Increased Activation of Stromal Interaction Molecule-1/Orai-1 in Aorta From Hypertensive Rats
A Novel Insight Into Vascular Dysfunction

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Abstract—Disturbances in the regulation of cytosolic calcium (Ca\(^{2+}\)) concentration play a key role in the vascular dysfunction associated with arterial hypertension. Stromal interaction molecules (STIMs) and Orai proteins represent a novel mechanism to control store-operated Ca\(^{2+}\) entry. Although STIMs act as Ca\(^{2+}\) sensors for the intracellular Ca\(^{2+}\) stores, Orai is the putative pore-forming component of Ca\(^{2+}\) release–activated Ca\(^{2+}\) channels at the plasma membrane. We hypothesized that augmented activation of Ca\(^{2+}\) release–activated Ca\(^{2+}\)/Orai-1, through enhanced activity of STIM-1, plays a role in increased basal tonus and vascular reactivity in hypertensive animals. Endothelium-denuded aortic rings from Wistar-Kyoto and stroke-prone spontaneously hypertensive rats were used to evaluate contractions because of Ca\(^{2+}\) influx. Depletion of intracellular Ca\(^{2+}\) stores, which induces Ca\(^{2+}\) release–activated Ca\(^{2+}\) activation, was performed by placing arteries in Ca\(^{2+}\)-free-EGTA buffer. The addition of the Ca\(^{2+}\) regular buffer produced greater contractions in aortas from stroke-prone spontaneously hypertensive rats versus Wistar-Kyoto rats. Thapsigargin (10 \(\mu\)mol/L), an inhibitor of the sarcoplasmic reticulum Ca\(^{2+}\) ATPase, further increased these contractions, especially in stroke-prone spontaneously hypertensive rat aorta. Addition of the Ca\(^{2+}\) release–activated Ca\(^{2+}\) channel inhibitors 2-aminoethoxydiphenyl borate (100 \(\mu\)mol/L) or gadolinium (100 \(\mu\)mol/L), as well as neutralizing antibodies to STIM-1 or Orai-1, abolished thapsigargin-increased contraction and the differences in spontaneous tone between the groups. Expression of Orai-1 and STIM-1 proteins was increased in aorta from stroke-prone spontaneously hypertensive rats when compared with Wistar-Kyoto rats. These results support the hypothesis that both Orai-1 and STIM-1 contribute to abnormal vascular function in hypertension. Augmented activation of STIM-1/Orai-1 may represent the mechanism that leads to impaired control of intracellular Ca\(^{2+}\) levels in hypertension. (Hypertension. 2009;53[part 2]:00-00.)

Key Words: Ca\(^{2+}\) regulation ■ STIM-1 ■ Orai-1 ■ SOC ■ CRAC channel ■ hypertension ■ vascular smooth muscle cell

On stimulation, most excitable cells display a biphasic increase in cytosolic calcium (Ca\(^{2+}\)) concentrations. The initial transient increase is attributed to inositol triphosphate–mediated release of endoplasmic reticulum (ER) Ca\(^{2+}\). The subsequent prolonged increase requires extracellular Ca\(^{2+}\) influx through various pathways. In smooth muscle cells (SMCs), Ca\(^{2+}\) entry mechanisms responsible for sustained cellular activation are normally mediated by either voltage-operated or receptor-operated Ca\(^{2+}\) channels.

Depletion of ER Ca\(^{2+}\) stores is able to gate the entry of extracellular Ca\(^{2+}\). This mechanism, by which the ER acts as a capacitor, leads to the term “store-operated Ca\(^{2+}\) (SOC) entry.” Recently, the discoveries of the stromal interacting molecules (STIMs) and Orai proteins have provided important information into the possible mechanisms by which this process is regulated.

