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Effects of CP-060*S* on membrane channels in vascular smooth muscle cells from guinea pig

Yusuke Ohya *, Natsuko Adachi, Motoko Setoguchi, Isao Abe, Masatoshi Fujishima

Second Department of Internal Medicine, Faculty of Medicine, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-82, Japan

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Abstract

The newly developed cardioprotective drug, CP-060*S*, (−)-(*S*)-2-[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]-3-[3-[*N*-methyl-*N*-[2-(3,4-methylenedioxyphenoxy) ethyl] amino] propyl]-1,3-thiazolidin-4-one hydrogen fumarate, is reported to possess a vasodilating action. Our objective was to examine the effects of CP-060*S* on the membrane channels in mesenteric arterial cells from guinea pigs, using whole-cell patch-clamp techniques. CP-060*S* inhibited the Ca^{2+} channel current in a concentration-dependent manner (ED_{50} = 1.7 μM at a holding potential of −80 mV and a stimulation frequency of 0.1 Hz). The inhibition was potentiated by a more depolarized holding potential and a higher stimulation frequency. These effects of CP-060*S* resembled those of diltiazem and gallopamil more than to those of nifedipine; the inhibition was more frequency dependent and less holding-potential dependent than with nifedipine. Higher concentrations of CP-060*S* also inhibited the delayed K^+ channel currents (ED_{50} = 18 μM). The present observations suggest that CP-060*S* exhibits the profile of a Ca^{2+} channel antagonist, similar to that of diltiazem and gallopamil. © 1997 Elsevier Science B.V.

Keywords: Ca^{2+} channel; CP-060*S*; Smooth muscle, vascular; Electrophysiology; Voltage clamp; Ca^{2+} channel antagonist

1. Introduction

Ca^{2+} channel antagonists are used clinically as antihypertensive agents and antianginal agents. The various cardiovascular actions of different Ca^{2+} channel antagonists are partly attributable to their different voltage-dependent and frequency-dependent features for Ca^{2+} channel inhibition and to their different potencies for the inhibition of other channels such as K^+ channels (Lee and Tsien, 1983; Fleckenstein, 1983; Kuga et al., 1990; Hume, 1985). Therefore, the evaluation of these characteristics is important for the understanding of the in vivo action of each Ca^{2+} channel antagonist.

The newly synthesized cardioprotective drug, CP-060*S*, (−)-(*S*)-2-[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]-3-[3-[*N*-methyl-*N*-[2-(3,4-methylenedioxyphenoxy) ethyl] amino] propyl]-1,3-thiazolidin-4-one hydrogen fumarate, exhibits vasodilating and antiarrhythmic actions (Tamura et al., 1996; Koga et al., 1996). Since CP-060*S* shifts the concentration-response curve for Ca^{2+} to the right in the depolarized rat aortic strip, this agent is considered to cause vasodilatation via inhibition of Ca^{2+} channels, although its chemical structure differs from that of any known Ca^{2+} channel antagonist (Tamura et al., 1996).

The present study was performed to investigate the effects of CP-060*S* on Ca^{2+} channels, delayed-K^+ channels and Na^+ channels in vascular smooth muscle cells, since such information is not presently available. The modes of the inhibitory action of CP-060*S* on the Ca^{2+} channels, especially the voltage-dependent and frequency-dependent features, were also evaluated. The results were then compared with those obtained for the well-characterized Ca^{2+} channel antagonists, nifedipine, diltiazem, and gallopamil.

2. Materials and methods

2.1. Single-cell dispersion

Female guinea pigs (body weight 250–300 g) were anesthetized with ether and then decapitated. Single smooth muscle cells were obtained by collagenase treatment (collagenase; Wako, Tokyo, Japan) from the mesenteric arterial branch (diameter < 300 μm) and portal vein using

* Corresponding author. Tel.: (81-92) 642-5256; Fax: (81-92) 642-5271.

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methods described previously (for mesenteric artery: Ohya et al., 1993, for portal vein: Ohya and Sperelakis, 1991).

