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Selectivity of dihydropyridines for cardiac L-type and sympathetic N-type Ca^{2+} channels

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Abstract

The blocking effects of cilnidipine and other dihydropyridines on L-type cardiac Ca^{2+} channels ($I_{Ca,L}$) and N-type sympathetic Ca^{2+} channel currents ($I_{Ca,N}$) were studied using a whole-cell patch-clamp technique. At −80 mV, cilnidipine had little inhibitory effect below concentrations of 1 μM on $I_{Ca,L}$ (IC_{50} value; 17 μM). However, 1 μM cilnidipine strongly shifted the steady-state inactivation curve of $I_{Ca,L}$ toward negative potentials without changing the current–voltage relationship. Each action of cilnidipine was characterized by a high affinity for the inactivated channel in preference to the resting channel. The IC_{50} values of dihydropyridines for $I_{Ca,L}$ were in the range between 0.01 and 10 μM, and those for $I_{Ca,N}$ were between 3 and 30 μM. Cilnidipine had the strongest affinity for $I_{Ca,N}$ among the dihydropyridines tested. These results suggest that cilnidipine did not cause hypotension-evoked tachycardia deficiency by depression of cardiac L-type channels but by sympathetic N-type channels blockade.

Keywords: Dihydropyridine; Cilnidipine (FRC-8653); Ca^{2+} channel; Sympathetic neuron; Ventricular myocyte

1. Introduction

Dihydropyridine-type Ca^{2+} channel antagonists have been widely used in the treatment of arterial hypertension. Their pharmacological and therapeutic properties were, to date, thought to be attributed to the blockade of Ca^{2+} influx through L-type Ca^{2+} channels, and not to interaction with other Ca^{2+} channel subtypes. However, recent electrophysiological data have revealed that some second-generation dihydropyridine derivatives (cilnidipine, nimodipine, amlodipine) have a blocking potency for N-type as well as L-type Ca^{2+} channels (Furukawa et al., 1997; Uneyama et al., 1997) and discussion has started about the possible involvement of non-L-type actions of dihydropyridines in their therapeutic features. Oral administration of cilnidipine, for instance, attenuates stress-induced hypertension which is not effectively treated with clinically available dihydropyridines (Saihara et al., 1993; Hosono et al., 1995b). We have already a wide variety of data on the pattern of selectivity of the dihydropyridines for L-type Ca^{2+} channels with different pharmacological characteristics (Wei et al., 1988), but we have two little information about most dihydropyridines concerning their ability to cause N-type Ca^{2+} channel blockade and their therapeutic potential.

A fast-acting dihydropyridine such as nifedipine causes a baroreceptor-mediated reflex increase in sympathetic tone and activates neurohormonal systems such as the adrenergic system and the renin–angiotensin system. Increased plasma levels of these neurohormones result in tachycardia, increased myocardial contractility and stroke volume, to increase the workload of the heart as well as an increase in the myocardial oxygen demand (Kimichi and Lewis, 1991). The Secondary Prevention Reinfarction Israel Nifedipine Trial 2 (SPRINT 2) study reported that early administration of nifedipine increases the risk of mortality in patients with suspected acute myocardial infarction (Goldbourt et al., 1993). In contrast, a slow-acting dihydropyridine such as amlodipine can, in a way, overcome the clinical disadvantage of the fast-acting dihydro-

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pyridine, namely, the attenuation of the sympathetic nerve hyperactivation, by slowing its rapid hypotensive effects (Haria and Wagstaff, 1995).

Cilnidipine (FRC-8653) is an antihypertensive dihydropyridine known to possess an antagonistic effect on L- and N-type Ca^{2+} channels (Yoshimoto et al., 1991). The N-type Ca^{2+} channel-blocking action of cilnidipine has, in particular, been extensively examined in various neuronal cells (Fujii et al., 1997; Uneyama et al., 1997, 1998). In in vitro experiments, cilnidipine blocks catecholamine secretion from vessel walls elicited by electrical stimulation (Nakashima et al., 1991) or that from nerve growth factor (NGF)-differentiated PC12 cells elicited by high K^+ stimulation (Uneyama et al., 1998). Oral administration of cilnidipine reduced blood pressure without increasing the heart rate or blood catecholamine levels (Hosono et al., 1995a). This reduction in hypotension-induced baroreflex activity is thought to be due to attenuation of sympathetic nerve activation by N-type Ca^{2+} channel blockade. However, it is not clear whether this might be caused simply by indirect N-type blocking action or by a cardiac depressant action, such as elicited by diltiazem (Walsh, 1987; Michalewicz and Messerli, 1997), because the direct action of cilnidipine on cardiac L-type Ca^{2+} channels has not been investigated. In the present experiments, we examined the effects of cilnidipine on cardiac L-type Ca^{2+} channel currents and compared these with the effects of other clinically available dihydropyridines, using the conventional whole-cell patch-clamp technique. At the same time, we also evaluated the inhibitory effects of these dihydropyridines on the sympathetic N-type Ca^{2+} channel currents to clarify their Ca^{2+} channel characteristics for cardiac L- and the N-type channels, and discuss the possible merit of dihydropyridine-induced blockade of N-type Ca^{2+} channels in hypertension therapy.

