Inhibitory Effect of Cilnidipine on Vascular Sympathetic Neurotransmission and Subsequent Vasoconstriction in Spontaneously Hypertensive Rats

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ABSTRACT—We reported previously that cilnidipine inhibited increases in blood pressure and plasma norepinephrine (NE) level in response to cold stress in spontaneously hypertensive rats (SHRs). In the present study, we investigated the effect of cilnidipine on sympathetic neurotransmission and subsequent vasoconstriction in SHRs. In pithed SHRs, electrical sympathetic nerve stimulation (ESNS) elevated blood pressure, and this pressor response was abolished by guanethidine. Cilnidipine at 10 μg/kg, i.v. and phentolamine at 1 mg/kg, i.v. suppressed the pressor response to ESNS by 28 ± 6% and 67 ± 3%, respectively. Neither nifedipine nor nicardipine inhibited it. The pressor response to exogenous NE was not influenced by cilnidipine. α,β-Methylene ATP inhibited the pressor response to ESNS in the presence or absence of phentolamine. Cilnidipine also attenuated the phentolamine-resistant pressor response to ESNS. In SHR mesenteric vasculatures preloaded with [3H]-NE, cilnidipine (10⁻⁷ M) as well as ω-conotoxin significantly inhibited the ³H overflow evoked by periarterial nerve stimulation. In radioligand binding experiments, cilnidipine inhibited [¹²⁵I]-ω-conotoxin binding to rat synaptosomes, but it did not inhibit [³H]-prazosin binding to rat cortex membranes. These results suggest that cilnidipine may reduce electrically stimulated NE release from the sympathetic nerve endings of SHR vasculatures probably through its N-type Ca channel blocking action and that cilnidipine may also inhibit the vasoconstriction induced by ATP released concomitantly during nerve stimulation.

Keywords: Cilnidipine, Pithed spontaneously hypertensive rat, Norepinephrine release, α,β-Methylene ATP, ω-Conotoxin

Cilnidipine is a new 1,4-dihydropyridine (DHP) derivative calcium (Ca) channel blocker that has a slow-onset and long-lasting hypotensive action (1). Cilnidipine has also been reported to inhibit the pressor response to acute cold stress in spontaneously hypertensive rats (SHRs) (2). It has been suggested that the inhibition of the pressor response by cilnidipine may be due to reduced sympathetic nerve activity, because cilnidipine attenuated the cold stress-induced increase in plasma norepinephrine (NE) concentration in SHRs (2).

In the present study, in order to further elucidate the mechanism of the inhibition by cilnidipine of cold stress-induced elevation in blood pressure, we investigated the effects of cilnidipine on both the pressor response to electrical sympathetic nerve stimulation (ESNS) in pithed SHR and the ³H overflow evoked by periarterial nerve stimulation (PNS) from perfused SHR mesenteric vasculatures preloaded with [³H]-NE. In some experiments, the effects of cilnidipine on [³H]-prazosin and [¹²⁵I]-ω-conotoxin (ω-CTx) bindings to rat cortex membranes and rat synaptosomes, respectively, were also studied.

MATERIALS AND METHODS

Animals used
Male 18- to 27-week-old SHRs (Charles River Japan, Yokohama) weighing 322–420 g were used. Male Sprague-Dawley (SD) rats (Charles River Japan) weighing 160–180 g were also used for binding assays.

