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Short communication

Effects of a dual L/N-type Ca^{2+} channel blocker cilnidipine on neurally mediated chronotropic response in anesthetized dogs

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Abstract

We investigated the effects of an L-type and N-type Ca^{2+} channel blocker, cilnidipine, on neurally mediated chronotropic responses to clarify the anti-autonomic profile of cilnidipine in anesthetized dogs. Pretreatment with cilnidipine (0.3, 1.0 and 3.0 μg/kg, i.v.), which decreased mean blood pressure by 5 to 31 mm Hg, inhibited the changes in heart rate and plasma norepinephrine concentration induced by bilateral carotid artery occlusion, whereas it had no effect on vagal nerve stimulation-induced bradycardia. These results suggest that antihypertensive and antisympathetic doses of cilnidipine fail to influence chronotropic responses mediated by parasympathetic nerve activation in the in vivo canine heart.

Keywords: N-type Ca^{2+} channel; Cilnidipine; Bilateral carotid artery occlusion; Vagal nerve stimulation

1. Introduction

While a wide range of Ca^{2+} channel blockers are used for the treatment of cardiovascular diseases such as hypertension and angina pectoris, recent patch-clump studies have revealed that some Ca^{2+} channel blockers, such as cilnidipine (Uneyama et al., 1999a), affect N-type Ca^{2+} channels in addition to L-type Ca^{2+} channels (Uneyama et al., 1997; Fujii et al., 1997). N-type Ca^{2+} channels have been widely recognized to control neurotransmitter release from sympathetic nerves (Hirning et al., 1988; Uneyama et al., 1999b), and cilnidipine has been shown to suppress norepinephrine release from an isolated rat vascular preparation and in vivo canine kidney (Hosono et al., 1995; Takahara et al., 1997). Furthermore, the role of N-type Ca^{2+} channels in humans has been clarified in the isolated human heart atrium and in patients with essential hypertension by cardiac imaging (Sakata et al., 1999; Moldering et al., 2000). Thus, Ca^{2+} channel blockers with a sympatholytic action are expected to be promising drugs for the treatment of cardiovascular diseases, unlike nifedipine, which increases mortality in patients with coronary heart disease, possibly due to sympathetic activation (Furberg et al., 1995).

Although the role of Ca^{2+} channels in neurotransmitter release from cardiac parasympathetic nerves has been demonstrated using a specific L-, N- or P-type Ca^{2+} channel blocker in isolated heart preparations, it is now controversial (Uneyama et al., 1999b). Since parasympathetic activity has been recognized to be of importance in avoiding sudden or arrhythmic death following myocardial infarction (Farrell et al., 1991), the influence of Ca^{2+} channel blockers with an N-type Ca^{2+} channel blocking action on parasympathetic nerves in the in vivo heart should be clarified. In the present study, we investigated the effects of cilnidipine on chronotropic responses induced by vagal nerve stimulation in comparison with those induced by sympathetic nerve activation in anesthetized, closed-chest dogs to clarify the anti-autonomic profile of cilnidipine in antihypertensive doses.

2. Materials and methods

All experiments were conducted according to the Animal Ethics Committee of Ajinomoto (Tokyo, Japan).

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2.1. Animal preparation

Ten male Beagle dogs weighing 9 to 12 kg were anesthetized with pentobarbital sodium (30 mg/kg, i.v.). After intubation with an endotracheal tube, the dogs were artificially ventilated with room air. A catheter was placed in the left cephalic vein for infusion of pentobarbital sodium at a rate of 6 mg/kg/h to maintain anesthesia throughout the experiments. Another catheter was placed in the right cephalic vein for drug administration. Blood pressure was measured with a pressure transducer (TP-400T, Nihon Kohden, Tokyo, Japan) through a heparinized catheter placed in the left brachial artery. Heart rate was measured by a heart rate counter (AT-601G, Nihon Kohden) triggered by the blood pressure. Another arterial catheter for blood sampling was placed in the right brachial artery for measurement of plasma norepinephrine concentration. Both cervical vagi were isolated from the common carotid artery and cut at the midcervical level. Dogs were divided into two groups. In group 2, bipolar platinum electrodes were attached to the distal end of the vagus nerve on the right side.

2.2. Experimental protocol

2.2.1. Effects on bilateral carotid artery occlusion-induced cardiovascular responses (group 1)

After a stabilization period, the vehicle of cilnidipine was administered. Two minutes later, a blood sample was collected from the right brachial artery, and the bilateral carotid arteries were occluded for 2 min to induce sympathetic activation. Before termination of bilateral carotid artery occlusion, a blood sample was obtained again. After the cardiovascular parameters returned to baseline, an initial dose of cilnidipine (0.3 μg/kg) was administered. Two minutes later, blood sampling and bilateral carotid artery occlusion were performed as described above. Then, we administered 1 μg/kg of cilnidipine and assessed its effect in the same manner. After assessment of the effect of the second dose of cilnidipine, we additionally administered a third dose (3 μg/kg) of cilnidipine and assessed its effect in the same manner.

