Cardiovascular Drug Reviews
Vol. 17, No. 4, pp. 341–357

Cilnidipine: Preclinical Profile and Clinical Evaluation

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Key Words: Antihypertensive Ca^{2+} channel antagonists—Cilnidipine—Dihydropyridine

INTRODUCTION

Cilnidipine (FRC-8653) is a dihydropyridine (DHP) type of calcium channel antagonist originally developed in Japan. Unlike other calcium channel antagonists, cilnidipine blocks the influx of Ca^{2+} ions into both vascular smooth muscle at the level of L-type Ca^{2+} channels and neuronal cells at the level of N-type Ca^{2+} channels. The L-type Ca^{2+} channel blockade by cilnidipine affects predominantly vascular smooth muscle, thereby producing vasodilation of peripheral resistance vessels and coronary arteries. The blockade of N-type Ca^{2+} channels affects predominantly peripheral nerve endings of sympathetic neurons, thereby dilating blood vessels by lowering plasma catecholamine levels. Cilnidipine produced greater reductions in blood pressure in patients with hypertension than in healthy volunteers. Although increases in heart rate were noted in studies with conventional L-type selective DHPs, the changes in heart rate with cilnidipine were negligible, even in patients with rapid blood pressure reduction. Thus, it appears that the hypotension-induced baroreflex sympathetic stimulation is attenuated by this inhibitory action on N-type Ca^{2+} channels and that even greater blood pressure-lowering effects are achieved with cilnidipine. Furthermore, cilnidipine antagonized the increase in blood pressure in response to acute cold stress, which is not usually depressed by L-type Ca^{2+} channel antagonists. Direct negative inotropic effects were not, however, detected in hypertensive patients who received cilnidipine.

Recent developments of antihypertensive Ca^{2+} channel antagonists have focused on achieving two desirable goals: 1) tissue selectivity among L-type Ca^{2+} channels to reduce the likelihood of undesirable side effects, and 2) a gradual onset and long duration of action to improve compliance and also to reduce sympathetic stimulation due to hypotensive baroreflexes. However, complete management of this sympathetic stimulation

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seems to be very difficult because of widespread tissue distribution of L-type Ca^{2+} channels. As a result of our experience with cilnidipine, we now believe that it is possible to manage these sympathetic problems by modulating N-type Ca^{2+} channels as well. We suggest that cilnidipine, a dual antagonist for both L-type and N-type Ca^{2+} channels, is potentially useful as a prototype for the next generation of antihypertensive therapeutic agents.

CHEMISTRY

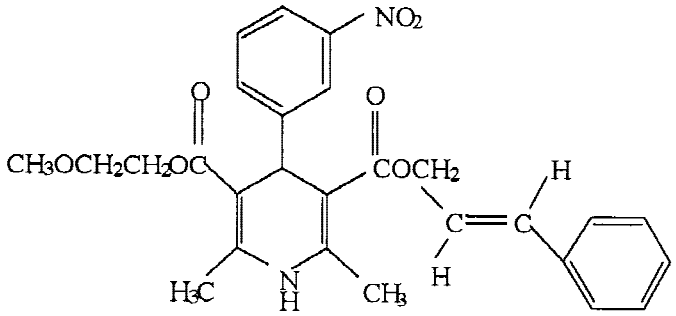
Cilnidipine (FRC-8653) was originally synthesized in the laboratories of Fuji & Rebio Pharmaceutical Co., Ltd. (Hino, Japan). The chemical name is 2-methoxyethyl (E)-3-phenyl-2-propenyl (±)-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate. The structure is shown in Fig. 1. Cilnidipine is the first dihydropyridine with an antagonistic action on both N-type and L-type Ca^{2+} channels.

PRECLINICAL PHARMACOLOGY

In Vitro Pharmacology

Ca^{2+} Channel Antagonistic Effects

Receptor-binding studies on different membrane preparations, using [^{3}H]nitrendipine (a specific ligand for L-type Ca^{2+} channels) or ω-[^{3}H]conotoxin GVIA (a specific ligand for N-type Ca^{2+} channels), showed that cilnidipine was as potent as nifedipine in binding to L-type Ca^{2+} channels. The IC_{50} values of cilnidipine, nifedipine, and nicardipine were about 2, 1, and 0.4 nmol, respectively. For N-type Ca^{2+} channels, however, nicardipine failed to displace ω-[^{3}H]conotoxin binding at concentrations up to 10 μM, while cilnidipine showed a partial affinity for these channels, displacing ω-[^{3}H]conotoxin by only 25% at concentrations up to 10 μM (Fig. 2). Evaluation of the functional effects of



Chemical name: 2-Methoxyethyl (E)-3-phenyl-2-propen-1-yl (±)-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyrimidine-3,5-dicarboxylate
Molecular Formula: $C_{27}H_{28}N_2O_7$
Molecular Weight: 492.53
Patent Status: US 4,672,068
EP 161,877
JP 602333058

FIG. 1. Chemical structure of cilnidipine (FRC-8653).

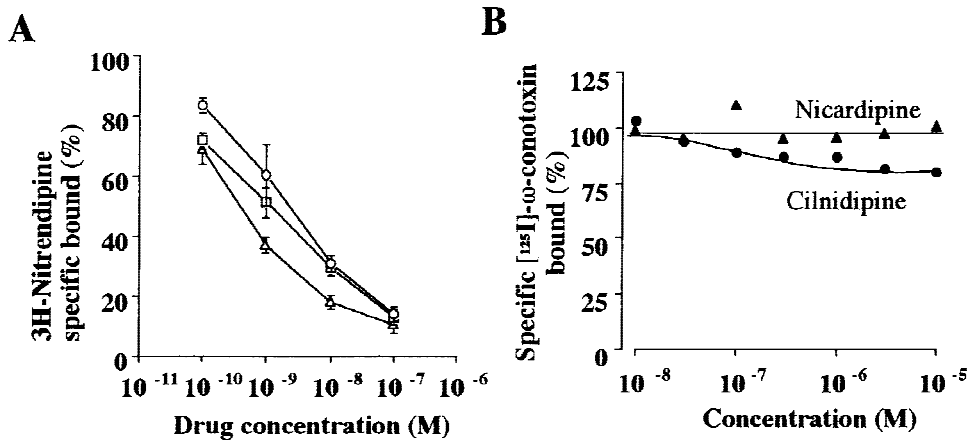


FIG. 2. Inhibitory actions of cilnidipine on nitrendipine- and ω-conotoxin-binding sites. *A*, effects of cilnidipine (○), nifedipine (□), and nicardipine (△) on specific [^{3}H]nitrendipine binding to the membrane preparations of rat heart. Each point represents the mean ± S.E. of four experiments. *B*, effects of cilnidipine (●) and nicardipine (▲) on specific ^{125}I-labeled ω- conotoxin GVIA binding to rat brain synaptosomes. Each point represents a mean of four to five separate experiments performed in duplicate. Data are from refs. 6 and 8.

