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Antiarrhythmic properties of tedisamil (KC8857), a putative transient outward K^+ current blocker

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1 Rats were used to evaluate the antiarrhythmic properties of tedisamil, a novel agent with the electrophysiological properties of a Class III antiarrhythmic drug. Tedisamil was tested against coronary artery occlusion-induced arrhythmias in conscious animals.

2 The actions of tedisamil on the ECG, as well as responses to electrical stimulation, were compared with those on the configuration of epicardial intracellular action potentials recorded *in vivo*.

3 Tedisamil (1–4 mg kg^{-1}, i.v.) caused bradycardia, elevated blood pressure and dose-dependently reduced ventricular fibrillation (VF) induced by occlusion of the left anterior descending coronary artery. Other ischaemia-associated arrhythmias were not so well suppressed. Antiarrhythmic activity was greatest when the tedisamil-induced bradycardia was prevented by electrically-pacing the left ventricle.

4 Tedisamil dose-dependently lengthened the effective refractory period and prevented electrically-induced VF. *In vivo*, tedisamil (0.5–4 mg kg^{-1}, i.v.) prolonged the duration of epicardial intracellular action potentials by up to 400%.

5 Results showed that tedisamil possessed antifibrillatory actions in rats that were related to Class III electrophysiological actions as revealed by electrical stimulation and electrophysiological analyses.

Introduction

Vaughan Williams (1984) classified antiarrhythmic drugs on the basis of effects of action potential morphology. The clinical and experimental usefulness of the classification is constantly under review, particularly for Class III antiarrhythmics which act mainly to prolong action potential duration (APD) presumably by inhibition of repolarizing cardiac K^+ currents. The testing of Class III drugs against arrhythmias is limited by the lack of adequate models and selective drugs. Clinically available Class III agents have mixed electrophysiological actions although newer drugs with greater Class III selectivity are claimed e.g. (+)-sotalol plus its imadizolium derivatives (Lis *et al.*, 1987), N-acetylprocainamide (Dangman & Hoffman, 1981) and its stable amide (methanesulphonamide) sematilide, (Lumma *et al.*, 1987), as well as radically new agents such as tedisamil, UK 68,798 and risotilide (Walker & Beatch, 1988; Gwilt *et al.*, 1989; Colatsky *et al.*, 1989).

Tedisamil appears to inhibit selectively the transient outward potassium current, i_{to}, in cardiac tissue (Dukes & Morad, 1989). As a result it is bradycardic in all species tested (Buschmann *et al.*, 1989a) but markedly increases the Q-T_c interval as well as APD in the rat (Walker & Beatch, 1988; Beatch *et al.*, 1990). It also widens the Q-T_c interval in primates (Buschmann *et al.*, 1989b).

In view of the above, the present study was undertaken to assess the electrophysiological and antiarrhythmic actions of tedisamil in the rat. We have previously used rat models to demonstrate the dose-related efficacy of Class I and Class IV agents against ischaemia-induced arrhythmias (Abraham *et al.*, 1989; Curtis *et al.*, 1986).

The following experiments were performed in rats: (i) Evaluation of antiarrhythmic effectiveness against occlusion-induced arrhythmia. (ii) Evaluation of effects on the ECG and responses to electrical stimulation. (iii) Evaluation of actions on intracellular potentials *in vivo*.

The occurrence and density of specific potassium channels are tissue- and species-dependent (Irisawa, 1987). Thus we considered it important to conduct all studies in a single species. In a systematic manner we hoped to establish that tedisamil increased action potential duration *in vivo*, resulting in Q-T_c widening and increasing ventricular refractoriness. An increase in refractoriness would be expected to inhibit the occurrence and severity of ischaemia-induced arrhythmias. Thus, tedisamil might be a useful drug in rats with which to investigate the antifibrillatory and antiarrhythmic effectiveness of a potent and efficacious Class III compound.

Methods

Male Sprague-Dawley rats (250–350 g) were used throughout. When required, pentobarbitone (45 mg kg^{-1}) was used for anaesthesia and animals artificially ventilated with O_2 at a stroke volume of 10 ml kg^{-1}, 60 min^{-1}; a regimen which has been found to keep blood gases at normal levels (Maclean & Hiley, 1988). Body temperature was maintained at 37.0 ± 0.5°C by means of a thermocouple linked to a heating lamp. Tedisamil (KC8857), i.e. N,N'dicyclopropylmethyl-9, 9-tetramethylene-3,7- diazabicyclo(3,3,1) nonane 2 HCl, was supplied by Kali-Chemie, FRG. It was dissolved in saline.

