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Antisympathetic and hemodynamic property of a dual L/N-type Ca^{2+} channel blocker cilnidipine in rats

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Abstract

The in vivo antisympathetic property of a dual L/N-type Ca^{2+} channel blocker cilnidipine compared with that of typical N-type Ca^{2+} channel blockers has never been clarified. We investigated the effects of the drug on a sympathetic nerve-mediated vascular response and vasodilating action in rats in comparison with those of an N-type Ca^{2+} channel blocker ω-conotoxin MVIIA. In pithed rats, ω-conotoxin MVIIA preferentially suppressed the sympathetic nerve stimulation-induced pressor response, whereas cilnidipine suppressed the pressor response induced by sympathetic nerve stimulation and angiotensin II. In anesthetized rats, cilnidipine or ω-conotoxin MVIIA decreased mean blood pressure, while heart rate was decreased by ω-conotoxin MVIIA, but slightly increased by cilnidipine. These results suggest that cilnidipine can affect sympathetic N-type Ca^{2+} channels in addition to vascular L-type Ca^{2+} channels in antihypertensive doses in the rat in vivo. The antisympathetic activity of cilnidipine is not excessive for an antihypertensive drug in comparison with that of ω-conotoxin MVIIA.

Keywords: Cilnidipine; ω-Conotoxin MVIIA; Sympathetic nerve; Ca^{2+} channel, N-type

1. Introduction

Cilnidipine is a Ca^{2+} channel blocker, whose action lasts longer than that of the first generation of Ca^{2+} channel blockers including nifedipine, and the drug is used for the treatment of essential hypertension (Uneyama et al., 1999a,b). In patch-clamp studies, cilnidipine has been shown to suppress neuronal N-type Ca^{2+} channel currents in sympathetic cervical ganglion cells (Uneyama et al., 1997), dorsal ganglion cells (Fujii et al., 1997) and functionally expressed N-type Ca^{2+} channels in the *Xenopus* oocytes (Furukawa et al., 1999) in addition to its L-type Ca^{2+} channel-blocking action. Since N-type Ca^{2+} channels are predominantly located on sympathetic nerve endings and regulate neurotransmitter release (Hirning et al., 1988), cilnidipine is expected to be a favorable drug that overcomes some of the deficiencies of the first generation of Ca^{2+} channel blockers (Goldbourt et al., 1993) by inhibiting neurotransmitter release from sympathetic nerve endings (Takahara et al., 1997; Sakata et al., 1999). However, the in vivo antisympathetic property of cilnidipine compared with that of typical N-type Ca^{2+} channel blockers, ω-conotoxins, has never been clarified.

The purpose of the present study is to assess and compare the in vivo antisympathetic potential of cilnidipine and ω-conotoxin MVIIA (McGuire et al., 1997), both of which are clinically used N-type Ca^{2+} channel blockers. We evaluated the pithed rat model, in which the other N-type Ca^{2+} channel blocker, ω-conotoxin GVIA, has been tested to characterize its antisympathetic action (Pruneau and Angus, 1990). However, since the mechanism of the vascular contraction induced by sympathetic nerve activation possibly involves the opening of L-type Ca^{2+} channels on vascular smooth muscle cells, dual L/N-type Ca^{2+} channel blockers such as cilnidipine may not be analyzed adequately in this model. In the present study, we compared the effect of cilnidipine on the pressor response to sympathetic nerve activation and angiotensin II injection to explore the vascular L-type and sympathetic N-type Ca^{2+} channel-blocking actions of the drugs.

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2. Methods

All experiments were performed according to Guidelines for Animal Experiments, Pharmaceutical Research Laboratories (Kawasaki, Japan).

2.1. Anti-sympathetic effects in pithed rats

Male Sprague–Dawley rats (Charles River Japan, Yokohama, Japan) weighing 350–550 g were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). The right carotid artery was cannulated for the measurement of blood pressure with a pressure transducer (TP-400T; Nihon Kohden, Tokyo, Japan), while the right jugular vein was also cannulated for drug administration. Heart rate was measured using a heart rate counter (AT-601G; Nihon Kohden) triggered by blood pressure. Blood pressure and heart rate were recorded using a polygraph system (RM-6000; Nihon Kohden). After a bilateral vagotomy was performed in the cervical region, the rats were pithed by inserting a steel rod through the orbit down into the spinal canal. Immediately after pithing, the rats were artificially ventilated with room air through the tracheal cannula attached to the rodent ventilator (7025 Rodent Ventilator; Ugo Basile, Comerio, Italy) at a rate of 60 cycles/min with a tidal volume of 10 ml/kg. Another steel rod was subcutaneously inserted into the back to serve as an indifferent electrode. To paralyze the animals, *d*-tubocurarine in a dose of 3 mg/kg was intravenously administered. The body temperature was kept at 37–38 °C by a heating lamp.

