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The mechanism of action of the optical enantiomers of verapamil against ischaemia-induced arrhythmias in the conscious rat

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1 The actions of (−)-verapamil (0.2–6 mg kg^{-1}) and (+)-verapamil (0.4–12 mg kg^{-1}) against arrhythmias induced by coronary artery occlusion were studied in conscious rats.

2 Intravenously administered (−)- and (+)-verapamil dose-dependently reduced ventricular arrhythmias. (−)-Verapamil was consistently 4 times more potent than (+)-verapamil.

3 In the same animals, (−)-verapamil was approximately 4 times more potent than (+)-verapamil for effects on heart rate and blood pressure. Both enantiomers prolonged P-R interval, but had no effect on QRS interval.

4 In separate groups of conscious rats, neither enantiomer influenced the threshold voltage and pulse width required to elicit fibrillo-flutter, or altered the maximum following frequency, during electrical stimulation of the left ventricle.

5 In isolated, paced, Langendorff-perfused ventricles of the rat, both enantiomers dose-dependently reduced contractility, (−)-verapamil being 8–21 times more potent than (+)-verapamil; both absolute and relative potencies were dependent on potassium concentration.

6 These results are compatible with the hypothesis that calcium antagonism in the ischaemic ventricular myocardium is antiarrhythmic during acute myocardial ischaemia.

Introduction

We have recently demonstrated that racemic (±)-verapamil dose-dependently reduces arrhythmias resulting from permanent coronary occlusion in conscious rats, and on the basis of this, and supplementary studies, we hypothesized that the antiarrhythmic actions occurred by virtue of calcium antagonism in the ventricles (Curtis *et al.*, 1984). To test this hypothesis, we have compared the antiarrhythmic actions of the optical enantiomers of verapamil. The hypothesis predicts an antiarrhythmic potency ratio equal to the calcium antagonist potency ratio in the ventricular myocardium, based on the reported potency difference between the enantiomers for calcium antagonism (Bayer *et al.*, 1975; Nawrath *et al.*, 1981; Ferry *et al.*, 1985).

The enantiomers have been previously shown to be equipotent blockers of the fast inward current (Nawrath *et al.*, 1981), but effective concentrations were greater than those required to reduce ventricular contractility to below 50%. If the antiarrhythmic actions of (±)-verapamil are a result of Na^+ channel blocking properties, then the optical enantiomers should be equally antiarrhythmic. As an indirect measure of the Na^+ channel blocking-dependent antiarrhythmic potency ratio, we measured the effects on the QRS of the ECG, and electrically-induced fibrillo-flutter in conscious rats, variables which are sensitive to Na^+ channel blocking agents such as quinidine (Curtis *et al.*, 1984).

Verapamil was recently found to exhibit stereoselective plasma protein binding and hepatic metabolism in humans (Eichelbaum *et al.*, 1984; Vogelgesang *et al.*, 1984; Echizen *et al.*, 1985). If the same phenomena occur in rats there is a possibility that calcium antagonist potency ratios *in vivo* and *in vitro* may be different. Therefore, we compared the potency ratios of the enantiomers *in vivo* (reductions in blood pressure, and P-R interval prolongation) with those obtained *in vitro* (negative inotropic actions) in isolated Langendorff-perfused ventricles. Since ex-

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tracellular potassium (K^+) concentration was shown to rise rapidly following coronary artery occlusion (Hirche *et al.*, 1980), *in vitro* experiments were carried out using a range of K^+ concentrations in the perfusing solution, in order to examine the dependence of absolute and relative potencies of the enantiomers on K^+ concentration.

Methods

Coronary occlusion in conscious rats

Most of the methods used have been described previously (Johnston *et al.*, 1983; Curtis *et al.*, 1984). Male Sprague-Dawley rats (250–320 g) were prepared with a loose indwelling coronary occluder, V_3 ECG leads and aortic blood pressure lines. The occluder was implanted close to the origin of the left anterior descending coronary artery in order to produce large occluded zones. An i.v. cannula (vena caval) was implanted for drug administration. After 6–8 days, the conscious rats were pretreated and subjected to coronary occlusion. Two groups of 9 rats received saline (controls). The remaining groups of 9 rats received either (−)-verapamil (0.2, 0.6, 2 or 6 mg kg^{-1}) or (+)-verapamil (0.4, 4, 8 or 12 mg kg^{-1}). Drugs were dissolved in saline and administered in a volume of 0.25 ml kg^{-1} by slow i.v. injection over 10 min, beginning 15 min before coronary occlusion. Haemodynamic and ECG responses to coronary artery occlusion were monitored for 4 h. An oscilloscope (Honeywell Type E for M) was used to assist the diagnosis of arrhythmias. If a rat experienced ventricular fibrillation (VF) or tachycardia (VT) lasting longer than 10 s, an attempt was made to restore sinus rhythm by tapping the chest. Upon death, or 24 h after occlusion, (whichever sooner) occlusion was verified *ex vivo* by dye exclusion (Fast green dye; B.D.H.). In rats surviving 24 h, the extent of infarction was determined by incubating ventricular tissue with 2,3,5-triphenyltetrazolium. Randomization to treatment, monitoring and analysis of records were carried out blind.

