(+)-[³H]-PN 200-110 binding to cell membranes and intact strips of portal vein smooth muscle: characterization and modulation by membrane potential and divalent cations

C. Dacquet, G. Loirand, L. Rakotoarisoa, ¹C. Mironneau & J. Mironneau

Laboratoire de Physiologie Cellulaire et Pharmacologie Moléculaire, INSERM JF 88.13, Université de Bordeaux II, 3 Place de la Victoire, 33076 Bordeaux, France

1 Specific binding of the calcium-antagonist dihydropyridine derivative, (+)-[³H]-PN 200-110 (isradipine), to cell membranes of equine portal vein smooth muscle was compared with binding to intact strips isolated from rat portal veins.

2 Specific binding to vascular smooth muscle membranes was of high affinity, saturable and reversible. The dissociation constant obtained from association and dissociation kinetics of (+)-[³H]-PN 200-110 was similar to that obtained from equilibrium binding and competition experiments.

3 Specific binding of (+)-[³H]-PN 200-110 was completely displaced by unlabelled dihydropyridines. Among other calcium antagonists, D888 and (+)-*cis*-diltiazem partially inhibited the binding at 25°C. At 37°C, only (+)-*cis*-diltiazem stimulated the binding. LaCl₃, CdCl₂, NiCl₂, CoCl₂ had inhibitory effects, whereas KCl and NaCl had no effect.

4 When intact strips of portal vein were incubated in high external potassium concentrations for 30 min, the *K_d* was lowered to 0.04 ± 0.01 nM from the control value of 0.14 ± 0.02 nM (*n* = 5), thereby indicating that (+)-[³H]-PN 200-110 bound to voltage-dependent calcium channels, with a higher affinity, in the depolarized state.

5 When external Ca²⁺ was removed or substituted with Ba²⁺ or Sr²⁺, *K_d* values increased suggesting that the dihydropyridine binding to intact strips was modulated by binding of Ca²⁺ ions to voltage-dependent calcium channels.

Introduction

A better understanding of the voltage-dependent calcium channels in smooth muscles can be obtained by use of the three methodologies described below.

(1) The voltage clamp method studies both activation and inactivation parameters of calcium channels responding to depolarization, and to the effects of calcium antagonists on calcium currents (Tsien, 1983; Dacquet *et al.*, 1988). Calcium channels may be in one of at least three states: resting, open, or inactivated and each state is determined by the membrane potential. Binding of a calcium antagonist to a site located within the channel is influenced by the

state of the channel. (2) In contraction experiments, the voltage-dependence of calcium channel blockade is indicated by a shift to the left of the dose-inhibition curves measured in physiological solution or in K⁺-rich depolarizing solution (Dacquet *et al.*, 1987). (3) The third method is to use radioligand binding techniques. With radiolabelled calcium antagonists, the binding studies revealed the affinities and maximal binding capacities of the calcium antagonists, and the interactions with various drugs and polyvalent cations (Fosset *et al.*, 1983; Fosset & Lazdunski, 1987). As binding studies are generally carried out using cell membranes, demonstration of a correlation with binding to calcium channels under

¹ Author for correspondence.

physiological conditions is difficult to obtain. Accordingly, methods for binding of calcium antagonists to isolated cells or intact tissue preparations have been developed (Kokubun *et al.*, 1986; Morel & Godfraind, 1987; Sumimoto *et al.*, 1988). We describe the properties of the high affinity (+)-[³H]-PN 200-110 binding to vascular smooth muscle membranes. The findings are compared with the binding to intact smooth muscle strips which is modulated by both the membrane potential and the ionic environment.

Methods

Membrane preparation

Microsomes from fresh equine portal vein were prepared in the presence of 1 mM iodoacetamide and 0.1 mM phenylmethylsulphonylfluoride to minimize proteolytic degradation (Dacquet *et al.*, 1988). Protein was determined with use of gamma-globulins as standard (Bradford, 1976).