2.2. Electrical recordings

The conventional whole-cell voltage clamp was performed with a patch pipette through a voltage-clamp amplifier (Axopatch 1-D, Axon Instruments, Foster City, CA, USA), as previously described (Ohya et al., 1993; Setoguchi et al., 1995). Current recording was performed at room temperature (22–24°C). Membrane currents were low-pass filtered at 2 kHz, digitized with a sampling frequency of 5–10 kHz, and stored in a personal computer system for subsequent analysis. Command potentials were applied every 10 s, if not otherwise stated. Data were obtained after the current amplitude had been stabilized (usually 3–4 min after the whole-cell configuration was obtained). The Ca^{2+} channel current apparently did not run down over the next 15 min under these conditions (Ohya and Sperelakis, 1989a; Setoguchi et al., 1995). The liquid junction potential of 10 mV was corrected, and the leak and residual capacitive currents were subtracted using the P/4 protocol for the recordings of Ca^{2+} and Na^{+} channel currents.

2.3. Solutions and chemicals

To isolate the inward Ca^{2+} channel current or Na^{+} channel current, the pipette was filled with a high-Cs^{+} solution of the following composition (mM): Cs aspartate, 130; CsCl, 20; $ATPNa_2$, 3; $MgCl_2$, 3; EGTA, 10; HEPES, 10; pH 7.3 titrated with CsOH. The bath solution contained (mM): for the Ca^{2+} channel current, $BaCl_2$, 10; TrisCl, 140; glucose, 5.4; HEPES, 5; pH 7.3 titrated with CsOH; and for the Na^{+} channel current, NaCl, 150; KCl, 6; $CoCl_2$, 2; $MgCl_2$, 2; glucose 5.4; HEPES 5; pH 7.3 titrated with NaOH. For the recording of the K^{+} channel currents, the pipette solution contained (mM): KCl, 150; $MgCl_2$, 1; EGTA, 10; HEPES, 10; pH 7.3 titrated with KOH, and the bath solution was the same as that for the Na^{+} channel current except that 2 mM $CaCl_2$ was included instead of $CoCl_2$.

The drugs used were: CP-060*S* (a gift from Chugai, Tokyo, Japan); diltiazem (Calbiochem, San Diego, CA, USA); nifedipine (a gift from Bayer, Osaka, Japan); and gallopamil (D600) (Sigma, St. Louis, MO, USA). CP-060*S* and nifedipine were dissolved in 100% dimethyl sulphoxide (DMSO) and 100% ethanol, respectively, and diluted at least 1000 times when used. This concentration of DMSO or ethanol (below 0.1%) did not alter the currents. Gallopamil and diltiazem were dissolved in deionized water. Final drug concentrations are stated in the text.

2.4. Curve-fitting and statistics

Fitting of the data to each equation was performed using the non-linear least-squares method. The data were expressed as means ± S.E. Statistical significance was determined by means of Student's *t*-test (unpaired) or one-way analysis of variance. A P value of less than 0.05 was considered statistically significant.

3. Results

3.1. Effects on Ca^{2+} channels in mesenteric arterial cells

Fig. 1 shows the effects of CP-060*S* on Ca^{2+} channel currents in mesenteric arterial cells. Ca^{2+} channel currents

