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Pranidipine enhances relaxation produced by endothelium-derived relaxing factor in carotid artery

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Abstract

The effects of pranidipine, a novel dihydropyridine-type Ca^{2+}-channel antagonist, on acetylcholine-induced endothelium-dependent relaxation were investigated in isolated carotid artery of the guinea-pig. In arteries contracted with high-K^+ solution ($[K^+]_0 = 28.8$ mM) containing noradrenaline, the relaxation was inhibited by N^{ω}-nitro-L-arginine, indicating an involvement of endothelium-derived relaxing factor. Pranidipine (10^{-9}–10^{-7} M) augmented the relaxation in a concentration-dependent manner. Sodium nitroprusside produced a relaxation in arteries contracted with high-K^+ solution containing noradrenaline, in an endothelium-independent manner, and the relaxation was enhanced by pranidipine. 1*H*-[1,2,4] oxadiazolo [4,3-*a*] quinoxalin-l-one (ODQ), an inhibitor of nitric oxide-sensitive guanylate cyclase, attenuated the relaxation produced by acetylcholine or sodium nitroprusside. In the presence of ODQ, pranidipine did not enhance the acetylcholine-induced relaxation. The relaxation produced by endothelium-derived hyperpolarizing factor was inhibited by pranidipine, with no alteration of the hyperpolarization. Thus, pranidipine augments the nitric oxide-induced relaxation, possibly by enhancing the mechanisms related to cyclic GMP.

Keywords: Carotid artery, (guinea-pig); Relaxation; EDRF (endothelium-derived relaxing factor); Ca^{2+}-channel antagonist; cGMP

1. Introduction

Most vascular smooth muscles relax in response to acetylcholine indirectly by the release of relaxing factors from endothelial cells (Furchgott, 1983). The factors involve mainly three substances, endothelium-derived relaxing factor (EDRF), endothelium-derived hyperpolarizing factor (EDHF) and prostacyclin (Moncada et al., 1991). EDRF is possibly nitric oxide which induces vasodilatation through enhanced production of cyclic GMP (Ignarro et al., 1987; Moncada et al., 1991). EDHF relaxes vascular smooth muscle by hyperpolarizing the membrane through activation of K^+-channels (Chen et al., 1988; Suzuki and Chen, 1990). Prostacyclin elevates production of cyclic AMP and relaxes vascular smooth muscle (Moncada et al., 1991). The production of these endothelial factors requires an increase in Ca^{2+} in endothelial cells (Moncada et al., 1991). The membrane of endothelial cells does not possess functional voltage-sensitive Ca^{2+}-channels, and as a consequence depolarization of the membrane by high K^+ solution actually reduces the influx of Ca^{2+} due to a reduced potential gradient across the membrane (Laskey et al., 1990). In fact, many types of Ca^{2+}-channel antagonist are unable to modulate the EDRF-induced relaxation (Furchgott, 1983; Griffith et al., 1986).

Pranidipine is a novel dihydropyridine-type Ca^{2+}-channel antagonist and possesses long-term anti-hypertensive and anti-anginal actions (Nakayama et al., 1990, 1991). This Ca^{2+}-channel antagonist enhances endothelium-dependent relaxation in the rat thoracic aorta, and this property differs from other Ca^{2+}-channel antagonists such as nifedipine, verapamil and diltiazem (Nakayama et al., 1993; Hirano et al., 1997; Mori et al., 1998). The properties of vascular smooth muscle differ between regions and species, therefore attempts were made to observe whether endothelium-dependent relaxation in the guinea-pig carotid artery is also affected by pranidipine.

The carotid artery of guinea-pig produces an endothelium-dependent relaxation in response to acetylcholine, and the factors involved are EDRF and EDHF (Zhang et

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al., 1994). The EDRF-induced relaxation could be produced in muscles contracted with high K^+ solution after preventing the EDHF-induced component of the relaxation (Chen and Suzuki, 1989). The EDHF-induced relaxation could be isolated after inhibiting the production of EDRF and prostacyclin with N^{ω}-nitro-L-arginine and indomethacin, respectively (Suzuki et al., 1992). The experiments were carried out to observe the effects of pranidipine on relaxation produced by EDRF and EDHF, and also on the EDHF-induced hyperpolarization produced by acetylcholine in the guinea-pig carotid artery. The results indicate that pranidipine enhanced the EDRF-induced component and inhibited the EDHF-induced component of the acetylcholine-induced relaxation, with no alteration of the acetylcholine-induced hyperpolarization. Possible involvement of cyclic GMP in the augmenting actions of pranidipine on relaxation mediated by nitric oxide was considered.

