I.

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EFFECT OF ALMOKALANT A SPECIFIC INHIBITOR OF I_{Kr} ON MYOCARDIAL ISCHAEMIA-REPERFUSION INDUCED ARRHYTMIAS IN RABBITS

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Abstract: The antiarrhythmic effect of almokalant, a new type III antiarrhythmic agent, was examined by occluding and releasing the left circumflex coronary artery for 10 min, respectively, in open-chest, pentobarbital-anaesthetized albino rabbits. Almokalant pretreatment increased the number of animals developing no arrhythmias (5/9 vs. 1/12 in controls), and decreased the incidence of ventricular fibrillation (1/9 vs. 9/12) during reperfusion. According to our results almokalant can protect the heart against arrhythmias induced by ischaemia and reperfusion.

Keywords: almokalant, antiarrhythmic effect, ischaemia-reperfusion, rabbit

Introduction

Almokalant, a new type III antiarrhythmic compound (3), blocks mainly the rapid component of the voltage dependent delayed rectifier potassium current (I_{Kr}). Although this agent has undergone extensive observation, there are relatively few data gained from in vivo experiments about its antiarrhythmic effectiveness. Thus the aim of present experiments was to examine the effect of almokalant on arrhythmias induced by coronary artery occlusion and reperfusion in rabbits.

Methods

New Zealand White rabbits (n=24) weighing 1.5 to 2.9 kg from either sex were anaesthetized with pentobarbital sodium (30 mg/kg, i.v.). Catheters were implanted into the right carotid artery and into the marginal vein of the left ear for recording arterial blood pressure and infusion of drugs. After tracheotomy, the animals were mechanically ventilated with a Harvard respirator. Having performed left thoracotomy and pericardiotomy, the first branch of the left circumflex coronary artery was ligated just under its origin. Saline or almokalant (250 nmol/kg) was administered intravenously for 10 min in continuous infusion (infusion volume 2 ml) right before the occlusion. Coronary artery was occluded and released for 10 min, respectively. ECG was registered during the experiments. At the end of reperfusion (or after three min ventricular fibrillation) heparin sodium (500 U.I./kg, i.v.) was administered and the rabbits were killed with an overdose of pentobarbital. The heart was cut out from the chest in order to determine the size of the ischaemic zone. After occluding the coronary branch, the heart was perfused retrogradely with saline and ethanol. The non-denatured area (area at risk) was excised and its extent was expressed in percentage of the weight of ventricles. If the area at risk was less than 16% or was bigger than 32%, then the animal was excluded from the final evaluation. Data are presented as mean±SEM and *n* indicates the number of observations. Student's paired *t* test and χ^2 test with Yates' correction were applied for statistical analysis. Differences were considered statistically significant when $p<0.05$.

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Results

Almokalant pretreatment in a dose of 250 nmol/kg modestly reduced the heart rate (278±7 vs. 269±9 min^{-1}) and lengthened both the QT interval (153±3 vs. 170±7 ms) and the rate corrected QT interval (329±4 vs. 357±12 ms). This dose of almokalant significantly increased the proportion of animals developing no arrhythmias during reperfusion (5/9 vs. 1/12 in control group), and significantly decreased the incidence of ventricular fibrillation (1/9 vs. 9/12). Table 1 shows the incidence of arrhythmias in the control group and in the treated group during occlusion and reperfusion.

Table 1

Effect of almokalant (250 nmol/kg, i.v.) on the incidence of arrhythmias during coronary artery occlusion and reperfusion in anaesthetised rabbits

	Occlusion		Reperfusion	
	Control	Almokalant	Control	Almokalant
n	15	9	12	9
no arrhythmia	47%	67%	8%	56%*
ES	47%	33%	58%	44%
VT	0%	0%	42%	0%
VF	27%	0%	75%	11%*
died	20%	0%	58%	11%

n = number of animals; no arrhythmia = animals developing no arrhythmias; ES = ventricular extrasystoles; VT = ventricular tachycardia; VF = ventricular fibrillation; died = died because of VF; * $p<0.05$ vs. control

Discussion

As a result of delayed rectifier potassium channel blockade, almokalant lengthens the action potential duration and the refractory period of atrial and ventricular muscle cells (2). Previous clinical trials (1, 4) showed that almokalant lengthened the QT (and QTc) interval dose dependently and exhibited dose dependent antiarrhythmic effect in patients with ventricular premature contractions or with supraventricular reciprocating tachycardias. In the present study, almokalant also widened the QT and QTc interval on the ECG and parallel to this action, protected the heart against arrhythmias induced by coronary artery occlusion and reperfusion in anaesthetized rabbits.

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II.

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Comparison of the antiarrhythmic and the proarrhythmic effect of almokalant in anaesthetised rabbits

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Abstract

In this study the antiarrhythmic and the proarrhythmic activities of almokalant, a selective class III antiarrhythmic agent, were compared. The antiarrhythmic effect of the drug was tested in pentobarbital-anaesthetised rabbits. Arrhythmia was evoked by occluding and releasing the left circumflex coronary artery. Almokalant in a dose of 250 nmol/kg i.v., significantly decreased the incidence of reperfusion induced ventricular fibrillation (21% vs. 75% in the control group) and increased the proportion of surviving animals during reperfusion (86% vs. 42%). The proarrhythmic effect of almokalant was examined during α_1-adrenoceptor stimulation in chloralose-anaesthetised rabbits. Almokalant (75 nmol/kg per min) triggered torsade de pointes arrhythmias in 8 animals out of 11. The dose of almokalant (mean ± S.E.M.) required to produce this effect was 1181 ± 519 nmol/kg. It is concluded that, although almokalant is an effective antiarrhythmic agent against ischaemia-reperfusion induced arrhythmias, it has marked proarrhythmic activity during α_1-adrenoceptor stimulation. © 1998 Elsevier Science B.V.

Keywords: Almokalant; Antiarrhythmic effect; Reperfusion arrhythmias; Proarrhythmia; Torsade de pointes; (Anaesthetised rabbit)

1. Introduction

After the Cardiac Arrhythmia Suppression Trial (Cardiac Arrhythmia Suppression Trial investigators, 1989) the use of sodium channel blockers in the treatment of patients convalescing from myocardial infarction became limited and the attention was focused on the repolarisation prolonging agents, which exert their effects mostly by blocking potassium channels (Colatsky and Follmer, 1989). These agents prolong the atrial and the ventricular action potential duration and the corresponding effective refractory period and prevent or terminate reentrant ventricular tachycardias leading to ventricular fibrillation (Hondeghem and Snyders, 1990; Katritsis and Camm, 1993; Roden, 1993). The use of these agents in the therapy, however, might also result in severe proarrhythmias.

The typical proarrhythmia observed with the use of drugs delaying ventricular repolarisation is the provocation of polymorphic ventricular tachycardia denoted torsade de pointes (Jackman et al., 1988). Initiation of torsade de pointes is usually associated with a pause or slowing of heart rate and a concomitant increase in QT interval, followed by a series of rapid repetitive polymorphic QRS complexes with characteristic undulating peaks (Roden et al., 1986; Cranefield and Aronson, 1988). Sometimes torsade de pointes can deteriorate into ventricular fibrillation, a mechanism undoubtedly responsible for sudden cardiac death in a number of patients (Bayes de Luna et al., 1989).

Almokalant is a pure class III antiarrhythmic agent, which blocks selectively the rapid component of the delayed rectifier potassium current (I_{Kr}; Wettwer et al., 1992). Clinical studies have already proved that almokalant is an effective antiarrhythmic agent in patients with supraventricular reciprocating tachycardias, atrial flutter and fibrillation and in post-myocardial infarction patients with ventricular extrasystoles (Darpö and Edvardsson, 1995; Crijns et al., 1995; Wiesfeld et al., 1992). Almokalant as well as other selective I_{Kr} blockers can trigger torsade de pointes both in experimental animals (Carlsson et al., 1993a; Verduyn et al., 1995) and in human (Wiesfeld et al., 1993, Darpö et al., 1996).

The aim of the present study was to examine both the antiarrhythmic and the proarrhythmic effect of almokalant, as a representative agent of the pure class III antiarrhythmics, in the same species under well defined in vivo

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experimental conditions, and to determine the safety of the cardiac action of this agent.

2. Materials and methods

2.1. Animals

The experiments were performed on New Zealand White rabbits from either sex weighing 1.5 to 2.9 kg. The animals were allowed to have tap water and laboratory rabbit chow (Altromin, Gödöllő, Hungary) ad libitum until the experiment. The animals were handled according to a protocol reviewed and approved by the Ethical Committee for the Protection of Animals in Research of the Albert Szent-Györgyi Medical University, Szeged, Hungary.

2.2. Examination of the antiarrhythmic activity

Acute coronary artery ligation and reperfusion were performed according to Thiemermann et al. (1989). Rabbits ($n = 59$) were anaesthetised with pentobarbital sodium (30 mg/kg intravenously into the marginal vein of the right ear). A catheter was introduced into the right carotid artery in order to measure blood pressure (Blood Pressure Monitor BP-1, World Precision Instruments, Berlin, Germany). The catheter was filled with isotonic saline containing heparin (500 I.U./ml), but the animals were not heparinised. Another catheter was introduced into the marginal vein of the left ear for infusion of drugs.

After tracheal cannulation, left thoracotomy was performed and artificial ventilation was immediately started with room air (Harvard rodent ventilator, model 683, Harvard Apparatus, South Natick, MA, USA). Respiratory volume and rate (7 ml/kg/stroke, 40 stroke/min, respectively) were subsequently adjusted to keep the blood gases and pH within a normal range. After pericardiotomy, a loose loop of 4-O atraumatic silk (Ethicon, Edinburgh, UK) was placed around the first branch of the left circumflex coronary artery just under its origin. Both ends of the ligature were led out of the thoracic cavity through a flexible tube.

After 10 min stabilising period saline or almokalant (100 or 250 nmol/kg) was administered intravenously for 10 min in continuous infusion (infusion volume 2 ml) right before the coronary artery occlusion.

After the pretreatment, the loose loop was tightened and fixed by clamping on the silk, and thus local myocardial ischaemia was produced. After 10 min coronary artery occlusion, the ligature was released and 10 min reperfusion followed.

The electrocardiogram (lead I, II, III) was registered during the experiments by a thermographic recorder (ESC 110 4 CH, Multiline KFT, Esztergom, Hungary) using subcutaneous needle electrodes. The length of QT intervals was measured in predetermined intervals. QT interval was defined as the time between the first deviation from the isoelectric line during the PR interval until the end of TU wave. Rate corrected QT interval (QTc) was calculated subsequently by using the equation: $QTc = QT - 0.175(RR-300)$, where RR is the cycle length (Carlsson et al., 1993a).

Arrhythmias were detected during ischaemia and reperfusion and diagnosed in accordance with the Lambeth conventions as ventricular tachycardia, ventricular fibrillation and other types of arrhythmias including single extrasystoles, bigeminy, salvos and bradycardia (Walker et al., 1988). The onset and duration of arrhythmias were also measured.

At the end of reperfusion, heparin sodium (500 U.I./kg, i.v.) was administered and the animals were killed with an overdose of pentobarbital. The heart was cut out from the chest in order to determine the size of the occluded zone. After retightening the ligation coronary arteries were perfused retrogradely with 20 ml saline and 10 ml of 96% ethanol through the aorta (Leprán et al., 1983). The non-denatured area (occluded zone) was excised and its extent was expressed in percentage of the total weight of ventricles. Generally, in about 75% of the rabbits the main supplying artery of the left ventricle is the left circumflex coronary artery, whereas in the rest of the rabbits (in about 25% of the animals) the left ventricle is supplied mainly by the left anterior descending coronary artery (Toyo-oka et al., 1984). In accordance with this, in our experiments there were 15 rabbits out of 59 (25%), in which the occluded zone was less than 16% of the total weight of ventricles, because the occluded left circumflex coronary artery was poorly developed and the frontal wall and the apex of the heart were supplied by the non-occluded left anterior descending coronary artery. In these animals only ischaemic ECG changes (e.g., ST elevation, QRS distortion) occurred and arrhythmias did not develop. In 2 other animals the whole left circumflex coronary artery was occluded proximally to the origin of the first branch, therefore the occluded zone was almost doubled compared to the highest occluded zone measured after occluding only the first branch (53% and 56% vs. 32%, respectively). In these 2 animals the mean arterial blood pressure fell severely right after coronary artery occlusion and remained in a low level until the occurrence of irreversible ventricular fibrillation in the 6th and 7th min of coronary artery occlusion. Thus the animals, in which the occluded zone was less than 16% or was larger than 32%, i.e., altogether 17 animals were excluded from the final evaluation.

2.3. Examination of the proarrhythmic activity

The proarrhythmic activity of almokalant was examined in an animal model of acquired long QT syndrome (Carlsson et al., 1990). After sedation with pentobarbital sodium (5 mg/kg, i.v.), male rabbits ($n = 21$) were anaesthetised with α-chloralose (100 mg/kg intravenously into the

Table 1
Heart rate, mean arterial blood pressure and rate corrected QT values before and during ischaemia and reperfusion in anaesthetised rabbits

	n	Basal	Pretreat.	n1	Ischaemia (min)					n2	Reperfusion (min)				
					1	3	5	7	10		1	3	5	7	10
Control															
HR		288±7.3	288±7.3		284±7.6	281±8.4	281±8.4	275±9.0	276±9.9		273±20.3	280±19.4	282±17.1	281±16.0	282±15.2
MBP	15	86±3.0	86±3.1	12	77±3.4	78±3.4	78±3.3	78±3.5	80±3.6	5	71±11.0	78±6.4	80±4.6	79±4.6	81±4.3
QTc		163±3.3	164±3.6		167±3.8	169±4.3	172±3.5	174±4.7	172±4.2		159±10.0	173±10.5	172±11.0	174±9.2	172±10.6
Alm 100															
HR		278±8.7	282±6.7		274±6.2	270±6.7	268±6.8	269±6.3	263±7.3		276±19.2	254±8.4	255±8.5	260±9.9	261±8.4
MBP	13	89±3.1	90±3.2	13	83±2.7	83±2.6	83±2.8	82±2.6	82±2.8	8	72±6.1	78±3.2	76±4.7	79±3.9	81±3.1
QTc		165±6.4	167±5.9		178±3.7[b]	176±3.3	179±4.1	179±3.5	177±3.4		174±6.3	178±4.9	186±5.1	182±3.3	178±3.4
Alm 250															
HR		280±6.6	270±5.8[a]		264±5.6	265±5.5	264±5.9	265±5.3	266±5.3		265±5.9	263±6.6	264±6.5	269±5.9	271±6.4
MBP	14	88±3.0	88±4.1	14	74±4.5	81±3.2	80±3.3	77±3.7	79±3.2	12	77±4.4	81±3.8	78±3.2	78±3.0	78±3.2
QTc		159±4.2	175±6.5[a]		184±4.8[b]	182±4.5	184±4.4[b]	181±4.8	183±5.1		182±6.0	180±5.9	179±5.3	178±5.1	178±5.0

Alm 100 and Alm 250, groups of animals pretreated with almokalant in a dose of 100 or 250 nmol/kg; HR, heart rate (min^{-1}); MBP, mean arterial blood pressure (mmHg); QTc, rate corrected QT interval (ms); *n*, number of animals; Pretreat., values measured after the pretreatment; *n*1, number of animals surviving ischaemia; *n*2, number of animals surviving reperfusion; [a] $P < 0.05$ compared to the basal value; [b] $P < 0.05$ compared to the control group.

marginal vein of the right ear, in 10 ml/kg infusion volume, at a rate of 1 ml/min).

Catheters were introduced into the right carotid artery, the right jugular vein and the marginal vein of the left ear for recording arterial blood pressure and infusion of drugs, respectively. After tracheal cannulation, the animals were mechanically ventilated with room air as described in Section 2.2. Blood pressure and electrocardiogram was registered during the experiments as in the occlusion–reperfusion model. After 10 min stabilising period, continuous phenylephrine infusion at a rate of 15 μg/kg per min was administered into the right jugular vein of the animals for 80 min (in 2 ml infusion volume as a whole). Ten minutes after the beginning of the phenylephrine infusion, simultaneous almokalant infusion was given into the marginal vein of the left ear at a rate of 25 nmol/kg per min or 75 nmol/kg per min for 70 min (also in 2 ml infusion volume as a whole). Isotonic saline (2 ml over a period of 70 min) was administered to the animals in the control group instead of almokalant. During the experiment the onset and duration of arrhythmias were measured. Torsade de pointes was considered to have occurred if five or more closely coupled repetitive extrasystoles with a twisting or torsioning QRS morphology was observed. Heart rate, blood pressure, QT and QTc intervals and the total accumulated dose of almokalant at the first incidence of torsade de pointes or monomorphic ventricular tachycardia were also measured.

2.4. Drugs

The following drugs were used: almokalant (Astra Hässle, Mölndal, Sweden), phenylephrine (L-Phenylephrine HCl, Koch-Light Laboratories, Colnbrook-Bucks, England), heparin-sodium (Richter Gedeon RT, Budapest, Hungary), pentobarbital-sodium (Nembutal, Phylaxia-Sanofi, Budapest, Hungary), α-chloralose (Fluka Chemie, Buchs, Switzerland). Almokalant was prepared as a concentrated stock solution (100 mmol/ml) by Astra Hässle. The stock solution was diluted further with isotonic saline. All other drugs were dissolved in isotonic saline. Each dose was prepared on the day of the experiment and all doses in the text refer to bases of the compounds.

2.5. Statistical evaluation

The percentage incidence of arrhythmias was calculated and compared by using Fisher's exact probability test. Continuous data were expressed as mean ± standard error of the mean (S.E.M.). Means from the same sample were compared with Student's paired-samples *t*-test. Means from independent samples were compared with one way analysis of variance and if significant, multiple comparisons were performed with modified *t*-test according to the 'Least Significant Difference' method to assess which group was significantly different. Differences were considered statistically significant when $P < 0.05$.

3. Results

3.1. The antiarrhythmic activity of almokalant

Almokalant in a lower dose (100 nmol/kg) did not have any effect on the heart rate, the mean arterial blood pressure and the QTc intervals, whereas pretreatment with

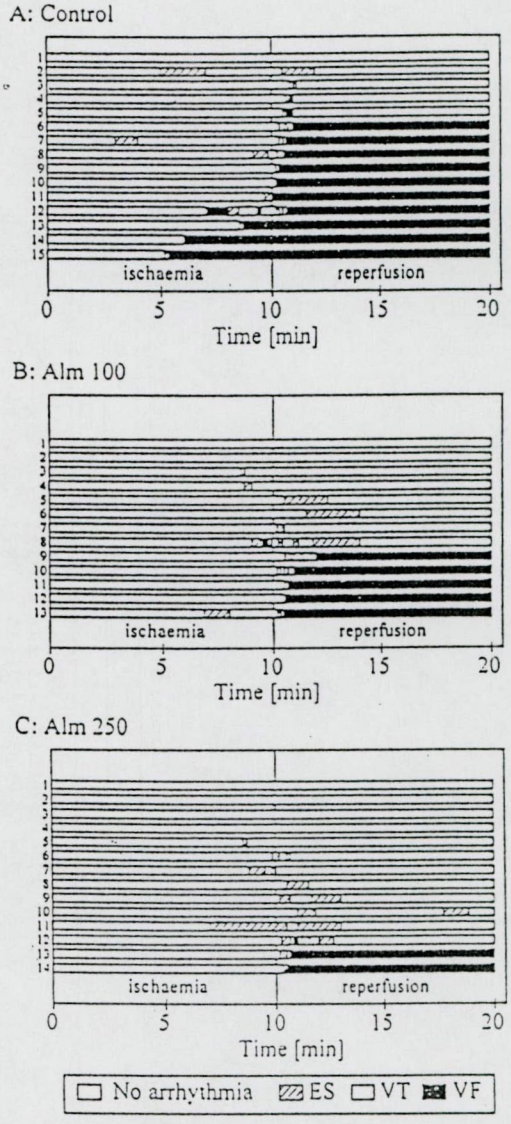

Fig. 1. The arrhythmia map of the control group (A) and groups of animals pretreated with almokalant in a dose of 100 or 250 nmol/kg (B and C, respectively). Each number of the ordinates refers to a separate animal and every row shows the arrhythmias of the given animal during the 10-min ischaemia and the 10-min reperfusion. In every group the animals are listed in order of severity of their arrhythmias. No arrhythmia, periods without arrhythmia; ES, extrasystoles, salvos, and/or bigeminy; VT, monomorphic ventricular tachycardia; VF, ventricular fibrillation.

Table 2
Effect of almokalant on the survival rate and incidence of arrhythmias during ischaemia and reperfusion in anaesthetised rabbits

	n	Survived (%)	Incidence of arrhythmias (%)			
			None	VF	VT	Other
Ischaemia						
Control	15	80	47	27	0	47
Alm 100	13	100	69	8	0	31
Alm 250	14	100	71	0	0	29
Reperfusion						
Control	12	42	8	75	42	58
Alm 100	13	62	31	39	31	46
Alm 250	14	86[a]	43	21[a]	14	57

Alm 100 and Alm 250, groups of animals pretreated with almokalant in a dose of 100 or 250 nmol/kg; *n*, number of animals; VF, ventricular fibrillation; VT, monomorphic ventricular tachycardia; Other, extrasystoles, salvos, and/or bigeminy; [a] $P < 0.05$ compared to the control group.

a dose of 250 nmol/kg decreased the heart rate modestly, while did not have significant effect on the mean arterial blood pressure (Table 1). The higher dose of almokalant prolonged the QTc intervals significantly, compared to the basal values (Table 1). None of the pretreatments induced any arrhythmia before the coronary artery ligation.

In the control group the heart rate (during sinus rhythm) did not change significantly in the course of coronary artery occlusion and reperfusion, while the mean arterial blood pressure fell right after the coronary artery ligation and remained at a lower level till the end of reperfusion (Table 1). In both groups pretreated with almokalant the heart rate and blood pressure response was not significantly different from those in the control group during coronary artery occlusion and reperfusion (Table 1). The longer QTc intervals were present throughout the ischaemia and reperfusion in the group of animals pretreated with almokalant in a dose of 250 nmol/kg per min (Table 1).

Arrhythmia maps of the three groups show the arrhythmias of each animal during the 10 min ischaemia and the 10 min reperfusion (Fig. 1), and the incidence of arrhythmias is shown in Table 2. During the 10 min occlusion period 3 animals died in the control group due to irreversible ventricular fibrillation, whereas all of the animals pretreated with almokalant survived occlusion. Ventricular fibrillation did not appear at all during occlusion in the group of animals pretreated with the higher dose of almokalant (Table 2).

In the control group the incidence of ventricular fibrillation was very high and the survival rate was low during reperfusion. In contrast, in the group of animals pretreated with almokalant in a dose of 250 nmol/kg the incidence of ventricular fibrillation was significantly lower and the survival rate was significantly higher during reperfusion than in the control group (Table 2).

3.2. The proarrhythmic activity of almokalant:

In the first 10 min, when only phenylephrine was administered to every animal, the heart rate decreased and the mean arterial blood pressure increased significantly in all three groups (Table 3). In both almokalant treated groups the heart rate values were statistically not different from those in the control group. In contrast, the blood pressure was significantly lower in the 80th min in the group of animals treated with almokalant infusion at a rate of 25 nmol/kg per min and from the 20th min in the group of animals treated with almokalant infusion at a rate of 75 nmol/kg per min, as compared to the control group (Table 3).

There was a significant QTc prolongation in all three groups in the first 10 min, when only phenylephrine was infused to the animals (Fig. 2). Almokalant infusion produced a dose related further prolongation of the QTc interval compared to the control group (Fig. 2).

Table 3
Effect of almokalant on the heart rate and the blood pressure in anaesthetised rabbits during continuous phenylephrine infusion

	n	Basal	Phe	Phe + drug						
			10 min	20 min	30 min	40 min	50 min	60 min	70 min	80 min
Control										
HR	10	284 ± 10.8	217 ± 7.5[a]	192 ± 10.2	195 ± 9.0	192 ± 10.8	190 ± 12.1	192 ± 12.1	188 ± 14.0	184 ± 14.7
MBP		87 ± 4.3	120 ± 2.8[a]	117 ± 3.0	118 ± 2.3	118 ± 2.3	116 ± 2.3	116 ± 3.1	118 ± 3.3	117 ± 2.6
Alm 25										
HR	10	289 ± 11.4	219 ± 16.4[a]	209 ± 15.7	213 ± 16.1	203 ± 13.6	211 ± 11.4	218 ± 10.2	212 ± 13.3	220 ± 13.7
MBP		98 ± 3.4	117 ± 3.2[a]	117 ± 2.9	114 ± 2.6	109 ± 3.6	106 ± 4.6	105 ± 4.7	106 ± 4.4	80 ± 6.0[b]
Alm 75										
HR	11	289 ± 10.0	204 ± 15.1[a]	186 ± 14.0	182 ± 12.4	193 ± 15.6	197 ± 14.2	196 ± 15.9	198 ± 12.8	222 ± 14.6
MBP		93 ± 3.6	108 ± 3.4[a]	98 ± 6.4[b]	94 ± 4.7[b]	92 ± 4.1[b]	87 ± 5.7[b]	87 ± 6.3[b]	82 ± 6.4[b]	77 ± 3.1[b]

Alm 25 and Alm 75, groups of animals treated with almokalant infusion in a rate of 25 or 75 nmol/kg per min; HR, heart rate (min^{-1}); MBP, mean arterial blood pressure (mmHg); *n*, number of animals; Phe, phenylephrine infusion in a rate of 15 mg/kg per min; [a] $P < 0.05$ compared to the basal value; [b] $P < 0.05$ compared to the control group.

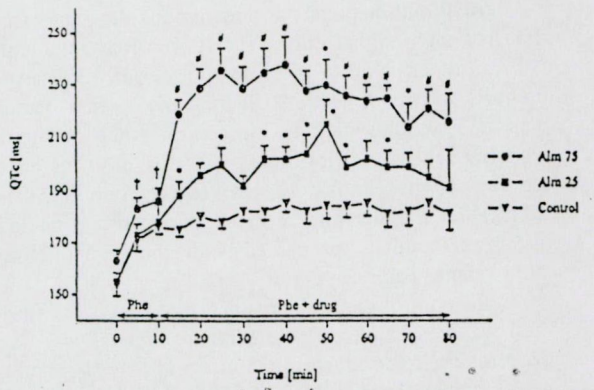

Fig. 2. The effect of almokalant on the rate corrected QT intervals during phenylephrine infusion. Alm 25 and Alm 75, groups of animals treated with almokalant infusion in a rate of 25 or 75 nmol/kg per min; QTc, rate corrected QT interval (ms); Phe, phenylephrine infusion in a rate of 15 mg/kg per min; $^{\dagger}P < 0.05$ compared to the basal value in all three groups; $^{*}P < 0.05$ compared to the control group; $^{\#}P < 0.05$ compared to the control and the Alm 25 group.

In each group every animal survived the first 10 min, when only phenylephrine was administered, but only 26% (8 out of 31) of them had no any arrhythmia. Sporadic extrasystoles, bigeminy and salvos appeared in 55% (17 out of 31) of the animals. The incidence of bradycardia was 39% (12 out of 31). Torsade de pointes or monomorphic ventricular tachycardia or ventricular fibrillation did not occur in this period.

