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In vitro assessment for vascular selectivity of a new dihydropyridine derivative, NB-818

Yuichi Hattori *, Mitsuhiro Fukao, Hideki Houzen, Fazhi Qi, Yoichi Yamada, Morio Kanno

Department of Pharmacology, Hokkaido University School of Medicine, Sapporo 060, Japan

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Abstract

The vascular selectivity of NB-818 (isopropyl methyl 2-carbamoyloxymethyl-6-methyl-4-(2,3-dichlorophenyl)-1,4-dihydropyridine-3,5-dicarboxylate), a newly synthesized dihydropyridine derivative, was evaluated in in vitro experiments. NB-818 and nifedipine concentration dependently caused a relaxant effect in rabbit femoral arteries precontracted with 60 mM K^+, a negative inotropic effect in guinea-pig papillary muscles, and a negative chronotropic effect in guinea-pig right atria. The onset of these inhibitory effects of NB-818 was much slower than that of nifedipine when compared at concentrations producing the same inhibition. The relaxant effect of NB-818 was about 10 times more potent than that of nifedipine, while the negative inotropic effect of NB-818 was about 100 times less potent than that of nifedipine. As a result, NB-818 showed about 300 times higher vascular selectivity than nifedipine. The two drugs exhibited a similar potency for the negative chronotropic effect. In a whole-cell configuration with voltage clamp, the blocking effect of NB-818 on L-type Ca^{2+} current (I_{Ca}) in guinea-pig ventricular cells appeared much more slowly than that of nifedipine and was hardly washed out. The potency of NB-818 to block I_{Ca} was markedly enhanced under depolarized conditions (i.e. at a holding potential of -30 mV) compared to that under polarized conditions (i.e. at a holding potential of -70 mV). Such a voltage-dependent blocking action on I_{Ca} was less pronounced for nifedipine. These results indicate that NB-818 is a slow-acting Ca^{2+} channel antagonist with much high vascular selectivity. Its vascular selectivity may be at least in part related to the marked voltage-dependent inhibition of I_{Ca}.

Keywords: Ca^{2+} channel antagonist; Vascular relaxation; Negative inotropic action; Negative chronotropic action; Ca^{2+} current, L-type; NB-818

1. Introduction

A number of structurally diverse compounds have the ability to inhibit the transmembrane Ca^{2+} influx by blocking L-type Ca^{2+} channels. Of these, 1,4-dihydropyridines such as nifedipine preferentially act on vascular smooth muscle (Kazda et al., 1983; Godfraind et al., 1986; Vanhoutte and Paoletti, 1987). At least under clinical conditions their cardiodepressant effects are slight or negligible (Sorkin et al., 1985). Nevertheless, research has been focussing on the development of even more vasoselective dihydropyridine Ca^{2+} channel antagonists. In addition, the recently developed dihydropyridines differ from their prototype, nifedipine, in having a slow onset and long duration of action (Burges et al., 1985; Micheli et al., 1990; Masuda et al., 1990; Okamiya et al., 1992).

NB-818, isopropyl methyl 2-carbamoyloxymethyl-6-methyl-4-(2,3-dichlorophenyl)-1,4-dihydropyridine-3,5-dicarboxylate, is a newly synthetized dihydropyridine derivative. This compound causes a selective and long-lasting increase in vertebral blood flow in anesthetized dogs (Nagura et al., 1989). In isolated canine vessels, the vasorelaxant action of NB-818 has been shown to be very slow in onset and long-lasting (Nishikibe et al., 1989). Radioligand binding studies have demonstrated that NB-818 slowly associates with and slowly dissociates from the dihydropyridine binding sites in rat brain membranes (Ihara et al., 1988). These pharmacological properties may be important in a clinical setting, especially for the long-term use of NB-818 as an antihypertensive agent. However, the mechanisms contributing to the vascular selectivity of NB-818 remain elusive. Furthermore, it has not been clarified whether the slow onset and long-lasting effect of NB-818 is attributable to its functional interaction with L-type Ca^{2+} channels.

The purpose of the present study was to investigate in detail the cardiovascular profile of NB-818 and to charac-

* Corresponding author. Tel.: +81-11-706-6920; fax: +81-11-717-1139.

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terize more clearly its tissue-selective and time-dependent Ca^{2+} antagonistic action. We also examined the inhibitory effect of NB-818 on the L-type Ca^{2+} current (I_{Ca}) electrophysiologically by the patch clamp method in order to clarify whether the characteristics of NB-818 found in vitro result from the action of this compound on L-type Ca^{2+} channels.