Measurement of (+)-[³H]-PN 200-110 binding to membranes

Membrane proteins (0.08 to 0.15 mg ml⁻¹) were incubated for 45 min at 25°C with various concentrations of (+)-[³H]-PN 200-110 in 1 ml of 20 mM HEPES buffer (pH 7.4) containing 0.1% bovine serum albumin (Dacquet *et al.*, 1988). Non specific binding was defined as the amount of radioligand bound in the presence of 2 µM unlabelled nifedipine and subtracted from the total binding. All binding experiments were performed under a dim light.

Measurement of (+)-[³H]-PN 200-110 binding to rat portal vein strips

Studies of binding to intact strips of rat portal vein (0.5–2 mg wet weight) were performed under conditions similar to those described for mesenteric arteries (Morel & Godfraind, 1987). Strips were incubated for 90 min at 37°C in a physiological solution (mM): NaCl 130, KCl 5.6, CaCl₂ 2, MgCl₂ 0.24, HEPES 8.3, glucose 11, at pH 7.4, (polarized veins) or for 60 min in physiological solution followed by 30 min in a 135 mM K⁺ depolarizing solution obtained by substituting NaCl for KCl in equimolar amounts (depolarized veins). At the end of the incubation time, each strip was dried on filter paper, and then weighed. Radioactivity was revealed after dissolving the veins in 100 µl of a mixture of perchloric acid and H₂O₂ (1:1).

Figure 1 Kinetic constants of [³H]-PN 200-110 binding to vascular smooth muscle membranes. Cell membranes (0.08 mg ml⁻¹) were incubated with different concentrations of (+)-[³H]-PN 200-110 at 25°C in 1 ml buffer containing 1 mM CaCl₂, 20 mM HEPES NaOH (pH 7.4). (a) Association time course with three different concentrations of (+)-[³H]-PN 200-110: (■) 0.015 nM, (□) 0.190 nM and (●) 0.393 nM for different times. Each point represents the mean response of 4 experiments with s.e.mean shown by vertical lines. (b) The association (k_1) and dissociation (k_{-1}) rate constants were determined by linear regression of the k_{obs} value versus (+)-[³H]-PN 200-110 concentration. The k_1 and k_{-1} values were $0.178 \times 10^9 \text{ M}^{-1} \text{ min}^{-1}$ and 0.018 min^{-1} , respectively. The equilibrium dissociation constant K_d was $k_{-1}/k_1 = 0.101 \text{ nM}$, $n = 2$.

Table 1 Inhibition of (+)-[³H]-PN 200-110 binding to membranes of portal vein smooth muscles in the presence of calcium channel antagonists

	IC_{50} or K_i	n Hill	Maximal inhibition (%)
Nitrendipine	0.45 ± 0.05 nM	1.08	100
D888	0.25 ± 0.02 μ M	0.50	55 ± 5
(+)- <i>cis</i> -Diltiazem	50.00 ± 5.00 μ M	1.25	65 ± 7
La ³⁺	10.00 ± 2.00 μ M	0.55	100
Cd ²⁺	25.00 ± 4.00 μ M	0.75	100
Ni ²⁺	1.00 ± 0.50 mM	0.42	70 ± 8
Co ²⁺	2.00 ± 0.80 mM	0.40	70 ± 7

Each result is mean $\pm$ s.e.mean of 5 experiments.

K_i values were calculated for competitive inhibitors from inhibition curves of (+)-[³H]-PN 200-110 specific binding according to the equation of Cheng & Prusoff (1973).

Drugs

(+)-[³H]-PN 200-110 (specific activity 84 Ci mmol⁻¹) was obtained from Amersham (France). (+)-PN 200-110 (isradipine), was from Sandoz (France); ($\pm$)-nifedipine and ($\pm$)-nitrendipine were from Bayer (F.R.G.); (+)-*cis*-diltiazem was from Synthelabo (France) and (–)-D888 (desmethoxy verapamil) and ($\pm$)-D600 (methoxyverapamil) were from Knoll (F.R.G.).

