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Contribution of nitric oxide and K^+ channel activation to vasorelaxation of isolated rat aorta induced by procaine

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Abstract

The endothelium-dependent and -independent relaxant effect of procaine was examined in isolated rat aortic rings. Procaine induced relaxation of arteries precontracted with phenylephrine or with 60 mM K^+ in a concentration-dependent manner (0.01–3 mM). Procaine (1 mM) inhibited the transient contraction induced by caffeine (10 mM) in Ca^{2+}-free Krebs solution. Removal of the endothelium caused a rightward shift of the concentration-response curve for procaine. N^G-Nitro-L-arginine (L-NNA, 10–100 μM), N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) and methylene blue (1–10 μM) significantly attenuated the procaine-induced relaxation without affecting the maximal response. L-Arginine (1 mM) partially but significantly antagonized the effect of L-NAME (100 μM). Pretreatment of endothelium-intact aortic rings with procaine (1 mM) or with acetylcholine (10 μM) significantly elevated the tissue contents of cyclic GMP and this increase was inhibited in the presence of 100 μM L-NNA. Tetrapentylammonium ions (1–3 μM) reduced the procaine-induced relaxation in both endothelium-intact and -denuded arteries. Tetrapentylammonium ions (3 μM) did not affect the procaine-induced relaxation of 60 mM K^+-contracted arteries. Tetraethylammonium ions (3 mM) inhibited the procaine-induced relaxation. In contrast, iberiotoxin (100 nM), glibenclamide (3 μM), 4-aminopyridine (3 mM) and indomethacin (10 μM) had no effect. These results indicate that the procaine-induced relaxation may be mediated through multiple mechanisms. A substantial portion of the procaine-induced relaxation in rat aorta was caused by nitric oxide but not by other endothelium-derived factors. The activation of tetrapentylammonium- and tetraethylammonium-sensitive K^+ channels contributes in part to the procaine-induced vasorelaxation. Besides, procaine may directly inhibit both external Ca^{2+} entry and internal Ca^{2+} release in aortic smooth muscle cells.

Keywords: Procaine; Nitric oxide (NO); Endothelium; K^+ channel; Relaxation; Aorta; (Rat)

1. Introduction

Procaine, a local anaesthetic, is known for its inhibitory action on K^+ channel activators in smooth muscle cells of rabbit pulmonary artery (Ito et al., 1977), rabbit mesenteric artery (Wilson et al., 1988), guinea-pig bladder (Fujii et al., 1990) and pig coronary artery (Cowan and Cohen, 1992). Depolarization of the cell membrane of guinea-pig mesenteric artery (Itoh et al., 1981) and porcine coronary artery (Itoh et al., 1982) by procaine is probably through inhibition of K^+ channels. Procaine was also shown to reduce muscle tension in dog coronary artery (Imai et al., 1984), rabbit intestine (Ahn and Karaki, 1988), and rat vas deferens (Khoyi et al., 1993; Huang, 1995).The muscle relaxant effect of procaine may be caused by its inhibitory action on Ca^{2+}-induced Ca^{2+} release (Endo, 1977; Itoh et al., 1982; Khoyi et al., 1993; Huang, 1995) because procaine abolishes the transient contractile response of smooth muscle to caffeine in a Ca^{2+}-free solution (Itoh et al., 1981; Imai et al., 1984; Ahn and Karaki, 1988). In addition, non-selective inhibition of Ca^{2+} influx was also shown for the procaine-induced relaxation of arteries precontracted with high-K^+ solution (Imai et al., 1984; Ahn and Karaki, 1988). Both endothelium and membrane potential are important in the regulation of vascular tone (Feletou and Vanhoutte, 1988; Moncada et al., 1991; Nelson and Quayle, 1995). However, a possible role of factors derived from the endothelium and K^+ channel activation in procaine-induced relaxation in arteries has not been explored.

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In the present study, a number of pharmacological agents previously known to inhibit endothelium-dependent vasorelaxation and various vascular K^+ channels were examined for their effects on the relaxant action of procaine in rat isolated aortic rings. The results indicated that both nitric oxide (NO/cyclic GMP) and activation of K^+ channels contribute towards procaine-induced relaxation in rat isolated aortic rings. Besides, procaine could interfere directly with extracellular Ca^{2+} influx and intracellular Ca^{2+} release.

2. Materials and methods

2.1. Tissue preparation

Male Sprague–Dawley rats (250–300 g) were killed by cervical dislocation. The thoracic segment of the aorta was dissected out and cut into rings 3 mm in length. The preparation was then transferred to a 10-ml organ bath filled with Krebs solution, and bubbled with a mixture of 95% O_2 and 5% CO_2. The aortic rings were mounted between two L-shaped stainless-steel hooks. One of the hooks was mounted at the bottom of the bath while the other was connected to a FT03 force-displacement transducer (Grass Instruments). Krebs solution contained (mM): 119 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 25 $NaHCO_3$,1.2 KH_2PO_4, 11 D-glucose, 0.2 ascorbic acid. A basal tension of 1.5 g was applied in all experiments. All experiments were performed at 37 ± 1°C.

