Correlation Between the Plasma Concentration of Mepirodipine and Its Occupancy of Ca^{2+} Antagonist Receptors in Rats

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The relationship between the plasma concentration of mepirodipine (1,4-dihydropyridine Ca^{2+} antagonist) and its occupancy of cardiac and cerebral Ca^{2+} antagonist receptors in rats has been characterized by a radioreceptor assay technique using (+)-[^{3}H]PN 200-110. Oral administration of mepirodipine in rats produced a dose-dependent and sustained decrease in the number of specific (+)-[^{3}H]PN 200-110 binding sites in both tissues, and the effect was more pronounced in the cardiac tissue than in the cerebral cortex. The occupancy of cardiac and cerebral Ca^{2+} antagonist receptors by mepirodipine correlated well with its plasma concentration, whereas a 20-fold higher plasma concentration of this drug was necessary to occupy Ca^{2+} antagonist receptors in the cerebral cortex. Thus, these data suggest that mepirodipine occupies Ca^{2+} antagonist receptors in cardiovascular tissue selectively over those in brain tissue.

KEY WORDS: Ca^{2+} antagonist; plasma concentration; receptor occupancy; heart; brain.

INTRODUCTION

Ca^{2+} channel antagonists are used clinically in the treatment of angina pectoris and systemic hypertension, and they produce therapeutic effects by blocking the slow inward current of Ca^{2+} in cardiovascular tissues. Nifedipine, the prototype of 1,4-dihydropyridine (DHP) Ca^{2+} antagonist, has a relatively short duration of antihypertensive effect. Thus, novel 1,4-DHP derivatives possessing long-lasting antihypertensive effect and tissue selectivity have been introduced (1–4). Some 1,4-DHP derivatives are subject to extensive and variable first pass metabolism by the liver (5,6), which reduces systemic bioavailability and drug efficacy after oral administration. Studies on the pharmacokinetics and pharmacodynamics of 1,4-DHP derivatives have clarified the relationship between the cardiovascular effect of 1,4-DHP Ca^{2+} antagonists and their plasma concentrations in experimental animals and in humans (7–9).

The pharmacological effects of 1,4-DHP Ca^{2+} antagonists are produced by an interaction with specific receptors *in vivo* in the cardiovascular system. By using radiolabeled 1,4-DHP derivatives, the Ca^{2+} antagonist receptor sites were characterized extensively *in vitro* in brain, cardiac, and smooth muscles (10–12), and thus the binding affinities of unlabeled Ca^{2+} antagonists to the receptors were determined. However, the *in vitro* receptor affinities of Ca^{2+} antagonists do not appear to correlate with the tissue selectivity *in vivo* of these drugs inferred from pharmacological data (13). Sustained occupancy *in vivo* of cardiovascular Ca^{2+} antagonist receptors by 1,4-DHP derivative in spontaneously hypertensive rats (SHR) correlated significantly with its long-lasting hypotension (14). Thus, *in vivo* characterization of Ca^{2+} antagonist binding to the receptors in different tissues is important for the analysis of pharmacokinetics and pharmacodynamics of these drugs. However, little is known about the relationship between the occupancy of Ca^{2+} antagonist receptors by 1,4-DHP derivatives and their plasma concentrations or pharmacokinetics. Mepirodipine [(+)3′*S*,4*S*)-3-(1′-benzyl-3′-pyrrolidinyl methyl 2,6-dimethyl-4-(*m*-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate hydrochloride] is a novel light-resistant 1,4-DHP Ca^{2+} antagonist possessing potent hypotensive action of a long duration (3,15,16). The purpose of the present study was to characterize the relationship between the plasma concentration of mepirodipine and its occupancy of Ca^{2+} antagonist receptors in rats.

MATERIALS AND METHODS

Drug Administration

Male Wistar rats weighing 200 to 300 g were housed three or four per cage in the laboratory with free access to food and water and maintained on a 12-hr dark–light cycle in a room with controlled temperature (24 ± 1°C) and humidity (55 ± 5%). They were fasted for 16 hr before drug administration and administered orally with mepirodipine hydrochloride (1, 3, and 10 mg/kg) dissolved in water containing ethanol (10%) and polyethylene glycol 400 (10%) as solvents. Control animals were administered with the vehicle. At 0.5 to 12 hr after the drug administration, rats were killed by taking the blood from descending aorta under light anesthesia with ethyl ether, and the heart and brain were perfused with 0.9% saline from the aorta. Then both tissues were removed, and fat and blood vessels were removed. The plasma from rat blood was separated by centrifugation.

