Calcium Cycling in Synthetic and Contractile Phasic or Tonic Vascular Smooth Muscle Cells

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Additional information is available at the end of the chapter

1. Introduction

Calcium ions (Ca^{2+}) are present in low concentrations in the cytosol (~100 nM) and in high concentrations (in mM range) in both the extracellular medium and intracellular stores (mainly sarco/endo/plasmic reticulum, SR). This differential allows the calcium ion to be a ubiquitous 2nd messenger that carries information essential for cellular functions as diverse as contraction, metabolism, apoptosis, proliferation and/or hypertrophic growth. The mechanisms responsible for generating a Ca^{2+} signal greatly differ from one cell type to another. In the different types of vascular smooth muscle cells (VSMC), enormous variations do exist with regard to the mechanisms responsible for generating Ca^{2+} signal. In each VSMC phenotype (synthetic/proliferating[1] and contractile[2] [1], tonic or phasic), the Ca^{2+} signaling system is adapted to its particular function and is due to the specific patterns of expression and regulation of Ca^{2+} handling molecules (**Figure 1**). For instance, in contractile VSMCs, the initiation of contractile events is driven by membrane depolarization; and the principal entry-point for extracellular Ca^{2+} is the voltage-operated L-type calcium channel (LTCC). In contrast, in synthetic/proliferating VSMCs, the principal way-in for extracellular Ca^{2+} is the store-operated calcium (SOC) channel. Whatever the cell type, the calcium signal consists of limited elevations of cytosolic free calcium ions in time and space. The calcium pump, sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA), has a critical role in determining the frequency of SR Ca^{2+} release by controlling the velocity of Ca^{2+} upload into the sarcoplasmic reticulum (SR) and the Ca^{2+} sensitivity of SR calcium channels, Ryanodin Receptor, RyR and Inositol tri-Phosphate

[1]Synthetic VSMCs have a fibroblast appearance, proliferate readily, and synthesize increased levels of various extracellular matrix components, particularly fibronectin, collagen types I and III, and tropoelastin [1].
[2]Contractile VSMCs have a muscle-like or spindle-shaped appearance and well-developed contractile apparatus resulting from the expression and intracellular accumulation of thick and thin muscle filaments [1].

Receptor, IP$_3$R. Therefore, it is a major player in determining the spacio-temporal patterns of intracellular calcium signaling. This chapter focuses on the changes in Ca^{2+} signaling associated with different VSMC phenotypes. We will discuss the physiological implications of altered expressions of Ca^{2+} channels and pumps (referred to as Ca^{2+} handling proteins) and how they contribute to VSMC dysfunction in vascular disease.
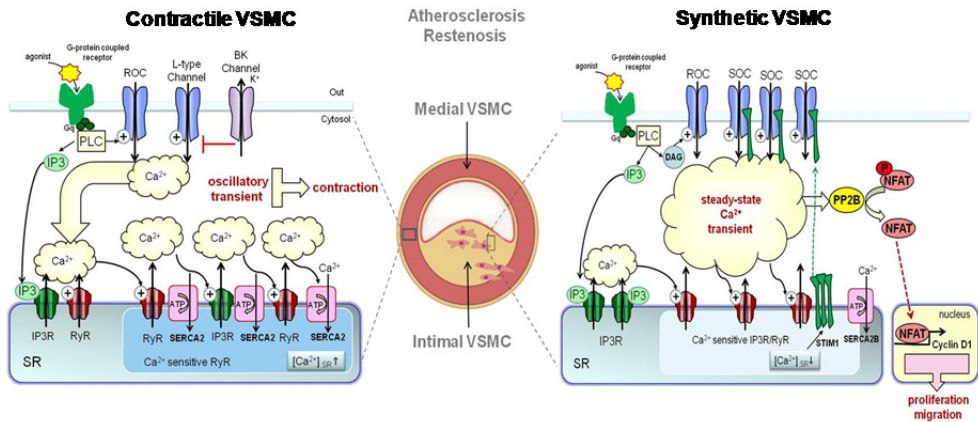
Figure 1. Schematic representation of Calcium Cycling in Contractile and Proliferating VSMCs. Left panel: schematic representation of calcium cycling in quiescent /contractile VSMCs. Contractile response is initiated by extracellular Ca^{2+} influx due to activation of Receptor Operated Ca^{2+} channels (through phosphoinositol-coupled receptor) or to activation of L-Type Calcium channels (through an increase in luminal pressure). Small increase of cytosolic due IP$_3$ binding to IP$_3$R (puff) or RyR activation by LTCC or ROC-dependent Ca^{2+} influx leads to large SR Ca^{2+} release due to the activation of IP$_3$R or RyR clusters ("Ca^{2+}-induced Ca^{2+}release" phenomenon). Cytosolic Ca^{2+} is rapidly reduced by SR calcium pumps (both SERCA2a and SERCA2b are expressed in quiescent VSMCs), maintaining high concentration of cytosolic Ca^{2+} and setting the sensitivity of RyR or IP$_3$R for the next spike. Contraction of VSMCs occurs during oscillatory Ca^{2+} transient. Middle panel: schematic representation of atherosclerotic vessel wall. Contractile VSMC are located in the media layer, synthetic VSMC are located in sub-endothelial intima. Right panel: schematic representation of calcium cycling in quiescent /contractile VSMCs. Agonist binding to phosphoinositol-coupled receptor leads to the activation of IP$_3$R resulting in large increase in cytosolic Ca^{2+}. Calcium is weakly reduced by SR calcium pumps (only SERCA2b, having low turnover and low affinity to Ca^{2+} is expressed). Store depletion leads to translocation of SR Ca^{2+} sensor STIM1 towards PM, resulting in extracellular Ca^{2+} influx though opening of Store Operated Channel (CRAC). Resulted steady state Ca^{2+} transient is critical for activation of proliferation-related transcription factors 'NFAT). Abbreviations: PLC - phospholipase C; PM - plasma membrane; PP2B - Ca^{2+}/calmodulin-activated protein phosphatase 2B (calcineurin); ROC- receptor activated channel; IP$_3$ - inositol-1,4,5-trisphosphate, IP$_3$R - inositol-1,4,5-trisphosphate receptor; RyR - ryanodine receptor; NFAT - nuclear factor of activated T-lymphocytes; VSMC - vascular smooth muscle cells; SERCA - sarco(endo)plasmic reticulum Ca^{2+} ATPase; SR - sarcoplasmic reticulum.