2. Materials and methods

2.1. Rabbit femoral artery

Male New Zealand White rabbits (1.8–2.5 kg) were anesthetized with sodium pentobarbital (30 mg/kg i.v.), and the femoral arteries were dissected. The arteries were cleaned of adherent fat and surrounding tissue and cut into rings of 3–5 mm length. The intimal surface of the arterial rings was gently rubbed with a wooden rod to remove the inhibitory influence of the vascular endothelium. Each ring was suspended by a pair of stainless steel hooks under a resting tension of 1 g in a water-jacketed bath filled with 25 ml of normal physiological salt solution. The composition of the solution was (in mM): NaCl 118.2, KCl 4.7, $MgCl_2$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25.0 and glucose 10.0. The solution in the bath was gassed with 95% O_2 and 5% CO_2, and its temperature was maintained at 37°C. Force generation was monitored using an isometric transducer (Sanei-Sokki 45196, Tokyo, Japan) and a carrier amplifier (Sanei-Sokki 1236). The output of the force transducer was registered on a pen recorder (TOA Electronics ERP-241A, Tokyo, Japan) through a polygraph recorder (Sanei-Sokki 142-8).

Following the equilibration period of at least 60 min, the rings were exposed several times to high K^+ (60 mM) solution until reproducible contractile responses were obtained. High K^+ solution was prepared by replacement of NaCl with equimolar KCl in order to avoid a change in tonicity of the solution. Once contractions stabilized, the following protocol was designed to test the relaxant effects of NB-818 and nifedipine on high K^+-induced contractions. The arterial rings were exposed to high K^+ solution and allowed to reach a steady state of contraction. Twenty to thirty minutes after the stable contraction was obtained, a single concentration of NB-818 or nifedipine was added to the bath. The relaxant effects of the drugs were evaluated for 4 h after their addition. The results were expressed as a percentage of the amplitude of the sustained contraction in response to high K^+ solution.

2.2. Guinea-pig papillary muscle

Guinea-pigs of either sex, weighing 400–600 g, were killed by a blow on the head. The hearts were placed in a dissection bath containing oxygenated Krebs-Henseleit solution. The composition of the solution was (in mM): NaCl 119, KCl 4.8, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 24.9 and glucose 10.0. The right ventricular papillary muscles, less than 1 mm in diameter, were carefully dissected from the hearts. The muscle was mounted under 0.5 g of resting tension in a water-jacketed organ bath containing 10 ml of Krebs-Henseleit solution. The solution in the bath was bubbled with 95% O_2 and 5% CO_2, and its temperature was kept at 30°C. The muscle was stimulated by rectangular pulses of 1 Hz in frequency, 5 ms in duration and 1.5 times the threshold voltage, delivered by a pair of spiral platinum electrodes connected to an electronic stimulator (Sanei-Sokki 3F46) through an isolation unit (Sanei-Sokki 5361). Isometric tension developed in the preparation was measured with a force transducer (Nihon Kohden TB612T, Tokyo, Japan) and recorded on a thermal array recorder (Nihon Kohden RTA-1200) through a preamplifier (Nihon Kohden RP-5). After the preparations were allowed to equilibrate for at least 120 min, a single concentration of NB-818 or nifedipine was added to the bath, and the changes in force of contraction were monitored for 120 min after drug addition. The results were expressed as a percentage of basal values recorded before the administration of the drugs.

2.3. Guinea-pig right atrium

The spontaneously beating right atrium was isolated from the guinea-pig heart as described for the papillary muscle preparations. The right atrium was suspended in a 50-ml water-jacketed bath containing Krebs-Henseleit solution gassed with 95% O_2 and 5% CO_2. The resting tension applied to the preparation was adjusted to 2 g. The bath was kept at a temperature of 35°C. The spontaneous rate was determined by counting the mechanical activity which was monitored with a transducer. Following an equilibration period of at least 120 min, a single concentration of NB-818 or nifedipine was added to the bath, and the changes in spontaneous rate were monitored for 90 min after drug addition. The results were expressed as a percentage of basal values recorded before the administration of the drugs.

2.4. Guinea-pig ventricular cells

Single ventricular myocytes of the guinea-pig were obtained essentially by the same technique as described previously (Tohse, 1990). Briefly, collagenase (0.02% w/v, Wako Pure Chemical, Osaka, Japan) in a low Ca^{2+} (less than 60 μM) Tyrode solution (50 ml) was perfused for 40 min through the coronary artery using a Langendorff apparatus. The collagenase solution was washed out by KB solution whose composition was (in mM): KOH 70, *l*-glutamic acid 50, KCl 40, taurine 20, KH_2PO_4 20, $MgCl_2$ 3, glucose 10, EGTA 0.5 and Hepes 5 (pH 7.4). The ventricular tissue was cut into small pieces, agitated gently in a small beaker with KB solution, and then

filtered through a 100-μm stainless steel mesh. The cell suspension was stored in a refrigerator (4°C) for later use.

