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The role of STIM1 and SOCE in smooth muscle contractility

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Abstract

Contraction is a central feature for skeletal, cardiac and smooth muscle; this unique feature is largely dependent on calcium (Ca^{2+}) signaling and therefore maintenance of internal Ca^{2+} stores. Stromal interaction molecule 1 (STIM1) is a single-pass transmembrane protein that functions as a Ca^{2+} sensor for the activation store-operated calcium channels (SOCCs) on the plasma membrane in response to depleted internal sarco(endo)plasmic (S/ER) reticulum Ca^{2+} stores. STIM1 was initially characterized in nonexcitable cells; however, evidence from both animal models and human mutations suggests a role for STIM1 in modulating Ca^{2+} homeostasis in excitable tissues as well. STIM1-dependent SOCE is particularly important in tissues undergoing sustained contraction, leading us to believe STIM1 may play a role in smooth muscle contraction. To date, the role of STIM1 in smooth muscle is unknown. In this review, we provide a brief overview of the role of STIM1-dependent SOCE in striated muscle and build off that knowledge to investigate whether STIM1 contributes to smooth muscle contractility. We conclude by discussing the translational implications of targeting STIM1 in the treatment of smooth muscle disorders.

Keywords

Stromal interaction molecule 1 (STIM1); Store-operated calcium entry; Store-operated calcium channels; Smooth muscle contraction

1. Introduction

1.1. Store-operated calcium entry and STIM1

The concept of store-operated calcium entry (SOCE) was first introduced in 1986 with a series of experiments by the Putney lab suggested that depletion of internal calcium (Ca^{2+})

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stores controlled the extent of Ca^{2+} influx in nonexcitable cells [1]. This mechanism of Ca^{2+} entry served as a link between extracellular Ca^{2+} and intracellular Ca^{2+} stores. When the stores were full, no Ca^{2+} influx was detected, but when the stores were emptied, Ca^{2+} entry developed. Indeed, SOCE is now recognized as a ubiquitous pathway that functions to maintain Ca^{2+} homeostasis in response to depletion of internal sarco(endo)plasmic (S/ER) Ca^{2+} stores [1,2]. Extensive research over the last decade has now defined key aspects of SOCE including a Ca^{2+} sensor for store depletion and the Ca^{2+} entry pore. Stromal interaction molecule 1 (STIM1) is a single-pass transmembrane protein that acts as a Ca^{2+} sensor by activating store-operated calcium channels (SOCCs) following S/ER Ca^{2+} store depletion [3,4]. SOCCs are highly selective Ca^{2+} channels at the plasma membrane comprised of Orai1 multimers [5,6]. In the presence of adequate S/ER Ca^{2+} stores, Ca^{2+} ions bind to an EF-hand motif in the luminal domain of STIM1 [3,4]. Depletion of S/ER Ca^{2+} stores results in STIM1 aggregation (puncta formation) and subsequent allosteric activation of SOCCs [6–8]. The function of STIM1 was initially characterized in non-excitable cells; however, evidence from animal models and human mutations suggests a role for STIM1-dependent SOCE in a variety of excitable tissues. The purpose of this review is to provide a brief overview of the role of STIM1-dependent SOCE in striated muscle and build on that knowledge to investigate the lesser known role of STIM1 and SOCE in smooth muscle contractility.

1.2. SOCE in skeletal muscle

Despite early descriptions of a non-voltage dependent Ca^{2+} entry pathway in skeletal muscle fibers, the physiologic role for SOCE in skeletal muscle had been controversial [9]. Since the identification of STIM1 and Orai1 as components of SOCE, evidence has emerged that STIM1 and Orai1 are expressed in muscle where they control SOCE in order to refill SR Ca^{2+} stores [10]. Moreover, mutations in STIM1 and Orai1 in humans and mice cause two different forms of skeletal myopathies. Loss of function mutations in Orai1 and STIM1 lead to a phenotype that includes hypotonia and muscle atrophy of slow twitch fibers [11,12]. In contrast, gain of function mutations in STIM1 and Orai1 confer constitutive Ca^{2+} entry in muscle fibers even in the absence of store depletion. These patients develop a tubular aggregate myopathy that results from the accumulation of SR membrane [13–18]. Mouse models with identical mutations in STIM1 and Orai1 strongly resemble these human myopathies. It is clear from studies using these mouse models that SOCE has prominent role in muscle performance and function. It is also evident from these studies that regulation of SOCE in skeletal muscle is critically important for muscle development and contractility.

