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# Antiarrhythmic actions of verapamil against ischaemic arrhythmias in the rat

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**1** The actions of intravenous verapamil against arrhythmias induced by occlusion of a coronary artery were investigated in conscious rats.

**2** Verapamil (2–20 mg $kg^{-1}$, i.v. given pre-occlusion) dose-dependently reduced arrhythmias in rats with either large or small occluded zones at an $ED_{50}$ of 6 mg $kg^{-1}$. This dose was effective when given immediately post-occlusion.

**3** Severe arrhythmias, as opposed to PVC, were preferentially reduced.

**4** In conscious, and pentobarbitone-anaesthetized rats, verapamil (6 mg $kg^{-1}$) had different effects on electrically-induced arrhythmias, and the ECG, from an equi-effective anti-arrhythmic dose of quinidine (20 mg $kg^{-1}$, i.v.). Quinidine decreased following frequency, but increased threshold current and pulse width, whereas verapamil did not. Both drugs increased P–R interval, but only quinidine increased QRS and Q–T intervals.

**5** Thirty minutes post-occlusion, the verapamil content of tissue and blood was determined after a 6 mg $kg^{-1}$ dose given pre- or post-occlusion. Measurable levels of verapamil were found in both normal and ischaemic myocardium. Plasma and plasma water concentrations were $3.6 \pm 0.8$ $\mu$mol $l^{-1}$ and $0.6 \pm 0.1$ $\mu$mol $l^{-1}$ ($\bar{x} \pm$ s.e.mean), respectively following post-occlusion administration vs. $2.7 \pm 1.2$ and $0.24 \pm 0.04$ for pre-occlusion administration.

**6** Plasma water concentrations were close to $IC_{50}$ values for inhibition of contractility in rat atria and ventricles. Similar concentrations depressed slow action potentials induced in rat ventricles by raised $K^+$

**7** We suggest that the ability of verapamil to prevent severe ventricular arrhythmias following myocardial ischaemia in the conscious rat is largely due to the calcium antagonist effects of the drug.

## Introduction

A variety of drugs have been shown to reduce experimental arrhythmias due to irreversible myocardial ischaemia. Unfortunately in many experiments only single doses were studied by methods of uncertain precision and accuracy. Our method of occluding a coronary artery in conscious rats allows responses to ischaemia and infarction to be assessed with precision and accuracy. The model has been used previously to assess established antiarrhythmics (Johnston *et al.*, 1983a), prostacyclin and aspirin (Johnston *et al.*, 1983b), halothane (MacLeod *et al.*, 1983) and similar anaesthetics (Jang *et al.*, 1983). Arrhythmias in this model were not reduced by β-blockade or sympathectomy (Botting *et al.*, 1983). We have now investigated the antiarrhythmic actions of verapamil in this model in conjunction with studies into other actions of verapamil on rat myocardium.

Verapamil is well-established as a treatment for supra-ventricular antiarrhythmias (Surawicz, 1982). Antiarrhythmic activity has also been demonstrated in a variety of experimental preparations. Verapamil (0.1 to 0.5 mg $kg^{-1}$) has antiarrhythmic actions against arrhythmias induced by coronary occlusion in dogs (Brooks *et al.*, 1980; Temesy *et al.*, 1982; Thale *et al.*, 1982). Verapamil has also been reported to be antiarrhythmic following coronary artery occlusion in the acutely prepared anaesthetized rat (Bernauer, 1982; Mertz & Kaplan, 1982). Attempts were not made to determine the mechanism or site of action of verapamil. Unfortunately in these, and other studies, a range of doses of verapamil was not studied. The doses which were studied appeared to correspond with those used clinically for supraventricular arrhythmias. Bolus injections of verapamil (0.1 mg $kg^{-1}$) may transiently raise plasma concentrations sufficiently to reduce calcium currents in the

atrioventricular node, and so abruptly terminate supra-ventricular tachyarrhythmias, but there is no reason to assume that such a bolus will continuously suppress ischaemia-induced ventricular arrhythmias. In order to reduce ischaemia-induced ventricular arrhythmias it is possible that verapamil concentrations exceeding those of benefit in supraventricular arrhythmias must be maintained.

To investigate verapamil's possible antiarrhythmic actions against ischaemic-induced ventricular arrhythmias we performed a dose-response study of verapamil's antiarrhythmic action in conscious rats. We subsequently analysed further the action of verapamil (at an antiarrhythmic $ED_{50}$) by pharmacological and pharmacokinetic studies.

## Methods

### *Occlusion in conscious rats*

Our method of producing coronary occlusion in conscious rats has been used in a number of studies (see above for references). The model's precision and accuracy, have been described in detail (Johnston *et al.*, 1981; 1983a). In brief, a coronary artery occluder, aortic and venous cannulae, and permanent ECG leads were surgically implanted under halothane anaesthesia seven days before testing. The occlusion site was varied to produce either large (LOZ), or small occluded zones (SOZ). Zone size was pre-determined by choosing a high or low occlusion site on the LAD coronary tree.

On the day of study the animal was placed in its home cage with leads and cannulae connected. After 30 min, appropriate control or verapamil solutions were given by intravenous infusion over 10 min in a total volume of 1 ml or less. Thereafter, a further 10 min elapsed before occlusion. One group of SOZ rats received verapamil 6 mg $kg^{-1}$ infusions, beginning immediately post-occlusion, and continuing for 15 min. Infusions were temporarily stopped if diastolic blood pressure fell below 60 mmHg.

Different occluded zone size groups were used since previous studies (Johnston *et al.*, 1983a) showed that arrhythmia scores following occlusion are a square root function of the occluded zone volume (i.e., a linear function of the interface area). Furthermore, non-arrhythmic mortality is low in SOZ rats. Treatments, procedures and record analyses were performed blind and randomized.

### *Antiarrhythmic studies*

*Ischaemic arrhythmias* Four doses of verapamil (0.2, 2, 6 and 20 mg $kg^{-1}$) were tested in LOZ (greater than 30% of total ventricular weight) rats. Two, 6 and 20 mg $kg^{-1}$ were also tested in SOZ (less than 30% of total ventricular weight) rats. Five control groups (2 of SOZ, and 3 of LOZ) were used. All groups, treated and control, contained nine rats.

After occlusion rats were continuously monitored for 4 h, or until death. Survivors for 24 h were monitored for another 30 min before they were killed and their hearts excised. Perfusion of the heart with dye was used to determine the occluded zone (OZ) followed by tetrazolium for determination of the infarct zone (IZ) (Johnston *et al.*, 1983a). In animals dying before 24 h only OZ was determined.

Arrhythmias, arrhythmia score, occluded and infarcted zones, blood pressure, heart rate, ECG and mortality were recorded. Statistical analyses was applied as previously described (Johnston *et al.*, 1981; 1983a).

*Electrically-induced arrhythmias* Verapamil and quinidine were tested against electrically-induced arrhythmias in conscious and anaesthetized rats by methods similar to those of Martinez & Crampton (1981) and Marshall *et al.* (1983). Stainless steel electrodes were permanently implanted, 0.3 cm apart, into the left ventricle, and their free ends exteriorised in the neck region. Stimulation parameters for fibrillo-flutter were measured in chronically prepared conscious, and acutely prepared pentobarbitone (50 mg $kg^{-1}$ i.p.) anaesthetized rats. The threshold current (at 50 Hz and 0.8 ms) inducing a precipitous fall in blood pressure was determined together with the threshold pulse width (at 50 Hz and 2 × threshold current) and maximum following frequency (at 0.8 ms, 2 × threshold current). The latter was revealed as an abrupt large increase in pulse pressure as the heart failed to follow, on a 1 : 1 basis, a steadily increasing frequency of stimulation from 5 to 30 Hz. ECG and blood pressure records showed that, as the frequency of stimulation increased, the heart was eventually 'captured' with resulting changes in the axis of the ECG. This ECG change was maintained as the frequency increased while pulse and mean blood pressures fell. Eventually the heart failed to follow stimulation on a 1 : 1 basis and, as a result of the dropped beat, the heart was able to fill during diastole resulting in a large increase in pulse pressure for the next beat. The ECG showed the missing beat, and sometimes a change in complex shape. As this first missed beat produced such an easily discernible change in blood pressure it was used as the clearest end point. The mean of three determinations were obtained 5 min before, and 5, 15, 45 and 60 min after drug administration.