The whole-cell membrane currents were recorded by the patch-clamp method, using glass patch electrodes with a tip diameter 3–4 μm and a resistance of 2–5 MΩ. The electrode was connected to the input of a patch-clamp amplifier (Nihon Kohden CEZ 2300). The signals were displayed on an oscilloscope (Sony-Tektronix 5113 OP 03, Tokyo, Japan) and were simultaneously fed to a data recording system, consisting of a video cassette recorder (National NV-F1, Osaka, Japan) and a PCM converter system (Sony PCM-501ES, Osaka, Japan) as a back-up. The current and voltage signals were filtered at 2 kHz, digitalized by an AD converter (Canoopus Electronics ADX-98, Kobe, Japan) at 2 kHz and stored in the 20 MByte hard disk of a personal computer (NEC PC-98XA, Tokyo, Japan) for later analysis.

The pipette solution contained (in mM): CsOH 110, KCl 20, *l*-aspartic acid 100, $MgCl_2$ 1.0, Na_2-ATP 5.0, Na_2-creatine phosphate 5.0, EGTA 10 and Hepes 5.0 (pH 7.4). The composition of the external solution was (in mM): NaCl 143, CsCl 5.4, $MgCl_2$ 0.5, $CaCl_2$ 1.8, NaH_2PO_4 0.33, glucose 5.5 and Hepes 5.5 (pH 7.4). The compositions of the external and pipette solutions were formulated to eliminate the involvement of K^+ currents in the whole-cell membrane currents. In general, I_{Ca} was elicited by a 300-ms depolarizing test pulse to +10 mV from a holding potential (V_H) of −30 mV. The inihibitions of I_{Ca} induced by NB-818 and nifedipine were evaluated in a single concentration-effect manner. The maximum inhibition at a given concentration was obtained by repetitive application of the command pulses at 0.1 Hz. We have confirmed that there was no significant rundown of I_{Ca} at least through our experimental protocols (Tohse et al., 1993). In some experiments, the effects of the drugs on I_{Ca} were tested by voltage-clamp under polarized conditions, i.e. at a V_H of −70 mV, where a 20-ms prepulse to −30 mV was given just before depolarization of 300 ms to +10 mV in order to inactivate the Na^+ current. All experiments were carried out at a temperature of 35–37°C.

2.5. Drugs

NB-818 was supplied by Kowa Shinyaku Co. (Nagoya, Japan). Nifedipine was purchased from Sigma Chemical (St. Louis, MO, USA). Other chemicals used in this study were of the highest purity available from Sigma or Wako. NB-818 and nifedipine were dissolved in 100% polyethyleneglycol 400 as stock solutions (10 mM), before dilution in distilled water. Further dilutions to the desired concentrations were made with the suitable buffer solution. All experiments were performed in the dark, and the solution bottles and tubing were covered with aluminum foil.

2.6. Statistics

The data are expressed as means ± S.E.M. Statistical analysis was performed using Student's *t*-test for unpaired observations. Differences were considered to be statistically significant when $P < 0.05$.

3. Results

3.1. Vasorelaxant effects of NB-818 and nifedipine

The relaxant effect of NB-818 (0.1–10 nM) on K^+-induced contractions in rabbit femoral arteries was examined in comparison with that of nifedipine (1–30 nM). Exposure of the arteries to high K^+ (60 mM) solution resulted in sustained contractions that were averaged to be 4.0 ± 0.3 g ($n = 46$). Against these contractions, both NB-818 and nifedipine caused relaxations in a concentration-dependent

