HYPOTHESIS

STIM and Orai proteins: players in sexual differences in hypertension-associated vascular dysfunction?

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ABSTRACT

Sex-associated differences in hypertension have been observed repeatedly in epidemiological studies; however, the mechanisms conferring vascular protection to females are not totally elucidated. Sex-related differences in intracellular Ca\textsuperscript{2+} handling or, more specifically, in mechanisms that regulate Ca\textsuperscript{2+} entry into vascular smooth muscle cells have been identified as players in sex-related differences in hypertension-associated vascular dysfunction. Recently, new signalling components that regulate Ca\textsuperscript{2+} influx, in conditions of intracellular store depletion, were identified: STIM1 (stromal interaction molecule 1), which works as an intracellular Ca\textsuperscript{2+} sensor; and Orai1, which is a component of the CRAC (Ca\textsuperscript{2+} release-activated Ca\textsuperscript{2+}) channels. Together, these proteins reconstitute store-operated Ca\textsuperscript{2+} channel function. Disturbances in STIM1/Orai signalling have been implicated in pathophysiological conditions, including hypertension. In the present article, we analyse evidence for sex-related differences in Ca\textsuperscript{2+} handling and propose a new hypothesis where sex-related differences in STIM/Orai signalling may contribute to hypertension-associated vascular differences between male and female subjects.

INTRODUCTION

Defective regulation of intracellular Ca\textsuperscript{2+} is a hallmark of hypertension-associated vascular dysfunction and plays a key role in the augmented vascular reactivity, characteristic of clinical and experimental hypertension. The recent identification of new signalling components linking intracellular Ca\textsuperscript{2+} stores to plasma membrane Ca\textsuperscript{2+} entry has brought a new insight into the understanding of Ca\textsuperscript{2+} homoeostasis. STIM1 (stromal interaction molecule 1) is the Ca\textsuperscript{2+} sensor protein that triggers Ca\textsuperscript{2+} influx in response to Ca\textsuperscript{2+} store depletion, whereas Orai is an essential component of CRAC (Ca\textsuperscript{2+} release-activated Ca\textsuperscript{2+}) channels. Although research on STIM1/Orai signalling is entering an exponential phase of growth, the role of these proteins in vascular dysfunction is unknown.

Mechanisms contributing to hypertension and its associated end-organ dysfunction are differentially regulated in males and females. Critical sex differences are observed in the intrinsic vascular mechanisms that regulate total peripheral resistance, namely that gonadally intact females display less vascular dysfunction associated with experimental hypertension [1–6], compared with gonadally intact males. Although the existence of sex differences in vascular Ca\textsuperscript{2+} handling is well established [7–10], no studies have addressed differences in vascular STIM1/Orai signalling in male and female hypertensive animals.

Key words: hypertension, Orai, sex difference, stromal interaction molecule (STIM).

Abbreviations: CRAC, Ca\textsuperscript{2+} release-activated Ca\textsuperscript{2+}; ER, endoplasmic reticulum; IC\textsubscript{CRAC}, CRAC current; SHR, spontaneously hypertensive rats; SHRSP, stroke-prone SHR; SOCE, store-operated Ca\textsuperscript{2+} entry; SR, sarcoplasmic reticulum; STIM, stromal interaction molecule; VSMC, vascular smooth muscle cell; WKY, Wistar–Kyoto.

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Given the importance of STIM1/Orai signalling in intracellular Ca\(^{2+}\) homoeostasis, it seems plausible that increased activation of STIM1/Orai contributes to increased vascular contraction in the vasculature of hypertensive animals.

In the present article, we will review evidence supporting these hypotheses. First, we will address the participation of STIM1 and Orai1 on Ca\(^{2+}\)-handling mechanisms during physiological conditions, as well as in hypertension. Then, we will focus on how alteration of STIM1/Orai signalling contributes to differences in Ca\(^{2+}\) handling, and how this impairment may contribute to sex-related differences in vascular function in hypertension.

**STIM1/Orai1 pathway represents a key Ca\(^{2+}\)-handling mechanism**

Nearly all cell types depend on cytoplasmic Ca\(^{2+}\) signals to trigger specific responses [11]. Upon stimulation, most excitable cells display a biphasic increase in cytosolic Ca\(^{2+}\) concentrations. The initial transient increase is due to Ca\(^{2+}\) release from intracellular stores, e.g. Ins(1,4,5)P\(_3\)-mediated release of ER (endoplasmic reticulum) Ca\(^{2+}\), whereas the subsequent prolonged increase requires extracellular Ca\(^{2+}\) influx through various pathways. Upon depletion of Ca\(^{2+}\) from the ER, Ca\(^{2+}\) channels are activated in the plasma membrane to refill internal Ca\(^{2+}\) stores. This mechanism, by which the ER acts as a capacitor, leads to the term SOCE (store-operated Ca\(^{2+}\) entry) [12,13]. SOCE carries a highly Ca\(^{2+}\)-selective, non-voltage-gated, inwardly rectifying current termed the CRAC current or \(I_{\text{CRAC}}\) [13,14].