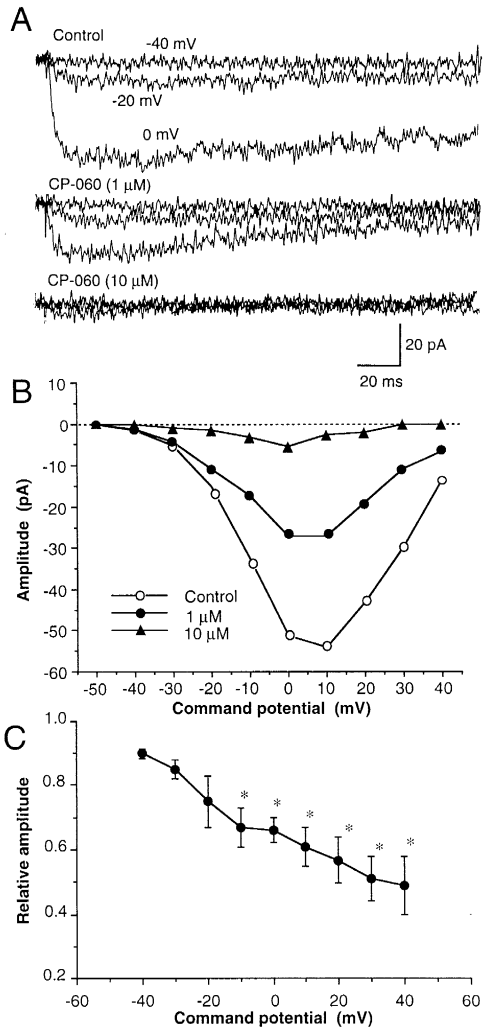

Fig. 1. Inhibitory effect of CP-060*S* on voltage-dependent Ca^{2+} channel currents recorded from mesenteric arterial cells, with the voltage-clamp technique. (A) Current traces obtained at command potentials of −40 mV, −20 mV, and 0 mV from a holding potential of −80 mV. (B) Amplitudes of the currents obtained in the absence (Control, open circle) and presence of CP-060*S* (closed circle, 1 μM; closed triangle, 10 μM), plotted against command potentials. (C) The current inhibition at various command potentials. The amplitudes at any given command potential in the absence of CP-060*S* are normalized to 1.0, and those observed at the same potentials in the presence of 1 μM CP-060*S* are expressed relative to the former amplitudes. Data are expressed as means ± S.E. from 5 cells. Bath filled with the Ba^{2+}-containing solution, and pipette contained the high-Cs^{+} solution. $^{*}P < 0.05$ compared with 1.0.

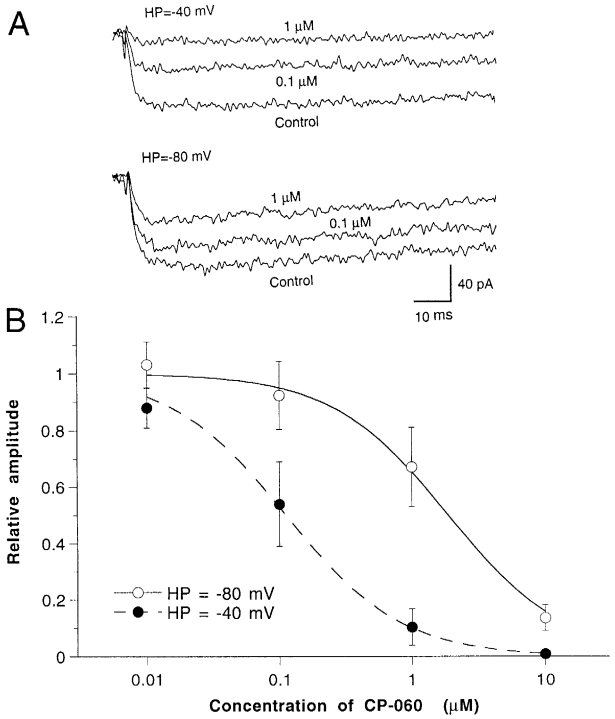

Fig. 2. Dose–response relationships of CP-060*S* action on the Ca^{2+} channel currents at holding potentials of −40 mV and −80 mV. (A) Currents recorded before (Control) and after the application of CP-060*S* (1 μM and 10 μM). Currents were evoked by a command potential of 10 mV from a holding potential (HP) of either −40 mV (upper panel) or −80 mV (lower panel). (B) The relative amplitudes of the Ca^{2+} channel currents are plotted against the CP-060*S* concentration (open circle, HP of −80 mV; closed circle, HP of −40 mV). Control amplitude (before application of the drug) was normalized to 1.0. A continuous curve was obtained by fitting the data to the Michaelis-Menten equation: $I_{drug}/I_{control} = 1/(1+[drug]/ED_{50})$, where I_{drug} is the current amplitude recorded with a given concentration of drug. $I_{control}$ is the current amplitude recorded before application of the drug, and ED_{50} is the drug concentration required for half-inhibition (0.11 μM with HP of −40 mV and 17 μM with HP of −80 mV). Each point represents the mean ± S.E. of 6–7 values.

were evoked by depolarizing command pulse to −40 mV or more. CP-060*S* inhibited the Ca^{2+} channel current in a concentration-dependent manner (Fig. 1A). The current-voltage curves before and after the application of CP-060*S* (1 μM and 10 μM) obtained from this cell are shown in Fig. 1B. The same experiments were performed on 5 cells, and the relative amplitudes with 1 μM CP-060*S* at given command potentials are shown in Fig. 1C. Greater inhibition was observed at the more depolarized command potentials ($P < 0.05$).

The dose–response relationship for the CP-060*S* action on Ca^{2+} channel currents is shown in Fig. 2. The current was evoked by a command potential of 10 mV from the holding potential of either −80 mV or −40 mV. The stimulation interval was 10 s. The inhibition was greater with the holding potential of −40 mV than with that of −80 mV. The concentrations for half-inhibition of the current (ED_{50}) with the holding potentials of −80 mV and −40 mV were 1.7 μM and 0.11 μM, respectively.

