European Journal of Pharmacology 610 (2009) 81–86

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Cardiovascular Pharmacology

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

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ARTICLE INFO

Article history:
Received 8 February 2009
Accepted 9 March 2009
Available online 16 March 2009

Keywords:
Cyclopiazonic acid
Vascular contraction
Ca^{2+} extrusion
Long-lasting upregulation
Rat aorta

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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0014-2999/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.ejphar.2009.03.030

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

of SR Ca^{2+}-ATPases. Like CPA, thapsigargin also caused contractile effects in endothelium-denuded rat aortic rings (data not shown). Unlike CPA, the contraction due to thapsigargin cannot be washed off with normal PSS due to its irreversible nature (data not shown). This is consistent with a previous observation (Mikkelsen et al., 1988; Low et al., 1993), and this is due to tight and irreversible binding of thapsigargin with SR Ca^{2+}-ATPases (Sagara et al., 1992).

3.2. Contractile effects of CPA in different blood vessels

Our earlier studies on contractile effects of CPA in different blood vessels isolated from different animal species indicated that the magnitudes and the temporal patterns of CPA-induced tension development were dependent on vessel size (Low et al., 1996), and these data are consistent with the studies indicating that there are significant species and tissue differences for contractile effects of CPA (Petkov and Boev, 1996). We therefore examined contractile effects of CPA in the thoracic aorta, the abdominal aorta, and the superior mesenteric artery as shown in Fig. 2. 10 μM CPA upon the first application caused a biphasic contraction in thoracic segments of the aorta (which include the aorta proximal to the heart in Fig. 2A and the thoracic aorta before the diaphragm in Fig. 2B), which was characterized by a slowly developing humpy contraction followed by a smaller and slowly diminishing contraction over a prolonged period. In the abdominal aorta (Fig. 2C) and the superior mesenteric artery (Fig. 2D), 10 μM CPA, however, induced a slowly developing monophasic transient contraction which returned to basal tension. It was also confirmed that 10 μM CPA upon the second application only produced a slowly developing tension with much diminished magnitude in the thoracic aortic rings (Fig. 2), and it is interesting to note that CPA upon the second application caused little or no contractions in the abdominal aorta and the superior mesenteric artery. It appears that the initial phases of the contractions were totally inhibited upon the second exposure to CPA. To maintain consistency of data collection, only thoracic segments of the aorta were used for all other studies.

