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Enhanced production and action of cyclic ADP–ribose during oxidative stress in small bovine coronary arterial smooth muscle

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Abstract

Recent studies in our lab and by others have indicated that cyclic ADP–ribose (cADPR) as a novel second messenger is importantly involved in vasomotor response in various vascular beds. However, the mechanism regulating cADPR production and actions remains poorly understood. The present study determined whether changes in redox status influence the production and action of cADPR in coronary arterial smooth muscle cells (CASMCs) and thereby alters vascular tone in these arteries. HPLC analyses demonstrated that xanthine (X, 40 μM)/xanthine oxidase (XO, 0.1 U/ml), a superoxide-generating system, increased the ADP–ribosyl cyclase activity by 59% in freshly isolated bovine CASMCs. However, hydrogen peroxide (H_2O_2, 1–100 μM) had no significant effect on ADP–ribosyl cyclase activity. In these CASMCs, X/XO produced a rapid increase in $[Ca^{2+}]_i$ ($\Delta[Ca^{2+}]_i = 201$ nM), which was significantly attenuated by a cADPR antagonist, 8-Br-cADPR. Both inhibition of cADPR production by nicotinamide (Nicot) and blockade of Ca^{2+}-induced Ca^{2+} release (CICR) by tetracaine (TC) and ryanodine (Rya) significantly reduced X/XO-induced rapid Ca^{2+} responses. In isolated, perfused, and pressurized small bovine coronary arteries, X at 2.5–80 μM with a fixed XO level produced a concentration-dependent vasoconstriction with a maximal decrease in arterial diameter of 45%. This X/XO-induced vasoconstriction was significantly attenuated by 8-Br-cADPR, Nicot, TC, or Rya. We conclude that superoxide activates cADPR production, and thereby mobilizes intracellular Ca^{2+} from the SR and produces vasoconstriction in coronary arteries.

Keywords: Cyclic adenosine diphosphate-ribose; Oxidative stress; Ryanodine receptors; Calcium; Coronary artery; Smooth muscle

Introduction

Cyclic ADP–ribose (cADPR), an endogenous metabolite of nicotinamide adenine dinucleotide (NAD) via ADP–ribosyl cyclase, has been identified as an intracellular second messenger in a variety of mammalian cells (Galione, 1993; Koshiyama et al., 1991; Lee, 1993; Lee and Aarhus, 1991, 1993; Lee, et al., 1993; Takesawa et al., 1993). This signaling nucleotide initiates Ca^{2+}-induced Ca^{2+} release (CICR) through activation of ryanodine (Rya) receptors (RyRs), which is completely independent of 1, 4, 5-triphosphate (IP_3) signaling pathway (Galione et al., 1991; Lee, 1993; Lee et al., 1995). Functionally, this cADPR-mediated signaling has been indicated to participate in the regulation of many cell functions or physiological processes, including insulin secretion, egg fertilization, cell proliferation, and nitric oxide-induced Ca^{2+} movement (Galione, 1993; Lee et al., 1993; Li et al., 2001; Okamoto, 1999; Yu et al., 2000). Intracellular ADP–ribosyl cyclase has been reported to serve as a putative NAD^+ sensor, which acts to produce cADPR to regulate cytosolic Ca^{2+} concentration, thereby altering cell function. Since NAD^+ is a product of cellular intermediary metabolism, cADPR production may be linked to metabolic activity (Sun et al., 2002). Recent studies in our laboratory and by others have demonstrated that cADPR-mediated Ca^{2+} signaling plays an important role in the control of basal vascular tone and in the mediation of vasomotor response to vasoactive agonists such as acetylcholine, nitric oxide (NO), and endothelium-derived hyperpolarization factor, epoxyeicosatrienoic acids (EETs) (Ge et al., 2003; Li et al., 2002; White et al., 2002; Yu et al., 2000). However, it remains unclear how cADPR production is regulated in vascular smooth muscle cells and whether this novel Ca^{2+} signaling pathway is involved in cell adaptation process to various stimuli such as oxidative stress, hypoxia, metabolic activities, or others.

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Recent studies have highlighted that redox status importantly contributes to the control of vascular tone or vasomotor response in different vascular beds (Chakraborti et al., 1998; Rathaus and Bernheim, 2002; Schnackenberg, 2002a). It has been demonstrated that reactive oxygen species (ROS) serve as a vascular O_2-sensing factor to activate the vascular reactivity in response to tissue metabolic activity (Wolin, 2000). An important mechanism by which ROS regulate the vascular tone may be due to the changes in the intracellular Ca^{2+} homeostasis. In previous studies, ROS have been shown to cause Ca^{2+} mobilization in cardiac, skeletal, and smooth muscles (Chakraborti et al., 1998; Kawakami and Okabe, 1998; Kourie, 1998; Suzuki and Ford, 1999). This ROS-induced change in Ca^{2+} homeostasis in muscles could be mediated via a variety of mechanisms such as inhibition of Ca^{2+}-ATPase of sarcolemmal and sarcoplasmic reticulum (SR), modification in gating activities of SR Ca^{2+} release channels, or alteration of Ca^{2+} release from either inositol-1,4,5-triphosphate (IP_3)-sensitive or ryanodine-sensitive Ca^{2+} stores (Chakraborti et al., 1998; Clementi, 1998; Kawakami and Okabe, 1998; Kourie, 1998; Okabe et al., 2000). However, the underlying mechanisms by which ROS exert their actions on Ca^{2+} homeostasis in vascular smooth muscle are still poorly understood. Given the important role of cADPR in regulating intracellular Ca^{2+} levels, basal vascular tone, and vasomotor response, we hypothesized that changes in cell redox status may also enhance cADPR-mediated Ca^{2+} signaling and thereby produce vasoconstriction in coronary circulation. To test this hypothesis, we first examined whether a challenge of coronary arterial smooth muscle cells (CASMCs) by O_2^--generating system, X/XO, alters ADP–ribosyl cyclase activity and cADPR production in bovine CASMCs. Then, we examined whether altered cADPR production in response to oxidative stress contributes to Ca^{2+} mobilization and vasoconstriction in coronary arterial smooth muscle.

