized as the mean ± s.e.mean for all rabbits included in the study ($n=105$). The effects of treatments are expressed as mean per cent change ± s.e.mean, $n=42$ for vehicle controls and $n=7$ for each treated group. Post-infusion values were measured 5 min after commencing the infusion. Statistical significance was tested using ANOVA at a significance level of $P<0.05$ followed by Tukey's test for differences. Significant differences from control are indicated by (*). Differences between the lowest and highest dose of each drug is indicated by (#) and differences between the two lower doses and the highest dose is indicated by a (†).

effects on the QT interval of the ECG. Lidocaine had no effect on MAP duration at 90% repolarization ($MAPD_{90\%}$) before or after induction of ischaemia (data not shown). Tedisamil prolonged $MAPD_{90\%}$ before induction of ischaemia in a dose-related fashion; however, such prolongation was rapidly lost after induction of ischaemia (Figure 2). No significant differences in $MAPD_{90\%}$ were found between vehicle control and tedisamil treated rabbits 5 min after induction of ischaemia. In contrast, RSD1019 significantly prolonged $MAPD_{90\%}$ in normal myocardial tissue but only at the highest dose tested (Figure 3). This prolongation was partly maintained after induction of ischaemia. Compared to vehicle controls, RSD1019 significantly prolonged $MAPD_{90\%}$ after 5 min of ischaemia (Figure 3). This effect was dose-related. MAP duration at times greater than 5 min after the induction of ischaemia were also prolonged; however, statistics were not performed on these data due to deterioration of MAPs recorded from ischaemic tissue as previously reported (Barrett *et al*., 1997b; 1998). In summary, the AP prolonging effects of tedisamil were lost under conditions of myocardial ischaemia while those of RSD1019 were partly maintained.

Drug effects on MAP activation alternans

Time dependent changes in MAP morphology were observed after induction of ischaemia. These changes included alternans in MAP duration, activation alternans and multiple wave forms. In vehicle controls, the number of rabbits in which activation alternans could be observed increased with time after induction of ischaemia. The occurrence of activation alternans commenced as early as 2 min after the induction of ischaemia and peaked at approximately 10 min. After 15 min of ischaemia MAPs were no longer recognisable. Multiple MAP wave forms were rarely observed in vehicle controls (two of 42). When they were observed, they occurred transiently between activation alternans and MAPs becoming unrecognizable.

Lidocaine increased the occurrence of activation alternans and reduced the time to the first occurrence. The time to the first occurrence of activation alternans in the control group was 8.0 ± 1.0 min (mean $\pm$ s.e.mean, $n=12$) while in the 2.5, 5 and 10 μmol kg^{-1} min^{-1} lidocaine groups it was 6.0 ± 0.6 ($n=5$), 5.2 ± 1.0 ($n=5$) and 2.3 ± 0.4 min ($n=7$, $P<0.01$ vs control), respectively. The median time after occlusion to the

