Antiarrhythmic drugs: limited effectiveness and proarrhythmia

PhD thesis

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To my grandpa and my family

THE THESIS IS BASED ON THE FOLLOWING PAPERS

1. Farkas A, Leprán I, Papp JG. Effect of almokalant a specific inhibitor of I_{Kr} on myocardial ischaemia-reperfusion induced arrhythmias in rabbits. *Acta Physiol Hung* 1996; 84: 281-2.

2. Farkas A, Leprán I, Papp JG. Comparison of the antiarrhythmic and the proarrhythmic effect of almokalant in anaesthetised rabbits. *Eur J Pharmacol* 1998; 346: 245-53.

3. Farkas A, Qureshi A, Curtis MJ. Inadequate ischaemia-selectivity limits the antiarrhythmic efficacy of mibefradil during regional ischaemia and reperfusion in the rat isolated perfused heart. *Br J Pharmacol* 1999; 128: 41-50.

4. Baczkó I, El-Reyani NE, Farkas A, Virág L, Iost N, Leprán I, Mátyus P, Varró A, Papp JGy. Antiarrhythmic and electrophysiological effects of GYKI-16638, a novel N-(phenoxyalkyl)-N-phenylalkylamine, in rabbits. *Eur J Pharmacol* 2000; 404: 181-90.

5. Farkas A, Leprán I, Papp JGy. The proarrhythmic effects of intravenous quinidine, amiodarone, d-sotalol and almokalant in the anesthetized rabbit model of torsade de pointes. *J Cardiovasc Pharmacol.* In Press.

6. Farkas A, Curtis MJ. Limited antifibrillatory effectiveness of clinically-relevant concentrations of Class I antiarrhythmics in isolated perfused rat hearts. *J Cardiovasc Pharmacol.* In Press.

SUMMARY

Large clinical trials and meta-analytic studies revealed that most of the antiarrhythmic drugs are associated with proarrhythmia and do not increase long-term survival in man. Thus, better understanding of proarrhythmic events and better antiarrhythmic drugs are required.

The antiarrhythmic activity of almokalant and d-sotalol (selective K^+ channel inhibitors, Class III antiarrhythmics) were tested in anesthetized rabbits subjected to coronary artery occlusion and reperfusion. Both drugs prevented reperfusion-induced ventricular fibrillation (VF). The proarrhythmic effects of continuous almokalant infusions and increasing doses of d-sotalol, almokalant, quinidine and amiodarone were examined in α_1-adrenoceptor stimulated anesthetized rabbits. Almokalant and d-sotalol evoked not only torsade de pointes (TdP), but also ventricular tachycardia (different from TdP) and VF. The high proarrhythmic activity of these drugs compromises their antiarrhythmic efficacy and precludes their use as antifibrillatory agents.

Mibefradil, a blocker of both L and T Ca^{2+} channels, was compared with the (±)-verapamil for effects on ischemia- and reperfusion-induced VF in isolated rat hearts, and the role of putative ischemia-selective L-channel block was also examined. Mibefradil was less potent than (±)-verapamil as an antiarrhythmic drug. As mibefradil is the more potent T channel blocker, the T channel is unlikely to represent the molecular target for the antiarrhythmic effects of the drug. The effects of both drugs can be explained on the basis of L-channel blockade within the ischemic region. Neither drug prevents VF at concentrations devoid of hazardous vascular and atrioventricular nodal effects. This precludes the safe use of mibefradil in VF suppression, and explains the lack of clinical effectiveness of (±)-verapamil.

The antifibrillatory and proarrhythmic effects of representative Na^+ channel blockers (Class Ia, Ib and Ic drugs) were examined in isolated rat hearts subjected to coronary artery occlusion and reperfusion. The ischemia-selective VF suppression by flecainide, and the ineffectiveness of clinically relevant concentrations of quinidine and lidocaine during both ischemia and reperfusion confirms that the spectrum of antiarrhythmic activity of Class I agents is narrow and weak at clinically safe 'therapeutic' concentrations. Moreover, there was a tendency for proarrhythmia with flecainide. These findings may explain the limited effectiveness of these Na^+ channel inhibitors against sudden cardiac death in man.

These results gave experimental evidence of proarrhythmic activity or limited effectiveness of representative agents of three major classes of antiarrhythmics and the methods applied provide useful experimental tool for examining both the effectiveness and the harmful effects of newly developed antiarrhythmic agents.

ACRONYMS AND ABBREVIATIONS

AV	atrioventricular
EAD	early afterdepolarization
ECG	electrocardiogram
i.p.	intraperitoneal(ly)
i.v.	intravenous(ly)
QTc	heart rate corrected QT interval
TdP	torsade de pointes
VF	ventricular fibrillation
VPB	ventricular premature beat
vs.	versus
VT	ventricular tachycardia
$I_{Ca\text{-}L}$	inward L-type calcium current
I_{Na}	inward sodium current
I_K	delayed rectifier potassium current
I_{Kr}	the rapid component of the delayed rectifier potassium current
I_{K1}	inward rectifier potassium current
I_{to}	transient outward potassium current

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1. INTRODUCTION

1.1. Background of the study

Ischemic heart disease and myocardial infarction are still the leading causes of death in modern societies. Thus, sudden cardiac death, which is attributable in many instances to life threatening arrhythmias, remains an important pharmacotherapeutic target.

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 showed that Class Ic type Na^+ channel blockers increased mortality in survivors of myocardial infarction [1, 2]. The results of these trials and those of the "Electrophysiologic Study Versus Electrocardiographic Monitoring" (ESVEM) [3] and the "Cardiac Arrest Study in Seattle: Conventional Versus Amiodarone Drug Evaluation" (CASCADE) trials [4] shifted the attention to Class III antiarrhythmics.

In the CASCADE trial amiodarone, a repolarization prolonging (Class III) antiarrhythmic drug with complex effects, provided better survival rate than the conventional antiarrhythmic drug therapy in post-myocardial infarction patients [4]. In the ESVEM trial d,l-sotalol, another Class III antiarrhythmic drug with antiadrenerg effects, was more effective than other six antiarrhythmic drugs in preventing death and recurrences of arrhythmia [3]. However, the "European Myocardial Infarct Amiodarone Trial" (EMIAT) and the "Canadian Amiodarone Myocardial Infarction Trial" (CAMIAT) demonstrated that amiodarone reduced arrhythmic but not overall mortality in post-myocardial infarction patients [5, 6]. Likewise, d,l-sotalol has been shown to have neutral effects on overall mortality in post-myocardial infarction patients in another large clinical trial [7]. Moreover, long-term amiodarone treatment leads to severe side effects [8] and d,l-sotalol causes torsade de pointes arrhythmias (TdP) [9]. Thus, more selective Class III drugs have been developed with the expectation that the use of these agents will provide better efficacy and safer use.

As a disappointment, in the "Survival With Oral d-Sotalol" (SWORD) trial d-sotalol, a selective Class III antiarrhythmic, was shown to increase mortality in subsets of patients with myocardial infarction and lowered ejection fraction [10]. On the contrary, in the "Danish Investigations of Arrhythmia and Mortality on Dofetilide" (DIAMOND) trial dofetilide, another selective Class III antiarrhythmic, did not affect mortality in patients with severe left-ventricular dysfunction and recent myocardial infarction [11]. However, dofetilide therapy is associated with an increased risk of TdP [12-14] and proarrhythmic sudden cardiac death [13].

In patients with coronary disease nifedipine, a dihydropyridine calcium agonist, causes an increase in total mortality [15]. On the contrary, data from trials of non-dihydropyridine

calcium agonists such as diltiazem and verapamil have not demonstrated increased mortality rates in patients with coronary artery disease and good left ventricular function [16-19]. Nevertheless, convincing evidence of reduced mortality with the use of non-dihydropyridine Class IV antiarrhythmics is unavailable in most cardiovascular conditions.

These and other clinical trials and meta-analytic studies indicate that better understanding of proarrhythmic events and more effective antiarrhythmics devoid of serious side effects are required for prevention of life threatening arrhythmias.

This thesis summarizes the results of experimental studies, which examined the anti and proarrhythmic effects of both novel antiarrhythmic agents and widely used clinical antiarrhythmic drugs of three major classes of antiarrhythmics i.e. K^+ channel blockers (Class III), Ca^{2+} channel blockers (Class IV) and Na^+ channel blockers (Class I).

1.2. Potassium channel blockers

Repolarization prolonging drugs (Class III antiarrhythmics) exert their effects mostly by blocking K^+ channels [20]. These agents prolong the atrial and the ventricular action potential duration and the corresponding effective refractory period and prevent or terminate reentrant ventricular tachycardias (VT) leading to ventricular fibrillation (VF) [21-23]. The use of these agents in the therapy, however, may also result in severe proarrhythmia.

The typical proarrhythmia observed with the use of drugs delaying ventricular repolarization is the provocation of polymorphic ventricular tachycardia denoted torsade de pointes (TdP) [24]. This arrhythmia can be evoked by various drugs and toxins in man [25, 26], 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 [27, 28]. The underlying mechanism of this arrhythmia is still not known fully, but early afterdepolarizations (EAD) and dispersion of ventricular repolarization are suspected to be the main electrophysiologic substrates for TdP generation [28, 29]. Initiation of TdP is also 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 [30, 31]. Sometimes TdP can deteriorate into VF, a mechanism undoubtedly responsible for sudden cardiac death in a number of patients [32].

Quinidine, a combined K^+ and Na^+ channel blocker, was by far the most frequently reported drug associated with TdP in the clinical practice [25]. On the contrary, amiodarone a combined K^+, Na^+ and Ca^{2+} channel blocker with antiadrenerg effects was found to have a remarkably low potential for inducing TdP [33, 34].