cilnidipine on isolated Ca^{2+} channel currents using the conventional patch-clamp technique showed that cilnidipine also potently blocks vascular L-type Ca^{2+} channels (19) and sympathetic neuronal N-type Ca^{2+} channels (24), but that cilnidipine is only a weak inhibitor of cardiac L-type Ca^{2+} channel currents (25) (Fig. 3). The pIC_{50} values of cilnidipine were 6.0 for L-type Ca^{2+} channels of rabbit basilar artery, 6.1 for N-type Ca^{2+}

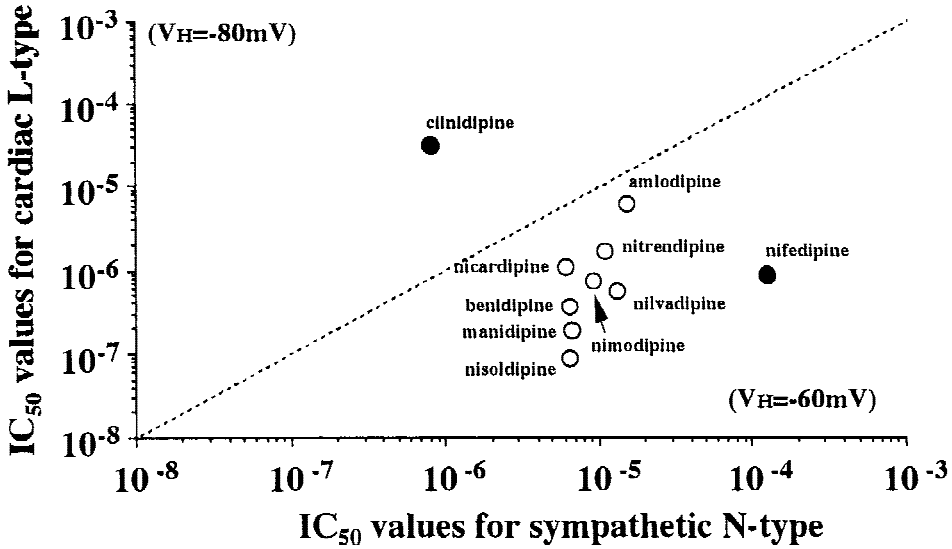


FIG. 3. IC_{50} value of each dihydropyridine (DHP) for cardiac L-type and sympathetic N-type Ca^{2+} channel currents. The blocking actions of 10 clinically available DHPs on cardiac L-type and sympathetic N-type Ca^{2+} channel currents were examined by the use of the conventional whole-cell patch-clamp technique. Each point represents a mean of four to six separate experiments. Data are from ref. 25.

channel currents of rat superior ganglion neuron, and 4.8 for L-type Ca^{2+} currents of rat ventricular myocyte (Table 1). Detailed pharmacological studies on neuronal N-type Ca^{2+} channels have also been performed with the voltage-clamping technique using rat dorsal root ganglion cells (1) and nerve growth factor (NGF)-differentiated pheochromocytoma PC12 cells (26). In Fig. 3, we summarize cilnidipine's blocking potency for cardiac L-type and sympathetic ganglionic N-type Ca^{2+} currents, compared with other clinically available DHPs, all assayed under the same experimental conditions. These results clearly demonstrate that cilnidipine is the only DHP having potent sympathetic N-type Ca^{2+} channel blockade at doses without significant cardiac L-type blockade. The relative selectivity of cilnidipine for sympathetic N-type Ca^{2+} channels over cardiac L-type ones is at least 10 times greater than that of other Ca^{2+} channel antagonists. Furthermore, cilnidipine preferentially inhibits vascular as compared with cardiac L-type Ca^{2+} channels.

As observed with conventional DHPs, cilnidipine showed a marked voltage-dependent block of vascular L-type Ca^{2+} channel currents. The IC_{50} value of cilnidipine was, for instance, shifted from 1.0 μM ($V_H = -80$ mV) to 1.0 nM ($V_H = -40$ mV) (Fig. 4). The midpoint of the voltage-dependent steady-state inactivation (Vh) was shifted by 21 mV to more hyperpolarizing potentials in the presence of 0.1 μM for the arterial L-type Ca^{2+} channels (19). For the L-type currents in cardiac myocytes, the hyperpolarizing shift of Vh was somewhat less, being only 17 mV in the presence of 1 μM cilnidipine (25). Thus, this Vh change might be related to the potent selectivity of cilnidipine for vascular L-type channels. In contrast to L-type currents, the block by cilnidipine of the neuronal N-type Ca^{2+} channel currents did not show this voltage dependence. This finding was confirmed by Fuji et al. (1) using rat DRG neurons. They estimated that the Vh values in the presence and absence of 0.5 μM cilnidipine were 44 mV and 45 mV, respectively.

The functional effects of cilnidipine blockade of N-type Ca^{2+} channels were investigated in *in vitro* experiments using sympathetic neuron-like PC12 cells (26). These cells are derived from a rat pheochromocytoma cell line and are very popular for investigating neuronal differentiation. In response to externally applied NGF, these PC12 cells acquire the characteristics of sympathetic neurons such as neurite extension, increased catecholamine synthesis, and expression of neuronal types of voltage-dependent N-type Ca^{2+} channels (3). With these model cells, we examined the effects of cilnidipine on high K^+-evoked catecholamine secretion, which is closely linked to the intracellular Ca^{2+} concentration. Cilnidipine potently depressed dopamine release in both NGF-treated and -untreated PC12 cells, whereas inhibition of release by nifedipine was markedly decreased in the differentiated PC12 cells (Fig. 5*A*). Increases in the intracellular Ca^{2+} concentration

TABLE 1. *Inhibitory potency of cilnidipine for various Ca^{2+} channel currents*[a]

	DHPs[b]	Ca^{2+} channel current pIC_{50}	Ref.
Rabbit basilar arterial cells	Cilnidipine	6.01	19
Rat ventricular myocytes	Cilnidipine	4.77	24
	Nifedipine	5.97	24
Rat sympathetic neurons	Cilnidipine	6.08	25
	Nifedipine	3.90	25

[a] Ca^{2+} channel currents were monitored with the conventional whole-cell patch-clamp technique. Ca^{2+} channel current was evoked by 50- to 200-ms depolarizing pulses from −80 mV to 0 mV.
[b] Abbreviation: DHP, dihydropyridine.

