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BRIEF COMMUNICATION

Calcium-Induced Calcium Release and Cyclic ADP-Ribose-Mediated Signaling in the Myocytes from Small Coronary Arteries

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Cyclic ADP-ribose (cADPR), an endogenous metabolite of nicotinamide adenine dinucleotide, was first reported to be present in sea urchin eggs and to possess Ca^{2+} mobilizing activity (Clapper *et al.*, 1987; Lee *et al.*, 1989). Recent studies have indicated that cADPR is produced in a variety of mammalian tissues and cells, including heart, liver, spleen, brain and red blood cells, pituitary cells, as well as renal epithelial cells (Beers *et al.*, 1995; Koshiyama *et al.*, 1991; Takesawa *et al.*, 1993; White *et al.*, 1993). Basal concentrations of cADPR in cardiac muscle, liver, and brain are estimated to be 100–200 nM (Galione, 1994; Lee, 1994). Like sea urchin eggs, cADPR also causes Ca^{2+} release from the endoplasmic reticulum in these mammalian tissues and cells. It mobilizes intracellular Ca^{2+} by a mechanism completely independent of inositol 1,4,5-trisphosphate (IP_3), since the IP_3 receptor antagonist heparin cannot block the effect of cADPR (Galione, 1994; Galione *et al.*, 1993; Lee and Aarhus, 1991, 1993). This cADPR-induced Ca^{2+} release may mediate the secretion of hormones such as insulin and catecholamines, the fertilization of eggs, the estrogen response in rat uterus, and the effects of nitric oxide (NO), serotonin, and acetylcholine in nonmuscle tissues (Chini *et al.*, 1997; Galione, 1993; Galione *et al.*, 1993; Lee, 1993; Lee and Aarhus, 1991, 1993; Lee *et al.*, 1989; Morita *et al.*, 1997; Takesawa *et al.*, 1993).

There are two major Ca^{2+} release mechanisms in excitable and nonexcitable mammalian cells: one dependent on the second messenger IP_3 and another sensitive to Ca^{2+} and known as calcium-induced calcium release (CICR) (Berridge, 1997). Recent studies have indicated that inhibitors of CICR, such as tetracaine, ryanodine, and ruthenium red, selectively inhibit cADPR-sensitive Ca^{2+} release and agonists of CICR, such as caffeine and Ca^{2+}, potentiate the Ca^{2+} releasing activity of cADPR (Galione *et al.*, 1993; Lee, 1993, 1994; Lee *et al.*, 1995), indicating that cADPR may be a novel endogenous Ca^{2+} mobilizing molecule mediating or modulating CICR. However, this role of cADPR in CICR has not yet been studied in vascular smooth muscle cells. Recently, studies in our laboratory and by others have demonstrated that cADPR can be produced in coronary arterial smooth muscles and

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that cADPR activates reconstituted ryanodine receptors (RyR) and induces Ca^{2+} release from intracellular stores of these coronary myocytes (Kannan *et al.*, 1996; Li *et al.*, 1998, 2001; Yu *et al.*, 2000). It remains unknown whether the effect of cADPR is attributed to CICR in vascular smooth muscle cells and whether cADPR- or RyR-mediated CICR plays an important role in the vasomotor response of these arteries. The present study was designed to test the hypothesis that cADPR- and RyR-mediated CICR is functioning in coronary arterial smooth muscle cells, which contributes to the control of intercellular Ca^{2+} concentrations and vasoconstrictor response in these vessels. We first determined the role of CICR in KCl- and caffeine-induced $[Ca^{2+}]_i$ increase in coronary arterial smooth muscle cells using single cell fluorescent microscopic spectrometry and then examined the contribution of CICR to the KCl-induced contractile response in isolated and pressurized small coronary arteries. By measuring the Ca^{2+} release response in single coronary myocytes and the contractile response to KCl, we also examined the role that cADPR plays in CICR of these coronary arteries.

MATERIALS AND METHODS

Isolation of coronary arterial smooth muscle cells (CASMCs). The small coronary arteries (200–500 μm i.d.) from fresh bovine hearts were dissected and pooled. The arterial segments were incubated for 10 min at room temperature (RT) in dissociation solution containing (mM) NaCl, 145; KCl, 5.6; $CaCl_2$, 0.05; $MgCl_2$, 1; $NaHCO_3$, 4.2; glucose, 10; and Hepes, 10, and in albumin, 1 mg/ml (pH 7.4). The segments were then incubated for 30 min at 37°C in 1 ml of dissociation solution containing 1.5 mg/ml of papain and 1 mg/ml of dithiothreitol, followed by an incubation for 30 min at 37°C in 1 ml of dissociation solution containing 2 mg/ml of collagenase, 0.5 mg/ml of elastase, and 1 mg/ml of soybean trypsin inhibitor. The supernatant was collected and the cells were spun down at 500*g* for 5 min, resuspended in fresh dissociation solution, and stored at 4°C until used.

