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Effect of nitric oxide on calcium-induced calcium release in coronary arterial smooth muscle

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Abstract

The present study was designed to determine whether nitric oxide (NO)-induced reduction of $[Ca^{2+}]_i$ is associated with Ca^{2+}-induced Ca^{2+} release (CICR) in coronary arterial smooth muscle cells (CASMCs). Caffeine was used as a CICR activator to induce Ca^{2+} release in these cells. The effects of NO donor, sodium nitroprusside (SNP), on caffeine-induced Ca^{2+} release were examined in freshly dissociated bovine CASMCs using single cell fluorescence microscopic spectrometry. The effects of NO donor on caffeine-induced coronary vasoconstriction were examined by isometric tension recordings. Caffeine, a CICR or ryanodine receptor (RYR) activator, produced a rapid Ca^{2+} release with a 330 nM increase in $[Ca^{2+}]_i$. Pretreatment of the CASMCs with SNP, CICR inhibitor tetracaine or RYR blocker ryanodine markedly decreased caffeine-induced Ca^{2+} release. Addition of caffeine to the Ca^{2+}-free bath solution produced a transient coronary vasoconstriction. SNP, tetracaine and ryanodine, but not guanylyl cyclase inhibitor, ODQ, significantly attenuated caffeine-induced vasoconstriction. These results suggest that CICR is functioning in CASMCs and participates in the vasoconstriction in response to caffeine-induced Ca^{2+} release and that inhibition of CICR is of importance in mediating the vasodilator response of coronary arteries to NO.

Keywords: Endothelium-derived relaxing factor; Calcium mobilization; Ca^{2+}-induced Ca^{2+} release; Coronary artery; Vascular tone; Heart

1. Introduction

It has been reported that nitric oxide (NO) serves as an endothelium-derived relaxing factor (EDRF) to play an important role in the control of vascular tone (Hutchinson et al., 1987; Ignarro et al., 1987). In spite of intensive studies, the precise mechanisms by which NO causes vasodilation have not yet been fully understood. The activation of soluble guanylyl cyclase and production of cGMP have been indicated to mediate vasodilator action of NO (Murad et al., 1987; Murphy and Walker, 1998). However, recent studies have challenged the role of cGMP/PKG in mediating the effects of NO. It has been reported that blockade of cGMP/PKG pathway does not alter NO-induced vasodilation, especially in the resistance arteries or arterioles (Loscalzo and Welch, 1995; Weisbrod et al., 1998; Wong et al., 1995). This led to the hypothesis that the cGMP/PKG-independent mechanisms may participate in the action of NO on vascular smooth muscle. In this regard, activation of K_{Ca} channels (Bolotina et al., 1994), inhibition of vasoconstrictor, 20-HETE production (Alonso-Galicia et al., 1997; Sun et al., 1999) and direct effect on Ca^{2+} influx and Ca^{2+} release have been reported to be the possible mechanisms mediating the action of NO on vascular smooth muscle (Blatter and Wier, 1994; Garg and Hassid, 1991; Kannan et al., 1997; Kasai et al., 1997; Wang et al., 1996).

With respect to its effects on $[Ca^{2+}]_i$, NO may produce activation of Ca^{2+} ATPase and resulting increase in the sequestration of Ca^{2+} from the cytoplasm, inhibition of inositol 1,4,5-triphosphate (IP_3) formation, inactivation of voltage-dependent Ca^{2+} channels and decrease in Ca^{2+} influx (Abe et al., 1989; Blatter and Wier, 1994; Cohen et al., 1999; McDaniel et al., 1992). However, it appears that the mechanisms mediating the effects of NO on $[Ca^{2+}]_i$ are different, depending upon vascular beds or VSMC preparations.

Recently, CICR and cADP-ribose (cADPR)-mediated Ca^{2+} signaling mechanisms have been established to play an important role in the control of basal vascular tone and the mediation of vasomotor response (Berridge, 1997). In

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some vascular beds, ryanodine receptor (RYR)-mediated Ca^{2+} release or Ca^{2+}-induced Ca^{2+} release (CICR) predominantly contributes to the control of basal vascular tone or vasoconstrictor response to different stimuli. In cerebral circulation, RYR mediates Ca^{2+} release, which can activate K_{Ca} channel, and participates in the control of basal vascular tone. This Ca^{2+} release response largely contributes to vasoconstrictor response of cerebral resistance arteries to stimuli (Kamishima and McCarron, 1997, 1998). In pulmonary vascular bed, RYR-mediated CICR is crucial for the development of basal vascular tone and production of hypoxic vasoconstriction (Abdalla et al., 1994; Vandier et al., 1997). More recently, we reported that cADPR as a second messenger is importantly involved in the vasoconstrictor response through RYR, and blockade of cADPR produced vasodilation in coronary arteries (Geiger et al., 2000; Li et al., 1998a,b). All these findings indicate that CICR or RYR-mediated Ca^{2+} release is an important mechanism mediating global increase in $[Ca^{2+}]_i$ and activation of vascular smooth muscle (Ito et al., 1991; Berridge, 1997). Yet, it remains unknown whether the vasodilator effect of NO is associated with alteration of CICR.

