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# Effects of calcium entry blockers on calcium-dependent contractions of rat portal vein

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**1** The effects of six calcium entry blockers belonging to the dihydropyridine (isradipine or PN 200-110, nifedipine, nicardipine), verapamil (D888 or desmethoxyverapamil, D600 or gallopamil) and diltiazem classes were investigated on isometric spontaneous contractions and contractions induced by high-$K^+$ solutions, noradrenaline, acetylcholine and caffeine.

**2** The rank order of potency was PN 200-110>nicardipine = nifedipine = D888>D600>diltiazem from experiments on spontaneous contractions and high-$K^+$ induced contractions. With depolarized preparations, the concentration-response curves for nicardipine, PN 200-110, nifedipine and D600 were significantly shifted to the left indicating that the calcium entry blockers show voltage-dependent inhibitory properties. This effect was not significant with D888 and diltiazem.

**3** All the calcium entry blockers strongly reduced the noradrenaline (NA)- and acetylcholine (ACh)-induced contractions at concentrations which produced complete inhibition of spontaneous contractions. They had a slight effect on caffeine-induced contractions.

**4** In $Ca^{2+}$-free, EGTA-containing solutions, both ACh, NA and caffeine produced transient contractions, the amplitude of which could be taken as a measurement of the amount of internal calcium present in a drug-sensitive calcium store. The filling of the calcium store was maximal after 10–12 min of calcium loading in 2.1 mM $Ca^{2+}$, while the depletion was complete after 4–6 min of perfusion in $Ca^{2+}$-free solution.

**5** At concentrations which abolished spontaneous contractions, PN 200-110, nifedipine, D888 and D600 had no appreciable effect on contractions evoked in $Ca^{2+}$-free solutions by ACh, NA and caffeine. When added in $Ca^{2+}$-containing solutions diltiazem and, to a lesser extent, nicardipine strongly reduced the contractions evoked in $Ca^{2+}$-free solutions, suggesting that they inhibited the filling of the internal calcium store.

**6** These results indicate that the six calcium entry blockers are potent inhibitors of calcium influx through voltage-dependent calcium channels. Two of them (diltiazem and nicardipine) may exert an additional effect to depress contractions dependent on intracellular calcium release.

## Introduction

On voltage-clamped portal vein smooth muscle, it has been shown that there are two sources of intracellular ionized calcium. One is the influx of $Ca^{2+}$ ions from outside through voltage-dependent calcium channels, and the other is the release of calcium from intracellular storage sites (Mironneau & Gargouil, 1979). The release of internal calcium is also demonstrated by stimulating portal vein strips in $Ca^{2+}$-free solutions with different neurotransmitters (Bond *et al.*, 1984; Nanjo, 1984). Organic calcium entry blockers such as dihydropyridines are known to reduce the inward current through slow calcium channels in isolated cells from rat portal vein (Loirand *et al.*, 1986). Other substances possessing calcium channel blocking properties may interfere with calcium release from the intracellular calcium store of smooth muscles, as previously shown with pinaverium bromide (Mironneau *et al.*, 1984) or indapamide (Mironneau *et al.*, 1986).

These considerations led us to study the effects of organic calcium entry blockers belonging to the dihydropyridine, verapamil and diltiazem classes on calcium-dependent contractions of rat portal vein. This was done on isometric spontaneous contractions

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and on contractions induced by high-$K^+$ solutions, neurotransmitters or caffeine. Perfusion with $Ca^{2+}$-free, EGTA-containing solution was used to determine whether the organic calcium entry blockers may affect contractions induced by noradrenaline, acetylcholine or caffeine.

A preliminary account of these findings has been presented (Dacquet *et al.*, 1986).

## Methods

All experiments were performed on Wistar rats weighing between 250 to 300 g. Isolated longitudinal strips (100–200 μm in diameter; 3–4 mm in length) were obtained from rat portal vein. After a stabilizing period (approximately 20 min) in the reference solution the preparation was ready for mechanical recordings and study of the effects of calcium entry blockers.

### *Mechanical recordings*

The experimental chamber consisted of a 3 × 3 × 20 mm open-topped channel, connected at one end to a four way tap opening directly into the channel, allowing rapid change of the perfusing solution. The solution entered the channel at a rate of 10 ml $min^{-1}$ so that the liquid was completely changed in about 1 s. About 2 mm away from the tap, one end of the strip was tied to the floor of the chamber by means of a nylon loop. The other end of the strip was fixed to the lever of a highly sensitive isometric force transducer (Akers 801 AME, Horten, Norway) with a very low drift, good linearity and high sensitivity. A resting tension of 50 mg was applied to each strip at the beginning of the experiment.

