

# Store-Operated Ca<sup>2+</sup> Entry in Muscle Physiology

Marco Brotto, Noah Weisleder and Jianjie Ma\*

*Department of Physiology and Biophysics, UMDNJ-Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 08854, USA*

**Abstract:** Store-operated Ca<sup>2+</sup> entry (SOCE) represents a unique Ca<sup>2+</sup> entry mechanism, where Ca<sup>2+</sup> channels located on the plasma membrane sense the Ca<sup>2+</sup> filling status of intracellular Ca<sup>2+</sup> stores and gate the entry of Ca<sup>2+</sup> from the extracellular reservoir to replenish intracellular Ca<sup>2+</sup> storage. This pathway has received great interest not only because of its unusual nature as a retrograde signal, but also due to its wide occurrence in both excitable and non-excitabile cells and its potential role in various physiological and pathophysiological situations. In skeletal muscle, contractility is contingent upon the maintenance of intracellular Ca<sup>2+</sup> homeostasis, which requires the preservation of low levels of resting cytosolic Ca<sup>2+</sup>, readily available releasable pool of Ca<sup>2+</sup> from the sarcoplasmic reticulum, as well as functional Ca<sup>2+</sup> uptake and extrusion mechanisms. Recent studies have demonstrated that SOCE is present in skeletal muscle, and may play a significant role in muscle physiology. While the need for SOCE is increased during strenuous muscle exercise and fatigue, disruption of this process can lead to pathophysiological conditions. Repressed SOCE activity has been linked to aging related dysfunction. Elevated SOCE could lead to elevated intracellular Ca<sup>2+</sup> in dystrophic muscle cells and progression of muscular dystrophy. The role of SOCE in the physiology and pathophysiology of skeletal muscle is a subject of increasing interest in the muscle biology field. Manipulation of SOCE by chemical, pharmacological and genetic approaches should have great potential in the treatment of muscle disorders that involve dysfunctional Ca<sup>2+</sup> homeostasis.

## STORE OPERATED CA<sup>2+</sup> ENTRY: A LINK BETWEEN INTRACELLULAR CA<sup>2+</sup> STORES AND THE EXTRACELLULAR CA<sup>2+</sup> RESERVOIR

Store-operated Ca<sup>2+</sup> entry (SOCE), or capacitative Ca<sup>2+</sup> entry (CCE), was originally proposed by Dr. James Putney as an extracellular Ca<sup>2+</sup> ([Ca<sup>2+</sup>]<sub>o</sub>) entry pathway in non-excitabile cells that is stimulated by the reduction of intracellular Ca<sup>2+</sup> stores [1, 2]. Since this initial finding, SOCE has been observed in many different cell types, including skeletal muscle [3, 4]. When the intracellular Ca<sup>2+</sup> stores of a cell, and in particular the stores within the endoplasmic reticulum (ER) or sarcoplasmic reticulum (SR), are reduced due to cellular activity, the cell must refill these stores to maintain Ca<sup>2+</sup> homeostasis and effective cellular function.

During the course of normal cellular physiology, Ca<sup>2+</sup> is released from the ER/SR into the cytosol primarily through the inositol 1,4,5-trisphosphate receptor (InsP3R) or ryanodine receptor (RyR) and the majority of this Ca<sup>2+</sup> returns to the ER/SR through the sarco/endoplasmic Ca<sup>2+</sup>-ATPase (SERCA) pump. A fraction of Ca<sup>2+</sup> is lost from the cell through the action of Ca<sup>2+</sup>-ATPase pumps in the plasma membrane. Thus, as these release and uptake cycles continue, there would be an eventual depletion of ER/SR Ca<sup>2+</sup> store without the intervention of Ca<sup>2+</sup> entry mechanisms. While multiple mechanism of Ca<sup>2+</sup> entry are present in many cell types, SOCE is one process by which the cell senses reductions in the ER/SR Ca<sup>2+</sup> store and induces Ca<sup>2+</sup> entry into the cytosol to compensate for the Ca<sup>2+</sup> lost to the extracellular space [5]. SOCE allows Ca<sup>2+</sup> entry into the cell from the vast reservoir of [Ca<sup>2+</sup>]<sub>o</sub> in response to depletion of

Ca<sup>2+</sup> within the ER/SR. The value of such a mechanism to the cell is obvious, as it provides a gate to refill the intracellular Ca<sup>2+</sup> storage. This pathway has received great interest not only because of its unusual nature as a retrograde signal, but also due to its wide occurrence in both excitable and non-excitabile cells and its potential role in various physiological and pathophysiological situations [6].

Interest in SOCE grew after the initial observation of a highly selective, non-voltage activated inward current that was activated by depletion of the ER Ca<sup>2+</sup> store of mast cells [7]. This Ca<sup>2+</sup> release activated Ca<sup>2+</sup> current (I<sub>CRAC</sub>) was subsequently identified in many cell types, particularly those of hematopoietic origin [8-10]. While I<sub>CRAC</sub> has been extensively studied as a model for SOCE, other currents with distinct conductance properties appear to account for SOCE in various cell types. These so called store-operated Ca<sup>2+</sup> currents, or I<sub>SOC</sub>, have been identified in many cell types [5], including smooth muscle cells [11, 12]. Studies from our laboratory and those of other investigators demonstrate that SOCE is present in skeletal muscle cells [3, 4, 13], and that SOCE may function during strenuous muscle exercise and fatigue [14-16].

Two major obstacles have slowed progress in the study of SOCE. The first is the lack of high specificity inhibitors of SOCE. While econazole, 1- [β- [3-(4-Methoxyphenyl)propoxy]-4-methoxyphenethyl]-1H-imidazole (SKF-96365) and 2-aminoethoxydiphenylborane (2-APB) have all been used to block SOCE in multiple cell types, they all display some degree of off-target effects [17]. A second major obstacle is the difficulty in resolving the molecular identity of store-operated Ca<sup>2+</sup> channels. In the search for these channels, much of the interest has focused on some members of the transient receptor potential (TRP) family of channel proteins. Many studies indicate that various TRP proteins contribute to SOCE activity in some cell types, while other studies show that these TRP proteins cannot reproduce all aspects

\*Address correspondence to this author at the Department of Physiology and Biophysics, UMDNJ-Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 08854, USA; Tel: (732) 235-4494; Fax: (732) 235-4483; E-mail: maj2@umdnj.edu

required of SOC [18]. Thus, the precise role for TRP proteins in mediating SOCE has not yet been clearly defined and has remained a target of significant study. It is possible that multiple proteins form a complex of regulatory factors and a pore conduction unit that vary in different cell types in order to facilitate SOCE. In this view, store-operated channels would consist of a macromolecular complex containing the membrane resident  $\text{Ca}^{2+}$  permeable pore subunit, plus cytosolic factors that integrate the retrograde signal from ER/SR to activation of SOCE. Such an arrangement would explain the difficulty in molecular determination of store-operated channels and that different current characteristics are observed in many cell types under various conditions.

Recent screenings of the *Drosophila* genome have revealed a gene, Orai1 [19] or CRACM1 [20], that is a strong candidate for a molecular component of SOCE, and perhaps even the pore conduction unit of the CRAC channel. Disruption of this gene results in ablation of  $I_{\text{CRAC}}$  in several cell types. This gene codes for a protein that is predicted to contain four transmembrane domains, however there is no clear pore structure to be resolved in the secondary structure of this protein and it does not contain the canonical glutamate ring sequence found in many  $\text{Ca}^{2+}$  selective channels. Considering that store-operated channels have different conduction properties than previously identified  $\text{Ca}^{2+}$  channels, it would not be surprising if the structure of this channel also diverged from the current understanding of  $\text{Ca}^{2+}$  channel structure. It is interesting to note that genetic disruption of Orai1/CRACM1 results in immune deficiency and no other obvious phenotypes in affected patients. This provides further evidence of heterogeneity of store-operated channels in different cell types and suggests that other family members, such as Orai2 and Orai3 may provide for similar functions in other cell types [21].

