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Tedisamil and lidocaine enhance each other's antiarrhythmic activity against ischaemia-induced arrhythmias in rats

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1 Combinations of the action potential-widening drug tedisamil (Class III antiarrhythmic activity), and the inactivated state sodium channel blocker lidocaine (Class Ib antiarrhythmic activity) were assessed for antiarrhythmic actions in a rat model of ischaemia-induced arrhythmias and for electrophysiological actions in normal rat myocardial tissue.

2 Both tedisamil and lidocaine dose-dependently suppressed ischaemia-induced arrhythmias. The ED_{50} values were 3.0 ± 1.3 and $4.9 \pm 0.6\,\mu mol\,kg^{-1}\,min^{-1}$, respectively.

3 Combinations of the two drugs acted synergistically such that the ED_{50} for tedisamil was reduced to $0.8 \pm 0.2\,\mu mol\,kg^{-1}\,min^{-1}$ in the presence of $2\,\mu mol\,kg^{-1}\,min^{-1}$ lidocaine. Similarly, the ED_{50} for lidocaine was reduced to $0.7 \pm 0.2\,\mu mol\,kg^{-1}\,min^{-1}$ in the presence of $2\,\mu mol\,kg^{-1}\,min^{-1}$ tedisamil (both $P < 0.05$).

4 In a separate series of experiments in which normal ventricular tissue was electrically stimulated, $2\,\mu mol\,kg^{-1}\,min^{-1}$ lidocaine produced a leftward shift in the dose–response curve for tedisamil's effect on effective refractory period ($P < 0.05$). This dose of lidocaine had no effect on its own. These data indicate that the synergistic actions of combinations of tedisamil and lidocaine were mediated, at least in part, by extension of effective refractory period in normal myocardial tissue.

5 In contrast to the strategy of developing drugs that are selective for a single electrophysiological mechanism, the results of the present study suggest that effective antiarrhythmic drugs might be developed by optimising the combination of two complimentary electrophysiological mechanisms (i.e., action potential-prolonging activity and inactivated state sodium channel blockade).
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Abbreviations: ANOVA, analysis of variance; $AS_{control}$, arrhythmia score in the control group; AS_{Tx}, arrhythmia score in the treated group; *D*, infused dose of the drug; ED_{50}, dose producing 50% of the maximum response; *h*, slope factor of the dose–response curve; $\%P_{Tx}$, per cent protection produced by a treated group; PVB, premature ventricular beat; VF, ventricular fibrillation; VT, ventricular tachycardia

Introduction

Suppression of cardiac arrhythmias due to myocardial ischaemia and infarction remains a major clinical problem for which no complete solution has been found. Clinical trials with antiarrhythmic drugs have not only failed to demonstrate benefit, but more commonly reveal that such drugs actually increase mortality. Examples of this include the use of quinidine for prophylaxis of atrial fibrillation (Morganroth & Goin, 1991), flecainide and encainide for suppression of premature ventricular beats (The CAST Investigators, 1989) and d-sotalol for the suppression of postinfarct arrhythmias (Waldo *et al*., 1996). Only β-adrenoceptor blockers (Yusuf *et al*., 1985) and amiodarone (Connolly, 1999) have been demonstrated to reduce arrhythmic death. Amiodarone is unique among antiarrhythmic drugs in that it has properties characteristic of all four of the categories described by Vaughan-William's antiarrhythmic classification scheme. Given the failure of selective sodium (CAST, 1989) and potassium (Waldo *et al*., 1996; Torp-Pedersen *et al*., 1999) channel-blocking drugs, and the effectiveness of the nonselective drug amiodarone, it might be more rational to develop ion channel-blocking drugs that have more than one electrophysiological action.

Combinations of antiarrhythmic drugs have been tested in man (e.g., Duff *et al*., 1983; Stroobandt *et al*., 1987; Wagner *et al*., 1987; Dorian *et al*., 1993) and in animal models (e.g., Duff & Gault, 1986; Duff, 1989) to assess their antiarrhythmic efficacy. Early studies by Duff *et al*. demonstrated that combinations of quinidine and mexiletine were effective (Duff & Gault, 1986; Duff, 1989) and the combination was better tolerated in man (Duff *et al*., 1983). These studies demonstrated an interaction between action potential prolongation produced by quinidine and inactivated state sodium channel blockade produced by mexiletine. The observed synergy was attributed to increased refractoriness in the peri-infarcted zone of the evolving infarct (Duff & Gault, 1986; Duff, 1989).

Despite the studies of Duff's group and others, it is not clear if combining these two antiarrhythmic mechanisms is more

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effective for suppression of arrhythmias than either alone. The modulated receptor hypothesis predicts that inactivated state sodium channel block will be increased by prolonging action potential duration (Hondeghem & Katzung, 1984). Thus, the effective refractory period of normal myocardial tissue would be prolonged by two mechanisms: (1) prolongation of action potential duration and (2) delaying the recovery of sodium channels beyond action potential duration (i.e., extension of postrepolarisation refractoriness).

The aim of the present study was to examine the antiarrhythmic actions of combinations of the Class III antiarrhythmic drug tedisamil (Dukes & Morad, 1989; Beatch *et al*., 1991) and the Class Ib antiarrhythmic drug lidocaine, which have been demonstrated to block sodium channels in the inactivated state (Hondeghem & Katzung, 1984), over a wide range of effective doses in a rat model of ischaemia-induced arrhythmias. While the primary electrophysiological effect of tedisamil is to prolong action potential duration, it should be noted that this drug blocks a number of ion currents including the delayed rectifier K^+ current (Dukes & Morad, 1989), the rapid and slow transient outward currents (Dukes & Morad, 1989; Berger *et al*., 1998), the ATP-dependent K^+ current, cyclic AMP-activated Cl^- current (Faivre *et al*., 1998) and at higher concentrations, the inward sodium current (Beatch *et al*., 1991; Nemeth *et al*., 1996). Thus, while tedisamil is not absolutely selective in this regard, it is very effective in prolonging cardiac action potentials in the rat (Beatch *et al*., 1991). Further studies were conducted with effective combinations of these two drugs, to evaluate whether extension of effective refractory period in normal cardiac tissue could account for the synergistic antiarrhythmic activity observed. A preliminary report of this work has been presented (Sarraf *et al*., 1998).

Methods

Guidelines for the use of animals

Rats were housed in the animal care facility in the Department of Pharmacology and Therapeutics at the University of British Columbia (Vancouver, BC, Canada) or at Johnson & Johnson Pharmaceutical Research and Development (La Jolla, CA, U.S.A.). All animals were housed with a 12 h light/dark cycle. Food (standard rat chow) and water were available *ad libitum*. Studies were approved by The University of British Columbia's or Johnson & Johnson Pharmaceutical Research and Development's institutional animal care and use committee. All the experiments described in this study were carried out in accordance with internationally accepted guidelines.