Doses and route of administration of tedisamil

In a separate study we determined the maximum tolerated dose of tedisamil. Tedisamil at 4 mg kg^{-1}, i.v. (given over 2 min) produced no symptoms of adverse effects, whereas 8 mg kg^{-1} produced respiratory symptoms of gasping and disturbed breathing. None of these doses produced arrhythmias. Adverse effects were attenuated when the drug was injected more slowly. The antiarrhythmic actions of tedisamil in conscious rats were therefore studied at 1, 2 and 4 mg kg^{-1} with doses administered as an infusion over 10 min. In electrical stimulation studies, tedisamil was administered in a cumulative manner with doses of 0.5, 0.5, 1, 2, and 4 mg kg^{-1} given 15 min apart; each dose was infused over 2 min. The same dosing schedule was used for *in vivo* intracellular studies. In order to allow for comparisons between the different dosing regimens, equivalent Q-T_c widenings in different preparations

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were assumed to indicate equivalence of plasma concentrations.

Evaluation of antiarrhythmic properties

Ischaemic arrhythmias were produced in conscious rats surgically implanted 7 days previously with a loosely-applied left anterior descending (LAD) coronary artery occluder, ECG electrodes (approximate lead V3) as well as arterial and venous cannulae as described previously (Johnston *et al.*, 1983). Experiments were performed one week after surgery. The measured variables included serum potassium, blood pressure (BP), heart rate (HR) and ECG changes as well as arrhythmias before and after occlusion of the LAD coronary artery. Arrhythmias were recorded 0–0.5 h, 0.5–4 h and 24 h after occlusion plus, in some cases, one week and one month later. As a summary of arrhythmic history, arrhythmia scores (AS) were calculated according to the occurrence of ventricular premature beats (VPBs) and the number and duration of ventricular tachycardia (VT), and VF episodes as justified elsewhere (Curtis & Walker, 1988). One day after occlusion, rats were killed and their occluded zone (OZ or zone-at-risk) estimated as described previously (Johnston *et al.*, 1983).

Doses of 1, 2, and 4 mg kg^{-1} of tedisamil were administered to groups of 9 rats as a 10 min infusion with a repeat dose 1 h after occlusion. Control rats ($n = 9$) received saline. The design of the experiments and the analysis of arrhythmias followed the Lambeth Conventions (Walker *et al.*, 1988). In keeping with the Conventions, the number of VPB in any time period were log transformed before statistical analysis in order to normalize statistically such data.

As a control for tedisamil-induced bradycardia, separate groups of rats had their ventricles electrically-stimulated so as to overcome the bradycardia. On the day of occlusion, chronically prepared rats were fitted with silver wire intraventricular electrodes, under pentobarbitone anaesthesia, in order to pace their hearts at 6.5 Hz, i.e. the rate found in control rats. Tedisamil (2 mg kg^{-1}, i.v.) was infused as above. Ventricles were stimulated from 4–15 min following occlusion, i.e., the vulnerable period for arrhythmias.

In separate experiments, animals with one day, one week, and one month old infarcts were found to have variable incidences of ventricular arrhythmias. The effects of tedisamil against these arrhythmias was tested using the cumulative dose regimen previously described, with 15 min between doses. Drug-effects were assessed in terms of an increase, or decrease (by 90%) in the control rate of appearance of spontaneous arrhythmias.

Electrical stimulation

The technique of ventricular electrical stimulation in intact rats was used as an index of the electrophysiological actions of tedisamil. To control for possible effects of anaesthesia and acutely-performed surgery, electrical stimulation was performed in acutely prepared pentobarbitone-anaesthetized rats, and chronically prepared halothane-anaesthetized rats.

In pentobarbitone-anaesthetized and artificially ventilated rats, the right jugular vein and left carotid artery were cannulated for drug administration and blood pressure recording, respectively. Teflon coated silver wire electrodes were implanted in the left ventricle as described previously (Curtis & Walker, 1986). The suitability of electrode location was confirmed by stable threshold values for stimulation, and verified by post mortem examination.

The following were measured: (a) Current threshold for single ventricular extra-systoles and for VF (VF_t) at 50 Hz and 2 times threshold pulse width as determined for the animal being investigated; (b) maximum following frequency (MFF) obtained by smoothly increasing pacing frequency (at twice threshold current and pulse width) from 6 Hz until the heart failed to follow, on a 1:1 basis, the increasing stimulation rate; (c) effective refractory period (ERP) obtained by pacing the heart at 7.5 Hz (at 2 times threshold pulse width and current) and randomly adding an extra stimulus at an increasing delay. The shortest interval required to produce a premature extrasystole was taken as the effective refractory period.

