KCl-Depolarization Potentiates the Ca^{2+} Sensitization by Endothelin-1 in Canine Coronary Artery

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ABSTRACT—In Ca^{2+} -free solution containing endothelin-1 (ET-1), depolarization by high KCl increased the force of contraction without any changes in intracellular calcium concentration ($[\text{Ca}^{2+}]_i$) in canine coronary artery. The $[\text{Ca}^{2+}]_i$ -force relationships were examined in the absence or presence of ET-1 in 5 or 90 mM KCl. The relation curve in the presence of ET-1 in 90 mM KCl-PSS was shifted to the left and upward compared with that in 5 mM KCl. These results suggest that KCl-depolarization potentiates the Ca^{2+} sensitization by ET-1.

Keywords: KCl-depolarization, Endothelin-1, Ca^{2+} sensitivity

Mechanisms involved in contractions following receptor stimulation by Ca^{2+} -mobilizing agonists are far more complex than those following simple membrane depolarization in vascular smooth muscle. There are at least three major components: Ca^{2+} influx, intracellular Ca^{2+} release and an increase in Ca^{2+} sensitivity of the contractile elements (Ca^{2+} sensitization) (1). However, no investigators have measured intracellular Ca^{2+} concentrations ($[\text{Ca}^{2+}]_i$) to determine the influence of changes in the membrane potential on the Ca^{2+} sensitization by agonists. In the present study, we examined whether the depolarization induced by high KCl affects the Ca^{2+} sensitization induced by the agonist ET-1 by the simultaneous measurement of both force of contraction and $[\text{Ca}^{2+}]_i$ in canine coronary arteries loaded with fura-2.

Hearts were obtained from mongrel dogs of either sex, weighing 10 to 16 kg, anesthetized with sodium pentobarbital (30 mg/kg, i.v.). Endothelium-denuded coronary arterial rings (0.7–1.2 mm in diameter, about 1 mm in width) were used. We performed fluorescence measurements by using fura-2 as previously described (2–4). We have used the relative F_{340}/F_{380} ratio as an indicator of $[\text{Ca}^{2+}]_i$.

The composition of 5 mM KCl physiological salt solution (5K-2.5Ca-PSS) was as follows: 140 mM NaCl, 5 mM KCl, 2.5 mM CaCl_2 , 2.5 mM MgCl_2 , 11.1 mM glucose

and 3 mM HEPES (4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid), pH 7.4 (37°C). High KCl-PSS was made by substituting NaCl with equimolar KCl. In the experiment to reduce extracellular NaCl concentration, NaCl was substituted with equimolar choline chloride. Low Ca^{2+} -PSS was made by reducing (0.1 mM, designated as 0.1Ca) or by removing CaCl_2 from normal PSS and adding 1 mM EGTA (ethyleneglycol-bis-(β -aminoethylether)*N,N,N',N'*-tetra-acetate) to the solution (0Ca).

Drugs and chemicals were obtained from the following sources: ET-1 (Peptide Institute, Osaka), choline chloride (Tokyo Kasei Kogyo, Tokyo), EGTA, HEPES and fura-2 AM (Dojin, Kumamoto). ET-1 was dissolved in distilled water at 10 μM . The stock solutions were diluted to the desired concentrations by PSS.

Each experimental value is expressed as a percentage of that in 5K-2.5Ca-PSS in the presence of ET-1 and given as the mean $\pm$ S.E.M. Statistical significance of results was evaluated by the paired *t*-test; *P* values < 0.05 were considered to indicate a significant difference.

In canine coronary arteries, the perfusion of 5 mM KCl and Ca^{2+} -free (5K-0Ca)-PSS reduced $[\text{Ca}^{2+}]_i$ below the resting level without changing force. In 0Ca-PSS, depolarization with 90 mM KCl (90K) did not change $[\text{Ca}^{2+}]_i$ and force at all (Fig. 1A). The application of ET-1 (1 nM) produced an increase in $[\text{Ca}^{2+}]_i$ and a gradual development of contraction (Fig. 1B).

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Fig. 1. Typical traces of the force and $[Ca^{2+}]_i$ (indicated as fluorescence ratio) in canine coronary arteries loaded with fura-2. (A) Effects of depolarization by 90K in 0Ca-PSS. (B) Effects of depolarization by 90K in the presence of 1 nM ET-1 in 0Ca-PSS. (C) Effects of depolarization by 90K in the presence of 1 nM ET-1 in 0.1Ca-PSS.

