In Vivo Measurement of 1,4-Dihydropyridine Receptors in Mesenteric Arteries of Spontaneously Hypertensive Rats and Effect of Nifedipine and Cilnidipine

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The present study was undertaken to measure 1,4-dihydropyridine (DHP) receptor binding sites in vivo in the mesenteric artery and other tissues of spontaneously hypertensive rats (SHR) and to examine the effect of nifedipine and cilnidipine. Specific in vivo binding of (+)-[^3H]PN 200-110 in the SHR mesenteric artery was dose dependently reduced by oral administration of nifedipine at relatively low doses. Oral administration of cilnidipine (6.09 μmol/kg) significantly reduced the specific in vivo binding of (+)-[^3H]PN 200-110 in the mesenteric artery, aorta, and myocardium. A significant reduction in (+)-[^3H]PN 200-110 binding was seen at 1—12 h in the mesenteric artery and at 1—7 h in the aorta and myocardium. In contrast, oral administration of nifedipine (28.9 μmol/kg) markedly reduced in vivo (+)-[^3H]PN 200-110 binding in all tissues of SHR at 1—6 h, and the degree and time course of the reduction did not differ much among the tissues. The area under the curve (AUC) for receptor occupancy vs. time was calculated from the reduction rate (%) of specific in vivo (+)-[^3H]PN 200-110 binding. The ratio (1.4 or 1.7) of the AUCmesenteric artery to AUCaorta or AUCmyocardium after oral administration of cilnidipine was greater than the corresponding value (1.1) for nifedipine. In conclusion, the present study demonstrates that cilnidipine, but not nifedipine, may occupy 1,4-DHP receptors in the small artery in a more selective and sustained manner than in other tissues of SHR, and thus such receptor binding specificity may be responsible for the long-lasting hypotensive effect of this drug.

Key words nifedipine; cilnidipine; mesenteric artery; 1,4-DHP receptor occupancy

1,4-Dihydropyridine (DHP) calcium channel antagonists bind to specific receptors in cardiac and smooth muscles to inhibit influx of extracellular calcium through the voltage-operated channels involved in the excitation-contraction process. Nifedipine, the prototype of 1,4-DHP calcium channel antagonists, has short-acting cardiovascular effects. Cilnidipine has been clinically used as a long-acting 1,4-DHP calcium channel antagonist and this drug has been reported to differ from nifedipine in having a slower onset and longer duration of action.1—3 These pharmacological data indicate that there may be significant differences between cilnidipine and nifedipine in terms of cardiovascular 1,4-DHP receptor binding characteristics. The receptor sites of 1,4-DHP calcium channel antagonists in several tissues have been identified and characterized extensively by the in vitro radioligand binding technique,4—7 and the affinities of a number of compounds with the 1,4-DHP structure for the binding sites have been screened in vitro. However, the data obtained with the in vitro radioligand binding assay may not necessarily reflect in vivo pharmacological specificity because they do not take both pharmacokinetic and pharmacodynamic factors into account.8,9 In addition, the time-course and duration of the cardiovascular effects of 1,4-DHP drugs cannot be predicted by in vitro receptor binding data.

We have previously characterized the in vivo receptor binding of mepredipine and gianidipine in the aorta, myocardium, and brain of spontaneously hypertensive rats (SHR)9—11 It is evident that the major target organ for 1,4-DHP drugs in reducing arterial blood pressure is represented by resistant arterioles rather than the aorta and myocardium. Although it is more important to characterize their in vivo receptor binding in hypertensive-resistant arterioles, to our knowledge such studies with 1,4-DHP drugs have received little attention so far. Thus the aim of the present study was to measure 1,4-DHP receptor sites in vivo in the mesenteric arterial tissues of SHR, an animal model of essential hypertension in humans, and to characterize the binding properties of cilnidipine and nifedipine on these mesenteric arterial receptors.

MATERIALS AND METHODS

Materials Isopropyl-4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-5-methoxycarbonyl-2,6-dimethyl-3-pyridinecarboxylate (3027 GBq/mmol) (+)-(5-methyl[^3H]PN 200-110) was purchased from DuPont-NEN Co., Ltd. (Boston, MA, U.S.A.). Cilnidipine was kindly donated by Ajinomoto Co., Ltd. (Tokyo, Japan). Nifedipine hydrochloride was purchased from Sigma Chemical Co., Ltd. (St. Louis, MO, U.S.A.). All other chemicals were obtained from commercial sources.