Almokalant is a novel class III antiarrhythmic agent, which blocks selectively the rapid component of the delayed rectifier K^+ current (I_{Kr}) [35]. Clinical studies have already proved that almokalant is an effective antiarrhythmic agent in patients with supraventricular reciprocating tachycardias [36] and in post-myocardial infarction patients with ventricular premature beats (VPB) [37]. On the other hand, almokalant can evoke TdP both in experimental animals [38, 39] and in man [40, 41].

d-Sotalol, another Class III antiarrhythmic agent, inhibits multiple cardiac K^+ channels e.g. I_{Kr}, transient outward current (I_{to}) and inward rectifier current (I_{K1}) [42]. d-Sotalol has been shown to exert antiarrhythmic effects in a number of animal studies [43-45] and in man [46, 47]. On the other hand, d-sotalol can evoke TdP both in experimental animals [39, 48] and in man [42].

Though most of the K^+ channel blockers possess the described two-faced nature i.e. both prevent and induce severe arrhythmias in man, no experimental studies have been published in which both the antiarrhythmic and the proarrhythmic effect of these drugs would have been examined in the same species under well defined *in vivo* experimental conditions.

Although useful canine *in vivo* experimental arrhythmia [49, 50] and proarrhythmia [51, 52] models have been developed, there are some disadvantages of these models e.g. the extensive collateral circulation of the canine heart [53] and the high expenses. There have been *in vivo* experimental arrhythmia [54] and proarrhythmia [55] models developed for the rabbit too. 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 [53], and thus, myocardial infarction in the rabbit can mimic better human myocardial infarction. Moreover, rabbit is a relatively inexpensive small laboratory animal and its cardiac electrophysiology is reported to have similar depolarizing and repolarizing currents to that of man [56, 57]. Though it needs skill to perform coronary artery occlusion in the rabbit, the proarrhythmia model [55], unlike in the dog [51, 52], is quite simple in this species, and does not need difficult surgical techniques.

Several animal models have been developed with TdP as the endpoint [58-60]. The method developed by Carlsson et al. [55] 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 [38, 48].

Although Carlsson's model has been used to examine the proarrhythmic activity of novel antiarrhythmic agents [48, 61, 62], the model has not been characterized sufficiently for its clinical relevance. Importantly, the information on the effects of long-established drugs with clinically well-characterized proarrhythmic activity is sparse.

1.3. Calcium channel blockers

Of the major classes of antiarrhythmic drugs, class IV agents (calcium antagonists) can suppress ischemia-induced VF in animal models [63]. (±)-Verapamil possesses selectivity for L-channels in ischemic versus (vs.) non-ischemic myocardium, and this is determined in part by the facilitatory effect of extracellular K^+, which increases in concentration locally during acute ischemia [64]. However, (±)-verapamil's ischemia-selectivity is inadequate since marked atrioventricular (AV) nodal effects and catastrophic hypotension occur at the doses necessary for VF suppression [64], which would explain why (±)-verapamil fails to prevent sudden cardiac death in man [65].

(+)- and (-)-verapamil reduce developed pressure and increase diastolic pressure in perfused rat hearts, and this action is enhanced by increasing the K^+ content of the perfusion solution to mimic the rise in K^+ that occurs in acute ischemia [63, 64]. This negative inotropic activity of (+)- and (-)-verapamil is fully attributable to L-type calcium antagonist activity [63]. Thus, measuring the effect of a Ca^{2+} channel inhibitor on ventricular contractile function during varying perfusion K^+ content can determine whether the drug has greater or lesser putative ischemia-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.

Mibefradil is a calcium antagonist that blocks both L and T-channels [66-69]. Although it is known that mibefradil can suppress exercise related arrhythmias in dogs with healed myocardial infarction [70], it is not known whether the drug can suppress arrhythmias induced by sustained acute ischemia, whether it can do so without causing severe AV block or vasodilatation, or whether any effects are T or L-channel-mediated.

1.4. Sodium channel blockers

Although there is no accurate information on the time-course of susceptibility to VF following coronary artery obstruction in man, animal studies show that VF susceptibility has two peaks during ischemia, one during the first 30 min (phase-1), and one after 90 min (phase-2), and another during reperfusion [71, 72]. Each of these, if they occur in man, could contribute to sudden cardiac death.

Class I antiarrhythmic agents block Na^+ channels, and can be subdivided into Ia, Ib and Ic on the basis of their binding characteristics and selectivity for Na^+ vs. K^+ channels [73]. Despite their extensive clinical use, and evidence of suppression of certain types of ventricular arrhythmias [74-76], meta-analysis of cumulative data from large numbers of patients showed

that these drugs are ineffective in the prevention of sudden cardiac death [65, 77]. Furthermore, Class Ic drugs actually increased mortality in patients with coronary artery disease [1, 2]. 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 [78], which means that there is a need for more extensive assessment of drugs in a range of animal models.

The isolated rat heart model with regional ischemia caused by coronary artery occlusion allows the generation and examination of ischemia- and reperfusion-induced VF [71, 79]. Phase-1 VF peaks after 7-15 min of ischemia, and reperfusion causes VF any time between 5 and 40 min after the start of ischemia [72, 80].

Interestingly, the effects of Na^+ channel blockers on phase-1 VF have not been examined in the isolated rat heart model [72]. The lack of clinical effectiveness of Class I drugs [65, 77] implies that at least one of the forms of VF that contributes 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.

1.5. Aims of the study

The primary goal of the K^+ channel blocker study was to examine both the antiarrhythmic and the proarrhythmic effect of newly developed K^+ channel blockers in the same species under well defined *in vivo* experimental conditions. The first set of experiments assessed the antiarrhythmic activity of almokalant and d-sotalol against reperfusion induced VF in anesthetized rabbits subjected to coronary artery occlusion and reperfusion. In order to examine the proarrhythmic profile of almokalant, the effects of continuous almokalant infusions were studied in anesthetized rabbits during α_1-adrenoceptor stimulation by phenylephrine. Another set of experiments examined the proarrhythmic effects of quinidine and amiodarone, two widely used antiarrhythmic drugs, and compared them with d-sotalol and almokalant in anesthetized rabbits during α_1-adrenoceptor stimulation by phenylephrine.

The aim of the Ca^{2+} channel blocker study was to examine whether mibefradil can suppress ischemia 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. 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 [72]. Arrhythmia data were contrasted with effects in separate groups of hearts on ventricular contractile function, varying perfusion K^+ content to determine whether mibefradil has greater or lesser putative ischemia-selective L-channel blocking activity than (±)-verapamil.

In the sodium channel study a set of experiments examined the antifibrillatory effect of representative Class Ia, Ib and Ic drugs, (quinidine, lidocaine and flecainide) each at two concentrations equivalent to the clinically relevant 'therapeutic' unbound and total plasma concentrations in isolated Langendorff perfused rat hearts. In order to detect antifibrillatory effects, the perfusion solutions contained 3 mM K^+, which allows high incidence of VF in controls [71, 79]. Additionally, a limited number of experiments was performed to examine the proarrhythmic effects of the three drugs using perfusion solutions containing 5 mM K^+, which give rise to a low incidence of ischemic VF in control hearts [79] and hence scope to reveal proarrhythmia.

2. MATERIALS AND METHODS

2.1. Animals

The anti- and proarrhythmic activity of K^+ channel inhibitors were examined *in vivo* in New Zealand White rabbits from either sex weighing 1.5-3.0 kg. *In vitro* investigations were performed on hearts excised from male Wistar rats weighing 180-250 g to assess the anti- and proarrhythmic activity of Ca^{2+} and Na^+ channel inhibitors.

2.2. Examination of the anti- and proarrhythmic activity of K^+ channel inhibitors *in vivo*

2.2.1. Myocardial ischemia and reperfusion in anesthetized rabbits

Acute coronary artery occlusion and reperfusion were performed as described by Coker [54]. Rabbits (n=87) were anesthetized with pentobarbital-Na (30 mg kg^{-1} i.v.). 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-Na (500 I.U. ml^{-1}), but the animals were not heparinized. 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) with a respiratory volume and rate of 7 ml kg^{-1} per stroke and 40 stroke min^{-1}, respectively.

After 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. Tightening the loose loop with a clamp fixed on the silk induced regional ischemia. Releasing the occluder induced reperfusion.

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.

2.2.2. Experimental protocol of the rabbit ischemia-reperfusion experiments

In the first set of experiments isotonic saline or almokalant (35 or 88 μg kg^{-1}) was administered intravenously over 10 min in continuous infusion in a volume of 2 ml right before the coronary artery occlusion.

In the second set of experiments saline or d-sotalol (1 or 3 mg kg^{-1}) was administered intravenously with a short infusion over 1 min in a volume of 2 ml kg^{-1} 5 min prior to coronary artery occlusion.

After drug treatment coronary artery was occluded to achieve myocardial ischemia for 10 min. At the end of ischemia the ligature was released and 10 min of reperfusion followed.

2.2.3. Occluded zone size and exclusion criteria in anesthetized rabbits

At the end of reperfusion, heparin-Na (500 U.I. kg^{-1}, i.v.) was administered and the animals were killed with an overdose of pentobarbital-Na. The hearts were excised and the size of the occluded zone (the region subjected to ischemia and reperfusion) was quantified using the ethanol exclusion method [81] and expressed as per cent total ventricular weight. All rabbits with an occluded zone less than 16% or larger than 32% were excluded from the final evaluation.

2.2.4. Preparation of anesthetized rabbits for the proarrhythmia experiments

The proarrhythmic activity of K^+ channel inhibitors was examined in an animal model of acquired long QT syndrome [55]. After sedation with pentobarbital-Na (5 mg kg^{-1}, i.v.), male rabbits (n=76) were anesthetized with α-chloralose (100 mg kg^{-1}, i.v.).