## MECHANISMS OF SOCE REGULATION

A major remaining question in the study of SOCE is the molecular mechanism responsible for the retrograde signaling from ER/SR  $\text{Ca}^{2+}$  store depletion to activation of SOCs on the plasma membrane. There have been several theories presented to account for this signal transduction, most of which fall into one of four major categories [22]. In the first, a diffusible messenger is released from the ER/SR to induce activation of SOCE. The diffusible messenger theory for SOCE activation was first proposed based on the finding that extracts from Jurkat cells with depleted  $\text{Ca}^{2+}$  stores could be used to activate SOCE in other cell types [23]. This undefined low-molecular weight factor was termed  $\text{Ca}^{2+}$  influx factor (CIF). Despite intensive studies following these findings, the role of CIF in the activation of SOCs is still not clear. However, a recent study has shown that CIF mediated activation of SOCE is likely dependent on a signaling cascade that involves  $\text{Ca}^{2+}$ -insensitive phospholipase A2 ( $\text{iPLA}_2$ ) [24]. While CIF is the most studied diffusible regulator of SOCE, numerous other factors have been suggested to regulate SOCE activation in several cell types. These include lysophospholipids [25] and protein kinase C [26] in smooth muscle cells, and calmodulin in skeletal muscle cells [27].

Another theory for the activation of SOCE is that store-operated channels are sequestered from the plasma mem-

brane into intracellular vesicles until ER/SR  $\text{Ca}^{2+}$  stores are depleted, which would result in the translocation of these vesicles to the membrane, much like the insulin activated translocation of GLUT4 glucose transporters. Much of the support for this theory is derived from the translocation of TRP channels to the plasma membrane following ligand-induced activation [28]. While early studies suggested that exocytosis may be important in regulation of SOCE [9, 29, 30], other studies have provided alternative interpretations of these findings [31-33]. Other more recent studies have suggested that exocytosis is not a major component in the regulation of SOCE in some cell types [34, 35].

Some have proposed that SOC may be inhibited by local  $\text{Ca}^{2+}$  concentration at the site of the channel on the plasma membrane [36]. In this case, SOCs would remain inactive as long as the  $\text{Ca}^{2+}$  concentration near their plasma membrane location was above a certain threshold. Reduction of the intracellular  $\text{Ca}^{2+}$  store would result in reduced  $\text{Ca}^{2+}$  concentrations beneath the plasma membrane and removal of this inhibition on SOCE.

The fourth major theory for SOCE regulation is one involving direct conformational or secretion coupling between the  $\text{Ca}^{2+}$  release channels on the ER/SR, particularly InsP3R, and the SOCs on the plasma membrane [37]. Direct coupling between ER/SR  $\text{Ca}^{2+}$  release channels and SOCs would likely result in rapid activation of SOCE following store depletion. This appears to be the case in mammalian skeletal muscle fibers [16, 38], but not the case in several other cell types [31]. This slow activation in some cell types lead to the hypothesis that remodeling of the ER/SR might follow depletion of the internal  $\text{Ca}^{2+}$  stores, allowing a slow translocation of InsP3R channels to a close physical proximity to the SOC on the plasma membrane, a so-called secretion-like mechanism [39]. These two versions are currently the most generally accepted mechanism for SOCE activation [22], however there are studies that suggest that these mechanisms are not at work in some cell types following store  $\text{Ca}^{2+}$  depletion [40] and that InsP3R is not required for SOCE activation in other cell types [41, 42]. Currently, the field has not reached a strong consensus as to the primary mechanism of SOCE activation. While current results point towards a more specific role for CIF in SOCE activation [43], the large number of studies conflicting with and in support of various models further suggests a heterogeneity of store-operated channels and regulatory processes for proper function of SOCE in various cell types. This likely reflects the specific kinetic and spatial requirements of different cells for maintenance of intracellular  $\text{Ca}^{2+}$  stores. While some common molecular machinery may prove to underlie the store-operated channels activity and/or SOCE regulation in different cell types, it is probable that the molecular machinery would be significantly different comparing a skeletal muscle fiber versus cells of hematopoietic origin. Thus, determination of the role of SOCE in cellular physiology and pathophysiology will require intensive efforts in several complementary cell model systems.

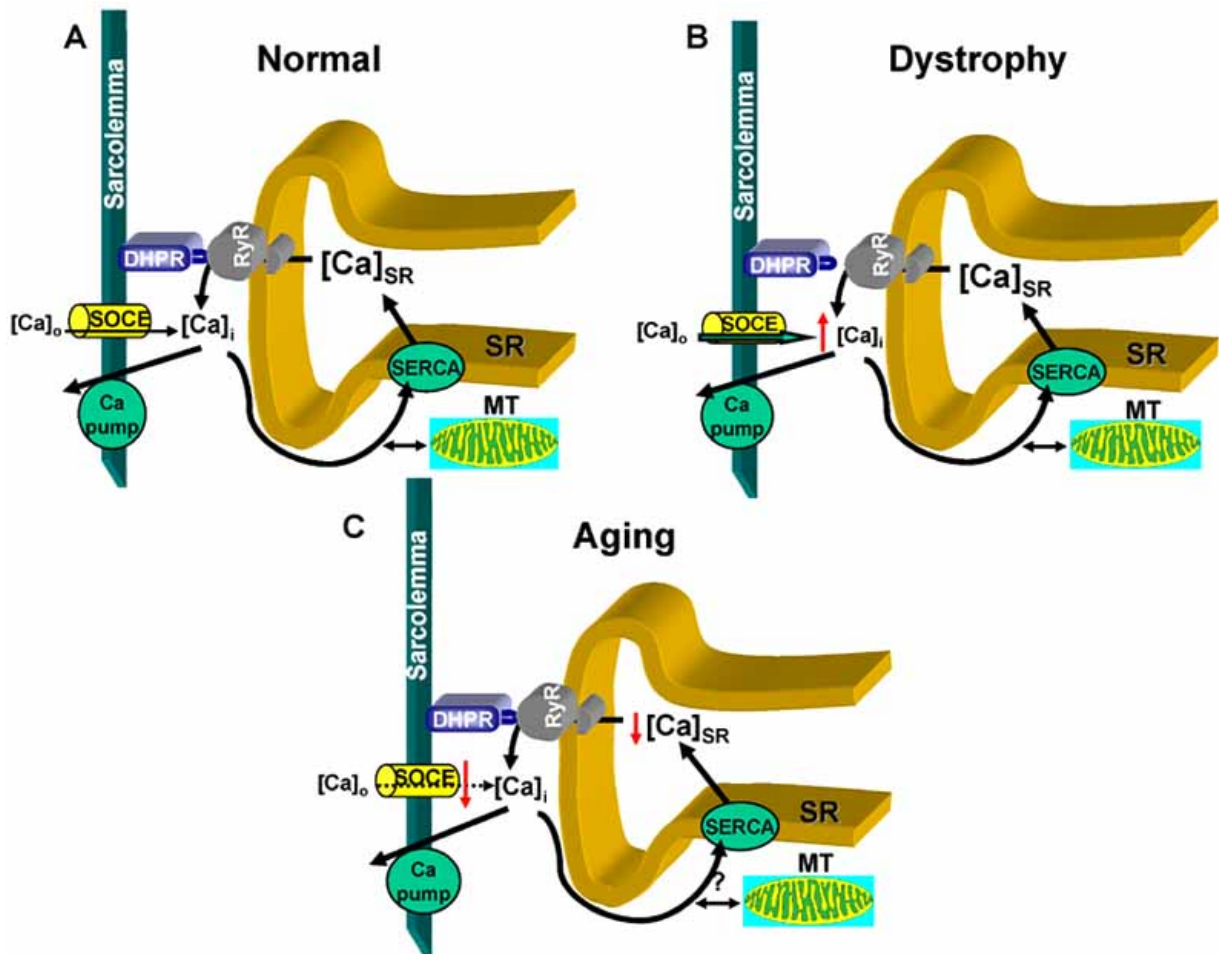
Recent studies have determined that specific intracellular factors, including  $\text{iPLA}_2$  and stromal interaction molecules (STIM1) [44-46] participate in the regulation of SOCE. STIM1 is a transmembrane protein found to localize throughout the ER/SR at the resting state, which contains a

$\text{Ca}^{2+}$  binding motif thought to act as a sensor for the loading state of the  $\text{Ca}^{2+}$  store within the ER/SR. Upon  $\text{Ca}^{2+}$  store depletion, STIM-1 translocates from a diffuse pattern in the ER/SR to a punctate pattern near the plasma membrane. This translocation appears to be essential, but perhaps not sufficient [47], to activate SOCE. Identification of these important SOCE regulatory factors provides an entry point for further dissection of the molecular mechanisms at work in SOCE regulation.