2.2. Effects of nitric oxide synthesis inhibitors and K^+ channel blockers on procaine-induced relaxation

Twenty minutes after being set up in organ baths, the preparations were first contracted with a single concentration of phenylephrine (0.3 μM, an approximate EC_{80} concentration) to test their contractile responses after which time they were rinsed several times in normal Krebs solution to restore the initial tension. The basal tone was always monitored and adjusted to 1.5 g. After a 60-min equilibration period, sustained contraction of aortic rings with intact endothelium in response to 0.3 μM phenylephrine or of rings without endothelium to 0.1 μM phenylephrine was obtained, and procaine was applied cumulatively to induce concentration-dependent relaxation. In experiments testing possible roles of endothelium-derived vasoactive factors, aortic rings were first exposed to the inhibitor (10–100 μM N^G-nitro-L-arginine (L-NNA), 100 μM N^G-nitro-L-arginine methyl ester (L-NAME), 1–10 μM methylene blue, 10 μM indomethacin, 3 μM glibenclamide, 100 nM iberiotoxin, 3 mM tetraethylammonium, 1–3 μM tetrapentylammonium or 3 mM 4-aminopyridine) for 30 min before they were contracted with phenylephrine to establish a steady tone, procaine was then added cumulatively (0.01–10 mM). The effect of the vehicle was also tested. In some experiments the endothelium was mechanically disrupted by rubbing the lumen of the artery with plastic tubing. Successful removal of the endothelium was demonstrated by the lack of a relaxant response of the preparation to acetylcholine (3 μM) at the beginning of each experiment. The removal of endothelium was also evaluated and confirmed by light microscopy of the histological sections of aorta. Since pretreatment with some inhibitors of nitric oxide activity and K^+ channels could increase the amplitude of phenylephrine (0.3 μM)-induced contraction, 0.1 μM phenylephrine was used to induce a similar amplitude of muscle tension in different groups. In some experiments, the effect of L-NNA on sodium nitroprusside-induced relaxation and the effect of tetrapentylammonium on forskolin-induced relaxation were also examined.

2.3. Effect of procaine on contraction induced by high K^+

In another set of experiments, a sustained contraction of both endothelium-intact and -denuded arteries in response to 60 mM K^+ was obtained and procaine was applied cumulatively (0.01–10 mM). In some experiments, tetrapentylammonium (3 μM) was tested on procaine-induced relaxation of arteries precontracted with 60 mM K^+. In experiments using high-K^+ solution, Na^+ in the bathing medium was replaced by an equimolar concentration of K^+ to maintain a constant ion strength.

2.4. Effect of procaine on intracellular Ca^{2+}-mediated contraction

The tissues were exposed to a Ca^{2+}-free solution containing 0.3 mM Na_2-EGTA and washed with this solution twice and left for 15 min before application of caffeine. The tissues were then washed twice with normal Krebs solution (30-min contact time for Ca^{2+} refilling of the intracellular stores) and then twice with Ca^{2+}-free solution (15-min contact time). The second contractile response as a time control contraction evoked by caffeine was tested in the absence and presence of 1 mM procaine (15-min contact time). The ratio of the second contraction to the first contraction in a Ca^{2+}-free solution was $107 \pm 9.8\%$ ($n = 5$) for 10 mM caffeine.

2.5. Cyclic GMP assay

After 60-min equilibration under an initial tension of 1.5 g at 37°C, the arterial tissues were first incubated with phenylephrine for 10 min, then with 1 mM procaine for 10 min in the absence and presence of L-NNA. At the end of the reaction, arterial rings were rapidly frozen in liquid nitrogen and stored at −70°C until homogenization in 0.5 ml of ice-cold 6% trichloroacetic acid, using a glass homogenizer. The homogenate was centrifuged at $2000 \times g$ for 10 min at 4°C. The supernatant was extracted three times with three volumes of diethyl ether before lyophili-