Ischaemia-induced arrhythmias

Myocardial ischaemia was induced by occlusion of the left anterior descending artery in rats weighing between 200 and 350 g (Barrett *et al*., 1995). Rats were anaesthetised with 65 mg kg^{-1} pentobarbital i.p. A tracheotomy was performed to allow artificial ventilation with oxygen at a rate of 60 cycles min^{-1} and 10 ml kg^{-1}. Core body temperature was measured with a rectal thermometer and maintained at 35–37°C with a heating lamp. The jugular vein and carotid artery were cannulated with polyethylene tubing (PE50, Intramedic) for injection of drugs and measurement of blood pressure, respectively. Blood pressure was displayed on Labview software, utilising Statham pressure transducer, and was monitored for the duration of the experiment.

The chest was opened at the fourth intercostal space and a pericardial sling was made. A snare was loosely placed around the proximal left anterior descending artery. After placing the snare, the chest was closed with a purse string suture (3-0 silk, Ethicon). An ECG was recorded from subcutaneous pin electrodes in approximately a lead V3 configuration. The ECG was recorded using Labview software, and monitored for the duration of the experiment.

Blood [K^+] was measured 15 min before and after occlusion, wherever possible. Blood [K^+] was determined using a K^+-selective electrode (Ionetics), calibrated with three standards (2.0, 4.0 and 8.0 mM, Ionetics K^+ standards).

After completing the experiment, the occluded zone size (zone at risk) was measured as previously described (Curtis *et al*., 1987). The heart was excised and perfused *via* the aortic root with saline followed by a saline solution containing indocyangreen (0.5 mg ml^{-1}). The perfusion pressure was ~100 mmHg. Occluded zone size was defined as the percentage of the ventricular mass not dyed green.

Inclusion criteria were applied to ensure equality of arrhythmogenic stimulus and quality of the preparation. The inclusion criteria were: (1) mean arterial blood pressure greater than 70 mmHg before starting the experiment, (2) mean blood pressure not less than 25 mmHg for more than 2 min after occlusion, except when caused by arrhythmias, (3) no atrioventricular block unless it was due to prolonged global ischaemia caused by arrhythmias, (4) occluded zone size between 25 and 50% of the total ventricular mass, (5) blood [K^+] between 2.5 and 4.5 mM before starting the experiment and (6) not more than 15 PVBs, and no episodes of VT or VF could occur before drug administration and subsequent coronary artery occlusion.

Drugs and doses

Dose–response curves were constructed for the antiarrhythmic and electrophysiological actions of tedisamil, lidocaine and various combinations of the two drugs. A randomised and blind experimental design was used. Tedisamil was tested at doses of 0.125, 0.25, 0.5, 1, 2 and 4 μmol kg^{-1} min^{-1}, while lidocaine was tested at doses of 2, 4, 6 and 8 μmol kg^{-1} min^{-1}. These doses were selected on the basis of our previous studies (Beatch *et al*., 1991; Barrett *et al*., 1995). The influence of lidocaine on tedisamil's antiarrhythmic and electrophysiological actions was assessed by coadministration of 2 μmol kg^{-1} min^{-1} lidocaine with tedisamil at doses of 0.125, 0.25, 0.5, 1 and 2 μmol kg^{-1} min^{-1} and by coadministration of 4 or 6 μmol kg^{-1} min^{-1} lidocaine with tedisamil at doses of 0.063, 0.125, 0.25, 0.5 and 1 μmol kg^{-1} min^{-1}. The influence of tedisamil on lidocaine's antiarrhythmic and electrophysiological actions was assessed by coadministration of 0.5 μmol kg^{-1} min^{-1} tedisamil with lidocaine at doses of 1, 2, 4, and 6 μmol kg^{-1} min^{-1} and by coadministration of 1 μmol kg^{-1} min^{-1} tedisamil with lidocaine at doses of 0.5, 1, 2, 4 and 6 μmol kg^{-1} min^{-1} as well as coadministration of 2 μmol kg^{-1} min^{-1} tedisamil with lidocaine at doses of 0.25, 0.5, 1 and 2 μmol kg^{-1} min^{-1}.The group size for each treatment was 5. In total, 85 vehicle-treated rats were used in the various

control groups. The common vehicle used for these studies was 10% dimethylsulphoxide, 30% ethanol and 60% distilled water.

Experimental protocol

After completion of the surgical preparation, 15 min was allowed for stabilisation before starting the experiment. During this time, an arterial blood sample was obtained as described above. Thereafter, the test solution was infused (Harvard infusion pump, 1 ml h^{-1}) continuously throughout the experiment, starting 5 min before coronary artery occlusion. The effect of treatments on blood pressure, heart rate and ECG variables (PR, QRS and QT intervals) was assessed before coronary artery occlusion. Coronary artery occlusion was produced by tightening the snare around the artery. Arrhythmias were diagnosed based on their ECG appearance according to the definitions in the Lambeth conventions (Walker *et al*., 1988). Each experiment was monitored for 15 min following coronary artery occlusion, for the occurrence of arrhythmias. After completing the protocol, occluded zone size was measured as described above.

Electrical stimulation studies

Electrical stimulation studies were carried out to assess drug-induced changes in the threshold current for pacing, effective refractory period and maximum following frequency of the ventricle in anaesthetised rats as previously described (Walker & Beatch, 1988; Barrett *et al*., 1995). Rats were anaesthetised with 65 mg kg^{-1} pentobarbital i.p. The right and left jugular veins and the right carotid artery were cannulated with polyethylene tubing for injection of drugs and measurement of blood pressure, respectively. Blood pressure was monitored for the duration of the experiment. A tracheotomy was performed and an endotracheal tube inserted (Jelco™ 14-gauge needle sheath). Rats were not artificially ventilated, but were allowed to continue to breathe spontaneously. An ECG was recorded from subcutaneous pin electrodes in approximately a lead V3 configuration. The ECG was displayed on Labview or PowerLab software.

An incision was made over the fourth intercostal space to expose the chest wall. A pair of Teflon™-coated silver wires was inserted through the chest wall into the left ventricle using a 23-gauge needle as a guide. The end of each wire was stripped of its Teflon™ coating and bent to form a barb. When the needle was withdrawn, the electrode remained anchored in the left ventricle. A constant current stimulator (Grass, model SD9 or S88) was used to stimulate the left ventricle with square wave pulses.