Measurement of $[Ca^{2+}]_i$ in CASMCs. Fura2-AM (Molecular Probes) was used for monitoring intracellular $[Ca^{2+}]_i$ (Tsien, 1989). Freshly dissociated CASMCs were incubated with 5 μM fura2-AM in Hanks' buffer containing (mM) NaCl, 130; KCl, 5.4; $CaCl_2$, 1.25; $MgCl_2$, 1; glucose, 5.5; and Hepes, 20, pH 7.4. After incubation for 30 min at RT, an aliquot of cell suspension was pipetted onto the cover glass bottom of a 0.5-ml flow-through chamber and mounted on the stage of an inverted microscope equipped with a 40X objective (Nikon Diaphot). The cells were incubated with fresh Hanks' buffer and allowed to attach to the bottom of the chamber for 10–20 min. The fluorescence was measured with a PTI RatioMaster, a microscope photometry-based ratio fluorescence system. The $[Ca^{2+}]_i$ was calculated with the equation $[Ca^{2+}]_i$ (nM) $= K_d \times (R - R_{min})/(R_{max} - R) \times (F_0/F_S)$, where the R was a fluorescence ratio of fura-2 excited at 340 and 380 nm and recorded at 510 nm (F_{340}/F_{380}), R_{max} and R_{min} were the fluorescence ratios determined by adding 2 μM ionomycin (saturating Ca^{2+}) and subsequently adding 2 mM EGTA (zero Ca^{2+}), respectively, F_0 and F_S were the maximal (zero Ca^{2+}) and minimal (saturating Ca^{2+}) signal intensities at 380 nm, respectively, and K_d was the dissociation constant of fura-2 for Ca^{2+}, assumed to be 224 nM (Tsien, 1989).

Intracellular Ca^{2+} release from the intracellular store was stimulated by adding KCl (80 mM) or caffeine (1 mM) into the bath solution 15 min after incubation of the cells in Ca^{2+}-free Hanks' buffer consisting of (mM) NaCl, 130; KCl, 5.4; EGTA, 1; glucose, 5.5; and Hepes, 20, pH 7.4. To study the effects of CICR modulators on this calcium release, ryanodine (50 μM) or tetracaine (50 μM), two CICR inhibitors (Gyorke and Palade, 1992; Li *et al.*, 2001; Venosa and Hoya, 1999), thapsigargin (2 μM), a Ca^{2+}-ATPase inhibitor (Gonzales *et al.*, 1995), xestospongin (2 μM), an IP_3 receptor blocker (Bishara *et al.*, 2001; Gafni *et al.*, 1997), or 8-bromo-cADP-ribose (30 μM), a cell permeable cADPR antagonist (Lee, 1994), was added into the bath solution 10 min before caffeine- or KCl-induced Ca^{2+} release was determined.

Isolated small coronary artery preparation. Fresh bovine hearts were obtained from a local abattoir. Small intramural coronary arteries from the left anterior descending artery were carefully dissected. Segments of small arteries (100–200 μm i.d.) were cannulated with two glass micropipettes as described previously (Zhang *et al.*, 2001). The outflow cannula was clamped, and the arteries were pressurized to 60

mm Hg. The arteries were bathed in the PSS containing (mM) NaCl, 119; KCl, 4.7; $CaCl_2$, 1.6; $MgSO_4$, 1.17; NaH_2PO_4, 1.18; $NaHCO_3$, 24; EDTA, 0.026; and glucose, 5.5, pH 7.4, equilibrated with 95% O_2/5% CO_2, and maintained at 37°C. The vasoconstrictor or vasodilator responses were determined based on changes in the internal diameter.