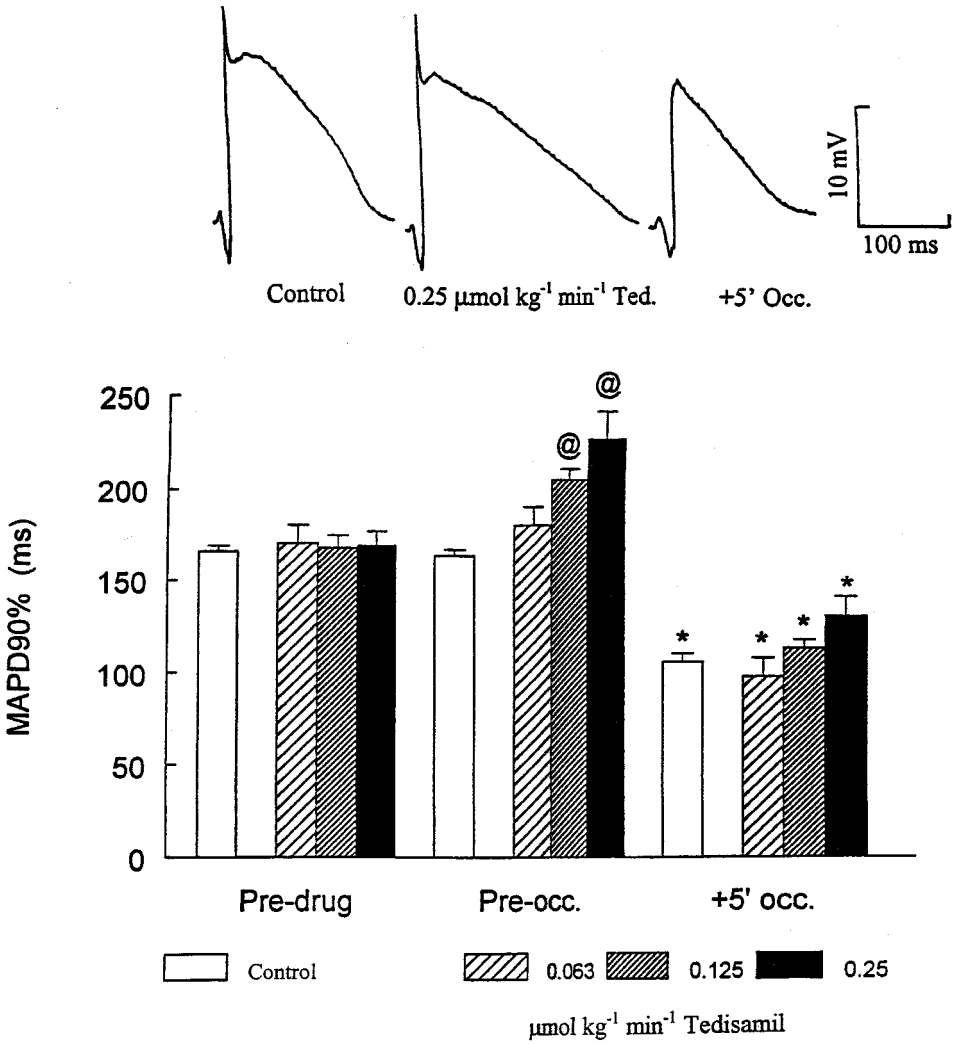

Figure 2 The effect of tedisamil on epicardial MAP duration before and after induction of ischaemia in anaesthetized rabbits. The top panel shows MAP recordings that illustrate the effect of tedisamil. The lower panel summarizes the group data as the mean $MAPD_{90\%} \pm$ s.e.mean. The open bars show data for control rabbits, $n=42$, and treated rabbits, $n=7$. Tedisamil was infused i.v. starting 5 min before coronary artery occlusion and maintained for the duration of the experiment. Statistical significance was determined by ANOVA ($P<0.05$) followed by a Tukey test for differences. The asterisk (*) indicates statistical significance between the +5′-occlusion value and the respective pre-drug and pre-ischaemia values. The symbol (@) indicates a difference between the pre-drug and pre-occlusion values.

