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

Fernanda R.C. Giachini^{1,2}, R. Clinton Webb¹, and Rita C. Tostes^{1,2}

¹ Department of Physiology, Medical College of Georgia – Augusta – GA – United States

² Department of Pharmacology, University of Sao Paulo – Sao Paulo – SP – Brazil

Abstract

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

Keywords

STIM; Orai; hypertension; sexual differences

1.0 - Introduction

Defective regulation of intracellular calcium (Ca^{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 discovery of new signaling components linking intracellular Ca^{2+} stores to plasma membrane Ca^{2+} entry brought a new insight into the understanding of Ca^{2+} homeostasis. Stromal interaction molecule 1 (STIM1) is the Ca^{2+} sensor protein that triggers Ca^{2+} influx in response to Ca^{2+} store depletion, whereas Orai is an essential component of CRAC (Ca^{2+} release-activated Ca^{2+}) channels. Although research on STIM1/Orai signaling 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 to gonadally intact males. Although the existence of sex differences in vascular Ca^{2+} handling is well established [7–10], no studies have addressed differences in vascular STIM1/Orai signaling in male and female hypertensive animals.

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

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

2.0 - STIM1-Orai1 pathway represents a key Ca^{2+} handling mechanism

Nearly all cell types depend on cytoplasmatic 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. inositol trisphosphate (IP_3)-mediated release of ER 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, lead to the term store-operated Ca^{2+} entry (SOCE) [12,13]. SOCE carries a highly Ca^{2+} -selective, non-voltage-gated, inwardly rectifying current termed CRAC (Ca^{2+} release-activated Ca^{2+}) current, or I_{CRAC} [13,14].

The discovery of new signaling components linking intracellular Ca^{2+} stores to plasma membrane Ca^{2+} entry brought a new insight into the understanding of Ca^{2+} homeostasis. Stromal interaction molecule 1 (STIM1) was identified as a Ca^{2+} sensor essential for Ca^{2+} -store depletion-triggered Ca^{2+} influx [15,16]. Roos and colleagues (2005) showed that knockdown of STIM in *Drosophila* S2 cells significantly reduced thapsigargin-dependent Ca^{2+} entry and completely suppressed I_{CRAC} [15]. In addition to being an ER Ca^{2+} sensor, STIM1 functions within the plasma membrane to control 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 coordinated 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].

2.1 - STIM1-Orai1 pathway alterations and hypertension

Since a rise in intracellular free Ca^{2+} concentration is the principal process that initiates contraction of vascular smooth muscle cells (VSMCs) [23], the maintenance of the steady-state Ca^{2+} is critically important to maintain vascular tone and, consequently, total peripheral resistance [24]. Defective regulation of intracellular Ca^{2+} plays a major role in the augmented vascular reactivity [25]. Augmented Ca^{2+} levels in VSMCs from hypertensive animals can be attributed to various mechanisms, including increased Ca^{2+} release from intracellular stores [26]; reduced Ca^{2+} uptake by the SR [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].

Here we will focus on how increased Ca^{2+} influx through STIM1 and Orai1 pathway may contribute to augmented vascular reactivity in hypertension. We first demonstrated that aortas from stroke-prone spontaneously hypertensive rats (SHRSP), displayed 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 was shown that depletion of ER Ca^{2+} stores induces greater SOCE activation in vascular myocytes from SHRSP, compared to that in control Wistar Kyoto (WKY - Figure 1) rats [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 signaling. In addition, vascular expression of STIM1 (Figure 2) and Orai proteins is increased in SHRSP versus WKY. Thus, augmented STIM1/Orai signaling may represent a mechanism leading to impaired control of intracellular Ca^{+2} in hypertension.

3.0 - 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 gender-associated differences during hypertension have been repeatedly observed in epidemiological studies, the mechanisms for gender 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.