STIMs are a family of single-transmembrane domain proteins found both in the plasma membrane and the ER, and their involvement with Ca\(^{2+}\) entry regulation has been described recently. It has been proposed that one of its isoforms, STIM-1, functions as a sensor for the ER Ca\(^{2+}\). The suppression of STIM-1 expression prevents SOC entry and eliminates the store-dependent activation of Ca\(^{2+}\) release-activated Ca\(^{2+}\) (CRAC) channels. Furthermore, Ca\(^{2+}\) depletion from ER results in a profound intracellular redistribution of STIM-1, from a uniform ER pattern to spatially discrete areas close to the plasma membrane.

Another protein family, Orai, which presently has 3 members, seems to be an essential component of CRAC channels, and Orai-1 has been suggested to be the pore-forming component of CRAC channels. In addition, STIM-1 and...
Orai-1 are sufficient for the generation of functional CRAC channels.\textsuperscript{12,14}  

Ca\textsuperscript{2+} is elevated in various cells from patients with hypertension and from spontaneously hypertensive rats. Because Ca\textsuperscript{2+} is a central component in the control of vascular contraction, abnormal handling of this cation by vascular myocytes has been suggested to account for the increased responses of vascular SMCs (VSMCs) to constrictor stimuli and augmented myogenic tone,\textsuperscript{15–19} key markers of arterial hypertension.

The magnitude of contraction is associated with the concentration of free Ca\textsuperscript{2+}. So, we used the contractile responses to reflect vascular Ca\textsuperscript{2+} influx or mobilization. This technique has been validated by Karaki et al\textsuperscript{20} and previous work from our laboratory.\textsuperscript{21,22} In addition, the development of active tone in stroke-prone spontaneously hypertensive rat (SHRSP) aortas is brought by changes in Ca\textsuperscript{2+} influx, and changes in tone and intracellular Ca\textsuperscript{2+} levels are positively correlated.\textsuperscript{23}

Therefore, considering that hypertension is associated with an imbalance in Ca\textsuperscript{2+} homeostasis, and STIM and Orai proteins seem to be fundamental to intracellular Ca\textsuperscript{2+} regulation, we hypothesized that increased activation of CRAC/Orai-1, through the Ca\textsuperscript{2+} sensor STIM-1, plays a role in augmented reactivity in aortas from hypertensive animals.

**Methods**

**Animals**

Five- to 6-month-old male SHRSPs were obtained from the breeding colony at the Medical College of Georgia. Age-matched male Wistar-Kyoto (WKY) rats were purchased from Harlan (Indianapolis Ind). Rats were maintained on a 12-hour light:dark cycle, housed 2 per cage, and allowed access to normal chow and water ad libitum. Systolic blood pressure was measured in nonanesthetized animals by tail cuff using a RTBP1001 blood pressure system (Kent Scientific Corporation). All of the procedures were performed in accordance with the Guiding Principles in the Care and Use of Animals, approved by the Medical College of Georgia Committee on the Use of Animals in Research and Education.

**Vascular Functional Studies**

After euthanasia, thoracic aortas were rapidly excised and placed in ice-chilled (\textasciitilde4°C) physiological salt solution. Segments of thoracic aorta were carefully dissected, and the endothelium was removed by gently rubbing the lumen side of the vessels with a metallic pin. Aortic rings (4 mm in length) were mounted on 2 stainless-steel wires in standard organ chambers (model 610 mol/L, Danish MyoTech) for isometric tension recording, as described previously.\textsuperscript{21,22} After stabilization, arterial integrity was assessed first by stimulation of vessels with 120 mmol/L of KCl and, after washing and a new stabilization period, by contracting the segments with phenylephrine (PE; 1 \textmu mol/L). Endothelium denudation was assessed by the absence of a relaxation response to acetylcholine (1 \textmu mol/L) during PE-induced contraction.
Experimental Protocols