In the control group, after the first 10 min, when saline was administered simultaneously with phenylephrine, only sporadic extrasystoles, bigeminy and salvos occurred and none of the animals died, whereas in the groups of animals treated with almokalant simultaneously with phenylephrine even torsade de pointes, monomorphic ventricular tachycardia and ventricular fibrillation appeared (Table 4). The incidence of torsade de pointes was significantly higher in the group of animals treated with almokalant infusion at a rate of 75 nmol/kg per min. Torsade de pointes usually ended spontaneously after several seconds. Sometimes monomorphic ventricular tachycardia and torsade de pointes were attached for a short period or transformed into each other continuously for a longer time. There was one animal treated with almokalant at lower infusion rate in which only monomorphic ventricular tachycardia developed as a malignant ventricular arrhythmia and torsade de pointes did not. One animal died in both almokalant treated groups due to deterioration of torsade de pointes into irreversible ventricular fibrillation (Table 4).

After 35.3 ± 17.6 min infusion of almokalant at a rate of 25 nmol/kg per min, i.e., a dose of 883 ± 440 nmol/kg (min. = 125; max. = 1650 nmol/kg) the drug induced torsade de pointes or monomorphic ventricular tachycardia in 3 animals out of 10. In the group of animals treated with almokalant infusion at a rate of 75 nmol/kg per min infusion of 1181 ± 519 nmol/kg (min. = 75; max. = 4500 nmol/kg) drug over a period of 15.8 ± 6.9 min produced torsade de pointes or monomorphic ventricular tachycardia in 8 out of 11 animals.

Table 4
The survival rate and incidence of arrhythmias in anaesthetised rabbits treated with almokalant during continuous phenylephrine infusion

	n	Survived (%)	Incidence of arrhythmias (%)					
			None	VF	VT	TdP	Bradycardia	Other
Control	10	100	10	0	0	0	90	60
Alm 25	10	90	10	10	10	20	40	80
Alm 75	11	91	0	18	18	73[a]	73	91

Alm 25 and Alm 75, groups of animals treated with almokalant infusion in a rate of 25 or 75 nmol/kg per min; n, number of animals; VF, ventricular fibrillation; VT, monomorphic ventricular tachycardia; TdP, torsade de pointes; Bradycardia, heart rate < 200 min^{-1}; Other, extrasystoles, salvos, and/or bigeminy; [a] $P < 0.05$ compared to the Alm 25 and control group.

4. Discussion

In this study the antiarrhythmic and the proarrhythmic effects of almokalant have been examined in rabbits. Although, useful arrhythmia and also proarrhythmia models have been developed for several other species, including the dog (Vos et al., 1995), rabbit was preferred by us. The anatomy of the coronary arteries of the rabbit is rather variable, but there is no collateral circulation in the rabbit's heart unlike in dog's heart (Maxwell et al., 1987), and thus, myocardial infarction in the rabbit can mimic better human myocardial infarction. Though it needs skill to perform coronary artery occlusion in rabbits, the proarrhythmia model, unlike in the dog (Vos et al., 1995), is quite simple in this species, and does not need difficult surgical techniques.

The present study demonstrated that intravenous almokalant prevent reperfusion induced ventricular fibrillation. Other blockers of the delayed rectifying potassium current (I_K), such as D-sotalol, dofetilide, sematilide, E-4031 and UK66,914 vary in their effectiveness against reperfusion arrhythmias. For example, Pasnani and Ferrier (1994) found D-sotalol to be ineffective against ischaemia and reperfusion induced arrhythmias in isolated guinea pig right ventricular free wall preparations. In contrast, UK66,914 possessed marked antiarrhythmic effect on reperfusion arrhythmias in isolated rabbit hearts (Rees and Curtis, 1993). It was published recently that D-sotalol, E-4031 and MS-551 (a non-selective potassium channel blocker) are effective against reperfusion arrhythmias and arrhythmias induced by programmed electrical stimulation, whereas dofetilide and sematilide prevent only arrhythmias

induced by programmed electrical stimulation but did not suppress reperfusion arrhythmias in anaesthetised dogs (Chen et al., 1996; Xue et al., 1996).

Reperfusion induced arrhythmias may be mediated both via reentry mechanism (Coronel et al., 1992) and triggered activities (Pogwizd and Corr, 1992; Hayashi et al., 1996). Selective prolongation of repolarisation (class III effect) is one possibility to prevent and terminate reentrant arrhythmias, but it has no effect on arrhythmias induced by triggered activities. Abrahamsson et al. (1993) showed that almokalant prolongs the action potential duration in a dose dependent manner both in isolated Purkinje and in ventricular muscle cells of the rabbit by recording transmembrane action potentials. Duker et al. (1992) found that almokalant (1.0 mmol/kg, i.v.) significantly prolonged the epicardial monophasic action potential duration and the atrial and ventricular effective refractory period but it had no effect on the atrial and ventricular conduction in anaesthetised dogs. In this study almokalant pretreatment (in a dose of 250 nmol/kg) significantly prolonged QTc interval, i.e., ventricular repolarisation. Thus, the possible mechanism by which almokalant prevented reperfusion arrhythmias is the lengthening of action potential duration and the refractory period of myocardial fibres (achieved by selective blockade of I_{Kr}) in the reentrant circuit to such an extent that the propagating reentrant impulse no longer finds excitable myocardium but blocks in refractory tissue. The effectiveness of I_K block as a mechanism for prevention of both ischaemia and reperfusion induced arrhythmias was demonstrated also by Rees and Curtis (1993).

In contrast to its effectiveness on reperfusion induced ventricular fibrillation almokalant was found to elicit torsade de pointes in the Carlsson et al. (1990). The torsadogenic potential of several selective class III agents, i.e., clofilium, sematilide, almokalant, D-sotalol, ibutilide, E-4031 and dofetilide, has been tested during α_1-adrenoceptor stimulation (Carlsson et al., 1990, 1993a; Buchanan et al., 1993). All of the examined class III agents induced torsade de pointes in this model. The underlying mechanisms of torsade de pointes are not yet known fully but appearance of early afterdepolarisations and enhanced dispersion of ventricular repolarisation are supposed to be the main electrophysiologic prerequisites for initiation of torsade de pointes especially in the presence of predisposing factors like low heart rate, electrolyte abnormalities (hypokalemia and hypomagnesemia), lengthening of repolarisation, depressed left ventricular function and/or life threatening arrhythmias in the case history (Hohnloser and Singh, 1995).

In our experiments the antiarrhythmic dose of almokalant (250 nmol/kg) was administered at a slow infusion rate for 10 min or at high infusion rate for 3.33 min. The high infusion rate of a class III agent increases markedly the incidence of torsade de pointes, probably by increasing the dispersion of repolarisation (Carlsson et al., 1993a). In our hands almokalant infusion at a higher rate prolonged QTc interval more than the infusion of almokalant at lower rate, and this marked QTc prolongation coincided with high incidence of torsade de pointes. These findings are in discordance with the results of Carlsson et al. (1993a), because they found no significant difference between the corresponding QTc values of the high and low infusion rate group, despite the former having a higher propensity to cause torsade de pointes. The data about the importance of QTc lengthening are quite contradictory and the question whether there is a relationship between any critical QTc prolongation and proarrhythmias is not yet answered. Buchanan et al. (1993) also found no correlation between the proarrhythmic potential of class III agents and the degree of QTc (or QT) interval prolongation in their experiments with the same model of acquired long QT syndrome. These findings are in agreement with others' conclusion that the incidence of torsade de pointes is not quantitatively related to the degree of QTc prolongation caused by repolarisation delaying agents (Soffer et al., 1982; Lazzara, 1993). Thus, the predictive value of the QTc interval prolongation for developing proarrhythmia needs further examination.

Class III agents typically produce torsade de pointes. However, in a clinical study ibutilide and sotalol induced not only torsade de pointes but also monomorphic ventricular tachycardia in patients with atrial fibrillation or flutter (Kowey et al., 1996). Darpö et al. (1996) reported a case in which an almokalant treated patient with WPW syndrome developed torsade de pointes after a pacing induced pause, and this tachycardia degenerated into ventricular fibrillation that required immediate defibrillation. In our experiments almokalant infusion (during α_1-adrenoceptor stimulation) produced monomorphic ventricular tachycardia and irreversible ventricular fibrillation as well as torsade de pointes. Carlsson et al. (1990, 1993a) reported only on premature ventricular complexes and torsade de pointes induced by class III agents in rabbits. Maybe this discrepancy is attributable to the fact that the latter authors terminated their experiments at the time of the first appearance of torsade de pointes. In our study two animals developed monomorphic ventricular tachycardia prior to the first torsade de pointes and one developed monomorphic ventricular tachycardia without the occurrence of torsade de pointes. Likewise, Buchanan et al. (1993) observed frequently the development of wide complex tachycardia, which was not pause dependent like torsade de pointes following administration of class III agents.

Carlsson et al. (1993b, 1996) and Hallman and Carlsson (1995) found that both pretreatment and acute intervention with lidocaine, nisoldipine or flecainide prevent torsade de pointes induced by almokalant infusion. In our experiments in the high infusion rate group, in 3 out of 8 animals with torsade, the short repetitive sequences of torsade de pointes occurred within 2 min period and in one additional animal within 6 min period. After these short attacks no more ventricular tachycardia, but just premature ventricu-

lar complexes developed in these animals, though the infusion of almokalant was not terminated and no suppressive agent was administered. These observations suggest that acute intervention with antiarrhythmic agents in order to suppress torsade de pointes induced by a class III agent in this model may give false positive results, and pretreatment should be preferred to investigate any intervention for influencing the torsadogenic potential of antiarrhythmic agents.

As a result of our study the margin of safety of almokalant was estimated by comparing the proarrhythmic dose (during α_1-adrenoceptor stimulation) of almokalant to the antiarrhythmic dose, which was effective against ischaemia-reperfusion induced arrhythmias. The proarrhythmic dose of almokalant was about 4–6 times higher than the antiarrhythmic dose under the conditions of this study. We do not want to overemphasise this finding, because it may not be valid in clinical setting, but it could be useful for comparison of new antiarrhythmic agents under similar experimental conditions. Though different anaesthetic agents were used in the two applied experimental models in our study, we think that the differences of the results were not attributable to the use of different anaesthesia. Recently, Bril et al. (1996) suggested that intravenous pentobarbital sodium can be used instead of α-chloralose without altering the proarrhythmic response to repolarisation prolonging agents in Carlsson's torsade model.

In conclusion, our study has provided evidence that almokalant, a selective class III antiarrhythmic agent, is effective against reperfusion arrhythmias, though it has a marked proarrhythmic effect during α_1-adrenoceptor stimulation in anaesthetised rabbits. We demonstrated experimentally that a class III drug is able to produce not only torsade de pointes as a malignant proarrhythmia, but also monomorphic ventricular tachycardia and ventricular fibrillation. The proarrhythmic response to almokalant is, in fact, quite complex in the applied rabbit model of acquired long QT syndrome. Furthermore, our study demonstrated that the combination of the 'coronary artery occlusion–reperfusion model' and the 'acquired long QT syndrome model' in rabbits is suitable to assess and compare experimentally the antiarrhythmic efficacy and the proarrhythmic activity of repolarisation delaying agents.

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Inadequate ischaemia-selectivity limits the antiarrhythmic efficacy of mibefradil during regional ischaemia and reperfusion in the rat isolated perfused heart

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1 Mibefradil was compared with (±)-verapamil for effects on ischaemia- and reperfusion-induced ventricular fibrillation (VF), and the role of ischaemia-selective L-channel block was examined. Langendorff perfused rat hearts ($n=12$/group) were used.

2 Neither drug at up to 100 nM reduced the incidence of VF during 30 min regional ischaemia. 300 and 600 nM (±)-verapamil abolished VF ($P<0.05$); mibefradil was effective only at 600 nM ($P<0.05$). Reperfusion-induced VF incidence was reduced only by 600 nM (±)-verapamil ($P<0.05$). Both drugs at ⩾100 nM increased coronary flow ($P<0.05$) with a similar potency and maximum effectiveness.

3 In separate hearts perfused with Krebs' solution containing 3 mM K^+ (the same as that used for arrhythmia studies) neither drug at up to 600 nM affected ventricular contractility. With K^+ raised to 6 mM, (±)-verapamil ⩾30 nM reduced developed pressure ($P<0.05$); mibefradil did so only at 600 nM ($P<0.05$). With K^+ raised to 10 mM the effects of (±)-verapamil were further increased ($P<0.05$) and mibefradil became active at ⩾100 nM ($P<0.05$). Likewise both drugs impaired diastolic relaxation, with raised K^+ exacerbating the effects and (±)-verapamil being more potent and its effects more greatly exacerbated by K^+. In contrast, when K^+ was normal (3 mM), coronary flow was increased by each drug at ⩾30 nM ($P<0.05$) indicating a marked vascular:myocardial selectivity.

4 In conclusion, mibefradil differed from (±)-verapamil in its myocardial effects only in terms of its lower potency. As mibefradil is the more potent T-channel blocker, the T-channel is unlikely to represent the molecular target for these effects. The K^+ elevations that occur in the ischaemic milieu determine the ability of both drugs to block myocardial L-channels; this is sufficient to account for the drugs' actions on VF. Neither drug possesses sufficient selectivity for ischaemic myocardium versus blood vessels to permit efficacy (VF suppression without marked vasodilatation) and so inappropriate hypotension is likely to preclude the safe use of mibefradil (or similar analogue) in VF suppression, and explains the lack of clinical effectiveness of (±)-verapamil.

Keywords: Cardiac contractility; ischaemia-selectivity; L-channel; mibefradil; myocardial ischaemia; potassium; T-channel; ventricular fibrillation; (±)-verapamil

Abbreviations: ECG, electrocardiogram; VF, ventricular fibrillation; VPB, ventricular premature beat; VT, ventricular tachycardia

Introduction

Prevention of ventricular fibrillation (VF) and sudden cardiac death represents a continuing challenge in drug development. The findings of the CAST (1989) and SWORD studies (see Cobbe 1996) illustrate that better antiarrhythmic agents devoid of serious side effects are required. Of the major classes of antiarrhythmic drugs (Vaughan Williams, 1970), class IV agents (calcium antagonists) can suppress ischaemia-induced VF in animal models (Curtis, 1990). (±)-Verapamil possesses selectivity for L-channels in ischaemic versus non-ischaemic myocardium, and this is determined in part by the facilitatory effect of extracellular K^+ which increases in concentration locally during acute ischaemia (Curtis & Walker, 1986b). However, (±)-verapamil's ischaemia-selectivity is inadequate since marked AV nodal effects and catastrophic hypotension occur at the doses necessary for VF suppression (Curtis & Walker, 1986b), which would explain why (±)-verapamil fails to prevent sudden cardiac death in man (Antman *et al.*, 1992).

Mibefradil is a calcium antagonist that blocks both L- and T-channels (Osterrieder & Holck, 1989; Mishra & Hermsmeyer, 1994; Rutledge & Triggle, 1995; Bezprozvanny & Tsien, 1995). Although it is known that mibefradil can suppress exercise related arrhythmias in dogs with healed myocardial infarction (Billman, 1991), it is not known whether the drug can suppress more clinically relevant arrhythmias induced by sustained acute ischaemia, whether it can do so without causing severe AV block or vasodilatation, or whether any effects are T- or L-channel-mediated. We have therefore examined whether mibefradil can suppress ischaemia and reperfusion arrhythmias in a controlled *in vitro* setting that allows for precise determination of concentration response relationships for actions on ventricles, the AV node, and coronary vessels. (±)-Verapamil was used as a positive control. If T-channels are a more useful target than L-channels then mibefradil should be more selective than (±)-verapamil for suppression of VF.

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The model chosen was the isolated perfused rat heart (Langendorff preparation) which has been shown to detect significant antiarrhythmic effects of a variety of agents (Curtis, 1998).

(+)- and (−)-verapamil reduce developed pressure and increase diastolic pressure in perfused rat hearts, and these actions are enhanced by increasing the K^+ content of the perfusion solution to mimic the rise in K^+ that occurs in acute ischaemia (Curtis & Walker, 1986b; Curtis, 1990). The negative inotropic activity of (+)- and (−)-verapamil is fully attributable to L-type calcium antagonist activity (Curtis, 1990). We therefore contrasted arrhythmia data with effects in separate groups of hearts on ventricular contractile function, varying perfusion K^+ content to determine whether mibefradil has greater or lesser putative ischaemia-selective L-channel blocking activity than (±)-verapamil, under the assumption that the potentiation by K^+ of effects on contractile function is indicative of a common mechanism, namely L-channel blockade.

Methods

Animals and general methods for arrhythmia experiments

Rats (male Wistar; Bantin and Kingman, U.K. 180–250 g; $n = 12$ per group) were anaesthetized with pentobarbitone (60 mg kg^{-1} i.p.) mixed with 250 I.U. sodium heparin to prevent blood clot formation in the coronary vasculature. Hearts were excized and placed into ice-cold solution containing (in mM): NaCl 118.5, $NaHCO_3$ 25.0, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $CaCl_2$ 1.4, KCl 3 and glucose 11.1, then perfused according to Langendorff, with solution delivered at 37°C and pH 7.4. All solutions were filtered (5 μm pore size) before use. Perfusion pressure was maintained constant at 70 mmHg. A unipolar electrogram (ECG) was recorded by implanting one stainless-steel wire electrode into the centre of the region to become ischaemic with a second connected to the aorta. A traction-type coronary occluder consisting of a silk suture (Mersilk, 4/0) threaded through a polythene guide was used for coronary occlusion. The suture was positioned loosely around the left main coronary artery beneath the left atrial appendage. Regional ischaemia and reperfusion were induced by tightening the occluder and by releasing it.

Experimental protocol

Hearts were perfused for an initial 5 min with control solution, then solution was switched in a blinded fashion to one of 11 solutions: control (vehicle), 10, 30, 100, 300 or 600 nM mibefradil or 10, 30, 100, 300 and 600 nM (±)-verapamil. The choice of solution was made by reference to a randomization table. After a further 5 min perfusion, the left coronary artery was occluded. After 30 min ischaemia the occluder was released to achieve reperfusion. Randomization was achieved by coding each group with a letter of meaning that was unknown to the operator. Blinded analysis was achieved by using stock solutions prepared by a second operator who did not participate in heart perfusion or data analysis.

Individual measures of coronary flow and ECG variables were taken 1 min before and 1 min after the introduction of drug perfusion or vehicle, 1 min before and each min after coronary occlusion for 5 min, then every 5 min thereafter for 25 min, and again 1 min before reperfusion, and after 1 and 5 min of reperfusion.

The choice of drug concentrations was based on the following. Both (±)-verapamil and mibefradil are highly plasma protein bound: >80% (Curtis *et al.*, 1984) and more than 99% (Clozel, personal communication) respectively. The unbound plasma concentration of (±)-verapamil associated with a 50% reduction in severity of ischaemia-induced arrhythmias in conscious rats in 600 nM (Curtis *et al.*, 1984). Therefore, the initial plan was to study a range of concentrations of (±)-verapamil with 600 nM as the median. However, we found in preliminary studies that >600 nM (±)-verapamil reduced developed pressure in rat hearts by more than 50%, and caused AV block in some of the hearts. In contrast, in conscious rats mean blood pressure was reduced by less than 50%, and AV block did not occur when unbound blood levels were ~600 nM (Curtis *et al.*, 1984). The explanation for this discrepancy is likely to be that the potency of (±)-verapamil increases when sympathetic tone is removed (Curtis & Walker, 1986a), i.e., by cardiac excision. Thus, 600 nM was chosen as the maximum (±)-verapamil concentration for the present experiments. This is sufficient to cause L-channel blockade in isolated ventricular myocytes (Lee & Tsien, 1983).

For mibefradil, the mean plasma concentration in man following a 100 mg p.o. dose is 870–1200 nM (Clozel, personal communication; Welker, personal communication). This means that mean unbound concentrations are in the region of 10 nM. In order to ensure that mibefradil and (±)-verapamil could be contrasted at identical concentrations, and taking these other factors into consideration, we opted to test 10, 30, 100, 300 and 600 nM of each drug.

Measurement of involved zone size and coronary flow

At the end of 5 min of reperfusion the size of the involved zone (the region subjected to ischaemia and reperfusion) was quantified using the disulphine blue dye exclusion method (Curtis & Hearse, 1989a) and expressed as per cent total ventricular weight. Coronary flow was measured by timed collection of coronary effluent. Values of coronary flow in the uninvolved tissue and the reperfused zone were calculated from the total coronary flow and the weights of the involved zone and the uninvolved zone, as described previously (Curtis & Hearse, 1989b).

Exclusion criteria

Any heart with a sinus rate of less than 250 beats min^{-1}, or a coronary flow more than 18 ml $min\ g^{-1}$ or less than 8 ml $min\ g^{-1}$ at 6 min before the onset of ischaemia (before the start of perfusion with drug or vehicle) or an involved zone of less than 30% or more than 50% of total ventricular weight was excluded. Excluded hearts were replaced to maintain equal group sizes. Any heart not in sinus rhythm during the 2 s before the start of reperfusion was excluded from the reperfusion sample, but was not replaced.

Arrhythmia diagnosis and ECG analysis

The ECG was recorded using a MacLab system. Arrhythmias were defined according to the Lambeth Conventions (Walker *et al.*, 1988) with slight modification (Tsuchihashi & Curtis, 1991). The QT interval at the point of 90% repolarization was

not corrected for heart rate as it is not rate-dependent in perfused rat hearts (Rees & Curtis, 1993).

Measurement of all variables was performed in a blinded manner.

Assessment of ischaemia-selective L-channel blocking activity

Hearts ($n = 10$ per group) were perfused with standard solution (see above) containing 3, 6 or 10 mM K^+, and a compliant non-elastic balloon (Curtis *et al.*, 1986) was inflated in the ventricle so as to give a developed pressure of more than 100 mmHg at a diastolic pressure of less than 5 mmHg. To standardize the experiment, the balloon was inflated with an added volume of 0.12 ml, which obtains a developed pressure under baseline conditions of about 70% of the maximum achievable in a heart weighing 0.6–0.7 g (Ellwood & Curtis, 1996).

Table 1 Arrhythmia incidences

Group	*Ischaemia-induced VF* (%)	*Reperfusion-induced VF* (%)	*AV block* (%)
Control	92	100	0
(±)-Verapamil 10 nM	92	100	0
(±)-Verapamil 30 nM	83	100	0
(±)-Verapamil 100 nM	75	83	0
(±)-Verapamil 300 nM	0*	73	12
(±)-Verapamil 600 nM	0*	42*	58*
Mibefradil 10 nM	83	100	0
Mibefradil 30 nM	100	88	0
Mibefradil 100 nM	100	100	0
Mibefradil 300 nM	75	75	17
Mibefradil 600 nM	17*	75	83*

Data are % incidence of VF during ischaemia and during reperfusion, and AV block during ischaemia. *$P<0.05$ versus control.

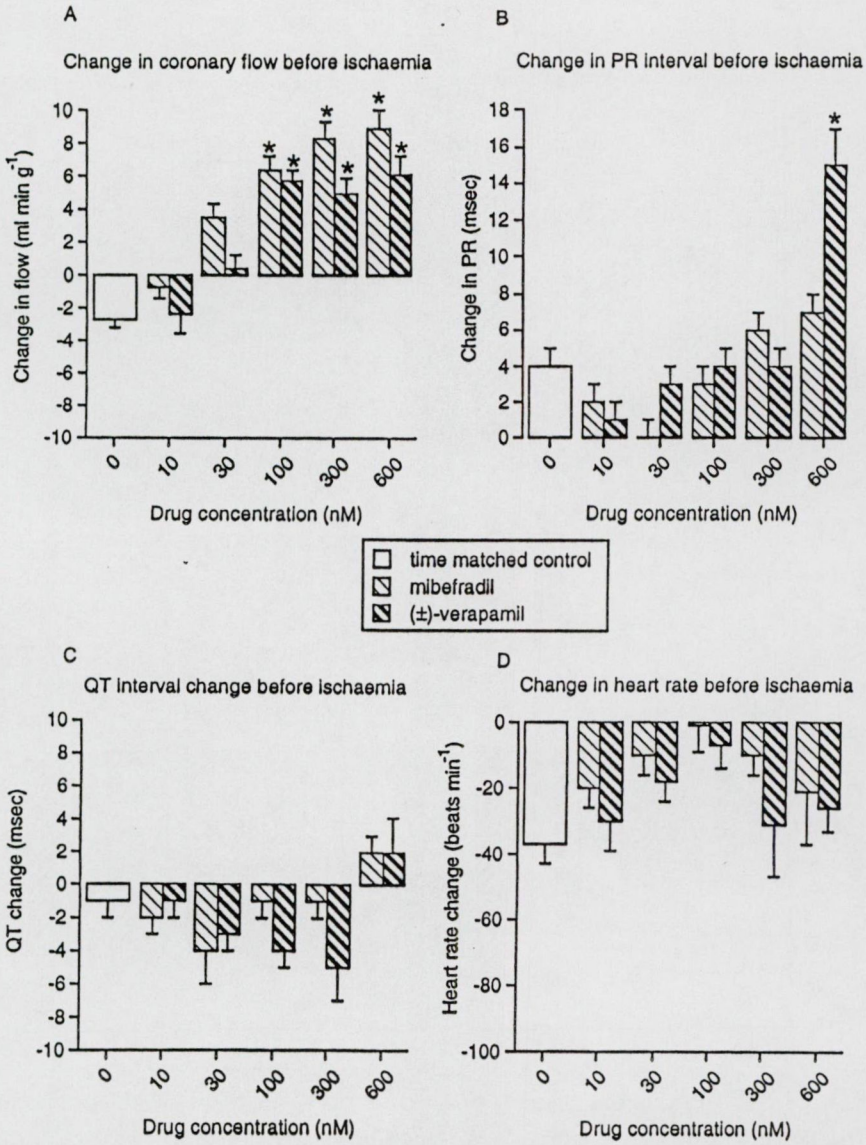

Figure 1 Change in coronary flow (A), PR interval (B) QT interval (C) and heart rate (D) induced by switching from control solution to intervention 5 min before the start of ischaemia. Values are changes measured at 1 min before the start of ischaemia. *$P<0.05$ versus 0 nM group (time matched control).

The hearts were initially perfused for 15 min with drug-free solution, then exposed to 30, 100, 300 and 600 nM mibefradil or (±)-verapamil, sequentially, 5 min per concentration (10 nM was not used because this concentration was several orders of magnitude below that found to affect arrhythmias – see Results). Exposure was continuous, and separate perfusion reservoirs were used for each solution. Preliminary studies established that this was ample time for drug effects to peak. Separate hearts were used for each drug. A time-matched control group was used for each K^+ concentration. In these controls the perfusion delivery was switched every 5 min between reservoirs each containing drug vehicle solution.