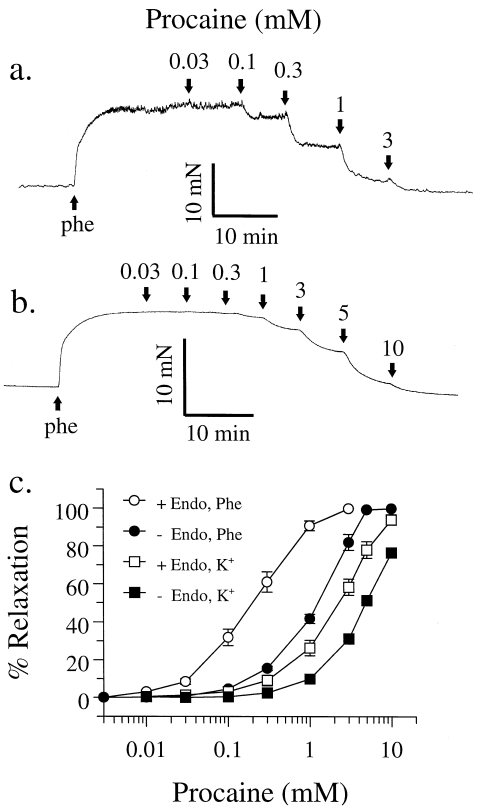

Fig. 1. Representative traces showing the relaxant effect of procaine in phenylephrine (Phe)-contracted rat isolated aortic rings with endothelium (a) and without endothelium (b). (c) Concentration-effect curves for the relaxant effect of procaine in aortic rings precontracted with phenylephrine (○, $n=8$ with endothelium; ●, $n=14$, without endothelium) or with 60 mM K^+ (□, $n=9$ with endothelium; ■, $n=7$, without endothelium). Results are means ± S.E.M. of n experiments.

zation. Cyclic GMP was assayed by radioimmunoassay with a [^{125}I]cyclic GMP RIA kit (DuPont, Wilmington, DE, USA). The tissue residue was dissolved in 2 M NaOH and the protein content was determined using a protein assay kit (Sigma, St. Louis, MO, USA) with bovine serum albumin as the standard. The concentration of cyclic GMP is presented as pmol/mg protein.

2.6. Drugs

The following drugs were used: phenylephrine hydrochloride, acetylcholine hydrochloride, procaine, indomethacin, glibenclamide, L-arginine, tetraethylammonium chloride, caffeine, 4-minopyridine, sodium nitroprusside, nifedipine (Sigma); L-NNA, L-NAME, N^G-nitro-D-arginine, methylene blue, iberiotoxin, forskolin (Research Biochemical, Natick, MA, USA); tetrapentylammonium bromide (Aldrich Chem., St. Louis, MO, USA). Glibenclamide, forskolin, indomethacin and nifedipine were dissolved in dimethyl sulfoxide. Other drugs were dissolved in distilled water and further dilution was made with Krebs solution.

2.7. Analysis of data

Relaxant responses to procaine in the absence and presence of various inhibitors were expressed as percentages of the phenylephrine-induced contraction. IC_{50} values (concentration producing half-maximal relaxation) were calculated by non-linear regression analysis of the concentration-relaxation curves using all the data points. Data are presented as means ± S.E.M. of n experiments. Statistical analysis of results was performed with Student's unpaired t-test. A probability value of $P<0.05$ was regarded as significant.

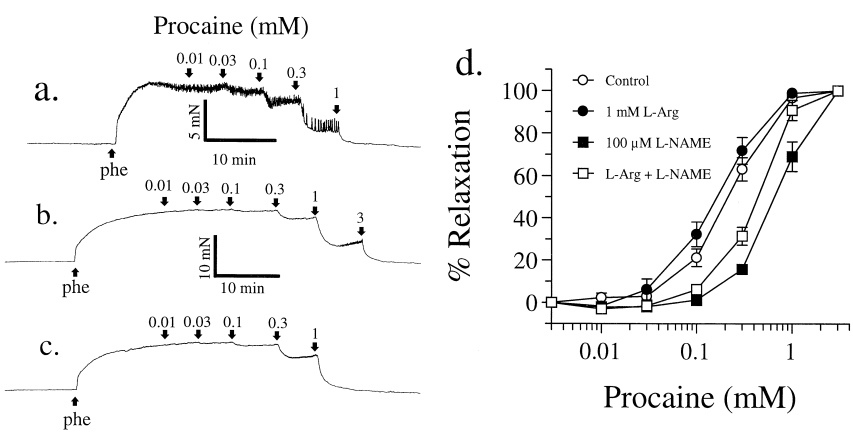

Fig. 2. Traces showing the representative records for procaine-induced relaxation in endothelium-intact aortic rings for the control (a), in the presence of 100 μM L-NAME (b) and in the presence of 1 mM L-arginine plus 100 μM L-NAME (c). Tissues were incubated with L-NAME for 30 min prior to application of phenylephrine (Phe) and L-arginine was added 10 min before application of L-NAME. (d) The log contraction-response curves for different treatments (○, $n=7$ in the presence of endothelium; ●, $n=5$ for 100 μM L-NAME; □, $n=5$ in 1 mM L-arginine; ■, $n=5$ in L-arginine plus L-NAME). Results are means ± S.E.M. of n experiments.