The effects of CP-060*S* on voltage-dependent inactivation of the Ca^{2+} channels were investigated using the double-pulse protocol. Fig. 3A shows the actual current traces with various holding potentials before and after the application of 1 μM CP-060*S*. The steady-state inactivation curves were obtained by plotting the amplitude against the conditioning potentials (Fig. 3B). CP-060*S* shifted the steady-state inactivation relationship in a negative direction; the V_h shifted by −16.4 mV.

Dissociation constants for the resting channels (K_R) and inactivated channels (K_I) were obtained according to the modulated-receptor hypothesis (Bean, 1984), i.e., the shift in V_h with the drug (ΔV_h) was as follows: $\Delta V_h = k \ln\{(1+[drug]/K_I)/(1+[drug]/K_R)\}$, where k was the Boltzman coefficient (6.1 mV) and [drug] was the drug concentration. In the present study, K_R was considered as the ED_{50} value obtained at a holding potential of −80 mV (1.7 μM). K_I was calculated as 0.04 μM from the above equation.

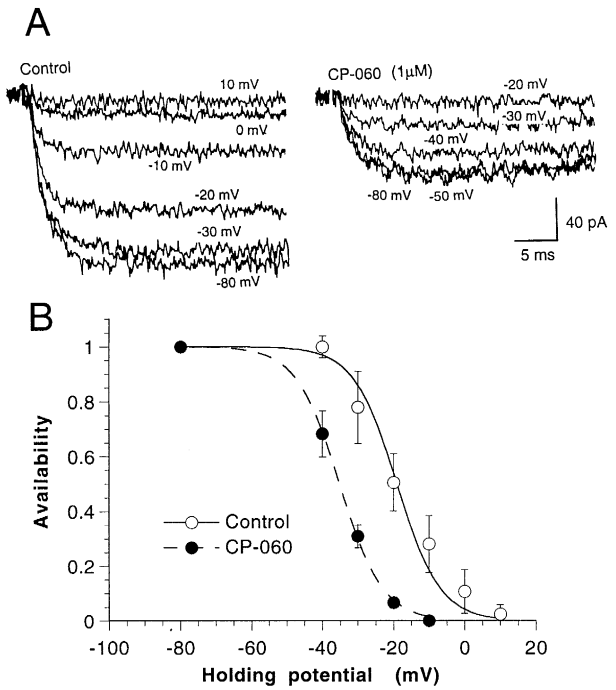

Fig. 3. Effects of holding potentials on the CP-060*S* action on the Ca^{2+} channels. (A) Current traces evoked by a test potential of 10 mV with various levels of the holding potential (duration of 10 s) in the absence (Control) or presence of 1 μM CP-060*S*, using the double-pulse protocol. The label for each tracing indicates the level of the holding potential. (B) The steady-state inactivation curves obtained in the absence (open circle) and presence of 1 μM CP-060S (closed circle). The normalized amplitudes of the test pulse (Availability) are plotted against the conditioning potential (Holding potential). The amplitude of the current evoked with a holding potential of −80 mV was defined as 1.0. Each point represents the mean ± S.E. of 4–6 values. The curves were obtained by fitting data to the Boltzman distribution: $P = 1/[1+\exp\{(V-V_h)/k\}]$, where P is the availability, V is the conditioning potential, V_h is the potential required for half-inhibition of the current, and k is the Boltzman coefficient. The V_h of control and CP-060S were −19.0 mV and −35.2 mV, respectively, and the k value was 6.1 mV.