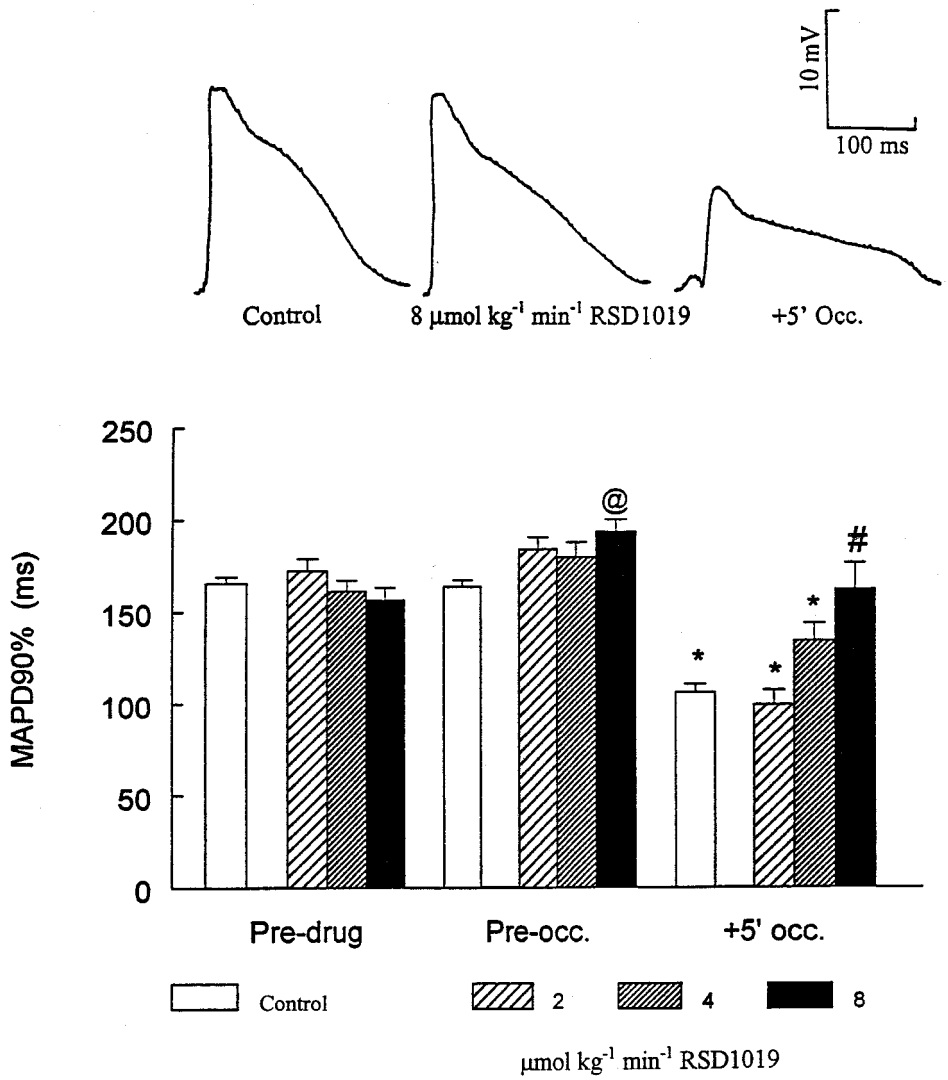

Figure 3 The effect of RSD1019 on epicardial MAP duration before and after induction of ischaemia in anaesthetized rabbits. The format for this figure is the same as for Figure 2. RSD1019 was infused iv starting 5 min before coronary occlusion and maintained for the duration of the experiment. Statistical significance was determined by ANOVA ($P<0.05$) followed by a Tukey test for differences. The asterisk (*) indicates statistical significance between the +5′-occlusion value and the respective pre-drug and pre-occlusion values as well as versus vehicle control. The symbol (@) indicates a difference between the pre-drug and pre-occlusion values while the symbol (#) indicates a difference between groups 5 min after occlusion.

occurrence of activation alternans in vehicle controls was 7 min; in rabbits treated with 2.5, 5 and 10 μmol kg^{-1} min^{-1} lidocaine this value was reduced to 6, 6 and 2 min, respectively. Activation alternans quickly became more complex such that multiple wave forms were commonly observed in lidocaine-treated rabbits (Figure 1). Multiple wave forms were observed in all lidocaine-treated rabbits (21 of 21) while they were only observed in two of 42 vehicle controls rabbits ($P<0.05$). Tedisamil and RSD1019 had no effect on the occurrence, or the time distribution, of activation alternans relative to vehicle controls and multiple MAP wave forms were not observed (data not shown).

Drug effects on ischaemia-induced arrhythmias

Ischaemia-induced arrhythmias were influenced differently by lidocaine, tedisamil and RSD1019 (Table 2). Lidocaine increased the occurrence ventricular tachycardia (VT) and ventricular fibrillation (VF) and reduced the time to the occurrence of VF (both $P<0.05$; Table 2). Neither of these effects were clearly dose-related. The proarrhythmic effects of lidocaine were seen as a statistically significant increase in arrhythmia score ($P<0.05$, Table 2).

Tedisamil increased the occurrence of VT at the lowest dose tested (0.063 μmol kg^{-1} min^{-1}), but was without statistically significant antiarrhythmic effects at any dose. The possible proarrhythmic actions of the low dose of tedisamil occurred within the first 2 min of ischaemia. In vehicle controls, 10 of 42 had VT and none had VF within this time period. Over the same time period, VT occurred in four of seven rabbits treated with 0.063 μmol kg^{-1} min^{-1} tedisamil ($P<0.08$, not significant) and one of seven had VF.

RSD1019 completely prevented the occurrence of VT and VF at doses of 4 and 8 μmol kg^{-1} min^{-1} (Table 2). These antiarrhythmic effects were reflected as a significant reduction in arrhythmia score ($P<0.05$). The time to the occurrence of VF, where it occurred, was similar to that observed in vehicle controls.

The pre-drug blood [K^+] for all groups was 2.5 ± 0.1 mM ($n=105$) and occluded zone size was 49 ± 1% of the ventricular

Table 2 The effect of lidocaine, tedisamil and RSD1019 on ischaemia-induced arrhythmias in anaesthetized rabbits

		Ischaemia-induced arrhythmias			
Drug, dose (μmol kg^{-1} min^{-1})	*AS*	*VPB*	*VT*	*VF*	*Time to VF* (min)
Vehicle control	2.6±0.3	39/42	24/42	10/42	14.5±1.4
Lidocaine, 2.5	5.5±0.7*	7/7	6/7*	7/7*	8.4±1*
Lidocaine, 5	4.0±1.0	5/7	6/7*	5/7*	9.3±1.7*
Lidocaine, 10	5.4±1.0*	7/7	6/7*	6/7*	9.1±1.9*
Tedisamil, 0.063	4.4±1.0	7/7	6/7*	3/7	10±5.2
Tedisamil, 0.125	1.7±0.7	7/7	1/7	3/7	14.9±1.8
Tedisamil, 0.25	1.8±0.7	6/7	2/7	4/7	12.6±3.4
RSD1019, 2	1.9±0.6	7/7	4/7	1/7	15.3
RSD1019, 4	0.2±0.2*	4/7	0/7	0/7	/
RSD1019, 8	0.0±0.0*	6/7	0/7	0/7	/