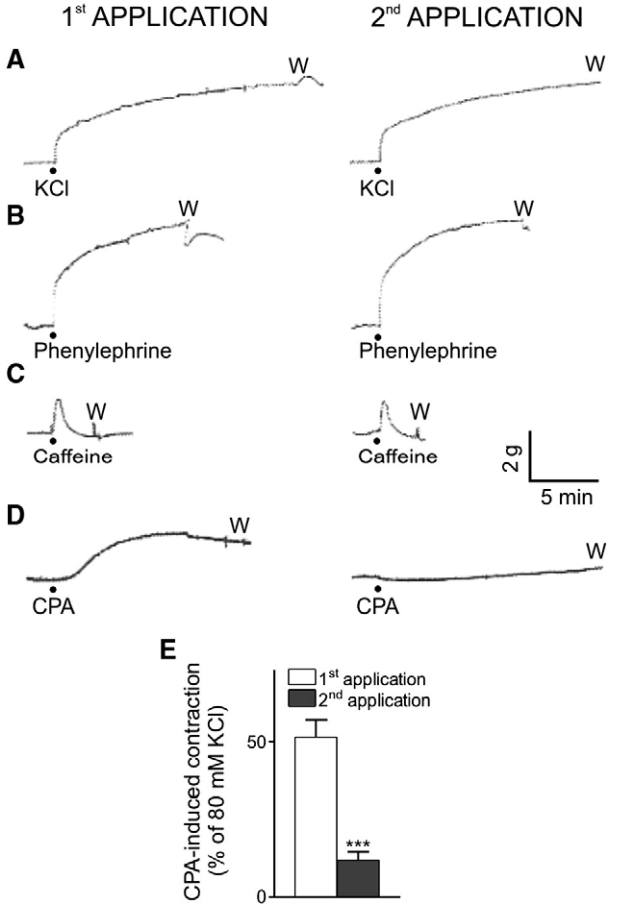

Fig. 1. Typical tracings showing contractile effects of 80 mM KCl (A), 1 μM phenylephrine (B), 20 mM caffeine (C), and 10 μM cyclopiazonic acid (CPA; D) in endothelium-denuded rat aortic rings (W: wash). (E) Summary of CPA-induced contractions ($n=11$, $P<0.0001$) in endothelium-denuded rat aortic rings.

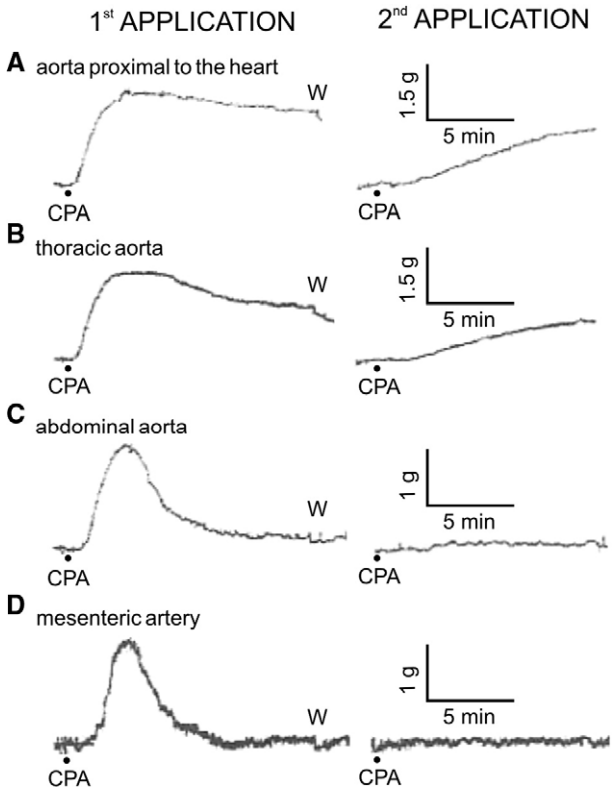

Fig. 2. Tracings show contractile effects of CPA in rat aorta proximal to the heart (A), the thoracic aorta before the diaphragm (B), the abdominal aorta (C), and the superior mesenteric artery (D) without endothelia.

3.3. Contractile effects of CPA with different doses

Fig. 3 shows the contractions evoked by 4 concentrations of CPA (2, 5, 10, and 30 μM) in thoracic segments of rat aorta without endothelium. At all concentrations, CPA upon the second application at the same concentrations failed to generate contractions with the same pattern and level as those upon the first application. Only at a very low introductory concentration (2 μM), CPA, which was increased to 10 μM upon the second application, caused an additional transient contraction. CPA-evoked contractions upon the second application were measured from the baseline to the peak of contractions caused by the final doses.

3.4. Roles of extracellular Ca^{2+} in contractile effects of CPA

Fig. 4 shows the roles of extracellular Ca^{2+} in CPA-induced contractions in endothelium-denuded rat aortic rings. Vascular segments, after equilibrated in a Ca^{2+}-free solution over 10 min and then washed with a Ca^{2+}-containing medium to induce Ca^{2+} entry, still responded well to 10 μM CPA (Fig. 4A) and displayed contractile responses of $42.5 \pm 1.2\%$ ($n=3$) of 80 mM KCl-induced contractions in these pretreated rings, which is similar to the percentage of CPA-induced contractile responses upon its first application ($51.4 \pm 5.7\%$; Fig. 1E) in a Ca^{2+}-containing medium. As compared to the control