Tissue Preparation

The cardiac tissue from rats was minced with scissors and homogenized by a Kinematica Polytron homogenizer (type PT 10/35) in 20 vol of ice-cold 50 m*M* Tris–HCl buffer (pH 7.5). The cardiac homogenate was centrifuged at 500*g* for 10 min, and the supernatant, after filtration through three layers of cheese cloth, was centrifuged at 40,000*g* for 15 min. The pellet was resuspended in the ice-cold buffer, and the suspension was centrifuged again at 40,000*g* for 15 min. The resulting pellet was finally suspended in the buffer for the binding assay. The cerebral cortex was homogenized in 20 vol of 50 m*M* Tris–HCl buffer with a Polytron homogenizer, and the homogenate was centrifuged at 40,000*g* for 15 min. The pellet was washed twice by centrifugation. The pellet was finally resuspended in the original volume of the buffer.

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All steps were performed at 4°C. Protein concentration was measured according to the method of Lowry *et al.* (17).

(+)-[^{3}H]PN 200-110 Binding Assay

Membranes (400–600 μg of protein) prepared from cardiac and brain tissues were incubated with (+)-[^{3}H]PN 200-110 in 50 m*M* Tris–HCl buffer (18). Incubation was carried out in the dark with a sodium lamp for 60 min at 37°C. The reaction was terminated by rapid filtration (Cell Harvester, Brandel Co., Gaithersburg, MD) through Whatman GF/B glass-fiber filters, and filters were rinsed three times with 4 mL of ice-cold buffer. Tissue-bound radioactivity was extracted from the filters overnight in scintillation fluid and the radioactivity was determined by a liquid scintillation counter. Specific (+)-[^{3}H]PN 200-110 binding was determined experimentally from the difference between counts in the absence and presence of 3 μ*M* nifedipine. All assays were conducted in duplicate.

Determination of the Plasma Concentration of Mepirodipine

The concentration of mepirodipine in the rat plasma was determined by a radioreceptor assay as described previously (19). The lower limit of detection by this method, defined as the amount of mepirodipine hydrochloride displacing approximately 25% of specific (+)-[^{3}H]PN 200-110 binding, was 0.2 pmol/assay.

Data Analysis

The analysis of binding data was performed as described previously (20). The apparent dissociation constant (K_d) and maximal number of binding sites (B_{max}) for (+)-[^{3}H]PN 200-110 were estimated by Rosenthal analysis of the saturation data (21).

Materials

(+)-[^{3}H]PN 200-110 (87.0 Ci/mmol) was purchased from Dupont-NEN Co. Ltd. (Boston, MA). The following drugs were kindly donated by the companies indicated: mepirodipine hydrochloride, Yamanouchi Pharmaceutical Company (Tokyo, Japan); and nifedipine hydrochloride, Bayer Pharmaceutical Company (Osaka, Japan). All other chemicals were obtained from commercial sources.

RESULTS

Occupancy of Cardiac and Cerebral Ca^{2+} Antagonist Receptors by Mepirodipine

The equilibrium binding isotherms for specific (+)-[^{3}H]PN 200-110 (0.02–2.4 n*M*) binding in cardiac and cerebral cortical membranes were determined at 0.5 to 24 hr after an oral administration of mepirodipine (1, 3, and 10 mg/kg) in rats. The Rosenthal plots (at 3 hr) for cardiac (+)-[^{3}H]PN 200-110 binding following the mepirodipine administration, and the K_d and B_{max} values, are shown in Fig. 1 and Table I, respectively. At 0.5 and 3 hr after an oral administration of mepirodipine at a dose of 1 mg/kg in rats, there was a significant (68 and 43%, respectively) decrease in the B_{max} values without a significant change in the K_d values for cardiac

