Role of store-operated Ca\textsuperscript{2+} entry in adenosine-induced vasodilatation of rat small mesenteric artery

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Wang S, Zhang Y, Wier WG, Yu X, Zhao M, Hu H, Sun L, He X, Wang Y, Wang B, Zang W. Role of store-operated Ca\textsuperscript{2+} entry in adenosine-induced vasodilatation of rat small mesenteric artery. Am J Physiol Heart Circ Physiol 297: H347–H354, 2009. First published May 8, 2009; doi:10.1152/ajpheart.00060.2009.—Store-operated Ca\textsuperscript{2+} entry (SOCE) has recently been proposed to contribute to Ca\textsuperscript{2+} influx in vascular smooth muscle cells (VSMCs). Adenosine is known for its protective role against hypoxia and ischemia by increasing nutrient and oxygen supply through vasodilatation. This study was designed to examine the hypothesis that SOCE have a functional role in adenosine-induced vasodilatation. Small mesenteric arteries and mesenteric VSMCs were obtained from rats. Isometric tensions of isolated artery rings were measured by a sensitive myograph system. Laser-scanning confocal microscopy was used to determine the intracellular Ca\textsuperscript{2+} concentration of fluo-3-loaded VSMCs. Adenosine (0.1–100 μM) relaxed artery rings that were precontracted by phenylephrine in a concentration-dependent manner. In cultured mesenteric VSMCs, passive store depletion by thapsigargin and active store depletion by phenylephrine both induced Ca\textsuperscript{2+} influx due to SOCE. Adenosine inhibited SOCE-mediated increases in cytosolic Ca\textsuperscript{2+} levels evoked by the emptying of the stores. In isolated artery rings, adenosine inhibited SOCE-induced contractions due to store depletion. A\textsubscript{2a} receptor antagonism with SCH-58261 and adenylyl cyclase inhibition with SQ-22536 largely attenuated adenosine responses. The cAMP analog 8-bromo-cAMP mimicked the effects of adenosine on SOCE. Our results indicate a novel mechanism of vasodilatation by adenosine that involves regulation of SOCE through the cAMP signaling pathway due to activation of adenosine A\textsubscript{2a} receptors.

Materials and Methods

Solutions, drugs, and chemicals. Krebs solution contained the following (in mM): 119 NaCl, 4.7 KCl, 2.5 CaCl\textsubscript{2}, 1 MgCl\textsubscript{2}, 25 NaHCO\textsubscript{3}, 1.2 KH\textsubscript{2}PO\textsubscript{4}, and 11 d-glucose. Ca\textsuperscript{2+}-free Krebs solution was prepared by omitting CaCl\textsubscript{2} and adding 0.5 mM EGTA instead. Fluo-3-AM was purchased from Molecular Probes (Leiden, The Netherlands). Collagenase was purchased from Worthington (Lakewood, NJ). All other reagents were obtained from Sigma (St. Louis, MO).

Artery ring preparation. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication No. 85-23, Revised 1996) and approved by the Ethical Committee of Xi’an Jiaotong University. Male Sprague-Dawley rats weighing 180–230 g were killed by cervical dislocation under pentobarbital sodium anesthesia. Small mesenteric resistance arteries were gently isolated and immersed immediately in cold oxygenated Krebs solution. The arteries were carefully cleaned of fat and connective tissue and then cut into 1- to 2-mm-length rings. In most preparations, the endothelium was mechanically removed by rubbing the luminal surface with a stainless steel wire. The removal of the endothelium was confirmed by a lack of relaxation response to 1 μM acetylcholine.

Isometric tension measurement. Isometric tension was measured as previously described (36). Briefly, the artery rings (internal diameter ~150 μm) were mounted in a Multi Myograph System (Danish Myo Technology, Aarhus, Denmark), and changes in isometric tension for activation of SOCCs (8, 20, 29). There is accumulating evidence suggesting that canonical transient receptor potential (TRPC) proteins are important components of SOCCs (21, 40). An antibody against TRPC5 protein inhibits Ca\textsuperscript{2+} influx via SOCCs, providing direct evidence that SOCCs play a functional role in VSMCs (37). However, considerably less is known about the physiological properties of SOCCs in smooth muscle cells from arterioles and small resistance arteries. Many of these arteries, such as the mesenteric artery, are critically involved in regulating local blood flow and blood pressure.