The present study was designed to test the hypothesis that NO inhibits CICR and thereby produces vasodilation in coronary arteries. We determined the effect of NO donor on caffeine-induced increase in $[Ca^{2+}]_i$ in coronary arterial smooth muscle cells (CASMCs) exposed to Ca^{2+}-free solution using single cell fluorescent microscopic spectrometry. Based on the results in single cell experiments, we went on to examine the effects of NO donor on caffeine-induced vasoconstriction in coronary arteries.

2. Materials and methods

2.1. Isolation of CASMCs

Bovine hearts obtained from a local slaughterhouse were cut into $2 \times 3 \times 1$-cm pieces and sliced into 300-μm-thick tissue sections. Under a dissecting stereomicroscope, coronary arteries were dissected, pooled and stored in ice-cold PSS as we described previously (Li et al., 1997). The dissected coronary arteries (OD 500–1000 μm) were first incubated for 30 min at 37°C with collagenase type II (340 U/ml) (Worthington), elastase (15 U/ml) (Worthington, NJ), dithiothreitol (1 mg/ml) and soybean trypsin inhibitor (1 mg/ml) in HEPES buffer consisting of (mM): NaCl, 119; KCl, 4.7; $CaCl_2$, 0.05; $MgCl_2$, 1; glucose, 5; HEPES, 10 (pH 7.4). The digested tissue was then agitated with a glass pipette to free the arterial 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.

2.2. Measurement of $[Ca^{2+}]_i$ in intact CASMCs

Fura 2-AM (Molecular Probes, Eugene, OR) was used for monitoring intracellular $[Ca^{2+}]_i$ (Tsien, 1989; Inscho et al., 1996). Freshly dissociated CASMCs on glass coverslips were washed with Hanks' buffer (mM): NaCl, 130; KCl, 5.4; HEPES, 20; glucose, 5.5; $CaCl_2$, 1.25; $MgCl_2$, 1; and incubated with 5 μM fura 2-AM at 37°C for 30 min. After washing out the free fura 2-AM, the coverslip was mounted on a perfusion chamber and then on the stage of an inverted microscope (Nikon Diaphot, NY). Cells were incubated with Hanks' buffer for 20 min to allow for complete hydrolysis of intracellular fura 2-AM to fura 2, and then the ratio of fura 2 emissions when excited at 340 and 380 nm was monitored in the perfusion chamber with temperature control system (37°C) by using a fluorescence microscopic spectrometric system (PTI). $[Ca^{2+}]_i$ was calculated from the ratio of F_{340}/F_{380} using the following equation:

$$[Ca^{2+}]_i \text{ (nM)} = K_d(F_0/F_S)(R - R_{min})/(R_{max} - R)$$

where R is the ratio of F_{340}/F_{380}; K_d is a dissociation constant of Ca^{2+}; R_{min} and R_{max} are minimal and maximal ratios of F_{340}/F_{380}, respectively; F_0 and F_S represent the maximal and minimal signal intensities at 380 nm, respectively. The dissociation constant (K_d) was 224 nM. R_{max}, R_{min} and F_0/F_S were calculated from in situ calibrations. R_{max} was calculated from the fluorescence intensity after permeabilizing the cells with 5 mM ionomycin and represented maximal $[Ca^{2+}]_i$. R_{min} was obtained by addition of 2 mM EGTA in permeabilized cells and represented the minimal $[Ca^{2+}]_i$ (Cornfield et al.,1994; Tsien, 1989).

Caffeine-induced intracellular Ca^{2+} release was recorded by addition of caffeine (1 mM) into the bath solution 30 min after incubation of the cells in Ca^{2+}-free solution, and the effects of sodium nitroprusside (SNP), CICR inhibition, guanylyl cyclase inhibition and NO trapping on caffeine-induced Ca^{2+} release were examined by pretreatment of the cells with SNP (500 μM), tetracaine (50 μM), ryanodine (50 μM), ODQ (20 μM) or oxyhemoglobin (OxyHb, 10 μM) for 10 min. Doses of these compounds were chosen based on previous studies showing significant effects on vascular tone or CICR, respectively (Alonso-Galicia et al., 1999; Buus et al., 2000; Csernoch et al., 1999; Li et al., 1998a,b, 2001; Venosa and Hoya, 1999; Weisbrod et al.,1998; Watson et al., 1999; Yang and Steele, 2000)

2.3. Vascular reactivity studies

Vascular reactivity in bovine coronary arteries was determined as previously described (Geiger et al., 2000). Briefly, the epicardial left anterior descending coronary artery was dissected, cleaned of adhering fat and connective tissue, and placed in a Krebs' bicarbonate solution containing (mM): NaCl, 119; KCl, 5; $NaHCO_3$, 24; KH_2PO_4, 1.2; $MgSO_4$, 1.2; glucose, 11; EDTA, 0.02; $CaCl_2$, 3.2. The arteries were cut

into rings (2–3 mm length, 1–2 mm diameter), which were suspended horizontally between two stainless steel hooks in a 6-ml water-jacked organ chamber filled with Krebs' bicarbonate buffer that was bubbled with 95% O_2/5% CO_2 and maintained at 37°C. Isometric tension was measured with force displacement transducer (model FT-03C; Grass Instrument, RI) and recorded on a polygraph (model 7D; Grass Instrument, RI). After an equilibration period of 1.5 h under a resting tension of $3.5 \times g$, the rings were challenged with KCl (60 mmol/l) until reproducible and stable contractions were elicited. Then, the rings were assigned to different protocols as below. Vasoconstrictor response was expressed as percentage of contraction. One hundred percent represents maximum contraction induced by KCl.

