Br. J. Pharmac. (1986), **88**, 355–361

Actions of the verapamil analogues, anipamil and ronipamil, against ischaemia-induced arrhythmias in conscious rats

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1 Two analogues of verapamil, ronipamil and anipamil, were tested for their ability to reduce arrhythmias induced by occlusion of the left anterior descending coronary artery in conscious rats.

2 Only anipamil (50 and 150 mg kg^{-1} orally) produced a statistically significant reduction in arrhythmias; it was most effective against ventricular fibrillation. Ronipamil at the same doses had limited antiarrhythmic actions.

3 Only anipamil delayed the development of ECG signs of ischaemia, while both drugs reduced the magnitude of such changes.

4 Anipamil has a more favourable ratio of antiarrhythmic to hypotensive effects when compared with verapamil.

Introduction

We have recently demonstrated that the calcium antagonist verapamil has a dose-related effectiveness against the arrhythmias induced in conscious rats by occlusion of a coronary artery (Curtis *et al.*, 1984). On the basis of pharmacokinetic and pharmacological studies we postulated that the antiarrhythmic activity of verapamil occurred by virtue of calcium antagonism in the ventricular myocardium (Curtis *et al.*, 1984). If this pharmacological property is responsible for the antiarrhythmic effect of verapamil, then all calcium antagonists with such a myocardial action should be antiarrhythmic in our model.

Other workers have demonstrated that verapamil (Fagbemi *et al.*, 1984) and other calcium antagonists (Fagbemi & Parratt, 1981) have antiarrhythmic actions in acutely prepared anaesthetized rats. In such preparations, verapamil has been reported to produce a bell-shaped dose-effect curve against ischaemia-induced arrhythmias (Fagbemi *et al.*, 1984). In contrast with anaesthetized rats, we found that much higher doses of verapamil were tolerated in conscious rats, and that the resultant antiarrhythmic log dose-response relationship was sigmoidal (Curtis *et al.*, 1984). The beneficial antiarrhythmic activity of verapamil was, however, offset by profound hypotension, which, in rats with large occluded zones (35–40% of the ventricles by weight), was associated with cardiogenic shock, and related mortality, particularly at the highest dose studied (20 mg kg^{-1}, i.v.). It was of interest, therefore, to determine whether verapamil analogues exist which are equally antiarrhythmic without producing excessive hypotension. Unfortunately, most calcium antagonists have selectivity for vasculature versus the myocardium, and produce profound hypotension at doses below those affecting calcium currents in the heart (see Briscoe & Smith, 1982; Van Zwieten & Timmermans, 1983).

Anipamil and ronipamil (this name replaces the former proposed name, fedopamil (Lu 38425)) can be regarded as verapamil (1,7-bis-(3,4-dimethoxyphenyl)-3-methylaza-7-cyano-8-methylnonane) analogues. With anipamil the main chain of verapamil has been extended by 10 C-atoms and the methoxy substituents removed from the 4 position on the phenyl rings so as to give 1,7-bis-(3-methoxyphenyl)-3-methylaza-7-cyano-nonadecane. Ronipamil has the same structure as anipamil except for the lack of methoxy substituents on the phenyl rings, i.e. it is 1,7-bisphenyl-3-methylaza-7-cyano-nonadecane. Both compounds have been demonstrated to have antiischaemic effects in a variety of preparations (Kovach, 1984; Kretzschmar & Raschack, 1984; Raschack, 1984; Urbanics & Kovach, 1984). Both possess the 1,7-bisphenyl-3-methylaza-7-cyano structure similar to verapamil. The lipophilicity conferred by the straight 10 C-chain extension on C-9 may be responsible for

the long durations of anti-ischaemic action seen with anipamil and ronipamil. Anipamil appears similar to verapamil in having calcium antagonist activity in the myocardium (Raschack, 1984). The lack of methoxy substituents in ronipamil may account for the very limited haemodynamic effects seen with this drug (Kretzschmar & Raschack, 1984). In view of the differences between the analogues, they were tested for their ability to reduce ischaemia-induced arrhythmias in conscious rats.