All of the methods outlined above, with the exception of the vena caval intravenous line, have been described in detail previously (Johnston *et al.*, 1983; Curtis *et al.*, 1984). In addition, we evaluated the effects of drug treatment on the ECG (P-R, QRS) by comparing values 15 min before occlusion (pre-drug) with those 1 min before occlusion (4 min after drug administration). Also, we expressed S-T segment elevation as %R-wave amplitude (ST%) according to Bernauer (1982), since we found the coefficient of variation of ST% to be significantly larger than that of the previously used measure, dSTR, in conscious rats (unpublished observations). Statistical analysis was carried out as previously described (Johnston *et al.*, 1983) with the supplementary use of chi^2 for ordered variables (Armitage, 1955). Gaussian distributed variables were expressed as mean ± s.e.mean. No differences were seen between the 2 control groups for any variable, and these groups were therefore amalgamated.

Electrically-induced arrhythmias in conscious rats

Male Sprague-Dawley rats (250–320 g) were prepared in a manner identical with that for rats used in the occlusion study, with the exception that instead of a coronary occluder, teflon-coated stainless steel wire electrodes (0.32 Ω cm^{-1} at 25°C) were implanted 0.3 cm apart into the left ventricle. All leads and lines were exteriorized at the subscapular region. After 6–8 days recovery from surgery, the threshold voltage and threshold pulse width for induction of fibrillo-flutter, and the maximum following frequency were determined in the conscious rats as previously described (Curtis *et al.*, 1984). Since we previously showed (Curtis *et al.*, 1984) that 6 mg kg^{-1} (±)-verapamil did not influence the variables under investigation (in contrast to quinidine), we evaluated only the equivalent of the higher doses used in the occlusion study. Mean % changes in variables were compared by analysis of variance and Duncan's multiple range test.

Contractility studies in isolated perfused ventricles

Hearts were excised from male Sprague-Dawley rats (250–320 g) and mounted for Langendorff perfusion using a 9 chamber, sealed, pressurised perfusion apparatus (Curtis *et al.*, 1986), designed for rapid switching between perfusing solutions (dead space approximately 0.1 ml). The perfusion pressure was set at 95 mmHg by gassing the chambers with 95% O_2 plus 5% CO_2. The atria were removed and the ventricles were paced at 4 V and 1 ms (supramaximal threshold voltage and pulse width) at 300 min^{-1}, using teflon-coated stainless steel plunge electrodes (0.32 Ω cm^{-1} at 25°C). The perfusing solution was a modified Krebs-Henseleit buffer comprising (in mmol l^{-1}) $CaCl_2$ 0.7, NaCl 118, KCl 1.8–8.8, $MgCl_2$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and dextrose 11, at 37°C and pH 7.4. We used 0.7 mmol l^{-1} $CaCl_2$ because in previous studies we have found the EC_{50} for positive inotropism in perfused rat ventricles to be 0.5 ± 0.1 mmol l^{-1} (Curtis *et al.*, 1984). KCl was varied at the expense of NaCl to produce K^+ concentrations from 3 to 10 mequiv. l^{-1} in order to simulate K^+ elevation seen in the extracellular fluid during the early period of myocardial ischaemia (Hirche *et al.*, 1980). Isochoric contractility was determined at a diastolic pressure of 15 mmHg using a non-elastic water-filled balloon connected to a pressure transducer. Perfusate temperature was monitored by insert-

ing a wire thermocouple into the pulmonary artery. Following stabilization, the ventricles were perfused with (+)- or (−)-verapamil (in half $\log_{10}$ increments) and steady state developed pressures recorded. Coronary flow was estimated by measuring the volume of perfusion fluid passing from the graduated reservoir chambers, under the assumption that if end diastolic pressure remained constant when perfusion pressure was varied from 80 to 140 mmHg, then the aortic valve was competent (Curtis *et al.*, 1986). This relation was tested during the stabilization period. Each preparation was used for a single drug at a single K^+ buffer concentration (3, 5.9, 8 or 10 mequiv. l^{-1}); therefore 8 groups of preparations ($n = 6$ per group) were used. Records were analysed blind. Concentration-response curves were constructed and EC_{50} and slope values were estimated for individual preparations. Potency ratios at each K^+ concentration were determined by contrasting every EC_{50} for (+)-verapamil with every EC_{50} for (−)-verapamil. Slopes, EC_{50} values and potency ratios were compared by analysis of variance and Duncan's multiple range test.

Results

Coronary occlusion

The effects of the enantiomers on blood pressure and heart rate immediately before and after coronary occlusion are shown in Figure 1. Pre-drug values were not statistically significantly different from one another. Before occlusion, blood pressure was reduced dose-dependently by both enantiomers. Only 0.2 mg kg^{-1} (−)- and 0.4 mg kg^{-1} (+)-verapamil did not produce statistically significant reductions compared with controls. (−)-Verapamil (Figure 1a) was approximately 4 times more potent than (+)-verapamil (Figure 1b). Both enantiomers elicited a small increase in heart rate at low doses, and a dose-dependent decrease in heart rate at higher doses. The heart rate changes between the enantiomers occurred in parallel with an approximate potency ratio of 4 in favour of (−)-verapamil.

