Br. J. Pharmacol. (1988), **94**, 1275–1286

The mechanism of action of calcium antagonists on arrhythmias in early myocardial ischaemia: studies with nifedipine and DHM9

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1 Nifedipine and DHM9 (carboxymethyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate) were studied for their effects on arrhythmias resulting from regional myocardial ischaemia in conscious rats, and for their effects on left ventricular developed pressure *in vitro*.

2 Nifedipine possessed antiarrhythmic activity at a high dose of $10\,mg\,kg^{-1}$ i.v., but not at 0.5 or $2\,mg\,kg^{-1}$. Ventricular fibrillation (VF), tachycardia (VT), and ventricular premature beats (VPB) were all attenuated to a similar degree; nifedipine did not have a selectivity of action for high frequency arrhythmias.

3 Before coronary occlusion, the three doses of nifedipine reduced arterial blood pressure by a similar magnitude, indicating a similar (maximal) degree of systemic vasodilatation. The reductions in blood pressure were accompanied by reflex tachycardia. Heart rate and blood pressure did not correlate with the incidence or severity of arrhythmias.

4 DHM9 had no influence on arrhythmias, haemodynamic variables or the ECG, even at $20\,mg\,kg^{-1}$ i.v.

5 Nifedipine concentration-dependently reduced contractility in perfused paced (5 Hz) rat ventricles *in vitro*. Raising the concentration of K^+ in the perfusion fluid from 3 to 10 mequiv.l^{-1} increased the potency ($-\log_{10} EC_{50}$) of nifedipine up to four fold, and caused a significant depression in excitability.

6 DHM9 at up to 3×10^{-5} M had no significant influence on ventricular contractility *in vitro*.

7 The results provided indirect evidence in support of the hypothesis that calcium antagonists inhibit ischaemia-induced arrhythmias by virtue of inhibition of the slow inward current (I_{si}) in the ischaemic ventricular myocardium.

Introduction

Calcium antagonists are drugs which inhibit the slow inward current (I_{si}; Fleckenstein, 1983). There has been considerable disagreement concerning their antiarrhythmic activity in the setting of early regional myocardial ischaemia. Phenethylalkylamines such as verapamil have been shown to possess marked antiarrhythmic actions by some investigators (e.g., Kaumann & Aramendia, 1968; Bren *et al.*, 1982; Bergey *et al.*, 1984), but not by others (e.g., Mertz & Kaplan, 1982; Mueller & Wilsmann, 1982; Fagbemi *et al.*, 1984). In the case of verapamil, the minimum effective dose is approximately $0.5\,mg\,kg^{-1}$ in acutely prepared anaesthetized animals and $2\,mg\,kg^{-1}$ in conscious animals (Curtis *et al.*, 1984).

The two optical enantiomers of verapamil have different potencies as calcium antagonists (Ferry *et al.*, 1985). By comparing their antiarrhythmic activity, Kaumann & Serur (1975) suggested that calcium antagonism was responsible for their antiarrhythmic effects during early ischaemia in anaesthetized dogs. We recently confirmed this finding in conscious rats (Curtis & Walker, 1986). Moreover, we found that the correlation between calcium antagonist activity and antiarrhythmic activity improved if ischaemia-

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induced regional elevations in extracellular K^+ concentration (Hill & Gettes, 1980) were taken into consideration (Curtis & Walker, 1986).

1,4-Dihydropyridine calcium antagonists like nifedipine have also been examined for their antiarrhythmic activity in early myocardial ischaemia. Negative (e.g., Verdouw & Wolffenbuttel, 1983; Coker & Parratt, 1985; Curtis *et al.*, 1985a) and positive (e.g., Fagbemi & Parratt, 1981; Bergey *et al.*, 1984; Endo *et al.*, 1985) results have been obtained, and no clear pattern has emerged. We have attempted to resolve this issue by using the conscious rat preparation in conjunction with *in vitro* experiments in the same way that we examined the optical enantiomers of verapamil (Curtis & Walker, 1986).

Although the closed-chest conscious rat preparation is a reproducible bioassay for examining antiarrhythmics (see Curtis *et al.*, 1987 for review) it does not permit direct analysis of mechanisms via mapping of conduction pathways or recording of intracellular potentials, hence the inclusion of *in vitro* studies. We examined nifedipine, the prototype 1,4-dihydropyridine calcium antagonist (Fleckenstein *et al.*, 1972) and DHM9 (carboxymethyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylate), a new 1,4-dihydropyridine with selectivity of action on ventricular versus vascular tissue (Clarke *et al.*, 1984). Part of this study has been communicated to the British Pharmacology Society (Curtis & Walker, 1987).

Methods

Coronary occlusion in conscious rats

The general experimental approach conformed with the guidelines of the Lambeth Conventions (Walker *et al.*, 1988).

The use of rats for the study of ischaemia-induced arrhythmias has recently been reviewed (Curtis *et al.*, 1987). The methods used in the present study were identical to the methods used to examine (+)- and (−)-verapamil (Curtis & Walker, 1986).