Anaesthetized rats received only one drug with no saline control, while each conscious animal, acting as its own control, received each drug, and saline, with 3 days between tests.

Verapamil (6 mg kg$^{-1}$) was compared with a dose of quinidine (20 mg kg$^{-1}$) previously shown to reduce ischaemia-induced arrhythmias by 50% in our model (Johnston *et al.*, 1983a). Quinidine was given by slow injection over 5–10 min whereas verapamil was infused slowly to avoid hypotension. Verapamil infusions took 40 min in anaesthetized rats but only 5 min in conscious rats.

From ECG records obtained on a Grass polygraph (Model 79B pre-amplifier type 7P1A, band width 0.8–50 Hz), P–P, P–R, QRS and Q–T intervals were measured. The first two variables were conventionally measured whereas QRS was measured from Q to the isoelectric point on the RS wave. The T wave is not easily seen in the rat ECG and so a standardized postion on the S–T complex was taken as a measure of the position of T. ECG records were analysed in a blind manner from records obtained at 100 mm s$^{-1}$ chart speed.

*Tissue and Blood levels of verapamil*

Tissue and blood levels of verapamil were determined 30 min post-occlusion after 6 mg kg$^{-1}$ i.v. given either pre- or post-occlusion in SOZ rats. Thirty minutes after occlusion, which is after the major period for arrhythmias, animals were killed and heart and blood collected. On the basis of visual inspection hearts were divided into ischaemic and non-ischaemic tissue. Small samples were also taken from the centre of the ischaemic zone. Verapamil concentrations were determined in myocardial tissue, in blood, in plasma, and in plasma ultrafiltrates (plasma centrifuged with CF25 Centriflo Amicon membranes at 2100 *g*) by modifications of the published techniques for extraction of blood (Cole *et al.*, 1981) and tissue (McAllister & Howell, 1976) with subsequent detection by h.p.l.c. (Kuwada *et al.*, 1981).

In vitro *studies on contractility and intracellular potentials*

These were designed to determine the myocardial effects of a concentration range of verapamil commensurate with those found *in vivo*. Right and left atria were dissected from hearts of 300 g Sprague-Dawley rats and suspended in Krebs-Henseleit solution at 35° C as previously described (Au *et al.*, 1980). Initial cumulative calcium concentration-response curves were obtained (for inotropism) and

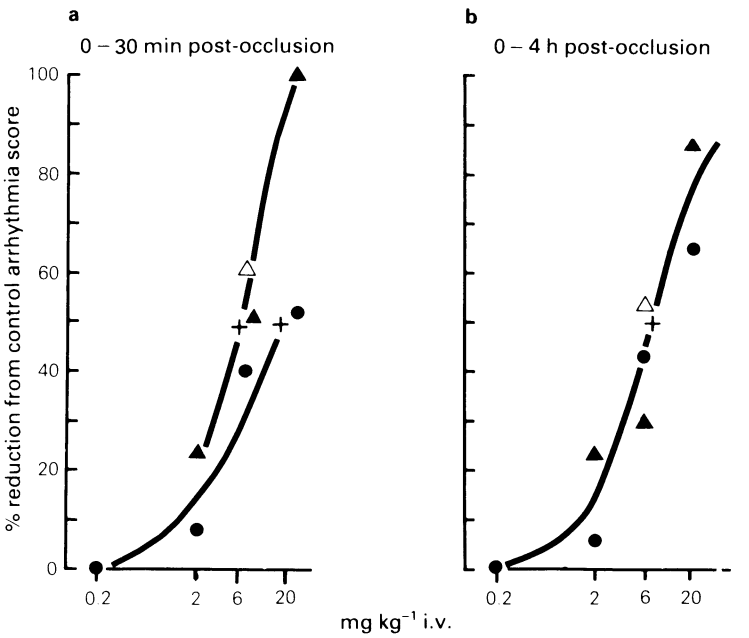


**Figure 1** The effect of various doses of verapamil on mean arrhythmia scores in rats with large or small occluded zones. Various doses of verapamil were given i.v. pre-occlusion to rats with large (●) or small (▲) occluded zones. Symbol (△) indicates 6 mg kg$^{-1}$ verapamil given post-occlusion. The % reduction in arrhythmia score from the appropriate mean control value (LOZ or SOZ) is given on the ordinate and $\log_{10}$ dose in the abscissa scale. Arrhythmia scores (see Methods) were measured for the 0–30 min (a) and 0–4 h (b) post-occlusion periods. Altogether 13 groups of rats (9 rats per group) were studied and values for the 2 SOZ and 3 LOZ groups of controls were accumulated. The crosses indicate estimates of $ED_{50}$ values.

then, in the presence of the determined $EC_{50}$ concentration of calcium, cumulative negative inotropism curves were obtained for verapamil. Calcium and verapamil dose-response curves were also determined for perfused rat ventricles stimulated at 180 or 400 beats $min^{-1}$. Developed pressure within ventricles was measured with intraventricular balloons at a diastolic pressure of 15 mmHg.

The method of Inoue *et al.* (1979) was used to ascertain the effect of verapamil on slow calcium potentials generated in the guinea-pig papillary, and ventricle muscle, by exposure to 20 mM potassium and $10^{-6}$ M isoprenaline.

## Results

### *Dose-response data for antiarrhythmic efficacy of verapamil*

Verapamil 0.2–20 mg $kg^{-1}$ i.v. produced a dose-dependent reduction in the arrhythmias induced by coronary occlusion as judged by reductions in arrhythmia score from control values (Figure 1a and b). The arrhythmia score summarises the arrhythmic history following coronary occlusion. Twenty mg $kg^{-1}$ was almost 100% effective in suppressing occlusion-induced arrhythmias in both the 0–30 min