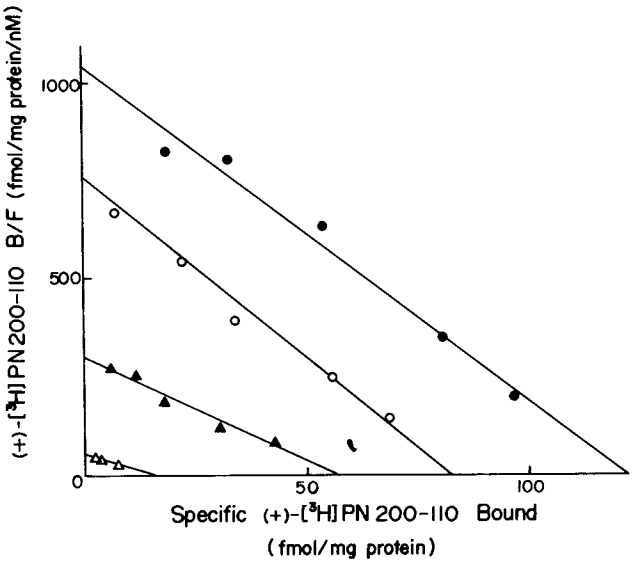

Fig. 1. Scatchard plots of specific (+)-[^{3}H]PN 200-110 binding to cardiac membranes from control (●) and mepirodipine (○, 1 mg/kg; ▲, 3 mg/kg; △, 10 mg/kg) administered rats. Rats were sacrificed at 3 hr after the oral administration of mepirodipine. Each point represents the mean of duplicate determinations from 3 to 18 rats.

(+)-[^{3}H]PN 200-110 binding compared to the control value. Further, a significant decrease in B_{max} values was also observed at 3 and 6 hr (54 and 38%, respectively) after the administration at the dose of 3 mg/kg, while the K_d values increased only at 3 hr. Following the administration of the highest dose (10 mg/kg) of mepirodipine, there was a marked loss of B_{max} values for cardiac (+)-[^{3}H]PN 200-110 binding at 3, 6, and 12 hr (85, 58, and 21%, respectively). Further, the K_d values for the (+)-[^{3}H]PN 200-110 binding at 3, 6, and 12 hr were significantly increased. The reduction of B_{max} values by mepirodipine was most pronounced at 0.5 or 3 hr, and the effect decreased significantly with time. The K_d and B_{max}

Table I. Effects of Oral Administration of Mepirodipine on K_d and B_{max} Values of Specific (+)-[^{3}H]PN 200-110 Binding to Rat Heart

	K_d (n*M*)[a]	B_{max} (fmol/mg protein)[a]
Control	0.11 ± 0.01	120 ± 5
Mepirodipine		
1 mg/kg		
0.5 hr	0.16 ± 0.03	38 ± 5***
3 hr	0.09 ± 0.003	68 ± 5**
3 mg/kg		
3 hr	0.20 ± 0.03***	55 ± 4**
6 hr	0.12 ± 0.01	74 ± 11**
12 hr	0.10 ± 0.002	101 ± 16
10 mg/kg		
3 hr	0.30 ± 0.04***	18 ± 2***
6 hr	0.38 ± 0.09***	50 ± 9***
12 hr	0.18 ± 0.02**	95 ± 14*
24 hr	0.09 ± 0.01	107 ± 11

[a] Mean ± SE of duplicate determinations from 3 to 18 rats.
* Significantly different from control values, $P < 0.05$.
** Significantly different from control values, $P < 0.01$.
*** Significantly different from control values, $P < 0.001$.

values for specific (+)-[^{3}H]PN 200-110 binding at 12 (3 mg/kg) and 24 (10 mg/kg) hr after an oral administration of mepirodipine in rats returned close to the control values.

The K_d and B_{max} values for cerebral (+)-[^{3}H]PN 200-110 binding in rats administered orally with mepirodipine at the low dose (1 mg/kg) were not significantly different from the control value (K_d = 0.09 ± 0.01 n*M*, B_{max} = 119 ± 3 fmol/mg protein; mean ± SE; n = 18). Following the mepirodipine administration of 3 and 10 mg/kg, however, there was a significant reduction ($P < 0.01$; 28 and 46%, respectively) of B_{max} values for cerebral (+)-[^{3}H]PN 200-110 binding at 3 hr after the administration of 3 mg (B_{max} = 97.1 ± 4.0 fmol/mg protein; n = 8) and 10 mg (B_{max} = 64.1 ± 6.1 fmol/mg protein; n = 6). A 40% decrease in the B_{max} by mepirodipine at the dose of 10 mg/kg persisted after 6 hr but not 12 hr. The K_d value in the (+)-[^{3}H]PN 200-110 binding was significantly ($P < 0.05$) increased only at 6 hr after an oral administration of mepirodipine at the dose of 10 mg/kg (K_d = 0.24 ± 0.07 n*M*; n = 5).