Adenosine produces potent vasodilatory effects in the majority of vasculatures, including the mesenteric arteries. Despite a growing understanding of the mechanisms by which adenosine is formed in the vasculature, the precise molecular mechanisms through which adenosine relaxes vascular smooth muscle are not fully understood. Cytoplasmic Ca\textsuperscript{2+} is a primary determinant of vascular smooth muscle contraction. There is now considerable evidence supporting the ability of adenosine to interfere with Ca\textsuperscript{2+} mobilization in several kinds of cells, including VSMCs (12, 26). However, there is a paucity of information that relates the intracellular Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]) to regulation of adenosine to its vasodilatory effect. We assessed the hypothesis that functional modulation of SOCE by adenosine contributes to regulation of adenosine-induced vasodilatation in isolated mesenteric artery.

IN NONEXCITABLE CELLS, the release of Ca\textsuperscript{2+} from intracellular stores is often followed by a sustained phase of Ca\textsuperscript{2+} entry from the extracellular space. The activation of Ca\textsuperscript{2+} entry dependent on, and subsequent to, the depletion of intracellular Ca\textsuperscript{2+} stores was originally termed “capacitative Ca\textsuperscript{2+} entry.” Today, it is most often referred to as store-operated Ca\textsuperscript{2+} entry (SOCE) (27, 30). In excitable cells, such as vascular smooth muscle cells (VSMCs), Ca\textsuperscript{2+} entry has long been considered to occur through either voltage-operated Ca\textsuperscript{2+} channels (VOCCs) or receptor-operated Ca\textsuperscript{2+} channels (ROCCs) (5). Our previous study and recent evidence indicate that store-operated Ca\textsuperscript{2+} channels (SOCCs) play an important role in replenishing Ca\textsuperscript{2+} stores. Furthermore, SOCCs contribute to Ca\textsuperscript{2+} entry in VSMCs and may regulate vascular tone (4, 28, 41). It is clear that the signal for activation of SOCCs is generated by the depleted store itself. Recently, two essential proteins, STIM1 and Orai1, were identified to be essential for activation of SOCCs (8, 20, 29). There is accumulating evidence suggesting that canonical transient receptor potential (TRPC) proteins are important components of SOCCs (21, 40). An antibody against TRPC5 protein inhibits Ca\textsuperscript{2+} influx via SOCCs, providing direct evidence that SOCCs play a functional role in VSMCs (37). However, considerably less is known about the physiological properties of SOCCs in smooth muscle cells from arterioles and small resistance arteries. Many of these arteries, such as the mesenteric artery, are critically involved in regulating local blood flow and blood pressure.

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were continually recorded. Two tungsten wires were passed through the lumen of the ring. One of the wires was fixed to a micrometer for length adjustments, and the other was connected to a force transducer for isometric force measurements. Each ring was bathed in an organ chamber containing Krebs solution, maintained at 37°C, and continuously bubbled with 95% O2 plus 5% CO2 (pH 7.4). After being mounted with a previously determined optimal resting tension of 4 mN for 60 min, each ring was first contracted by 10 μM phenylephrine (PE) and subsequently challenged with 1 μM acetylcholine to confirm the vessel’s contractility and the integrity of its endothelium. Then the rings were washed to restore tension to baseline level and allowed to stabilize for 60 min.

