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Contractions and relaxations accompanied by endothelial nitric oxide production induced in the porcine coronary artery by Ca^{2+}, Ba^{2+} and Sr^{2+}

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Abstract

In order to investigate the effects of Ca^{2+}, Ba^{2+} and Sr^{2+} on vascular endothelial nitric oxide (NO) production, contractile and relaxant responses of porcine depolarized coronary arteries to these divalent cations were compared. In the presence of diltiazem, Ba^{2+} induced NO-dependent relaxation, Sr^{2+} did slightly and Ca^{2+} did not; however all three cations increased cGMP levels in endothelium-intact arteries to similar extents. In the absence of diltiazem, these cations evoked contractions: the EC_{50} of Ca^{2+} for endothelium-denuded arteries was lower than those of Ba^{2+} and Sr^{2+}. The IC_{50} of diltiazem for arteries precontracted with Ca^{2+} was higher than for arteries precontracted with Ba^{2+} and Sr^{2+}. These results suggest that Ba^{2+} and Sr^{2+}, as well as Ca^{2+}, activate coronary arterial NO production, and also that the different responses of coronary arteries to these divalent cations can be explained, in part, by the different sensitivities of the smooth muscle to these cations and by the different potencies of diltiazem to inhibit the contractions the cations induced.

Keywords: Ca^{2+}; Ba^{2+}; Sr^{2+}; Nitric oxide (NO); Coronary artery, porcine; Diltiazem

1. Introduction

Calcium ions (Ca^{2+}) activate the contractile proteins in vascular smooth muscle cells (Barron et al., 1980; Driska et al., 1981). However, elevation of cytosolic Ca^{2+} is an essential step in the synthesis of prostacyclin and endothelium-derived relaxing factor (EDRF)/nitric oxide (NO) by vascular endothelial cells (Lückhoff et al., 1988; Busse et al., 1988). Therefore, both contractile and relaxant mechanisms of vascular smooth muscle appear to be regulated by Ca^{2+}.

Two other alkaline earth metal ions, barium (Ba^{2+}) and strontium (Sr^{2+}), have been used to study properties of contractile proteins in smooth muscle (Ebashi and Endo, 1968), and were shown to mimic the contractile effects of Ca^{2+} on depolarized vascular smooth muscle (Uvelius et al., 1974; Uchida, 1975). Ebeigbe and Aloamaka (1985) showed that the order of sensitivity of rat depolarized tail arterial smooth muscle to divalent cations was $Ca^{2+} > Sr^{2+} = Ba^{2+}$. In their study, however, the endothelial effects of divalent cations were not examined. As the endothelium is known to modulate the tone and contractile responses of vascular smooth muscle, it is important to elucidate whether the effects of these cations are modified by the endothelium.

We reported that Ca^{2+} induced EDRF-dependent relaxations of canine depolarized coronary arteries in the presence of Ca^{2+} channel antagonists (Kikkawa et al., 1989). In the absence of Ca^{2+} channel antagonists, however, the Ca^{2+}-induced contractions overcame this EDRF-dependent relaxant effect. These results suggested that Ca^{2+} channel antagonists do not affect the Ca^{2+} influx into endothelial cells necessary for the synthesis of EDRF/NO. Contractions induced by Ba^{2+} and Sr^{2+} were found to be inhibited by Ca^{2+} channel antagonists through the blockade of Ba^{2+} and Sr^{2+} influx via voltage-dependent Ca^{2+} channels (Karaki et al., 1986; Barreda and Anselmi, 1989). If Ba^{2+} and

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Sr^{2+} act on the endothelium in a similar manner to Ca^{2+}, this would enable EDRF-dependent relaxant effects of divalent cations in the presence of Ca^{2+} channel antagonists to be evaluated.

In the present study, in order to investigate the effects of divalent cations on vascular endothelial NO production, we compared the contractile and relaxant responses of porcine depolarized coronary arteries to Ca^{2+}, Ba^{2+} and Sr^{2+}, and examined the effects of these cations on cGMP formation by these arteries. We also compared the sensitivities of this smooth muscle to NO and the inhibitory effects of diltiazem on the contractions induced by Ca^{2+}, Ba^{2+} and Sr^{2+}.