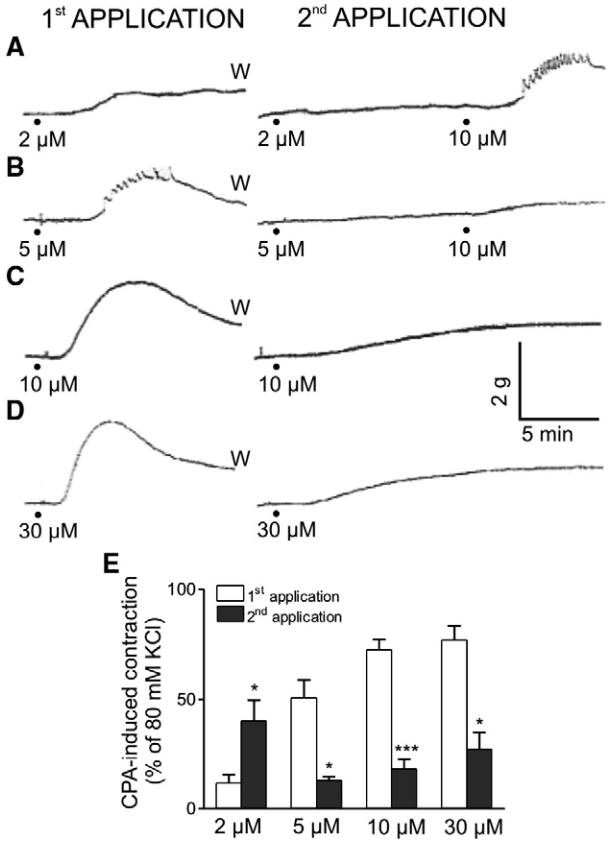

Fig. 3. Tracings show contractile effects of 2 μM (A), 5 μM (B), 10 μM (C), and 30 μM (D) CPA in rat aortic rings without endothelia. (E) Summary of contractions induced by 2 μM ($n=4$, $P<0.05$), 5 μM ($n=4$, $P<0.05$), 10 μM ($n=4$, $P<0.001$), and 30 μM ($n=4$, $P<0.05$) CPA. CPA-evoked contractions upon the second application were measured from the baseline to the peak of contractions caused by the final doses.

rings in the presence of extracellular Ca^{2+} and in the absence of extracellular Ca^{2+} channel blockers (Fig. 1D), the aortic rings preincubated with 10 μM CPA in the absence of extracellular Ca^{2+} only displayed little or no elevation of resting tension (Fig. 4B and C, left), However, the aortic ring pretreated with CPA in Ca^{2+}-free solution, following a thorough wash first with a Ca^{2+}-free medium to remove CPA and then with a Ca^{2+}-containing medium to induce Ca^{2+} entry, indeed displayed a slowly developing and subsequently diminishing characteristic contraction after the second application of CPA in a Ca^{2+}-containing medium (Fig. 4C, right).

We further investigated that the roles of extracellular Ca^{2+} in contractile effects of CPA by using Ca^{2+} channel blockers (Fig. 5). 0.3 μM nicardipine, a selective blocker of L-type Ca^{2+} channels, did not exert any inhibitory effects on CPA-induced contractions, and a contraction induced by CPA upon the second application did not occur in nicardipine-treated rat aortic ring (Fig. 5A). However, La^{3+}, a non-selective inorganic blocker of Ca^{2+} entry (Kwan and Putney, 1990), totally inhibited CPA-induced contractions at 10 μM (Fig. 5B, left). In addition, following a thorough wash in the ring treated by La^{3+} to remove La^{3+} and CPA with Ca^{2+}-free and subsequently Ca^{2+}-containing solutions, CPA upon the second application in La^{3+}-treated rings, however, induced a contraction (Fig. 5B, right), with the same feature as that in the tissue, which was stimulated by CPA in a Ca^{2+}-free medium and was then re-exposed to CPA in a Ca^{2+}-containing medium following a thorough wash with Ca^{2+}-free and subsequently Ca^{2+}-containing solutions (Fig. 4C). Other inorganic cationic Ca^{2+} antagonists such as Ni^{2+} and Co^{2+} also displayed the same effects as that induced by La^{3+} on CPA-induced contractions in endothelium-denuded rat aortic rings (data not shown).