2.4. Experimental protocols for vascular reactivity

2.4.1. Effect of SNP and/or tetracaine on caffeine-induced vasoconstriction

Caffeine (1 mM), as a CICR or RYR activator (Kang et al., 1995; Ricard et al., 1997), was added into the bath solution 20 min following the incubation of the arterial rings in Ca^{2+}-free buffer. To examine the effect of NO and/or CICR inhibition on caffeine-induced contraction, the rings were incubated with SNP (500 μM) and/or tetracaine (50 μM) for 20 min, and then caffeine-induced contraction was elicited and monitored.

2.4.2. Effect of SNP and/or ryanodine on caffeine-induced vasoconstriction

Caffeine (1 mM), as a CICR or RYR activator, was added into the bath solution 20 min following the incubation of the arterial rings in Ca^{2+}-free buffer. To examine the effect of NO and/or RYR blockade on caffeine-induced contraction, the rings were incubated with SNP (500 μM) and/or ryanodine (50 μM) for 20 min, and then caffeine-induced contraction was elicited and monitored.

2.4.3. Effect of SNP and/or ODQ on caffeine-induced vasoconstriction

Caffeine (1 mM), as a CICR or RYR activator, was added into the bath solution 20 min following the incubation of the arterial rings in Ca^{2+}-free buffer. To examine the effect of NO and/or guanylyl cyclase inhibition on caffeine-induced contraction, the rings were incubated with SNP (500 μM) and/or ODQ (20 μM) for 20 min, and then caffeine-induced contraction was elicited and monitored.

2.5. Statistics

Data are presented as mean ± S.E.M. The significance of the 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. Student's *t* test was used to evaluate statistical significance of differences between two paired or unpaired observations. $P < .05$ was considered statistically significant.

3. Results

3.1. Effect of SNP on caffeine-induced Ca^{2+} release response in CASMCs

Fig. 1A presents a typical fluorescent microscopic spectrometric recording depicting Ca^{2+} release response induced by caffeine (1 mM) from the SR of CASMCs.

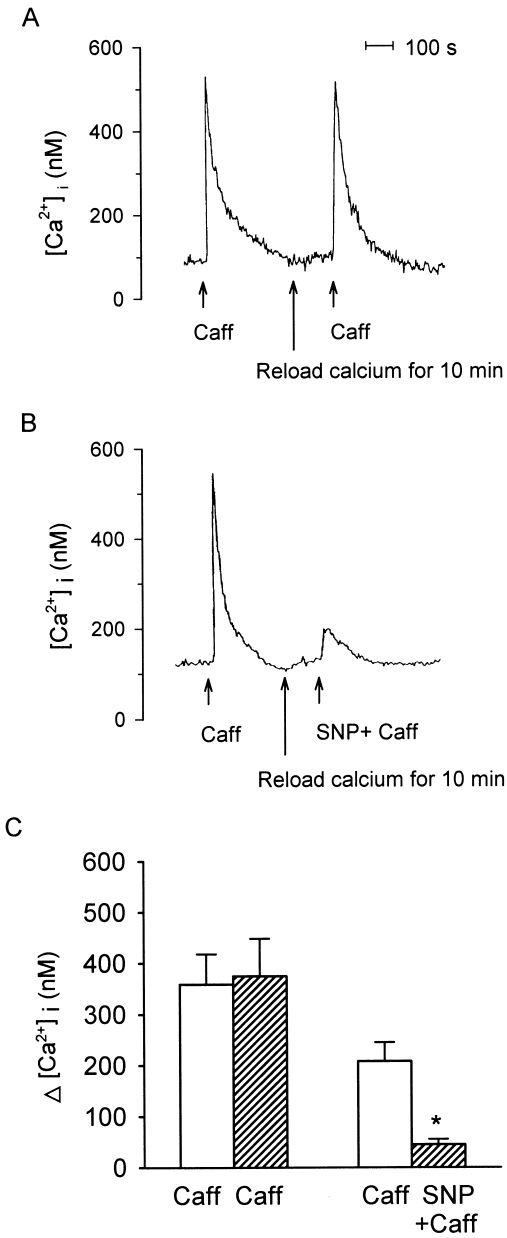

Fig. 1. Effect of SNP on caffeine-induced intracellular Ca^{2+} release in CASMCs. (A) Representative recordings of intracellular Ca^{2+} release in response to caffeine (1 mM). (B) Representative recordings depicting inhibition of caffeine-induced Ca^{2+} release by SNP (500 μM). (C) Summarized data showing caffeine-induced intracellular Ca^{2+} release in the absence or presence of SNP ($n = 16$ cells from eight bovine hearts). * Indicates significant difference ($P < .05$) compared with the values in the absence of SNP.

