British Journal of Pharmacology (1997) 122, 37–42

Blockade of N-type Ca^{2+} current by cilnidipine (FRC-8653) in acutely dissociated rat sympathetic neurones

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1 The inhibitory effects of cilnidipine (FRC-8653) and various organic Ca^{2+} channel blockers on high voltage-activated Ba^{2+} currents (HVA I_{Ba}) in rat sympathetic neurones were examined by means of the conventional whole-cell patch-clamp recording mode under voltage-clamped conditions.

2 HVA I_{Ba} was classified into three different current components with subtype selective peptide Ca^{2+} channel blockers. No ω-Agatoxin IVA-sensitive (P-type) or ω-conotoxin MVIIC-sensitive (Q-type) current components were observed. Most (>85%) I_{Ba} was found to consist of ω-conotoxin GVIA-sensitive N-type components.

3 The application of cilnidipine inhibited HVA I_{Ba} in a concentration-dependent manner. The K_d value for cilnidipine was 0.8 μM. Cilnidipine did not shift the current-voltage (*I*-V) relationship for HVA I_{Ba}, as regards the threshold potential and peak potential where the amplitude reached a maximum.

4 High concentrations of three hypotensive Ca^{2+} channel blockers, nifedipine, diltiazem and verapamil, all inhibited HVA I_{Ba} in a concentration-dependent manner. The K_d values for nifedipine, diltiazem and verapamil were 131, 151 and 47 μM, respectively. A piperazine-type Ca^{2+} channel blocker, flunarizine, showed a relatively potent blocking action on I_{Ba}. The K_d value was about 3 μM.

5 These results thus show that cilnidipine potently inhibits the sympathetic Ca^{2+} channels which predominantly consist of an ω-Cg-GVIA-sensitive component. This blockade of the N-type Ca^{2+} channel, as well as the L-type Ca^{2+} channel by cilnidipine suggests that it could be used therapeutically for treatment of hypersensitive sympathetic disorders associated with hypertension.

Keywords: Cilnidipine (FRC-8653); N-type Ca^{2+} channel; sympathetic neurones

Introduction

In excitatory cells such as smooth and cardiac muscle cells, and neurones, high voltage-activated (HVA) Ca^{2+} channels are well known to regulate a variety of cellular events, which include muscle contraction, neuronal electrical activity, the release of neurotransmitters and hormones as well as gene expressions (Tsien *et al*., 1988; Bean, 1989). The HVA Ca^{2+} channels are now classified into at least five different subtypes (L, N, P, Q and R-type) based on molecular biological and electrophysiological studies and are known to show tissue specific distributions (Tsien *et al*., 1991; Miller, 1992). The L-type, which is predominantly expressed in cardiac and smooth muscle cells, regulates muscle contraction and is therefore the target of organic Ca^{2+} channel blockers developed for the treatment of cardiovascular disease such as hypertension. The other four types are mainly localized in the peripheral and central nervous system. In particular, at the nerve endings of the sympathetic neurones, it has been well established that the N-type is localized and regulates cardiovascular functions via the release of catecholamines (Hirning *et al*., 1988).

Cilnidipine (FRC-8653) is a newly synthesized 1,4-dihydropyridine (DHP) type of organic Ca^{2+} channel blocker that has been developed as a slow-onset and long-lasting antihypertensive drug in Japan (Yoshimoto *et al*., 1991; Hosono *et al*., 1992). In man and rodents, cilnidipine depressed the pressor response to acute cold stress but failed to induce tachycardia evoked by hypotensive baro-reflexes (Saihara *et al*., 1993; Hosono *et al*., 1995c). In spontaneously hypertensive rats (SHRs) and dogs, vasoconstriction induced by electrical sympathetic nerve stimulation was also blocked by cilnidipine (Hosono *et al*., 1995b). In *in vitro* experiments, cilnidipine inhibited KCl-induced vasoconstriction and also inhibited [^{3}H]-noradrenaline (NA) release evoked by electrical stimulation in the rat aorta (Nakashima *et al*., 1991). Moreover, a recent electrophysiological study on rat sensory neurones revealed that cilnidipine blocked both the ω-conotoxin-sensitive and DHP-insensitive Ca^{2+} channels (Fujii *et al*., 1997). Recent clinical findings and basic observations thus suggest that this antihypertensive drug, cilnidipine, reduces blood pressure not only by blocking L-type Ca^{2+} channels in vascular smooth muscle but also depresses sympathetic nerve tonus by modulating the N-type Ca^{2+} channel in the endings of the sympathetic peripheral nervous system. However, a direct action of cilnidipine on the HVA Ca^{2+} channels in the sympathetic neurones has yet to be elucidated.