SOCE is present in resting muscle fibers and is needed to maintain Ca^{2+} homeostasis. Loss of SOCE does not however directly impact excitation contraction coupling (ECC), the Ca^{2+} signaling process that triggers muscle contractility. Single muscle contractions remain intact for muscle fibers lacking STIM1 or Orai1 indicating Ca^{2+} release from a single membrane depolarization remains intact. In contrast, high frequency stimulation of muscle activates SOCE in order to sustain muscle contraction. It is likely that this level of stimulation is seen during muscle development and mature muscle during exercise. Development of inducible, skeletal muscle specific mouse models offers the opportunity to address remaining questions. An important observation by our group and others is that the skeletal muscle

phenotype described for the STIM1 null mice is far more severe than that described for the Orai1 null mice. Indeed, blocking or deleting Orai1 channels from skeletal muscles result in a mild myopathy that involves loss of slow twitch fibers. The myopathy results from the developmental deletion of Orai1 and reduced Ca^{2+} dependent growth signaling during periods of intense neonatal muscle growth. Deletion of STIM1 from skeletal muscle leads to a reduction in muscle contractility, distorted muscle structures and a drastic reduction in survival. One reason for this difference between the Orai1 and STIM1 null phenotype is that STIM1 is a multifunction signaling scaffold that may influence target proteins beyond the well-established interaction with Orai channels. We used a phage display to screen for novel STIM1 interacting partners. We found that STIM1 interacts with phospholamban in cardiomyocytes in order to enhance Ca^{2+} store refilling by S/R Ca^{2+} ATPase (SERCA2a) [19]. These studies have opened key insight to the putative function of STIM1 in cardiac muscle. Our studies implicate STIM1 dependent Ca^{2+} signaling in cardiac pacemaking through the coordinated refilling of SR stores.

1.3. SOCE in cardiac muscle

Following the description of STIM1 in skeletal muscle, several groups proposed a similar role for SOCE in cardiac muscle [20–22]. Changes in the frequency and amplitude of intracellular calcium transients are altered by neural or hormonal inputs to cardiomyocytes to modify the rate and/or the force of muscle contraction. Recent studies describe how SOCE is induced by the neural and hormonal inputs. Using gene delivery techniques and loss of function mouse models it appears that STIM1 induction and SOCE are critical for the development of heart failure [20,23]. Here, STIM1 and SOCE are induced as part of the fetal gene program that is known to be expressed in hypertrophied and the failing heart. STIM1 seems to be a primary event in this process as the transgenic overexpression in the heart leads to cardiac failure [24]. An interesting concept has emerged that although STIM1 is operational in the cardiomyocyte this action is independent of Ca^{2+} entry through Orai1 [19]. While STIM1 KO cardiomyocytes displayed altered SR Ca^{2+} signaling, we did not detect SOC currents. Rather, we found that STIM1 interacts with phospholamban to regulate Ca^{2+} refilling of SR Ca^{2+} stores. We identified the interaction of STIM1 and phospholamban in a screen using phage display. Along the same lines the Chatham laboratory has proposed that STIM1 functions to mitigate the ER stress in response to hypertrophic agonists [25]. Another possibility is that STIM1 interacts and regulates the voltage gated Ca^{2+} currents in cardiomyocytes (cav1.2). Although this interaction was mapped out in neurons and smooth muscle cells, it has been difficult to demonstrate the relevance in STIM1 mutant mice [26,27]. Separating STIM1 from SOCE is consistent with the longstanding belief that STIM1 is a multifunctional signaling scaffold for Ca^{2+} signaling. We propose that much like described for skeletal muscle, STIM1 Ca^{2+} signaling is required during fast heart rate where SOCE is needed to refill the SR Ca^{2+} stores of sinoatrial cells. It is likely that SOCE is a key component of the Ca^{2+} clock mechanism during cardiac pacemaking [28].