Variables (diastolic pressure, developed pressure and coronary flow) were recorded 1 min before exposure to each concentration of drug and, to maintain the timing, 4 min after introduction of the highest concentration of drug. Coronary flow was calculated as ml min g^{-1}, thus taking into account any differences in weight between individual hearts. All values have been expressed as change from baseline (the value recorded 1 min before the first change of perfusion solution, which did not differ significantly between groups).

Drugs and materials

Mibefradil and (±)-verapamil drug stocks were prepared fresh each week and perfusion solutions were prepared fresh each day. 'Vehicle stock' was 2 ml of plain water. The control solution contained 0.5 ml of this in 2 l of modified Krebs' solution. The 600 nM solutions were prepared from 2 ml of a 'drug stock' consisting of 4800 nmol drug dissolved in 2 ml water, such that 0.5 ml of this stock dissolved in 2 l of Krebs' solution obtained a 600 nM solution. The other solutions were

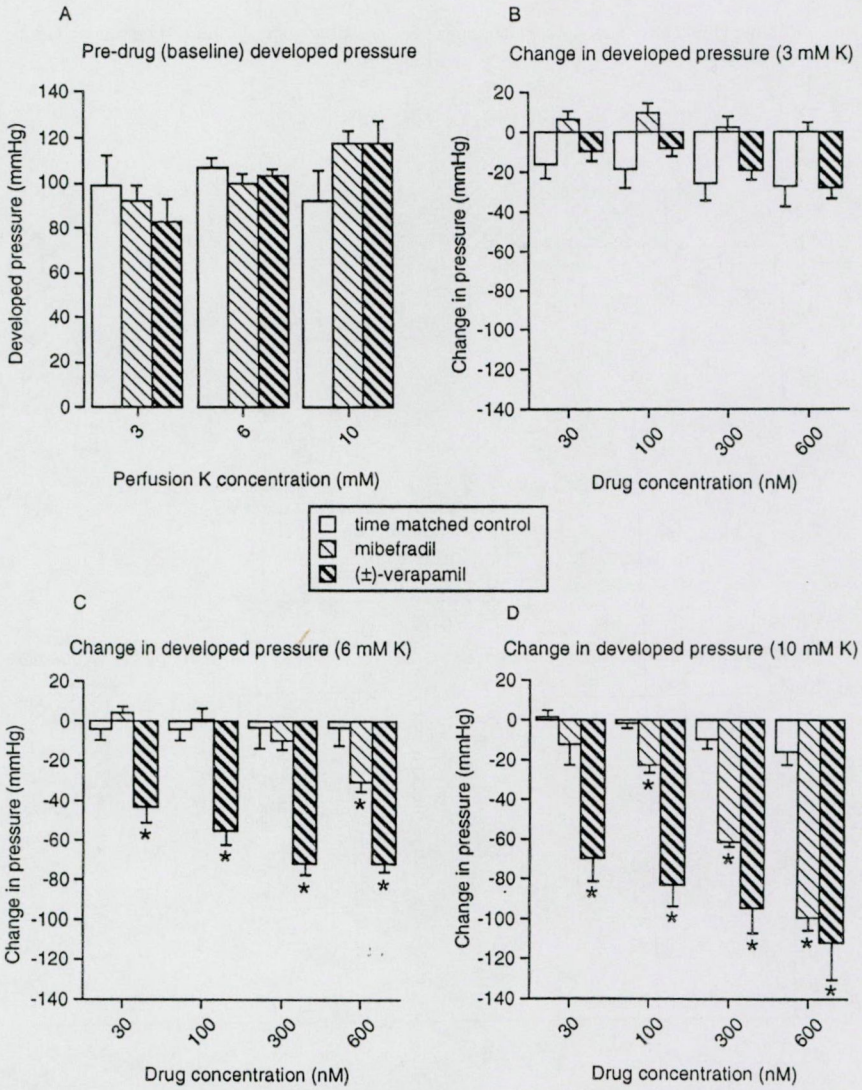

Figure 2 Pre-drug (baseline) left ventricular developed pressure in hearts perfused with different K^+ concentrations (A), and changes in developed pressure following introduction of drug solutions in hearts perfused with 3 mM K^+ solution (B), with 6 mM K^+ solution (C) or with 10 mM K^+ solution (D). Values are mmHg (A) or changes measured 4 min after the introduction of each drug solution (B–D). *$P<0.05$ versus 0 nM group (time matched control).

prepared in an equivalent manner, using stocks made by serial dilution of main stocks in 'vehicle stock' when necessary, so as to maintain constant the volume of stock added to prepare each solution.

All salts were reagent grade chemicals from Sigma Chemical Co. (U.S.A.). Water for preparing perfusion solution was supplied using a reverse osmosis system (Milli-RO 10 and Milli-Q 50, Millipore Ltd) and had a specific resistivity of more than 18 M Ohm.

Statistics

Gaussian distributed variables, expressed as mean ± s.e.mean, were subjected to analysis of variance followed by Dunnett's test when appropriate. The time to onset of first arrhythmia was $\log_{10}$ transformed to generate Gaussian distributed variables (Curtis & Hearse, 1989a). Binomially distributed variables were compared using Chi^2 test with Yates' correction where appropriate (Gad & Weil, 1989). $P<0.05$ was taken as indicative of a statistically significant difference between values.

Results

Arrhythmia studies

Ischaemia and reperfusion arrhythmia incidences Neither drug at 10, 30 or 100 nM reduced VF incidence (Table 1). VF was, however, abolished by higher concentrations of (±)-verapamil (300 and 600 nM). Mibefradil was less potent than (±)-verapamil, protective only at 600 nM. There were no significant drug effects on the incidence of ischaemia-induced ventricular tachycardia (VT; 100% in all groups), bigeminy, salvos or ventricular premature beats (VPBs) (data not shown). The onset times of the first episode of ischaemia-induced arrhythmias (all types) and VF were neither hastened nor delayed by mibefradil or (±)-verapamil (data not shown).

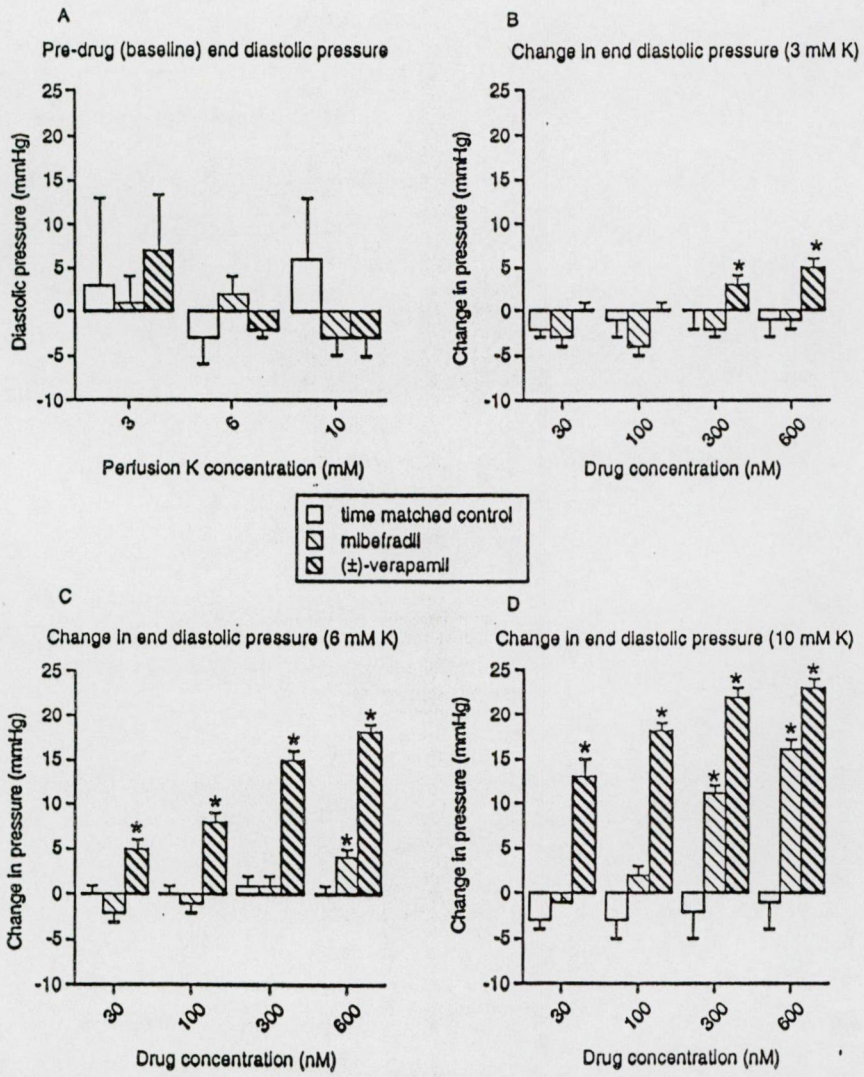

Figure 3 Pre-drug (baseline) left ventricular diastolic pressure in hearts perfused with different K^+ concentrations (A), and changes in diastolic pressure following introduction of drug solutions in hearts perfused with 3 mM K^+ solution (B), with 6 mM K^+ solution (C) or with 10 mM K^+ solution (D). Values are mmHg (A) or changes measured 4 min after the introduction of each drug solution (B–D). *$P<0.05$ versus 0 nM group (time matched control).

The majority of hearts were in sinus rhythm 30 min after the start of ischaemia, permitting assessment of reperfusion-induced VF. Only the highest concentration of (±)-verapamil reduced reperfusion-induced VF incidence ($P<0.05$), and mibefradil was ineffective at all five concentrations (Table 1).

Neither drug at up to 100 nM caused AV block at any time during the experiment, and fewer than 20% of hearts in either drug group had AV block at 300 nM. However, 600 nM mibefradil and 600 nM (±)-verapamil caused AV block in most hearts during ischaemia (Table 1), and this was most commonly a mixture of Möbitz I and II. AV block progression usually began with 1st degree block, followed by 2nd degree block (first Möbitz I then Möbitz II) and finally 3rd degree block in some hearts.

Mean size of the involved region was not affected by either drug and values ranged from 36–41% (data not shown).

Coronary flow and ECG intervals Group mean baseline coronary flows 1 min before perfusion with drugs ranged from 12±0.5 to 15.1±1.3 ml min g^{-1} (no significant difference between groups). There was a small time-dependent fall in flow in controls (Figure 1A). This is typical of the model, and tends to bottom-out at this time (Curtis & Hearse, 1989a,b). Since controls were time-matched, this is of no importance. Both drugs increased coronary flow before the onset of ischaemia, with effects significant at ⩾100 nM (Figure 1A). Although the flow increases produced by 300 and 600 nM mibefradil tended to be greater than those elicited by equivalent concentrations of (±)-verapamil, the differences were not significant. During ischaemia, flow fell step-wise to a similar extent in all groups and, during reperfusion, flow recovered to values at least as great as those before the onset of ischaemia in all groups (data not shown).

Pre-drug mean PR intervals ranged from 35±1 to 39±2 msec, and did not differ significantly between groups. The ability to detect changes in PR interval in hearts perfused with 300 nM, and particularly 600 nM mibefradil and (±)-verapamil, was limited during ischaemia by the occurrence of

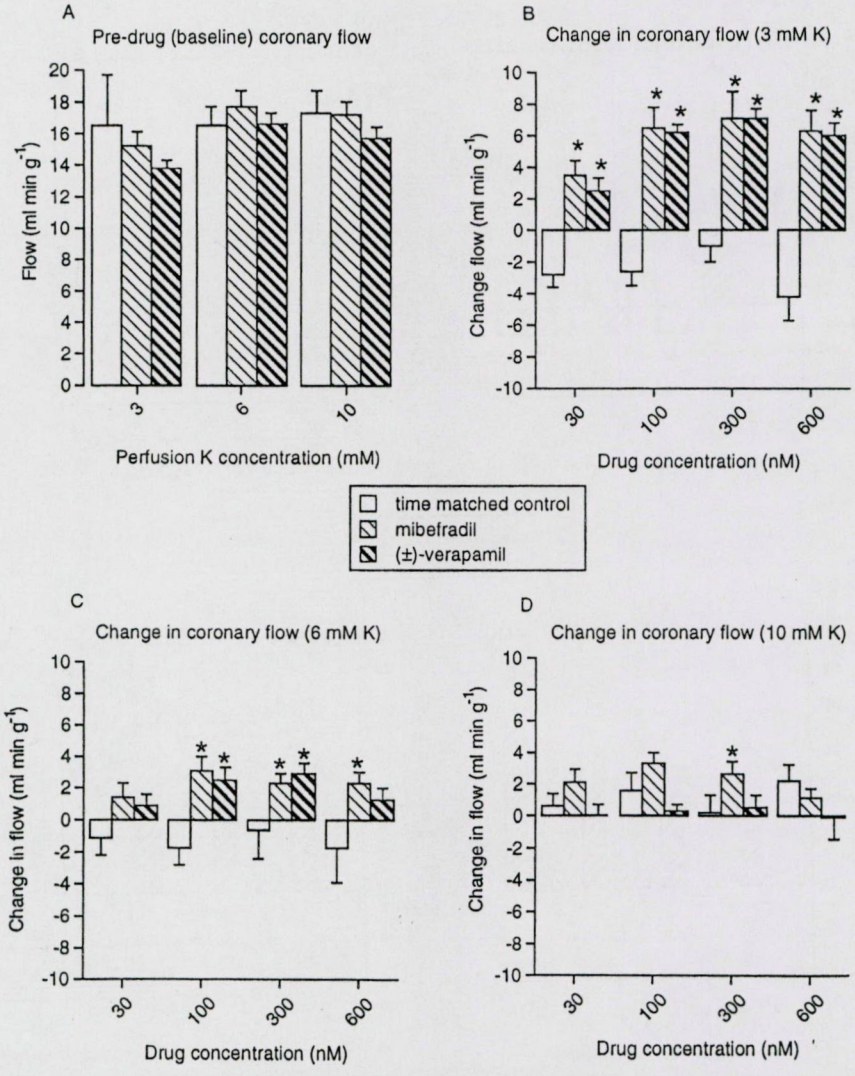

Figure 4 Pre-drug (baseline) coronary flow in hearts perfused with different K^+ concentrations (A), and changes in flow following introduction of drug solutions in hearts perfused with 3 mM K^+ solution (B), with 6 mM K^+ solution (C) or with 10 mM K^+ solution (D). Values are ml min g^{-1} (A) or changes measured 4 min after the introduction of each drug solution (B–D). *$P<0.05$ versus 0 nM group (time matched control).

Möbitz I AV block, since beat-to-beat changes in PR interval were interspersed with ventricular arrhythmias. Thus, we have presented PR data only for intervals recorded 1 min before the start of ischaemia. PR interval was widened ($P<0.05$) at this time only by 600 nM (±)-verapamil (Figure 1B). It would be wrong, however, to assume that mibefradil had no effect on AV node conduction since during ischaemia both mibefradil and (±)-verapamil at 600 nM caused AV block (Table 1).

Pre-drug mean QT intervals ranged from 59±2 to 66±2 msec (no significant differences between groups). At 1 min before the onset of ischaemia, neither drug affected QT interval (Figure 1C) but mibefradil at 600 nM widened QT interval from 15 min after the start of ischaemia (101±6 versus 79±2 msec in controls; $P<0.05$); the effect was still present after 5 min of reperfusion. (±)-Verapamil had no such effect (data not shown).

Pre-drug heart rates varied from 319±8 to 356±9 beats min^{-1}, and did not differ significantly between groups. There was a small time-dependent fall in heart rate in controls from 326±11 to 301±7 beats min^{-1} 1 min before the start of ischaemia, but neither drug affected heart rate at this time (Figure 1D), and there was no trend to an effect of either drug during the remainder of the experiment (data not shown).

Contractile function studies

Developed ventricular pressure In a separate set of hearts, pre-drug baseline left ventricular developed pressure values were similar in each group, and were not dependent on perfusion K^+ concentration (Figure 2A). With the same inflation of the intraventricular balloon in each heart, the pressure development was equivalent to about 70% of maximum attainable for the preparation, as desired. Neither drug at up to 600 nM affected developed pressure when hearts were perfused with Krebs' solution containing 3 mM K^+ (Figure 2B). In contrast, when 6 mM K^+ was used, 30, 100, 300 and 600 nM (±)-verapamil reduced developed pressure ($P<0.05$) concentration-dependently and by a maximum of approximately 70 mmHg (Figure 2C). However, mibefradil reduced pressure only at 600 nM ($P<0.05$) and by only approximately 30 mmHg. When 10 mM K^+ was used, the effects of (±)-verapamil were further increased ($P<0.05$) and mibefradil, though less potent than (±)-verapamil, was active at ⩾100 nM ($P<0.05$) (Figure 2D).

Diastolic ventricular pressure In the same hearts, negative lusitropic effects on diastolic pressure (relaxation impairment, manifesting as an increase in end-diastolic pressure) mirrored changes in developed pressure, with high K^+ exacerbating the effects of both drugs, and (±)-verapamil being the more potent drug at each K^+ concentration. Baseline diastolic pressures were similar in each group, and were unrelated to perfusion K^+ concentration (Figure 3A). (±)-Verapamil at 300 and 600 nM caused a small (maximum of approximately 5 mmHg) increase in diastolic pressure ($P<0.05$) when hearts were perfused with Krebs' solution containing 3 mM K^+ (Figure 3B), whereas mibefradil was inactive. When 6 mM K^+ was used, 30, 100, 300 and 600 nM (±)-verapamil increased diastolic pressure ($P<0.05$) concentration-dependently and by a maximum of approximately 18 mmHg (Figure 3C). However, mibefradil increased pressure only at 600 nM ($P<0.05$) and by less than 5 mmHg. When 10 mM K^+ was used, the effects of (±)-verapamil and mibefradil were further increased ($P<0.05$) although mibefradil remained less effective than (±)-verapamil at each concentration (Figure 3D).

Coronary flow Baseline coronary flow values in these hearts were not related to perfusion K^+ (Figure 4A). The range of means (13.8±0.5 to 17.7±1 ml $min\ g^{-1}$) was slightly higher than that in the arrhythmia study, presumably reflecting a slight vasodilatory response to the ventricular loading caused by balloon inflation. Flow was increased significantly to a similar extent by both drugs when K^+ was 3 mM (Figure 4B), just as it was in the earlier arrhythmia study (Figure 1A). The maximum increase in flow was ~6 ml $min\ g^{-1}$, and only 100 nM drug was required to achieve this. In contrast to effects on ventricular contractile function, the vascular effects of both drugs were diminished by raising K^+ to 6 mM (Figure 4C), and were absent at 10 mM K^+ (Figure 4D), and there was no difference between (±)-verapamil and mibefradil in terms of these effects and their modification by K^+.

Discussion

The aim of this study was to compare mibefradil with (±)-verapamil for effects on arrhythmias induced by ischaemia and by reperfusion. By considering effects on the AV node and the coronary vasculature, and the ability of elevated extracellular K^+ to influence the actions of the drugs on ventricular contractile function, we attempted to link the suppression of VF with blockade of L- and T-channels.

Actions of (±)-verapamil

(±)-Verapamil is not selective for the myocardial L-channel. However, there is compelling evidence that actions such as alpha receptor blockade, sodium channel block, recruitment of collateral flow and bradycardia do not contribute to its effects on VF when examined in conscious rats (Curtis & Walker, 1986b). Importantly, the (+)- to (−)-verapamil potency ratio for effects on ischaemia-induced VF *in vivo* correlates with the negative inotropic potency ratio in hearts perfused with high, but not low K^+ containing solution; this, together with other observations (Curtis, 1990), indicates that L-channel blockade within the ischaemic region (in which extracellular K^+ levels are elevated) fully accounts for (±)-verapamil's effects on VF during ischaemia in conscious rats. Therefore we anticipated that (±)-verapamil could be used to provide, for the isolated rat heart, a similar template response profile for a drug that prevents VF and modulates contractility and other cardiac variables by blocking cardiac L-channels. The present data (PR widening, coronary vasodilatation, K^+-dependent negative inotropy and lusitropy, no effect on QT or heart rate) supported this notion.

The lack of effect of low concentrations of (±)-verapamil on VF was unsurprising, despite evident coronary vasodilatation. The rat heart is collateral-deficient (Maxwell *et al.*, 1987), so vasodilatation does not confer protection against ischaemia-induced VF in this species (Curtis & Walker, 1986b; Curtis, 1998). The lack of effect of (±)-verapamil on QT interval rules out the possibility that unforeseen Class III and bradycardic actions contributed to its effects on VF. Likewise the data prove unequivocally that a reduction in afterload (impossible in the Langendorff preparation) did not contribute to the effects on VF.

The effects of (±)-verapamil on systolic and diastolic pressure were similar to those observed previously with its (+) and (−) enantiomers (Curtis & Walker, 1986b). Developed pressure was reduced by more than 90% only in hearts perfused with 10 mM K^+, and only by 300 or 600 nM (±)-verapamil. It is noteworthy that only these higher concentra-

tions of (±)-verapamil reduced ischaemia-induced VF in the parallel study. The importance of this is that during early myocardial ischaemia, local extracellular K^+ concentration in the involved region rises to 10 mM and beyond (Hill & Gettes, 1980).

The data therefore illustrates, for the first time, that (±)-verapamil's protective effects on ischaemia-induced VF in conscious rats (Curtis & Walker, 1986a; Curtis, 1990) are mirrored by similar actions *in vitro*, and appear to be mediated by the same mechanism, namely L-channel blockade in the involved region.

Actions of mibefradil

T-channels are not considered to play any significant role in human ventricular myocardium (Cremers *et al.*, 1997). In the rat ventricle, unpublished studies have failed to detect measurable T-channel activity (Shattock, personal communication). Thus, any effect of mibefradil on VF would be expected to be more likely to result from L-channel blockade. If the response profile of mibefradil differed qualitatively from that of (±)-verapamil, we would have required to question this notion. However, we found that mibefradil exhibited a pattern of activity on most variables that was qualitatively identical to that of (±)-verapamil.

Mibefradil suppressed ischaemia-induced VF, but it was less potent than (±)-verapamil. Both drugs produced similar significant effects on coronary flow before ischaemia, and both caused a similar degree of AV block. Mibefradil also resembled (±)-verapamil in terms of its lack of effect on heart rate. Mibefradil's profile of activity is therefore similar to that of (±)-verapamil. However, this is insufficient in itself to prove that mibefradil suppressed VF solely by blocking L-channels.

Mechanism of action of mibefradil on ischaemia-induced VF

The role of L-channel blockade is much strengthened by considering the effects of K^+ on the inotropic and lusitropic effects of mibefradil compared with (±)-verapamil. Mibefradil had little or no effect on contractility when K^+ was normal. Likewise, only concentrations in excess of those used in the present study affected cardiac contractility in human (Cremers *et al.*, 1997) and guinea-pig (Hoischen *et al.*, 1998) studies. The negative inotropic and lusitropic effects of mibefradil were exacerbated by high K^+, but the magnitudes of the responses were less than those produced by (±)-verapamil. Importantly, each drug reduced developed pressure by more than 90 mmHg only in hearts perfused with 10 mM K^+, and the concentrations achieving this were the only ones that significantly reduced the incidence of ischaemia-induced VF in the parallel study.

Mibefradil does not affect IK_1, a current important in arrhythmogenesis in rat heart (Rees & Curtis, 1993) at up to 30,000 nM (Nilius *et al.*, 1997). Nor does it reduce evoked norepinephrine release from sympathetic nerves at a concentration (IC_{50} 1000 nM) relevant to present findings (Gothert & Molderings, 1997). Likewise, the IC_{50} for its effects on free-radical mediated cellular injury is 2000 nM (Mason *et al.*, 1998). This limits the scope for L-channel-independent effects of mibefradil on VF in the present study.

Thus, the overall response profile suggests that the antiarrhythmic effect of mibefradil was mediated by the same molecular mechanism as that of (±)-verapamil, and that this mechanism was the same as that responsible for effects on contractile function. Thus, we propose that mibefradil reduced ischaemia-induced VF by K^+-dependent L-channel blockade within the involved region, without any contribution from additional actions (including T-channel blockade), differing from (±)-verapamil only in terms of potency.

Properties limiting efficacy of (±)-verapamil and mibefradil on ischaemia-induced VF

Both (±)-verapamil and mibefradil were compromised by their ability to elicit AV block and increase coronary flow. The latter effect of (±)-verapamil is matched, *in vivo*, by a tendency to dilate other blood vessels leading to a sharp fall in blood pressure at doses associated with VF suppression (Curtis & Walker, 1986b).

The ratio of vascular to myocardial selectivity has been examined previously for mibefradil and (±)-verapamil, and mibefradil was found to be approximately 200 times more vascular selective than (±)-verapamil (Sarsero *et al.*, 1998). Likewise, in the present study both drugs affected coronary flow at concentrations much lower than those affecting myocardial contractile function and VF, and (±)-verapamil was more potent for effects on contractility and VF. These differences can be explained entirely on the basis of differences between the drugs in the manner in which they interact with L-channels. Coronary vasculature has a more positive average membrane potential even than ischaemic myocardium. Single cell voltage clamp studies have shown that mibefradil is a more potent L-channel blocker at depolarized holding potentials, shows preferential affinity for activated and inactivated state channels versus the rested state (Bezprozvanny & Tsien, 1995) and unbinds more slowly from the open channel (Aczel *et al.*, 1998). Thus, the lack of cardioselectivity and resultant low potency of mibefradil as an anti VF agent can be explained by its unfavourable profile of selectivity for the different states of the L-channel in ischaemic (depolarized) myocardium.

Reperfusion-induced VF and other observations

The effect of the drugs on reperfusion-induced VF was a minor focus of the study. It was interesting to note that both drugs were much less effective in suppressing reperfusion-induced VF than ischaemia-induced VF, and significant activity was observed only with the highest concentration of (±)-verapamil. Reperfusion causes rapid wash-out of K^+ from the extracellular space (Hill & Gettes, 1980), and this resolves the ischaemia-induced diastolic depolarization. In view of the voltage dependence of the actions of the drugs on L-channels, blockade by each drug can be expected to be diminished by reperfusion. This would explain the limited ability of each drug to affect reperfusion-induced VF compared with ischaemia-induced VF.

The present data may also explain an earlier observation that electrically-induced arrhythmias in non-ischaemic hearts are resistant to suppression by mibefradil and (±)-verapamil, whereas electrically-induced arrhythmias in ischaemic hearts are suppressed by both drugs (Billman & Hamlin, 1996). Furthermore, they are in agreement with an observation that arrhythmias during ischaemia in dogs are suppressed by lower doses of mibefradil than those required to suppress electrically-induced arrhythmias (Müller *et al.*, 1998). Each of these observations further point to an ischaemia-selective L-channel dependent mechanism of action.