To determine the role of CICR in the development of basal vascular tone, the arteries were incubated with Bay K 8644 (2 nM), a Ca^{2+} channel agonist, to elicit an optimal basal tone (10–15% decrease in internal diameter). Then, the responses to ryanodine (10–50 μM) or tetracaine (10–50 μM) were examined. To determine the role of CICR in agonist-induced contraction, the arteries were pretreated with ryanodine (50 μM), tetracaine (50 μM), xestospongin (2 μM), 8-bromo-cADP-ribose (30 μM, 8-br-cADPR), or vehicle for 20 min before the contractile response to KCl (40 mM) was examined. As a control, the contractile responses to U46619 (100 nM) were examined before and after the treatment of the arteries with xestospongin (2 μM) for 15 min. U46619, a thromboxane A_2 analogue, has been reported to produce vasoconstriction through increase in intracellular IP_3 concentration and/or opening of the membrane Ca^{2+} channels (Tosun *et al.*, 1998; Yamagishi *et al.*, 1992).

Statistics. Data are presented as means ± SEM. The significance of differences in mean values between and within multiple groups was examined using an analysis of variance for repeated measures followed by Duncan's multiple range test (Sigmastat). Student's *t* test was used to evaluate the significance of differences between two paired observations. $P < 0.05$ was considered statistically significant.

RESULTS

Effects of ryanodine and tetracaine on KCl- and caffeine-induced Ca^{2+} release in CASMCs. Figure 1A presents representative recordings depicting the changes in Ca^{2+} induced by KCl in CASMCs. The resting $[Ca^{2+}]_i$ was 143 ± 14 nM in Ca^{2+}-containing Hanks' buffer. Addition of KCl (80 mM) produced a large rapid increase in $[Ca^{2+}]_i$, followed by a tonic increase in $[Ca^{2+}]_i$. The initial peak of the Ca^{2+} transient primarily represented the sarcoplasmic reticulum (SR) Ca^{2+} release as described in previous studies (Katsuyama *et al.*, 1991; Mellors *et al.*, 1999), while the sustained increase in $[Ca^{2+}]_i$ corresponded to Ca^{2+} influx (Ethier *et al.*, 2001; Fukao *et al.*, 2001; Liu *et al.*, 2001; Yu *et al.*, 2000). Therefore, the peak increase in this Ca^{2+} transient was calculated as the Ca^{2+} release from SR. To determine whether CICR participates in this KCl-induced Ca^{2+} release response, the cells were pretreated with the specific CICR inhibitor ryanodine (50 μM) or tetracaine (50 μM) for 20 min. In the presence of these inhibitors, the KCl-induced Ca^{2+} release was significantly attenuated, but the sustained increase in $[Ca^{2+}]_i$ still exhibited. Figure 1B summarizes the effects of these inhibitors on KCl-induced Ca^{2+} release in CASMCs. Ryanodine and tetracaine inhibited the increase in $[Ca^{2+}]_i$ induced by KCl to 279 ± 75 and 304 ± 69 nM, which were much lower than the increase of 533 ± 77 nM in controls.

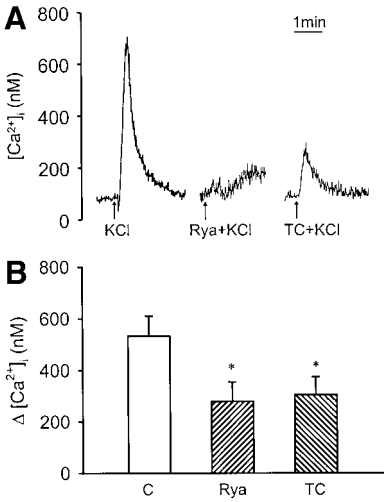

FIG. 1. Effect of ryanodine (Rya) and tetracaine (TC) on KCl-induced Ca^{2+} release in CASMCs. (A) Representative recordings of a transient increase in $[Ca^{2+}]_i$ induced by KCl (80 mM) under control conditions or after pretreatment with Rya (50 μM) or TC (50 μM). (B) Summarized data showing the average peak changes in $[Ca^{2+}]_i$ in response to KCl in the absence or the presence of Rya or TC (n = 9). *$P < 0.05$ vs control.