### *Spontaneous contractions in rat portal vein*

Spontaneous twitch contractions were recorded during an initial 20 min stabilization period before adding the calcium antagonistic compounds in a non-cumulative manner. The perfusion times to obtain maximal inhibitory effects were about 7 min for dihydropyridines and about 10 min for D600, D888 and diltiazem. These equilibration times were used for the following experiments. Complete reversibility of contractions was observed within 10 min for dihydropyridines and for times longer than 25 min with D600, D888 and diltiazem. A recovery period of 30 min was allowed between responses and normally doses were given at regular intervals in a random order of concentrations. Spontaneous contractions recorded during the last 5 min of recovery period were taken as control responses and subsequent responses were expressed as a percentage of this control. Inhibitory concentration-effect curves were obtained by plotting isometric peak contraction (as a %) as a function of the external compound concentration.

### *High $K^+$-induced contractions in rat portal vein*

Application of a 60 mM potassium solution induced a phasic peak contraction followed by a sustained plateau, the amplitudes of which were dependent on the external calcium concentration (Godfraind & Kaba, 1969; Gabella, 1978). At the beginning of the experiment, three successive applications of 60 mM potassium solution were made in order to obtain at least two contractions similar in amplitude and kinetics. The last contraction was considered as the control response. The compounds were added either before the $K^+$-induced contraction, for their respective equilibration times, or during the plateau of the $K^+$-induced contraction and inhibitory concentration-effect curves were performed. During each recovery period, control high $K^+$ responses were recorded; it may be noted that the amplitude of contractions remained similar for experiments lasting several hours.

### *Drug-induced contractions*

Concentrations of calcium entry blockers producing a complete inhibition of spontaneous contractions were tested on contractions induced by noradrenaline, acetylcholine and caffeine. After recording of three successive drug-induced contractions of similar amplitude, the calcium antagonist was added for a period corresponding to its equilibrium time. Then, the drug was added at the same concentration as in control and the reduction in maximal amplitude measured as a percentage of the control. After 30 min of washing in reference solution, complete recovery of drug-induced contractions was obtained.

### *Contractions induced in $Ca^{2+}$-free solutions*

The presence of an internal calcium store that can be released by various drugs has been demonstrated previously in vascular smooth muscles (Deth & Van Breemen, 1977; Droogmans *et al.*, 1977; Casteels & Droogmans, 1981). This was usually shown by washing the preparation in a $Ca^{2+}$-free, EGTA-containing solution and inducing a contraction in response to a supra-maximal concentration of drug. The drug-induced contraction was evoked only once in $Ca^{2+}$-free solution. The response reappeared after refilling the internal store by exposure of the preparation to a $Ca^{2+}$-containing solution. The size of the contraction was largely dependent on the time interval between removal of calcium and application of the drug. The effects of the calcium entry blockers on the drug-induced contractions elicited in $Ca^{2+}$-free solutions

were studied. The calcium entry blockers were added either during the calcium loading (effect on the filling of the internal calcium store) or during the perfusion in $Ca^{2+}$-free solutions (effect on the depletion of the internal calcium store). In the latter case, the time in $Ca^{2+}$-free solution was too short in comparison with the equilibration time of the drugs. After complete calcium loading in $Ca^{2+}$-containing solution, the test calcium entry blocker was applied in the same $Ca^{2+}$-containing solution for 9 min and maintained during perfusion in $Ca^{2+}$-free solution. Control contractions were recorded before and after addition of a calcium entry blocker (Figures 6 and 7).

*Solutions*

The physiological solutions had the following composition: (a) reference solution (mM):NaCl 130, KCl 5.6, $CaCl_2$ 2.1, $MgCl_2$ 0.24, glucose 11; this solution was buffered with Tris HCl (8.3 mM) at pH 7.4. (b) In calcium-free solution, $CaCl_2$ was omitted and 0.5 mM EGTA was added. At this concentration EGTA was able to chelate superficial $Ca^{2+}$ ions from the cell membrane in less than 1 min, as triggered action potentials were suppressed. (c) High potassium solutions were prepared by substituting NaCl for KCl in equimolar amounts. It has been shown that the amplitude of potassium contractions was maximal with external potassium concentrations of 40–60 mM (Gabella, 1978). The different solutions were maintained at 36 ± 1°C by means of a heated bath or at 20°C (room temperature) for experiments in $Ca^{2+}$-free, EGTA-containing solutions.