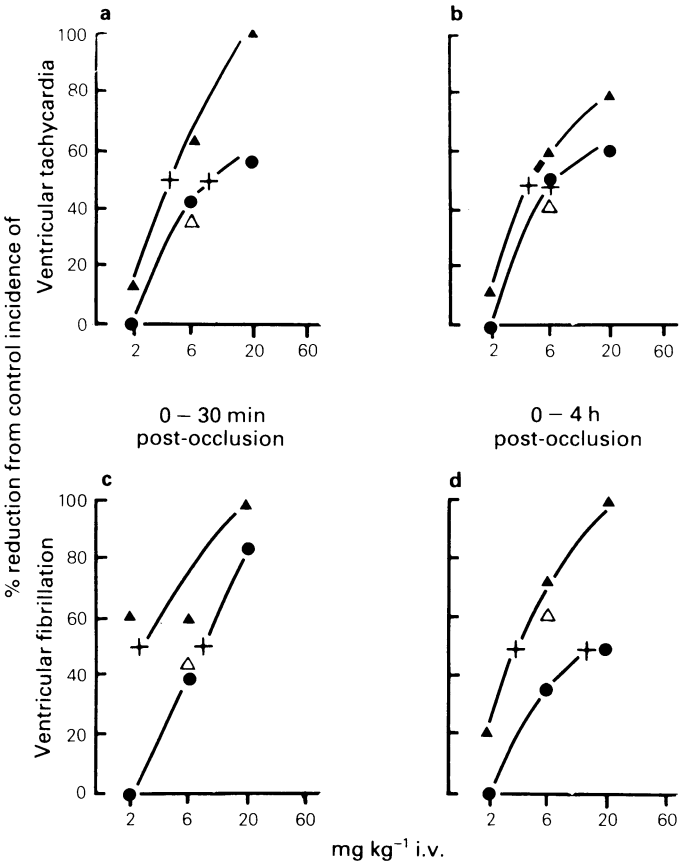


**Figure 2** The effect of various doses of verapamil on the incidence of ventricular flutter (a and b) and fibrillation (c and d) in rats with large or small occluded zones. In (a) and (b) the incidence of ventricular flutter in groups of LOZ or SOZ rats given 2, 6, or 20 mg $kg^{-1}$ verapamil pre-occulsion is expressed as the percentage reduction from control incidence (LOZ or SOZ) 0–30 min (a) or 0–4 h (b) after occlusion. Data from the 0.2 mg $kg^{-1}$ LOZ group is excluded from the figure. The symbol (●) indicates LOZ rats and (▲) indicates SOZ rats. In (c) and (d) the incidences of ventricular fibrillation are shown: 0–30 min (c) and 0–4 h post-occlusion (d). The open symbol refers to SOZ rats given 6 mg $kg^{-1}$ post-occlusion. Crosses indicate estimates of $ED_{50}$ values. The control incidences of ventricular flutter for SOZ were 67% (0–30 min) and 78% (0–4 h). For ventricular fibrillation the incidences were 56% and 89% respectively. For LOZ the corresponding figures were 74% and 82% for tachycardia and 74% and 89% for fibrillation.

and 0–4 h post-occlusion arrhythmic periods (see also Johnston *et al.*, 1981; 1983a). The $ED_{50}$ (i.e., the dose producing a 50% reduction in arrhythmia score from control) was approximately 6 mg $kg^{-1}$, in both SOZ and LOZ rats. In the LOZ groups, animals were lost from the study because of non-arrhythmic deaths. These deaths occurred at 30, 30 and 180 min post-occlusion in rats given 2 mg $kg^{-1}$ and 16, 21, 25, 28, 31 and 34 min post-occlusion with 20 mg $kg^{-1}$. Thus most deaths occurred after the first major arrhythmic period (4–15 min post-occlusion) in the 0–30 min post-occlusion observation period. Arrhythmias were suppressed by verapamil in both SOZ and LOZ rats, despite differences in control arrhythmia scores for the two groups. Control arrhythmia scores in SOZ rats were $3.3 \pm 0.5$ ($x \pm$ s.e.mean) and $4.2 \pm 0.5$ for the 0–30 min and 0–4 h periods, respectively, versus $4.8 \pm 0.5$ and $5.3 \pm 0.4$ in LOZ. Verapamil (6 mg $kg^{-1}$) given post-occlusion to SOZ rats was just as effective as when given pre-occlusion.

Previous studies (Johnston *et al.*, 1983a) showed the relationship between arrhythmia score and occluded zone size to be such that arrhythmia score (AS) = a + bI, where a = intercept, b = slope and I = estimated area of interface between ischaemic and normal tissue. Control rats in this study obeyed the same relationship with coefficients of a = 0 $cm^2$ and b = 6.5 AS/$cm^2$ for an assumed average ventricle wall thickness of 0.25 cm and the radius of a 50% OZ to be 0.65 cm. Data for verapamil also obeyed the same linear relationship with a common slope and variable extrapolated intercepts (a) of 0.2 $cm^2$ for 2 mg $kg^{-1}$ verapamil, 0.3 $cm^2$ for 6 mg $kg^{-1}$ verapamil and 0.6 $cm^2$ for 20 mg $kg^{-1}$ verapamil.

The antiarrhythmic effectiveness of verapamil was also seen as a reduced incidence of ventricular flutter (a,b) and fibrillation (c,d) (Figure 2). Verapamil was most effective in reducing the incidence of these severe arrhythmias in SOZ rats. Twenty mg $kg^{-1}$ verapamil prevented almost all severe arrhythmias, whether flutter (a,b) or fibrillation (c,d), especially in SOZ versus LOZ rats. The 6 mg $kg^{-1}$ post-occlusion dose in SOZ rats was less effective against severe arrhythmias than the pre-occlusion dose. The group incidence of fatal irreversible ventricular fibrillation was also reduced by verapamil (see Table 2).

While verapamil markedly reduced arrhythmia score, and the incidence of ventricular flutter and fibrillation, it had a lesser effect on the number of premature ventricular contractions (PVC) (Table 1). Even at the highest dose, verapamil did not markedly reduce the number of PVCs in the large infarct group (25% reduction only). The only dose which reduced the number of PVCs significantly was 20 mg $kg^{-1}$ in SOZ rats.

**Table 1** The effect of various doses of verapamil on the number of premature ventricular contractions (PVC) (as $log_{10}$) induced by occlusion

| *Treatment* | *$log_{10}$ PVC 0–30 min post-occlusion* | *$log_{10}$ PVC 0–4 h post-occlusion* |
|---|---|---|
| *Large occluded zones* (LOZ) | | |
| Control ($n = 27$) | $1.4 \pm 0.1$ | $1.7 \pm 0.4$ |
| 2 mg $kg^{-1}$ ($n = 9$) | $1.5 \pm 0.2$ | $1.9 \pm 0.4$ |
| 6 mg $kg^{-1}$ ($n = 9$) | $1.2 \pm 0.3$ | $1.7 \pm 0.4$ |
| 20 mg $kg^{-1}$ ($n = 9$) | $1.2 \pm 0.2$ | $1.3 \pm 0.2$ |
| *Small occluded zones* (SOZ) | | |
| Control ($n = 18$) | $1.5 \pm 0.1$ | $2.4 \pm 0.2$ |
| 2 mg $kg^{-1}$ ($n = 9$) | $1.0 \pm 0.2$ | $2.3 \pm 0.3$ |
| 6 mg $kg^{-1}$ ($n = 9$) | $1.3 \pm 0.2$ | $2.4 \pm 0.3$ |
| 6 mg $kg^{-1}$ Post ($n = 9$) | $1.3 \pm 0.2$ | $2.4 \pm 0.3$ |
| 20 mg $kg^{-1}$ ($n = 9$) | $0.4 \pm 0.1$* | $1.1 \pm 0.3$* |

Values are given as the mean ($\pm$ s.e.mean) of $log_{10}$ PVC in the 0–30 min and 0–4 h post-occlusion periods. Statistical significance for differences from control is indicated by * for $P < 0.05$. Control groups for SOZ (2) and LOZ (3) were amalgamated. Deaths limited PVC occurrence in LOZ rats. All doses were given pre-occlusion except for 6 mg $kg^{-1}$ Post where the drug was given post-occlusion.

*Effects of verapamil on infarcted zone size and mortality*

None of the verapamil treatments changed the size of the occluded or infarcted zones (Table 2) in either SOZ or LOZ rats although the drug produced a dose-dependent increase in non-arrhythmic deaths in LOZ animals (e.g., 6/9 rats at 20 mg $kg^{-1}$) while reducing the number of deaths due to irreversible ventricular fibrillation from 10/27 in controls to 0/9. Non-arrhythmic deaths were associated with profound falls in blood pressure and were considered to be due to cardiac output failure. Non-arrhythmic mortality in SOZ rats was not increased by verapamil although the occurrence of irreversible ventricular fibrillation was reduced (by 100% for 6 and 20 mg $kg^{-1}$).

*Effects of verapamil on the post-occlusion ECG*

The changes in the S–T segment expressed as dSTR and R wave size following occlusion were not influenced in a predictable manner by verapamil treatment (Table 2).