Animals (male Sprague-Dawley rats, Charles River, 240–320 g) were housed individually during the days (5–8) between preparative surgery and coronary occlusion, and fed Purina rat chow and tap water *ad libitum*. Six groups of nine rats were used. Drugs were dissolved in 20% ethanol in saline and administered at 0.25 ml $100\,g^{-1}$ body weight. Stock solutions were prepared in advance of the study, coded, refrigerated and stored in light-proof containers. Care was taken to prevent exposure of the drug-containing syringes to light before administration. Drug doses were nifedipine 0.5, 2 or $10\,mg\,kg^{-1}$ and DHM9 5 or $20\,mg\,kg^{-1}$. Controls received the ethanol/saline vehicle. Drug administration was by slow i.v. injection into the vena cava over a 10 min period, beginning at 15 min before coronary occlusion. Drug administration was carried out blind, and a randomization protocol was used. Analysis of records was carried out blind.

Detailed descriptions of preparative surgery (Au *et al.*, 1983), experimental design and statistical analysis (Johnston *et al.*, 1983), quantification of infarct size (Curtis *et al.*, 1985a) and measurement of serum potassium concentration (Curtis *et al.*, 1985b) have been described previously. Animals experiencing ventricular fibrillation (VF) were defibrillated by 'thump-version' (delivery of a blow to the chest with an index finger) if VF lasted for more than 10 s (Johnston *et al.*, 1983).

Calcium antagonist potency in rat ventricles

Isochoric left ventricular developed pressure was measured in rat ventricles, perfused *in vitro* via the aorta (Langendorff mode), exactly according to the protocol described recently (Curtis & Walker, 1986). The ventricles were paced at 5 Hz, and the K^+ concentration of the perfusion fluid was varied in order to simulate some of the changes in extracellular K^+ concentration which occur during the early phase of myocardial ischaemia (Hill & Gettes, 1980). Four concentrations of K^+ were used, 3, 5.9, 8 and $10\,mequiv.l^{-1}$. KCl was varied at the expense of NaCl. The other perfusion fluid constituents were standard (Krebs-Henseleit solution) with the exception of $CaCl_2$, which was 0.7 mM to give a developed pressure of approximately 50% of maximum as described previously (Curtis *et al.*, 1984). The apparatus used for the delivery of perfusion fluid permitted the generation of cumulative concentration-response data from individual preparations, owing to its facility for rapid switching between fluid storage vessels (Curtis *et al.*, 1986b). Excitability was measured after stabilization, followed by measurement of the effects of nifedipine and DHM9 on developed pressure by the construction of six-point concentration-response curves, in the manner described previously (Curtis & Walker, 1986). Records were analysed blind. Mean values of $-\log_{10} EC_{50}$, gradient and maxima data were calculated and the effects of K^+ were examined.

Statistics

The general approach to statistical analysis conformed with the guidelines of Wallenstein *et al.* (1980). Gaussian-distributed variables (including haemodynamic variables within discrete time periods and some transformed arrhythmia data such as $\log_{10}$ ventricular premature beat (VPB) number; Johnston

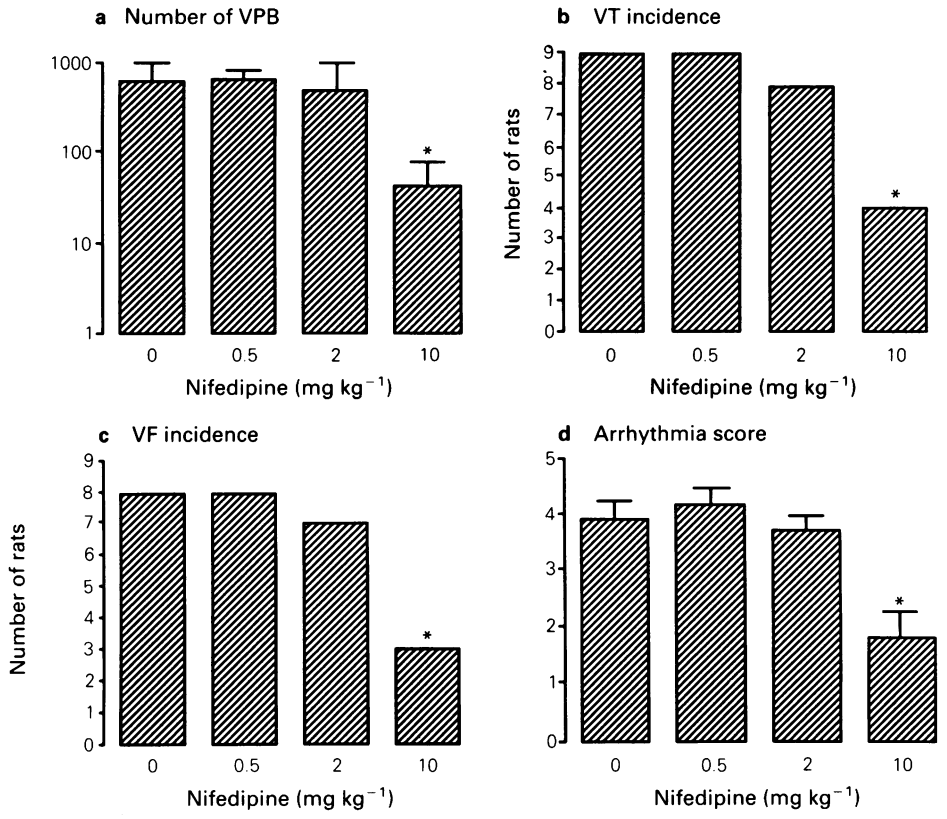

Figure 1 The effects of nifedipine on ischaemia-induced arrhythmias in the 4 h period following coronary occlusion. the number of ventricular premature beats is shown in (a); values are mean ± s.e.mean of the $\log_{10}$-transformed data. The incidence of ventricular tachycardia (VT) is shown in (b); values are incidence out of $n = 9$ rats per group. The incidence of ventricular fibrillation (VF) is shown in (c); values are incidence out of $n = 9$ rats per group. Mean arrhythmia scores are shown in (d); values are mean ± s.e.mean. In each part, statistical significance is indicated by $*P < 0.5$ versus the control (0 mg kg^{-1}) group.

et al., 1983) were subjected to analysis of variance. All six groups were included in each analysis of variance; differences between means were examined (by Duncan's multiple range test) only when treatment was found to be a significant source of variance. Mainland's contingency tables were used to compare binomially-distributed variables such as the incidences of ventricular tachycardia (VT) and VF (Mainland *et al.*, 1956). Statistical significance was taken as P less than 0.05.