The ability of K^+ to increase the inotropic and lusitropic effects of (±)-verapamil and mibefradil, yet diminish the coronary vasodilator effects appears at first to be anomalous. The likely explanation is that the observation is a quirk of the model. Raised K^+ concentrations increased the negative

lusitropic action of both drugs. In hearts containing balloons inflated to a fixed volume, negative lusitropy will inevitably give rise to an increase in ventricular intramural pressure during diastole. This may be sufficient to result in compression of coronary arterioles. Although this was evidently insufficient to reduce coronary flow to below baseline, it may nevertheless have limited the scope for any increase in flow in response to vasodilatory effects of (±)-verapamil and mibefradil. Thus, the 'blockade' by raised K^+ of the drug-induced flow increases almost certainly reflects a 'functional antagonism' secondary to an increase in average intramural pressure, resulting from negative lusitropy, rather than a genuine antagonism of drug-induced vasodilatation by K^+, mediated at the molecular level. Since coronary flow was not actually reduced below baseline by either drug, we can rule out the possibility that the uninvolved regions were underperfused at any time and, hence, ischaemic, during administration of the higher concentrations of the drugs. The presence of the balloon itself tended to increase flow (compared with the unloaded hearts used for the arrhythmia study) so any notion of the hearts being ischaemic as a result of the balloon is also untenable.

Conclusion

Mibefradil is less potent than (±)-verapamil as an antiarrhythmic in ischaemic rat ventricle. The effects of both drugs can be explained on the basis of L-channel blockade within the ischaemic region. Neither drug is sufficiently ischaemia-selective to achieve protection against VF at concentrations devoid of potentially hazardous vascular and AV nodal effects. This not only serves to explain the lack of efficacy of (±)-verapamil in preventing sudden cardiac death in man (Antman *et al.*, 1992) but also excludes the possibility of mibefradil (or a pharmacologically similar analogue) possessing better efficacy. Nevertheless, the data indicate that should a drug be found that possesses a greater degree of ischaemia-selective L-channel block than either of these agents, it may have potential value in prophylaxis against ischaemia-induced VF and sudden cardiac death. It also appears from the present study that the T-channel is unlikely to represent a useful molecular target for VF suppression, an observation reinforced by the apparent unimportance of the T-channel in human ventricle (Cremers *et al.*, 1997).

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Antiarrhythmic and electrophysiological effects of GYKI-16638, a novel *N*-(phenoxyalkyl)-*N*-phenylalkylamine, in rabbits

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Abstract

The effect of *N*-[4-[2-*N*-methyl-*N*-[1-methyl-2-(2,6-dimethylphenoxy)ethylamino]-ethyl]-phenyl]-methanesulfonamide.hydrochloride (GYKI-16638; 0.03 and 0.1 mg/kg, i.v.), a novel antiarrhythmic compound, was assessed and compared to that of D-sotalol (1 and 3 mg/kg, i.v.) on arrhythmias induced by 10 min of coronary artery occlusion and 10 min of reperfusion in anaesthetized rabbits. Also, its cellular electrophysiological effects were studied in rabbit right ventricular papillary muscle preparations and in rabbit single isolated ventricular myocytes. In anaesthetized rabbits, intravenous administration of 0.03 and 0.1 mg/kg GYKI-16638 and 1 and 3 mg/kg D-sotalol significantly increased survival during reperfusion (GYKI-16638: 82% and 77%, D-sotalol: 75% and 83% vs. 18% in controls, $P < 0.05$, respectively). GYKI-16638 (0.1 mg/kg) significantly increased the number of animals that did not develop arrhythmias during reperfusion (46% vs. 0% in controls, $P < 0.05$). In isolated rabbit right ventricular papillary muscle, 2 μM GYKI-16638, at 1 Hz stimulation frequency, lengthened the action potential duration at 50% and 90% repolarization (APD_{50-90}) without influencing the resting membrane potential and action potential amplitude (APA). It decreased the maximal rate of depolarization (V_{max}) in a use-dependent manner. This effect was statistically significant only at stimulation cycle lengths shorter than 700 ms. The offset kinetics of this V_{max} block were relatively rapid, the corresponding time constant for recovery of V_{max} was 328.2 ± 65.0 ms. In patch-clamp experiments, performed in rabbit ventricular myocytes, 2 μM GYKI-16638 markedly depressed the rapid component of the delayed rectifier outward and moderately decreased the inward rectifier K^+ current without significantly altering the slow component of the delayed rectifier and transient outward K^+ currents. These results suggest that in rabbits, GYKI-16638 has an in vivo antiarrhythmic effect, comparable to that of D-sotalol, which can be best explained by its combined Class I/B and Class III actions.

Keywords: Antiarrhythmic drug; Reperfusion arrhythmia; Action potential duration; V_{max}

1. Introduction

The analysis of the Cardiac Arrhythmia Suppression Trials (CAST-I and CAST-II) prompted the reconsideration of prophylactic antiarrhythmic treatment after myocardial infarction. The results shed light on the controversy that Class I/C type Na^+ channel blockers, i.e. flecainide and encainide, increased mortality in survivors of myocardial infarction despite their ability to reduce the number of premature ventricular beats (The Cardiac Arrhythmia Suppression Trial (CAST) Investigators, 1989; The Cardiac Arrhythmia Suppression Trial II Investigators, 1992). The results of these trials and those of the ESVEM and CASCADE trials shifted the attention to cardiac K^+ channel blockers (Mason, 1993; The CASCADE Investigators, 1993).

As a disappointment, in the SWORD trial D-sotalol, a so-called 'pure' Class III antiarrhythmic drug, which is known to block cardiac K^+ channels selectively, was shown to increase mortality in subsets of patients with myocardial infarction and lowered ejection fraction (Waldo et al., 1996).

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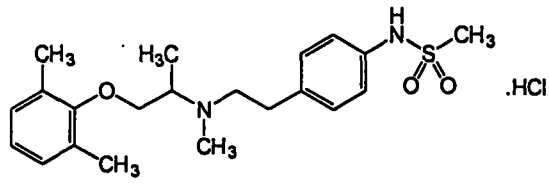

Fig. 1. Chemical structure of GYKI-16638.

Accordingly, special attention has been paid to antiarrhythmic drugs with complex effects on different ion channels and receptors. These include D,L-sotalol (a delayed rectifier K^+ channel blocker and β-adrenoceptor antagonist) and amiodarone (a K^+ channel blocker possessing Na^+ and Ca^{2+} channel blocking properties and antiadrenergic activity). Amiodarone has been shown to exert a strong antiarrhythmic effect in a number of studies and is currently considered to be one of the most efficacous antiarrhythmic drugs available in clinical practice. Long-term treatment with amiodarone, however, leads to the development of serious extracardiac side effects (Hilleman et al., 1998). Therefore, it seems worthwhile to pursue the development of novel amiodarone-like compounds with marked antiarrhythmic potency but without unwanted extracardiac side effects.

N-[4-[2-*N*-methyl-*N*-[1-methyl-2-(2,6-dimethylphenoxy) ethylamino]-ethyl]-phenyl]-methanesulfonamide. hydrochloride (GYKI-16638; Fig. 1) is a novel *N*-(phenoxyalkyl)-*N*-phenylalkylamine that has been developed recently. Although it is not an amiodarone congener, based on its chemical structure, the compound is expected to show amiodarone-like electrophysiological effects, i.e. both Class I/B and Class III properties.

In the present study, we investigated the effect of GYKI-16638 and D-sotalol on the incidence of coronary artery occlusion and reperfusion-induced arrhythmias in anaesthetized rabbits. We also studied the cellular electrophysiological effects of GYKI-16638 in rabbit right ventricular papillary muscle and in rabbit single isolated ventricular myocytes.

2. Materials and methods

2.1. Animals

Male rabbits weighing 2–3 kg were used for the experiments. The animals were allowed to have tap water and laboratory rabbit chow (Altromin, Gödöllő, Hungary) ad libitum until the experiment. The animal handling protocol was reviewed and approved by the Ethics Committee for the Protection of Animals in Research of the Faculty of Medicine, University of Szeged, Szeged, Hungary.

2.2. Coronary artery ligation and reperfusion

The animals were anaesthetized with 30 mg/kg pentobarbitone-Na given intravenously in a volume of 1 ml/kg into the marginal vein of the right ear. Acute coronary artery occlusion and reperfusion were performed as described by Coker (1989). To measure blood pressure, a catheter filled with isotonic saline containing 500 IU/ml heparin (the animals were not heparinized) was introduced into the right carotid artery. The catheter was connected to a pressure transducer (Gould-Statham, P23ID, Hugo Sachs Electronik, March-Hugstetten, Germany) and blood pressure was recorded on an oscillographic recorder (Watanabe, WTR 331, Hugo Sachs Electronik). For the infusion of drugs, another catheter was introduced into the marginal vein of the left ear.

After tracheal cannulation, thoracotomy was performed in the fourth intercostal space and artificial ventilation was started with room air (Harvard rodent ventilator, model 683, Harvard Apparatus, South Natick, MA, USA), with respiratory volume and rate subsequently adjusted to keep blood gases and pH within the normal range (7 ml/kg/stroke, 40 strokes/min, respectively). Following pericardiotomy, a loose loop of 4–0 atraumatic silk (Ethicon, Edinburgh, UK) was placed around the first branch of the left circumflex coronary artery just under its origin. Both ends of the ligature were led out of the thoracic cavity through a flexible tube.

After stabilization of blood pressure and heart rate (approximately 10 min), saline or 0.03 or 0.1 mg/kg GYKI-16638 or 1 or 3 mg/kg D-sotalol was administered i.v. during a 1-min infusion in a volume of 2 ml/kg, 5 min prior to coronary artery occlusion.

Coronary artery occlusion and, thus, local myocardial ischaemia, was produced by tightening the loose loop and clamping on the silk. After 10 min of coronary artery occlusion, the ligature was released to permit reperfusion for 10 min.

The electrocardiogram (lead I, II, III) was registered using a thermographic recorder (ESC 110 4 CH, Multiline, Esztergom, Hungary) with subcutaneous needle electrodes. QT interval was defined as the time between the first deviation from the isoelectric line during the PR interval until the end of the TU wave. QT interval corrected for heart rate (QT_c) was calculated using the following equation of Carlsson et al. (1993a): $QT_c = QT - 0.175 \times (RR - 300)$.

Arrhythmias were detected and diagnosed in accordance with the Lambeth conventions as ventricular tachycardia, ventricular fibrillation and other types of arrhythmias, including single extrasystoles, bigeminy, salvos and bradycardia (Walker et al., 1988).

At the end of the experiment, heparin-Na (500 IU/kg, i.v.) was administered and the animals were killed. The hearts were cut out from the chest in order to determine the size of the occluded zone. After the ligation was

tightened, the hearts were retrogradely perfused via the aorta with 20 ml saline and 10 ml of 96% ethanol as previously described by Leprán et al. (1983). The non-denaturated area (occluded zone) was excised and its extent is expressed as a percentage of the total wet weight of the ventricles. Four animals with an occluded zone less than 16% or larger than 32% were excluded from the final evaluation.

2.3. Drug administration protocol

D-Sotalol (1 or 3 mg/kg) was dissolved in saline, and GYKI-16638 (0.03 or 0.1 mg/kg) was dissolved in propylene glycol/saline, 1:1 mixture. Both drugs were applied 5 min prior to coronary artery ligation in a volume of 2 ml/kg. Each dose was prepared on the day of the experiment. Control animals received propylene glycol/saline, 1:1 mixture in a volume of 2 ml/kg.

2.4. Measurement of action potential parameters in rabbit right ventricular papillary muscle

Following cervical dislocation, the heart of each animal was rapidly removed through a right lateral thoracotomy. The hearts were immediately rinsed in oxygenated Tyrode's solution containing (in mM): NaCl, 115; KCl, 4; $CaCl_2$, 1.2; $MgCl_2$, 1; $NaHCO_3$, 21.4; and glucose, 11. The pH of this solution was 7.35–7.45 when gassed with 95% O_2 and 5% CO_2 at 37°C. The papillary muscles from the right ventricle were individually mounted in a tissue chamber (volume ≈ 50 ml). Each preparation was initially stimulated (HSE [Hugo Sachs Electronik] stimulator type 215/II, March-Hugstetten, Germany) at a basic cycle length of 500 ms (frequency = 2 Hz), using 2-ms long rectangular constant voltage pulses isolated from ground and delivered across bipolar platinum electrodes in contact with the preparation. We applied the following types of stimulation in the course of the experiments: stimulation with a constant cycle length of 500 ms (2 Hz); stimulation with different constant cycle lengths ranging from 300 to 5000 ms taking the measurement after the 25th beat.

To determine the recovery of V_{max}, extra test action potentials were elicited using single test pulses (S_2) in a preparation driven at a basic cycle length of 500 ms. The S_1–S_2 coupling interval was increased progressively from the end of the effective refractory period up to 10 s. The time constant for recovery of V_{max} was fitted to a single exponential function, starting at the 40 ms diastolic interval and ending at 5 s.

Before the control measurement, at least 1 h was allowed for each preparation to equilibrate while being continuously superfused with Tyrode's solution. The temperature of the superfusate was kept constant at 37°C. Transmembrane potentials were recorded using a conventional microelectrode technique. Microelectrodes filled with 3 M KCl and having tip resistances of 5–20 mΩ were connected to the input of a high impedance electrometer (HSE microelectrode amplifier type 309), which was referenced to the ground. The first derivative of transmembrane potentials (V_{max}) was electronically derived by an HSE differentiator (type 309). The voltage outputs from all amplifiers were displayed on a dual-beam memory oscilloscope (Tektronix 2230 100 MHz digital storage oscilloscope, Beaverton, OR).

The maximum diastolic potential (MDP), action potential amplitude (APA), and action potential duration measured at 50% and 90% repolarization (APD_{50-90}) were obtained using software developed in our department (HSE-APES). GYKI-16638 was dissolved in dimethyl sulfoxide (DMSO) as a 1-mM stock solution. After the control measurements, GYKI-16638 was added to the tissue bath to obtain a final concentration of 2 μM and the measurements were repeated after a 30-min incubation time.

To select the single in vitro concentration, we were guided by pharmacokinetic studies with GYKI-16638. In these measurements, the obtained plasma concentration after GYKI-16638 administration correlated well with the concentration used in our in vitro studies with GYKI-16638.

2.5. Whole-cell configuration of the patch-clamp technique

Single ventricular myocytes were obtained by enzymatic dissociation from New Zealand rabbits (1–2 kg) by a technique described earlier in detail (Varró et al., 1996).

One drop of cell suspension was placed in a transparent recording chamber mounted on the stage of an inverted microscope (TMS; Nikon, Tokyo, Japan), and at least 5 min were allowed for individual myocytes to settle and adhere to the bottom of the chamber before superfusion was started. Myocytes that were used were rod-shaped with clear striations. HEPES-buffered Tyrode solution served as the normal superfusate in all experiments. This solution contained (mM): NaCl 144, NaH_2PO_4 0.33, KCl 4.0, $CaCl_2$ 1.8, $MgCl_2$ 0.53, glucose 5.5, and HEPES 5.0 at pH 7.4.

Patch-clamp micropipettes were made from borosilicate glass capillaries (Clark, Reading, UK) using a P-97 Flaming/Brown micropipette puller (Sutter Instrument Co, Novato, CA, USA). These electrodes had resistances between 1.5 and 2.5 MΩ when filled with pipette solution containing (in mM): K-aspartate 100, KCl 45, K_2ATP 3, $MgCl_2$ 1, EGTA 10, and HEPES 5. The pH of this solution was adjusted to 7.2 by addition of KOH. Nisoldipine (1 μM; Bayer, Leverkusen, Germany) in the external solution eliminated the inward Ca^{2+} current (I_{Ca}). An Axopatch-1D amplifier (Axon Instruments, Foster City, CA, USA) was used to record the membrane current in the whole-cell

Table 1
Effect of intravenous administration of D-sotalol and GYKI-16638 on mean arterial blood pressure, heart rate, QT and QT_c intervals in anaesthetized rabbits

Group	Dose (mg/kg)		n	Before infusion	5 min after infusion
Control		MBP	19	101±2.8	100±2.6
		HR		271±7.2	268±6.6
		QT		149±4.0	149±4.4
		QT_c		162±3.4	162±3.8
D-sotalol	1.0	MBP	13	97±3.2	97±2.7
		HR		272±9.4	252±8.9[a]
		QT		142±4.7	162±5.2[a]
		QT_c		156±3.9	172±4.0[a]
	3.0	MBP	13	95±3.5	100±3.3
		HR		265±9.7	247±7.9[a]
		QT		150±6.8	167±6.7[a]
		QT_c		163±5.9	176±5.8[a]
GYKI-16638	0.03	MBP	14	93±3.4	93±2.8
		HR		273±5.2	259±6.3[a]
		QT		150±4.6	159±7.3
		QT_c		164±4.1	171±6.5
	0.1	MBP	17	94±2.7	93±2.5
		HR		270±8.7	253±7.3[a]
		QT		140±4.4	166±6.4[a,b]
		QT_c		153±3.4	173±4.7[a]

n = Number of animals, MBP = mean blood pressure (mm Hg), HR = heart rate (1/min), QT = QT interval (ms), QT_c = QT_c interval.
[a] $P < 0.05$, compared to the preinfusion value of the same group.
[b] $P < 0.05$, compared to the control group.

configuration of the patch-clamp technique. After a high (1–10 GΩ) resistance seal was established by gentle suction, the cell membrane beneath the tip of the electrode was disrupted by further suction or by application of 1.5 V electrical pulses applied for 1–5 ms. Series resistance was typically 4–8 MΩ prior to compensation (50–80%, depending on the voltage protocol utilized). Experiments, where the series resistance was high, or where it increased substantially during measurement, were terminated and the data were discarded. Membrane currents were digitized using a 333-kHz analog-to-digital converter (Digidata 1200, Axon Instruments) under software control (pClamp 6.0, Axon Instruments). Analyses were performed using Axon (pClamp 6.0) software after low-pass filtering at 1 kHz. All patch-clamp data were collected at 37°C.

GYKI-16638 was diluted at the time of use from a 10-mM stock solution containing 100% DMSO. DMSO at the resulting concentrations (0.2%) produced no discernible effect on APD or the membrane currents assessed. All stock solutions were prepared using HEPES-buffered Tyrode solution as the solvent.

2.6. Statistical evaluation

For the evaluation of data obtained from the cellular electrophysiology experiments, Student's t-test for paired data was used. All data are expressed as means ± standard error of the mean (S.E.M.).

The incidence of arrhythmias was calculated and compared by using the χ^2 method. All other variables are expressed as means ± S.E.M. and, after analysis of variance, were compared by means of the modified t statistic of Wallenstein et al. (1980). Differences were considered significant when P values were less than 0.05.

3. Results

3.1. Effect of GYKI-16638 on haemodynamic variables in anaesthetized rabbits

There were no significant differences between the mean arterial blood pressures of control and D-sotalol- or GYKI-16638-treated animals. Mean arterial blood pressure fell significantly in all groups due to coronary artery occlusion as compared to preocclusion values (74 ± 3.9 vs. 101 ± 2.8 mm Hg, 78 ± 4.5 vs. 97 ± 3.2 mm Hg, 84 ± 2.6 vs. 95 ± 3.5 mm Hg, 69 ± 3.8 vs. 93 ± 3.4 mm Hg and 74 ± 3.9 vs. 94 ± 2.7 mm Hg in controls, 1 and 3 mg/kg D-sotalol-, 0.03 and 0.1 mg/kg GYKI-16638-treated animals, respectively, all $P < 0.05$).

The infusion of 1 and 3 mg/kg D-sotalol, as well as 0.03 and 0.1 mg/kg GYKI-16638, significantly decreased the heart rate of rabbits compared to the basal values

Table 2
Effect of D-sotalol and GYKI-16638 on the incidence of arrhythmias during 10 min of coronary artery occlusion in anaesthetized rabbits

Group	Dose (mg/kg)	n	Incidence of arrhythmias (N/%)			
			None	VF	VT	Other
Control		19	4/19 (21%)	8/19 (42%)	2/19 (11%)	14/19 (74%)
D-Sotalol	1.0	13	5/13 (38%)	1/13 (8%)	0/13 (0%)	8/13 (62%)
	3.0	13	7/13 (54%)	1/13 (8%)	0/13 (0%)	6/13 (46%)
GYKI-16638	0.03	14	3/14 (21%)	3/14 (21%)	2/14 (14%)	11/14 (79%)
	0.1	17	5/17 (29%)	4/17 (24%)	0/17 (0%)	12/17 (71%)

n = Total number of animals; N = number of animals exhibiting the given response; % = percentage of the animals exhibiting the given response. VF = ventricular fibrillation; VT = ventricular tachycardia; Other = extrasystoles, salvos, and/or bigeminy.

Table 3
Effect of D-sotalol and GYKI-16638 on the incidence of arrhythmias during 10 min of reperfusion following 10 min of coronary occlusion in anaesthetized rabbits

Group	Dose (mg/kg)	n	Incidence of arrhythmias (N/%)			
			None	VF	VT	Other
Control		11	0/11 (0%)	9/11 (82%)	7/11 (64%)	5/11 (46%)
D-Sotalol	1.0	12	4/12 (33%)	3/12 (25%)[a]	4/12 (33%)	8/12 (67%)
	3.0	12	4/12 (33%)	2/12 (17%)[a]	4/12 (33%)	9/12 (75%)
GYKI-16638	0.03	11	3/11 (27%)	2/11 (18%)[a]	4/11 (36%)	9/11 (82%)
	0.1	13	6/13 (46%)[a]	3/13 (23%)[a]	6/13 (46%)	8/13 (62%)

n = Total number of animals; N = number of animals exhibiting the given response; % = percentage of the animals exhibiting the given response. VF = ventricular fibrillation; VT = ventricular tachycardia; Other = extrasystoles, salvos, and/or bigeminy.
[a] $P < 0.05$.

(Table 1). Coronary occlusion did not change heart rate significantly compared to preocclusion values. No significant changes occurred in the heart rate of animals during reperfusion.

3.2. Effect of GYKI-16638 on QT and QT_c intervals in anaesthetized rabbits

D-Sotalol infusion, in the dose of 1 and 3 mg/kg, significantly lengthened QT and QT_c intervals (Table 1). GYKI-16638, in the dose of 0.03 mg/kg, had no effect on QT and QT_c intervals, but caused a significant increase of both variables in the dose of 0.1 mg/kg. No significant changes occurred in the QT or QT_c intervals during reperfusion.

3.3. Arrhythmias during 10 min of myocardial ischaemia

In all groups, arrhythmias did not develop either during the 1-min infusion of drugs or vehicle, or between the infusion of drugs and coronary occlusion.

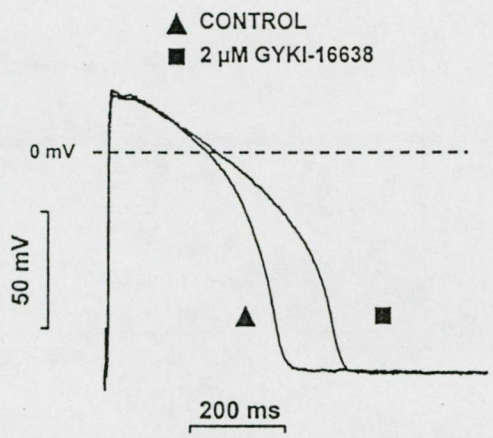

Fig. 2. Effect of 2 μM GYKI-16638 on the action potential in rabbit right ventricular papillary muscle (stimulation frequency: 2 Hz).

The incidence of arrhythmias in the control, D-sotalol- and GYKI-16638-treated groups during 10 min of coronary artery occlusion is shown in Table 2. The incidence of ventricular fibrillation was not statistically different in the D-sotalol- or GYKI-16638-treated animals compared to the control group.

There were no significant differences in the treated and control groups with respect to the incidence of other types of arrhythmias during 10 min of coronary artery ligation.

3.4. Reperfusion-induced arrhythmias

Arrhythmias induced by reperfusion appeared within 10–30 s following the release of the coronary artery ligature.

D-Sotalol (1 and 3 mg/kg) and 0.03 mg/kg GYKI-16638 pretreatment significantly reduced the incidence of reperfusion-induced ventricular fibrillation (Table 3). All drug pretreatments significantly increased the number of animals surviving reperfusion (75% and 83% with 1 and 3 mg/kg D-sotalol, 82% and 77% with 0.03 and 0.1 mg/kg GYKI-16638 vs. 18% in controls, $P < 0.05$, respectively).

The number of animals that did not develop any arrhythmia during reperfusion was significantly higher in the

Table 4
The effect of 2 μM GYKI-16638 on the action potential parameters in rabbit right ventricular papillary muscle

$n = 6$	Control	2 μM GYKI-16638
RP (mV)	−89 ± 1.5	−91 ± 0.8
APA (mV)	111.7 ± 2.1	112 ± 2.8
APD_{50} (ms)	158.3 ± 12.4	194.5 ± 12.7[a]
APD_{90} (ms)	205.8 ± 15.9	254.8 ± 14.9[a]
V_{max} (V/s)	208.3 ± 32.8	169.2 ± 20.8[a]

RP = resting potential; APA = action potential amplitude; APD_{50} = 50% repolarization time; APD_{90} = 90% repolarization time; V_{max} = maximum upstroke velocity; stimulation frequency: 2 Hz.
[a] $P < 0.05$.

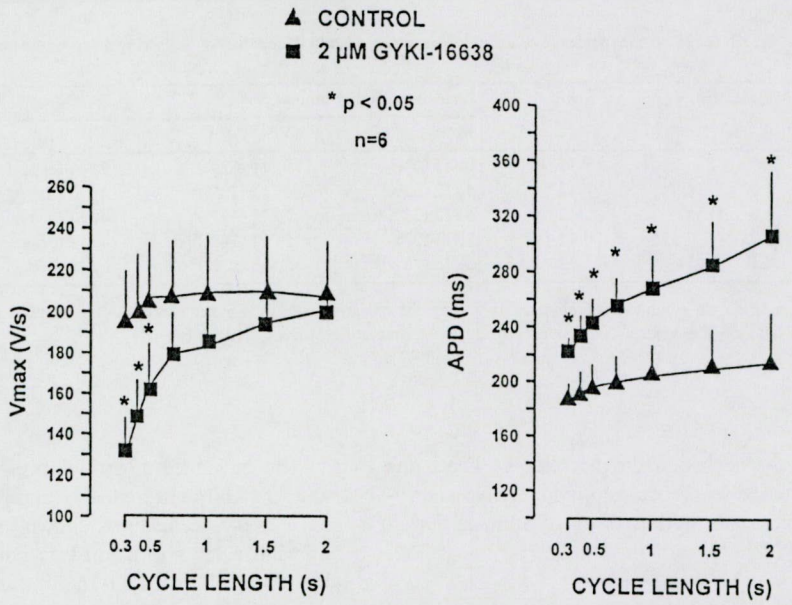

Fig. 3. Frequency-dependent effect of 2 μM GYKI-16638 on maximum upstroke velocity (V_{max}) and APD in rabbit right ventricular papillary muscle.