2. STIM1 and SOCE in smooth muscle

2.1. Overview

Smooth muscle contraction is driven predominantly by Ca^{2+} flux. Increased cytoplasmic Ca^{2+} concentrations ($[\text{Ca}^{2+}]_i$) initiate a cascade of intracellular events resulting in cross-bridge cycling and subsequent cellular contraction (reviewed by Webb et al. [29]). In brief, cytoplasmic Ca^{2+} binds to calmodulin; the Ca^{2+} -calmodulin complex activates myosin light chain kinase, which results in phosphorylation of the myosin light chain and resultant contraction [29]. Persistent low levels of myosin light chain phosphorylation in the absence of external stimuli are thought to contribute to smooth muscle tone [29]. Multiple pathways contribute to increased $[\text{Ca}^{2+}]_i$ including voltage-operated calcium channels (VOCCs), receptor-operated calcium channels (ROCCs) and store-operated calcium channels (SOCCs) [29,30]. The degree to which each pathway contributes to Ca^{2+} flux in smooth muscle is unknown and likely tissue-dependent.

The advent of the S/ER Ca^{2+} -ATPase inhibitors cyclopiazonic acid (CPA) and thapsigargin (TG) has led to the isolation and study of store depletion-induced Ca^{2+} flux [31]; thus allowing for the investigation of SOCE in smooth muscle. STIM1 has been identified as a mediator of smooth muscle proliferation and remodeling [32–35]; however, whether STIM1-dependent SOCE contributes to smooth muscle contractility remains debated [35].

The effects of STIM1 mutations on smooth muscle are unclear. Evidence from case reports suggests at least some involvement of smooth muscle in patients with loss-of function (LoF) mutations including iris hypoplasia and mydriasis [12]. Reports also exist of primary enuresis [36], suggesting the possible involvement of the bladder wall. Unfortunately, given the severity of disease conferred by STIM1 deficiency, no data is available regarding fertility or reproductive status of these patients. Furthermore, the severity of syndrome makes it difficult to assess the functions of other smooth muscles such as blood vessel and gastrointestinal tract. Persistent and severe infection secondary to SCID results in a variety of confounding factors that may influence smooth muscle contractility. Similarly, not much is known about the effect of STIM1 GoF on smooth muscle contractility. Whether the myopathy characteristic of STIM1 GoF extends to smooth muscle has not been investigated; however, reports of severe congenital miosis in patients with GoF mutations [13–15] suggest a defect in pupillary smooth muscle. Herein, we examine evidence from a variety of animal models supporting a role for SOCE and STIM1 in modulating smooth muscle contractility.

2.2. Vascular smooth muscle

A series of studies (summarized in Table 1) performed prior to the molecular identification of STIM1 and Orai1 suggest a role for capacitative Ca^{2+} entry in regulating vascular smooth muscle (VSM) contractility and tone [31,37–42]. The specific contribution of SOCCs (versus VOCCs) to Ca^{2+} flux is vessel dependent [31]. For example, SOCCs appear to contribute little to aortic smooth muscle Ca^{2+} flux; in aortic rings harvested from rats, CPA-induced Ca^{2+} flux was abolished by the voltage-sensitive Ca^{2+} channel blocker nifedipine [38,39,42]. In contrast, CPA-induced contractions of muscle strips harvested from rat pulmonary artery were resistant to treatment with both nifedipine and the calcium channel

blocker verapamil [37]. CPA-induced contraction in muscle strips harvested from rat carotid [41] and femoral [40] arteries were only partially inhibited by nifedipine, suggesting a role for both SOCCs and VOCCs in store depletion-induced Ca^{2+} in these tissues.

More recent studies have focused on using mouse models of STIM1 deficiency to investigate the role of STIM1 on VSM contraction. Mancarella et al. [43] developed a smooth muscle-specific STIM1 knockout mouse (sm-STIM1-KO) using cre-lox technology. Sm-STIM1-KO mice are smaller in size than WT littermates (sm-STIM1-WT) and have a 30–40% mortality rate within the first month of life [43]. Histological analysis of aortic tissue from sm-STIM1-KO mice shows significant thinning of the tunica media [43]. Aortic rings were harvested from sm-STIM1-KO mice and contraction was compared to that from sm-STIM1-WT littermates; both store-dependent and α 1-adrenergic agonist-induced contraction were decreased in sm-STIM1-KO tissue, however, KCl-induced contraction was unchanged [43]. These findings suggest diversity in the activation signal for muscle contraction that may or may not involve STIM1 and SOCE.

STIM1 and Orai1 are upregulated in the tunica media of the aorta in spontaneously hypertensive rats [44]. Contractile analysis of aortic rings revealed increased TG-induced contraction and basal tone in hypertensive animals compared to that of WT [44]. Interestingly, the differences in both TG-induced contraction and basal tone were abolished by treating tissues with either STIM1 or Orai1 neutralizing antibodies [44], suggesting STIM1-dependent SOCE as a potential target for the treatment of spontaneous hypertension.

