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Endothelium-dependent rhythmic contractions induced by cyclopiazonic acid in rat mesenteric artery

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Abstract

The action of cyclopiazonic acid, the putative inhibitor of the Ca^{2+}-ATPase of endoplasmic reticulum, on phenylephrine-evoked isometric contractions in rat isolated mesenteric arteries were investigated. Cyclopiazonic acid (3 μM) induced an initial relaxation followed by rhythmic contractions of the phenylephrine-precontracted arteries with intact endothelium. Removal of endothelium abolished the effect of cyclopiazonic acid. Pretreatment of tissues with N^G-nitro-L-arginine (100 μM) abolished the initial relaxation but not the rhythmic contractions. Indomethacin and glibenclamide did not affect the cyclopiazonic acid-induced response. Charybdotoxin (100 nM) converted the cyclopiazonic acid-induced rhythmic contractions to the sustained tension in the absence or presence of N^G-nitro-L-arginine (100 μM). Pretreatment of charybdotoxin (100 nM) abolished cyclopiazonic acid-induced rhythmic activity but not the initial relaxation. Nifedipine (10 nM) abolished the effect of cyclopiazonic acid. Moderate increase of extracellular K^+ (20 mM) reduced the initial relaxation but completely abolished rhythmic contractions induced by cyclopiazonic acid. The remaining relaxation was reversed or prevented by N^G-nitro-L-arginine (100 μM). The results of the present investigation indicate that cyclopiazonic acid caused endothelium-dependent response in rat isolated mesenteric arteries probably by releasing nitric oxide responsible for the initial relaxation, and probably by releasing endothelium-derived hyperpolarizing factors primarily responsible for activation of charybdotoxin-sensitive K^+ channels and induction of rhythmic contractile activity. © 1997 Elsevier Science B.V.

Keywords: Cyclopiazonic acid; Nitric oxide (NO); Endothelium; K^+ channel; Contractions, rhythmic; Mesenteric artery, rat

1. Introduction

Contractility of vascular smooth muscle is normally regulated by changes of intracellular Ca^{2+} concentration that is controlled by either vasoconstrictors or vasodilators. The importance of the endothelium in regulating muscle activity is well described in many vascular beds (Furchgott, 1983; Dainty et al., 1990; Garland et al., 1995). Vascular smooth muscle cells display different patterns of rhythmic contractions. Such oscillations of muscle tone are attributed to fluctuations of $[Ca^{2+}]_i$ that can originate either from Ca^{2+} influx (membrane oscillator) or from Ca^{2+} release from the endoplasmic reticulum (cytosolic oscillator) or from both combined (Berridge and Galione, 1988). Oscillatory changes either in the membrane potential or in Ca^{2+} release mainly from the endoplasmic reticulum have been proposed to explain agonist-induced oscillations in smooth muscle tone.

The rabbit mesenteric and ear arteries display rhythmic contractile responses when stimulated with phenylephrine (Omote and Mizusawa, 1993a, 1994). Such oscillations depend on the presence of endothelium. It was demonstrated that rhythmic activity of the ear artery was completely inhibited by charybdotoxin, a selective blocker of large conductance Ca^{2+}-activated K^+ (K_{Ca}) channels, and by nifedipine, a Ca^{2+} channel blocker (Omote and Mizusawa, 1993b). Interestingly, cyclopiazonic acid, a highly selective inhibitor of the Ca^{2+}-ATPase of endoplasmic reticulum in vascular smooth muscle (Deng and Kwan, 1991; Low et al., 1992; Inesi and Sagara, 1994; Zhang et al., 1994b) can convert phenylephrine-induced sustained tension of endothelium-denuded arteries into rhythmic contractions as seen in endothelium-intact arteries (Omote and Mizusawa, 1994). In contrast to the observations of Omote and Mizusawa (1993a, 1994), the results obtained from the present study show a different type of rhythmic contrac-

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tions in rat isolated mesenteric arteries. Phenylephrine only caused a sustained increase of muscle tone without inducing visible rhythmic activity. Cyclopiazonic acid, however, induced rhythmic contractions of phenylephrine-precontracted arteries, which were dependent on the presence of endothelium. An attempt was made to investigate the hypothesis that endothelium-derived factors are involved in cyclopiazonic acid-induced rhythmic contractions in rat mesenteric arteries, and to examine the possible ionic mechanisms underlying such oscillations of muscle tone.