### EXTRACELLULAR $\text{Ca}^{2+}$ ENTRY AND SKELETAL MUSCLE CONTRACTILITY

In mammalian skeletal muscles, opening of the skeletal muscle RyR isoform (RyR1) channel is directly coupled to conformational changes of voltage sensors (i.e., dihydropyridine receptors, DHPR) located on the sarcolemmal

membrane [48, 49]. Upon depolarization of the sarcolemmal membrane,  $\text{Ca}^{2+}$  is rapidly released from the SR through RyR1. Consequently, the major resource for contraction in skeletal muscle originates from the SR  $\text{Ca}^{2+}$  store, not from  $[\text{Ca}^{2+}]_o$  entry. After the  $\text{Ca}^{2+}$  release phase, intracellular  $\text{Ca}^{2+}$  must be quickly returned to nanomolar levels for muscle relaxation to occur, a process that is primarily accomplished by the highly effective SERCA pump in the SR and also by  $\text{Ca}^{2+}$ -ATPase pumps located in the sarcolemmal membrane [50, 51] (Fig. 1). While the SERCA pump recycles the vast majority of  $\text{Ca}^{2+}$  from the cytoplasm back to the SR store, not all  $\text{Ca}^{2+}$  released during a contraction cycle returns to the SR, since a fraction of the  $\text{Ca}^{2+}$  buffered by mitochondria and another fraction is unavoidably lost through the SERCA pump in the sarcolemmal membrane, as illustrated in Fig. 1A. In fact, Hemmings has demonstrated that the use of  $\text{Ca}^{2+}$



**Fig. (1). Possible role of SOCE in skeletal muscle physiology and pathophysiology.**

**A.** Under normal, physiological conditions, depolarization of the sarcolemmal membrane leads to opening of the RyR  $\text{Ca}^{2+}$  release channel through conformational coupling to the DHPR.  $\text{Ca}^{2+}$  release from the SR constitutes the major source for skeletal muscle contraction. Sequestration of cytosolic  $\text{Ca}^{2+}$  back into the SR through SERCA leads to muscle relaxation.  $\text{Ca}^{2+}$  transport into and out of the mitochondria (MT) provides certain buffering capacity for the changes in cytosolic  $\text{Ca}^{2+}$ . With each contraction-relaxation cycle, a portion of cytosolic  $\text{Ca}^{2+}$  is pumped out of the muscle fiber by the sarcolemmal-membrane localized the  $\text{Ca}^{2+}$ -ATPase. SOCE provides an important  $\text{Ca}^{2+}$  entry pathway in response to increased muscle activity, e.g. exercise and fatigue. SOCE also serves to replenish the decreased SR  $\text{Ca}^{2+}$  store associated with muscle contraction. **B.** Elevated  $\text{Ca}^{2+}$  entry through SOCE leads to a drastic increase in  $[\text{Ca}^{2+}]_i$ , and contributes to certain dystrophic phenotype in muscular dystrophy. **C.** Compromised SOCE in aged skeletal muscle could play a role in the reduced contractility of aged skeletal muscle. Many other aspects of dysfunctional  $\text{Ca}^{2+}$  signaling, such as reduced efficacy of excitation-contraction coupling, segregation of intracellular  $\text{Ca}^{2+}$  release, or altered mitochondria  $\text{Ca}^{2+}$  signaling, could all contribute to the overall muscle wasting and weakness during aging.

pumps evolved from dependence on the SERCA pump in amphibian skeletal muscle to the reliance on the  $\text{Ca}^{2+}$  pumps in both the SR and the sarcolemmal membrane in mammalian skeletal muscle [52]. Thus, a  $\text{Ca}^{2+}$  entry mechanism must help maintain intracellular  $\text{Ca}^{2+}$  storage and homeostasis in skeletal muscle; otherwise the SR  $\text{Ca}^{2+}$  store would become chronically depleted and skeletal muscle would weaken until it would be unable to perform basic contractile tasks.

Although SOCE appears to be ubiquitous in non-excitable cells, recent experimental evidence indicates that SOCE is also present in skeletal muscle [3], smooth muscle [26], and neurons [53]. While SOCE is a mechanism present in excitable and non-excitable cells, it has been more extensively explored in the latter. Interestingly, it has been recently shown that activation of RyR opens human TRPC3 channels (hTRPC3) in HEK293 cells [54], suggesting a functional coupling between RyR and hTRPC3 similar to what has been previously reported for hTRPC3 and InsP3R [55]. Furthermore, depletion of the ER *via* activation of RyR in cells lacking InsP3R initiates SOCE, illustrating functional coupling between RyR and store-operated channels that is independent of InsP3R action [56]. Additional physiological evidence highlighting the contribution of  $\text{Ca}^{2+}$  entry and SOCE to muscle physiology and pathophysiology has emerged. For example, exaggerated  $[\text{Ca}]_o$  entry has been linked to muscular dystrophy [57, 58], while our recent study shows that increased muscle activity requires increased  $[\text{Ca}]_o$  entry for maintenance of contractile activity [16].

While SOCE is an interesting candidate to refill the SR  $\text{Ca}^{2+}$  store in skeletal muscle, there are several other  $\text{Ca}^{2+}$  entry pathways that have been identified in skeletal muscle fibers. While L-type  $\text{Ca}^{2+}$  channel could provide one such alternative, previous studies have suggested that that L-type  $\text{Ca}^{2+}$  channel does not contribute significantly to maintaining repetitive muscle contraction [59]. It is well known that the L-type  $\text{Ca}^{2+}$  channel in skeletal muscle has slow activation kinetics [60, 61], suggesting that significant  $\text{Ca}^{2+}$  entry is unlikely to occur through the L-type  $\text{Ca}^{2+}$  channel. In addition, a role for L-type  $\text{Ca}^{2+}$  entry on skeletal muscle contractility is improbable as L-type  $\text{Ca}^{2+}$  channel blockers do not significantly affect contractility in mammalian muscle preparations [62-65]. Therefore, other  $\text{Ca}^{2+}$  entry mechanisms are likely responsible for maintaining proper intracellular  $\text{Ca}^{2+}$  storage and homeostasis during skeletal muscle contraction.

Recent studies have suggested a process of  $[\text{Ca}^{2+}]_o$  entry in cultured myotubes, named excitation-coupled  $\text{Ca}^{2+}$  entry (ECCE), which is coupled to conformational changes of the RyR that follow membrane depolarization [66, 67]. Currently, this mechanism has not yet been demonstrated in adult skeletal muscle fibers. It is possible that ECCE may play a role during the maturation of myoblasts to myotubes, since skeletal muscle contractility is more dependent on  $[\text{Ca}^{2+}]_o$  entry during earlier stages of development [68-70]. In addition, the physiological relevance of ECCE to adult muscle contraction is not clear since activation of ECCE is mainly governed by membrane depolarization and is independent of the  $\text{Ca}^{2+}$  storage within the SR [66, 67], thus a mechanism that would allow for ECCE termination has not been defined.