Methods

Our usual procedure was used to prepare 45 rats for occlusion of the left anterior descending coronary artery (Johnston *et al.*, 1983). A loose coronary artery occluder, permanent aortic cannula and ECG leads were implanted 7 days before occlusion. The occluder was placed close to the origin of the left anterior descending coronary artery in order to produce a large occluded zone (30–45% of the ventricles by weight).

After 7 days recovery from preparative surgery, rats were randomised to 5 groups (all $n = 9$). Anipamil and ronipamil were administered at 50 or 150 mg kg^{-1} orally, 4 h before coronary artery occlusion. Both drugs were administered in distilled water at a volume of 0.25 ml kg^{-1} body weight. Suspensions of 20 or 60 mg ml^{-1} of either drug were gently heated to 60°C to facilitate solution, and then allowed to cool slightly before oral administration. Control animals received an equivalent volume of water at the same temperature as the drug suspensions. After occlusion, animals were observed and monitored continuously for a further 4 h in the manner described for previous studies (Johnston *et al.*, 1983; Curtis *et al.*, 1984).

The variables recorded were arrhythmias, blood pressure, heart rate and our standard V_3 ECG. As in previous studies, animals experiencing episodes of ventricular fibrillation (VF) lasting longer than 10 s were subjected to chest taps in an attempt to restore sinus rhythm. Arrhythmias were summarized by our standardized arrhythmia score method (Johnston *et al.*, 1983) and sub-analysed in terms of the incidence of premature ventricular contractions (PVC) ventricular tachycardia (VT) and VF, as well as the number of episodes and the duration of such events. As described previously, numbers of episodes and duration of arrhythmias were expressed as $\log_{10}$ values such correction produces normally distributed (Gaussian) variables.

In all rats, irrespective of the time of death, the occluded zone was measured by dye exclusion *ex vivo*, whereas infarct zones were determined only for rats surviving 24 h. The techniques used for these determinations, and the statistical methods employed for analysis of all the variables measured have been described in full elsewhere (Botting *et al.*, 1983; Johnston *et al.*, 1983; Curtis *et al.*, 1984).

To ensure that the drugs were being absorbed following oral administration, plasma anipamil levels were measured (by Dr Brode, Knoll A.G., Ludwigshafen, using a technique developed in his laboratory) in separate groups of rats given either 50 or 150 mg kg^{-1} orally.

Results

Mean arterial blood pressure and heart rate changes at various times pre- and post-occlusion are shown in Table 1. Prior to oral administration of drugs the group mean heart rate ranged from 376 ± 19 beats min^{-1} ($\bar{x}$ ± s.e.mean) in the control group to 432 ± 20 beats min^{-1} in the high-dose anipamil group. The corresponding mean arterial blood pressures ranged from 103 ± 9 to 117 ± 4 mm Hg. There were no statistically significant differences between the means for the various pre-drug groups. Immediately before occlusion, and for 4 h after drug administration, neither drug, at either 50 or 150 mg kg^{-1}, had marked actions on heart rate or blood pressure when compared with the untreated animals. The small reductions in blood pressure seen prior to occlusion, expressed as percentage changes from pretreatment values, were not statistically different. In the animals treated with anipamil, heart rate and blood pressure were reduced by occlusion to a greater degree than occurred in control rats although the only statistically significant differences occurred in mean blood pressure of anipamil-treated rats, 4 h after occlusion. Thus ronipamil had no marked actions on blood pressure and heart rate while anipamil had limited cardiovascular depressant actions, and lowered heart rate and blood pressure.