The changes in $[Ca^{2+}]_i$ induced by the CICR activator caffeine in CASMCs are shown in Fig. 2. Caffeine (1 mM) induced a rapid increase in $[Ca^{2+}]_i$ with much less tonic increase in $[Ca^{2+}]_i$ compared to that induced by KCl. Lower concentrations of caffeine (0.01 and 0.1 mM) had no significant effect on $[Ca^{2+}]_i$ (data not

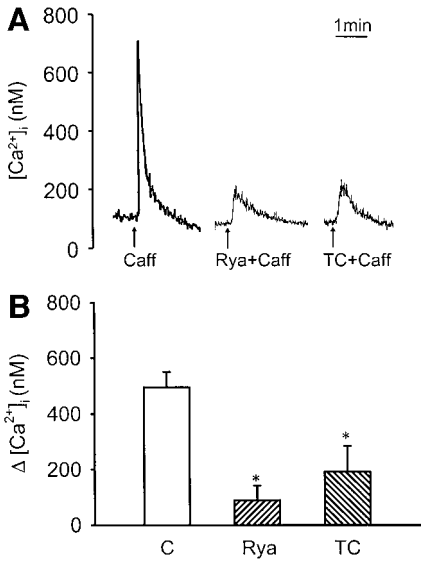

FIG. 2. Effect of Rya and TC on caffeine-induced Ca^{2+} release in CASMCs. (A) Representative recordings of a transient increase in $[Ca^{2+}]_i$ induced by caffeine (1 mM) under control conditions or after pretreatment with Rya (50 μM) or TC (50 μM) in Ca^{2+}-free Hanks' buffer. (B) Summarized data showing the average peak changes in $[Ca^{2+}]_i$ in response to caffeine in the absence or the presence of Rya or TC ($n = 9$). *$P < 0.05$ vs control.

shown). When the cells were pretreated with ryanodine (50 μM) or tetracaine (50 μM), caffeine-induced Ca^{2+} release was also significantly blocked. As summarized in Fig. 2B, caffeine produced a peak increase in $[Ca^{2+}]_i$ by 495 ± 77 nM in controls, but only by 89 ± 52 and 192 ± 93 nM in the presence of ryanodine and tetracaine, respectively.

Effect of xestospongin on KCl- and caffeine-induced Ca^{2+} release in CASMCs. To examine whether KCl- and caffeine-induced Ca^{2+} release is also related to the IP_3 pathway, the cells were pretreated with xestospongin (2 μM, for 20 min), an IP_3 antagonist. It was found that xestospongin had no significant effect on the increase in $[Ca^{2+}]_i$ induced by KCl (80 mM) and caffeine (1 mM) (Fig. 3). The KCl- and caffeine-induced Ca^{2+} increases were 474 ± 79 and 445 ± 139 nM in the presence of xestospongin. However, xestospongin blocked U46619-induced Ca^{2+} response in these cells (data not shown).

Effect of thapsigargin on KCl- and caffeine-induced Ca^{2+} release in CASMCs. To confirm that KCl- and caffeine-induced Ca^{2+} increase is from the Ca^{2+}-ATPase-related SR store in CASMCs, the cells were

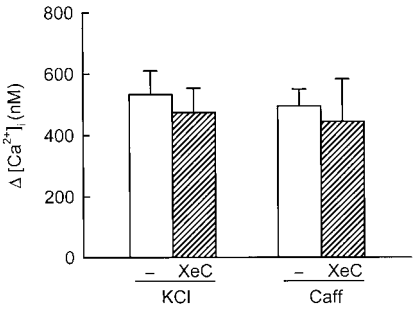

FIG. 3. Effect of xestospongin (XeC) on KCl- and caffeine-induced Ca^{2+} release in CASMCs. The transient increase in $[Ca^{2+}]_i$ induced by KCl (80 mM) or caffeine (Caff, 1 mM) was determined under control conditions or after pretreatment with XeC (2 μM) ($n = 8$).

pretreated with thapsigargin (2 μM, for 20 min), a Ca^{2+}-ATPase inhibitor that can deplete the SR Ca^{2+}. Similar to CICR inhibitors, thapsigargin also markedly blocked the increase in $[Ca^{2+}]_i$ induced by KCl (80 mM) and caffeine (1 mM). As shown in Fig. 4, both KCl- and caffeine-induced Ca^{2+} increases were decreased to 246 ± 92 and 116 ± 53 nM in the presence of thapsigargin.

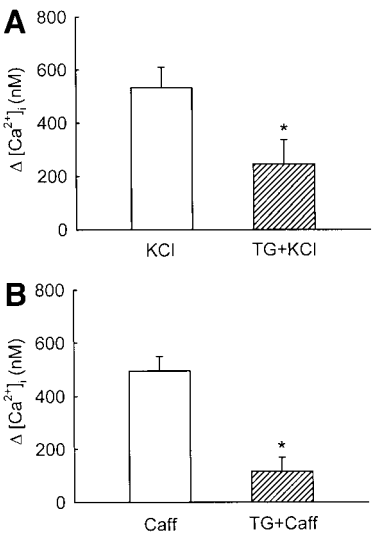

FIG. 4. Effect of thapsigargin (TG) on KCl- and caffeine-induced Ca^{2+} release in CASMCs. (A) Representative recordings of a transient increase in $[Ca^{2+}]_i$ induced by KCl (80 mM) or caffeine (1 mM) under control conditions or after pretreatment with TG (2 μM). (B) Summarized data showing the average peak changes in $[Ca^{2+}]_i$ in response to KCl or caffeine in the absence or the presence of TG ($n = 9$). *$P < 0.05$ vs control.