The end points for electrical stimulation variables were determined from the ECG. The threshold current for stable capture of the ventricle on a 1 : 1 basis was determined at a stimulation frequency of 7.5 Hz. Effective refractory period was determined by inserting a single extra stimulus at a variable-coupling interval after the last beat in the train. The coupling interval of the extra beat was progressively increased until it was possible to insert an extra beat. The longest coupling interval that failed to produce a propagated response was taken as the effective refractory period. Each measurement was repeated in triplicate before proceeding to the next dose.

Two studies were conducted to examine the effects of combinations of tedisamil and lidocaine on effective refractory period. In the first study, effective refractory period was measured at 7.5 Hz (cycle length 133 ms) and lower stimulation frequencies were used when drug-induced prolongation of the effective refractory period precluded pacing at this rate. A second series of experiments were conducted to directly assess the effects of combinations of the two drugs at stimulation rates of 7.5, 9.5 and 11.5 Hz (cycle lengths of 133, 105 and 87 ms, respectively). In this study, lower stimulation rates were not used if drug-induced prolongation of the effective refractory period precluded pacing at the specified rate.

Drugs and doses

The right and left jugular veins were used to administer two infusion regimens. One vein was used to administer a fixed dose of 2 μmol kg^{-1} min^{-1} lidocaine or lidocaine–vehicle (10% dimethylsulphoxide, 10% ethanol and 80% saline). The other vein was used to infuse doses of tedisamil between 0.016 and 2 μmol kg^{-1} min^{-1}, or its corresponding vehicle (10% ethanol and 90% saline). The dose of lidocaine was selected on the basis of the antiarrhythmic studies, which demonstrated that 2 μmol kg^{-1} min^{-1} lidocaine was the optimal dose to observe the interaction, and previous studies that demonstrated that this dose had little or no effect on effective refractory period when administered alone (Barrett *et al*., 1995).

Rats were assigned to one of the following four treatment groups: (1) lidocaine–vehicle and tedisamil–vehicle, (2) 2 μmol kg^{-1} min^{-1} lidocaine and tedisamil–vehicle, (3) lidocaine–vehicle and progressively increasing doses of tedisamil (0.016–2 μmol kg^{-1} min^{-1}, (4) 2 μmol kg^{-1} min^{-1} lidocaine and progressively increasing doses of tedisamil (0.016–2 μmol kg^{-1} min^{-1}). The initial infusion rate was 1 ml h^{-1} for both infusions. In order to limit the total volume infused over the course of the experiment, two stock solutions of tedisamil were used. In this way, the total infused volume did not exceed 10% of the animals' calculated blood volume.

Experimental protocol

Before starting the experiment, the electrical stimulation protocol was repeated at 5 min intervals until stable values were obtained. In most cases, this required three repetitions before starting the experiment. Determination of all electrophysiological variables was complete within 2 min. The same infusion regimen was used for both electrical stimulation studies.

After establishing stable baseline values, infusion of 2 μmol kg^{-1} min^{-1} lidocaine or lidocaine–vehicle was started. Effects on electrical stimulation variables were assessed after 5 min and every 5 min thereafter. Infusion of lidocaine or lidocaine–vehicle was continued throughout the remainder of the experiment. Infusion of the initial dose of tedisamil, or tedisamil–vehicle, was started 5 min after starting the infusion of lidocaine or lidocaine–vehicle. Thereafter, the dose of tedisamil was doubled every 5 min until the maximum dose of 2 μmol kg^{-1} min^{-1} was reached.

Dose–response curve analysis and statistics

Group data are summarised as the mean ± s.e.m. For antiarrhythmic studies, $n = 5$ in treated groups and $n = 85$ in vehicle controls, while for electrophysiological studies $n = 3$ or

$n=5$ for all groups. Analysis of variance (ANOVA) followed by Duncan's multiple range test was performed to assess differences between groups. An arrhythmia score was used to summarise the arrhythmic history of each animal (see below). Since the Lambeth conventions (Walker *et al*., 1988) recommend that arrhythmia scores be used to summarise, but not replace arrhythmia incidence data, the latter were analysed for statistical significance using a Fisher's exact test and the data are presented separately. All statistical tests were performed using Sigma Stat version 2.0 with statistical significance at $P<0.05$. All tests were two-tailed.

The arrhythmic history of each rat was summarised using the arrhythmia score described below (score A, Curtis & Walker, 1988). The following values were assigned according to the incidence, time of occurrence and duration of arrhythmias: 0 = 0–49 premature ventricular beats (PVBs), 1 = 50–499 PVBs, 2 = >499 PVBs and/or 1 episode of spontaneously reverting VT or VF, 3 = >1 episode of VT or VF or both with a total duration <60 s, 4 = VT or VF or both 60–120 s total duration, 5 = VT or VF or both >120 s duration, 6 = fatal VF starting at >15 min after occlusion, 7 = fatal VF starting between 4 and 14 min 59 s, 8 = fatal VF starting between 1 and 3 min 59 s, 9 = fatal VF starting <1 min after occlusion. These data were then transformed such that the response produced by each treatment ($\%P_{Tx}$) was expressed as a per cent of the maximum possible response (Equation (1)). The arrhythmia score in vehicle-treated rats was defined as 0% response ($AS_{control}=5.8\pm0.2$, mean ± s.d., $n=85$) and the maximum response possible (100%) was defined as the complete suppression of arrhythmias (AS = 0).

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

where AS_{Tx} and $AS_{control}$ are the arrhythmia scores in the test group and vehicle control groups, respectively. Dose–response curves were constructed and fit according to a logistics function (Equation (2)) shown below using Slidewrite version 2 software.

$$\%P = 100*(D^h/(D^h + ED_{50}^h)) \quad (2)$$

where $\%P$ is the per cent antiarrhythmic protection, D is the infused dose of the drug, h is the slope factor and ED_{50} is the dose required to produce 50% of the maximum effect. For the purposes of curve fitting, the maximum response was defined to be 100% antiarrhythmic protection and dose–response curves were forced to pass through this point. The slope of the dose–response curve was allowed to vary.

A two-way analysis of variance was employed to assess the interaction between tedisamil alone and combinations of tedisamil and lidocaine.

Results

Drug effects on blood pressure, heart rate and ECG intervals

Infusion of lidocaine, 1–8 μmol kg^{-1} min^{-1}, caused a dose-related decrease in heart rate and blood pressure (Table 1), while infusion of tedisamil, 0.25–4 μmol kg^{-1} min^{-1}, caused a dose-related decrease in heart rate. The 2 μmol kg^{-1} min^{-1} dose of tedisamil increased blood pressure, while the other doses were without effect.