Drug Administration Male SHR at 14 to 16 weeks of age (Japan SLC Inc., Shizuoku, Japan) were housed, 3 to 4 animals per cage, in the laboratory and had free access to food (normal rat chow) and water, and were maintained on a 12-h dark/light cycle in a room with controlled temperature (24±2°C) and humidity (55±5%). They were fasted for 12—18 h before drug administration and then received oral cilnidipine (6.09 μmol/kg) or nifedipine hydrochloride (2.89—28.9 or 116 μmol/kg). Cilnidipine was suspended in 5% gum arabic, and nifedipine hydrochloride was dissolved in 90% (v/v) PEG 400. Control animals received the vehicle alone.

Total Radioactivity and Specific in Vivo Binding of (+)-[^3H]PN 200-110 In vivo measurement of 1,4-DHP calcium channel antagonist receptors using (+)-[^3H]PN 200-110 was performed as previously described.10,11 Nifedipine and clini-
dipine were administered orally to SHR 1—18 h before injection of 555 kBq of (+)-[3H]PN 200-110 (66.0 ng, 0.18 nmol i.v.) into the femoral vein under temporary anesthesia with ethyl ether. Blood was taken from the descending aorta of rats 10 min after intravenous injection of (+)-[3H]PN 200-110, and the mesenteric artery, aorta, heart, and brain (cerebral cortex) were dissected and the fat tissues removed. These tissues were homogenized in ice-cold 50 mM Tris–HCl buffer to a final tissue concentration of 10 mg/ml (mesenteric artery, aorta, and myocardium) and 20 mg/ml (cerebral cortex) with a Physocotron homogenizer (Nition Co., Ltd., Tokyo, Japan). The radioactivity in plasma and aliquots of tissue homogenates (as the total amount of radioactivity in tissue) was measured in a liquid scintillation counter.

The particulate-bound radioactivity was determined by rapid filtration of 1 or 1.5 ml of each tissue homogenate through Whatman GF/C filters, which were subsequently washed with 3 ml of ice-cold buffer. The total and particulate-bound radioactivity in each tissue was measured in a liquid scintillation counter after addition of scintillation fluid.

Based on the data for pharmacological specificity,9—11 the particulate-bound radioactivity in each tissue from vehicle- and nifedipine- (116 μmol/kg, i.p., 0.5 h-pretreatment) treated SHR was defined as total binding and nonspecific binding, respectively, and the difference represented specific binding in vivo (+)-[3H]PN 200-110 binding. In a preliminary experiment, it was shown that there was no significant difference in the amount of specific in vivo (+)-[3H]PN 200-110 binding between one and two washings with 3 ml of ice-cold buffer of Whatman GF/C filters after the filtration of tissue homogenates. Thus we considered that the nonspecifically bound radioactivity could be removed by a single washing with 3 ml of buffer under the present assay conditions. The data were expressed as Bq per gram of tissue (wet weight). All procedures were performed in the dark with a sodium lamp.

Data Analysis The area under the curve (AUC) for the receptor occupancy vs. time were calculated from the rates (%) for the reduction of specific in vivo (+)-[3H]PN 200-110 binding in the mesenteric artery, aorta, and myocardium after oral administration of cilnidipine (6.09 μmol/kg, 0—18 h) and nifedipine (28.9 μmol/kg, 0—12 h), using the trapezoidal rule. Ratios of AUCmesenteric artery to AUCaorta or AUCmyocardium for each drug were calculated.

All data are presented as mean±S.E. Statistical analysis of the data was performed using Student’s two-tailed t-test and Dunnett’s test for single and multiple comparisons. Statistical significance was accepted at p<0.05.

RESULTS

Specific in Vivo Binding of (+)-[3H]PN 200-110 in the Mesenteric Artery and Other SHR Tissues The specific in vivo binding of (+)-[3H]PN 200-110 in the mesenteric artery of SHR was measured. Ten minutes after intravenous injection of (+)-[3H]PN 200-110 (555 kBq) to control SHR, the total amounts of radioactivity in the plasma and mesenteric artery were 4465±203 Bq/ml and 1932±126 Bq/g tissue (n=7), respectively, and the particulate-bound radioactivity in the tissue was 1301±110 Bq/g tissue (Fig. 1). To measure the nonspecific in vivo binding of (+)-[3H]PN 200-110 to the mesenteric artery, nifedipine (116 μmol/kg, i.p.) was administered 0.5 h before intravenous injection of (+)-[3H]PN 200-110 in SHR. The total radioactivity in the plasma and mesenteric artery of nifedipine-pretreated SHR was similar to that in control rats, but the particulate-bound radioactivity was markedly reduced. A significant amount of specific (+)-[3H]PN 200-110 binding, defined as the difference in particulate-bound radioactivity between vehicle- and nifedipine-pretreated SHR, was demonstrated in the SHR mesenteric artery. Simultaneously, there were significant amounts of specific (+)-[3H]PN 200-110 binding in the aorta, myocardium, and cerebral cortex of these SHR (Fig. 2), as previously reported.9—11 As shown in Fig. 3, there was a dose-dependent reduction in the specific in vivo binding of (+)-[3H]PN 200-110 in each SHR tissue 3 h after oral administration of nifedipine at doses of 2.89, 8.67, and 28.9 μmol/kg. Nifedipine 2.89 μmol/kg significantly reduced (53.6% and 39.6%, respectively) in vivo (+)-[3H]PN 200-110 binding in the mesenteric artery and cerebral cortex, and nifedipine 8.67 μmol/kg reduced it by 48.6—71.4% in each tissue.
Effect of Oral Administration of Nifedipine and Cilnidipine on Specific in Vivo (\(1\))-[\(3H\)]PN 200-110 Binding