Caffeine produced a large rapid increase in $[Ca^{2+}]_i$ from baseline of 104 to 471 nM. This caffeine-induced Ca^{2+} release response was observed repeatedly when the cells were washed and recovered with Hanks' buffer to reload Ca^{2+} into the SR. When the cells were pretreated with SNP (500 μM) after reloading Ca^{2+} into the SR, caffeine-induced Ca^{2+} release was substantially blocked (Fig. 1B). As summarized in Fig. 3C, in the absence of tetracaine, both caffeine stimulations repeatedly produced Ca^{2+} release (358.4 and 374.8 nM), and SNP inhibited caffeine-induced Ca^{2+} release by 80% (from 207.9 to 44.7 nM; $n=16$).

3.2. Reversal by OxyHb of SNP-mediated inhibitory effect on caffeine-induced Ca^{2+} release response in CASMCs

To determine whether the effect of SNP on CICR is induced by NO, rather than its other end products, we examined the influence of NO-trapping reagent, OxyHb, on SNP-induced inhibition on Ca^{2+} release response to caffeine. These results are presented in Fig. 2. Caffeine produced a large rapid increase in $[Ca^{2+}]_i$. When the cells were pretreated with SNP (500 μM) after reloading Ca^{2+} into the SR, caffeine-induced Ca^{2+} release was substantially blocked ($\Delta[Ca^{2+}]_i$ from 249 of control to 78 nM). In the presence of OxyHb, SNP did not significantly reduce caffeine-induced Ca^{2+} release ($\Delta[Ca^{2+}]_i$ from 249 to 212 nM), suggesting that the effect of SNP is dependent on the production of NO ($n=7$).

In addition, we performed experiments to determine the effect of guanylyl cyclase inhibition on caffeine-induced Ca^{2+} release. A specific guanylyl inhibitor, ODQ, was not found to have effect on caffeine-induced Ca^{2+} release. Increases in $[Ca^{2+}]_i$ in response to caffeine were 366.5 ± 34.7 and 332.4 ± 34.5 nM, respectively, in the absence and presence of ODQ. Moreover, ODQ had no significant effect on SNP-induced inhibition of caffeine-induced Ca^{2+} release response ($n=6$).

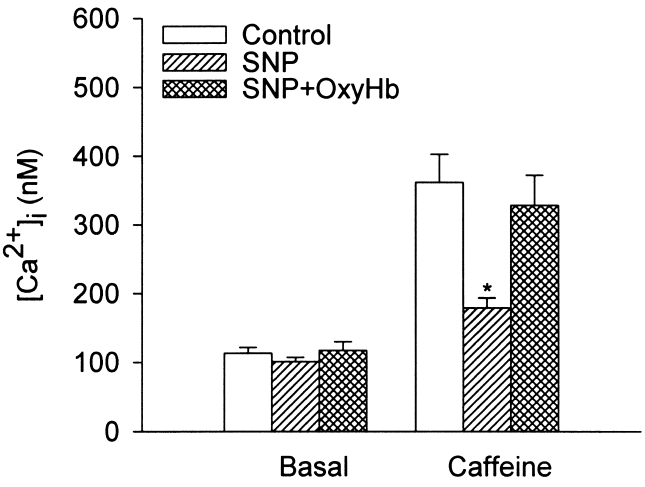

Fig. 2. Reversal by OxyHb of SNP inhibition on caffeine-induced intracellular Ca^{2+} release in CASMCs. The effects of SNP on caffeine-induced intracellular Ca^{2+} release were examined in the absence or presence of OxyHb (HbO) ($n=7$ cells from seven bovine hearts). * Indicates significant difference ($P<.05$) compared with control caffeine Ca^{2+} release.