Pithed rat model is useful for investigating the effects of vasoactive substances on systemic peripheral vascular vessels in vivo. Diastolic blood pressure, where there is smaller influence of a heart function on the blood pressure, is usually used as a parameter of peripheral vascular contraction in this model. Electrical sympathetic nerve stimulation (5–10 Hz, 1 ms duration, 70 V) was carried out for 30 s through the pithing rod using an electrical stimulator (SEN-3201; Nihon Kohden) and isolation unit (SS-201J; Nihon Kohden) to obtain pressor responses of 80–90 mm Hg (diastolic pressure). When blood pressure returned to baseline, angiotensin II (1 μg/kg) was intravenously administered. After stable pressor responses were obtained, 3 μg/kg of cilnidipine ($n=6$) was intravenously administered through the cannula. Two minutes later, sympathetic nerve stimulation and angiotensin II were reapplied. The second dose (10 μg/kg) of cilnidipine was administered, and pressor responses were observed. The third dose of cilnidipine (30 μg/kg) was similarly administered, and pressor responses were observed. The effects of ω-conotoxin MVIIA (3, 10, 30 and 100 μg/kg; $n=5$) and nifedipine (10, 30 and 100 μg/kg; $n=5$) were also evaluated in the same manner.

2.2. Hemodynamic study in anesthetized rats

Male Sprague–Dawley rats (Charles River Japan) weighing 350–550 g were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). The right femoral artery was cannulated for the measurement of blood pressure with a pressure transducer (TP-400T, Nihon Kohden), and the right femoral vein was also cannulated for drug administration. Heart rate was measured using a heart rate counter (AT-601G; Nihon Kohden) triggered by the blood pressure. Mean blood pressure and heart rate were recorded using a polygraph system (RM-6000; Nihon Kohden). The body temperature was kept at 37–38 °C by a heating lamp.

After a stabilization period, 1 μg/kg of cilnidipine was intravenously administered through the cannula ($n=5$), and changes in blood pressure and heart rate were observed. Three to four minutes after its administration, the second dose (3 μg/kg) of cilnidipine was administered, and hemodynamic changes were observed. The third, fourth and fifth doses of cilnidipine (10, 30 and 100 μg/kg, respectively) were similarly administered, and hemodynamic changes were observed. The effects of ω-conotoxin MVIIA (3, 10, 30 and 100 μg/kg; $n=3$) and nifedipine (3, 10, 30, 100 and 300 μg/kg; $n=6$) were also evaluated in the same manner.

2.3. Drugs

Cilnidipine was synthesized at Ajinomoto (Kawasaki). Nifedipine and *d*-tubocurarine were purchased from Sigma (St. Louis, MO, USA), while ω-conotoxin MVIIA and angiotensin II were purchased from the Peptide Institute (Osaka, Japan). Cilnidipine and nifedipine were initially dissolved in 50% hydrogenated castor oil (HCO-60; Nikko Chemicals, Tokyo, Japan)–ethanol solution and then diluted with saline, as previously reported (Hosono et al., 1995a). Pentobarbital sodium (Tokyo Kasei, Tokyo, Japan), ω-conotoxin MVIIA, angiotensin II and *d*-tubocurarine were dissolved in saline.

2.4. Statistics

Data are expressed as the means ± S.E.M. The significant difference between two groups was determined using a paired Student's *t*-test. Dunnett's multiple comparison was used for the multiple intergroup test. A *P*-value less than 0.05 was considered to be statistically significant.

3. Results

3.1. Anti-sympathetic effects of Ca^{2+} channel blockers

The basal diastolic blood pressure of the pithed rats was 42 ± 2 mm Hg ($n=16$). The control pressor response to sympathetic nerve stimulation was 86 ± 3 mm Hg, and that to angiotensin II was 85 ± 3 mm Hg ($n=16$). The reproducibility of these pressor responses was confirmed during vehicle administration (data not shown).

