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Unrepeatable extracellular Ca^{2+}-dependent contractile effects of cyclopiazonic acid in rat vascular smooth muscle

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ABSTRACT

Cyclopiazonic acid (CPA), a specific reversible inhibitor of Ca^{2+}-pumps in sarcoplasmic reticulum, causes a slowly developing and subsequently diminishing characteristic contraction in endothelium-denuded rat vascular smooth muscle. We recently found that CPA-induced contractions were not completely repeatable in endothelium-denuded rat aorta and superior mesenteric artery. 10 μM CPA-induced contractions expressed as a percentage of 80 mM KCl-induced contraction were significantly decreased from 51.4 ± 5.7% to 11.8 ± 2.6% ($P<0.0001$) upon the second application in endothelium-denuded rat aorta, and this was not due to any irreversible cytotoxic effects of CPA. The decrease of CPA-induced contractile responses upon the second application was dependent on both types of blood vessels and doses of CPA upon the first application. CPA upon the second application in Ca^{2+}-containing solutions did induce its characteristic contractions in the rings pretreated with Ca^{2+}-free solutions or Ca^{2+} entry blockers before and during its first application, suggesting that capacitative mode of Ca^{2+} influx during the application of CPA might be responsible for the diminishment of contractions upon the second application. These data suggest that CPA by inducing a transient rise in cytosolic Ca^{2+} level might cause a long-lasting upregulation of Ca^{2+} extrusion across the plasma membrane in vascular smooth muscle cells and thus accelerate Ca^{2+} efflux over a prolonged period, leading to unrepeatable contractile effects of CPA. Such long-lasting upregulation of Ca^{2+} extrusion may contribute to the regulation of excitability of vascular smooth muscle cells and protect the cells against excitotoxic injury.

1. Introduction

Vascular smooth muscle (VSM) controls blood pressure and flow by a contractile state in response to cytosolic Ca^{2+}, which regulates the tonus of the VSM as an important carrier of cellular signals, and therefore it is critically important that the resting VSM cells maintain concentration of cytosolic Ca^{2+} ($[Ca^{2+}]_i$) at a very low level (Carafoli, 2002; Fray et al., 1986; Missiaen et al., 1992). Thus, function of Ca^{2+} handling in the VSM cells is important in regulating cellular excitability, blood pressure, and development of hypertension. Two types of specific transmembrane proteins, plasma membrane Ca^{2+}-ATPases (PMCA), which include four isoforms (PMCA1, PMCA2, PMCA3, and PMCA4) and over 30 splice variants (Domi et al., 2007), and Na^+/Ca^{2+} exchangers (NCX), which include three isoforms (NCX1, NCX2, and NCX3; Nakasaki et al., 1993; Quednau et al., 2004), conduct Ca^{2+} exit across the plasma membrane and keep $[Ca^{2+}]_i$ at a very low level in the VSM cells (Saris and Carafoli, 2005; Strehler and Zacharias, 2001; Szewczyk et al., 2007). This leads to a great electrochemical gradient for Ca^{2+} across the plasma membrane. An elevation of $[Ca^{2+}]_i$ causes a rapid reduction of the gradient level and a contraction in the VSM, and is mediated primarily by two pathways (van Breemen and Saida, 1989), Ca^{2+} entry across the plasma membrane and Ca^{2+} release from internal Ca^{2+} stores. Ca^{2+} entry is mainly conducted by voltage-gated Ca^{2+} channels, receptor-operated Ca^{2+} channels, and store-operated Ca^{2+} channels in the VSM cells. The NCX in a reverse mode can also contribute to Ca^{2+} entry across the plasma membrane. In addition, sequestration of intracellular Ca^{2+} plays an important role in regulating $[Ca^{2+}]_i$ and excitability of the VSM cells (Carafoli, 2002; Kwan et al., 1994). Sarcoplasmic reticulum (SR) in the VSM cells contributes to cytosolic Ca^{2+} homeostasis by using internal membrane Ca^{2+}-ATPases to pump Ca^{2+} into stores or by using Ca^{2+} release channels to release Ca^{2+} from the stores into the cytosol (Misquitta et al., 1999). Cyclopiazonic acid (CPA), a specific reversible inhibitor of SR Ca^{2+}-ATPases (Seidler et al., 1989) by causing conformational changes in the pump structure and by blocking the pumps (Moncoq et al., 2007), inhibits Ca^{2+} uptake into the stores, depletes the stores, and causes Ca^{2+} entry (Deng and Kwan, 1991). CPA was first shown from our laboratory to induce a contractile effect in the VSM (Deng and Kwan, 1991), and its action is attributed to capacitative Ca^{2+} entry or store-operated Ca^{2+} entry via the inhibition of SR Ca^{2+}-ATPases (Asano et al., 1998; Leung and