Results

DHM9

DHM9 had no effect on any of the variables studied in conscious rats. In the perfused rat ventricles, DHM9 had no effect on developed pressure at up to 3×10^{-5} M. For this reason, DHM9 has not been mentioned further in the results, although some of the data are included in the tables.

Arrhythmias resulting from coronary occlusion

The numbers of VPB and the incidences of VT and VF were not reduced by nifedipine at up to 2 mg kg^{-1} (Figure 1 and Table 1). However, all three variables were significantly reduced by the highest dose, 10 mg kg^{-1}. The arrhythmia score was reduced correspondingly (Figure 1d). The incidence of VF requiring defibrillation was significantly reduced by 10 mg kg^{-1} nifedipine, but the incidence of short bursts of VF lasting for less than 10 s and reverting spontaneously to sinus rhythm was not significantly reduced, since the control incidence was too low for an effect to be revealed (Table 1). During the first 4 h

Table 1 Ventricular fibrillation (VF) and mortality following coronary occlusion

Dose (mg kg^{-1})		*SVF incidence* 0–0.5 h	0–4 h	*NVF incidence* 0–0.5 h	0–4 h	*Mortality* 0–4 h
Controls	$n = 9$	3/9	5/9	5/9	7/9	0/9
DHM9 5.0	$n = 9$	1/9	6/9	8/9	8/9	0/9
DHM9 20.0	$n = 9$	1/9	5/9	5/9	7/9	0/9
Nifedipine 0.5	$n = 9$	1/9	4/9	3/9	8/9	0/9
Nifedipine 2.0	$n = 9$	1/9	3/9	4/9	6/9	1/9
Nifedipine 10.0	$n = 9$	0/9	1/9	0/9*	2/9*	2/9

Values are incidence (out of n) for mortality, VF reverting spontaneously within 10 s of onset (SVF) and VF not reverting spontaneously within 10 s of onset and requiring defibrillation (NVF). The 3 deaths indicated in the table resulted from pulmonary oedema, not VF. * Indicates $P < 0.05$ versus controls.

after coronary occlusion, the $\log_{10}$ transform of the number of VPBs, the incidence of VT, the incidence of VF and the arrhythmia score were all reduced by approximately 50% by 10 mg kg^{-1} nifedipine (Figure 1). Almost all of the rats which survived for 24 h had VPBs when monitored for a 30 min period at this time, but the incidence and number were not different between the groups (not shown).