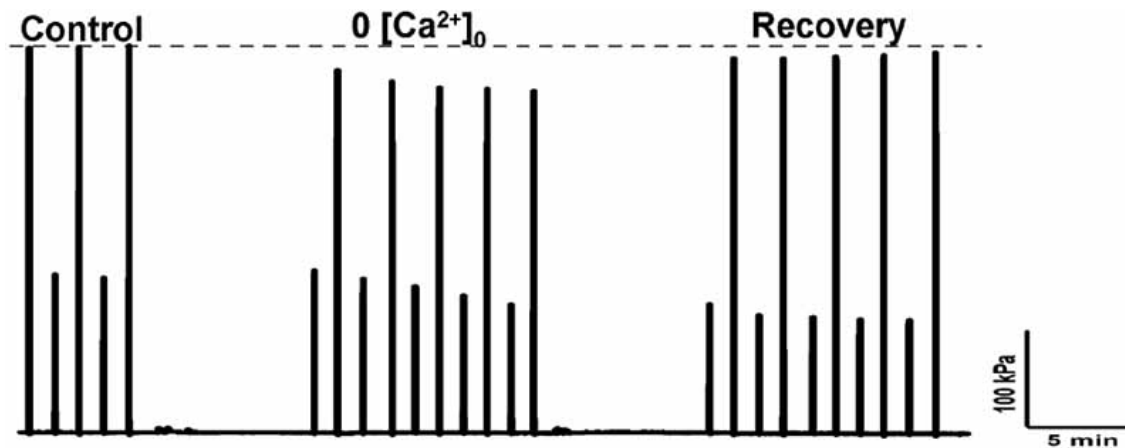
Therefore, it seems that SOCE represents a relevant  $[\text{Ca}^{2+}]_o$  entry mechanism in both non-excitable cells [5, 22,

71, 72] and in skeletal muscles, as it provides a mechanism where  $\text{Ca}^{2+}$  channels located on the sarcolemmal membrane sense the  $\text{Ca}^{2+}$  filling status of the SR and gate the entry of  $\text{Ca}^{2+}$  from the extracellular reservoir to replenish and maintain intracellular  $\text{Ca}^{2+}$  storage. In fact, while many studies have identified SOCE in skeletal muscle, the role of SOCE in skeletal muscle physiology and pathophysiology have just begun to be recognized [3, 13, 16, 51, 73].

SOCE seems to play an important role under conditions of higher  $\text{Ca}^{2+}$  demand, such as muscle exercise and aging [16, 74]. While elevated SOCE seems required under conditions of increased muscle activity for maintenance of contractile activity [16], reduced SOCE has been recently reported in aged human fibroblasts [75] and in aged rat cervical ganglion cells [74]. In contrast, a physiological role of SOCE under normal contractile conditions that do not engage higher  $\text{Ca}^{2+}$  demand is uncertain. Therefore, we have conducted an extensive series of experiments in an effort to determine if  $[\text{Ca}^{2+}]_o$  entry through SOCE plays a role in skeletal mammalian muscle contractility under non-fatiguing conditions. Our experimental design involved fulfillment of four essential conditions. First, our experiments were designed to mimic working muscles under physiological conditions where the duty cycle duration does not induce fatigue (1 tetanic stimulation/min). Second, to inhibit  $[\text{Ca}^{2+}]_o$  entry, we removed  $\text{Ca}^{2+}$  from the bathing solution. As shown in Fig. 2, removal of  $[\text{Ca}^{2+}]_o$  results in a quick drop in the contractile force with this inhibitory effect continuing to develop with prolonged exposure to 0  $[\text{Ca}^{2+}]_o$ . Here, it is important to observe that our 0  $[\text{Ca}^{2+}]_o$  solution also contained 0.1 mM EGTA, as we have found this to be essential to unequivocally observe the  $[\text{Ca}^{2+}]_o$  dependence of normal contractility in skeletal muscles. We have concluded that by simply removing  $\text{Ca}^{2+}$  from the extracellular solution without adding an effective  $\text{Ca}^{2+}$  chelator, contaminating levels of  $[\text{Ca}^{2+}]_o$  could easily reach  $\sim 20 \mu\text{M}$ . Since under physiological conditions the transverse-tubular invagination of the sarcolemmal membrane stores more than 1 mM  $\text{Ca}^{2+}$ , these contamination levels of  $\text{Ca}^{2+}$  would maintain threshold levels of  $\text{Ca}^{2+}$  within the transverse-tubules, thereby shrouding a clear observation for a role of SOCE on skeletal muscle contractility. Third, since physiological mechanisms display obvious dependence on temperature, it is indispensable to establish the extent to which SOCE affects contraction of working muscle at physiological temperatures. Our experiments at  $37^\circ\text{C}$  revealed an essentially similar effect of  $[\text{Ca}^{2+}]_o$  entry on contractility at both room and physiological temperatures. Lastly, we confirmed the role of SOCE on physiological muscle contractility by incubating skeletal muscles with pharmacological blockers of SOCE, such as  $\text{NiCl}_2$  and 2-APB. The effects of  $\text{NiCl}_2$  and 2-APB on muscle contractility are similar to that observed with 0  $[\text{Ca}^{2+}]_o$ , suggesting that SOCE is likely involved in the normal contractile function of skeletal muscle.

## SOCE AND MUSCLE FATIGUE

Fatigue is an important functional hallmark of skeletal muscles, and is defined as a reversible decrease in contractile force in response to an increase in the frequency or duration of stimulation. Muscle fatigue is still a research area in need of additional investigation, as the cellular and molecular mechanisms are not yet fully established [76-78]. Some of



**Fig. (2).** Dependence of skeletal muscle contraction on extracellular  $\text{Ca}^{2+}$  entry. (a) An Intact EDL muscle was electrically stimulated with low (40-60 Hz) and high frequency (100-130 Hz) in a bath solution with either 2.5 mM extracellular  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_o$ ) (control) or 0  $[\text{Ca}^{2+}]_o$ +0.1 mM EGTA. Fast recovery upon return of preparation to 2.5 mM  $[\text{Ca}^{2+}]_o$  is observed.

the current theories of muscle fatigue include: a) Disruption of intracellular  $\text{Ca}^{2+}$  homeostasis, leading to failure in the effective communication between the transverse-tubules and  $\text{Ca}^{2+}$  release from the SR (discussed below), b) Sodium theory: changes in the muscle cell ionic concentration leading to failure of action potential propagation. This is thought to occur only during continuous fatigue, but not during intermittent fatigue; c) Activation of  $\text{Ca}^{2+}$  proteases: fatiguing stimulation leads to a rise in intracellular  $\text{Ca}^{2+}$ , activation of  $\text{Ca}^{2+}$  proteases and subsequent cleavage of essential excitation-contraction (E-C) coupling related proteins, d) Reactive oxygen species (ROS) theory: increased muscle activity leads to increased mitochondrial respiration, causing a net increase in superoxide, hydrogen peroxide and free radicals that can directly modify protein function, e) Accumulation of metabolites theory: a number of metabolites such as ROS, inorganic phosphate, ADP, AMP, etc, accumulate during fatigue and cause both a decrease in the amount of  $\text{Ca}^{2+}$  release from the SR and functional inhibition of the myosin-actin interaction. It is fascinating that for more than 60 years a dominant theory in muscle research has been that lactate accumulation was a major cause of muscle fatigue [79]. In the last 10 years this concept has evolved significantly from initial suggestions that lactate did not play such a prominent role in muscle fatigue [80], to a more recent concept that lactate may even play a protective role during fatigue [81, 82]. This example reveals the dependence of our knowledge on technical advancements that can either support or challenge theories. Our current understanding of fatigue may further develop as a greater understanding of the physiological and pathophysiological role of SOCE in skeletal muscle is developed.

A consensus view in muscle physiology research is that optimal muscle performance revolves around the maintenance of intracellular  $\text{Ca}^{2+}$  homeostasis, as inadequate  $\text{Ca}^{2+}$  release from the SR leads to decreased force output, a phenomenon observed when muscles are exposed to fatiguing stimulation [78, 83]. This deficient  $\text{Ca}^{2+}$  release process can result from improper coupling between the transverse-tubules and RyR, reduction of the SR  $\text{Ca}^{2+}$  store and/or direct modification of RyR function [76, 84, 85]. Since SR  $\text{Ca}^{2+}$  depletion is the cellular signaling mechanism that activates