Methods
Effect on pressor response to ESNS in pithed SHR:
SHRs were anesthetized with urethane (Tokyo Kasei Kogyo Co., Tokyo) at 1.2 g/kg, i.p. and the trachea was cannulated. Then the rats were pithed by inserting a 1.5-mm diameter steel rod down into the spinal cord to the first sacral vertebra. The stimulating electrode, which was uninsulated up to 1.0 cm from the end, was placed at a lower thoracic position (Th7–9). A steel needle was inserted into the skin of the back to serve as an indifferent electrode. Immediately after pithing, the tracheal cannula was connected to a rodent respirator (SN-480-7; Shinano, Tokyo) and the rats were ventilated artificially at a frequency of 60 cycles/min with a tidal volume of 2 ml/100 g body weight. The body temperature of the animals was kept at 37–38°C by a heating lamp. Systemic arterial blood pressure was measured from the left femoral artery via a pressure transducer (DX-200; Nihon Kohden, Tokyo), and the heart rate was measured by means of a tachometer (AT-601G, Nihon Kohden) triggered by the pressure pulse. These parameters were recorded continuously on a recorder (N4428; NEC San-ei, Tokyo). The left femoral vein was cannulated for intravenous drug administration. The animal was allowed to stabilize for at least 120 min after pithing and subsequently received 1 mg/kg, i.v. of tubocurarine. ESNS of spinal sympathetic outflow (Th7–9) was carried out by an electrical stimulator (SEN-3201, Nihon Kohden) via the pithing rod. Stimulation parameters were 0.1 msec duration and 60 V for 30 sec. In our preliminary experiments, ESNS at 0.1–20 Hz caused a frequency-dependent pressor response, and ESNS at 2 Hz increased the mean blood pressure by 74±9 mmHg (n=6), which corresponded to a nearly half-maximal response in pithed SHRs. Therefore, we used a stimulation frequency of 2 Hz in the subsequent experiments. To stabilize the response to ESNS, ESNS was preloaded repeatedly 10 times every 5 min, and then the preparation was equilibrated until the experiments were started. The following four series of experiments were carried out.

In the first series of experiments, ESNS was repeated twice at 30-min intervals. Guanethidine (3 mg/kg, i.v.) or phentolamine (1 mg/kg, i.v.) was administered 5 min before the onset of the 2nd ESNS. α,β-Methylene ATP (m-ATP) treatment was performed by the method of Bulloch and McGrath (3); the first 3 boluses of m-ATP at 50 μg/kg, i.v. were given, which was followed by 4 boluses of m-ATP at 500 μg/kg, i.v. at 1-min intervals. This P2-purinoceptor desensitizing procedure was started 12 min before the onset of the 2nd ESNS, because it spanned a 7-min period.

In the second series of experiments, ESNS was repeated 5 times every 30 min. Each drug or vehicle at stepwise increasing doses was administered 5 min before each onset of the 2nd to the 5th ESNS. The effects of drug and vehicle were expressed as a percentage of the pressor response to the 1st ESNS.

In the third series of experiments, ESNS was repeated 3 times every 30 min. Pretreatment with phentolamine (1 mg/kg, i.v.) was performed 5 min before the onset of the 2nd ESNS. Cilnidipine, m-ATP or vehicle was administered 5, 12 or 5 min before the onset of the 3rd ESNS, respectively. The effects of drug and vehicle were expressed as a percentage of the pressor response to the 2nd ESNS.

In the fourth series of experiments, intravenous administration of NE (3 μg/kg) was repeated 3 times every 30 min. Vehicle and cilnidipine were given 5 min before the 2nd and the 3rd NE administration, respectively.

Effect on 3H overflow induced by PNS in isolated perfused SHR mesenteric vasculatures preloaded with [3H]-NE