3.1 - 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 [49–53], 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 to that in male SHR [10]. In addition, VSMCs from female rats display reduced Ca^{2+} entry and reduced depolarization-induced Ca^{2+} levels, compared to those in male [8]. Differences in intracellular Ca^{2+} increases in VSMC 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 VSMC, we hypothesize that vascular protection in hypertensive females reflects an attenuated signaling through STIM1/Orai in vascular myocytes from hypertensive females (figure 3).

In agreement with this hypothesis, we showed that upon store depletion, force development during Ca^{2+} loading period is augmented in aortas from male SHRSP, compared to female SHRSP. Interestingly, pharmacological blockade of CRAC channel is able to abolish sex-differences in spontaneous contractions during Ca^{2+} loading period [40]. Additionally, after

store depletion, neutralizing antibodies against STIM1 and Orai1 abolish sex-differences in spontaneous contractions.

It is possible that augmented STIM1/Orai signaling represents a mechanism for increased activation of Ca^{2+} -dependent signaling pathways in arteries from hypertensive animals. Identification of mechanisms leading to sex differences in hypertension may uncover a regulatory mechanism that may be used to confer cardiovascular protection.

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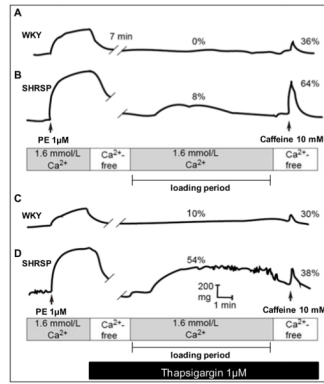


Fig.1. SHRSP aortas display augmented spontaneous tone after depletion of Ca^{2+} stores
 Inhibition of the SR Ca^{2+} -ATPase with thapsigargin augmented contractile responses to Ca^{2+} with greater effects in SHRSP. Representative traces.

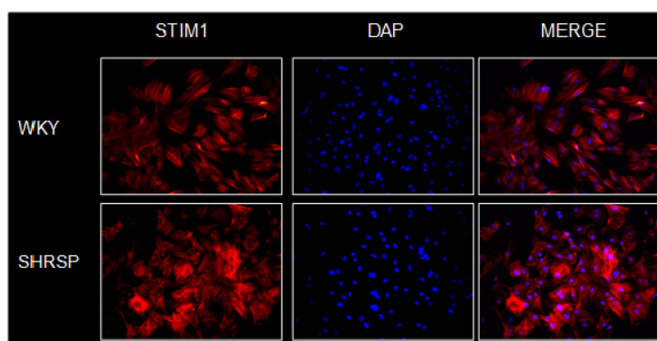


Fig. 2. SHRSP displays increased vascular expression of STIM-1

Immunocytochemistry performed in rat aorta vascular smooth muscle cells. Cells were incubated with primary antibodies rabbit anti-STIM1 and counterstained with a Cy3-conjugated anti-mouse IgG secondary antibody. Cells were incubated with 4',6-diamidino-2-phenylindole (DAPI, D-9564) to detect nuclei.

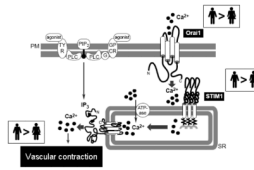


Fig. 3. Putative mechanism by which augmented STIM-1/Orai1 signaling contributes to abnormal Ca^{2+} homeostasis in vascular myocytes

Compared to female, arteries from male subjects display increased activation of STIM1/Orai1. In conditions of depletion or decrease of intracellular Ca^{2+} stores, STIM1 is activated, resulting in the dimerization of Orai1, and the formation of CRAC channels. Ca^{2+} influx, which is increased in VSMCs from hypertensive males compared to hypertensive females, is stimulated via STIM1 and CRAC channels (Orai1). Therefore, arteries from hypertensive male subjects display increased Ca^{2+} influx. SR Ca^{2+} ATPase is activated and the intracellular Ca^{2+} stores are refilled. However, the SR in VSMCs of female and male hypertensive displays similar buffering capacity. Consequently, arteries from hypertensive male subjects show increased free cytosolic Ca^{2+} , which can lead to increased vascular contraction.