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The effects of verapamil, prenylamine, flunarizine and cinnarizine on coronary artery occlusion-induced arrhythmias in anaesthetized rats

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1 In male rats, anaesthetized with pentobarbitone, ligation of the main left coronary artery causes an early phase of ventricular arrhythmias which last about 30 min. In approximately 60% of control animals, ventricular fibrillation occurs but since spontaneous reversion to sinus rhythm may occur, mortality is of the order of 30%.

2 When administered intravenously 15 min prior to ligation, verapamil (0.01 and 0.05 mg kg^{-1}), prenylamine (0.5 mg kg^{-1}), flunarizine (0.1, 0.25, 0.5 and 1.0 mg kg^{-1}) and cinnarizine (0.25, 0.5 and 1.0 mg kg^{-1}) protected against these arrhythmias.

3 Higher doses of verapamil (0.1 and 0.5 mg kg^{-1}), prenylamine (5 mg kg^{-1}) and flunarizine (2.5 mg kg^{-1}) did not afford a similar protection and mortality was increased to or above control values. Death was due in prenylamine-treated rats to atrioventricular block leading to asystole whereas in those administered verapamil or flunarizine it was a consequence of persistent ventricular fibrillation.

4 Prior to ligation, a sustained fall in mean arterial blood pressure was observed only following the administration of the highest doses of prenylamine, flunarizine and cinnarizine. Heart rate was reduced by administration of only the highest dose of prenylamine.

5 These studies show that although the four calcium antagonists studied, i.e. verapamil, prenylamine, flunarizine and cinnarizine do suppress ischaemia-induced arrhythmias, this protective effect may be limited to a narrow concentration range.

Introduction

Kaumann & Aramendia (1968) demonstrated that verapamil, a drug capable of blocking the slow calcium channels in cardiac and vascular tissue, reduced the severity of the early arrhythmias that result from coronary artery ligation in the anaesthetized dog. In that study, verapamil was administered intravenously prior to ligation in a dose of 0.79 mg kg^{-1}. A similar protection against coronary artery occlusion-induced ventricular fibrillation in the dog by pretreatment with a lower dose of verapamil (0.2 mg kg^{-1}), has also been reported (Elharrer *et al.*, 1977).

In contrast to these findings in the dog, our preliminary studies in the anaesthetized rat model, showed that verapamil in doses of 0.1 and 0.5 mg kg^{-1} also given prior to ligation, increased the resultant mortality (Kane *et al.*, 1981). In view of these conflicting results, and the observation by Hearse *et al.* (1980) that in the isolated heart of the rat, verapamil does not exhibit a linear dose-response curve with respect to its cardioprotective action against the calcium paradox, we designed the present study to extend our preliminary experiments with verapamil. The aim of this study was, therefore, to examine the antiarrhythmic effects of a wider range of concentrations of verapamil against early ischaemia-induced arrhythmias in the anaesthetized rat and to compare its actions with other slow channel blocking agents, namely prenylamine, flunarizine and cinnarizine. A preliminary report of these findings has been published (Fagbemi *et al.*, 1983).

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Methods

The experiments were performed on male Sprague-Dawley rats weighing between 200 and 400 g. The animals were anaesthetized with pentobarbitone sodium (6 mg 100 g^{-1} intraperitoneally) and catheters were placed in a carotid artery (for blood pressure measurement) and in a femoral vein (for vehicle or drug administration). The rats were subjected to coronary artery ligation as previously described in detail (Clark *et al.*, 1980). During the first 30 min post-occlusion, the percentage mortality and the percentage incidence of ventricular fibrillation (VF) was noted together with, in the survivors, the number of ectopic beats (including those which occurred as isolated extrasystoles and as ventricular tachycardia) and the duration of both ventricular tachycardia (VT) and ventricular fibrillation (VF). VT was defined as a run of 7 or more consecutive ectopic beats at a rate faster than sinus rhythm.

Drugs and doses used were (±)-verapamil HCl (Knoll, A.G.) 0.01, 0.05, 0.1 and 0.5 mg kg^{-1}, flunarizine HCl (Janssen) 0.1, 0.25, 0.5, 1.0 and 2.5 mg kg^{-1}, prenylamine gluconate (Hoechst AG) 0.5 and 5.0 mg kg^{-1} and cinnarizine (Janssen) 0.1, 0.25, 0.5 and 1 mg kg^{-1}. Verapamil and flunarizine were dissolved in saline, cinnarizine in 0.1 M tartaric acid and prenylamine 0.9% w/v gluconate. All drugs and vehicles were administered as bolus injections 15 min before ligation.