STIM1 may also play a role in modulating splenic smooth muscle contraction. Burt et al. demonstrated that CPA-induced contraction of the rat spleen that was resistant to nifedipine but sensitive to SKF96365 [45], suggesting a role for SOCCs in tonic contraction of the spleen.

2.3. Gastrointestinal smooth muscle

Gastrointestinal (GI) smooth muscle regulates the passage of food and waste through the body and its appropriate function is critical to preventing malabsorption, diarrhea and a large variety of other GI smooth muscle-associated pathologies. Little is known about the role of STIM1 in GI smooth muscle contraction; however, evidence from animal models suggests a role for SOCE in maintaining GI smooth muscle tone. Ohta et al. [46] found that muscle strips isolated from the longitudinal smooth muscle layer of the rat ileum exhibited CPA-induced contraction that was resistant to both nifedipine and methoxyverapamil. Further studies performed in smooth muscle strips from both cat and guinea pig stomach smooth muscle showed CPA-induced tonic contractions that were only partially inhibited by nifedipine [47]. These findings are further supported by evidence of thinning of the muscularis propria in the stomach in sm-STIM1-KO mice, as well as general distention and dilatation of their GI tract [43]. Taken together, these findings suggest that STIM1 is required for maintaining tone in the GI smooth muscle and that STIM1 deficiency results in both morphological and functional aberrations.

2.4. Genitourinary smooth muscle

2.4.1. Male reproductive tract—Little is known about the role of STIM1-dependent SOCE in the male reproductive tract; however, a recent study by Sung et al. [48] suggests a role for STIM1 in moderating penile erection. Adequate function of the cavernous smooth muscle (CSM) of the penis is required for erection. In the flaccid state, CSM is tonically contracted, restricting blood flow [49]; CSM must relax in order for erection to occur. STIM1 is present and functionally active in human penile cavernous smooth muscle (CSM) [48]. Interestingly, transfection of dominant negative mutants of either STIM1 or Orai1 into the penile corpeal tissue was sufficient to restore erectile function in a rat model of diabetes-induced erectile dysfunction [48], suggesting inhibition of SOCE could be used clinically to treat pathologies of impaired smooth muscle relaxation such as erectile dysfunction, hypertension or preeclampsia.

2.4.2. Female reproductive tract—STIM1 has been identified in human myometrial samples [50]; however, the role of STIM1 in the female reproductive tract remains largely unknown. Noble et al. [51] demonstrated that CPA-induced Ca^{2+} flux in term-pregnant rat myometrial strips are only partially sensitive to nifedipine (20%); interestingly, the remaining 80% is sensitive to SKF96365 [51], indicating a potential role for SOCE in the pregnant myometrium. Our preliminary data confirms the presence of STIM1 in both the human and murine myometrium, and also shows significant STIM1 expression in the smooth muscle layer of the fallopian tubes (*unpublished data*), suggesting a possible role for STIM1 in modulating fertility.

STIM1 null ($\text{STIM1}^{\text{LacZ/LacZ}}$) mice were generated as previously described [10]. Examination of uterine horns from non-pregnant STIM1 KO mice show thinning of both the outer transverse and inner circular layers of the myometrium compared to that of WT ($\text{STIM1}^{+/+}$) littermates (Fig. 1). Anecdotal evidence suggests STIM1 null females die during parturition, suggesting a role for STIM1 in myometrial contraction. To investigate this, myometrial strips were harvested from WT and KO mice. Representative myographs of spontaneous contraction (Fig. 2) suggest a severe contractile deficit conferred by STIM1 deficiency consistent with our anecdotal findings. Further studies are required to understand the role of STIM1-dependent SOCE in parturition.