2. Materials and methods

2.1. Preparations and general procedures

Porcine hearts were obtained from a slaughterhouse, and washed with cold physiological salt solution (PSS) of the following composition (mM): 147.2 NaCl, 5.4 KCl, 14.5 $NaHCO_3$, 1.0 $MgCl_2$, 2.2 $CaCl_2$, 5.4 glucose. The left branches of the circumflex coronary arteries were isolated carefully, cleaned of any adhering connective tissue, and cut into 4-mm-long rings. Each ring was mounted between a pair of triangular stainless steel hooks, one of which was stationary and the other was connected to a strain-gauge transducer (UL10GR, Minebea), and the isometric tension was recorded (MC6625, Graphtec, Tokyo, Japan). The rings, placed in 15-ml organ baths containing PSS maintained at 37°C and aerated continuously with 95% v/v O_2-5% v/v CO_2, were stretched to a resting tension of 1.5 g and allowed to equilibrate for at least 1 h with occasional changes of the PSS. After equilibration, the rings were exposed to nominally Ca^{2+}-free PSS containing 80 mM KCl (equimolar replacement for NaCl). No Ca^{2+}-chelating agent was present. The endothelium was removed from some preparations by gentle rubbing of the intimal surface with a cotton swab. The presence or absence of endothelium was confirmed by examining the relaxations in response to bradykinin (10^{-7} and 10^{-6} M) of arterial rings precontracted by U46619 (9,11-dideoxy-9α,11α-methanoepoxy-prostaglandin $F_{2\alpha}$) (10^{-7} M).

2.2. Concentration-response curves for Ca^{2+}, Ba^{2+} and Sr^{2+}

Ca^{2+}, Ba^{2+} or Sr^{2+} (10^{-5}–10^{-2} M) was added cumulatively to the organ baths containing the porcine coronary arteries. Diltiazem (3×10^{-7}–3×10^{-5} M) was added 10 min before, indomethacin (5×10^{-6} M) and N^G-monomethyl-L-arginine (L-NMMA) (10^{-4} M) were added 15 and 5 min, respectively, before the cumulative administration of the required divalent cations.

2.3. Relaxations induced by NO solution, sodium nitroprusside and diltiazem

Nitric oxide gas was dissolved in 2 ml distilled water deoxygenated by bubbling with helium gas for at least 30 min. Appropriate volumes were removed with an air-tight syringe and added to fresh distilled water to produce stock solutions of NO: according to the solubility of NO in water at 20°C, the concentration of NO in the prepared saturated solution was considered to be 2×10^{-3} M (Ishii et al., 1992). Nitric oxide (10^{-9}–10^{-5} M), sodium nitroprusside (10^{-8}–10^{-4} M) and diltiazem (10^{-9}–3×10^{-5} M) were added cumulatively to K^+-depolarized coronary arteries precontracted with Ca^{2+}, Ba^{2+} and Sr^{2+} (10^{-3} M).

2.4. Measurement of cGMP

Coronary arterial segments were washed repeatedly with nominally Ca^{2+}-free, K^+-depolarizing PSS, incubated first with diltiazem (3×10^{-6} M) for 10 min, and then challenged with the required divalent cation (10^{-3} M) for 5 min; L-NMMA (10^{-4} M) was added 15 min before the addition of each cation. After incubation with the required divalent cation, each preparation was frozen quickly by immersion in liquid nitrogen, and stored at −70°C until analyzed. Each frozen ring was homogenized in 0.5 ml trichloroacetic acid (6% v/v) with a glass-glass homogenizer, the homogenate was centrifuged at $2000 \times g$ for 10 min at 4°C, and the protein content of the pellet was assayed as described by Lowry et al. (1951). Each supernatant was extracted 3 times with 2 volumes of water-saturated ether, lyophilized samples were reconstituted in 100 μl distilled water, and the cGMP content was determined using a cGMP radioimmunoassay kit. The content of cGMP was expressed as mol/mg protein.

2.5. Data analysis

The data are expressed as means ± S.E. The concentration-response curve data for each individual preparation were fitted to a general logistic function to determine the EC_{50} and the IC_{50}. Student's or Welch's t-test was used to compare the means of two groups and analysis of variance, followed by Bonferroni's t-test, was performed to compare the results of three groups. Differences at P values of less than 0.05 were considered to be significant.

2.6. Materials

The drugs used were U46619 (Cayman Chemical Co.), bradykinin acetate, indomethacin (Sigma Chemi-

cal Co.), diltiazem hydrochloride (Tanabe Seiyaku, Co.), $CaCl_2 \cdot 2H_2O$ (the highest grade, Wako Pure Chemical Co.), $BaCl_2 \cdot 2H_2O$ (the highest grade, Kanto Chemical Co.), $SrCl_2 \cdot 2H_2O$ (the highest grade, Hanni Kagaku, Co.), L-NMMA (synthesized by the organic chemistry research laboratory, Tanabe Seiyaku, Co. Ltd.), L-arginine hydrochloride, sodium nitroprusside (Nacalai Tesque) and NO gas (Sumitomo Seika, Co.). U46619 (10^{-4} M) and indomethacin (10^{-3} M) were dissolved in 99.5% v/v ethanol, bradykinin (10^{-3} M) was dissolved in 70% v/v ethanol, and the other agents were dissolved in distilled water.