The effects of ω-conotoxin MVIIA and nifedipine on pressor responses to sympathetic nerve stimulation and

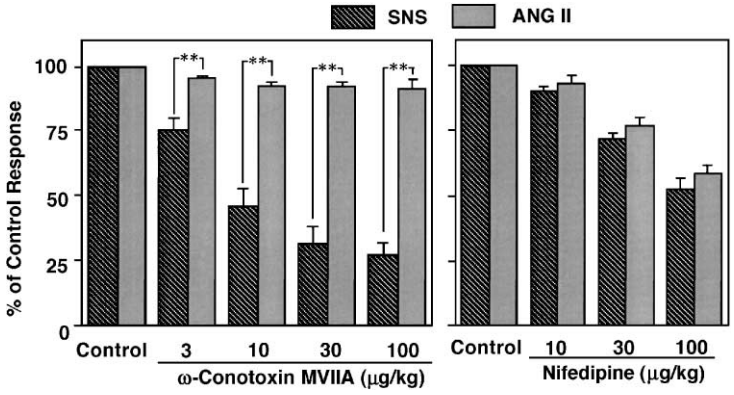

Fig. 1. Effects of ω-conotoxin MVIIA ($n=5$) and nifedipine ($n=5$) on the pressor responses to sympathetic nerve stimulation (SNS) and angiotensin II (ANG II) in pithed rats. Data are expressed as means ± S.E.M. $^{**}P<0.01$, significantly different between SNS and ANG II responses.

angiotensin II are shown in Fig. 1. ω-Conotoxin MVIIA in doses of 3 to 100 μg/kg significantly suppressed the sympathetic nerve stimulation-induced pressor response in a dose-dependent manner, whereas the angiotensin II-induced pressor response was only slightly attenuated by the peptide at the highest dose. Thus, the inhibitory effect of ω-conotoxin MVIIA on the pressor response induced by sympathetic nerve stimulation was greater than that induced by angiotensin II. Nifedipine in doses of 10 to 100 μg/kg significantly suppressed both sympathetic nerve stimulation- and angiotensin II-induced pressor responses in a dose-dependent manner. The inhibitory effect on the pressor response induced by sympathetic nerve stimulation was not significantly different compared with that induced by angiotensin II.

The effects of cilnidipine on pressor responses to sympathetic nerve stimulation and angiotensin II are shown in Fig. 2. Cilnidipine in doses of 3 to 30 μg/kg significantly suppressed both sympathetic nerve stimulation- and angiotensin II-induced pressor responses in a dose-dependent manner. The inhibitory effect on the pressor response induced by sympathetic nerve stimulation was significantly greater than that induced by angiotensin II in doses of 3 and 10 μg/kg.

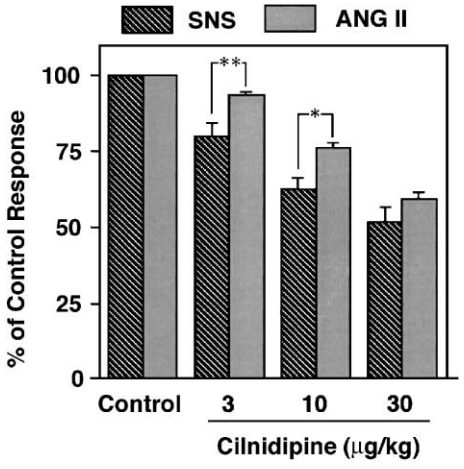

Fig. 2. Effects of cilnidipine ($n=6$) on the pressor responses to sympathetic nerve stimulation (SNS) and angiotensin II (ANG II) in pithed rats. Data are expressed as means ± S.E.M. $^{*}P<0.05$ and $^{**}P<0.01$, significantly different between SNS and ANG II responses.

3.2. Hemodynamic study

The effects of the drugs on systemic hemodynamics in anesthetized rats are summarized in Fig. 3. The basal mean blood pressure and heart rate were 102 ± 4 mm Hg and

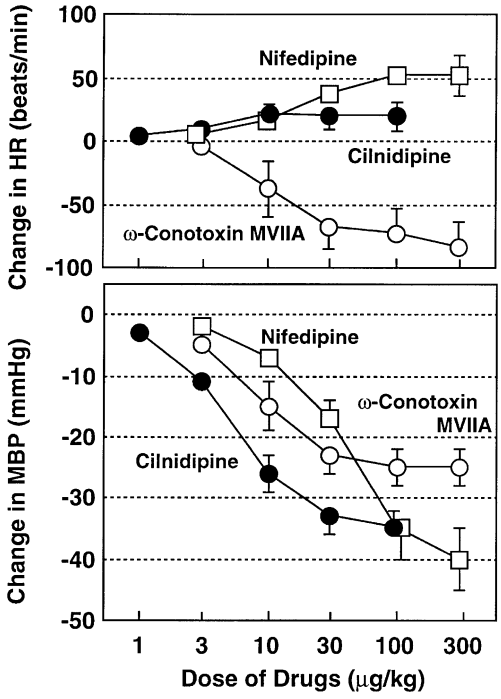

Fig. 3. Effects of cilnidipine ($n=5$), ω-conotoxin MVIIA ($n=3$) and nifedipine ($n=6$) on mean blood pressure (MBP) and heart rate (HR) in anesthetized rats. Data are expressed as means ± S.E.M.