Materials and methods

Dissection of small coronary arteries

Fresh bovine hearts were obtained from a local abattoir. The left ventricular wall was rapidly dissected and immersed in ice-cold phosphate-buffered saline (PBS) (Sigma, pH 7.4). A myocardial section was transported immediately to the laboratory and used to dissect intramyocardial coronary arteries from the branches of left anterior descending artery. For dissociation of smooth muscle cells, the arteries were cut into small pieces (0.5–1 mm in length) and then incubated with enzyme solution as described below. For microperfusion experiments, the artery segments (5–8 mm in length) were cleaned off of fat and connective tissues under a dissection microscope and placed in cold PBS. The rings of small coronary arteries were passed through its lumen by a segment of hair. The endothelium was removed by gently rotating the hair (Ge et al., 2003).

Dissociation of fresh single CASMCs

Single smooth muscle cells from small bovine coronary arteries were freshly prepared by enzymatic dissociation as we previously described (Li et al, 2001). Briefly, dissected small coronary arteries were first incubated for 25 min at 37°C with collagenase type II (340 U/ml) (Worthington), elastase (15 U/ml) (Worthington), dithiothreitol (1 mg/ml), and soybean trypsin inhibitor (1 mg/ml) in HEPES buffer consisting of (in mM): NaCl, 119; KCl, 4.7; $CaCl_2$, 0.05; $MgCl_2$, 1; glucose, 5; HEPES, 10 (pH 7.4). The digested arterial tissue was then agitated with a glass pipette to free vascular smooth muscle cells and the supernatant was collected. Remaining tissue was further digested with fresh enzyme solution, and the supernatant was collected at 5-min intervals for an additional 15 min. The supernatants were pooled and diluted 1:10 with HEPES buffer and stored at 4°C until used.

HPLC assay of ADP–ribosyl cyclase activity in freshly isolated CASMCs

Homogenates were prepared from freshly isolated CASMCs with a modified method as we described previously (Li et al., 1994, 1997). Briefly, freshly isolated CASMCs were rinsed with chilled PBS (pH 7.0, Sigma). After removing the PBS, the cells were resuspended and homogenized in HEPES buffer (pH 7.0) containing (in mM) HEPES, 10; NaCl, 148; KCl, 5; $CaCl_2$, 1.8; $MgCl_2$, 0.3, and glucose, 5.5; sonicated three times with a sonifier cell disruptor (Branson, Model 185) for 20 s and then centrifuged at 6000 rpm for 10 min at 4°C. The supernatant was considered as the cell homogenate. To determine ADP–ribosyl cyclase activity, the homogenate (100 μg protein) was incubated with β-nicotinamide guanine dinucleotide (β-NGD^+) of 100 μM at 37°C for 20 min. β-NGD^+ was used as a substrate to determine ADP–ribosyl cyclase activity, because this enzyme converts NGD into cGDPR, but unlike cADPR, cGDPR cannot be hydrolyzed by cADPR hydrolase. The reaction mixtures were centrifuged at 4°C through an Amicon microultrafilter at 13,800 × *g* to remove proteins, and then analyzed by HPLC with a fluorescence detector (Hewlett-Packard 1090 HPLC system and 1046A spectrofluorometer). The excitation wavelength of 300 nm and the emission wavelength of 410 nm were used to detect the fluorescent products. All HPLC data were collected and analyzed by a Hewlett-Packard Chemstation.

Nucleotides were resolved on a 3-μm Supelcosil LC-18 column (4.6 × 150 mm) with a 5-μm Supelcosil LC-18 guard column (4.6 × 20 mm, Supelco, Bellefonte, BA). The

injection volume was 20 μl. The mobile phase consisted of 150 mM ammonium acetate (pH 5.5) containing 5% methanol (solvent A) and 50% methanol (solvent B). The solvent system was a linear gradient of 5% solvent B in A to 30% solvent B in A over 1 min, held for 25 min, and then increased to 50% solvent B over 1 min. The flow rate was 0.8 ml/min. Peak identities were confirmed by comigration with known standards. Quantitative measurements were performed by comparison of known concentrations of standards (Yu et al., 2000).

