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

Fernanda R.C. Giachini, Chin-Wei Chiao, Fernando S. Carneiro, Victor V. Lima, Zidonia N. Carneiro, Anne M. Dorrance, Rita C. Tostes, R. Clinton Webb

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 μmol/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 μmol/L) or gadolinium (100 μmol/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]:409-416.)

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+}/H\textsubscript{11001} is elevated in various cells from patients with hypertension and from spontaneously hypertensive rats. Because Ca\textsuperscript{2+}/H\textsubscript{11001} 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 (∼4°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 μmol/L). Endothelium denudation was assessed by the absence of a relaxation response to acetylcholine (1 μmol/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 nonspecific CRAC channel blockers, 2-aminophosphoryl benzoate (2-ABP, 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 cell²⁴–²⁶ 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 microscope, 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. Nonspecific binding sites were blocked with PBS-T plus 1% BSA for 1 hour at 20°C, 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

Immunofluorescence Microscopy Analysis

STIM-1 and Orai-1 expression in endothelium-denuded aortas from SHRSsPs and WKY rats was determined by immunofluorescence staining technique. Aortas were frozen in optimal cutting temperature, and sections were obtained. Briefly, aorta sections were washed with PBS with 0.2% Triton X (PBS-T) for 15 minutes at room temperature and fixed in aceton 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 588 (1:1000, green fluorescence, Invitrogen) and probed with goat antiantibat Alexa Fluor 656 (1:1000, red fluorescence, Invitrogen), which was followed by another rinse with TBS-T. 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 number of experiments. 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, SHRSPs 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
CRAC channel blockade with 2-APB and Gd3⁺ vs WKY rats (n = 6) displayed greater contractions during the Ca²⁺ loading period, which is greater in SHRSPs (n = 6) vs WKY rats (n = 6), was significantly inhibited after CRAC channel blockade with 2-APB and Gd3⁺ (Figure 2A and 2B, vehicle). After thapsigargin treatment, aortic rings from WKY rats (n = 5) and SHRSPs (n = 5) displayed greater contractions during the Ca²⁺ loading period. CRAC channel blockade with 2-APB and Gd3⁺ decreased contractions and abolished differences between the groups. Values are expressed as means ± SEMs, n = 6. *P < 0.05 vs WKY rats. †P < 0.05 vs vehicle.

**Force Development During the Ca²⁺ Loading Period**

Figure 1 illustrates the protocol used to evaluate force development in response to Ca²⁺ influx after depletion of intracellular Ca²⁺ stores (loading period) and after caffeine stimulation to measure the functional capacity of the sarcoplasmic reticulum (SR) to release Ca²⁺. After an initial response to 120 mmol/L of KCl (SHRSP, 14.1 ± 3.2 mN versus WKY, 22.7 ± 5.3 mN; n = 10) and a new stabilization period, aortas were stimulated with PE (1 µmol/L), and the contraction was allowed to reach a plateau. PE-induced contractions were similar in aortas from SHRSPs (20.8 ± 1.7 mN; n = 12) compared with WKY rats (15.6 ± 2.7 mN; n = 12). After PE contraction, aortas were washed in Ca²⁺-free EGTA buffer either in the absence (Figure 1A and 1B, vehicle) or presence (Figure 1C and 1D) of thapsigargin (1 µmol/L).

Figure 2A shows that, during the Ca²⁺-loading period, force development was augmented in aortas from SHRSPs (10.0 ± 0.9 mN; n = 6) compared with WKY rats (4.8 ± 1.0 mN; n = 6). CRAC channel blockade with 2-APB and Gd³⁺ significantly inhibited contraction in SHRSP aortas during the Ca²⁺ loading period (3.9 ± 0.6 and 6.3 ± 0.4 mN, respectively) but had no significant effects in WKY aortas.

Thapsigargin was used to inhibit the SR Ca²⁺-ATPase and to promote the depletion of intracellular Ca²⁺ stores. Accordingly, this would result in continuous stimulation of the SR Ca²⁺ sensor, STIM-1, and, consequently, activation of SOC entry through CRAC channels. As shown in Figure 2B, thapsigargin incubation augmented contractions during the Ca²⁺ loading period in aortic rings from both groups. However, contractions were greater in SHRSP aortas (16.5 ± 0.9 mN versus WKY, 10.7 ± 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.