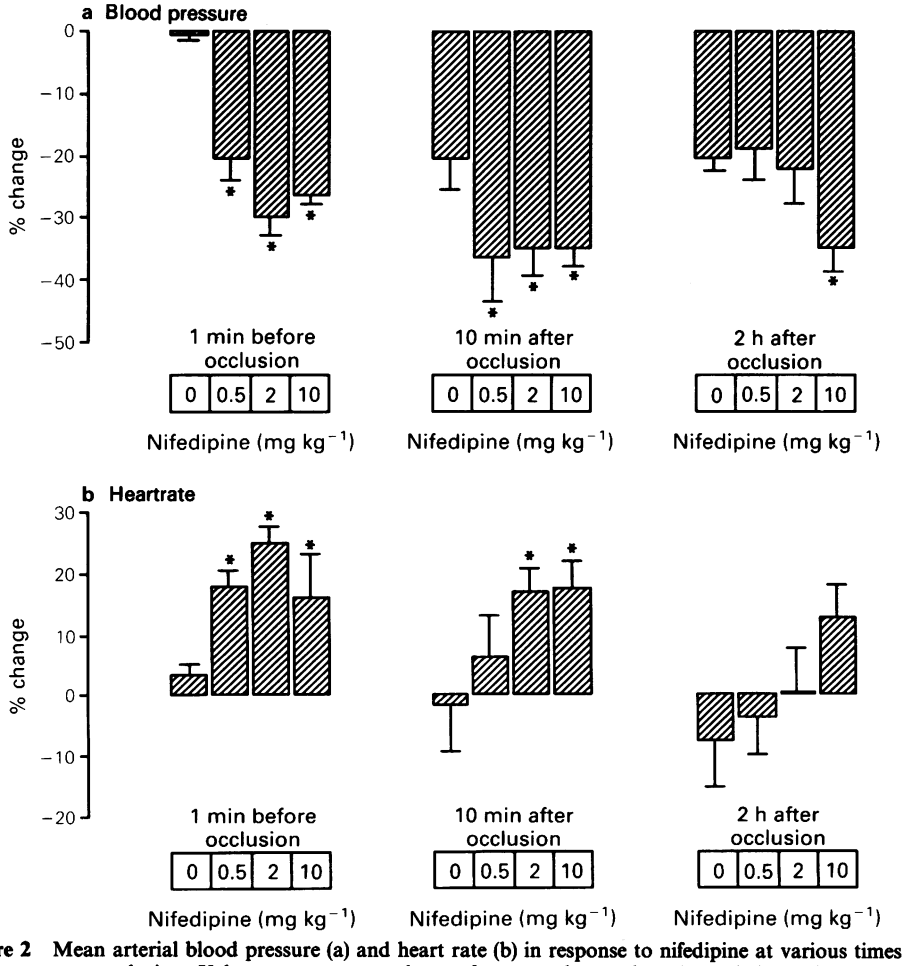

Figure 2 Mean arterial blood pressure (a) and heart rate (b) in response to nifedipine at various times before and after coronary occlusion. Values are mean % change from pre-drug values (recorded 15 min before occlusion); vertical lines indicate s.e.mean. *$P < 0.05$ versus the control group.

Table 2 Size of occluded zone and infarct zone

Dose ($mg\,kg^{-1}$)		*OZ* (% of ventricle weight)	*IZ* (% of ventricle weight)	*IZ* (% of OZ)
Controls	$n = 9$	39 ± 3	32 ± 2	81 ± 4
DHM9 5.0	$n = 9$	35 ± 2	30 ± 3	86 ± 8
DHM9 20.0	$n = 9$	35 ± 2	25 ± 3	72 ± 5
Nifedine 0.5	$n = 9$	37 ± 3	29 ± 3	80 ± 7
Nifedipine 2.0	$n = 9$	38 ± 2	25 ± 3	70 ± 10
Nifedipine 10.0	$n = 9$	36 ± 3	24	82

The occluded zone (OZ) was expressed as % ventricular weight. The infarct zone (IZ) was expressed as % ventricular weight and also as % OZ weight. Values (mean ± s.e.mean) are for group sizes of n for all variables except IZ which was measured in 24 h survivors only (in which case s.e.mean data have been omitted in one group in which n was less than 5).

Haemodynamic and ECG variables before occlusion

Before drug administration there were no significant differences between the groups with regard to mean aortic blood pressure (109 ± 2 mmHg in controls) and heart rate (401 ± 17 beats min^{-1} in controls). Blood pressure was reduced by all three doses of nifedipine, and heart rate was increased ($P < 0.05$) (Figure 2). These haemodynamic effects were not dose-dependent in that all three doses produced similar changes.

Before drug administration there were no significant differences in PR interval (43 ± 1 ms in controls) and QRS interval (29 ± 1 ms in controls) between the groups. Neither variable was affected by the drug vehicle, but both were reduced dose-independently by nifedipine (not shown). These effects were commensurate with an indirect effect mediated by an increase in sympathetic tone, but were not statistically significant.

Blood pressure and heart rate following coronary occlusion

Blood pressure fell considerably in all groups within 1 min of occlusion. At 10 min after occlusion, at the time of the first peak of ischaemia-induced arrhythmias in the rat (Johnston *et al.*, 1983), the difference in blood pressure between the controls and the treated groups were similar to the differences observed before occlusion (Figure 2) and there were no significant differences between the three doses of nifedipine. By 2 h after occlusion, during the second peak of ischaemia-induced arrhythmias (Johnston *et al.*, 1983), blood pressure remained significantly reduced only with the highest dose of nifedipine (Figure 2a).

Coronary occlusion had no significant effect on heart rate in control rats (Figure 2b), except that values appeared to have fallen as a trend by approximately 2 h after occlusion (although there was much variability at this time). The dose-independent sinus tachycardia produced by nifedipine before occlusion was still apparent 1 min after occlusion (not shown), and occlusion neither added to, nor subtracted from, this effect. By 10 min after occlusion, a significant sinus tachycardia was present only in the groups given 2 or 10 $mg\,kg^{-1}$ nifedipine (Figure 2b). By 2 h after occlusion, heart rate differences between the groups were not statistically significant, owing to an increase in variability with time (Figure 2b).

Occluded zone, infarction, mortality and ECG changes after occlusion

Nifedipine had no significant influence on the size of the occluded zone (OZ) or the extent of infarction (Table 2). Mortality during the first 4 h after occlusion (Table 1) was extremely low (3 deaths out of 54 rats). Thump-version was successful in defibrillating every episode of VF which had not self-terminated within 10 s of onset.

There were no statistically significant effects of treatment on the magnitude of maximum S-T segment elevation or maximum R wave amplitude in the V_3 lead following occlusion (Table 3). However, the time at which these events reached their maxima and the time at which a pathognomic Q wave became evident were all delayed by the highest dose of nifedipine.

Serum K^+ concentration following coronary occlusion

Nifedipine had no significant influence on serum K^+ concentration measured 2 h after occlusion. Values ranged from 3.6 ± 0.2 mequiv.l^{-1} (0.5 $mg\,kg^{-1}$ nifedipine group) to 4.3 ± 0.2 mequiv.l^{-1} (10 $mg\,kg^{-1}$ nifedipine group). Values in the control group (4 ± 0.1 mequiv.l^{-1}) were similar to previous control values (Curtis *et al.*, 1985b,c).