2. Materials and methods

2.1. Isolation of rat ventricular myocytes

Single ventricular myocytes were obtained from adult Wistar rats by enzymatic dispersion as previously described (Those et al., 1987). Briefly, male Wistar rats (6 to 8 weeks old) were anesthetized with pentobarbital (50 mg/kg) and hearts were quickly removed. The heart was mounted on a modified Langendorff perfusion system for retrograde perfusion of the coronary circulation with a normal Tyrode solution. About 5 min after the perfusion, the heart was then perfused with a nominally Ca^{2+}-free Tyrode solution for 15 min and subsequently perfused with a solution containing type 1 collagenase (0.1 mg/ml) and 1% bovine serum albumin for 15 min. Then, the enzyme was washed out by perfusion with a modified Krebs' buffer (KB) solution for 10 min. The temperature of all solutions was kept at 35–37°C. Myocytes were obtained by a mechanical dissociation of ventricular tissue from the heart in the modified KB solution, and stored in the solution at a low temperature (4°C) for 1 h. Single ventricular myocytes were placed in a small plastic dish (Falcon Primaria) attached to an inverted microscope (Olympus IMT-2, Japan) and superfused with the normal Tyrode solution.

2.2. Isolation of superior cervical ganglion neurons

The superior cervical ganglion neurons were isolated as previously described (Uneyama et al., 1997). In brief, male Wistar rats (4–6 weeks old) were decapitated after a lethal injection of sodium pentobarbital (75 mg/kg). The superior cervical ganglia were dissected, connective tissues were removed, and three to four cuts were made in each ganglion. The ganglia were incubated at 37°C for 20 min in a Ca^{2+}-free Tyrode solution containing 20 U/ml papain. The papain was then replaced by a mixture of 500 U/ml collagenase and 16 mg/ml dispase. Sixty minutes later, the ganglia were rinsed with the Ca^{2+}-free Tyrode solution and triturated gently through a small-pore glass pipette.

2.3. Electrical measurements

All electrical measurements were performed using a conventional whole-cell patch recording technique under voltage-clamped conditions (Hamill et al., 1981; Uneyama et al., 1992). The pipettes were pulled from 1.5-mm capillary glass [Narishige] in two stages with a vertical pipette puller [Narishige, PB-7]. The pipette tip was fire-polished before use. The resistance between the recording electrode filled with pipette solution and the reference electrode in external solution was 3–5 MΩ. The current and voltage were measured with a patch-clamp amplifier [List Medical, EPC-7], and monitored on both a storage oscilloscope [Iwatsu DS-9121] and a pen recorder [Sanei, RECTI-HORIZ-8K], and then stored on DAT tape with a PCM processor [TEAC RD-120TE] after being filtered at 1 kHz [NF Instruments]. Then, analog signals were reconverted into digital signals with an AD converter at a sampling frequency of 3 kHz, and were stored in an IBM-compatible computer [Deskpro, Compac] by using pClamp software [Axon Instruments]. The value of the maximum voltage-clamp error in the present study was about 4.0 mV. All experiments were performed at room temperature (20–22°C).

2.4. Solutions

The ionic composition of the normal external solution was (in mM): 150 NaCl, 5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10

N-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) and 10 glucose. The composition of the modified KB solution was (in mM): 70 L-glutamic acid, 50 KOH, 30 KCl, 15 taurine, 10 KH_2PO_4, 0.5 $MgCl_2$, 11 glucose, 0.5 ethylene glycol-bis(±-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetra-acetic acid (EGTA) and 5 HEPES–KOH buffer (pH 7.3). The Na^+/K^+ free solution for sympathetic Ca^{2+} current measurement contained (in mM): 130 tetraethylammonium chloride, 4 CsCl, 1 $MgCl_2$, 10 $BaCl_2$, 10 HEPES, 10 glucose. For cardiac Ca^{2+} current measurement, 130 mM tetraethylammonium chloride and 10 mM $BaCl_2$ were replaced by 150 mM tetraethylammonium chloride and 1.8 mM $CaCl_2$. The pH was adjusted to 7.4 with tris(hydroxymethyl)aminomethane (Tris)–OH. The conventional patch-pipette solution contained (in mM): 75 CsCl, 75 Cs-methanesulfonate, 2 ATP-Mg, 5 EGTA and 10 HEPES. The pH was adjusted to 7.2 with Tris–OH.