3. Results

3.1. Responses of porcine coronary arteries to divalent cations

Cumulative application of Ca^{2+}, Ba^{2+} or Sr^{2+} (10^{-5}–2×10^{-2} M) induced concentration-dependent contractions of porcine K^+ (80 mM)-depolarized coronary arteries with endothelium (Fig. 1a). The EC_{50}s for Ca^{2+}, Ba^{2+} and Sr^{2+} were $1.24 \pm 0.36 \times 10^{-4}$, $2.12 \pm 0.20 \times 10^{-4}$ and $2.04 \pm 0.30 \times 10^{-4}$ M, respectively, and did not differ significantly ($n = 6$).

As shown in Fig. 1, Ca^{2+}-induced contractions were inhibited by pretreatment with diltiazem (3×10^{-6} M and 3×10^{-5} M). Ba^{2+} evoked a marked relaxation in the presence of diltiazem (3×10^{-6} and 3×10^{-5} M). Sr^{2+} also evoked a small but significant ($P < 0.05$) relaxation in the presence of diltiazem (3×10^{-5} M). Maximal relaxations were obtained in response to 10^{-3} M Ba^{2+} in the presence of diltiazem (3×10^{-6} M), and to 5×10^{-3} M Ba^{2+} and 2×10^{-3} M Sr^{2+} with a higher concentration of diltiazem (3×10^{-5} M). The magnitudes of the maximal relaxations were dependent on the concentration of diltiazem and were in the following order: $Ba^{2+} > Sr^{2+}$.

3.2. Effects of endothelial removal, L-NMMA and indomethacin

In order to determine whether the relaxations were due to endothelial NO release, the effects of endothelial removal, L-NMMA and indomethacin, in the presence of diltiazem (3×10^{-6} M), were studied. Alone, L-NMMA (10^{-4} M) had no effect on the basal tension. The contractions induced by Ca^{2+} in the presence of diltiazem (3×10^{-6} M) were augmented slightly by endothelial removal and L-NMMA pretreatment (Fig. 2a), whereas the Ba^{2+}-induced relaxations were inhibited significantly by both these measures (Fig. 2b). Indomethacin (5×10^{-6} M), which increased the resting tension by $8.9 \pm 0.4\%$, had no effect on Ba^{2+}-induced relaxations (data not shown). The Sr^{2+}-induced

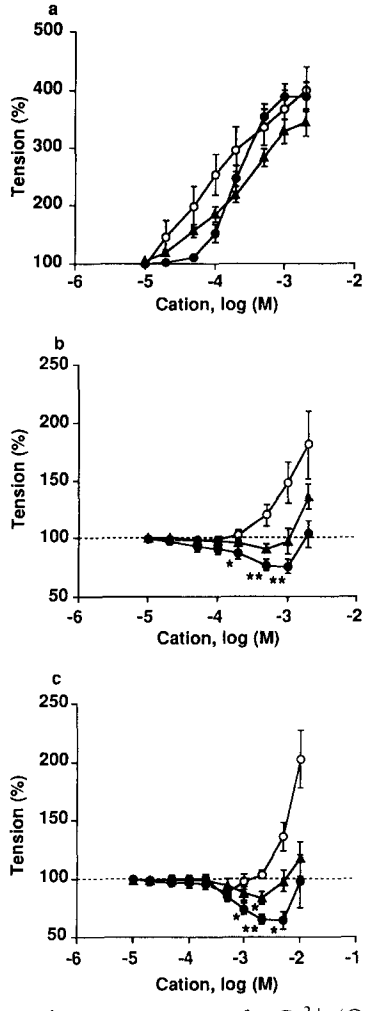

Fig. 1. Concentration-response curves for Ca^{2+} (○), Ba^{2+} (●) and Sr^{2+} (▲) of porcine K^+ (80 mM)-depolarized endothelium-intact coronary arteries in the absence (a) and presence (b, c) of diltiazem. Diltiazem (3×10^{-6}, b, and 3×10^{-5} M, c) was added to the preparations 10 min before cumulative administration of Ca^{2+}, Ba^{2+} or Sr^{2+}. The responses are expressed as percentages of the resting tension; each point represents the mean of four to seven experiments and the vertical bars represent S.E.; the broken lines indicate the resting tension; $^*P < 0.05$, $^{**}P < 0.01$ vs. the resting tension in b and c.

contractions were augmented by L-NMMA pretreatment (Fig. 2c).