2. General aspects of calcium cycling and signaling in vascular smooth muscle cells

Besides maintaining vascular tone in mature vessels, VSMCs also preserve blood vessel integrity [2]. In other words, VSMCs are also instrumental for vascular remodeling and repair associated with VSMCs proliferation and migration. Interestingly, Ca^{2+} plays a central role in both physiological processes. In VSMCs, calcium signaling involves a cross-regulation of Ca^{2+} influx, sarcolemmal membrane signaling molecules and Ca^{2+} release and uptake from the sarco/endo/plasmic reticulum and mitochondria, which plays a central role in both vascular tone and integrity.

2.1. Calcium handling by the plasma membrane's calcium channels and pumps

Membrane depolarization is believed to be a key process for the activation of calcium events in mature VSMCs. Thus, much attention has been given to uncovering the various mechanisms responsible for triggering this depolarization. Increased intra-vascular pressure of resistance arteries stimulates gradual membrane depolarization in VSMCs, increasing the probability of opening L-type high voltage-gated Ca^{2+} channels (Cav1.2) (LTCC) [3, 4]. Alternatively, the calcium-dependent contractile response can be induced through the activation of specific membrane receptors coupled to phospholipase C (PLC) isoforms[3]. The various isoforms of transient receptor potential (TRP) ion channel family, particularly TRPC3, TRPC6 and TRPC7 possibly activated directly by diacyl glycerol (DAG), can also contribute to initial plasma membrane Ca^{2+} influx and subsequent membrane depolarization [5-8]. Non-selective receptor-activated canonical TRPC6 channel, that conduct large sodium (Na^{2+}) currents was also suggested to contribute to membrane depolarization and subsequent L-type channel activation [9, 10]. Membrane depolarization can spread to neighboring cells by current flow through gap junctions providing a synchronization mechanism for VSMC membrane depolarization within the vessel wall [11, 12].

Among voltage-insensitive calcium influx pathways, the store-operated Ca^{2+} channels (SOC), maintain a long-term cellular Ca^{2+} signal. They are activated upon a decrease of internal store Ca^{2+} concentration resulting from a Ca^{2+} release via the opening of SR Ca^{2+} release channels. SOC was first hypothesized in 1986 [13], a paradigm that was confirmed by the identification of its two essential regulatory components, the SR/ER located Ca^{2+} sensor STIM1 (stromal interaction molecule) and the Ca^{2+} channels Orai1 [14-17]. Upon decrease of $[Ca^{2+}]$ in the reticulum (<500μM), Ca^{2+} dissociates from STIM1; then STIM1 molecules oligomerize and translocate to specialized cortical reticulum compartments adjacent to the plasma membrane [18, 19]. There, the STIM1 cytosolic activating domains bind to and cluster the Orai proteins into an opened archaic Ca^{2+} channel known as $\underline{C}a^{2+}$-$\underline{r}$elease $\underline{a}$ctivated $\underline{C}a^{2+}$ $\underline{c}$hannel (CRAC) [4]. Furthermore, transient receptor potential ion

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[3]All isoforms of PLC, catalyze the hydrolysis of phosphatidylinositol4,5-biphosphate (PIP_2) to produce the intracellular messengers IP_3 increase and diacylglycerol (DAG); both of which promote cytosolic Ca^{2+} rise through activation of plasma membrane or sarcoplasmic reticulum calcium channels.