Some lines of evidence suggest that the Ca^{2+} channels of the superior cervical ganglion (SCG) neurones predominantly consist of ω-Cg-GVIA-sensitive channels and that the activation of the channel evokes catecholamine release (Hirning *et al*., 1988; Lipscombe *et al*., 1989). A wide variety of electrophysiological experiments to examine the function of N-type Ca^{2+} channel have, therefore, also been performed on the SCG neurone (Ikeda & Schofield, 1989; Plummer *et al*., 1989; Regan *et al*., 1991; Sah & Bean, 1994). In the present experiments, in order to elucidate further the inhibitory mechanism of cilnidipine on sympathetic nerve activity, the effects of cilnidipine on HVA Ba^{2+} current in freshly dissociated sympathetic neurones were investigated by using whole-cell patch recording under voltage-clamped conditions and then were compared with other organic Ca^{2+} channel blockers.

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Methods

Isolation of SCG neurones

Male Wister rats (4–6 weeks old) were decapitated after a lethal injection of sodium pentobarbitone (75 mg kg^{-1}). The superior cervical ganglia (SCG) 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 20 u ml^{-1} papain containing a Ca^{2+}-free Tyrode solution. The papain was then replaced by a mixture of 500 u ml^{-1} collagenase and 16 mg ml^{-1} dispase. Sixty minutes later, the ganglia was rinsed with the Ca^{2+}-free Tyrode solution and triturated gently through a small-pore glass pipette. The isolated SCG neurones were plated on a recording chamber (Falcon, 35 mm) and were used for recording up to 10 h after dissociation.

Solutions

The ionic composition of normal external solution was (in mM): NaCl 150, KCl 5, $MgCl_2$ 1, $CaCl_2$ 2, N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (HEPES) 10 and glucose 10. K^+-free external solution was (in mM): TEACl 130, CsCl 4, $MgCl_2$ 1, $BaCl_2$ 10, HEPES 10, glucose 10 and tetrodotoxin 0.001. The pH was adjusted to 7.4 with tris(hydroxymethyl)aminomethane (Tris)-OH. The conventional patch-pipette solution contained (in mM): CsCl 75, Cs-methanesulphonate 75, ATP-Mg 2 and HEPES 10. The pH was adjusted to 7.2 with Tris-OH.

Electrical measurements

All electrical measurements were performed by means of a conventional whole-cell patch recording under voltage-clamped conditions (Hamill *et al*., 1981; Uneyama *et al*., 1993a,b). The pipettes were pulled from 1.5 mm capillary glass (Narishige) in two stages by 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 10 kHz (NF Instruments). Then, analogue signals were reconverted into digital signals by an AD converter at a sampling frequency of 3 kHz after being refiltered at 1 kHz, and were stored in an IBM compatible computer (Deskpro, Compac) by using pClamp software (Axon Instruments). The capacitative and leak currents were subtracted by hyperpolarizing pulses. When the series resistance was measured, by a circuit for series resistance compensation system in the amplifier, the values ranged from 13 to 16 MΩ. The value of compensation of the series resistance was 30–80%. The value of the maximum voltage-clamp error was 4.8 mV. All experiments were performed at room temperature (20–22°C).

Drugs

ω-Conotoxin GVIA (ω-Cg-GVIA), ω-conotoxin-MVIIC (ω-Cg-MVIIC) and ω-agatoxin-GIVA (ω-Aga-IVA) were purchased from the Peptide Institute (Osaka, Japan) while calciseptine came from Research Biochemical Internationals (RBI). Nifedipine, diltiazem, verapamil, flunarizine and all other chemicals were obtained from Sigma (St. Louis, U.S.A.). 2-Methoxyethyl (E)-3-phenyl-2-propen-1-yl (±)-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl) pyridine-3,5-dicarboxylate (cilnidipine) was synthesized at the Ajinomoto central research laboratories (Kawasaki, Japan). All drugs were dissolved in external solution just before use. The drugs were applied by means of a rapid application method termed the 'Y-tube' method, as described elsewhere (Nakagawa *et al*., 1991; Uneyama *et al*., 1993a,b). By using this technique, the solution surrounding a dissociated neurone could be exchanged within 20 ms.