3. Results

3.1. Relaxant response to procaine

Phenylephrine caused a sustained increase of tension in rat isolated thoracic aorta (6.96 ± 0.5 mN, $n = 27$, by 0.3 μM phenylephrine, with endothelium, and 8.37 ± 0.9 mN, $n = 14$, by 0.1 μM phenylephrine, without endothelium). Representative traces in Fig. 1 show that cumulative addition of procaine reduced the phenylephrine-evoked contraction in a concentration-related manner. Procaine caused full relaxation in both endothelium-intact (Fig. 1a) and -denuded arteries (Fig. 1b), but a higher concentration of procaine was needed to cause complete relaxation in the endothelium-denuded artery (Fig. 1c). IC_{50} values for the relaxant effect of procaine were 0.21 ± 0.01 mM ($n = 8$) and 1.33 ± 0.16 mM ($n = 14$) in endothelium-intact and -denuded tissues, respectively (Fig. 1c). Similarly, procaine also concentration dependently relaxed the artery precontracted with 60 mM K^+ and the relaxant effect of procaine was greater in arteries with endothelium than in those without endothelium (Fig. 1c). The IC_{50} values were 1.88 ± 0.15 mM ($n = 9$) and 3.44 ± 0.22 mM ($n = 7$) in endothelium-intact and -denuded tissues, respectively (Fig. 1c). It is evident that procaine was less effective against the high-K^+-induced contraction in rat aorta. The contractile response to 60 mM K^+ was abolished by 10 nM nifedipine ($n = 4$) or in Ca^{2+}-free Krebs solution (containing 0.3 mM Na_2-EGTA, $n = 4$), whereas caffeine (10 mM) induced a transient increase of muscle tension (1.1 ± 0.2 mN, $n = 5$) and procaine at 1 mM inhibited the caffeine-induced response by approximately 45% ($n = 4$).

3.2. Effects of inhibitors of nitric oxide activity

Since procaine caused both endothelium-dependent and -independent relaxation in rat aorta, attempts were made to examine the possible involvement of endothelium-derived factors in the vascular response to procaine. Traces in Fig. 2b show that pretreatment of endothelium-intact rings with L-NAME (100 μM) markedly reduced the procaine-induced relaxant response without affecting the maximal response, while L-arginine (1 mM) alone did not influence the procaine-induced relaxation (Fig. 2d) but it partially reversed the inhibitory effect of L-NAME (Fig. 2c and d). Pretreatment with L-NNA (10–100 μM) also significantly inhibited the procaine-induced relaxation (Table 1). In contrast to L-NNA, its enantiomer N^G-nitro-D-arginine (D-NNA, 100 μM) did not affect the relaxant response to procaine (Table 1). L-NNA at 100 μM completely inhibited the endothelium-dependent relaxation induced by acetylcholine ($n = 5$, data not shown) while it had no effect on the relaxant response to sodium nitroprusside (IC_{50}: 10.4 ± 0.5 nM for control and 10.2 ± 0.8 nM in the presence of L-NNA, $n = 5$ in each case). Besides, methylene blue (1–10 μM) induced a significant rightward shift of the concentration-relaxation curves for procaine in endothelium-intact preparations without affecting the maximum response (Table 1).

Table 1
Effects of various inhibitors on procaine-mediated relaxation

Treatment	IC_{50} (mM)	n
Control	0.21 ± 0.01	10
L-NNA (10 μM)	1.23 ± 0.14[a]	6
L-NNA (100 μM)	1.89 ± 0.07[a]	6
D-NNA (100 μM)	0.22 ± 0.02	6
Control	0.21 ± 0.01	10
MB (1 μM)	1.01 ± 0.05[a]	6
MB (10 μM)	2.43 ± 0.37[a]	6
Control	0.22 ± 0.02	6
IBX (100 nM)	0.21 ± 0.02	5
4-AP (3 mM)	0.32 ± 0.04	6
Glib (3 μM)	0.28 ± 0.04	5
Indomethacin (10 μM)	0.19 ± 0.02	5

Significant difference ([a]$P < 0.05$) between the control and treatment groups is indicated.

3.3. Effect of K^+ channel blockers on procaine-induced relaxation

The reduced potency of procaine to relax the high-K^+-contracted arteries indicates that activation of K^+ channels may also be involved. Various inhibitors of K^+ channels