2. Materials and methods

2.1. Preparation

Male guinea-pigs, weighing 250–300 g, were anesthetized with ether and then exsanguinated by cutting the femoral artery. The protocols used conformed with guidelines on the conduct of animal experiments issued by government, and were approved by The Committee on the Ethics of Animal Experiments in Nagoya City University Medical School. Common carotid arteries were dissected and cleaned by removing surrounding connective tissues in Krebs' solution at room temperature. In some experiments, the endothelial cells were removed mechanically by rubbing the internal surface of the artery with stainless steel wire. The successful removal of functional endothelial cells from the artery was confirmed by the absence of electrical and mechanical responses to acetylcholine (10^{-6} M) or substance P (10^{-7} M), agonists which produce endothelium-dependent responses in this artery (Zhang et al., 1994).

2.2. Recording methods for electrical responses

Electrical responses of smooth muscle cells elicited by acetylcholine were recorded from the carotid artery using conventional microelectrode techniques. The artery, 1–1.5 cm long, was immobilized on a rubber plate which was fixed at the bottom of the recording chamber, and superfused with warmed (35°C) Krebs' solution at a constant flow rate of about 3 ml/min. Glass capillary microelectrodes with a tip resistance of 50–80 MΩ were used to impale the smooth muscle cells from the adventitial side.

2.3. Recording methods for mechanical responses

Mechanical responses were measured isometrically in ring preparations of the carotid artery. Isolated ring segments of the artery with a length of about 1 mm were mounted between a pair of stainless wires with diameter of about 50 μm inserted into the lumen of the artery; one wire was fixed at the bottom and the other was connected to the lever of a mechano-transducer (FD-pick up, TB-612T, Nihon-kohden, Tokyo, Japan). The contractile forces of the ring segment due to contraction of the circular muscle were measured isometrically. The ring segment was placed under a resting tension of about 0.1 g, and was perfused with oxygenated warmed (35°C) Krebs' solution at a constant flow rate of about 3 ml/min. After stabilization, the rings were stimulated with solution containing high K^+ ($[K^+]_0 = 28.8$ mM) several times until the peak amplitude of contraction reached a steady value (usually three times). In the measurement of mechanical responses, the Ca^{2+}-channel antagonistic actions of pranidipine remained effective even after removal of pranidipine from the superfusate for several hours. The successful removal of the effects of previously used pranidipine from the recording system required washing with 99.5% ethanol for 30 min and then further washing with distilled water for 4–6 h. These washing procedures were applied at the end of every experiment.

2.4. Solutions

The ionic composition of the Krebs' solution was as follows (in mM); Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, HCO_3^- 15.5, $H_2PO_4^-$ 1.2, Cl^- 134, glucose 11.5. High K^+ solution containing 28.8 mM $[K^+]_0$ (high-K^+ solution) was prepared by replacing NaCl with KCl isoosmotically. The solution was aerated with O_2 containing 5% CO_2. The pH of the solution was 7.2–7.3.

2.5. Drugs

Drugs used were acetylcholine chloride, noradrenaline hydrochloride (Sigma, St. Louis, USA), pranidipine (Otsuka Pharmac., Tokyo, Japan), N^{ω}-nitro-L-arginine (Peptide Institute, Osaka, Japan), indomethacin (Sigma), and 1 *H*-[1,2,4] oxadiazolo [4,3-*a*] quinoxalin-1-one (ODQ) (Research Biochem. Int., MA, USA). These drugs were dissolved in distilled water (acetylcholine, noradrenaline, N^{ω}-nitro-L-arginine), 5×10^{-3} M Na_2CO_3 solution (indomethacin) or dimetyl sulfoxide (ODQ, pranidipine), and added to the Krebs' solution to obtain the desired concentration. The final concentration of these solvents in the Krebs' solution did not exceed 1:1000, and no alteration of the pH of the solution was detected following these procedures.