SHRs were anesthetized with sodium pentobarbital (Nembutal®; Abbott Laboratories, North Chicago, IL, USA) at 50 mg/kg, i.p., and the abdomen was opened by midline incision. The mesenteric-intestinal loop was prepared according to the method described by Castellucci et al. (4). The aorta was ligated both proximal and distal to the superior mesenteric artery, and a polyethylene cannula was inserted into the superior mesenteric artery through the aorta. The mesenteric vasculature preparation with the whole intestine was isolated immediately after flushing with 2 ml of physiological salt solution (PSS) containing 100 U/ml of heparin (Novo Nordisk, Bagsvaerd, Denmark). The composition of the PSS was as follows: 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM MgSO4, 1.2 mM KH2PO4, 25 mM NaHCO3 and 10 mM glucose. The PSS was aerated with a gas mixture of 95% O2 and 5% CO2 and maintained at 37°C. The isolated preparation was incubated at 37°C for 60 min in the PSS containing 10−7 M [3H]-NE (440.3–458.8 GBq/mmol; New England Nuclear (NEN), Boston, MA, USA) in the presence of 1.14×10−4 M l-ascorbic acid (Wako Pure Chemical Ind., Osaka) and then rinsed repeatedly with [3H]-free PSS. The preparation preloaded with [3H]-NE was placed in a 50-ml organ bath maintained at 37°C and perfused with the PSS at a constant flow rate of 5 ml/min by a peristaltic pump (SJ-1211H; Atto, Tokyo). The preparation was simultaneously superfused at a rate of 1 ml/min with the same PSS to prevent drying. After a 60-min equilibration period, PNS was derived by the electrical stimulator at 5 Hz, 1 msec pulse duration and 50 V for 30 sec via bipolar platinum electrodes placed around the superior mesenteric artery. PNS was applied twice at 90-min intervals. The mixture sample of both perfusate and superfusate was continuously collected at 5 min intervals from 10 min before the
1st PNS through the experiment. At the end of experiments, each preparation was dissolved in Soluene-100 (Packard Instrument Co., Inc., Downers Grove, IL, USA) for the determination of the amount of $^{3}$H retained in the tissue. To measure the radioactivity in the 5-min samples and preparations, 1 ml of each sample was mixed with 9 ml of Clear-sol I (Nacalai Tesque, Kyoto) and then counted in a liquid scintillation counter (LSC-1000; Aloka, Tokyo). The amount of $^{3}$H in each mixture sample of perfusate and superfusate was expressed as a percentage of the amount of $^{3}$H present in the preparation at the beginning of the respective sample collection. The PNS-evoked $^{3}$H overflow was determined by subtracting the radioactivity obtained in the 5-min sample just before PNS from that in the 5-min sample during PNS. The radioactivity obtained from the 5-min sample just before PNS was considered to be the spontaneous $^{3}$H outflow. Each drug or vehicle was perfused continuously from 85 min before the 2nd PNS. The effects of drug and vehicle are expressed as percentages of the 1st PNS-evoked $^{3}$H overflow.

**Displacement of $[^{3}H]$-prazosin binding to rat brain membranes**

The inhibition of $[^{3}H]$-prazosin binding to rat brain membranes was assessed by a modification of the method of Greengrass and Brenner (5). SD rats were sacrificed by decapitation, and the brain of each rat was quickly removed. The cerebral cortex was isolated and homogenized in 10 volumes of ice-cold 0.32 M sucrose using a polytron (PT-10-35; Kinematica, Lucerne Switzerland). The homogenate was centrifuged for 30 min at 50,000 × g. The pellet was suspended in 50 mM Tris-HCl buffer (pH 7.4) and centrifuged again under the same conditions as described above. The resulting pellet was washed with 50 mM Tris-HCl buffer (pH 7.4) and resuspended to a final concentration of 3 mg protein/ml in 50 mM Tris-HCl buffer (pH 7.4). The membrane preparation was frozen rapidly and stored at −80°C.

In a final volume of 1 ml, 300 μg protein of the membrane preparation was incubated at 25°C for 30 min in 50 mM Tris-HCl buffer (pH 7.4) containing 0.5 nM $[^{3}H]$-prazosin (2819.4 GBq/mmol, NEN). A 10-μl aliquot of drug solution or vehicle was also added to the buffer. The binding reaction was terminated by adding 3 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.4), and the reaction mixture was rapidly filtered under vacuum through Whatman GF/B glass fiber filters followed by rinsing the filters 4 times, each time with 3 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.4). The filters were dipped in 10 ml of Clear-sol I, and then the radioactivity retained on the filter was counted by a liquid scintillation counter. Nonspecific binding was determined in the presence of 10 μM prazosin. The results are expressed as percentages of the value obtained by using vehicle instead of drug. All assays were carried out in duplicate.