ECG and blood pressure changes were recorded throughout. Tedisamil was administered by a cumulative dose regimen and determinations made 10–15 min after each dose. Triplicate determinations were made at each dose level and six animals were tested per group.

In chronically-prepared animals, abdominal aortic and inferior vena caval cannulae were implanted together with a loose electrode carriage (Walker & Beatch, 1988) one week prior to testing. This carriage, fashioned out of PE 10 polyethylene tubing, was designed to reversibly position stainless steel electrodes (2.5 mm apart) against the left ventricular epicardium. On the day of experiment, animals were anaesthetized with halothane (1%) and blood pressure and ECG recorded. The electrode was positioned against the ventricle wall by gentle traction on the electrode assembly. Lead II was used to assess effects on the ECG, while lead III served to detect electrically-induced arrhythmias. The above protocol for acutely prepared rats was followed, except that only doses of 0.5, 0.5, 1 and 2 mg kg^{-1} tedisamil were studied.

Epicardial intracellular action potential recording in vivo

Under pentobarbitone anaesthesia and artificial ventilation, the carotid artery and jugular vein were cannulated, and ECG electrodes placed subcutaneously. The heart was exposed through an incision at the level of 4th–5th intercostal space and a portion of the left ventricular epicardial surface immobilized by suturing it to a looped silver/silver chloride reference electrode. Epicardial action potentials were recorded with 3 M KCl fibre-filled microelectrodes and a floating-tip technique. This technique has been used in the evaluation of the effects of tetrodotoxin on action potential configuration (Abraham *et al.*, 1989). Doses of tedisamil (0.5, 0.5, 1, 2 and 4 mg kg^{-1}, i.v.) were administered consecutively every 15 min. A multiple impalement technique was used to assess drug effects upon action potential height, maximum value of dV/dt for the rising phase of the action potential (dV/dt_{max}) and APD at 10, 25, 50 and 75% of repolarization (APD_{10}, etc).

Data analysis

Most studies were conducted according to blind and random protocols. Statistical analysis was performed by ANOVA. Differences between means was determined by Duncan's multiple range test. Contingency tables were used to determine significance for nonparametric data.

Results

Occlusion study

The time course of tedisamil and occlusion effects on blood pressure and heart rate were summarized in Figure 1 which illustrates that tedisamil produced a dose-dependent bradycardia and an increase in mean arterial blood pressure. At 30 min post-occlusion the bradycardic effect of tedisamil was still apparent, whereas the pressor response was lost. At 1 h and 4 h post-infusion, heart rate and blood pressure were restored to control values. Serum potassium levels were not different in any of the groups with average values of 3.4–3.6 mM.

The effects of tedisamil on P–R, QRS and Q–T_c intervals of the ECG prior to occlusion are shown in Table 1. Tedisamil significantly widened the Q–T_c interval, and prolonged P–R duration, but had no effect on QRS interval. Tedisamil also influenced the ECG after occlusion by increasing the degree of 'S–T' segment elevation induced by occlusion (Figure 2). Similar effects on R-waves were not noted.

The effects of tedisamil on arrhythmias as measured by the incidence of VT, VF, as well as $\log_{10}$ of total VPB in any time