Cell culture. Mesenteric VSMCs were prepared using a modified version of previously described procedures (16). After removing the endothelium, small (1-cm²) mesenteric artery segments (internal diameter, ~150 μm) were incubated in Ca²⁺-free Krebs solution containing 1 mg/ml papain, 1 mg/ml BSA, and 1 mg/ml diithioerythritol at 37°C for 4–6 min. The tissues were then washed several times with ice-cold solution to remove any enzymes. Next, they were incubated in a digestive solution containing 1 mg/ml collagenase, 1 mg/ml BSA, and 1 mg/ml diithioerythritol at 37°C for 4–6 min. The tissues were then washed several times with ice-cold solution to remove any enzymes. Next, they were incubated in a digestive solution containing 1 mg/ml collagenase, 1 mg/ml BSA, and 1 mg/ml diithioerythritol at 37°C for 4–6 min. The tissues were then washed several times with ice-cold solution to remove any enzymes. Next, they were incubated in a digestive solution containing 1 mg/ml collagenase, 1 mg/ml BSA, and 1 mg/ml diithioerythritol at 37°C for 4–6 min. The tissues were then washed several times with ice-cold solution to remove any enzymes.

Intracellular Ca²⁺ measurements. Ca²⁺ concentrations in VSMCs were monitored using fluo 3-AM, a fluorescent Ca²⁺ indicator. Briefly, cells on cover slips were incubated in the dark for 60 min at 25°C in Krebs solution containing 5 μM fluo 3-AM. The cover slips were washed in dye-free Krebs solution to remove extracellular fluo 3. [Ca²⁺]i concentration in arbitrary cell was monitored by a laser confocal scanning microscopy system (TCS-SP2; Leica, Heidelberg, Germany) with an excitation of 488 nm and an emission of 530 nm. Images were obtained at a rate of one image per 2 s and were analyzed with Leica confocal software.

**RESULTS**

Relaxant effect of adenosine. The trace in Fig. 1A shows that adenosine (0.1–100 μM) caused a concentration-dependent relaxation of PE (1 μM)-contracted endothelium-intact rings. The pEC₅₀ value was 4.79 ± 0.07, and the maximal response was 95.4 ± 4.8%. This relaxation was unaltered in endothelium-denuded rings (Fig. 1A, right). Adenosine (0.1–100 μM) failed to relax the 60-mM KCl-induced contraction that was abolished by 0.3 μM nifedipine (L-type VOCCs blocker) (Fig. 1B). As shown in Fig. 1C, nifedipine (0.3 μM) caused partial and sustained reduction of PE-induced contraction. Addition of adenosine (10 μM) completely inhibited the remaining nifedipine-insensitive contraction.

[Ca²⁺]i response to SOCE after store depletion. Recent evidence indicates that SOCE contributes to Ca²⁺ entry in vascular smooth muscle and plays a role in the maintenance of vascular tone (28). Therefore, we first determined whether rat mesenteric VSMCs contain a functional SOCE pathway. In VSMCs loaded with fluo 3, and in Ca²⁺-free solution, application of thapsigargin (TG) induced a slow transient rise in [Ca²⁺]i. Considering that store depletion may activate VOCCs by membrane depolarization, SOCE was studied in the presence of nifedipine to block VOCC-mediated Ca²⁺ influx. As shown in Fig. 2A, subsequent restoration of extracellular Ca²⁺ (2.5 mM) in the presence of 0.3 μM nifedipine was followed by a large increase in [Ca²⁺]i. The large TG-induced [Ca²⁺]i