[4] The CRAC is responsible for the "2h cytosolic Ca^{2+} increase" required to induce VSMCs proliferation [57].

channel (TRPC) family members have also been demonstrated to participate in SOC channels functioning via interactions with STIM1 and Orai proteins [20-22].

The calcium signal is terminated by membrane hyper-polarization and cytosolic Ca^{2+} removal. First, calcium sparks resulting from the opening of sub-plasmalemmal clusters of RyR activate large-conductance Ca^{2+} sensitive K^+ (BK) channels. Then, the resulting spontaneous transient outward currents (STOC) hyperpolarize the membrane and decrease the open probability of L-type Ca^{2+} channels [23]. Cytosolic calcium is extruded at the level of plasma membrane by plasma membrane Ca^{2+} ATPase (PMCA) and the Na^+/Ca^{2+} exchanger (NCX) [24, 25]. The principal amount of cytosolic Ca^{2+} (> 70%) is re-uploaded to the internal store.

## 2.2. Calcium handling by the sarco/endoplasmic reticulum's calcium channels and pumps

The initial entry of Ca^{2+} through plasma membrane channels triggers large Ca^{2+} release from the internal store via the process of Ca^{2+}-induced Ca^{2+}-release (CICR). The mechanism responsible for initiating Ca^{2+} release depends on Ca^{2+} sensitive SR calcium channels, the ryanodin receptor (RyR)[5] or the IP_3 receptor (IP_3R). Indeed, IP_3R and RyR are highly sensitive to cytosolic Ca^{2+} concentrations and when cytosolic Ca^{2+} concentration ranges from nM to μM, they open up. On the contrary, a higher cytosolic Ca^{2+} concentration (from μM to mM) closes them [26]. In other words, cytosolic Ca^{2+} increase first exerts a positive feedback and facilitates SR channels opening whereas a further increase has an opposite effect and actually inhibits the SR channels opening [27-29]. Importantly enough to be mentioned, RyR phosphorylation by the second messenger cyclic ADP ribose (cADPR) and protein kinase A (PKA) enhances Ca^{2+} sensitivity, the phosphorylation induced by the protein kinase C (PKC) decreases RyR sensitivity to Ca^{2+} [29, 30]. The initial release occurs in the vicinity of the plasma membrane. It spreads into the cell through the regenerative release of Ca^{2+} by the RyR and /or the IP_3R in the form of an intracellular Ca^{2+} wave travelling down the length of the cell [31-33]. When $[Ca^{2+}]i$ is integrated over an entire cell with time, these Ca^{2+} waves appear as rhythmical oscillations [34].

Sarco/Endoplasmic Ca^{2+}ATPases (SERCA), the only calcium transporters expressed within sarco/endoplasmic reticulum (SR), serve to actively return calcium into this organelle. In mammals, three SERCA genes ATP2A1, ATP2A2 and ATP2A3 coding for SERCA1, SERCA2 and SERCA3 isoforms respectively have been identified [35]. Each gene gives rise to a different SERCA isoform through alternative splicing (**Figure 2**); they all have discrete tissue distributions and unique regulatory properties, providing a potential focal point within the cell for the integration of diverse stimuli to adjust and fine-tune calcium homeostasis in the SR/ER [36]. In VSMCs, SERCA2a and the ubiquitous SERCA2b isoforms are expressed; besides vascular smooth muscle, SERCA2a is preferentially expressed in cardiac and skeletal muscles. SERCA2b differs from SERCA2a by an extension of 46 amino acids that forms an

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[5] RyR are structurally and functionally analogous to IP_3R, although they are approximately twice as large and have twice the conductance of IP_3R [27]; RyR channels are sensitive to store loading and IP_3R channels are sensitized by the agonist-dependent formation of IP_3.