2.5. Chemicals

Nimodipine and nitrendipine were purchased from Research Biochemical Internationals (RBI). Nifedipine, nicardipine and all other chemicals were obtained from Sigma [St. Louis, USA]. Manidipine, benidipine, nilvadipine, amlodipine and 2-Methoxyethyl (*E*)-3-phenyl-2-propen-1-yl (±)-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl) pyridine-3,5-dicarboxylate (cilnidipine) were synthesized at the Ajinomoto central research laboratories [Kawasaki, Japan]. The drugs were applied using a rapid application method termed the 'Y-tube' method, as described elsewhere (Murase et al., 1990; Uneyama et al., 1992).

2.6. Statistical analysis

All experimental values are presented as the means ± standard error of the mean (S.E.M.). For the evaluation of the half-maximal inhibitory concentration (IC_{50}) and Hill coefficient (n) of the concentration–inhibition curve, the data were fitted to a mirror image of the Michaelis–Menten equation using a least-squares fitting procedure,

$$I/I_{max} = 1 - C^n/(Kd^n + C^n) \quad (1)$$

where I is the current, I_{max} is the maximum response, and C is the concentration of the antagonist. The data for the concentration–inhibition curve were fitted to Eq. (1) by the least-squares fitting to obtain the half-maximal inhibition concentration. The voltage of half-maximal inactivation (V_h) and the slope factor (k) describing the steepness of the inactivation curve were obtained by using a computer-calculated Boltzmann fit according to Eq. (2):

$$I/I_{max} = 1/\left(1 + \exp\left((V_m - V_h)/k\right)\right) \quad (2)$$

where V_m is the prepulse potential.

3. Results

3.1. Effects of cilnidipine on the cardiac L-type Ca^{2+} channel currents

For the measurement of cardiac Ca^{2+} channel currents ($I_{Ca,L}$), we used 1.8 mM Ca^{2+} as a charge carrier through Ca^{2+} channels since Ba^{2+} sometimes causes irreversible myocyte contractions. Fig. 1A shows the time course of inhibitory action of 1 μM cilnidipine and nifedipine on $I_{Ca,L}$. The $I_{Ca,L}$ was evoked by a 200-ms depolarizing pulse from −80 mV to 0 mV every 10 s. The application of 1 μM cilnidipine showed a small inhibitory effect (17 ± 3% inhibition, $n = 4$), but subsequent application of the same concentration of nifedipine induced a substantial decrease in $I_{Ca,L}$ (51 ± 8% inhibition, $n = 4$).

Next, the effects of nifedipine (1 μM, Fig. 1B) or cilnidipine (10 μM, Fig. 1C) on the current–voltage relationships (I–V curves) for $I_{Ca,L}$ were examined. In these experiments, myocytes were depolarized for 200 ms from the V_h of −80 mV to various potentials ranging from −70 mV to +60 mV every 10 s. Neither 10 μM cilnidipine nor nifedipine affected the shape of the I–V curve, such as the threshold and the peak potentials. In Fig. 2, the concentration-dependent effects of cilnidipine and nifedipine on the cardiac $I_{Ca,L}$ are summarized. The IC_{50} value of nifedipine was 1.06 ± 0.27 μM ($n = 5$). However, 50% inhibition of the peak $I_{Ca,L}$ could not be obtained even with 10 μM cilnidipine (44 ± 16% inhibition at 10 μM; $n = 6$). The estimated IC_{50} for cilnidipine was 16.91 ± 6.94 ($n = 5$). Thus, the action of cilnidipine on cardiac L-type currents was markedly weaker than that of nifedipine under these experimental circumstances.

3.2. Voltage-dependent effect of cilnidipine on the cardiac Ca^{2+} channel currents

In Fig. 3A, we examined the effects of cilnidipine on $I_{Ca,L}$ with two different holding potentials. Cells were voltage-clamped at a V_h of −80 mV or −60 mV, and $I_{Ca,L}$ was obtained with depolarizing pulses from the potential to ±0 mV. In the absence of cilnidipine, peak amplitudes of $I_{Ca,L}$ were not so different between the two potentials. However, in the presence of cilnidipine, the peak amplitudes of $I_{Ca,L}$ were markedly reduced by changing the holding potential from −80 mV to −60 mV. The inhibitory percentages at V_h's of −80 mV and −60 mV were 7 ± 5% and 57 ± 9% ($n = 4$), respectively.