The effect of lidocaine, tedisamil and RSD1019 on ischaemia-induced arrhythmias in pentobarbital anaesthetized rabbits. The arrhythmia score (AS) is shown as the mean±s.e.mean, $n=42$ for controls whereas $n=7$ for each treated group. The arrhythmia score data was analysed using ANOVA followed by Dunnett's test for differences. Arrhythmia incidence data is shown as the number of rabbits having one episode (or greater) of the indicated arrhythmia over the total number in the group. VPB stands for ventricular premature beats, VT for ventricular tachycardia and VF for ventricular fibrillation. The time to the first occurrence of VF, in min, is expressed as the mean±s.e.mean for the number of rabbits in which VF occurred. Arrhythmia occurrence data was analysed using Fisher's exact test. The asterisk (*) indicates a statistically significant difference from vehicle control at $P<0.05$

mass. These values are consistent with previous reports (Barrett *et al*., 1997b; Barrett & Walker, 1998) and no differences were found between groups.

Exclusions

Rabbits not meeting the inclusion criteria were excluded and replaced. All exclusions were made on the basis of severe hypotension after coronary artery occlusion. In all, 12 animals were excluded from the following groups: two, one and three rabbits from the 2.5, 5 and 10 μmol kg^{-1} min^{-1} lidocaine groups; one in the 0.25 μmol kg^{-1} min^{-1} tedisamil group; one, one and three from the 2, 4, and 8 μmol kg^{-1} min^{-1} RSD1019 groups. More lidocaine and RSD1019 treated rabbits were replaced than vehicle control or tedisamil-treated rabbits.

Discussion

Comparison of the antiarrhythmic and electrophysiological actions of lidocaine, tedisamil and RSD1019 in anaesthetized rabbits illustrates the potential benefits and risks of ischaemia-selectivity as an antiarrhythmic approach. The ischaemia-dependent actions of lidocaine (i.e., induction of activation alternans and multiple MAP wave forms) clearly led to an undesirable arrhythmic end. On the other hand, the lack of selectivity shown by tedisamil paralleled its lack of effect on ischaemia-induced arrhythmias. In contrast, RSD1019's actions were at least partly ischaemia-selective and were associated with antiarrhythmic actions.

MAP recordings have been used to examine the electrophysiological characteristics of ischaemic myocardial tissue by others (Dilly & Lab, 1987; Franz 1991). Despite the demonstrated usefulness of this method there are a number of nuances that must be considered when interpreting such data. MAPs were recorded from an intact beating heart and, as a result, the signal can be contaminated by the local electrogram. The remnant of this electrogram (i.e., the QRS complex) can be seen at the beginning of the MAP upstroke. This artefact can complicate measurement of MAP variables with the maximum upstroke velocity being the most sensitive to contamination and $MAPD_{90\%}$ the least.

Lidocaine had profibrillatory actions in the present study and this effect was associated with marked electrophysiological derangement of ischaemic myocardial tissue. The proportion of rabbits in which activation alternans could be observed was increased and the time to the occurrence of activation alternans was decreased by lidocaine. Moreover, lidocaine caused multiple wave forms in ischaemic tissue. While the interpretation of the electrophysiological significance of multiple wave forms is complicated, an increase in their occurrence clearly represents an increase in the heterogeneity of the electrophysiological properties of the ischaemic myocardium. Thus, lidocaine's ischaemia-selective conduction slowing actions, and the resulting derangement of the electrophysiological properties of the ischaemic myocardium were associated with an increase in the incidence of VF and a decrease in the latency to VF. The profibrillatory actions of lidocaine after induction of myocardial ischaemia have been reported by others (Bergey *et al*., 1982; Carson *et al*., 1986).

Since the actions of lidocaine are rate- and voltage-dependent (Hondeghem & Katzung, 1984), beat to beat differences in AP morphology (i.e., AP amplitude and duration) can be expected to give rise to beat to beat variations in the degree of Na^+ channel block produced. In turn, the amount of Na^+ channel recovery will depend on heart rate as well as AP duration and amplitude. These AP characteristics will in turn vary in a manner which depends on the degree of Na^+ channel block produced during the preceding beat. Once initiated, positive feedback is an inherent property of this mechanism. Regional differences in the degree and electrophysiological consequences of ischaemia result in spacial dispersion of AP morphology, as well as effective refractory period, both of which set the stage for VF.

In the present study, the AP prolonging effects of tedisamil were not maintained in ischaemic tissue. Such data are generally in agreement with the literature for I_{Kr} blockers (Culling *et al*., 1984; Cobbe *et al*., 1985a,b). Other studies, using a selection of I_{Kr} blockers, suggest that this is a common finding for this class of drug (MacKenzie *et al*., 1993; Baskin & Lynch, 1994). Duff *et al*. (1997) demonstrated that the loss of effectiveness of I_{Kr} blockers under conditions of ischaemia is mediated by K^+-induced antagonism of the binding of these drugs to the ion channels that underlay I_{Kr}.