*Drugs*

The calcium entry blockers were dissolved in dimethylsulphoxide (DMSO, Sigma): PN 200-110 (isradapine) and nicardipine (Sandoz), nifedipine (Bayer), D600 (methoxyverapamil) and D888 (desmethoxyverapamil) (Knoll), D-*cis*-diltiazem (Synthelabo). Noradrenaline, acetylcholine and ethyleneglycol bis (B-aminoethylether)-N, N'-tetraacetic acid (EGTA) were obtained from Sigma. Caffeine was obtained from Merck. DMSO (up to 1/5,000) had no effect on either spontaneous or $K^+$-induced contractions.

*Calculations and statistical analysis*

The experimental results are expressed as mean ± s.d. and significance was tested by means of Student's *t* test. A microcomputer (Tektronix 4052) was used for calculation of mechanical parameters.

The drug potencies were expressed as $-\log$ M concentration which produced 50% inhibition of contraction ($IC_{50}$) for each test calcium entry blocker. Relative potencies between drugs on polarized and depolarized preparations were calculated as the antilog of the differences in $IC_{50}$ against the plateau of $K^+$-induced contractions and against spontaneous contractions (Granger *et al.*, 1985).

## Results

*Effects of calcium entry blockers on spontaneous contractions of rat portal vein*

All the compounds produced a concentration-dependent inhibition of the amplitude of spontaneous contractions. An example is shown in Figure 1 where a low concentration of PN 200-110 (0.3 nM) produces a strong inhibition of contraction. It should be noted that complete reversibility was obtained within 10 min of washing out in reference solution. Figure 2 shows the concentration-response curves obtained with several calcium entry blockers. The antagonist concentrations required to produce 50% inhibition of the maximal contraction ($IC_{50}$) were: PN 200-110, 9.87 ± 0.21; nicardipine, 8.77 ± 0.30; nifedipine, 8.69 ± 0.16; D888, 8.59 ± 0.45; D600, 7.67 ± 0.16; diltiazem, 6.90 ± 0.43; ($-\log$ M, means ± s.d., $n$ = 5–8). The differences in $IC_{50}$ were significant ($P < 0.001$) except between nicardipine, nifedipine and D888 ($P > 0.05$). The rank order of potency was, therefore, PN 200-110 > nicardipine = nifedipine = D888 > D600 > diltiazem.

*Effects of calcium entry blockers on $K^+$-induced contractions*

When the calcium entry blockers were added 10 min before the development of the 60 mM $K^+$-induced contraction, the inhibitory action of the compounds was measured on the phasic peak contraction. The concentration-response curves of the inhibitory effects of calcium entry blockers lay to the right of those derived from spontaneous contractions for all the compounds. The antagonist concentrations required to produce 50% inhibition of the peak $K^+$ contraction ($IC_{50}$) were: PN 200-110, 9.30 ± 0.10; nicardipine, 8.34 ± 0.05; nifedipine, 8.29 ± 0.33; D888, 8.15 ± 0.50; D600, 7.22 ± 0.25; diltiazem, 6.05 ± 0.25


**Figure 1** Effect of PN 200-110 (0.3 nM) on isometric contractions in a polarized rat portal vein strip bathed in the reference salt solution. Maximal inhibition and recovery were obtained within 10 min.

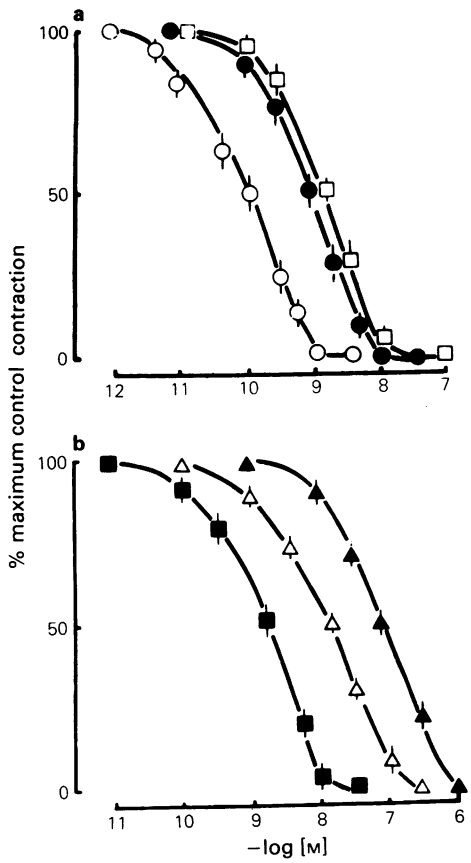


**Figure 2** Effects of calcium entry blockers on spontaneous contractions in portal vein strips bathed in the reference solution. (a) Concentration-response curves for PN 200-110 (○), nicardipine (●), nifedipine (□). (b) Concentration-response curves for D888 (■), D600 (△) and diltiazem (▲).Ordinate scales: percentage of maximal control contraction. Abscissa scales: −log of drug concentration (M). Each point represents mean of 5–8 preparations; vertical lines show s.d.mean.