The discovery of new signalling components linking intracellular Ca\(^{2+}\) stores to plasma-membrane Ca\(^{2+}\) entry brought a new insight into the understanding of Ca\(^{2+}\) homoeostasis. STIM1 was identified as a Ca\(^{2+}\) sensor essential for Ca\(^{2+}\)-store-depletion-triggered Ca\(^{2+}\) influx [15,16]. Roos et al. [15] showed that knockdown of STIM in Drosophila S2 cells significantly reduced thapsigargin-dependent Ca\(^{2+}\) entry and completely suppressed \(I_{\text{CRAC}}\). In addition to being an ER Ca\(^{2+}\) sensor, STIM1 functions within the plasma membrane to control the operation of the Ca\(^{2+}\) entry channel itself [17], and STIM1 migrates from the Ca\(^{2+}\) store to the plasma membrane in conditions of store depletion [18].

It was later demonstrated that Orai1 is an essential pore subunit of the CRAC channel [19]. Accordingly, upon depletion of ER Ca\(^{2+}\) stores, STIM1 and Orai move in a co-ordinated fashion to form closely apposed clusters in the ER and plasma membrane [20], creating the elementary unit of SOCE [21]. In addition, the interaction between STIM1 and Orai1 is greatly enhanced after thapsigargin treatment, which acts as selective inhibitor of the ER Ca\(^{2+}\)-ATPase, resulting in depletion of ER Ca\(^{2+}\) stores [22].

**Figure 1**  Aortas from SHRSP have augmented spontaneous tone after depletion of Ca\(^{2+}\) stores

The tracings illustrate the protocol used to evaluate force in response to Ca\(^{2+}\) influx after depletion of intracellular Ca\(^{2+}\) stores (loading period). Briefly, aortic rings from WKY rats (A and C) and SHRSP (B and D) were stimulated with 1 \(\mu\)mol/l PE (phenylephrine) for 15 min. Aortas were washed in Ca\(^{2+}\)-free EGTA buffer, in order to deplete intracellular Ca\(^{2+}\) stores, for 20 min. During the depletion period, arteries were incubated with vehicle (A and B) or 1 \(\mu\)mol/l thapsigargin (C and D). After Ca\(^{2+}\) depletion, intracellular Ca\(^{2+}\) stores were loaded (loading period) by placing the aortas in 1.6 mmol/l Ca\(^{2+}\) buffer for 15 min. Contractile responses during the loading period, taken as a functional measure of Ca\(^{2+}\) influx, were determined. The bathing medium was then replaced with Ca\(^{2+}\)-free buffer and, after 2 min, aortas were stimulated with caffeine (10 mmol/l), in order to deplete intracellular Ca\(^{2+}\) stores, which resulted in a transient contraction. The magnitude of this last response was taken as a measure of the functional capacity of the SR to release Ca\(^{2+}\).
from intracellular stores [26], reduced Ca\(^{2+}\) uptake by the SR (sarcoplasmic reticulum) [27], impaired function of Ca\(^{2+}\)-binding proteins [28], decreased Ca\(^{2+}\) extrusion mechanisms in the plasma membrane [26], and increased Ca\(^{2+}\) influx [29,30].

In the present article, we will focus on how increased Ca\(^{2+}\) influx through the STIM1/Orai1 pathway may contribute to augmented vascular reactivity in hypertension. We have demonstrated that aortas from SHRSP (stroke-prone SHR (spontaneously hypertensive rats)) had increased force development during Ca\(^{2+}\) loading, upon depletion of intracellular Ca\(^{2+}\) stores [31]. The SR Ca\(^{2+}\) store is larger in aortas from SHRSP due to an enhanced influx of Ca\(^{2+}\) across the sarcolemma, rather than an impaired recycling of the cation by the SR Ca\(^{2+}\)-ATPase [32].

It has been shown that depletion of ER Ca\(^{2+}\) stores induces greater SOCE activation in vascular myocytes from SHRSP compared with that in control WKY (Wistar–Kyoto) rats (Figure 1) [30]. This is associated with augmented vascular contractile responses to Ca\(^{2+}\), which is blocked by molecular (neutralizing antibodies against STIM1 and Orai1) and pharmacological (2-APB and Gd\(^{3+}\)) inhibition of STIM1/Orai signalling. In addition, vascular expression of STIM1 (Figure 2) and Orai proteins is increased in SHRSP compared with WKY rats. Thus augmented STIM1/Orai signalling may represent a mechanism leading to impaired control of intracellular Ca\(^{2+}\) in hypertension.

The microvasculature network plays an important role in blood pressure control. During hypertension, the microvasculature displays both functional and structural changes that have been implicated in the augmented peripheral resistance. Additionally, the microvasculature displays myogenic activity in response to increases in blood pressure. It seems likely that increased activation of the STIM1/Orai1 pathway can be a contributor of vascular dysfunction during hypertension. Therefore studies addressing the STIM1/Orai1 pathway in the microvasculature represent an exciting topic that should be carefully evaluated.