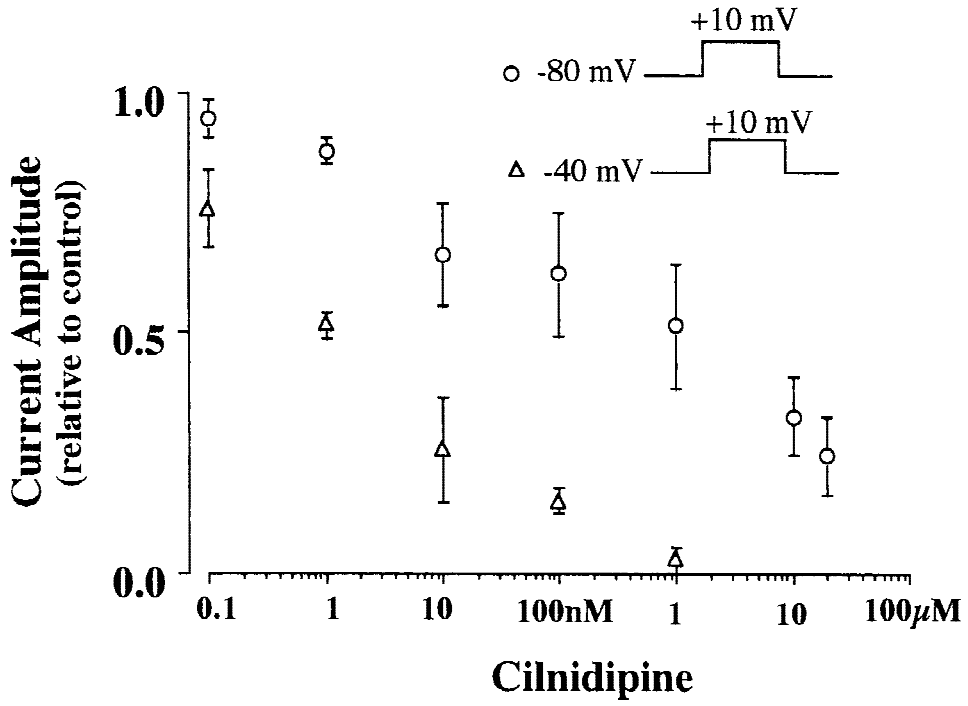


FIG. 4. Resting membrane potential-dependent effects of cilnidipine on vascular L-type Ca^{2+} channel currents. Single smooth muscle cells were isolated from rabbit basilar arteries, and L-type Ca^{2+} channel currents were monitored under voltage-clamped conditions. The Ca^{2+} channel current was induced at two V_H of −80 mV and −40 mV in the presence and absence of each concentration of cilnidipine. Data are from ref. 19.

($[Ca^{2+}]_i$), measured using Fura-2, were also resistant to nifedipine. The remaining component of $[Ca^{2+}]_i$ increase was blocked by cilnidipine as well as by ω-conotoxin-GVIA (Fig. 5*B*), implicating N-type channels in catecholamine secretion. Nakashima et al. (17) also reported that cilnidipine, at submicromolar concentrations, inhibited electrical stimulation-evoked [^{3}H]norepinephrine (NE) release in the rabbit mesenteric artery.

In Vivo Pharmacology

Antihypertensive Effect

Intravenous administration of cilnidipine, nifedipine, or nicardipine dose dependently decreased the mean blood pressure in conscious and catheterized spontaneously hypertensive rats (SHRs) (27, 29). The doses causing a reduction in mean blood pressure of about 25 mmHg were 10 μg/kg for cilnidipine and nicardipine and 30 μg/kg for nifedipine. The doses causing a 45-mmHg reduction in mean blood pressure were 30 μg/kg for cilnidipine and nicardipine and 100 μg/kg for nifedipine (Table 2). These results showed that the decrease in mean blood pressure produced by intravenous administration of cilnidipine was equal to that caused by nicardipine and greater than that produced by nifedipine.

By oral administration, cilnidipine (1 to 10 mg/kg) dose dependently reduced systolic