Since the size of the ischaemic zone (occluded zone size) is a determinant of occlusion-induced arrhythmias (Johnston *et al.*, 1983; Curtis *et al.*, 1984), this variable was determined for all rats and was not found to be statistically significantly altered by treatment. The means of group infarct sizes at 24 h could not be compared since, with the exception of high dose anipamil, too few animals survived 24 h. The mean occluded zone size, as a percentage of ventricular weight, was 42 ± 4% ($\bar{x}$ ± s.e.mean) for controls *vs* 41 ± 4, 40 ± 2, 39 ± 4 and 36 ± 3% for low and high doses of ronipamil, and low and high dose anipamil, respectively. The mean infarct size in the high dose anipamil group was 29 ± 4% of total ventricular weight which was comparable to values in previous control groups (Curtis *et al.*, 1984). We were unable to obtain enough estimates of infarct size in the other groups to allow useful comparisons.

Arrhythmias following occlusion in control rats

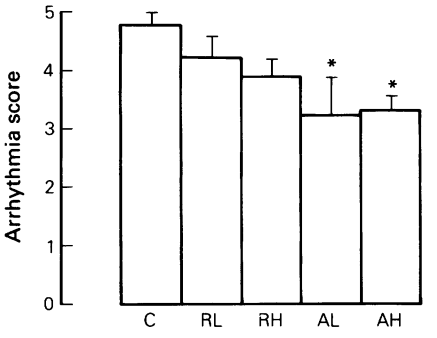

Figure 1 Effect of ronipamil and anipamil on arrhythmia score. The mean arrhythmia scores are shown for the 4 h period following occlusion; s.e.mean shown by vertical lines. The treatments were control (C), 50 mg kg^{-1} ronipamil (RL), 150 mg kg^{-1} ronipamil (RH), 50 mg kg^{-1} anipamil (AL) and 150 mg kg^{-1} anipamil (AH); $n = 9$ for each group. *Indicates $P < 0.05$ versus controls.

have been shown to be bi-modally distributed with time. Peaks occur at 15 min and around 2 h post-occlusion, with a quiescent interval lasting from 20 min to around 1.5 h post-occlusion. As arrhythmia incidence within groups was similar during both the 0–30 min, and 0.5–4 h periods post-occlusion, arrhythmia data are presented for the overall 0–4 h period. The arrhythmia scores for the different groups are shown in Figure 1. Both doses of anipamil caused a statistically significant reduction in arrhythmia score whereas the effect of ronipamil was smaller and the arrhythmia score was not statistically different from control.

With regard to individual types of arrhythmias, the incidence of VT was high in all groups. In those rats having VT, the duration of such events (Figure 2a), was less in the treatment groups but reductions were not statistically significant. The number of episodes of VT (as $\log_{10}$) in those having VT was 1.5 ± 0.2 ($\bar{x} \pm$ s.e.mean) in controls 1.4 ± 0.2 and 1.1 ± 0.2 with low and high dose ronipamil, and to 1.1 ± 0.2 and 1.0 ± 0.2 with low and high dose anipamil respectively. Most importantly, anipamil reduced both the incidence (Figure 2b) and duration (Figure 2c) of VF, particularly at the high dose. PVC were not reduced to a statistically significant degree by anipamil (Figure 2d) although means were lower with all treatments. In summary, while anipamil was an effective antiarrhythmic, particularly against VF, ronipamil was far less effective and failed to produce any statistically significant antiarrhythmic effects.

Despite a high incidence of VF, particularly in control rats, it was found possible to defibrillate manually all episodes of VF lasting longer than 10 s

Table 1 The effects of treatment with ronipamil (R) and anipamil (A) at a low oral dose (L) of 50 mg kg^{-1} and a high dose (H) of 150 mg kg^{-1} on mean arterial pressure (MAP) and heart rate (HR)