2.6. Statistics

Data obtained were expressed by the mean ± standard deviation (S.D.). Statistical significance of the values was

tested using Student's *t*-test, and probabilities of less than 5% ($P < 0.05$) were considered significant.

3. Results

3.1. Effects of pranidipine on contractions produced by noradrenaline and high-K^+ solution

Ring preparations of guinea-pig carotid artery were contracted by high-K^+ solution ($[K^+]_0 = 28.8$ mM) or high-K^+ solution containing 10^{-6} M noradrenaline. Each of these solutions produced a contraction which reached a stable value within 10 min; the amplitude produced by high-K^+ solution containing noradrenaline was 1.42 ± 0.20 times ($n = 28$) that produced by high-K^+ solution alone. The effects of pranidipine on these contractions were observed after the arteries had been pretreated with the drug for at least 30 min. Pranidipine (10^{-7} M) inhibited the contractions produced by both high-K^+ solution and high-K^+ solution containing noradrenaline (Fig.1). The summarized data indicated that significant inhibition by pranidipine of contractions produced by high-K^+ solution or high-K^+ solution containing noradrenaline appeared at concentrations of 10^{-8} M and reached about 60% inhibition at 10^{-7} M (Fig. 2).

3.2. Effects of pranidipine on acetylcholine-induced relaxation

The acetylcholine-induced relaxation was produced in ring preparations of the guinea-pig carotid artery contracted by high-K^+ solution containing noradrenaline or by solution containing noradrenaline alone, in the absence or presence of 10^{-5} M N^{ω}-nitro-L-arginine and 5×10^{-6} M indomethacin, the objective being to generate relaxation mainly by EDRF or EDHF, respectively. In muscles contracted by high-K^+ solution containing noradrenaline,

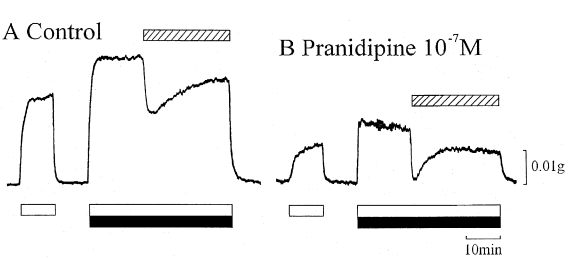

Fig. 1. Effects of pranidipine on mechanical responses produced by high-K^+ solution, noradrenaline and acetylcholine in ring preparation of the carotid artery. Ring preparation of the guinea-pig carotid artery was stimulated by high-K^+ solution (open column) and 10^{-6} M noradrenaline (filled column) separately or together, before (A) and after application of 10^{-7} M pranidipine (B). Acetylcholine (10^{-6} M) was applied for 25 min during stimulation with high-K^+ solution containing noradrenaline (oblique line column). A and B were recorded from the same tissue.

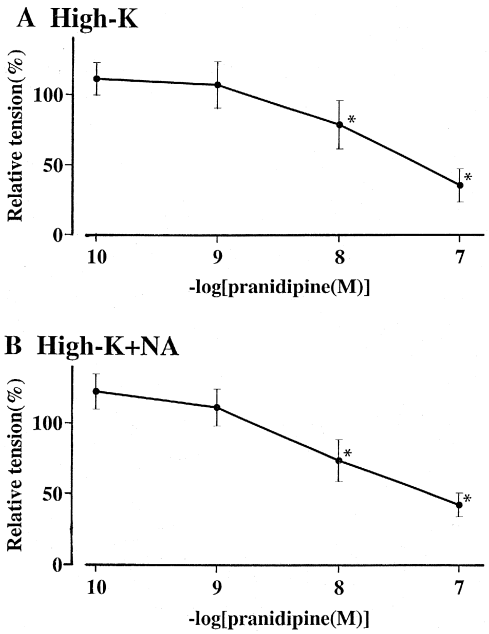

Fig. 2. Effects of pranidipine on contractions produced by high-K^+ solution with or without noradrenaline. Ring preparation of the guinea-pig carotid artery was contracted by high-K^+ solution (High-K, A) or High-K^+ solution containing 10^{-6} M noradrenaline (B, High-K^+ NA), in the presence of various concentrations (10^{-10}–10^{-7} M) of pranidipine. Preparation was pretreated with pranidipine for at least 30 min before application of stimulants, and peak amplitude of contractions relative to those before application of pranidipine was measured. Mean ± S.D. ($n = 14$). *, significant difference from control.