The removal of extracellular Ca^{2+} 30 min after the application of ET-1 reduced $[Ca^{2+}]_i$ below the resting level, but the force was inhibited incompletely ($31.4 \pm 8.0\%$, $n = 5$). In the presence of ET-1 in 0Ca-PSS, the depolarization with 90K increased the force ($42.5 \pm 9.7\%$, $P < 0.05$) without any changes in $[Ca^{2+}]_i$ (Fig. 1B). The reduction of NaCl concentration in PSS to 55 mM by replacing choline chloride did not change $[Ca^{2+}]_i$ and force in the presence of ET-1 in 0Ca-PSS (data not shown). Since it had become clear that the depolarization by 90K-0Ca-PSS increased the force produced by ET-1 without changes in $[Ca^{2+}]_i$, the effects of depolarization were also examined in reduced Ca^{2+} -PSS (0.1 mM $CaCl_2$, 0.1Ca). After examining the effects of 90K-2.5Ca-PSS for 10 min, the application of ET-1 (1 nM) was started. The reduction of Ca^{2+} from 2.5 to 0.1 mM in PSS reduced $[Ca^{2+}]_i$ below the resting level and reduced the force in the presence of ET-1 (Fig. 1C). The depolarization by 90K partially recovered $[Ca^{2+}]_i$ to $45.6 \pm 11.4\%$ ($n = 6$), whereas the force significantly increased up to $242.7 \pm 47.8\%$ ($P < 0.01$) compared with that in 5K-2.5Ca-PSS (Fig. 1C). Figure 2 shows the $[Ca^{2+}]_i$ -force relationships in the absence or presence of ET-1 in 5K- or 90K-PSS. The relation curve in the presence of ET-1 in 90K-PSS was shifted to the left and upward compared with that in

Fig. 2. Relationship between $[Ca^{2+}]_i$ and force in the absence or presence of 1 nM ET-1 in 5 or 90 mM KCl-PSS. The numbers in the figure indicate extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$), 1: Ca^{2+} -free + 1 mM EGTA, 2: 0.03 mM, 3: 0.1 mM, 4: 0.3 mM, 5: 1.0 mM, 6: 2.5 mM. $[Ca^{2+}]_i$ and force were normalized by the responses to 90K-2.5Ca-PSS for 10 min in each experiment. Each point represents the mean of five to six experiments $\pm$ S.E.M. (vertical and horizontal bars).

5K-PSS. The increase in $[Ca^{2+}]_i$ by 90K-PSS with various concentrations of $[Ca^{2+}]_o$ in the presence of ET-1 was almost the same as that in the absence.

In this study, in the presence of ET-1, depolarization by high KCl increased the force of contraction in spite of a reduced Ca^{2+} influx or even in the absence of Ca^{2+} influx in canine coronary artery. In the absence of agonists, since the force of the contraction produced by depolarization is solely dependent on the verapamil-sensitive influx of Ca^{2+} (2), 90K did not change $[Ca^{2+}]_i$ and force at all in 0Ca-PSS. The vasoconstricting mechanisms of ET-1 have been shown to involve its ability to increase Ca^{2+} influx, release Ca^{2+} from intracellular stores and produce the Ca^{2+} sensitization (5). There have also been several reports showing the Ca^{2+} sensitization in contractions induced by various agonists (1, 3, 6, 7). The increase in $[Ca^{2+}]_i$ by ET-1 was abolished by 0Ca and $[Ca^{2+}]_i$ decreased below the resting level, as it was in the absence of ET-1, but the force of contraction was inhibited only partially. The force induced by agonists in 0Ca-PSS is highly sensitive to $[Ca^{2+}]_i$ (8). The mechanisms to increase Ca^{2+} sensitivity may involve G-protein-coupled mechanisms (6) and/or the activation of protein kinase C derived from phospholipase C (7) or D (3) stimulated by agonists. In our previous study, the potentiation of U46619 (a thromboxane A_2 analogue)-induced contraction by high KCl-PSS was dependent on the KCl concentration (3). Thus, the depolarization-induced potentiation seems to be a consistent phenomenon. In the presence of ET-1 in 0Ca-PSS, the depolarization by 90K increased force without any changes in $[Ca^{2+}]_i$. Although, in the presence of ET-1, the increased level of $[Ca^{2+}]_i$ induced by 90K-0.1Ca-PSS was lower than that in 5K-2.5Ca-PSS, the force induced by depolarization with 90K-0.1Ca-PSS exceeded that in 5K-2.5Ca-PSS. Such results may indicate the voltage-dependence of the mechanisms regulating the Ca^{2+} sensitivity of the contractile elements. The potentiating effect of depolarization seems to be large when $[Ca^{2+}]_i$ is higher than the resting level. Thus, in the presence of agonists, depolarization of the plasma membrane potentiates Ca^{2+} sensitization. In line with membrane potential-regulated influences on the Ca^{2+} sensitivity of the contractile elements, a recent report has shown that membrane hyperpolarization induced by the K^+ channel opener lemakalim may inhibit an increase in $[Ca^{2+}]_i$ and force related to phosphoinositides breakdown induced by noradrenaline (9). Furthermore, we have demonstrated that hyperpolarization induced by the K^+ channel opening action of E4080 reduces the Ca^{2+} sensitivity of contractile ele-

ments in 30K-PSS (4). Interestingly, it has been reported that a hyperpolarization-activated K^+ efflux appears to directly regulate adenylyl cyclase activity in *Paramecium* (10). These findings suggest the possible regulation of activity of membrane-associated enzymes by membrane potential. The depolarization-induced potentiation of the Ca^{2+} sensitization by agonists may involve some membrane-associated enzymes or proteins under the influence of membrane potential.

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