Measurement of $[Ca^{2+}]_i$ in single CASMCs

Determination of $[Ca^{2+}]_i$ in freshly dissociated single CASMCs was performed using fura-2 as an indicator. The cells were first loaded with fura-2 acetoxymethyl ester (fura-2 AM, 5 μM) (Molecular Probes, Eugene, OR) at 37°C for 45 min. A polylysine-coated glass cover slip was mounted at the bottom of a chamber, which was mounted horizontally on a Nikon Diaphoto TMD inverted microscope (Nikon, Japan). The cells on a cover slip were incubated with a Ca^{2+}-free Hanks' buffer for 20 min to allow the complete hydrolysis of intracellular fura-2-AM to fura-2. X/XO (X, 40 μM) was applied to the bath solution to induce Ca^{2+} release. To examine the specificity of X/XO and the role of cADPR signaling pathway in X/XO-induced Ca^{2+} release, CASMCs were pretreated for 15 min with 8-bromo-cADPR (8-Br-cADPR, a cADPR antagonist) (30 μM), nicotinamide (Nicot, an inhibitor of ADP−ribosyl cyclase) (6 mM), ryanodine (Rya, an antagonist of ryanodine receptors) (30 μM), or tetracaine (TC, an CICR inhibitor) (2 μM). The concentrations of these inhibitors were based on the results obtained from in vitro microperfusion of coronary arteries and their ability to inhibit X/XO-induced vasoconstriction. The ratio of fura-2 emissions, when excited at the wavelength of 340 and 380 nm, was monitored using a fluorescent ratiometric spectrofluorometry microscope (PTI) (Yu et al., 2000).

Vascular reactivity in in vitro perfused small coronary arteries

Dissected small coronary arteries (approximately 200 μm) were transferred to a water-jacketed perfusion chamber and cannulated with two glass micropipettes as described previously (Zhang et al., 2002b).The outflow cannula was clamped, and the arteries bathed under the transmural pressure of 60 mm Hg in physiological saline solution (PSS) containing (in 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). PSS in the bath was continuously bubbled with a gas mixture of 95% O_2 and 5% CO_2 and maintained at 37 ± 0.1°C throughout the experiment. The internal diameter of the arteries was measured with a video system composed of a stereomicroscope (Leica MZ8), a charge-coupled device camera (KP-MI AU, Hitachi), a video monitor (VM-1220U, Hitachi), a video measuring apparatus (VIA-170, Boeckeler Instrument), and a video printer (UP890 MD, Sony). The arterial images were recorded continuously with a videocassette recorder (M-674, Toshiba).

After a 60-min equilibration period, X/XO (X, 2.5 to 80 μM) was cumulatively added to the bath solution to induce vasoconstriction. When the X/XO-induced contraction reached a stable plateau at each concentration, the internal diameter of vessel was measured and recorded. To examine the contribution of cADPR signaling pathway to X/XO-induced vasoconstriction, the arteries were pretreated for 30 min with one of the following compounds: 8-Br-cADPR (30 μM), Nicot (6 mM), Rya (30 μM), or TC (2 μM). Then, X/XO was added, and the vasoconstrictor response was determined as described above. The contractile responses to X/XO were expressed as percent reduction in vascular internal diameters.

Statistics analysis

Data are expressed as mean ± standard error of mean (SEM). The significance of the differences in mean values

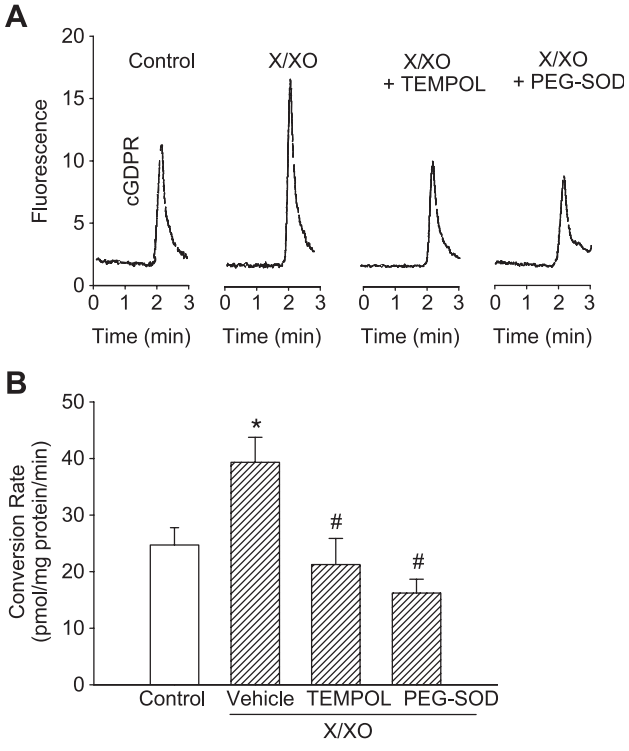

Fig. 1. Effect of X/XO on ADP-ribosyl cyclase activity in freshly dissociated CASMCs. (A) Typical fluorescence chromatograms depicting the effects of X/XO on cGDPR production in homogenates from freshly dissociated CASMCs in the absence or presence of SOD mimetic, TEMPOL (1 mM), or PEG-SOD (1 mM). (B) Summarized data showing the effects of X/XO on the conversion rate of NGD^+ to cGDPR by ADP-ribosyl cyclase in the absence or presence of TEMPOL or PEG-SOD. X/XO: xathane/xanthine oxidase. *$P < 0.05$, compared to control ($n = 6$); #$P < 0.05$, compared to vehicle ($n = 6$).