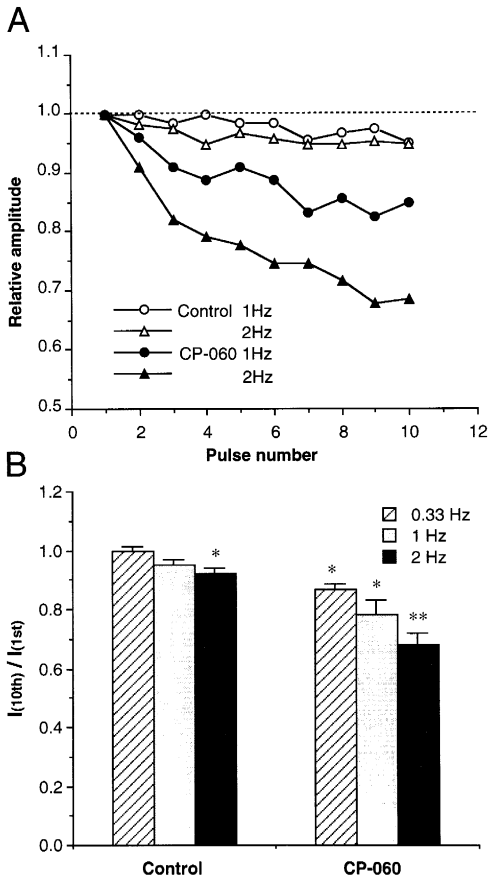

Fig. 4. Effect of repetitive depolarizations on the CP-060*S* action on the Ca^{2+} channels. (A) The relative amplitudes of the current during repetitive stimulation at frequencies of 1 Hz (circle) and 2 Hz (triangle) before (open symbols) and after the application of CP-060*S* (1 μM; closed symbols) are shown. The amplitude of the current evoked by the first pulse was normalized to 1.0. The current was elicited by a command potential of 10 mV (duration of 100 ms) from a holding potential of −80 mV. (B) Relative amplitudes of the current evoked by the 10th pulse in the repetitive depolarizations (I_{10}/I_1) at 0.33 Hz (hatched bar), 1 Hz (shaded bar), and 2 Hz (closed bar) in the absence or presence of 1 μM CP-060S. Data are expressed as means ± S.E. of 4–5 experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. 1.0.

To evaluate the frequency-dependence feature of the CP-060*S* action, the inhibition was tested during repetitive stimulation at various frequencies. Fig. 4A depicts typical results at the stimulatory frequencies of 1 and 2 Hz. Before application of the drug (Control), the repetitive stimulations caused only slight inhibition of the amplitude. With 1 μM CP-060*S*, the current inhibition showed an accumulation during the repetitive stimulations at both frequencies; however, the higher frequency caused the greater inhibition. The frequency-dependent inhibition was evaluated from the current amplitude obtained at the 10th stimulation (Fig. 4B). With CP-060*S*, the amplitude at the 10th stimulation was significantly smaller than that at the 1st stimulation for all frequencies used ($P < 0.05$). In addition, this accumulation of the inhibition was significantly greater at a higher frequency ($P < 0.05$).

The effects of CP-060*S* on the decay of the Ca^{2+} channel current were also examined (Fig. 5). To emphasize the difference in the current decay, the after-drug trace was magnified so that peak amplitudes of the traces before and after drug are matched (Fig. 5A). CP-060*S* accelerated the decay of the Ba^{2+} current. To clarify the change in the current decay further, the amplitude of the current at 100 ms relative to the peak amplitude ($I_{100\,ms}/I_{peak}$) was compared before and after the application of 1 μM CP-060*S* in 5 cells (Fig. 5B). The relative amplitude at 100 ms was significantly smaller with CP-060*S* than that without drug ($P < 0.05$).

3.2. Effects on the delayed-K^+ channels in mesenteric arterial cells

The effects of CP-060*S* on the K^+ channels were also examined in mesenteric arterial cells (Fig. 6A and Fig. 7). The major component of the outward current was a delayed K^+ current, that appeared as a sustained component when evoked by the depolarizing command pulse to −30 mV or more. On the other hand, the Ca^{2+}-dependent K^+ current was decreased by 10 mM EGTA in the pipette solution. The application of 20 μM CP-060*S* nearly halved