acetylcholine (10^{-6} M) produced a relaxation with two components; an initial transient relaxation followed by some recovery of force to a sustained relaxation with amplitude being 30%–50% smaller than the initial peak relaxation. The initial relaxation reached a peak amplitude at about 2 min, and then the following relaxation decayed slowly with time to reach a stable amplitude at 15–20 min (Fig.1A). The relaxation was confirmed to be produced mainly by EDRF from evidence that it was inhibited to less than 10% by 10^{-5} M N^{ω}-nitro-L-arginine ($n = 3$)(data not shown). Acetylcholine also produced relaxation in the presence of pranidipine, but the amplitude relative to the contraction produced by high-K^+ solution containing noradrenaline was larger in the presence than in the absence of pranidipine (Fig.1B). The relaxation was quantified by expressing the amplitude as relative to the contraction produced before application of acetylcholine. In muscles contracted with high-K^+ solution containing noradrenaline, the amplitude of the initial component of acetylcholine-induced relaxation was about 40%, and it decayed to about 7% at 25 min (Fig.3A). In muscles contracted with noradrenaline in the presence of N^{ω}-nitro-L-arginine and indomethacin, acetylcholine again produced a biphasic relaxation. However, in this case the amplitude of the relaxation was inhibited by 10^{-8} M pranidipine (Fig.3B),

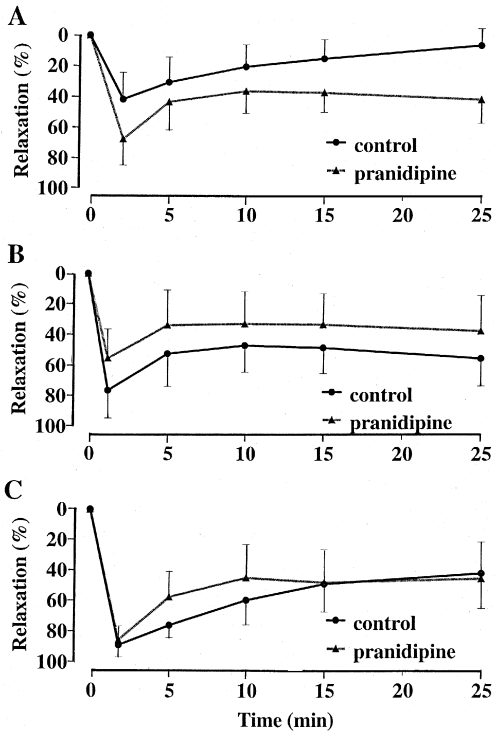

Fig. 3. Effects of pranidipine on acetylcholine-induced relaxation. Ring preparation of the guinea-pig carotid artery was contracted by (A) high-K^+ solution containing 10^{-6} M noradrenaline, (B) solutions containing 10^{-6} M noradrenaline, 10^{-5} M N^{ω}-nitro-L-arginine and 5×10^{-6} M indomethacin, and (C) 10^{-6} M noradrenaline alone, and 10^{-6} M acetylcholine was added for 25 min to induce relaxation in the absence (control) and presence of 10^{-8} M pranidipine. Acetylcholine was added at time 0. Mean ± S.D., $n = 6$ for A, $n = 7$ for B, $n = 4$ for C.

indicating that the relaxation produced by EDHF was inhibited by pranidipine.

In the guinea-pig carotid artery contracted with noradrenaline, the acetylcholine-induced relaxation is produced by both EDRF and EDHF (Suzuki et al., 1992; Zhang et al., 1994). Thus, the present results indicate that pranidipine augments the relaxation produced by EDRF and inhibits the relaxation produced by EDHF in this artery. If this is the case, the acetylcholine-induced relaxation produced in muscles contracted with noradrenaline alone may not be significantly altered by pranidipine, due to concomitant counteracting effects of pranidipine on EDRF- and EDHF-induced relaxation. The results shown in Fig. 3C indicate that the acetylcholine-induced relaxation produced in muscles contracted by noradrenaline was not significantly altered by pranidipine. The amplitude of contraction produced by noradrenaline was not significantly changed by pranidipine ($106.1 \pm 25.1\%$, $n = 5$, $P > 0.05$).