Combinations of tedisamil and lidocaine had effects on blood pressure and heart rate that could readily be explained by the additive effects of the two drugs. Tedisamil partially prevented lidocaine-induced hypotension; however, the highest dose of lidocaine in combination with tedisamil still reduced blood pressure relative to vehicle control ($P<0.05$; Table 1). Higher doses of the two drugs in combination had a greater bradycardic effect than either drug alone.

Infusion of tedisamil produced a large, dose-dependent prolongation of the QT interval ($P<0.05$, Table 1). Higher doses of tedisamil prolonged both the PR interval and the QRS duration of the ECG to a small degree ($P<0.05$). In contrast, infusion of lidocaine produced only a small increase in the PR interval of the ECG ($P<0.05$), while the QRS duration and the QT interval were unchanged relative to vehicle control (Table 1).

Compared to vehicle control, combinations of tedisamil and lidocaine prolonged the PR interval and the QRS duration of the ECG ($P<0.05$; Table 1). These effects could largely, but not completely, be attributed to tedisamil. Only the highest doses of the two drugs produced greater prolongation of the PR interval and the QRS duration than either drug alone. Coadministration of lidocaine did not have a consistent effect on the QT prolongation produced by tedisamil.

Ischaemia-induced arrhythmias

Infusion of tedisamil, 0.125–4 μmol kg^{-1} min^{-1}, or lidocaine, 2–8 μmol kg^{-1} min^{-1}, provided dose-related protection against arrhythmias induced by myocardial ischaemia (Figures 1, 2). At a high dose (8 μmol kg^{-1} min^{-1}), lidocaine completely prevented the occurrence of ischaemia-induced VF, whereas tedisamil did not completely suppress ischaemia-induced VF at any dose (Table 2).

Coadministration of tedisamil and lidocaine resulted in a leftward shift in the antiarrhythmic dose–response curve relative to either drug alone (Figures 1, 2). In addition, the maximum response to tedisamil was increased by coadministration of lidocaine. Thus, for tedisamil, the antiarrhythmic ED_{50} was 3.0 ± 1.3 *versus* 0.5 μmol kg^{-1} min^{-1} when 6 μmol kg^{-1} min^{-1} lidocaine was coadministered (Table 3). Similarly, for lidocaine, the ED_{50} was 4.9 ± 0.6 *versus* 0.7 ± 0.2 μmol kg^{-1} min^{-1} when 2 μmol kg^{-1} min^{-1} tedisamil was coadministered ($P<0.05$, Table 3). The shift in the ED_{50}'s demonstrates that tedisamil and lidocaine interact in a way that is greater than expected if the two drugs act *via* a mutually additive mechanism. That is to say that the antiarrhythmic effects of tedisamil and lidocaine were synergistic.

Isobolograms were constructed using the various doses of tedisamil and lidocaine to further examine the possible synergistic actions between these two agents in terms of ED_{50} values (Figure 3). If the drugs have an additive interaction, then the data would be expected to fall on a straight line between the ED_{50}'s for the two drugs alone. However, if the two drugs act synergistically, then the data would be expected to fall below the line connecting the two ED_{50}'s. Thus, the isobologram (Figure 3) suggests that the two agents interacted synergistically in that five points in the figure were on or below the line of additivity, while only one point was above it. The optimal dose to observe the synergy was 2 μmol kg^{-1} min^{-1} of the two drugs.

Table 1 Effect of tedisamil and lidocaine on mean blood pressure, heart rate and ECG intervals

(a) Effect of tedisamil and lidocaine alone on mean blood pressure, heart rate and ECG intervals

Tedisamil	*BP* (mmHg)	*HR* (beats min^{-1})	*PR* (ms)	*QRS* (ms)	*QT* (ms)
Control	111±2	359±4	62±1	16±1	29±1
Tedisamil (μmol kg^{-1} min^{-1})					
0.125	122±5	339±27	66±2	16±2	38±1
0.25	124±3	312±17*	68±2	20±3	53±3
0.5	130±3	302±13*	75±1*	25±4*	67±9
1	114±3	237±12*	70±4*	16±1	91±5*
2	134±4*	199±16*	77±2*	23±3*	121±20*
4	115±14	108±9*	74±4*	21±1*	201±18*
Lidocaine (μmol kg^{-1} min^{-1})					
2	111±6	321±21*	63±1	18±1	30±1
4	75±9*	298±29*	68±2*	18±1	32±1
6	73±5*	266±8*	68±2*	18±1	29±1
8	75±7*	245±18*	69±2*	17±1	30±1

(b) Effect of combinations of tedisamil and varied lidocaine on mean blood pressure, heart rate and ECG intervals

Tedisamil (μmol kg^{-1} min^{-1})	*Lidocaine* (μmol kg^{-1} min^{-1})	*BP* (mmHg)	*HR* (beats min^{-1})	*PR* (ms)	*QRS* (ms)	*QT* (ms)
0.5	1	113±4*	287±17*	70±2*	22±2*	66±5*
0.5	2	109±3*	248±9*#	74±1*	21±1*	69±7*
0.5	4	117±6	238±10*#	74±1*	20±1*	71±4*
0.5	6	107±5*	211±5*#	75±2*	20±2*	71±2*
1	0.5	132±4	232±13*	70±1*	24±3*	84±4*
1	1	114±6	204±8*	72±2*	22±2*	87±10*
1	2	115±6	197±12*	70±3*	20±1*	107±20*
1	4	114±5	205±6*	76±1*	26±3*#	88±5*
1	6	117±7	167±9*#	79±2*	22±2*	94±4*
2	0.25	109±1*	164±11*	74±2*	24±2*	119±8*
2	0.5	126±4	171±12*	75±4*	25±2*	154±19*#
2	1	115±5*	164±3*	74±1*	22±1*	116±12*
2	2	126±7	152±13*	73±3*	22±2*	112±8*

(c) Effect of combinations of lidocaine and varied tedisamil on mean blood pressure, heart rate and ECG intervals

0.125	2	111±9	335±9	70±2*	17±1	35±2*
0.25	2	126±4	307±15*	69±1*	19±2	56±9*#
0.5	2	112±8	267±10*	72±1*#	19±1	64±4*#
1	2	113±5	233±5*#	69±2*	18±1	99±5*#
2	2	87±5*#	142±7*#	74±3*#	26±4*	105±4*#
0.063	4	107±5*	303±21*	72±2*	19±1	33±2*
0.125	4	98±6*#	273±19*	69±2*	20±2*	40±2*
0.25	4	112±7#	258±24*	70±3*	19±2	58±8*#
0.5	4	104±6#	241±12*	75±3*	23±4*	62±3*#
1	4	98±5*#	205±7*#	73±3*	18±2	88±6*#
0.063	6	88±5*	272±9*	69±1*	18±1	37±2*
0.125	6	88±2*	262±7*	67±2	17±1	39±1*
0.25	6	97±3*#	249±11*	75±2*#	20±2*	51±3*#
0.5	6	106±8*#	222±11*#	76±3*	21±3*	66±5*#
1	6	107±7*#	191±6*#	77±1*#	21±1	80±3*#

Effects of tedisamil and lidocaine, alone or in combination, on blood pressure (BP–mmHg), heart rate (HR–beats min^{-1}), and PR, QRS and QT interval of the ECG (ms). *denotes statistical significance at $P<0.05$ from vehicle control, while # denotes a significant difference at the dose specified from at least one of the two drugs.