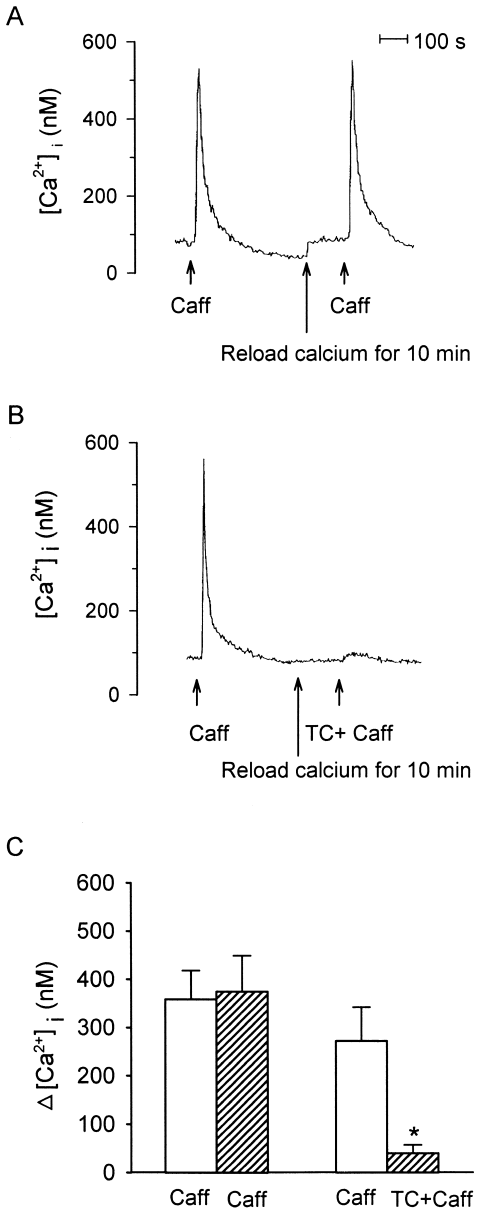

Fig. 3. Effect of tetracaine on caffeine-induced intracellular Ca^{2+} release in CASMCs. (A) Representative recordings of intracellular Ca^{2+} release in response to caffeine (1 mM). (B) Representative recordings depicting inhibition of caffeine-induced Ca^{2+} release by tetracaine (50 μM). (C) Summarized data showing caffeine-induced intracellular Ca^{2+} release in the absence or presence of tetracaine ($n=16$ cells from eight bovine hearts). * Indicates significant difference ($P<.05$) compared with the values in the absence of tetracaine.

3.3. Effect of tetracaine on caffeine-induced Ca^{2+} release response in CASMCs

Tetracaine is a specific inhibitor of CICR. In this group of experiments, tetracaine was used to confirm that caffeine-

induced Ca^{2+} release is through CICR (Kang et al., 1995; Ricard et al., 1997). The results of these experiments are presented in Fig. 3. Caffeine-induced Ca^{2+} release response in the absence (Panel A) and presence (Panel B) of tetracaine was monitored. Pretreatment of cells with tetracaine (50 μM) significantly decreased caffeine-induced Ca^{2+} release from SR (Fig. 3B). Fig. 3C summarizes the effects of tetracaine on caffeine-induced Ca^{2+} release. In the absence of tetracaine, caffeine repeatedly produced Ca^{2+} release (358.4 and 374.8 nM). In the presence of tetracaine, caffeine-induced Ca^{2+} release was decreased by 86% (from 271.7 to 39.4 nM) ($n = 16$).

3.4. Effect of ryanodine on caffeine-induced Ca^{2+} release response in CASMCs

Caffeine has been reported to activate RYRs in a variety of mammalian cells (Kang et al., 1995; Ricard et al., 1997). However, there is no direct evidence showing that RYRs mediate the caffeine effect in coronary arterial smooth muscle. This protocol was designed to determine the role of RYRs in mediating caffeine-induced Ca^{2+} release. The results of these experiments are presented in Fig. 4. Caffeine-induced Ca^{2+} release responses in the absence and presence of ryanodine were monitored. Fig. 4A presents a typical fluorescence microscopic recording showing that pretreatment of cells with ryanodine (50 μM) significantly decreased caffeine-induced Ca^{2+} release from SR. Fig. 4B summarizes the effects of ryanodine on caffeine-induced Ca^{2+} release ($n = 14$). In the presence of ryanodine, caffeine-induced Ca^{2+} release was decreased by 74% (from 379.2 to 97.9 nM).

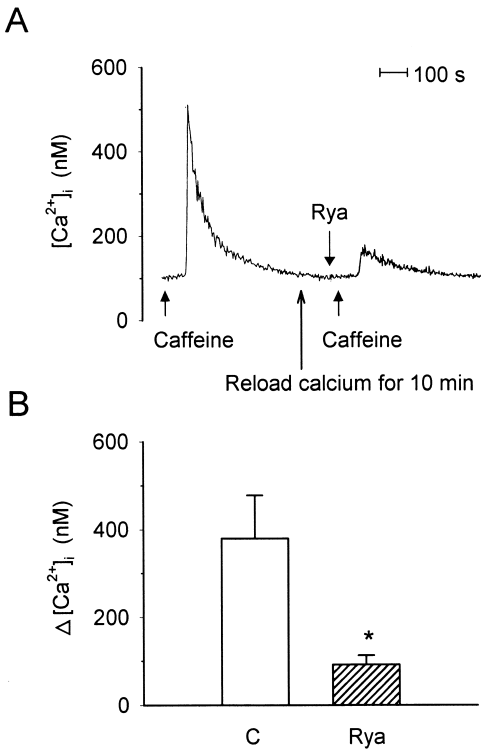

Fig. 4. Effect of ryanodine on caffeine-induced intracellular Ca^{2+} release in CASMCs. (A) Representative recordings depicting inhibition of caffeine-induced Ca^{2+} release by ryanodine (50 μM). (B) Summarized data showing caffeine-induced intracellular Ca^{2+} release in the absence or presence of ryanodine ($n = 14$ cells from seven bovine hearts). * Indicates significant difference ($P < .05$) compared with the values in the absence of ryanodine.