Fig. 4 summarizes the effects of pranidipine on the acetylcholine-induced relaxation in muscles contracted with high-K^+ solution containing noradrenaline. Both initial and sustained components of the relaxation were augmented by pranidipine (10^{-9}–10^{-7} M), in a concentration-dependent manner.

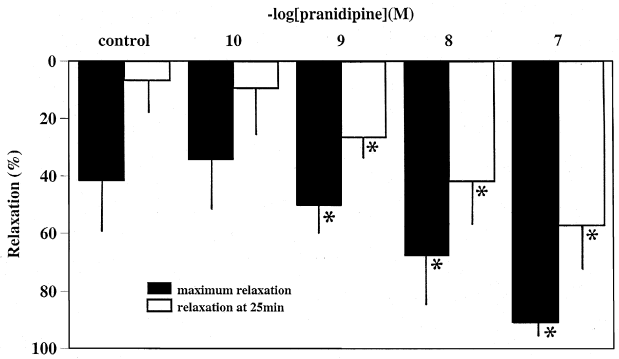

Fig. 4. Effects of pranidipine on relaxation produced by EDRF. Ring preparation of the guinea-pig carotid artery was contracted by high-K^+ solution containing 10^{-6} M noradrenaline, and then 10^{-6} M acetylcholine was applied for 25 min to induce relaxation, in the absence (control) and presence of pranidipine (10^{-10}–10^{-7} M). Amplitude of relaxation (peak, filled column; at 25 min, open column) was shown by the mean ± S.D. ($n = 6$). *, significant difference from control ($P < 0.05$).

3.3. Effects of pranidipine on relaxation produced by sodium nitroprusside

Effects of pranidipine on relaxation produced by sodium nitroprusside, a nitric oxide donor, were investigated in the guinea-pig carotid artery contracted with high-K^+ solution containing noradrenaline. As shown in Fig. 5, sodium nitroprusside (10^{-9}–10^{-5} M) applied cumulatively produced a relaxation, in a concentration-dependent manner. Sodium nitroprusside also relaxed the arterial ring preparations which had been denuded of the endothelial cells in a concentration-dependent manner, and the amplitude of relaxation was much larger than that observed in intact

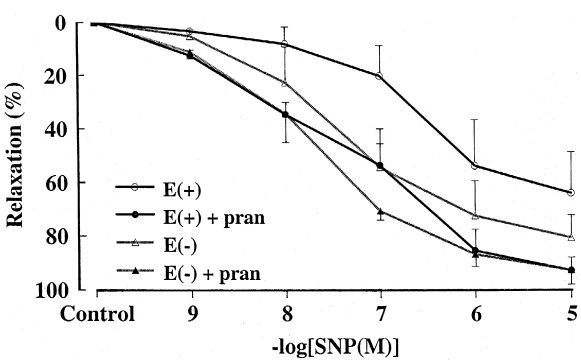

Fig. 5. Effects of pranidipine on relaxation produced by sodium nitroprusside. Ring preparations of the guinea-pig carotid artery (intact and endothelium-removed preparations) were contracted with high-K^+ solution containing 10^{-6} M noradrenaline, and then sodium nitroprusside (SNP) was added cumulatively, in the absence and presence of 10^{-8} M pranidipine. Amplitude of contraction in the presence of sodium nitroprusside was measured as relative to that before application of sodium nitroprusside. Mean ± S.D. ($n = 5$–10).

arteries. The maximum relaxation elicited by 10^{-5} M sodium nitroprusside was about 60% in intact artery and about 80% in the denuded artery. Pranidipine (10^{-8} M) augmented the relaxation produced by sodium nitroprusside in both intact and endothelium-denuded arteries; the effects appeared more pronounced in the former than in the latter.