2. Materials and methods

2.1. Preparation

Male Sprague–Dawley rats (about 400 g) were killed with an overdose of pentobarbitone. The main branch of the superior mesenteric arteries was dissected and the surrounding connective tissues were carefully removed. The artery was cut into rings of about 3 mm in length. Arterial rings were then mounted in 10 ml organ baths filled with Krebs–Henseleit solution oxygenated with a mixture of 95% O_2 and 5% CO_2. The Krebs–Henseleit solution had the following compositions (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. Tissues were allowed to equilibrate under 0.5 g resting tension for about 90 min. In some experiments the endothelium was mechanically disrupted by rubbing the lumen of the artery with plastic tubing. Successful removal of the endothelial layer was verified by the lack of the relaxant response of the preparation to acetylcholine (1 μM) at the beginning of each experiment. The isometric tension was measured with FT03 force-displacement transducer (Grass Instruments). All experiments were performed at 37°C.

2.2. Experimental protocol

20 min after setting up in organ baths the preparations with and without endothelium were first contracted by a single concentration of phenylephrine (3 μM) to test for their contractile response, after which they were rinsed three times in the Krebs–Henseleit solution to restore tension to the basal level. After a 90 min equilibration period, mesenteric arteries were again contracted with phenylephrine (3 μM, approximately EC_{80} value). Cyclopiazonic acid was then added to the bath to investigate its endothelium-dependent effect on the phenylephrine-precontracted arteries. Effects of N^G-nitro-L-arginine, charybdotoxin and indomethacin were examined by adding these compounds 10 min prior to application of phenylephrine. Effects of blockers of Ca^{2+} and K^+ channels, or tetrodotoxin were examined by applying the blockers to the bath after cyclopiazonic-acid-induced rhythmic contractile activity of the phenylephrine-precontracted arteries had appeared.

2.3. Drugs and chemicals

The following chemicals were used: phenylephrine hydrochloride, acetylcholine chloride, prazosin hydrochloride, glibenclamide, nifedipine, A23187, tetrodotoxin (Sigma, St. Louis, MO, USA). Cyclopiazonic acid, charybdotoxin, sodium nitroprusside, N^G-nitro-L-arginine, indomethacin, (Research Biochemicals International, Natick, MA, USA). Cyclopiazonic acid, prazosin and nifedipine were dissolved in dimethyl sulfoxide. Dimethyl sulfoxide at final concentration of 0.2% (v/v) did not affect rhythmic contractions induced by cyclopiazonic acid. The other chemicals were dissolved in Krebs solution.

2.4. Statistical analysis

The induced rhythmic activity was quantified as the products of the mean amplitude in g and frequency in cycles/min of rhythmic contractions in a 10 min record. The data were expressed as mean ± S.E.M. of n measurements. A level of probability of less than 0.05 obtained from Students' t-test was considered as significant.

3. Results

3.1. Effect of cyclopiazonic acid on phenylephrine-precontracted rat mesenteric arteries

A sustained contraction was induced by phenylephrine (3 μM) in rat isolated mesenteric arteries with or without endothelium. Fig. 1a shows that cyclopiazonic acid at 3 μM induced an initial relaxation (85 ± 12% reduction of evoked tension) followed by rhythmic contractions (mean amplitude: 0.28 ± 0.03 g, frequency: 2.73 ± 0.16 cycles/min, $n = 8$) in the phenylephrine-precontracted artery with endothelium. Cyclopiazonic acid at 10 μM caused a complete initial relaxation followed by low-frequency rhythmic activity (1.21 ± 0.39 cycles/min, $n = 5$). Acetylcholine (0.3 μM, $n = 9$) or A23187 (0.3 μM, $n = 5$, data not shown) transiently suppressed the rhythmic activity. The effect of cyclopiazonic acid was not seen in the endothelium-denuded artery ($n = 6$, Fig. 1b). Appearance of the rhythmic activity in the presence of cyclopiazonic acid was independent of the degree of induced tension when phenylephrine was used at concentration greater than 0.1 μM. However, the rhythmic activity increased as the induced tone increased by phenylephrine at concentrations of up to 10 μM (Fig. 1c). Tetrodotoxin (3 μM) did not affect the cyclopiazonic acid-induced rhythmic activity (mean amplitude: 0.31 ± 0.04 g, frequency: 2.53 ± 0.26 cycles/min, in control; and 0.29 ± 0.04 g, 2.39 ± 0.29 cycles/min, after addition of tetrodotoxin,

$n = 4$, $P > 0.05$, paired data), indicating that involvement of nerve activity was negligible.