	% change in MAP					*% change in HR*				
Group	−30 min	−1 min	+1 min	+30 min	+1 h	+4 h	−30 min	−1 min	+1 min	+30 min	+1 h	+4 h
Control	0 ± 3	3 ± 3	−5 ± 7	−10 ± 5	−8 ± 5	−17 ± 6	−1 ± 2	2 ± 3	9 ± 4	−4 ± 4	−5 ± 5	−1 ± 6
RL	4 ± 4	5 ± 4	−3 ± 5	−4 ± 6	−7 ± 6	−9 ± 8	0 ± 6	2 ± 7	3 ± 5	0 ± 6	−4 ± 5	−15 ± 9
RH	2 ± 6	4 ± 4	−15 ± 7	−15 ± 4	−16 ± 4	−24 ± 4	−5 ± 6	−5 ± 5	1 ± 6	−13 ± 5	−16 ± 5	−14 ± 5
AL	−5 ± 4	−7 ± 3	−22 ± 6	−22 ± 6	−22 ± 5	−41 ± 4*	−5 ± 5	1 ± 4	8 ± 4	−1 ± 5	−7 ± 6	−18 ± 4
AH	−6 ± 3	−5 ± 2	−21 ± 7	−22 ± 4	−21 ± 5	−36 ± 4*	−5 ± 4	−4 ± 4	2 ± 6	−12 ± 7	−15 ± 9	−19 ± 10

Control values were recorded at the time of drug administration (4 h before coronary occlusion), and the percentage changes in these values ($\bar{x} \pm$ s.e.mean) are shown for various time points before (−30 and −1 min) and after (+1 min to +4 h) occlusion. *Indicates $P < 0.05$ versus the control group at a particular time point, by ANOVA and Duncan's range test.

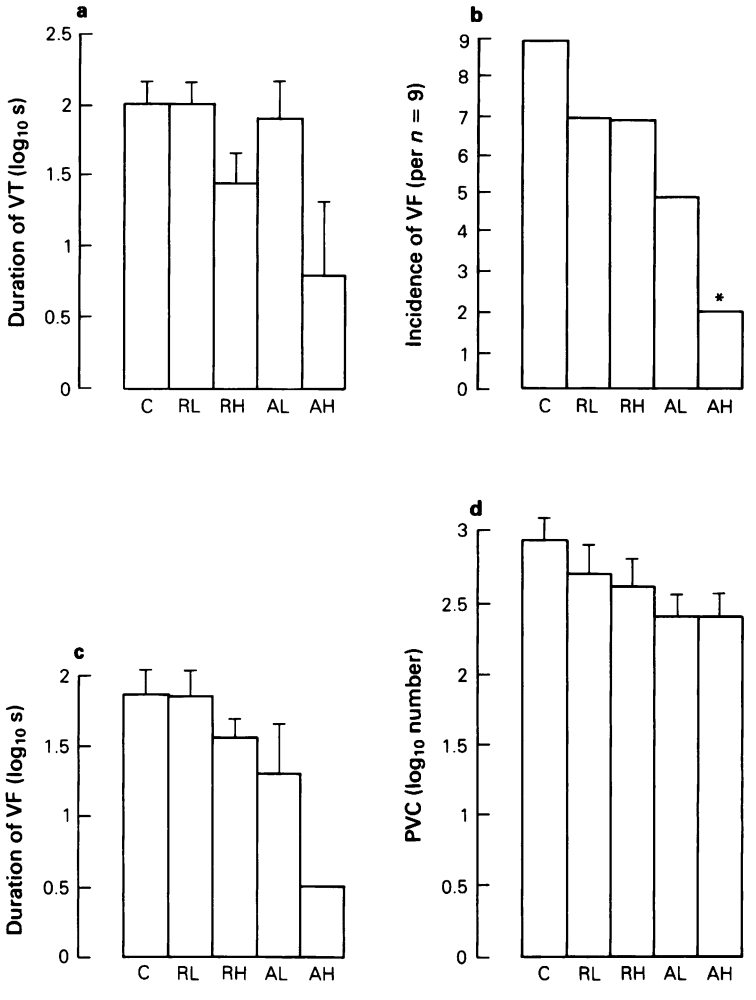

Figure 2 Effect of ronipamil and anipamil on the incidence and duration of various arrhythmias during the first 4 h following coronary occlusion. The treatments were control (C), 50 mg kg^{-1} ronipamil (RL), 150 mg kg^{-1} ronipamil (RH), 50 mg kg^{-1} anipamil (AL) and 150 mg kg^{-1} anipamil (AH). (a) Illustrates duration (log_{10} s) of ventricular tachycardia (VT) (mean, with s.e.mean shown by vertical lines) in those animals having this arrhythmia. The number of rats per group ($n = 9$) which had ventricular fibrillation (VF) is given in (b); (c) illustrates duration (log_{10} s) of ventricular fibrillation in those animals having this arrhythmia; (d) shows the mean of log_{10} premature ventricular contractions (PVC) occurring in the 0–4 h post-occlusion period. The s.e.mean data are omitted where n was less than 5, otherwise they are shown by vertical lines. *Indicates $P < 0.05$ versus controls.