4. Discussion

CPA has broadly been used to modulate $[Ca^{2+}]_i$ and excitability of smooth muscle cells. CPA is able to inhibit the uptake of Ca^{2+} into the intracellular Ca^{2+} stores by SR Ca^{2+}-ATPases in VSM (Deng and Kwan, 1991), which contribute to the regulation of $[Ca^{2+}]_i$, and such inhibition causes Ca^{2+} entry via the depletion of the Ca^{2+} store; a mechanism termed capacitative (Putney, 1986) or store-operated Ca^{2+} entry, or upregulation of NCX activity in a reverse mode (Poburko et al., 2006; Zhang et al., 2007). Thus, CPA causes transient contractile effects in vascular smooth muscle (Low et al., 1996). In addition, CPA can also cause Ca^{2+} entry in vascular endothelial cells by inhibiting Ca^{2+}-ATPases in endoplasmic reticulum, and such activation increases nitric oxide synthesis by activating Ca^{2+}/calmodulin-dependent nitric oxide synthase (Zheng et al., 1993; Moritoki et al., 1994). Our studies have shown that CPA, which causes a slowly developing and subsequently diminishing characteristic contraction in rat resistance arteries, displays unrepeatable vascular contractile effects and only evokes a greatly diminished yet sustained contraction upon its second application. This is the first report that CPA-induced contractions are not repeatable in rat resistance arteries. The decrease of CPA-induced contractile responses upon the second application is dependent on the doses of CPA upon the first application. The higher the dose applied upon the first application of CPA is, the smaller the contractile effect of CPA upon the second application is. The decrease of CPA-induced contractile responses upon the second application is also dependent on the types of blood vessels. The closer to the heart the tissue is, the larger the contractile effect of CPA upon the second application is. This may also explain why our data about the contractile effects of CPA in rat aorta and superior mesenteric artery are inconsistent with the studies from the other laboratory (Maggi et al., 1995). Maggi et al. (1995) reported that excitatory effects of CPA were reversible in guinea-pig ureter. Diminishing magnitudes of CPA-induced