**Displacement of $[^{125}I]$-ω-conotoxin binding to rat synaptosomes**

SD rats were sacrificed by decapitation, and their brains were quickly removed. The cerebral cortex was isolated and homogenized in 9 volumes of ice-cold 0.32 M sucrose–10 mM Tris-HCl (pH 7.0) using the polytron. The homogenate was centrifuged for 10 min at 1,000 × g. The supernatant was centrifuged for 30 min at 15,000 × g. This pellet was suspended in 0.32 M sucrose–10 mM Tris-HCl (pH 7.0) and centrifuged again for 30 min at 15,000 × g. The resulting pellet was resuspended in 0.32 M sucrose–10 mM Tris-HCl (pH 7.0). The resuspended pellet was overlaid above a discontinuous sucrose gradient, consisting of 0.8 M and 1.2 M sucrose, and then centrifuged at 63,000 × g for 90 min. The membrane fraction at the 0.32 M/0.8 M interface, the synaptosome fraction, was collected and suspended in 20 mM Tris-HCl (pH 7.4). The suspension was centrifuged for 30 min at 15,000 × g. The pellet was resuspended in 20 mM Tris-HCl (pH 7.4) at a concentration of 20 μg protein/ml. The preparation was frozen rapidly and stored at −80°C.

The inhibition of $[^{125}I]$-ω-conotoxin GVIA (ω-CTx) binding was assessed by a modification of the method described by Abe et al. (6). In a final volume of 2.5 ml, a sample of the synaptosome preparation (1 μg protein) was incubated at 4°C for 90 min in 20 mM Tris-HCl–0.1% BSA buffer (pH 7.4) containing 5 pM $[^{125}I]$-ω-CTx (81.4 TBq/mmol, NEN), and then 25 μl of drug solution or vehicle was added to the medium followed by further incubation for 90 min. The binding reaction was terminated by rapid filtration under vacuum through Whatman GF/C glass fiber filters. The filters were washed 4 times with 4 ml of ice-cold 20 mM Tris-HCl–0.2 M NaCl–0.1% BSA buffer (pH 7.4). The radioactivity retained on each filter was determined by an autowell gamma counter (ARC-300, Aloka). Nonspecific binding was determined in the presence of 10 nM ω-CTx. The results are expressed as percentages of the value obtained with vehicle instead of drug. All assays were carried out in duplicate.

**Drugs used**

Cilnidipine (Fujirebio, Inc., Tokyo), nifedipine and nicardipine hydrochloride (Sigma Chemical Co., St. Louis, MO, USA) were dissolved in 50% HCO-60 (Nikkol Chemicals, Tokyo) ethanol solution, 1.5% polyvinylpyrrolidone K-30 ethanol solution and distilled water, respectively. These solutions were diluted with saline before intravenous injection. Guanethidine sulfate (Sigma), m-ATP (Sigma) and NE (Wako) were dissolved in saline.
Phentolamine (Regitin®; Ciba-Geigy Japan, Takarazuka) was diluted with saline. In the perfusion experiments, drug solutions were diluted with the PSS. In the binding assay, Ca channel blockers and prazosin hydrochloride (Tokyo Kasei) were dissolved in dimethyl sulfoxide (Wako), and α-CTx (Peptide, Institute Inc., Osaka) was dissolved in distilled water.

Statistical analyses

Results are expressed as the mean ± S.E. The significant difference between two groups was determined using an unpaired Student's t-test. Dunnett's multiple comparison was used for the multiple intergroup test. In each case, P values less than 0.05 were considered to indicate a significant difference.

RESULTS

Effects on pressor response to ESNS in pithed SHR

The basal value of mean blood pressure in pithed SHRs was 60 ± 1 mmHg (n = 53). As shown in Fig. 1, the pressor response to ESNS at 2 Hz was almost abolished by 3 mg/kg, i.v. of guanethidine. Phentolamine at 1 mg/kg, i.v. and m-ATP at the total dose of 2.15 mg/kg, i.v. reduced significantly the pressor response by 67 ± 3% and 48 ± 5%, respectively (Fig. 1). Cilnidipine at 3 and 10 μg/kg, i.v. reduced dose-dependently the pressor effect of ESNS, and cilnidipine at the higher dose reduced it significantly by 28 ± 6% (Fig. 2). In contrast, neither nifedipine nor nicardipine at doses up to 10 μg/kg, i.v. affected the elevation in blood pressure by ESNS (Fig. 2).