Tedisamil is also a potent blocker of $I_{K(ATP)}$ ($IC_{50\%}$ for $I_{K(ATP)}$ ~50 nM vs I_{to} ~3 μM; Bray & Quast, 1992; Dukes & Morad, 1989). Differences in its pharmacology under certain conditions suggest that it acts at a different site than sulphonylurea drugs, such as glibenclamide (Bray & Quast, 1992; Guillemare *et al*., 1995). These results may be taken as

further support for the lack of effect of blockers of $I_{K(ATP)}$ on ischaemia-induced AP shortening *in vivo* (at least when administered as an acute i.v. infusion).

Tedisamil increased the occurrence of VT at the lowest dose tested but had no antiarrhythmic effects in the present study. However, its antifibrillatory actions have previously been reported in rabbits (Chi *et al*., 1996) and other species (Beatch *et al*., 1991; Adaikan *et al*., 1992; Wallace *et al*., 1995). The doses (concentrations) producing antifibrillatory actions in those studies were higher and resulted in greater AP widening than seen in the present study. This observation likely explains the lack of antiarrhythmic effects reported here. There is one study in which the antifibrillatory actions of tedisamil cannot be attributed to AP prolongation in normal tissue (Friedrichs *et al*., 1998). In this study, a low dose of tedisamil was given orally for 3 days prior to experiment. The authors attributed the antifibrillatory action of tedisamil to $I_{K(ATP)}$ blockade in the ischaemic myocardium. This discrepancy remains to be resolved.

RSD1019 prolonged AP duration before and after induction of ischaemia and abolished ischaemia-induced tachyarrhythmias. While this result did not reach statistical significance in the present study, our previous study in this species demonstrated a statistically significant reduction in VF (Bain *et al*., 1997). Similarly, RSD1019 prevented ischaemia-induced VF in anaesthetized rats subjected to coronary artery occlusion over the same dose range (Bain *et al*., 1997; Barrett, 1999). It is noteworthy that RSD1019 was much more effective as an antifibrillatory agent than the $I_{K(ATP)}$ blocker glibenclamide in same preparation (Barrett & Walker, 1998).

In the present study, only the highest dose of RSD1019 prolonged the QT interval of the ECG and $MAPD_{90\%}$ before induction of ischaemia. Tedisamil produced much larger increases in the QT and $MAPD_{90\%}$ duration. Thus by comparison with tedisamil, the antiarrhythmic actions of RSD1019 cannot be attributed to its AP prolonging effects in normal tissue. Thus, while the effects of RSD1019 were not absolutely selective for the conditions of myocardial ischaemia, they were maintained under such conditions unlike the actions of tedisamil.

Ischaemia-selective AP prolongation as an antiarrhythmic principle

Myocardial ischaemia causes AP shortening in all species and is associated with the occurrence of re-entrant arrhythmias (Janse & Wit, 1989; Pogwizd & Corr, 1987). Therefore, prolonging APs in ischaemic tissue, or simply preventing AP shortening in ischaemic tissue, might prevent arrhythmias of this aetiology. Confining the AP prolonging effects to ischaemic tissue might offer distinct advantages over a similar effect in normal tissue.

The antiarrhythmic actions of ischaemia-selective AP prolongation might be explained by examining the vulnerable window and wave-length of re-entry hypotheses. The vulnerable window hypothesis postulates that changes in excitability after induction of ischaemia range from minimal effects early on, to complete suppression (Hondeghem & Cotner, 1978). These two extremes are separated by a time window within which tissue is critically suppressed such that unidirectional block can occur. As unidirectional block is one of the fundamental requirements for re-entry, this time window is therefore the vulnerable window for arrhythmogenesis. If the wave length of a re-entry circuit is taken as the product of conduction velocity and the refractory period, then ischaemia-induced AP shortening allows re-entry to occur more easily (i.e., when conduction is faster; Adaikan *et al*., 1992). Re-entry cannot occur when conduction is too fast and/or the refractory period is too long. Whilst AP duration and refractory period are closely linked under normal circumstances, myocardial ischaemia prolongs refractoriness beyond AP duration (i.e., postrepolarization refractoriness develops). As recovery of excitability occurs at negative membrane potentials, preventing AP shortening in the ischaemic myocardium can be expected to further prolong postrepolarization refractoriness. In this way, preventing AP shortening in ischaemic tissue can be expected to prevent arrhythmias. A longer AP duration in ischaemic tissue requires that conduction be that much slower in order for re-entrant arrhythmias to occur. Such tissue may become inexcitable before AP duration is sufficiently short to allow re-entry to occur. In summary, prolonging AP duration in ischaemic tissue can be expected to delay the onset of, but not extend, the vulnerable window for arrhythmogenesis.