**Table 2** The effect of various doses of verapamil on occluded and infarct zone sizes, on mortality and on ECG changes induced by occlusion

| Dose | n | Occluded zone (as % ventricular weight) | Infarct zone | Mortality post-occlusion as number in group 0–4 h NA | 0–4 h A | 0–24 h | ECG (1 h) dSTR | ECG (1 h) R |
|---|---|---|---|---|---|---|---|---|
| *Large occluded zone* (LOZ) | | | | | | | | |
| Control | 27 | 40 ± 1 | 25 ± 4 | 3 | 10 | 16 | 0.29 ± 0.09 | 0.63 ± 0.07 |
| 2 mg kg$^{-1}$ | 9 | 40 ± 3 | 32 | 3 | 3 | 7 | 0.29 ± 0.03 | 0.52 ± 0.05 |
| 6 mg kg$^{-1}$ | 9 | 38 ± 2 | 26 | 2 | 2 | 6 | 0.34 ± 0.05 | 0.74 ± 0.05 |
| 20 mg kg$^{-1}$ | 9 | 40 ± 2 | 31 | 6 | 0 | 6 | 0.22 ± 0.02 ($n$ = 4–22) | 0.64 ± 0.09 |
| *Small occluded zone* (SOZ) | | | | | | | | |
| Control | 18 | 22 ± 1 | 16 ± 1 | 1 | 5 | 9 | 0.23 ± 0.03 | 0.64 ± 0.06 |
| 2 mg kg$^{-1}$ | 9 | 23 ± 1 | 16 ± 2 | 1 | 1 | 2 | 0.25 ± 0.04 | 0.63 ± 0.08 |
| 6 mg kg$^{-1}$ | 9 | 23 ± 1 | 17 ± 1 | 1 | 0 | 2 | 0.33 ± 0.09 | 0.59 ± 0.13 |
| 6 mg kg$^{-1}$ Post | 9 | 22 ± 3 | 13 ± 3 | 1 | 1 | 3 | 0.19 ± 0.06 | 0.66 ± 0.08 |
| 20 mg kg$^{-1}$ | 9 | 21 ± 1 | 14 ± 1 | 1 | 0 | 1 | 0.21 ± 0.04 ($n$ = 6–13) | 0.34 ± 0.11 |

Control groups for LOZ (3) and SOZ (2) were amalgamated to give a single control group for each occluded zone size (large or small) because analysis showed no differences between groups within the LOZ or SOZ groupings. Occluded and infarcted zones were estimated as indicated in Methods. Non-arrhythmic mortality (NA), in the 0–4 h period, is given as the number of dead animals in the group ($n$ originally = 9 except for control LOZ where $n$ = 27 and control SOZ where $n$ = 18). Arrhythmic deaths by 4 h post-ligation are indicated by A. Total mortality by 24 h (arrhythmic and non-arrhythmic) is given in the third column. ECGs at 1 h post-occlusion were analysed as described in Methods for S–T segment elevation and increase in R-wave size (R). The S–T segment elevations were corrected for R-wave size (dSTR). All doses were given pre-occlusion, except for 6 mg kg$^{-1}$ Post where drug treatment was started immediately post-occlusion. Values are $\bar{x}$±s.e.mean except for mortality figures. The s.e.mean is not given where $n < 4$.

*Effects of verapamil on heart rate and blood pressure*

In conscious rats verapamil infusion sometimes reduced diastolic blood pressure to 55–60 mmHg, but despite this, animals remained conscious although relatively immobile with their heads held down. At this blood pressure infusions were stopped, but they could be recommenced within 5 min as blood pressure recovered quite rapidly. In both SOZ and LOZ rats, 1 min pre-occlusion, verapamil produced a dose-dependent fall in blood pressure (Figure 3a) with an $ED_{25}$ of 8 mg kg$^{-1}$ and an $ED_{50}$ of 30 mg kg$^{-1}$. After 1 h of occlusion (Figure 3b), blood pressure was still reduced by verapamil in a dose-related manner, particularly in LOZ rats. Verapamil also caused a dose-related bradycardia prior to occlusion (Figure 3c) but this relationship was lost 1 h after occlusion (Figure 3d). The $ED_{50}$ for heart rate reduction in the pre-occlusion period was approximately 30 mg kg$^{-1}$. A slight tachycardia was seen with the lowest dose of verapamil. The ratios of $ED_{50}$ values were estimated to be at least 5 for vasodepressor to antiarrhythmic effect, and 5 for heart rate to antiarrhythmic effect.

*Effects of verapamil and quinidine on electrically-induced arrhythmias*

As verapamil at high concentrations is known to have quinidine-like actions on the rise rate of myocardial intracellular potentials *in vitro* (Bayer *et al.*, 1975), we determined whether 6 mg kg$^{-1}$ verapamil (i.e., the $ED_{50}$ against ischaemia-induced arrhythmias) had quinidine-like effects on electrically-induced arrhythmias, and on the ECG. Rise rate depression should reduce sensitivity to electrically-induced arrhythmias and widen the QRS interval. Quinidine also widens the Q–T interval and lowers following frequency by virtue of prolonging action potential duration. We determined whether verapamil at 6 mg kg$^{-1}$ had the same actions as 20 mg kg$^{-1}$ quinidine (Figure 4).

Pre-drug values for the stimulation thresholds (Table 3a) varied significantly between conscious

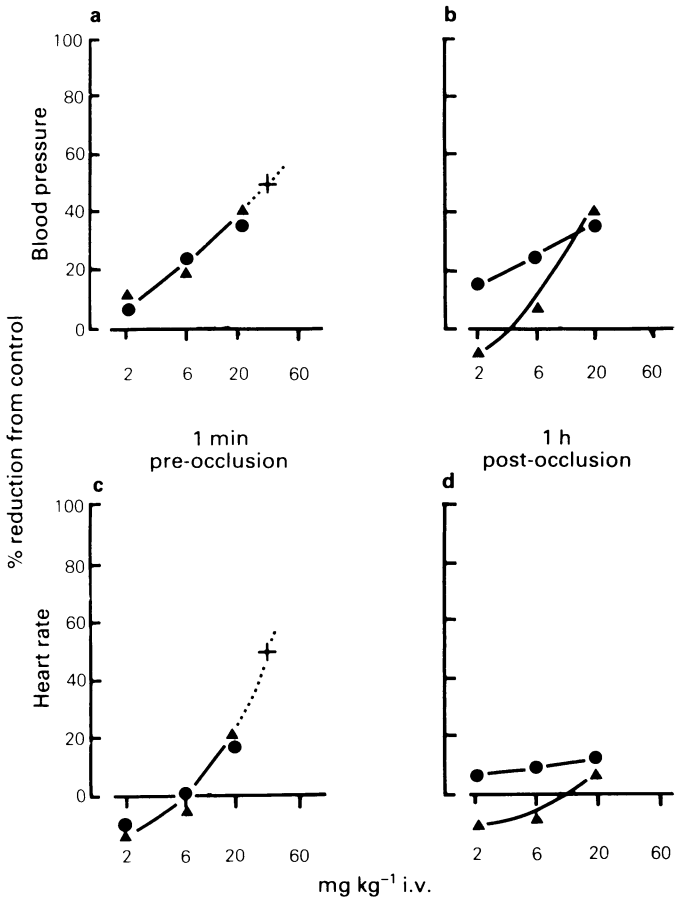


**Figure 3** The effect of verapamil on blood pressure (a and b) and heart rate (c and d) before (a and c), and 1 h after occlusion (b and d). Three different doses of verapamil were given to groups of rats with large (●) or small (▲) occluded zones. Blood pressure and heart rate data 1 min before occlusion were used to express the effects of verapamil as percentage reduction in blood pressure (a), and heart rate (c) from control group value. Additionally, 1 h post-occlusion values are given in (b) and (d). The dotted lines indicate extrapolations necessary to give estimates of $ED_{50}$ values (indicated by crosses). Control values in SOZ rats for mean blood pressure were $122 \pm 3$ mmHg at $-1$ min and $113 \pm 4$ mmHg at 1 h while values for heart rate were $400 \pm 9$ and $360 \pm 15$ beats $min^{-1}$ respectively. Values for LOZ rats were $113 \pm 3$ mmHg at $-1$ min, $102 \pm 3$ mmHg at 1 h, $405 \pm 10$ beats $min^{-1}$ and $380 \pm 20$ beats $min^{-1}$.

and anaesthetized rats. Threshold pulse width was longer in conscious versus anaesthetized controls. In conscious rats, threshold current could not be determined due to limitations in our stimulator and so threshold voltage was used instead. Quinidine produced the expected increases in threshold current, and pulse width, with a reduction in following frequency (Figure 4a); changes were most marked in anaesthetized rats. Verapamil had different effects; it increased following frequency without altering threshold current or pulse width; again changes were greatest in anaesthetized rats. Thus verapamil had a different spectrum of activity from quinidine and failed to show quinidine-like effects on thresholds or following frequency.