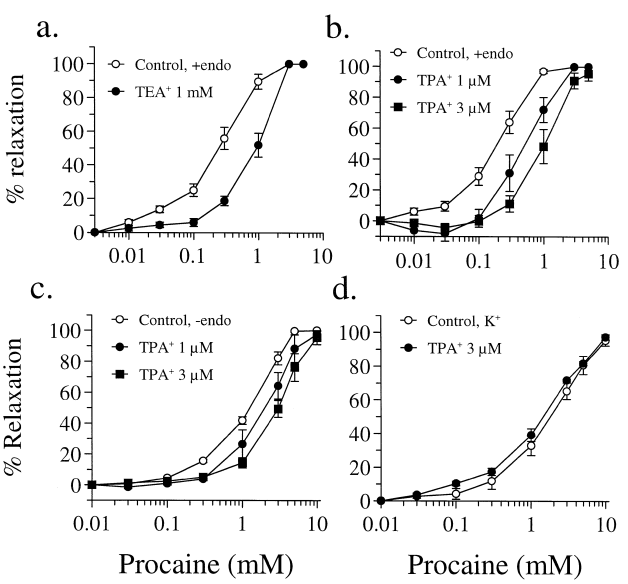

Fig. 3. (a) Log concentration-effect response curves for the relaxant effect of procaine in endothelium-intact aortic rings in the absence (○, $n = 7$) and presence of 3 mM tetraethylammonium (TEA^+, ●, $n = 5$). Log concentration-effect curves for the relaxant effect of procaine in endothelium-intact (b) and endothelium-denuded (c) aortic rings in the absence (○, $n = 14$) and presence of tetrapentylammonium (TPA^+) (●, $n = 6$ for 1 μM and ■, $n = 6$ for 3 μM TPA^+). (d) Log concentration-effect response curves for the relaxant effect of procaine in 60 mM K^+-contracted endothelium-intact arteries in the control (○, $n = 6$) and in the presence of 3 μM TPA^+ (●, $n = 5$). TPA^+ or TEA^+ was applied to the bath 30 min before addition of phenylephrine. Results are means ± S.E.M. of n experiments.

were examined for their possible inhibitory actions on procaine-mediated relaxation. Pretreatment of tissues with tetraethylammonium (3 mM) inhibited the procaine-induced relaxation (Fig. 3a). Tetrapentylammonium (1–3 μM), another quaternary ammonium agent also attenuated the procaine-induced relaxation in both endothelium-intact (Fig. 3b) and -denuded arteries (Fig. 3c). However, tetrapentylammonium (3 μM) did not alter the relaxant effect of procaine in 60 mM K^+-contracted arteries (IC_{50}: 1.58 ± 0.15 mM for control and 1.37 ± 0.13 mM for tetrapentylammonium, $n = 5$ in each case, $P > 0.05$, Fig. 3d). In contrast, the procaine-induced relaxant response was unaffected by iberiotoxin (100 nM, $n = 5$), glibenclamide (3 μM, $n = 5$) or 4-aminopyridine (3 mM, $n = 6$) (Table 1). In addition, exposure of tissues to 10 μM indomethacin did not affect the procaine-induced relaxant response in endothelium-intact arteries ($n = 5$, Table 1). Tetrapentylammonium (3 μM) did not change the relaxation induced by forskolin (IC_{50}: 36.3 ± 6.1 nM for control and 46.7 ± 7.4 nM for tetrapentylammonium, $n = 5$ in each case, $P > 0.05$).

3.4. Effect of procaine on tissues cyclic GMP content

Fig. 4 shows that the tissue content of cyclic GMP was significantly increased from the control value after the endothelium-intact aortic rings were exposed to 1 mM procaine ($n = 5$) for 10 min or to 10 μM acetylcholine ($n = 4$) for 5 min. Pretreatment of tissues with 100 μM L-NNA markedly reduced the cyclic GMP level that had been increased by either procaine or acetylcholine (Fig. 4).

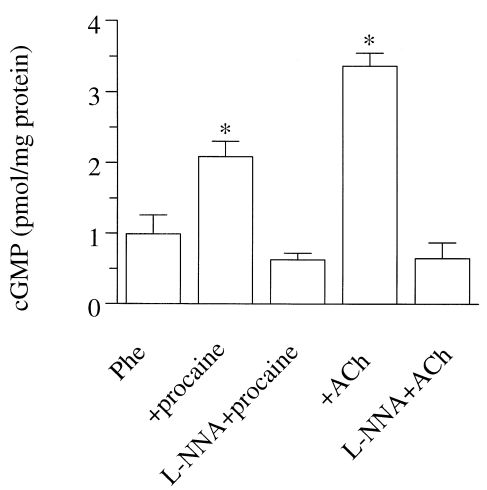

Fig. 4. Effects of 1 mM procaine (10-min contact time) and 10 μM acetylcholine (ACh, 5 min contact time) on the content of cyclic GMP in endothelium-intact aortic rings contracted with phenylephrine (Phe, 0.3 or 0.1 μM) in the absence and presence of 100 μM L-NNA. Tissues were exposed to L-NNA for 30 min before addition of 0.1 μM phenylephrine (20-min contact time) Data are means ± S.E.M. from 4–5 experiments. (* $P < 0.01$ Students' *t*-test).