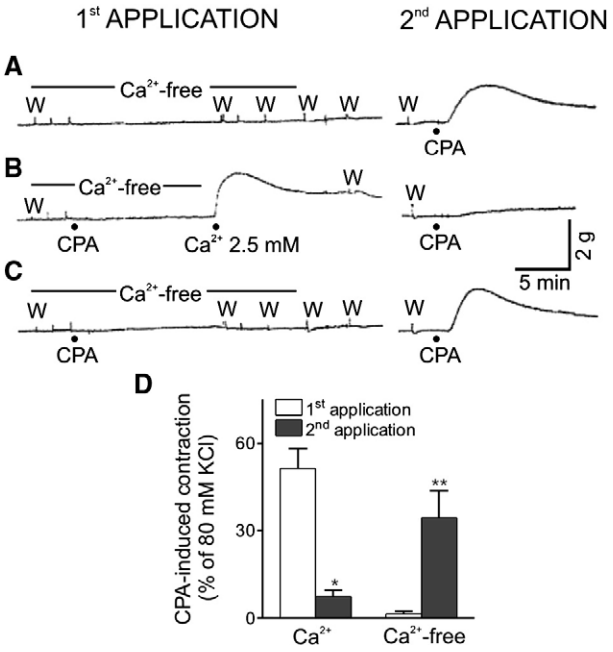

Fig. 4. Roles of extracellular Ca^{2+} in contractile effects of CPA. (A) Tracings showing the contractile effect of CPA in an endothelium-denuded rat aortic ring pretreated with a Ca^{2+}-free solution for 10 min and then washed with a Ca^{2+}-containing medium. (B) Contractile effect of CPA in an endothelium-denuded rat aortic ring, which was pretreated with 10 μM CPA in the absence of extracellular Ca^{2+} and then stimulated by 2.5 mM Ca^{2+} before a thorough wash with a Ca^{2+}-containing medium. (C) Contractile effect of CPA in an endothelium-denuded ring, which was pretreated with 10 μM CPA in the absence of extracellular Ca^{2+} and then washed with Ca^{2+}-free and subsequently Ca^{2+}-containing solutions. (D) Summary of CPA-induced contractions in B ($n=3$, $P<0.05$) and C ($n=3$, $P<0.01$).

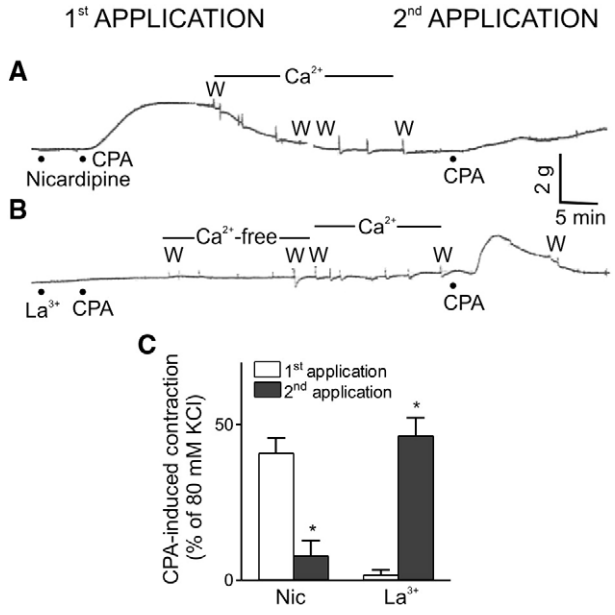

Fig. 5. Tracings showing the effects of 0.3 μM nicardipine (A) and 10 μM La^{3+} (B) on CPA-induced contractions in rat aortic rings without endothelia. (C) Summary of the effects of nicardipine ($n=3$, $P<0.05$) and La^{3+} ($n=3$, $P<0.05$) on CPA-induced contractions.

contraction have been observed in vascular smooth muscle of decreasing size and were first reported by us earlier (Low et al., 1996). We have attributed it to the fact that the plasmalemmal Ca^{2+}-extrusion capability was greater than the SR Ca^{2+}sequestaration capability as the blood vessels became smaller (Low et al., 1996).