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 2.2.1. Blood pressure and electrocardiogram (ECG) were registered during the experiments as in the occlusion-reperfusion model.

2.2.5. Experimental protocol of the rabbit proarrhythmia experiments

In the first set of proarrhythmia experiments continuous i.v. phenylephrine infusion, at the rate of 15 μg kg^{-1} min^{-1}, was administered for 80 min. Ten min after beginning of phenylephrine infusion, simultaneous and continuous i.v. almokalant infusion was given at the rate of 8.8 or 26 μg kg^{-1} min^{-1} for 70 min. Equivalent volume of isotonic saline was administered to the animals in the control group instead of almokalant. The infusion rates were chosen to administer the antiarrhythmic dose of almokalant (88 μg kg^{-1}) at the slow infusion rate over 10 min and at the high infusion rate over 3.33 min.

In the second set of proarrhythmia experiments continuous i.v. phenylephrine infusion, at the rate of 15 μg kg^{-1} min^{-1}, was begun, and was continued for 85 min. Ten min after the beginning of phenylephrine infusion, increasing doses of almokalant (26, 88, 260 μg kg^{-1}), d-sotalol, quinidine, amiodarone (3, 10, 30 mg kg^{-1}) or isotonic saline were administered intravenously. Each dose was given over a period of 5 min and there was 20 min interval between each dose of antiarrhythmic. The doses were chosen to span the antiarrhythmic dose range used by our group (see section 2.2.2.) and others [82-84].

2.2.6. Arrhythmia diagnosis and ECG analysis in the rabbit in vivo experiments

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 [85]. When continuous VF lasted longer than 120 s then VF was defined as sustained VF. Torsade de pointes (TdP) was defined as an arrhythmia in which five or more repetitive VPB were coupled, and for which the QRS complex showed a cyclic variation in size and shape. Every run of 4 VPB with twisting QRS morphology and every run of 4 or more VPB 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 (AV) blocks and intraventricular conduction defects (right or left bundle branch block).

Blood pressure, PP, RR and QT intervals were also measured at predetermined intervals. These parameters were also measured at the first incidence of TdP in the proarrhythmia experiments. In the second proarrhythmia study, where the proarrhythmic effects of d-sotalol, almokalant, quinidine and amiodarone were compared, 2:1 AV block

occurred frequently in some drug treated groups, therefore both sinus rate (SR=60/PP) and ventricular rate (VR=60/RR) were calculated subsequently instead of calculating a single heart rate value. Rate corrected QT interval (QTc) was calculated subsequently by using both Carlsson's equation [38]: QTc(Carlsson)=QT-0.175(RR-300) and Bazett's formula: QTc(Bazett)=QT/square root RR.

2.2.7. Drugs in the rabbit in vivo experiments

The following drugs were used: almokalant (AstraZeneca, Mölndal, Sweden), d-sotalol (Bristol-Myers Squibb, Wallingford, CT, USA), quinidine (Alkaloida, Tiszavasvári, Hungary), amiodarone (Cordarone injection, Sanofi Pharma, France), phenylephrine (L-Phenylephrine HCl, Koch-Light Laboratories LTD, Colnbrook-Bucks, England). Almokalant was prepared as a concentrated stock solution (35 mg ml^{-1}) by AstraZeneca. The stock solution was diluted further with isotonic saline (saline). d-Sotalol and quinidine were dissolved also in saline. Amiodarone was prepared by diluting Cordarone injection (150 mg amiodarone in 3 ml) with saline. Each solution was prepared on the day of the experiment.

2.3. Assessment of the anti- and proarrhythmic effects of Na^+ and Ca^{2+} channel blockers *in vitro*

2.3.1. Myocardial ischemia and reperfusion in isolated rat hearts

Rats were anesthetized with pentobarbital-Na (60 mg kg^{-1} i.p.) mixed with 250 I.U. heparin-Na 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. Perfusion pressure was maintained constant at 70 mmHg. A unipolar ECG was recorded by implanting one stainless-steel wire electrode into the center of the region to become ischemic 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. Regional ischemia and reperfusion were induced by tightening the occluder and by releasing it.

2.3.2. Experimental protocol of the sodium channel inhibitor study

Four sets of experiments were performed and each of them consisted of four groups (quinidine, lidocaine, flecainide or vehicle control). 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 to one of the four test solutions: control (vehicle), quinidine, lidocaine or flecainide. After a further 5 min perfusion, the left main coronary artery was occluded. After 30 min of ischemia the occluder was released to achieve reperfusion. Individual measures of coronary flow, and ECG variables were taken at predetermined intervals.

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 [76], 12.93 μM for lidocaine [74] and 1.48 μM for flecainide [75]. These concentrations are within the so-called human therapeutic range for these agents [86]. All three drugs are plasma protein bound: quinidine 90%, lidocaine 70% and flecainide 50% [87]. 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).

2.3.3. Experimental protocol of the calcium channel inhibitor study

Hearts (n=12/group) were perfused for an initial 5 min with control solution, then solution was switched to one of 11 solutions: control (vehicle), 10, 30, 100, 300 or 600 nM mibefradil or 10, 30, 100, 300 and 600 nM (±)-verapamil. After a further 5 min perfusion, the left coronary artery was occluded. After 30 min ischemia the occluder was released to achieve reperfusion. Individual measures of coronary flow, and ECG variables were taken at predetermined intervals.

The choice of drug concentrations was based on the following. Both (±)-verapamil and mibefradil are highly plasma protein bound: >80% [88] and more than 99% (Clozel, personal communication) respectively. The unbound plasma concentration of (±)-verapamil associated with a 50% reduction in severity of ischemia-induced arrhythmias in conscious rats is 600 nM [88]. 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 [88]. The explanation for this discrepancy is likely to be that the potency of (±)-verapamil increases when sympathetic tone is removed [89], 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 [90].

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.

2.3.4. Occluded zone size, coronary flow and exclusion criteria in isolated rat hearts

At the end of five minutes of reperfusion the size of the occluded zone was quantified using the disulphine blue dye exclusion method [71] and expressed as per cent total ventricular weight. Coronary flow was measured by timed collection of coronary effluent.

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 ischemia (before the start of perfusion with drug or vehicle) or an occluded 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 2 s 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.

2.3.5. Arrhythmia diagnosis and ECG analysis in the isolated rat heart studies

The ECG was recorded using a MacLab system. Arrhythmias were defined according to the Lambeth Conventions [85]. 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 [91].

2.3.6. Assessment of ischemia-selective L-channel blocking activity of Ca^{2+} antagonists

Isolated rat hearts (n=10/group) were perfused with standard solution (see above) containing 3, 6 or 10 mM K^+, and a compliant non-elastic balloon [92] 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 [93].

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. 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 4 min after introduction of the highest concentration of drug.

2.3.7. Drugs in the isolated rat heart studies

All salts were reagent grade chemicals from Sigma Chemical Co. Drug stocks of Na^+ and Ca^{2+} channel inhibitors were prepared fresh each week and perfusion solutions were prepared fresh each day.

2.4. Statistical evaluation of the results of the *in vivo* and *in vitro* studies

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.). Gaussian distributed variables from the same sample were compared with Student's paired-samples "t" test. Gaussian distributed variables from independent samples were compared with one way analysis of variance followed by multiple comparisons according to the "Least Significant Difference" method or Dunnett's test when appropriate. Comparisons within group were made using Wilcoxon's signed ranks test and comparisons between groups

were made using Kruskal-Wallis test on any data that was not normally distributed. Differences were considered statistically significant when $P<0.05$.

3. RESULTS

3.1. Examination of the antiarrhythmic activity of potassium channel blockers

3.1.1. Effect of almokalant and d-sotalol on the ischemic and reperfusion arrhythmias

None of the drug infusions evoked arrhythmias before coronary artery occlusion. During ischemia the incidence of VF was not statistically different in the almokalant- or d-sotalol-treated animals from the appropriate control groups (Table 1). There were no significant differences between the treated and control groups with respect to the incidence of other types of arrhythmias during 10 min of ischemia (Table 1).

Table 1. Effect of almokalant and d-sotalol on the survival rate and incidence of arrhythmias during ischemia and reperfusion in anesthetized rabbits

				Incidence of arrhythmias (%)			
	Dose	n	Survived (%)	None	VF	VT	Other
Ischemia							
Control	0 μg kg^{-1}	15	80	47	27	0	47
Almokalant	35 μg kg^{-1}	13	100	69	8	0	31
Almokalant	88 μg kg^{-1}	14	100	71	0	0	29
Control	0 mg kg^{-1}	19	58	21	42	11	74
d-Sotalol	1.0 mg kg^{-1}	13	92	38	8	0	62
d-Sotalol	3.0 mg kg^{-1}	13	92	54	8	0	46
Reperfusion							
Control	0 μg kg^{-1}	12	42	8	75	42	58
Almokalant	35 μg kg^{-1}	13	62	31	39	31	46
Almokalant	88 μg kg^{-1}	14	86*	43	21*	14	57
Control	0 mg kg^{-1}	11	18	0	82	64	46
d-Sotalol	1.0 mg kg^{-1}	12	75*	33	25*	33	67
d-Sotalol	3.0 mg kg^{-1}	12	83*	33	17*	33	75

n, number of animals; VF, ventricular fibrillation; VT, ventricular tachycardia; Other, ventricular premature beat, salvo, and/or bigeminy; *$P<0.05$ vs. control.

Almokalant in a dose of 88 μg kg^{-1} and d-sotalol (1 and 3 mg kg^{-1}) significantly reduced the incidence of reperfusion-induced VF (Table 1) and increased the number of animals surviving reperfusion (Table 1). There were no differences in the incidence of other types of arrhythmias between drug treated and control rabbits during reperfusion (Table 1).

