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

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

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

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

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

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

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

Introduction

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

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

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

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

Methods

Animals and general methods for arrhythmia experiments

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

Experimental protocol

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

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

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

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

Measurement of involved zone size and coronary flow

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

Exclusion criteria

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

Arrhythmia diagnosis and ECG analysis

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

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

Measurement of all variables was performed in a blinded manner.

Assessment of ischaemia-selective L-channel blocking activity

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

Table 1 Arrhythmia incidences

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

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

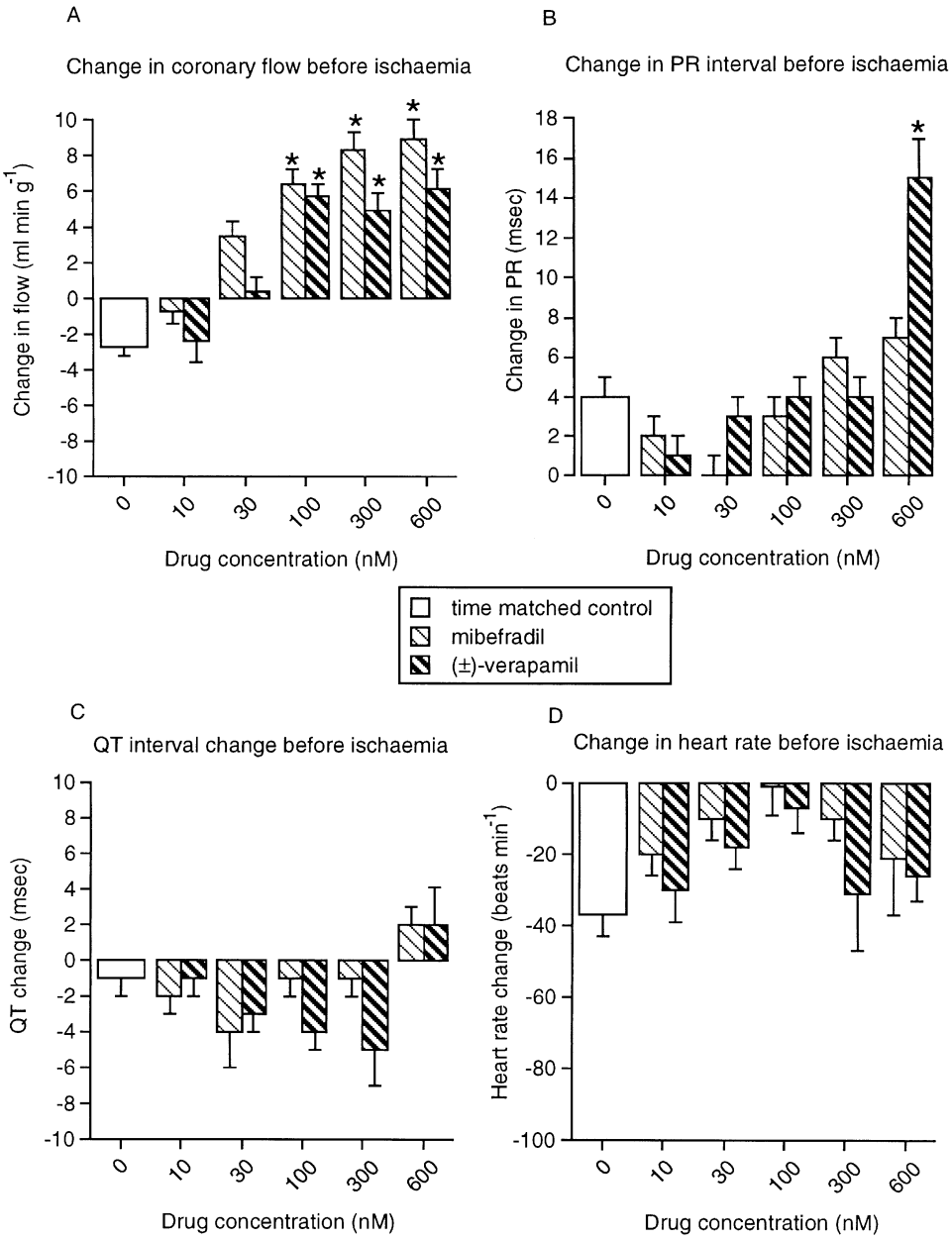

Figure 1 Change in coronary flow (A), PR interval (B) QT interval (C) and heart rate (D) induced by switching from control solution to intervention 5 min before the start of ischaemia. Values are changes measured at 1 min before the start of ischaemia. *$P<0.05$ versus 0 nM group (time matched control).