(−log M, means ± s.d., $n = 5–8$). There was no difference in the $IC_{50}$ between nicardipine and nifedipine ($P > 0.05$). The differences were significant between nifedipine and D888 ($P < 0.01$), and between the other calcium entry blockers ($P < 0.001$). The rank order of potency was, therefore, PN 200–110>nicardipine = nifedipine > D888 > D600 > diltiazem.

When the calcium entry blockers were added during the plateau of the $K^+$ contraction, the concentration-response curves for each compound (Figure 3) lay to the left of those obtained against spontaneous contractions. The antagonist concentrations required to

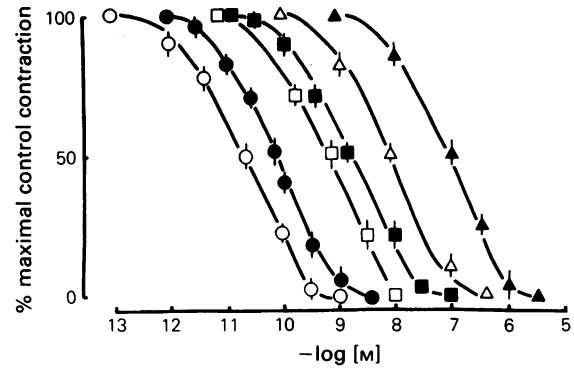


**Figure 3** Concentration-response curves for the effects of calcium entry blockers on sustained contractions induced by 60 mM $K^+$ solutions: PN 200-110 (○), nicardipine (●), nifedipine (□), D888 (■), D600 (△) and diltiazem (▲). Ordinate scale: percentage of maximal control contraction. Abscissa scale: −log of drug concentration (M). Each point represents mean 4–8 preparations; vertical lines show s.d.mean.

produce 50% inhibition of the plateau of the $K^+$ contraction were: PN 200–110, 10.58 ± 0.62; nicardipine, 10.01 ± 0.23; nifedipine, 9.17 ± 0.17; D888, 8.82 ± 0.20; D600, 8.20 ± 0.34; diltiazem, 7.0 ± 0.3 (−log M, means ± s.d., $n = 4–8$). The differences in $IC_{50}$ were significant between all the calcium entry blockers ($P < 0.001$). The rank order of potency was, therefore, PN 200-110 > nicardipine > nifedipine > D888 > D600 > diltiazem.

The relative potencies of calcium entry blockers calculated from the $IC_{50}$ against spontaneous contractions and against the plateau of $K^+$-induced contractions were: nicardipine, 17; PN 200–110, 5; nifedipine, 3; D600, 3; D888, 2; diltiazem, 1. Nicardipine, PN 200-110, nifedipine and D600 were more potent against depolarized preparations than against polarized preparations ($P < 0.01$) while D888 and diltiazem exhibited a similar potency ($P > 0.05$).

### *Effects of calcium entry blockers on drug-induced contractions*

Noradrenaline (NA, 10 μM), acetylcholine (ACh, 0.1 mM) and caffeine (25 mM) produced large and complex contractions in $Ca^{2+}$-containing solutions (Nanjo, 1984). At concentrations which produced complete inhibition of spontaneous contractions, all the calcium antagonists reduced the NA- or ACh-induced contractions. For example (Figure 4), the reduction of the peak contractions induced by NA was 81 ± 9% ($n = 5$, $P < 0.001$) with nicardipine (10 nM), 71 ± 6% ($n = 4$, $P < 0.01$) with PN 200-110 (5 nM),