Statistical analysis

Scatchard plots for binding of (+)-[³H]-PN 200-110 were obtained by a non linear least-square ligand programme (Munson & Rodbard, 1980). The experimental results were expressed as mean $\pm$ s.e.mean and significance was tested by Student's *t* test.

Results

Association and dissociation kinetics of (+)-[³H]-PN 200-110 binding to membranes

Specific binding of (+)-[³H]-PN 200-110 was determined as a function of time for different concentrations of labelled dihydropyridine (Figure 1a). Association reached a plateau after 40–50 min incubation at 25°C. The data for association kinetics were plotted according to a pseudo first order equation (Weiland & Molinoff, 1981). The slope of the plot, *k* observed (k_{obs}), was calculated for each radioligand concentration and plotted as a function of the (+)-[³H]-PN 200-110 concentration (Figure 1b). This allowed a direct determination of both association rate constant (0.178×10^9 M⁻¹ min⁻¹) and dissociation rate constant (0.018 min⁻¹). After 60 min

incubation of membranes with 0.4 nM (+)-[³H]-PN 200-110, the rate of dissociation of the complex was measured after addition of 2 μ M unlabelled nifedipine. The data, plotted according to first order kinetics, gave a rate constant for dissociation (k_{-1}) of 0.014 min⁻¹ that was similar to that obtained from Figure 1b. The dissociation constant from kinetic data $K_d = k_{-1}/k_1$, was estimated to be 0.08–0.10 nM. This value is similar to the dissociation constant obtained from equilibrium binding data (0.09 ± 0.03 nM, $n = 7$; Dacquet *et al.*, 1988).

Effects of organic calcium antagonists and polyvalent cations on the high affinity binding sites of (+)-[³H]-PN 200-110 to membranes

Increasing concentrations of unlabelled drugs gradually inhibited the (+)-[³H]-PN 200-110 binding (Figure 2a). The concentration producing half-maximal inhibition (IC_{50}) or the inhibition constant (K_i) and the slope factor of inhibition curves are listed in Table 1. The increase in (+)-[³H]-PN 200-110 binding by (+)-*cis*-diltiazem was observed only at 37°C. To elucidate the mode of binding action by these drugs, Scatchard analysis of the (+)-[³H]-PN 200-110 binding was carried out in the presence or absence of calcium antagonists (Table 2). Nitrendipine (2–4 nM) increased the K_d of [³H]-PN 200-110 binding without affecting the B_{max} suggesting that nitrendipine behaved as an apparent competitive inhibitor. A change in both K_d and B_{max} values was observed when D888 (50–100 nM) was added. This was a typical result showing that inhibition of (+)-[³H]-PN 200-110 binding was not of the competitive type. At 37°C, (+)-*cis*-diltiazem (50 μ M) increased B_{max} with no significant change in K_d . It was noted that temperature had no significant effect on the (+)-[³H]-PN 200-110 binding in the absence of (+)-*cis*-diltiazem as well as on the binding inhibi-

Table 2 Parameters of (+)-[³H]-PN 200-110 binding to membranes in the presence of calcium channel antagonists

	K_d (nM)	B_{max} (fmol mg ⁻¹ protein)
Control	0.09 ± 0.01	55 ± 10
Nitrendipine (2 nM)	0.44* ± 0.02	50 ± 4
D888 (100 nM)	0.22* ± 0.02	35* ± 2
(+)- <i>cis</i> -Diltiazem (50 μM)	0.11 ± 0.03	33* ± 4
La ³⁺ (10 μM)	0.10 ± 0.02	32* ± 3
Cd ²⁺ (10 μM)	0.11 ± 0.01	45* ± 2
Ni ²⁺ (2 mM)	0.10 ± 0.01	38* ± 3
Co ²⁺ (5 mM)	0.11 ± 0.01	40* ± 4

* Significant change ($P < 0.05$) caused by calcium antagonists. Each result is mean ± s.e.mean of 5 experiments.

tion induced by 100 nM (–)-D888. These results indicate that (+)-*cis*-diltiazem behaves as a non competitive inhibitor of (+)-[³H]-PN 200-110 binding to vascular smooth muscles membranes.