Data analysis

All results are presented as the mean ± s.e.mean. For the evaluation of the half-maximal effective concentration (EC_{50}) and Hill coefficient (n_H) of a concentration-response curve, the data were fitted to the Michaelis-Menten equation by a least-squares fitting, $I=(I_{max}\ C^n)/C^n+K_d^n)$ (Eqn. 1), where I is current, I_{max} is the maximum response, and C is the concentration of agonist. The equation for the concentration-inhibition curve is the mirror image of the Michaelis-Menton equation $I/I_{max}=1\text{-}C^n(K_d^n+C^n)$ (Eqn. 2) where C is the concentration of the antagonist. Assuming that the value of I_{max} is 1, then $I=1\text{-}C^n/(K_d^n+C^n)$ (Eqn. 3), $I=K_d^n/(K_d^n+C^n)$ (Eqn. 4). The data for the concentration-inhibition curve were fitted to Eqn. 4 by the use of the least-squares fitting to obtain the concentration for half-maximal inhibition.

Results

Characteristics of HVA Ca^{2+} channels in the rat SCG neuronal cell body

To isolate the HVA Ba^{2+} current (I_{Ba}) carried through the HVA Ca^{2+} channels, the dissociated SCG neurone was held at a holding potential (V_H) of −60 mV. At this potential, the

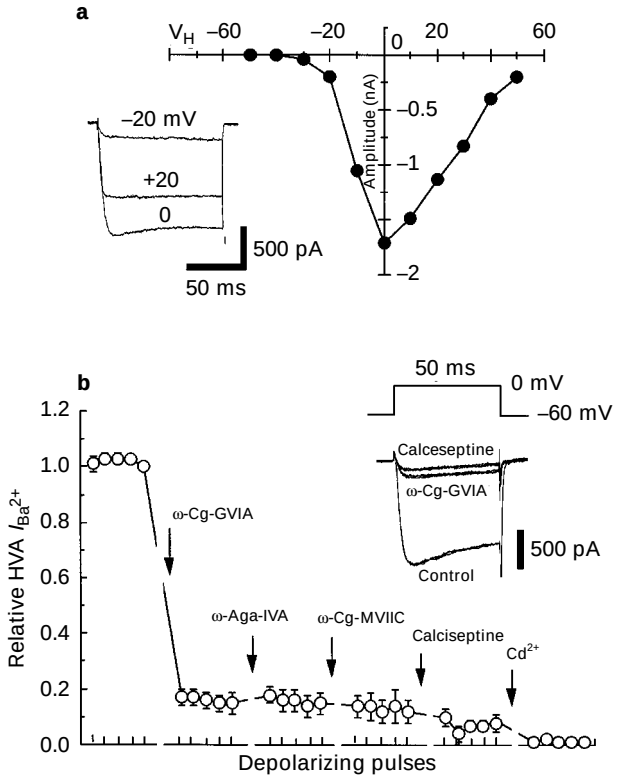

Figure 1 The HVA Ca^{2+} channel current in rat SCG neurones. (a) The current-voltage relationship for HVA I_{Ba}. HVA I_{Ba} was elicited by a 100 ms depolarizing pulse from −60 mV to various potentials every 15 s. The amplitude of I_{Ba} was measured at the peak. The figure is typical of three reproducible observations. (b) The effects of specific peptide inhibitors for various types of HVA Ca^{2+} channels. The neurone was held at a V_H of −60 mV and HVA I_{Ba} was elicited by 50 ms depolarizing pulses to 0 mV. Each drug was applied to the neurone 2 min before the subsequent five depolarizing pulses were applied every 15 s.