Force development in response to a specific experimental protocol was evaluated in aortas from both rat groups, as described in Figure 1. Briefly, aortic rings were contracted with PE (1 μmol/L). When the contraction reached a plateau, aortas were washed in Ca²⁺-free-EGTA buffer, to deplete intracellular Ca²⁺ stores (depletion period), for 20 minutes. During the depletion period, aortas were incubated with vehicle or thapsigargin (1 μmol/L), a putative selective Ca²⁺-ATPase inhibitor, and some rings were treated with nonselective CRAC channel blockers, 2-aminophenoxydiphenyl borate (2-APB, 100 μmol/L) or gadolinium (Gd³⁺; 100 μmol/L). After Ca²⁺ depletion, intracellular Ca²⁺ stores were loaded (loading period) by placing the aortas in 1.6 mmol/L of Ca²⁺ buffer for 15 minutes. Contraction responses during the loading period were determined. The bathing medium was then replaced with Ca²⁺-free buffer, and after 2 minutes, the aortic segments were stimulated with caffeine (20 mmol/L) to deplete intracellular Ca²⁺ stores, which resulted in a transient contraction.²⁰,²¹

Antibody Delivery by the Chariot Technique

Antibodies against STIM-1 and Orai-1 (ProSci) were intracellularly delivered by the Chariot technique (Chariot Protein Delivery Reagent, Active Motif). This transfection reagent is able to deliver antibodies into cells while preserving their ability to localize to the proper cellular compartment and to recognize antigens within the cell.²²-²⁶ and in our experiments was used to directly inhibit STIM-1 and Orai-1 proteins. Chariot/antibody complexes were prepared and used according to the manufacturer’s instructions. Briefly, aortic rings were incubated in Eagle’s minimum essential medium containing L-glutamine (1.0%), PBS (10.0%), and penicillin and streptomycin (0.5%) for 30 minutes at 37°C. For each transfection, 12 μL of Chariot in 100 μL of 40% dimethyl sulfoxide were mixed with 6 μg of antibody in 100 μL of PBS and incubated at room temperature for 30 minutes to allow the complex to form. The aortas were transferred to a sterile 24-well cell culture plate, overlaid with 200 μL of Chariot/antibody complex, and mixed gently. Eagle’s minimum essential medium (400 μL) was added, and the tissues were incubated for 1 hour at 37°C. Then, Eagle’s minimum essential medium (750 μL) was added, and tissues were further incubated for 2 hours at 37°C. After this period, rings were mounted in the myograph, and functional studies were performed.

Western Blotting

Aortas from hypertensive and control rats were isolated, cleaned from fat, dissected, and frozen in liquid nitrogen. Extracted proteins (40 μg) were separated by electrophoresis on a 10% polyacrylamide gel and transferred to a nitrocellulose membrane. Non-specific binding sites were blocked with 5% skim milk in Tris-buffered saline solution with Tween (0.1%) for 1 hour at 24°C. Membranes were then incubated with antibodies overnight at 4°C. Antibodies were as follows: anti–STIM-1 and anti–Orai-1 (1:1000, ProSci), as well as β-actin (1:1000, Sigma-Aldrich). Human ovary and mouse thymus tissue lysate (ProSci) and anti–Orai-1 (1:100, mouse polyclonal, Abcon), respectively. After incubation with secondary antibodies, signals were revealed with chemiluminescence, visualized by autoradiography, and quantified densitometrically. Results were normalized by β-actin expression and expressed as units of change from the control.