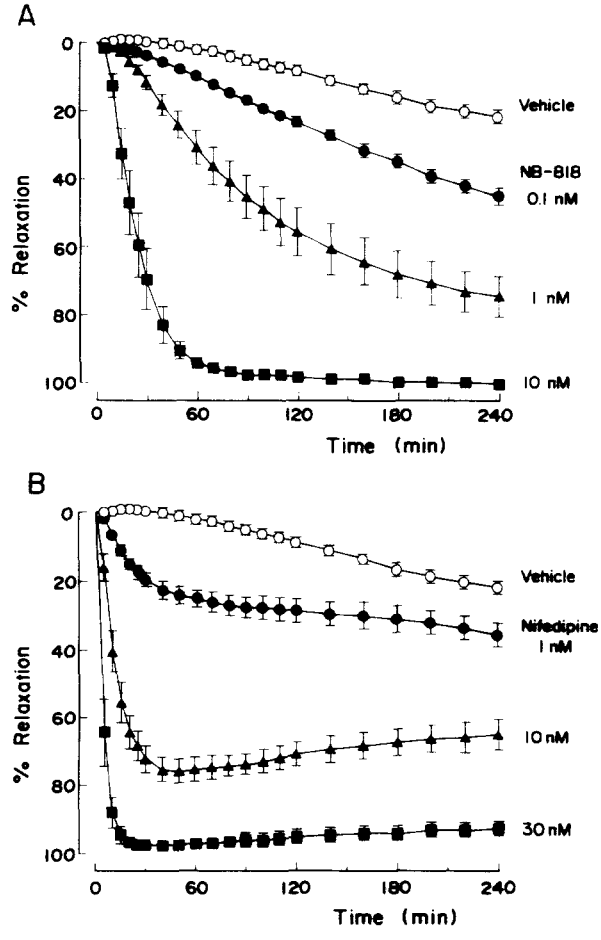

Fig. 1. Time courses of the relaxations induced by NB-818 (A) and nifedipine (B) in rabbit femoral arteries precontracted with 60 mM K^+. Ordinates: percentage relaxation of high K^+-induced contraction. Abscissae: time (min) of exposure to the drug. Points are means ± S.E. of six to seven experiments. The contractions induced by 60 mM K^+ were not significantly different between each group of preparations.

manner (Fig. 1). The rate of relaxation induced by both drugs also depended on their concentrations. When compared at the concentrations that produced the same degree of relaxation, NB-818 was much slower in reaching a steady state of relaxation than nifedipine. Indeed, the time course of the relaxant effect of a low concentration of NB-818 (0.1 nM) was very slow and did not reach a steady level even after exposure for 4 h. The high K^+-induced contractions were inhibited by 45 ± 3, 74 ± 6 and $100 \pm 0\%$ with respective half times ($T_{1/2}$) of 119 ± 5, 78 ± 8 and 23 ± 4 min at 0.1, 1 and 10 nM of NB-818, respectively. The inhibition induced by nifedipine at 1, 10 and 30 nM was 36 ± 4, 76 ± 3 and $98 \pm 1\%$ with $T_{1/2}$ of 24 ± 3, 10 ± 1 and 4 ± 1 min, respectively.

When parallel control preparations were treated with the amount of polyethyleneglycol 400 identical to that used as solvent for the maximum concentration of the drug, they exhibited a gradual and small decline in high K^+-evoked contractions (Fig. 1). A similar decline was observed in the untreated preparations. Thus, the IC_{50} values of NB-818 and nifedipine for the inhibitory effect against high K^+-induced contractions were calculated from the data points which were corrected by subtracting the spontaneous decline. Based on the IC_{50} values (Table 1), the relaxant effect of NB-818 was about 3 times more potent than that of nifedipine.

3.2. Negative inotropic effects of NB-818 and nifedipine

The negative inotropic activity of NB-818 (0.3–10 μM) in guinea-pig papillary muscles was examined in comparison with that of nifedipine (10–300 nM). The basal force of contraction of papillary muscles electrically paced at 1 Hz was 727 ± 69 mg ($n = 54$). The vehicle polyethyleneglycol 400 had no direct effect on force of contraction. As shown in Fig. 2, both NB-818 and nifedipine reduced force of contraction in a concentration-dependent manner. However, the negative inotropic effect of NB-818 turned out to be less than that of nifedipine. The force of contraction was decreased by $70 \pm 2\%$ ($n = 6$) when NB-818 was given at a concentration of 10 μM, above which NB-818 was incompletely dissolved in the bathing solution. Nifedipine at a concentration of 300 nM inhibited force of contraction by $94 \pm 1\%$ ($n = 6$). Thus, as shown in Table 1, NB-818 was about 100 times less potent than nifedipine. In addition, the time course of the negative inotropic effect of NB-818 was much slower than that of nifedipine. When compared at a given decrease in force of contraction (70–75%) caused by these drugs, the $T_{1/2}$ was 36 ± 3 min ($n = 6$) for 10 μM NB-818 and 13 ± 2 min ($n = 6$) for 100 nM nifedipine.