While evidence for the antiarrhythmic efficacy of ischaemia-selective AP prolongation is scarce, there is evidence from *in vitro* studies to support the hypothesis. Lucchesi's group (Friedrichs *et al*., 1994; Chi *et al*., 1996) has shown that drugs which can be expected to prevent AP shortening under 'simulated ischaemic' conditions effectively prevent VF. The conditions they used to simulate ischaemia are of major importance when interpreting their results. Ischaemia was simulated by using a combination of hypoxia and the $I_{K(ATP)}$ activator pinacidil. Under these conditions, $I_{K(ATP)}$ blockers have been shown to prevent AP shortening caused by organic $I_{K(ATP)}$ activators (Sanguinetti *et al*., 1988).

In summary, RSD1019 suppressed ischaemia-induced MAP shortening with minimal effects on MAP duration in normal tissue. By comparison to tedisamil, the MAP prolongation produced in normal tissue by RSD1019 cannot explain the antifibrillatory actions observed. The present study demonstrates that effective antiarrhythmic drugs can be developed by considering the aetiology of the arrhythmia and selecting a drug that is selective for the pathology.

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References

ADAIKAN, G., BEATCH, G.N., LEE, T.L., RATNAM, S.S. & WALKER, M.J.A. (1992). Antiarrhythmic actions of tedisamii: studies in rats and primates. *Cardiovasc. Drugs Ther.,* **6,** 345–352.

BAIN, A.I., BARRETT, T.D., BEATCH, G.N., FEDIDA, D., HAYES, E.S., PLOUVIER, B., PUGSLEY, M.K., WALKER, M.J.A., WALKER, M.L., WALL, R.A., YONG, S.L. & ZOLOTOY, A. (1997). Better anitarrhythmics? Development of antiarrhythmic drugs selective for ischaemia-depend arrhythmias. *Drug Dev. Res.,* **42,** 198–210.

BARRETT, T.D. (1999). Relationship between ischaemia-selective drug action and antiarrhythmic efficacy. Ph.D. Thesis. University of British Columbia.

BARRETT, T.D., HAYES, E.S., YONG, S.L., WALKER, M.L., BEATCH, G.N. & WALKER, M.J.A. (1997a). Ischemia selective electrophysiological and antiarrhythmic actions of RSD1019 in ischemic cardiac tissue. *J. Mol. Cell. Cardiol.,* **29,** 197.

BARRETT, T.D., MACLEOD, B.A. & WALKER, M.J.A. (1997b). A model of myocardial ischemia for the simultaneous assessment of electrophysiological changes and arrhythmias in intact rabbits. *J. Pharmacol. Toxicol. Meth.,* **37,** 27 – 36.

BARRETT, T.D., MACLEOD, B.A. & WALKER, M.J.A. (1998). Mechanism of lidocaine induced proarrhythmia in the setting of acute myocardial ischaemia: Use of monophasic action potentials to assess the effects of drugs on ischaemic myocardial tissue. *Proc. West. Pharmacol. Suppl.,* **T-06**.

BARRETT, T.D. & WALKER, M.J.A. (1998). Glibenclamide does not prevent action potential shortening induced by ischaemia in anaesthetized rabbits but reduced ischemia-induced arrhythmias. *J. Mol. Cell. Cardiol.,* **30,** 999 – 1008.

BASKIN, E.P. & LYNCH, JR J.J. (1994). Comparative effects of increased extracellular potassium and pacing frequency on the Class III activities of methanesulfonanilide I_{Kr} blockers dofetilide, D-sotalol, E-4031, and MK-499. *J. Cardiovasc. Pharmacol.,* **24,** 199 – 208.

BEATCH, G.N., ABRAHAM, S., MACLEOD, B.A. & WALKER, M.J.A. (1991). Antiarrhythmic properties of tedisamil (KC8857), a putative transient outward K^+ channel blocker. *Br. J. Pharmacol.,* **102,** 13 – 18.

BELLEMIN-BAURREAU, J., POIZOT, A., HICKS, P.E. & ARMSTRONG, J.M. (1994). An in vitro method for the evaluation of antiarrhythmic and antiischaemic agents by using programmed electrical stimulation of rabbit heart. *J. Pharmacol. Toxicol. Meth.,* **31,** 31 – 40.

BERGEY, J.L., NOCELLA, K. & MCCALLUM, J.D. (1982). Acute coronary artery occlusion-reperfusion-induced arrhythmias in rats, dogs and pigs: antiarrhythmic evaluation of quinidine, procainimide and lidocaine. *Eur. J. Pharmacol.,* **81,** 205 – 216.

BRAY, K. & QUAST, U. (1992). Differentail inhibition by tedisamil (KC 8857) and glibenclamide of the responses to cromakalim and minoxidil sulpate in rat isolated aorta. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **345,** 244 – 250.