3.1.2. Effect of almokalant and d-sotalol on the blood pressure, heart rate and QTc intervals

Almokalant and d-sotalol pretreatment had no effect on the blood pressure (Table 2). However, mean arterial blood pressure fell significantly in all groups due to coronary artery occlusion as compared to pre-occlusion values (77±3 vs. 86±3 mmHg, 83±3 vs. 90±3 mmHg, 74±5 vs. 88±4 mmHg in controls, 35 and 88 μg kg^{-1} almokalant treated animals, respectively, and 74±4 vs. 100±3 mmHg, 78±5 vs. 97±3 mmHg, 84±3 vs. 100±3 mmHg in controls, 1 and 3 mg kg^{-1} d-sotalol-treated animals, respectively, all $P<0.05$).

Table 2. Effect of almokalant and d-sotalol on the mean arterial blood pressure, heart rate, QT and QTc intervals in anesthetized rabbits

	Dose	n		Before drug	Pre-occlusion
Control	0 μg kg^{-1}	15	MBP	86±3	86±3
			HR	288±7	288±7
			QTc	163±3	163±4
Almokalant	35 μg kg^{-1}	13	MBP	89±3	90±3
			HR	278±9	282±7
			QTc	165±6	167±6
Almokalant	88 μg kg^{-1}	14	MBP	88±3	88±4
			HR	280±7	270±6#
			QTc	159±4	175±6#
Control	0 mg kg^{-1}	19	MBP	101±3	100±3
			HR	271±7	268±7
			QTc	162±3	162±4
d-Sotalol	1.0 mg kg^{-1}	13	MBP	97±3	97±3
			HR	272±9	252±9#
			QTc	156±4	172±4#
d-Sotalol	3.0 mg kg^{-1}	13	MBP	95±4	100±3
			HR	265±10	247±8#
			QTc	163±6	176±6#

n, number of animals; MBP, mean arterial blood pressure (mmHg); HR, heart rate (min^{-1}); QTc, Carlsson's rate corrected QT interval (ms); #$P<0.05$ vs. the preinfusion value of the same group

Almokalant in a dose of 88 μg kg^{-1} and d-sotalol (1 and 3 mg kg^{-1}) significantly decreased the heart rate of rabbits compared to the basal values (Table 2). Coronary occlusion did not change heart rate significantly compared to pre-occlusion values. No significant changes occurred in the heart rate of animals during reperfusion.

Almokalant in a dose of 88 μg kg^{-1} and d-sotalol (1 and 3 mg kg^{-1}) significantly lengthened QTc intervals (Table 2). No significant changes occurred in the QTc intervals during reperfusion.

3.2. Examination of the proarrhythmic profile of a selective potassium channel blocker

3.2.1. Effects of phenylephrine before almokalant infusions

In the first 10 min, when only phenylephrine was administered to every animal, the heart rate decreased, the mean arterial blood pressure increased and the QTc intervals were prolonged significantly in all three groups (Table 3). Every animal survived this period, but only 26% (8 out of 31) of them had no any arrhythmia. VPB, bigeminy and salvo appeared in 55% (17 out of 31) of the animals. TdP, VT or VF did not occur in this period.

3.2.2. Effect of almokalant infusions on the blood pressure, heart rate and QTc interval

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 the rate of 8.8 μg kg^{-1} min^{-1} and from the 20th min in the group of animals treated with almokalant infusion at the rate of 26 μg kg^{-1} min^{-1}, as compared to the control group (Table 3).

Table 3. The effect of continuous almokalant infusions on the heart rate, blood pressure and the rate corrected QT intervals in anesthetized rabbits primed with phenylephrine

			Phe	Phe + drug						
	n	Basal	10 min	20 min	30 min	40 min	50 min	60 min	70 min	80 min
Control										
HR	10	284±11	217±8#	192±10	195±9	192±11	190±12	192±12	188±14	184±15
MBP		87±4	120±3#	117±3	118±2	118±2	116±2	116±3	118±3	117±3
QTc		154±5	176±3#	180±4	181±3	185±4	183±4	184±5	181±5	180±6
Alm 8.8										
HR	10	289±11	219±16#	209±16	213±16	203±14	211±11	218±10	212±13	220±14
MBP		98±3	117±3#	117±3	114±3	109±4	106±5	105±5	106±4	80±6*
QTc		155±3	177±7#	196±4	192±4	202±5*	215±8*	202±6	198±6	191±13
Alm 26										
HR	11	289±10	204±15#	186±14	182±12	193±16	197±14	196±16	198±13	222±15
MBP		93±4	108±3#	98±6*	94±5*	92±4*	87±6*	87±6*	82±6*	77±3*
QTc		163±4	185±6#	228±8*†	228±9*†	238±10*†	230±10*	223±7*†	215±7*	216±10*†

Control, time matched control (0 μg kg^{-1} min^{-1}) group; Alm 8.8 and Alm 26, groups of animals treated with almokalant infusion at the rate of 8.8 or 26 μg kg^{-1} min^{-1}; HR, heart rate (min^{-1}); MBP, mean arterial blood pressure (mmHg); QTc, Carlsson's rate corrected QT interval (ms); n, number of animals; Phe, phenylephrine infusion at the rate of 15 μg kg^{-1} min^{-1}; #$P<0.05$ vs. basal value; *$P<0.05$ vs. control group; †$P<0.05$ vs. the QTc of Alm 8.8 group.

Saline infusion did not add to the QTc widening caused by phenylephrine (Table 3). However, almokalant infusion produced a dose related further prolongation of the QTc interval compared to the control group (Table 3).

3.2.3. Incidence of arrhythmias evoked by continuous almokalant infusions

In the control group, after the first 10 min, when saline was administered simultaneously with phenylephrine, only VPB, bigeminy and salvos occurred and none of the animals died, whereas in the groups of animals treated with almokalant simultaneously with phenylephrine even TdP, VT (different from TdP) and VF appeared (Table 4). The incidence of TdP was significantly higher in the group of animals treated with almokalant infusion at the rate of 26 μg kg^{-1} min^{-1}. TdP usually ended spontaneously after several seconds. Sometimes monomorphic VT and TdP 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 VT developed as a malignant ventricular arrhythmia and TdP did not. One animal died in both almokalant treated groups due to deterioration of TdP into sustained VF (Table 4).

Table 4. The effect of continuous almokalant infusions on the survival rate and incidence of arrhythmias in anesthetized rabbits primed with phenylephrine

			Incidence of arrhythmias (%)				
	n	Survived (%)	None	VF	VT	TdP	Other
Control (0 μg kg^{-1} min^{-1})	10	100	10	0	0	0	60
Almokalant 8.8 μg kg^{-1} min^{-1}	10	90	10	10	10	20	80
Almokalant 26 μg kg^{-1} min^{-1}	11	91	0	18	18	73*	91

n, number of animals; VF, ventricular fibrillation; VT, ventricular tachycardia; TdP, torsade de pointes; Other, ventricular premature beat, salvo, and/or bigeminy; *$P<0.05$ vs. either control group or the group treated with the low infusion rate of almokalant (8.8 μg kg^{-1} min^{-1}).

3.3. Comparison the proarrhythmic effects of different potassium channel inhibitors

The effect of the first 10 min of phenylephrine infusion was qualitatively and quantitatively nearly identical to that seen in the first proarrhythmia study (section 3.2.1), thus numerical data of this period is not detailed again.

3.3.1. Incidences of arrhythmias evoked by potassium channel inhibitors and onset times

Two animals died from sustained VF 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 5). Quinidine 30 mg kg^{-1} induced VT in 2 out of 7 animals. However, this drug did not cause TdP. VT was also induced by 10 and 30 mg kg^{-1} of d-sotalol and each dose of almokalant (Table 5). TdP was evoked only by d-sotalol and almokalant. 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. VPB, bigeminy and salvo) and the

incidence of VT and evoked TdP in 60% of animals (Table 5). Likewise, the 2nd and 3rd doses of almokalant increased significantly the cumulative incidence of less complex arrhythmias and the incidence of VT and all three doses induced TdP (Table 5). Quinidine, d-sotalol and almokalant evoked conduction blocks in a dose related manner, whereas blocks never occurred after amiodarone and saline administration (Table 5).

Table 5. The incidence of arrhythmias in phenylephrine primed anesthetized rabbits treated with d-sotalol, almokalant, quinidine or amiodarone

		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; VF, ventricular fibrillation; TdP, torsade de pointes; VT, ventricular tachycardia; Block, conduction block; Others, ventricular premature beat, 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.

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 5).

There was a significant difference between d-sotalol and almokalant in terms of ventricular tachyarrhythmia i.e. VT 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 vs. almokalant).

3.3.2. Effect of potassium channel inhibitors on the 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 6).

Addition of d-sotalol reduced blood pressure and ventricular rate dose relatedly (Table 6). There was dissociation between sinus and ventricular rate after administration of 30 mg kg^{-1} d-sotalol due to frequent occurrence of 2:1 AV block.

Table 6. Effect of d-sotalol, almokalant, quinidine and amiodarone treatment on the sinus rate, ventricular rate and the blood pressure in anesthetized rabbits primed with phenylephrine

		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 vs. the value measured right before the beginning of the administration of the given dose; *P<0.05 vs. 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.

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 AV blocks (Table 6). 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} and 240±12 vs. 188±14 min^{-1}, respectively). These differences remained significant till the end of the 20 min drug free intervals (Table 6). 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 6). 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.

The blood pressure drop after each dose of quinidine was significantly greater than those at the same time-points in all other groups (Table 6). 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) AV blocks allowing dissociation of sinus and ventricular rates after which sinus rate increased and ventricular rate decreased significantly compared to control (Table 6).