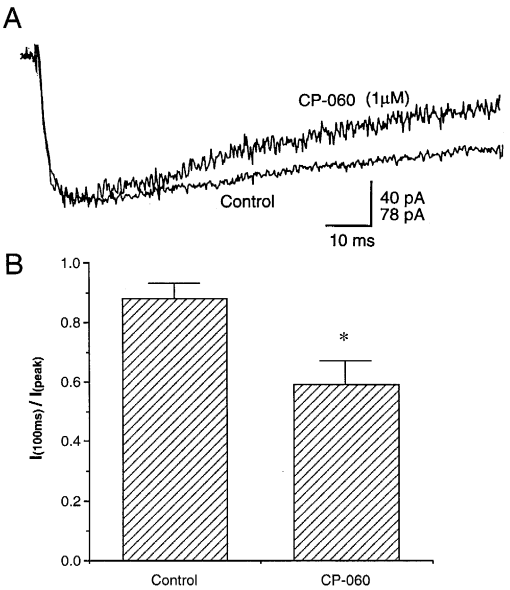

Fig. 5. Effects of CP-060*S* on the decay of the Ca^{2+} channel currents in mesenteric arterial cells. (A) Comparison of the current decay over 100 ms in the absence or presence of 1 μM CP-060*S*. The drug trace is magnified so that the peak amplitudes of the control current and the drug current are matched. The vertical scale for the control currents is 40 pA and that for CP-060*S* is 78 pA. Current was evoked by a command potential of 10 mV from a holding potential of −80 mV. (B) The amplitude of Ba^{2+} currents measured at 100 ms, which were normalized to their corresponding peak amplitudes ($I_{100\,ms}/I_{peak}$) (mean ± S.E., $n = 5$). $^{*}P < 0.05$ vs. control.

the delayed K^+ currents (Fig. 6A). The ED_{50} obtained at the command potential of 20 mV was 18 μM ($n = 5$) (Fig. 7).

3.3. Effects on voltage-dependent Na^+ channels in portal venous cells

Since mesenteric arterial cells do not have voltage-dependent Na^+ channels, the action of CP-060*S* on voltage-dependent Na^+ channels was examined in portal venous cells (Fig. 6B and Fig. 7). The voltage-dependent Na^+ channel currents were evoked by depolarizing command pulses to −40 mV and more, from a holding potential of −80 mV. The Ca^{2+} channel current was abolished by replacing Ca^{2+} with Co^{2+} in the bath solution. This Na^+ channel was sensitive to tetrodotoxin, and was similar to that in rat myometrial cells (Ohya and Sperelakis, 1989b) and rabbit pulmonary arterial cells (Okabe et al., 1987). The application of CP-060*S* inhibited the Na^+ channel currents. The ED_{50} value for Na^+ channels obtained at a command potential of −10 mV was 2.8 μM ($n = 5$) (Fig. 7).

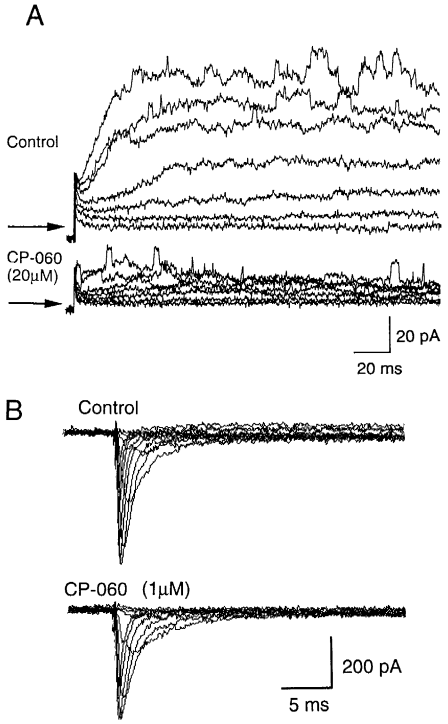

Fig. 6. Effect of CP-060*S* on the delayed-K^+ channel currents in mesenteric arterial cells (A) and on the voltage-dependent Na^+ channel currents in portal venous cells (B). A: K^+ channel currents recorded before (Control) and after application of 20 μM CP-060*S*. The currents were evoked by the command pulse to between −20 and 40 mV in a 10-mV increment. The holding potential was −60 mV. The pipette contained high-K^+ solution, and the bath contained physiological salt solution. Leak subtraction was not performed. The arrow indicates the zero current level. B: Na^+ channel currents evoked by command pulses to between −50 mV and 50 mV in a 10-mV increment before (Control) and after the application of 1 μM CP-060*S*. The holding potential was −80 mV. The bath solution was a physiological salt solution with $CoCl_2$ instead of $CaCl_2$. The pipette solution contained high-Cs^+ solution. Cells with short lengths (< 80 μm) were used in the experiments for Na^+ channels.