3. Summary

Since the description of SOCE more than three decades ago, our fundamental knowledge about this pathway has grown enormously. The physiologic cellular function of SOCE is to sustain low levels of Ca^{2+} entry over an expanded time period to refill S/ER Ca^{2+} stores, to activate Ca^{2+} signal transduction cascades and maintain Ca^{2+} homeostasis. It is important to point out that understanding the role SOCE plays in excitable cells is important given the rapid development of highly selective Orai inhibitors. As SOCE inhibitors are developed clinically to treat a number of inflammatory conditions, the potential for off target side effects is high in striated and smooth muscle cells. For excitable cells, it is likely that SOCE is activated during intense stimulation, conditions that deplete internal Ca^{2+} stores for the whole cell but it is entirely possible that depletion of local S/ER stores is sufficient to

activate SOCE. In particular, the kinetics of SOCE are much faster in muscle fibers. How this is accomplished is not fully understood at present, but may involve pre-positioning of STIM1 in the S/ER near the SOCC in the T-Tubule membranes. It is also interesting to point out that STIM1 and SOCE contribute to the phenotypic differences within muscles. For example, STIM1 and SOCE are enriched in type IIa and I muscle fibers. These fibers, unlike fast glycolytic type Ib fibers, utilize SOCE during intense stimulations which might explain why deletion of STIM1 or Orai from mouse and human skeletal muscle leads to atrophy of these specific fiber types. In analogous fashion, STIM1 and SOCE are likely to have important role in the phenotypic transition of smooth muscle, such as in the myometrium. STIM1 and the Ca^{2+} signaling are likely to have key roles in the transition from the quiescent to contractile smooth muscle. STIM1 and SOCE in muscle must distinguish themselves from that described in non-excitabile cells by adapting different properties. To begin to understand these differences, we have attempted to define the components of the STIM1 and SOCE complexes in muscle fibers, as compared to non-excitabile cells. Other issues that need to be resolved is whether STIM1 activates targets other than Orai channels and may therefore direct the refilling of organelles beyond that described for S/ER (e.g. mitochondria). We are particularly interested to know if the properties defined in skeletal muscle can be extended to smooth muscle. With the development of new selective Orai1 inhibitors and mouse models that enable inducible deletion of STIM1 and Orai1 from adult skeletal and smooth muscle, we are now poised to address these critical questions.

Abbreviations:

Ca^{2+}	calcium
CSM	cavernous smooth muscle
CPA	cyclopiazonic acid
$[\text{Ca}^{2+}]$	icytoplasmic calcium concentration
GoF	gain of function
GI	gastrointestinal
LoF	loss of function
ROCC	sreceptor-operated calcium channels
S/ER	sarco(endo)plasmic reticulum
SCID	severe-combined immunodeficiency
STIM1	stromal interaction molecule 1
SOCC	sstore-operated calcium channels
SOCE	store-operated calcium entry
TG	thapsigargin

VSM	vascular smooth muscle
VOCC	svoltage-operated calcium channels

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Fig. 1.

Representative cross-sectional images of uterine horns harvested from non-pregnant (A) STIM1-WT (STIM1^{+/+}) and (B) STIM1-null (STIM1^{LacZ/LacZ}) mice. Tissues were paraffin embedded and stained with eosin; all images were obtained using bright-field microscopy at 20× magnification. Thinning of both the outer transverse and inner circular layers of the myometrium were observed in STIM1-null mice (arrows).

Fig. 2. Representative myographs of spontaneous uterine contractions in tissues harvested from (A) STIM1-WT (STIM1^{+/+}) and (B) STIM1-null (STIM1^{LacZ/LacZ}) mice. Uterine horns were isolated from non-pregnant STIM1-WT and STIM1-null mice and suspended in a tissue organ bath at 0.5 g of tension. Following a 20-min equilibration period, spontaneous contraction was recorded for analysis.

Table 1

Tissues showing increased $[Ca^{2+}]_E$ -dependent tone in response to Ca^{2+} -ATPase inhibitors.

Tissue	Species	Nifedipine	Verapamil	SKF96365	Refs.
Aorta	Rat	Sensitive			[38,39,42]
Carotid artery	Rat		Partial		[41]
Femoral artery	Rat		Partial		[40]
Pulmonary artery	Rat	Resistant	Resistant		[37]
Anococcygeus	Mouse	Partial		Sensitive	[52]
Gastric fundus	Cat	Partial			[47,53]
	Guinea pig	Partial			[47]
Ileum	Rat	Resistant			[46]
Spleen	Rat	Resistant		Sensitive	[45]
Urinary bladder	Rat				[54]

Adapted from Gibson et al. [31]. Tissues were treated with the Ca^{2+} -ATPase inhibitors cyclopiazonic acid (CPA) or thapsigargin. Nifedipine and verapamil were used to inhibit voltage-dependent Ca^{2+} channels; SKF96365 was used to inhibit SOCE.