To investigate the potent voltage-dependent inhibitory action of cilnidipine, the effect of cilnidipine on the steady-state inactivation kinetics of $I_{Ca,L}$ were examined using a gapped double-pulse protocol before and after a 4-min exposure to cilnidipine. Myocytes were given a 1 s prepulse to potentials between −80 and +10 mV, followed by a 10 ms return to the V_h of −80 mV and then a fixed 200 ms test pulse to +10 mV. The pulse protocol

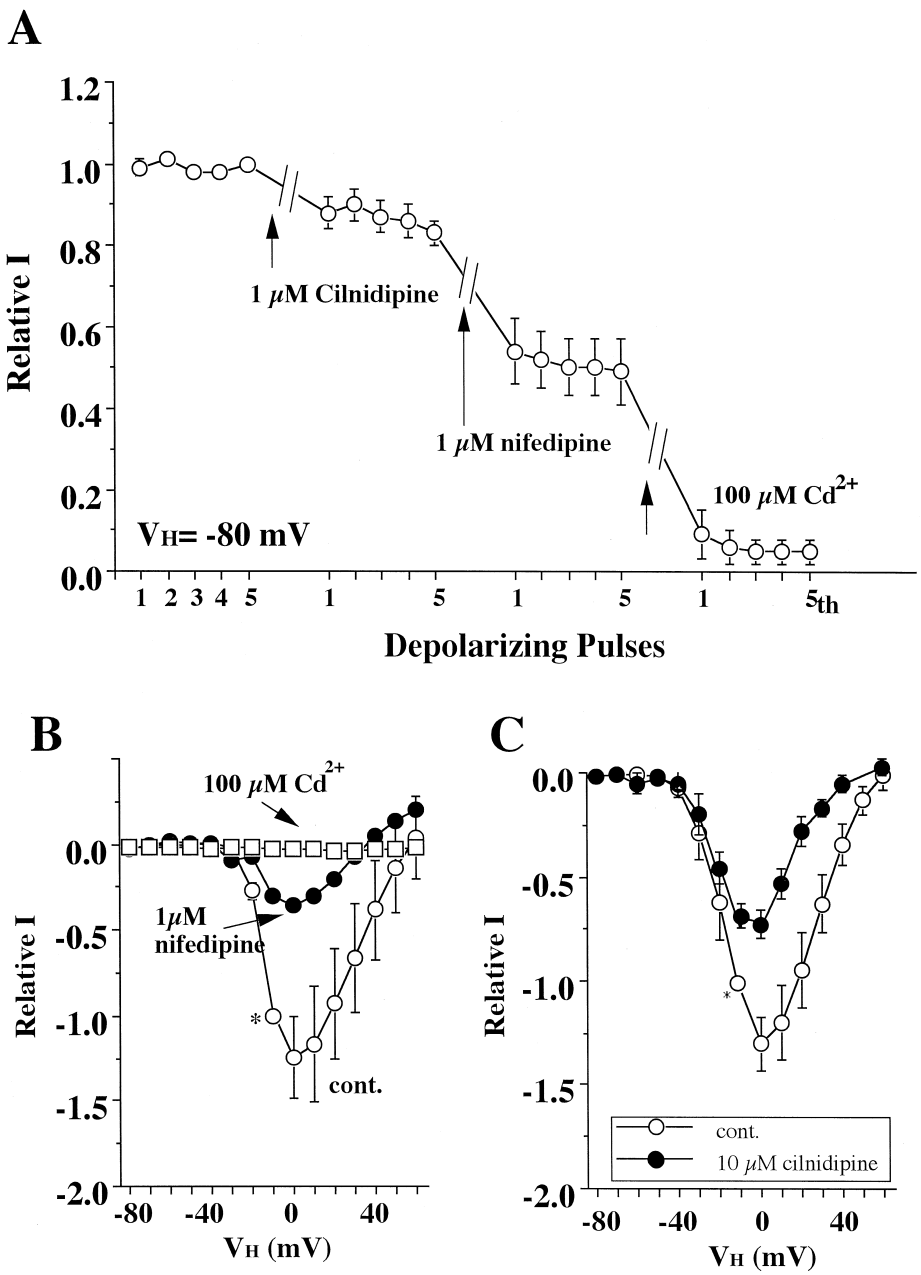

Fig. 1. Effects of cilnidipine on the cardiac $I_{Ca,L}$. (A) Effects of cilnidipine and nifedipine. Myocytes were depolarized for 200 ms from the V_h of −80 mV to various potentials ranging from −70 mV to +60 mV every 10 s. Each drug was applied to the neuron 2 min before the subsequent five depolarizing pulses were applied. (B and C) Effects of nifedipine and cilnidipine on *I–V* curve of $I_{Ca,L}$. $I_{Ca,L}$ was elicited by a 200-ms depolarizing pulse from −80 mV to various potentials every 10 s. The amplitude of $I_{Ca,L}$ was measured at the peak. All responses were normalized to the peak current amplitude at −20 mV (*) in the absence of each drug. $I_{Ca,L}$ was completely blocked by an inorganic ion, Cd^{2+}. Each point and vertical bar represents the mean ± S.E.M. from four different experiments.

was initiated once every 10 s. As shown in Fig. 3B, 1 μM cilnidipine elicited a strong hyperpolarizing shift (about 17 mV) of the steady-state inactivation curve. In the absence of cilnidipine, the steady-state inactivation curve was described by a V_h of -30.4 ± 0.8 mV and a slope factor (k) of 3.1 ± 0.3 mV/e-fold change ($n = 4$). In the presence of cilnidipine, it was described by a V_h of -47.2 ± 0.5 mV and k of 5.9 ± 0.3 mV ($n = 4$). In contrast, 0.3 μM nifedipine shifted left the steady-state inactivation curve to about 11 mV. The values of V_h (and k) in the presence and absence of nifedipine were -28.6 ± 5.0 mV (3.8 ± 0.4) and -40.3 ± 4.3 mV (5.2 ± 0.8), respectively (each $n = 3$).