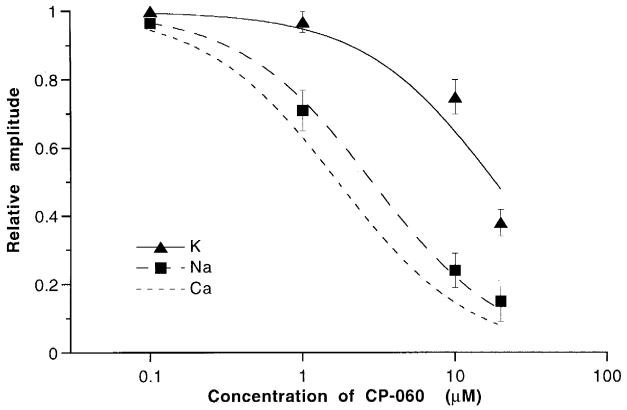

Fig. 7. Dose–response relationships for the inhibitory action of CP-060*S* on delayed-K^+ channels (closed triangle) and Na^+ channels (closed square). The relative amplitudes of the delayed-K^+ channel current evoked by a command potential of 20 mV and those of the Na^+ channel current evoked by a command potential of −10 mV are plotted against the CP-060*S* concentration. Control amplitude (before application of CP-060*S*) was normalized to 1.0. Continuous curves were obtained by fitting the data to the Michaelis-Menten equation as in the legend to Fig. 2B (the ED_{50} was 18 μM for the delayed-K^+ channels and 2.8 μM for the Na^+ channels). Each point represents the mean ± S.E. of 4–6 values. The dose-response curve for Ca^{2+} channels at a holding potential of −80 mV (obtained in Fig. 2B) is superimposed for reference.

4. Discussion

The main finding in the present study was that CP-060*S* inhibited the Ca^{2+} channel currents recorded from arterial smooth muscle cells. The mode of the inhibitory action of this drug was basically the same as that of other organic Ca^{2+} channel antagonists (Lee and Tsien, 1983; Terada et al., 1987a; Kuga et al., 1990; Setoguchi et al., 1995), i.e., these drugs inhibit the current in a frequency-dependent fashion (use-dependent block), shift the steady-state inactivation curve in a negative direction, and accelerate the decay of the Ba^{2+} current. This inhibitory effect of CP-060*S* on the Ca^{2+} channels would explain its vasodilator action (Tamura et al., 1996). In addition, CP-060*S* inhibited the delayed-K^+ channel currents with a relatively low potency, and inhibited the voltage-dependent Na^+ channel currents with approximately the same potency as that required for the Ca^{2+} channels.

CP-060*S* shifted the steady-state inactivation curve in a negative direction. Thus, the inhibition was more potent at more depolarized holding potentials. This result could be explained by postulating that CP-060*S* binds with high affinity to the inactivated channels and with low affinity to the resting channels, according to the modulated receptor hypothesis (Bean, 1984). The estimated dissociation constants of CP-060*S* for resting channels (K_R) and inacti-

vated channels (K_I) were 1.7 μM and 0.04 μM, respectively. Hence, CP-060*S* has an about 40-fold higher affinity to the inactivated channels than to the resting channels.

CP-060*S* exhibited a frequency-dependent inhibition of Ca^{2+} channels. In addition, CP-060*S* accelerated the decay of the Ba^{2+} current. Since these results are interpreted as resulting from the higher affinity of the drug for the open channels, we consider that CP-060*S* also possesses a high affinity for the open channels.

The inhibition of Ca^{2+} channel currents by CP-060*S* was greater with the depolarized command potentials. This command-potential dependence may be a result of the voltage-dependent nature of CP-060*S* action on Ca^{2+} channels. This observation may also be explained by the presence of T-type currents which are resistant to Ca^{2+} channel antagonists. However, we did not examine the action of CP-060*S* on the isolated T-type current, because the amplitude of the T-type currents is very small in the guinea-pig mesenteric artery (Ohya and Sperelakis, 1989a).

The inhibitory action of CP-060*S* on Ca^{2+} channel currents was compared with that of other types of Ca^{2+} channel antagonists, nifedipine (dihydropyridine), diltiazem (benzothiazepine), and gallopamil (phenylalkylamine). Table 1 summarizes the characteristics of the action of CP-060*S*, nifedipine, diltiazem and gallopamil, all determined on the same preparation, single cells from guinea-pig mesenteric arteries. The data for ΔV_h and K_R of nifedipine and diltiazem are cited from our previous study (Setoguchi et al., 1995). The potency to inhibit Ca^{2+} channels was as follows: nifedipine > CP-060*S* ≥ gallopamil ≥ diltiazem, as assessed by K_R and K_I. The potency to shift the steady-state inactivation curve was as follows: nifedipine > diltiazem ≥ CP-060*S* ≥ gallopamil. The potency for causing the frequency-dependent inhibition was as follows: CP-060*S* = diltiazem = gallopamil > nifedipine. Accordingly, the inhibitory action of CP-060*S* on Ca^{2+} channels resembled that of diltiazem and gallopamil more than that of nifedipine.