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

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

Drugs and materials

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

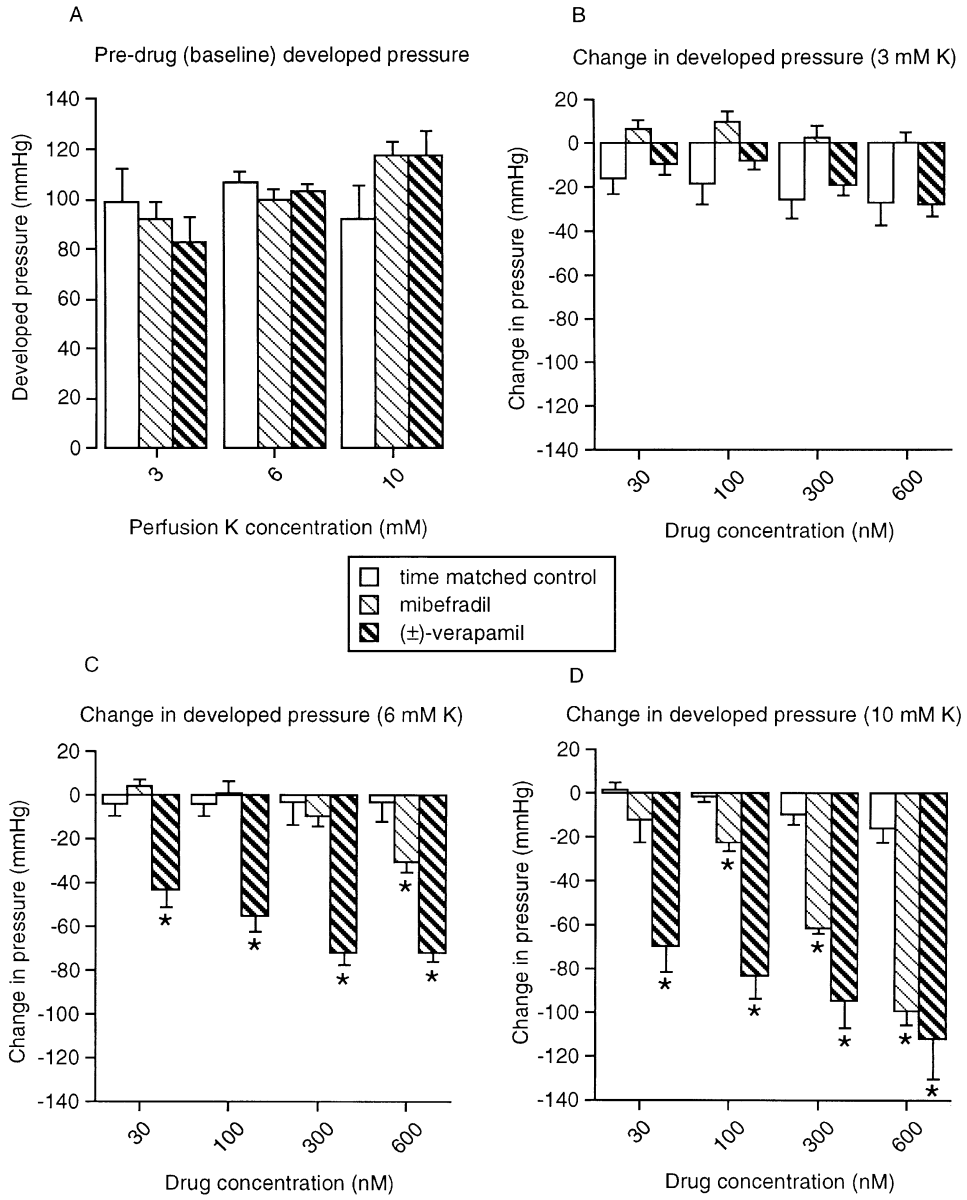

Figure 2 Pre-drug (baseline) left ventricular developed pressure in hearts perfused with different K^+ concentrations (A), and changes in developed pressure following introduction of drug solutions in hearts perfused with 3 mM K^+ solution (B), with 6 mM K^+ solution (C) or with 10 mM K^+ solution (D).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