Both tri- and divalent cations dose-dependently decreased the binding of (+)-[³H]-PN 200-110 obtained in the presence of 1 mM CaCl₂ (Figure 2b). The concentrations producing half maximal inhibition (IC₅₀) and the slope factor of inhibition curves are listed in Table 1. In contrast, monovalent cations such as Na⁺ and K⁺ were without effect on the binding of (+)-[³H]-PN 200-110, whatever the concentrations used. Scatchard representations of the effects of inorganic calcium entry antagonists on (+)-[³H]-PN 200-110 binding obtained in the presence of 1 mM CaCl₂ showed that calcium antagonists lowered the B_{max} with no significant change of the K_d , indicating that they acted as non competitive inhibitors (Table 2).

Effects of membrane depolarization on specific binding of (+)-[³H]-PN 200-110 to intact portal vein strips

Figure 3a illustrates the equilibrium binding of (+)-[³H]-PN 200-110 to polarized intact portal vein strips (5 mM external K⁺) at various concentrations of labelled PN 200-110 in the absence or presence of 2 μM unlabelled nifedipine. The non specific binding increased linearly with the concentration of (+)-[³H]-PN 200-110. The specific binding accounted for 75–80% of the total binding at a concentration close to the K_d value. Scatchard analysis of the specific binding of (+)-[³H]-PN 200-110 resulted in a linear plot, thereby indicating specific binding to a single class of sites (Figure 3b). The K_d and B_{max} values were 0.14 ± 0.02 nM and 12.0 ± 1.5 fmol mg⁻¹

Figure 2 Effects of organic calcium channel antagonists and polyvalent cations on (+)-[³H]-PN 200-110 binding to vascular smooth muscle membranes. (a) Membranes (0.1 mg ml⁻¹) were incubated with 0.15 nM (+)-[³H]-PN 200-110 for 45 min in the absence or presence of unlabelled nitrendipine (●), (–)-D888 (□), and (+)-*cis*-diltiazem at 25°C (■) and 37°C (Δ). Results are expressed as a percentage of specific binding obtained in the absence of unlabelled drugs, and are means of 4 experiments with s.e.mean shown by vertical lines. (b) Specific bindings (+)-[³H]-PN 200-110 (0.1 nM) to membranes (0.1 mg ml⁻¹) was measured under equilibrium conditions in presence of 1 mM Ca²⁺ and after addition of indicated concentrations of La³⁺ (□), Cd²⁺ (■), Ni²⁺ (Δ), Co²⁺ (▲) and K⁺ (●). Binding is expressed as a percentage of that occurring in the presence of 1 mM CaCl₂. Each point represents the mean response of 4–6 experiments with s.e.mean shown by vertical lines.

Figure 3 Specific binding of (+)-[³H]-PN 200-110 to polarized and depolarized intact strips from rat portal vein. (a) Saturation curve of (+)-[³H]-PN 200-110 binding to polarized rat portal vein. Saturation binding experiments were carried out on portal strips (0.5 to 1.2 mg) with increasing concentrations of (+)-[³H]-PN 200-110 for 90 min at 37°C. Non specific binding was defined with 2 μ M nifedipine. (○) Total binding, (●) specific binding and (□) non specific binding. Each point represents the mean response of 5 experiments with s.e.mean shown by vertical lines. (b) Scatchard analysis of specific binding to polarized strips in 5 mM K⁺ (●) and depolarized strips in 135 mM K⁺ solution (○). For depolarized strips, a first incubation for 60 min in 5 mM K⁺ was made in presence of radioligand followed by a 30 min incubation in 135 mM K⁺ with the same concentration of radioligand. To obtain high K⁺ solution, NaCl was replaced with an equimolar concentration of KCl. Similar estimates were obtained from 5 separate experiments; duplicate estimates were used for each point.