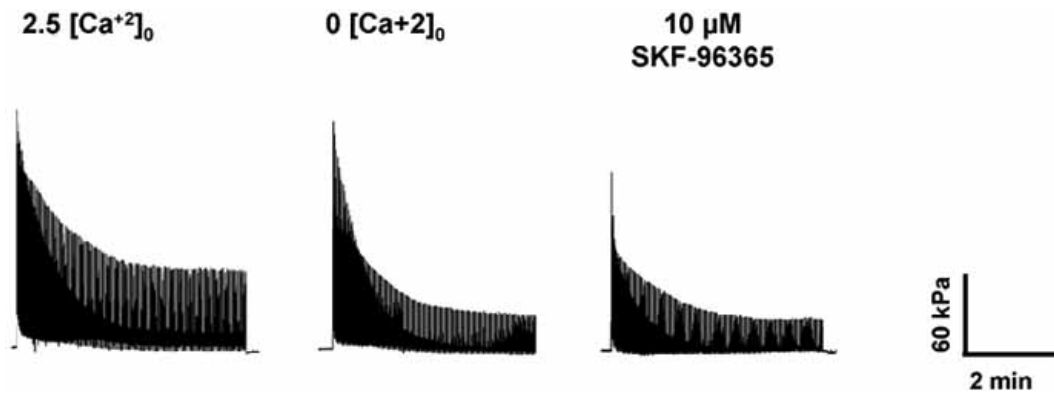
SOCE, and SOCE is essential for proper maintenance of SR  $\text{Ca}^{2+}$  storage, a role of SOCE during muscle fatigue must be carefully considered as part of the cellular mechanisms resulting in reduced force output that occurs during fatigue and recovery from fatigue. Thus, SOCE not only provides a mechanism for refilling of intracellular  $\text{Ca}^{2+}$  stores [5, 22, 71, 72], it can certainly add to the  $\text{Ca}^{2+}$  required for muscle contraction under conditions of muscle fatigue and intensive exercise when the SR  $\text{Ca}^{2+}$  store becomes naturally reduced. Therefore, reduced SOCE should exaggerate muscle fatigue, because the SR would deplete of  $\text{Ca}^{2+}$  during fatiguing stimulation at a higher rate, while the sarcolemmal  $\text{Ca}^{2+}$  pumps would extrude at higher rates, leaving less  $\text{Ca}^{2+}$  available for  $\text{Ca}^{2+}$  release during repetitive contraction cycles.

To directly test the contribution of SOCE to the acute fatigue behavior of skeletal muscle, intact muscle fibers from wild type mice were fatigued under conditions where the SOCE pathway is inhibited. Fig. 3 shows that contractile force is diminished and fatigability is enhanced in soleus muscles when  $\text{Ca}^{2+}$  was removed from the extracellular solution. In addition, the addition of SKF-96365 (10  $\mu\text{M}$ ) resulted in significantly reduced contractile force in soleus and concomitant enhancement of fatigability.

The role of SOCE in muscle contractility has just begun to be appreciated and the establishment of the physiological relevance of SOCE to skeletal muscle  $\text{Ca}^{2+}$  signaling and contractility is an exciting area of future research. SOCE may represent a unique therapeutic target for improvement of muscle performance during health and diseased conditions.

## SOCE AND MUSCLE AGING

Recent studies have indicated that SOCE is significantly reduced in aged cell preparations derived from both non-excitable and excitable cells. Using the broadly accepted technique of  $\text{Mn}^{2+}$  quenching of Fura-2 fluorescence to quantify unidirectional ion flux through SOCE, Vanterpool *et al.* demonstrated that aging reduces both rapid and spontaneous refilling of caffeine-sensitive  $\text{Ca}^{2+}$  stores in sympathetic superior cervical ganglion cells from aged rats, suggesting that SOCE is compromised in mammalian neuronal cells during



**Fig. (3).** Contribution of  $[Ca^{2+}]_o$  and SOCE to muscle fatigue. Records show fatiguing stimulation (tetanic stimulation trains of 80 Hz, 500 msec, every second) of isolated mouse soleus in the presence of 2.5 mM  $[Ca^{2+}]_o$ , 0  $[Ca^{2+}]_o$  muscle, and 10  $\mu$ M SKF. Vertical bar denotes normalized force in kPa and horizontal bar denotes time in minutes.

aging [74]. In humans, a similar phenomenon has been recently demonstrated by Papazafiri and Kletsas as they reported a diminished SOCE function in aged fibroblasts [75].

In aged skeletal muscle, changes in contractile apparatus function cannot fully explain the decrease in the specific force [86, 87]. Since reduced contractile force appears before muscle wasting is prevalent, disruption of E-C coupling and cellular  $Ca^{2+}$  handling may contribute to decreased function during muscle aging. Studies by Delbono and collaborators [87, 88] have suggested that disruption of E-C coupling is a central contributor to reduced contractility in aged muscle. Specifically, a population of aged skeletal muscle fibers has been shown to exhibit increased dependence on  $Ca^{2+}$  entry to maintain contractile function [89]. This finding suggests that compromised  $Ca^{2+}$  entry may contribute to the reduced muscle function in aging. Our recent studies revealed that muscle aging is associated with compromised  $Ca^{2+}$  spark signaling, the elemental process of RyR-mediated  $Ca^{2+}$  release, accompanied with the development of a segregated  $Ca^{2+}$  pool that uncouples for the normal E-C coupling process [90].

In agreement, Fraysee *et al.* recently reported the impact of aging on  $Ca^{2+}$  homeostasis in soleus and EDL muscles of aged rats by using intracellular  $Ca^{2+}$  measurements with fura-2 [91]. They reported that aging increases the resting cytosolic calcium concentration in both EDL and soleus muscles. Furthermore, these authors found that this effect was independent of  $Ca^{2+}$  influx since a reduced resting permeability of sarcolemma to divalent cations was observed in aged muscles. These authors concluded that the observed reduced resting permeability was likely due to a decreased activity of leak channels in the sarcolemmal membrane. We believe that what these authors defined as leak channels likely constitute SOCE and may point towards a reduced SOCE function during aging. We have unpublished data that suggests SOCE is significantly compromised in aged skeletal muscle. One can speculate that a defective SOCE may contribute to the reduced specific contractile force in aged skeletal muscle, as a reduced SOCE will contribute to a reduced availability of SR  $Ca^{2+}$  during repetitive contraction cycles (Fig. 1C).

### SOCE IN MUSCULAR DYSTROPHY

Muscular dystrophy is characterized by reduced contractile function and atrophy due in part to the death of skeletal muscle fibers. The most common form, Duchenne muscular

dystrophy (DMD), results from a loss of function of dystrophin [92], a high molecular weight structural protein that stabilizes the sarcolemma of muscle fibers by linking cytoskeletal actin to laminin in the extracellular matrix through the dystroglycan complex [93]. As a result, dystrophin protects the muscle against various mechanical stresses, maintaining sarcolemmal integrity [94]. While the exact cause of muscle fiber death is a matter of some controversy, there is an increasing body of evidence that a defect in  $Ca^{2+}$  homeostasis is a causal factor in muscular dystrophy [95].

One of the first cellular defects observed in dystrophic muscle fibers was a determination that the resting intracellular  $Ca^{2+}$  concentration was elevated above that seen in normal muscle fibers, which are thought to result from an increased influx of  $Ca^{2+}$  through stretch-activated  $Ca^{2+}$  channels and/or store-operated  $Ca^{2+}$  channels [57, 58, 96, 97]. Studies from other laboratories also find that SOCE may contribute to  $Ca^{2+}$  entry and elevated intracellular  $Ca^{2+}$  levels in dystrophic muscle fibers. A previous study showed that SOCE induced by either thapsigargin (TG) or caffeine was elevated in dystrophic mouse (*mdx*) fibers and that this elevated  $Ca^{2+}$  entry facilitated the increased intracellular  $Ca^{2+}$  observed in *mdx* muscle fibers [98]. This activity was attributed, at least in part, to the TRP family of proteins, as an antisense oligonucleotide approach against TRPC1 and TRPC4 resulted in attenuation of the exaggerated  $Ca^{2+}$  entry. An additional study by the same group concludes elevated SOCE is directly related to the absence of dystrophin, as elevated SOCE can be observed in dystrophin negative myotubes and transient expression of dystrophin into these myotubes can prevent the elevation of SOCE activity [99]. While there had been no mechanism proposed to explain the activation of SOCE in dystrophic muscle, our recent findings [100] suggest that aberrant  $Ca^{2+}$  spark activity in dystrophic muscle can lead to localized reduction of SR  $Ca^{2+}$  stores, resulting in sustained activation of SOCE, increased intracellular  $Ca^{2+}$  levels and muscular dystrophy. Fig. 1C illustrates the essential mechanisms involved with this upregulation of SOCE in dystrophic muscles.

It is not completely clear to what extent that SOCE contributes to the elevated intracellular  $Ca^{2+}$  levels observed in dystrophic muscle fibers. Other  $Ca^{2+}$  entry pathways aside from SOCE have been implicated in the elevation of intracellular  $Ca^{2+}$  in muscular dystrophy. As previous studies

have shown that Ca<sup>2+</sup> leak channels have altered open probabilities in dystrophic fibers [57] and contribute to Ca<sup>2+</sup>-dependent proteolysis [101]. These channels may also be regulated by membrane stretch [102, 103]. Recent molecular studies have linked the activity of these channels with TRP channels [104], which are associated with SOCE in several cell types. This creates the possibility that these supposedly separate Ca<sup>2+</sup> entry pathways could converge on similar molecular machinery at the sarcolemma.