The effects of cilnidipine and m-ATP on the pressor response to ESNS in the pithed SHRs pretreated with phentolamine (1 mg/kg, i.v.) are shown in Fig. 3. Cilnidipine (10 μg/kg, i.v.) as well as m-ATP significantly reduced the phentolamine-resistant pressor response to ESNS.

The 1st administration of NE at 3 μg/kg, i.v. increased blood pressure by 68 ± 4 mmHg (n = 4), which was comparable to the pressor response to ESNS at 2 Hz. Cilnidipine at 10 μg/kg, i.v. did not influence the exogenous NE-induced increase in blood pressure (Fig. 4), whereas phentolamine at 1 mg/kg, i.v. abolished it (data not shown).

Effects on PNS-evoked 3H overflow

The spontaneous 3H outflow before the 1st PNS and the 1st PNS-evoked 3H overflow above the spontaneous outflow were 0.65 ± 0.02% and 0.39 ± 0.02% of the total tissue radioactivity (n = 48), respectively. Guanethidine at 3 x 10⁻⁶ M almost abolished the PNS-evoked 3H overflow (data not shown). Figure 5 showed the effect of cilnidipine at 3 x 10⁻⁸ and 10⁻⁷ M on the PNS-evoked 3H over-
flow. At $10^{-7} \text{M}$, cilnidipine significantly reduced the PNS-evoked $^3\text{H}$ overflow by 22±3%. In contrast, nicardipine at $10^{-6} \text{M}$ produced no significant effect on the PNS-evoked $^3\text{H}$ overflow. $\omega$-CTx at $5 \times 10^{-10} - 5 \times 10^{-9} \text{M}$ reduced concentration-dependently the PNS-evoked $^3\text{H}$ overflow (Fig. 6). None of the drugs used affected the spontaneous $^3\text{H}$ outflow (data not shown).

**Displacement of $[^3\text{H}]$-prazosin binding to rat brain membranes**

Displacement curves for cilnidipine, nicardipine and prazosin in $[^3\text{H}]$-prazosin binding to the rat cortex membranes are shown in Fig. 7. Cilnidipine at $10^{-7} - 10^{-4} \text{M}$ had no effect on $[^3\text{H}]$-prazosin binding. On the other hand, prazosin and nicardipine inhibited $[^3\text{H}]$-prazosin binding in a concentration-dependent manner. The concentrations of prazosin and nicardipine required to

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**Fig. 3. Effects of cilnidipine (10 μg/kg, i.v.) and $\alpha,\beta$-methylene ATP (m-ATP, total dose of 2.15 mg/kg, i.v.) on the phenolamine-resistant pressor response to electrical sympathetic nerve stimulation (ESNS) in pithed SHRs. ESNS was repeated 3 times every 30 min. Pretreatment with phenolamine (1 mg/kg, i.v.) was performed 5 min before the 2nd ESNS. Cilnidipine, m-ATP and vehicle was administered 5, 12 and 5 min before the 3rd ESNS, respectively. The effects of drug and vehicle were expressed as a percentage of the pressor response to the 2nd ESNS (control). The figure in parenthesis indicates the number of experiments. Each value represents a mean±S.E. *P<0.05 and **P<0.01, significantly different from the vehicle group.**

**Fig. 4. Effects of cilnidipine (10 μg/kg, i.v.) on the pressor response to exogenous norepinephrine (NE, 3 μg/kg, i.v.) in pithed SHRs. NE was injected 3 times every 30 min. Vehicle and cilnidipine were administered 5 min before the 2nd and the 3rd NE administration, respectively. The effects were expressed as a percentage of the pressor response to the 1st NE administration (control). Each value represents a mean±S.E. of 4 experiments. n.s. indicates no significant difference.**