wet weight ($n = 5$), respectively. The inhibition induced by various calcium antagonists was similar to that reported with cell membranes. In depolarized (135 mM external K⁺) strips (Figure 3b) the Scat-

chard analysis showed that the K_d was reduced to 0.04 ± 0.01 nM ($P < 0.01$, $n = 5$) but the B_{max} was unchanged. As (+)-PN 200-110 blocks the calcium channels with a higher affinity in a more depolarized state (Kokubun *et al.*, 1986; Dacquet *et al.*, 1988), these results indicate that (+)-[³H]-PN 200-110 preferentially binds to inactivated calcium channels rather than to rested channels. Affinities of (+)-PN 200-110 obtained from electrophysiological, mechanical and binding experiments on both cell membranes and muscle strips are summarized in Table 3. It can be observed that the relative potency of (+)-PN 200-110 against rested slow calcium channels and against inactivated slow calcium channels ranged between 3.5 and 5.

Effects of divalent cations on (+)-[³H]-PN 200-110 binding to polarized or depolarized intact strips

Figure 4a shows that in 5 mM external K⁺, the K_d value increased from 0.14 ± 0.02 nM in 2 mM Ca²⁺ solution to 0.26 ± 0.01 nM when external CaCl₂ was replaced by BaCl₂ or SrCl₂ and when CaCl₂ was removed in the presence of 0.1 mM EGTA ($P < 0.001$, $n = 5-15$) without significant variations in the B_{max} values (between 11.5 and 14.5 fmol mg⁻¹ wet weight, $P > 0.05$). When intact strips were bathed in 135 mM external K⁺ (Figure 4b), the K_d value was increased from 0.04 ± 0.01 nM to 0.11 ± 0.02 nM after removal of external calcium (in the presence of 0.1 mM EGTA) and when CaCl₂ was replaced by BaCl₂ or SrCl₂ ($P < 0.01$, $n = 5-15$), without significant variations in the B_{max} values ($P > 0.05$).

Discussion

Several studies have suggested that the high affinity binding site identified in radioligand binding experiments is the receptor for the pharmacological action of the dihydropyridine calcium channel antagonists in skeletal (Fosset *et al.*, 1983), cardiac (Lee *et al.*, 1984), and smooth muscles (Sarmiento *et al.*, 1984). Using membranes of portal vein smooth muscle, we demonstrated that the (+)-[³H]-PN 200-110 binding was of high affinity, saturable and reversible, and correlated well with the pharmacological activities determined from inhibition of the mechanical and electrical responses (Dacquet *et al.*, 1987; 1988). In portal vein smooth muscle strips, the affinity of (+)-[³H]-PN 200-110 increased as the membrane depolarized suggesting that (+)-[³H]-PN 200-110 bound to calcium channels in the inactivated state with a higher affinity than in the rested state. The effect of membrane depolarization in decreasing the

Table 3 Binding affinities and pharmacological potencies of PN 200-110 in rat portal vein

	K_d (nM)		IC_{50}^{**} (nM)	
	Microsomes	Strips	Ca current	Contraction
Membrane potential (mV)	0	0	0	-20
Inactivated Ca channels	0.09 ± 0.03	$*0.04 \pm 0.01$	$*0.03 \pm 0.01$	$*0.03 \pm 0.01$
Membrane potential (mV)	—	-45	-50	-50
Resting Ca channels	—	0.14 ± 0.02	0.15 ± 0.02	0.13 ± 0.02
Relative potency	—	3.52	5.00	4.33

Results were obtained from binding, electrophysiological and contraction experiments. Relative potency is expressed as the ratio of affinities against polarized preparations and against depolarized preparations.