Statistics

Data are expressed as mean ± standard error of the mean (s.e.mean). For the number of extrasystoles and durations of ventricular tachycardia and fibrillation, the mean values ± s.e.mean were derived only from those animals surviving the 30 min post-occlusion period, in which that particular type of arrhythmia occurred. Statistical significance of differences between mean values was calculated using a one way analysis of variance together with a modified *t*-statistic provided that the variances of the groups were similar. If this was not the case the Kruskal-Wallis test was applied and the statistical significance of differences between means was calculated using a Mann-Whitney U test. For evaluation of differences between mortalities and incidences of fibrillation a Chi-squared test was used.

Results

Antiarrhythmic effects of verapamil, prenylamine, flunarizine and cinnarizine

Table 1 summarizes the severity of the arrhythmias observed upon coronary artery ligation in control and verapamil pretreated rats. Although 60% of the control animals fibrillated, mortality was of the order of 30% since spontaneous reversion to sinus rhythm often occurred. The two lower doses of verapamil (0.01 and 0.05 mg kg^{-1}) had a marked protective effect against these ischaemic arrhythmias; the mortality, incidence of VF, the number of extrasystoles and the duration of VT and VF were all reduced. However, with the two higher doses (0.1 and 0.5 mg kg^{-1}) the incidence of VF was identical to that of the control group; mortality was significantly increased because spontaneous defibrillation no longer occurred (Table 1). The number of extrasystoles and the duration of VT both appeared to be reduced by the highest dose of verapamil.

Prenylamine also exhibited antiarrhythmic activity in this model (Table 2). There was a dose-dependent reduction in the incidence of VF and in the duration of VT. Nevertheless, mortality tended to be greater in the group administered 5 mg kg^{-1} prenylamine compared with control; this was due to an increased incidence of atrioventricular block leading to asys-

Table 1 The effect of verapamil on early ischaemic arrhythmias in anaesthetized rats

Treatment	n	*% VF*	*% mortality*	*No. of ectopic beats*	*Duration of VT*(s)	*Duration of VF*(s)
Saline	16	62.5	31	1321 ± 199	82 ± 18	81 ± 38
Verapamil (mg kg^{-1})						
0.01	10	50	20	769 ± 144*	49 ± 15	2.8 ± 0.5
0.05	10	0**	10*	460 ± 154**	18 ± 8*	0
0.1	10	60	50*	881 ± 320	44 ± 22	161,9
0.5	10	60	60**	349 ± 79*	17 ± 4*	76

In this and in all subsequent tables *n* is the number of animals; individual values are quoted when the number of animals exhibiting a particular type of arrhythmia is less than 3. *$P < 0.05$; **$P < 0.01$

Table 2 The effect of prenylamine on early ischaemic arrhythmias in anaesthetized rats

Treatment	n	*%VF*	*% mortality*	*No. of ectopic beats*	*Duration of VT*(s)	*Duration of VF*(s)
Gluconate	14	57	29	1501 ± 297	98 ± 23	2 ± 7
Prenylamine (mg kg^{-1})						
0.5	10	30	20	616 ± 102**	40 ± 10*	26
5.0	10	0*	50	354 ± 217*	10 ± 7*	0

*$P < 0.05$; **$P < 0.01$.

tole. Thus, 5 out of 10 drug-treated animals exhibited A-V block on ligation compared with 1 of 14 in the controls. Moreover, an additional 4 animals given the highest dose of prenylamine were excluded from this study due to the occurrence of persistent A-V block prior to ligation.

The results obtained with flunarizine are shown in Table 3. Pretreatment with 0.25, 0.5 and 1.0 mg kg^{-1} of the drug significantly reduced all of the arrhythmic indices and there were no deaths in these drug-treated animals. However, in the group given 2.5 mg kg^{-1} flunarizine, the incidence of ventricular fibrillation was similar to that observed in control animals and, in surviving animals, the number of ectopic beats and the duration of VT were not statistically significantly different from control.