These results suggest that in the guinea-pig carotid artery, the relaxation produced by endogenous and exogenous nitric oxide is augmented by pranidipine. The relaxation produced by nitric oxide is believed to be related to the enhanced production of cyclic GMP due to stimulation of guanylate cyclase (Moncada et al., 1991). Attempts were therefore made to observe the effects of pranidipine on relaxation produced by 10^{-6} M acetylcholine or 10^{-6} M sodium nitroprusside in the presence of ODQ, an inhibitor of guanylate cyclase (Garthwaite et al., 1995).

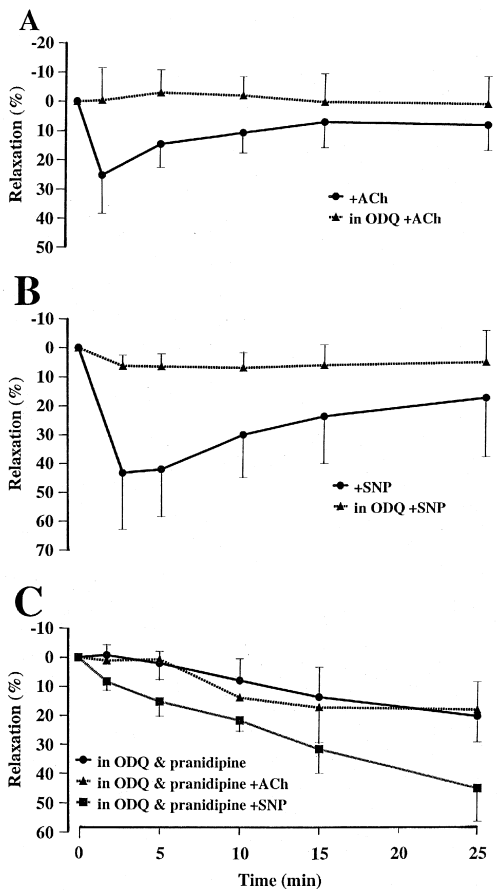

Fig. 6. Effects of ODQ on acetylcholine- or sodium nitroprusside-induced relaxation. The ring preparations with intact endothelial cells were contracted by high-K^+ solution containing noradrenaline, and the relaxation produced by 10^{-6} M acetylcholine (ACh, A) or 10^{-6} M sodium nitroprusside (SNP, B) was measured in the absence or presence of 10^{-5} M ODQ. (C) Contractions produced by high-K^+ solution containing 10^{-7} M noradrenaline in the presence of 10^{-5} M ODQ and 10^{-8} M pranidipine, and addition of 10^{-6} M acetylcholine or 10^{-6} M sodium nitroprusside. ODQ was added in the superfusate at least 30 min before starting experiments. Acetylcholine or sodium nitroprusside was added at time 0. Amplitude of relaxation relative to that before application of acetylcholine or sodium nitroprusside was measured for 25 min (mean ± S.D., $n = 5$ for A, $n = 7$ for B, $n = 3$ for C).

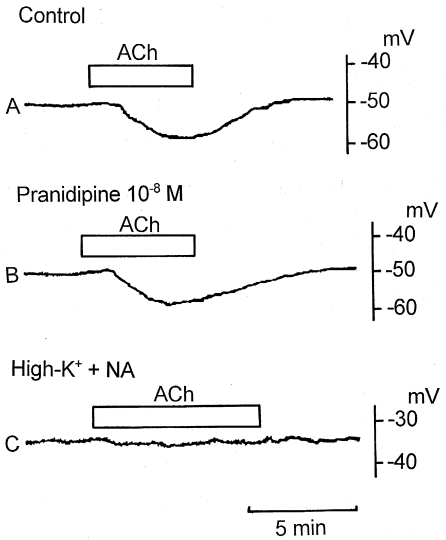

Fig. 7. Acetylcholine-induced hyperpolarization. Membrane responses of smooth muscle elicited by 10^{-6} M acetylcholine (ACh) were measured from the guinea-pig carotid artery, in the absence (A) and presence of 10^{-8} M pranidipine (B). (C) The responses produced by acetylcholine in the presence of high-K^+ solution containing 10^{-6} M noradrenaline (High-K^+ + NA).