Electrical stimulation studies

Continuous infusion of 2 μmol kg^{-1} min^{-1} lidocaine had no effect on either effective refractory period relative to vehicle control. In contrast, tedisamil produced a dose-related increase in effective refractory period (Figure 4) with a reciprocal depression of maximum following frequency (data not shown). Coadministration of 2 μmol kg^{-1} min^{-1} lidocaine shifted the dose–response curve for tedisamil's effect on these two variables to the left (Figure 4). As lidocaine itself had no

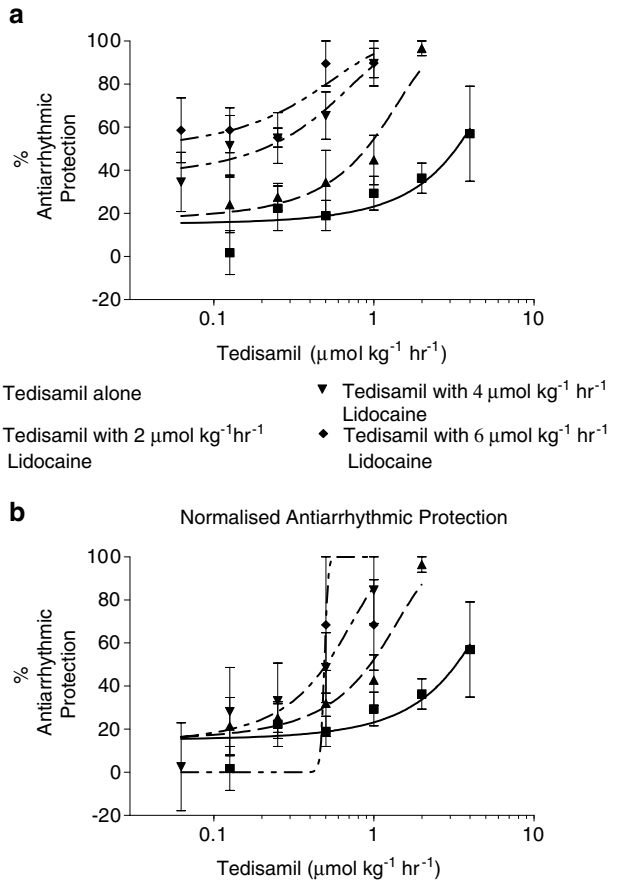

Figure 1 Antiarrhythmic dose–response curves for tedisamil and combinations of tedisamil and lidocaine as compared to vehicle control (a), or relative to the antiarrhythmic protection produced by the dose of lidocaine that was coadministered (b). Each point is the mean per cent protection ± s.e.m., $n = 5$. The data were fit to a two-parameter logistic function as described in the Methods section. The curve fit parameters are summarised in Table 3.

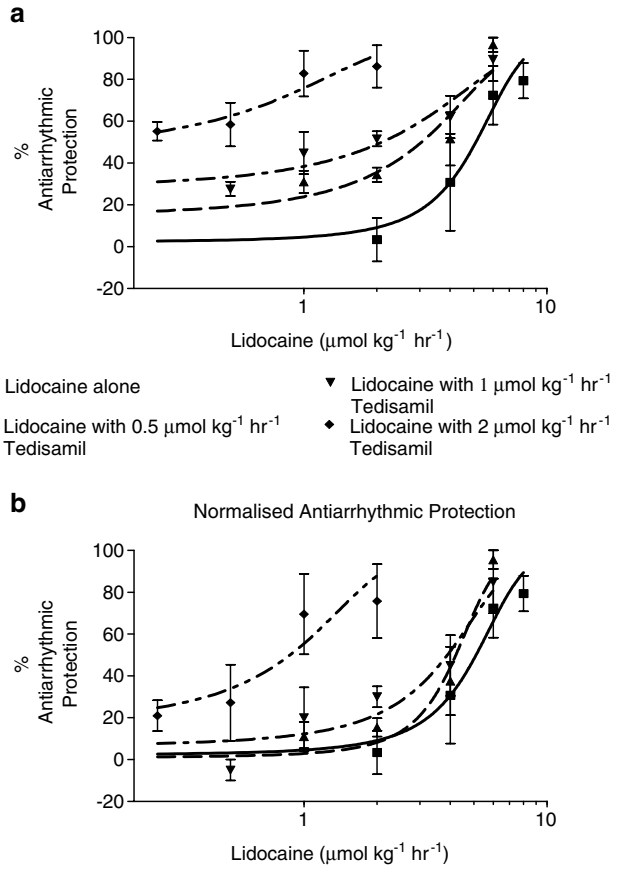

Figure 2 Antiarrhythmic dose–response curves for lidocaine and combinations of tedisamil and lidocaine as compared to vehicle control (a), or relative to the antiarrhythmic protection produced by the dose of tedisamil that was coadministered (b). Each point is the mean per cent protection ± s.e.m., $n = 5$. The data were fit to a two-parameter logistic function as described in the Methods section. The curve fit parameters are summarised in Table 3.

effect on either variable when administered alone, the leftward shift of the dose–response curve can be directly attributed to synergistic actions. This study likely underestimates the extent of the leftward shift in the dose–response curve as effective refractory periods >200 ms could not be measured. At the highest dose of tedisamil tested, 1/5 rats had an effective refractory period >200 ms. In contrast, at the highest dose of tedisamil tested in those rats in which 2 μmol kg^{-1} min^{-1} lidocaine was coadministered, 4/5 had an effective refractory period >200 ms.

Drug effects on the threshold current for capture were confined to the highest doses of tedisamil tested. At a dose of 2 μmol kg^{-1} min^{-1}, tedisamil increased the threshold current for capture from 120 ± 13 to 192 ± 30 μA ($P < 0.05$). Coadministration of 2 μmol kg^{-1} min^{-1} lidocaine and tedisamil increased the threshold current for capture from 118 ± 14 to 300 ± 79 μA ($P < 0.05$).