4. Discussion

Procaine induced a concentration-dependent relaxation of rat isolated aortic rings with a functional endothelium. Removal of the endothelium partially, but significantly, reduced the relaxation induced by procaine at submaximal concentrations, while the exogenous nitric oxide donor, sodium nitroprusside, induced a nitric oxide/endothelium-independent relaxation which was insensitive to L-NNA. These results suggest that procaine-induced relaxation involves endothelium-dependent and -independent mechanisms. Pretreatment of endothelium-intact tissues with L-NNA, L-NAME, or methylene blue, which are inhibitors of endothelium-derived relaxing factors, attenuated the procaine-induced relaxation to the same as with endothelium-denuded arteries. Neither D-NNA, an inactive form of L-NNA, nor indomethacin, an inhibitor of prostacyclin formation, affected the relaxant response to procaine. In addition, pretreatment with L-arginine, the nitric oxide precursor, partially reversed the inhibitory effect of L-NAME on the procaine-induced relaxation. These results indicate that nitric oxide produced by the endothelium was primarily responsible for the endothelium-dependent relaxation induced by procaine. Nitric oxide released from the endothelium has been shown to diffuse into the underlying vascular smooth muscle and to stimulate cyclic GMP accumulation which mediates the nitric oxide-dependent relaxation (Ignarro et al., 1984). The present study showed that the tissue content of cyclic GMP in endothelium-intact aortic rings increased in response to 1 mM procaine or to 10 μM acetylcholine, and that this increase was significantly suppressed by pretreatment with L-NNA. These findings support the hypothesis that the procaine-induced endothelium-dependent relaxation is partially mediated by nitric oxide production. However, it remains to be elucidated how procaine would elicit nitric oxide release from the endothelium.

In aortic smooth muscle cells, Ca^{2+}-activated K^+ (K_{Ca}) channels may contribute to maintenance of the membrane potential (Shoemaker and Worrel, 1991), which plays an important role in the regulation of vascular tone (Nelson and Quayle, 1995). Charybdotoxin-sensitive K_{Ca} channels were found to be activated in association with myogenic tone in pressurized rabbit cerebral arteries (Brayden and Nelson, 1992), indicating that modulation of K_{Ca} channel activity may be an important mechanism that controls the level of arterial tone. In many instances, the vasorelaxation induced by membrane hyperpolarization of arterial smooth muscle is caused by an increase in K^+ permeability. In the present study, when contractions of similar size induced by 60 mM K^+ or by phenylephrine, the former were less strongly inhibited by procaine. The procaine-induced aortic relaxation was inhibited by tetraethylammonium or tetrapentylammonium. These two quaternary ammonium agents are putative blockers of arterial K_{Ca} channels and a concentration of 1.49 mM was needed for tetrapentylam-

monium ions to cause 50% reduction of the mean unitary current of K_{Ca} channels in rat mesenteric artery (Langton et al., 1991). However, iberiotoxin, a more potent blocker of large-conductance K_{Ca} channels in vascular smooth muscle (Brayden and Nelson, 1992), had no effect on procaine-induced relaxation, indicating that activation of K_{Ca} channels may not be involved in the relaxant response to procaine in the present preparations. In arteries contracted with 60 mM K^+, the relaxation induced by procaine was not affected by tetrapentylammonium (3 μM) while this concentration significantly reduced the relaxant effect of procaine at submaximal concentrations on the phenylephrine-induced contraction in normal K^+-containing solution. In contrast, the procaine-induced relaxation was unaffected by glibenclamide or 4-aminopyridine. Glibenclamide had been demonstrated to inhibit the activity of arterial ATP-sensitive K^+ channels (Standen et al., 1989), while 4-aminopyridine, the voltage-sensitive K^+ channel blocker, attenuated β_1-adrenoceptor-mediated relaxation in rat aortic rings (Satake et al., 1996). These results indicate that neither glibenclamide- nor 4-aminopyridine-sensitive K^+ channels contributed to the relaxant response to procaine in rat aorta. The inhibitory effects of tetraethylammonium and tetrapentylammonium on the procaine-induced relaxation contrast with the reported blocking activity of K^+ channels by procaine. For example, procaine reduced the vasodilator response to cromakalim in rabbit mesenteric artery (Wilson et al., 1988) and in the rat perfused heart (Fulton et al., 1994). In non-vascular smooth muscle of the guinea-pig bladder, procaine prolonged the falling phase of the action potential and abolished the cromakalim-mediated membrane hyperpolarization, K^+ efflux and relaxation (Fujii et al., 1990). These findings indicate that procaine may have multiple sites of action that depend on the type of smooth muscle and pharmacological treatment used. Nevertheless, further electrophysiological study is needed to confirm the possible activator effect of procaine on the K^+ channels in arterial smooth muscle cells.

Nitric oxide was shown to activate charybdotoxin-sensitive K_{Ca} channels either directly (Archer et al., 1994) and/or through cyclic GMP-dependent mechanisms (Robertson et al., 1993). Tetraethylammonium and tetrapentylammonium attenuated the relaxation induced by nitric oxide donors in the same preparation (Huang, 1998). In order to test whether the tetrapentylammonium-sensitive component of procaine-induced relaxation was induced by nitric oxide, the effects of quaternary ammonium compounds were examined in endothelium-denuded arteries. It was found that tetrapentylammonium was still able to attenuate the procaine-induced relaxation, which suggests that procaine may have a direct stimulatory effect on K^+ channels in arterial smooth muscle.