In the presence of diltiazem (3×10^{-6} M), the contractions of endothelium-intact depolarized arteries induced by Ca^{2+}, Ba^{2+} and Sr^{2+} (2×10^{-3} M) were increased by the subsequent addition of L-NMMA (10^{-4} M, data not shown). Furthermore, when L-arginine (10^{-3} M) was added during plateau responses to L-NMMA, these tension increases were partially reversed.

3.3. Formation of cGMP induced by Ca^{2+}, Ba^{2+} and Sr^{2+}

In depolarized coronary arteries with endothelium, treatment with Ca^{2+}, Ba^{2+} and Sr^{2+} (10^{-3} M) for 5

min in the presence of diltiazem (3×10^{-6} M) increased the cGMP levels significantly to similar extents (Fig. 3). Treatment with L-NMMA (10^{-4} M) had no effect on the basal level of cGMP, but it inhibited the increases in cGMP levels induced by these divalent cations, significantly, back to the control levels. In endothelium-denuded arteries, these cations failed to increase the cGMP levels.

3.4. Effects of NO and sodium nitroprusside on divalent cation-induced contractions

The effects of NO (10^{-9}–10^{-5} M) and sodium nitroprusside (10^{-8}–10^{-4} M) on depolarized arteries

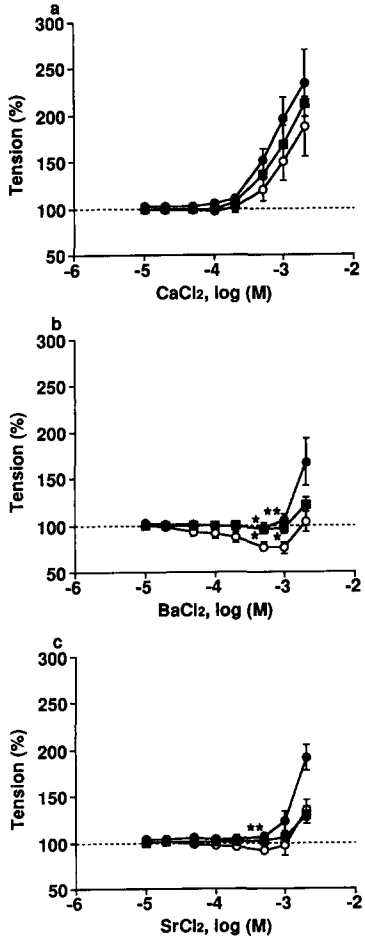

Fig. 2. Effects of L-NMMA pretreatment and endothelial removal on contractile and relaxant responses to divalent cations of porcine K^+ (80 mM)-depolarized coronary arteries in the presence of diltiazem. (○) Controls (endothelium-intact preparations); (●) L-NMMA-treated endothelium-intact preparations; (■) endothelium-denuded preparations. Diltiazem (3×10^{-6} M) was added to the preparations 10 min before cumulative administration of Ca^{2+} (a), Ba^{2+} (b) or Sr^{2+} (c); L-NMMA (10^{-4} M) was added to the preparations 5 min before cumulative administration of these cations. The responses are expressed as percentages of the resting tension; each point represents the mean of four to seven experiments and the vertical bars represent S.E. The broken lines indicate the resting tensions. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control groups.

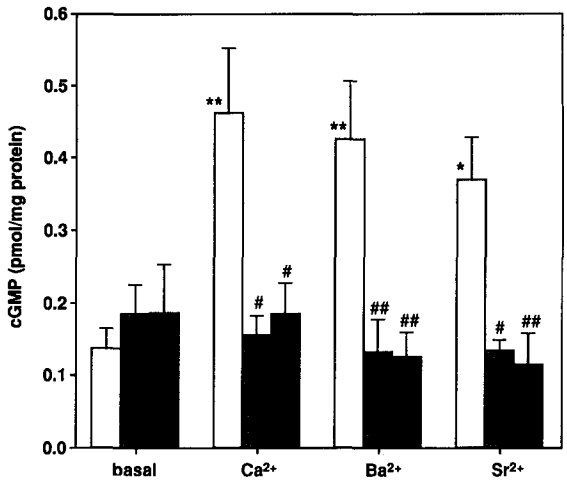

Fig. 3. Effects of divalent cations on cGMP formation by porcine K^+ (80 mM)-depolarized coronary arteries. Open columns: controls (endothelium-intact preparations); hatched columns: L-NMMA-treated endothelium-intact preparations; black columns: endothelium-denuded preparations. The amounts of cGMP were measured after incubation with diltiazem (3×10^{-6} M) for 10 min and subsequent co-incubation with the required divalent cations (10^{-3} M) for 5 min; L-NMMA (10^{-4} M) was added to the preparations 5 min before administration of divalent cations. Each column represents the mean of five to nine experiments and the vertical bars represent S.E. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the basal level; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. the control level for each cation.