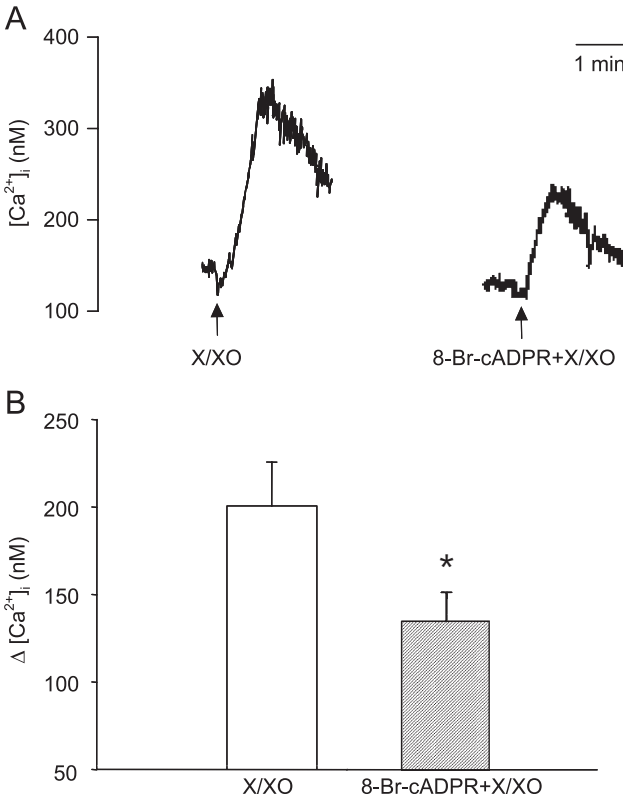

Fig. 2. Inhibition of X/XO-induced Ca^{2+} release by 8-Br-cADPR in freshly dissociated CASMCs. (A) Representative recordings of $[Ca^{2+}]_i$ transients. (B) Summarized data showing the changes ($\Delta[Ca^{2+}]_i$) from baseline $[Ca^{2+}]_i$ to the peak $[Ca^{2+}]_i$ in response to X/XO in the absence or presence of 8-Br-cADPR (30 μM). *$P < 0.05$ compared to control ($n = 18$).

between and within multiple groups was examined using an analysis of variance for repeated measures followed by Duncan's multiple range tests. Student's t test was used to evaluate statistical significance of differences between two paired observations. $P < 0.05$ was considered statistically significant.

Results

Effect of X/XO and H_2O_2 on ADP–ribosyl cyclase activity in freshly isolated CASMCs

Fig. 1A presents a typical fluorescence HPLC chromatograph showing cyclic GDP-ribose (cGDPR) production from β-NGD^+ by freshly isolated CASMCs. This product co-eluted with cGDPR standard at a retention time of 2.2 min. Under control conditions, the conversion rate of β-NGD^+ to cGDPR was 24.7 ± 2.7 pmol/min/mg protein ($n = 6$). When CASMCs were pre-incubated with X (40 μM)/XO (0.1 U/ml) at 37 °C for 30 min, the conversion rate of β-NGD^+ to cGDPR was significantly increased to 39.3 ± 4.0 pmol/min/mg protein ($n = 6$) (Fig. 1B). When CASMCs were pre-treated with a cell-permeable O_2^- dismutase (SOD) mimetic, 4-hydroxyl-tetramethylpiperidin-oxyl (TEMPOL, 1 mM) or PEG-SOD (1 mM), the conversion rate was significantly decreased to 21.3 ± 4.1 pmol/min/mg protein and 16.2 ± 2.2 pmol/min/mg protein, respectively (Fig. 1B). To determine whether H_2O_2 has any effect on the activity of ADP–ribosyl cyclase, we incubated the CASMCs with H_2O_2 (1–100 μM). The conversion rate of β-NGD^+ to cGDPR was not significantly altered even at the highest concentration of H_2O_2 (100 ìM) studied (data not shown).