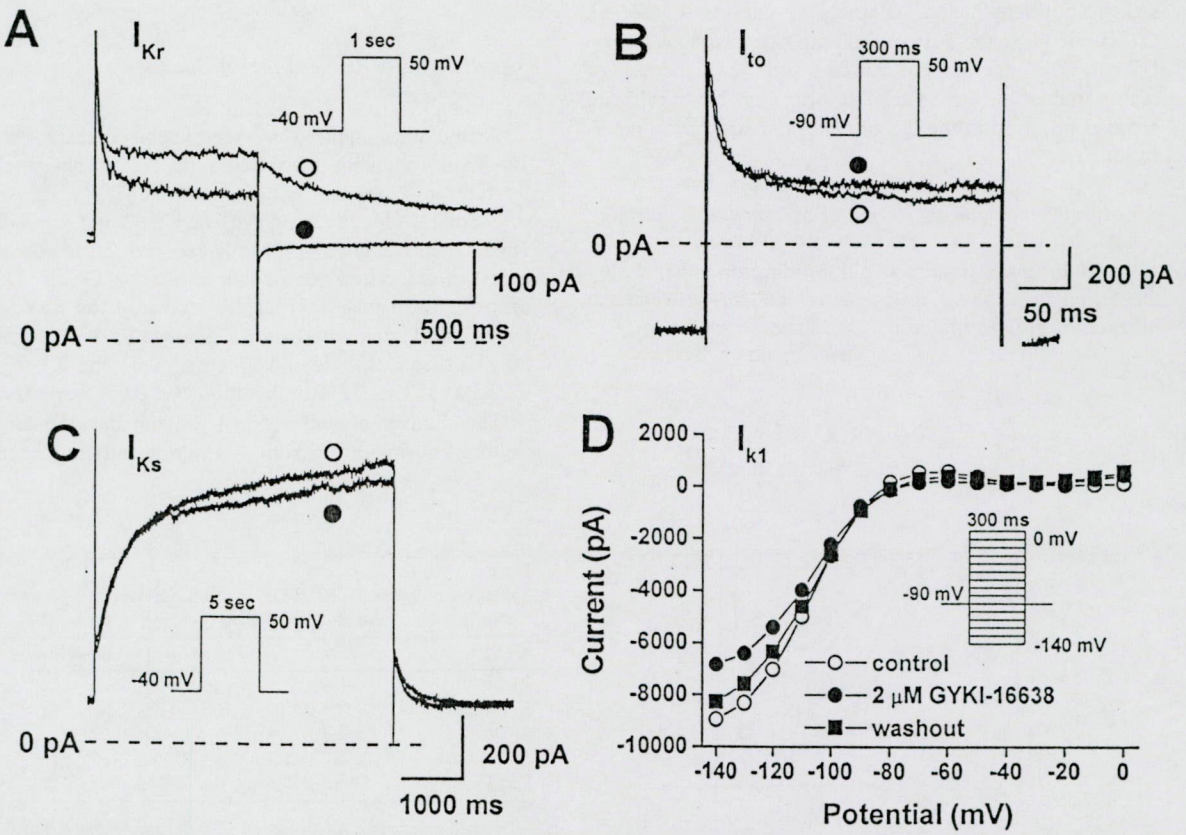

Fig. 4. Effect of 2 μM GYKI-16638 (A) on the rapid component of the delayed rectifier outward K^+ current (I_{Kr}), (B) on the transient outward current (I_{to}), (C) on the slow component of the delayed rectifier K^+ current (I_{Ks}), and (D) on the inward rectifier potassium current (I_{k1}). Pulse protocols are shown as insets.

0.1 mg/kg GYKI-16638-treated group (Table 3). There were no differences in the incidence of other types of arrhythmias between the animals receiving pretreatment and control rabbits during reperfusion (Table 3).

3.5. Effect of GYKI-16638 on the action potentials in rabbit papillary muscle

The effect of 2 μM GYKI-16638 on the action potentials at 2 Hz stimulation frequency in rabbit right ventricular papillary muscle is shown in Fig. 2 and Table 4. At 2 Hz stimulation frequency, 2 μM GYKI-16638 did not significantly influence the resting membrane potential and the APA, but it lengthened repolarization, measured as APD_{50} and APD_{90}. The maximal rate of depolarization (V_{max}) was also significantly reduced. The observed decrease of V_{max} in the presence of 2 μM GYKI-16638 was use-dependent and became significant only at stimulation cycle lengths shorter than 700 ms (Fig. 3). This was consistent with a delayed recovery of V_{max} measured in the presence of the drug ($\tau < 30$ ms in controls, and 328.2 ± 65.0 ms, ($n = 4$) with 2 μM GYKI-16638). The APD prolongation induced by 2 μM GYKI-16638 was reverse use-dependent: the slower the stimulation frequency, the more pronounced the APD prolongation (Fig. 3).

3.6. Effect of GYKI-16638 on various transmembrane K^+ currents in isolated rabbit ventricular myocytes

The effect of GYKI-16638 on various K^+ currents was studied in isolated single rabbit ventricular myocytes (Fig. 4). The rapid component of the delayed rectifier outward K^+ current (I_{Kr}) was elicited from −40 mV holding potential to various 1-s long test pulses ranging from −20 to +50 mV and then returning back to −40 mV. The amplitude of the deactivating tail current at this potential was measured as the difference between the peak tail current and the holding current level and was attributed to I_{Kr} (at +50 mV, it was 86.9 ± 16.6 pA, $n = 4$). Because of the very slow deactivation of I_{Kr}, the pulsing frequency in these experiments was 0.01 Hz. As Fig. 4A shows, 2 μM GYKI-16638 completely inhibited the I_{Kr} tail current. Similar results were obtained in three other cells.

Fig. 4B and C shows that 2 μM GYKI-16638 did not change or only minimally affected the transient outward (I_{to}) and the slow component of the delayed rectifier K^+ currents. Similar results were found in three other cells.

The effect of 2 mM GYKI-16638 on the inward rectifier K^+ current (I_{k1}) was studied at a holding potential of −80 mV and was elicited by 300-ms long voltage pulses to various potentials ranging from −140 to 0 mV. I_{k1} was determined as the steady-state current at the end of the voltage pulses. As a result of a representative experiment, Fig. 4D shows that 2 mM GYKI-16638 moderately decreased the amplitude of the steady state current–voltage relationship attributed to inhibition of I_{k1}. This effect was reversible upon 5 min of washout. The average value of the I_{k1} current ($n = 7$) at −100 mV before drug superfusion was −2648 ± 399 pA, which was significantly reduced to −2152 ± 401 pA after 5 min of superfusion with 2 mM GYKI-16638.

4. Discussion

The recently developed GYKI-16638 is a member of a new series of *N*-(phenoxyalkyl)-*N*-phenylalkylamine compounds. Its structure combines Class I/B and Class III structural elements, i.e. those of D-sotalol and mexiletine.

In the present study, the antiarrhythmic effect of GYKI-16638 in anaesthetized rabbits and its electrophysiological effects in rabbit right ventricular papillary muscle preparations were investigated. We compared the antiarrhythmic effect of GYKI-16638 to that of D-sotalol, a well-known pure Class III antiarrhythmic agent.

GYKI-16638 exerted an antiarrhythmic effect in our experiments that was comparable to that of D-sotalol. Both compounds significantly decreased the number of animals that died due to lethal ventricular arrhythmias during reperfusion after 10 min of regional myocardial ischaemia. The significant improvement of survival during reperfusion occurred in spite of the fact that there were animals that had reversible ventricular fibrillation in the control group as well. This is a well-known phenomenon in experimental arrhythmia studies, i.e. relatively small hearts can recover from ventricular fibrillation, while in human and large animal hearts, this arrhythmia is irreversible (Botting et al., 1986).

The antiarrhythmic activity of GYKI-16638 was already observed after the administration of the lower dose which did not influence QT and QT_c intervals. This may suggest that GYKI-16638 has a mechanism of action that is based not solely on the prolongation of repolarization. Indeed, it was found that GYKI-16638 not only caused a significant increase in APD and, consequently, in the effective refractory period, but that it also significantly reduced the maximum upstroke velocity (V_{max}) in rabbit right ventricular papillary muscles, reflecting its fast Na^+ channel (I_{Na}) blocking ability. However, it was found that, in a higher dose, it significantly prolonged the QT and QT_c intervals in anaesthetized rabbits, as was expected from its in vitro effect on the APD. The Na^+ channel blocking effect was significant only at cycle lengths shorter than 700 ms. This was consistent with the measured time constant for recovery of V_{max}, which resembled that of Class I/B type drugs (Campbell, 1983) and amiodarone (Varró et al., 1985). Such an effect may have therapeutic importance in the inhibition of arrhythmias due to early afterdepolarizations (Papp et al., 1996).

D-Sotalol has been shown to exert an antiarrhythmic effect in a number of animal (Lynch et al., 1985; Usui et

al., 1993; Hashimoto et al., 1995) and human studies (Hohnloser et al., 1995; Koch et al., 1995) with a proposed mechanism of action of terminating re-entry (Fei and Frame, 1996). However, it was shown in the SWORD trial that D-sotalol increased mortality in patients with myocardial infarction (Waldo et al., 1996). The results shifted attention towards antiarrhythmic compounds with a combined mechanism of action. As an example, amiodarone, an antiarrhythmic agent with a complex mode of action, has attracted a great deal of interest recently. It has been shown to decrease ventricular fibrillation vulnerability in rabbit hearts following long-term pretreatment (Behrens et al., 1997), to be protective against ischaemia- and reperfusion-induced arrhythmias (Varró and Rabloczky, 1986; Coker and Chess-Williams, 1991; Li and Northover, 1992), and to be effective in the treatment of life-threatening ventricular arrhythmias in humans (Singh, 1999). Also, some multicenter clinical trials have shown that amiodarone may reduce the incidence of arrhythmia-related sudden death (Julian et al., 1997; Cairns et al., 1997). Several electrophysiological studies showed that amiodarone possessed both Class I/B and Class III antiarrhythmic properties (Singh and Vaughan Williams, 1970; Varró et al., 1985; Honjo et al., 1991; Maruyama et al., 1995), as well as Ca^{2+} channel blocking (Nattel et al., 1987) and sympatholytic effects (Polster and Broekhuysen, 1976). While effectively diminishing the development of re-entry arrhythmias, selective I_{Kr} blockers can increase the incidence of arrhythmias, by increasing the interventricular dispersion of repolarization and initiating early afterdepolarizations, leading to torsade de pointes tachycardia (Verduyn et al., 1997; Hohnloser, 1997). It was demonstrated that almokalant, a selective I_{Kr} blocker, significantly reduced the incidence of coronary artery occlusion/reperfusion-induced arrhythmias but also showed marked proarrhythmic activity (Carlsson et al., 1993a; Farkas et al., 1998). D-Sotalol has also been shown to induce torsades de pointes in animals (Buchanan et al., 1993; Vos et al., 1995) and humans (Gottlieb et al., 1997).

Amiodarone was found to have a remarkably low potential for inducing torsades de pointes tachyarrhythmias despite its ability to prolong the QT_c interval (Hohnloser et al., 1994). The decrease in the transmural dispersion of ventricular repolarization and the consequent inhibition of the development of early afterdepolarization can possibly explain this effect of amiodarone (Sicouri et al., 1997). Class I/B antiarrhythmics may reduce the occurrence of this arrhythmia. Mexiletine (Shimizu and Antzelevitch, 1997) and lidocaine in both animal (Carlsson et al., 1993b) and human studies (Assimes and Malcolm, 1998) were shown to suppress torsades de pointes induced by D-sotalol. Antiarrhythmic drugs with a Class I/B action have also been shown to be effective against coronary artery occlusion/reperfusion-induced arrhythmias (Bonaduce et al., 1986; Uematsu et al., 1986; He et al., 1992; Komori et al., 1995). Also, the combination of mexiletine and sotalol prevented ventricular tachycardia induced by programmed stimulation in dogs with chronic infarction (Chezalviel et al., 1993), and Luderitz et al. (1991) concluded in their review that in humans, the combination of mexiletine and sotalol suppressed both premature ventricular beats and complex ventricular arrhythmias more effectively than sotalol alone. These results suggest that an antiarrhythmic compound with combined Class III and Class I/B effects could reduce the incidence of re-entry arrhythmias without a high risk of inducing torsades de pointes arrhythmias.

The exact ionic mechanism of the electrophysiologic and antiarrhythmic effects of GYKI-16638 is not fully understood. As mentioned above, the use-dependent depression of V_{max} strongly argues for inhibition of the fast inward Na^+ current. The APD lengthening effect of the compound can be best explained by the marked depression of I_{Kr} and, to a lesser extent, by the decrease of the I_{k1}. Therefore, based on the cellular electrophysiological measurements, GYKI-16638 can be regarded as an antiarrhythmic compound which — like amiodarone (Varró et al., 1996; Kodama et al., 1997) — interferes with multiple transmembrane ion channels.

When administered chronically, amiodarone exhibits serious extracardiac side effects that limit its use (Hilleman et al., 1998). GYKI-16638 shares some (Class I/B + Class III), but not all of the electrophysiological properties of amiodarone and its chemical structure is also different. Based on its different chemical structure, it can be reasonably expected that this compound, unlike amiodarone, will be relatively free of extracardiac side effects. Due to its Class I/B action, it is also expected that the compound will lack the significant inhibitory effect on conduction at a normal heart rate. The compound also showed reverse frequency-dependent prolongation of APD in rabbit papillary muscle (Fig. 3), an effect which resembles that of D-sotalol or any specific I_{Kr} blocker. Therefore, further studies are needed to elucidate the possible side effects of GYKI-16638, including its capability to induce torsades de pointes or conduction disturbance-related arrhythmias.

The haemodynamic side effects of antiarrhythmic agents are of particular importance. GYKI-16638 did not change the mean arterial blood pressure, but decreased the heart rate of anaesthetized rabbits. We also found that the administration of D-sotalol significantly decreased heart rate in rabbits. A similar heart rate decreasing effect of D-sotalol has been shown by Schwartz et al. (1987), although this compound lacks the antiadrenergic properties of D,L-sotalol. A moderate decrease in heart rate may be beneficial, especially in the setting of myocardial ischaemia and reperfusion-induced arrhythmias (Bernier et al., 1989).

In conclusion, we demonstrated that GYKI-16638, a novel antiarrhythmic drug candidate, protected against coronary artery occlusion and reperfusion-induced arrhythmias in anaesthetized rabbits. This protection was already noticed at a lower dose, which did not lengthen the QT_c interval significantly. Based on the results of our cellular

electrophysiological investigations in rabbit right ventricular papillary muscle, it can be assumed that GYKI-16638 exerts its antiarrhythmic effect through combined Class I/B and Class III actions.

Acknowledgements

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V.

The proarrhythmic effects of intravenous quinidine, amiodarone, d-sotalol and almokalant in the anesthetized rabbit model of torsade de pointes

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Running header: Proarrhythmic effect of antiarrhythmic drugs

ABSTRACT

The proarrhythmic effects of quinidine, amiodarone, d-sotalol and almokalant were examined during α_1-adrenoceptor stimulation in chloralose-anesthetized rabbits. Each dose of almokalant (26, 88 and 260 $\mu g\ kg^{-1}$), d-sotalol, quinidine or amiodarone (each 3, 10 and 30 mg kg^{-1}) was infused intravenously over 5 min and there was 20 min interval between each infusion. Only d-sotalol and almokalant evoked torsade de pointes (TdP) (6 out of 10 and 4 out of 10 animals, respectively). The incidences of TdP were 0%, 50%, and 40% after administering the 1st, 2nd and 3rd doses of the non-selective I_{Kr} inhibitor d-sotalol, respectively. Similarly, these values were 20%, 40% and 33% after administering the 1st, 2nd and 3rd doses of the selective I_{Kr} inhibitor almokalant, respectively. The 2nd dose of d-sotalol and almokalant increased the incidence of non-TdP ventricular tachycardia (VT) and the cumulative incidence of ventricular premature beats, bigeminy and salvo [$P<0.05$ versus time matched controls (n=10)]. Quinidine evoked only VT (2 out of 7 animals), but not TdP. Quinidine, d-sotalol and almokalant evoked conduction blocks in a dose related manner ($P<0.05$) and prolonged QT and QTc intervals ($P<0.05$). Amiodarone (n=8) neither prolonged QT and QTc nor evoked ventricular tachyarrhythmias, blocks or other proarrhythmia. All three doses of quinidine reduced blood pressure greater than the other three drugs ($P<0.05$). In conclusion, these results show no direct correlation between the occurrence of TdP and the infusion rate or dose of antiarrhythmics. Furthermore, the lack of TdP with quinidine warns of false negatives in the applied model.

Key words: torsade de pointes, d-sotalol, almokalant, quinidine, amiodarone, α_1-adrenoceptor stimulation.

INTRODUCTION

Torsade de pointes (TdP) can be evoked by various drugs and toxins in man (1, 2, 3), especially in the presence of predisposing factors like bradycardia, electrolyte abnormalities (i.e. hypokalemia and hypomagnesemia), prolonged repolarization, depressed left ventricular function and/or a history of life threatening arrhythmias (3). The underlying mechanism of this arrhythmia is still not known fully, but early afterdepolarisations (EADs) and dispersion of ventricular repolarisation are suspected to be the main electrophysiologic substrates for initiation of TdP (3).

Drugs that delay repolarisation have a tendency to cause TdP. Quinidine is by far the most frequently reported drug associated with this arrhythmia (1). Although most studies say that intravenous amiodarone does not widen QT or cause TdP in most patients, there is some disagreement about this (4, 5, 6). Therefore intravenous amiodarone was included in the study. Almokalant and d-sotalol, which differ from quinidine and amiodarone by virtue of their greater selectivity for cardiac potassium channels, can also evoke TdP in man (7, 8).

Several animal models have been developed with TdP as the endpoint (9, 10, 11). The method developed by Carlsson et al. (12) of the acquired long QT syndrome utilizes anesthetized rabbits, and TdP is evoked by co-administration of a test agent with the selective α_1-agonist, methoxamine. Almokalant and d-sotalol are highly proarrhythmic and prone to evoke TdP in rabbits (13, 14, 15).

Although this model has been used to examine the proarrhythmic activity of novel antiarrhythmic agents (14, 16, 17), the model has not been characterized sufficiently for its clinical relevance to be certain. Importantly, the information on the effects of long-established drugs with clinically well-characterized proarrhythmic activity is sparse. Therefore, in the present study we examined the effects of quinidine and amiodarone, two widely used

antiarrhythmic drugs, and compared them with d-sotalol and almokalant in anesthetized rabbits during α_1-adrenergic receptor stimulation by phenylephrine.

METHODS

Animals

The experiments were performed on male New Zealand White rabbits weighing 2.1 to 2.9 kg. The animals were handled according to a protocol reviewed and approved by the Ethical Committee for the Protection of Animals in Research of the University of Szeged, Szeged, Hungary.

Animal preparation

The proarrhythmic activity of antiarrhythmic compounds was examined by using the method of Carlsson et al. (12, 13) with minor modification. After sedation with pentobarbital sodium (5 mg kg^{-1}, i.v.), animals were anesthetized with α-chloralose (100 mg kg^{-1} i.v. into the marginal vein of the right ear, in 10 ml kg^{-1} infusion volume, at a rate of 1 ml min^{-1}). A catheter was introduced into the right carotid artery in order to measure blood pressure (Blood Pressure Monitor BP-1, World Precision Instruments, Sarasota, FL, USA). The catheter was filled with isotonic saline containing heparin (500 I.U. ml^{-1}), but the animals were not pretreated with heparin i.v.. Two other catheters were introduced into the right jugular vein and the marginal vein of the left ear for infusion of drugs. After tracheal cannulation, the animals were mechanically ventilated with room air at 7 ml kg^{-1} per stroke and 40 stroke min^{-1} (Harvard rodent ventilator, model 683, Harvard Apparatus, South Natick, MA, USA). The electrocardiogram (lead I, II, III) was registered during the experiments by a thermographic recorder (ESC 110 4 CH, Multiline KFT, Esztergom, Hungary) using subcutaneous needle electrodes.

Experimental protocol

The drug administration protocol is shown in Fig. 1. Ten min after preparation, continuous phenylephrine infusion at a rate of 15 $\mu g\ kg^{-1}\ min^{-1}$ via the right jugular vein was begun, and was continued for 85 min (total infusion volume 2 ml). Phenylephrine, an α_1-adrenoceptor agonist (18) was used, as it facilitates almokalant to evoke TdP (15) to an extent almost identical to that seen by Carlsson et al. (13) with methoxamine. Thus, methoxamine and phenylephrine are pharmacologically equivalent in terms of sensitizing rabbit heart to TdP.

The animals were divided randomly into 5 groups, and 10 min after the beginning of phenylephrine infusion, increasing doses of almokalant (26, 88, 260 $\mu g\ kg^{-1}$), d-sotalol, quinidine, amiodarone (3, 10, 30 $mg\ kg^{-1}$) or isotonic saline were administered via the marginal vein of the left ear of the animals. Each dose was given over a period of 5 min and there was 20 min interval between each dose of antiarrhythmic. The doses of almokalant, d-sotalol and quinidine were chosen to span the antiarrhythmic dose range in rabbits used by our group and others (15, 19, 20). Since no in vivo experimental data have been published about the antiarrhythmic activity of intravenous amiodarone in rabbits so far, pilot experiments were performed in phenylephrine primed anesthetized rabbits to choose the maximum dose of the drug that exerts tolerable hemodynamic effects. According to these experiments 30 $mg\ kg^{-1}$ (i.v.) amiodarone possesses marked bradycardic and blood pressure lowering effect (data not shown), therefore this dose was taken as the highest dose in the present study.

Arrhythmia diagnosis and ECG analysis

From the ECG, the incidence, the time to onset and the duration of ventricular arrhythmias were obtained. Ventricular premature beats (VPB), bigeminy, salvo and ventricular fibrillation (VF) were defined in accordance with the definitions of the Lambeth Convention (21). When continuous VF lasted longer than 120 s then the experiment was terminated and VF was defined as sustained VF (SVF). Torsade de pointes (TdP) was defined

as an arrhythmia in which five or more repetitive extrasystoles (ventricular premature beats) were coupled, and for which the QRS complex showed a cyclic variation in size and shape. Every run of 4 VPBs with twisting QRS morphology and every run of 4 or more VPBs without the torsade-like twisting QRS morphology were differentiated from TdP and were defined as ventricular tachycardia (VT). Blocks in the conduction system were also monitored. Conduction disturbances included atrioventricular blocks and intraventricular conduction defects (right or left bundle branch block).

Blood pressure, PP, RR and QT intervals were also measured at predetermined intervals and at the first incidence of TdP. At least four ECG complexes were measured and averaged at each time point. Only sinus-origin R waves were used for measuring RR intervals. Since 2:1 atrioventricular block occurred frequently in some drug treated groups, both sinus rate (SR = 60 / PP) and ventricular rate (VR = 60 / RR) were calculated subsequently instead of calculating a single heart rate value. QT interval was defined as the time between the first deviation from the isoelectric line during the PR interval until the end of TU wave. T (or U) wave frequently overlaps the P wave of the following sinus-origin beat due to relative high heart rate of the rabbit or excessive QT prolongation. In this case the end of TU wave was extrapolated from the curve of the TU wave to the isoelectric line under the P wave. Rate corrected QT interval (QTc) was calculated subsequently by using both Carlsson's equation (13): QTc(Carlsson) = QT-0.175(RR-300) and Bazett's formula: QTc(Bazett) = QT / square root RR.

Drugs

The following drugs were used: d-sotalol (Bristol-Myers Squibb, Wallingford, CT, USA), almokalant (AstraZeneca, Mölndal, Sweden), quinidine (Alkaloida, Tiszavasvári, Hungary), amiodarone (Cordarone injection, Sanofi Pharma, France), phenylephrine (L-Phenylephrine HCl, Koch-Light Laboratories LTD, Colnbrook-Bucks, England), heparin-

sodium (Richter Gedeon, Budapest, Hungary), pentobarbital-sodium (Nembutal, Phylaxia-Sanofi, Budapest, Hungary), α-chloralose (Fluka Chemie AG, Buchs, Switzerland). Almokalant was prepared as a concentrated stock solution (100 μmol ml^{-1}) by AstraZeneca. The stock solution was diluted further with isotonic saline (saline). d-Sotalol and quinidine were dissolved also in saline. The three doses of each antiarrhythmic agent (except amiodarone) were administered in a total volume of 2 ml. The same volume of saline (2 ml) was injected in three portions at comparable rates to the antiarrhythmic agents to the animals in the control group. Amiodarone was prepared by diluting Cordarone injection (150 mg amiodarone in 3 ml) with saline, and the three doses of amiodarone were infused in a total volume of 3 ml. Each solution was prepared on the day of the experiment and all doses in the text refer to bases of the compounds.

Statistical evaluation

The percentage incidence of arrhythmias was calculated and compared by using Fisher's exact probability test. Continuous data were expressed as mean ± standard error of the mean (S.E.M.). Comparisons within group were made using Wilcoxon's signed ranks test. Comparisons between groups were made using Kruskal-Wallis test. Differences were considered statistically significant when $P < 0.05$.

RESULTS

Effects of phenylephrine before antiarrhythmic drug administrations

After 10 min of phenylephrine infusion (n=45), mean arterial blood pressure increased from 85 ± 2 to 123 ± 1 mmHg ($P < 0.05$) and heart rate (both sinus and ventricular rates) decreased from 290 ± 5 to 227 ± 6 min^{-1} ($P < 0.05$). QT intervals increased from 157 ± 2 to 188 ± 4 ms ($P < 0.05$), QTc (Carlsson) increased from 173 ± 2 to 193 ± 4 ms ($P < 0.05$) and QTc (Bazett) increased from 343 ± 3 to 362 ± 6 ms ($P < 0.05$). All animals survived the infusion of phenylephrine. Premature ventricular beats, bigeminy or salvo appeared in 60 %, ventricular tachycardia occurred in 11 %, whereas TdP, ventricular fibrillation and conduction blocks were absent during this period.

Arrhythmia incidences and onset times

The ventricular premature beats, bigeminies or salvos elicited by phenylephrine persisted during the superimposition of saline infusion; there were no episodes of more serious arrhythmias, and no deaths in this control group (Table 1). Two animals died from sustained ventricular fibrillation due to administration of antiarrhythmic drugs. One of them died after administration of 88 μg kg^{-1} almokalant and the other one died after administration of 30 mg kg^{-1} d-sotalol (Table 1). Amiodarone and quinidine did not evoke TdP. Amiodarone did not evoke any arrhythmias in animals that had not developed arrhythmias during infusion with phenylephrine alone, whereas the other 3 drugs evoked arrhythmias in some animals that had hitherto been arrhythmia-free (data not shown). Quinidine 30 mg kg^{-1} induced ventricular tachycardia in 2 out of 7 animals. However, it did not cause TdP. Ventricular tachycardia was also induced by 10 and 30 mg kg^{-1} of d-sotalol and each dose of almokalant (Table 1). In contrast to amiodarone and quinidine, d-sotalol and almokalant additionally evoked TdP (Fig. 2). The 2nd and 3rd doses of d-sotalol (10 and 30 mg kg^{-1}) significantly increased the

cumulative incidence of less complex arrhythmias (i.e. ventricular premature beats, bigeminy and salvo) and the incidence of ventricular tachycardia and evoked TdP in 60% of animals ($P < 0.05$ versus saline controls) (Table 1). Likewise, the 2nd and 3rd doses of almokalant increased significantly the cumulative incidence of less complex arrhythmias and the incidence of ventricular tachycardia and all three doses induced TdP (Table 1). Quinidine, d-sotalol and almokalant evoked conduction blocks in a dose related manner, whereas blocks never occurred after amiodarone and saline administration (Table 1).