contracted by divalent cations (10^{-3} M) were studied (Fig. 4a,b). The addition of Ca^{2+}, Ba^{2+} and Sr^{2+} increased the tension by 4.18 ± 0.42, 2.69 ± 0.21 and 3.09 ± 0.27 g, respectively ($n = 8$). The IC_{50}s of NO for the arteries precontracted by Ca^{2+}, Ba^{2+} and Sr^{2+} were $6.81 \pm 2.01 \times 10^{-7}$, $4.51 \pm 0.98 \times 10^{-7}$ and $7.08 \pm 2.10 \times 10^{-7}$ M, respectively ($n = 4$). The corresponding respective values for sodium nitroprusside were $3.30 \pm 0.97 \times 10^{-6}$, $3.10 \pm 1.94 \times 10^{-6}$ and $3.23 \pm 0.87 \times 10^{-6}$ M ($n = 4$). No significant differences among the IC_{50} values of NO or sodium nitroprusside for the arteries precontracted by these three cations were observed.

3.5. Effects of diltiazem on divalent cation-induced contractions

The direct contractile effects of the divalent cations on vascular smooth muscle were examined, first. Cumulative application of Ca^{2+}, Ba^{2+} and Sr^{2+} (10^{-5}–2×10^{-3} M) to endothelium-denuded arteries induced concentration-dependent contractions in K^+ (80 mM)-depolarizing PSS (Fig. 5a). The contractions induced by Ca^{2+} were somewhat higher than those induced by the same concentrations of Ba^{2+} and Sr^{2+}. The EC_{50} of Ca^{2+} ($1.25 \pm 0.23 \times 10^{-4}$ M) was significantly lower than those of Ba^{2+} and Sr^{2+} ($2.63 \pm 0.32 \times 10^{-4}$ and $2.20 \pm 0.35 \times 10^{-4}$ M, respectively, $P < 0.05$, $n = 6 \sim 7$).

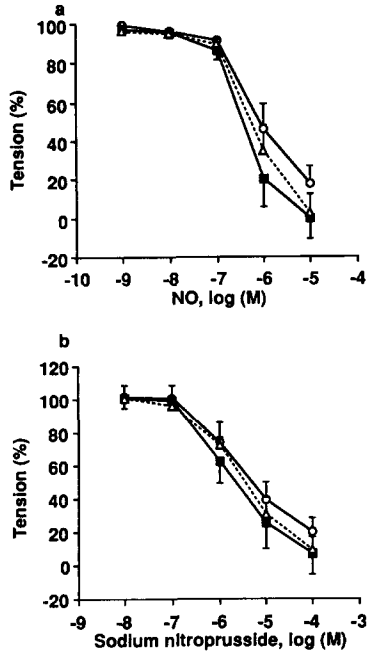

Fig. 4. Concentration-response curves for NO solution (a) and sodium nitroprusside (b) with porcine K^+ (80 mM)-depolarized coronary arteries contracted by Ca^{2+} (○), Ba^{2+} (■) and Sr^{2+} (△) (10^{-3} M). The responses are expressed as percentages of the maximum tension induced by the divalent cations; each point represents the mean of four experiments and the vertical bars represent S.E.

Pretreatment with diltiazem (3×10^{-7} and 3×10^{-6} M) inhibited the responses of endothelium-denuded arteries to Ca^{2+}, Ba^{2+} and Sr^{2+} (Fig. 5b,c). In the presence of diltiazem (3×10^{-7} M), the contractions induced by Ca^{2+} were somewhat higher than those induced by the same concentrations of Ba^{2+} and Sr^{2+} (Fig. 5b). Even in the presence of diltiazem (3×10^{-6} M), Ca^{2+} (10^{-3} M) still evoked contractions, but the Ba^{2+} (10^{-3} M)- and Sr^{2+} (10^{-3} M)-induced contractions were virtually abolished (Fig. 5c). These results show that the contractile effects of Ca^{2+} on depolarized arteries in both the absence and presence of diltiazem are stronger than those of Ba^{2+} and Sr^{2+}.

The inhibitory effects of diltiazem on divalent cation (10^{-3} M)-induced contractions of endothelium-denuded depolarized arteries were compared (Fig. 6). The addition of Ca^{2+}, Ba^{2+} and Sr^{2+} increased the tension by 2.76 ± 0.42, 1.96 ± 0.33 and 2.54 ± 0.07 g, respectively ($n = 4$). The IC_{50}s of diltiazem for the arteries precontracted by Ca^{2+}, Ba^{2+} and Sr^{2+} were $3.27 \pm 0.39 \times 10^{-7}$, $8.66 \pm 2.02 \times 10^{-8}$ and $9.83 \pm 1.17 \times 10^{-8}$ M, respectively. The IC_{50} for the artery precontracted by Ca^{2+} was significantly higher than those for arteries precontracted by Ba^{2+} and Sr^{2+} ($P < 0.01$), which indicates that diltiazem is a less potent inhibitor of Ca^{2+}-induced than of Ba^{2+}- and Sr^{2+}-induced contractions.