A dose-dependent reduction in the severity of the early ischaemia-induced arrhythmias was also observed following the administration of cinnarizine (Table 3). Animals pretreated with the lowest dose of cinnarizine (0.1 mg kg^{-1}) only exhibited a reduced duration of tachycardia when compared with control rats but with higher doses marked antiarrhythmic activity was observed.

Drug-induced changes in blood pressure and heart rate

In the lowest doses used, verapamil, flunarizine and cinnarizine did not significantly alter either mean arterial blood pressure or heart rate. A fall in mean arterial blood pressure was observed immediately following the administration of the higher concentrations of these drugs. In the case of verapamil, this hypotension was marked (from 106 ± 9 to 41 ± 5 mmHg at 0.5 min post-injection following a dose of 0.5 mg kg^{-1}). However, recovery was rapid and by the time the coronary artery was ligated there were no significant differences in either arterial blood pressure or heart rate between verapamil-treated animals and the controls. Arterial blood pressure of animals given the highest dose of flunarizine and cinnarizine did not fully recover before ligation; it

Table 3 The effect of flunarizine and cinnarizine on early ischaemic arrhythmias in anaesthetized rats

Treatment	n	*%VF*	*% mortality*	*No. of ectopic beats*	*Duration of VT*(s)	*Duration of VF*(s)
Saline	10	60	20	1027 ± 91	62 ± 11	34 ± 4
Flunarizine (mg kg^{-1})						
0.1	10	10*	0*	903 ± 79	51 ± 7	15 ± 7*
0.25	10	10*	0*	673 ± 104*	32 ± 8*	5 ± 5*
0.5	10	0**	0*	617 ± 157*	30 ± 8*	0**
1.0	10	0**	0*	384 ± 81**	19 ± 4*	0**
2.5	10	50	10	1096 ± 107	56 ± 8	13 ± 6*
Solvent	10	50	20	1149 ± 159	66 ± 9	26 ± 4
Cinnarizine (mg kg^{-1})						
0.1	10	60	20	953 ± 131	40 ± 7*	18 ± 8
0.25	10	40	10	759 ± 157*	40 ± 11*	11 ± 6*
0.5	10	0*	0*	510 ± 141*	17 ± 6*	0*
1.0	10	0*	0*	403 ± 107*	17 ± 4*	0*

*$P < 0.05$; **$P < 0.01$.

Table 4 The effect of prenylamine (given immediately after time 0) on mean arterial blood pressure (MABP) and heart rate in anaesthetized rats subjected to coronary artery occlusion (at 15 min)

		MABP Time (min)					*HR* Time (min)				
Treatment	n	0	0.5	15	20	45	0	0.5	15	20	45
Gluconate	14	96 ± 10	95 ± 11	94 ± 5	74 ± 8	94 ± 8	436 ± 22	430 ± 18	429 ± 19	421 ± 25	428 ± 19
Prenylamine (mg kg^{-1})											
0.5	10	87 ± 10	67 ± 7*	91 ± 8	78 ± 8	92 ± 8	419 ± 29	404 ± 27	425 ± 20	417 ± 25	427 ± 20
5.0	10	97 ± 11	41 ± 7**	67 ± 8**	53 ± 14*	71 ± 15	430 ± 18	308 ± 30**	364 ± 78	382 ± 26	386 ± 61

0 min represents the pretreatment values. *$P < 0.05$; **$P < 0.01$.

was about 20 mmHg below that in the controls. Both doses of prenylamine significantly reduced arterial blood pressure on injection and with the highest dose this was sustained and accompanied by a decrease in heart rate (Table 4).

Following coronary artery ligation, heart rate did not change significantly in control or drug-treated groups (Table 4). Mean arterial pressure fell transiently on ligation in all the animals but by the end of the 30 min arrhythmic period it was similar to preligation values.

Discussion

In this study we have shown that verapamil, prenylamine, flunarizine and cinnarizine may all protect against ischaemia-induced arrhythmias in anaesthetized rats. Following coronary artery ligation there were fewer ectopic beats and, more importantly, a reduced incidence of ventricular fibrillation and an improved survival in those rats pretreated with the calcium antagonists. These results confirm previous observations in anaesthetized dogs, in which verapamil (Kaumann & Aramendia, 1968; Elharrar *et al.*, 1977) prenylamine (Marshall & Parratt, 1977) and flunarizine (Tobia, A.J., personal communication) have all been shown to be effective against coronary artery occlusion-induced arrhythmias.