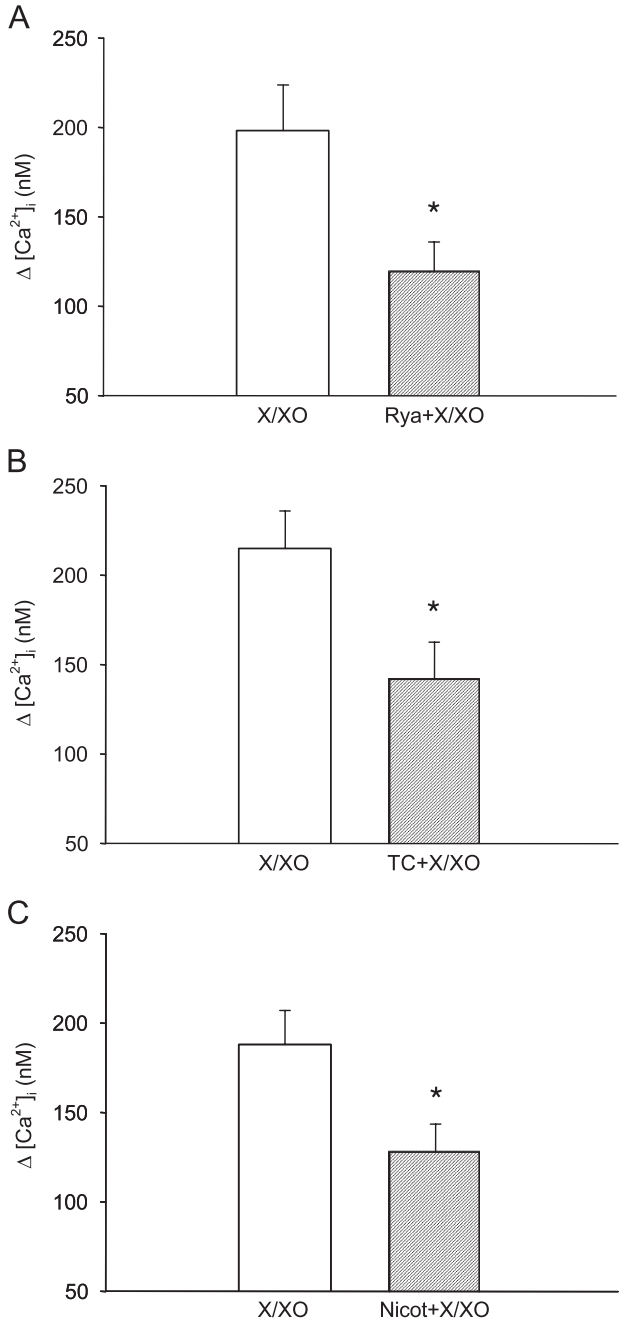

Fig. 3. X/XO-induced Ca^{2+} release in the absence or presence of (A) RyR blocker, Rya (30 μM, $n = 16$); (B) CICR inhibitor, TC (2 μM, $n = 15$); (C) ADP-ribosyl cyclase inhibitor, Nicot (6 mM, $n = 17$). *$P < 0.05$ compared to control.

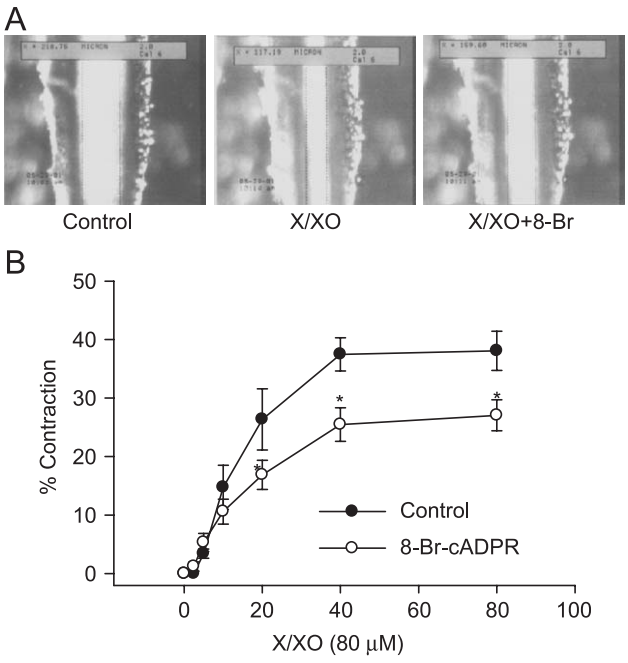

Fig. 4. X/XO-induced vasoconstriction in endothelium-denuded small coronary arteries in the absence or presence of cADPR blocker, 8-Br-cADPR. (A) Representative photo prints showing the changes in internal diameters of small coronary arteries treated with X/XO (80 μM/0.1U/ml) in the absence or presence of 8-Br-cADPR (8-Br, 30 μM). (B) Summarized data showing the inhibitory effect of 8-Br-cADPR on X/XO-induced concentration-dependent contraction. *$P < 0.05$, compared to control ($n = 5$).

X/XO-induced Ca^{2+} release in the presence or absence of cADPR antagonist

Fig. 2A presents typical Ca^{2+} transient recordings when the cells were incubated with X/XO. It shows that X/XO produced a rapid Ca^{2+} rise in CASMCs exposed to Ca^{2+}-free Hank's solution with 1mM EGTA and that pretreatment of the cells with 8-Br-cADPR markedly attenuated X/XO-induced Ca^{2+} response. The basal $[Ca^{2+}]_i$ in bovine CASMCs was 115.6 ± 5.6 nM ($n = 17$). X/XO (40 μM/0.1 mU/ml) evoked a peak $[Ca^{2+}]_i$ to 305.3 ± 18.4 nM ($n = 17$). After CASMCs were pretreated with 8-Br-cADPR (30 μM) for 15 min, the basal $[Ca^{2+}]_i$ was not significantly altered, but the X/XO-induced increase in $[Ca^{2+}]_i$ ($\Delta[Ca^{2+}]_i$) was significantly attenuated from 201 ± 23 nM to 136.5 ± 17 nM, a reduction of 32.3% ($n = 18$) (Fig. 2B).