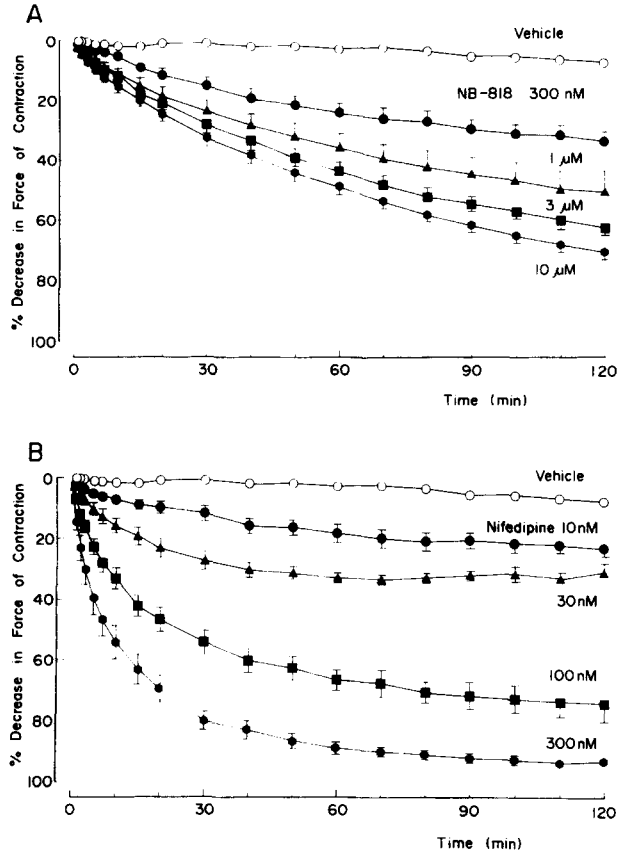

Fig. 2. Time courses of the negative inotropic effects of NB-818 (A) and nifedipine (B) in guinea-pig papillary muscles electrically driven at 1 Hz. Ordinates: percentage decrease in basal force of contraction. Abscissae: time (min) of exposure to the drug. Points are means ± S.E. of six experiments. The basal values of force of contraction were not significantly different between each group of preparations.

Table 1
IC_{50} values (nM) for NB-818 and nifedipine in isolated cardiovascular tissues

	NB-818	Nifedipine
Rabbit femoral artery	0.35	1.1
Guinea-pig papillary muscle	5100	55
Guinea-pig right atrium	310	370

IC_{50} values are defined as drug concentrations required to relax high K^+ (60 mM)-induced contractions to the half, to decrease force of contraction to half of the basal values and to decrease the spontaneously beating rate to half of the basal values in rabbit femoral arteries, guinea-pig papillary muscles and guinea-pig right atria, respectively. To determine IC_{50} values, the concentration-response curves were prepared from the data points derived from the time-response curves shown in Figs. 1, 2 and 3.

3.3. Negative chronotropic effects of NB-818 and nifedipine

The negative chronotropic activity of NB-818 (30 nM to 1 μM) in guinea-pig right atria was examined in comparison with that of nifedipine (30 nM to 1 μM). The basal rate of spontaneously beating right atria was 191 ± 3 beats/min ($n = 56$). The vehicle polyethyleneglycol 400 slightly increased the spontaneous rate ($10 \pm 1\%$, $n = 6$). As shown in Fig. 3, both NB-818 and nifedipine produced a reduction of the spontaneous rate in a concentration-de-

pendent manner. The IC_{50} value for the negative chronotropic effect of NB-818 was comparable to that for nifedipine (Table 1). Again, the time course of the negative chronotropic effect of NB-818 was much slower than that of nifedipine. NB-818 (1 μM) and nifedipine (1 μM) caused complete arrest of spontaneous beating at 64 ± 8 ($n = 6$) and 18 ± 3 min ($n = 6$) after drug addition, respectively.

3.4. Inhibitory effects of NB-818 and nifedipine on I_{Ca}

Fig. 4 shows the time courses of the inhibitory effects of NB-818 and nifedipine on L-type I_{Ca} in guinea-pig ventricular myocytes. I_{Ca} gradually decreased after application of NB-818 and reached a trough 6 min later. Its effect persisted even after 30 min of washout of the drug. In contrast, the inhibitory effect of nifedipine reached its maximum within 2 min and was almost washed out within a few minutes.

In order to test whether the inhibitory effects of NB-818 and nifedipine on I_{Ca} are voltage-dependent, we investigated the effects of these drugs on I_{Ca} under polarized conditions, i.e. at a V_H of -70 mV and under depolarized conditions ($V_H = -30$ mV). The concentration-response curves for the effects of NB-818 and nifedipine on I_{Ca} at two different potential levels are presented in Fig. 5. Under depolarized conditions, the inhibitory potencies of both drugs were enhanced. However, such a voltage-dependent effect was more marked for NB-818. Thus, under polarized conditions, NB-818 was a little more active than nifedipine ($IC_{50} = 0.15$ μM and 0.21 μM, respectively), while NB-818 was much more potent than nifedipine under depolarized conditions ($IC_{50} = 2.8$ nM and 66 nM, respectively).