In a second series of electrical stimulation experiments, similar results were found, although the protocol limited the range of doses over which observations could be made. Tedisamil produced a dose-related increase in effective refractory period at stimulation rates of 7.5, 9.5 and 11.5 Hz. Coadministration of 2 μmol kg^{-1} min^{-1} lidocaine shifted the dose–response curve for tedisamil to the left at all the three of these stimulation rates (Figure 5). While the shift in the dose–response curve was statistically significant ($P < 0.05$), the Tukey test failed to detect a significant difference between individual points. This was partly due to the smaller window over which observations could be made. In this experiment, effective refractory period could only be extended to the pacing cycle length before it was no longer possible to measure its value. Thus, at high doses of tedisamil (>0.5 μmol kg^{-1} min^{-1}), drug-induced increases in effective refractory period precluded pacing at rates of 7.5, 9.5 and 11.5 Hz in all animals. For example, it was only possible to pace one of three rats at 11.5 Hz after infusion of the 0.5 μmol kg^{-1} min^{-1} dose of tedisamil and it was not possible to pace any of the animals infused with this dose of tedisamil when 2 μmol kg^{-1} min^{-1} lidocaine was coadministered.

Discussion

Combinations of the action-prolonging agent, tedisamil, and the inactivated state sodium channel blocker, lidocaine, were found to be more effective in suppressing ischaemia-induced arrhythmias than either agent alone. This synergistic interaction is predicted by the modulated receptor hypothesis which

Table 2 Effect of tedisamil and lidocaine on the incidence of ischaemia-induced tachyarrhythmias

(a) Effect of tedisamil and lidocaine alone on ischaemia-induced tachyarrhythmias

	VT incidence	*VF incidence*
Control	82/85	68/85
Tedisamil (μmol kg^{-1} min^{-1})		
0.125	5/5	5/5
0.25	5/5	4/5
0.5	5/5	4/5
1	5/5	4/5
2	5/5	2/5
4	3/5*	0/5*
Lidocaine (μmol kg^{-1} min^{-1})		
2	5/5	4/5
4	4/5	2/5
6	2/5*	1/5*
8	1/5*	0/5*

(b) Effect of combinations of tedisamil and lidocaine on the incidence of ischaemia-induced tachyarrhythmias

Tedisamil (μmol kg^{-1} min^{-1})	*Lidocaine* (μmol kg^{-1} min^{-1})	*VT incidence*	*VF incidence*
Control	Control	82/85	68/85
1	0.5	5/5	4/5
1	1	4/5	3/5
1	2	5/5	2/5
1	4	4/5	1/5#
1	6	1/5*#	0/5*#
2	0.25	5/5	0/5*#
2	0.5	4/5	0/5*#
2	1	2/5*#	1/5*#
2	2	1/5*#	0/5*#
0.125	2	5/5	5/5
0.25	2	5/5	4/5
0.5	2	5/5	4/5
1	2	4/5	3/5
2	2	0/5*#	0/5*#
0.063	4	4/5	2/5
0.125	4	4/5	1/5*
0.25	4	4/5	2/5
0.5	4	4/5*	1/5*
1	4	1/5*	1/5*
0.063	6	3/5*	0/5*#
0.125	6	3/5*	0/5*#
0.25	6	5/5	0/5*#
0.5	6	1/5*#	0/5*#
1	6	1/5*#	0/5*#

Effects of combinations of tedisamil and lidocaine on the incidence of ischaemia-induced ventricular tachycardia (VT) and ventricular fibrillation (VF). *denotes statistical significance at $P<0.05$ (Fisher's exact test) from vehicle control, while # denotes a significant difference at the dose specified from at least one of the two drugs.

contends that combining these two activities should culminate in an extension of effective refractory period (Hondeghem & Katzung, 1984). Prolongation of effective refractory period in normal tissue can suppress cardiac arrhythmias by reducing the time during the cardiac cycle during which arrhythmias can occur. Alternatively, increased refractoriness prolongs the path length of re-entry circuits to such an extent that re-entry circuits cannot be sustained (Adaikan *et al*., 1992; Janse, 1992). Studies conducted in normal rat hearts confirm that the synergistic actions of combinations of tedisamil and lidocaine can be explained, at least in part, by extension of effective refractory period in normal cardiac tissue.

The modulated receptor hypothesis predicts a synergistic interaction between action potential prolongation and inactivated state sodium channel block based on the time- and voltage dependence of the interaction of the drug with the sodium channel (Hondeghem & Katzung, 1984). Action potential prolongation maintains cardiac cells in a depolarised state for a longer time. As sodium channels are predominately in the inactivated state at depolarised potentials, this increases the availability of inactivated sodium channels. Thus, with action potential prolongation, the number of inactivated state sodium channels available to a drug that binds selectively to this state is increased and the effective refractory period is prolonged proportionally.

The evidence for the enhanced antiarrhythmic and electrophysiological actions of combinations of tedisamil and lidocaine in the present study are four-fold. Firstly, ED_{50}s were reduced by combinations of the two drugs relative to either drug alone. Secondly, the analysis of variance for an interaction between the two drugs was statistically significant. Thirdly, isobologram analysis suggested that the two drugs interact in a manner that was more than additive. Fourthly, a dose of lidocaine (2 μmol kg^{-1} min^{-1}) that had no effect on the effective refractory period of normal cardiac tissue when administered alone, produced a leftward shift in the dose–response curve for tedisamil's effect on this variable.

While interpretation of the dose–response data and the statistical analysis is self-evident, the use of isobolograms warrants further comment. The premise underlying isobolograms is that combinations of drugs that act at the same receptor will, for a constant response, produce a response that is the arithmetic sum of the two responses. If the two drugs act synergistically, responses are greater than expected from additivity and therefore the ED_{50} for combinations is lower than expected. As such, the ED_{50} for combinations of the two drugs fall below the line of additivity that connects the ED_{50}'s for the two drugs alone. While it is clear that tedisamil and lidocaine have different molecular mechanisms of actions (i.e., blockade of the transient outward current and sodium current, respectively), the effect of combinations of the two drugs results in an extension of effective refractory period. It might therefore be possible to view the hypothetical 'single site' of action for the interaction to be an extension of the effective refractory period.