In the rat ECG before drug treatment (Table 3a), the QRS and Q–T intervals were longer in anaesthetized than in conscious rats. These differences were probably due to differences in heart rate (Table 3b) between the two groups. Drug-induced changes (Figure 4b) were potentiated in anaesthetized animals and such potentiation was greatest for quinidine. Both quinidine and verapamil prolonged P–R intervals. Quinidine failed to give an atropine-like shortening. Only quinidine produced the large increase in Q–T interval (uncorrected for rate) ex-

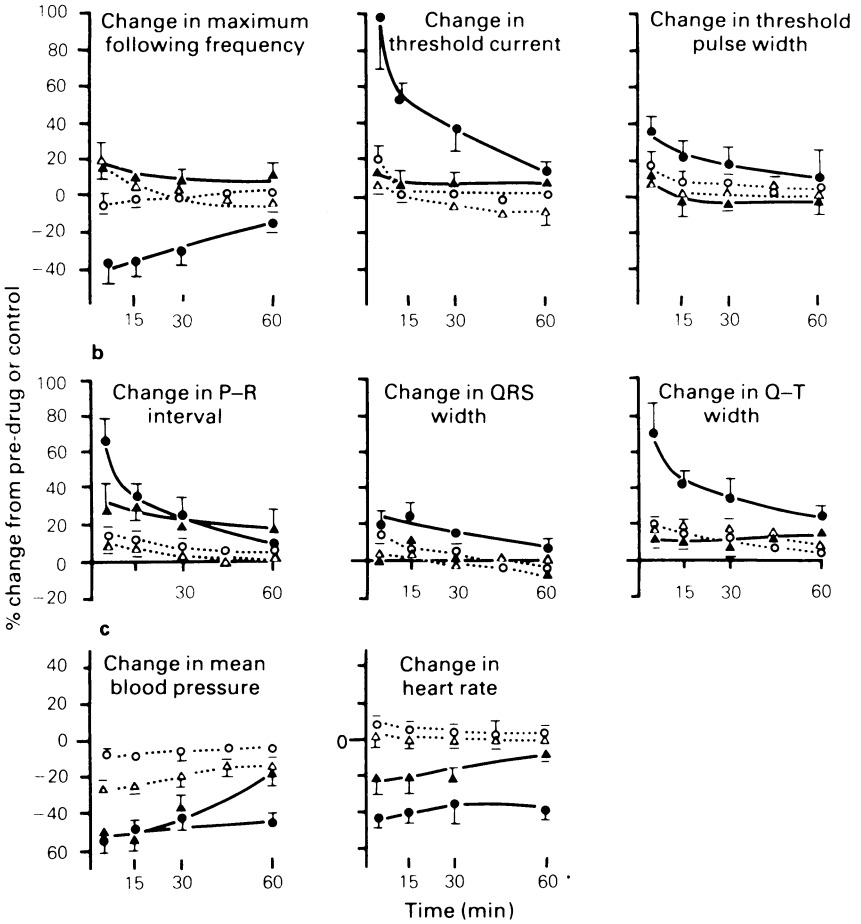


**Figure 4** The effect of verapamil and quinidine treatment on stimulation variables, ECG, blood pressure and heart rate in anaesthetized and conscious rats. Rats were treated with verapamil 6 mg kg$^{-1}$ (triangles) or quinidine 20 mg kg$^{-1}$ (circles) according to the schedules given in Methods. Injections were completed at time zero and changes in (a) the stimulation variables (following frequency, threshold current and pulse width), (b) ECG (P–R, QRS and Q–T intervals) and (c) blood pressure and heart rate are expressed as percentage change from pre-drug values for anaesthetized animals (filled symbols), and from saline control values for conscious rats (open symbols). Control values are given in Table 4.

pected of a drug that prolongs action potentials. Quinidine, presumeably via depression of action potential rise rate, also widened the QRS in both anaesthetized and conscious rats (Figure 3a) whereas verapamil did not. As was the case with electrically-induced arrhythmias, verapamil failed to show quinidine-like effects on the ECG.

Blood pressure and heart rate varied between conscious and anaesthetized rats (Table 3b). Marked hypertension occurred in anaesthetized animals. Both variables were lowered by verapamil and quinidine (Figure 4c) to approximately equal degrees in anaesthetized rats. In conscious rats, quinidine was not as effective as verapamil in lowering blood pressure and neither drug reduced heart rate.

As mentioned above, marked differences between conscious and anaesthetized animals were seen in those responses changed by verapamil, and/or quinidine (Figure 4). Pentobarbitone appeared to both potentiate and prolong the action of both drugs. Interpolation in Figure 4, gave pharmacological half-lives of 30–40 min for quinidine's effects on ECG and stimulation variables. Data for verapamil could not be so readily interpolated. Thus verapamil (6 mg kg$^{-1}$, i.v.) and quinidine (20 mg kg$^{-1}$, i.v.) had different effects both on the ECG and on sensitivity to electrical induction of arrhythmias.

**Table 3** Electrically-induced ventricular flutter variables, blood pressure and heart rate in anaesthetised and conscious rats prior to drug treatment

**A. Stimulation characteristics**

| | | *Thresholds for:* | | *ECG* (ms) | | |
|---|---|---|---|---|---|---|
| *Group* | *Maximum follow. freq.* (Hz) | *Current* (μA) | *Pulse width* (ms) | *P–R* | *QRS* | *Q–T* |
| | | | *Pentobarbitone-anaesthetized rats* | | | |
| Pre-verapamil | 17±1 | 219±15 | 0.18±0.01 | 46±2 | 25±1 | 79±5 |
| Pre-quinidine | 20±2 | 209±10 | 0.17±0.01 | 46±2 | 24±2 | 79±5 |
| | | | *Conscious rats* | | | |
| | | *Volts* | | | | |
| Pre-verapamil | 17±1 | 2.6±0.4 | 0.21±0.02* | 45±1 | 21±1* | 60±3* |
| Pre-quinidine | 18±2 | 2.4±0.3 | 0.26±0.04* | 46±1 | 22±1* | 64±4* |

**B. Blood pressure and heart rates**

| *Group* | *Blood pressure* (mmHg) | *Heart rate* |
|---|---|---|
| | *Pentobarbitone-anaesthetized Rats* | |
| Pre-verapamil | 160±6/137±5 (sys/dias) | 384±10 |
| Pre-quinidine | 160±7/130±9 (sys/dias) | 392±15 |
| | *Conscious rats* | |
| Pre-verapamil | 113±2* (mean pressure) | 413±14 |
| Pre-quinidine | 122±3* (mean pressure) | 416±17 |

Values are given as $\bar{x}$±s.e.mean for 5–6 rats; they were measured prior to admiistration of the drug indicated. The threshold stimulation variables were determined by means of silver electrodes inserted in the ventricles, and using the technique described in Methods. The ECG readings were from traces recorded after testing stimulation characteristics. Intervals were measured as indicated in Methods. *indicates $P<0.05$, for difference between conscious and anaesthetized rats.