![Fig. 1. Relaxation effect of adenosine in isolated rat mesenteric artery. A: tracing on left shows the tension changes in response to the cumulative addition of adenosine (Ado) in phenylephrine (PE) (1 μM) contracted artery rings. Panel on right shows the dose-response curves for adenosine-induced relaxation on endothelium-intact and endothelium-free arteries (n = 16). B: representative tracing and summarized data of 60 mM KCl-induced contraction in the presence of nifedipine (Nif, 0.3 μM) or adenosine (n = 8). Con, control. C: adenosine (10 μM) relaxed PE-induced contraction in the presence of nifedipine (n = 12). All of the summarized data are expressed as means, with error bars representing SE. *P < 0.05 vs. control.](http://ajpheart.physiology.org/Downloadedfrom)
rise in the presence of nifedipine was 68 ± 9.7% of the \([\text{Ca}^{2+}]_i\) rise in the absence of nifedipine. The store depletion-induced \([\text{Ca}^{2+}]_i\) rise was 85.4 ± 7.9% inhibited by 75 \(\mu\text{M}\) 2-aminoethoxydiphenyl borate (2-APB, a nonselective SOCC inhibitor) and 60.2 ± 6.8% inhibited by 1 mM Ni\(^{2+}\) (a nonspecific cation channel blocker). As shown in Fig. 2B, PE (1 \(\mu\text{M}\)) elicited a small and transient increase in \([\text{Ca}^{2+}]_i\) in mesenteric VSMCs in Ca\(^{2+}\)-free solution. The \([\text{Ca}^{2+}]_i\) response was absent when PE was administered for the second time, suggesting that the intracellular Ca\(^{2+}\) stores were empty. After washout, addition of Ca\(^{2+}\) in the presence of nifedipine induced a large increase in \([\text{Ca}^{2+}]_i\) with a long-lasting plateau. The summarized data (Fig. 2B, right) illustrate that 75 \(\mu\text{M}\) 2-APB and 1 mM Ni\(^{2+}\) caused 84.3 ± 9.2 and 55 ± 7.2% reduction in the \([\text{Ca}^{2+}]_i\) rise induced by PE-mediated store depletion, respectively.

Contractile response to SOCE after store depletion. We next investigated whether the Ca\(^{2+}\) influx via SOCCs is involved in smooth muscle contractions. As shown in Fig. 3A, PE (1 \(\mu\text{M}\)) elicited a transient contraction in isolated mesenteric artery rings in Ca\(^{2+}\)-free solution; this transient contraction had an

Fig. 2. Intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) response to store-operated Ca\(^{2+}\) entry (SOCE) after store depletion in fluo 3-loaded vascular smooth muscle cells (VSMCs). A: representative tracing showing the inhibitory effect of 75 \(\mu\text{M}\) 2-aminoethoxydiphenyl borate (2-APB) on the [Ca\(^{2+}\)]\(_i\) response to 10 \(\mu\text{M}\) thapsigargin (TG) in the absence (0Ca\(^{2+}\)) or presence (2.5 mM Ca\(^{2+}\)) of extracellular Ca\(^{2+}\) and nifedipine (0.3 \(\mu\text{M}\)). B: representative tracing showing 1 \(\mu\text{M}\) PE-induced transient [Ca\(^{2+}\)]\(_i\) rises in Ca\(^{2+}\)-free conditions. Readministering Ca\(^{2+}\) in the presence of 0.3 \(\mu\text{M}\) nifedipine increased [Ca\(^{2+}\)]\(_i\). Panel on right shows summarized data of the SOCE-induced change of [Ca\(^{2+}\)]\(_i\) fluorescence in the presence of 75 \(\mu\text{M}\) 2-APB or 1 mM Ni\(^{2+}\). All summarized data are expressed as means (\(n = 8–30\)) with error bars representing SE. *\(P < 0.05\) vs. control.