2.2.2. Effects on vagal nerve stimulation-induced bradycardia (group 2)

After a stabilization period, vagal nerve stimulation was applied as rectangular pulses of 1-ms duration and 20 V (supramaximal voltage) at 2 Hz, the stimulation parameters being adopted to avoid sinus arrest, for 30 s using an electronic stimulator (SEN-3300, Nihon Kohden) and an isolation unit (SS-201J, Nihon Kohden). Two minutes after the administration of vehicle, vagal nerve stimulation was applied for 30 s, and the maximum change in heart rate was recorded. After the cardiovascular parameters returned to baseline, an initial dose of cilnidipine (0.3 μg/kg) was administered and vagal nerve stimulation was again applied for 30 s. Then, 1 μg/kg of cilnidipine was administered and its effect was assessed in the same manner. After the assessment of the effect of the second dose of cilnidipine, we additionally administered a third dose (3 μg/kg) of cilnidipine and assessed its effect in the same manner.

2.3. Measurement of plasma norepinephrine concentration

Arterial blood, 3 ml, was drawn before and during bilateral carotid artery occlusion. Blood samples were centrifuged at 3000 rpm for 15 min at 4°C. Plasma norepinephrine concentration was determined by high-performance liquid chromatography, as previously reported (Takahara et al., 1997).

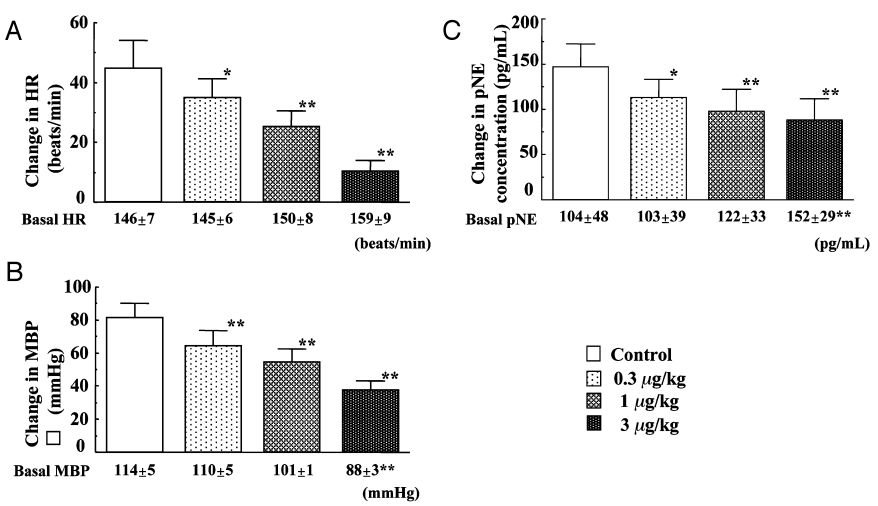

Fig. 1. Effects of cilnidipine on the change in (A) heart rate, (B) mean blood pressure and (C) plasma norepinephrine concentration induced by bilateral carotid occlusion in anesthetized dogs. Data represent the means ± S.E.M. of six experiments. The values at the bottom of each graph represent the basal values of each parameter. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with control. HR, heart rate; MBP, mean blood pressure; pNE, plasma norepinephrine.

2.4. Drugs

Cilnidipine (Ajinomoto) was dissolved in vehicle (ethanol: polyethylene glycol 400: saline = 15:15:70), while pentobarbital sodium (Tokyo Kasei, Tokyo, Japan) was dissolved in saline.

2.5. Data analysis

All values are expressed as means ± S.E.M. One-way analysis of variance was used for overall statistical analysis, followed by Dunnett's test for statistical analysis between control values and others using SuperANOVA (Abacus Concepts, Berkley, CA, USA). Differences at a *P*-value of less than 0.05 were considered to be statistically significant.

3. Results

3.1. Effects on bilateral carotid artery occlusion-induced cardiovascular responses (group 1)

The effects of cilnidipine on the change in heart rate, mean blood pressure and plasma norepinephrine concentration induced by bilateral carotid artery occlusion are summarized in Fig. 1. Bilateral carotid artery occlusion increased heart rate by 45 ± 9 beats/min and mean blood pressure by 81 ± 9 mm Hg with an elevation of plasma norepinephrine concentration from 104 ± 48 to 251 ± 59 pg/ml. Cilnidipine (0.3, 1.0 and 3.0 μg/kg) significantly suppressed bilateral carotid artery occlusion-induced tachycardia and pressor responses and the change in plasma norepinephrine concentration in a dose-dependent manner. As shown in Fig. 1, basal mean blood pressure and plasma norepinephrine concentration, which were recorded 2 min after cilnidipine administration, were significantly changed after the highest dose of cilnidipine.