RNA Extraction, cDNA Synthesis, and Quantitative Real-Time RT-PCR

Total RNA was extracted from aorta using the RNeasy kit (Qiagen Sciences). The quantity, purity, and integrity of all of the RNA samples were determined by NanoDrop spectrophotometry (NanoDrop Technologies). One microgram of total RNA was reverse transcribed in a final volume of 50 μL using the High-Capacity cDNA Archive kit (Applied Biosystems), and single-strand cDNA was stored at −20°C. Primers for STIM-1 (catalog No. Rn00968446_m1) and Orai-1 (catalog No. Rn02397170_m1) mRNA were obtained from Applied Biosystems. Real-time RT-PCR (quantitative PCR) reactions were performed using the 7500 Fast Real-Time PCR System (Applied Biosystems) in a total volume of 20 μL of reaction mixture following the manufacturer’s protocol, using TaqMan Fast Universal PCR Master Mix (2×; Applied Biosystems), and 0.1 μmol/L of each primer. Negative controls contained water instead of first-strand cDNA. Each sample was normalized on the basis of its 18S ribosomal RNA content. The 18S quantification was performed using a TaqMan Ribosomal RNA Reagent kit (Applied Biosystems), following the manufacturer’s protocol. Relative gene expression for STIM-1 and Orai-1 mRNA was normalized to a calibrator that was chosen to be the basal condition (untreated sample) for each time point. Results were calculated with the ΔΔCt method and expressed as n-fold differences in preproET-1 gene expression relative to 18S rRNA and to the calibrator and were determined as follows: n-fold=2−ΔΔCt sample−ΔCt calibrator, where the parameter Ct (threshold cycle) is defined as the fractional cycle number at which the PCR reporter signal passes a fixed threshold. ΔΔCt values of the sample and the calibrator were determined by subtracting the average Ct value of the transcript under investigation from the average Ct value of the 18S rRNA gene for each sample.

Immunofluorescence Microscopy Analysis

STIM-1 and Orai-1 expression in endothelium-denuded aortas from SHRSRs and WKY rats was determined by immunofluorescence staining technique. Aortas were frozen in optimal cutting temperature, and sections were obtained. Briefly, aortic sections were washed with PBS with 0.2% Triton X (PBS-T) for 15 minutes at room temperature and fixed in acetone for 5 minutes at −20°C, and nonspecific binding sites were blocked with PBS-T plus 1% BSA for 10 minutes at room temperature and with 10% goat serum in PBS-T/BSA for 30 minutes at room temperature. Subsequently, STIM-1 and Orai-1 expression was determined by incubating the vascular sections with anti–STIM-1 (1:100, rabbit polyclonal, ProSci) and anti–Orai-1 (1:100, mouse polyclonal, Abcon), respectively, overnight at 4°C. Aortic sections were then placed at room temperature, rinsed with PBS-T for 15 minutes, probed with goat antiamouse Alexa Fluor 586 (1:1000, green fluorescence, Invitrogen) and probed with goat antirabbit Alexa Fluor 568 (1:1000, red fluorescence, Invitrogen), which was followed by another rinse with PBS-T/B. Images were acquired with the use of the confocal microscope (LSM 510 Meta 3.2, Zeiss). Magnification power was set at ×10.

Drugs and Solutions

Physiological salt solution of the following composition was used: 130 mmol/L NaCl, 14.9 mmol/L NaHCO₃, 4.7 mmol/L KCl, 1.18 mmol/L KH₂PO₄, 1.17 mmol/L MgSO₄, 7 H₂O, 5.5 mmol/L glucose, 1.56 mmol/L CaCl₂, 2 H₂O, and 0.026 mmol/L EDTA. PE and acetylcholine were purchased from Sigma Chemical Co. All of the reagents were of analytic grade. Stock solutions were prepared in deionized water. Control solutions containing vehicle (DMSO) were also used through the experimental protocols.

Data Analysis

Results are presented as means ± SEMs. Contractions were recorded as changes in the displacement (mN) from baseline and are represented as mN per mm of experimenters’ figures. Statistically significant differences were calculated by ANOVA (Newman-Keuls posthoc test) or by Student’s t test where appropriate. P < 0.05 was considered significant.

Results

Systolic Blood Pressure and Body Weight of the Rats

At 24 weeks, SHRSRs displayed higher systolic blood pressure (mm Hg), in comparison with WKY rats (211.0±7.6, n=15, versus 119.0±1.8, n=15, respectively). Body weight

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CRAC channel blockade with 2-APB and Gd³⁺/H₁₁₀₀₁⁻/H₁₁₀₀₅⁻ intracellular Ca²⁺ to promote the depletion of intracellular Ca²⁺ displayed greater contractions during the Ca²⁺ influx (10.0±6.0 mN compared with WKY rats (4.8±0.9 mN; n=6). CRAC channel blockade with 2-APB and Gd³⁺, displayed greater contractions and abolished differences between the groups. Values are expressed as means±SEMs. *P<0.05 vs WKY rats. †P<0.05 vs DMSO.