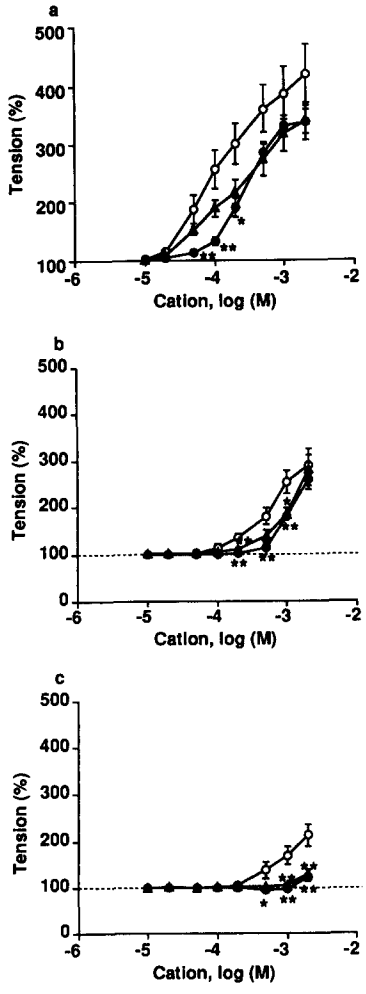

Fig. 5. Concentration-response curves for Ca^{2+} (○), Ba^{2+} (●) and Sr^{2+} (▲) with porcine K^+ (80 mM)-depolarized endothelium-denuded coronary arteries in the absence (a) and presence (b,c) of diltiazem. Diltiazem (3×10^{-7} M, b, and 3×10^{-6} M, c) was added to the preparations 10 min before cumulative administration of Ca^{2+}, Ba^{2+} and Sr^{2+}. The responses are expressed as percentages of the resting tension; each point represents the mean of four to six experiments and the vertical bars represent S.E. The broken lines represent the resting tensions. $^*P < 0.05$, $^{**}P < 0.01$ vs. Ca^{2+}.

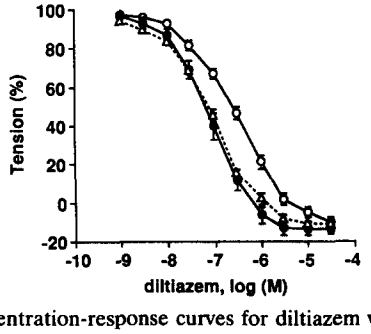

Fig. 6. Concentration-response curves for diltiazem with porcine K^+ (80 mM)-depolarized endothelium-denuded coronary arteries contracted by Ca^{2+} (○), Ba^{2+} (●) and Sr^{2+} (△) (10^{-3} M). The responses are expressed as percentages of the maximum tension induced by the divalent cations; each point represents the mean of four experiments and the vertical bars represent S.E.

4. Discussion

The major finding of this study was that Ba^{2+} and Sr^{2+} acted on the endothelium, and, in the presence of diltiazem, evoked relaxations of porcine depolarized coronary arteries. This is, to our knowledge, the first evidence that Ba^{2+} and Sr^{2+} can activate NO production by the porcine coronary arterial endothelium.

It should be noted that the magnitudes of the maximal relaxations induced by these divalent cations were dependent on the diltiazem concentration. The inhibitory effect of diltiazem on arterial smooth muscle contraction appears to reveal the relaxations induced by divalent cations. Therefore, the experimental conditions used in this study are suitable for the evaluation of contractile and relaxant effects of divalent cations on vascular preparations in vitro.

The effects of endothelial removal, of L-NMMA, an inhibitor of NO synthesis, and of indomethacin, a cyclooxygenase inhibitor, on Ba^{2+}-induced relaxations were examined. These relaxations were significantly inhibited by endothelial removal and by pretreatment with L-NMMA, but not indomethacin. These results indicate that Ba^{2+}-induced relaxations are mediated by endothelium-derived NO, but not by prostacyclin.