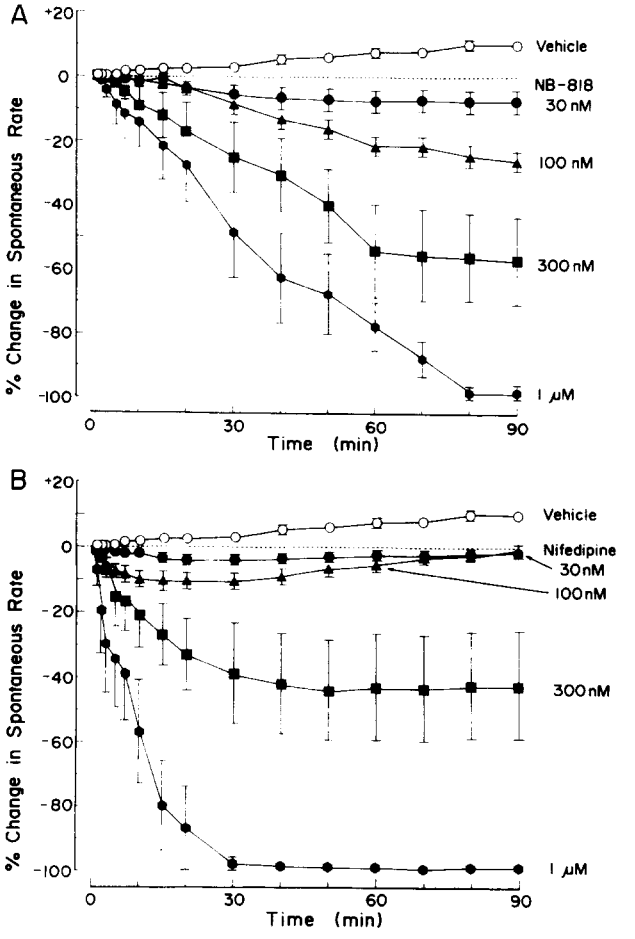

Fig. 3. Time courses of the negative chronotropic effects of NB-818 (A) and nifedipine (B) in spontaneously beating guinea-pig right atria. Ordinates: percentage change in basal beating rate. Abscissae: time (min) of exposure to the drug. Points are means ± S.E. of six to seven experiments. The basal spontaneous rates were not significantly different between each group of preparations.

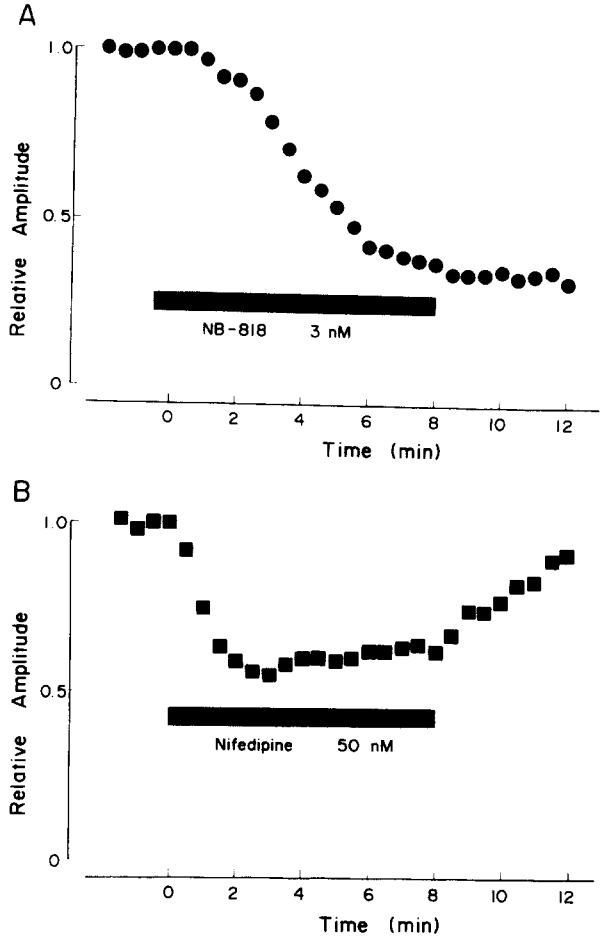

Fig. 4. Time courses of the effects of NB-818 (3 nM; panel A) and nifedipine (50 nM; panel B) on I_{Ca} in guinea-pig ventricular cells. The test pulses of 300 ms to +10 mV from a holding potential of −30 mV were applied at a rate of 0.1 Hz. The amplitude of I_{Ca} just before application of the drug was normalized as 1.0. The drug was applied for the time indicated by the bar in each tracing.