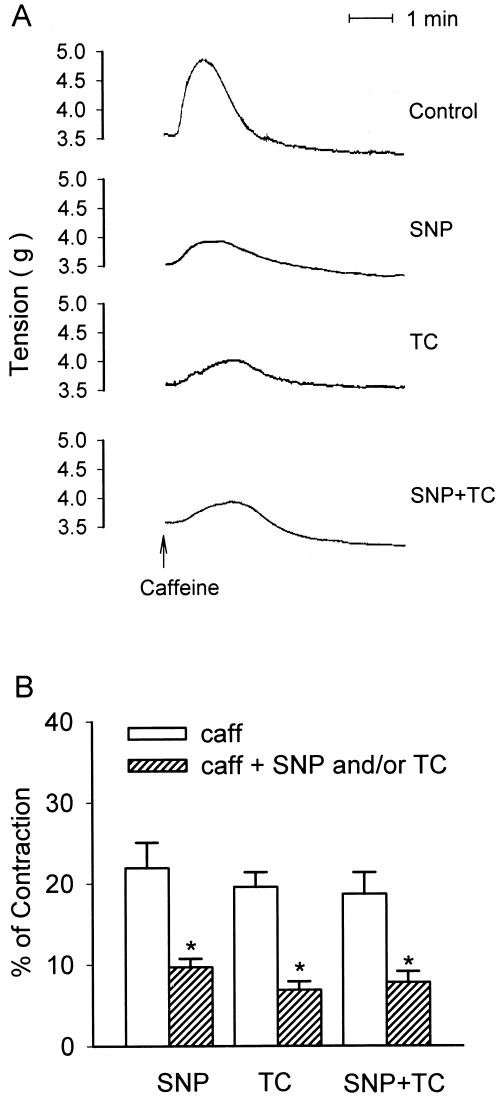

Fig. 5. Effect of SNP and/or tetracaine on caffeine-induced vasoconstriction. (A) Typical recording of coronary arterial reactivity to caffeine in the absence or presence of SNP and/or tetracaine. (B) Summarized data showing inhibition effect of SNP and/or tetracaine on caffeine-induced vasoconstriction ($n = 14$ arterial rings from 14 bovine hearts). * Significant difference from control (caffeine alone).

3.5. Effect of SNP and/or tetracaine on caffeine-induced vasoconstriction

As shown in Fig. 5, caffeine induced a transient vasoconstriction (Panel A). The maximal vasoconstriction reached 3 min following administration of caffeine. This caffeine-induced temporary vasoconstriction represents

Ca^{2+} activation of smooth muscle cells. Pretreatment of the vessels with SNP and/or tetracaine significantly inhibited caffeine-induced vasoconstriction. Panel B summarizes the results of these experiments ($n = 14$). SNP and tetracaine, alone or in combination, attenuated caffeine-induced vasoconstriction to a similar extent.

3.6. Effect of SNP and/or ryanodine on caffeine-induced vasoconstriction

As shown in Fig. 6, similar to the effect presented in Fig. 5, caffeine induced a transient vasoconstriction (Panel A). The maximal vasoconstriction reached 3 min following administration of caffeine. Pretreatment of the artery rings with SNP or ryanodine significantly inhibited caffeine-induced vasoconstriction. In the presence of ryanodine, SNP did not have further effect on caffeine-induced vasoconstriction. Panel B summarizes the results of these experiments ($n = 10$). SNP and ryanodine, alone or in combination, attenuated caffeine-induced vasoconstriction to a similar extent.

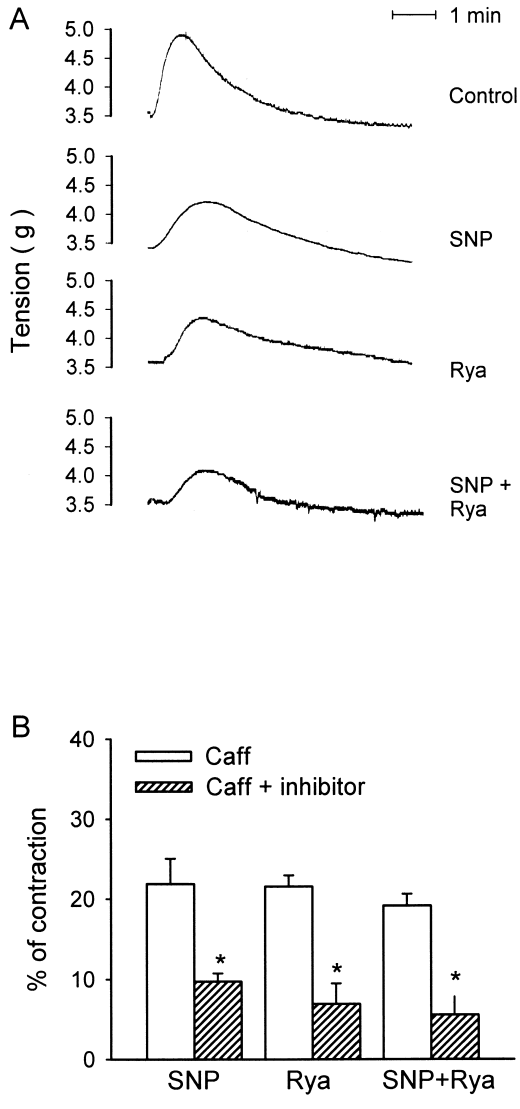

Fig. 6. Effect of SNP and/or ryanodine on caffeine-induced vasoconstriction. (A) Typical recording of coronary arterial reactivity to caffeine in the absence or presence of SNP and/or ryanodine. (B) Summarized data showing inhibition effect of SNP and/or ryanodine on caffeine-induced vasoconstriction ($n = 10$ arterial rings from six bovine hearts). * Significant difference from control (caffeine alone).