Examination of the antiarrhythmic dose–response curves for the coadministration of lidocaine with tedisamil and tedisamil with lidocaine suggests that the two are in fact different. Coadministration of lidocaine increased the maximum response produced by tedisamil and caused a leftward shift in the dose–response curve. In contrast, coadministration of tedisamil appeared to selectively cause a leftward shift in lidocaine's dose–response curve without affecting the maximum. Perhaps, this difference is not surprising given that lidocaine (Barrett *et al*., 1995) and other sodium channel-blocking drugs such as tetrodotoxin (Abraham *et al*., 1989) can

Table 3 Antiarrhythmic ED_{50}'s for tedisamil and lidocaine, alone or in combination

	ED_{50} (μmol kg^{-1} min^{-1})	Ratio		ED_{50} (μmol kg^{-1} min^{-1})	Ratio
Tedisamil alone	3.0±1.3		Lidocaine alone	4.9±0.6	
With lidocaine			With tedisamil		
2 μmol kg^{-1} min^{-1}	0.8±0.2*	3.8	0.5 μmol kg^{-1} min^{-1}	4.2±0.2*	1.2
4 μmol kg^{-1} min^{-1}	0.4±0.1*	7.5	1 μmol kg^{-1} min^{-1}	3.4±0.6*	1.4
6 μmol kg^{-1} min^{-1}	0.5	6	2 μmol kg^{-1} min^{-1}	0.7±0.2*	7

Dose–response curves were fit to a two-parameter logistics function as follows: per cent antiarrhythmic protection = $100*(D^h/(D^h + ED_{50}{}^h))$, where D is dose (μmol kg^{-1} min^{-1}), h is the slope factor and ED_{50} is the dose required to produce 50% of maximum response. The maximum response was defined as the complete elimination of arrhythmias whereas the minimum response was defined to be the occurrence of arrhythmias in vehicle controls. ED_{50} values are summarised as the mean±s.e.m. for each of the curves. *denotes a significant difference between combinations of drug relative to that drug administered alone (ANOVA at $P<0.05$).

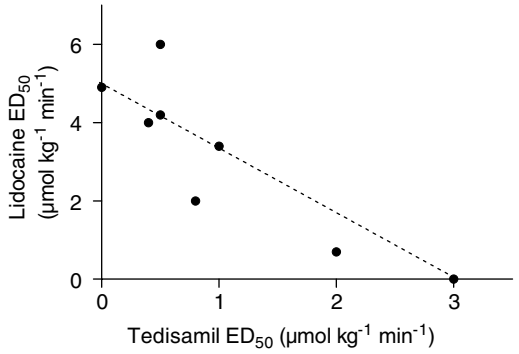

Figure 3 Isobologram for combinations of lidocaine and tedisamil. Each point is the antiarrhythmic ED_{50} determined from the antiarrhythmic dose–response curves shown in Figures 1 and 2. The hatched line connecting the ED_{50}'s for tedisamil and lidocaine is the line of additivity. Points below the line of additivity indicate synergy, while points above indicate functional antagonism.

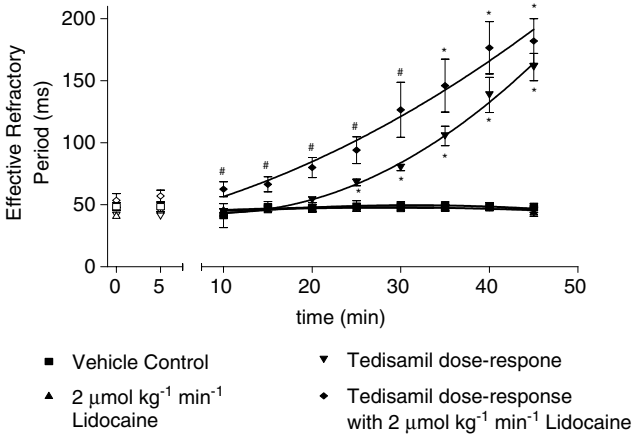

Figure 4 Dose–response curves for the effect of tedisamil and lidocaine, alone or in combination, on effective refractory period. Each point is the mean±s.e.m., $n=5$, as described in the Methods section. The open symbols at time 0 and 5 min show the values before starting the experiment and after administration of lidocaine or lidocaine–vehicle, respectively. Infusion of lidocaine or lidocaine–vehicle was maintained throughout the remainder of the protocol and was followed by increasing infused doses of tedisamil (0.016–2 μmol kg^{-1} min^{-1}). *denotes statistical significance at $P<0.05$ from vehicle control (repeated measures ANOVA), while # denotes statistical significance between tedisamil alone and tedisamil plus lidocaine groups as well as from vehicle control.

completely suppress the occurrence of ischaemia-induced arrhythmias at high doses, whereas tedisamil does not produce this maximal response in the rat model (Beatch *et al*., 1991). Regardless, it is remarkable that lidocaine confers this property to tedisamil at a relatively low dose (2 μmol kg^{-1} min^{-1}). This dose of lidocaine does not itself suppress ischaemia-induced arrhythmias or produce marked electrophysiological effects (in normal myocardial tissue).

In the studies by Duff and co-workers, quinidine was used to prolong cardiac action potentials (Duff *et al*., 1983; 1986; Duff, 1989). While quinidine is effective in this regard, it is also a potent sodium channel blocker and the modulated receptor hypothesis also predicts a complicated interaction between sodium channel-blocking drugs (Hondeghem & Katzung, 1984). Tedisamil is more selective than quinidine for prolonging cardiac action potentials (Dukes & Morad, 1989) and is therefore a better pharmacological tool to explore this interaction. In the rat model of ischaemia-induced arrhythmias, tedisamil prolongs the QT interval and action potential duration to a much greater extent than quinidine at equieffective antiarrhythmic doses (compare Beatch *et al*. (1991) and Barrett *et al*. (1995)). These data suggest that the sodium channel-blocking actions of quinidine contribute to the antiarrhythmic actions of this drug (and combinations of quinidine and mexiletine) and complicate interpretation of the results presented by Duff and co-workers. While tedisamil is more selective than quinidine in this regard, it is not devoid of sodium channel-blocking actions (Beatch *et al*., 1991; Nemeth *et al*., 1996). The sodium channel-blocking effects of tedisamil are apparent in the present studies in prolongation of the PR and QRS intervals of the ECG. The present studies cannot completely exclude the possibility that the interaction between the two drugs might be partly explained by addition of the two drugs' sodium channel-blocking effects.

Duff and co-workers attributed the synergistic actions of combinations of quinidine and mexiletine to prolongation of effective refractory period in peri-infarcted tissue (Duff & Gault, 1986; Duff, 1989). Results of the present study demonstrate that the extension of effective refractory period predicted by the modulated receptor hypothesis occurs in normal cardiac tissue. Moreover, this effect in normal cardiac tissue can account, at least partly, for the enhanced antiarrhythmic activity of combinations of tedisamil and lidocaine.