Fig. 3. Contractile response to SOCE after store depletion in isolated artery rings. A: representative tracing showing 1 \(\mu\text{M}\) PE-induced contractions in Ca\(^{2+}\)-free solution. Readministering Ca\(^{2+}\) in the presence of 0.3 \(\mu\text{M}\) nifedipine resulted in tonic contraction. Panel on right shows contractions when the rings were challenged with PE (\(n = 16\)) the 1st, 2nd, and 3rd times. *\(P < 0.05\) vs. 1st. B: panel on left compares SOCE and PE-induced contractions in the presence and absence of Ca\(^{2+}\) (\(n = 12\)). *\(P < 0.05\) vs. 2.5 Ca. Panel on right shows SOCE-induced contractions in the presence of 75 \(\mu\text{M}\) 2-APB or 1 mM Ni\(^{2+}\) (\(n = 12\)). *\(P < 0.05\) vs. control. C: representative tracing and summarized data showing tension changes in response to 2-APB on PE-contracted rings (\(n = 16\)). *\(P < 0.05\) vs. control. All summarized data are expressed as means, with error bars representing SE.
amplitude of 20.1 ± 6.4% of the contraction that occurs in the presence of extracellular Ca2+. The contractile effect was absent when PE was administered for the third time (Fig. 3A, right), indicating that the intracellular Ca2+ stores had been emptied. The subsequent addition of Ca2+ in presence of 0.3 µM nifedipine led to a contraction with an amplitude of 53 ± 8.6% of PE-induced contraction in normal Krebs solution (Fig. 3B, left). The contraction induced by Ca2+ restoration was inhibited by 87.2 ± 9.3 and 60.4 ± 8.1% when treated with 2-APB (75 µM) and Ni2+ (1 mM), respectively (Fig. 3B, right). Furthermore, the PE-induced contraction in normal Krebs solution was dose-dependently inhibited by 2-APB (Fig. 3C).

Role of SOCE in adenosine-induced vasorelaxation. We next investigated whether adenosine-induced relaxation was mediated through SOCE inhibition. The effect of adenosine on [Ca2+]i responses to SOCE are shown in Fig. 4, A and B. After Ca2+ stores were depleted in mesenteric VSMCs by TG, readministering Ca2+ in the presence of nifedipine resulted in a [Ca2+]i rise via SOCE. The addition of 10 µM adenosine during the plateau phase of this [Ca2+]i rise immediately decreased the [Ca2+]i by 75.4 ± 8.6% (Fig. 4A). Adenosine also caused a 77.3 ± 9.2% depression on SOCE-induced [Ca2+]i rise due to PE-mediated store depletion (Fig. 4B). As shown in Fig. 4C, readministering Ca2+ after complete Ca2+ store depletion by PE resulted in a tonic contraction. Adenosine (10 µM) produced an 80.6 ± 9.2% decrease in this SOCE-induced tension.

Effect of adenosine receptor blockade on adenosine-induced SOCE reduction. Next, we tested which adenosine receptor subtypes were involved in SOCE regulation. As shown in Fig. 5A, in endothelium-denuded rings, the relaxant effect of adenosine (10 µM) on SOCE-induced contraction was markedly inhibited in the presence of the adenosine A2A receptor antagonist SCH-58261 (100 nM). In contrast, neither the adenosine A2B receptor antagonist alloxazine (1 µM) nor the A1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; 1 µM) altered adenosine-induced relaxant response on SOCE-contracted rings (Fig. 5, B and C). The summarized data in Fig. 5D indicate that the maximum tensions induced by PE were decreased by 74.5 ± 7.2, 68.9 ± 7.9, and 79.4 ± 6.9% in adenosine (without antagonists), alloxazine-treated, and DPCPX-treated rings, respectively. However, the tension decreased by only 28.4 ± 9.8% in SCH-52861-treated rings. As shown in Fig. 5, E and F, the [Ca2+]i response to adenosine during PE-induced SOCE was also markedly inhibited by SCH-58261.

Role of cAMP in adenosine-induced SOCE reduction. Based on the previous studies, the cAMP signaling pathway is believed to be involved in adenosine-induced vasodilatation (32). Therefore, we examined whether adenosine regulates SOCE by utilizing the same pathway. As shown in Fig. 6A, adenylyl cyclase inhibitor SQ-22536 (1 µM) markedly inhibited the relaxant effect of adenosine on mesenteric artery rings that were contracted by SOCE. In VSMCs, the inhibitory effect of adenosine against SOCE-induced [Ca2+]i rise was also markedly depressed by SQ-22536 (Fig. 6, C and D). The cAMP analog 8-bromo-cAMP (8-Br-cAMP; 100 µM) mimicked adenosine responses in mesenteric artery and VSMCs (Fig. 6, B and D). The summarized data shown in Fig. 6E indicate that SOCE-mediated mesenteric artery maximum contractions were decreased by 74.9 ± 9.6% when treated with adenosine, whereas they were only 21.7 ± 8.3% decreased by adenosine in the presence of SQ-22536.