At 1, 3, 7, and 12 h after oral administration of cilnidipine 6.09 \(\mu\)mol/kg, specific in vivo binding of (\(1\))-[\(3H\)]PN 200-110 in the mesenteric artery of SHR was significantly reduced (55.6%, 47.0%, 83.3%, and 52.7%, respectively) at 1, 3, and 7 h, there was also a significant (45.5—57.6%) reduction in (\(1\))-[\(3H\)]PN 200-110 binding in the aorta and myocardium. The administration of this drug has little effect on the cerebral cortical (\(1\))-[\(3H\)]PN 200-110 binding, except for a significant (40.0%) decrease at 7 h. The AUC for 1,4-DHP receptor occupancy vs. time was calculated from the degree of reduction (%) by cilnidipine (0 to 18 h) in the specific in vivo (\(1\))-[\(3H\)]PN 200-110 binding in the mesenteric artery, aorta, and myocardium of SHR. The ratio of AUC_{mesenteric artery} to AUC_{aorta} and AUC_{myocardium} for the receptor occupancy vs. time after oral administration of each drug may express the selectivity of peripheral arterial to aortic or to myocardial receptor sites, based on the in vivo receptor occupancy. Following oral administration of cilnidipine (6.09 \(\mu\)mol/kg), the values (%·h) of AUC\(_{0—18}\) in the mesenteric artery, aorta, and myocardium were 924, 647, and 558, respectively. The ratios of AUC_{mesenteric artery} to AUC_{aorta} and AUC_{myocardium} were 1.4 and 1.7, respectively.

Oral administration of nifedipine at a dose of 28.9 \(\mu\)mol/kg markedly reduced (78.2—100%) in vivo (\(1\))-[\(3H\)]PN 200-110 binding in the mesenteric artery, aorta, and myocardium of SHR at 1, 3, and 6 h, and the degree and time-course of this reduction did not differ among these tissues. The reduction rates in the mesenteric artery at 1, 3, and 6 h were 87.2\(\pm\)9.1%, 92.4\(\pm\)6.8%, and 85.3\(\pm\)10.2%, respectively. The data at 3 h in each tissue are shown in Fig. 3. The (\(1\))-[\(3H\)]PN 200-110 binding in each tissue returned to the control level at 12 h. The cerebral cortical (\(1\))-[\(3H\)]PN 200-
AUC_{mesenteric artery} to AUC_{aorta} or AUC_{myocardium} was 1.1.

**DISCUSSION**

Specific binding of (+)-[3H]PN 200-110 in particulate fractions of the aorta, myocardium, and brain of rats after intravenous injection was shown to reflect the predominant in vivo labeling of 1,4-DHP receptors, because it was markedly inhibited by pretreatment with several 1,4-DHP calcium channel antagonists at doses exhibiting pharmacological effects.9–11 However, none of the previous studies examined the in vivo receptor binding of 1,4-DHP drugs in the hypertensive-resistant arteriole. The present study has demonstrated that specific in vivo (+)-[3H]PN 200-110 binding in the mesenteric artery of SHR selectively labels 1,4-DHP receptors because it was dose dependently reduced by the oral administration of nifedipine at relatively low doses.