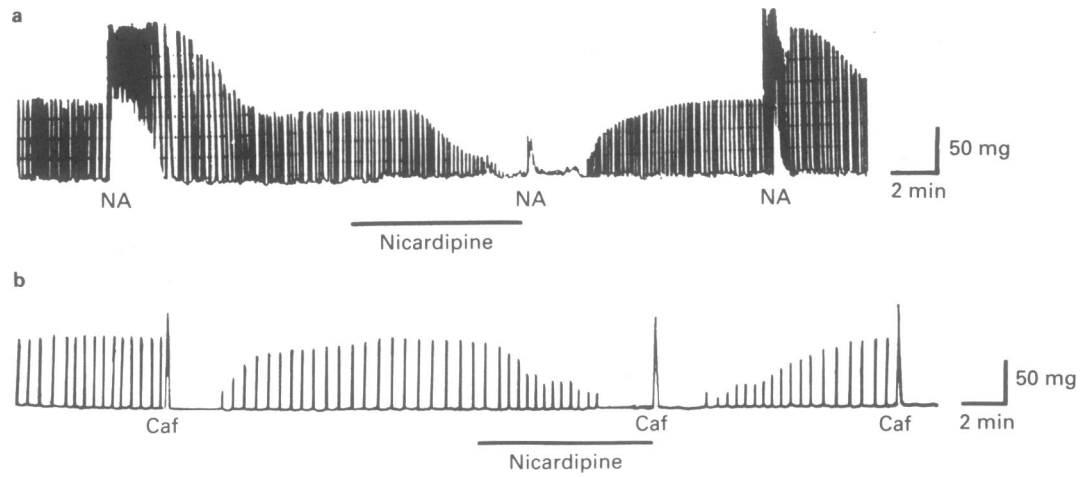


**Figure 4** Effect of nicardipine (10 nM) on contractions induced by noradrenaline (NA, 10 μM), a) and caffeine (Caf, 25 mM), b). The NA- and caffeine-induced contractions were reduced by 81 ± 9% ($n = 5$) and 5 ± 3% ($n = 3$), respectively.

65 ± 5 ($n = 5$, $P < 0.001$) with D888 (50 nM), 60 ± 7 ($n = 3$, $P < 0.02$) with D600 (0.5 μM), 58 ± 8% ($n = 4$, $P < 0.01$) with nifedipine (50 nM) and 46 ± 6% ($n = 4$, $P < 0.01$) with diltiazem (1 μM). In contrast, the inhibitory action of the calcium entry blockers on the caffeine-induced contractions was not significant ($n = 12$). For example, PN 200-110 and diltiazem reduced the contraction by 3 ± 2% ($n = 3$, $P > 0.05$) and 7 ± 3% (n = 3, $P > 0.05$), respectively.

*Internal calcium store sensitive to drugs*

Characteristics of the intracellular calcium store sensitive to neurotransmitters and caffeine were studied in $Ca^{2+}$-free, 0.5 mM EGTA solution on the assumption that the contraction amplitude represented a measurement of the intracellular calcium store (Casteels & Droogmans, 1981). The concentrations of NA, ACh and caffeine used in $Ca^{2+}$-free solutions were 10 μM, 0.1 mM and 25 mM, respectively. The transient NA, ACh and caffeine-induced contractions decreased as a function of time of perfusion in $Ca^{2+}$-free solution (Figure 5a); their amplitudes were expressed as a percentage of control tension elicited by individual drugs, after 30 s of perfusion in $Ca^{2+}$-free solutions. These results show that contractions decreased in a similar way as a function of time, and were lost within 4–6 min (21 preparations). The time at which the contraction was decreased by 50% was about 2 min. It has to be noted that the contraction obtained with NA (42 ± 2.4 mg, $n = 7$) was larger than those obtained with ACh (33 ± 1.3 mg, $n = 7$) and caffeine (30 ± 1.5 mg, $n = 7$). After complete disappearance of the drug-induced contraction in $Ca^{2+}$-free solutions, preparations were incubated in $Ca^{2+}$-containing solutions. This loading period was followed by a 1.5 min wash in $Ca^{2+}$-free, EGTA-containing solutions. Then, ACh, NA or caffeine was applied for 10 s in order to produce a transient contraction. As the drug-induced contractions were largely dependent on the contractile state of the preparation during the calcium loading period, the incubation in $Ca^{2+}$-containing solutions was carried out on quiescent preparations, at 20°C, for different periods of time. In Figure 5b, the amplitude of the contraction is plotted as a function of the $Ca^{2+}$-loading time in 2.1 mM $Ca^{2+}$. The contractions, which were expressed as a percentage of the maximal response obtained with each drug after incubation in 2.1 mM $Ca^{2+}$, reached a plateau value after a loading period in $Ca^{2+}$-containing solution exceeding 10–12 min. The contraction reached 50% of its maximal value in about 2–3 min.