**SEX DIFFERENCES IN HYPERTENSION**

Blood pressure is higher in men than in age-matched women, and there is a lower incidence of hypertension in pre-menopausal women than men [33–36]. Although sex-associated differences during hypertension have been repeatedly observed in epidemiological studies, the mechanisms for sex differences in blood pressure control are not totally elucidated.

Additionally, because Ca\(^{2+}\) triggers VSMC function and its regulation is highly controlled, differences in Ca\(^{2+}\)-handling mechanisms have been proposed to explain sex-related differences in vascular function during hypertension [37], as will be discussed.

**Sex differences in hypertension and Ca\(^{2+}\) handling by vascular myocytes**

Although sex-associated differences in hypertension are well established, with important differences in the neural, renal and vascular mechanisms associated with blood pressure homeostasis, the mechanisms that determine differences in blood pressure control in males and females are not totally elucidated. Considering that sex-related differences in mechanisms that regulate Ca\(^{2+}\) entry and storage in VSMCs have been identified, differential Ca\(^{2+}\) handling in VSMCs from males and females may explain sex-related differences in vascular function in hypertension [37]. Accordingly, in aorta from both normotensive and female SHR, Ca\(^{2+}\) influx upon contractile stimuli is decreased compared with that in male SHR [10]. In addition, VSMCs from female rats have reduced Ca\(^{2+}\) entry and decreased depolarization-induced Ca\(^{2+}\) levels compared with those in males [8]. Differences in intracellular Ca\(^{2+}\) increases in VSMCs from male and female SHR are abolished in the absence of extracellular Ca\(^{2+}\) [37–39].

Since augmented STIM1/Orai function may represent one mechanism that contributes to abnormal Ca\(^{2+}\) in VSMCs, we hypothesize that vascular protection in hypertensive females reflects an attenuated signalling through STIM1/Orai in vascular myocytes from hypertensive females (Figure 3).

In agreement with this hypothesis, we have shown that, upon store depletion, force development during Ca\(^{2+}\) loading period is augmented in aortas from male SHRSP compared with female SHRSP [40]. Interestingly, pharmacological blockade of the CRAC channel is able to abolish sex differences in spontaneous contractions during the Ca\(^{2+}\) loading period [40]. Additionally, after store depletion, neutralizing antibodies against STIM1 and Orai1 abolish sex-related differences in spontaneous contractions.

Considering that the female sex hormone oestrogen and progesterone have protective effects in the cardiovascular system [41,42], it will be interesting to determine whether sex steroids modulate STIM1/Orai1 activity/expression. In addition, studies showing whether the effects of sex steroids on this pathway are genomic or non-genomic will help to provide a better understanding of the mechanisms associated with female vascular protection. It is possible that augmented STIM1/Orai signalling represents a mechanism for the increased activation of Ca\(^{2+}\)-dependent signalling pathways in arteries from hypertensive animals. Identification of the mechanisms leading to sex differences in hypertension may uncover a regulatory mechanism that may be used to confer cardiovascular protection.
**Figure 2** SHRSP have increased vascular expression of STIM-1

Immunocytochemistry for STIM1 was performed in cultured rat aorta VSMCs plated on glass coverslips (5000 cells/cm²). After 24 h in serum-free medium, cells were washed, fixed in 4% paraformaldehyde for 10 min, permeabilized (0.1% Nonidet P40) and incubated in blocking buffer [1% (w/v) BSA in PBS] for 30 min at room temperature (25°C). Cells were incubated with the primary antibodies rabbit anti-STIM (1:100 dilution; ProSci) for 1 h at 37°C and counterstained with a FITC-conjugated anti-(rabbit IgG) secondary antibody (1:500 dilution; Jackson Immunochemistry) at 4°C overnight. Cells were then incubated with 5 μl/ml 4′,6-diamidino-2-phenyindole (DAP; Sigma) for 20 min to detect nuclei. Coverslips were mounted, and labelled cells were examined using a Zeiss microscope and software.

**Figure 3** Putative mechanism by which augmented STIM-1/Orai1 signalling contributes to abnormal Ca²⁺ homoeostasis in vascular myocytes

We hypothesized that, compared with females, arteries from male subjects have increased activation of STIM1/Orai1 (highlighted in the circles). In conditions of depletion or decrease of intracellular Ca²⁺ stores, STIM1 is activated, resulting in the dimerization of Orai1 and the formation of CRAC channels. Ca²⁺ influx, which is increased in VSMCs from hypertensive males compared with hypertensive females, is stimulated via STIM1 and CRAC channels (Orai1). Therefore arteries from hypertensive male subjects have increased Ca²⁺ influx. SR Ca²⁺-ATPase is activated and the intracellular Ca²⁺ stores are refilled. However, the SR in VSMCs of female and male hypertensive subjects have similar buffering capacity. Consequently, arteries from hypertensive male subjects have increased free cytosolic Ca²⁺, which can lead to increased vascular contraction. G, G-protein; GPCR, G-protein-coupled receptor; IP₃, Inos(1,4,5)P₃; PIP₂, PtdIns(4,5)P₂; PLC, phospholipase C; PM, plasma membrane; TYR, receptor tyrosine kinase.
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**REFERENCES**