Cilnidipine is a useful antihypertensive agent with a potential for once-daily administration.12–14) Pharmacologic studies have shown that this drug differs from nifedipine in the very slow rate of onset and cessation of cardiovascular effects both in vitro and in vivo.1,2,15) In the present study, the in vivo 1,4-DHP receptor binding of cilnidipine and nifedipine in the mesenteric artery of SHR was investigated and compared with that in the aorta, myocardium, and cerebral cortex. Oral doses of these drugs exhibiting a significant hypotensive effect in conscious SHR were used in the present study.2,3,15)

A significant reduction in specific in vivo (+)-[3H]PN 200-110 in the mesenteric artery of SHR was also observed following oral administration of cilnidipine (6.09 μmol/kg). Although the magnitude of the reduction produced by cilnidipine was not very different between the mesenteric artery and aorta, there was a significant difference in the time-course. The (+)-[3H]PN 200-110 binding in the mesenteric artery was significantly reduced up to at least 12 h after oral administration of cilnidipine at this dosage, whereas in the aorta it had already returned to the control level at this time. In other words, a significant reduction in aortic (+)-[3H]PN 200-110 binding was observed at 1–7 h. This was more clearly shown by the ratio (1.4) of AUC_{mesenteric artery} to AUC_{aorta} greater than unity for this drug. These data clearly indicate that cilnidipine occupies 1,4-DHP receptors for a significantly longer duration in the mesenteric artery of SHR than in the aorta in vivo. To our knowledge, this is the first in vivo evidence demonstrating that long-acting 1,4-DHP drugs such as cilnidipine bind selectively to receptors in the hypertensive small artery. The time course of arterial 1,4-DHP receptors agreed closely with that of hypotensive effect at the same oral dose of cilnidipine in conscious SHR.5) This prolonged occupancy of mesenteric arterial receptors could not be explained by the short plasma half-life (2 h) of radioactivity after oral administration of [14C]cilnidipine in rats.16) It is considered that the dissociation of this drug from mesenteric arterial receptors in SHR may be slow. Alternatively, there is a possibility that the relatively high concentration of cilnidipine was maintained in the mesenteric arterial tissues, as previously reported in the same tissue of SHR after oral administration of benidipine.17)

The in vivo myocardial (+)-[3H]PN 200-110 binding was significantly reduced by oral administration of cilnidipine. Thus it is possible that cilnidipine has some myocardial effects at dosages exhibiting hypotensive effects. Urayama et al.19) have shown that long-term administration of cilnidipine improved calcium deposition and necrosis in the myocardium from dilated cardiomyopathic hamsters. Furthermore, no consistent reduction in (+)-[3H]PN 200-110 binding in the SHR cerebral cortex by cilnidipine might be supported by the extremely low level of distribution of radioactivity in the brain of rats after oral administration of [14C]cilnidipine.16)

Oral administration of nifedipine at the hypotensive dose (28.9 μmol/kg) markedly reduced in vivo (+)-[3H]PN 200-110 binding in all SHR tissues examined, and the extent and duration of this reduction did not differ markedly among tissues. The duration of arterial receptor occupancy was clearly shorter than that of cilnidipine. Based on the ratio (1.1) of AUC_{mesenteric artery} to AUC_{aorta} or AUC_{myocardium}, it was evident that there was no tissue selectivity associated with the hypotensive dose of nifedipine. Nifedipine at the lowest dose (2.89 μmol/kg at 3 h) administered showed significant inhibition of arterial (+)-[3H]PN 200-110 binding, but this dose of nifedipine is known to exhibit little hypotensive effect in conscious SHR.15) In addition, there was a similar degree of reduction in (+)-[3H]PN 200-110 binding in each tissue 3 h after oral administration of nifedipine at the intermediate dose (8.67 μmol/kg) which caused significant hypotension in SHR.15) Thus nifedipine at hypotensive doses exhibited little selectivity for arterial receptors in SHR. This differs from the case of cilnidipine, which exerted arterial receptor selectivity at the hypotensive dose. Although we have no clear explanation for the lack of hypotensive effect at the lowest dose of nifedipine in spite of considerable inhibition of arterial (+)-[3H]PN 200-110 binding in SHR, prolonged occupation of arterial receptors for a certain period may be necessary, or there may be a threshold in the degree of receptor occupation, to produce a hypotensive effect. Further detailed studies are required to clarify this issue. Oral administration of nifedipine significantly reduced in vivo (+)-[3H]PN 200-110 binding in the cerebral cortex of SHR, and this observation agreed with the previous reports by Janicki et al.19) and Uchida et al.20) who showed that nifedipine could easily cross the blood–brain barrier in rats.

In conclusion, cilnidipine exhibits a selective and sustained occupation of 1,4-DHP calcium channel antagonist receptors in the SHR mesenteric artery in vivo, and such receptor binding specificity may be responsible for the long-lasting cardiovascular effects of this drug. Therefore the in vivo measurement of receptor occupancy by 1,4-DHP calcium antagonists in the SHR mesenteric artery may be extremely useful for elucidating the tissue selectivity and duration of action of these drugs under physiological and pathological conditions.

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