*Effects of calcium entry blockers on the internal calcium store sensitive to drugs*

The effects of calcium entry blockers were studied on the drug-induced contractions elicited after 1.5 min of perfusion in $Ca^{2+}$-free solutions. In Figure 6, calcium antagonists were added during calcium loading for a period of 10 min in order to reach a steady-state inhibitory effect. PN 200-110 (5 nM), nifedipine (50 nM), D888 (50 nM) and D600 (0.5 μM) had no

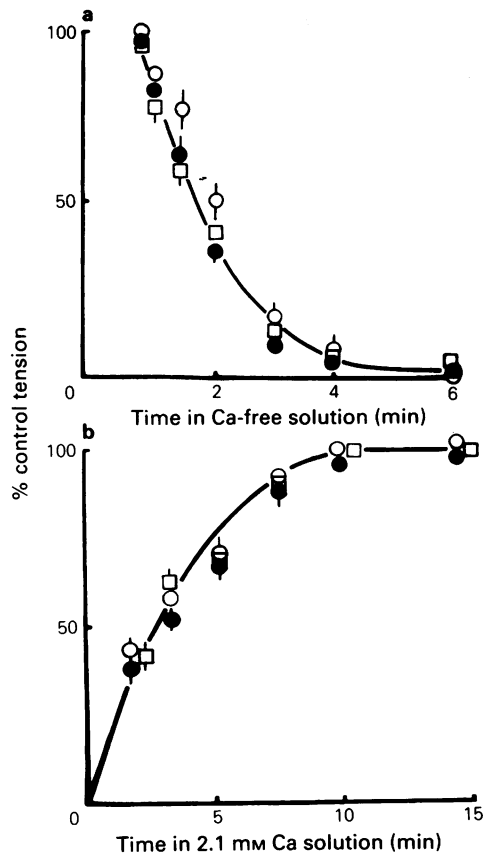


**Figure 5** Transient contractions induced by supramaximal doses of acetylcholine (0.1 mM, ●), noradrenaline (10 μM, ○) and caffeine (25 mM, □) in $Ca^{2+}$-free, 0.5 mM EGTA-containing solutions. (a) Peak contractions are plotted against time of exposure to $Ca^{2+}$-free solutions preceding the addition of the drugs. Responses are expressed as a percentage of control tension of individual drugs, obtained after 30 s of perfusion in $Ca^{2+}$-free solutions. The drugs were applied for 10 s. Each point represents the mean of 9–12 preparations; vertical lines show s.d.mean. (b) Peak contractions are plotted as a function of the loading time of $Ca^{2+}$-depleted preparations in solutions containing 2.1 mM $Ca^{2+}$. The tension is expressed as a percentage of the maximal response obtained with each drug after a calcium loading time of 15 min. Each point represents the mean of 9–12 preparations; vertical lines show s.d.mean.

significant effect on the contractions induced by NA, ACh or caffeine. For example, the PN 200-110 effect was $2 \pm 1\%$ ($n = 4$, $P > 0.05$). In contrast, nicardipine (10 nM) and diltiazem (1 μM) inhibited the drug-induced contractions by $33 \pm 7\%$ ($n = 6$, $P < 0.01$) and $75 \pm 10\%$ ($n = 5$, $P < 0.01$), respectively. In Figure 7, calcium entry blockers were added after a complete calcium loading period of 10 min for 8 min in $Ca^{2+}$-containing solution and maintained during the perfusion in $Ca^{2+}$-free solution. PN 200-110, Nifedipine, D888 and D600 had no significant effect on the drug-induced contractions. For example, the PN 200-110 effect was $2 \pm 0.9\%$ ($n = 5$, $P > 0.05$). Nicardipine and diltiazem reduced the drug-induced contractions by $16 \pm 6\%$ ($n = 5$) and $11 \pm 5\%$ ($n = 5$), respectively, but these changes were not significant ($P > 0.05$).

## Discussion

The present study shows that several calcium entry blockers are able to inhibit a variety of calcium-dependent contractions in rat portal vein. Against spontaneous contractions, the rank order of potency of these drugs was PN 200-110 > nicardipine = nifedipine = D888 > D600 > diltiazem. As contraction of portal vein is believed to be triggered by a calcium inward current (Mironneau & Gargouil, 1979), the conclusion of such observations is that the site of action of the calcium entry blockers is likely to be located at the voltage-dependent slow calcium channels. Several of our results are in good agreement with the values published by Granger *et al.* (1985). Against phasic peak contraction induced by 60 mM $K^+$ solutions, the rank order of potency of the calcium entry blockers was similar to that obtained with spontaneous contractions. However, the dose-response curves were shifted to the right indicating a decreased potency of the blockers. As the inhibitory effect of calcium entry blockers is studied after complete inhibition of spontaneous contractions, it is likely that the resting membrane potential is at its maximal negative value (near −60 mV). Thus, the $K^+$-induced depolarization may involve more fast $Ca^{2+}$ channels which are less sensitive to calcium entry blockers than slow $Ca^{2+}$ channels (Loirand *et al.*, 1986). Maintained $K^+$ contractions are gradually dependent on the external $Ca^{2+}$ concentration in the absence of action potentials, indicating that they may be produced, in part, by a maintained calcium influx through partially inactivated slow calcium channels (G. Loirand, P. Pacaud, C. Mironneau & J.Mironneau, unpublished results). During maintained depolarization (near −20 mV, the rank order of potency of the calcium entry blockers remained PN 200-110 > nicardipine > nifedipine > D888 > D600 > diltiazem. Against depolarized preparations, the potencies of nicardipine, PN 200-110, nifedipine and D600 were significantly increased while those of D888 and diltiazem were not significantly modified. In cardiac and smooth muscle cells, the action of dihydropyridines has been decribed as