low-voltage activated Ca^{2+} channel was completely inactivated (Akaike *et al*., 1989). Figure 1a shows the current-voltage (*I*-V) relationship for HVA I_{Ba}. The HVA I_{Ba} was activated at potentials more positive than -40 mV and reached a maximum amplitude close to 0 mV. The HVA I_{Ba} had a rapid rising phase (<5 ms) and decayed in a time-dependent manner. The peak HVA I_{Ba} was fractionated into N, L, P, Q and R types by using selective peptide Ca^{2+} channel antagonists (Figure 2). The N-type current was defined as that blocked by 10^{-6} M ω-Cg-GVIA. L-type, P-type and Q-type currents were defined as those blocked by 3×10^{-6} M calciseptine, 10^{-7} M ω-Aga-IVA and 3×10^{-6} M ω-Cm-MVIIC, respectively (Ishibashi *et al*., 1995). Figure 1b shows the time course of the inhibitory action of the HVA I_{Ba} by each peptide. The depolarizing pulses of 50 ms duration to ±0 mV at a V_H of -60 mV were applied every 15 s. Consequently, calciseptine-sensitive L-, ω-Cg-GVIA-sensitive N-, ω-Aga-GIVA-sensitive P- and ω-Cm-MVIIC-sensitive Q-type components were $3\pm2\%$, $85\pm4\%$, $0\pm2\%$ and $3\pm2\%$, respectively. The component resistant to the four peptides was $9\pm3\%$ ($n=6$). The present HVA Ca^{2+} channels of the SCG neurones thus predominantly consisted of ω-Cg-IVA-sensitive N-type Ca^{2+} channels, as previously found (Hirning *et al*., 1988; Ikeda & Schofield, 1988; Plummer *et al*., 1989; Regan *et al*., 1991).

Block of HVA I_{Ba} *by cilnidipine*

At a concentration of 10^{-6} M, a new dihydropyridine (DHP)-type Ca^{2+} channel blocker, cilnidipine (FRC-8653), showed a potent blocking action on HVA I_{Ba}. The inhibitory effect of cilnidipine on HVA I_{Ba} was irreversible for at least 20 min after washing out the drugs (data not shown). Figure 2a shows a typical current trace of HVA I_{Ba} evoked by long-depolarizing pulses (800 ms) before and after the application of 1 μM cilnidipine. The percentage inhibition of the peak amplitude and pulse-end amplitude by cilnidipine was 55 ± 7 and $61\pm5\%$, respectively ($n=4$). Thus, the blocking potency of cilnidipine for the peak current was not significantly different from that for the pulse-end current. Next, the effects of cilnidipine on the current voltage (*I*-V) relationship for HVA I_{Ba} were examined (Figure 2b). Cells were depolarized for 100 ms from V_H of -60 mV to various potentials ranging from -50 mV to $+50$ mV (10 mV steps) every 15 s. Consequently, cilnidipine did not affect the threshold potential and the potential at which HVA I_{Ba} reached a maximum. In Figure 3, the concentration-dependent inhibitory effect of cilnidipine on the HVA I_{Ba} is shown. The cumulative application of cilnidipine inhibited HVA I_{Ba} in not only a concentration- but also a time-dependent manner. The K_d value and Hill coefficient (n_H) were 8.3×10^{-7} M and 0.53 ($n=7$), respectively.

As shown in Figure 1, the HVA I_{Ba} contains the residual currents that were not blocked by both ω-Cg-GVIA and calciseptine. Therefore, we examined the effects of cilnidipine on the residual currents. In this experiment, the N- and L-type current components of the HVA I_{Ba} were completely blocked by treating the cell with 1 μM ω-Cg-GVIA and 3 μM calciseptine. Consequently, as shown in Figure 4a, the residual currents were not inhibited by 1 μM cilnidipine but were completely blocked by the further administration of 100 μM Cd^{2+}. However, cilnidipine at 3 μM and 10 μM blocked the residual currents con-