4. Discussion

The present study demonstrates that NB-818, a new dihydropyridine derivative, has a much more potent va-

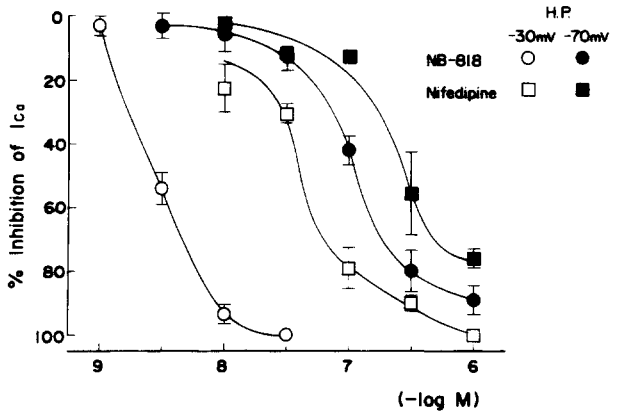

Fig. 5. Concentration-dependent inhibitor effects of NB-818 (○,●) and nifedipine (□,■) on I_{Ca} at holding potentials of −30 mV (○,□) and −70 mV (●,■). I_{Ca} was elicited by a 300-ms depolarizing test pulse to +10 mV from each holding potential. The amplitudes of I_{Ca} just before application of the drug at holding potentials of −30 and −70 mV were 7.54±0.66 (n = 36) and 5.17±0.55 pA/pF (n = 36), respectively, and were normalized as 100%. Points are means±S.E. of three to four experiments.

sorelaxant activity than cardiodepressant activity as compared to its prototype nifedipine. In agreement with the results of numerous previous investigators (Godfraind et al., 1984; Van Amsterdam et al., 1987; Hof, 1987; Nakaya et al., 1988), nifedipine showed a selectivity for arterial smooth muscle over cardiac muscle; it inhibited the K^+-induced contractions of rabbit femoral arteries more potently than it did the contractile force of guinea-pig papillary muscles. However, the vascular relaxant effect of NB-818 was about 3 times more potent than that of nifedipine, while the negative inotropic effect of NB-818 was about 100 times less potent than that of nifedipine. Thus, when the vascular selectivity was expressed as the ratio of the IC_{50} values for the negative inotropic activity and the vasodilating activity, NB-818 (ratio = 14,571) exhibited about 300 times higher vascular selectivity than nifedpine (ratio = 50). Thus, NB-818 can be characterized as a potent Ca^{2+} channel antagonist which is much more selective for vascular smooth muscle.

The differences in potency of dihydropyrines in cardiac and vascular smooth muscle may be due to voltage-dependent binding to L-type Ca^{2+} channels. According to the 'modulated receptor theory' (Hondeghem and Katzung, 1984), the affinity of a drug is modulated by the states of the channel, i.e. open, resting and inactivated state, which are determined by the membrane potential. Electrophysiological studies have revealed that dihydropyridines cause a voltage-dependent block of these Ca^{2+} channels (Bean, 1984; Hess et al., 1984). Thus, these compounds preferentially bind with high affinity to the inactivated Ca^{2+} channel and with low affinity to the resting channel (Sanguinetti and Kass, 1984; Rogart et al., 1986), possibly due to structural modifications during the voltage-dependent change in the Ca^{2+} channel states. In the present study, both nifedipine and NB-818 inhibited L-type I_{Ca} in guinea-pig ventricular cells in a voltage-dependent manner. When passing from a V_H of −70 mV to −30 mV, the concentration-response curves for the inhibitory effects of these compounds on I_{Ca} were shifted to the left. Thus, the inhibitory effects of nifedipine and NB-818 were stronger when the membrane was more depolarized. The inhibition of I_{Ca} by NB-818 was much more voltage-sensitive than that by nifedipine, indicating that NB-818 blocks more preferentially the inactivated state of Ca^{2+} channels. In general, vascular smooth muscles have a lower resting membrane potential than cardiac muscles. Especially in vascular smooth muscles exposed to high K^+, the resting membrane potential is more depolarized, resulting in an increased proportion of inactivated Ca^{2+} channels. In contrast, in cardiac muscles the more negative resting potential results in more Ca^{2+} channels being in the resting state. Therefore, the higher vascular selectivity of NB-818 may be partly attributed to its much higher affinity for Ca^{2+} channels in the inactivated state than in the resting state as compared to nifedipine.