3.3. Blocking activity of dihydropyridines on rat cardiac L-type Ca^{2+} channel currents

Fig. 4 (left) summarizes the blocking potency of each dihydropyridine at concentrations of 1 and 10 μM. At

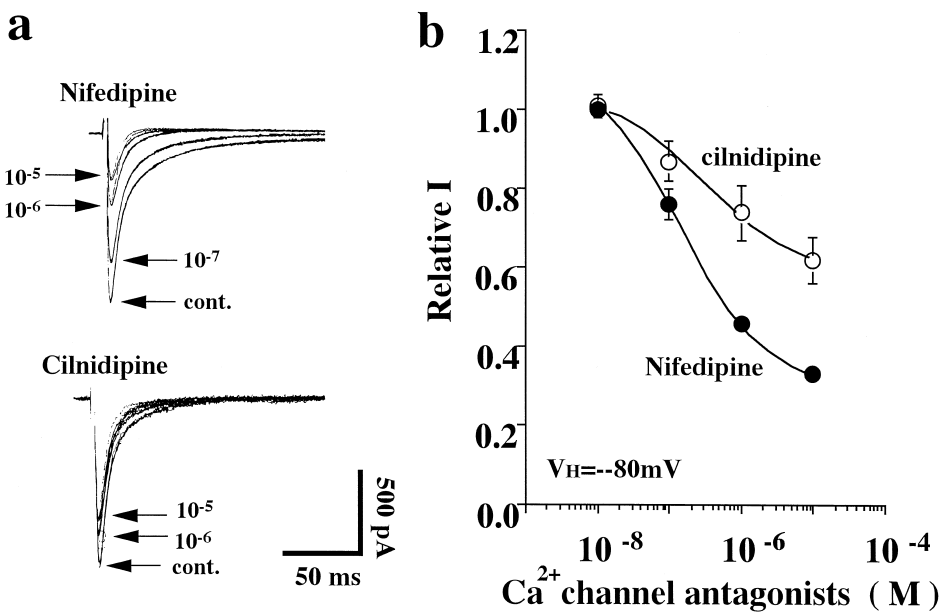

Fig. 2. Concentration-dependent effects of cilnidipine and nifedipine on cardiac $I_{Ca,L}$. (A) Typical current traces of $I_{Ca,L}$ in the presence and absence of nifedipine or cilnidipine. V_h was −80 mV. (B) Concentration–inhibition curves of cilnidipine and nifedipine for $I_{Ca,L}$. Each point and vertical bar represents the mean ± S.E.M. from four to six different experiments.

these concentrations, all dihydropyridine compounds showed strong inhibitory potencies for $I_{Ca,L}$, with the exception of cilnidipine and amlodipine. The IC_{50} value (in μM) for each dihydropyridine was as follows: nimodipine (0.68 ± 0.14; $n = 4$), nitrendipine (1.33 ± 0.07; $n = 5$), nisoldipine (0.09 ± 0.01; $n = 5$), nilvadipine (1.44 ± 0.78; $n = 5$), manidipine (0.39 ± 0.07; $n = 4$), benidipine (0.40 ± 0.17; $n = 4$), amlodipine (7.80 ± 2.03 $n = 4$) and nicardipine (1.92 ± 1.35; $n = 4$).

3.4. Blocking activities of dihydropyridines on rat sympathetic N-type Ca^n channel currents

In previous reports, we showed that cilnidipine also had potent blocking activity on N-type Ca^{2+} channel currents