Immediately following occlusion, blood pressure fell and heart rate increased in all groups. Changes

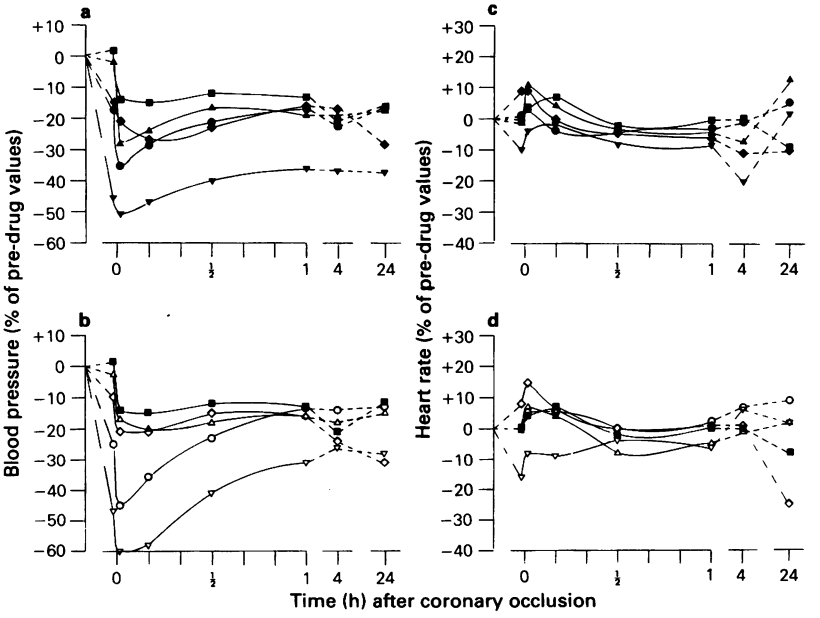

Figure 1 Mean % changes in blood pressure (a and b) and heart rate (c and d) before and after coronary occlusion in the conscious rat, and the effects of (+)- and (−)-verapamil. The s.e.mean values have been omitted for clarity. The abscissa scales indicate time (min) with respect to coronary occlusion. Broken lines indicate time discontinuity. The effects of (+)-verapamil (solid symbols) are shown in (a) and (c) and the effects of (−)-verapamil (open symbols) are shown in (b) and (d), (■) represents control values in (a–d). The symbols correspond with dose (in mg kg^{-1}); for (+)-verapamil (▲) 0.4, (◆) 4, (●) 8, (▼) 12, and for (−)-verapamil (Δ) 0.2, (◇) 0.6, (○) 2, (▽) 6. Statistical significance symbols have been omitted for clarity. A summary of full ANOVA data is available on request.

were essentially additive with the changes seen immediately before occlusion. By 30 min after occlusion, heart rate was similar in all groups, while blood pressure remained low only with 12 mg kg^{-1} (+)- and 6 mg kg^{-1} (−)-verapamil (the highest doses). By 4 h after occlusion, there were no ordered dose-related differences in blood pressure and heart rate between groups.

The effects of the enantiomers on P-R and QRS intervals are shown in Figure 2. Only the high dose of (+)-verapamil (12 mg kg^{-1}) and (−)-verapamil (6 mg kg^{-1}) prolonged P-R interval immediately before occlusion ($P < 0.05$). Neither enantiomer influenced QRS interval.

Treatment did not influence occluded zone (OZ) size, or infarct zone (IZ) size (whether expressed as a percentage of ventricular weight or as a percentage of OZ) (Table 1).

Mortality (Table 2) was low during the 4 h period following occlusion (12 deaths out of 90 rats) and occurred as a result of severe pulmonary oedema or cardiogenic shock. Manual defibrillation always resulted in the restoration of sinus rhythm. There were no differences in mortality between the groups.

Arrhythmias were reduced in incidence and severity in a dose-dependent manner by both enantiomers.

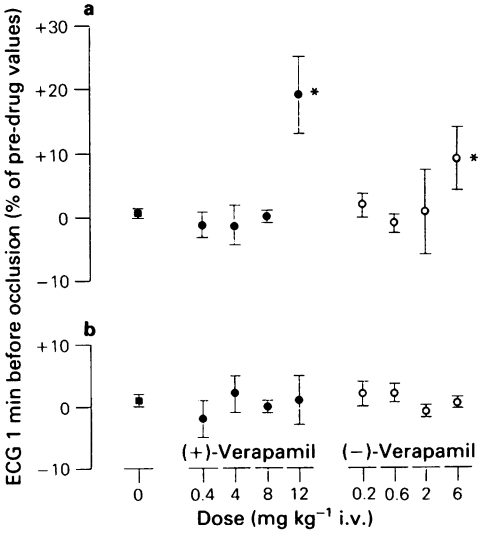

Figure 2 Effect of (+)- and (−)-verapamil on the P-R (a) and QRS (b) intervals. Values shown are mean % changes 1 min before occlusion with respect to pre-drug values. Symbols: (■) controls, (●) (+)-verapamil, (○) (−)-verapamil, and *$P < 0.05$ versus controls. The vertical lines represent s.e.mean.

Arrhythmias are summarized in terms of arrhythmia score (Johnston *et al.*, 1983) in Figure 3. Arrhythmia score was regressed with $\log_{10}$ dose, assuming linearity, parallelism and a potency ratio of 4, whether 0–30 min or 0–4 h data were considered. ED_{50} values were determined by interpolation. During the 0–30 min period following occlusion (Figure 3a) ED_{50} values were 2.4 mg kg^{-1} for (−)- and 9.6 mg kg^{-1} for (+)-verapamil. Corresponding ED_{50} values for the 0–4 h period (Figure 3b) were 5 and 20 mg kg^{-1}, respectively.