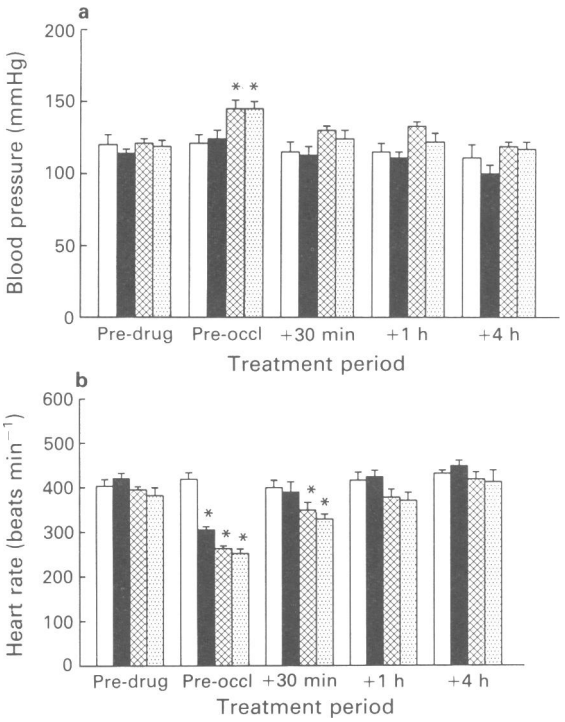

Figure 1 Effects of treatment and coronary artery occlusion on mean arterial blood pressure (a) and heart rate (b) in conscious chronically prepared rats. Tedisamil was administered as a 10 min infusion and occlusion performed 5 min after infusion. Open columns = saline; closed columns = 1 mg kg^{-1}; cross-hatched = 2 mg kg^{-1} and stippled = 4 mg kg^{-1} tedisamil. Values were determined before drug (pre-drug), before occlusion (pre-occl) and 30 min, 1 and 4 h after occlusion of the LAD coronary artery. Each column is the mean of $n = 5$–9 rats/group; s.e.mean shown by vertical bars. * $P < 0.05$ vs. control.

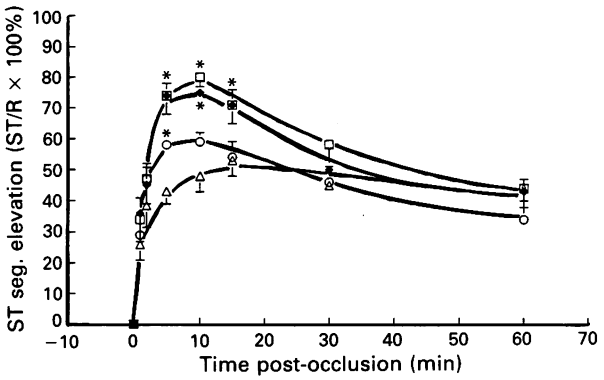

Figure 2 Effects of tedisamil on 'S–T' segment changes induced by LAD coronary occlusion in conscious chronically prepared rats. Drug was administered as a 10 min i.v. infusion and occlusion performed 15 min after beginning infusion. Symbol (△) indicates saline; (○) 1 mg kg^{-1}; (□) 2 mg kg^{-1} and (◆) 4 mg kg^{-1} tedisamil. 'S–T' segment amplitude is presented as percentage of R-wave amplitude. Each point is a mean of $n = 9$ rats/group; s.e.mean shown by vertical bars. For the sake of clarity only the first hour of occlusion is shown. * $P < 0.05$ vs. control.

period are summarized in Table 2. Tedisamil caused a dose-dependent reduction in the occurrence of VF, completely preventing this arrhythmia at 4 mg kg^{-1}. However, despite suppressing VF, the less serious arrhythmias (VT and VPB) were not obtunded. While tedisamil did not decrease the incidence of VT, it did modify its morphology. In control animals, and in those given 1 mg kg^{-1} tedisamil, VT patterns were of short cycle length and wide complex and were accompanied by precipitous falls in blood pressure (data not shown). In contrast, with 2 and 4 mg kg^{-1} tedisamil, VT was of a narrow-complex type with a long cycle length (e.g., 140 ms = 430 b min^{-1}) and was accompanied by minimal reductions in BP. Thus, although the incidence of VT in all groups was similar, the episodes were not equivalent. AS values, calculated according to the occurrence of VPBs and number and duration of VT and VF (Johnston *et al.*, 1983), were dose-dependently reduced by tedisamil: in the 0–30 min post occlusion period, 2 mg kg^{-1} tedisamil reduced AS to 2.4 ± 0.4, and 4 mg kg^{-1} tedisamil to 1.2 ± 0.5 ($P < 0.05$ for both effects), from the control value of 4.1 ± 0.8. For the 0.5–4 h time period, 2 mg kg^{-1} tedisamil reduced AS to 1.3 ± 0.4, from a control value of 2.6 ± 0.7 ($P < 0.05$).

Table 1 Effects of tedisamil on ECG variables in conscious rats

Dose (mg kg^{-1})	*P–R* (ms) *Pre*	*Post*	*QRS* (ms) *Pre*	*Post*	*Q–T$_c$* (ms) *Pre*	*Post*
Saline	41 ± 1	42 ± 1	22 ± 1	22 ± 1	215 ± 10	210 ± 10
1	38 ± 1	44 ± 1	22 ± 1	23 ± 1	220 ± 5	280 ± 9*
2	38 ± 2	47 ± 1*	23 ± 1	23 ± 1	205 ± 5	320 ± 7**
4	44 ± 2	53 ± 2*	22 ± 1	23 ± 1	195 ± 5	330 ± 9*

Tedisamil was administered to conscious rats as a 10 min i.v. infusion (see Methods). ECG values were recorded before (pre) and 4 min after (post) the end of infusion. Values are mean ± s.e.mean for $n = 9$ rats per group.
* $P < 0.05$; ** $P < 0.01$ compared with saline.