DISCUSSION

This study focused on the role of SOCE in adenosine-induced relaxation of an isolated rat small mesenteric resistance artery. Significant findings were as follows. 1) Adenosine produced a concentration-dependent relaxation in PE-contracted artery rings but not in KCl-contracted rings, and removing the endothelium did not affect the relaxant response to adenosine. 2) Ca2+ readmission after Ca2+ store depletion induced a marked and sustained increase in [Ca2+]i in mesenteric VSMCs. 3) Adenosine not only reduced the [Ca2+]i increase as a result of TG-mediated passive store depletion but also inhibited the [Ca2+]i increase after PE-mediated active store depletion. 4) Readmission of extracellular Ca2+ resulted in a sustained contraction after complete Ca2+ store depletion by PE in mesenteric arteries. Adenosine markedly relaxed this contraction. 5) Adenosine relaxation was mimicked by the
cAMP analog 8-Br-cAMP and unaffected by the adenosine A₁ antagonist DPCPX or the A₂B antagonist alloxazine; however, the adenosine A₂A antagonist SCH-58261 and the adenylate cyclase inhibitor SQ-22536 markedly depressed adenosine relaxation. To the best of our knowledge, this is the first study to establish that adenosine regulates vascular tone through a SOCE-based mechanism.

The influx of extracellular Ca²⁺ through specific Ca²⁺ channels plays a pivotal role in vascular smooth muscle contraction. VOCCs, activated by plasma membrane depolarization, were thought to be the major Ca²⁺ influx pathway in most VSMCs (5). In rat small mesenteric arteries, external high-K⁺ solution caused sustained contraction due to membrane depolarization, which leads to Ca²⁺ influx through VOCCs. We showed that adenosine had a negligible effect on this contraction. Similar observation has been made before in rat renal artery (10). In contrast, some investigators found that adenosine relaxed high K⁺-induced contraction in other blood vessels, such as coronary artery (31, 32). However, direct evidence supporting that adenosine inhibits VOCCs by means of ion channel recording and intracellular Ca²⁺ imaging in VSMCs has scarcely been reported. It is possible that adenosine may target different Ca²⁺ channels in different arteries. Furthermore, we found adenosine fully relaxed the remaining PE contraction in the presence of nifedipine. These results suggest that adenosine might cause relaxation by VOCC-independent mechanisms.

There is now considerable evidence that the voltage-independent channels, such as ROCCs and SOCCs, play an important role in Ca²⁺ entry into VSMCs and smooth muscle contraction (4, 28, 41). ROCCs are receptor-operated Ca²⁺ entry channels activated by agonist and coupled to G proteins; they do not necessarily depend on depletion of internal Ca²⁺ stores (18). In contrast, SOCCs refer to store-operated Ca²⁺ entry channels which are proposed to be stimulated in response to Ca²⁺ store depletion (27). In the present study, the role of SOCC-mediated Ca²⁺ entry in adenosine-induced vasorelaxation was investigated by active and passively depleting the Ca²⁺ stores with PE and TG. According to the SOCE model, SOCCs can be activated not only by the active emptying of Ca²⁺ stores with agonists like PE but also by passively depleting the Ca²⁺ stores after inhibition of sarcoplasmic reticulum Ca²⁺ ATPase (SERCA) with TG (27, 30). PE activation of membrane receptors, such as a-adrenoceptors, increases intracellular inositol 1,4,5-trisphosphate (IP₃) production. IP₃ activates its receptors on the sarcoplasmic reticulum (SR) membrane, induces Ca²⁺ release, and ultimately depletes Ca²⁺ from the SR. In turn, this phenomenon enables Ca²⁺ influx from the extracellular space via SOCCs when this ion is added to the medium (3, 22). TG, a selective SERCA inhibitor, depleted SR Ca²⁺ stores by inhibiting sequestration of Ca²⁺ and allowing activation of SOCCs without activating G protein-coupled receptors (38). Our proposal that adenosine modulates SOCE is based on two findings. First, adenosine depressed the [Ca²⁺]ᵢ increase that is due to TG- or PE-induced SOCE in mesenteric VSMCs. Second, adenosine inhibited the contraction that occurs as a result of SOCE induced by PE-mediated store depletion in isolated mesenteric arteries.