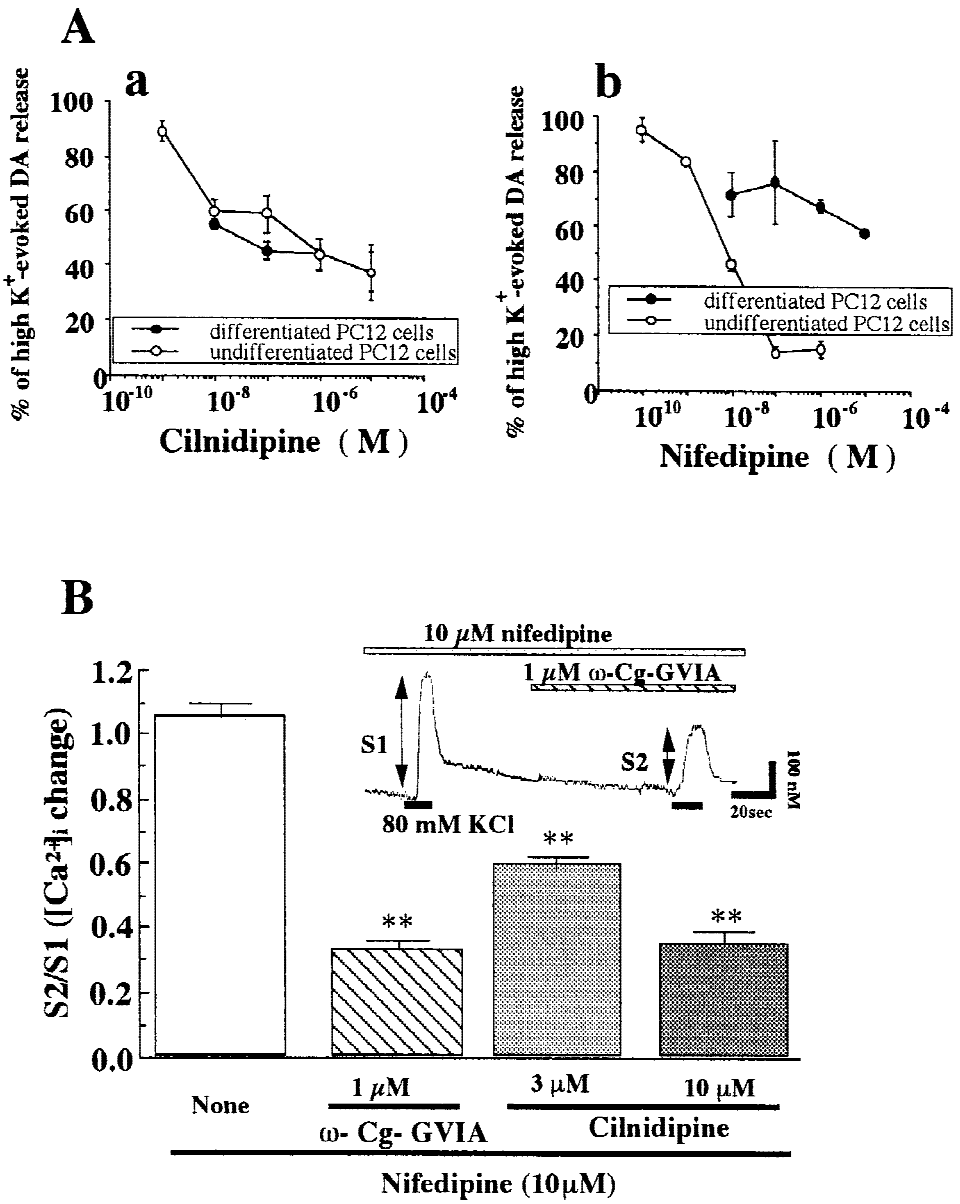


FIG. 5. Cilnidipine blocks catecholamine secretion from nerve growth factor (NGF)-differentiated PC12 cells. *A*, effects of cilnidipine (*a*) and nifedipine (*b*) on the high K^+-evoked dopamine (DA) release from undifferentiated (○) and differentiated (●) rat PC12 cells. Cells were preincubated with various concentrations of the Ca^{2+} channel antagonists for 4 min. After preincubation, the cells were stimulated with 80 mM KCl for 1 min in the continued presence of the drugs. The amount of dopamine in the supernatants and cell lysates was measured with an HPLC/ECD system. Data are shown as percentages of the amount of DA released by high K^+. Each point and its vertical bar represent the mean ± S.E.M., respectively, from three distinct determinations. *B*, effects of cilnidipine on the nifedipine-resistant $[Ca^{2+}]_i$ elevation in differentiated rat PC12 cells. Cells were preloaded with the Ca^{2+} indicator, Fura-2, and changes in $[Ca^{2+}]_i$ were measured fluorometically. Each point and its vertical bar represent the mean ± S.E.M. from three experiments. $^{**}P < 0.01$ vs. the nifedipine-treated (control) group (Dunnett's test for multiple comparisons). Data are from ref. 26.

TABLE 2. *Effects of intravenous administration of cilnidipine, nifedipine, and nicardipine on the mean blood pressure in conscious, spontaneously hypertensive rats*[a]

Drugs	Dose (μg/kg)	Blood pressure (mm Hg)		Peak time (min)[b]	Duration 50 (min)[c]
		Initial	Maximal decrease		
Cilnidipine	10	151.2 ± 3.8	−24.5 ± 3.2	1.1 ± 0.3	7.2 ± 1.0
	30		−43.5 ± 2.1	1.7 ± 0.1	20.4 ± 4.1
Nifedipine	30	150.5 ± 4.3	−26.3 ± 1.5	0.6 ± 0.1	5.4 ± 0.7
	100		−44.3 ± 1.7	0.6 ± 0.1	7.5 ± 0.9
Nicardipine	10	145.2 ± 4.0	−23.0 ± 2.1	0.6 ± 0.1	2.5 ± 0.5
	30		−51.5 ± 3.2	0.8 ± 0.1	6.8 ± 1.4

[a] Each value represents the mean ± S.E. of six experiments.
[b] Time required to achieve the maximal decrease of blood pressure.
[c] Time required for recovery to half of the maximal decrease.

blood pressure in conscious SHRs, renal hypertensive rats (RHRs), and DOCA-salt hypertensive rats (DHRs) (9). In each hypertensive model, the maximal reduction in systolic blood pressure was observed 3 h after administration of the drug. In normotensive rats (NTRs), oral administration of cilnidipine, even at a high dose of 100 mg/kg, produced only a mild dose-dependent decrease in systolic blood pressure. The ED_{20} values of cilnidipine in SHRs, RHRs, DHRs, and NTRs are listed in Table 3.

In anesthetized dogs, intravenous administration of cilnidipine produced a dose-dependent decrease in mean arterial blood pressure and a slight increase in heart rate. A marked tachycardia, which was observed after administration of either nifedipine or nicardipine, was not obseved even at high doses of cilnidipine. The ED_{40} values (doses causing a 40% reduction in mean blood pressure) for cilnidipine, nifedipine, and nicardipine were 24.3, 20.1, and 23.3 μg/kg, respectively. These results indicate that in dogs, cilnidipine had almost the same hypotensive activity as did nifedipine or nicardipine.

The antihypertensive activity of cilnidipine in rats and dogs is characterized by a particular slow-onset and long-lasting time course of action. In conscious SHRs, the effect of cilnidipine at 30 μg/kg reached a maximum at 1.7 min after intravenous administration, whereas the peak effects of nifedipine (100 μg/kg) and nicardipine (30 μg/kg), at doses causing a similar maximal decrease, were observed at 0.6 and 0.8 min after intravenous administration, respectively. The half-life of the hypotensive effect was 20.4 min for cilnidipine (30 μg/kg, i.v.) and 6 to 8 min for nifedipine (100 μg/kg, i.v.) and nicardipine

TABLE 3. *Comparison of the antihypertensive effects of orally administered cilnidipine, nifedipine, or nicardipine in normotensive and hypertensive rats*[a]

Compounds	ED_{20}[b] (mg/kg, p.o.)			
	Normotensive rat	Spontaneously hypertensive rat	Renal hypertensive rat	DOCA-salt hypertensive rat
Cilnidipine	9.4 (6.3–13.8)[c]	2.3 (1.7–3.5)	1.9 (1.0–2.9)	2.5 (1.6–3.6)
Nifedipine	7.7 (5.8–10.3)	2.0 (1.5–2.5)	1.4 (1.0–1.9)	2.2 (1.8–2.7)
Nicardipine	5.7 (4.6–6.7)	2.0 (1.5–2.5)	1.6 (1.3–2.0)	1.5 (1.2–1.8)

[a] Values are calculated from data from 10 to 12 experiments.
[b] Abbreviation: ED_{20}, dose causing a reduction of systolic blood pressure by 20%.
[c] Numbers in parentheses, 95% confidence interval.

(30 μg/kg, i.v.) (27). In anesthetized dogs, similar hypotensive time courses were obtained after intravenous administration of cilnidipine, nifedipine, or nicardipine. The effects of 30 μg/kg (approximately the ED_{40} values) of the three drugs reached their peak at 1.8, 0.7, and 0.9 min, respectively. The onset of cilnidipine action was about twice as slow as that of nifedipine or nicardipine, while the duration of its hypotensive effect was twice as long as that of the other two drugs. Thus, it appears that cilnidipine has a slow onset and long-lasting action *in vivo*.