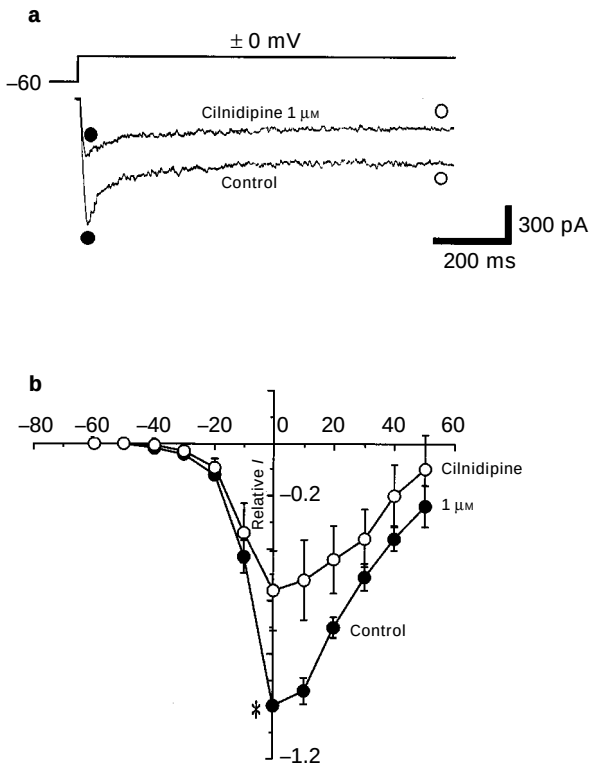

Figure 2 The effects of cilnidipine on HVA I_{Ba}. (a) A typical current trace of HVA I_{Ba} in the presence and absence of 1 μM cilnidipine. HVA I_{Ba} was elicited by 800 ms depolarizing pulses from a V_H of -60 mV to 0 mV. Cilnidipine was pretreated for 2 min just before stimulation. (b) The current (*I*)-voltage (V) relationship for HVA I_{Ba} in the presence and absence of 1 μM cilnidipine. HVA I_{Ba} was elicited by 50 ms depolarizing pulse from -60 mV to various potentials every 15 s. The amplitude of I_{Ba} was measured at the peak. All responses were normalized to the peak current amplitude at 0 mV (*) in the absence of cilnidipine. Each point and vertical lines represents the mean and s.e.mean, respectively, from 4 different experiments.

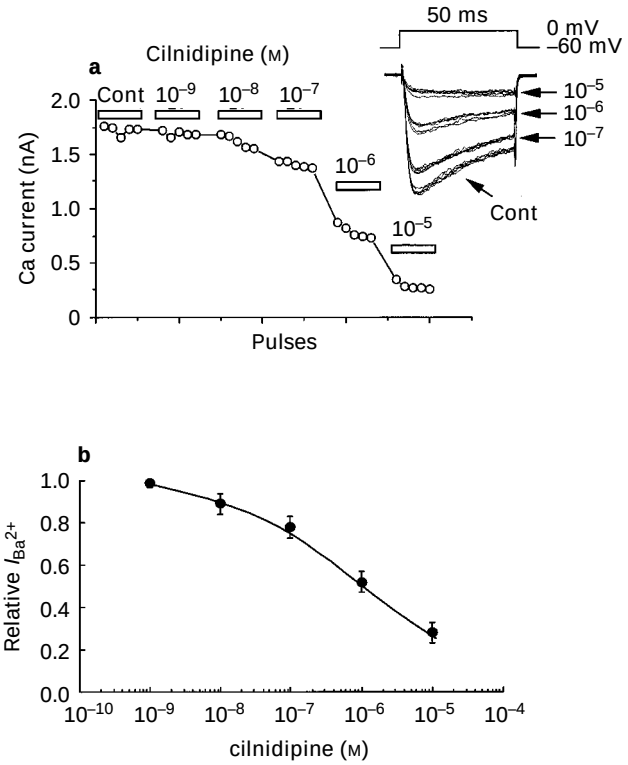

Figure 3 The effects of cilnidipine on HVA I_{Ba}. (a) The concentration- and time-dependent effects of cilnidipine. Neurone was held at a V_H of -60 mV and HVA I_{Ba} was elicited by 50 ms depolarizing pulses to 0 mV. Each concentration of cilnidipine was applied to the neurone 2 min before subsequent five depolarizing pulses were applied every 15 s. The amplitude of I_{Ba} was measured at the peak. All recordings were obtained from the same neurone. (b) Concentration-inhibition curve of cilnidipine for HVA I_{Ba}. Inhibitory effects of cilnidipine were evaluated when the maximum inhibition was attained by the 5th pulse. All responses were normalized to the peak current amplitude in the absence of cilnidipine. Each point and vertical lines represents the mean and s.e.mean, respectively, from 6 experiments.