Figure 2. CRAC channel blockade partially prevents contraction during Ca²⁺ loading and abolishes differences in spontaneous tone between arteries from WKY rats and SHRSPs. A, Contraction during Ca²⁺ loading period, which is greater in SHRSPs (165.0±3.2 mN; n=6) versus WKY rats (107.7±1.0 mN; n=6). During thapsigargin incubation, simultaneous inhibition of CRAC channels by 2-APB and Gd³⁺ significantly decreased Ca²⁺ loading–induced contractions both in WKY rats (2.4±0.2 and 4.3±0.9 mN, respectively) and SHRSPs (3.9±0.1 and 5.9±0.4 mN, respectively), abolishing the differences between the groups. Collectively, these results suggest that CRAC channel activation is increased in aortas from the hypertensive animals, contributing to augmented extracellular Ca²⁺ influx.

Figure 3. Neutralizing antibodies against STIM-1 and Orai-1, delivered with the Chariot technique, partially prevent contraction during the Ca²⁺ loading period. Aortic rings from WKY rats (n=5) and SHRSPs (n=5), transfected with antibodies anti-STIM-1 or anti-Orai-1, displayed reduced contraction during the Ca²⁺ loading period either in the presence of vehicle (A) or after thapsigargin treatment (B). Values are expressed as means±SEMs. *P<0.05 vs WKY rats. †P<0.05 vs DMSO.
Ca\textsuperscript{2+} greater in SHRSP aortas, confirming that inhibition of the SR
produced compared with those in vehicle-treated vessels, but still
incubation, caffeine-induced contractions were already re-
duced compared with WKY rats and SHRSPs. On thapsigargin
incubation, caffeine-induced contractions were already re-
duced in aortas from both groups on CRAC channel blockade. Treatment
with 2-APB and Gd\textsuperscript{3+} practically abolished contractions in
both groups (Figure 4B). These results confirm that CRAC
channel activation contributes to SR Ca\textsuperscript{2+} loading.

**Intracellular Ca\textsuperscript{2+} Stores Depletion**
The functional capacity of the SR to release Ca\textsuperscript{2+} was
evaluated by placing the aortas in Ca\textsuperscript{2+}-free buffer for 2
minutes and by stimulation with caffeine (20 mmol/L). As observed in Figure 4A, SHRSP aortas displayed increased
caffeine-induced contractions compared with WKY rats. CRAC channel blockade with 2-APB and Gd\textsuperscript{3+} during the
Ca\textsuperscript{2+}-loading period reduced contractions induced by caffeine, both in WKY rats and SHRSPs. On thapsigargin
incubation, caffeine-induced contractions were already re-
duced compared with those in vehicle-treated vessels, but still
greater in SHRSP aortas, confirming that inhibition of the SR
Ca\textsuperscript{2+}-ATPase activity decreases intracellular Ca\textsuperscript{2+} storage. Caffeine-induced contractions were further reduced in aortas from both groups on CRAC channel blockade. Treatment
with 2-APB and Gd\textsuperscript{3+} practically abolished contractions in
both groups (Figure 4B). These results confirm that CRAC
channel activation contributes to SR Ca\textsuperscript{2+} loading.

**STIM-1 and Orai-1 Expression**
Aortas from SHRSPs exhibited increased protein levels of
STIM-1 (1.61±0.14) and Orai-1 (1.23±0.06) compared with
WKY rats (Figure 6A-B). In addition, STIM-1 and Orai-1
mRNA levels were augmented in aortas from SHRSPs
(1.25±0.3, respectively) compared with WKY patients (Figure 6C and 6D). Immunohistochemistry analysis
confirmed increased expression of STIM-1, but not Orai-1, in aortas from SHRSPs in comparison with WKY rats (Figure 6E).