Sympatho-Inhibitory Activity

In conscious and free-moving SHRs, the effects of cilnidipine on the mean blood pressure, heart rate, and plasma norepinephrine concentration were investigated and compared with other DHP calcium antagonists (5). After oral administration of the short-acting DHPs, nifedipine and nicardipine (3 mg/kg), rapid decreases and increases in the heart rate and plasma norepinephrine concentrations were observed. The same dose (3 mg/kg, p.o.) of long-lasting DHP calcium antagonists (manidipine, benidipine, and amlodipine) caused a slow onset and long-lasting hypotension, but at the same time, these calcium antagonists significantly increased the heart rate and plasma norepinephrine concentrations. On the contrary, cilnidipine (3 mg/kg, p.o.), in spite of causing a similar decrease in mean blood pressure as the above DHP calcium antagonists, had no significant

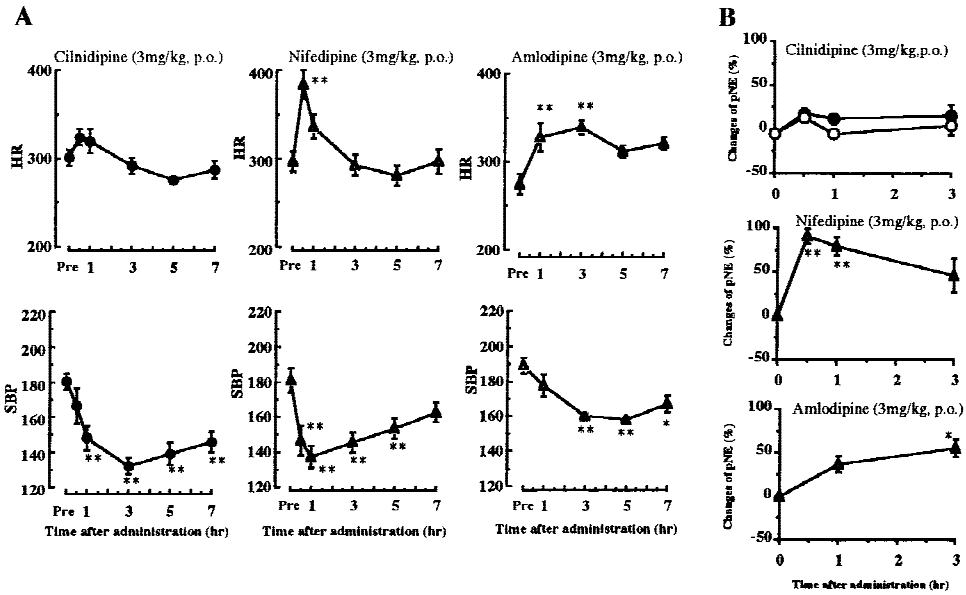


FIG. 6. Changes in heart rate (HR) and plasma norepinephrine (pNE) levels in response to a single oral dose of cilnidipine, nifedipine, or amlodipine. *A*, systolic blood pressure (SBP) and heart rate were measured telemetrically in conscious spontaneously hypertensive rats (SHRs) before and after administration of each drug. Separate from this procedure, plasma norepinephrine levels were measured before and after administration of each drug in SHRs fitted with indwelling cannulae for blood collection (*B*). Each point and its vertical bar represent the mean ± S.E. from 6 to 11 animals. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. values before administration (Dunnett's multiple comparison test). From ref. 5.

effect on the heart rate and plasma norepinephrine concentration (Fig. 6). This may be attributable to not only the slow onset of action but also the N-type Ca^{2+} channel blocking action.

Some clinical trials, such as the cold pressor test, arithmetic test, and hand grip test, are used to examine the cardiovascular and neuroendocrine responses to stress. Many clinicians have reported that subjects experienced an increase in blood pressure and plasma catecholamine levels in response to these stress tests. In laboratory studies, these stress-mediated cardiac events can be mimicked using cold stress-loaded SHRs (7). Conscious and free-moving SHRs are each placed in individual cages, and their blood pressure and heart rate are continuously measured using a telemetry system. The blood pressure and heart rate of SHRs are rapidly elevated by this cold stress and recover to normal values upon removing the stress. Using this model, we can easily examine cardiovascular responses to acute cold stress in a good reproducible manner. Oral administration of prazosin (1 mg/kg), an α_1-adrenoceptor antagonist, virtually abolishes these pressor responses, indicating that these pressor responses are evoked via sympathetic nerve activation. Oral administration of cilnidipine (1 and 3 mg/kg, p.o.) caused a reduction in the acute cold stress-loaded pressor responses in a dose-dependent manner. The inhibitory effects of cilnidipine (3 mg/kg, p.o.) reached a peak of 78 ± 6% inhibition at 1.5 h after administration, and the inhibition lasted over 3 h after administration. In contrast, nifedipine, nicardipine, and manidipine (3 mg/kg, p.o.), at a dose showing a similar hypotensive effect to that of cilnidipine (5), had no significant effects on the pressor responses (Fig. 7). In addition, in studies evaluating the effect of DHP Ca^{2+} channel antagonists on the pressor responses to electrical sympathetic nerve stimulation (ESNS) in pithed SHRs, cilnidipine (10 μg/kg, i.v.) significantly reduced the pressor responses by 28 ± 6%, while neither nifedipine nor nicardipine, at doses up to 10 μg/kg, i.v., had any effect. Moreover, cilnidipine did not affect the pressor responses to exogenous norepinephrine (6), suggesting that its site of action may be the neuronal release of norepinephrine.

The effects of cilnidipine on sympathetic nerve-regulated renal function have also been studied in anesthetized dogs (22). The kidney is known to be an organ that is densely innervated by adrenergic fibers. As shown in Fig. 8, the renal nerve stimulation-induced increase in the norepinephrine secretion rate was attenuated by intraarterial infusion of cilnidipine. By intraarterial (i.a.) administration, cilnidipine (0.1 to 0.3 μg/kg/min) also suppressed the decrease in renal blood flow induced by electrical stimulation of the renal nerve, demonstrating that it can interfere with neurally regulated renal vasoconstriction in the dog kidney. On the contrary, nifedipine (0.1 to 0.3 μg/kg/min, i.a.) failed to inhibit the electrical stimulation-induced renal blood flow responses.

Renal nerve stimulation is also known to enhance renal tubular sodium reabsorption (4). Cilnidipine attenuated these renal nerve stimulation-induced changes in urinary sodium concentration. In contrast, other calcium channel antagonists, such as nifedipine (18), diltiazem, and nicardipine (11), have been reported not to affect the renal nerve stimulation-induced changes in urinary parameters at natriuretic doses.

These *in vivo* and *in vitro* studies demonstrate that cilnidipine reduces sympathetic nerve activation, and it is thought that N-type Ca^{2+} channel blockade by cilnidipine may, at least in part, contribute to this reduction in sympathetic activation.