3.2. Effects of N^G-nitro-L-arginine and indomethacin

The endothelium-dependent effect of cyclopiazonic acid indicates the involvement of endothelium-derived factors. Fig. 2a shows that N^G-nitro-L-arginine (100 μM) abolished the initial relaxation but not subsequent oscillations of muscle tone induced by 3 μM cyclopiazonic acid (mean amplitude: 0.284 ± 0.024 g, frequency: 2.63 ± 0.11 cycles/min, $n = 25$, in control; mean amplitude: 0.256 ± 0.032 g, frequency: 2.22 ± 0.28 cycles/min, $n = 7$, in 100 μM N^G-nitro-L-arginine, $P > 0.05$, unpaired data). The lack of effect of 1 μM indomethacin ($70 \pm 16\%$ initial relaxation, mean amplitude: 0.262 ± 0.029 g, frequency: 2.78 ± 0.22 cycles/min, $n = 5$, Fig. 2b), an inhibitor of cyclooxygenase, suggests that prostanoids might not be involved in the cyclopiazonic acid-induced responses in rat mesenteric arteries. In addition, sodium nitroprusside relaxed the phenylephrine-precontracted artery with IC_{50} value of 2.3 ± 0.25 nM ($n = 4$) without causing any oscillations of muscle tone.

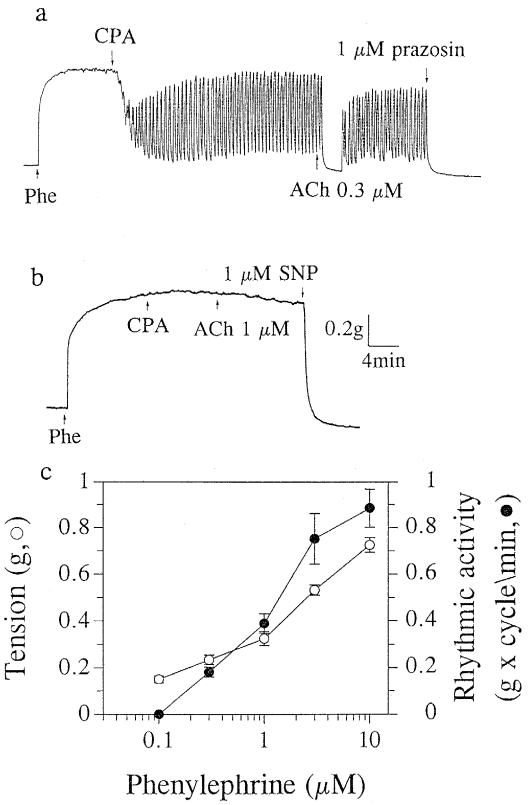

Fig. 1. Effect of cyclopiazonic acid (CPA) on phenylephrine (Phe)-precontracted arteries. (a) CPA (3 μM) relaxed the Phe (3 μM)-precontracted rat mesenteric artery with intact endothelium and subsequently induced rhythmic contractions which were transiently inhibited by acetylcholine (Ach, 0.3 μM) and completely inhibited by prazosin (1 μM). (b) Removal of the endothelium abolished the effect of CPA and Ach (1 μM). Each trace was representative of 6–9 experiments. Calibration bars apply to both traces. (c) Effect of the degree of phenylephrine-induced tone on the phasic activity induced by cyclopiazonic acid (3 μM). The amplitude of phenylephrine-induced tension was presented in g (○) and the rhythmic contractile activity was the product of mean amplitude and frequency of phasic contractions measured in a 10 min record (g cycles/min, ●). Data are the mean ± S.E.M. of 6–8 experiments.