345 ± 16 beats/min, respectively ($n = 14$). Intravenous administration of cilnidipine (1, 3, 10, 30 and 100 μg/kg) and nifedipine (3, 10, 30, 100 and 300 μg/kg) significantly lowered the mean blood pressure in a dose-dependent manner, while the positive chronotropic effects of nifedipine were greater than those of cilnidipine. ω-Conotoxin MVIIA (3, 10, 30, 100 and 300 μg/kg) significantly lowered the mean blood pressure and heart rate in a dose-dependent manner.

4. Discussion

The cardiovascular effect of cilnidipine was quite different from that of ω-conotoxin MVIIA in anesthetized rats. While ω-conotoxin MVIIA markedly decreased both blood pressure and heart rate, cilnidipine decreased only blood pressure, suggesting that the contribution of antisympathetic activity associated with the blockade of N-type Ca^{2+} channels to the changes in cardiovascular variables may be different between the two drugs. To assess and compare the direct in vivo antisympathetic actions of cilnidipine and ω-conotoxin MVIIA, we evaluated the effects of the drugs on the sympathetic nerve stimulation-induced pressor response in pithed rats, a model used to evaluate sympathetic N-type Ca^{2+} channel-blocking drugs (Pruneau and Angus, 1990). However, since an L-type Ca^{2+} channel blocker nifedipine suppressed the pressor response in the present study, the sympathetic N-type Ca^{2+} channel-blocking action of dihydropyridines with a strong L-type Ca^{2+} channel-blocking property like cilnidipine appears to be hardly evaluated in this model. Thus, we attempted to compare the suppressive effects of the drugs on the angiotensin II-induced pressor response with those on the sympathetic nerve stimulation-induced response in the pithed rats because angiotensin II-induced vasoconstriction in vivo has been shown to be mediated by L-type Ca^{2+} channel activation (Takahara et al., 1990, 1997).

As shown in the results, ω-conotoxin MVIIA suppressed the sympathetic nerve stimulation-induced pressor response but did not affect the angiotensin II-induced one, which strongly indicates that ω-conotoxin MVIIA suppressed norepinephrine release by inhibition of N-type Ca^{2+} channels located on sympathetic nerve endings but did not affect L-type Ca^{2+} channels on vascular smooth muscle in the in vivo model. However, cilnidipine in antihypertensive doses suppressed the sympathetic nerve stimulation-induced pressor response more potently than the angiotensin II-induced one, in contrast to nifedipine, suggesting that cilnidipine can suppress norepinephrine release from sympathetic nerve endings through the activation of N-type Ca^{2+} channels in addition to vascular contraction through activation of L-type Ca^{2+} channels on vascular smooth muscle. The results can explain the hemodynamic actions of ω-conotoxin MVIIA assessed in anesthetized rats, in which it decreased both blood pressure and heart rate, probably due to a sympatholytic action, and those of cilnidipine, which decreased blood pressure but only slightly affected heart rate, unlike nifedipine.

The present study using ω-conotoxin MVIIA shows that the doses that inhibited the sympathetic nerve stimulation-induced pressor response by less than 25% in pithed rats failed to affect hemodynamic variables in anesthetized rats. Although the antisympathetic potential of cilnidipine was not quantitatively determined in the current pithed animal model, it seems to be less potent than that of ω-conotoxin MVIIA. It has been previously reported that cilnidipine can suppress catecholamine release from the sympathetic nerve endings by 20–25%, an effect which may be associated with partial blockade of ω-conotoxin GVIA binding (Hosono et al., 1995a; Takahara et al., 1997). From this evidence, the N-type Ca^{2+} channel-blocking property of cilnidipine may not positively regulate blood pressure under physiological conditions because of its partial antisympathetic action. Furthermore, the differences between the antisympathetic property of cilnidipine and ω-conotoxin MVIIA can explain why cilnidipine does not induce orthostatic hypotension (Ishii et al., 1993; Hosono et al., 1995b), in contrast to ω-conotoxins (Hawkes et al., 1995; McGuire et al., 1997), in clinical and experimental settings.

In conclusion, cilnidipine can affect sympathetic N-type Ca^{2+} channels in addition to vascular L-type Ca^{2+} channels in antihypertensive doses. Its antisympathetic activity is not excessive for an antihypertensive drug in comparison with that of ω-conotoxin MVIIA.

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