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}). 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 6).

3.3.3. Effect of potassium channel inhibitors on the QT and QTc intervals

Saline infusion had no significant effect on the QT and QTc widening effect of the maintained infusion of phenylephrine (Table 7). Amiodarone did not add to the QT and QTc widening caused by phenylephrine. However, d-sotalol and almokalant increased the QT and QTc in a dose related manner (Table 7) 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 7). 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 amiodarone (10 mg kg^{-1})(Table 7).

3.3.4. 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 (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, QTc-Carlsson 255±8 vs. 178±4 ms, QTc-Bazett 453±15 vs. 353±8 ms, all P<0.05).

Table 7. Effect of d-sotalol, almokalant, quinidine and amiodarone treatment on the QT and the rate corrected QT intervals in anesthetized rabbits primed with phenylephrine

		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), Carlsson's rate corrected QT interval (ms); QTc (B), Bazett's rate corrected QT interval (ms); Ventricular heart rate (see Table 6) was used for calculating rate corrected QT intervals. #P<0.05 vs. the value measured right before the beginning of the administration of the given dose; *P<0.05 vs. control group; †P<0.05 vs. quinidine and amiodarone. See other details in Table 6.

3.4. Examination of the antiarrhythmic effects of calcium channel blockers

3.4.1. Effect of mibefradil and (±)-verapamil on the ischemic and reperfusion arrhythmias

Neither (±)-verapamil nor mibefradil at 10, 30 or 100 nM reduced VF incidence (Table 8). 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 ischemia-induced VT (100% in all groups), bigeminy, salvos or VPBs (data not shown). The onset times of the first episode of ischemia-induced arrhythmias (all types) and VF were neither hastened nor delayed by mibefradil or (±)-verapamil (data not shown).

Only the highest concentration of (±)-verapamil reduced reperfusion-induced VF incidence significantly, and mibefradil was ineffective at all 5 concentrations (Table 8).

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 ischemia (Table 8), and this was most commonly a mixture of Mobitz I and II.

Mean occluded zone size was not affected by either drug and values ranged from 36 to 41% (data not shown).

Table 8. Arrhythmia incidences in isolated rat hearts perfused with calcium channel blockers during coronary artery occlusion and reperfusion

		Ischemia			Reperfusion	
	Concentration	n	AV block (%)	VF (%)	n	VF (%)
Control	0 nM	12	0	92	10	100
Verapamil	10 nM	12	0	92	6	100
	30 nM	12	0	83	4	100
	100 nM	12	0	75	6	83
	300 nM	12	12	0*	11	73
	600 nM	12	58*	0*	12	42*
Mibefradil	10 nM	12	0	83	8	88
	30 nM	12	0	100	7	100
	100 nM	12	0	100	4	100
	300 nM	12	17	75	8	75
	600 nM	12	83*	17*	12	75

n, number of hearts; AV block, atrioventricular conduction block; VF, ventricular fibrillation; Verapamil, (±)-verapamil; *P<0.05 vs. control.

3.4.2. Effect of mibefradil and (±)-verapamil on the coronary flow and ECG intervals

Mean baseline coronary flow (n=132) 1 min before perfusion with drugs was 13.4±0.3 ml min^{-1} g^{-1} and there were no significant differences between groups. There was a small time-dependent fall in flow in controls (Table 9). Both drugs increased coronary flow before the onset of ischemia, with effects significant at ≥100 nM (Table 9). 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 ischemia, 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 ischemia in all groups (data not shown).

Pre-drug mean PR interval was 37±0.4 ms (n=132), and there were no significant differences between groups. PR interval was widened significantly only by 600 nM (±)-verapamil 1 min before the start of ischemia (Table 9). During ischemia PR intervals could not be measured due to frequent occurrence of Mobitz I AV block.

Pre-drug mean QT interval was 63±0.6 ms (n=132) and there were no significant differences between groups. At 1 min before the onset of ischemia, neither drug affected QT

interval (Table 9) but mibefradil at 600 nM widened QT interval from 15 min after the start of ischemia (101±6 vs. 79±2 ms 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 rate was 336±3 min^{-1} (n=132) and there were no significant differences between groups. There was a small time-dependent fall in heart rate in controls 1 min before the start of ischemia (Table 9), but neither drug affected heart rate at this time, and there was no trend to an effect of either drug during the remainder of the experiment (data not shown).

Table 9. Change in coronary flow, PR interval, QT_{90} interval and heart rate induced by switching from control solution to intervention 5 min before the start of ischemia

			Change in			
	Concentration	n	Coronary flow (ml min^{-1} g^{-1})	PR interval (ms)	QT90 interval (ms)	Heart rate (min^{-1})
Control	0 nM	12	-2.8±0.6	4±1	-1±1	-37±7
Verapamil	10 nM	12	-2.6±1.1	1±1	-1±1	-31±8
	30 nM	12	0.3±0.8	3±1	-3±1	-19±5
	100 nM	12	5.7±0.6*	4±1	-4±1	-7±7
	300 nM	12	4.8±0.9*	4±1	-5±2	-32±16
	600 nM	12	6.2±1.0*	15±2*	2±2	-27±7
Mibefradil	10 nM	12	-0.8±0.6	2±1	-2±1	-20±6
	30 nM	12	3.4±0.8	0±1	-4±2	-10±6
	100 nM	12	6.3±0.8*	3±1	-1±1	-1±7
	300 nM	12	8.2±1.0*	6±1	-1±1	-10±6
	600 nM	12	8.7±1.2*	7±1	2±1	-22±16

n, number of hearts; QT90 interval, QT interval measured at 90% repolarization; Verapamil, (±)-verapamil; Values are changes measured at 1 min before the start of ischemia. *$P<0.05$ vs. control.

3.5. Effect of elevated K^+ concentrations on the activity of calcium channel blockers

3.5.1. Effect of mibefradil and (±)-verapamil on the 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 (Table 10). 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' containing 3 mM K^+ (Table 11). In contrast, when 6 mM K^+ was used, 30, 100, 300 and 600 nM (±)-verapamil reduced developed pressure significantly and concentration-dependently and by a maximum of approximately 70 mmHg (Table 11). However, mibefradil reduced pressure significantly only at 600 nM and by only approximately 30 mmHg. When 10 mM K^+ was used,

the effects of (±)-verapamil were further increased and mibefradil, though less potent than (±)-verapamil, was active at ≥100 nM (Table 11).

Table 10. Pre-drug (baseline) left ventricular developed and diastolic pressures and coronary flow in rat hearts perfused with 3, 6 and 10 mM K^+ solutions

		Developed pressure			Diastolic pressure			Coronary flow		
	n	3 mM K	6 mM K	10 mM K	3 mM K	6 mM K	10 mM K	3 mM K	6 mM K	10 mM K
Control	10	99±12	106±5	92±13	3±10	-3±3	6±7	16.5±2.3	16.5±1.2	17.1±1.7
Mibef	10	91±8	100±3	117±6	1±3	2±2	-3±2	15.1±0.9	17.7±1.0	17.0±0.8
Verap	10	82±10	102±4	117±9	7±6	-2±1	-3±2	13.8±0.5	16.5±0.7	15.4±1.0

n, number of hearts at each K^+ concentration; Control, vehicle groups (time and K^+ matched control groups); Mibef, mibefradil groups; Verap, (±)-verapamil groups. Pressure values are mmHg, coronary flow values are ml min^{-1} g^{-1}.

3.5.2. Effect of mibefradil and (±)-verapamil on the 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 (Table 10). (±)-Verapamil at 300 and 600 nM caused a small (maximum of approximately 5 mmHg) increase in diastolic pressure when hearts were perfused with Krebs' containing 3 mM K^+ (Table 11), whereas mibefradil was inactive. When 6 mM K^+ was used, 30, 100, 300 and 600 nM (±)-verapamil increased diastolic pressure significantly and concentration-dependently and by a maximum of approximately 18 mmHg (Table 11). However, mibefradil increased pressure significantly only at 600 nM and by less than 5 mmHg. When 10 mM K^+ was used, the effects of (±)-verapamil and mibefradil were further increased although mibefradil remained less effective than (±)-verapamil at each concentration (Table 11).

3.5.3. Effect of mibefradil and (±)-verapamil on the coronary flow

Baseline coronary flow in these hearts was not related to perfusion K^+ (Table 10). The baseline values were slightly higher than those in the arrhythmia study (see section 3.4.2.), 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 (Table 11), just as it was in the earlier arrhythmia study (Table 9). The maximum increase in flow was ~6 ml min^{-1} 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 (Table 11), and were absent at 10 mM K^+ (Table 11), and

there was no difference between (±)-verapamil and mibefradil in terms of these effects and their modification by K^+.