Table 2 Effects of tedisamil on the occurrence of occlusion-induced arrhythmias in conscious rats

	Arrhythmias in 0–0.5 h and 0.5–4 h post-occlusion periods						
	Incidence						
	VT		*VF*			*log$_{10}$ VPB*	
Dose	*Time post occlusion* (h)						
(mg kg^{-1})	*0–0.5*	*0.5–4*	*0–0.5*	*0.5–4*	*Mort*	*0–0.5*	*0.5–4*
Saline	8/9	3/5	7/9	3/5	4	1.5 ± 0.1	2.0 ± 0.1
1	7/9	5/7	6/9	3/7	2	1.8 ± 0.2	2.5 ± 0.2
2	8/9	2/8	4/9	1/8	1	1.6 ± 0.2	2.1 ± 0.4
4	4/9	4/9	1/9*	0/9	0*	1.4 ± 0.2	2.1 ± 0.3

Tedisamil was administered to conscious chronically prepared rats as a 10 min i.v. infusion as indicated in Methods. Occlusion was performed 5 min after the end of infusion. A repeat dose was given 1 h after occlusion. Arrhythmias occurring in the 0–0.5 h and 0.5–4 h periods are expressed as incidence (number of rats per group having one or more events) of VT or VF and the mean ± s.e.mean of $\log_{10}$ VPB. Initial group size was 9, but animals dying (Mort) in the first post-occlusion period (0–0.5 h) reduced the number of survivors in the second period (0–4 h).
* $P < 0.05$ compared with saline.

Variations in occluded zone size may account for variations in the incidence and severity of arrhythmias and so these were measured in all animals and were not found to be altered by tedisamil treatment. Values ranged from a mean of 34 to 37% and were not substantially different from each other. The similarity in occluded zone size might have been expected to result in similar 'S–T' segment changes being induced by occlusion but this was not found to be the case (see above).

To compensate for tedisamil-induced bradycardia, a separate group of paced rats was administered 2 mg kg^{-1} tedisamil. Ventricular pacing at 6.5 Hz appeared to increase the efficacy of tedisamil such that VT incidence (2/11), VF incidence (3/11) and AS (1.0 ± 0.4) were significantly lower ($P < 0.05$) in the electrically-paced rats than in controls (8/9, 9/9 and 3.8 ± 0.4, respectively). When comparison was made between paced and unpaced rats treated with 2 mg kg^{-1}, the VT incidence was reduced significantly (see Table 2 and above) in paced rats whereas VF incidence was unaffected. In the pacing study, the total duration of the ECG complex almost equalled the cycle length, i.e., Q–T/R–R = 0.95.

Effects in infarcted rats

Administration of tedisamil to rats with one-day, one-week, or one-month old infarcts did not affect blood pressure but produced bradycardias similar in magnitude to those seen in other rats. For example, control values for heart rate were 440 ± 12, 410 ± 15 and 330 ± 30 beats min^{-1} in one-day, one-week and one-month infarcted rats, respectively. At 4 mg kg^{-1} of tedisamil, heart rate in the three groups fell to 259 ± 9, 245 ± 16 and 225 ± 18 beats min^{-1}, respectively. Tedisamil exhibited antiarrhythmic activity in one-day infarcted animals suppressing VPBs in 8/14 rats. The effective dose ranged from 1–4 mg kg^{-1}. In one-week and one-month infarcted rats the incidence of arrhythmias was too low to test for antiarrhythmic effects. Proarrhythmic actions, consisting of an increase in VPB, bigeminy, or alternating bradytachycardia, were only encountered in 8/14 one-day infarcted rats. This occurred at a median cumulative dose of 7 mg kg^{-1}. Proarrhythmic effects of increased VPB and short episodes of VT were seen in 1/5 rats that had been infarcted for one-week. These occurred after cumulative doses of 1 and 4 mg kg^{-1} tedisamil. A similar finding occurred in 1/5 rats infarcted for 1 month after it had received a cumulative dose of 8 mg kg^{-1}. Tedisamil also elevated the 'S–T' segment in infarcted rats as is illustrated in Figure 3.