**Fig. 5. Effects of cilnidipine and nicardipine on the periarterial nerve stimulation (PNS)-evoked $^3\text{H}$ overflow from perfused SHR mesenteric vasculatures preloaded with $[^3\text{H}]$-norepinephrine. PNS was applied twice at 90-min intervals. Each drug or vehicle was perfused continuously from 85 min before the 2nd PNS. The effects of drug and vehicle were expressed as a percentage of the 1st PNS-induced $^3\text{H}$ overflow. Each value represents a mean±S.E. of 6 experiments. *P<0.05, significantly different from the vehicle group.**

**Fig. 6. Effects of $\omega$-conotoxin on the periarterial nerve stimulation (PNS)-evoked $^3\text{H}$ overflow from perfused SHR mesenteric vasculatures preloaded with $[^3\text{H}]$-norepinephrine. PNS was applied twice at 90-min intervals. Each drug or vehicle was perfused continuously from 85 min before the 2nd PNS. The effects of drug and vehicle were expressed as a percentage of the 1st PNS-induced $^3\text{H}$ overflow. Each value represents a mean±S.E. of 6 experiments. *P<0.05 and **P<0.01, significantly different from the vehicle group.**
produce a 50% inhibition of \(^{3}\text{H}\)-prazosin binding were \(1.1 \times 10^{-9}\) and \(4.5 \times 10^{-6}\) M, respectively.

**Displacement of \([^{125}\text{I}]\)-\(\omega\)-CTx binding to rat synaptosomes**

Displacement curves for cilnidipine, nicardipine and \(\omega\)-CTx in \([^{125}\text{I}]\)-\(\omega\)-CTx binding to the rat synaptosomes are shown in Fig. 8. \(\omega\)-CTx at \(10^{-14} - 3 \times 10^{-12}\) M inhibited concentration-dependently \([^{125}\text{I}]\)-\(\omega\)-CTx binding to synaptosomes. Cilnidipine at \(10^{-8} - 10^{-5}\) M slightly inhibited \([^{125}\text{I}]\)-\(\omega\)-CTx binding in a concentration-dependent manner. At the maximal concentration used, \(10^{-5}\) M, cilnidipine reduced \([^{125}\text{I}]\)-\(\omega\)-CTx binding by 25% or less. On the other hand, nicardipine at concentrations up to \(10^{-5}\) M did not influence \([^{125}\text{I}]\)-\(\omega\)-CTx binding to the rat synaptosomes.

**DISCUSSION**

The previous reports have demonstrated that cilnidipine, a novel DHP Ca channel blocker, inhibited the pressor response to acute cold stress of SHRs (2). It is known that cold stress causes an increased plasma NE level due to enhanced sympathetic nerve activity, resulting in an elevation in blood pressure (7). So, we first examined the effect of cilnidipine on the pressor response to ESNS in pithed SHRs. ESNS elevated blood pressure with little effect on heart rate. The pressor response to ESNS was abolished by guanethidine, indicating that the response was a consequence of neural stimulation. In addition, phentolamine markedly suppressed the pressor response to ESNS. Therefore, it seems that the elevated blood pressure in response to ESNS under the present conditions may be due partly to \(\alpha\)-adrenergic receptor-mediated vasconstriction induced by NE released from sympathetic nerve endings.

Cilnidipine, but not nifedipine and nicardipine, inhibited dose-dependently the pressor response to ESNS, suggesting that the inhibitory action of cilnidipine may not be attributable to a vasoconstriction based on its L-type...
Ca channel blocking action. Cilnidipine at a dose (10 μg/kg, i.v.) sufficient to inhibit the ESNS-induced pressor effect, failed to inhibit the exogenous NE-induced elevation in blood pressure, although the amplitude of the pressor responses to ESNS and exogenous NE was similar. This finding is in agreement with our previous in vitro observation that cilnidipine did not inhibit the NE-induced vasoconstriction in the rabbit aorta (8). Furthermore, the present radioligand binding study indicated that cilnidipine has no antagonistic action on α₁-adrenoceptors. These results, together with no inhibition of the cat sympathetic ganglion transmission by cilnidipine (9), strongly suggest that this Ca channel blocker may reduce the pressor response to ESNS via postsynaptic mechanisms rather than presynaptic α-adrenergic receptor blocking action.