In the presence of diltiazem, endothelial removal or L-NMMA pretreatment increased the contraction induced by Ca^{2+} and Sr^{2+} slightly. The contractions induced by Ca^{2+}, Ba^{2+} and Sr^{2+} were increased by post-treatment with L-NMMA and these tension increases were partially reversed by subsequent treatment with L-arginine. Importantly, Ca^{2+}, Ba^{2+} and Sr^{2+} increased the cGMP levels in endothelium-intact arteries to similar extents, and these increases were abolished by L-NMMA pretreatment and endothelial removal. These results suggest that Ca^{2+}, Ba^{2+} and Sr^{2+} induce NO release from the endothelium in a similar manner.

Endothelium-dependent relaxations of porcine coronary arteries were shown to be partially mediated by endothelium-dependent hyperpolarization (Nagao and Vanhoutte, 1992). In the present study, however, the effect of endothelium-dependent hyperpolarization on divalent cation-induced relaxations could be ignored, because we examined them using high K^+ (80 mM)-depolarizing PSS.

Endothelial cellular NO synthesis requires Ca^{2+} and calmodulin (Busse and Mülsch, 1990; Förstermann et al., 1991). As Ba^{2+} has been shown to bind to calmodulin with an affinity much lower than that of Ca^{2+} (Chao et al., 1984), it is interesting that Ba^{2+} was found to activate NO synthesis. The results of the present study are not consistent with those reported previously by Ku (1988), who found that Ca^{2+} and Sr^{2+}, but not Ba^{2+}, evoked endothelium-dependent relaxation of canine Ca^{2+}- and Mg^{2+}-deficient coronary arteries. In Ku's preparation, NO-mediated relaxation may have been masked by the contractions induced by Ba^{2+}. With regard to the mechanism responsible for Ba^{2+}- and Sr^{2+}-induced relaxations, our results suggest that Ba^{2+} and Sr^{2+} enter the endothelial cells and activate NO synthesis, although it is not clear whether this effect is due to intracellular calcium release. The precise mechanism responsible remains to be elucidated and further studies using a simpler system such as cultured endothelial cells should be carried out.

In the present study, the order of magnitude of the maximum relaxations of the porcine coronary arterial rings was $Ba^{2+} > Sr^{2+}$ and Ca^{2+} did not evoke relaxation, although all three cations increased the cGMP levels in endothelium-intact arteries to similar extents. Several possible mechanisms may account for the different responses of coronary arteries to divalent cations: (1) different effects of the cations on the sensitivity of the smooth muscle to NO, (2) different effects of the cations on the contractile process and (3) differential inhibitory effects of diltiazem on divalent cation-induced contractions.

As Ca^{2+} and Sr^{2+} were shown to inhibit bovine coronary arterial soluble guanylate cyclase (Gruetter et al., 1980), the effects of divalent cations on the sensitivity of the smooth muscle to NO may differ. However, in the present study, the relaxations of the arteries contracted by these divalent cations induced by NO and sodium nitroprusside were of the same magnitude, which suggests that the effects of divalent cations on NO-dependent relaxation did not differ.

The direct contractile effects of divalent cations on contraction of endothelium-denuded arteries were examined. The EC_{50} of Ca^{2+} was lower than those of Ba^{2+} and Sr^{2+}, suggesting that the sensitivity of the smooth muscle to Ca^{2+} was greater than it was to Ba^{2+} and Sr^{2+}. Therefore, the different responses of coronary arteries to these divalent cations may be accounted for, in part, by the different magnitudes of the contraction they evoked. Ca^{2+} and calmodulin are known to play a crucial role in smooth muscle contraction (Kamm and Stull, 1985). Therefore, the different affinities of these cations for binding to calmodulin could reflect the cation sensitivities of the smooth muscle to them. Different membrane permeability of these cations may also explain the cation sensitivity.

Our results also showed that the IC_{50} of diltiazem for arteries precontracted by Ca^{2+} was higher than the IC_{50} for arteries precontracted by Ba^{2+} and Sr^{2+}, which showed clearly that the inhibitory effect of diltiazem is weaker on arteries precontracted by Ca^{2+} than on those contracted by Ba^{2+} and Sr^{2+}. The different inhibitory potencies of diltiazem on divalent cation-induced contractions were probably due to the differential inhibition of the entry of the divalent cations into

the smooth muscle cells of porcine coronary arteries through L-type Ca^{2+} channels.

Both the sensitivity of the coronary arterial smooth muscle to the cations and different inhibitory potencies of diltiazem would explain why Ca^{2+} did not evoke relaxation: both mechanisms would account for the observation that Ca^{2+} still induced contractions of endothelium-denuded arteries in the presence of diltiazem, whereas the Ba^{2+}- and Sr^{2+}-induced contraction were diminished. In the present study, however, the reason for the difference between Ba^{2+}- and Sr^{2+}-induced relaxations was not clear, and further study is needed to elucidate this.