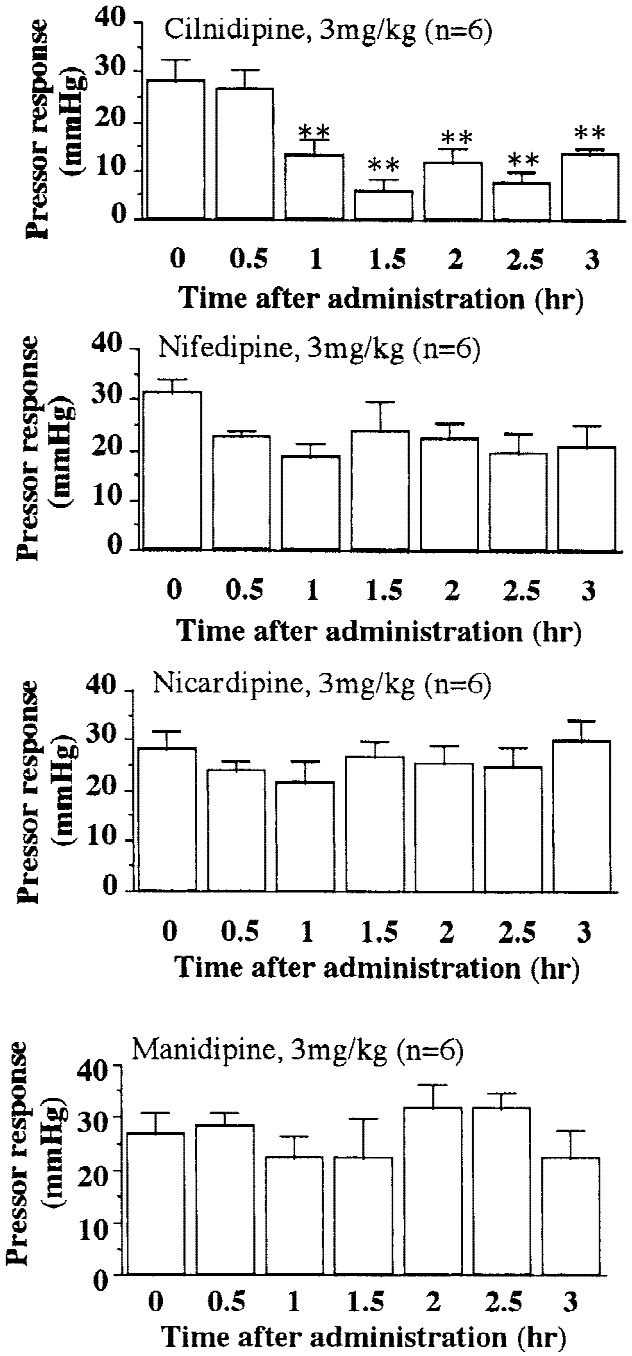


FIG. 7. The effects of cilnidipine, nifedipine, nicardipine, or manidipine, each at 3 mg/kg, p.o., on the pressor response to acute cold stress in conscious and unrestrained spontaneously hypertensive rats (SHRs). Each SHR was placed in an individual cage, and blood pressure and heart rate were continuously measured using the telemetry system. The cage was submerged by 2 cm into ice-cold water, sufficient to immerse all four paws. The blood pressure and heart rate of the SHRs were rapidly elevated by this cold stress and recovered to normal values upon removing the stress. Each dihydropyridine was administrated orally at the indicated dose just before starting the experiment. Each value represents the mean ± S.E.M. from six experiments. $^{*}P < 0.05$ and $^{**}P < 0.01$, statistically significant differences from the control group. Data are from ref. 7.

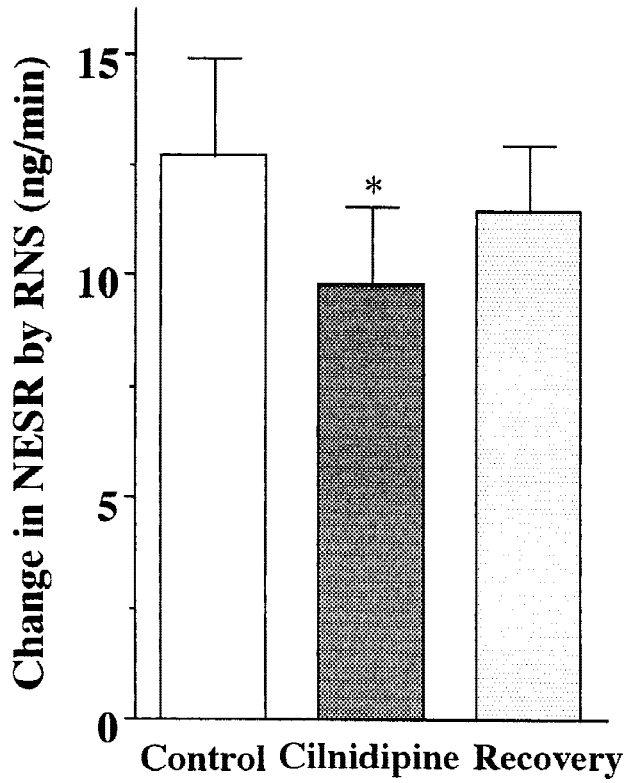


FIG. 8. Effects of intrarenal artery infusion of cilnidipine at 0.3 μg/kg/min on renal nerve stimulation (RNS)-induced increase in the norepinephrine secretion rate (NESR) in anesthetized dogs ($n = 5$). Each value represents the mean ± S.E. $^{*}P < 0.05$ vs. the control group. Data are from ref. 22.

Clinical Profile

Antihypertensive Activity in Patients with Essential Hypertension

In patients with essential hypertension, cilnidipine, at a single daily oral dose of 5, 10, or 20 mg, exerted a significant decrease in systolic, diastolic, and mean blood pressures when measured at 24 h after treatment. Cilnidipine did not, however, affect the circadian profile of blood pressure fluctuations (10). In a comparative study of cilnidipine and amlodipine in patients with essential hypertension (15), the daily pattern of blood pressure was measured during a 4-w drug-free period, during a 4-w treatment period with cilnidipine (5 or 10 mg once daily), and during a 4-w treatment period with amlodipine (2.5 or 5 mg once daily). Cilnidipine and amlodipine both showed a slow onset and long-lasting time course of their hypotensive action. The hypotensive action of cilnidipine, as well as that of amlodipine, is clear from the power spectra of the blood pressure frequency components shown in Table 4. Cilnidipine and amlodipine significantly lowered the average values for the entire 24 h to a similar extent (cilnidipine: −11.0 ± 2.5/−5.2 ± 1.3 mmHg; amlodipine: −12.7 ± 2.8/−4.6 ± 1.8 mmHg). Average reductions for daytime and nighttime blood pressures are presented separately in Table 4 to demonstrate that the

TABLE 4. *Power spectral data during the cilnidipine, amlodipine, and drug-free periods*[a]

	Cilnidipine	Amlodipine	Drug free
24 h			
LF,[b] ln (ms^2)	4.2 ± 0.1	4.1 ± 0.2	4.4 ± 0.2
HF, ln (ms^2)	3.7 ± 0.2	3.7 ± 0.2	4.1 ± 0.2
ln (LF/HF)	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.2
Daytime			
LF, ln (ms^2)	4.2 ± 0.1	4.2 ± 0.2	4.4 ± 0.1
HF, ln (ms^2)	3.4 ± 0.2	3.5 ± 0.3	3.9 ± 0.2
ln (LF/HF)	0.7 ± 0.1	0.6 ± 0.1	0.5 ± 0.2
Nighttime			
LF, ln (ms^2)	4.3 ± 0.2	4.1 ± 0.2	4.5 ± 0.3
HF, ln (ms^2)	4.3 ± 0.3	4.0 ± 0.2	4.4 ± 0.3
ln (LF/HF)	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2

[a] Values expressed as the mean ± S.E.
[b] Abbreviations: LF, low-frequency component; HF, high-frequency component.

hypotensive effects of these drugs were present during both waking and sleeping hours. These results indicate that both cilnidipine and amlodipine are equally effective as once daily antihypertensive agents for hypertensive patients.