Effects of tedisamil on electrical stimulation

The effects of tedisamil on Q–T intervals, blood pressure, heart rate and electrical stimulation variables in chronically-prepared halothane anaesthetized rats are summarized in Table 3. Tedisamil lacked marked effects on blood pressure, but decreased heart rate as seen in the previous experiment. At doses of 4 mg kg^{-1} or greater, tedisamil completely inhibited electrical-induction of VF despite only moderately increasing VF_t threshold. At 4 mg kg^{-1}, tedisamil reduced MFF to 7 Hz and increased ERP to twice control values. The above responses were accompanied by marked $Q–T_c$ prolongation and insignificant P–R and QRS prolongation of the ECG. The effects of tedisamil on electrically-induced arrhythmias were quantitatively and qualitatively similar in acutely-prepared pentobarbitone anaesthetized rats although control ERP and VF_t values tended to be lower.

Effects on intracellular action potentials recorded in vivo

Representative epicardial transmembrane potentials before and after tedisamil are illustrated in Figure 4. As shown in the figure, tedisamil caused a marked prolongation of the epicardial intracellular potential. Table 4 shows that tedisamil prolonged APD by up to 500% at the higher dose levels. In addition, at the highest doses, tedisamil depressed the maximum rise rate (dV/dt_{max}) of phase 0 of the action poten-

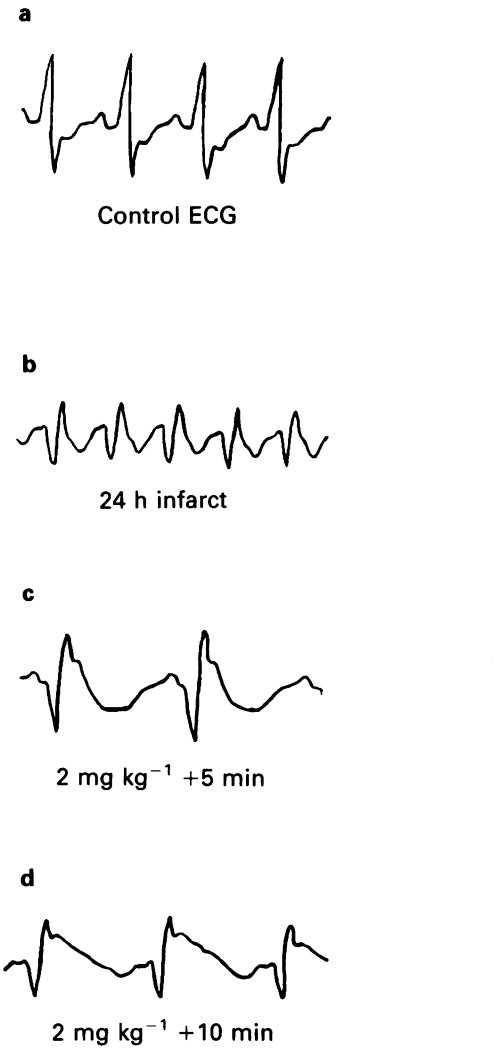

Figure 3 Effects of tedisamil on S–T segment elevation in 1-day infarcted rats. Representative ECG traces were sampled (a) before occlusion (control), (b) 24 h post occlusion and (c) 5 and (d) 10 min after administration of 2 mg kg^{-1} of tedisamil. Chart speed 100 mm s^{-1}.

Table 3 Effects of tedisamil on electrical stimulation characteristics of halothane-anaesthetized rats

Dose (mg kg^{-1})	*MFF* (Hz)	*ERP* (ms)	*VF_t* (μA)
0	13 ± 1	64 ± 7	230 ± 40
0.5	12 ± 2	68 ± 10	230 ± 50
1	11 ± 2	76 ± 12	250 ± 50
2	9 ± 2*	94 ± 26*	270 ± 60
4	7 ± 2**	107 ± 39*	VF not inducible

Dose (mg kg^{-1})	*Q–T* (ms)	*BP* (mmHg)	*HR* (beats min^{-1})
0	55 ± 3	105 ± 6	410 ± 15
0.5	100 ± 3**	106 ± 7	360 ± 20*
1	115 ± 5**	110 ± 6	335 ± 13**
2	130 ± 6**	104 ± 6	290 ± 25**
4	155 ± 8**	109 ± 10	245 ± 16**

Tedisamil was administered cumulatively every 15 min with each dose infused over 2 min. Doses are expressed cumulatively. Measurements were made 10 min after injections. All values represent mean ± s.e.mean for 6 rats per group. Effective refractory period (ERP), maximum following frequency (MFF) and current threshold for ventricular fibrillation (VF_t) were measured as detailed in Methods.
* $P < 0.05$; ** $P < 0.01$ from pre-drug.