additional trans-membrane domain setting the SERCA2b C terminus in the SR lumen [37]. Functional characterization of SERCA2 isoforms performed in transfected HEK-298 cells clearly indicated that the SERCA2a isoform displays a lower affinity for Ca^{2+} ($K_{0.5}$ = 0.985 µM) but has a higher turnover rate (ATP hydrolysis 70 s^{-1}) compared to SERCA2b ($K_{0.5}$ = 0.508 µM; 35 s^{-1}) [38]. Diversity of SERCA isoforms in the same cell suggests that each of them could be responsible for controlling unique cell functions.
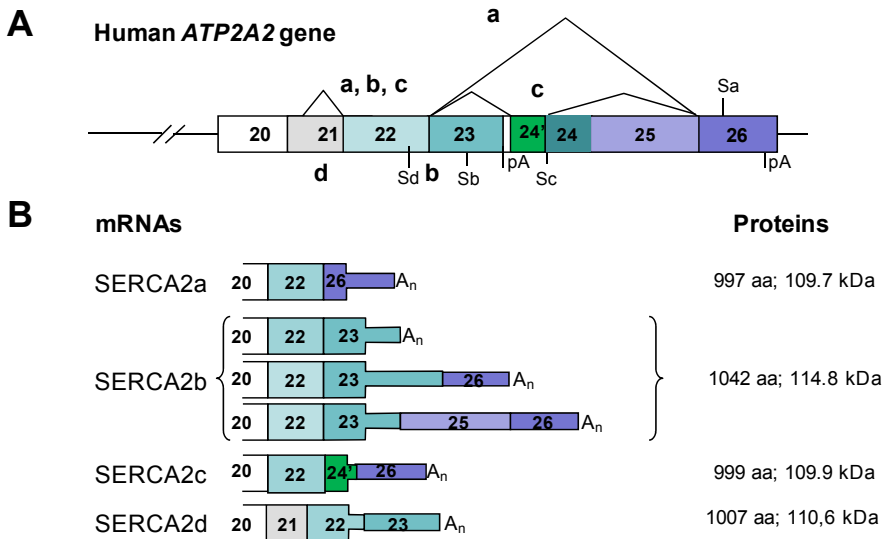
Figure 2. Alternative splicing of the human ATP2A2 genes, protein sizes of the SERCA2 isoforms. A. Representation of the 3'-end of the human ATP2A2 gene. Broken lines with letters represent alternative splicings. The position of stop codons for the corresponding isoforms and polyadenylation site are indicated by Sa-Sd & pA, respectively. B. Representation of the 3'-end of SERCA2a, SERCA2b, SERCA2c and SERCA2d mRNAs expressed in human cells and the sizes of the corresponding proteins. Wide boxes represent translated segments; the less wide boxes represent untranslated segments.

SERCA2's activity depends on its interaction with phospholamban and is inhibitory in its de-phosphorylated form. PKA phosphorylation of phospholamban results in its dissociation from SERCA2, thus activating the Ca^{2+} pumps [39]. Cyclic ADP-ribose was also reported to stimulate SERCA pump activity [40].

As previously mentioned, SR Ca^{2+} content controls the sensitivity of SR Ca^{2+} channels, RyR and IP_3R, as well as functioning of SOC-mediated Ca^{2+} entry, thereby determining the type of intracellular calcium transient. Since SOCs opening depends on Ca^{2+} content of the store, one may suggest that SERCA participates to its regulation. Consistent with this, SOCs open up when the leak of Ca^{2+} from intracellular stores is not compensated with SERCA activity; SERCA inhibitors such as thapsigargin which prevent Ca^{2+} uptake are commonly used to chemically induce SOC currents; several works have established that SERCA can cluster with STIM1 and Orai1 in various cellular types [41, 42].

2.3. Mechanisms of cytosolic Ca^{2+} oscillations in VSMC

Ca^{2+} oscillations are one of the ways that VSMCs respond to agonists [43-47]. These Ca^{2+} oscillations are maintained during receptor occupancy and are driven by an endogenous pacemaker mechanism, called the cellular Ca^{2+} oscillator [12, 33, 34]. Ca^{2+} oscillators were classified into two main types, the membrane oscillators and the cytosolic oscillators [48].

Membrane oscillators are those which generate oscillations at the cell membrane by successive membrane depolarization. In most small resistance arteries, inhibitors of plasma membrane voltage-dependent channels reduce or even abolish the membrane potential oscillations which precede rhythmical contractions. This suggests that rhythmic extracellular Ca^{2+} influx can be required for calcium oscillatory transient [34]. Besides, membrane oscillators greatly depend on Ca^{2+} entry in order to provide enough Ca^{2+} to charge up the intracellular stores for each oscillatory cycle. Some of the entry mechanisms characterized include the Na^+/Ca^{2+} exchanger (NCX) operating in its reverse mode [32, 44, 49], the ATP-sensitive P2X receptor responsible for generating junctional Ca^{2+} transients (jCaTs) [50] or the L-type Cav1.2 channels operating as clusters in a "high open probability mode" to produce persistent Ca^{2+} sparklets [51]. In the case of the latter, entry of Ca^{2+} through these L-type channel clusters does not directly activate RyR to produce sparks, but provides the necessary amount of Ca^{2+} to charge up the ER and sensitize the RyR [52]. Another way of internal store reloading is achieved by the various isoforms of the transient receptor potential (TRP) ion channel family, activated directly by DAG [5, 6].