In summary, we found that Ba^{2+} and Sr^{2+}, as well as Ca^{2+}, activated NO production by the porcine coronary arterial endothelium. However, the responses of coronary artery to these divalent cations differed, which can be explained, in part, by different sensitivities of the smooth muscle to these cations and the differential inhibitory effects of diltiazem on the contractions they induced. The responses of porcine coronary arteries to Ca^{2+}, Ba^{2+} and Sr^{2+} are the result of the combined effects of these cations on both smooth muscle and endothelial components.

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References

Barreda, A. and E. Anselmi, 1989, Study of the strontium response in isolated rat aorta, Arch. Int. Pharmacodyn. 299, 210.

Barron, J.T., M. Barany, K. Barany and R.V. Storti, 1980, Reversible phosphorylation of the 20,000-dalton light chain of myosin during the contraction-relaxation-contraction cycle of arterial smooth muscle, J. Biol. Chem. 255, 6238.

Busse, R. and A. Mülsch, 1990, Calcium-dependent nitric oxide synthase in endothelial cytosol is mediated by calmodulin, FEBS Lett. 256, 133.

Busse, R., H. Fichtner, A. Lückhoff and M. Kohlardt, 1988, Hyperpolarization and increased free calcium in acetylcholine-stimulated endothelial cells, Am. J. Physiol. 255, H965.

Chao, S.-H., Y. Suzuki, J.R. Zysk and W.Y. Cheung, 1984, Activation of calmodulin by various metal cations as a function of ionic radius, Mol. Pharmacol. 26, 75.

Driska, S.P., M.O. Aksoy and R.A. Murphy, 1981, Myosin light chain phosphorylation associated with contraction in arterial smooth muscle, Am. J. Physiol. 240, C222.

Ebashi, S. and M. Endo, 1968, Calcium ion and muscle contraction, Prog. Biophys. Mol. Biol. 18, 125.

Ebeigbe, A.B. and C.P. Aloamaka, 1985, Barium and strontium as calcium substitutes for contractile responses in the rat tail artery, Comp. Biochem. Physiol. 82C, 213.

Förstermann, U., J.S. Pollock, H.H.H.W. Schmidt, M. Heller and F. Murad, 1991, Calmodulin-dependent endothelium-derived relaxing factor/nitric oxide synthase activity is present in the particulate and cytosolic fractions of bovine aortic endothelial cells, Proc. Natl. Acad. Sci. USA 88, 1849.

Gruetter, D.Y., C.A. Gruteter, B.K. Barry, W.H. Baricos, A.L. Hyman, P.J. Kadowitz and L.J. Ignarro, 1980, Activation of coronary arterial guanylate cyclase by nitric oxide, nitroprusside, and nitrosoguanidine – inhibition by calcium, lanthanum, and other cations, enhancement by thiols, Biochem. Pharmacol. 29, 2943.

Ishii, K., H. Sheng, T.D. Warner, U. Förstermann and F. Murad, 1991, A simple and sensitive bioassay method for detection of EDRF with RFL-6 rat lung fibroblasts, Am. J. Physiol. 261, H598.

Kamm, K.E. and J.T. Stull, 1985, The function of myosin and myosin light chain kinase phosphorylation in smooth muscle, Ann. Rev. Pharmacol. Toxicol. 25, 593.

Karaki, H., N. Satake and S. Shibata, 1986, Mechanism of barium-induced contraction in the vascular smooth muscle of rabbit aorta, Br. J. Pharmacol. 88, 821.

Kikkawa, K., S. Murata and T. Nagao, 1989, Endothelium-dependent calcium-induced relaxation in the presence of Ca^{2+}-antagonists in canine depolarized coronary arteries, Br. J. Pharmacol. 98, 700.

Ku, D.D., 1988, Divalent cation regulation of endothelial-dependent relaxation in coronary blood vessels, Microcirc. Endoth. Lymphatics 5, 99.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193, 265.

Lückhoff, A., U. Pohl, A. Mülsch and R. Busse, 1988, Differential role of extra- and intracellular calcium in the release of EDRF and prostacyclin from cultured endothelial cells, Br. J. Pharmacol. 95, 189.

Nagao, T. and P.M. Vanhoutte, 1992, Hyperpolarization as a mechanism for endothelium-dependent relaxations in the porcine coronary artery, J. Physiol. 445, 355.

Uchida, E., 1975, Contraction and relaxation of vascular smooth muscle and calcium, strontium, barium, Jpn. Circ. J. 39, 565.

Uvelius, B., S.B. Sigurdsonn and B. Johansson, 1974, Strontium and barium as substitutes for calcium on electrical and mechanical activity in rat portal vein, Blood Vessel 11, 245.