In carotid artery of the guinea-pig, 10^{-5} M ODQ caused an increase in amplitude of noradrenaline-induced contraction by 1.5–2 times. Therefore, in the presence of ODQ the concentrations of noradrenaline were reduced to $1–3 \times 10^{-7}$ M to obtain contractions with comparable amplitude with those in the absence of ODQ. In the presence of ODQ, high-K^+ solution containing noradrenaline produced a sustained contraction, and the relaxation produced by acetylcholine (Fig.6A) or sodium nitroprusside (Fig.6B) was strongly attenuated. In the presence of 10^{-5} M ODQ and 10^{-8} M pranidipine, amplitude of contractions produced by high-K^+ solution containing noradrenaline did not remain stable, but decayed slowly with time. Addition of acetylcholine did not, but sodium nitroprusside did, facilitate significantly the decline of tension (Fig.6C). This suggests that the relaxation elicited by acetylcholine is mainly produced by a cyclic GMP-dependent mechanism and that pranidipine augments this mechanism. Sodium nitroprusside facilitated the inhibition of contraction produced with high-K^+ solution containing noradrenaline by pranidipine in the presence of ODQ, indicating that the later part of the relaxation produced by sodium nitroprusside may be independent of cyclic GMP.

3.4. Effects of pranidipine on the acetylcholine-induced hyperpolarization

The resting membrane potential of smooth muscle cells in the guinea-pig carotid artery ranged between −50 and

−55 mV (mean, -52.3 ± 3.2 mV, $n = 21$), the value being similar to that reported previously (Suzuki et al., 1992; Zhang et al., 1994). Application of 10^{-6} M acetylcholine hyperpolarized the membrane by 10.1 ± 3.7 mV ($n = 3$). Application of 10^{-8} M pranidipine did not alter the resting membrane potential (-52.9 ± 3.1 mV, $n = 12$) or the amplitude of acetylcholine-induced hyperpolarization (11.3 ± 6.7 mV, $n = 3$; $P > 0.05$ for both) (Fig.7A and B). High-K^+ solution containing noradrenaline depolarized the membrane to -36.7 ± 5.5 mV ($n = 20$), and the value was not altered by additional application of 10^{-8} M pranidipine (-35.8 ± 4.8 mV, $n = 8$, $P > 0.05$). In high-K^+ solution containing noradrenaline, acetylcholine (10^{-6} M) did not produce any hyperpolarization ($n = 5$) (Fig.7C). Thus, the effects of pranidipine appear with no relation to the membrane potential.

4. Discussion

The present experiments showed that in the guinea-pig carotid artery, pranidipine augments nitric oxide-induced relaxation and inhibits EDHF-induced relaxation. The augmentation of the nitric oxide-induced relaxation by pranidipine was also observed after removal of endothelial cells, indicating that the actions of pranidipine appear in an endothelium-independent manner and are, therefore, most probably exerted at the level of smooth muscle cells. It seems likely that these effects of pranidipine are causally related to the actions of cyclic GMP, since inhibition of guanylate cyclase by ODQ abolished the actions of pranidipine. These results differ from those reported in the rat aorta in which the augmentation by pranidipine of the endothelium-dependent relaxation produced by acetylcholine is not causally related to the production of cyclic GMP or the activity of nitric oxide synthase (Mori et al., 1998). The difference between these two arteries also appeared in the effects of pranidipine on the initial component of the EDRF-induced relaxation; the amplitude of the initial relaxation was augmented in the guinea-pig carotid artery but not in the rat aorta. It remains unclear why the effects of pranidipine differ between these two arteries.

Pranidipine has Ca^{2+}-channel antagonistic actions (Nakayama et al., 1990), and these actions are also confirmed in the present experiments, as the contractions produced by high-K^+ or high-K^+ solution containing noradrenaline are inhibited. It remains unclear whether the actions of pranidipine in the augmentation of nitric oxide-dependent relaxation have any causal relationship with inhibition of voltage-sensitive Ca^{2+}-channels, although this was not found to be the case in the rat aorta (Mori et al., 1998). In vascular smooth muscles, increases in cyclic GMP levels reduce the cytosolic Ca^{2+} concentrations due to accelerated activation of Ca^{2+} extrusion pumping mechanisms (Moncada et al., 1991). The present experiments could not reveal the cellular mechanisms related to the augmentation by pranidipine of the endothelium-dependent relaxation. The gradual decrease in amplitude of contraction produced with high-K^+ solution containing noradrenaline in the presence of both pranidipine and ODQ, but not in the presence of ODQ alone, suggests that pranidipine may have unidentified actions to inhibit contraction of vascular smooth muscle cells. In skinned smooth muscle of rat aorta, pranidipine has been shown to have no inhibitory action on the Ca^{2+}-sensitivity of the contractile proteins (Mori et al., 1998).