Sympatho-Inhibitory Activity

Cilnidipine (5 or 10 mg once daily), as well as amlodipine (2.5 or 5 mg once daily), did not change the average daytime, night-time, or 24-h heart rate (Table 4) (15). In contrast, nifedipine retard (10 mg or 20 mg twice daily), which reduced the 24-h blood pressure to a similar extent as cilnidipine (5 or 10 mg once daily), significantly increased the average heart rate (for the entire 24 h: +3.3 ± 1.4 beats/min; for the daytime: +3.5 ± 1.2 beats/min) (13). The heart rate after nisoldipine (5 to 20 mg once daily) was also significantly higher than that after cilnidipine (5 to 20 mg once daily) during the morning (by 4.1 ± 1.3 beats/min) or the afternoon (by 6.4 ± 3.6 beats/min), although the blood pressure levels were similar in these two groups (Figs. 9 and 10) (14).

Furthermore, autonomic nerve activity was evaluated by a power spectral analysis of heart rate variability, using the ratio of the low-frequency (LF) component to the high-frequency (HF) component as an index of sympathovagal balance in which increases indicate the activation of the sympathetic nervous system. Nifedipine retard significantly increased the LF/HF ratio during the day or night; this effect was significantly greater than that observed with cilnidipine. During the night, the LF/HF ratio after nifedipine retard was significantly higher than that after cilnidipine (Table 5) (13).

In a repeated dose study in patients with essential hypertension, cilnidipine (5 or 10 mg once daily) did not significantly affect the plasma renin activity or aldosterone concentration. In addition, the same study found no significant effect by cilnidipine on plasma epinephrine or norepinephrine concentrations (23).

In a single-blind, placebo-controlled study in healthy volunteers, cilnidipine (10 mg) significantly reduced the increase in systolic blood pressure induced by cold stress loading (by placing one hand in ice water up to the wrist) for up to 2 h after a single administration (cilnidipine: +21.9 ± 7.4 mmHg; placebo: +32.1 ± 7.2 mmHg) (Fig. 11) (20).

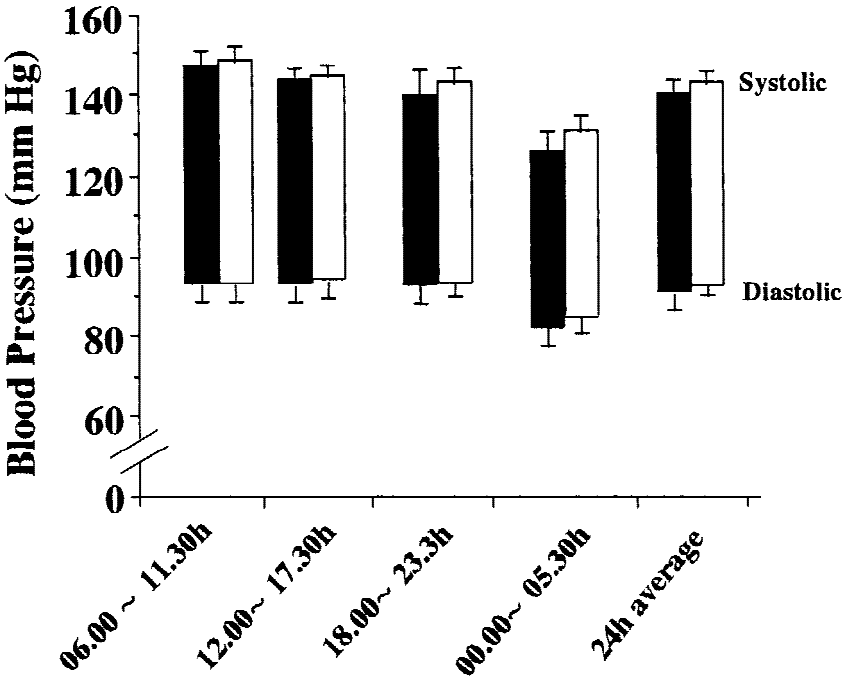


FIG. 9. The 6-h and 24-h averaged blood pressures (means ± S.E.M.) following a single dose of cilnidipine (closed bars) and nisoldipine (open bars). Blood pressure levels were similar during the two treatment periods for each 6-h segment of the day and throughout the entire 24-h period. Data are from ref. 14.

These inhibitory effects of cilnidipine on sympathetic nerve activation and reflex tachycardia could provide an additional clinical advantage in the treatment of hypertension by reducing the incidence of ischemic heart or cerebral diseases. This effect may be, at least in part, attributable to cilnidipine's N-type Ca^{2+} channel blocking action.

Conclusion

Currently a wide variety of DHPs (nifedipine, nicardipine, amlodipine, manidipine, nitrendipine, nisoldipine, felodipine, etc.) are available or are being developed for the treatment of hypertension. These DHPs are L-type Ca^{2+} channel antagonists that lower arterial blood pressure by decreasing total peripheral vascular resistance. Consequently, reflex tachycardia and increased cardiac output are commonly seen, particularly in response to the initial dose of these drugs (28). According to the Secondary Prevention of Reinfarction Israel Nifedipine Trial 2 (SPRINT 2), early treatment with nifedipine increased the risk ratio for mortality in suspected acute myocardial infarction (2). This undesirable effect sometimes limits the clinical use of these DHPs. We present here data suggesting that a newer DHP Ca^{2+} channel antagonist, cilnidipine, has a blocking action not only on vascular L-type Ca^{2+} channels but also on neuronal N-type Ca^{2+} channels. This profile is unique and, from a practical viewpoint, may overcome some of the undesirable effects of such conventional DHPs as nifedipine. In support, we present some new therapeutic evidence showing that cilnidipine depresses the pressor response to acute cold