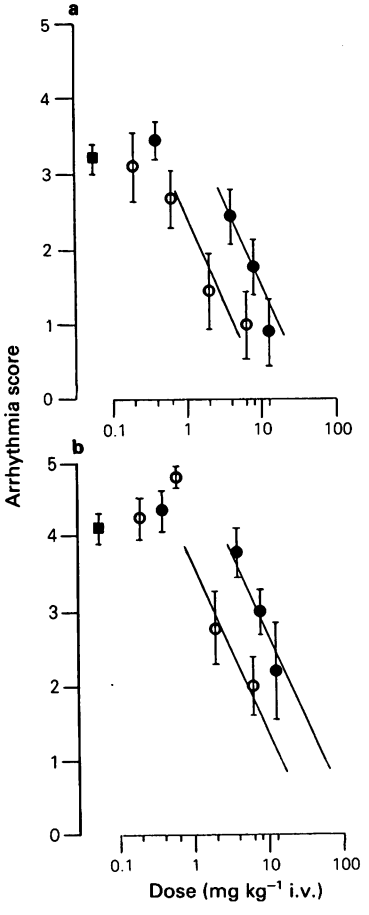

Figure 3 Mean arrhythmia scores for the 0–30 min (a) and 0–4 h (b) periods following coronary occlusion. Vertical lines show s.e.mean. The abscissa scales give dose in $\log_{10}$ scale. Symbols: (■) controls, (●) (+)-verapamil and (○) (−)-verapamil. Scores during both periods were significantly reduced ($P < 0.05$) versus controls for the 2 highest doses of both enantiomers (not shown for clarity). The 4 parallel lines were constructed assuming a potency ratio of 4 in favour of (−)-verapamil in order to estimate ED_{50} values.

Table 1 The extent of ischaemia and infarction, and ECG changes after occlusion

Dose of verapamil (mg kg^{-1})	n	*OZ%* (% ventricular weight)	*IZ%* (% ventricular weight)	*IZ* (%OZ)	*max ST%* (% of R)	*Time of max ST%* (min)	*Max R* (mV)	*Time of max R* (min)	*Time of Q-wave* (h)
Controls	18	39 ± 1	31 ± 1	81 ± 4	68 ± 4	8 (7– 9)	1.15 ± 0.07	3 (2– 4)	1.9 (1.7–2.2)
(+)-Verapamil									
0.4	9	37 ± 2	32 ± 1	86 ± 3	74 ± 5	11 (9–13)	1.02 ± 0.09	3 (2– 5)	2.6 (2.3–2.9)
4.0	9	40 ± 2	29 ± 2	75 ± 4	74 ± 7	9 (8–11)	1.31 ± 0.12	5 (4– 7)	1.7 (1.4–2.0)
8.0	9	39 ± 3	22 ± 4	59 ± 8	89 ± 10	11(9–14)	1.13 ± 0.14	4 (3– 7)	2.4 (2.1–2.7)
12.0	9	35 ± 2	29 ± 2	88 ± 6	87 ± 4	33 (24–46)*	1.47 ± 0.16	4 (3– 5)	2.8 (2.3–3.3)
(−)-Verapamil									
0.2	9	34 ± 3	29 ± 3	88 ± 7	64 ± 5	11 (9–13)	1.08 ± 0.08	7 (5–10)	1.8 (1.4–2.3)
0.6	9	37 ± 2	25 ± 4	70 ± 10	79 ± 5	12 (9–16)	1.06 ± 0.9	10 (9–11)	1.5 (1.3–1.9)
2.0	9	38 ± 3	27 ± 1	79 ± 6	72 ± 7	25 (18–36)*	1.00 ± 0.13	10 (6–16)	2.4 (2.0–2.9)
6.0	9	39 ± 3	27 ± 1	79 ± 4	70 ± 7	28 (16–49)*	0.98 ± 0.07	8 (5–13)	3.2 (2.7–3.7)

The occluded zone size (OZ) was measured by dye exclusion *ex vivo* and expressed as % ventricular weight. The infarct zone (IZ) was expressed as % ventricular weight and also as % OZ weight. Times to max ST%, max R and Q-wave development were calculated in $\log_{10}$ time, but expressed as mean (− 1 and + 1 s.e.mean) of real time for clarity. Other variables are mean ± s.e.mean. *Indicates $P < 0.05$ versus controls.

The incidences per group of spontaneously reverting ventricular fibrillation (SVF) and non-spontaneously reverting VF (NVF) are shown in Table 2. Both enantiomers dose-dependently reduced SVF and NVF. Since the control incidence of SVF was low, particularly during the first 30 min after occlusion, the reductions were statistically significant for NVF but not SVF, in general. The effects of the enantiomers on both types of VF considered together, and ventricular tachycardia (VT) are also shown in Table 2. ED_{50} values for reduction in VT and VF incidence were determined by interpolation. Both VT and VF incidence were reduced dose-dependently during the 0–30 min and 0–4 h periods following occlusion. (−)-Verapamil reduced VT and VF incidence more potently than (+)-verapamil, (potency ratio approximately 4). In contrast with the large reductions in VT and VF, the enantiomers produced only small reductions in the incidence of $\log_{10}$ premature ventricular contraction ($\log_{10}$ PVC) during the 0–30 min period following occlusion, and this effect was lost when the entire 0–4 h period following occlusion was considered