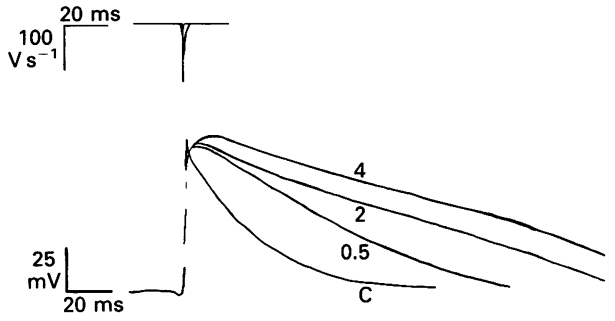

Figure 4 Representative epicardial action potential recorded *in vivo*, before (C) and 5 min after administration of tedisamil 0.5, 2 and 4 mg kg^{-1}, i.v. Redrawn from original records.

tial. The depression of dV/dt_{max} was not associated with a decrease in action potential height, instead (as shown in Figure 4) tedisamil tended to round-off the action potential spike without reducing its ultimate height.

Discussion

In rats, antifibrillatory doses of tedisamil widened the Q–T_c interval of the ECC and prolonged APD which justifies its classification as a Class III antiarrhythmic drug in this species. Effects on Q–T_c intervals, intracellular potentials, and responses to electrical stimulation were probably due to potassium channel blockade (see later). Prolongation of APD widened the Q–T_c interval and prolonged ventricular refractoriness. An increased refractoriness accounted for the observed decrease in MFF. Presumably, tedisamil prevented both ischaemia and electrically-induced VF by reducing the ability of the heart to follow high rates of stimulation.

Studies in rat isolated myocytes indicate that tedisamil speeds inactivation of the transient outward current (i_{to}) (Dukes & Morad, 1989), a major repolarizing current in rat ventricle (Irisawa, 1987). Blockade of this current would therefore widen the action potential in the rat. Blockade of i_{to} also explains why tedisamil is bradycardic in various species (Buschmann *et al.*, 1989a). Tedisamil prolongs sinoatrial node potentials (Oexle *et al.*, 1987) since i_{to} contributes to repolarization in these cells (Irisawa, 1987). In our study, Q–T_c prolongation paralleled bradycardia. Bradycardia alone does not widen rat (unlike guinea-pig) ventricular epicardial action potentials recorded *in vivo* (unpublished observations from our laboratory). Thus, the antifibrillatory activity of tedisamil is most easily explained by its Class III action.

Tedisamil widens Q–T_c interval in rats and baboons (Buschmann *et al.*, 1989b) but there is debate as to whether drugs which prolong Q–T interval prevent, or precipitate, arrhythmias. Association of Q–T prolongation with bradycardia and hypokalaemia has been clinically associated with arrhythmias, particularly Torsade de Pointes (Bacaner *et al.*, 1986; Singh, 1987; Surawicz, 1987; 1989).

The importance of K^+ channels in maintaining and terminating cardiac action potentials is recognized (Noma, 1987; Cook, 1988). The antiarrhythmic efficacy, or arrhythmogenic potential, of selective K^+ blockade merits investigation. Ideal antiarrhythmic drugs have yet to be found (Brugada, 1987), particularly those for ischaemia-induced arrhythmias. Tedisamil, with its marked Class III effects and lack of action on blood pressure, is thus useful for antiarrhythmic studies in rats subjected to myocardial ischaemia.

The *in vivo* electrophysiological actions of tedisamil were consistent with the findings of Dukes & Morad (1989) regarding action potential widening plus Class I actions at the higher doses or concentrations. The slight reduction of dV/dt and prolongation of P–R interval suggested ventricular sodium channel blockade (Buchanan *et al.*, 1985) at higher doses. However, any blockade must have been slight since neither elevations in thresholds for stimulation-induced VF, nor QRS prolongation, occurred. A fall in dV/dt normally reduces action potential height, but this did not occur in this study, presumably because the reductions in repolarizing potassium currents allowed the action potential to approach closer to the sodium equilibrium potential. In conclusion, the limited Class I actions of tedisamil did not contribute to its antiarrhythmic profile. The antiarrhythmic profile of tedisamil was markedly different from that for Class I drugs. The latter do not preferentially suppress VF but do increase VF_t (Walker & Beatch, 1988). Furthermore, the antiarrhythmic actions of Class I drugs do not convert VF to the particular form of VT seen with tedisamil. Another mechanism unlikely to have contributed to the antiarrhythmic actions of tedisamil was bradycardia. Previous studies have failed to show a relationship between bradycardia and antiarrhythmic actions (Curtis *et al.*, 1987; Abraham *et al.*, 1989).