As an alternative approach to the pharmacological inhibition with 2-APB and Gd³⁺, neutralizing antibodies against STIM-1 and Orai-1 were intracellularly delivered by the Chariot technique. Figure 3A shows that transfection with Orai-1 or STIM-1 antibodies resulted in decreased contractile responses in SHRSP aortas were similar to those in the control group, reinforcing the notion that these proteins play a role in augmented tone development in vessels from SHRSPs. On thapsigargin inhibition, anti–Orai-1 and anti–STIM-1 antibodies resulted in decreased 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.
Ca²⁺ greater in SHRSP aortas, confirming that inhibition of the SR induced compared with those in vehicle-treated vessels, but still incubation, caffeine-induced contractions were already re-
duced in aortas from hypertensive rats, through mechanisms dependent on STIM-1 and Orai-1 activation, results in exacerbated aortas from WKY rats and SHRSPs. On thapsigargin incubation, caffeine-induced contractions were de-
duced in vessels from WKY rats and SHRSPs. Aortic rings from WKY rats (Figure 3B). Empty Chariot or incubation of vessels with 2-APB and Gd³⁺ from both groups on CRAC channel blockade. Treatment with 2-APB and Gd³⁺ practically abolished contractions in both groups (Figure 4B). These results confirm that CRAC channel activation contributes to SR Ca²⁺ loading.

Figure 5A shows that transfection of Orai-1 or STIM-1 antibodies decreased the SR Ca²⁺ loading capacity, resulting in smaller transient contractions to caffeine in both groups. Furthermore, differences in caffeine-induced contractions between aortas from WKY rats and SHRSPs were abolished after transfection of Orai-1 or STIM-1 antibodies. On thapsigargin incubation, caffeine-induced contractions were decreased in vessels from SHRSPs and WKY rats, and no differences were found among the groups (Figure 5B). In this condition, transfection of Orai-1 or STIM-1 antibodies had no further effects. These data reinforce the suggestion that STIM-1 and Orai-1 activation is important during the SR Ca²⁺ reloading period and indicate that aortas from hypertensive rats display increased activation of STIM-1 and Orai-1–related pathways.

**Intracellular Ca²⁺ Stores Depletion**

The functional capacity of the SR to release Ca²⁺ was evaluated by placing the aortas in Ca²⁺-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 Gd³⁺ during the Ca²⁺-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 Ca²⁺-ATPase activity decreases intracellular Ca²⁺ storage. Caffeine-induced contractions were further reduced in aortas from both groups on CRAC channel blockade. Treatment with 2-APB and Gd³⁺ practically abolished contractions in both groups. Figure 4A shows that transfection of Orai-1 or STIM-1 antibodies decreased the SR Ca²⁺ loading capacity, resulting in smaller transient contractions to caffeine in both groups. Furthermore, differences in caffeine-induced contractions between aortas from WKY rats and SHRSPs were abolished after transfection of Orai-1 or STIM-1 antibodies. On thapsigargin incubation, caffeine-induced contractions were decreased in vessels from SHRSPs and WKY rats, and no differences were found among the groups (Figure 5B). In this condition, transfection of Orai-1 or STIM-1 antibodies had no further effects. These data reinforce the suggestion that STIM-1 and Orai-1 activation is important during the SR Ca²⁺ reloading period and indicate that aortas from hypertensive rats display increased activation of STIM-1 and Orai-1–related pathways.

**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 ± 0.1 and 1.69 ± 0.3, respectively) compared with WKY rats (Figure 6C and 6D). Immunohistochemistry analysis

---

**Figure 4.** CRAC channels contribute to the SR Ca²⁺ loading. Caffeine-induced contractions after loading of intracellular Ca²⁺ stores are prevented by CRAC channel blockade, both in aortas from WKY rats and SHRSPs. On thapsigargin incubation, caffeine-induced contractions were already re-
duced compared with those in vehicle-treated vessels, but still incubation, caffeine-induced contractions were de-
duced in aortas from hypertensive rats, through mechanisms dependent on STIM-1 and Orai-1 activation, results in exacerbated aortas from WKY rats and SHRSPs. On thapsigargin incubation, caffeine-induced contractions were de-
duced in vessels from WKY rats and SHRSPs. Aortic rings from WKY rats (Figure 3B). Empty Chariot or incubation of vessels with 2-APB and Gd³⁺ from both groups on CRAC channel blockade. Treatment with 2-APB and Gd³⁺ practically abolished contractions in both groups. Figure 4B. These results confirm that CRAC channel activation contributes to SR Ca²⁺ loading.