Fig. 5. The adenosine receptor mediates adenosine-induced inhibition of SOCE. Representative tracing showing tension changes during contraction induced by SOCE in the presence of adenosine (10 µM) and adenosine receptor antagonists, including SCH-58261 (SCH, 1 µM; A), alloxazine (Allo, 10 µM; B), and DPCPX (10 µM; C). D. summarized data (n = 12–18) showing maximum tensions of SOCE-induced contractions in the presence of adenosine and adenosine receptor antagonists. Representative tracing (E) and summarized data (F) showing [Ca²⁺]ᵢ fluorescence intensity changes in response to adenosine in the presence of SCH-58261 during SOCE. Summarized data are expressed as means, with error bars representing SE. P < 0.05 vs. control (*) and vs. adenosine-treated arteries (#).
should be noted that store depletion by PE or TG may activate nonselective cation channels which cause membrane depolarization, thereby activating VOCCs to further facilitate Ca\(^{2+}\)/H\(^{+}\) influx (25). Thus all experiments were carried out in the presence of nifedipine to block L-type Ca\(^{2+}\)/H\(^{+}\) channel activation during instances when SR Ca\(^{2+}\)/H\(^{+}\) stores are depleted.

A useful tool in elucidating the coupling mechanism for SOCC activation has been the cell-permeable IP\(_{3}\) receptor blocker 2-APB. Though the lack of specificity of 2-APB, it remains a reliable and widely used inhibitor of SOCCs (1, 23). 2-APB has been demonstrated to effectively inhibit SOCCs in many cell types, including VSMCs (6, 11, 17). In this study, the inhibitory effect of 2-APB on [Ca\(^{2+}\)]\(_{i}\) increase and contraction induced by Ca\(^{2+}\) store depletion under conditions of L-type Ca\(^{2+}\) channel blockade further supports the proposal that the channels that are activated by store depletion are SOCCs. Interestingly, we also found that 2-APB dose-dependently inhibited PE-induced vasoconstriction in Ca\(^{2+}\)-containing medium. This suggests a role for SOCCs in agonist-induced vasoconstriction. This result is in agreement with the study of rat coronary artery by Smani et al. (34). However, in addition to its IP\(_{3}\) receptor antagonism effect, a number of TRPC channels are inhibited by 2-APB (19). These channels include TRPC6, which likely contributes to receptor-operated Ca\(^{2+}\) entry (14). Thus 2-APB may inhibit PE-induced contraction by blocking receptor-operated Ca\(^{2+}\) entry evoked by \(\alpha\)-adrenoceptor activation.

Four adenosine receptor subtypes have been cloned (A\(_1\), A\(_{2A}\), A\(_{2B}\), and A\(_3\)). Adenosine A\(_{2A}\) receptors are generally considered the most important subtype, and they account for adenosine-induced vasodilatation; however, A\(_{2B}\) receptors may also play a role in some instances (7, 33). In this study, the adenosine A\(_1\) receptor antagonist DPCPX did not modify adenosine response in rings contracted by SOCE. This finding ruled out the involvement of adenosine A\(_1\) receptors in the relaxant actions of adenosine. We found that 10 \(\mu\)M adenosine A\(_{2B}\) receptor antagonist alloxazine slightly (but not significantly) depressed the relaxation response to adenosine. This depression raised the possibility that adenosine A\(_{2B}\) receptors may play a lesser role in adenosine-induced relaxation. It is important to note that alloxazine at this concentration also has some selectivity for adenosine A\(_{2A}\) receptors (2). Furthermore, rat mesenteric arteries show more extensive expression of adenosine A\(_{2A}\) receptors than A\(_{2B}\) receptors (15). Therefore, the action of the 10 \(\mu\)M alloxazine in this study may be mediated by adenosine A\(_{2A}\) receptors. SCH-58261, a highly selective antagonist against adenosine A\(_{2A}\) receptors, significantly depressed the adenosine response in this study. Although we cannot exclude a possible lesser role of adenosine A\(_{2B}\) receptors in the adenosine response, the results of this study indicate that A\(_{2A}\) receptors are the dominant receptor subtype responsible for the inhibitory effect of adenosine on SOCE in rat mesenteric arteries. The cAMP signaling pathways have been demonstrated to be involved in the vasodilatation mediated by adenosine receptors that are coupled to \(G_{s}\) proteins (32, 35). In support of this is the finding that adenosine-induced tension and [Ca\(^{2+}\)]\(_{i}\) reduction is significantly depressed by SQ-22536, a selective adenylate cyclase inhibitor. However, we did not directly measure intra-