Table 3 Extent of ECG changes after occlusion

Dose ($mg\,kg^{-1}$)		*Max ST%* (% of R)	*Time of max ST%* (min)	*Max R* (mV)	*Time of max R* (min)	*Time of Q-wave* (h)
Controls	$n = 9$	77 ± 4	9 (8–11)	1.12 ± 0.06	5 (3–9)	2.1 (1.7–2.5)
DHM9 5.0	$n = 9$	82 ± 8	6 (5–7)	1.29 ± 0.08	7 (5–10)	1.7 (1.4–2.2)
DHM9 20.0	$n = 9$	63 ± 7	11 (8–15)	1.42 ± 0.15	14 (9–22)	1.7 (1.3–2.2)
Nifedipine 0.5	$n = 9$	85 ± 6	11 (9–14)	1.26 ± 0.13	6 (4–11)	1.7 (1.4–2.1)
Nifedipine 2.0	$n = 9$	74 ± 6	13 (10–18)	1.06 ± 0.09	7 (4–12)	2.8 (2.4–3.2)
Nifedipine 10.0	$n = 9$	68 ± 5	44 (35–55)*	1.11 ± 0.11	15 (8–31)	3.9 (3.4–4.4)*

Times to max ST%, max R and Q-wave development were calculated in $\log_{10}$ time, but expressed as mean (-1 and $+1$ s.e.mean) of real time for clarity. Other variables are mean $\pm$ s.e.mean. *Indicates $P < 0.05$ versus controls. For all variables $n = 9$.

Actions of nifedipine in rat isolated perfused ventricles

Figures 3 and 4 show the relationship between the negative inotropic activity of nifedipine and the concentration of K^+ present in the perfusion solution. Increasing the K^+ concentration from 3 to 10 mequiv.l^{-1} caused a four fold increase in nifedipine's potency ($-\log_{10}$ EC_{50}). The $-\log_{10}$ EC_{50} values were all statistically significantly different from one another. The relationship between K^+ and $-\log_{10}$ EC_{50} was linear (Figure 4).

The slopes of the concentration-response curves for nifedipine were significantly greater than 1 at 3, 5.9 and 8 mequiv.l^{-1} K^+ (values being 1.46 ± 0.14, 1.7 ± 0.11 and 1.39 ± 0.12, respectively). However, at 10 mequiv.l^{-1} K^+, the slope was not different from 1 (it was 1.01 ± 0.03). Therefore it is not strictly appropriate to extrapolate from the $-\log_{10}$ EC_{50} data and state that K^+ had changed 'potency'. Nevertheless, the differences in slope were not particularly large (slope values were always less than 2), and variance values for $-\log_{10}$ EC_{50} were always small (Figure 4).

Nifedipine caused increases in coronary flow which were not clearly concentration-related nor related to the K^+ concentration (not shown). Pre-drug flows were 9.3 ± 0.6, 11.3 ± 1.2, 8.9 ± 0.9 and 11.2 ± 0.7 ml min^{-1} in the 3, 5.9, 8 and 10 mequiv.l^{-1} K^+ groups, respectively.

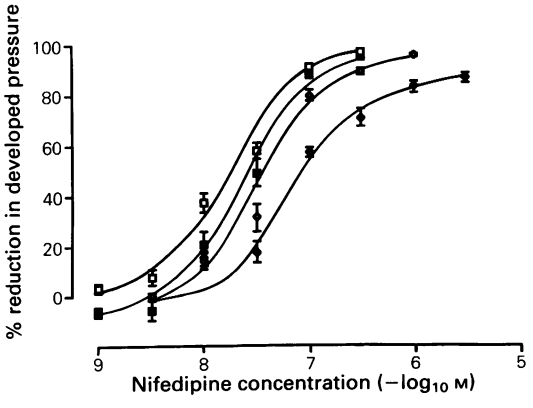

Figure 3 Concentration-response curves for the effect of nifedipine on developed pressure (systolic minus diastolic) in Langendorff-perfused rat ventricles. The abscissa scale shows % reduction in developed pressure from pre-drug value, and the ordinate scale indicates the $-\log_{10}$ concentration of nifedipine (M). Values are mean and vertical lines s.e.mean of $n = 6$ preparations. The K^+ concentration in the perfusion fluid was 3, 5.9, 8, or 10 mequiv.l^{-1} (◆, ◇, ■ and □, respectively).

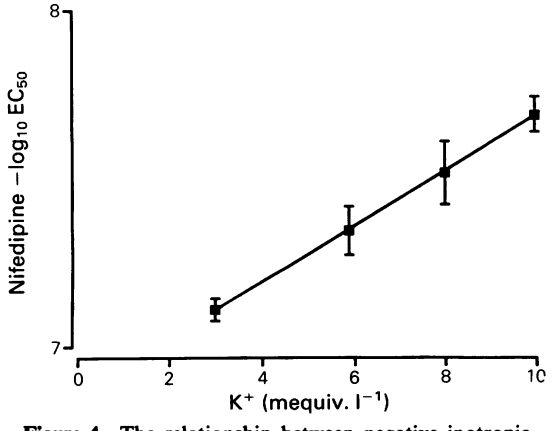

Figure 4 The relationship between negative inotropic potency of nifedipine and K^+ concentration (see Figure 3 for raw data). The abscissa scale is K^+ concentration (mequiv.l^{-1}) in the perfusion fluid, and the ordinate scale is $-\log_{10}$ EC_{50} for negative inotropism (mean $\pm$ s.e.mean) for $n = 6$ preparations per value. The values were all significantly different from one another ($P < 0.05$).