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Kwan, 1999; Putney, 1986). In addition, CPA can cause Ca^{2+} entry by upregulating NCX activity in a reverse mode (Poburko et al., 2006; Zhang et al., 2007).

CPA has broadly been used as an important tool in regulating $[Ca^{2+}]_i$ and excitability of cells in biology and medicine. Studies from our laboratory have shown that CPA in many types of vascular smooth muscle tissues causes a slowly developing and subsequently diminishing contraction, which gradually diminishes to a relatively stable state (Low et al., 1996). However, CPA has been reported to display multiple effects in modulating the excitability of smooth muscle cells (Ferrusi et al., 2004; Fukao et al., 1995; Inesi and Sagara, 1994; Maggi et al., 1995; Petkov and Boev, 1996; Suzuki et al., 2002). We here report that although CPA induced a slowly developing and subsequently diminishing characteristic contraction, the CPA-induced contraction upon its second application was significantly decreased, or even disappeared in rat aorta and superior mesenteric artery.

2. Methods and materials

2.1. Animals and vascular preparations

Male Sprague-Dawley rats weighing 250–300 g, were anaesthetized with sodium pentobarbital (50 mg/kg), and then killed by bleeding from the abdominal aorta in accordance with the guideline of the Canadian Council on Animal Care and our University Ethics Committee. A total of 20 Sprague-Dawley rats at age of 10–12 weeks were involved in this study. Vascular tissues were prepared as previously described (Zhang et al., 2004). Briefly, the thoracic aorta, the abdominal aorta, and the superior mesenteric artery were removed and placed in cold Kreb's physiological saline solution (PSS, pH 7.4) composed of (mM): NaCl 115.5, KCl 4.6, NaH_2PO_4 1.3, $NaHCO_3$ 22, $CaCl_2$ 2.5, $MgSO_4$ 1.2, and Glucose 11.1, and solution was aerated with a 95% O_2 and 5% CO_2 gas mixture. Fats and connective tissues surrounding the blood vessels were removed, and the blood vessels were then cut into 3–4 mm-wide ring segments. The endothelium was denuded by using a cotton-covered wire stick to rub the inner surfaces of the rings. An inability of 3 μM carbamylcholinel chloride to elicit an endothelium-dependent relaxation confirmed that the endothelium was successfully denuded in the ring. Each ring was vertically mounted in an organ bath with 4 ml PSS maintained at 37 °C, and the PSS in the organ bath was continuously aerated with a 95% O_2 and 5% CO_2 gas mixture. A Beckman R-411 Dynograph recorder was used for recording. A resting tension was set optimally at 2.5 g for the aortic rings and 1.5 g for the mesenteric arterial rings, and these values were determined by preliminary studies using the optimal contraction to 80 mM KCl against passive tension. The rings were allowed to equilibrate in aerated PSS for at least 90 min. The PSS in the organ bath was changed every 20 min. After the equilibration, KCl at 80 mM was added to stimulate the rings. The stimulation, following a thorough wash, was repeated until KCl-induced contractions became stable in the rings.