Fig. 6. Adenosine inhibits SOCE via the cAMP signaling pathway. A and B: representative tracing showing tension changes in SOCE-contracted rings in response to adenosine (10 \(\mu\)M) in the presence of SQ-22536 (SQ: 100 \(\mu\)M) (A) and 100 \(\mu\)M 8-bromo-cAMP (8-Br-cAMP) (B). C: representative tracing showing [Ca\(^{2+}\)]\(_{i}\), fluorescence intensity changes in response to adenosine in the presence of SQ-22536 (100 \(\mu\)M) during SOCE. D, right: [Ca\(^{2+}\)]\(_{i}\) fluorescence intensity of SOCE in the presence of 8-Br-cAMP. E and F: summarized data (\(n = 12–18\)) showing SOCE-induced contraction and [Ca\(^{2+}\)]\(_{i}\), fluorescence intensity in the presence of adenosine, 8-Br-cAMP, or adenosine plus SQ-22536. The summarized data are expressed as means, with error bars representing SE. P < 0.05 vs. control (*) and vs. adenosine-treated arteries (#).
cellular cAMP levels following the addition of adenosine, and the precise mechanisms underlying the involvement of cAMP in adenosine-induced vasorelaxation remains to be established. The results in cultured cells provided direct evidence that SOCE played a functional role in VSMCs. However, VSMCs may present a different electrophysiological phenotype once cultured in the presence of serum. For example, cultured VSMCs rapidly lose their L-type voltage-operated channels when they switch to the proliferating phenotype (13, 24). Meanwhile, one of the characteristics of cultured VSMCs is a marked upregulation of TRPC channels (9). In cultured VSMCs, it has been shown that proliferation is associated with upregulation of several TRPC channels, such as TRPC1, TRPC4, and TRPC6 channels, resulting in enhanced store-operated Ca\(^{2+}\) influx via L-type Ca\(^{2+}\) channels and much more dependent on TRPC channels compared with native cells. The most commonly used means to stop VSMC proliferation in vitro is to culture them in serum-free medium, which can force VSMCs to differentiate. The differentiated VSMCs obtained by serum starvation have been shown less modulation of Ca\(^{2+}\) signaling when compared with those grown in serum medium (13). However, it is not clear whether the in vitro differentiated VSMCs are like their in vivo counterpart. It therefore becomes necessary to verify mechanisms deduced from cultured VSMCs by data obtained from acutely isolated VSMCs or VSMCs in vivo. Considering those electrophysiological changes in cultured VSMCs, isolated artery was used in the present study, and much of our understanding regarding the SOCE pathway has been based on studies using isolated artery. In conclusion, the data from this study provide evidence that adenosine potentially caused endothelium-independent relaxation of rat small mesenteric artery by inhibiting SOCE in VSMCs. This inhibition is predominantly mediated by adenosine A2A receptors and appears to be dependent on cAMP signaling. Store-operated channels could provide a novel approach to regulating the tone of small resistant arteries. These channels may represent realistic targets for therapeutic intervention in diseases such as hypertension, which involve excessive smooth muscle contraction.

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