X/XO-induced Ca^{2+} release during RyR blockade, CICR suppression, or ADP–ribosyl cyclase inhibition

To further determine whether X/XO-induced Ca^{2+} release is associated with RyR-mediated Ca^{2+} mobilization from the SR, Rya as a RyR blocker and TC as a CICR inhibitor were used to pretreat CASMCs, and then the Ca^{2+} release response of those cells to X/XO was measured. In the presence of Rya (30 μM) and TC (2 μM), X/XO-

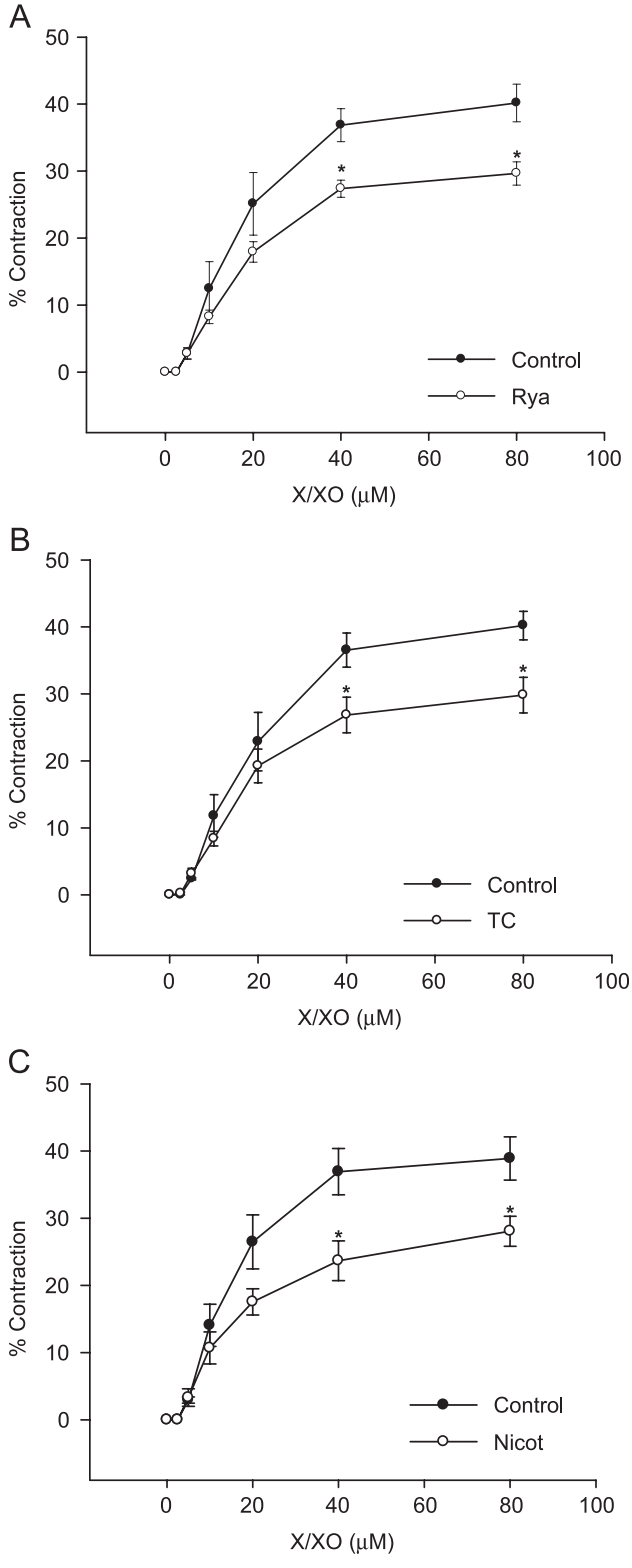

Fig. 5. X/XO-induced vasoconstriction in endothelium-denuded small coronary arteries during RyR blockade, CICR suppression or ADP-ribosyl cyclase inhibition. X/XO-induced concentration-dependent vasoconstriction in the absence or presence of (A) RyR blocker, Rya (30 μM, $n = 6$); (B) CICR inhibitor, TC (2 μM, $n = 6$); (C) ADP-ribosyl cyclase inhibitor, Nicot (6 mM, $n = 5$). *$P < 0.05$, compared to control.

stimulated Ca^{2+} release was significantly attenuated by 40% (n = 16) and 33% (n = 15), respectively (Figs. 3A and B). Inhibition of cADPR production in CASMCs with Nicot (6 mM) also significantly reduced X/XO-induced Ca^{2+} release by 31% (n = 17). These results were summarized in Fig. 3C.

X/XO-induced contraction in endothelium-denuded small coronary arteries

Fig. 4A shows typical photomicrograph of a coronary artery depicting X/XO-induced vasoconstriction and 8-Br-cADPR-induced blockade of X/XO response. During a 60-min equilibration period, the internal diameters of small coronary arteries were not significantly changed. There were no significant differences in baseline internal diameters between groups of vessels in each experiment. In these experiments, cumulative addition of X (2.5–80 μM) with a fixed XO level (0.1 U/ml) induced a concentration-dependent vasoconstriction. After washing and equilibrating, repeated addition of X/XO produced similar contraction to that observed in the first experiment, indicating that X/XO-induced coronary vasoconstriction is repeatable in this preparation.