Role of CICR in the development of vascular tone in small coronary arteries. The average resting diameter of six pressurized small bovine coronary arteries averaged 120 ± 15 μm. Addition of deta NONOate (100 μM), an NO donor, increased the arterial diameter to 149 ± 17 μm, indicating the presence of basal vascular tone in these arteries. Using these arterial preparations, the role of CICR in the development of basal vascular tone was determined. Ryanodine (10–50 μM) or tetracaine (10–50 μM) resulted in a dose-dependent vasodilation in these arteries. The maximal increases in arterial diameter were 10 ± 2 and 11 ± 2% in the presence of ryanodine and tetracaine, respectively.

Role of CICR in KCl-induced contraction in small coronary arteries. The effects of CICR inhibitors on KCl-induced contractile response of small bovine coronary arteries are shown in Fig. 5. Figure 5A presents the representative video recordings of the arteries under control conditions or after treatment with KCl, ryanodine + KCl, or tetracaine + KCl. As summarized in Fig. 5B, KCl (40 mM) alone contracted the arteries by 64 ± 11% relative to resting controls. After incubation of arteries with the CICR inhibitor ryanodine (50 μM) or tetracaine (50 μM) for 20 min, the contractile responses to KCl were significantly inhibited, with maximal contractions of 41 ± 11 and 35 ± 6%, respectively. Corresponding to the results of Ca^{2+} measurement described above, pretreatment of the arteries with the IP_3 inhibitor xestospongin (2 μM, for 20 min) had no effect on KCl-induced contraction. Xestospongin also had no significant effect on the basal vascular tone. However, it markedly attenuated the contraction induced by U46619, an agonist known to act through the IP_3 pathway (Fig. 5C).

Effect of 8-bromo-cADP-ribose on KCl-induced Ca^{2+} release in CASMCs and contraction in small coronary arteries. All of the above experiments indicate that KCl-induced Ca^{2+} release in CASMCs and contraction in small bovine coronary arteries is at least partially mediated by RyR-mediated CICR. However, the mechanism by which KCl activates the CICR has yet to be determined. Because cADPR can activate the RyR and modulate the CICR, a selective cADPR antagonist, 8-br-cADPR, was used to determine whether the Ca^{2+} release and contraction induced by KCl was dependent on the action of cADPR. As shown in Fig.