In spontaneously beating right atria of guinea-pigs, NB-818 produced a negative chronotropic effect which was equipotent to that induced by nifedipine. This was in sharp contrast with the finding that in guinea-pig papillary muscles NB-818 produced a much weaker negative inotropic effect than nifedipine. The resting membrane potential of the sinoatrial node is more positive than −60 mV, whereas that of the papillary muscle is around −90 mV (Nakaya et al., 1986). Thus, in cardiac tissues having low membrane potentials, NB-818 is likely to display a pronounced Ca^{2+} channel antagonistic action. The result obtained in spontaneously beating right atria again reflects the fact that the ability of NB-818 to block cardiac Ca^{2+} channels is much more voltage-sensitive than that of nifedipine.

It has been assumed that dihydropyridines penetrate into the lipid bilayer, diffuse laterally and interact with the specific binding site (Rhodes et al., 1985). A recent report has proposed that the dihydropyridine binding site resides within the lipid bilayer or channel pore closer to the extracellular membrane space (Kwan et al., 1995). Lipophilicity may influence the biological potency of dihydropyridines. Rodenkirchen et al. (1979) have shown a decrease in the negative intropic action with increasing lipophilicity of various nifedipine derivatives in isolated cat papillary muscles. However, Bossert et al. (1979) have demonstrated an increase in vasodilator potency in parallel with an increase in the lipophilicity of ester-substituted nitrendipine derivatives. These differential influences of lipophilicity on the activities of dihydropyridines in cardiac and vascular tissues suggest that the structural properties of the lipid bilayer matrix surrounding the dihydropyridine binding site might be different between cardiac and vascular smooth muscle. Thus, the lipophilicity of dihydropyridines appears to be important as a determinant

factor in vascular selectivity. NB-818 is a relatively highly lipophilic compound as predicted from its *n*-octanol-water partition coefficent (log $P = 4.3$; technical report from Banyu Pharmaceutical Co.), a value which is larger than those for other dihydropyridines such as nifedipine (log $P = 2.6 \sim 3.1$) (Pang and Sperelakis, 1984; Diez et al., 1991). Therefore, the high vascular selectivity of NB-818 may be at least in part due to its lipophilic property affecting tissue penetration in cardiac and vascular smooth muscle in a different way.

In addition, the vascular selectivity of NB-818 might be dependent on differences in the structure of L-type Ca^{2+} channels within various tissues. Thus, vascular Ca^{2+} channels and cardiac Ca^{2+} channels might have different sensitivities for NB-818. However, the characteristics of L-type Ca^{2+} channels in vascular smooth muscle cells are more like those of cardiac cells (Sanguinetti and Kass, 1984; Bean et al., 1986). Furthermore, the binding sites show similar affinities for dihydropyridines in membranes from different tissues (Sarmiento et al., 1984). We cannot exclude the possibility that a distinct distribution of L-type Ca^{2+} channels in cardiac and vascular smooth muscle might contribute to the vascular selectivity of dihydropyridines including NB-818.

Besides the vascular selectivity, another characteristic of NB-818 was its slow onset of action. The relaxant effect of NB-818 in high K^+-contracted arteries was much slower in onset than that of nifedipine. The slow onset of action of NB-818 was also recognized in isolated cardiac tissues when its negative inotropic and chronotropic effects were evaluated. Our present results obtained with the voltage-clamp technique showed that NB-818 inhibited I_{Ca} in guinea-pig ventricular cells much more slowly than nifedipine did, thus indicating that the slow onset of its action is due to the slow binding of the compound to Ca^{2+} channels. This is consistent with the radioligand binding data showing that NB-818 slowly associates with the dihydropyridine binding sites in rat brain membrane (Ihara et al., 1988). While the inhibitory effect of nifedipine on I_{Ca} was almost washed out within a few minutes, that of NB-818 was hardly washed out within 30 min, an observation which supports the long-lasting action of NB-818 in in vivo experiments (Nagura et al., 1989). The results obtained in single cells may not quantitatively account for the slow onset and long-lasting action of NB-818 in in vivo experiments, since in the whole body the pharmacokinetic property of the compound may become the important one of the factors to determine the duration of action. However, the slow onset and long-lasting property of NB-818 would be relevant to its clinical use as documented for other dihydropyridines with a similar profile to NB-818 (Opie, 1988; Reicker-Reiss and Barasch, 1991).

In conclusion, NB-818 is a highly vascular selective Ca^{2+} channel antagonist. The vascular selectivity of NB-818 may be in part attributed to its strong voltage-dependent inhibition of I_{Ca} and its lipophilicity. In addition, the Ca^{2+} channel antagonistic action of NB-818 was very slow in onset and prolonged after withdrawal. These unique pharmacological profiles may be of great advantage when this compound is used for the treatment of hypertension.

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