## CONCLUSIONS

Through recent advances in several laboratories, our understanding of the role of SOCE in skeletal muscle physiology and pathophysiology has begun to come into focus. SOCE acts to replenish the SR Ca<sup>2+</sup> store in skeletal muscle, compensating for the loss of Ca<sup>2+</sup> during cycles of SR Ca<sup>2+</sup> release and uptake associated with muscle contraction. As a result, SOCE appears to play a role in the response to fatiguing stimulation of skeletal muscle, where repetitive contraction increases the need for Ca<sup>2+</sup> entry into the muscle fiber. In pathophysiology, alteration of the normal level of SOCE can result in disruption of skeletal muscle function. Aged skeletal muscle appears to display reduced SOCE, which correlates with the diminished contractile force observed in skeletal muscle from aged animals. In dystrophic skeletal muscle, increased SOCE may produce the elevated intracellular Ca<sup>2+</sup> levels thought to contribute to the progression of the pathology of muscular dystrophy. Determination of the molecular identity of the regulatory factors that control SOCE, as well as the pore-conducting unit responsible for SOCE, in skeletal muscle will be a vital next step to understand the role of SOCE in muscle function. Establishing the molecular mechanisms contributing to SOCE regulation in skeletal muscle will provide excellent candidates for therapeutic intervention by chemical or pharmacological approaches that will apply to muscle disorders that involve altered muscle fiber Ca<sup>2+</sup> homeostasis.

## REFERENCES

- Putney JW, Jr. A model for receptor-regulated calcium entry. *Cell Calcium* 1986; 7(1): 1-12.
- Putney JW, Jr. Capacitative calcium entry revisited. *Cell Calcium* 1990; 11(10): 611-24.
- Kurebayashi N, Ogawa Y. Depletion of Ca<sup>2+</sup> in the sarcoplasmic reticulum stimulates Ca<sup>2+</sup> entry into mouse skeletal muscle fibres. *J Physiol* 2001; 533(Pt 1): 185-99.
- Pan Z, Yang D, Nagaraj RY, *et al.* Dysfunction of store-operated calcium channel in muscle cells lacking mg29. *Nat Cell Biol* 2002; 4(5): 379-83.
- Parekh AB, Putney JW, Jr. Store-operated calcium channels. *Physiol Rev* 2005; 85(2): 757-810.
- Carafoli E. Calcium signaling: a tale for all seasons. *Proc Natl Acad Sci USA* 2002; 99(3): 1115-22.
- Hoth M, Penner R. Depletion of intracellular calcium stores activates a calcium current in mast cells. *Nature* 1992; 355(6358): 353-6.
- Parekh AB, Penner R. Depletion-activated calcium current is inhibited by protein kinase in RBL-2H3 cells. *Proc Natl Acad Sci USA* 1995; 92(17): 7907-11.
- Somasundaram B, Norman JC, Mahaut-Smith MP. Primaquine, an inhibitor of vesicular transport, blocks the calcium-release-activated current in rat megakaryocytes. *Biochem J* 1995; 309 (Pt 3): 725-9.
- Rychkov G, Brereton HM, Harland ML, Barritt GJ. Plasma membrane Ca<sup>2+</sup> release-activated Ca<sup>2+</sup> channels with a high selectivity for Ca<sup>2+</sup> identified by patch-clamp recording in rat liver cells. *Hepatology* 2001; 33(4): 938-47.
- Trepakova ES, Gericke M, Hirakawa Y, Weisbrod RM, Cohen RA, Bolotina VM. Properties of a native cation channel activated by Ca<sup>2+</sup> store depletion in vascular smooth muscle cells. *J Biol Chem* 2001; 276(11): 7782-90.
- Albert AP, Large WA. Activation of store-operated channels by noradrenaline via protein kinase C in rabbit portal vein myocytes. *J Physiol* 2002; 544(Pt 1): 113-25.
- Ma J, Pan Z. Retrograde activation of store-operated calcium channel. *Cell Calcium* 2003; 33(5-6): 375-84.
- Brotto MA, Nagaraj RY, Brotto LS, Takeshima H, Ma JJ, Nosek TM. Defective maintenance of intracellular Ca<sup>2+</sup> homeostasis is linked to increased muscle fatigability in the MG29 null mice. *Cell Res* 2004; 14(5): 373-8.
- Nagaraj RY, Nosek CM, Brotto MA, *et al.* Increased susceptibility to fatigue of slow- and fast-twitch muscles from mice lacking the MG29 gene. *Physiol Genomics* 2000; 4(1): 43-9.
- Zhao X, Yoshida M, Brotto L, *et al.* Enhanced resistance to fatigue and altered calcium handling properties of sarcalumenin knockout mice. *Physiol Genomics* 2005; 23(1): 72-8.
- Franzius D, Hoth M, Penner R. Non-specific effects of calcium entry antagonists in mast cells. *Pflugers Arch* 1994; 428(5-6): 433-8.
- Petersen OH, Fedirko NV. Calcium signalling: store-operated channel found at last. *Curr Biol* 2001; 11(13): R520-3.
- Feske S, Gwack Y, Prakriya M, *et al.* A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature* 2006; 441(7090): 179-85.
- Vig M, Peinelt C, Beck A, *et al.* CRACM1 is a plasma membrane protein essential for store-operated Ca<sup>2+</sup> entry. *Science* 2006; 312(5777): 1220-3.
- Parekh AB. Cell biology: cracking the calcium entry code. *Nature* 2006; 441(7090): 163-5.
- Putney JW, Jr, Broad LM, Braun FJ, Lievreumont JP, Bird GS. Mechanisms of capacitative calcium entry. *J Cell Sci* 2001; 114(Pt 12): 2223-9.
- Randriamampita C, Tsien RY. Emptying of intracellular Ca<sup>2+</sup> stores releases a novel small messenger that stimulates Ca<sup>2+</sup> influx. *Nature* 1993 Aug 26; 364(6440): 809-14.
- Smani T, Zakharov SI, Csutura P, Leno E, Trepakova ES, Bolotina VM. A novel mechanism for the store-operated calcium influx pathway. *Nat Cell Biol* 2004; 6(2): 113-20.
- Smani T, Zakharov SI, Leno E, Csutura P, Trepakova ES, Bolotina VM. Ca<sup>2+</sup>-independent phospholipase A2 is a novel determinant of store-operated Ca<sup>2+</sup> entry. *J Biol Chem* 2003; 278(14): 11909-15.
- Albert AP, Large WA. Store-operated Ca<sup>2+</sup>-permeable non-selective cation channels in smooth muscle cells. *Cell Calcium* 2003; 33(5-6): 345-56.
- Vazquez G, de Boland AR, Boland RL. Involvement of calmodulin in 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> stimulation of store-operated Ca<sup>2+</sup> influx in skeletal muscle cells. *J Biol Chem* 2000; 275(21): 16134-8.
- Bezzrides VJ, Ramsey IS, Kotecha S, Greka A, Clapham DE. Rapid vesicular translocation and insertion of TRP channels. *Nat Cell Biol* 2004; 6(8): 709-20.
- Holda JR, Blatter LA. Capacitative calcium entry is inhibited in vascular endothelial cells by disruption of cytoskeletal microfilaments. *FEBS Lett* 1997; 403(2): 191-6.
- Alderton JM, Ahmed SA, Smith LA, Steinhardt RA. Evidence for a vesicle-mediated maintenance of store-operated calcium channels in a human embryonic kidney cell line. *Cell Calcium* 2000; 28(3): 161-9.
- McDonald TV, Premack BA, Gardner P. Flash photolysis of caged inositol 1,4,5-trisphosphate activates plasma membrane calcium current in human T cells. *J Biol Chem* 1993; 268(6): 3889-96.
- Gregory RB, Barritt GJ. Store-activated Ca<sup>2+</sup> inflow in *Xenopus laevis* oocytes: inhibition by primaquine and evaluation of the role of membrane fusion. *Biochem J* 1996; 319 (Pt 3): 755-60.
- Yao Y, Ferrer-Montiel AV, Montal M, Tsien RY. Activation of store-operated Ca<sup>2+</sup> current in *Xenopus* oocytes requires SNAP-25 but not a diffusible messenger. *Cell* 1999; 98(4): 475-85.
- Scott CC, Furuya W, Trimble WS, Grinstein S. Activation of store-operated calcium channels: assessment of the role of snare-mediated vesicular transport. *J Biol Chem* 2003; 278(33): 30534-9.
- Bakowski D, Burgoyne RD, Parekh AB. Activation of the store-operated calcium current ICRAC can be dissociated from regulated