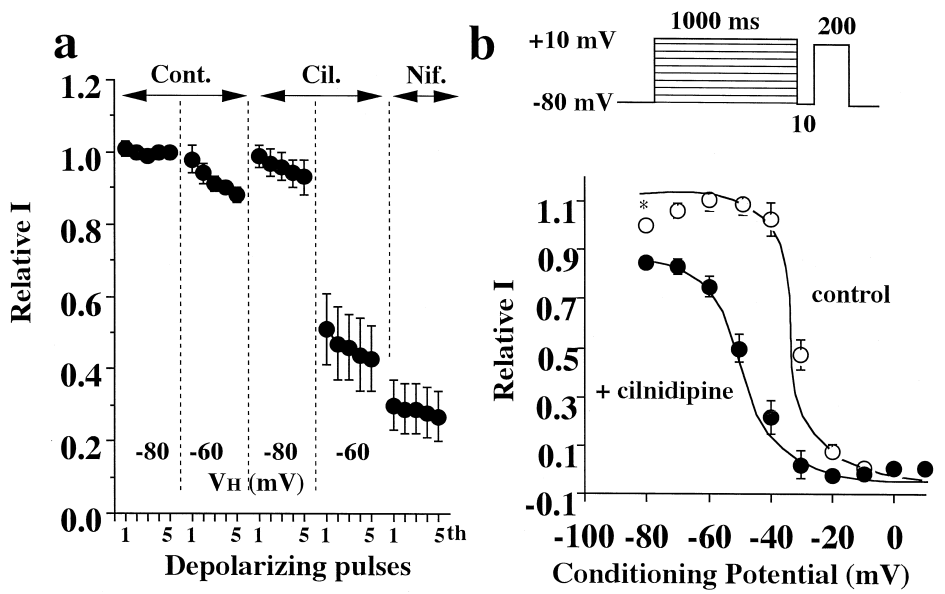

Fig. 3. Voltage-dependent effects of cilnidipine on the cardiac $I_{Ca,L}$. (A) Effects of V_h change. $I_{Ca,L}$ was induced at two V_h's of −80 mV and −60 mV in the presence and absence of 1 μM cilnidipine. All responses were normalized to the peak current amplitude at the fifth control pulse (*). Cont.; Control, Cil.; 1 μM Cilnidipine, Nif; 1 μM nifedipine. (B) Steady-state inactivation curves were obtained using a gapped double-pulse protocol before and after a 4-min exposure to 1 μM cilnidipine. Myocytes were given a 1-s prepulse to potentials between −80 and +10 mV, followed by a 10-ms return to the V_h of −80 mV and then a fixed 200-ms test pulse to +10 mV. The pulse protocol was initiated once every 10 s. All responses were normalized to the peak current amplitude at a conditioning potential of −80 mV (*) in the absence of cilnidipine. Each point and vertical bar represents the mean and ±S.E.M. from four different experiments.

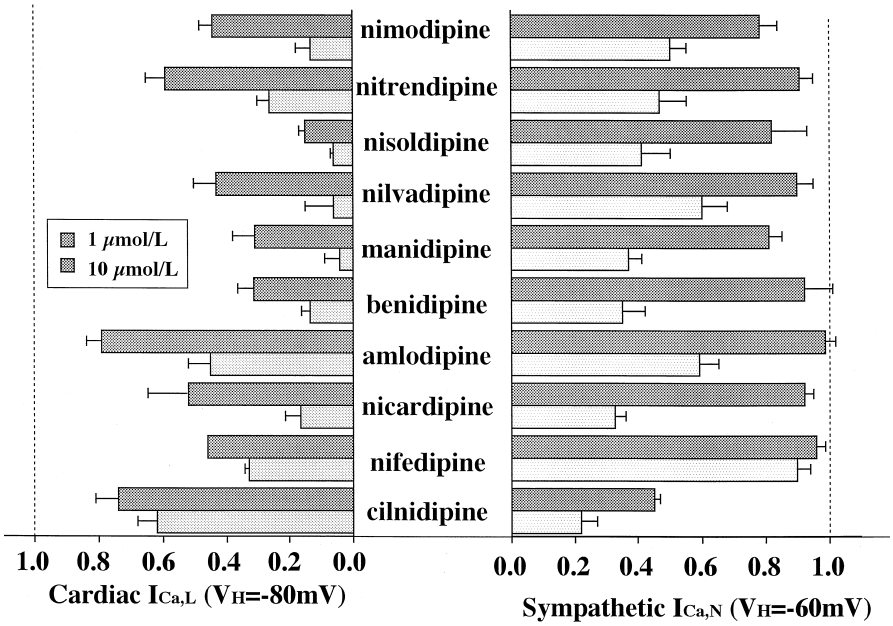

Fig. 4. Effects of clinically available dihydropyridines on cardiac $I_{Ca,L}$ and sympathetic $I_{Ca,N}$. Cardiac $I_{Ca,L}$ was evoked by 200-ms depolarizing pulses at a V_h of −80 mV to 0 mV, and sympathetic $I_{Ca,N}$ was induced by 50-ms depolarizing pulses at −60 mV to 0 mV. Each concentration (0.1 to 10 μM) of Ca^{2+} antagonists was cumulatively applied to cells at 3 min interval. Each column and horizontal bar represents the mean ± S.E.M. of the data obtained with each drug at concentrations of 1 and 10 μM from four to six different experiments.