Table 11. Changes in left ventricular developed and diastolic pressures and in coronary flow following introduction of solutions of Ca^{2+} channel blockers in rat hearts perfused with 3, 6 and 10 mM K^+ solutions

K+	Conc	Change in developed pressure			Change in diastolic pressure			Change in coronary flow		
(mM)	(nM)	Control	Mibef	Verap	Control	Mibef	Verap	Control	Mibef	Verap
3	30	-17±7	6±4	-11±4	-2±1	-3±1	0±1	-2.9±0.8	3.4±0.9*	2.4±0.8*
	100	-19±10	8±7	-9±4	-1±2	-4±1	0±1	-2.8±0.8	6.3±1.5*	6.1±0.5*
	300	-26±9	2±5	-20±5	0±2	-2±1	3±1*	-1.0±1.0	6.9±1.7*	6.9±0.7*
	600	-28±9	0±4	-29±5	-1±2	-1±1	5±1*	-4.3±1.6	6.2±1.5*	6.0±0.8*
6	30	-4±6	4±3	-44±8*	0±1	-2±1	5±1*	-1.1±1.2	1.2±1.1	0.8±0.7
	100	-4±6	1±5	-55±9*	0±1	-1±1	8±1*	-1.8±1.1	3.0±1.0*	2.4±0.8*
	300	-3±11	-10±5	-72±5*	1±1	1±1	15±1*	-0.8±1.8	2.2±0.7*	2.9±0.7*
	600	-3±10	-31±5*	-72±3*	0±1	4±1*	18±1*	-1.9±2.1	2.2±0.8*	1.1±0.8
10	30	1±4	-13±10	-70±12*	-3±1	-1±1	13±2*	0.5±0.9	2.1±0.7	0.0±0.6
	100	-1±2	-23±4*	-84±10*	-3±2	2±1	18±1*	1.5±1.2	3.1±0.9	0.2±0.4
	300	-10±4	-63±2*	-95±13*	-2±3	11±1*	22±1*	0.2±1.1	2.4±1.0*	0.5±0.7
	600	-16±7	-100±7*	-113±18*	-1±3	16±1*	23±1*	2.1±1.0	1.0±0.7	-0.1±1.4

Conc, concentration; Control, vehicle group (time and K^+ matched control group); Mibef, group of hearts perfused with sequentially increasing concentrations of mibefradil; Verap, group of hearts perfused with sequentially increasing concentrations of (±)-verapamil. Changes measured 4 min after the introduction of each drug solution. Pressure values are mmHg, coronary flow values are ml min^{-1} g^{-1}; n=10 hearts/each drug at each K^+ concentration. *P<0.05 vs. control.

3.6. Examination of the anti- and proarrhythmic effects of sodium channel blockers

3.6.1. Effects of sodium channel blockers on the ischemic and reperfusion arrhythmias

When perfusion solution with 3 mM K^+ was used, VF developed in almost all control hearts during ischemia (Table 12). In hearts in which VF was transient, subsequent reperfusion evoked a further episode of VF. Of the three Class I drugs, only flecainide reduced ischemia-induced VF incidence significantly at the lower concentration (Table 12). In contrast all three drugs at the higher concentration reduced VF incidence during ischemia (Table 12). None of the drugs, even at higher concentration, had any effect on ischemia-induced VT, salvo, bigeminy or VPB incidences (data not shown).

The incidence of reperfusion-induced VF was reduced significantly only by lidocaine and quinidine at the higher concentrations in hearts perfused with 3 mM K^+ (Table 12). 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 12). There were no statistically significant proarrhythmic effects of any of the drugs during ischemia or reperfusion, i.e. VF incidence was not increased (Table 12). Flecainide prevented ischemia-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). There were no significant drug effects on the incidence of ischemia-induced salvo, bigeminy or VPB 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.

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

Table 12. The incidences of ischemic and reperfusion-induced ventricular fibrillation in isolated rat hearts perfused with sodium channel blockers

	3 mM K^+ in perfusate				5 mM K^+ in perfusate			
	ischemia		reperfusion		ischemia		reperfusion	
	n	VF(%)	n	VF(%)	n	VF(%)	n	VF(%)
Control (0 μM)	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 (0 μM)	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

VF, ventricular fibrillation; n, number of hearts; *$P<0.05$ vs. control.

3.6.2. Proarrhythmic events evoked by sodium channel blockers

A hastening of the mean onset time of the first ischemia-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 ischemia-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 s after the onset of ischemia (the equivalent value in K^+-matched controls being 480 s). 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 s after the onset of ischemia (the equivalent value in K^+-matched controls being 1013 s).

Monomorphic VT lasting longer than 120 s 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^+, monomorphic VT with a frequency of approximately 650 min^{-1} and with a duration longer than 120 s 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).

3.6.3. Effects of sodium channel blockers on the 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 ischemia in all groups (data not shown).

3.6.4. Effects of sodium channel blockers on the heart rate and ECG intervals

In hearts perfused with Krebs' containing 3 mM K^+, quinidine and (to a lesser extent) flecainide slowed heart rate during (but not before) ischemia with effects similar at the lower and higher concentrations (Table 13). 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 flecainide) were lost (Table 13).

The effects of the drugs on PR interval were clearly concentration- and K^+-dependent. When Krebs' contained 3 mM K^+, all three drugs at the higher concentration widened PR interval significantly, with quinidine eliciting the greatest effect (Table 13); the lower concentrations had no effect. When Krebs' contained 5 mM K^+, the effectiveness of the higher concentration of flecainide was increased, its actions becoming similar to those of quinidine, 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 (Table 13).

Table 13. Heart rate, PR interval and QT_{90} interval before and during coronary artery occlusion in isolated rat hearts

K+ (mM)		Conc (μM)	n	Heart rate (min^{-1})			PR interval (ms)			QT90 interval (ms)		
				Baseline	-1 min	15 min	Baseline	-1 min	15 min	Baseline	-1 min	15 min
3	Control	0	12	332±8	309±7	283±10	37±1	38±1	40±2	65±2	64±2	74±1
	Quinidine	0.79	12	321±7	283±11	224±10*	39±1	39±1	40±2	65±2	70±2	88±4*
	Lidocaine	3.88	12	335±10	294±10	258±9	37±1	40±1	41±1	64±2	66±2	81±3
	Flecainide	0.74	12	333±10	302±9	251±6*	37±1	40±1	44±1	65±1	66±1	79±2
	Control	0	12	357±11	317±12	285±13	39±1	41±1	40±1	65±2	65±2	70±2
	Quinidine	7.90	12	379±9	284±12	243±12*	40±1	50±2*	55±2*	61±2	85±3*	109±5*
	Lidocaine	12.93	12	364±10	293±10	274±9	39±2	42±2	46±2*	66±1	66±3	76±2
	Flecainide	1.48	12	340±13	290±12	247±13	42±1	47±2*	48±2*	67±3	74±2	85±3*
5	Control	0	6	369±25	367±25	336±36	36±2	37±3	34±2	57±2	56±2	58±3
	Quinidine	0.79	6	401±15	354±18	319±25	37±1	39±1	42±2*	52±2	55±1	66±2
	Lidocaine	3.88	6	347±25	314±21	307±16	36±1	37±1	40±1	60±2	59±2	68±1
	Flecainide	0.74	6	407±28	355±35	320±20	35±3	41±3	45±2*	54±1	57±2	63±3
	Control	0	6	400±18	364±17	321±17	41±1	44±1	43±2	56±3	57±3	66±1
	Quinidine	7.90	6	371±16	297±16*	248±9*	43±3	55±3*	57±1*	55±3	73±3*	81±3*
	Lidocaine	12.93	6	365±15	298±9*	271±16	41±2	47±2	47±2	55±1	62±3	72±2
	Flecainide	1.48	6	356±25	306±25	290±23	41±2	52±3	54±3*	56±3	61±3	72±2

Conc, concentration; n, number of hearts; QT90 interval, QT interval measured at 90% repolarization; Baseline, values measured before switching Krebs' solution to test solution; -1 min, values measured 1 min before coronary artery occlusion; 15 min, values measured in the 15th min of ischemia; Control, vehicle groups (time and K^+ matched control groups); *$P<0.05$ vs. control.

QT interval (measured at 90% repolarization) was mildly prolonged by the lower concentration of quinidine, when Krebs' solution contained 3 mM K^+ (Table 13). However, the higher concentration of quinidine markedly and significantly widened QT interval (Table 13). This effect was substantially diminished, and its onset delayed, by perfusion with a higher concentration of K^+ (5 mM) (Table 13). Additionally, this high K^+ concentration itself shortened QT interval with all three drugs (Table 13). Flecainide mildly prolonged QT interval at higher concentration, when Krebs' solution contained 3 mM K^+, but this effect was significant only at 15 min of ischemia (Table 13).

4. DISCUSSION

4.1. The antiarrhythmic activity of potassium channel inhibitors

In these studies the antiarrhythmic activity of intravenous almokalant and d-sotalol have been assessed in rabbits, and the results demonstrated that these drugs prevent reperfusion-induced VF. Interestingly, K^+ channel blockers e.g. d-sotalol, dofetilide, sematilide, E-4031and UK66,914 vary in their effectiveness against reperfusion arrhythmias. For example, in one study d-sotalol was found to be ineffective against ischemia and reperfusion induced arrhythmias in isolated guinea pig right ventricular free wall preparations [94]. In contrast, UK66,914 possessed marked antiarrhythmic effect on reperfusion arrhythmias in isolated rabbit hearts [95]. In another study d-sotalol, E-4031 and MS-551 (a non-selective K^+ channel blocker) were effective against reperfusion arrhythmias and arrhythmias induced by programmed electrical stimulation, whereas dofetilide and sematilide prevented only arrhythmias induced by programmed electrical stimulation but did not suppress reperfusion arrhythmias in anesthetized dogs [96, 97].

4.1.1. The possible mechanism of antiarrhythmic action of almokalant and d-sotalol

Reperfusion induced arrhythmias may be mediated both via reentry mechanism [98] and triggered activities [99, 100]. Selective prolongation of repolarization (class III antiarrhythmic effect) is one possibility to prevent and terminate reentrant arrhythmias, but it has no effect on arrhythmias induced by triggered activities. Abrahamsson et,al. showed [101] 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. [102] found that almokalant (1.0 mmol kg^{-1}, 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 anesthetized dogs. Similarly to almokalant, d-sotalol has been shown to prolong action potential duration and the corresponding refractory period (Class III antiarrhythmic effect) without affecting depolarization in guinea-pig papillary muscles, sheep and rabbit Purkinje fibers [103]. In the present study 88 $\mu g\ kg^{-1}$ almokalant and d-sotalol (in the dose of 1 and 3 mg kg^{-1}) significantly prolonged QTc interval, i.e. ventricular repolarization. Thus, the possible mechanism by which these drugs prevented reperfusion arrhythmias is the lengthening of the action potential duration and the refractory period of myocardial fibers (achieved by selective blockade of K^+ channels) in the reentrant circuit to such an extent that the propagating reentrant impulse no longer finds excitable myocardium but blocks in refractory tissue.