One limitation of the present study that must be considered is the species dependence in the mechanism through which cardiac action potentials are prolonged. While the rat

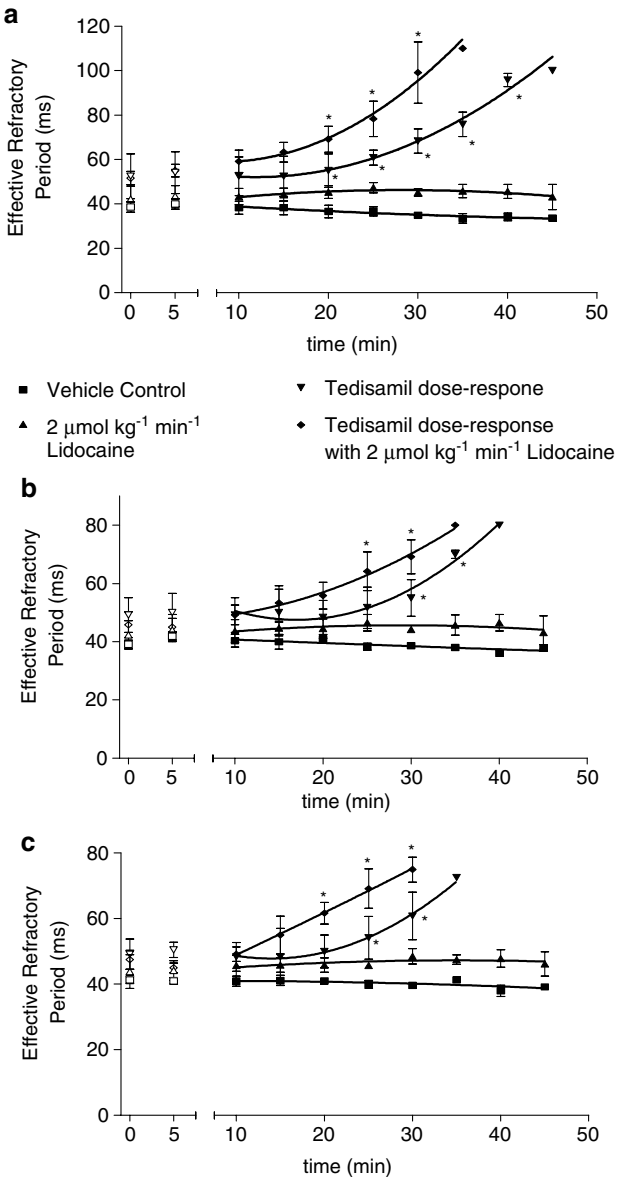

Figure 5 Dose–response curves for the effect of tedisamil and lidocaine, alone or in combination, on effective refractory period determined at pacing rates of 7.5(a), 9.5 (b) and 11.5 Hz (c). Each point is the mean ± s.e.m., $n=3$, except where no error bars are presented in which case $n=1$. The open symbols at time 0 and 5 min show the values before starting the experiment and after administration of lidocaine or lidocaine–vehicle, respectively. Infusion of lidocaine or lidocaine–vehicle was maintained throughout the remainder of the protocol and was followed by increasing infused doses of tedisamil ($0.016-2\,\mu mol\,kg^{-1}\,min^{-1}$). *denotes statistical significance at $P<0.05$ from vehicle control (repeated-measures ANOVA). While the tedisamil and tedisamil plus lidocaine groups were significantly different by ANOVA ($P<0.05$). at each pacing rate, the Tukey test did not have the power to detect which points were significantly different.

myocardium relies primarily on the transient outward current for repolarisation of the ventricle, the human ventricular myocardium is more dependent on the delayed rectifier potassium current with the transient outward current making a variable contribution. Thus, while tedisamil prolongs cardiac action potentials in both species, it likely does so by blocking the transient outward current in the rat ventricle and the delayed rectifier current in the human ventricle. However, the results of the present study are applicable to treatment of ischaemia-induced arrhythmias in humans, as the interaction described herein should occur regardless of the mechanism through which cardiac action potentials are prolonged.

It should be noted that a drug with the properties of combinations of tedisamil and lidocaine would be anomalous in the Vaughan–William's classification scheme. Such a drug would slow conduction in the heart but only at high rates (and in ischaemic myocardial tissue) and prolong cardiac action potentials. The combination of these two activities would be classified as a Class Ia antiarrhythmic. However, the state dependence of sodium channel blockade and the kinetics of sodium channel block would differ from existing Class Ia antiarrhythmic drugs such that no effect would be seen at normal heart rates. Thus, combinations of tedisamil and lidocaine differ from quinidine in that quinidine blocks sodium channels in the activated (open) state and the prolongation of effective refractory period produced by quinidine is therefore solely attributed to action potential prolongation (Campbell, 1983). This differs from combinations of tedisamil and lidocaine in that prolongation of effective refractory period occurs by two mechanisms with the combination: prolongation of action potential duration, and delaying recovery of sodium channels beyond repolarisation. Of the antiarrhythmic drugs currently in use, combinations of tedisamil and lidocaine resemble amiodarone the most. While the pharmacology of amiodarone is complex, it prolongs cardiac action potentials (Singh & Vaughan-Williams, 1970) and blocks sodium channels in the inactivated state (Mason *et al*., 1984). This parallel, along with the results of the present study, suggests that more effective antiarrhythmic drugs might be developed by optimising combinations of two complementary electrophysiological mechanisms.

Class III antiarrhythmics exhibit reverse rate dependence in their effects on action potential duration (Hondeghem & Snyders, 1990). Thus, their action potential-prolonging effects are reduced at high heart rates and exacerbated at low heart rates. The excessive prolongation of action potential duration at low heart rates increases the risk of torsades de pointes and limits the utility of Class III antiarrhythmic drugs (Hondeghem & Snyders, 1990). Indeed, tedisamil was recently shown to cause torsades de pointes in an isolated rabbit heart model (Barrett *et al*., 2001). Lidocaine has been demonstrated to reduce the incidence of torsades de pointes caused by action potential-prolonging drugs in experimental animals (Carlsson *et al*., 1993; Chezalviel-Guilbert *et al*., 1995) and has been used to treat torsades de pointes in man (Assimes & Malcolm, 1998). This suggests that the addition of inactivated state sodium channel-blocking activity to a Class III drug might provide additional benefit by reducing the proarrhythmic effects associated with action potential prolongation.

In summary, combinations of tedisamil and lidocaine were more effective than either agent alone for suppression of ischaemia-induced arrhythmias. The enhanced antiarrhythmic activity of combinations of the two agents can be accounted for, at least partly, by extension of effective refractory period in normal cardiac tissue.

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