*Blood and tissue levels of verapamil in coronary occluded rats*

The results of pharmacokinetic studies in rats are summarised in Figure 5. Concentrations of verapamil in the occluded tissue were, even when given post-occlusion, close to concentrations found in normal tissue. Occluded tissue contained 86% of the verapamil content of normal. Although a degree of cross-contamination could be expected because of

**Table 4** *In vitro* sensitivity of atrial and ventricular tissue to calcium and verapamil and effect of verapamil on slow calcium potentials

| | *Sensitivity to calcium* | | *Sensitivity to verapamil* | |
|---|---|---|---|---|
| *Tissue* | $ED_{50}$ (mM) | Slope | $ED_{50}$ (μM) | Slope |
| **(A) Inotropic responses** | | | | |
| Right atria | 2.7±0.5 | 2.6±0.4 | 0.35±0.10 | 1.3±0.3 |
| Left atria (180 b $min^{-1}$) | 2.7±0.4 | 2.5±0.2 | 0.40±0.05 | 0.8±0.1 |
| (400 b $min^{-1}$) | — | — | 0.19±0.11 | — |
| Ventricles (180 b $min^{-1}$) | 0.5±0.1 | 1.2±0.2 | 0.14±0.08 | 1.0±0.1 |
| (400 b $min^{-1}$) | — | — | 0.06±0.03 | — |

**(B) Slow calcium potentials**

(Effective verapamil concentration range for inhibition)

| | |
|---|---|
| Rat ventricle muscle | 0.1 to 1 μM |
| Guinea pig papillary muscle | 0.1 to 1 μM |

The values in (A) are $\bar{x}$±s.e.mean ($n$=5–6) calculated from cumulative dose-response curves for calcium and verapamil obtained as explained in Methods. The slope value is the Hill coefficient for the curves. Values in **(B)** are a range of effective concentrations obtained for 4–6 preparation with one cell per preparation examined. The height of the slow response was depressed by 20–80%.

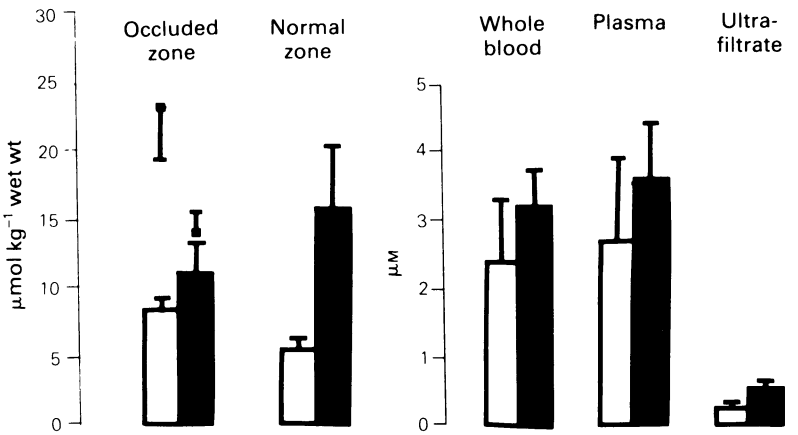


**Figure 5** Verapamil content of heart tissue and blood matrices 30 min after occlusion and after 6 mg $kg^{-1}$ given i.v. pre- or post-occlusion. Values are given as $\bar{x} \pm$s.e.mean for cardiac tissue (normal or occluded zone), and for whole blood, plasma and plasma ultrafiltrate ($n = 5-6$). Values for small samples from the centre of the occluded zone are indicated by the symbol (■). Values obtained when verapamil was given pre-ligation are shown in open columns whereas values from post-occlusion treatment are shown in filled columns.

imperfections in the method of separating ischaemic from normal tissue, it appears that verapamil distributes into the occluded zone. Surprisingly, samples taken from the centre of the zone had the highest verapamil contents. This was particularly so when verapamil was given pre-occlusion.

Despite the large dose, the plasma concentration of verapamil 30 min post-occlusion was below $10^{-5}$ M. Verapamil was plasma-protein bound. Binding was $82 \pm 4$ and $84 \pm 4\%$ for the two groups. The mean plasma water verapamil concentrations were $0.6 \pm 0.1$ μM for the post group vs. $0.2 \pm 0.04$ μM for the pre values which corresponded with *in vitro* concentrations supressing contractility (Table 4).

*Verapamil metabolites*

In animals given 6 mg $kg^{-1}$ verapamil pre-occlusion, a number of metabolites were found. The two major metabolites (as verapamil equivalents) accumulated in the ischaemic tissue to $9.7 \pm 3.1$ μmol $kg^{-1}$ ($\bar{x} \pm$s.e.mean) and $5.4 \pm 1.3$ respectively versus $1.5 \pm 0.7$ and $1.2 \pm 0.2$ μmol $kg^{-1}$ in normal tissue. These metabolites were not plasma-protein bound; plasma concentrations were $0.53 \pm 0.09$ and $0.28 \pm 0.04$ μM.

*Effects of verapamil on myocardial tissue*

Both atrial and ventricular tissue showed dose-related positive inotropic responses to low concentrations of extracellular calcium. In atrial tissues the calcium $EC_{50}$ (Table 4) was close to the concentration (2.5 mM) in our Krebs-Henseleit solution whereas, in ventricles, the $EC_{50}$ was less. $EC_{50}$ estimations for verapamil's suppression of contractility (Table 4) were made in the presence of the appropriate calcium $EC_{50}$. Verapamil was equi-effective in paced and unpaced atrial tissue and more active in ventricular versus atrial tissue, despite the differences (slope and $EC_{50}$ values) in calcium-response curves for the two tissues. $EC_{50}$ values for verapamil were lower at higher stimulation frequencies except between 400 vs. 600 beats per min.

In electrophysiological experiments, verapamil was found to inhibit slow calcium potentials (induced by 15 mM $K^+$ and isoprenaline) at concentrations similar to those inhibiting contractility (Table 4a vs. 4b).

## Discussion

The aim of this study was to demonstrate antiarrhythmic actions for verapamil against ischaemia-induced arrhythmias, and to determine the various factors involved. The results demonstrated unambiguously that verapamil is antiarrhythmic in the rat whether given pre- or post-occlusion. The rat is now frequently used for ischaemia and infarction studies (Au *et al.*, 1979; Clark *et al.*, 1980; Marshall *et al.*, 1981). In conscious rats we demonstrated antiarrhythmic activity for a number of drugs (MacLeod *et al.*, 1983; Jang *et al.*, 1983; Johnston *et al.*, 1983b) including quinidine (Johnston *et al.*, 1983a). Despite a small heart size and differences in cardiac physiology, the rat is sensitive to antiarrhythmics and is suitable for the qualitative and quantitative evaluation of drugs

active against ischaemic-arrhythmias. Comparison between anaesthetized ischaemic rats, pigs and dogs showed no fundamental differences between the three species (Bergey *et al.*, 1982).

In anaesthetized rats, Kane *et al.* (1981) could not show antiarrhythmic activity for 0.1 mg $kg^{-1}$ verapamil but were able to show it for niludipine, nisoldipine and nifedipine (Fagbemi & Parratt, 1981). In a similar study 0.2 mg $kg^{-1}$ verapamil was moderately antiarrhythmic (Mertz & Kaplan, 1982). Bernauer (1982) found verapamil to be antiarrhythmic at 1 and 2 mg $kg^{-1}$ while Muller & Wilsmann (1982) showed minimal activity for 0.5 mg $kg^{-1}$ verapamil; it was less effective than 5 mg $kg^{-1}$ quinidine. The authors stressed the antiarrhythmic importance of sodium channel blockade.

The above rat studies used low doses of verapamil compared with those used in the present study. This may account for equivocal evidence of verapamil's effectiveness. Previous studies used acutely prepared anaesthetized animals in which high doses of verapamil are poorly tolerated. We found anaesthetized rats to be very sensitive to the adverse cardiovascular effects of verapamil and quinidine. The resistance of conscious animals possibly reflects a superior cardiovascular status, increased drug metabolism, and/or intact reflexes.