(Uneyama et al., 1997, 1998). In the present experiment, we evaluated the blocking effects of the above mentioned compounds on N-type Ca^{2+} channel currents ($I_{Ca,N}$), using rat superior cervical ganglion neurons. For measuring sympathetic Ca^{2+} channel currents, we used 10 mM Ba^{2+} instead of Ca^{2+} to prevent Ca^{2+}-dependent run-down phenomena of $I_{Ca,N}$ by repetitive stimulation. To isolate $I_{Ca,N}$ carried through high-voltage activated Ca^{2+} channels, the dissociated superior cervical ganglion neuron was held at a holding potential (V_h) of −60 mV. At this potential, the low-voltage activated T-type Ca^{2+} channel is completely inactivated (Ishibashi et al., 1995). $I_{Ca,N}$ was elicited in a reproducible manner by 50-ms depolarizing pulse from −60 mV to 0 mV every 10 s.

Fig. 4 (right) summarizes the blocking potency of each dihydropyridine at concentrations of 1 and 10 μM. All of the dihydropyridines, except cilnidipine, showed a small inhibitory effect on $I_{Ca,N}$ at 1 μM, but at 10 μM, all blocked $I_{Ca,N}$. The IC_{50} value for each dihydropyridine other than cilnidipine and nifedipine was as follows: nimodipine (8.3 ± 3.4; $n = 4$), nitrendipine (13.0 ± 3.44; $n = 4$), nisoldipine (8.04 ± 4.1; $n = 5$), nilvadipine (13.1 ± 3.74; $n = 4$), manidipine (7.10 ± 1.79; $n = 5$), benidipine (7.96 ± 3.85; $n = 4$), amlodipine (18.1 ± 5.99; $n = 4$), and nicardipine (5.60 ± 0.67; $n = 4$). The values for cilnidipine and nifedipine were reported previously (0.8 μM for cilnidipine and 131 μM for nifedipine). Thus, it was revealed that, at high concentrations (more than 10 μM), all dihydropyridines possess sympathetic N-type Ca^{2+} channel-blocking actions.

We plotted IC_{50} values of each dihydropyridine for $I_{Ca,L}$ and $I_{Ca,N}$ in Fig. 5. Among these dihydropyridines, the selectivity of each drug for $I_{Ca,L}$ and $I_{Ca,N}$ was markedly different. The ratio for $I_{Ca,N}/I_{Ca,L}$ of each dihydropyridine was about 0.082 (nimodipine), 0.10 (nitrendipine), 0.01 (nisoldipine), 0.01 (nilvadipine), 0.055 (manidipine), 0.050 (benidipine), 0.43 (amlodipine), 0.34 (nicardipine), 0.008 (nifedipine) and 21 (cilnidipine). Cilnidipine has a preference for sympathetic N-type Ca^{2+}

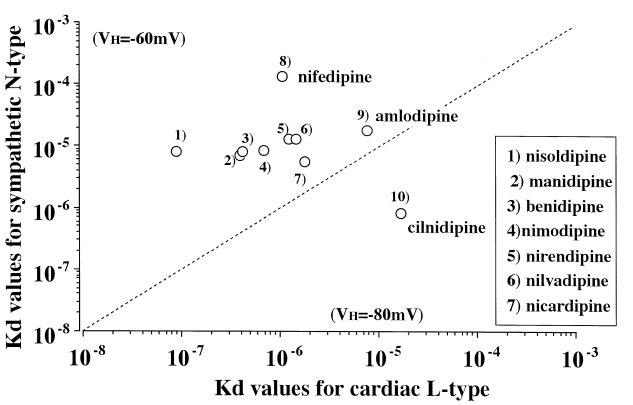

Fig. 5. Ratio of IC_{50} value of each dihydropyridine for cardiac $I_{Ca,L}$ and sympathetic $I_{Ca,N}$. IC_{50} values of each dihydropyridine for $I_{Ca,L}$ and $I_{Ca,N}$ were calculated by least-squares fitting (see Section 2) and plotted. Each point represents mean of four to six different experiments. The values of cilnidipine and nifedipine for $I_{Ca,N}$ were quoted from the previous report (1).

channels, and nifedipine has a preference for cardiac L-type Ca^{2+} channels under an experimental conditions.