The augmentation by pranidipine of the acetylcholine-induced relaxation was observed under circumstances where contractile tension was reduced, possibly due to the Ca^{2+}-channel antagonistic actions of this drug on smooth muscle cells. It is possible that the amplitude of relaxation could be influenced by the pre-existing level of tension, since the values are expressed relative to this level. In aortic rings of the rabbit, the amplitude of relaxation is related to the level of muscle tension, and it reduces when the tension increases over a certain level while it remains unaltered when the tension reduces below this level (Ibengwe and Suzuki, 1986). This is also the case in the guinea-pig carotid artery, and reduction of tension by 20%–30% does not alter the relative amplitude of the acetylcholine-induced relaxation (Suzuki et al., 1992). Thus, the augmentation by pranidipine of the relaxation produced by acetylcholine or sodium nitroprusside may not merely reflect the reduction in amplitude of contraction.

The inhibition by pranidipine of the EDHF-induced relaxation is unlikely to be associated with reduced amplitude of endothelium-dependent hyperpolarization in the guinea-pig carotid artery. In vascular smooth muscles, hyperpolarization induces relaxation by inhibiting the influx of Ca^{2+} through voltage-sensitive Ca^{2+}-channels (Nelson et al., 1990) or the production of $InsP_3$ (Itoh et al., 1992). Although the mechanism remains unclear, the inhibition of the EDHF-induced relaxation by pranidipine may not be causally related to the inhibition of voltage-sensitive Ca^{2+}-channels or production of $InsP_3$, since the acetylcholine-induced hyperpolarization was not altered by pranidipine. The concomitant effects of pranidipine on EDRF and EDHF resulted in the absence of significant alteration in the acetylcholine-induced relaxation in muscles contracted with noradrenaline alone (Fig.3C). The contribution of EDRF and EDHF differ between vascular beds, and EDRF is the major factor in large arteries whereas EDHF is relatively more important in the peripheral arteries (Garland et al., 1995). If this is the case, pranidipine may augment vasodilatation in large arteries and may attenuate it in peripheral arteries.

Endothelial cells are the source of endogenous nitric oxide in vascular tissues (Moncada et al., 1991), and this is also the case in the guinea-pig carotid artery (Zhang et al., 1994). Sodium nitroprusside is a nitric oxide donor, and the relaxation of vascular smooth muscle by sodium nitro-

prusside is elicited in an endothelium-independent manner (Moncada et al., 1991). The present experiments confirmed that the sodium nitroprusside-induced relaxation was observed after rubbing the endothelial cells in the guinea-pig carotid artery. However, there was a difference in sensitivity and potency of sodium nitroprusside in the relaxation; it was larger in the rubbed artery than in the intact. In the present experiments, the effects of sodium nitroprusside were observed in muscles contracted with high-K^+ solution containing noradrenaline, indicating that the production of EDRF might be also enhanced by this solution through activation of α-adrenoceptors on the endothelial membrane (Cocks and Angus, 1983). It is speculated that the relaxing actions of nitric oxide are diminished in the presence of a trace of cyclic GMP in smooth muscle cells, possibly as a feedback inhibition (auto-inhibition) of the release of nitric oxide (Bauersachs et al., 1996). Thus, it is possible that the difference in the potency of sodium nitroprusside (i.e., the sodium nitroprusside-induced relaxation is larger in the denuded artery than in the intact) is causally related to the amount of cyclic GMP produced or alternatively to background activity of guanylate cyclase.

It is concluded that in the guinea-pig carotid artery, pranidipine augments EDRF-induced relaxation, possibly though mechanisms related to cyclic GMP. The EDHF-induced relaxation is inhibited by pranidipine, with no significant alteration of the hyperpolarization.

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