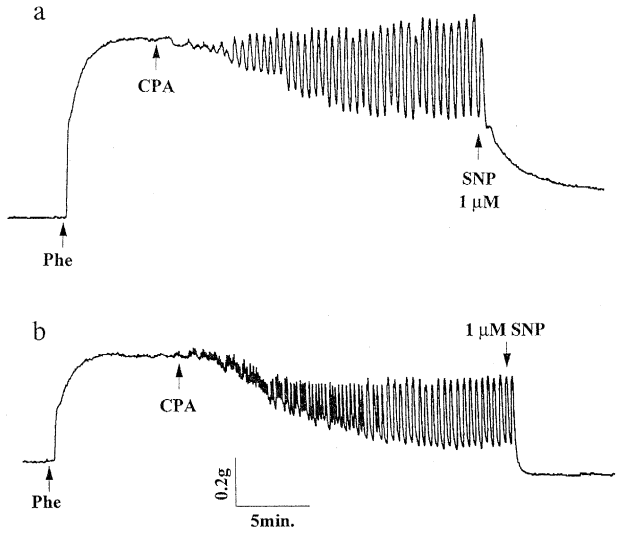

Fig. 2. Effect of CPA (3 μM) on phenylephrine (Phe)-precontracted rat mesenteric arteries with intact endothelium in the presence of 100 μM N^G-nitro-L-arginine (a) and of 1 μM indomethacin (b). The artery was incubated with each drug for 10 min prior to addition of 3 μM Phe. Sodium nitroprusside (SNP, 1 μM) completely relaxed the artery. Each trace was representative of 5–7 experiments. Calibration bars apply to both traces.

3.3. Effects of raised extracellular K^+ concentration and K^+ channel blockers

Since inhibitors of nitric oxide synthase and cyclooxygenase did not inhibit cyclopiazonic acid-induced endothelium-dependent rhythmic contractions, it was considered worthwhile testing whether other factors such as the endothelium-derived hyperpolarizing factor might be involved. Raising extracellular K^+ concentration would both depolarize the cell membrane and bring the membrane potential closer to the new equilibrium potential for K^+ ions. Fig. 3b shows that a moderate increase of extracellular K^+ (20 mM) totally abolished cyclopiazonic acid-induced rhythmic contractions in phenylephrine-precontracted arteries and reduced by approximately 39% the initial relaxation which was reversed by N^G-nitro-L-arginine (100 μM). In the presence of both 20 mM external K^+ and 100 μM N^G-nitro-L-arginine, cyclopiazonic acid (3 μM) failed to relax the artery, instead, it caused a small increase ($9.5 \pm 2.1\%$, $n = 8$) of the sustained tension induced by phenylephrine (Fig. 3c). In addition, cyclopiazonic acid at 3 μM did not induce any rhythmic activity but a small increase ($12 \pm 2.9\%$, $n = 5$) in tension of the artery contracted with 60 mM K^+. In order to identify the types of K^+ channels involved in

evoked rhythmic contractions, charybdotoxin, a selective blocker of large conductance K_{Ca} channels (Miller et al., 1985), and glibenclamide, a selective ATP-sensitive K^+ channel blocker (Standen et al., 1989; Ashcroft and Ashcroft, 1990) were tested for their effects on the cyclopiazonic acid-induced response. Fig. 4a and b show that charybdotoxin at 100 nM converted the cyclopiazonic acid-induced rhythmic contractions into the sustained tension in the absence ($n = 5$) or presence ($n = 7$) of N^G-nitro-L-arginine (100 μM). In addition, in arteries pretreated with 100 nM charybdotoxin, cyclopiazonic acid (3 μM) did not induce rhythmic contractions, instead it caused 76 ± 10% ($n = 4$) relaxation followed by slow recovery of muscle tension (Fig. 4c). In contrast, the arterial response to cyclopiazonic acid (3 μM) was unaffected by glibenclamide (mean amplitude: 0.265 ± 0.03 g, frequency: 2.58 ± 0.19 cycles/min before glibenclamide and mean amplitude: 0.242 ± 0.028 g, frequency: 2.49 ± 0.23 cycles/min after glibenclamide, $n = 4$, $P > 0.05$, paired data, Fig. 4d) at 1 μM, a concentration that entirely prevented vasorelaxation induced by cromakalim (0.1 μM), an ATP-sensitive K^+ channel opener, in the same preparations.