Table 2 Arrhythmias and mortality following coronary occlusion

Dose of verapamil		*VT incidence*		*VF incidence*		*SVF incidence*		*NVF incidence*		*$\log_{10}$ PVC*		*Mortality*	
(mg kg^{-1})	n	*0–30-min*	*0–4 h*	*0–30-min*	*0–4 h*	*0–30-min*	*0–4 h*	*0–30-min*	*0–4 h*	*0–30 min*	*0–4 h*	*0–4 h*	*0–24 h*
Controls	18	17/18	17/18	14/18	18/18	5/18	12/18	13/18	18/18	1.8 ± 0.1	2.8 ± 0.2	2/18	8/18
(+)-Verapamil													
0.4	9	9/9	9/9	8/9	8/9	3/9	6/9	8/9	8/9	1.8 ± 0.2	2.8 ± 0.2	3/9	5/9
4.0	9	8/9	9/9	5/9	8/9	1/9	5.9	4.9	8/9	1.5 ± 0.1	2.7 ± 0.2	2/9	4/9
8.0	9	4/9*	8/9	2/9*	4/9*	1/9	2/9	1/9*	3/9*	1.4 ± 0.3	2.6 ± 0.2	0/9	1/9
12.0	9	2/9*	4/9*	1/9*	3/9*	0/9	3/9	1/9*	3/9*	0.8 ± 0.2*	2.3 ± 0.3	1/9	1/9
(−)-Verapamil													
0.2	9	8/9	9/9	6/9	9/9	3/9	7/9	5/9	8/9	1.5 ± 0.2	2.5 ± 0.3	0/9	4/9
0.6	9	7/9	9/9	6/9	9/9	1/9	8/9	6/9	8/9	1.5 ± 0.2	2.9 ± 0.1	1/9	5/9
2.0	9	4/9*	7/9	2/9*	5/9*	1/9	5/9	1/9*	4/9*	1.3 ± 0.3	2.3 ± 0.1	1/9	2/9
6.0	9	3/9*	5/9	1/9*	2/9*	1/9	1/9*	0/9*	1/9*	0.9 ± 0.2*	2.1 ± 0.2	2/9	5/9

Values are incidence (out of *n*) of mortality, ventricular tachycardia (VT), ventricular fibrillation (VF), spontaneously reverting VF (SVF) and non-spontaneously reverting VF (NVF), and mean ± s.e.mean for other variables. Premature ventricular contractions (PVC) are expressed as $\log_{10}$ PVC number, since the number of PVC is a $\log_{10}$ normally distributed variable. *Indicates $P < 0.05$ versus controls.

(Table 2). Neither enantiomer delayed the onset of arrhythmias (not shown).

In 24 h survivors (10/18 controls, 25/36 (+)-verapamil-treated, 19/36 (−)-verapamil-treated), we observed frequent multifocal PVCs, but no VT or VF (not shown). The incidences of PVCs were 18/18, 19/25 and 15/19 in controls, (+)- and (−)-verapamil-treated groups, respectively (not statistically significant).

In addition to the antiarrhythmic actions of the enantiomers the ECG changes caused by coronary occlusion were delayed (Table 1), perhaps reflecting delays in the development of ischaemia. Both enantiomers delayed the development of 'S-T' segment elevation (max ST%), elevation in R-wave amplitude (max R) and the development of a Q-wave following coronary occlusion (Table 1), although the effects were only statistically significant in the case of time to max ST%. Neither maximum R-wave amplitude following coronary occlusion (max R) nor max ST% were statistically significantly altered by either enantiomer (Table 1).

Electrically-induced arrhythmias in conscious rats

At 5 min after drug administration, neither (+)- nor (−)-verapamil produced statistically significant alterations in threshold voltage or pulse width for induction of fibrillo-flutter, or maximum following frequency in conscious rats during electrical stimulation of the left ventricle (Figure 4).

Contractility studies in isolated perfused ventricles

Both enantiomers dose-dependently reduced contractility in paced rat ventricles. The slopes for both enantiomers were not significantly different from 1.0 at any K^+ concentration. However, EC_{50} values were dependent on buffer K^+ concentration (Figure 5). At 3 mequiv. l^{-1} K^+, mean EC_{50} values (and ± 1 s.e.mean from antilog) were 2.8 (2.4–3.3) μM for (+)- and 0.16 (0.13–0.2) μM for (−)-verapamil. At 10 mequiv. l^{-1} K^+, (+)-verapamil was 70 times more potent than it had been at 3 mequiv. l^{-1} K^+, while the potency of (−)-verapamil was increased 33 fold. Raising the

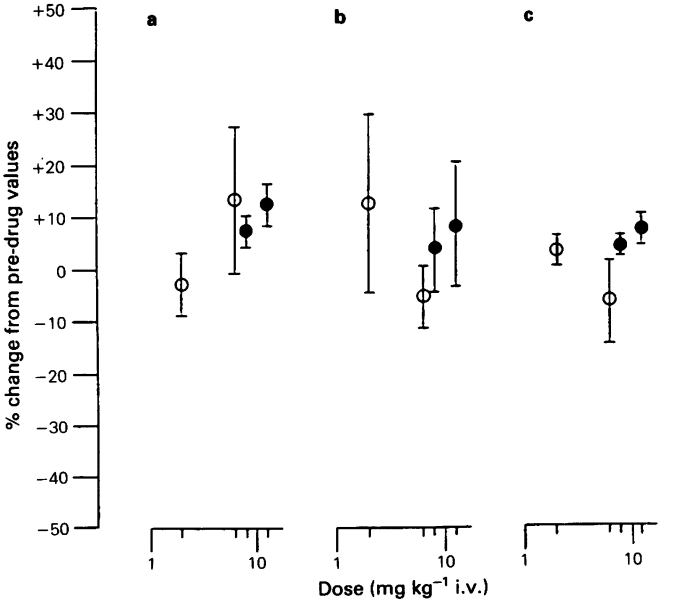

Figure 4 Effects of (+)-verapamil (●) and (−)-verapamil (○) on threshold voltage (a) and threshold pulse width (b) for induction of fibrillo flutter, and maximum following frequency (c), in conscious rats subjected to electrical stimulation of the left ventricle. Values shown are mean % changes 5 min after drug administration with respect to pre-drug values. Vertical lines indicate s.e.mean. The abscissae indicate dose in $\log_{10}$ scale. Treatment was not a statistically significant source of variance.