There was no direct correlation between the occurrence of TdP and the infusion rate or the dose of d-sotalol and almokalant, since the percentage incidences of this arrhythmia were greatest after the administration of the second doses of the drugs (Table 1).

There was a significant difference between d-sotalol and almokalant in terms of ventricular tachyarrhythmia e.g. ventricular tachycardia and TdP onset. Almokalant induced ventricular tachyarrhythmias shortly after the start of the administration of its 2nd dose (Log_{10} onset time 2.206 ± 0.089 s, n=6). In contrast, several minutes delay occurred before the onset of ventricular tachyarrhythmias after the start of the administration of the 2nd dose of d-sotalol (Log_{10} onset time 2.816 ± 0.084 s, n=6; $P < 0.05$ versus almokalant).

Blood pressure and heart rate

When saline infusion was begun (control group) during continuous phenylephrine infusion, the blood pressure and the heart rate (both sinus and ventricular) did not change further (Table 2).

Addition of d-sotalol reduced blood pressure and ventricular rate dose relatedly (Table 2). There was dissociation between sinus and ventricular rate after administration of 30 mg kg^{-1} d-sotalol due to frequent occurrence of 2:1 atrioventricular block (Table 2).

Similarly to d-sotalol, almokalant reduced blood pressure and there was dissociation between sinus and ventricular rate after administration of each dose of the drug due to frequent

2:1 atrioventricular blocks (Table 2). Five min after administration of 88 and 260 μg kg^{-1} almokalant sinus rate increased significantly compared to saline control (232 ± 9 vs. 196 ± 9 min^{-1}, $P < 0.05$ and 240 ± 12 vs. 188 ± 14 min^{-1}, $P < 0.05$, respectively). These differences remained significant till the end of the 20 min drug free intervals (Table 2). After the administration of the 2nd dose of almokalant, sinus rate was also significantly different from those after the administration of the 2nd dose of d-sotalol (Table 2). This difference remained significant between d-sotalol and almokalant nearly by the end of the experiment. On the other hand, there was no significant difference between the effect of almokalant and d-sotalol on the blood pressure and ventricular rate during the whole experiment.

Quinidine treatment decreased the blood pressure markedly. The pressure drop after each dose of quinidine was significantly greater than those at the same time-points in all other groups (Table 2). The 1st dose of quinidine (3 mg kg^{-1}) elevated heart rate, whereas the 3rd dose (30 mg kg^{-1}) caused 2:1 (and total) atrioventricular blocks allowing dissociation of sinus and ventricular rates (Table 2). After administration of 30 mg kg^{-1} qiunidine ventricular rate decreased and sinus rate increased significantly compared to control (Table 2).

Amiodarone lowered blood pressure in a dose-related manner. Ten min after administration of 10 mg kg^{-1} amiodarone, heart rate (both sinus and ventricular rate) decreased significantly compared to saline control (170 ± 9 vs. 197 ± 9 min^{-1}, $P < 0.05$). From this time-point heart rate decreased constantly in amiodarone treated animals and remained significantly different from control till the end of the experiment (Table 2).

QT prolongation

Saline infusion had no significant effect on the QT and QTc widening effect of the maintained infusion of phenylephrine (Table 3). Amiodarone did not add to the QT and QTc widening caused by phenylephrine (Table 3). However, d-sotalol and almokalant increased the QT and QTc in a dose related manner (Table 3) and there was no difference between the time

course of QT and QTc prolonging effects of the two drugs. Interestingly, only the 3rd dose of quinidine (30 mg kg^{-1}) prolonged QT, whereas the rate corrected QT intervals were already widened by the 1st dose of the drug (3 mg kg^{-1}) (Table 3). The 2nd dose of d-sotalol and almokalant (10 mg kg^{-1} and 88 μg kg^{-1}, respectively) widened QT and QTc to such an extent that these were significantly different from those after administration of the 2nd dose of quinidine and amidarone (10 mg kg^{-1})(Table 3).

Variables measured before TdP occurrence

Since there was no statistical difference between the d-sotalol and almokalant treated animals in terms of blood pressure, ventricular rate, QT and QTc, these data of the animals with TdP induced by either d-sotalol or almokalant were summarized. The values measured before the first TdP at the last sinus-origin beats were compared to those measured before phenylephrine was begun. In animals with TdP (n=10) mean arterial blood pressure was elevated (120 ± 3 vs. 85 ± 2 mmHg at baseline, $P < 0.05$) and ventricular rate was reduced significantly (183 ± 13 vs. 286 ± 9 min^{-1}, $P < 0.05$) at the first incidence of TdP. Furthermore, QT and the rate corrected QT intervals were prolonged markedly at this time (QT 262 ± 8 vs. 162 ± 5 ms at baseline, $P < 0.05$; QTc-Carlsson 255 ± 8 vs. 178 ± 4 ms, $P < 0.05$; QTc-Bazett 453 ± 15 vs. 353 ± 8 ms, $P < 0.05$).

DISCUSSION

The present study has demonstrated that in anesthetized rabbits out of four intravenously administered antiarrhythmic agents only d-sotalol and almokalant induced TdP, whereas quinidine and amiodarone did not. Moreover, the present results showed no direct correlation between the occurrence of TdP and the infusion rate or the dose of antiarrhythmics.

In our investigation we modified the protocol of Carlsson et al. (12, 13). Instead of giving repolarisation prolonging drugs in continuous infusions, we applied stepwise elevation of doses with an interval between each dose. This was for the following reasons: (i) According to Carlsson et al. (13) the infusion rate of drugs is an important predisposing factor for TdP. With our protocol, three different infusion rates were tested in one animal, which decreased the total number of animals required. (ii) Not only the dose dependence, but also the time dependence of the occurrence of arrhythmias can be compared between different treatment groups with this protocol as there is an interval between the administration of increasing doses of drugs.

No torsade de pointes with quinidine

In our experiments quinidine did not evoke TdP, though this drug is by far the most frequently reported drug associated with this arrhythmia (1). In patients with TdP due to quinidine, the plasma level of the drug is usually within or below the therapeutic range (22). Quinidine's clinical proarrhythmic profile (especially the dose-dependence) is not mimicked in any of the presently available animal proarrhythmia models. In anesthetized dogs Inoue and Sugimoto (23) used toxic dose of qiuinidine (30 mg kg^{-1} over 5 min, i.v.) to evoke TdP. Despite the observation that this dose prolonged QT in their study, TdP never occurred spontaneously and required additional programmed electrical stimulation to evoke it. Likewise, in conscious hypokalemic dogs with chronic complete AV block TdP did not occur

spontaneously during or after a continuous infusion of quinidine (~20 mg kg^{-1} over 3 hour), though QT interval was widened significantly by this dose (24); and an additional propranolol infusion was necessary to allow the generation of TdP in that study. In methoxamine-primed anesthetized rabbits continuous quinidine infusion (1.25 mg kg^{-1} min^{-1} for 60 min) did not evoke TdP despite of increasing QT, QTc and QT dispersion significantly, but on the other hand, the drug evoked conduction blocks frequently (25). Thus, the lack of quinidine induced TdP in the present study is in a good accordance with the results of the previous in vivo animal studies.

In our investigations, in contrast to d-sotalol and almokalant, only the highest dose of quinidine prolonged QT intervals whereas QTc was already prolonged by the lower doses. Moreover, the 2nd dose of quinidine was less potent in prolonging either QT or QTc intervals than the middle dose of almokalant and d-sotalol. This smaller QT and QTc prolonging potency might be a reason for the little proarrhythmic activity of the lower doses of quinidine in the present study. However, TdP was also absent even when marked QT and QTc prolongation were achieved by the highest dose of quinidine. This observation accords well with that of Carlsson et al. (13) and suggests that QT and QTc prolongation are not the only contributing factors to TdP generation in the anesthetized rabbit model.

The high heart rate of the rabbit compared to that of man could also contribute to the blunted proarrhythmic activity of quinidine compared with its effects in man. At high frequencies quinidine's sodium channel inhibiting property is increased (use-dependent block) (26), which may prevent TdP at these high heart rates of the rabbit (27). In addition, after the administration of the 1st dose of quinidine, its antimuscarinic action on atrial muscarinic receptors (28) and/or its blood pressure lowering effect might prevent phenylephrine-induced reflex bradycardia, which could predispose to TdP. However, this lack of reflex bradycardia became irrelevant especially after the administration of the 3rd dose of quinidine, as this dose decreased the ventricular rate markedly due to frequent occurrence of 2:1 and total

atrioventricular blocks. However, this relatively low ventricular rate, which coincided with marked QT and QTc prolongation, was still insufficient to allow generation of TdP. Likewise, quinide and terfenadine reduced heart rate and prolonged QT and QTc markedly without evoking TdP in α_1-adrenoceptor stimulated rabbits (25). These suggest that low ventricular (or heart) rate and prolonged QT and QTc intervals are not the only contributing factors to TdP generation in the rabbit model of the acquired long QT syndrome.

In the present investigation quinidine dose dependently lowered blood pressure and the pressure drops were significantly greater than those with any other drug. In methoxamine sensitized anesthetized rabbits terfenadine and quinididne reduced blood pressure to a very low level and did not evoke TdP despite of increasing QT, QTc and QT dispersion and reducing heart rate (25). These suggest that marked blood pressure reduction may prevent TdP generation in the anesthetized rabbit model.

Quinidine inhibits α_1-adrenoceptors competitively at therapeutic blood levels (29), thereby preventing the complex sensitizing effect of α_1-adrenoceptor stimulation in this animal model. A similar effect was seen in a recent study in which cisapride, a potent inhibitor of the rapid component of delayed rectifier potassium current (I_{Kr}) was found to have very low proarrhythmic potential in the rabbit model of acquired long QT syndrome (30). This was attributed to the drug's high α_1-adrenoceptor blocking potency.

No torsade de pointes with amiodarone

Intravenous amiodarone did not induce ventricular tachyarrhythmias and did not prolong significantly QT and QTc in our experiments. The lack of intravenous amiodarone-induced QT and QTc prolongation in the present study is consistent with the findings of previous in vivo and in vitro rabbit studies. In anesthetized rabbits 10 mg kg^{-1} i.v. bolus amiodarone failed to exert any effect on ECG (31, 32). Likewise, perfusion of isolated rabbit

hearts with a buffer solution containing 1 μg ml^{-1} amiodarone had no effect on ECG intervals (33).

In anaesthetized rabbits, the time of maximal uptake of i.v. bolus amiodarone by the myocardium has been estimated as between 5 and 15 min (31). Therefore, low accumulation of amiodarone in our experiments cannot be responsible for the low proarrhythmic activity. Amiodarone dose relatedly lowered blood pressure and the highest dose of the drug (30 mg kg^{-1}) induced marked blood pressure drop and bradycardia. This indicates that higher doses of the drug could not have been administered to the animals without inducing catastrophic hemodynamic effects. This suggests that despite the lack of effect on QT/QTc interval amiodarone was not under-dosed in the present study.

Acute and chronic electrophysiologic effects of amiodarone differ substantially from each other (34, 35). Despite chronic administration of the drug prolongs repolarisation markedly, it induces TdP only very rarely in man (36). Though it is debatable whether acute amiodarone prolongs QT interval (5), TdP may be evoked by this administration of the drug in man (4, 5, 6). However, proarrhythmic events are very rare in patients treated with intravenous amiodarone (5) similarly to that seen in patients treated with long-term amiodarone therapy (36).

Intravenously administered amiodarone inhibits the delayed rectifier outward potassium current (I_K), the inward sodium current (I_{Na}) and the inward L-type calcium current ($I_{Ca\text{-}L}$) (34, 37). The simultaneous inhibition of I_K and I_{Na} or I_K and $I_{Ca\text{-}L}$, achieved by combination of selective inhibitors, has been shown to have low proarrhythmic activity in Carlsson's rabbit model (16, 17, 27, 38) probably due to the EAD suppressing or repolarisation prolongation limiting effect of $I_{Ca\text{-}L}$ and I_{Na} inhibition, respectively. Intravenous amiodarone inhibits α-adrenoceptors in a non-competitive manner (39), which could also contribute to the low proarrhythmic activity of the drug in this model.

Torsade de pointes with d-sotalol and almokalant

In the present study only d-sotalol and almokalant induced TdP. Moreover, these drugs induced large number of ventricular tachycardias and non-complex arrhythmias, e.g. ventricular premature beat, bigeminy and salvo. In fact, TdP was always preceded by frequent occurrence of non-complex arrhythmias and sometimes ventricular tachycardia. On the other hand, similarly to quinididne, d-sotalol and almokalant also induced conduction blocks in a dose related manner. This accords well with the results of Lu et al (25) who showed that the selective I_{Kr} blocker dofetilide and the non-selective I_{Kr} blocker clofilium, quinidine and terfenadine evoke conduction blocks frequently in α_1-adrenergically stimulated rabbits. The primary targets of I_{Kr} blockers are the cells of the conductive system and the M cells (3). Extreme repolarisation and refractory period prolongation of these cells may lead to blocks, especially at the relatively short cycle length of the rabbit. The initiating mechanism of TdP is probably related to ventricular premature beats whereas the maintenance mechanism related to reentry (3). Thus, frequent non-complex arrhythmias and conduction blocks may play a role in the generation of TdP and other reentrant arrhythmias e.g. ventricular tachycardia and ventricular fibrillation in the rabbit model of acquired long QT syndrome.

In the present study TdPs induced by either d-sotalol or almokalant were always preceded by markedly prolonged QT and QTc intervals, elevated blood pressure and relatively slow ventricular rate. In contrast, neither these factors coincided nor TdP developed in amiodarone and quinididne treated animals. These suggest that the coincidence of markedly prolonged QT and QTc intervals, elevated blood pressure and slow ventricular rate may be a prerequisite of TdP generation in the presently utilized animal model.

In the present investigation almokalant and d-sotalol showed different proarrhythmic profile in terms of ventricular tachyarrhythmia onset. These arrhythmias occurred earlier during or after the administration of the middle dose of almokalant than those after the administration

of the middle dose of d-sotalol. This earlier onset of ventricular tachyarrhythmias coincided with elevated sinus rate in the almokalant group, whereas the effects of d-sotalol and almokalant were not different on the QT and QTc intervals, ventricular rate and blood pressure. This suggests that not only QT and QTc prolongation, relatively low ventricular rate and elevated blood pressure play a role in arrhythmia genesis in the rabbit model of TdP. Similarly to our findings, the proarrhythmic effects of these two relatively selective potassium channel blockers were different in a dog torsade model (40). Although both drugs widened QT in that study (40) too, only almokalant evoked TdP spontaneously, while programmed electrical stimulation was necessary to initiate this arrhythmia in d-sotalol treated animals. In that study almokalant increased the interventricular dispersion of repolarisation and the number of EADs to a greater degree than d-sotalol.

Almokalant is a selective I_{Kr} inhibitor (41), whereas d-sotalol inhibits I_{Kr} and other potassium currents, i.e. transient outward current (I_{to}) and inward rectifier current (I_{K1}) (8). This difference in potassium channel selectivity, or d-sotalol's residual β-adrenoceptor blocking property (42) or possible differences between the time-course of the development of repolarisation dispersion between the drugs might play a role in this different proarrhythmic profile of the two drugs.

Infusion rate of antiarrhythmics and occurrence of torsade de pointes

In the present study there was no direct correlation between the occurrence of TdP and the infusion rate or the dose of d-sotalol and almokalant, since the percentage incidences of this arrhythmia were greatest after the administration of the middle doses of the drugs. Carlsson et al. (13) reported that the occurrence of TdP is dependent on infusion rate. Their suggestion was that this was fundamental to the occurrence of TdP. The present data demonstrate, that dependence on infusion rate may be particular to the continuous infusion (13, 15) and not to short-term infusions.

Both selective and non-selective I_{Kr} blockers evoke torsade de pointes in the rabbit

Recently Lu et al. (25) concluded that non-selective I_{Kr} blockers do not induce TdP in the rabbit model of long QT syndrome, as quinidine and terfenadine did not evoke this arrhythmia in their studies, whereas dofetilide and clofilium did so. However, clofilium is not a selective I_{Kr} blocker, as it affects I_{Na}, $I_{Ca\text{-}L}$, I_{to}, I_{Kr}, the slow component of the delayed rectifier potassium currents (I_{Ks}) and the ATP-dependent potassium current (I_{KATP}) in a similar concentration range (43, 44, 45, 46). Similarly to clofilium, other non-selective I_{Kr} blockers, e.g. the combined I_{Kr} and $I_{Ca\text{-}L}$ blocker BRL-32872 (16), the combined I_{Kr} and I_{Ks} blocker azimilide (47), the combined I_{Kr} and I_{K1} blocker sematilide (12, 47), the combined I_{Na} enhancer and I_{Kr} blocker ibutilide (14, 17) and the combined β-adrenoceptor and multiple potassium channel inhibitor d,l-sotalol (47) have hitherto been shown to induce TdP in Carlsson's rabbit model. In our study almokalant and d-sotalol evoked TdP. Almokalant is a selective I_{Kr} inhibitor (41), whereas d-sotalol inhibits I_{Kr}, I_{to} and I_{K1} (8). These results and the previously published studies with several non-selective I_{Kr} blockers show that I_{Kr} selectivity is not an exclusive factor of TdP generation in the rabbit model of acquired long QT syndrome.

CONCLUSIONS

Our results suggest that the incidence of TdP may not depend on the infusion rate or the dose of antiarrhythmics when graded doses were applied with an interval. Furthermore, TdP generation may be a multifactorial process in the rabbit model of TdP and the contributing factors may be slightly different from those in man. Thus, drugs which have different pharmacodynamic actions at high heart rates (seen in the rabbit) compared with effects at lower heart rates (seen in man), drugs which decrease blood pressure markedly or drugs, which possess α_1-adrenoceptor inhibitory effects, could elicit a false negative outcome (i.e., low proarrhythmic activity) in the rabbit model of TdP.

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Table 1. The incidence of arrhythmias in anesthetized rabbits treated with d-sotalol, almokalant, quinidine or amiodarone during continuous phenylephrine infusion

		Incidence of arrhythmias (%)					
	n	SVF	VF	TdP	VT	Block	Others
Control							
1st	10	0	0	0	0	0	60
2nd	10	0	0	0	0	0	10
3rd	10	0	0	0	0	0	20
total	10	0	0	0	0	0	60
d-Sotalol							
3 mg kg^{-1}	10	0	0	0	0	0	80
10 mg kg^{-1}	10	0	0	50 *	60 *	50 *	90 *
30 mg kg^{-1}	10	10	10	40	60 *	100 *	90 *
total	10	10	10	60 *	70 *	100 *	100
Almokalant							
26 μg kg^{-1}	10	0	0	20	40	10	80
88 μg kg^{-1}	10	10	10	40	60 *	40	80 *
260 μg kg^{-1}	9	0	0	33	67 *	78 *	100 *
total	10	10	10	40	70 *	70 *	100
Quinidine							
3 mg kg^{-1}	7	0	0	0	0	0	43
10 mg kg^{-1}	7	0	0	0	0	43	29
30 mg kg^{-1}	7	0	0	0	29	86 *	71
total	7	0	0	0	29	86 *	86
Amiodarone							
3 mg kg^{-1}	8	0	0	0	0	0	38
10 mg kg^{-1}	8	0	0	0	0	0	25
30 mg kg^{-1}	8	0	0	0	0	0	13
total	8	0	0	0	0	0	38

n, number of animals; SVF, sustained ventricular fibrillation i.e. ventricular fibrillation that lasts longer than 120 s; VF, ventricular fibrillation; TdP, torsade de pointes; VT, ventricular tachycardia different from TdP; Block, conduction block; Others, ventricular premature beats, bigeminy and salvo; Control, isotonic NaCl; total, the cumulative incidence of arrhythmias in the given group. The values in the rows of doses show the incidences of arrhythmias during the administration of the given dose and the subsequent 20 min interval. *$P < 0.05$ vs. control.

Table 2. Effect of d-sotalol, almokalant, quinidine and amiodarone treatment on the sinus rate, ventricular rate and the blood pressure in anesthetized rabbits during continuous phenylephrine infusion

		1st dose		2nd dose		3rd dose		
	n	0 min	5 min	25 min	30 min	50 min	55 min	75 min
Control								
SR		226±11	214±9	200±9	199±9	200±10	200±10	202±10
VR	10	226±11	214±9	200±9	199±9	200±10	200±10	202±10
MBP		123±3	122±4	121±3	121±3	119±3	121±3	119±3
d-Sotalol								
SR		240±13	212±10 †	213±11	192±8 †	208±12	200±14	220±19
VR	10	240±13	212±10 †	213±11	192±8 †	208±12	176±6 †	181±8
MBP		122±3	123±3 $^{\#}$	120±2	107±4 †$^{*\#}$	114±2 $^{\#}$	101±4 †$^{*\#}$	110±5 $^{\#}$
Almokalant								
SR		227±9	238±10	220±12	235±15 ‡	227±5 *‡	234±11 ‡	250±13 *
VR	10	227±9	219±16	220±12	195±15	227±5 *	202±18	203±18
MBP		121±3	117±3 $^{\#}$	120±4	109±2 *$^{\#}$	114±4 $^{\#}$	104±4 *$^{\#}$	107±4 $^{\#}$
Quinidine								
SR		211±18	240±14 †	215±14	225±14	219±16	242±23	263±13 *
VR	7	211±18	240±14 †	215±14	225±14	219±16	147±31 *	177±31
MBP		124±3	91±2 †*	117±3	59±2 †*	96±4 *	39±5 †*	76±3 *
Amiodarone								
SR		227±11	224±11	202±20	197±9	159±10 *	164±8 *	149±10 *
VR	8	227±11	224±11	202±20	197±9	159±10 *	164±8 *	149±10
MBP		123±2	113±3 †$^{*\#}$	121±3	91±5 †$^{*\#}$	116±4 $^{\#}$	79±5 †$^{*\#}$	107±6 $^{\#}$

SR, sinus rate (min^{-1}); VR, ventricular rate (min^{-1}); MBP, mean arterial blood pressure (mmHg); †$P < 0.05$ compared to the value measured right before the beginning of the administration of the given dose; *$P < 0.05$ compared to the control group. $^{\#}P < 0.05$ vs. quinidine MBP. ‡$P < 0.05$ almokalant vs. d-sotalol. 1st dose: 3 mg kg^{-1} (d-sotalol, quinidine, amiodarone) or 26 μg kg^{-1} (almokalant) i.v.; 2nd dose: 10 mg kg^{-1} (d-sotalol, quinidine, amiodarone) or 88 μg kg^{-1} (almokalant) i.v.; 3rd dose: 30 mg kg^{-1} (d-sotalol, quinidine, amiodarone) or 260 μg kg^{-1} (almokalant) i.v.; Control: isotonic NaCl.

Table 3. Effect of d-sotalol, almokalant, quinidine and amiodarone treatment on the QT and the rate corrected QT intervals in anesthetized rabbits during continuous phenylephrine infusion

		1st dose		2nd dose		3rd dose		
	n	0 min	5 min	25 min	30 min	50 min	55 min	75 min
Control								
QT		188±6	194±8	210±10	216±12	215±12	212±11	219±13
QTc (C)	10	193±4	197±7	209±8	214±9	213±9	211±9	218±10
QTc (B)		362±8	364±11	378±11	387±12	386±12	382±12	397±13
d-Sotalol								
QT		171±5	223±9 †	223±9	260±8 †*#	244±13	261±9 *	266±11
QTc (C)	10	178±4	225±8 †*	225±7	257±6 †*#	245±11	253±9 *	260±12
QTc (B)		339±7	415±12 †*	415±8	461±8 †*#	450±15 *	447±17 *	461±21 *
Almokalant								
QT		200±11	234±11	223±16	271±13 †*#	222±8	278±12 †*	263±20
QTc (C)	10	206±11	236±9 *	226±13	267±9 †*#	228±7	271±6 †*	259±15
QTc (B)		389±22	438±12 *	418±18	482±16 †*#	431±12 *	480±7 †*	463±19 *
Quinidine								
QT		182±11	205±13	214±15	225±13	229±13	321±35 †*	270±29
QTc (C)	7	182±7	213±10 †	216±12	230±10	232±10	291±23 †*	255±19
QTc (B)		334±8 *	406±15 †*	399±17	430±12 †*	431±12 *	471±21 †*	436±14
Amiodarone								
QT		204±12	207±13	220±15	221±11	236±13	235±9	254±16
QTc (C)	8	209±10	212±11	218±11	219±9	221±11	222±8	233±13
QTc (B)		391±14	394±16	394±15	396±13	380±16	386±14	394±18

QT, QT interval (ms); QTc (C), rate corrected QT interval (ms) according to Carlsson et al. (see Methods); QTc (B), rate corrected QT interval (ms) according to Bazett (see Methods); Ventricular heart rate (Table 1.) was used for calculating rate corrected QT intervals. †$P <$ 0.05 compared to the value measured right before the beginning of the administration of the given dose; *$P < 0.05$ compared to the control group; #$P < 0.05$ vs. quinidine and amiodarone. See other details in Table 2.

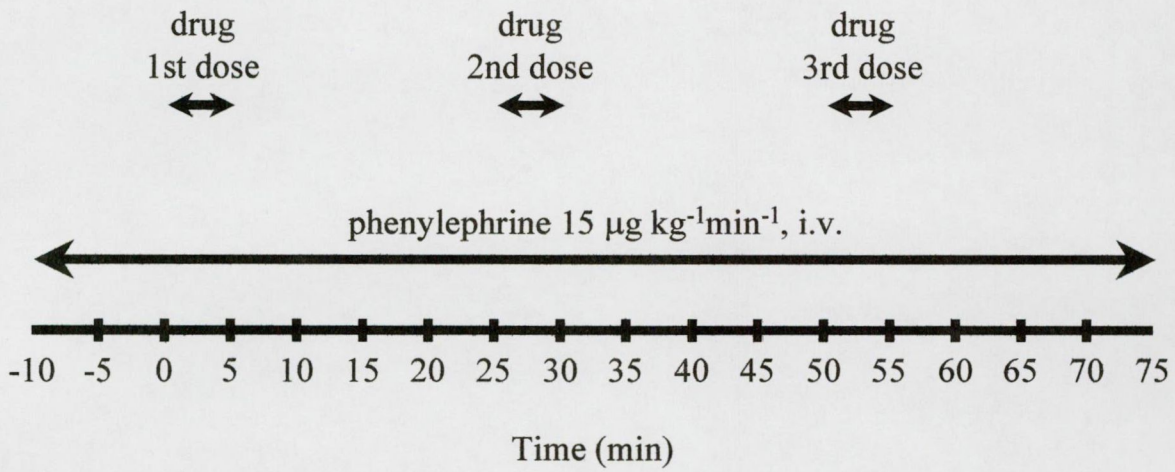

Fig. 1. Drug administration protocol. Increasing doses, i.e. 1st dose, 2nd dose, 3rd dose, of antiarrhythmic agents (almokalant in doses of 26, 88, 260 μg kg^{-1}, d-sotalol, quinidine, amiodarone in doses of 3, 10, 30 mg kg^{-1}) or isotonic saline were administered intravenously to the animals during continuous α_1-adrenoceptor stimulation with phenylephrine.