Re-entry and abnormal automaticity are arrhythmogenic mechanisms which differ in their response to different drugs (Pogwizd & Corr, 1987; Brugada, 1987; Gitant & Cohen, 1988). An increase in refractoriness may be antiarrhythmic by selectively abolishing re-entry or, alternatively, by reducing the time available for arrhythmias. Action potentials occupying the whole cycle would leave no 'free-time' for arrhythmias. In our 'unpaced' study the T–Q interval (normally 70 ms), a measure of 'free-time', was not reduced by tedisamil suggesting that a simple increase in refractoriness did not account for the antiarrhythmic actions observed. However, in rats subjected to pacing such that T–Q was less than 10 ms, both VT and VF were reduced.

Re-entry circuits can be abolished by prolonging refractoriness within the circuit. Increased refractoriness can be expressed as an increase in minimal cycle time and this results either in termination of the re-entry, or a circuit of longer path-length. With VT, an increased path-length results in a slower VT providing conduction velocity remains unchanged. Such a mechanism would explain the preferential abolition of VF and occurrence of slow VT seen in our study. Tedisamil, 4 mg kg^{-1}, specifically abolished VF (stimulation or ischaemia-induced), slowed VT, reduced MFF to 7 Hz and prolonged effective refractory period to 107 ms. If, under these conditions, the re-entrant circuit had a conduction velocity of 0.6 m s^{-1}, the minimal path-length would be 6.42 cm. In the absence of tedisamil, a refractory period of 64 ms and the

Table 4 Effects of tedisamil on epicardial action potential variables recorded *in vivo*

	APD				*Action potential*	
Dose (mg kg^{-1})	10%	25%	50% (ms)	75%	dV/dt (V s^{-1})	*AP height* (mV)
Control	4.8 ± 0.4	10 ± 0.4	19 ± 0.7	45 ± 3	183 ± 6	97 ± 3
0.5	8 ± 1	19 ± 2	41 ± 3	81 ± 5	173 ± 8	103 ± 3
1	11 ± 1	26 ± 2	58 ± 6	95 ± 4	175 ± 7	105 ± 2*
2	17 ± 1	37 ± 1	69 ± 2	114 ± 3	170 ± 15	110 ± 2*
4	24 ± 4	53 ± 8	100 ± 14	162 ± 22	115 ± 18*	102 ± 4
8	29	67	125	195	165	107

After pre-drug recording, tedisamil was injected every 15 min according to a cumulative dose regimen. Each point is a mean ± s.e.mean of 6 rats with values averaged 14–15 min after dosing. At the 8 mg kg^{-1} dose level error is not given since n was less than 6. APD is shown at 10, 25, 50 and 75% of repolarization. The trend for tedisamil to increase APD at all levels was statistically highly significant ($P < 0.001$); * indicates $P < 0.05$ for difference from control in all other cases.

above conduction velocity, the minimal path-length would be 3.84 cm. This path-length would allow for multiple re-entry (i.e., VF) in the rat heart.

It was notable that tedisamil (0–4 mg kg^{-1}) was not arrhythmogenic in non-occluded rats but was arrhythmogenic in animals subjected to myocardial infarction. In a previously reported series of experiments, proarrhythmic effects of tedisamil in non-infarcted rats were seen at doses above 15 mg kg^{-1} and these arrhythmias depended upon the presence of an intact autonomic nervous system and signs of gNa^+ blockade (Howard *et al.*, 1989). Thus bradycardia and Q–T prolongation alone were not sufficient to induce arrhythmias. A 'substrate' of pathology (i.e. infarction) had to be present to reveal the arrhythmogenic actions of high doses of tedisamil.

The 'S–T' segment elevation induced by tedisamil was probably due to alterations in myocardial repolarization patterns since all animals had the same size OZs. If coronary vasoconstriction was the cause of the elevated 'S–T' segment (Wergia *et al.*, 1949) elevation should have been present in non-occluded rats, but this was not the case. The 'S–T' elevating actions of tedisamil may render it useful in the diagnosis of ischaemia/infarction.

In conclusion, tedisamil had Class III antiarrhythmic actions in the rat and was antifibrillatory. These actions were associated with increased APD, Q–T interval and refractoriness. Antifibrillatory actions were only seen at doses giving a four fold increase in APD. Previously available Class III antiarrhythmics produced only limited AP widening and thus may have limited antifibrillatory activity in the setting of myocardial ischaemia.

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