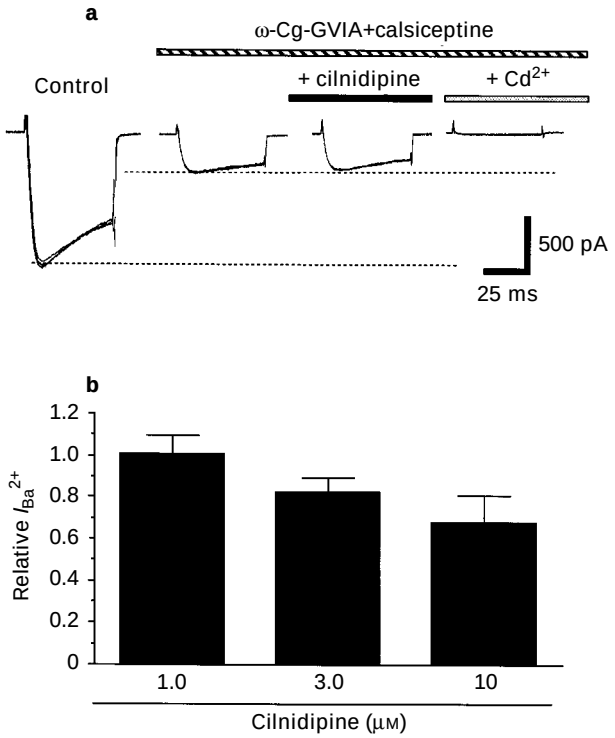

Figure 4 The effects of cilnidipine on the ω-Cg-GVIA- and calciseptine-resistant HVA I_{Ba}. (a) Effects of 1 μM cilnidipine and 100 μM Cd^{2+}. N-type and L-type current components were completely blocked by treatment with 1 μM ω-Cg-GVIA and 3 μM calciseptine. Each current trace was obtained from the same neurone. (b) Concentration-dependent effects of cilnidipine on the ω-Cg-GVIA- and calciseptine-resistant current. Each concentration of cilnidipine was applied to the neurone 2 min before subsequent five depolarizing pulses were applied every 15 s. The amplitude of I_{Ba} was measured at the peak. The current recorded before application of cilnidipine was normalized to 1.0. Each column and vertical lines represent the mean$\pm$s.e.mean from 4 experiments.

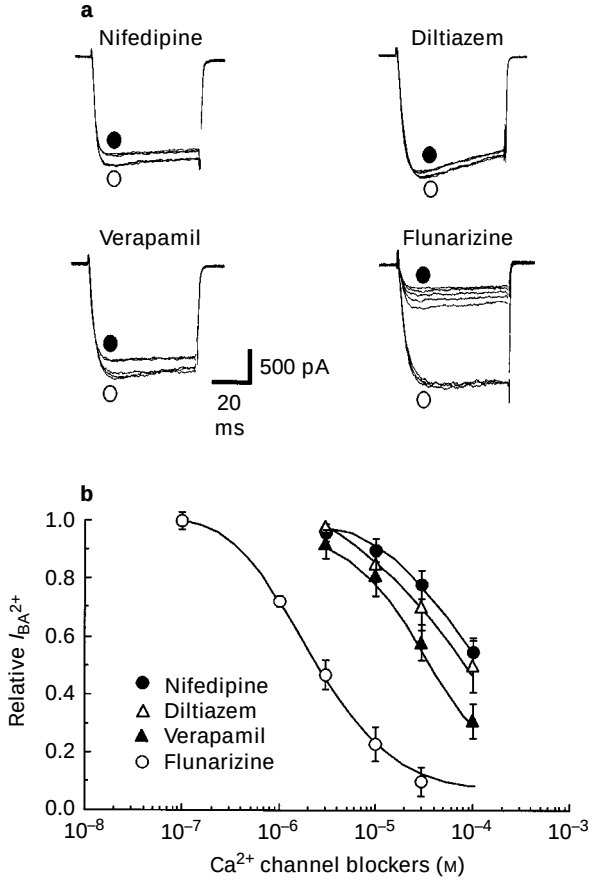

Figure 5 The effects of organic Ca^{2+} blockers on HVA I_{Ba}. (a) A typical current trace of HVA I_{Ba} in the presence (●) and absence (○) of blockers. HVA I_{Ba} was elicited by 50 ms depolarizing pulses from a V_H of -60 mV to 0 mV. Each blocker was pretreated for 2 min before five depolarizing pulses were subsequently applied every 15 s. (b) Concentration-inhibition curves for nifedipine, diltiazem, verapamil and flunarizine, of HVA I_{Ba}. The inhibitory effects of the blockers were evaluated when the maximum inhibition was attained by the 5th pulse. All responses were normalized to the peak current amplitude in the absence of each blocker. Each point and vertical lines represent the mean and s.e.mean, respectively, from 4 different experiments.