The present results also showed that procaine concentration dependently reduced the sustained contraction induced by 60 mM K^+ in rat aortic rings. This finding is in agreement with a previous report for dog coronary artery (Imai et al., 1984). Similarly, the voltage-sensitive Ca^{2+} channel blocker, nifedipine completely abolished the high-K^+ response. This indicates that procaine may inhibit Ca^{2+} influx through the plasma membrane of smooth muscle cells as proposed earlier for blood vessels (Imai et al., 1984; Ahn and Karaki, 1988), but this effect may be non-selective since procaine also inhibited Na^+ and K^+ influx into human isolated saphenous vein (Wali et al., 1987). In non-vascular smooth muscle cells, procaine was found to suppress the Ca^{2+} current (Zholos et al., 1991; Yoshino et al., 1996). It has been demonstrated that procaine inhibits the Ca^{2+}-induced Ca^{2+} release from intracellular stores in both vascular and non-vascular preparations (Endo, 1977; Itoh et al., 1982; Khoyi et al., 1993; Huang, 1995). Procaine inhibited the noradrenaline-elicited ^{45}Ca efflux (as indication of intracellular Ca^{2+} release) in rabbit aorta and intestine (Ahn and Karaki, 1988) and abolished the noradrenaline-induced increase in both phosphatidylinositol turnover and ^{45}Ca efflux in rat vas deferens (Khoyi et al., 1993). Also, in the present study, the caffeine-induced transient contractile response was reduced by procaine in the absence of extracellular Ca^{2+}. Caffeine was shown to release Ca^{2+} from the sarcoplasmic reticulum by enhancing the Ca^{2+}-induced Ca^{2+} release (Itoh et al., 1981). The present results do not suggest that procaine inhibits Ca^{2+} influx as the mechanism initiating the inhibition of the Ca^{2+}-induced Ca^{2+} release process. Instead, procaine may interfere directly with the caffeine-sensitive Ca^{2+} release (Antoniu et al., 1985; Zahradnikova and Palade, 1993; Garcia and Schneider, 1995). Since procaine at higher concentrations still induced full relaxation in the presence of various inhibitors, it appears that various vasorelaxant mechanisms could be recruited to mediate relaxation when a higher concentration of procaine was used.

Taken together, the current results show a multiplicity of the vascular response to procaine in rat isolated aortic rings. Procaine-induced relaxation comprises both endothelium-dependent and -independent components. The mechanism underlying the procaine-induced endothelium-dependent relaxation is likely through the endothelial nitric oxide release and cyclic GMP accumulation in arterial smooth muscle. Procaine-induced endothelium-independent relaxation may be partially mediated through the activation of K^+ channels which are sensitive to inhibition by tetraethylammonium and tetrapentylammonium, and partly through inhibition of nifedipine-sensitive Ca^{2+} influx or/and intracellular Ca^{2+} release.

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References

Ahn, H.Y., Karaki, H., 1988. Inhibitory effect of procaine on contraction and calcium movement in vascular and intestinal smooth muscles. Br. J. Pharmacol. 94, 789–796.

Antoniu, B., Kim, D.H., Morii, H., Ikemoto, N., 1985. Inhibitors of Ca^{2+} release from the sarcoplasmic reticulum. Biochem. Biophys. Acta 816, 9–17.

Archer, S.L., Huang, J.M.C., Hampl, V., Nelson, D.P., Shultz, P.J., Weir, E.K., 1994. Nitric oxide and cGMP cause vasorelaxation by activation of a charybdotoxin-sensitive K^+ channel by cGMP-dependent protein kinase. Proc. Natl. Acad. Sci. USA 91, 7583–7587.

Brayden, J.E., Nelson, M.T., 1992. Regulation of arterial tone by activation of calcium-activated potassium channels. Science 256, 532–535.

Cowan, C.L., Cohen, R.A., 1992. Different mechanisms of relaxation of pig coronary artery to bradykinin and cromakalim are distinguished by potassium channel blockers. J. Pharmacol. Exp. Ther. 260, 248–253.

Endo, M., 1977. Calcium release from the sarcoplasmic reticulum. Physiol. Rev. 57, 71–108.

Feletou, M., Vanhoutte, P.M., 1988. Endothelium-dependent hyperpolarization of canine coronary smooth muscle. Br. J. Pharmacol. 93, 515–524.

Fulton, D., McGiff, J.C., Quilley, J., 1994. Role of K^+ channels in the vasodilator response to bradykinin in the rat heart. Br. J. Pharmacol. 113, 954–958.

Fujii, K., Foster, C.D., Bragding, A.F., Parekh, A.B., 1990. Potassium channel blockers and the effects of cromakalim on the smooth muscle of the guinea-pig bladder. Br. J. Pharmacol. 99, 779–785.