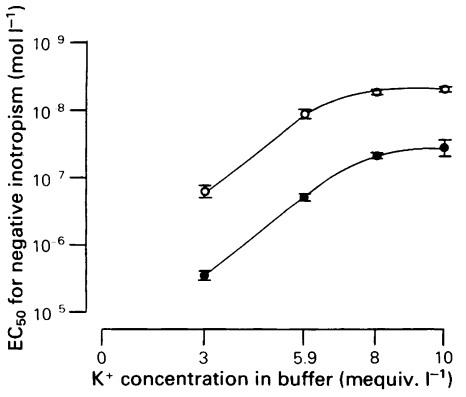

Figure 5 The relationship between negative inotropic potency of (+)-verapamil (●) and (−)-verapamil (○) and K^+ concentration in rat isolated Langendorff-perfused ventricles. The abscissa scale represents K^+ concentration in the perfusion and the ordinate scale mean EC_{50} for negative inotropism (calculated as $\log_{10}$ mol l^{-1} but shown as anti-log). Vertical lines indicate s.e.mean for $n = 6$ preparations.

buffer K^+ concentration increased the potency of (+)- more than (−)-verapamil, such that the potency ratio of (−) to (+) fell from 21.3 ± 2.6 at 3 mequiv. l^{-1} K^+, to 17.4 ± 1.1, 8.6 ± 0.4 and 8.3 ± 0.7 at 5.9, 8 and 10 mequiv. l^{-1} K^+, respectively. Ratios at 8 and 10 mequiv. l^{-1} K^+ were significantly smaller than those at 3 and 5.9 mequiv. l^{-1} K^+ ($P<0.05$). For both enantiomers, the relationship between EC_{50} and K^+ concentration was approximately linear between 3 and 8 mequiv. l^{-1}, whereupon the effect appeared to saturate (Figure 5). Neither control developed pressure nor control coronary flow correlated with K^+ concentration, although both tended to be lower at 3 mequiv. l^{-1} K^+ compared to the other K^+ concentrations. However, before the application of drugs, threshold voltage (at 1 ms) and threshold pulse width (at 4 mV) for capture were both elevated 3–4 fold (from 0.5 ± 0.04 mV and 0.068 ± 0.009 ms to 1.8 ± 0.1 mV and 0.31 ± 0.04 ms, respectively, $P<0.05$) by elevating K^+ from 3 to 8 mequiv. l^{-1}. We were unable to extend the study to K^+ values above 10 mequiv. l^{-1} since the ventricles either failed to capture, or only captured at unacceptably high voltages (>20V) under these conditions. Pulmonary artery temperature ranged from 36.8 ± 0.3 to 37.3 ± 0.3°C, and remained constant throughout each experiment. At 3 and 5.9 mequiv. l^{-1} K^+, episodes of PVC were observed (Table 3). PVC were absent at 8 and 10 mequiv. l^{-1} K^+. Both enantiomers dose-dependently reduced and abolished these PVC (Table 3), (−)-verapamil being much more potent than (+)-verapamil in this regard. In addition, the potency of both enantiomers for this antiarrhythmic action was enhanced by elevating K^+ from 3 to 5.9 mequiv. l^{-1} (Table 3).

Discussion

Both (+)- and (−)-verapamil possessed dose-dependent antiarrhythmic activity against coronary occlusion-induced arrhythmias in conscious rats (Figure 3 and Table 2). VT and VF appeared to be reduced more effectively than PVC. (−)-Verapamil was consistently 4 times more potent in this respect than (+)-verapamil, irrespective of whether the incidence of VT, VF or arrhythmia score was being considered. The antiarrhythmic potency ratio corre-

Table 3 Incidence of premature ventricular contractions (PVC) in isolated ventricles

	Drug concentrations									
	0	1 nM	3 nM	10 nM	30 nM	100 nM	300 nM	1 μM	3 μM	10 μM
Buffer K^+ (mequiv. l^{-1})	(−)-Verapamil									
3	–	–	6	6	4	0*	0*	0*	–	
5.9	6	6	5	2	0*	0*	0*	–	–	–
8	0	0	0	0	0	0	0	–	–	–
10	0	0	0	1	0	0	0	–	–	–
	(+)-Verapamil									
3	6	–	–	–	6	6	6	2	0*	0*
5.9	6	–	–	6	6	4	2	0*	0*	–
8	0	–	–	0	0	0	0	0	0	–
10	0	–	–	0	0	0	0	0	0	–

Incidence (out of $n = 6$) of spontaneous PVC in rat isolated ventricles perfused with 0.7 mmol l^{-1} $CaCl_2$ at 300 beats min^{-1} under various buffer K^+ conditions. *Indicates $P<0.05$ versus pre-drug incidence.

sponded with that for effects on heart rate and blood pressure seen immediately before coronary occlusion (Figure 1). The potency ratio for the antiarrhythmic actions corresponded reasonably well with recent estimates of the calcium antagonist potency ratio for the enantiomers in ventricular muscle, based on pharmacological and radioligand binding studies (Ferry *et al.*, 1985).