In addition, nifedipine, a voltage-sensitive Ca^{2+} channel blocker completely suppressed cyclopiazonic acid-induced rhythmic contractions at 10 nM ($n = 5$, data not shown).

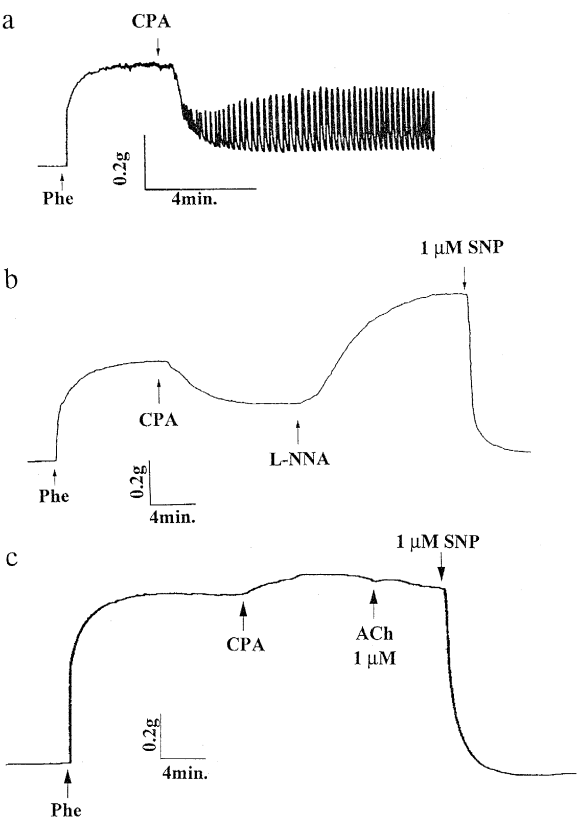

Fig. 3. Effect of CPA (3 μM) on phenylephrine (Phe)-precontracted rat mesenteric arteries with intact endothelium in 5.9 mM (a) and 20 mM external K^+ (b) and in the presence of both 100 μM L-NNA and 20 mM K^+ (c). SNP (1 μM) inhibited the sustained contraction. Each trace was representative of 5–8 experiments.

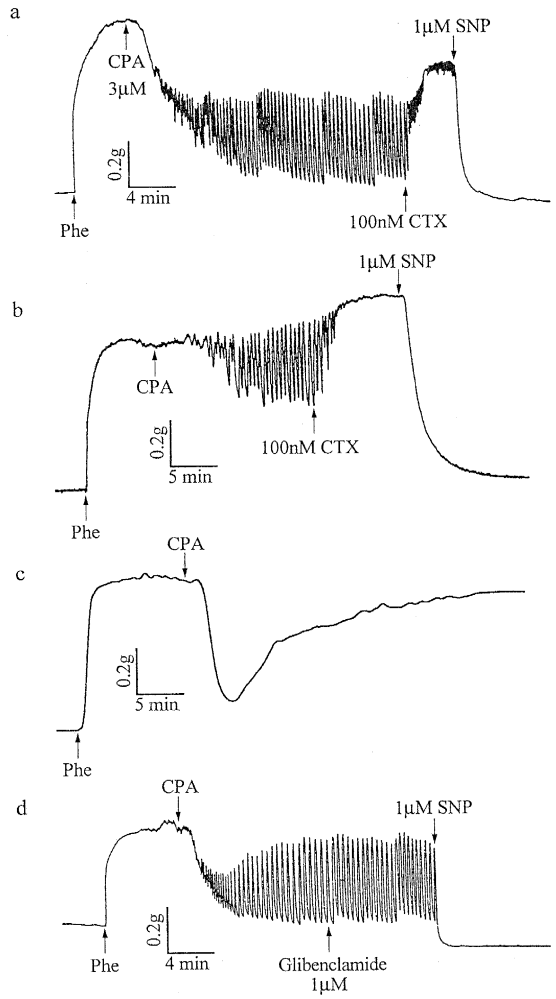

Fig. 4. Effects of K^+ channel blockers on CPA (3 μM)-induced response in endothelium-intact mesenteric arteries precontracted with 3 μM phenylephrine (Phe). Charybdotoxin (CTX, 100 nM) converted CPA-induced rhythmic contractions to sustained tension in the absence (a) and presence (b) of L-NNA (100 μM). SNP (1 μM) completely relaxed the artery. Effect of pretreatment with CTX (100 nM) on the CPA-induced arterial response (c). Lack of effect of glibenclamide (1 μM) on CPA-induced rhythmic activity (d). Each trace was representative of 4–7 experiments.