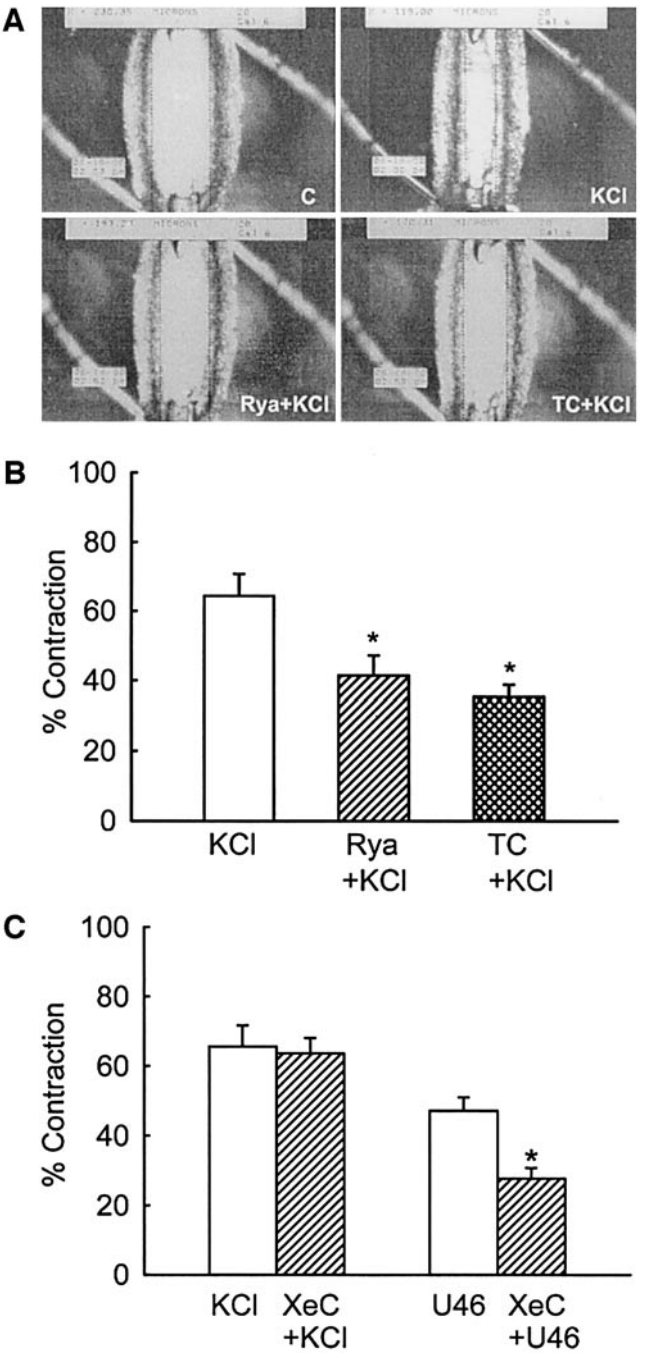

FIG. 5. Effect of Rya, TC, and XeC on KCl- or U46619-induced contractile responses in isolated small coronary arteries. (A) Representative video prints of the arteries under control conditions or after treatment with KCl, ryanodine + KCl, or tetracaine + KCl. (B and C) Summarized data showing that the vasoconstriction in response to KCl (40 mM) (n = 6) or U46619 (100 nM) (n = 6) were determined under control conditions or after pretreatment with Rya (50 μM), TC (50 μM), or XeC (2 μM). *$P < 0.05$ vs control.

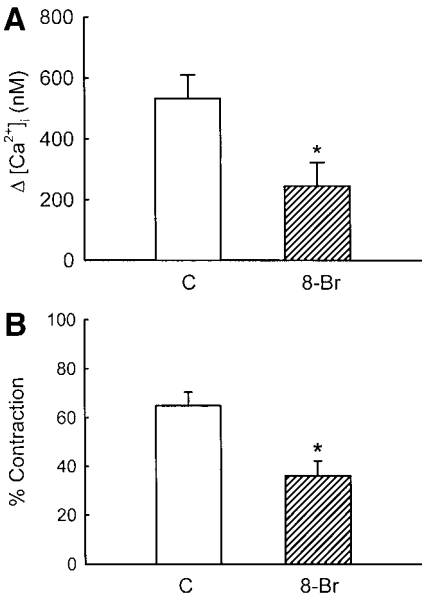

FIG. 6. Effect of 8-br-cADPR (8-Br) on KCl-induced Ca^{2+} release in CASMCs (A) and KCl-induced contractile responses in isolated small coronary arteries (B). The transient increase in $[Ca^{2+}]_i$ ($n = 12$) or the contractile responses induced by KCl ($n = 6$) were determined under control conditions or after pretreatment with 8-Br (30 μM). *$P < 0.05$ vs control.

6A, 8-br-cADPR (30 μM) significantly inhibited the increase in $[Ca^{2+}]_i$ induced by KCl (80 mM) in CASMCs to a similar extent to ryanodine and tetracaine. The KCl-induced Ca^{2+} increases were decreased to 246 $\pm$ 77 nM after pretreatment of arteries with 8-br-cADPR. In isolated and perfused small coronary arteries, 8-br-cADPR pretreatment also significantly attenuated the contraction induced by KCl (40 mM) (Fig. 6B).

DISCUSSION

In the present study, we found that both KCl and caffeine induced a rapid increase in $[Ca^{2+}]_i$ in freshly dissociated myocytes from small resistance coronary arteries, which was significantly inhibited by the CICR inhibitors ryanodine and tetracaine, but not by the IP_3 antagonist xestospongin. Blockade of CICR decreased the resting tone and attenuated KCl-induced contraction in pressurized small coronary arteries. Inhibition of cADPR actions by 8-br-cADPR also significantly blocked KCl-induced increase in $[Ca^{2+}]_i$ and contractile response in these arteries. These results indicate that CICR- and cADPR-mediated Ca^{2+} signaling are involved in the control of $[Ca^{2+}]_i$ in small coronary arterial smooth muscle and thereby contribute to vasoconstrictor response in these arteries.