Therefore, to examine whether cilnidipine inhibits sympathetic neurotransmission, we secondly investigated the effect of cilnidipine on electrically stimulated ³H overflow from rat perfused mesenteric vasculature preparation preloaded with [³H]-NE. Cilnidipine at 10⁻⁷ M significantly suppressed the PNS-evoked ³H overflow, whereas nicardipine even at 10⁻⁴ M failed to inhibit it significantly. Conflicting results have been reported with respect to the effects of Ca channel blockers on sympathetic neurotransmission; some authors (10-12) showed that nicardipine and verapamil inhibited depolarization-evoked release of NE, although the concentrations of Ca channel blockers required for the inhibition of sympathetic neurotransmission appear to be considerably higher than those needed to inhibit the KCl-induced vasoconstriction. In contrast, the other investigators (13, 14) demonstrated that nifedipine and verapamil failed to influence the Ca²⁺-dependent NE release.

Nowycky et al. (15) have shown the existence of three types of voltage-dependent Ca channels, L-, N- and T-type, based on electrophysiological characterization. DHP Ca channel blockers selectively block only L-type Ca channels. It has been reported that α₁-CTx blocked L- and N- but not T-type Ca channels (16), and that the neurotoxin inhibited the depolarization-evoked NE release (17, 18). These findings suggest that the depolarization-induced NE release from the sympathetic nerve endings may be triggered mainly by Ca²⁺ influx through N-type Ca channels. The present results also indicated that α₁-CTx but not nicardipine produced a marked and concentration-dependent reduction in the PNS-evoked ³H overflow from SHR mesenteric vasculatures preloaded with [³H]-NE. In radioligand binding experiments, α₁-CTx inhibited competitively specific [¹²⁵I]-α₁-CTx binding to rat synaptosomes. Cilnidipine also inhibited concentration-dependently specific [¹²⁵I]-α₁-CTx binding, although this inhibitory effect of cilnidipine was much weaker than that of α₁-CTx. On the other hand, nicardipine at concentrations up to 10⁻⁵ M failed to affect specific [¹²⁵I]-α₁-CTx binding. From these results, it is concluded that cilnidipine but not nicardipine may inhibit sympathetic neurotransmission by reducing Ca²⁺ influx via N-type Ca channels related to NE release from the nerve endings.

In the present study, the pressor response to ESNS of pithed SHRs could not be abolished completely by phentolamine, suggesting the existence of a phenolamine-resistant pressor response. It is well-known that ATP is a co-transmitter from the sympathetic nerve endings and causes vasoconstriction via P₂-purinoceptor (19). In this study, a P₂-purinoceptor desensitizing agent, m-ATP, suppressed the phenolamine-resistant pressor response. Thus, it appears that the pressor response to ESNS may be mediated by not only by α₁-adrenergic receptors but also by P₂-purinoceptors. Cilnidipine also significantly inhibited the phenolamine-resistant pressor response, suggesting the possibility that cilnidipine may attenuate the pressor response to ESNS by inhibiting postsynaptically the vasoconstriction in response to ATP released concomitantly during nerve stimulation. This view is supported by the result of Nakashima et al. (20), who reported that cilnidipine inhibited the vasoconstriction induced by ATP in the rabbit mesenteric artery.

In conclusion, these results suggest that cilnidipine may reduce electrically stimulated NE release from the sympathetic nerve endings of SHR vasculatures, probably through its N-type Ca channel blocking action, and that cilnidipine may also inhibit the vasoconstriction induced by ATP released concomitantly during nerve stimulation.

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