To determine the contribution of cADPR signaling pathway to X/XO-induced vasoconstriction, the vessels were first treated for 20 min with 8-Br-cADPR (30 μM). In the presence of 8-Br-cADPR, X/XO-induced contraction was significantly attenuated. The maximal contraction was changed from 38.1 ± 3.3% to 27.1 ± 2.6% (n = 5) (Fig. 4B).

X/XO-induced contraction in endothelium-denuded small coronary arteries during RyR blockade, CICR suppression, and ADP–ribosyl cyclase inhibition

Additional groups of bovine coronary arteries were used to test whether X/XO-induced vasoconstriction is associated with RyR and CICR. As shown in Fig. 5, in the presence of Rya and TC, X/XO-induced concentration-dependent contraction was significantly decreased from a maximal contraction of 46.2 ± 4.1% (n = 6), to 32.8 ± 3.5%, and to 27.7 ± 5.9% (n = 6), which represent a 30% and 40% reduction, respectively. In addition, inhibition of ADP–ribosyl cyclase by Nicot also markedly decreased X/XO-induced vasoconstriction from 43.0 ± 3.7% to 22.5 ± 5.3% (n = 5), a 49% decrease.

Discussion

In the present study, we demonstrated that O_2^- produced by X/XO significantly increased cADPR production and Ca^{2+} release in CASMCs and thereby led to coronary vasoconstriction. X/XO-induced Ca^{2+} release response was significantly attenuated by cADPR blocker or ADP–ribosyl cyclase inhibitor. Similarly, CICR inhibitor and ryanodine receptor (RyR) blockade also markedly decreased X/XO-induced Ca^{2+} response and vasoconstriction. These results suggest that cADPR importantly contributes to Ca^{2+} increases and contraction of small coronary arterial smooth muscle during oxidative stress.

In previous studies, ADP–ribosyl cyclase activity has been reported to be regulated by redox status in some tissues or cells. The production of cADPR is increased by intracellular oxidants and this redox regulation of cADPR production is dependent on a dimerization of ADP–ribosyl cyclase which enhances its catalytic activity (Burruet et al., 1998; Chidambaram et al., 1998; Franco et al., 1994; Guida et al., 1995; Munshi et al., 1998; Shubinsky and Schlesinger, 1997). Since the cysteine residues in the ADP–ribosyl cyclase determine the ability of this enzyme to function as either ADP–ribosyl cyclase or cADPR hydrolase, the oxidation of cysteine molecules, which leads to the formation of one or several disulfide bonds may result in the formation of ADP–ribosyl cyclase dimers, thereby increasing ADP–ribosyl cyclase activity (Tohgo et al., 1994). However, it remains unclear whether this redox response of cADPR production contributes to the regulation of vascular tone. A recent study has indicated that in pulmonary arteries, the cellular redox state may be coupled to cADPR synthesis and thereby to activation of ryanodine receptors and Ca^{2+} release. This redox coupling to Ca^{2+} mobilization may play an important role in regulating hypoxic pulmonary vasoconstriction (Wilson et al., 2001). In the present study, HPLC analyses demonstrated that incubation of CASMCs with O_2^- generating system X/XO produced activation of ADP–ribosyl cyclase, which is consistent with a previous study showing that O_2^- generated by X/XO stimulated synthesis of cADPR from NAD^+ in myocardium (Kumasaka et al., 1999). The increase in ADP–ribosyl cyclase activity was attributed to O_2^-, a major ROS produced by X/XO, since incubation of CASMCs with H_2O_2 did not significantly alter ADP–ribosyl cyclase activity even with the highest concentration (100 μM) studied. Our previous studies have demonstrated that another ROS, NO, decreased ADP–ribosyl cyclase activity and thereby reduced cADPR production in coronary arterial smooth muscle cells (Yu et al., 2000). Taken together, our results indicate that it is O_2^- that activates ADPR production in coronary arterial smooth muscle cells. This O_2^- induced activation of cADPR signaling may be importantly involved in regulation of intracellular Ca^{2+} mobilization in vascular smooth muscle during oxidative stress, and ADP–ribosyl cyclase may serve as a link between intracellular $[Ca^{2+}]_i$ and oxidants.

To test this hypothesis, we further examined rapid Ca^{2+} transient response to production or accumulation in single coronary arterial smooth muscle cells. It was demonstrated that X/XO induced a rapid and transient increase in $[Ca^{2+}]_i$ in freshly isolated single CASMCs. In these experiments, since the cells were bathed with Ca^{2+}-free solution, it is unlikely that Ca^{2+} entry participates in the effect of O_2^- production on Ca^{2+} increase. Therefore, Ca^{2+} release from intracellular store

may be the primary resource of X/XO-induced Ca^{2+} increase. In the presence of 8-Br-cADPR, a cell membrane-permeable cADPR antagonist, the effect of X/XO was significantly attenuated by 32.3%. Similar results were obtained when CASMCs were pretreated with nicotinamide (Nicot), an inhibitor of ADP−ribosyl cyclase. The results indicate that X/XO-induced $[Ca^{2+}]_i$ increase is associated with cADPR signaling mechanism. Previous studies in our laboratory and by others have shown that cADPR-mediated Ca^{2+} signaling functions through a CICR mechanism associated with RyRs activation (Galione et al., 1991; Kamishima and McCarron, 1997; Zhang et al., 2001, 2002a). In the present study, the contribution of CICR and RyRs to Ca^{2+} response during oxidative stress has also been determined. Either the blockade of RyR by ryanodine (Rya) or inhibition of CICR by tetracaine (TC) markedly reduced X/XO-induced Ca^{2+} release to a similar extent to cADPR inhibition, suggesting that this cADPR pathway-associated component in X/XO-induced Ca^{2+} response is CICR- and RyR-dependent.