4.2. The proarrhythmic effect of potassium channel inhibitors

These studies have demonstrated that in anesthetized rabbits only d-sotalol and almokalant induced TdP, whereas quinidine and amiodarone did not. Moreover, our results showed that not only TdP but also other types of arrhythmias can be evoked by K^+ channel inhibitors. Furthermore, we found no direct correlation between the occurrence of TdP and the infusion rate or the dose of antiarrhythmics when graded doses were applied with an interval between each dose.

In the second set of proarrhythmia experiments (section 3.3.) we modified the protocol of Carlsson et al. [38, 55]. Instead of giving repolarization 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. [38] and our results with continuous almokalant infusions (section 3.2.) the infusion rate of drugs is an important predisposing factor for TdP. With our modified 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.

4.2.1. No torsade de pointes with quinidine

In our experiments quinidine did not evoke TdP, though this drug was by far the most frequently reported drug associated with this arrhythmia [25]. In patients with TdP due to quinidine, the plasma level of the drug is usually within or below the therapeutic range [30]. 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 [104] used toxic dose of quinidine (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 [105]; 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 [106], but on the other hand, the drug evoked conduction blocks frequently [106]. 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 Lu et al. [106] and suggests that QT or QTc prolongation and increased QT dispersion [106] are not the only contributing factors to TdP generation in Carlsson's 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 Na^+ channel inhibiting property is increased (use-dependent block) [107], which may prevent TdP at these high heart rates of the rabbit [108]. In addition, after the administration of the 1st dose of quinidine, its antimuscarinic action on atrial muscarinic receptors [109] 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 AV blocks. However, this relatively low ventricular rate, which coincided with marked QT and QTc prolongation, was still insufficient to allow generation of TdP. Likewise, quinidine and terfenadine reduced heart rate and prolonged QT and QTc markedly without evoking TdP in α_1-adrenoceptor stimulated rabbits [106]. 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 [106] terfenadine and quinidine 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. In contrast, clofilium and dofetilide had much milder effect on the blood pressure and evoked TdP while having similar QT, QTc, QT dispersion and heart rate effect than quinidine and terfenadine in the same study [106]. 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 [110], 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 I_{Kr} was found to have very low proarrhythmic potential in the rabbit model of acquired long QT syndrome [111]. This was attributed to the drug's high α_1-adrenoceptor blocking potency.

4.2.2. No torsade de pointes with amiodarone

Intravenous amiodarone did not induce ventricular tachyarrhythmias and did not prolong significantly QT and QTc in our experiments. Although proarrhythmic events are very rare in patients treated with intravenous amiodarone [34], the drug can evoke TdP in man [34, 112, 113]. In rabbits, the time of maximal uptake of i.v. bolus amiodarone by the myocardium has been estimated as between 5 and 15 min [114]. Therefore, low accumulation of amiodarone in our experiments cannot be responsible for the low proarrhythmic activity. Intravenously administered amiodarone inhibits the delayed rectifier outward K^+ current (I_K), the inward Na^+ current (I_{Na}) and the inward L-type Ca^{2+} current ($I_{Ca\text{-}L}$) [115, 116]. 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 [61, 62, 108, 117] probably due to the EAD suppressing or repolarization prolongation limiting effect of $I_{Ca\text{-}L}$ and I_{Na} inhibition, respectively. Intravenous amiodarone inhibits α-adrenoceptors in a non-competitive manner [118], which could also contribute to the low proarrhythmic activity of the drug in this model.

4.2.3. Complex proarrhythmic response to K^+ channel blockers: not only torsade de pointes

Class III agents typically produce TdP. However, in a clinical study ibutilide and sotalol induced not only TdP but also monomorphic VT in patients with atrial fibrillation or flutter [119]. Darpö et al. [41] reported a case in which an almokalant treated patient with WPW syndrome developed TdP after a pacing induced pause, and this tachycardia degenerated into VF that required immediate defibrillation. In our experiments almokalant and d-sotalol (during α_1-adrenoceptor stimulation) produced monomorphic and polymorphic VT and sustained VF as well as TdP. Carlsson et al. [38, 55] reported only on premature ventricular complexes and TdP 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 TdP. However, in our proarrhythmia study with continuous almokalant infusions two animals developed VT prior to the first TdP and one developed VT without the occurrence of TdP. Likewise, Buchanan et al. [48] observed frequently the development of wide complex

tachycardia, which was not pause dependent like TdP following administration of class III agents.

4.2.4. Torsade de pointes with increasing doses of d-sotalol and almokalant

In the second proarrhythmia study only d-sotalol and almokalant induced TdP. Moreover, these drugs induced large number of VT and non-complex arrhythmias, e.g. VPB, bigeminy and salvo. In fact, TdP was always preceded by frequent occurrence of non-complex arrhythmias and sometimes VT. On the other hand, similarly to quinidine, d-sotalol and almokalant also induced conduction blocks in a dose related manner. This accords well with the results of Lu et al. [106] 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 [28]. Extreme repolarization 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 VPB whereas the maintenance mechanism related to reentry [26, 28]. Thus, frequent non-complex arrhythmias and conduction blocks may play a role in the generation of TdP and other reentrant arrhythmias e.g. VT and VF in the rabbit model of acquired long QT syndrome.

In our proarrhythmia 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 quinidine 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 K^+ channel blockers were different in a dog TdP model [39]. Although both drugs widened QT in that study [39] 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 repolarization and the number of EADs to a greater degree than d-sotalol.

Almokalant is a selective I_{Kr} inhibitor [35], whereas d-sotalol inhibits I_{Kr} and other K^+ currents, i.e. I_{to} and I_{K1} [42]. This difference in K^+ channel selectivity, or d-sotalol's residual β-adrenoceptor blocking property [120] or possible differences between the time-course of the development of repolarization dispersion between the drugs might play a role in this different proarrhythmic profile of the two drugs.

4.2.5. Infusion rate of antiarrhythmics and occurrence of torsade de pointes

In our first proarrhythmia experiments continuous almokalant infusion at a higher rate induced more TdP than the infusion of almokalant at a lower rate. This accords well with the findings of an earlier study with continuous almokalant infusions performed by Carlsson et al. [38]. They reported that the occurrence of TdP is dependent on infusion rate. Their suggestion was that this was fundamental to the occurrence of TdP [38]. Interestingly, in our second proarrhythmia study when graded doses of K^+ channel inhibitors were applied 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. Our data demonstrate, that dependence on infusion rate may be particular to the continuous infusion and not to short-term infusions.

4.3. The antiarrhythmic effects of calcium channel inhibitors

We compared mibefradil with (±)-verapamil for effects on arrhythmias induced by ischemia 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.

4.3.1. Actions of (±)-verapamil

(±)-Verapamil is not selective for the myocardial L-channel. However, there is compelling evidence that actions such as α-receptor blockade, Na^+ channel block, recruitment of collateral flow and bradycardia do not contribute to its effects on VF when examined in conscious rats [64]. Importantly, the (+)- to (-)-verapamil potency ratio for effects on ischemia-induced VF *in vivo* correlates with the negative inotropic potency ratio in hearts perfused with high, but not low K^+ containing solution. This finding, together with other

observations [63], indicates that L-channel blockade within the ischemic region (in which extracellular K^+ levels are elevated) fully accounts for (±)-verapamil's effects on VF during ischemia in conscious rats.

In the present study, the lack of effect of low concentrations of (±)-verapamil on VF was unsurprising, despite evident coronary vasodilatation. The rat heart is collateral-deficient [53], so vasodilatation does not confer protection against ischemia-induced VF in this species [64, 72]. 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.

The effects of (±)-verapamil on systolic and diastolic pressure were similar to those observed previously with its (+) and (-) enantiomers [64]. 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 concentrations of (±)-verapamil reduced ischemia-induced VF in the parallel study. The importance of this is that during early myocardial ischemia, local extracellular K^+ concentration in the involved region rises to 10 mM and beyond [121].

The data therefore illustrates, for the first time, that (±)-verapamil's protective effects on ischemia-induced VF in conscious rats [63, 89] are mirrored by similar actions *in vitro*, and appear to be mediated by the same mechanism, namely L-channel blockade in the involved region.

4.3.2. Actions of mibefradil

T-channels are not considered to play any significant role in human ventricular myocardium [122]. In the rat heart, 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 ischemia-induced VF, but it was less potent than (±)-verapamil. Both drugs produced similar significant effects on coronary flow before ischemia, 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.

4.3.3. Mechanism of action of mibefradil on ischemia-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 [122] and guinea pig [123] 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 ischemia-induced VF in the parallel study.

Mibefradil does not affect I_{K1}, a current important in arrhythmogenesis in rat heart [91] at up to 30,000 nM [124]. Nor does it reduce evoked norepinephrine release from sympathetic nerves at a concentration (IC_{50} 1000 nM) relevant to present findings [125]. Likewise, the IC_{50} for its effects on free-radical mediated cellular injury is 2,000 nM [126]. 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. The present findings imply that mibefradil reduced ischemia-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.

4.3.4. Properties limiting efficacy of (±)-verapamil and mibefradil on ischemia-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 [127]. 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 [128]. Likewise, in the present study both drugs affected coronary flow at concentrations much lower than those affecting VF. This marked vascular effect along with the AV block eliciting effects of these Ca^{2+} channel inhibitors preclude their use as antifibrillatory agents.