In dogs with myocardial ischaemia, verapamil has been shown to be antiarrhythmic in the limited dose range tested (see refs. in Introduction). Unfortunately, such studies lacked control for occluded zone size despite its importance in arrhythmogenesis (Austin *et al.*, 1982). Our rat model allows dose-response studies, with control for occluded zone size, while monitoring other responses to coronary occlusion.

We have shown that verapamil reduces arrhythmia score in a dose-related manner as well as reducing the incidence of major arrhythmias (ventricular flutter and fibrillation). PVCs were minimally reduced which perhaps reflects the different arrhythmogenic mechanisms for PVC, and/or the frequency-dependent nature of verapamil's action (Sanguinetti & West, 1982). While verapamil was antiarrhythmic against severe arrhythmias, and thus prevented deaths due to irreversible ventricular fibrillation, it also caused deaths by non-arrhythmic mechanisms. Doses preventing arrhythmias produced vasodilatation and cardiac depression sufficient, when coupled with ischaemia, to cause death due to reduced cardiac output. However, if the OZ size were limited, as in the SOZ animals, such deaths did not occur.

With the efficacy of verapamil established, we investigated various factors involved. Above $10^{-5}$ M *in vitro*, verapamil has some quinidine-like actions (Bayer *et al.*, 1975) but such a concentration is 100 × the $ED_{50}$ for inhibiting myocardial contractility (this study), or slow calcium currents (Sanguinetti & West, 1982; Mobyvda & Sperelakis, 1983). Verapamil, at $10^{-7}$ M, is antiarrhythmic in perfused rat hearts with coronary occlusion (Woodward, 1981). Plasma water concentrations of verapamil ($0.6 \pm 0.1$ μM post-occlusion and $0.24 \pm 0.04$ μM pre-occlusion) 30 min after 6 mg $kg^{-1}$ i.v. would indicate that only calcium antagonism was present in the rats used in this study. Verapamil's pharmacokinetics in rats indicates a very short half-life. In dogs, verapamil has a 2 min $t_{1/2}$ alpha phase and a 2 h $t_{1/2}$ β-phase (Hashimoto *et al.*, 1982) such that 0.3 mg $kg^{-1}$ gives a maximum beta phase plasma concentration of 0.3 μmol $l^{-1}$. Extrapolation of our 6 mg $kg^{-1}$ data suggests plasma concentrations at 30 min post-occlusion of 0.008 μmol $l^{-1}$ for 0.2 mg $kg^{-1}$, up to 0.8 μmol $l^{-1}$ for 20 mg $kg^{-1}$).

If inhibition of contractility (Table 4) involves inhibition of the same calcium currents as those involved in ischaemic arrhythmogenesis in the rat then verapamil's antiarrhythmic action may be due to calcium antagonism. The other evidence that supports this view is that verapamil did not have quinidine-like actions on electrical arrhythmias and ECG. Quinidine raised thresholds and lowered following frequency, whereas verapamil did not. Only quinidine widened QRS width and Q–T intervals. This latter data was similar to that of Tisné-Versailles *et al.* (1982). Muller & Wilsmann (1982) showed verapamil is 10–40 × more potent than quinidine in effects on blood pressure, heart rate, and PR interval but equipotent in widening the QRS width. QRS widening was not significant with 10 mg $kg^{-1}$ verapamil in anaesthetized rats. Pharmacokinetic evidence further suggested verapamil was antiarrhythmic by virtue of calcium antagonism. In order to reveal antiarrhythmic actions of verapamil high doses had to be given. These lowered blood pressure, reduced heart rate, and, in LOZ rats, increased non-arrhythmic mortality. However, the $ED_{50}$ values (Figure 2) for such effects were greater than the antiarrhythmic $ED_{50}$.

In view of high concentrations of metabolites found in the occluded zone, and the possibly greater antiarrhythmic efficacy of pre-occlusion administration, the possibility exists that a verapamil metabolite(s) was responsible for observed antiarrhythmic actions.

Three cardiac loci for verapamil's antiarrhythmic actions are obvious; the ischaemic zone, the interface zone and normal tissue. Extra-cardiac loci are possible although blood pressure lowering alone does not account for verapamil's antiarrhythmic actions since other vasodepressors are not antiarrhythmic (Au *et al.*,1983; Jang *et al.*, 1983). Furthermore, verapamil's vasodepressor dose-response curve was different from that for antiarrhythmic action.

Pharmacokinetic data did not exclude the occluded

zone since both verapamil and metabolites were found there. The high concentrations found cannot readily be explained although the rat heart has small (100 μM) collaterals which may deliver verapamil. Alternatively, venous drainage may result in verapamil entering the zone retrogradely. Whatever the cause, verapamil, and particularly its metabolites, are sequestered in both normal and ischaemic myocardium but it is unlikely that all this verapamil is available for effect. Possibly verapamil binds in a non-specific manner to myocardium.

With regard to the interface zone being a possible locus of action, our model of arrhythmogenesis (Johnston *et al.*, 1983) of $A = a + bI$, indicates that verapamil increased a. In effect this reduces the effectiveness of I since I (i.e. OZ or IZ) did not change (Table 2). It is possible that verapamil (or metabolites) reduces the interface arrhythmogenicity or reduces electrophysiological disparity between ischaemic and normal tissue, which amounts to the same thing. In dogs, verapamil reduces disparity between conduction times in normal and ischaemic tissue (Nakaya *et al.*, 1980; Moses, 1981; Fondacaro *et al.*, 1978) and normalizes electrophysiological changes induced by exposure to acidic, hyperkalaemic, and hypoxic conditions (Kimura *et al.*, 1982).

Finally, if occluded or interface zones are the site of action, and calcium blockade the mechanism, our study suggests a design specification for a more effective anti-fibrillatory drug. A myocardial calcium antagonist, without vascular effects, would be a particular effective antiarrhythmic if it preferentially accumulated at, or became activated by, the acid pH encountered in ischaemia. A frequency-dependent action would selectively reduce high frequency arrhythmias such as ventricular flutter and fibrillation.

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## References

AU, T.L.S., COLLINS, G.A. HARVIE, C. & WALKER, M.J.A. (1979). The actions of prostaglandins $I_2$ and $E_2$ on arrhythmias produced by coronary occlusion in the rat and dog. *Prostaglandins*, **18,** 707–720.

AU, T.L.S., COLLINS, G.A. & WALKER, M.J.A. (1980). Rate, force and cyclic adenosine 3', 5'-monophosphate responses to (−) adrenaline in neonatal rat heart tissue. *Br. J. Pharmac.*, **69,** 601–608.

AU, T.L.S., COLLINS, G.A., MACLEOD, B.A. & WALKER, M.J.A. (1983). Actions of prostaglandin $E_2$, propranolol, and nitroglycerine with halothane, pethidine or pentobarbitone anaesthesia on arrhythmias and other responses to ligation of coronary artery in rats. *Br. J. Pharmac.*, **79,** 929–937.

AUSTIN, M., WENGER, T.L., HARRELL, F.E., LUZZI, F.A. & STRAUSS, H.C. (1982). Effect of myocardium at risk on outcome after coronary occlusion and release. *Am. J. Physiol.*, **243,** H340–H345.

BAYER, R., KALUSCHE, D., KAUFMANN, R. & MANNHOLD, R. (1975). Inotropic and electrophysiological actions of verapamil and D-600 in mammalian myocardium. III Effects of the optical isomers on transmembrane action potentials. *Naunyn Schmiedebergs Arch. Pharmac.*, **290,** 81–97.

BERGEY, J.L., NOCELLA, K. & CALLUM, M.C. (1982). Acute coronary artery occlusion-reperfusion-induced arrhythmias in rats, dogs and pigs: antiarrhythmic evaluation of quinidine, procainamide and lidocaine. *Eur. J. Pharmac.*, **81,** 205–216.