and thus all deaths occurring during the 4 h period following occlusion were associated with hypotension (cardiogenic shock) or severe pulmonary oedema. In a previous study (Curtis *et al.*, 1984) we showed that verapamil at the highest dose of 20 mg kg^{-1} exacerbated the mortality associated with cardiogenic shock during the 4 h period following occlusion. In this study however, drug treatment did not consistently increase the number of such deaths. The number of deaths by 4 h post-occlusion was 1, 5, 1, 1, 1 for control, 50 mg kg^{-1} and 150 mg kg^{-1} ronipamil, 50 mg kg^{-1} and 150 mg kg^{-1} anipamil, respectively. The high value seen with the 50 mg kg^{-1} dose of ronipamil was not statistically significantly different from the other groups. By 24 h post-occlusion, mortality was 5, 6, 5, 5 and 3 respectively.

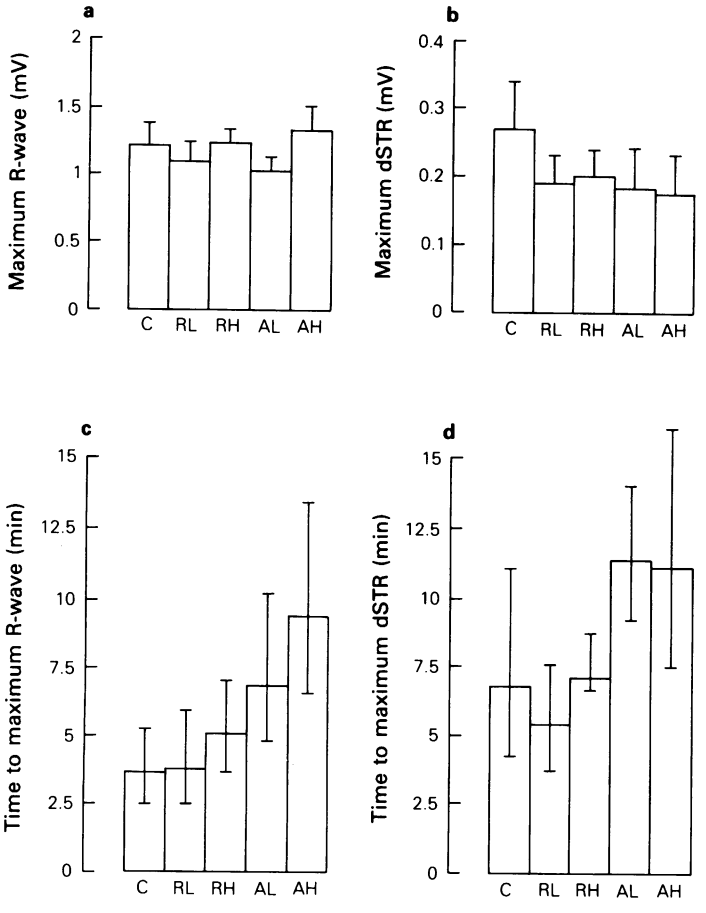

Figure 3 Effect of ronipamil and anipamil on the ECG changes (R-wave and 'S-T' segment elevation): (a) shows maximum R-wave amplitudes in mV; (c) shows the times at which maximum R-wave amplitudes were reached; (b) and (d) show corresponding values for 'S-T' segment elevation (dSTR). The times at which maximum R-wave (c) and dSTR (d) were reached were calculated as $\log_{10}$ min, but are illustrated as antilogs. $n = 8-9$ for all values; s.e.mean shown by vertical lines. *Indicates $P < 0.05$ versus controls. Treatments were as in previous figures.