2.2. Vascular reactivity studies

After the establishment of a stable contraction to 80 mM KCl, 3 μM carbamylcholinel was applied in a ring precontracted with 1 μM phenylephrine to test whether the endothelium was successfully denuded, and then CPA, following a thorough wash, was used to induce a contraction in the ring. Following a 30–40 min thorough wash, CPA was reapplied to induce a contraction in the ring. To observe vascular contractile effects of caffeine, the normal PSS in a bath was replaced with a PSS containing 20 mM caffeine to induce a transient contraction in a ring. In the experiments performed under Ca^{2+}-free condition, the vascular rings were equilibrated in a Ca^{2+}-free solution for 10 min before the application of CPA.

2.3. Chemicals

Phenylephrine, nicardipine, caffeine, carbamylcholinel chloride, cyclopiazonic acid, and $LaCl_3$, were the products of Aldrich-Sigma Chemical Co. (Oakville, ON, Canada) and CPA was dissolved in DMSO. Final concentration of DMSO was less than 0.1% and this concentration of DMSO did not affect contractile responses of the rings.

2.4. Statistical analysis

Since each ring at the beginning of each experiment was stimulated by 80 mM KCl several times until 80 mM KCl evoked a stable contraction in the ring, CPA-induced contractions were measured from the baseline to the peak and were expressed as a percentage of the contraction induced by 80 mM KCl as a routine procedure for data normalization. Data were entered in a Prism worksheet (GraphPad software). Results were shown as mean ± S.E. M., and "*n*" is from the number of rats. Statistical analysis was estimated by Student's *t*-test, and the difference was regarded to be significant when $P<0.05$.

3. Results

3.1. Vasoconstrictor effects of CPA in rat aorta

In addition to CPA, many vascular stimulants are able to cause increases in $[Ca^{2+}]_i$ and vascular contractile effects by multiple mechanisms. We first observed vascular contractile effects of different stimulants such as KCl utilizing Ca^{2+} entry via voltage-gated Ca^{2+} channels (Meisheri et al., 1981), phenylephrine utilizing Ca^{2+} release via inositol 1,4,5-trisphosphate-induced Ca^{2+} release channels (Khalil and van Breemen, 1988), and caffeine utilizing Ca^{2+} release via Ca^{2+}-induced Ca^{2+} release channels (Leijten and van Breemen, 1984) in endothelium-denuded rat aortic rings. After contractions induced by the stimulants, the aortic rings were washed thoroughly for 30–40 min, and a reproducible contractile response to 80 mM KCl (Fig. 1A), 1 μM phenylephrine (Fig. 1B), or 20 mM caffeine (Fig. 1C) could be obtained upon the second application of the same stimulants with equitant concentrations. When 10 μM CPA was used to induce a contraction in an endothelium-denuded rat aortic ring under the same experimental conditions, a slowly developing and subsequently diminishing characteristic contraction was observed (Fig. 1D, left). After a thorough wash, CPA upon the second application, however, failed to generate a contraction with the same pattern and level as that upon the first application. Instead, a very small yet sustained tension development was obtained (Fig. 1D, right). This, however, was not due to any irreversible cytotoxic effects of CPA because CPA-treated aortic rings still responded well to 80 mM KCl, 1 μM phenylephrine, or 20 mM caffeine (data not shown). Thus, KCl-, phenylephrine-, or caffeine-induced vascular contractions did not display significant changes upon the second application. CPA-induced vascular contractions, however, were decreased from 51.4 ± 5.7% to 11.8 ± 2.6% ($n=11$, $P<0.0001$) upon the second application in endothelium-denuded rat aortic rings (Fig. 1E), which corresponds to a 77% decrease.

Under normal *in vivo* condition, the vasculature contains intact endothelium. Therefore, we also examined contractile effects of CPA in endothelium-intact rat aortic rings. CPA (10 μM) induced a rapid transient endothelium-dependent relaxation as we have previously observed (Deng and Kwan, 1991) followed by a slowly developing and subsequently diminishing contraction similar to that in endothelium-denuded rat aortic rings, and this contraction was also not completely repeatable either. CPA (10 μM)-induced vascular contractions were decreased from 20.3 ± 3.9% to 1.1 ± 0.9% ($n=4$, $P<0.05$) upon the second application in endothelium-intact rat aortic rings.

Furthermore, we also tested vascular contractile effect of thapsigargin, which is also a specific and more potent irreversible inhibitor