CARDINAL, R., JANSE, M.J., VAN EEDEN, I., WENER, G., NAUMANN, C., D'ALNONCOURT, C.N. & DURRER, D. (1981). The effects of lidocaine on intracellular and extracellular potentials, activation, and ventricular arrhythmias during acute regional ischaemia in the isolated porcine heart. *Circ. Res.,* **49,** 792 – 806.

CARSON, D.L., CARDINAL, R., SAVARD, P., VASSEUR, C., NATTEL, S., LAMBERT, C. & NADEAU, R. (1986). Relationship between an arrhythmogenic action of lidocaine and its effects on excitation patterns in acutely ischaemic porcine myocardium. *J. Cardiovasc. Pharmacol.,* **8,** 126 – 136.

CHI, L., PARK, J.L., FRIEDRICHS, G.S., BANGLAWALA, Y.A., PEREZ, M.A., TANHEHCO, E.J. & LUCCHESI, B.R. (1996). Effects of tedisamil (KC-8857) on cardiac electrophysiology an ventricular fibrillation in the rabbit isolated heart model. *Br. J. Pharmacol.,* **117,** 1261 – 1269.

COBBE, S.M., MANLEY, S.B. & ALEXOPOULOS, D. (1985a). The influence of acute myocardial ischaemia on the Class III antiarrhythmic action of sotalol. *Cardiovasc. Res.,* **19,** 661 – 667.

COBBE, S.M., MANLEY, S.B. & ALEXOPOULOS, D. (1985b). Interaction of the effects of hypoxia, substrate depletion, acidosis and hyperkalemia on the class III antiarrhythmic properties of sotalol. *Cardiovasc. Res.,* **19,** 668 – 672.

CONNOLLY, S.J. (1999). Evidence-based analysis of amiodarone efficacy and safety. *Circulation,* **100,** 2025 – 2034.

CORONEL, R., FIOLET, J.W., WILMS-SCHOPMAN, F.J., SCHAAPHERDER, A.F., JOHNSON, T.A., GETTES, L.S. & JANSE, M.J. (1988). Distribution of extracellular potassium and its relation to electrophysiologic changes during acute myocardial ischemia in isolated perfused porcine heart. *Circulation,* **77,** 1125 – 1138.

CULLING, W., PENNY, W.J. & SHERIDAN, D.J. (1984). Effects of sotalol on arrhythmias and electrophysiology during myocardial ischaemia and reperfusion. *Cardiovasc. Res.,* **18,** 397 – 404.

CURTIS, M.J. & WALKER, M.J.A. (1988). Quantification of arrhythmias using scoring systems: an examination of seven scores in an in vivo model of regional myocardial ischemia. *Cardiovasc. Res.,* **22,** 656 – 665.

DICKENSON, D.R., DAVIS, D.R. & BEATCH, G.N. (1997). Development and evaluation of a fully automated monophasic action potential analysis program. *Med. Biol. Eng. Comput.,* **35,** 653 – 660.

DILLY, S.G. & LAB, M.J. (1987). Changes in monophasic action potential duration during the first hour of regional myocardial ischaeamia in the anaesthetised pig. *Cardiovasc. Res.,* **21,** 908 – 915.

DUFF, H.J., FENG, Z.P., FISET, C., WANG, L., LEES-MILLER, J. & SHELDON, R.S. (1997). [3H]Dofetilide binding to cardiac myocytes: modulation by extracellular potassium. *J. Mol. Cell. Cardiol.,* **29,** 183 – 191.

DUKES, I.D. & MORAD, M. (1989). Tedisamil inactivates transient outward K^+ current in rat ventricular myocytes. *Am. J. Physiol.,* **257,** H1746 – H1749.

FAIVRE, J.F., ROUANET, S. & BRIL, A. (1998). Comparative effects of glibenclamide, tedisamil, dofetilide, E-4031 and BRL-32872 on protein kinase A-activated chloride current in guinea pig myocytes. *J. Cardiovasc. Pharamcol.,* **31,** 551 – 557.

FRANZ, M.R. (1991). Method and theory of monophasic action potential recording. *Prog. Cardiovasc. Dis.,* XXXIII, **6,** 347 – 368.

FRIEDRICHS, G.S., ABREU, J.N., DRISCOLL, JR E.M., BORLAK, J. & LUCCHESI, B.R. (1998). Antifibrillatory efficacy of long term tedisamil administration in a post infarcted canine model of ischaemic ventricular fibrillation. *J. Cardiovasc. Pharamcol.,* **31,** 56 – 66.

FRIEDRICHS, G.S., CHI, L., GREEN, A.L. & LUCCHESI, B.R. (1994). Antifibrillatory effects of clofilium in the rabbit isolated heart. *Brit. J. Pharmacol.,* **113,** 209 – 215.