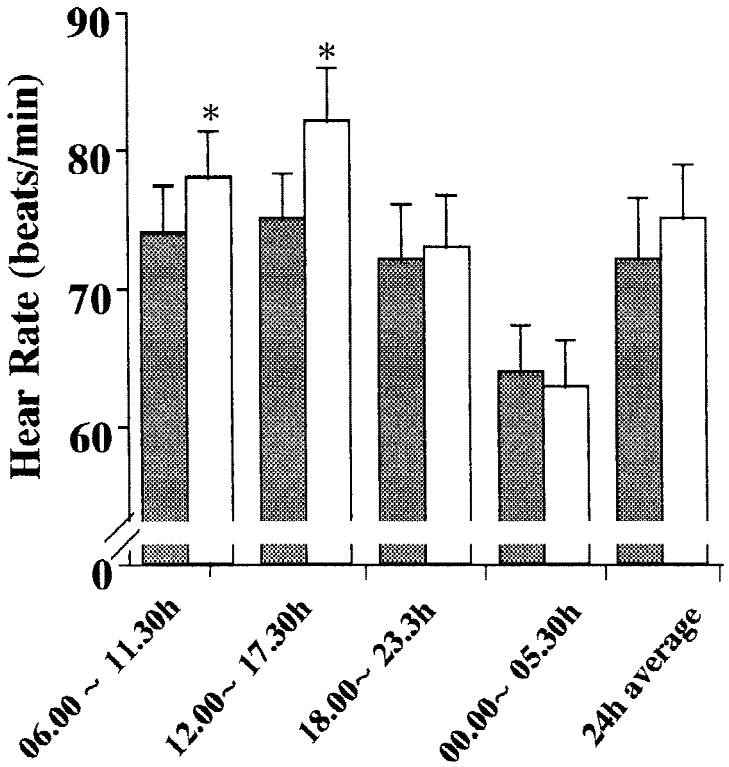


FIG. 10. The 6-h and 24-h averaged heart rate (means ± S.E.M.) following a single dose of cilnidipine (closed bars) or nisoldipine (open bars). The heart rate was significantly higher during treatment with nisoldipine than during treatment with cilnidipine in the morning segment (by 4.1 ± 1.3 beats/min ($P < 0.05$)) and the afternoon segment (by 6.4 ± 3.6 beats/min ($P < 0.05$)), whereas the 24-h average heart rate did not differ significantly between the two treatment periods. Data are from ref. 14.

stress and lowers blood pressure without baroreflex-induced tachycardia. The mechanism of cilnidipine's actions can be explained by the blockade of both peripheral N-type and L-type Ca^{2+} channels.

The sympathetic nervous system affects many aspects of cardiovascular functions. It is unfortunate but well documented that the incidence of acute myocardial infarction increased after the Gulf War (16) and the Hanshin-Awaji earthquake (21). These epidemiologic reports make us recognize again the importance of mental stress in the initiation or exacerbation of some cardiovascular diseases. Stress is both a risk factor for and a trigger of hypertension, cardiac arrhythmias, or sudden death. Ca^{2+} channel antagonists that block peripheral N-type and L-type Ca^{2+} channels are promising in that they may better control sympathetic nerve activity and reduce, therefore, the incidence and/or severity of stress-related diseases. In particular, we believe that an L-type Ca^{2+} channel antagonist, with additional N-type blocking action by cilnidipine, might be potentially useful in the treatment of hypertension as a prototype for the next generation of antihypertensive drugs.

TABLE 5. *Heart rate power spectral data analysis during the cilnidipine, nifedipine retard, and drug-free periods*[a]

	Cilnidipine	Nifedipine retard	Drug free
24 h			
LF, ln (ms^2)	4.24 ± 0.13	4.16 ± 0.11	4.43 ± 0.19
HF, ln (ms^2)	3.78 ± 0.14	3.76 ± 0.22	4.15 ± 0.19
ln (LF/HF)	0.45 ± 0.13	0.66 ± 0.07	0.27 ± 0.16
Daytime			
LF, ln (ms^2)	4.22 ± 0.14	4.11 ± 0.20	4.38 ± 0.16
HF, ln (ms^2)	3.52 ± 0.16	3.47 ± 0.31	3.99 ± 0.16
ln (LF/HF)	0.70 ± 0.13[c]	0.78 ± 0.10[d]	0.39 ± 0.16
Nighttime			
LF, ln (ms^2)	4.31 ± 0.28	4.33 ± 0.28	4.48 ± 0.35
HF, ln (ms^2)	4.25 ± 0.26	3.99 ± 0.24	4.41 ± 0.31
ln (LF/HF)	−0.04 ± 0.23[e]	0.42 ± 0.10	0.05 ± 0.22[e]

[a] Values expressed as the mean ± S.E.
[b] Abbreviations: LF, low-frequency component; HF, high-frequency component.
[c] $P < 0.05$ vs. drug free.
[d] $P < 0.01$ vs. drug free.
[e] $P < 0.05$ vs. nifedipine retard.

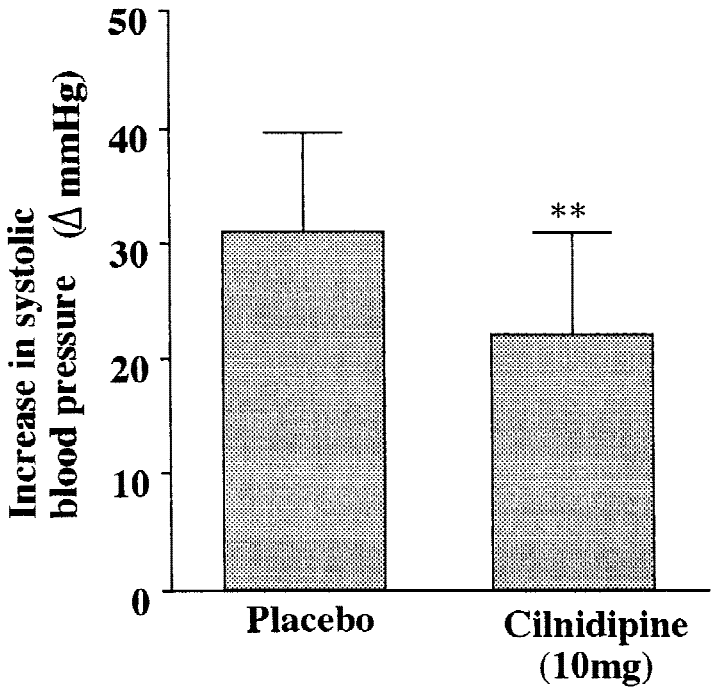


FIG. 11. Effects on systolic blood pressure induced by cold stress. Ten mg of cilnidipine or a placebo were given to normal human subjects who exhibited an increase of at least 20% in systolic blood pressure following cold stress (by placing one hand in ice water up to the wrist for 1 min). An increase in systolic blood pressure by cold stress was observed 2 h after administration of the drug. Each vertical column and bar represent the mean ± S.E.M. for 10 individuals. $^*P < 0.01$ vs. placebo (Student's *t* test). Data are from ref. 20.

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