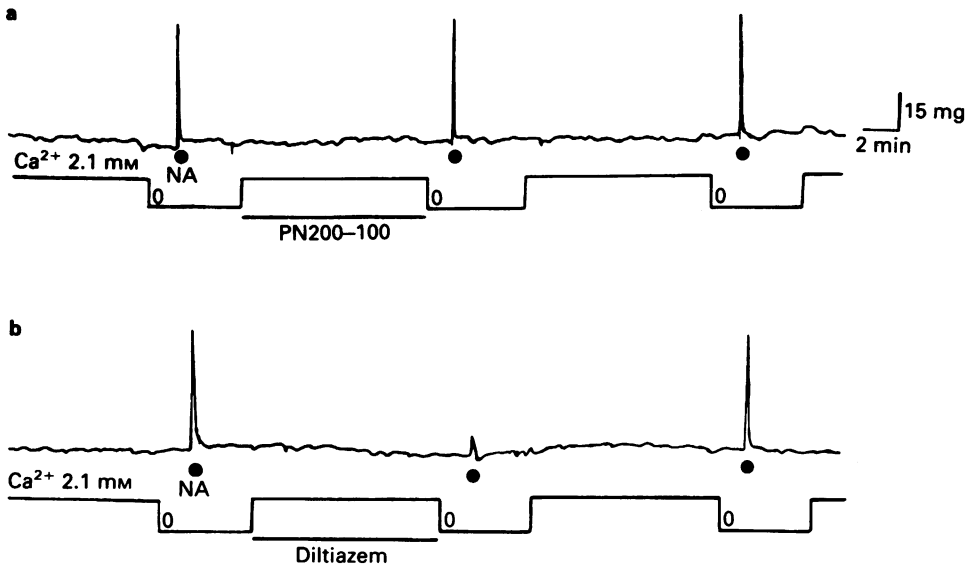


**Figure 6** Effects of PN 200-110 and diltiazem on the transient contractions induced by noradrenaline (NA, 10 μM) in $Ca^{2+}$-free solutions. (a) When PN 200-110 (5 nM) was added during calcium loading, the NA-induced contraction was not significantly modified. (b) When diltiazem (1 μM) was added during calcium loading, the NA-induced contraction was reduced by 75 ± 10% ($n$ = 5). NA was applied after 1.5 min in $Ca^{2+}$-free solutions.

voltage-dependent (Lee & Tsien, 1983; Sanguinetti & Kass, 1984; Uehara & Hume, 1985) while that of verpamil derivatives is generally considered to be less dependent on the membrane potential (Pelzer *et al.*, 1982; Jmari *et al.*, 1986). Our results indicate that only nicardipine, PN 200-110, nifedipine and D600 show a significant voltage-dependent inhibition in portal vein.

NA and ACh produced phasic and tonic contractions in $Ca^{2+}$ containing solutions, the latter ceased but the former were retained in $Ca^{2+}$-free solutions (Nanjo, 1984). The mechanisms underlying the rise in cytoplasmic calcium induced by neurotransmitters are complex and probably involve both voltage-dependent and receptor-operated calcium channels (Bolton, 1979), as well as release of intracellular $Ca^{2+}$ ions. At concentrations which completely inhibited both spontaneous and $K^+$-induced contractions, the calcium entry blockers were less potent on peak contractions induced by neurotransmitters. Derivatives of the dihydropyridine class show a higher potency than D888, D600 and diltiazem. It has been shown that nicardipine may also interact with α-adrenoceptors of vascular smooth muscle (Ruffolo *et al.*, 1984). However, the incomplete inhibition of drug-induced contractions could be explained either by a partial insensitivity of receptor-operated channels to calcium entry blockers or activation of agonist-sensitive intracellular calcium stores.

Caffeine activates the surface membrane as well as the internal calcium storage site in portal vein (Nanjo, 1984). However, the caffeine-induced depolarization peaks to 10 mV at maximal doses while both NA and ACh evoke membrane depolarizations as large as 40–45 mV. Thus, it is likely that the main effect of caffeine is to produce a release of $Ca^{2+}$ ions from the sacroplasmic reticulum, as shown with skinned portal vein strips. This could explain the absence of an important inhibitory effect of calcium entry blockers on the caffeine-induced contractions in $Ca^{2+}$-containing perfusion, after complete inhibition of spontaneous contractions.