GUILLEMARE, E., LUZDUNSKI, M. & HONORÉ. (1995). Glibenclamide opens ATP-sensitive potassium channels in xenopus oocyte follicular cells during metabolic stress. *J. Pharmacol. Exp. Ther.,* **47,** 588 – 594.

HONDEGHEM, L.H. & COTNER, C.L. (1978). Reproducible and uniform cardiac ischemia: effects of antiarrhythmic drugs. *Am. J. Physiol.,* **235,** H574 – H580.

HONDEGHEM, L.M. & KATZUNG, B.G. (1984). Antiarrhythmic agents: the modulated receptor mechanism of action of sodium and calcium channel-blocking drugs. *Ann. Rev. Pharmacol. Toxicol.,* **24,** 387 – 423.

HONDEGHEM, L.M. & SNYDERS, D.J. (1990). Class III antiarythmic agents have a lot of potential but a long way to go. Reduced effectiveness and dangers of reverse use dependence. *Circulation,* **81,** 686 – 690.

JANSE, M.J. & WIT, A.L. (1989). Electrophysiological mechanisms of ventricular arrhythmias resulting from myocardial ischemia and infarction. *Physiol. Reviews,* **69,** 1049 – 1169.

LUKAS, A. & ANTZELEVITCH, C. (1993). Differences in the electrophysiological response of canine ventricular epicardium and endocardium to ischemia. Role of the transient outward curret. *Circulation,* **88,** 2903 – 2915.

MACKENZIE, I., SAVILLE, V.L. & WATERFALL, J.F. (1993). Differential Class III and glibenclamide effects on action potential duration in guinea-pig papillary muscle during normoxia and hypoxia/ischaemia. *Br. J. Pharmacol.,* **110,** 531 – 538.

NATTEL, S. (1998). Experimental evidence for proarrhythmic mechanisms of antiarrhythmic drugs. *Cardiovasc. Res.,* **37,** 567 – 577.

POGWIZD, S.M. & CORR, P.B. (1987). Reentrant and nonreentrant mechanisms contribute to arrhythmogenesis during early myocardial ischaemia: results using three-dimentional mapping. *Circ. Res.,* **61,** 352 – 371.

RUIZ-PETRICH, E., PONCE ZUMINO, A. & SCHANNE, O.F. (1996). Early action potential shortening in hypoxic hearts: role of chloride current(s) mediated by catecholamine release. *J. Mol. Cell. Cardiol.,* **28,** 279 – 290.

SANGUINETTI, M.C. & JURKIEWICZ, N.K. (1990). Two components of cardiac delayed rectifier K^+ current. Differential sensitivity to block by class III antiarrhythmic agents. *J. Gen. Physiol.,* **96,** 195 – 215.

SANGUINETTI, M.C., SCOTT, A.L., ZINGARO, G.J. & SIEGL, P.K. (1988). BRL 34915 (cromakalim) activates ATP-sensitive K^+ current in cardiac muscle. *Proc. Nat. Acad. Sci. USA,* **85,** 8360 – 8364.

TORP-PEDERSEN, C., MOLLER, M., BLOCH-THOMSEN, P.E., KOBER, L., SANDOE, E., EGSTRUP, K., AGNER, E., CARLSEN, J., VIDEBAEK, J., MARCHANT, B. & CAMM, A.J. (1999). Dofetidide in patients with left ventricle dysfunctin and either heart failure of acute myocardial infarction: rationale, design, and patient characteristics of the DIAMOND studies. Danish Investigations on Arrhythmia and Mortality ON Dofetilide. *New Engl. J. Med.,* **341,** 857 – 865.

WALDO, A.L., CAMM, A.J., DE RUYTER, H., FRIEDMAN, P.L., MACNEIL, D.J., PAULS, J.F., PITT, B., PRATT, C.M., SCHWARTZ, P.J. & VELTRI, E.P. (1996). Effect of d-sotalol on mortality in patients with left ventricular dysfunction after recient and remote myocardial infarction. The SWORD investigators. *Lancet,* **348,** 7–12.

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., RUSSEL, R.C., SHERIDAN, D.J., WINSLOW, E. & WOODWARD, B. (1988). The Lambeth conventions: guidelines for the study of arrhythmias in ischaemia, infarction and reperfusion. *Cardiovasc. Res.,* **22,** 447–455.

WALLACE, A.A., STUPIENSKI, R.F. 3rd, BASKIN, E.P., APPLEBY, S.D., KOTHSTEIM, T., GEHRET, J.R., KING, S.W., REMY, D.C. & LYNCH, JR J.J. (1995). Cardiac electrophysiological and antiarrhythmic actions of tedisamil. *J. Pharmacol. Exp. Ther.,* **273,** 168–175.

YANG, T. & RODEN, D.M. (1996). Extracellular potassium modulation of drug block of I_{Kr}. Implications for torsade de pointes and reverse use-dependence. *Circulation,* **93,** 407–411.

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