Occlusion of the left anterior descending coronary artery in conscious rats produces changes in the ECG including an increase in R-wave size and a rise in the 'S-T' segment (corrected for concomitant changes in R wave-dSTR). When these variables were measured (Figure 3, a-d), it appeared that anipamil reduced the rate (Figure 3, c,d) at which both changes occurred while both anipamil and ronipamil reduced maximum dSTR (Figure 3c). However, the maximum change in R wave (Figure 3a) was the same for all groups. In this study the control value for dSTR was 0.27 ± 0.07 mV ($\bar{x} \pm$ s.e.mean) which is almost identical with the values found for controls in previous studies (Johnston *et al.*, 1983; Curtis *et al.*, 1984). Thus all treatments appeared to have reduced maximum dSTR.

The elevation of the 'S-T' segment of the ECG was also reduced by the drugs if changes in the segment were expressed as a percentage of R-wave amplitude (not shown) in the manner used by Bernauer (1982). The apparent delays in the development of ischaemia produced by both drugs were not associated with delays in the onset of arrhythmias.

Pooled plasma concentrations ($\mu g\,ml^{-1}$) of anipamil determined in separate groups of rats ($n = 5$) given 50 or 150 mg kg^{-1} anipamil were 3.2, 4 and 2.4 at 1, 3 and 5 h after administration of the low dose, and 5, 8.5 and 3.4, respectively, after administration of the high dose.

Discussion

Anipamil and ronipamil were developed as verapamil analogues with a long duration of action. Since both are new drugs, relatively little is known about their pharmacology. Both have been demonstrated to delay the depletion of myocardial enzymes in rat hearts induced by a period of hypoxia (Kretzschmar & Raschack, 1984; Raschack, 1984). In other tests similar anti-ischaemic or anti-hypoxic actions have been observed (Kovach, 1984; Ferrari *et al.*, 1984). Both anipamil and ronipamil were developed as verapamil analogues with a long duration of action. In the case of anipamil, calcium antagonism may be retained but this is much less with ronipamil (see Introduction). In keeping with this, we found that only anipamil produced statistically significant cardiovascular depressant effects in this study. In agreement with previously reported anti-ischaemic actions it is possible in this study that both anipamil and ronipamil moderated the ischaemia induced by occlusion since both reduced the maximum dSTR. Anipamil also delayed the development of dSTR and R-wave changes. However, any changes in occluded zone size were not statistically significant, and unlikely to account for the changes seen in the ECG.

Regardless of possible anti-ischaemic actions, it was apparent that while both drugs had some antiarrhythmic actions, only the effects of anipamil were statistically significant. The antiarrhythmic action of anipamil appeared to be most marked on VF compared with VT or PVC. Anipamil, at the high dose, reduced the incidence of VF from 9/9 in controls to 2/9. With respect to such a specific antifibrillatory action, we have previously suggested (Curtis *et al.*, 1984) that an ideal antifibrillatory drug would have frequency dependence leading to electrophysiological actions only at high frequencies (fibrillation). A description of the frequency-dependence of the action of anipamil on ventricular tissue is not, at present, available.

As previously indicated, neither ronipamil nor anipamil produced an excess of non-arrhythmic deaths. However, verapamil in our previous study (Curtis *et al.*, 1984) did produce such deaths in rats with large occluded zones (although this effect was mainly confined to the highest dose, 20 mg kg^{-1}). It was of interest, therefore, to compare anipamil with verapamil for their respective antiarrhythmic versus cardiovascular activity. Thus, anipamil reduced VF by about 50% and 80% at 50 and 150 mg kg^{-1}, respectively, doses which only reduced blood pressure before occlusion by 7% and 5%, respectively. By interpolation with previous data (Curtis *et al.*, 1984), verapamil, at a dose reducing the incidence of VF by 50%, reduced blood pressure before occlusion by 23%. Thus, when one considers that anipamil did not increase the incidence of non-arrhythmic deaths, it follows that the therapeutic ratio of anipamil is higher than that of verapamil. Since, at present, the major observed distinction between anipamil and ronipamil appears to be in cardiovascular depressant activity (anipamil being much more potent in this respect than ronipamil) we tentatively conclude from this study that the antiarrhythmic actions of anipamil were dependent on calcium antagonist activity. This antiarrhythmic effect may well only occur with concentrations above those responsible for any possible anti-ischaemic actions, since anipamil reduced maximum 'S-T' segment elevation to the same degree as ronipamil, but was more effective than ronipamil as an antiarrhythmic.