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

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

Statistics

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

Results

Arrhythmia studies

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

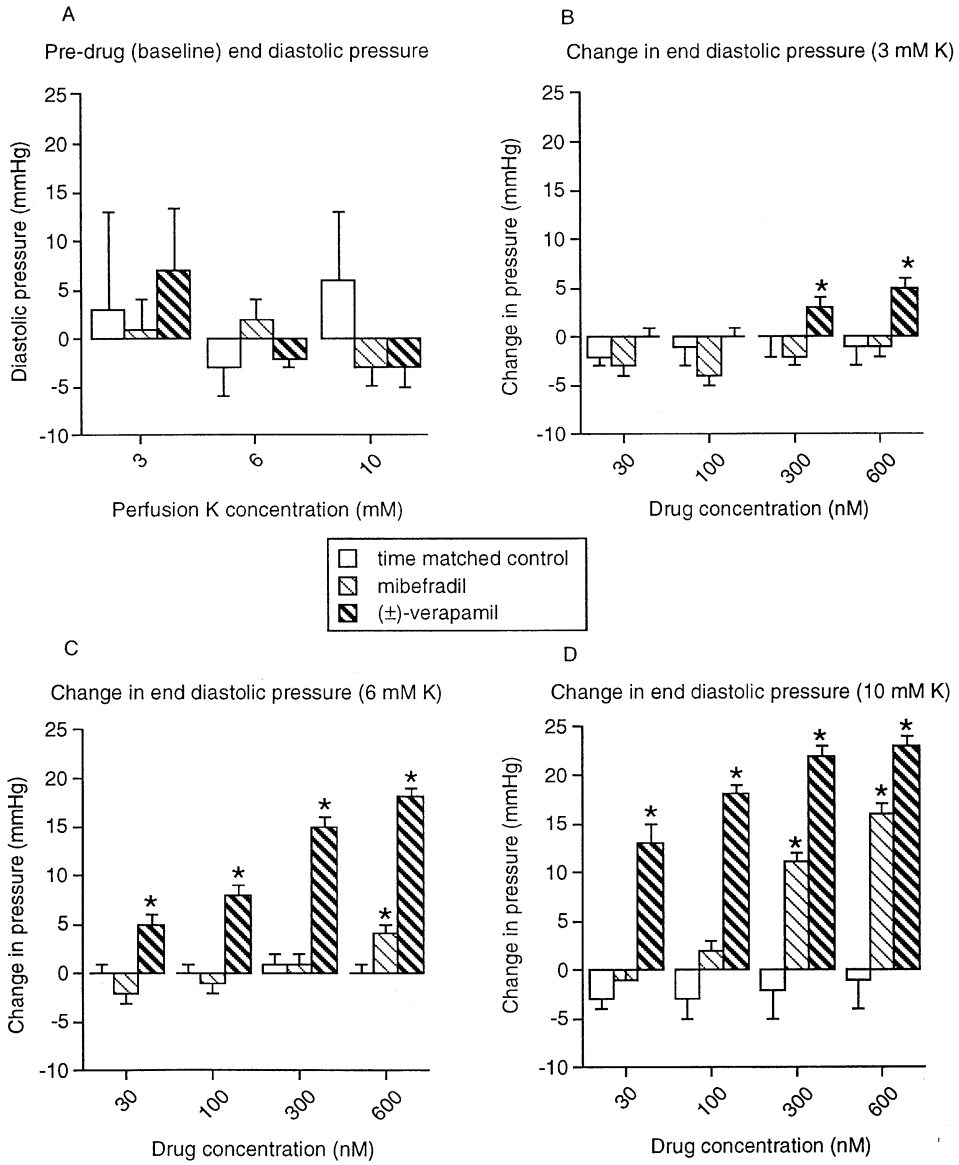

Figure 3 Pre-drug (baseline) left ventricular diastolic pressure in hearts perfused with different K^+ concentrations (A), and changes in diastolic pressure following introduction of drug solutions in hearts perfused with 3 mM K^+ solution (B), with 6 mM K^+ solution (C) or with 10 mM K^+ solution (D).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