3.2. Effects on vagal nerve stimulation-induced bradycardia (group 2)

The effects of cilnidipine on vagal nerve stimulation-induced bradycardia are shown in Fig. 2. Vagal nerve stimulation at 2 Hz decreased heart rate by 37 ± 3 beats/min in the control period, and vagal nerve stimulation-induced bradycardia was not affected by cilnidipine in doses of 0.3, 1.0 and 3.0 μg/kg. As shown in Fig. 2, basal heart rate, which was measured 2 min after cilnidipine administration, was not affected by cilnidipine.

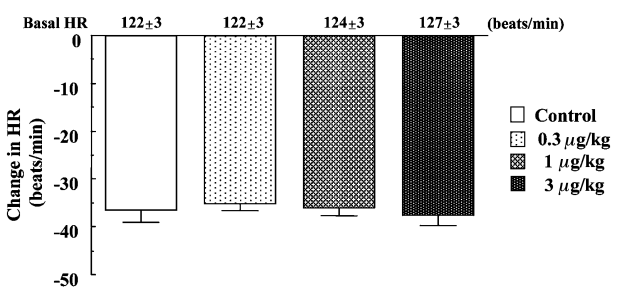

Fig. 2. Effects of cilnidipine on vagal nerve stimulation-induced bradycardia. Data represent the means ± S.E.M. of four experiments. Each value on the top of the graph represents the basal heart rate. No significant changes were observed. HR, heart rate.

4. Discussion

The present study was designed to clarify the anti-autonomic profile of cilnidipine by comparing its effects on sympathetic and parasympathetic nerve activation-induced chronotropic responses in anesthetized closed-chest dogs. Pretreatment with cilnidipine (0.3, 1 and 3 μg/kg, i.v.), which lowered the mean blood pressure by 5 to 31 mm Hg, inhibited bilateral carotid artery occlusion-induced tachycardia and elevation of plasma norepinephrine but failed to affect vagal nerve stimulation-induced bradycardia.

Since the antihypertensive and antisympathetic effects of cilnidipine have not been investigated in anesthetized dogs, we first examined the effects of the drug on bilateral carotid artery occlusion-induced cardiovascular responses, which reflect sympathetic nerve activation (Bradley et al., 1987). As shown in the results, cilnidipine effectively suppressed the bilateral carotid artery occlusion-induced increase in heart rate, mean blood pressure and plasma norepinephrine concentration in a dose-dependent manner whereas exogenous norepinephrine-induced responses were not affected by cilnidipine (data not shown), suggesting that cilnidipine can suppress sympathetic nerve activation-mediated cardiovascular responses in antihypertensive doses. Although the mechanisms of antisympathetic action cannot be analyzed exactly from this study, cilnidipine would seem to affect at least the sympathetic nerve endings because the drug can suppress norepinephrine release induced by electrical renal nerve stimulation in dogs (Takahara et al., 1997). The present results also support the previous clinical and experimental evidence that cilnidipine suppresses cardiac sympathetic overactivity and cold stress-induced pressor response (Sakata et al., 1999; Uneyama et al., 1999b).

Whereas sympathetic hyperactivity promotes the occurrence of life-threatening cardiovascular events, augmented vagal tone has been recognized to exert a protective and antifibrillatory effect (Schwartz et al., 1992). Thus, investigation of the effects of cilnidipine on parasympathetic nerve function is important to provide useful information about its potential as an antihypertensive drug. As shown in the results, antihypertensive and antisympathetic doses of cilnidipine failed to affect vagal nerve stimulation-induced negative chronotropic responses. Although some in vitro studies have shown that the parasympathetic nerve-mediated action is associated with N-type Ca^{2+} channels, in vivo studies have shown that a specific N-type Ca^{2+}

channel blocker, ω-conotoxin GVIA, at an antisympathetic dose fails to affect baro-reflex-induced bradycardia in conscious rabbits (Pruneau and Angus, 1990; Uneyama et al., 1999b). Therefore, these findings suggest that N-type Ca^{2+} channels are more predominant in sympathetic nerves than in parasympathetic nerves. Furthermore, since the lack of an antiparasympathetic action of cilnidipine may not worsen mortality by causing life-threatening cardiovascular events, the anti-autonomic action of cilnidipine will be expected to have cardioprotective actions like those of β-adrenoceptor antagonists (Squire and Barnett, 2000).

Baroreflex sensitivity is used as a clinical marker of vagal activity, which is expressed as the ratio of α-adrenoceptor agonist-induced changes in heart rate and blood pressure (Farrell et al., 1991). However, since the α-adrenoceptor agonist-induced pressor responses were prevented by a typical Ca^{2+} channel blocker nifedipine (Takahara et al., 1994), the present study showing that the negative chronotropic responses were directly induced by electrical nerve stimulation may be important for predicting the clinical effect of cilnidipine on vagal activity.

In conclusion, antihypertensive and antisympathetic doses of cilnidipine failed to influence chronotropic responses mediated by parasympathetic nerve activation in the in vivo canine heart, and thus the drug may contribute to the treatment of cardiovascular diseases.

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