**Figure 5.** STIM-1 and Orai-1 contribute to the SR Ca²⁺ loading. Neutralizing antibodies against STIM-1 and Orai-1, delivered with the Chariot technique, partially prevent contraction in response to caffeine after loading of intracellular Ca²⁺ stores. In the presence of vehicle (A) or after thapsigargin treatment (B), aortic rings from WKY rats (n = 5) and SHRSPs (n = 5) submitted to anti-STIM-1 or anti-Orai-1 transfection, displayed reduced contractions to caffeine after a depletion and reloading cycle, as described in Figure 1. Differences between SHRSPs and WKY rats were abolished after antibody transfection. Values are expressed as means ± SEMs. *P < 0.05 vs WKY. †P < 0.05 vs DMSO.
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\(^{2+}\) plays a role in the increased vascular reactivity in hypertension. The cellular mechanisms involved in abnormal Ca\(^{2+}\) handling in VSMCs are numerous and include increased Ca\(^{2+}\) entry, increased Ca\(^{2+}\) storage in the SR, or decreased Ca\(^{2+}\) extrusion. In this article, we focused on the possibility that increased Ca\(^{2+}\) influx mediated by a defective signal from intracellular Ca\(^{2+}\) stores contributes to abnormal vascular reactivity in hypertension. Increased activation of CRAC/Orai-1, through the Ca\(^{2+}\) sensor STIM-1, plays a role in augmented reactivity in aortas from hypertensive animals.

Augmented Ca\(^{2+}\) levels in SMCs from spontaneously hypertensive rats\(^{30}\) can be attributed to an increase in the amount of Ca\(^{2+}\) in the SR, which may contribute to enhanced vascular tone through Ca\(^{2+}\)-release mechanisms. Differences in SR Ca\(^{2+}\) levels in arteries from hypertensive and normotensive rats include various possibilities. First, it is possible that augmented Ca\(^{2+}\) uptake through the SR Ca\(^{2+}\)-ATPase-dependent mechanisms contributes to an increased Ca\(^{2+}\) store. Accordingly, Levitsky et al\(^{30}\) showed higher expression of the SR Ca\(^{2+}\)-ATPase 2a and 2b isoforms in VSMCs from SHRs, which may account for the increased responses to thapsigargin and enhanced Ca\(^{2+}\) stores in vascular myocytes from these animals. Furthermore, Cortes et al\(^{19}\) have shown that, in Ca\(^{2+}\)-free medium, Ca\(^{2+}\) release induced by thapsigargin, which represents Ca\(^{2+}\) release from SR/ER, was 2 times higher in rat aortic SMCs from SHRs than in those from WKY rats.\(^{29}\) 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 calsequestrin and calreticulin, 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.\(^{31}\) 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.\(^{33}\) 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
criteria of being the SOC moiety itself. 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. Peel et al. 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. 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 for the theory that Orai-1 forms the pore-forming subunits of CRAC/Orai activation in VSMCs from hypertensive animals has been reported by a doctoral fellowship from Fundação de Amparo à Pesquisa do Estado de São Paulo (São Paulo, Brazil).

We described previously that aortas from SHRSPs displayed increased force development during the Ca\(^{2+}\) influx during the Ca\(^{2+}\) loading period on the depletion of intracellular Ca\(^{2+}\) stores. 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. 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.

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. 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. 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.

**Sources of Funding**

This study was supported by grants from the National Institutes of Health (HL-71138 and HL-74167). F.R.C.G. and F.S.C. are supported by a doctoral fellowship from Fundação de Amparo à Pesquisa do Estado de São Paulo (São Paulo, Brazil).

**Disclosures**

None.

**References**


Hypertension. 2009;53:409-416; originally published online December 15, 2008; doi: 10.1161/HYPERTENSIONAHA.108.124404

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/53/2/409

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/