Cytosolic oscillators do not depend on the cell membrane to generate oscillations. Instead, they arise from intracellular store membrane instability. The pacemaker mechanism of cytosolic Ca^{2+} oscillator is based on the velocity of luminal Ca^{2+} loading and luminal Ca^{2+} content [53, 54]. The mechanism responsible for initiating Ca^{2+} release depends either on RyRs or IP$_3$R activation. As soon as stores are sufficiently charged with Ca^{2+}, the SR Ca^{2+} channels become sensitive to cytosolic Ca^{2+} and can participate to the process of Ca^{2+}-induced Ca^{2+}-release, which is responsible for orchestrating the regenerative release of Ca^{2+} from the SR/ER. Importantly, extracellular Ca^{2+} influx is not required for cytosolic oscillator function. Indeed, the Ca^{2+} oscillations can be observed in the absence of extracellular Ca^{2+} [43, 45, 55, 56].

Finally, it should be mentioned that SERCA proteins play a major role in the establishment of oscillatory Ca^{2+} transient required for phasic contractile response[6], independently from the type of oscillator. Indeed, the frequency of Ca^{2+} oscillations depends solely on the velocity with which Ca^{2+} is re-loaded to the SR. This allows the sensitization of SR release channels, which determine the timing of the next Ca^{2+} spike [54, 57]. Thus, as long as the IP$_3$ membrane receptor/Ca^{2+} channel is activated, the next spike is initiated as soon as the sensitivity of IP$_3$Rs (RyRs) is restored, resulting in oscillatory mode of calcium cycling.

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[6]Phasic contractions are apparent as rapid peaks, whereas tonic contractions cause gradual changes in force that can be maintained for prolonged periods.

3. Calcium cycling and signalling in vascular smooth muscle cells

3.1. Vascular smooth muscle cell phenotype diversity

VSMCs maintain a considerable plasticity throughout life, exhibiting a diverse range of phenotypes in response to local environmental changes [58, 59]. Because studies of smooth muscle phenotype have principally focused on mechanisms which control VSMC proliferation and differentiation [58], VSMC phenotypes are classified into two main categories: the synthetic/proliferating/migratory/inflammatory and the contractile/quiescent/differentiated phenotype. In mature vessels, most VSMCs exhibit a quiescent/contractile phenotype and control the vascular tone [58, 59]. Transition of contractile VSMCs towards a proliferating/migratory/inflammatory phenotype is one of the initial mechanisms leading to pathological vascular remodeling[7] [58, 60]. Culturing VSMCs *in vitro* mimics this progression, as primary cultures rapidly lose differentiation markers and exhibit a synthetic phenotype [60].

Another possibility for classifying VSMCs is to categorize them based on their contractile properties which determine whether the smooth muscle is considered phasic or tonic. Phasic vascular smooth muscle refers to blood vessels displaying rhythmic contractile activity whereas tonic vascular smooth muscle refers to blood vessels displaying continuous contractions [61]. Phasic contraction is the characteristic of small resistance arteries (SRA, 20-50 μm diameters) which predominantly regulates vascular functions such as pressure and flow. *In vivo* small arteries exhibit a mixture of tonic and phasic contractions (RW: [34, 45, 62, 63]) and/or conducted vasomotor response termed vasomotion[8] [64].

The contractile capacities of various VSMC phenotypes are determined by expression of different isoforms of contractile proteins. For instance, phasic contractions displayed by small arteries of the heart and lung, muscular femoral artery, small mesenteric arteries and renal afferent arteriole result from the VSMC expression of the fast isoform of smooth muscle myosin heavy chain (MHC) which determines the velocity of shortening during vasomotion (rev. [61]). Of note, lent isoform of smooth muscle myosin, so called "non muscular myosin, (NM-B)" is expressed in all types of VSMCs including synthetic/proliferating VSMC; it plays a significant role in force maintenance during tonic contraction [58, 61].

Contractile response is initiated by the rise in cytosolic $[Ca^{2+}]i$ leading to the activation of Ca^{2+}/calmodulin-dependent myosin light chain kinase (MLCK) [65]. However the mode of contraction, phasic or tonic, is determined by the type of cytosolic Ca^{2+} cycling. Different types of calcium cycling were observed in synthetic and contractile tonic or phasic VSMCs, in accordance with differential expression of calcium handling proteins [59, 60, 66].

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[7]Vascular remodeling was initially defined as the process of arterial enlargement to accommodate the plaque and maintain constant flow despite increases in atherosclerotic lesion mass. Experimental and clinical observations indicate that blood flow properties influence remodeling after angioplasty, hypertension, and flow diversion as well as atherosclerotic plaque progression [60].