4. Discussion

At a holding potential of −80 mV, cilnidipine had little inhibitory effect below concentrations of 1 μM on $I_{Ca,L}$ (IC_{50} value; 17 μM) when compared with nifedipine (IC_{50} value; 1 μM). Cilnidipine, as well as nifedipine, inhibited $I_{Ca,L}$ in a concentration-dependent manner without changing the current–voltage relationship. 1 μM cilnidipine shifted the steady-state inactivation curve drastically to negative potentials more than what 0.1 μM nifedipine did (Fig. 3B). Thus, the features of the blocking action of cilnidipine might be the same as those of dihydropyridines such as nifedipine. Thus, despite of having a higher high-affinity for inactivated channels to the resting channels, the features of the blocking action of cilnidipine might be the same as those of the classical dihydropyridine. In keeping with this strong shift of the steady-state inactivation curve, 1 μM cilnidipine caused subtle changes in $I_{Ca,L}$ when cells were voltage-clamped at a V_h of −80 mV. However, with a V_h of −60 mV, the same concentration of cilnidipine produced a marked reduction of $I_{Ca,L}$ (Fig. 3A). These results also suggest the following two possibilities: (1) Cilnidipine does not depress cardiac performance in normally polarized cardiac myocytes but if cardiac myocytes are depolarized by treatment with cardiac glycoside or ischemia, cilnidipine possibly depresses cardiac performance by decreasing oxygen demand. This might be useful for the treatment of an angina, because it is thought that part of the myocardium becomes ischemic and depolarized during an angina episode. (2) As reported for amlodipine (Kass and Arena, 1991) and felodipine (Sun and Triggle, 1995), cilnidipine has potent selectivity for L-type Ca^{2+} channels in cardiovascular smooth muscle cells when compared with those in cardiac myocytes.

In previous reports, we showed that cilnidipine had a potent blocking action on $I_{Ca,N}$ in rat sympathetic neurons (Uneyama et al., 1997) and differentiated rat PC12 cells (Uneyama et al., 1998). Recent reports reveal that some dihydropyridines such as amlodipine also block neuronal N-type Ca^{2+} channels (Furukawa et al., 1997). Therefore, in addition to cilnidipine and nifedipine, we estimated the blocking effects of seven clinically available dihydropyridines on $I_{Ca,N}$ in superior cervical ganglion neurons. Interestingly, in high concentrations of higher than several micromolar, all dihydropyridines clearly blocked $I_{Ca,N}$. However, it was cilnidipine alone that had obvious blocking potency for $I_{Ca,N}$ even at concentrations below 1 μM (Fig. 4).

The inhibitory effects of cilnidipine and amlodipine on the cardiac $I_{Ca,L}$ were markedly different from those of the other dihydropyridines at a holding potentials of −80 mV. The weak block by amlodipine under a negative V_h was also reported by Kass and Arena (1989). The blocking potency of cilnidipine for vascular L-type Ca^{2+} channels has been reported to be almost equal to that of other dihydropyridines (Oike et al., 1990). Now it was recognized that the strong voltage-dependent block might explain their selectivity for L-type channels of vascular tissues, because the resting membrane potential of vascular smooth muscle is somewhat higher (around −40 mV) (Wei et al., 1988). In our experiments, cilnidipine and amlodipine at 1 μM blocked $I_{Ca,L}$ by 60% when preclamped at a V_h of −60 mV (data not shown). Cilnidipine and amlodipine resemble each other in their strong vascular selectivity and weak negative inotropic effects, compared with other dihydropyridines.

The reflex increase in sympathetic activity induced by dihydropyridines may underlie their adverse actions besides the observed proischemic, negative inotropic, and arrythmogenic effects of these compounds. In particular, a short-acting dihydropyridine, nifedipine in moderate to high doses causes an increase in total mortality in patients with coronary disease (Furberg et al., 1995). Muller et al. (1989) hypothesized that the circadian variation in the onset of acute cardiovascular events is associated with alterations in sympathetic activity that may lead to plaque rupture. Therefore, to prevent hyperactivity of sympathetic nerve tonus evoked by a rapid reduction of blood pressure, slow-acting and long-lasting (second generation) dihydropyridines have been developed. In keeping with this strategy, a reduction in the risk of mortality in trials using the second-generation dihydropyridines is now being reported for amlodipine (Packer et al., 1996).

N-type Ca^{2+} channels play a key role in the regulation of sympathetic nerve activity. Activation of N-type Ca^{2+} channel leads to the release of catecholamines from sympathetic nerve endings (Hirning et al., 1988), and a peptide antagonist selective for N-type Ca^{2+} channel, ω-conotoxin-GVIA, blocks norepinephrine release from vessels (Nakashima et al., 1991). Thus, in addition to modifying the pharmacokinetics of dihydropyridines, the baroreflex evoked by dihydropyridines can be prevented by increasing affinity for N-type Ca^{2+} channels as well as L-type channels. We have already some preclinical experimental data to confirm this theoretical possibility. In spontaneously hypertensive rats, cilnidipine reduced blood pressure without elevating heart rate and plasma norepinephrine concentrations (Hosono et al., 1995a). In this model, other dihydropyridines (amlodipine, nifedipine, manidipine, nicardipine, benidipine) induced baroreflex tachycardia combined with plasma norepinephrine elevation. Furthermore, cilnidipine had a therapeutic effect even in the mental stress-induced hypertension model (cold stress model) whereas other dihydropyridines were ineffective (Hosono et al., 1995b). Thus, it is suggested that dual N- and L-type Ca^{2+} channel antagonists such as cilnidipine, may be antihypertensive Ca^{2+} channel antagonists to mini-

mize the unfavorable effects of antihypertensive Ca^{2+} channel antagonists.

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