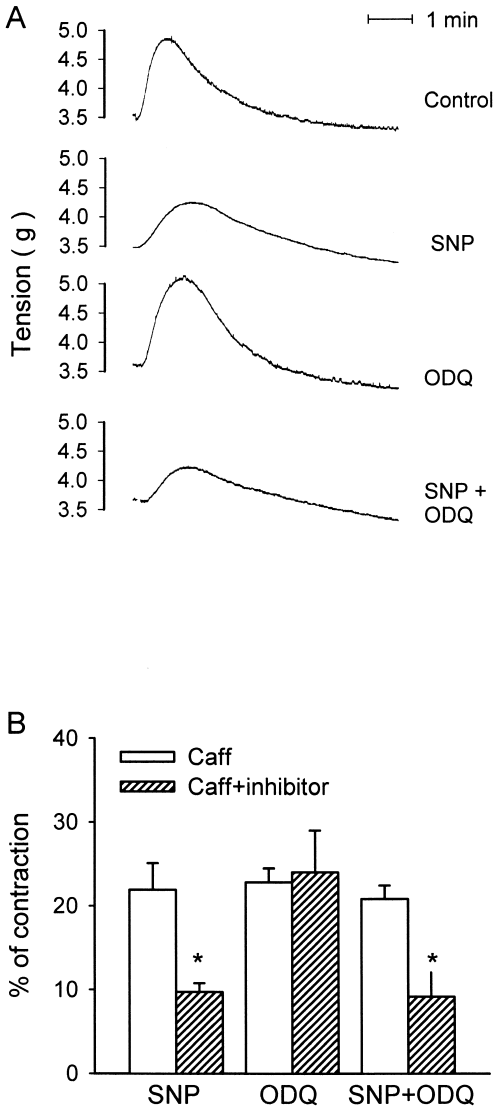

Fig. 7. Effect of SNP and/or ODQ on caffeine-induced vasoconstriction. (A) Typical recording of coronary arterial reactivity to caffeine in the absence or presence of SNP and/or ODQ. (B) Summarized data showing inhibition effect of SNP and/or ODQ on caffeine-induced vasoconstriction ($n = 10$ arterial rings from seven bovine hearts). * Significant difference from control (caffeine alone).

3.7. Effect of SNP and/or ODQ on caffeine-induced vasoconstriction

In different vascular beds, NO has been reproted to dilate vessels through cGMP signaling pathway. It is unknown whether this cGMP-mediated mechanism participates in caffeine-induced vasomotor response in coronary vascular

bed. In this group of experiments, we addressed this issue. As shown in Fig. 7, caffeine induced a transient vasoconstriction (Panel A). The maximal vasoconstriction reached 3 min following administration of caffeine. Pretreatment of the vessels with SNP significantly inhibited caffeine-induced vasoconstriction, but guanylyl cyclase inhibitor, ODQ, had no effect on caffeine-induced vasoconstriction. Panel B summarizes the results of these experiments ($n = 10$). SNP attenuated caffeine-induced vasoconstriction in coronary arteries, but ODQ did not alter the vasoconstrictor response to caffeine in these arteries. In the presence of ODQ, SNP-induced blockade of caffeine vasoconstriction was not changed.

4. Discussion

In the present study, we detected CICR in freshly isolated bovine CASMCs. These cells were first incubated with Ca^{2+}-free bath solution, and then caffeine as a CICR activator was added into the bath solution to activate Ca^{2+} release response from intracellular stores. Using fluorescent microscopic spectrometry, an intracellular Ca^{2+} transient response with a rapid peak increase was observed, and caffeine-induced Ca^{2+} increase can be repeatedly activated after the cells were reloaded with Hanks' buffer containing 1.25 mM $CaCl_2$. This caffeine Ca^{2+} response represents a typical Ca^{2+} release response from intracellular Ca^{2+} stores, since there is no Ca^{2+} in the extracellular solution. Previous studies have demonstrated that caffeine produces Ca^{2+} release from the sarcoplasmic reticulum through RYR in different cells including vascular smooth muscle cells (Kang et al., 1995; Ricard et al., 1997).

To determine the effect of NO on CICR, the present study examined caffeine-induced Ca^{2+} release response in the absence and presence of SNP in CASMCs. We found that in the presence of SNP, caffeine-induced Ca^{2+} release was significantly blocked, suggesting that NO may inhibit CICR in CASMCs. Since SNP has been reported to release other products such as superoxide, however, there is a concern regarding whether the effect of SNP on CICR is directly induced by NO. To address this issue, we examined the effect of NO-trapping reagent, OxyHb (Li et al., 1998a,b) on SNP-induced inhibition of caffeine response in CASMCs. In the presence of OxyHb, SNP did not inhibit caffeine-induced Ca^{2+} release response. This reversal of SNP inhibition on caffeine-induced Ca^{2+} release by OxyHb trapping of NO indicates that SNP attenuates caffeine response through NO production, rather than other end products.