[8] Vasomotion or pulsative flow is suggested to enhance blood flow or tissue oxygenation (rev.[61])

3.2. Calcium cycling in synthetic/proliferating VSMC

The hallmark of synthetic status of VSMCs is a lack of functional proteins entity associated with the contractile response [60]; we refer to voltage activated L-type calcium channels (LTCC), SR calcium release channel RyR and "fast" isoform of SR calcium pump SERCA2a (**Figure 3**). In line with this, large conductance K^+ channels (BK_{Ca}), which are involved in negative feedback regulation of LTCC activity through plasma membrane hyperpolarization, are also down-regulated in synthetic VSMC [67, 68]. On the other hand, the expression of the molecular enti-ties modulating the plasma membrane Ca^{2+}-release activated Ca^{2+} channel (CRAC) functioning [57, 69] are highly up-regulated; we refer to the proteins forming the CRAC complex or regulat-ing the I_{CRAC} (such as ORAI1-3 and STIM1) and to the IP_3R [70, 71]. Besides, the expression of TRPCs family members, particularly TRPC1 and TRPC6, dramatically increases in synthetic cells leading to the increase of whole cell Ca^{2+} current [72, 73].

In synthetic VSMC, agonist binding to PLC-coupled membrane receptors activates IP_3R, resulting in a drastic increase of cytosolic Ca^{2+} which is weakly pumped by the "slow" calci-um pump SERCA2b (the only isoform of SERCA expressed in synthetic VSMCs). The de-pleted store triggers the translocation of STIM1 towards the plasma membrane, which, through the opening of CRAC, induces an extracellular Ca^{2+} influx. This translates into a long lasting increase of cytosolic calcium critical for the activation of Ca^{2+}-sensitive transcrip-tion factor NFAT (nuclear factor of activated T lymphocytes), required for proliferation and migration of VSMCs [56, 74]. Since these cells express contractile proteins, such as NM-B [58, 61], one may suggest that long lasting increase of cytosolic calcium can also produces tonic contraction.

Importantly enough to be mentioned, the restoration of SERCA2a expression by gene trans-fer in synthetic VSMCs blocks their proliferation and migration via inhibition of transcrip-tion factor NFAT [56, 75]. Molecular mechanisms of this effect are related to the prevention of functional association between STIM1 and Orai1 (CRAC protein entity) which lead to the suppression of store-operated calcium influx [56]. It is worth mentioning that SOC influx following agonist stimulation is not observed in contractile VSMCs, naturally expressing SERCA2a (Bobe & Lipskaia, unpublished data), highlighting again the importance of the SERCA isoform(s) expressed in VSMCs.

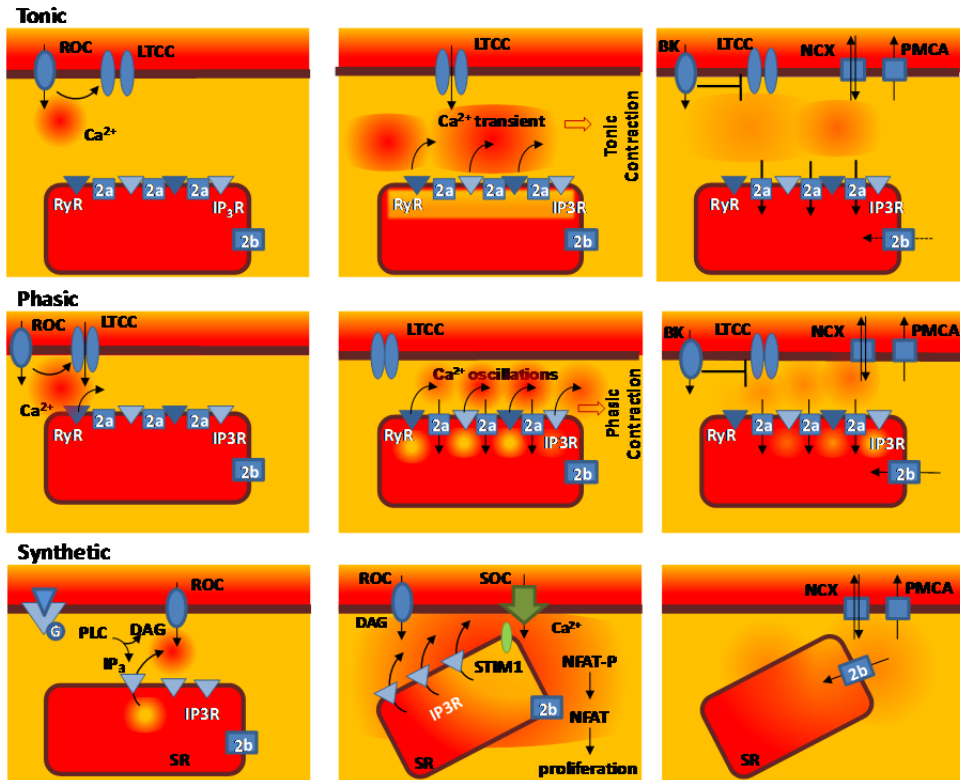## 3.3. Calcium cycling in contractile tonic and phasic VSMC