4.3.5. 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 ischemia-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 [121], and this resolves the ischemia-induced diastolic depolarization. In view of the voltage dependence of the actions of the drugs on L-channels (see above), 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 ischemia-induced VF.

The present data may also explain an earlier observation that electrically-induced arrhythmias in non-ischemic hearts are resistant to suppression by mibefradil and (±)-verapamil, whereas electrically-induced arrhythmias in ischemic hearts are suppressed by both drugs [129]. Furthermore, they are in agreement with an observation that arrhythmias during ischemia in dogs are suppressed by lower doses of mibefradil than those required to suppress electrically-induced arrhythmias [130]. Each of these observations further point to an ischemia-selective L-channel dependent mechanism of action.

4.4. The anti- and proarrhythmic effects of sodium channel blockers

Of the three representative Class I agents, only flecainide (Ic) prevented phase-1 ischemia-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 at the human 'therapeutic' free plasma 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 at concentrations equivalent to the 'therapeutic' free plasma concentration may explain their poor clinical efficacy.

4.4.1. The antifibrillatory effect of quinidine

The effect of quinidine on phase-1 ischemic 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 hemodynamic status in conscious and anesthetized rats [82, 131-133], anesthetized rabbits [54], pigs and dogs [82].

Quinidine reduced the incidence of reperfusion-induced VF only at a very high concentration (7.90 μM). This accords with other studies with isolated Langendorff-perfused rat hearts in which only inappropriately high concentrations of quinidine (4-30 μM) were found to prevent reperfusion VF after regional [134, 135] or global ischemia [136]. However, quinidine was ineffective at 'therapeutic' concentration in the present study and this lack of effect accords with the lack of clinical effectiveness against sudden cardiac death (and may contribute to the lack of clinical effectiveness). In studies *in vivo*, quinidine prevented reperfusion VF at a high dose of 10 mg kg^{-1} i.v. in anesthetized rats and dogs [82], while even this dose was ineffective against reperfusion-induced VF in anesthetized pigs [82]. Overall our results with quinidine accord with published *in vitro* and *in vivo* animal studies.

4.4.2. The antifibrillatory effect of lidocaine

Lidocaine did not influence the incidence of phase-1 ischemic 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 ischemia [137]. In contrast, a free concentration equivalent to the human 'therapeutic' free plasma concentration prevented phase-1 VF in blood perfused isolated pig hearts [138]. 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 ischemic VF in anesthetized [132, 133, 139, 140] and conscious [131] rats. Furthermore, even high doses of the drug did not prevent phase-1 VF in anesthetized rabbits [141] and pigs [82, 142]. Similarly, neither low nor high doses of lidocaine abolished phase-1 ischemic VF in anesthetized dogs [82, 143-146].

Like quinidine, lidocaine reduced the incidence of reperfusion VF only at the higher concentration (12.93 μM, 3 mM K^+ in Krebs') in our experiments. Similarly to our results, 10 μM lidocaine significantly decreased the incidence of reperfusion VF in isolated Langendorff-perfused [140] and working rat hearts [147] after regional ischemia. In other studies, only extremely high concentrations of lidocaine (30-35 μM) prevented reperfusion-induced VF in isolated rat hearts after regional ischemia [135, 148]. The minimum protective concentrations of lidocaine against reperfusion VF were also very high (15-20 μM) after regional ischemia in isolated working rabbit hearts [149] and after global ischemia in isolated Langendorff-perfused guinea pig hearts [150]. Interestingly, even 30 μM lidocaine did not decrease the incidence of

reperfusion-induced VF in one study after global ischemia in isolated Langendorff-perfused rat hearts [136]. *In vivo*, reperfusion VF was abolished by a relatively high dose (5 mg kg^{-1}, i.v.) of lidocaine after coronary artery occlusion in anesthetized rats [151]. On the contrary, this arrhythmia was not prevented even by 10 mg kg^{-1} i.v. lidocaine in anesthetized pigs [82]. Likewise, the drug had no protective effect against reperfusion-induced VF in anesthetized dogs [144-146, 152].

Thus lidocaine, like quinidine has little or no effect on ischemia-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.

4.4.3. The antifibrillatory effect of flecainide

The effect of flecainide on ischemia induced phase-1 VF has never been examined in isolated heart preparations. In our study, this drug was the only representative Class I antiarrhythmic that prevented ischemic 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 ischemic VF *in vivo*, in anesthetized rats [132, 133, 153]. In contrast, the same dose of the drug did not reduce the incidence of phase-1 ischemic VF in anesthetized pigs [154, 155] or anesthetized dogs [156].

Unlike the other two representative Class I agents, flecainide even at high concentration (1.48 μM) had no significant effect on reperfusion-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 ischemia in an other study in Langendorff-perfused rat hearts [157]. Similarly, the drug abolished reperfusion-induced VF *in vivo*, at 2 mg kg^{-1} i.v., in anesthetized dogs [156].

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 ischemia-induced and reperfusion-induced VF at clinically relevant concentrations.

4.4.4. The proarrhythmic effects of sodium channel inhibitors

In the present study none of the Class I antiarrhythmics increased the incidence of phase-1 ischemic 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 ischemia-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 [60]. 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 ischemic VF in a study *in vivo* in anesthetized rabbits [141]. Likewise, lidocaine and flecainide reduced the mean time to onset of phase-1 ischemic VF in anesthetized pigs [82, 155]. Furthermore, lidocaine increased the incidence of phase-1 ischemia-induced VF in anesthetized rabbits [141], pigs [158] and in anesthetized [159] and conscious dogs [160]. Lidocaine also increased the incidence of reperfusion VF in anesthetized dogs [82].

These studies suggest that proarrhythmic effects of Class I drugs during ischemia 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 [65, 77] 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 ischemia and an old infarct [161], a set of conditions that are not mimicked in isolated heart preparations subjected to acute ischemia and/or reperfusion. If this is correct then the isolated heart models of acute ischemia and reperfusion (such as the present) that show minimal proarrhythmia in response to Class I drugs may be better reflective of clinical susceptibility to proarrhythmia in the absence of chronic heart disease than the *in vivo* acute ischemia models that show greater susceptibility to Class I proarrhythmia (and which would therefore be inappropriately sensitive to proarrhythmic drug effects).

4.4.5. Ancillary pharmacological actions of sodium channel inhibitors

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 ischemia-induced and reperfusion-induced VF were not affected by clinically relevant drug concentrations (with the exception of flecainide's actions on ischemia-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 [86]. This is encouraging evidence that the drug concentrations studied were pharmacologically active at their primary molecular target, the Na^+ channel.

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^+ vs. K^+ channels, quinidine being the least selective [86]. In the rat heart, which does not express functional I_K [162], quinidine's QT widening effects are exclusively attributable to blockade of I_{to} [163]. In short, the QT and PR prolonging effect of quinidine shows that the concentrations of the drug studied were pharmacologically active at both Na^+ and K^+ channels.

5. CONCLUSIONS

Our K^+ channel blocker studies have provided evidence that almokalant and d-sotalol are effective against reperfusion VF, though they have marked proarrhythmic effects during α_1-adrenoceptor stimulation in anesthetized rabbits. Furthermore, these studies demonstrated experimentally that almokalant and d-sotalol are able to produce not only TdP as a malignant proarrhythmia, but also VT (different from TdP) and VF. This high propensity of these drugs to evoke severe proarrhythmia compromises their antiarrhythmic efficacy and precludes their use as antifibrillatory agents. Our results also suggest that the incidence of TdP may not depend on the infusion rate or the dose of antiarrhythmics when graded doses are applied with an interval between each dose. 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.

As a conclusion of the Ca^{2+} channel inhibitor study, mibefradil is less potent than (±)-verapamil as an antiarrhythmic agent in ischemic rat ventricle. The effects of both drugs can be explained on the basis of L-channel blockade within the ischemic region. Neither drug is sufficiently ischemia-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 [65], but also excludes the

possibility of mibefradil (or a pharmacologically similar analogue) possessing better efficacy. It also appears from the Ca^{2+} channel inhibitor study that the T-channel is unlikely to represent a useful molecular target for VF suppression.

Regarding the Na^+ channel inhibitor study, the ischemia-selective VF suppression by flecainide, and the ineffectiveness of clinically relevant concentrations of quinidine and lidocaine during both ischemia 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), despite an overall reduction in ischemic VF incidence. These findings are consistent with, and may explain, the limited effectiveness of these Na^+ channel inhibitors against sudden cardiac death in man. Since none of the Class I 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.

These results gave experimental evidence of proarrhythmic activity or limited effectiveness of representative agents of three major classes of antiarrhythmics and the methods applied provide useful experimental tool for examining both the effectiveness and the harmful effects of newly developed antiarrhythmic agents.

6. NEW FINDINGS

1. Almokalant and d-sotalol prevent reperfusion VF in anesthetized open chest rabbits. It appears that the incidence of TdP may not depend on the infusion rate or the dose of antiarrhythmics when graded doses are applied with an interval between each dose. 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.
2. Mibefradil is not sufficiently ischemia-selective to achieve protection against VF at concentrations devoid of potentially hazardous vascular and AV nodal effects in isolated Langendorff-perfused rat hearts. The antifibrillatory effect of mibefradil can be explained on the basis of L-channel blockade within the ischemic region and T Ca^{2+} channel is unlikely to represent a useful molecular target for VF suppression.
3. The spectrum of antiarrhythmic activity of representative Class I agents in the isolated rat heart is narrow and weak at the equivalent of clinically safe 'therapeutic' concentrations and the clinical proarrhythmia seen with these drugs may not be related to exacerbation of phase-1 ischemia-induced VF.

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