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

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

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

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

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

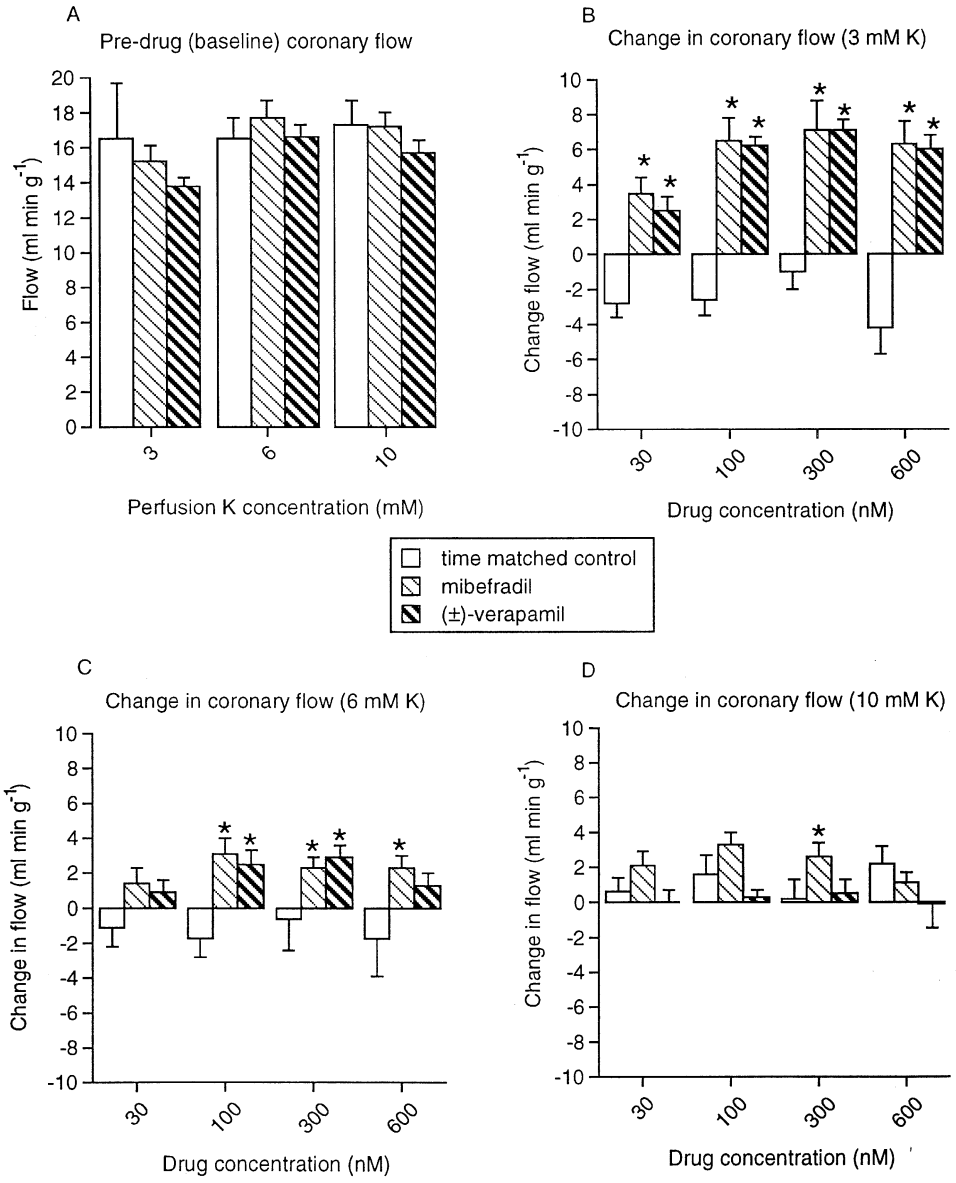

Figure 4 Pre-drug (baseline) coronary flow in hearts perfused with different K^+ concentrations (A), and changes in flow following introduction of drug solutions in hearts perfused with 3 mM K^+ solution (B), with 6 mM K^+ solution (C) or with 10 mM K^+ solution (D). Values are ml min g^{-1} (A) or changes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

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

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

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

Contractile function studies

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

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

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

Discussion

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

Actions of (±)-verapamil

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

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

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

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

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

Actions of mibefradil

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

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

Mechanism of action of mibefradil on ischaemia-induced VF

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

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

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

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

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

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

Reperfusion-induced VF and other observations

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

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

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

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

Conclusion

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

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