In mature vessels, VSMCs mainly exhibit a tonic or phasic contractile phenotype. In contractile VSMCs extracellular calcium influx predominantly takes place through the voltage-dependent L-type calcium channel, $LTCC^9$ (**Figure 3**). Extracellular Ca^{2+} influx causes a small increase of cytosolic Ca^{2+} generated by the opening of IP_3R clusters, called puff and/or RyR2 clusters, called spark [28, 57]. These local rises of cytosolic Ca^{2+} generate a larger SR Ca^{2+} release through the Ca^{2+}-induced Ca^{2+} release phenomenon. Elevation of free cytosolic calcium triggers VSMC contraction. The mode of intracellular calcium transient determines the type of contraction,

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[9] In contractile VSMCs, NFAT can be activated by sustained Ca^{2+} influx (persistent Ca^{2+} sparklets) mediated by clusters of L-type Ca^{2+} channels operating in a high open probability mode [76, 77].

tonic or phasic. Steady state increase in cytosolic Ca^{2+} triggers tonic contraction; oscillatory type of Ca^{2+} transient triggers phasic contraction. [34, 76]. It is worth mentioning that accumulating evidence indicate that SR Ca^{2+}ATPase functioning/location within the cell (which greatly influences the velocity of calcium upload) determines the mode of Ca^{2+} transient in VSMCs. Consistent with this, i) "phasic" VSMCs display a greater number of peripherally located SR than "tonic" VSMCs; indeed "tonic" VSMCs exhibit centrally located SR; (rev in [61, 77]); ii) drugs which interfere with the IP_3 pathway or intracellular stores abolish spontaneous vasomotion [11, 78]; iii) blocking SERCA strongly inhibits the Ca^{2+} oscillations, demonstrating that they are induced by SR Ca^{2+} release; this latter argument is further supported by the fact that oscillations are present even in the absence of extracellular Ca^{2+} [43, 45, 55, 56].

Figure 3. Dynamic schematic representation of calcium cycling in contractile phasic or tonic and synthetic VSMCs. Left panel: initiation of calcium event. Middle panel: resulting calcium transient and related physiological function. Right panel: termination of calcium event. The color intensity reflects Ca^{2+} concentrations. Abbreviations: BK - potassium channel; DAG - diacylglycerol; IP3 - inositol-1,4,5-trisphosphate; IP3R - inositol-1,4,5-trisphosphate receptor; LTCC - voltage-gated L-Type Calcium channel; NCX - Na^+/Ca^{2+} exchanger; NFAT - nuclear factor of activated T-lymphocytes; PLC - phospholipase C; PMCA – plasma membrane Ca^{2+} ATPase; ROC - receptor activated channel; RyR - ryanodine receptor; SERCA2a and SERCA2b - sarco(endo)plasmic reticulum Ca^{2+} ATPase type 2a and 2b; SOC - store operated Ca^{2+} channel; SR - sarcoplasmic reticulum, STIM1 - stromal interaction molecule 1.

The "fast" calcium pump SERCA2a, specifically expressed in contractile VSMCs can be responsible for the establishment of the "cytosolic oscillator". Several arguments are in agreement with this proposal: i) SERCA2a has a higher catalytic turnover when compared to SERCA2b due to a higher rate of de-phosphorylation and a lower affinity for Ca^{2+}; ii) SER-CA2a is absent in synthetic VSMCs, which only exhibit tonic contraction, iii) transferring the SERCA2a gene to synthetic cultured VSMCs modifies the agonist-induced calcium transient from steady-state to oscillatory mode [56]. Therefore, one might suggest that the physiological role of SERCA2a in VSMCs consists of controlling the "cytosolic oscillator", thereby determining phasic vs tonic type of smooth muscle contraction.

Despite the fact that agonist-induced Ca^{2+} oscillations are a characteristic feature of the activation mechanisms of VSMCs [43-47], oscillatory type of Ca^{2+} transient is poorly associated with phasic contractile response. In some vessels, asynchronous oscillations of individual VSMC maintain a particular vascular tonus. However, in small resistance vessels the oscillations of groups of cells are synchronized through gap-junctions resulting in the pulsative contractile response [34, 45, 79, 80]. This oscillatory activity can be regulated by variations of neurotransmitters following sympathetic activation and can affect contractile tone through the increase of frequency, thereby increasing blood flow or tissue oxygenation [43, 45, 46, 81]. This frequency modulation could result from PKA phosphorylation of RyR, PLB and contractile proteins, as it has been established for cardiomyocytes [82-84]. Micro-vascular dysfunction, defined as the intrinsic changes in VSMCs contractility (such as reduction of frequency and shortening velocity of phasic contractions), observed in the context of cardio-vascular [85, 86], may be related to reduced PKA phosphorylation of Ca^{2+} handling and contractile proteins, as observed in failing cardiomyocytes [82].