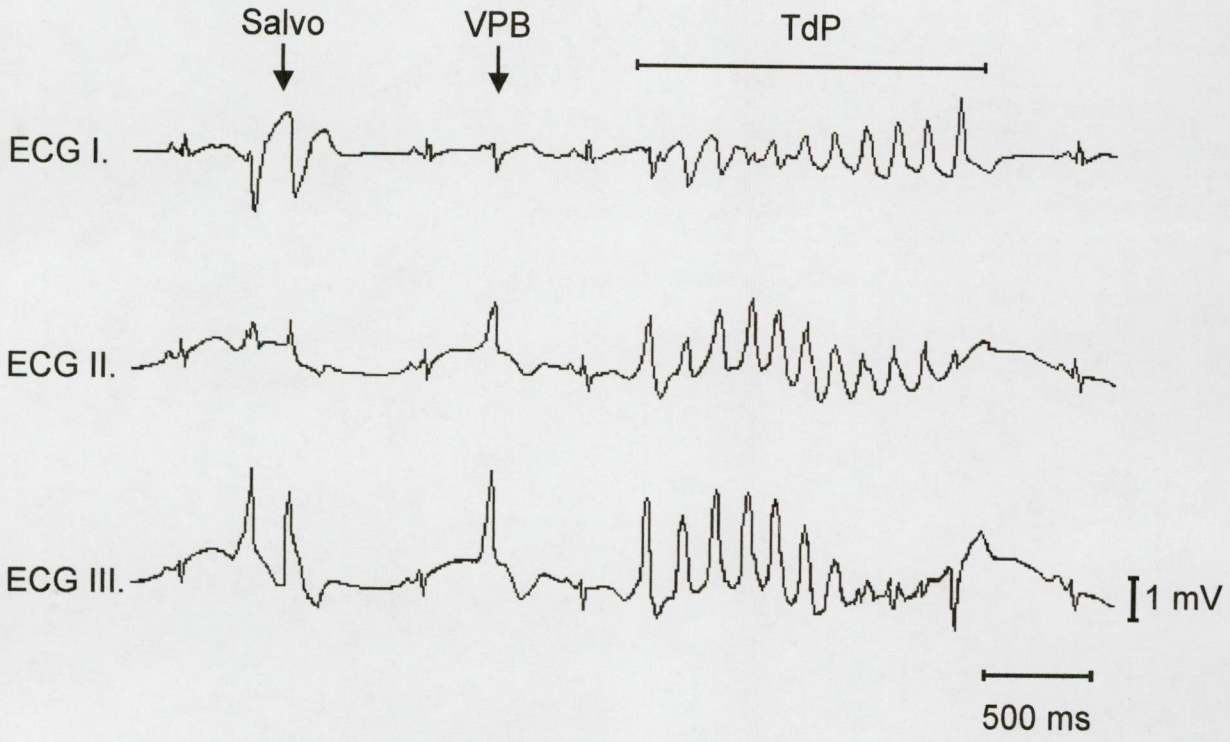

Fig. 2. d-Sotalol-induced torsade de pointes arrhythmia (TdP) with preceding salvo and ventricular premature beat (VPB). ECG I-III, electrocardiogram I-III.

VI.

Limited antifibrillatory effectiveness of clinically-relevant concentrations of Class I antiarrhythmics in isolated perfused rat hearts

Running title:

Class I antiarrhythmics lack effectiveness

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Abstract

The Langendorff-perfused rat heart with regional ischaemia is increasingly used for evaluating drugs for prevention of phase-1 ischaemia-induced ventricular fibrillation (VF). Surprisingly, the effectiveness of Class I antiarrhythmics has not been characterised in this model. One lower and one higher concentration of quinidine (0.79 and 7.90 μM), lidocaine (3.88 and 12.93 μM) and flecainide (0.74 and 1.48 μM), representing the peak unbound plasma and total blood concentrations, respectively, at 'therapeutic' dosage, were evaluated. The left main coronary artery was occluded for 30 min to elicit phase-1 VF, following which reperfusion-induced VF was examined. In hearts perfused with Krebs' solution containing 3 mM K^+, the higher concentrations of quinidine and lidocaine reduced the incidence of phase-1 VF from 92 % to 0 % and 17 % respectively, (each $P<0.05$). The lower drug concentrations were ineffective. Flecainide was equi-effective at low and high concentration, with VF incidence reduced from 92 % to 17 % ($P<0.05$). Neither low nor high concentrations of any of the drugs affected the incidence of reperfusion-induced VF. Using hearts perfused with Krebs' containing 5 mM K^+, sufficient to substantially reduce control phase-1 VF incidence, the experiment was repeated in order to test for possible proarrhythmic activity. None of the three drugs increased arrhythmia incidence. In this model, it was not possible to suppress both ischaemia- and reperfusion-induced VF with flecainide, lidocaine or quinidine at concentrations equivalent to peak unbound plasma levels following clinical administration. This may explain the lack of clinical benefit with these drugs against sudden cardiac death. Since none of the drugs were proarrhythmic in ischaemic hearts in which arrhythmia susceptibility had been lowered by high K^+, it would appear that clinical proarrhythmia seen with these drugs may not be related to exacerbation of phase-1 ischaemia-induced VF.

Keywords: Class I antiarrhythmics, Langendorff-preparation, myocardial ischaemia, proarrhythmia, reperfusion, ventricular fibrillation

Introduction

Sudden cardiac death, attributable in many instances to ventricular fibrillation (VF) remains an important pharmacotherapeutic target that is poorly controlled by drugs (1, 2). Although there is no accurate information on the time-course of VF susceptibility following coronary artery obstruction in man, animal studies show that VF susceptibility has two peaks during ischaemia, one during the first 30 min (phase-1), and one after 90 min (phase-2), and another during reperfusion (3, 4). Each of these, if they occur in man, could contribute to sudden cardiac death.

Class I antiarrhythmic agents block sodium channels, and can be subdivided into Ia, Ib and Ic on the basis of their binding characteristics and selectivity for Na^+ versus K^+ channels (5). Despite their extensive clinical use, and evidence of suppression of certain types of ventricular arrhythmias (6, 7, 8), meta-analysis of cumulative data from large numbers of patients showed that these drugs are ineffective in prevention of sudden cardiac death (1, 2). Furthermore, Class Ic drugs actually increased mortality in patients with coronary artery disease (9). As a consequence of these data and similar data sets for other classes of drugs, the consensus is that individual animal models cannot be used alone to predict clinical effectiveness against sudden cardiac death in man (10), meaning that there is a need for more extensive assessment of drugs in a range of animal models.

The isolated rat heart model with regional ischaemia caused by coronary artery occlusion allows the generation and examination of ischaemia- and reperfusion-induced VF (3, 11). Phase-1 VF peaks after 7-15 min of ischaemia, and reperfusion causes VF any time between 5 and 40 min after the start of ischaemia (4, 12). The isolated rat heart model has been used extensively for the study of the effects of drugs on ischaemia and reperfusion arrhythmias, and the advantages and limitations of the model have been described in detail (4).

Interestingly, the effects of sodium channel blockers on phase-1 VF have not been examined in the isolated rat heart model (4). The lack of clinical effectiveness of Class I drugs implies that at least one of the forms of VF that contribute to sudden cardiac death in man (phase-1, phase-2 or reperfusion-induced) is unaffected (or even exacerbated) by these drugs. If this is the case then, at the so-called human 'therapeutic' plasma concentrations, these drugs would be expected to fail to prevent phase-1 VF or reperfusion VF in isolated rat hearts (1).

As a test of this hypothesis, the present study examined the antifibrillatory effect of representative Class Ia, Ib and Ic drugs, (quinidine, lidocaine and flecainide) each at two concentrations equivalent to the unbound and total plasma clinical 'therapeutic' concentrations. In order to detect antifibrillatory effects, perfusion solutions contained 3 mM K^+, which allows high incidence of VF in control (3, 11). Additionally, a limited number of experiments were performed to examine the proarrhythmic effects of the three drugs using perfusion solutions containing 5 mM K^+, which gives rise to low incidence of ischaemic VF in control hearts (11) and hence scope to reveal proarrhythmia.

Methods

Animals and general experimental methods

Male Wistar rats (180-250g; Bantin and Kingman, UK) were anaesthetized with pentobarbitone (60 mg kg^{-1} i.p.) mixed with 250 I.U. sodium heparin to prevent blood clot formation in the coronary vasculature. Hearts were excised and placed into ice-cold solution containing (in mM), NaCl 118.5, $NaHCO_3$ 25.0, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $CaCl_2$ 1.4, KCl 3 (or 5 where indicated) and glucose 11.1, then perfused according to Langendorff, with solution delivered at 37°C and pH 7.4. All solutions were filtered (5 μm pore size) before use. Perfusion pressure was maintained constant at 70 mmHg. A unipolar electrogram (ECG) was recorded by implanting one stainless-steel wire electrode into the centre of the region to become ischaemic with a second connected to the aorta. A traction-type coronary occluder consisting of a silk suture (Mersilk, 4/0) threaded through a polythene guide was used for coronary occlusion. The suture was positioned loosely around the left main coronary artery beneath the left atrial appendage. Regional ischaemia and reperfusion were induced by tightening the occluder and by releasing it.

Experimental protocol

Four sets of experiments were performed and each of them consisted of four groups (quinidine, lidocaine, flecainide or vehicle control). The basis for choice of drug concentrations is explained later. In the first set of experiments hearts were perfused with the lower concentrations of the drugs, i.e. quinidine 0.79 μM, lidocaine 3.88 μM, flecainide 0.74 μM or vehicle (time-matched control). In the second set of experiments hearts were perfused with the higher concentrations of the drugs, i.e. quinidine 7.90 μM, lidocaine 12.93 μM, flecainide 1.48 μM or vehicle (time-matched control). In these first two sets of experiments all perfusates

were prepared by using modified Krebs' solution containing 3 mM K^+, and each group consisted of n=12 hearts. In the third and fourth sets of experiments again the same lower and higher drug concentrations were used, together with vehicle controls, but the Krebs' solution was modified to contain 5 mM K^+, and each group consisted of n=6 hearts.

In all four sets of experiments hearts were perfused for an initial 5 min with control solution, then solution was switched in a blinded fashion to one of the four test solutions: control (vehicle), quinidine, lidocaine or flecainide. The choice of solution was made by reference to a randomisation table. After a further 5 min perfusion, the left main coronary artery was occluded. After 30 min of ischaemia the occluder was released to achieve reperfusion. Randomisation was achieved by coding each group with a letter of meaning that was unknown to the operator. Blinded analysis was achieved by using stock solutions prepared by a second operator who did not participate in heart perfusion or data analysis.

Individual measures of coronary flow, and ECG variables were taken 1 min before and 1 min after the introduction of drug perfusion or vehicle, 1 min before and each min after coronary occlusion for 5 min, then every five min thereafter for 25 min, and again 1 min before reperfusion, and after 1 and 5 min of reperfusion.

The choice of drug concentrations was based on the following. In clinical studies aimed at evaluating drug effects on ventricular arrhythmias, peak blood concentrations (unbound fraction plus plasma-protein bound fraction) have been determined following typical drug dosage. These concentrations have been reported to be 7.90 μM for quinidine (8), 12.93 μM for lidocaine (6), and 1.48 μM for flecainide (7). These concentrations are within the so-called human therapeutic range for these agents (5). All three drugs are bound to plasma proteins: quinidine 90%, lidocaine 70% and flecainide 50% (13). Thus, the plasma-protein unbound concentrations associated with these clinically relevant dosage are approximately 0.79 μM, 3.88 μM and 0.74 μM for quinidine, lidocaine and flecainide, respectively. Therefore we chose

to study the mean peak unbound and mean peak total plasma concentrations (lower and higher concentrations, respectively).

Measurement of involved zone size and coronary flow

At the end of five minutes of reperfusion the size of the involved zone (the region subjected to ischaemia and reperfusion) was quantified using the disulphine blue dye exclusion method (3) and expressed as per cent total ventricular weight. Coronary flow was measured by timed collection of coronary effluent. Values of coronary flow in the uninvolved tissue and the reperfused zone were calculated from the total coronary flow and the weights of the involved zone and the uninvolved zone, as described previously (11).

Group sizes and exclusion criteria

Initially, hearts were randomised to 6 lines (n=6 per group). The study was blinded. Codes were broken and the experiment was terminated if it was apparent that an increase in group size to n=12 would be insufficient to reveal a statistically significant difference between groups for incidence of ischaemia-induced VF. If this were not the case, a second randomisation was undertaken, and group sizes were increased to n=12. This procedure was designed to preclude unnecessary use of animals.

Any heart with a sinus rate less than 250 min^{-1}, or a coronary flow more than 23 ml min^{-1} g^{-1} or less than 7.5 ml min^{-1} g^{-1} 6 min before the onset of ischaemia (before the start of perfusion with drug or vehicle) or an involved zone of less than 30% or more than 50% of total ventricular weight was excluded. All excluded hearts were replaced to maintain equal group sizes. Any heart not in sinus rhythm during the two seconds before the start of reperfusion was excluded from the reperfusion sample, but was not replaced. For this reason,

incidences of reperfusion-induced arrhythmias are assessed for groups of sizes that are may not be equal.

Arrhythmia diagnosis and ECG analysis

The ECG was recorded using a MacLab system. Arrhythmias were defined according to the Lambeth Conventions (14). Ventricular premature beats (VPB) were defined as discrete and identifiable premature QRS complexes and a run of 4 or more VPBs was defined as ventricular tachycardia (VT). Ventricular fibrillation (VF) was defined as a signal from which individual QRS deflections vary in amplitude and coupling interval on a cycle to cycle basis. From the ECG, the incidence and the time to onset of ventricular tachyarrhythmias, the PR interval, RR interval and the QT interval (measured at the point of 90% repolarization with on-screen cursors) were obtained. QT interval was not corrected for heart rate as it is not rate-dependent in perfused rat hearts (15). Measurement of all variables was performed in a blinded manner.

Drugs and materials

Drug stocks were prepared fresh each week and perfusion solutions were prepared fresh each day. "Vehicle stock" was an 8 ml solution containing 6 ml ethanol and 2 ml water. The control solution contained 0.24 ml of "vehicle stock" in 1l of modified Krebs (i.e., 0.018% ethanol). The drug solutions were prepared from 8 ml of a "drug stock". Drug stocks for low concentrations consisted of 10 mg quinidine or 35 mg lidocaine or 11.7 mg flecainide dissolved in 6 ml ethanol plus 2 ml water, while drug stocks for high concentrations consisted of 100 mg quinidine or 116.7 mg lidocaine or 23.4 mg flecainide dissolved in 6 ml ethanol plus 2 ml water, such that 0.24 ml of these stocks dissolved in 1l of modified Krebs obtained the drug

solutions (quinidine 0.79 or 7.90 μM, lidocaine 3.88 or 12.93 μM, flecainide 0.74 or 1.48 μM). Thus, all solutions contained the same amount of ethanol (0.018%).

All salts were reagent grade chemicals from Sigma Chemical Co. Water for preparing perfusion solution was supplied using a reverse osmosis system (Milli-RO 10 and Milli-Q 50, Millipore Ltd) and had a specific resistivity of more than 18 M Ohm.

Statistics

Gaussian distributed variables, expressed as mean ± s.e.mean, were subjected to analysis of variance. If treatment constituted a significant source of variance, each group was compared with the control using Dunnett's test. The time to onset of first arrhythmia is log10 transformed to generate Gaussian distributed variables (3). The incidences of arrhythmias were compared using Fisher's exact probability test with Bonferoni correction, i.e. the P values of Fisher's exact probability test were multiplied by 4 (the number of groups in each set of experiments) to allow for multiple comparisons (16). $P<0.05$ was taken as indicative of a statistically significant difference between values.

Results

Ischaemia and reperfusion arrhythmia incidences

When perfusion solution with 3 mM K^+ was used, VF developed in almost all control hearts during ischaemia (Table 1). In hearts in which VF was transient (a common occurrence in the rat heart) (4), subsequent reperfusion evoked a further episode of VF (Table 1). Of the three Class I drugs, only flecainide reduced ischaemia-induced VF incidence significantly at the lower concentration (Table 1). The actions of the drugs were dependent on the duration of ischaemia. All three drugs at the higher concentration reduced the incidence of bigeminy, salvos and VT during the first 10 min of ischaemia (Figure 1G-1I) but their effects were lost during the latter 20 min of ischaemia (Figure 1G-1I). In contrast all three drugs at the higher concentration reduced VF incidence during the entire 30 min period of ischaemia (Figure 1F).

The incidence of reperfusion-induced VF was reduced as a trend in hearts perfused with 3 mM K^+ by all three drugs at the higher concentration, whereas at the lower concentration only flecainide had a trend to an effect (Table 1). However, none of these 'effects' attained statistical significance. None of the drugs, even at higher concentration, had any effect on reperfusion-induced VT, salvo, bigeminy or VPB incidences (data not shown).

In separate groups of hearts perfused with Krebs' modified to contain 5 mM K^+, the control incidence of VF was low, as desired (Table 1). There were no statistically significant proarrhythmic effects of any of the drugs during ischaemia or reperfusion (Table 1). In fact, the trends to antiarrhythmic effects observed earlier in hearts perfused with 3 mM K^+ were replicated albeit, once again, this did not reach statistical significance in the case of VF. Flecainide prevented ischaemia-induced VT at the lower concentration (0 out of 6 vs. 6 out of 6 in control, $P<0.05$) although this effect was not significant for the higher concentration of the drug (3 out of 6 vs. 6 out of 6 in control, $P>0.05$). There were no significant drug effects on

the incidence of ischaemia-induced salvos, bigeminy or VPBs in these hearts (data not shown). Similarly, none of the drugs affected the incidences of reperfusion-induced ventricular arrhythmias (any kind) in this set of experiments (VF is shown in Table 1).

Mean involved zone size was not affected by any of the drugs and values ranged from 37-43 % of the total ventricular weight (data not shown).

Proarrhythmic events

A hastening of the mean onset time of the first ischaemia-induced arrhythmia is indicative of a proarrhythmic drug effect. Arrhythmia onset was neither hastened nor delayed by any of the three drugs, whether hearts were perfused with Krebs' containing 3 or 5 mM K^+ (data not shown).

However, an unusual very early onset of ischaemia-induced VF was observed in two hearts perfused with the higher concentration of flecainide. In one, a heart perfused with Krebs' containing 3 mM K^+, VF occurred 294 sec after the onset of ischaemia (the equivalent value in K^+-matched controls being 480 sec). This was by far the earliest onset in the whole study. In the other, a heart perfused with Krebs' containing 5 mM K^+, VF commenced 437 sec after the onset of ischaemia (the equivalent value in K^+-matched controls being 1013 sec).

Monomorphic VT lasting longer than 120 sec and having a frequency higher than 1000 min^{-1} was a rare event, occurring in only 3 hearts (all perfused with Krebs' containing 3 mM K^+), one during perfusion with the lower concentration of quinidine and one with the lower and one with the higher concentration of lidocaine. In addition, in one heart perfused with the higher concentration of flecainide and Krebs' containing 3 mM K^+, monomorpic VT with a frequency of approximately 650 min^{-1} and with a duration longer than 120 sec was observed. These unusual episodes of VT were not predictive of a susceptibility to any subsequent manifestation of VF.

Flecainide and lidocaine concentration-dependently evoked sinus arrhythmias, manifesting as apparently random beat-to-beat variations of RR interval. When the K^+ content of the Krebs' solution was 3 mM, sinus irregularity developed in 58, 33, 0 and 0% of hearts perfused with the higher concentration of flecainide, lidocaine, quinidine and vehicle, respectively ($P<0.05$ for flecainide compared to vehicle control). Irregular sinus rhythm developed in some hearts perfused with the lower concentration of flecainide and lidocaine, and in hearts perfused with Krebs' containing 5 mM K^+, but under these circumstances the incidences were not significantly different from zero (appropriate K^+-matched control group) (data not shown).

Coronary flow

When hearts were perfused with Krebs' containing 3 or 5 mM K^+, group mean baseline coronary flows 1 min before introducing drug-containing Krebs' ranged from 11.9±0.7 to 14.9±0.5 ml min^{-1} g^{-1} and 13.8±0.7 to 16.5±0.6 ml min^{-1} g^{-1}, respectively (no significant differences between drug groups and controls at either K^+ concentration). Subsequent perfusion with drugs had no significant effect on coronary flow prior to occlusion. Coronary flow fell to a similar extent in all groups during coronary artery occlusion (data not shown). During reperfusion flow recovered to values at least as great as those before the onset of ischaemia in all groups (data not shown).

Heart rate and ECG intervals

In all groups, heart rate fell during the interval between the start of perfusion and the initiation of ischaemia. This is a transient process in the Langendorff preparation and experience has shown that it is effectively complete 15 min after the start of perfusion (17). It plays no role in arrhythmogenesis. In hearts perfused with Krebs' containing 3 mM K^+,

quinidine and (to a lesser extent) flecainide slowed heart rate during (but not before) ischaemia with effects similar at the lower and higher concentrations (Figure 2A-2B). When perfusate contained 5 mM K^+, the bradycardic effect of the higher concentration of quinidine was exacerbated, and an equivalent effect of lidocaine was unmasked, whereas the effects of the lower concentration of quinidine (and effects of both concentrations of flecainide) were lost (Figure 2C-2D).

The effects of the drugs on PR interval were clearly concentration- and K^+-dependent (Figure 3). When Krebs' contained 3 mM K^+, all three drugs at the higher concentration widened PR interval significantly, with quinidine eliciting the greatest effect (Figure 3B); the lower concentrations had little effect (Figure 3A). When Krebs' contained 5 mM K^+, the effectiveness of the higher concentration of flecainide was increased, its actions becoming similar to those of quinidine, (Figure 3D) and the effects of the lower concentration of flecainide were potentiated to the extent that PR intervals exceeded those in hearts perfused with the lower concentration of quinidine (Figure 3C).

QT interval (measured at 90% repolarisation) was not affected by the lower drug concentrations, regardless of the K^+ content of the Krebs' solution (Figure 4A and 4C). However, the higher concentration of quinidine markedly and significantly widened QT interval (Figure 4B). This effect was substantially diminished, and its onset delayed, by perfusion with a higher concentration of potassium (5 mM K^+) (Figure 4D). Additionally, this high potassium concentration itself shortened QT interval prior to and during perfusion with all three drugs (Figures 4C and 4D).

Discussion

Of the three representative Class I agents, only flecainide (Ic) prevented phase-1 ischaemia-induced VF at the human 'therapeutic' free plasma concentration in isolated rat hearts. Quinidine (Ia) and lidocaine (Ib) prevented phase-1 VF only at the human 'therapeutic' *total* blood concentration, which is much greater than the free plasma concentration, and therefore inappropriately high in terms of clinical relevance. None of the three Class I antiarrhythmics prevented reperfusion-induced VF, even at an inappropriately high concentration. Since each of these types of VF (phase-1 and reperfusion-induced) potentially contribute to sudden cardiac death, the inability of these agents to achieve complete VF suppression even at borderline toxic concentrations may explain their poor clinical efficacy.

The antifibrillatory effect of quinidine

The effect of quinidine on phase-1 ischaemic VF has never been examined in isolated hearts before. In the present study quinidine prevented phase-1 VF only at an inappropriate high concentration (7.90 μM). This accords with published *in vivo* animal studies in which phase-1 VF was suppressed only by a very high dose (10 mg kg^{-1} i.v.) that adversely affected haemodynamic status in conscious and anaesthetized rats (18, 19, 20, 21), anaesthetized rabbits (22), pigs and dogs (19).

Quinidine had no statistically significant effect on the incidence of reperfusion-induced VF even at an inappropriately high concentration (7.90 μM). This contrasts with a published study in which 4 μM quinidine prevented reperfusion-induced VF in isolated rat hearts (23). The difference in outcome may relate to the timing of reperfusion, which was begun 30 min after the onset of ischaemia in the present study, but after only 15 min of ischaemia in the published study (23). This is because the mechanisms responsible for reperfusion-induced VF are different according to the duration of preceding ischaemia (11). Regardless, the reason for

the difference in outcome is less important than the fact that quinidine was ineffective in the present study since this lack of effect at 'therapeutic' concentrations accords with the lack of clinical effectiveness against sudden cardiac death (and may contribue to the lack of clinical effectiveness). This accords with other studies with isolated Langendorff-perfused rat hearts in which only inappropriately high concentrations of quinidine (30 μM) were found to prevent reperfusion VF after regional (24) or global ischaemia (25). In studies *in vivo*, quinidine prevented reperfusion VF at the high dose of 10 mg kg^{-1} i.v. in anaesthetized rats and dogs (19), while even this dose was ineffective against reperfusion-induced VF in anaesthetized pigs (19). Overall our results with quinidine accord with published *in vitro* and *in vivo* animal studies.

The antifibrillatory effect of lidocaine

Lidocaine did not influence the incidence of phase-1 ischaemic VF at the human 'therapeutic' free plasma concentration (3.88 μM), whereas the drug was effective at the human 'therapeutic' total blood concentration (12.93 μM). This finding is in agreement with some, but not all published studies. In isolated guinea pig hearts only an inappropriately high concentration (10 μM) abolished VF during low flow global ischaemia (26). In contrast, a free concentration equivalent to the human 'therapeutic' free plasma concentration prevented phase-1 VF in blood perfused isolated pig hearts (27). However the 'drug effect' may have been an artefact since the study design incorporated repetitive ligation and reperfusion, which could have preconditioned these pig hearts. In studies *in vivo*, only high and toxic doses of lidocaine (> 7-10 mg kg^{-1}) prevented phase-1 ischaemic VF in anaesthetized (20, 21, 28, 29) and conscious (18) rats. Furthermore, even high doses of the drug did not prevent phase-1 VF in anaesthetized rabbits (30) and pigs (19, 31). Similarly, neither low nor high doses of lidocaine abolished phase-1 ischaemic VF in anaesthetized dogs (19, 32, 33, 34, 35).

Like quinidine, lidocaine even at the higher concentration (12.93 μM) had no significant effect on the incidence of repefusion VF in our experiments. In contrast, 10 μM lidocaine significantly decreased the incidence of reperfusion VF in isolated Langendorff-perfused (29) and working rat hearts (36) after regional ischaemia. In other studies, only very high concentrations of lidocaine (30-35 μM) prevented reperfusion-induced VF in isolated rat hearts after regional ischaemia (24, 37). The minimum protective concentrations of lidocaine against reperfusion VF were also very high (15-20 μM) after regional ischaemia in isolated working rabbit hearts (38) and after global ischaemia in isolated Langendorff-perfused guinea pig hearts (39). Interestingly, even 30 μM lidocaine did not decrease the incidence of reperfusion-induced VF in one study after global ischaemia in isolated Langendorf-perfused rat hearts (25). In vivo, reperfusion VF was abolished by a relatively high dose (5 mg kg^{-1}, i.v.) of lidocaine after coronary artery occlusion in anaesthetized rats (40). On the contrary, this arrhythmia was not prevented even by 10 mg kg^{-1} i.v. lidocaine in anaesthetized pigs (19). Likewise, the drug had no protective effect against reperfusion-induced VF in anaesthetized dogs (33, 34, 35, 41).