The fact that all four drugs used in this study have in common the ability to reduce the slow inward calcium current would suggest that this action may be the basis of this antiarrhythmic activity. Indeed, the order of potency in protecting against ischaemia-induced arrhythmias (verapamil > flunarizine > cinnarizine) is similar to that for inhibition of the slow channel as assessed by their vascular smooth muscle relaxing properties (Tobia, A.J., personal communication). However, the precise mechanism of action of these calcium antagonists, whether it be suppression of slow response action potentials in the myocardium (Dersham & Han, 1981), improvement of conduction (Elharrar *et al.*, 1977), increase in myocardial blood flow (Henry *et al.*, 1978) or a reduction in the degree of ischaemic damage (Smith *et al.*, 1975) cannot be elucidated from these experiments. Moreover, all of these drugs have other pharmacological actions which may contribute to their protective effects during ischaemia. Thus, verapamil has α-adrenoceptor and sodium channel blocking activities (Bayer *et al.*, 1975; Brooks *et al.*, 1980) whilst prenylamine depletes myocardial catecholamine stores (Lindner, 1971) and inhibits the fast sodium current in cardiac tissue (Hasselbach *et al.*, 1968) and both flunarizine and cinnarizine are histamine antagonists (Van Nueten & Janssen, 1973). There is, therefore, a need for further experi-

mental studies to determine the contribution, if any, of each of the above properties to the antiarrhythmic effectiveness of these drugs.

The present study indicates that there may be an important species difference in sensitivity during ischaemia to the myocardial actions of calcium antagonists. Whereas in dogs the effective antiarrhythmic concentrations of verapamil, prenylamine and flunarizine were 0.2–0.79, 5 and 2.5 mg kg^{-1} i.v. respectively, administration of similar concentrations of all three agents to anaesthetized rats resulted in a loss of beneficial activity. For example, verapamil (in doses of 0.1 and 0.5 mg kg^{-1}) and flunarizine (in a dose of 2.5 mg kg^{-1}) did not protect rats against ventricular fibrillation; indeed, with verapamil, mortality was greater than in the corresponding control group. Although animals administered 5 mg kg^{-1} prenylamine did not fibrillate upon occlusion, there was a high mortality in this group due to an increased incidence of atrioventricular block leading to asystole.

Similar bell-shaped dose-response curves for the cardio-protective action of calcium antagonists have been reported previously. For example, verapamil does not exhibit a linear log dose-response curve with respect to protection against myocardial enzyme leakage during hypoxia (Nayler *et al.*, 1976) or ischaemia (Yamamoto *et al.*, 1983) nor against the calcium paradox (Hearse *et al.*, 1980). Furthermore, the ability of both prenylamine (Manning *et al.*, 1982) and nifedipine (Selwyn *et al.*, 1979) to reduce the extent of myocardial ischaemic damage is lost at higher concentrations. The reason(s) underlying the loss of this protective action of calcium antagonists at higher concentrations is not known. In our studies, the non-linearity of the log dose-response relationships could not readily be explained by drug-induced changes in arterial blood pressure or heart rate. Thus, although the higher concentrations of prenylamine and flunarizine caused a sustained hypotension, verapamil did not. Again, only prenylamine significantly altered heart rate prior to occlusion. It has been suggested by Selwyn *et al.* (1979) that during ischaemia high doses of calcium antagonists may, by virtue of a 'coronary steal' effect, cause a greater inhomogeneity of coronary blood flow between ischaemic and normal regions of the myocardium. If this is the case, it might be anticipated that all calcium antagonists would exhibit the same phenomenon. In our experiments, at least over the concentration-range studied, cinnarizine did not exhibit this non-linear dose-response relationship. However, since the highest cinnarizine concentration used was only four times the effective antiarrhythmic dose compared with a twenty-five fold difference for the structurally related, flunarizine (see Table 3) we cannot exclude the possibility that non-linearity would be observed with higher doses of cinnarizine.

These results suggest that, if feasible, full dose-response relationships of antiarrhythmic drugs should be examined, especially if there appears to be species-dependent differences in sensitivity. We have shown that, under conditions of myocardial ischaemia in anaesthetized rats, the beneficial effects of calcium antagonists may be limited to a narrow concentration-range, a finding which, if it can be extrapolated to man, has obvious clinical relevance.

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