4. SERCA2a as a potential target for treating vascular proliferative diseases

Abundant proliferation of VSMCs is an important component of the chronic inflammatory response associated to atherosclerosis and related vascular occlusive diseases (intra-stent restenosis, transplant vasculopathy, and vessel bypass graft failure). Great efforts have been made to prevent/reduce trans-differentiation and proliferation of synthetic VSMCs. Anti-proliferative therapies including the use of pharmacological agents and gene therapy approaches are, until now, considered as a suitable approach in the treatment of these disorders [87]. Indeed, coronary stenting is the only procedure that has been proven to reduce the incidence of late restenosis after percutaneous transluminal coronary angioplasty ([88]). Nevertheless, post-interventional intra-stent restenosis, characterized by the re-narrowing of the arteries caused by VSMC proliferation, occurs in 10 to 20 % of patients. These disorders remain the major limitation of revascularization by percutaneous transluminal angioplasty and artery bypass surgery. The use of drug-eluting stents (stent eluting anti-proliferative drug) significantly reduces restenosis but impairs the re-endothelialization process and subsequently often induces late thrombosis [89, 90]. In human, trans-differentiation of contractile VSMCs towards a synthetic/proliferating inflammatory/migratory phenotype after percutaneous transluminal angioplasty appears to be a fundamental process of vascular

proliferative disease [91]. In contrast, phenotypic re-differentiation of neo-intimal VSMCs after bare metal stent implantation was reported to be associated with a decline in platelet activation and inflammatory cell infiltration, and the regeneration of the endothelial cell layer [92]. Thus, defining novel molecular target(s) of DESs, that can simultaneously prevents VSMC proliferation and adverse vascular remodeling while facilitating re-endothelialization, is crucial. SERCA2a gene transfer prevents neo-intimal proliferation and intimal thickening in the rat carotid injury model by normalizing calcium cycling and inhibiting NFAT activity [75]. Furthermore, SERCA2a gene transfer prevents VSMC trans-differentiation in injured segments while allowing re-endothelialization [75]. Thus, SERCA2a can be considered as a potential and powerful target for treating vascular proliferative disease.

5. Concluding remarks

Over the last decade, great progress has been made in the understanding of the various intracellular molecular mechanisms in VSMCs which control calcium cycling and excitation/contraction or excitation/transcription coupling. VSMCs employ a great variety of Ca^{2+} signaling systems that are adapted to control their different contractile functions. Alterations in the expressions of Ca^{2+} handling molecules are closely associated with VSMC phenotype modulation. Furthermore, these changes in expression are inter-connected and each acquired or lost Ca^{2+} signaling molecule represents a component of signaling module functioning as a single unit.

In non-excitable synthetic VSMCs, calcium cycling results from the protein module ROC/IP$_3$R/STIM1/ORAI1 which controls SOC influx. Agonist stimulation of synthetic VSMCs translates into a sustained increase in cytosolic Ca^{2+}. This increase is required for the activation of NFAT downstream cellular signaling pathways inducing proliferation, migration and possibly an inflammatory response. Calcium cycling in excitable contractile VSMCs is governed by the protein module composed of ROC/LTCC/RyR2/SERCA2a and controls the contractile response. The location of particular ion channels within the smooth muscle cell with regards to internal stores, other membrane ion channels, gap junctions as well as the expression of fast isoforms of contractile proteins have a significant impact on the resulting phasic or tonic contractile response. Future studies unraveling the correlation between the dynamic changes in Ca^{2+} signaling protein expression and specific subcellular localization are needed to delineate the mechanisms by which Ca^{2+} signaling molecules produce a phasic or tonic contractile response.

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Acknowledgments

Abbreviations

BK – large-conductance Ca^{2+} sensitive K^+ channel

cADPR – cyclic Adenosine Diphosphate Ribose

CICR - Ca^{2+}- Induced Ca^{2+} Release

CRAC - Ca^{2+}- Release Activated Ca^{2+} Channels

DAG - Diacyl Glycerol

IP_3R - sarco/endoplasmic reticulum Ca^{2+} channel Inositol tri-Phosphate Receptor

LTCC - voltage-dependent L-type Ca^{2+} channels

NCX – Na^+/Ca^{2+} exchanger

PKA – Protein Kinase A (activated by cAMP, cyclic adenosine monophosphate)

PLC – Phospholipase C

PMCA – Plasmic Membrane Ca^{2+} ATPase

RyR - sarco/endoplasmic reticulum Ca^{2+} channel Ryanodin Receptor

SOC - Store-Operated Ca^{2+} Channels

SERCA - Sarco/Endoplasmic Reticulum Ca^{2+} ATPase

SRA - Small Resistance Arteries

SR/ER – Sarco/Endoplasmic Reticulum

STIM1 – Stromal Interaction Molecule 1, SR Ca^{2+} sensor

TRPC - Transient Receptor Potential ion Channel

VSMCs - Vascular Smooth Muscle Cells

6. References

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