* Significant change ($P < 0.01$) caused by membrane depolarization with respect to normal resting potential.

Each result is mean $\pm$ s.e.mean of 5–8 experiments.

** From Dacquet *et al.* (1987, 1988).

Figure 4 Modulation of (+)-[³H]-PN 200-110 affinity by divalent cations and membrane depolarization to intact strips from rat portal vein. (a) Scatchard analysis of specific binding to polarized strips in 5 mM K⁺ in the presence of 2 mM CaCl₂ (●), 2 mM BaCl₂ (▲), 2 mM SrCl₂ (△), after removal of external Ca²⁺ and addition of 0.1 mM EGTA (□). (b) Scatchard analysis of specific binding to depolarized strips in 135 mM K⁺ in the different ionic conditions as described in (a). Similar estimates were obtained from 5–7 separate experiments; duplicate estimates were used for each point.

K_d values of intact strips suggests that the K_d values in cell membranes are lower than those in intact polarized strips, because the calcium channels are in an inactivated state in cell membranes. Similar results showing that depolarization enhances the affinity of dihydropyridine binding have been reported in cardiac (Kokubun *et al.*, 1986) and vascular myocytes (Morel & Godfraind, 1987; Sumimoto *et al.*, 1988). The relative potencies of (+)-PN 200-110 calculated from the affinity against polarized preparations and against depolarized preparations were similar using binding and contraction responses in intact portal vein strips and calcium current recordings in isolated cells (Table 3).

The inhibition of (+)-[³H]-PN 200-110 binding to smooth muscle membranes induced by various calcium antagonists (nitrendipine, D888 and (+)-*cis*-diltiazem) suggest that there might be several sites for these structurally unrelated calcium antagonists and that they were allosterically regulated. Inorganic polyvalent cations are known to block the voltage-dependent calcium channels. In our results, the polyvalent cations inhibited (+)-[³H]-PN 200-110 binding to smooth muscle membranes with a rank order of potency: La³⁺ > Cd²⁺ > Ni²⁺ > Co²⁺, similar to that obtained from electrophysiological experiments (Jmari *et al.*, 1986a). The cations behaved as apparent non competitive inhibitors of (+)-[³H]-PN 200-110 binding suggesting that they did not bind at the same site on the calcium channel as (+)-PN 200-110.

In intact smooth muscle strips, the maximal binding capacity of (+)-[³H]-PN 200-110 was not affected by calcium removal (in the presence of 0.1 mM EGTA) or when CaCl₂ was replaced by BaCl₂ or SrCl₂ while the dissociation constant was significantly increased. The reduction in binding

affinity was observed whatever the membrane potential (polarized or depolarized preparations). Thus, the affinity of (+)-[³H]-PN 200-110 binding was increased by both membrane depolarization and addition of external calcium. These observations can be correlated to electrophysiological studies demonstrating that inactivation of calcium channels in smooth muscles is mediated by both calcium-dependent and membrane potential-dependent mechanisms (Jmari *et al.*, 1986b; Ohya *et al.*, 1988).

In conclusion, our studies on binding of (+)-[³H]-PN 200-110 to membranes and intact strips of portal

vein smooth muscle indicate that the dihydropyridine binding site may be associated with the voltage-dependent calcium channel and modulated by binding of calcium ions to binding sites such as those which regulate calcium-dependent inactivation.

This work was supported by grants from Centre National de la Recherche Scientifique (Pirmé), Etablissement Public régional d'Aquitaine, Institut National de la Santé et de la Recherche Médicale, (87.5011) and Fondation pour la Recherche Médicale, France. We thank N. Perrier for her secretarial assistance.