4. Discussion

The present study shows that cyclopiazonic acid, a selective inhibitor of the Ca^{2+}-pump of the sarcoplasmic reticulum (Deng and Kwan, 1991; Zhang et al., 1994a,b), induced an initial relaxation and subsequent rhythmic contractions of rat isolated mesenteric arteries precontracted by phenylephrine. The cyclopiazonic acid-induced rhythmic contractions appeared when the sustained tension was evoked by phenylephrine at concentrations greater than 0.1

μM, while mean amplitude and frequency of the rhythmic activity increased with increasing phenylephrine concentration. The response to cyclopiazonic acid was totally dependent upon the presence of endothelium. In contrast to this observation, another pattern of rhythmic contractions was reported in rabbit mesenteric and ear arteries when stimulated with the α_1-adrenoceptor agonist (Omote and Mizusawa, 1993a,b, 1994). In these arteries, phenylephrine induced endothelium-dependent oscillations of muscle tone. Interestingly, the phenylephrine-induced tonic contraction in the absence of endothelium was converted into rhythmic activity by cyclopiazonic acid (Omote and Mizusawa, 1994). Endothelium-independent rhythmic contractions induced by cyclopiazonic acid were, however, not seen in rat mesenteric arteries. In addition, cyclopiazonic acid only caused nitric oxide-mediated relaxation but not rhythmic contractions in rat aorta (Moritoki et al., 1994; Zhang et al., 1994b), indicating the fundamental difference in interaction between endothelium and arterial smooth muscle in the same artery of different species or different arteries of the same species. It appears that multiple factors released from endothelium might be involved in the complex effect of cyclopiazonic acid in rat mesenteric arteries. The initial relaxation induced by cyclopiazonic acid was abolished by the nitric oxide synthase inhibitor N^G-nitro-L-arginine at 100 μM while rhythmic contractile activity persisted. This concentration of N^G-nitro-L-arginine completely inhibited the endothelium-dependent relaxation induced by acetylcholine (Huang, 1997). In contrast, the inhibitor of nitric oxide synthesis completely eliminated phenylephrine-induced endothelium-dependent oscillations of muscle tone in the hamster aorta (Jackson et al., 1991). On the other hand, the amplitude of the initial relaxation induced by cyclopiazonic acid was similar in the absence and presence of charybdotoxin. In addition, low concentration of sodium nitroprusside did not induce rhythmic activity of the precontracted artery. Taken together, these results indicate that nitric oxide-mediated cyclic guanosine monophosphate-dependent pathway might play a little role in the cyclopiazonic acid-induced oscillations of muscle tone. Besides, prostacyclin might not be involved since indomethacin did not affect the cyclopiazonic acid-induced rhythmic contractions. Similarly, indomethacin did not change the cyclopiazonic acid-induced hyperpolarization in rat mesenteric arteries (Fukao et al., 1995). Cyclopiazonic acid at 10 μM, a concentration thought to maximally inhibit the Ca^{2+}-pump activity in smooth muscles (Uyama et al., 1992; Zheng et al., 1992), initially induced a complete inhibition of the evoked tension followed by rhythmic contractions at low frequency. It is likely that a greater amount of nitric oxide was released by cyclopiazonic acid to reduce the subsequent rhythmic activity of muscle tension.