centration-dependently (Figure 4b). In this experiment, cilnidipine was applied to the SCG neurone after the N- and L-type components of the HVA I_{Ba} had been abolished by the peptide inhibitors. Thus, in rat SCG neurones, it is suggested that cilnidipine selectively inhibits the N-type current component of the HVA I_{Ba} at concentrations less than 1 μM.

Effects of classical organic Ca^{2+} channel blockers on HVA I_{Ba}

The effects of four classical types of organic Ca^{2+} channel blockers on HVA I_{Ba} were investigated. As shown in Figure 5a, at 1×10^{-5} M nifedipine (dihydropyridine), diltiazem (benzothiazepine) and verapamil (phyenylalkylamine) all slightly inhibited the HVA I_{Ba} (10 ± 4, 15 ± 6 and 19 ± 7% inhibition, respectively; $n=4$). In contrast, flunarizine (piperazine-type) 1×10^{-5} M strongly inhibited the HVA I_{Ba} (77 ± 6% inhibition; $n=4$). Nifedipine, diltiazem and verapamil acted as reversible inhibitors of HVA I_{Ba}, while flunarizine acted as an irreversible inhibitor (data not shown). However, at high concentrations, these blockers all inhibited HVA I_{Ba} in a concentration-dependent manner (Figure 5b). The K_d values (and Hill coefficients) for nifedipine, diltiazem, verapamil and flunarizine were 1.27×10^{-4} M (0.87), 9.47×10^{-5} M (0.81), 4.3×10^{-5} M (0.96) and 2.75×10^{-6} M (0.97), respectively.

Discussion

These experiments with peptide Ca^{2+} channel inhibitors clearly showed the subtype of the high voltage-activated Ca^{2+} channels in the somata of rat SCG neurones to be predominantly composed of the ω-Cg-GVIA-sensitive Ca^{2+} channel (N-type). Hypotensive organic L-type Ca^{2+} channel blockers (diltiazem, verapamil and nifedipine) at 10 μM also had little inhibitory effect on the HVA I_{Ba} (Figure 5a). Even under such experimental conditions, submicromolar concentrations of a novel DHP type of Ca^{2+} channel blocker, cilnidipine, clearly inhibited the HVA channels (Figure 3). Therefore, cilnidipine showed a stronger inhibitory action on the sympathetic ω-Cg-GVIA-sensitive Ca^{2+} channel than classical antihypertensive Ca^{2+} channel blockers.

Several drugs have been shown to inhibit N-type Ca^{2+} channels in SCG neurones (i.e., dopamine receptor antagonists such as fluspiriline and chlorpromazine, and recently developed Ca^{2+} antagonist such as fantfarone) (Sah & Bean, 1994; Romey & Lanzdunski, 1994). However, no drugs based on DHP derivatives have been previously studied in this preparation. We showed that all the drugs studied, except cilnidipine, failed to inhibit HVA I_{Ba} below concentrations of 3×10^{-7} μM, and their K_d values were more than several micromolar. The present results thus suggest cilnidipine to be a potent N-type Ca^{2+} channel blocker based on DHP derivatives. In fact, in receptor-binding experiments with rat brain membranes, 10^{-5} M cilnidipine has been shown to displace [^{3}H]-ω-Cg-GVIA binding by only 25% (Hosono *et al*., 1995). ω-Cg-GVIA is known to bind to the

third loop of the α subunit (Ellinor *et al*., 1994). Therefore, cilnidipine might bind to a site different from the binding region. The inactivation kinetics of HVA I_{Ba} were not affected by the presence of cilnidipine (Figure 2a). In a previous study, D_2 blockers such as chlorpromazine were found to accelerate I_{Ba} inactivation (Sah & Bean, 1994), thus suggesting that cilnidipine might also bind to a site different from that of D_2 blockers. Determination of the cilnidipine binding region might thus make it possible to design a new type of N-type Ca^{2+} channel blocker.