Ventricular excitability and extracellular K^+ concentration

Preliminary evidence from our previous study with (+)- and (−)-verapamil (Curtis & Walker, 1986) suggested that increasing the K^+ concentration of the perfusion solution reduced the excitability of the ventricles (as determined by sampling the strength-duration curve at 4 V or 1 ms). This effect was confirmed in the present experiment. Raising the K^+ concentration caused a large increase in the threshold voltage for capture at 1 ms (Figure 5a) and a similar increase in the threshold pulse-width for capture at 4 V (Figure 5b). These effects appeared to be linear over the K^+ range studied, but there was a suggestion that the effects were beginning to saturate; with only 4 points, it was impossible to distinguish between the two models, therefore the points have not been connected in Figure 5.

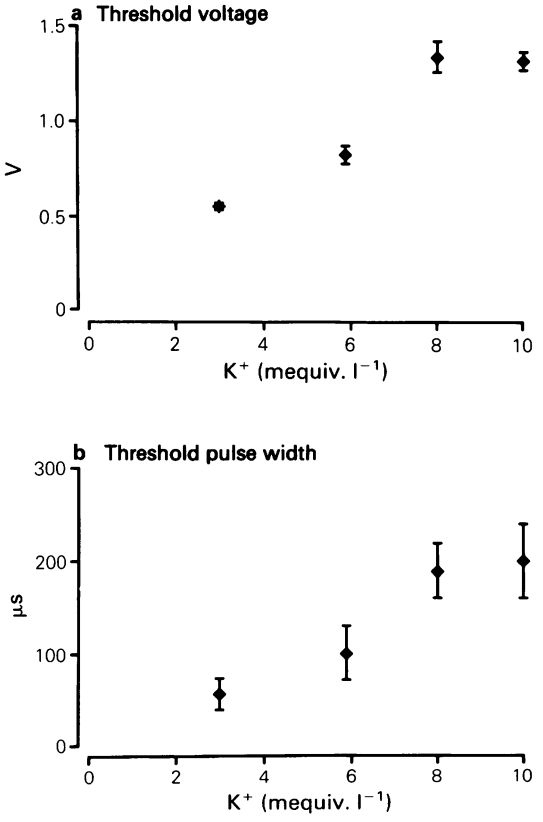

Figure 5 The effect of perfusion fluid K^+ concentration on (a) the threshold voltage (at 1 ms pulse width) and (b) the threshold pulse width (at 4 V) for capture in the Langendorff-perfused rat ventricle preparation (stimulation frequency 300 min^{-1}). Values are mean, and vertical lines s.e.mean, and were recorded after 10–15 min stabilization. Each preparation was subsequently used to measure the negative inotropic activity of nifedipine (see Figure 4). The values for both variables at 3 and 5.9 mequiv.l^{-1} K^+ were significantly different from each other and from the values at 8 and 10 mequiv.l^{-1} K^+ ($P < 0.05$).

Discussion

Antiarrhythmic activity of calcium antagonists in early ischaemia

The present studies demonstrated that nifedipine can inhibit ischaemia-induced ventricular arrhythmias in conscious rats, but only at a high dose of 10 mg kg^{-1} i.v.

In previous studies we suggested that the anti-arrhythmic effects of calcium antagonists occur entirely as a result of inhibition of I_{si} (Curtis *et al.*, 1984; 1985a; 1986b; Curtis & Walker, 1986; Au *et al.*, 1987), and that the site of action is most probably the ischaemic tissue. However, there appeared to be differences between the major classes of calcium antagonists, since we found that felodipine, a 1,4-dihydropyridine, had negligibile antiarrhythmic activity (Curtis *et al.*, 1985a), whereas verapamil and other phenethylalkylamines (Curtis *et al.*, 1984; 1986b; Curtis & Walker, 1986; Au *et al.*, 1987) were effective, yet both classes of drugs produced similar reductions in blood pressure.

These results led to the hypothesis that 1,4-dihydropyridine calcium antagonists are less anti-arrhythmic than phenethylalkylamines at equivalent vasodepressor doses because they inhibit I_{si} in the ventricles to a lesser degree at such doses (Curtis *et al.*, 1985a). The present study was carried out to test this hypothesis by examining whether nifedipine (which is similar to felodipine in its vascular-selectivity) can inhibit ischaemia-induced arrhythmias if a sufficiently high dose is given.

If calcium antagonists inhibit ischaemia-induced arrhythmias by inhibiting I_{si} in ventricles, then arrhythmias ought to be inhibited in a manner consistent with such an action. We examined this by considering tissue selectivity (vasculature versus myocardium), voltage-dependence (effects of variation in extracellular K^+) and frequency-dependence (as reflected by selectivity for high versus low frequency arrhythmias). Together, these properties have led us to propose the concept of site selectivity.

Tissue selectivity

In order to prove that inhibition of I_{si} in the ischaemic myocardium accounts for the antiarrhythmic effects of nifedipine and other calcium antagonists, it is necessary to compare their antiarrhythmic potencies with their I_{si}-blocking potencies in the ischaemic myocardium. In our studies we did not have a direct *in vivo* measure of I_{si} in the ischaemic myocardium. Extrapolation from indirect measurements (blood pressure or cardiac output, for example) is vulnerable to misinterpretation, partly because of differences in tissue selectivity.

Tissue selectivity (vascular versus myocardial) constitutes a major difference between nifedipine and (+)-verapamil. Nifedipine possesses a greater selectivity for the systemic vasculature versus the myocardium than (±)-verapamil (Fleckenstein-Grun *et al.*, 1976; Raschack, 1976; Briscoe & Smith, 1982). Therefore, doses of nifedipine and (±)-verapamil which have equivalent effects on the systemic vasculature will not similarly have equivalent effects in the myocardium. Our findings were consistent with this.