BERNAUER, W. (1982). Comparative investigations of the effects of metoprolol, propranolol, practolol and verapamil in the acute phase of experimental myocardial infarction. *Klin. Wochenschr.*, **60,** 87–96.

BOTTING, J.H., JOHNSTON, K.M., MACLEOD, B.A. & WALKER, M.J.A. (1983). The effect of modification of sympathetic activity on responses to ligation of a coronary artery in the conscious rat. *Br. J. Pharmac.*, **79,** 265–271.

BROOKS, W.W., VERRIER, R.L. & LOWN, B. (1980). Protective effect of verapamil on vulnerability to ventricular fibrillation during myocardial ischaemia and reperfusion. *Cardiovasc. Res.*, **14,** 295–302.

COLE, S.C.J., FLANAGAN, R.J., JOHNSTON, A. & HOLT, D.W. (1981). Rapid high performance liquid chromatographic method for the measurement of verapamil and nor-verapamil in blood plasma or serum. *J. Chromatog.*, **218,** 621–629.

CLARK, C., FOREMAN, M.I., KANE, K.A., MACDONALD, F.M. & PARRATT, J.R. (1980). Coronary artery ligation in anaesthetized rats as a method for the production of experimental dysrhythmias and for the determination of infarct size. *J. Pharmac. Methods*, **3,** 357–366.

FAGBEMI, O, & PARRATT, J.R. (1981). Marked suppression by orally-administered nifedipine, nisoldipine and niludipine on ventricular fibrillation resulting from acute myocardial ischaemia. *Br. J. Pharmac.*, **74,** 12–14.

FONDACARO, J.D., HAN, J. & YOON, M.S. (1978). Effects of verapamil on ventricular rhythm during acute coronary occlusion. *Am. Heart J.*, **96,** 81–86.

HASHIMOTO, K., SATOH, H., SHIBUYA, T. & IMAI, S. (1982). Canine-effective plasma concentrations of antiarrhythmic drugs on the two-stage coronary ligation arrhythmia. *J. Pharmac. exp. Ther.*, **223,** 801–810.

INOUE, F., McNEILL, J.H., & PUIL, E. (1979). Electropharmacological analysis of the actions of histamine in cardiac tissue. *Can. J. Physiol. Pharmac.*, **57,** 778–786.

JANG, T.L., MACLEOD, B.A. & WALKER, M.J.A. (1983). Effects of halogenated hydrocarbon anesthetics on responses to ligation of a coronary artery in chronically prepared rats. *Anesthesiology*, **59,** 309–315.

JOHNSTON, K.M., MACLEOD, B.A. & WALKER, M.J.A. (1981). ECG and other responses to ligation of a coronary artery in the conscious rat. In *Rat Electrocardiogram in Pharmacology Toxicology*, ed. Budden R., Detweiler D.K. & Zbinden G. pp. 243–252. Oxford: Pergamon Press.

JOHNSTON, K.M., MACLEOD, B.A. & WALKER, M.J.A. (1983a). Responses to ligation of a coronary artery in conscious rats and the actions of antiarrhythmics. *Can. J. Physiol. Pharmac.*, **61,** 1340–1353.

JOHNSTON, K.M., MACLEOD, B.A. & WALKER, M.J.A. (1983b). Effects of aspirin and prostacyclin on arrhythmias resulting from coronary artery ligation and on infarct size. *Br. J. Pharmac.*, **78,** 29–37.

KANE, K.A., MACDONALD, F.M. & PARRATT, J.R. (1981). What pharmacological properties are necessary for the prevention of early post-infarction ventricular arrhythmias? *Br. J. Pharmac.*, **73,** 512–513P.

KIMURA, S., NAKAYA, H. & KANNO, M. (1982). Effects of verapamil and lidocaine on changes in action potential characteristics and conduction time induced by combined hypoxia, hyperkalemia, and acidosis in canine ventricular myocardium. *J. Cardiovasc. Pharmac.*, **4,** 655–667.

KUWADA, M., TATEYAMA, T. & TSUTSUMI, J. (1981). Simultaneous determination of verapamil and its 7 metabolites by high performance liquid chromatography. *J. Chromatog.*, **222,** 507–511.

MACLEOD, B.A., AUGEREAU, P. & WALKER, M.J.A. (1983). Effects of halothane anesthesia and no anesthesia during coronary ligation in rats. *Anesthesiology*, **58,** 44–52.

MARSHALL, R.J. MUIR, A.W. & WINSLOW, E. (1981). Development of a severe model of early coronary artery ligation induced dysrhythmias in the anaesthetized rat. *Br. J. Pharmac.*, **73,** 951–959.

MARSHALL, R.J., MUIR, A.W. & WINSLOW, E. (1983). Effects of antiarrhythmic durgs on ventricular fibrillation thresholds of normal and ischaemic myocardium in the anaesthetized rat. *Br. J. Pharmac.*, **78,** 165–171.

MARTINEZ, T.T. & CRAMPTON, J.M. (1981). Prostaglandins show marked differences when tested against early ischaemic dysrhythmias in rat. *Life Sci.*, **29,** 1449–1456.

McALLISTER, F.J. & HOWELL, S.M. (1976). Fluorometric assay of verapamil in biological fluids and tissues. *J. Pharm Sci.*, **65,** 431–432.

MERTZ, T.E. & KAPLAN, H.R. (1982). Pirmenol hydrochloride (Cl-845) and reference antiarrhythmic agents: effect on early ventricular arrhythmias after acute coronary artery ligation in anesthetized rats *J. Pharmac. exp. Ther.*, **223,** 580–585.

MOBYVDA, P.-A. & SPERELAKIS, N. (1983). Comparison of effects of several calcium antagonistic drugs (slow-channel blockers) on the electrical and mechanical activities of guinea pig papillary muscle. *J. Cardiovasc. Pharmac.*, **5,** 162–169.

MOSES, J.W. (1981). Differing electrophysiologic effects of nifedipine and verapamil in acute ischaemia. *Clin. Res.*, **29,** 224A.

MULLER, B. & WILSMANN, K. (1982). Effects of the optical isomers of D 600 on cardiovascular parameters and on arrhythmias caused by aconitine and coronary artery ligation in anesthetized rats. *J. Cardiovasc. Pharmac.*, **4,** 615–621.

NAKAYA, H., HATTORI, Y. & KANNO, M. (1980). Effects of calcium antagonists and lidocaine on conduction delay induced by acute myocardial ischaemia in dogs. *Jap. J. Pharmac.*, **30,** 587–597.

SANGUINETTI, M.C. & WEST, T.C. (1982). Comparison of papaverine and verapamil on frequency-dependent changes in $V_{max}$ of K-depolarized ventricular tissue. *J. Cardiovasc. Pharmac.*, **4,** 791–802.

SURAWICZ, B. (1982). Role of calcium-blocking agents in treatment of cardiac arrhythmias related to myocardial ischaemia. *Am. Heart J.*, **103,** 698–706.

TEMESY, A.P., LEGENZA, M. & HOFFMAN, B.F. (1982). Effect of verapamil on arrhythmias during myocardial ischaemia. *Am. Heart J.*, **49,** 977.

THALE, J. GUELKER, H., HEUER, H., ZURSTEGGE, K.M. & BENDER, F. (1982). The influence of verapamil on the ventricular vulnerability of the heart after acute coronary occlusion and after reperfusion. *Z. Kardiol.*, **71,** 628.

TISNÉ-VERSAILLES, J.T., VERSCHEURE, Y., LE KIM, A. & POURRIAS, B. (1982). Comparison between the ventricular fibrillation thresholds of spontaneously hypertensive and normotensive rats – investigation of antidysrhythmic drugs. *J. Cardiovasc. Pharmac.*, **4,** 430–435.

WOODWARD, B. (1981). A model of ventricular fibrillation in the isolated rat heart. *J. Pharmac. Methods*, **6,** 219–231.

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