To further explore the possibility that CICR plays a role in mediating the action of NO, we examined the effects of CICR inhibition or RYR blockade on caffeine-induced Ca^{2+} release. Tetracaine is a specific inhibitor, which has been reported to inhibit CICR in various tissues or cells (Gyorke and Palade, 1992; Overend et al., 1998; Venosa and Hoya, 1999). Using this CICR inhibitor, we found that caffeine-induced Ca^{2+} release in CASMCs was markedly attenuated. However, in the presence of tetracaine, SNP did not have further effect on caffeine-induced Ca^{2+} release. It seems that SNP and tetracaine share the same mechanism to inhibit caffeine-induced Ca^{2+} release, suggesting that NO may be a CICR inhibitor. Since previous studies have demonstrated that CICR is primarily mediated by RYRs (Kang et al., 1995; Ricard et al., 1997), we wondered whether the effect of SNP on caffeine-induced Ca^{2+} release is attributable to the activity of RYRs. To answer this question, we tested the effect of ryanodine at a high concentration, a concentration that blocks RYRs, on SNP inhibition of caffeine-induced Ca^{2+} release. As expected, ryanodine substantially blocked caffeine-induced Ca^{2+} release, suggesting that the action of caffeine is associated with activation of RYRs in these CASMCs. In the presence of ryanodine, SNP did not have any effect on caffeine response. These results support the view that NO may decrease intracellular Ca^{2+} concentrations by inhibition of RYR-mediated CICR.

Although the present study demonstrated that NO inhibits CICR in bovine CASMCs, we cannot exclude the possible actions of NO on Ca^{2+} influx in the vascular smooth muscle cells from other vascular beds. In fact, SNP was found to inactivate voltage-gated Ca^{2+} channels, thereby decreasing $[Ca^{2+}]_i$ in cultured vascular smooth muscle cells (Blatter and Wier, 1994). However, there is accumulating evidence indicating that NO decreases $[Ca^{2+}]_i$ primarily through inhibition of intracellular Ca^{2+} mobilization in different smooth muscle cells (Kannan et al., 1997). It has been demonstrated that endothelium-dependent vasodilators and NO donors decreased $[Ca^{2+}]_i$ through inhibition of intracellular Ca^{2+} release from the SR in pulmonary, cerebral, aortic and mesenteric arterial smooth muscle cells (Meszaros et al., 1996; Tomioka et al., 1999; Yuan et al., 1997). This NO-sensitive Ca^{2+} release could be either IP_3-dependent or IP_3-independent, which depends upon the vessels from different vascular beds. To our knowledge, it remains unknown whether this effect of NO on IP_3-independent Ca^{2+} release is attributed to the action on CICR through RYR. The present findings indicate that NO may directly inhibit CICR, decrease $[Ca^{2+}]_i$ and consequently result in vasodilation in CASMCs, which probably represents one of IP_3-independent mechanism for the action of NO to decrease $[Ca^{2+}]_i$. This NO effect on CICR has been confirmed in other tissue preparations such as skeletal muscle SR, cardiac muscle SR, tracheal smooth muscle cells and reconstituted planar lipid bilayer (Kannan et al., 1997; Meszaros et al., 1996; Zahradnikova et al., 1997). Taken together, our results imply that CICR may be an important target for the action of NO on coronary vascular smooth muscle cells.

To determine the functional significance of NO inhibition of CICR in CASMCs, we examined the vascular reactivity of these arteries by isometric tension recording. Coronary arterial rings were preincubated in Ca^{2+}-free isotonic solu-

tion and caffeine was added to induce vasoconstriction. Caffeine was found to produce a temporary contraction in bovine coronary arteries exposed to a Ca^{2+}-free solution. Given that caffeine induces Ca^{2+} release in CASMCs as described above, these results suggest that caffeine may primarily produce a Ca^{2+} activation of coronary contraction and that a long-term sustained vasoconstriction in these arteries may depend upon other mechanisms. This caffeine-induced Ca^{2+} activation of coronary arteries is due to intracellular Ca^{2+} release or enhancement of CICR (Kang et al., 1995; Ricard et al., 1997).

Consistent with the effects of SNP on caffeine-induced Ca^{2+} release in CASMCs, SNP was found to significantly reduce caffeine-induced coronary vasoconstriction. These SNP effects on caffeine-induced vasoconstriction were demonstrated similar to those of CICR inhibition by tetracaine or RYR blockade by a high concentration of ryanodine. In the presence of either tetracaine or ryanodine, SNP had no further effect on this caffeine-induced vasoconstriction. Moreover, we demonstrated that guanylyl cyclase inhibition by ODQ altered neither caffeine-induced vasoconstriction nor SNP inhibition on this caffeine response. All these results indicate that NO inhibits CICR and thereby decreases Ca^{2+} activation in coronary arteries, which may be an important mechanism mediating vasodilator effect of NO in coronary circulation.

5. Summary

The present study demonstrates that CICR is functioning in CASMCs and participates in the Ca^{2+} activation in the coronary arteries. NO donor, SNP, inhibits CICR and thereby blocks CICR-mediated coronary arterial contraction. Our results indicate that inhibition of CICR may contribute to the vasodilator action of NO in coronary circulation.

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