Nifedipine reduced blood pressure independently of the dose, indicating that the maximum vasodilatation attainable with this drug was probably reached even by the lowest dose. However, only the highest dose reduced arrhythmias. In contrast, (±)-, (+)- and (−)-verapamil have been shown to reduce blood pressure dose-dependently without a maximum effect being reached over the dose-range which inhibits arrhythmias (Curtis *et al.*, 1984; Curtis & Walker, 1986). Therefore, as expected, nifedipine's vasodilator potency did not correlate with its antiarrhythmic activity.

This finding has two important ramifications. Firstly, when speculating on antiarrhythmic dose requirements for calcium antagonists, it is inappropriate to base expectations on their potency in vascular smooth muscle. Secondly, it appears that the actions that calcium antagonists have on blood vessels play a relatively unimportant role in their antiarrhythmic effects in comparison with their actions in the myocardium itself.

Frequency-dependence and voltage-dependence

There are important differences between (±)-verapamil and nifedipine, which make studies which compare their potencies *in vivo* difficult to interpret, even when differences in tissue selectivity are taken into account. Firstly, the higher degree of plasma-protein binding of nifedipine compared with (±)-verapamil (Hermann & Morselli, 1985) would be expected to reduce the apparent potency of nifedipine relative to (±)-verapamil *in vivo*. Secondly, there are differences in the way these drugs interact with the calcium channel.

Differences in the frequency-dependence and voltage-dependence of calcium antagonists have been invoked to explain the large discrepancy between binding study and pharmacological estimates of their potency, and also the differences in their tissue selectivity (Bean, 1984; Sanguinetti & Kass, 1984; Holck & Osterrieder, 1985). In theory, frequency-dependence and voltage-dependence will modulate not only calcium antagonist potency, but also the antiarrhythmic activity (Hondegham & Katzung, 1984).

Nifedipine has been shown to possess little (Woods & West, 1983) if any (Bayer *et al.*, 1977) frequency-dependence in cardiac tissues. However, (±)-verapamil possesses marked frequency-dependence (Ehara & Kaufmann, 1978; McDonald *et al.*, 1980; Sanguinetti & West, 1982). If calcium antagonists inhibit arrhythmias by blocking I_{si}, verapamil should possess selective activity on high frequency arrhythmias such as VF, whereas nifedipine should affect VT, VF and VPBs equally effectively, as suggested previously (Hondeghem & Katzung, 1984). Our results were consistent with these predictions, since nifedipine inhibited VF, VT and VPB to a similar degree whereas we found previously that (+)- (±)- and (−)-verapamil all inhibited VF more effectively than VT and VPB (Curtis *et al.*, 1984; Curtis & Walker, 1986). This evidence of a link between frequency-dependent inhibition of I_{si} and frequency-dependent antiarrhythmic activity is consistent with the hypothesis that calcium antagonists inhibit ischaemia-induced arrhythmias by inhibiting I_{si} in the ventricles.

Although most studies suggest that 1,4-dihydropyridines do not possess marked frequency-dependence (see above), they do exhibit voltage-dependence (Bean, 1984). The potency of nifedipine on I_{si} is increased by depolarization, and its negative inotropic potency is correspondingly increased by raising extracellular K^+ (Holck & Osterrieder, 1985). The difference between the frequency-dependence and the voltage-dependence of nifedipine would result if nifedipine has a higher affinity for its receptor when the channel is in the inactivated state versus the rested state (as suggested for nitrendipine; Bean, 1984), but a small dissociation rate constant (resulting in a relative lack of frequency-dependence). By comparison, although (±)-verapamil exhibits voltage-dependence for the same reason as nifedipine, it appears to differ from nifedipine by having a large dissociation rate constant resulting in frequency-dependence (Hondeghem & Katzung, 1984). In view of these findings, we have suggested that since regional ischaemia causes regional

increases in extracellular K^+ concentration and regional depolarization, voltage-dependent calcium antagonists should have site-selectivity for the ischaemic versus non-ischaemic myocardium (Curtis & Walker, 1986). This would explain the antiarrhythmic activity of nifedipine.

Site selectivity

The actions of nifedipine on developed pressure *in vitro* provided an independent estimate of calcium antagonist activity in ventricular tissue over a K^+ concentration range commensurate with the elevated extracellular K^+ concentrations seen in early ischaemia (Hill & Gettes, 1980). The effects of K^+ on the potency of nifedipine were consistent with the voltage-dependence of nifedipine (Holck & Osterrieder, 1985); increasing K^+ concentration from 3 to 10 mequiv.l^{-1} increased the potency of nifedipine four fold in the present studies. This compares with a thirty fold and seventy fold increase in the case of (−)- and (+)-verapamil, respectively (Curtis & Walker, 1986). Therefore, although K^+ did not potentiate the potency of nifedipine as much as that of the verapamil enantiomers, we would nevertheless predict that nifedipine should inhibit I_{si} more potently in the ischaemic myocardium than in the non-ischaemic myocardium. Thus, we suggest that regional elevations of K^+ concentration confer site-selectivity of action (in the ischaemic tissue) on nifedipine, and that the resultant inhibition of I_{si} in this region accounts for the antiarrhythmic activity.