Garcia, J., Schneider, M.F., 1995. Suppression of calcium release by calcium or procaine in voltage clamped rat skeletal muscle fibers. J. Physiol. 485, 437–445.

Huang, Y., 1995. $BaCl_2$- and 4-aminopyridine-evoked phasic contractions in the rat vas deferens. Br. J. Pharmacol. 115, 845–851.

Huang, Y., 1998. Hydroxylamine-induced relaxation inhibited by K^+ channel blockers in rat aortic rings. Eur. J. Pharmacol. 349, 53–60.

Ignarro, L.J., Burke, T.M., Wood, K.S., Wolin, M.S., Kadowitz, P.J., 1984. Association between cyclic GMP accumulation and acetylcholine-elicited relaxation of bovine intrapulmonary artery. J. Pharmacol. Exp. Ther. 228, 682–690.

Imai, S., Nakazawa, M., Imai, H., Nabata, H., 1984. Effects of procaine on the isolated dog coronary artery. Arch. Int. Pharmacodyn. Ther. 271, 98–105.

Ito, Y., Suzuki, H., Kuriyama, H., 1977. Effects of caffeine and procaine on the membrane and mechanical properties of the smooth muscle cells of the rabbit main pulmonary artery. Jpn. J. Physiol. 27, 467–481.

Itoh, T., Kuriyama, H., Suzuki, H., 1981. Excitation–contraction coupling in smooth muscle cells of the guinea-pig mesenteric artery. J. Physiol. 321, 513–535.

Itoh, T., Kajiwara, M., Kitamura, K., Kuriyama, H., 1982. Roles of stored calcium on the mechanical response in smooth muscle cells of the porcine coronary artery. J. Physiol. 322, 107–125.

Khoyi, M.A., Dalziel, H.H., Zhang, L., Bjur, R.A., Gerthoffer, W.T., Buxton, I.L.O., Westfall, D.P., 1993. $[Ca^{2+}]_i$-sensitive, IP_3-independent Ca^{2+} influx in smooth muscle of rat vas deferens revealed by procaine. Br. J. Pharmacol. 110, 1353–1358.

Langton, P., Nelson, M.T., Huang, Y., Standen, N., 1991. Block on calcium-activated potassium channels in mammalian arterial myocytes by tetraethylammonium ions. Am. J. Physiol. 260, H927–H934.

Moncada, S., Palmer, R.M.J., Higgs, E.A., 1991. Nitric oxide: physiology, pathophysiology and pharmacology. Pharmacol. Rev. 43, 109–142.

Nelson, M.T., Quayle, J.M., 1995. Physiological roles and properties of potassium channels in arterial smooth muscle. Am. J. Physiol. 268, C799–C822.

Robertson, B.E., Schubert, R., Hescheler, J., Nelson, M.T., 1993. cGMP-dependent protein kinase activates Ca-activated K channels in cerebral artery smooth muscle cells. Am. J. Physiol. 265, C299–C303.

Satake, N., Shibata, M., Shibata, S., 1996. The inhibitory effects of iberiotoxin and 4-aminopyridine on the relaxation induced by β_1- and β_2-adrenoceptor activation in rat aortic rings. Br. J. Pharmacol. 119, 505–510.

Shoemaker, R.L., Worrel, R.T., 1991. Ca^{2+}-activated K^+ channels in aortic smooth muscle of rats. Proc. Soc. Exp. Biol. Med. 196, 325–332.

Standen, N.B., Quayle, J.M., Davies, N.W., Brayden, J.E., Huang, Y., Nelson, M.T., 1989. Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. Science 245, 177–180.

Wali, F.A., Suer, A.H., Greenidge, E., Tugwel, A.C., Hayter, A., 1987. Local anesthetics inhibit influx of calcium, sodium and potassium into rat ileum, diaphragm and human isolated saphenous vein. Gen. Pharmacol. 18, 351–355.

Wilson, C., Coldwell, M.C., Howlett, D.R., Cooper, S.M., Hamilton, T.C., 1988. Comparative effects of K^+ channel blockade on the vasorelaxant activity of cromakalim, pinacidil and nicorandil. Eur. J. Pharmacol. 152, 331–339.

Yoshino, M., Matsufuji, Y., Yabu, H., 1996. Voltage-dependent suppression of calcium current by caffeine in single smooth muscle cells of the guinea-pig urinary bladder. Naunyn-Schmiedeberg's Arch. Pharmacol. 353, 334–341.

Zahradnikova, A., Palade, P., 1993. Procaine effects on single sarcoplasmic reticulum Ca^{2+} release channels. Biophys. J. 64, 991–1003.

Zholos, A.V., Baidan, L.V., Shuba, M.F., 1991. The inhibitory action of caffeine on calcium currents in isolated intestinal smooth muscle cells. Pflügers Arch. 419, 267–273.