- exocytosis in rat basophilic leukaemia (RBL-1) cells. *J Physiol* 2003; 553(Pt 2): 387-93.
- [36] Barritt GJ. Does a decrease in subplasmalemmal Ca<sup>2+</sup> explain how store-operated Ca<sup>2+</sup> channels are opened? *Cell Calcium* 1998; 23(1): 65-75.
- [37] Berridge MJ. Capacitative calcium entry. *Biochem J* 1995; 312 (Pt 1): 1-11.
- [38] Launikonis BS, Barnes M, Stephenson DG. Identification of the coupling between skeletal muscle store-operated Ca<sup>2+</sup> entry and the inositol trisphosphate receptor. *Proc Natl Acad Sci USA* 2003; 100(5): 2941-4.
- [39] Patterson RL, van Rossum DB, Gill DL. Store-operated Ca<sup>2+</sup> entry: evidence for a secretion-like coupling model. *Cell* 1999; 98(4): 487-99.
- [40] Bakowski D, Glitsch MD, Parekh AB. An examination of the secretion-like coupling model for the activation of the Ca<sup>2+</sup> release-activated Ca<sup>2+</sup> current I(CRAC) in RBL-1 cells. *J Physiol* 2001; 532(Pt 1): 55-71.
- [41] Sugawara H, Kurosaki M, Takata M, Kurosaki T. Genetic evidence for involvement of type 1, type 2 and type 3 inositol 1,4,5-trisphosphate receptors in signal transduction through the B-cell antigen receptor. *EMBO J* 1997; 16(11): 3078-88.
- [42] Fierro L, Parekh AB. On the characterisation of the mechanism underlying passive activation of the Ca<sup>2+</sup> release-activated Ca<sup>2+</sup> current ICRAC in rat basophilic leukaemia cells. *J Physiol* 1999; 520 Pt 2: 407-16.
- [43] Bolotina VM, Csutora P. CIF and other mysteries of the store-operated Ca<sup>2+</sup>-entry pathway. *Trends Biochem Sci* 2005; 30(7): 378-87.
- [44] Spassova MA, Soboloff J, He LP, Xu W, Dziadek MA, Gill DL. STIM1 has a plasma membrane role in the activation of store-operated Ca(2+) channels. *Proc Natl Acad Sci USA* 2006; 103(11): 4040-5.
- [45] Roos J, DiGregorio PJ, Yeromin AV, *et al.* STIM1, an essential and conserved component of store-operated Ca<sup>2+</sup> channel function. *J Cell Biol* 2005; 169(3): 435-45.
- [46] Zhang SL, Yu Y, Roos J, *et al.* STIM1 is a Ca<sup>2+</sup> sensor that activates CRAC channels and migrates from the Ca<sup>2+</sup> store to the plasma membrane. *Nature* 2005; 437(7060): 902-5.
- [47] Feske S, Prakriya M, Rao A, Lewis RS. A severe defect in CRAC Ca<sup>2+</sup> channel activation and altered K<sup>+</sup> channel gating in T cells from immunodeficient patients. *J Exp Med* 2005; 202(5): 651-62.
- [48] Schneider MF. Control of calcium release in functioning skeletal muscle fibers. *Annu Rev Physiol* 1994; 56: 463-84.
- [49] Dirksen RT. Bi-directional coupling between dihydropyridine receptors and ryanodine receptors. *Front Biosci* 2002; 7: d659-70.
- [50] Kurebayashi N, Ogawa Y. Calcium dynamics in skeletal muscle. *Clin Calcium* 2001; 11(11): 1417-23.
- [51] Weigl L, Zidar A, Gscheidlinger R, Karel A, Hohenegger M. Store operated Ca<sup>2+</sup> influx by selective depletion of ryanodine sensitive Ca<sup>2+</sup> pools in primary human skeletal muscle cells. *Naunyn Schmiedebergs Arch Pharmacol* 2003; 367(4): 353-63.
- [52] Hemmings SJ. New methods for the isolation of skeletal muscle sarcolemma and sarcoplasmic reticulum allowing a comparison between the mammalian and amphibian beta(2)-adrenergic receptors and calcium pumps. *Cell Biochem Funct* 2001; 19(2): 133-41.
- [53] Putney JW, Jr. Capacitative calcium entry in the nervous system. *Cell Calcium* 2003; 34(4-5): 339-44.
- [54] Kiselyov KI, Shin DM, Wang Y, Pessah IN, Allen PD, Muallem S. Gating of store-operated channels by conformational coupling to ryanodine receptors. *Mol Cell* 2000; 6(2): 421-31.
- [55] Boulay G, Brown DM, Qin N, *et al.* Modulation of Ca(2+) entry by polypeptides of the inositol 1,4, 5-trisphosphate receptor (IP3R) that bind transient receptor potential (TRP): evidence for roles of TRP and IP3R in store depletion-activated Ca(2+) entry. *Proc Natl Acad Sci USA* 1999; 96(26): 14955-60.
- [56] Kiselyov K, Shin DM, Shcheynikov N, Kurosaki T, Muallem S. Regulation of Ca<sup>2+</sup>-release-activated Ca<sup>2+</sup> current (Icrac) by ryanodine receptors in inositol 1,4,5-trisphosphate-receptor-deficient DT40 cells. *Biochem J* 2001; 360(Pt 1): 17-22.
- [57] Fong PY, Turner PR, Denetclaw WF, Steinhart RA. Increased activity of calcium leak channels in myotubes of Duchenne human and mdx mouse origin. *Science* 1990; 250(4981): 673-6.
- [58] Mallouk N, Jacquemond V, Allard B. Elevated subsarcolemmal Ca<sup>2+</sup> in mdx mouse skeletal muscle fibers detected with Ca<sup>2+</sup>-activated K<sup>+</sup> channels. *Proc Natl Acad Sci USA* 2000; 97(9): 4950-5.
- [59] Tanabe T, Mikami A, Niidome T, Numa S, Adams BA, Beam KG. Structure and function of voltage-dependent calcium channels from muscle. *Ann N Y Acad Sci* 1993; 707: 81-6.
- [60] Sanchez JA, Stefani E. Inward calcium current in twitch muscle fibres of the frog. *J Physiol* 1978; 283: 197-209.
- [61] Cota G, Stefani E. Voltage-dependent inactivation of slow calcium channels in intact twitch muscle fibers of the frog. *J Gen Physiol* 1989; 94(5): 937-51.
- [62] Dulhunty AF, Gage PW. Effects of extracellular calcium concentration and dihydropyridines on contraction in mammalian skeletal muscle. *J Physiol* 1988; 399: 63-80.
- [63] Viires N, Pavlovic D, Aubier M. Multiple effects of BAY K 8644 and nifedipine on isolated diaphragmatic fibers *in vitro*. *J Appl Physiol* 1991; 71(3): 841-6.
- [64] Miller MJ, Shannon K, Reid MB. Effect of nifedipine on the contractile function of the rat diaphragm *in vitro*. *Life Sci* 1989; 45(25): 2419-28.
- [65] Miller MJ, Shannon K, Reid MB. Inhibition by nifedipine of the indirectly induced contractile response of the rat diaphragm. *Life Sci* 1989; 45(25): 2429-35.
- [66] Cherednichenko G, Hurne AM, Fessenden JD, *et al.* Conformational activation of Ca<sup>2+</sup> entry by depolarization of skeletal myotubes. *Proc Natl Acad Sci USA* 2004; 101(44): 15793-8.
- [67] Hurne AM, O'Brien JJ, Wingrove D, *et al.* Ryanodine receptor type 1 (RyR1) mutations C4958S and C4961S reveal excitation-coupled calcium entry (ECCE) is independent of sarcoplasmic reticulum store depletion. *J Biol Chem* 2005; 280(44): 36994-7004.
- [68] Dangain J, Neering IR. Effect of low extracellular calcium and ryanodine on muscle contraction of the mouse during postnatal development. *Can J Physiol Pharmacol* 1991; 69(9): 1294-300.
- [69] Louboutin JP, Fichter-Gagnepain V, Noireaud J. Comparison of contractile properties between developing and regenerating soleus muscle: influence of external calcium concentration upon the contractility. *Muscle Nerve* 1995; 18(11): 1292-9.
- [70] Pereon Y, Louboutin JP, Noireaud J. Contractile responses in rat extensor digitorum longus muscles at different times of postnatal development. *J Comp Physiol [B]* 1993; 163(3): 203-11.
- [71] Elliott AC. Recent developments in non-excitable cell calcium entry. *Cell Calcium* 2001; 30(2): 73-93.
- [72] Zhu X, Birnbaumer L. Calcium Channels Formed by Mammalian Trp Homologues. *News Physiol Sci* 1998; 13: 211-7.
- [73] Vazquez G, de Boland AR, Boland RL. 1alpha,25-dihydroxyvitamin-D<sub>3</sub>-induced store-operated Ca<sup>2+</sup> influx in skeletal muscle cells. Modulation by phospholipase c, protein kinase c, and tyrosine kinases. *J Biol Chem* 1998; 273(51): 33954-60.
- [74] Vanterpool CK, Pearce WJ, Buchholz JN. Advancing age alters rapid and spontaneous refilling of caffeine-sensitive calcium stores in sympathetic superior cervical ganglion cells. *J Appl Physiol* 2005; 99(3): 963-71.
- [75] Papazafiri P, Kletsas D. Developmental and age-related alterations of calcium homeostasis in human fibroblasts. *Exp Gerontol* 2003; 38(3): 307-11.
- [76] Bruton JD, Lannergren J, Westerblad H. Mechanisms underlying the slow recovery of force after fatigue: importance of intracellular calcium. *Acta Physiol Scand* 1998; 162(3): 285-93.
- [77] Brotto M, van Leyen SA, Brotto LS, Jin JP, Nosek CM, Nosek TM. Hypoxia/fatigue-induced degradation of troponin I and troponin C: new insights into physiologic muscle fatigue. *Pflugers Arch* 2001; 442(5): 738-44.
- [78] Brotto MA, Nosek TM, Kolbeck RC. Influence of ageing on the fatigability of isolated mouse skeletal muscles from mature and aged mice. *Exp Physiol* 2002; 87(1): 77-82.
- [79] Fletcher WW, Hopflins FG. Lactic acid in mammalian muscle. *J Physiol (Lond)* 1907; 35: 247-303.
- [80] Bangsbo J, Madsen K, Kiens B, Richter EA. Effect of muscle acidity on muscle metabolism and fatigue during intense exercise in man. *J Physiol* 1996; 495 (Pt 2): 587-96.
- [81] Pedersen TH, Nielsen OB, Lamb GD, Stephenson DG. Intracellular acidosis enhances the excitability of working muscle. *Science* 2004; 305(5687): 1144-7.
- [82] Allen D, Westerblad H. Physiology. Lactic acid-the latest performance-enhancing drug. *Science* 2004; 305(5687): 1112-3.