Table 1
Comparison of inhibitory actions of CP-060*S*, nifedipine, diltiazem and gallopamil on Ca^{2+} channels

	CP-060*S*	Nifedipine	Diltiazem	Gallopamil
ΔV_h (mV)	−17.4	−24.1 [a]	−19.3 [a]	−16.6
K_R (μM)	1.7	0.17 [a]	65.0 [a]	27.4
K_I (μM)	0.04	0.002 [a]	1.2 [a]	1.6
I_{10}/I_1 (1 Hz)	0.78	0.95	0.84	0.77
(2 Hz)	0.68	0.86	0.70	0.70

[a] Data are cited from Setoguchi et al. (1995). ΔV_h, the shift in the holding potential for half-inhibition of the current in the steady-state inactivation curve; K_R, dissociation constant for the resting channels; K_I, dissociation constant for the inactivated channels; I_{10}/I_1, relative amplitude of the current evoked by the 10th pulse, normalized by that evoked by the 1st pulse in the repetitive stimulation. We used the ED_{50} obtained with a holding potential of −80 mV as the value for K_R. The K_I was obtained from the following equation: $\Delta V_h = k \ln\{(1+[drug]/K_I)/(1+[drug]/K_R)\}$, where k is the Boltzman coefficient obtained in the steady-state inactivation.

Ca^{2+} channel antagonists were initially defined as drugs that selectively block Ca^{2+} channels but not Na^+ channels. Later studies showed that several Ca^{2+} channel antagonists also inhibit Na^+ channels and K^+ channels (Hume, 1985; Terada et al., 1987b; Bustamane, 1985). Fleckenstein (1983) classified Ca^{2+} channel antagonists into two groups according to this selectivity: one is highly selective for Ca^{2+} channels, and the other inhibits Na^+ channels as well as Ca^{2+} channels. Terada et al. (1987b) examined the selectivity of various Ca^{2+} channel antagonists for Ca^{2+} channels against K^+ channels in guinea-pig intestinal smooth muscle cells, using a ratio of the ED_{50} for K^+ channels to that for Ca^{2+} channels. These authors reported that diltiazem and verapamil had a relatively lower selectivity (the ED_{50} ratio was 10 to 20) than had nicardipine (the ratio was about 200). Okabe et al. (1987) also reported that CV-4093, one of the dihydropyridine derivatives, had a high selectivity for Ca^{2+} channels against K^+ channels in rabbit pulmonary arterial cells (the ratio was about 3000). In the present study, the ratio of CP-060*S* was about 10. Thus, the selectivity of CP-060*S* for Ca^{2+} channels over K^+ channels is as low as that of verapamil and diltiazem. The inhibition of K^+ channels may provide additional cardiovascular effects by modulating the resting membrane potential and /or changing the repolarization.

We also demonstrated that CP-060*S* inhibited venous Na^+ channels with approximately the same potency as that required for Ca^{2+} channels. This finding indicates that CP-060*S* can be classified as the second type of Ca^{2+} channel antagonist, as described by Fleckenstein (1983). Since the involvement of Na^+ channels in the contraction of vascular smooth muscle cells is unknown, the hemodynamic implications of this Na^+ channel inhibition in vascular smooth muscle cells are not clear. In contrast, the inhibitory action of this drug on the cardiac channels that include the Na^+ channel is of great interest, since this drug has been reported to prevent the veratridine-induced Ca^{2+} overload and contraction in isolated guinea-pig cardiomyocytes, and to exhibit an antiarrhythmic action in anesthetized rats (Koga et al., 1996; Tamura et al., 1996).

In conclusion, CP-060*S* exhibited the profile of a Ca^{2+} channel antagonist in the voltage-clamp study. The mode of the inhibitory action of CP-060*S* on Ca^{2+} channels was closer to that of diltiazem and gallopamil than to that of nifedipine. The inhibition by this drug was more frequency dependent, less holding-potential dependent, and less selective for Ca^{2+} channels over other channels than the inhibition with nifedipine.

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