The present experiments showed that HVA Ca^{2+} channels can also be inhibited by various types of organic L-type Ca^{2+} channel blockers, including dihydropyridine (nifedipine), phyenylalkylamine (verapamil), benzothiazepine (diltiazem) and piperazine (flunarizine). The potency of the blocking action was cilnidipine > flunarizine > > diltiazem > verapamil > nifedipine. However, high concentrations (more than 10^{-5} M) of the compounds other than cilnidipine and flunarizine were needed to inhibit the HVA I_{Ba}. Flunarizine has been previously shown to inhibit Na^+ channels (Tytgat *et al*., 1990), T-type and L-type Ca^{2+} channels below concentrations of 10^{-6} M (Tytgat *et al*., 1990; Takahashi & Akaike, 1991) in cardiac and neuronal cells, thus indicating flunarizine to be a powerful non-selective channel blocker.

Cilnidipine inhibited HVA I_{Ba} even at 10^{-8} M and the K_d value was about 0.8 μM (Figure 3). In *in vitro* experiments, cilnidipine depressed NA release from vascular tissues at 10^{-7} M (Nakashima *et al*., 1991). In *in vivo* experiments, the oral administration (3 mg kg^{-1}, p.o.) of cilnidipine inhibited the hypotension-evoked baroreflexes and cold stress-evoked blood pressure elevation (Hosono *et al*., 1995c), under conditions where the plasma concentration of cilnidipine was estimated to reach more than several nanomolar (Matsuzawa *et al*., 1992). N-type Ca^{2+} channels have been thus proposed to play an important role in the release of noradrenaline from the sympathetic neurones (Hirning *et al*., 1988; Lipscombe *et al*., 1989). These observations thus suggest that the *in vivo* antisympathetic effects of cilnidipine are caused by it reducing NA release from the nerve endings of sympathetic neurones via blockade N-type Ca^{2+} channels.

N-type Ca^{2+} channels, which are widely distributed in both central and peripheral sympathetic neurones, might be involved in the neuronal regulation of cardiovascular functions (Touw *et al*., 1980; Fujita *et al*., 1993). In normotensive and spontaneously hypertensive rats (SHR), the baroreceptor-heart rate reflex evoked by an acute reduction of blood pressure could not be observed after a bolus injection of ω-Cg-GVIA (Pruneau & Angus, 1990; Pruneau & Belichard, 1992). This indicates that antihypertensive drugs demonstrating an N-type Ca^{2+} channel blockade might thus show baroreflex desensitization and direct sympatholytic effects. In addition, the hypotensive effects of ω-Cg-GVIA were stronger in SHR than normotensive rats (Pruneau & Belichard, 1992). These findings therefore strongly indicate an increased contribution of the sympathetic nervous system to the maintenance of elevated blood pressure and also the possible involvement of ω-Cg-GVIA-sensitive Ca^{2+} channels in hypertensive cardiovascular disorders. The successful therapeutic effect of cilnidipine on stress-mediated hypertension has already been confirmed in animals and man as previously mentioned.

In addition to such clinical benefits, N-type channel blockade by this drug could, possibly, evoke undesirable side effects, such as postural hypotension and CNS effects. However, in the rabbit model, administration of cilnidipine failed to induce postural hypotension even at doses which reduced the blood pressure (Hosono *et al*., 1995a). In man no differences, at present, have been found between cilnidipine and other DHP compounds as regards the appearance of these undesirable side effects. We need to perform further clinical and basic studies to clarify any undesirable actions mediated by N-type Ca^{2+} channel blockade. In contrast to these possible adverse reactions, N-type Ca^{2+} channel blockade might be also useful for protection against neuronal death in stroke, as described for the synthetic peptide N-type Ca^{2+} channel inhibitor, SNX-111 (Valentino *et al*., 1993). Many patients with hypertension develop stroke and suffer from recurrent ischaemia. We believe that L-type Ca^{2+} channel blockers which can also block N-type channels, such as cilnidipine, might be potentially useful in hypertension therapy as a prototype for the next generation of anti-hypertensive drugs.

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(Received January 2, 1997
Revised May 29, 1997
Accepted May 30, 1997)