The mechanism underlying the diminishment about CPA-induced contractions upon the second application in rat resistance arteries remains unknown. CPA upon the second application in a Ca^{2+}-containing medium induced its characteristic contraction in an aortic ring pretreated with a Ca^{2+}-free solution (Fig. 4C) or La^{3+} (Fig. 5B) before and during the first application of CPA, suggesting that it is not the first exposure of the aortic ring to CPA but capacitative mode of Ca^{2+} influx during the application of CPA to be responsible for the diminishment of contractions upon the second application. Since increases in $[Ca^{2+}]_i$ in vascular smooth muscle cells are absolutely essential to production of excitatory contractions (van Breemen and Saida, 1989) and CPA can reversibly inhibit SR Ca^{2+}-ATPases, CPA, which only causes a diminished contraction upon the second application, might cause a long-lasting activation in Ca^{2+} extrusion across the plasma membrane and thus accelerate Ca^{2+} efflux over a prolonged period, leading to unrepeatable contractile effects of CPA in rat aorta and superior mesenteric artery. Despite of the lack of experiments measuring cytosolic Ca^{2+} concentrations by using fluorescence dye technique, our observations from these studies suggest that there is a possibility that Ca^{2+} extrusion is upregulated after the application of CPA in rat aorta and superior mesenteric artery. If CPA does cause a long-lasting upregulation of Ca^{2+} extrusion by inhibiting SR Ca^{2+}-ATPases to deplete Ca^{2+} stores, it could be due to CPA-induced changes in level of cytosolic Ca^{2+} as an important carrier of cellular signals and subsequently in cellular signal transduction pathways, which regulate activities of many ion transporters, including PMCA (Guerini et al., 2005) and NCX (Dipolo and Beauge, 2006). If CPA does cause a long-lasting upregulation of Ca^{2+} extrusion by inducing a transient rise in $[Ca^{2+}]_i$, there are at least two possible mechanisms, since Ca^{2+} extrusion across the plasma membrane is conducted primarily by the PMCA and the NCX (Carafoli, 2002). One possibility is that CPA causes a long-lasting increase in the NCX activity in rat aorta and superior mesenteric artery. Such an increase accelerates Ca^{2+} efflux over a prolonged period and leads to decreased contractile effects of CPA upon the second application. Studies from other laboratories have shown that CPA can upregulate the NCX activity in both a forward mode (Maggi et al., 1995) and a reverse mode (Zhang et al., 2007). However, it is not known whether the upregulation in the studies above was long-lasting. The other possibility is that CPA causes a long-lasting upregulation of the PMCA activity in rat resistance arteries. A long-lasting activation of the PMCA activity up to 1 h has been reported in rat dorsal root ganglion neurons (Pottorf and Thayer, 2002). Such long-lasting activation was induced by a transient rise in $[Ca^{2+}]_i$ and might be attributed to a slow dissociation of calmodulin from the PMCA, as suggested by Pottorf and Thayer (2002). Calmodulin is an important regulator of the PMCA activity and the types of PMCA isoforms may determine efficacy of the dissociation of calmodulin from the pumps (Guerini et al., 2005). Ca^{2+} extrusion in different types of tissues may be conducted by different isoforms of the PMCA and/or the NCX. These may also help to explain why the contractile effect of CPA upon the second application is dependent on the types of blood vessels. Future studies will investigate whether a long-lasting upregulation of Ca^{2+} extrusion across the plasma membrane leads to decreased contractile effects of CPA upon the second application in rat aorta and superior mesenteric artery and explore whether modulation of cellular signaling system contributes to this long-lasting upregulation.

If CPA by inducing a transient rise in $[Ca^{2+}]_i$ does cause a long-lasting upregutation of Ca^{2+} extrusion, the physiological significance of the long-lasting upregutation is not yet known. Such long-lasting upregulation may be beneficial to the VSM cells. Such an effect may cause a more rapid and sustained removal of cytosolic Ca^{2+} in the VSM cells. Since cytosolic Ca^{2+} plays key roles in regulating excitability and tonus of the VSM, excessive retention of cytosolic Ca^{2+} causes excitotoxic injury and an increase in peripheral resistance and ultimately leads to elevation of blood pressure and other cardiovascular diseases (Kwan, 1985). CPA-induced more rapid and sustained removal of cytosolic Ca^{2+} from the VSM cells may help to keep a great electrochemical gradient for Ca^{2+} across the plasma membrane and thus protect the cells against excitotoxic injury.

In summary, we here report that a transient rise in $[Ca^{2+}]_i$ caused by CPA leads to a significantly diminished contraction upon its second application in rat aorta and superior mesenteric artery. This phenomenon could be due to CPA-induced long-lasting upregulation of Ca^{2+} extrusion across the plasma membrane and such upregulation may contribute to the maintenance of $[Ca^{2+}]_i$ at a very low level, control excitability of muscle cells, and protect the cells against excitotoxic injury.

Acknowledgements

This work was supported by a seeding grant awarded by the Faculty of Health Sciences, McMaster University, Hamilton, Canada, and a start-up grant from the China Medical University, Taichung, Taiwan. We thank Dr. Ashok K Grover for his helpful discussion.

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