**Discussion**

In smooth muscle, the activation of SOC entry is key to mediating long-term cytosolic Ca\(^{2+}\) signals and replenishing intracellular Ca\(^{2+}\) stores.\(^{6,27,28}\) In many cell types, SOC entry carries a highly Ca\(^{2+}\)-selective, nonvoltage-gated, inwardly rectifying current termed the "CRAC current."\(^{7,27,28}\) A defect in the regulation of Ca\(^{2+}\) storage in the SR, which may contribute to enhanced vascular reactivity in hypertension. The cellular mechanisms involved in abnormal vascular reactivity in hypertension. Increased active Ca\(^{2+}\) entry in SMCs involves functional STIM-1 proteins, which, on activation, translocate to plasma membrane domains and activate Ca\(^{2+}\) release from SR/ER, was 2 times higher in rat aortic SMCs from SHRs than in those from WKY rats. These data show that the thapsigargin-sensitive pool is greater in SHRs than in WKY rats. Second, alterations in Ca\(^{2+}\) storage proteins, such as calreticulin and calnexin, may also contribute to increased SR Ca\(^{2+}\) levels. Ca\(^{2+}\) storage proteins, characterized by high Ca\(^{2+}\)-binding capacity, keep high concentrations of Ca\(^{2+}\) inside the SR/ER. On inositol triphosphate stimulus, eg, Ca\(^{2+}\) is unbound from Ca\(^{2+}\) storage proteins and released to the cytoplasm. Furthermore, calreticulin is elevated in peripheral maternal blood during preeclampsia in humans,\(^{32}\) and cardiac hypertrophy causes alterations in the SR Ca\(^{2+}\)-storing proteins, a mechanism that may contribute to the contractile dysfunction of hypertrophied cardiac myocytes. A correlation between increased levels of calreticulin/calsequestrin and increased risk of adult heart disease has also been established.\(^{34}\) A third mechanism is represented by alterations in the Ca\(^{2+}\) ER sensor, STIM-1, which modulates/activates Ca\(^{2+}\) entry. SOC entry in SMCs involves functional STIM-1 proteins, which, on activation, translocate to plasma membrane domains and activate Ca\(^{2+}\) entry, similarly to what has been described in other tissues.\(^{35-37}\)

Recent literature has identified STIM and Orai proteins as key signaling players in the activation of SOC in a number of inflammatory cell types. Orai-1 protein fulfills all of the

- **Figure 6.** SHRPS display increased vascular Orai-1 and STIM-1 protein and mRNA levels. Top, representative Western blot images, and, bottom, corresponding bar graphs demonstrating that (A) STIM-1 and (B) Orai-1 protein and (C) STIM-1 and (D) Orai-1 mRNA levels are increased in SHRSP aortas (\(n=6\)) vs WKY rats (\(n=6\)). Densitometric analyses were performed, and values were normalized to β-actin protein expression. *P<0.05 vs WKY rats. E, Immunohistochemistry analysis using confocal microscopy confirmed increased expression of STIM-1 (red) and Orai-1 (green) in aortas from SHRSPs (\(n=3\)) vs WKY rats (\(n=3\)).
criteria of being the SOC moiety itself.11,13,14,38–41 In addition, combined expression of STIM-1 and Orai-1 resulted in a huge gain of SOC function and indicates that the 2 proteins are likely sufficient to mediate the operation of SOCs.13,14,38,39 Peel et al42 showed that Orai-1 small interfering RNA transfection on whole airway SMCs blocks inward currents initiated by Ca\(^{2+}\) store depletion. They found a similar result by using inhibitors of SOC Ca\(^{2+}\) influx, including 2-APB, Gd\(^{3+}\), and lanthanum. In addition, mutations in Orai-1 also result in significant changes to the electrophysiology properties of CRAC current, rendering the current less Ca\(^{2+}\) selective with outward rectification.11 Such studies have provided evidence for the theory that Orai-1 forms the pore-forming subunits of the CRAC channel. Orai-1 and STIM-1 may also contribute to the function of other SOC channels.43 In this sense, Orai-1 has been reported to interact with the TRPC1 channel and forms a ternary complex along with STIM-1 in the plasma membrane.44,45 Nailing down the identity of both the sensor and the channel itself has created new possibilities in the Ca\(^{2+}\) signaling field.