The present results show that appearance of the rhythmic activity was unaffected by inhibition of nitric oxide activity even though it can not be entirely ruled out that nitric oxide may be involved in the rhythmic contractions albeit to a lesser degree. This suggests that cyclopiazonic acid could release factors other than nitric oxide from endothelium to induce rhythmic contractions. Endothelium-derived hyperpolarizing factors (EDHF) were reportedly involved in the control of vascular tone (Garland et al., 1995). Cyclopiazonic acid was previously reported to increase Ca^{2+} influx through non-selective cation channels in human endothelium (Zhang et al., 1994a). More recently, cyclopiazonic acid was found to hyperpolarize the cell membrane of smooth muscles from rat mesenteric arteries with endothelium and the induced hyperpolarization was resistant to inhibition of nitric oxide synthesis (Fukao et al., 1995). The change in the smooth muscle membrane potential induced by EDHF is normally related to an increase in the membrane conductance, which largely reflects an increase in K^+ permeability (Chen and Suzuki, 1989). If K^+ channels are opened by hyperpolarizing factors, raising the external K^+ concentration would reduce the driving force for K^+ efflux and subsequent hyperpolarization. Indeed, a moderate increase of external K^+ (20 mM) totally abolished the rhythmic contractions but only reduced the nitric oxide-dependent initial relaxation induced by cyclopiazonic acid. Further supporting evidence comes from the experiments showing that charybdotoxin, a potent blocker of the large-conductance K_{Ca} channels, completely converted the cyclopiazonic acid-induced rhythmic contractions into the sustained tension in the absence or presence of N^G-nitro-L-arginine. Pretreatment of the artery with charybdotoxin totally abolished cyclopiazonic acid-induced rhythmic contractions. Under this condition, cyclopiazonic acid still caused a relaxation in contracted arteries, but this relaxation could not sustain, whilst glibenclamide, a selective blocker of ATP-sensitive K^+ channels, had no effect. Similar effects of charybdotoxin and glibenclamide were also found in the cyclopiazonic acid-induced rhythmic activity in the endothelium-denuded rabbit ear and femoral arteries (Omote and Mizusawa, 1993b, 1994). It appears that K_{Ca} channels might play a role in the rhythmic activity. However, at present the nature of endothelial factors which activate K_{Ca} channels on smooth muscles is unknown. Our results suggest that the rhythmic activity of muscle tone induced by cyclopiazonic acid might be set up by an interplay between Ca^{2+} channels and K_{Ca} channels. Nifedipine abolished the rhythmic contractions at a concentration of 10 nM which completely inhibited the high K^+ (60 mM)-induced contraction in the same preparation. The EDHF-induced hyperpolarization of the plasma membrane might initially inactivate the dihydropyridine-sensitive Ca^{2+} channels opened by the vasoconstrictor; the local $[Ca^{2+}]_i$ in proximity to K^+ channels will then fall below the threshold needed to maintain K_{Ca} channel activity; closure of K_{Ca} channels will, in turn, depolarize the plasma membrane in the presence of the α-adrenoceptor agonist and cyclopiazonic acid, and again open the voltage-sensitive Ca^{2+}

channels. As a result, the intracellular Ca^{2+} concentration will increase above the levels needed to activate K_{Ca} channels to initiate another cycle. Nevertheless, it can not be determined whether EDHF alone causes endothelium-dependent rhythmic contractions until selective inhibitors of EDHF become available. In addition, 20 mM extracellular K^+ inhibited both the rhythmic activity and the relaxation to a lesser degree, produced by CPA. This implies that nitric oxide may at least in part act through K^+ channels.

To summarize, we have shown that cyclopiazonic acid induced the initial relaxation followed by rhythmic contractile activity in rat mesenteric arteries precontracted with the α_1-adrenoceptor agonist, and this effect depended upon the presence of endothelium. The initial relaxation was most likely mediated by nitric oxide released from the endothelium and the subsequent rhythmic contractions were triggered probably by EDHF in the endothelium. The rhythmic contractile activity was completely abolished by blockers of both large-conductance K_{Ca} channels and voltage-sensitive Ca^{2+} channels as well as by raising the extracellular K^+ concentration.

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References

Ashcroft, S.J.H., Ashcroft, F.M., 1990. Properties and functions of ATP-sensitive K^+ channels. Cell. Signal. 2, 197–214.

Berridge, M.J., Galione, A., 1988. Cytosolic calcium oscillators. FASEB J. 2, 3074–3082.