In conclusion, the high therapeutic index and selective antifibrillatory actions of anipamil are encouraging indications that effective non-toxic antifibrillatory calcium antagonists may be developed.

Ronipamil and anipamil were kindly supplied by Dr Kretzschmar, Knoll A.G., Ludwigshafen. This study was funded in part by the British Columbia Heart Foundation. Elaine L. Jan and Lisa Heiduschka are thanked for their help in the preparation of this manuscript.

References

BERNAUER, W. (1982). Comparative investigation 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.

BRISCOE, M.G. & SMITH, H.J. (1982). Sensitivity of cat papillary muscles to verapamil and nifedipine: enhanced effects in acidosis. *Cardiovasc. Res.*, **16**, 173–177.

CURTIS, M.J., MACLEOD, B.A. & WALKER, M.J.A. (1984). Antiarrhythmic actions of verapamil against ischaemic arrhythmias in the rat. *Br. J. Pharmac.*, **83**, 373–385.

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.

FAGBEMI, O., KANE, K.A., MCDONALD, F.M., PARRATT, J.R. & ROTHAUL, A. (1984). The effects of verapamil, prenylamine, flunarizine and cinnarizine on coronary artery ligation induced arrhythmias in the anaesthetised rat. *Br. J. Pharmac.*, **83**, 299–304.

FERRARI, R., RADDINO, R., CECONI, C., CURELLO, S. & VISIOLI, O. (1984). Protective effect of anipamil pretreatment during myocardial ischaemia and reperfusion. In *International Symposium on Calcium Entry Blockers and Tissue Protection*. Rome, March 15–16, 1984. ed. Paoletti, R. & Godfraind, T. (in press).

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

KOVACH, A.G.B. (1984). Protective effects of gallopamil and new verapamil derivatives against brain and other vital organ damage developing during anoxia and hemorrhagic shock. In *International Symposium on Calcium Entry Blockers and Tissue Protection*. Rome, March 15–16, 1984. ed. Paoletti, R. & Godfraind, T. (in press).

KRETZSCHMAR, R. & RASCHACK, M. (1984). Cardioprotective effects of the new verapamil derivative fedopamil. In *International Symposium on Calcium Entry Blockers and Tissue Protection*. Rome, March 15–16, 1984. ed. Paoletti, R. & Godfraind, T. (in press).

RASCHACK, M. (1984). Anipamil, a novel calcium antagonist with long-lasting oral efficacy, as assessed against hypoxic and ischaemic damage of heart and kidney. In *International Symposium on Calcium Entry Blockers and Tissue Protection*. Rome, March 15–16, 1984. ed. Paoletti, R. & Godfraind, T. (in press).

URBANICS, R. & KOVACH, A.G.S. (1984). Microcirculatory effects of the calcium antagonists gallopamil and fedopamil in cat cortex under normoxia and hypoxia. In *International Symposium on Calcium Entry Blockers and Tissue Protection*. Rome, March 15–16, 1984. ed. Paoletti, R. & Godfraind, T. (in press).

ZWIETEN, P.A.VAN & TIMMERMANS, P.B.M.W.M. (1983). Calcium entry blockers: a review with an attempt at classification. *Eur. Heart J.*, **4**, Suppl. C, 18.

(Received September 20, 1985.
Revised January 22, 1986.
Accepted February 7, 1986.)