In $Ca^{2+}$-free, EGTA-containing solution, the amount of calcium in the intracellular store, as measured from the change in contraction amplitude to stimulant drugs, decreased as a function of time, indicating that the store was completely lost after 4–6 min of perfusion at 20°C. The filling of the internal calcium store in portal vein was dependent on both external and cytoplasmic calcium concentrations, as previously shown on other smooth muscles (Casteels & Raymaekers, 1979; Brading & Sneddon, 1980;

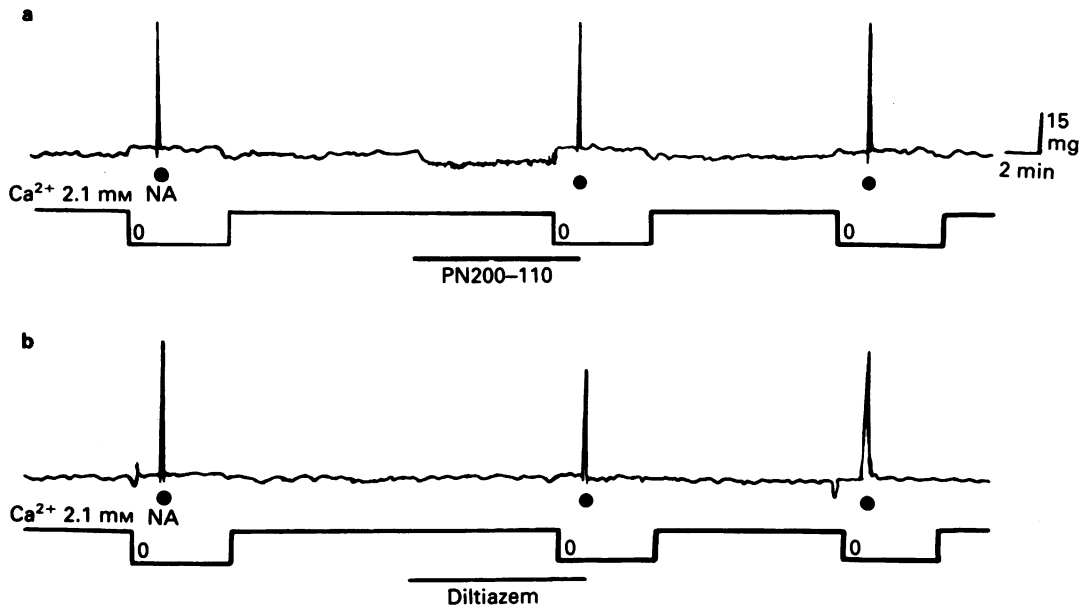


**Figure 7** Effects of PN 200-110 and diltiazem on the transient contractions induced by noradrenaline (NA, 10 μM) in $Ca^{2+}$-free solutions. (a) When added for 8 min in $Ca^{2+}$-containing solutions and maintained in $Ca^{2+}$-free solutions, PN 200-110 (5 nM) had no significant effect. (b) Applied under similar conditions, diltiazem (1 μM) reduced the response to NA by 11 ± 5% ($n$ = 5). NA was applied after 1.5 min in $Ca^{2+}$-free solutions.

Lalanne *et al.*, 1984). Decreasing the temperature of the perfusing solution to 20°C suppressed both electrical activity and calcium influx through voltage-dependent channels. Under these conditions the filling of the internal calcium store depends on the direct penetration of $Ca^{2+}$ ions from outside. A model has recently been proposed to account for such a mechanism (Putney, 1986). Calcium entry blockers exerted various effects on both direct calcium uptake into and calcium release from the internal store. PN 200-110, nifedipine, D888 and D600 had no inhibitory action on either calcium uptake or release. A similar absence of effect of nifedipine on both calcium accumulation into and calcium release from the internal store has been described in rabbit mesenteric artery (Kanmura *et al.*, 1983). In contrast, nicardipine and diltiazem strongly reduced the direct calcium uptake, diltiazem being more potent than nicardipine. In guinea-pig basilar artery, nicardipine also reduced the direct calcium uptake (Fujiwara & Kuriyama, 1983). Both drugs slightly affected the release of calcium induced by stimulant drugs in $Ca^{2+}$-free solutions.

In conclusion, the calcium entry blockers used in this work are potent inhibitors of calcium influx through voltage-dependent channels in rat portal vein. Two of them, diltiazem and nicardipine, may exert an additional effect to depress contractions dependent on intracellular calcium release.

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