It is obvious that X/XO-induced increase in $[Ca^{2+}]_i$ in CASMCs was not completely blocked by blockade of this cADPR/CICR signaling pathway. Previous studies have reported that IP_3-induced Ca^{2+} release was also potentiated by O_2^- (Suzuki and Ford, 1992). In addition, inhibition of the Ca^{2+}-ATPase by ROS is importantly involved in enhancement of Ca^{2+} release from the SR of vascular smooth muscle (Grover and Samson, 1988; Suzuki and Ford, 1991). Taken together, the results indicate that increase in Ca^{2+} release in vascular smooth muscle during oxidative stress or enhanced O_2^- production may be associated with several different mechanisms. cADPR-mediated Ca^{2+} signaling pathway represents one of these important mechanisms producing Ca^{2+} increase in response to oxidative stress.

Using isolated and perfused small coronary artery preparations, we have examined the functional significance of this redox response of cADPR-signaling pathway in the control of vascular tone. It was found that O_2^- generated from X/XO produced vasoconstriction in small coronary arteries. Pretreatment of the arteries with 8-Br-cADPR, Nicot, Rya, or TC significantly blunted X/XO-induced vasoconstriction in this preparation. These results provide direct evidence that the cADPR signaling pathway participates in the vasoconstrictor response to ROS in the coronary circulation, supporting the view that oxidative stress, especially increased O_2^- production results in vasoconstriction in part through cADPR/RyR activation pathway in CASMCs. However, a previous study reported that O_2^- generated by hypoxanthine/xanthine oxidase did not alter basal vascular tone or IP_3-induced contraction, but attenuated caffeine-induced contraction in á-toxin-permeabilized rabbit mesenteric arteries (Wada and Okabe, 1997). The reason for the discrepancy in two studies is unclear. It is possible that O_2^- may have different vasoactive actions in coronary and mesenteric arteries. In addition, species difference may be another reason for this discrepancy. Previous studies have indicated that O_2^- can cause muscle contraction via the interaction with endothelium-derived vasoactive compounds such as NO or via increasing production of endothelium-derived contraction factors such as thromboxane A_2 or endothelin in many vascular systems (Gupte et al., 1996; Liu et al., 2003; Rueckschloss et al., 2003; Schnackenberg, 2002b; Torrecillas et al., 2001). Moreover, O_2^- has been reported to induce ERK MAP-kinase-mediated vasoconstriction in rat thoracic aorta (Peters et al., 2000). In the present study, we have shown that ROS may exert their effect on Ca^{2+} signaling in vascular smooth muscle through cADPR/RyR activation. It appears that ROS serve as important mediators in regulating vascular reactivity in response to oxidative stress under physiological and pathological conditions.

The present study did not explore the mechanism by which O_2^- enhances ADP−ribosyl cyclase activity and thereby increases cADPR in coronary arterial smooth muscle cells. As discussed above, O_2^- may oxidize cysteine residues of this cyclase, resulting in dimer formation and enhancement of cADPR production. In addition, the interaction of O_2^- and NO in smooth muscle cells may be another important mechanism for activation of ADP−ribosyl cyclase. In recent studies, we have demonstrated that NO decreased ADP−ribosyl cyclase activity, resulting in decrease in Ca^{2+} mobilization and vasodilation (Yu et al., 2000). Considering a rapid reaction of NO with O_2^-, it is possible that NO decrease during increase in O_2^- levels may also lead to activation of ADP−ribosyl cyclase. Therefore, NO may serve as an antioxidative mechanism to antagonize the action of O_2^- in these coronary arterial smooth muscle cells. Along with this line, cADP−ribosyl cyclase may serve as a molecular switch between O_2^- and NO or oxidants and antioxidants in regulating intracellular Ca^{2+} level or vascular tone in coronary vascular bed.

In summary, the present study provided the first evidence that cADPR production was increased by O_2^- through the activation of ADP−ribosyl cyclase in coronary arterial smooth muscle cells. In both isolated single CASMCs, cADPR antagonism, RyR and CICR blockade, and inhibition of cADPR production were shown to significantly attenuate O_2^--induced Ca^{2+} release. Consistently, these inhibitors or blockers of cADPR/RyR signaling pathway could attenuate vasoconstriction in isolated and perfused small coronary arteries. These results indicate that cADPR/RyR-mediated Ca^{2+} release importantly participates in enhanced Ca^{2+} mobilization and consequent vasoconstriction in coronary arteries during oxidative stress.

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