Thus lidocaine, like quinidine has little or no effect on ischaemia-induced or reperfusion-induced VF when appropriate and tolerable concentrations or doses are examined in a wide range of models, including the isolated perfused rat heart.

The antifibrillatory effect of flecainide

The effect of flecainide on ischaemia induced phase-1 VF has never been examined in isolated heart preparations. In our study, this drug was the only representative Class I antiarhythmic that prevented ischaemic VF at the human 'therapeutic' free plasma concentration (0.74 μM). Similarly to our results flecainide (2 mg kg^{-1}, i.v.) reduced the incidence of ischaemic VF *in vivo*, in anaesthetized rats (20, 21, 42). In contrast, the same

dose of the drug did not reduce the incidence of phase-1 ischaemic VF in anaesthetized pigs (43, 44) or anaesthetized dogs (45).

Like the other two representative Class I agents, flecainide even at high concentration (1.48 μM) had no significant effect on repefusion-induced VF in our experiments. On the contrary, flecainide at an inappropriately high concentration (1 μM) decreased the incidence of reperfusion-induced VF after low flow global ischaemia in an other study in Langendorff-perfused rat hearts (46). Similarly, the drug abolished reperfusion-induced VF *in vivo*, at 2 mg kg^{-1} i.v., in anaesthetized dogs (45).

Although the results of the present study with flecainide differ from those in some previous animal experiments, neither this study nor the previous ones show an unequivocal antifibrillatory effect of flecainide, or comprehensive suppression of both phase-1 ischaemia-induced and reperfusion-induced VF at clinically relevant concentrations.

The proarrhythmic effects of sodium channel inhibitors

In the present study none of the Class I antiarrhythmics increased the incidence of phase-1 ischaemic VF (even as a trend) when 5 mM K^+ was used in the perfusate in order to keep the control VF incidence at a low level. However, sporadic proarrhythmic events (e.g. a very early onset of ischaemia-induced VF in a minority of hearts, and induction of sustained monomorphic VT) occurred. Despite the lack of attainment of statistical significance, an appearance of possible proarrhythmic events is regarded presently as a cause for concern in drug development (47). Interestingly, there are no published isolated heart studies to date showing statistically significant proarrhythmic effects of Class I agents on arrhythmia onset times or incidences. However, lidocaine reduced the mean time to onset of phase-1 ischaemic VF in a study *in vivo* in anaesthetized rabbits (30). Likewise, lidocaine and flecainide reduced the mean time to onset of phase-1 ischaemic VF in anaesthetized pigs (19, 44). Furthermore,

lidocaine increased the incidence of phase-1 ischaemia-induced VF in anaesthetized rabbits (30), pigs (48) and in anaesthetized (49) and conscious dogs (50). Lidocaine also increased the incidence of reperfusion VF in anaesthetized dogs (19).

These studies suggest that proarrhythmic effects of Class I drugs during ischaemia and reperfusion are easier to evoke in *in vivo* models compared with isolated perfused hearts. Perhaps the presence of an autonomic nervous system, hormones, and other events such as heart rate variability, are necessary for proarrhythmic drug effects to reach a threshold for arrhythmia manifestation. It is also possible that there are species differences in sensitivity to proarrhythmia, i.e., larger animals (e.g. rabbit, pig and dog) may be more sensitive than rats and guinea pigs.

It is well known that proarrhythmic drug effects in man are more common where there is chronic heart diseases (1, 2) such as established infarction. Indeed, the unexpected effects of flecainide in the Cardiac Arrhythmia Suppression Trial have been attributed to an interaction between the drug, a new episode of ischaemia and an old infarct (51), a set of conditions that are not mimicked in isolated heart preparations subjected to acute ischaemia and/or reperfusion.

In several instances, the experiments with high K^+ designed to test for proarrhythmia actually revealed trends towards VF suppression. These trends may have become statistically significant if group sizes had been increased to n=12. However this was not pursued since the objective of using 5 mM K^+ was to explore possible proarrhythmic drug effects.

Ancillary pharmacological actions

Drug effects on heart rate, QT and PR interval, and coronary flow can give an indication of mechanisms underlying drug actions on arrhythmias. In the present study ischaemia-induced and reperfusion-induced VF were barely affected by the drugs, and not

affected by clinically relevant drug concentrations (with the exception of flecainide's actions on ischaemia-induced VF). Thus, drug effects on ancillary variables are interesting only in relation to their presence being indicative of the presence of "pharmacologically active" drug concentrations.

There were no substantial or consistent drug effects on heart rate that related to VF suppression (or the lack of suppression) in any way. PR interval was widened in a concentration-dependent manner that was exacerbated by high K^+, and is thus consistent with Na^+ channel block in the AV node (5). This is encouraging evidence that the drug concentrations studied were pharmacologically active at their primary molecular target, the Na^+ channel. However, quinidine was the most effective PR widening agent, yet was largely ineffective as an antiarrhythmic, so it would be wrong to relate drug effects on the AV node to their actions (or lack of actions) on ventricular arrhythmias.

Of the 3 drugs, only quinidine had substantial effects on QT interval. This, together with the observation that elevating the K^+ content of the perfusion solution reversed the QT widening effect of quinidine, is consistent with the known relative selectivity of the three drugs for Na^+ versus K^+ channels, quinidine being the least selective (5). In the rat heart, which does not express functional delayed rectifier potassium current (52), quinidine's QT widening effects are exclusively attributable to blockade of transient outward potassium current (I_{to}) (53). I_{to} blockers widen QT interval in isolated rat hearts but are ineffective in preventing ischaemia-and reperfusion-induced VF (52). Thus, the changes in QT interval were unrelated to arrhythmia suppression (or lack of) in the present study.

Conclusions

Overall, the ischaemia-selective VF suppression by flecainide, the general Class I drug resistance of reperfusion-induced VF, and the ineffectiveness of quinidine and lidocaine during

both ischaemia and reperfusion, despite evidence of Na^+ channel blockade and (in the case of quinidine) K^+ channel blockade, confirms that the spectrum of antiarrhythmic activity of Class I agents in the isolated rat heart is narrow and weak at the equivalent of clinically safe 'therapeutic' concentrations. Moreover, there was a tendency for proarrhythmia with flecainide (early VF onset and sustained monomorphic VT), as predicted by Hondeghem (54), despite an overall reduction in VF incidence.

These findings are consistent with, and may explain, the limited effectiveness of these agents against sudden cardiac death in man. Assessment of phase-1 VF and reperfusion-induced VF in the perfused rat heart represents a potentially useful method of assessing the potential utility of novel agents for suppressing sudden cardiac death.

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Table 1. The incidences of ischaemic and reperfusion-induced VF.

	3 mM K+ in perfusate				5 mM K+ in perfusate			
	ischaemia		reperfusion		ischaemia		reperfusion	
Group	n	VF(%)	n	VF(%)	n	VF(%)	n	VF(%)
Control	12	92	5	100	6	50	5	60
Quinidine 0.79 μM	12	75	10	80	6	0	6	67
Lidocaine 3.88 μM	12	58	9	89	6	0	6	83
Flecainide 0.74 μM	12	17*	12	67	6	0	6	33
Control	12	92	7	100	6	17	5	20
Quinidine 7.90 μM	12	0*	12	42	6	0	6	0
Lidocaine 12.93 μM	12	17*	11	36	6	0	6	33
Flecainide 1.48 μM	12	17*	12	58	6	17	6	0

Values are percent incidence of ventricular fibrillation (VF). Group size is indicated by n.

Control, the time matched control group. *P < 0.05 versus the time matched control group.

Figure legends

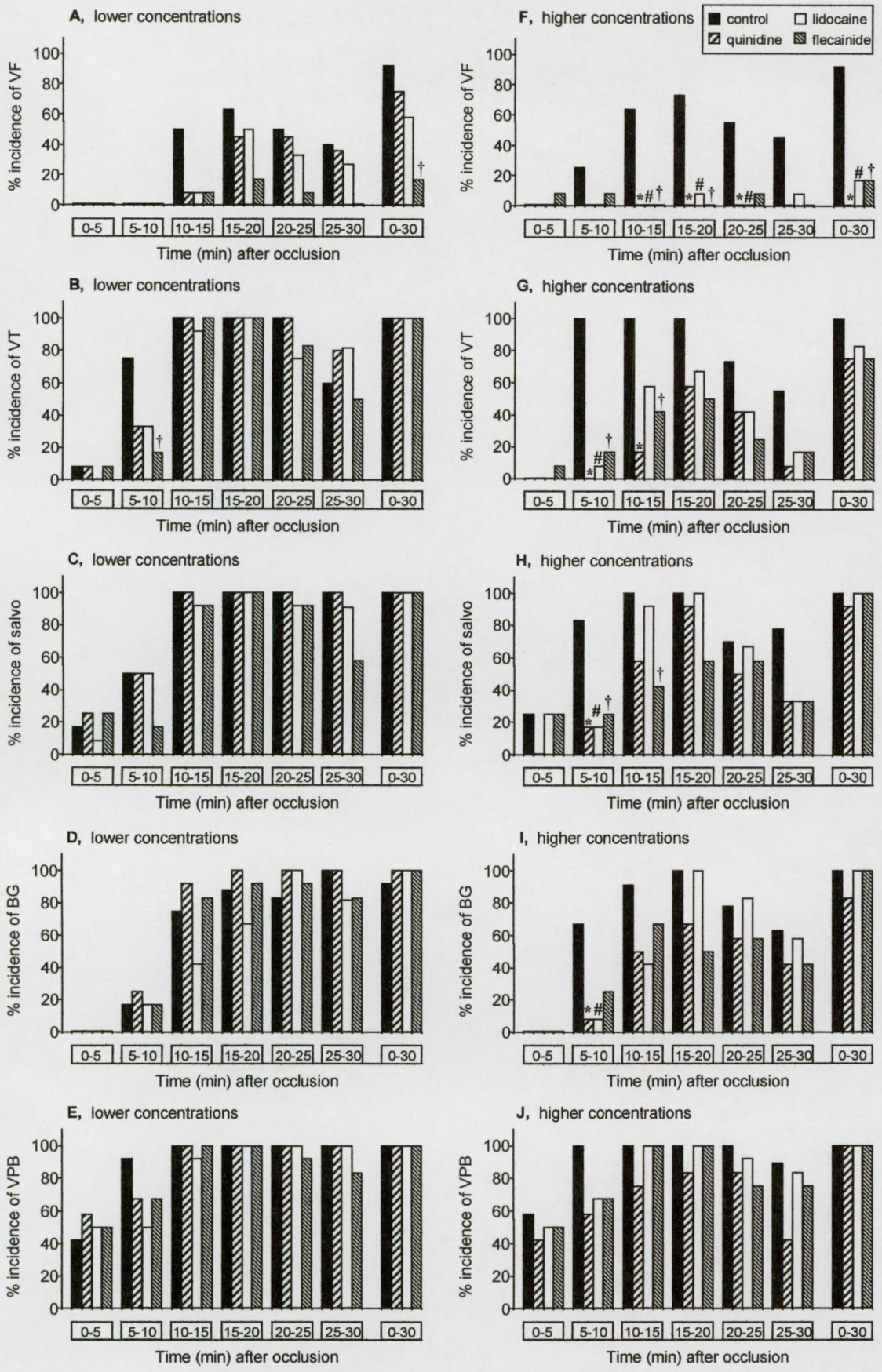
Figure 1. The time course and total incidence of ischaemia induced ventricular arrhythmias in isolated rat hearts (3 mM K^+ in perfusate). The abscissa scales show time intervals following the onset of ischemia. Lower concentrations: 0.79 μM quinidine, 3.88 μM lidocaine, 0.74 μM flecainide, vehicle (time matched control). Higher concentrations: 7.90 μM quinidine, 12.93 μM lidocaine, 1.48 μM flecainide, vehicle (time matched control). VF, ventricular fibrillation; VT, ventricular tachycardia; BG, bigeminy; VPB, ventricular premature beats. *$P < 0.05$ when quinidine treated group compared to control; $^{\#}P < 0.05$ when lidocaine treated group compared to control; $^{\dagger}P < 0.05$ when flecainide treated group compared to control.

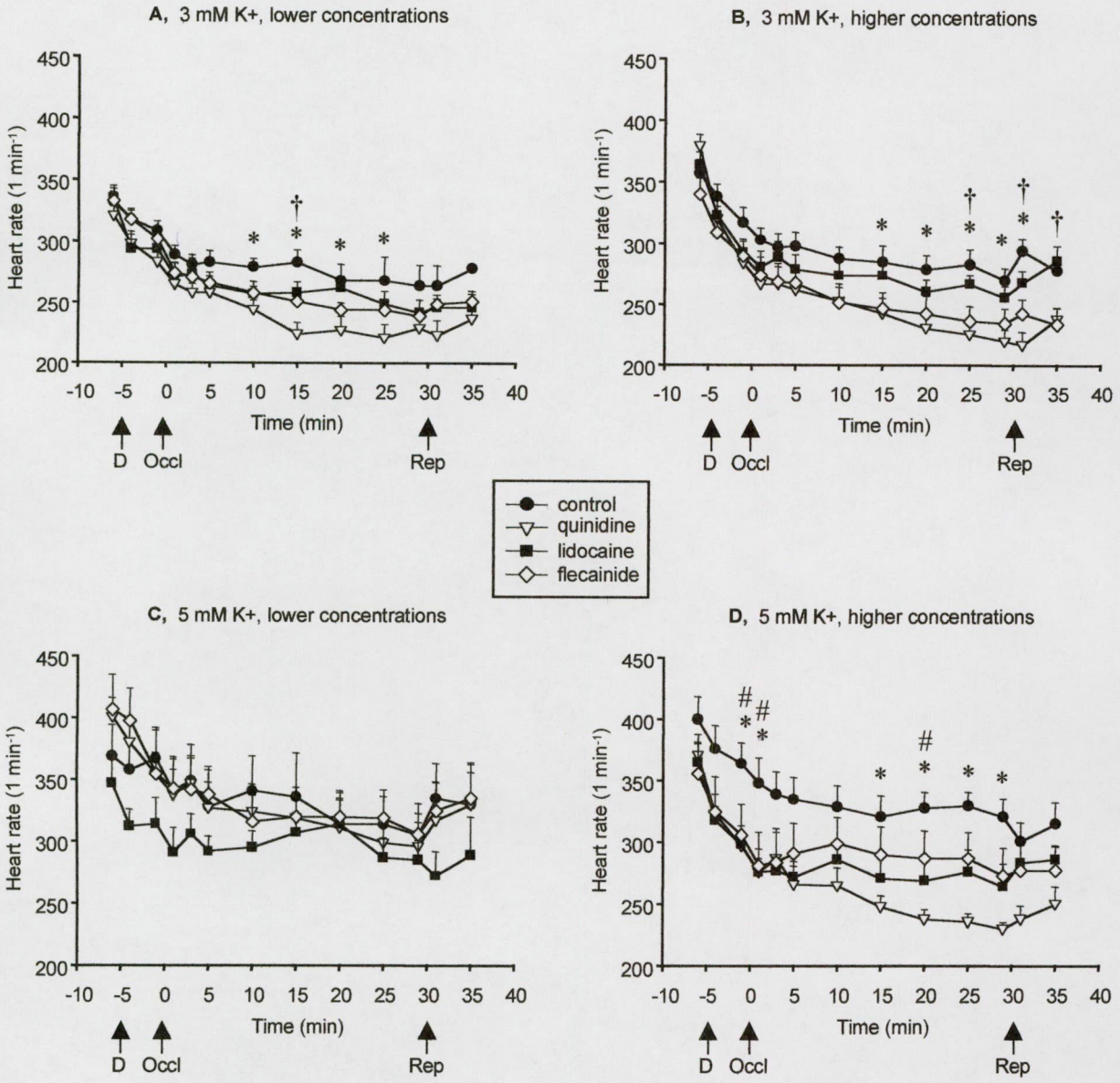
Figure 2. Heart rate during coronary artery occlusion and reperfusion. Part **A**, perfusate contained 3 mM K^+ and lower concentrations of drugs i.e. 0.79 μM quinidine or 3.88 μM lidocaine or 0.74 μM flecainide or vehicle (time matched control). Part **B**, perfusate contained 3 mM K^+ and higher concentrations of drugs i.e. 7.90 μM quinidine or 12.93 μM lidocaine or 1.48 μM flecainide or vehicle (time matched control). Part **C**, perfusate contained 5 mM K^+ and low concentrations of drugs (see above). Part **D**, perfusate contained 5 mM K^+ and high concentrations of drugs (see above). Switching the perfusate from Krebs solution to test solution is indicated by 'D' . Coronary artery occlusion is indicated by 'Occl'. Reperfusion is indicated by 'Rep'. Control, the time matched control group; quinidine, group of hearts perfused with solution containing quinidine; lidocaine, group of hearts perfused with solution containing lidocaine; flecainide, group of hearts perfused with solution containing flecainide.

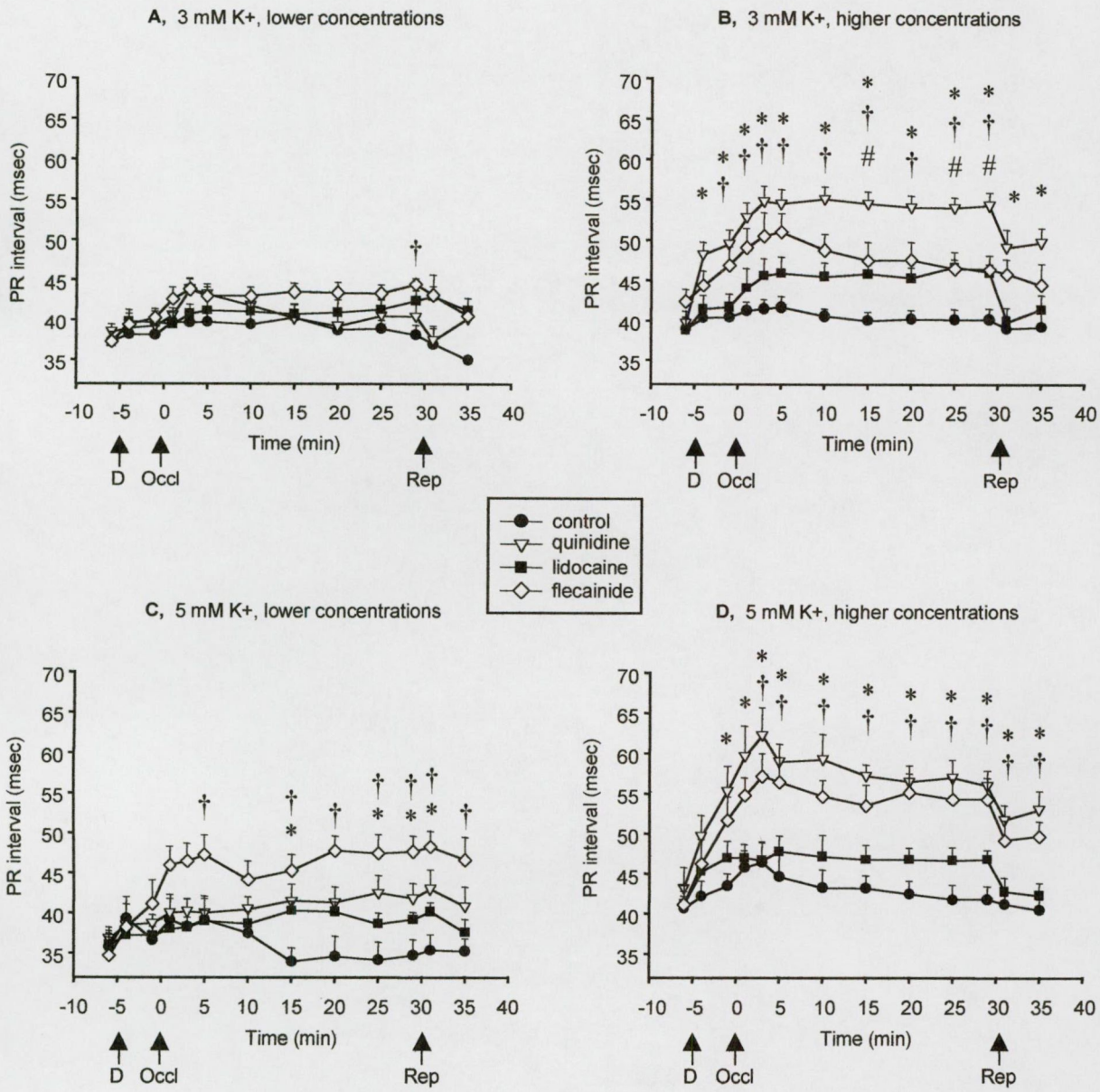
*P < 0.05 when quinidine treated group compared to control; #P < 0.05 when lidocaine treated group compared to control; †P < 0.05 when flecainide treated group compared to control. All values shown as mean + s.e.mean.

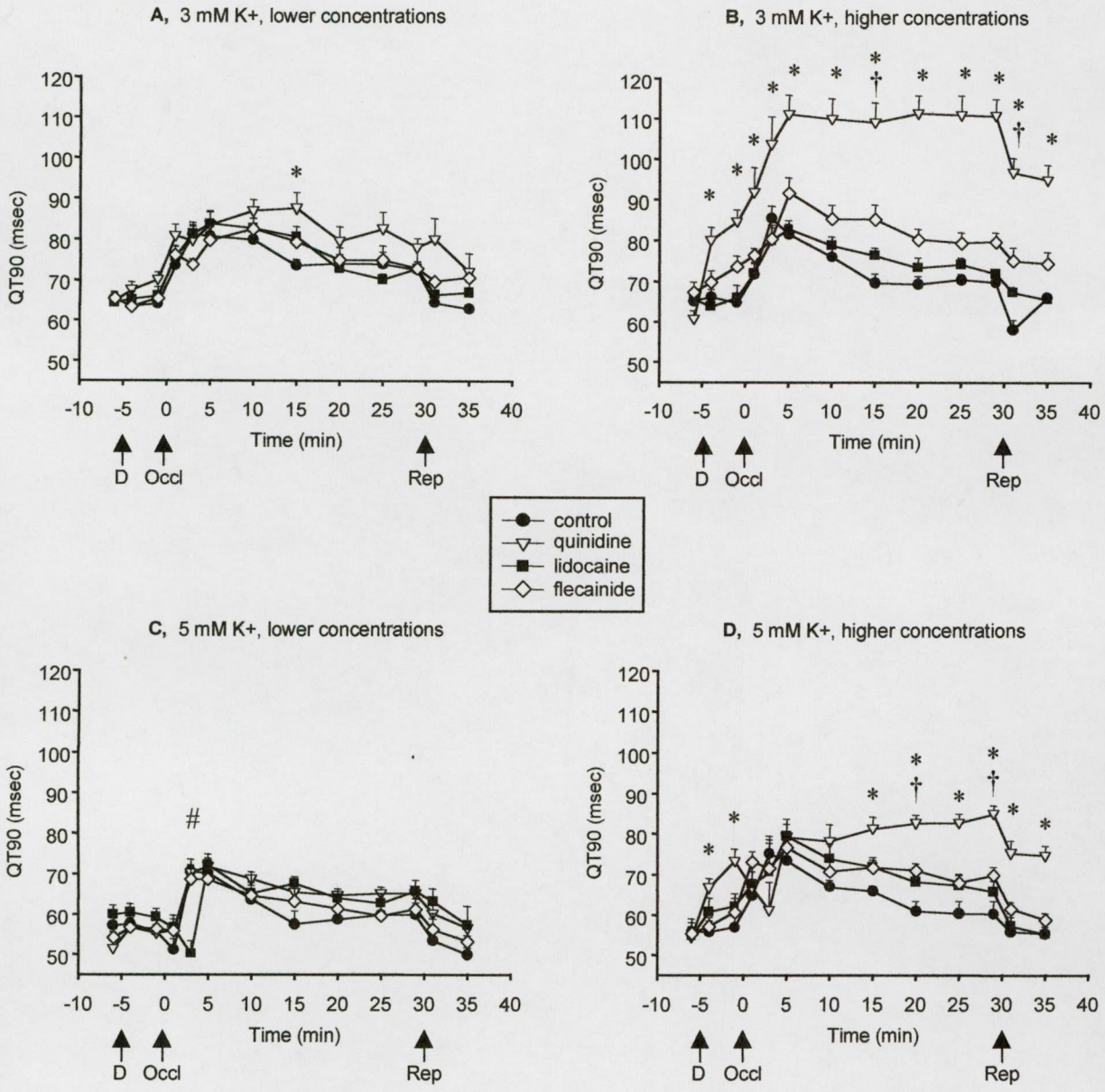
Figure 3. PR intervals during coronary artery occlusion and reperfusion in isolated rat hearts. Part **A**, perfusate contained 3 mM K^+ and lower concentrations of drugs. Part **B**, perfusate contained 3 mM K^+ and higher concentrations of drugs. Part **C**, perfusate contained 5 mM K^+ and low concentrations of drugs. Part **D**, perfusate contained 5 mM K^+ and high concentrations of drugs. *P < 0.05 when quinidine treated group compared to control; #P < 0.05 when lidocaine treated group compared to control; †P < 0.05 when flecainide treated group compared to control. See other details in Figure 2.

Figure 4. QT90 intervals during coronary artery occlusion and reperfusion. Part **A**, perfusate contained 3 mM K^+ and lower concentrations of drugs. Part **B**, perfusate contained 3 mM K^+ and higher concentrations of drugs. Part **C**, perfusate contained 5 mM K^+ and low concentrations of drugs. Part **D**, perfusate contained 5 mM K^+ and high concentrations of drugs. *P < 0.05 when quinidine treated group compared to control; #P < 0.05 when lidocaine treated group compared to control; †P < 0.05 when flecainide treated group compared to control. See other details in Figure 2.


A, lower concentrations
F, higher concentrations
control
lidocaine
quinidine
flecainide
% incidence of VF
B, lower concentrations
G, higher concentrations
% incidence of VT
C, lower concentrations
H, higher concentrations
% incidence of salvo
D, lower concentrations
I, higher concentrations
% incidence of BG
E, lower concentrations
J, higher concentrations
% incidence of VPB
0-5
5-10
10-15
15-20
20-25
25-30
0-30
Time (min) after occlusion


A, 3 mM K+, lower concentrations
B, 3 mM K+, higher concentrations
C, 5 mM K+, lower concentrations
D, 5 mM K+, higher concentrations
Heart rate (1 min-1)
Time (min)
D
Occl
Rep
control
quinidine
lidocaine
flecainide


A, 3 mM K+, lower concentrations
B, 3 mM K+, higher concentrations
C, 5 mM K+, lower concentrations
D, 5 mM K+, higher concentrations
PR interval (msec)
Time (min)
D
Occl
Rep
control
quinidine
lidocaine
flecainide


A, 3 mM K+, lower concentrations
B, 3 mM K+, higher concentrations
C, 5 mM K+, lower concentrations
D, 5 mM K+, higher concentrations
QT90 (msec)
Time (min)
D
Occl
Rep
control
quinidine
lidocaine
flecainide