References

- BRADFORD, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**, 248–253.
- CHENG, Y.C. & PRUSSOFF, W.H. (1973). Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition of an enzymatic reaction. *Biochem. Pharmacol.*, **22**, 3099–3108.
- DACQUET, C., MIRONNEAU, C. & MIRONNEAU, J. (1987). Effects of calcium entry blockers on calcium-dependent contractions of rat portal vein. *Br. J. Pharmacol.*, **92**, 203–211.
- DACQUET, C., PACAUD, P., LOIRAND, G., MIRONNEAU, C. & MIRONNEAU, J. (1988). Comparison of binding affinities and calcium current inhibitory effects of a 1,4-dihydropyridine derivative (PN 200-110) in vascular smooth muscle. *Biochem. Biophys. Res. Comm.*, **152**, 1165–1172.
- FOSSET, M., JAIMOVICH, E., DELPONT, E. & LAZDUNSKI, M. (1983). (³H) nitrendipine receptors in skeletal muscle properties and preferential localization in transverse tubules. *J. Biol. Chem.*, **258**, 6086–6092.
- FOSSET, M. & LAZDUNSKI, M. (1987). Biochemical characterization of the skeletal muscle Ca²⁺ channel. In *Structure and Physiology of the slow inward calcium channel receptor*, *Biochemistry and Methodology*, ed. Venter, J.C. & Triggle, D.J. Vol. 9, pp. 141–159. New York: Alan R. Liss Inc.
- JMARI, K., MIRONNEAU, C. & MIRONNEAU, J. (1986a). Canaux calciques des cellules musculaires lisses. *J. Physiol. (Paris)*, **81**, 168–176.
- JMARI, K., MIRONNEAU, C. & MIRONNEAU, J. (1986b). Inactivation of calcium channel current in rat uterine smooth muscle: evidence for calcium and voltage mediated mechanisms. *J. Physiol.*, **380**, 111–126.
- KOKUBUN, S., PROD'HOM, B., PORZIG, H. & REUTER, H. (1986). Studies on Ca channels in intact cardiac cells: voltage-dependent effects and cooperative interactions of dihydropyridine enantiomers. *Mol. Pharmacol.*, **30**, 571–584.
- LEE, H.R., ROESKE, W.R. & YAMAMURA, H.I. (1984). High affinity specific (³H) PN 200-110 binding to dihydropyridine receptors associated with calcium channels in rat cerebral cortex and heart. *Life Sci.*, **35**, 721–732.
- MOREL, N. & GODFRAIND, T. (1987). Prolonged depolarization increases the pharmacological effect of dihydropyridines and their binding affinity for calcium channels of vascular smooth muscle. *J. Pharmacol. Exp. Ther.*, **243**, 711–715.
- MUNSON, P.J. & ROBBARD, D. (1980). Ligand: a versatile computerized approach for characterization of ligand binding systems. *Anal. Biochem.*, **107**, 220–239.
- OHYA, Y., KITUMARA, K. & KURIYAMA, H. (1988). Regulation of calcium current by intracellular calcium in smooth muscle cells in rabbit portal vein. *Circ. Res.*, **62**, 375–383.
- SARMIENTO, J.G., JANIS, R.A., KATZ, A.M. & TRIGGLE, D.J. (1984). Comparison of the high affinity binding of calcium channel blocking drugs to vascular smooth muscle and cardiac sarcolemmal membranes. *Biochem. Pharmacol.*, **33**, 3119–3123.
- SUMIMOTO, K., HIRATA, M. & KURIYAMA, H. (1988). Characterization of [³H] nifedipine binding to intact vascular smooth muscle cells. *Am. J. Physiol.*, **254**, C2–C5.
- TSIEN, R.W. (1983). Calcium channels in excitable cell membranes. *Ann. Rev. Physiol.*, **45**, 341–358.
- WEILAND, G.A. & MOLINOFF, P.B. (1981). Quantitative analysis of drug-receptor interactions. Determination of kinetic and equilibrium properties. *Life Sci.*, **29**, 313–330.

(Received July 20, 1988
Revised December 12, 1988
Accepted January 5, 1989)