Considering that defects in Ca\(^{2+}\) handling in VSMCs are among the candidate cellular mechanisms for the pathogenesis of hypertension, STIM-1/Orai-1 represent important components to the intracellular Ca\(^{2+}\) regulation, and STIM-1/Orai activation in VSMCs from hypertensive animals has not been addressed, we hypothesized that increased activation of CRAC/Orai-1, through the Ca\(^{2+}\) sensor STIM-1, plays a role in augmented reactivity in aortas from hypertensive animals. As evidenced by our results, CRAC channel activation is increased in aortas from SHRSPs by mechanisms dependent on STIM-1 and Orai-1 activation, resulting in augmented extracellular Ca\(^{2+}\) influx, inferred from spontaneous enhancement in contractile function.

We described previously that aortas from SHRSPs displayed increased force development during the Ca\(^{2+}\) loading period on the depletion of intracellular Ca\(^{2+}\) stores.22 The SR Ca\(^{2+}\) store is larger in aortas from SHRSPs because of an enhanced influx of Ca\(^{2+}\) across the sarcolemma rather than an impaired recycling of the cation by the SR Ca\(^{2+}\)-ATPase.42 This study shows that prevention of STIM-1 activation by neutralizing antibodies prevents contractions induced by Ca\(^{2+}\) influx during the Ca\(^{2+}\)-loading period. Neutralization of Orai-1 also decreased Ca\(^{2+}\) influx, as indicated by our functional experiments. Furthermore, thapsigargin-mediated vascular Ca\(^{2+}\) influx was greatly reduced by transfection of STIM-1 and Orai-1 antibodies compared with that in negative transfected aortas.

2-APB, a nonselective blocker, and Gd\(^{3+}\), a selective and irreversible blocker of CRAC channels, were used to inhibit SOC entry.46 2-APB and Gd\(^{3+}\) inhibited Ca\(^{2+}\) entry, as evidenced by inhibition of contractile responses, in aortas from WKY rats and SHRSPs. The differences in spontaneous tone development during the Ca\(^{2+}\)-loading period between the groups were abolished after CRAC channel blockade. These results demonstrate that CRAC channels represent an important pathway for Ca\(^{2+}\) entry and that CRAC activation is enhanced in aortas from SHRSPs compared with that in WKY rats.

Possible mechanisms associated with an abnormal function of the Ca\(^{2+}\) sensor STIM-1 and CRAC channels include increased expression or activity of these proteins, defective activation of the sensor by Ca\(^{2+}\), or augmented activation/decreased inactivation of CRAC channels. In this study, we have observed that aortas from SHRSPs display increased expression of both STIM-1 and Orai-1. Although it is possible that increased protein levels may account for the increased functional contractile responses observed after intracellular Ca\(^{2+}\) depletion, other mechanisms may need to be taken into consideration.

Perspectives

Most of the work related to the characterization of STIM-1 and Orai-1 was performed in lymphocytes and T cells. A mutation in Orai-1 was observed in patients with severe combined immune deficiency, resulting in abrogated CRAC channel function.10 The effect of the mutation in the Orai-1 was not reverted by overexpression of Orai-2 or Orai-3. This important article showed that alterations in Orai-1 are related to pathophysiological conditions and called the attention of pharmaceutical companies that have begun to investigate CRAC channel blockers as an option to reduce the toxicity of immunosuppressant drugs, such as cyclosporine A.40,47 Here we show for the first time that STIM-1 and Orai-1 proteins are upregulated in vessels from hypertensive animals. Functional upregulation of SOC in vessels from SHRSPs was confirmed both by pharmacological and molecular approaches. CRAC channel inhibitors may represent a new therapeutic approach to treat vascular dysfunction or to prevent end-organ damage associated with arterial hypertension.

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Disclosures

None.

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