Chen, G., Suzuki, H., 1989. Some electrical properties of the endothelium-dependent hyperpolarization recorded from rat arterial smooth muscle cells. J. Physiol. 410, 91–106.

Dainty, I.A., Mcgrath, J.C., Spedding, M., Templeton, A.G., 1990. The influence of the initial stretch and the agonist-induced tone on the effect of basal and stimulated release of EDRF. Br. J. Pharmacol. 100, 767–773.

Deng, H.W., Kwan, C.Y., 1991. Cyclopiazonic acid is a sarcoplasmic reticulum Ca^{2+}-pump inhibitor of rat aortic muscle. Acta Pharmacol. Sin. 12, 53–58.

Fukao, M., Hattori, Y., Kanno, M., Sakuma, I., Kitabatake, A., 1995. Thapsigargin- and cyclopiazonic acid-induced endothelium-dependent hyperpolarization in rat mesenteric artery. Br. J. Pharmacol. 115, 987–992.

Furchgott, R.F., 1983. Role of endothelium in responses of vascular smooth muscle. Circ. Res. 53, 557–573.

Garland, C., Plane, F., Kemp, B.K., Cocks, T.M., 1995. Endothelium-dependent hyperpolarization: A role in the control of vascular tone. TiPS 16, 23–30.

Huang, Y., 1997. Influence of endothelium in contraction induced by phorbol ester in isolated rat aortic rings. Life Sci. 60, 1749–1756.

Inesi, G., Sagara, Y., 1994. Specific inhibitors of intracellular Ca^{2+} transport ATPase. J. Membr. Biol. 141, 1–6.

Jackson, W.F., Mulsch, A., Busse, R., 1991. Rhythmic smooth muscle activity in hamster aortas is mediated by continuous release of NO from the endothelium. Am. J. Physiol. 260, H248–H253.

Low, A.M., Kwan, C.Y., Daniel, E.E., 1992. Evidence for two types of internal Ca^{2+} stores in canine mesenteric artery with different refilling mechanisms. Am. J. Physiol. 262, H31–H37.

Miller, C., Moczydlowski, E., Latorre, R., Phillis, M., 1985. Charybdotoxin, a protein inhibitor of single Ca^{2+}-activated K^+ channels from skeletal muscle. Nature 313, 316–318.

Moritoki, H., Hisayama, T., Takeuchi, S., Kondoh, W., Imagawa, M., 1994. Relaxation of rat thoracic aorta induced by the Ca^{2+}-ATPase inhibitor, cyclopiazonic acid, possibly through nitric oxide formation. Br. J. Pharmacol. 111, 655–662.

Omote, M., Mizusawa, H., 1993a. The role of sarcoplasmic reticulum in endothelium-dependent and endothelium-independent rhythmic contractions in the rabbit mesenteric artery. Acta Physiol. Scand. 149, 15–21.

Omote, M., Mizusawa, H., 1993b. Phenylephrine-induced rhythmic activity in the rabbit ear artery. Jpn. J. Physiol. 43, 511–520.

Omote, M., Mizusawa, H., 1994. Effects of cyclopiazonic acid on phenylephrine-induced contractions in the rabbit ear artery. Br. J. Pharmacol. 111, 233–237.

Standen, D.N., 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.

Uyama, Y., Imaizumi, Y., Watanabe, M., 1992. Effects of cyclopiazonic acid, a novel Ca^{2+}-ATPase inhibitor, on contractile responses in skinned ileal smooth muscle. Br. J. Pharmacol. 106, 208–214.

Zhang, H., Inazy, M., Weir, B., Buchanan, M., Daniel, E., 1994a. Cyclopiazonic acid stimulates Ca^{2+} influx through non-specific cation channels in endothelial cells. Eur. J. Pharmacol. 251, 119–125.

Zhang, X.F., Kwan, C.Y., Daniel, E.E., 1994b. Role of intracellular Ca^{2+} in EDRF release in rat aorta. J. Vasc. Res. 31, 18–24.

Zheng, X.F., Guan, Y.Y., Kwan, C.Y., 1992. Cyclopiazonic acid causes endothelium-dependent relaxation in rat aorta. Acta Pharmacol. Sin. 14, 21–26.