Plasma Concentration of Mepirodipine

Figure 2 shows the plasma concentration–time curve of mepirodipine in rats at 0.5 to 12 hr after an oral administration of mepirodipine at the doses of 1, 3, and 10 mg/kg. Mepirodipine was rapidly absorbed. The plasma concentrations attained maximum levels (1 mg, 32 ± 7 ng/mL; 3 mg, 138 ± 22 ng/mL; 10 mg, 280 ± 38 ng/mL; mean ± SE; n = 3–7) at 0.5 hr and thereafter gradually decreased.

DISCUSSION

The major findings of this study are that (i) mepirodipine produced more selective occupancy of 1,4-DHP Ca^{2+} antagonist receptors in the cardiac tissue than in the cerebral cortex of rats and (ii) its receptor occupancy correlated with mepirodipine plasma concentration.

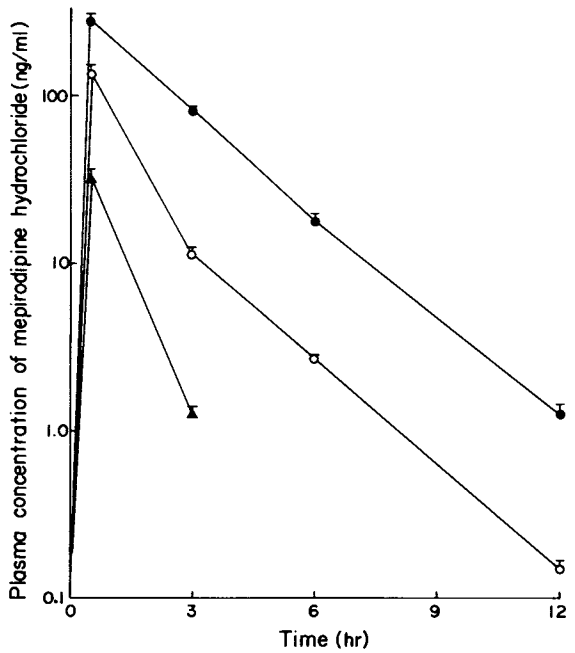

Fig. 2. Plasma concentrations of mepirodipine at 0.5 to 12 hr after its oral administration (▲, 1 mg/kg; ○, 3 mg/kg; ●, 10 mg/kg) to rats. Rats were sacrificed at 0.5, 3, 6, and 12 hr after the oral administration of mepirodipine. Each point represents the mean ± SE of duplicate determinations from three to seven rats.

The effect of mepirodipine on cardiac and cerebral Ca^{2+} antagonist receptors in rats has been investigated by an *ex vivo* radioreceptor assay technique. Following an oral administration of mepirodipine at the doses of 1, 3, and 10 mg/kg, there was a dose-dependent decrease in the number of cardiac (+)-[^{3}H]PN 200-110 binding sites at 0.5 to 12 hr, compared to control values. The effect of mepirodipine was most pronounced at 0.5 hr (1 mg/kg) and 3 hr (3 and 10 mg/kg) after oral administration, and the B_{max} value for cardiac (+)-[^{3}H]PN 200-110 binding returned to the control value at 12 or 24 hr after the mepirodipine administration. On the other hand, the K_d value was unchanged by the low dose of mepirodipine, whereas there was a significant increase in K_d only after the high dose. Therefore, mepirodipine caused mainly a change in the density of Ca^{2+} antagonist receptors. In the cerebral cortex, higher oral doses of mepirodipine were needed to produce a significant reduction of (+)-[^{3}H]PN 200-110 binding sites at 3 and 6 hr. Compared to the cardiac receptors, therefore, Ca^{2+} antagonist receptors in the cerebral cortex were less sensitive to the blockade by mepirodipine in rats. Our previous study demonstrated that the decrease in the *ex vivo* occupancy of cardiac Ca^{2+} antagonist receptors by mepirodipine in SHR correlated well with that in the *in vivo* occupancy of cardiac and vascular Ca^{2+} antagonist receptors determined by intravenous injection of (+)-[^{3}H]PN 200-110 (14). Thus, in the *ex vivo* experiment with mepirodipine, little dissociation of this drug from the receptor sites appears to occur during homogenization and incubation. Taken together, these data strongly suggest that mepirodipine produces more selective and sustained occupancy of 1,4-DHP Ca^{2+} antagonist receptors in cardiovascular target organs than in brain tissues of rats.