A variety of agonists, such as norepinephrine, acetylcholine, and ATP, have been reported to mobilize Ca^{2+} from the SR through the IP_3-mediated signaling pathway in vascular smooth muscles (Kobayashi *et al.*, 1988, 1989; Mellors *et al.*, 1999; Somlyo *et al.*, 1985; Utz *et al.*, 1999). However, the mechanisms by which other stimuli, such as membrane depolarization, Ca^{2+} influx, and some vasoactive agonists like endothelin, induce Ca^{2+} mobilization remain unknown. In the first series of experiments, we determined whether KCl-induced Ca^{2+} mobilization is associated with CICR in CASMCs. Using fluorescent microscopic spectrometry, KCl was found to induce an intracellular Ca^{2+} transient response that consisted of a rapid peak increase in $[Ca^{2+}]_i$ followed by a sustained rise in $[Ca^{2+}]_i$, which represent the Ca^{2+} release and influx, respectively (Ethier *et al.*, 2001; Fukao *et al.*, 2001; Katsuyama *et al.*, 1991; Liu *et al.*, 2001; Mellors *et al.*, 1999). Therefore, the present study observed both Ca^{2+} influx and Ca^{2+} release in CASMCs when KCl was added into the bath. Since the Ca^{2+} release was activated by the membrane depolarization and the subsequent entry of Ca^{2+} into the cells, it represented a Ca^{2+} influx-induced Ca^{2+} release.

In the presence of the RyR blocker ryanodine and the CICR inhibitor tetracaine, the KCl-induced rapid peak increase in $[Ca^{2+}]_i$ was substantially attenuated, while xestospongin was without effect on this KCl-induced peak Ca^{2+} increase. These results suggest that KCl-induced Ca^{2+} release is primarily from a ryanodine-sensitive but not IP_3-sensitive Ca^{2+} store and this Ca^{2+} release represents CICR. Furthermore, the present study demonstrated that depletion of the SR Ca^{2+} store by thapsigargin significantly decreased the KCl-induced peak increase in $[Ca^{2+}]_i$, confirming that KCl-induced Ca^{2+} release is from the Ca^{2+}-ATPase-related Ca^{2+} store in the SR.

To further confirm that CICR is functioning in CASMCs, caffeine, a well-characterized CICR activator (Hurley *et al.*, 1999; Janiak *et al.*, 2001), was employed to characterize this Ca^{2+} signaling mechanism

in the present study. As expected, caffeine primarily produced a rapid Ca^{2+} release response in CASMCs, which was markedly blocked by ryanodine, tetracaine, and thapsigargin, but not by xestospongin. Taking these results together, we conclude that CICR is functioning in CASMCs, which can be activated by membrane depolarization and consequent Ca^{2+} influx as well as direct binding of caffeine to RyR. This CICR in CASMCs is associated with activation of RyR, rather than IP_3 receptors on the SR and is sensitive to Ca^{2+}-ATPase inhibition.

To determine the physiological importance of this CICR in the control of vascular tone, the effects of CICR antagonists on the basal vascular tone and vasomotor reactivity were examined using an isolated and pressurized small coronary arterial preparation. Ryanodine and tetracaine were found to produce a dose-dependent vasodilation in these small coronary arteries. Pretreatment of the arteries with CICR antagonists significantly attenuated the vasoconstrictor response to KCl. In contrast, the IP_3 inhibitor xestospongin had no effect on KCl-induced vasoconstriction, but decreased the vasoconstrictor response to U46619, a vasoactive agent known to act through the IP_3 pathway (Tosun *et al.,* 1998; Yamagishi *et al.,* 1992). These results provide direct evidence that RyR-mediated CICR is involved in the development of basal vascular tone and the vasoconstrictor response in small coronary arteries. This finding is generally in agreement with previous reports indicating that RyR participates in the regulation of the vascular tone under basal conditions and after stimulation in other vascular beds, such as aorta and cerebral and pulmonary arteries (Ito *et al.,* 1991; Kamishima and McCarron, 1997; Vandier *et al.,* 1997; Watanabe *et al.,* 1993). Based on these findings, we propose that under the resting conditions, a small Ca^{2+} influx may produce Ca^{2+} release through RyR-mediated CICR, thereby developing basal vascular tone in these coronary arteries. When the cell membrane is depolarized and Ca^{2+} influx largely increased, a remarkable CICR would be activated, resulting in vasoconstriction. This CICR-mediated vasomotor response may represent another important mechanism to mediate the effects of vasoactive agents or other stimuli independent of the IP_3 pathway.