- [83] Westerblad H, Allen DG. Changes of myoplasmic calcium concentration during fatigue in single mouse muscle fibers. *J Gen Physiol* 1991; 98(3): 615-35.
- [84] Williams JH, Klug GA. Calcium exchange hypothesis of skeletal muscle fatigue: a brief review. *Muscle Nerve* 1995; 18(4): 421-34.
- [85] Allen DG, Lannergren J, Westerblad H. Muscle cell function during prolonged activity: cellular mechanisms of fatigue. *Exp Physiol* 1995; 80(4): 497-527.
- [86] Thompson LV, Brown M. Age-related changes in contractile properties of single skeletal fibers from the soleus muscle. *J Appl Physiol* 1999; 86(3): 881-6.
- [87] Delbono O. Molecular mechanisms and therapeutics of the deficit in specific force in ageing skeletal muscle. *Biogerontology* 2002; 3(5): 265-70.
- [88] Renganathan M, Messi ML, Delbono O. Dihydropyridine receptor-ryanodine receptor uncoupling in aged skeletal muscle. *J Membr Biol* 1997; 157(3): 247-53.
- [89] Payne AM, Zheng Z, Gonzalez E, Wang ZM, Messi ML, Delbono O. External Ca(2+)-dependent excitation-contraction coupling in a population of ageing mouse skeletal muscle fibres. *J Physiol* 2004; 560(Pt 1): 137-55.
- [90] Weisleder N, Brotto M, Komazaki S, *et al.* Muscle aging is associated with compromised Ca<sup>2+</sup> spark signaling and segregated intracellular Ca<sup>2+</sup> release. *J Cell Biol* 2006; 174(5): 639-45.
- [91] Fraysse B, Desaphy JF, Rolland JF, *et al.* Fiber type-related changes in rat skeletal muscle calcium homeostasis during aging and restoration by growth hormone. *Neurobiol Dis* 2006; 21(2): 372-80.
- [92] Hoffman EP, Brown RH, Kunkel LM. Dystrophin: the protein product of the Duchene muscular dystrophy locus 1987. *Biotechnology* 1992; 24: 457-66.
- [93] Ibraghimov-Beskrovnaya O, Ervasti JM, Leveille CJ, Slaughter CA, Sernett SW, Campbell KP. Primary structure of dystrophin-associated glycoproteins linking dystrophin to the extracellular matrix. *Nature* 1992; 355(6362): 696-702.
- [94] Lynch GS, Rafael JA, Chamberlain JS, Faulkner JA. Contraction-induced injury to single permeabilized muscle fibers from mdx, transgenic mdx, and control mice. *Am J Physiol Cell Physiol* 2000; 279(4): C1290-4.
- [95] Blake DJ, Weir A, Newey SE, Davies KE. Function and genetics of dystrophin and dystrophin-related proteins in muscle. *Physiol Rev* 2002; 82(2): 291-329.
- [96] Takagi A, Kojima S, Ida M, Araki M. Increased leakage of calcium ion from the sarcoplasmic reticulum of the mdx mouse. *J Neurol Sci* 1992; 110(1-2): 160-4.
- [97] Kumar A, Khandelwal N, Malya R, Reid MB, Boriek AM. Loss of dystrophin causes aberrant mechanotransduction in skeletal muscle fibers. *FASEB J* 2004; 18(1): 102-13.
- [98] Vandebrouck C, Martin D, Colson-Van Schoor M, Debaix H, Gailly P. Involvement of TRPC in the abnormal calcium influx observed in dystrophic (mdx) mouse skeletal muscle fibers. *J Cell Biol* 2002; 158(6): 1089-96.
- [99] Vandebrouck A, Ducret T, Basset O, *et al.* Regulation of store-operated calcium entries and mitochondrial uptake by minidystrophin expression in cultured myotubes. *FASEB J* 2006; 20(1): 136-8.
- [100] Wang X, Weisleder N, Collet C, *et al.* Uncontrolled calcium sparks act as a dystrophic signal for mammalian skeletal muscle. *Nat Cell Biol* 2005; 7(5): 525-30.
- [101] Alderton JM, Steinhardt RA. Calcium influx through calcium leak channels is responsible for the elevated levels of calcium-dependent proteolysis in dystrophic myotubes. *J Biol Chem* 2000; 275(13): 9452-60.
- [102] Franco A Jr, Lansman JB. Calcium entry through stretch-inactivated ion channels in mdx myotubes. *Nature* 1990; 344(6267): 670-3.
- [103] Franco-Obregon A, Lansman JB. Changes in mechanosensitive channel gating following mechanical stimulation in skeletal muscle myotubes from the mdx mouse. *J Physiol* 2002; 539(Pt 2): 391-407.
- [104] Maroto R, Raso A, Wood TG, Kurosky A, Martinac B, Hamill OP. TRPC1 forms the stretch-activated cation channel in vertebrate cells. *Nat Cell Biol* 2005; 7(2): 179-85.