The decrease in the density of cardiac (+)-[^{3}H]PN 200-110 binding sites by an oral administration of mepirodipine may be due to its slowly dissociating blockade of the receptors, as demonstrated previously in the nonequilibrium blockade of brain nicotinic receptors by neosurugatoxin (22). The *in vitro* blockade of cardiac (+)-[^{3}H]PN 200-110 binding induced by mepirodipine was not reversed by repeated washout procedures (suspension-centrifugation) with Tris–HCl buffer, whereas the blockade by nifedipine was easily reversible under these conditions (unpublished observation). These results indicate that, unlike nifedipine, mepirodipine may bind persistently to Ca^{2+} antagonist receptors.

As illustrated in Fig. 3, the plasma concentrations of mepirodipine in rats given mepirodipine orally correlated well with its occupancy of cardiac and cerebral Ca^{2+} antagonist receptors (heart, $P < 0.01$; cerebral cortex, $P < 0.05$). This receptor occupancy was determined from the decrease in B_{max} values for specific (+)-[^{3}H]PN 200-110 binding by the mepirodipine administration. Thus, it was shown that mepirodipine produced a plasma concentration-related decrease in the density of unoccupied receptors. The plasma concentrations of mepirodipine necessary to occupy 50% of the total number of Ca^{2+} antagonist receptors in the heart and cerebral cortex were approximately 6 and 120 ng/mL, respectively. The protein binding of mepirodipine in rats and

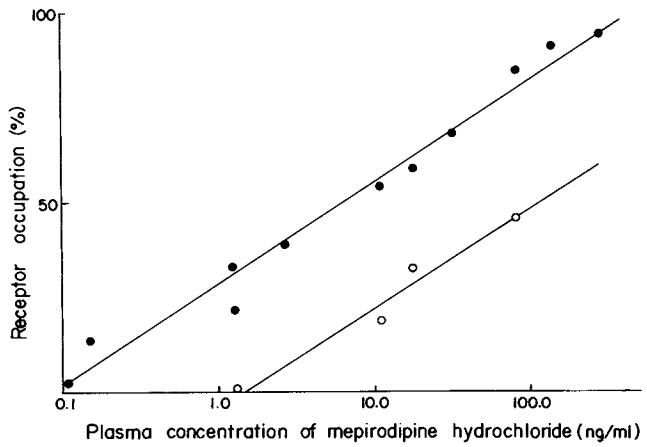

Fig. 3. Relationship between plasma concentration of mepirodipine and its occupancy of Ca^{2+} antagonist receptor in the heart (●) and cerebral cortex (○) in rats. The receptor occupancy was determined from the decrease in the B_{max} values for specific (+)-[^{3}H]PN 200-110 binding by the mepirodipine administration. Each point fits to the linear equation: heart, $y = 27.0x + 28.8$, $r = 0.99$; cerebral cortex, $y = 25.9x - 3.59$, $r = 0.98$. Here x is the concentration of mepirodipine and y is the percentage of receptor occupation.

humans is high (95–98%), and thus, the free fraction of this drug necessary to occupy 50% of cardiac Ca^{2+} antagonist receptors may be estimated to be 0.12–0.30 ng/mL (0.22–0.55 pmol/mL) of plasma. This plasma concentration (EC_{50} value) of free mepirodipine is similar to the K_i value (0.45 n*M*) of this drug to displace specific (+)-[^{3}H]PN 200-110 binding in cardiovascular tissues *in vitro* (18).

Our data demonstrated that a 20 times higher plasma concentration of mepirodipine is necessary to occupy Ca^{2+} antagonist receptors in brain tissues than in cardiac tissues, providing the first evidence that a 1,4-DHP derivative preferentially occupies Ca^{2+} antagonist receptors in cardiovascular target tissues. This result could account for the observation that few central effects occur with 1,4-DHP Ca^{2+} antagonists at the dosage used in the therapy of cardiovascular diseases (23). Since the occupancy of cardiovascular receptors by mepirodipine in SHR was shown to correlate with its hypotensive effect (14), a direct correlation between the plasma concentration of mepirodipine and its pharmacological effects can be assumed.

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