The potency ratio for the enantiomers determined in isolated ventricles was dependent on the concentration of K^+ in the perfusing buffer (Figure 5). The mechanism for this effect is unclear. Similar potentiating effects of small increases in K^+ have been found for the effect of lidocaine on V_{max} of the fast inward current (Singh & Vaughan Williams, 1971). Elevation of K^+ from 5.4 to 8 mequiv.l^{-1} has been previously shown to produce only a small depolarization of resting membrane potential in ventricular tissue (Buchanan *et al.*, 1985) of insufficient magnitude to affect steady state activation and inactivation of i_{si} (Reuter, 1979). Therefore the K^+ ion appeared to alter the potency of the verapamil enantiomers directly, perhaps associated with the observed reduction in excitability of the ventricles.

Elevations in K^+ reduced the potency ratio of the enantiomers (from 21 to 8), such that in raised K^+, the potency ratio was similar to the antiarrhythmic potency ratio during myocardial ischaemia. In this regard, it is important to consider that within 5–10 min of coronary occlusion, extracellular K^+ rises to 8–15 mequiv.l^{-1} in the ischaemic tissue (Hirche *et al.*, 1980). It is suggested, therefore, that in acutely ischaemic ventricular muscle, the calcium antagonist potency ratio of (−)- to (+)-verapamil should be 8 or less, if extracellular K^+ is an important determinant of potency.

The calcium antagonist potency ratio *in vivo* may be even less than 8 in the ischaemic myocardium, in terms of dose, if pharmacokinetic factors are considered. In humans, verapamil has been shown to exhibit stereoselective hepatic clearance (Vogelgesang *et al.*, 1984) and plasma protein binding (Echizen *et al.*, 1985). Since hepatic clearance of (−)-verapamil was much greater than that of (+)-verapamil, then, in rats, the more rapid metabolic rate may serve to reduce the potency ratio in terms of dose. In human tissue the potency ratio of the enantiomers *in vivo* for prolonging PR interval, in terms of unbound plasma concentration (Echizen *et al.*, 1985), and the negative inotropic potency ratio *in vitro* (Ferry *et al.*, 1985) have been found to be 4.5 and 8 in favour of (−)-verapamil, respectively. These values correspond almost exactly with the antiarrhythmic and *in vitro* negative inotropic potency ratios in raised K^+ solution, respectively, described here.

The spontaneous occurrence of PVC, and inhibition of such by the enantiomers, in isolated ventricles, were 2 phenomena which were K^+-dependent (Table 3). To our knowledge, such PVC have not been previously investigated. (−)-Verapamil was more potent than (+)-verapamil in reducing these PVC, in concordance with reductions of ischaemia-induced arrhythmias.

In rats, elevations in serum K^+ are associated with reductions in ischaemia-induced arrhythmias (Curtis *et al.*, 1985b). Myocardial ischaemia is, however, associated with regional elevations in extracellular K^+ (Hirche *et al.*, 1980). It is possible, therefore, that early arrhythmias during myocardial ischaemia depend, in part, on a heterogeneity of extracellular K^+ between the normal and ischaemic myocardium. Since the enantiomers clearly did not abolish conduction in the non-ischaemic ventricular tissue *in vivo*, a selective action in the ischaemic tissue leading to a reduction in arrhythmias is an obvious hypothesis. The high extracellular K^+ concentration seen in acutely ischaemic tissue is therefore a candidate for mediation of such selectivity of action, provided that the actions of verapamil on arrhythmogenesis during ischaemia depend on K^+, in a similar manner to the actions of verapamil on spontaneous PVC and the force of contraction in isolated ventricles.

Irrespective of the reasons for the small discrepancy in potency ratios *in vivo* versus *in vitro* in the present study, and the possible roles of extracellular K^+, it nevertheless remains that the antiarrhythmic potency ratio corresponded with the potency ratio for effects on blood pressure and heart rate *in vivo*, reductions in which were presumably a result of calcium antagonism (Nayler & Horowitz, 1983).

We consider the above findings to support the hypothesis that the antiarrhythmic actions of both enantiomers during acute myocardial ischaemia occur by virtue of calcium antagonism, rather than via quinidine-like Na^+ channel blockade. The latter property has previously been shown to be expressed by both enantiomers, but only at concentrations 35–150 fold in excess of those required to abolish i_{si}, and not in a stereoselective manner (Nawrath *et al.*, 1981). With regard to this property, we have previously shown that quinidine at doses reducing ischaemia-induced arrhythmias by 50% (20 mgkg^{-1}) protects against electrically-induced arrhythmias, and increases maximum following frequency and QRS interval (Curtis *et al.*, 1984). Neither enantiomer affected these variables at doses which protected against ischaemia-induced arrhythmias in the present study. In addition, Raschack (1976) showed that in anaesthetised rats, (+)-verapamil reduced aconitine-induced arrhythmias, while high doses of (−)-verapamil (which caused second degree atrioventricular block) were ineffective, suggesting that for (−)-verapamil, plasma concentrations effective in reducing the fast inward current (and associated aconitine-induced arrhythmias) cannot be safely reached.

There were 2 differences between this study and the previous one with (±)-verapamil (Curtis *et al.*, 1984). Firstly, arrhythmia-induced mortality was abolished (previous control incidence was 30%) by improving the defibrillation technique, in order to reduce the censoring of data produced by early death (this is the object of defibrillation in our studies), allowing for improved precision of variables. Secondly, non-arrhythmic mortality (cardiogenic shock) was lower in the present study. However, most non-arrhythmic deaths occurred previously with $20\,mgkg^{-1}$ (±)-verapamil (Curtis *et al.*, 1984) and the doses used in the present study were lower. Nevertheless, if the ED_{50} for (±)-verapamil during the 0–30 min period ($6\,mgkg^{-1}$) is compared with that for (−)-verapamil ($2\,mgkg^{-1}$) and (+)-verapamil ($10\,mgkg^{-1}$), it is apparent that the order of potency (− > ± > +) corresponds with that expected if calcium antagonism alone, rather than Na^+ channel blockade alone, or a combination of the 2 properties was responsible for the antiarrhythmic actions observed.