Previous studies have indicated that the Ca^{2+} signal induced by membrane influx may be attenuated by the SR Ca^{2+} uptake or accumulation. It seems that Ca^{2+} influx should not produce Ca^{2+} release in this type of myocytes (van Breemen *et al.,* 1995). However, recent studies demonstrate that the buffering of Ca^{2+} entry by the SR occurs mainly if there is a more gradual buildup of $[Ca^{2+}]_i$ resulting from a moderate stimulation of Ca^{2+} entry in the SR close to the cell membrane. When $[Ca^{2+}]_i$ rapidly increases in the vicinity of RyR on the SR, a Ca^{2+} release amplification response by CICR can be produced in vascular smooth muscle cells (van Breemen *et al.,* 1995). It is obvious that the role of CICR or superficial SR Ca^{2+} buffering in the regulation of $[Ca^{2+}]_i$ depends upon the magnitude and speed of Ca^{2+} influx. There is substantial evidence that a large Ca^{2+} influx primarily produces CICR, especially in small arteries or arterioles (Ganitkevich and Isenberg, 1995; Kamishima and McCarron, 1997, 1998). For example, by simultaneous recording of the voltage-gated Ca^{2+} channel currents and Ca^{2+} fluorescence imaging in the smooth muscle cells from the resistance arteries, previous studies have showed that opening of Ca^{2+} channels by a large pulse increase in membrane potential produced a Ca^{2+} release response, providing direct evidence of Ca^{2+} influx-induced Ca^{2+} release. The findings of the present study further support this view.

The next question we tried to answer was whether there is an endogenous mediator or modulator which is involved in CICR. Recent studies in our laboratory have shown that an enzyme pathway responsible for cADPR production and hydrolysis is present in coronary arterial smooth muscle cells (Li *et al.,* 1998). We wondered whether cADPR participates in CICR and thereby plays a role in the control of vascular tone. To address this question, we performed another series of experiments to examine the effects of a specific cell permeable cADPR antagonist, 8-br-CADPR, on CICR and CICR-related vasomotor responses in small coronary arteries. We found that blockade of cADPR actions by 8-br-cADPR significantly attenuated the Ca^{2+} release induced by KCl in single CASMCs and the vasoconstrictor response to KCl in pressurized small coronary arteries. These results support the view that cADPR participates in the regulation of vascular tone through a CICR mechanism in these small coronary arteries. This is consistent with the our previous report showing that the inhibition of cADPR formation produces vasorelaxation and blunts Bay-K8644-induced

vasoconstriction using bovine large coronary artery ring preparations (Geiger *et al.*, 2000).

The mechanism by which cADPR affects the CICR and vascular tone in coronary arteries remains unknown. However, two mechanistic models of cADPR action in smooth muscle cells and other cells have been proposed recently, including cADPR as mediator or modulator of CICR (Galione *et al.*, 1998; Lee *et al.*, 1995). As mediators, it is assumed that the agonists or stimuli induce the activation of ADP-ribosyl cyclase, an enzyme responsible for the production of cADPR from NAD, leading to an increase in the cytosolic cADPR and a subsequent activation of RyR-mediated Ca^{2+} release. In addition, intracellular Ca^{2+} increase may also activate the production of cADPR, thereby resulting in Ca^{2+} release. As a modulator, the cytosolic cADPR sensitizes the RyR, enhancing CICR activated by agonists or Ca^{2+} influx. The relative contribution of these two mechanisms may vary depending on the concentrations of intracellular cADPR, Ca^{2+}, and calmodulin and RyR functional status in different cells. Although the present study demonstrated that cADPR is involved in CICR in coronary arterial myocytes, it remains unknown whether it serves as a mediator or modulator or both in these cells. Further studies are needed to clarify this issue when a validated measurement of cADPR concentrations is available.

In summary, the present study demonstrated that KCl and caffeine induced Ca^{2+} release from intracellular stores of coronary arterial smooth muscle cells and this Ca^{2+} release was decreased in the presence of CICR and cADPR antagonists. Corresponding to these effects in single myocytes, inhibition of CICR or blockade of cADPR action induced vasorelaxation and attenuated the vasoconstrictor response to KCl in isolated and pressurized small coronary arteries. These results indicate that RyR- and cADPR-mediated CICR is involved in the control of $[Ca^{2+}]_i$ in coronary arterial smooth muscles, thereby participating in the vasomotor response in these arteries.

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