Although we did not directly measure the effect of K^+ on membrane potential, the evidence that K^+ reduced excitability was consistent with a reduction in resting membrane potential. Therefore, the K^+-induced site-selectivity of nifedipine (and verapamil) may be explained in terms of voltage-dependence (Lee & Tsien, 1983; Hondeghem & Katzung, 1984). Experiments were not carried out with higher K^+ concentrations because previous work had suggested that it was not possible to pace the ventricles efficiently when K^+ concentration was more than 10 mequiv.l^{-1} (Curtis & Walker, 1986). Studies with large mammalian hearts have revealed that conduction velocity becomes markedly depressed at K^+ concentrations exceeding 10 mequiv.l^{-1} (Kleber *et al.*, 1986).

In early ischaemia, K^+-depolarization-induced site-selectivity of I_{si} blockade by both nifedipine and (±)-verapamil may be enhanced by ischaemia-induced regional acidosis (Hill & Gettes, 1980), since the negative inotropic potencies of both nifedipine and (±)-verapamil are enhanced in ventricles by acidosis (Briscoe & Smith, 1982).

Other actions which may contribute to nifedipine's antiarrhythmic activity

Nifedipine appeared to have no direct effect on QRS interval in the present study. In this regard, nifedipine does not appear to affect sodium conductance (gNa) in ventricular tissue (Bayer *et al.*, 1977), unlike (±)-verapamil which can block gNa at high concentrations *in vitro* (Nawrath *et al.*, 1981). Nifedipine also failed to prolong PR interval. Thus nifedipine did not appear to have any direct effects on the electrophysiology of the normal (non-ischaemic) myocardium. Therefore it seems unlikely that the antiarrhythmic actions of nifedipine occurred via an action in the non-ischaemic myocardium.

Mild hyperkalaemia (serum K^+ 4–6 mequiv.l^{-1}) can lead to a significant reduction in the incidence of ischaemia-induced arrhythmias in rats (Curtis *et al.*, 1985b). However, nifedipine had no significant effect on serum K^+, in agreement with clinical findings (Sluiter *et al.*, 1985). Therefore it seems unlikely that the antiarrhythmic actions of nifedipine occurred via an action on serum K^+ concentration.

Ischaemia-induced arrhythmias do not correlate with heart rate or blood pressure in conscious rats (Johnston *et al.*, 1983). In agreement with this, we found that 2 and 10 mg kg^{-1} nifedipine had similar haemodynamic actions during early ischaemia but dissimilar effects on arrhythmias. Therefore it seems unlikely that the antiarrhythmic actions of nifedipine occurred via reduction in afterload or alteration in heart rate.

DHM9

DHM9, a metabolite of nicardipine, has been shown to inhibit I_{si}-dependent events selectively in ventricular muscle versus vascular smooth muscle (Clarke *et al.*, 1984). However, DHM9 is a very weak calcium antagonist, and is in fact less potent than both nifedipine and verapamil in ventricular tissue (Clarke *et al.*, 1984). This feature was reflected in our results. DHM9 was without antiarrhythmic activity. This was not surprising, since we found that DHM9 was without any activity on electrocardiographic or haemodynamic variables, either in rat isolated perfused ventricles (at up to 3×10^{-5} M) or in conscious rats (at up to 20 mg kg^{-1} i.v.).

Infarct size

Nifedipine (and DHM9) did not reduce infarct size. Nifedipine did, however, delay the process of isch-

aemia and infarction, as indicated by a delay in the development of pathognomic ECG signs. This pattern of response is almost exactly the same as the pattern described previously for other calcium antagonists in the conscious rat (Curtis *et al.*, 1984; 1985a; 1986b; Curtis & Walker, 1986) and is not related to antiarrhythmic activity. No class of drug has been consistently found to reduce infarct size in rats with permanent coronary occlusion; we have argued that this is expected (Curtis *et al.*, 1987), given the paucity of collateral anastamoses in rat ventricles. Nevertheless, based upon the indirect evidence of a delay in the infarct process in the present (and previous) studies, we hypothesize that calcium antagonists may be effective in reducing infarct size in rats with transient coronary occlusion and reperfusion.

Therapeutic implications

Although nifedipine (and verapamil; Curtis *et al.*, 1984) can inhibit ischaemia-induced arrhythmias, clinical application is limited as a result of high dose-requirements (and attendant side-effects). However, we predict that a therapeutically useful calcium antagonist for prophylaxis against VPB, VT and VF could be developed if voltage-dependence, frequency-dependence and site-selectivity are taken into consideration. The first requirement, *sine qua non*, would be voltage-dependence (even more than with verapamil) to confer site-selectivity in the ischaemic region. The second requirement would be a lack of frequency-dependence (even less than with nifedipine) to minimize unwanted actions outside the ischaemic region. However, (as a note of caution) if vascular selectivity is a manifestation of voltage-dependence (rather than channel sub-type, for example) it may ultimately prove impossible to design a safe (ischaemia-selective) antiarrhythmic calcium antagonist on the basis of these considerations alone.

We would like to thank Professor D.J. Hearse, Dr E. Riva, Dr R. Kempsford and Dr M. Avkiran for their helpful comments on the manuscript, and Mrs C. Erlebach for her assistance in its preparation. The work was funded by the British Columbia Heart Foundation (M.J.A.W. & M.J.C.) and the Canadian Heart Foundation (M.J.C.). Nifedipine was kindly supplied by Dr R. Kazda, Bayer AG, Wuppertal, F.R.G., and DHM9 was a gift of Dr R.L. Whiting, Syntex, Palo Alto Ca., U.S.A.

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(Received February 8, 1988
Revised March 23, 1988
Accepted April 6, 1988)