It was suggested by Mueller & Wilsmann (1982) that the antiarrhythmic actions of D-600 (desmethoxy-verapamil) during myocardial ischaemia resided mainly in the (+)-enantiomer. However, this conclusion was based on a single low dose study, carried out in anaesthetized rats. In our experience (Curtis *et al.*, 1984) anaesthetized rats are much more sensitive to the haemodynamic effects of phenethylamine calcium antagonists than conscious rats. In addition, according to Fagbemi *et al.* (1984), in anaesthetized rats, (±)-verapamil possesses a bell-shaped dose-response curve for the reduction of occlusion-induced arrhythmias. Therefore, since D-600 and verapamil possess qualitatively similar properties, the conclusions of Mueller & Wilsmann were by no means unequivocal. In conscious rats we administered much higher doses of (±)-verapamil, and the log dose-response curves for the reduction of ischaemia-induced arrhythmias were approximately sigmoidal (Curtis *et al.*, 1984). The present study demonstrated that this also appeared to be the case for the optical enantiomers of verapamil.

Although the present study supports the hypothesis that the enantiomers of verapamil exerted their antiarrhythmic actions via calcium antagonism rather than Na^+ channel blockade, other explanations may be considered, for example, α-adrenoceptor blockade. However, current evidence does not support such an explanation, for the following reasons. Firstly, it is unclear whether verapamil truly influences α-adrenoceptors. While it has been claimed that verapamil is a selective antagonist of α_2-adrenoceptors, having little effect on α_1-adrenoceptor mediated responses (Timmermans *et al.*, 1983; Cavero *et al.*, 1983), it has also been claimed that the opposite is the case (Vanhoutte, 1982). Proponents of the α_2-selective hypothesis suggest that the antagonist action of verapamil is non-competitive, and report that verapamil has almost negligible affinity for the α_2-receptors themselves (Van Meel *et al.*, 1981). It was also suggested that while verapamil inhibited α-adrenoceptor agonist-induced inotropic responses in atria, the effect was not mediated by α-adrenoceptor antagonism (Tung *et al.*, 1985). Secondly, it is doubtful whether α-adrenoceptor activation is of pathological importance in arrhythmogenesis during acute myocardial ischaemia. In non-ischaemic heart tissue, pharmacological and electrophysiological studies revealed that α-adrenoceptor agonism was mediated essentially by the α_1-subtype and was expressed as a prolongation of action potential duration (Dukes & Vaughan Williams 1984; Black *et al.*, 1985). It was argued that this electrophysiological effect should be antiarrhythmic rather than arrhythmogenic (Dukes & Vaughan Williams 1984). During myocardial ischaemia, α-adrenoceptor antagonism was shown to reduce arrhythmias in cats (Sheridan *et al.*, 1980), but identical or similar treatment was ineffective in dogs (Bolli *et al.*, 1984; Coker *et al.*, 1984, respectively). In addition, the reported antiarrhythmic actions of α-adrenoceptor antagonists in rat isolated ischaemic hearts was not attributed to α-adrenoceptor antagonism (Daugherty & Woodward, 1982), rather to 'membrane stabilizing' effects of the drugs studies (Bralet *et al.*, 1985). Finally, we have shown that in rats, neither combined α- and β-adrenoceptor blockade nor sympathectomy reduced arrhythmias (Botting *et al.*, 1983), while graded ablations in the CNS and catecholamine infusions did not influence arrhythmias in a manner consistent with a role for α- (or β-)adrenoceptors in arrhythmogenesis during acute myocardial ischaemia (Curtis *et al.*, 1985c). Therefore we consider it highly improbable that verapamil reduced ischaemia-induced arrhythmias in conscious rats via myocardial α-adrenoceptor antagonism.

It may be suggested that the verapamil enantiomers exerted their antiarrhythmic actions indirectly by reducing afterload or heart rate. However, this also may be ruled out since we have found no correlation between either blood pressure or heart rate and arrhythmias in conscious rats (Johnston *et al.*, 1983), and felodipine, a vascular selective calcium antagonist (Au & Sutter, 1984) produced large reductions in blood pressure without concomitant reductions in arrhythmias (Curtis *et al.*, 1985a).

In addition to their antiarrhythmic actions, both enantiomers delayed the development of ECG indices of ischaemia ('S-T' elevation, Table 1), although infarct size was not reduced, in agreement with previous studies with (±)-verapamil in the rat (Curtis *et al.*, 1984; Baur *et al.*, 1984; Evans *et al.*, 1985). The delay in 'S-T' segment elevation did not appear to correlate with arrhythmias. In this regard, felodipine

also delayed 'S-T' segment elevation in conscious rats but did not reduce arrhythmias during myocardial ischaemia (Curtis *et al.*, 1985a).

In conclusion, the antiarrhythmic actions of the optical enantiomers of verapamil during myocardial ischaemia corresponded with their calcium antagonist potency ratios in isolated ventricles under conditions of raised K^+. Calcium antagonism in the ischaemic ventricular myocardium appears to be the most likely explanation for the antiarrhythmic actions of verapamil in acute myocardial ischaemia.

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