[Frontiers in Bioscience 15, 1023-1039, June 1, 2010]

TRPC channels in smooth muscle cells

Jose C. Gonzalez-Cobos, Mohamed Trebak

The Center for Cardiovascular Sciences, Albany Medical College, Albany, NY

TABLE OF CONTENTS

1. ABSTRACT

Transient receptor potential canonical (TRPC) proteins constitute a family of seven (TRPC1-7) nonselective cation channels within the wider TRP superfamily. TRPC1, TRPC3, TRPC4, TRPC5 and TRPC6 channels are expressed in vascular smooth muscle cells from human vessels of all calibers and in smooth muscle from organs such as the uterus and the gastrointestinal tract. TRPC channels have recently emerged as important players in the control of smooth muscle function. This review will focus on the retrospective analysis of studies proposing contributions of TRPC channels to native calcium entry pathways in smooth muscle and to physiological and pathophysiological responses with emphasis on the vascular system.

2. CALCIUM SIGNALING IN SMOOTH MUSCLE CELLS

Calcium (Ca^{2+}) ions impact nearly every aspect of cellular life and are considered universal intracellular messengers controlling a diverse range of cellular processes, including skeletal, cardiac and smooth muscle contraction, neuronal growth and neurotransmitter release (1; 2; 3). The spatial localization of Ca^{2+} signals also contribute to increase the diversity of signals that can be successfully transmitted to downstream effectors (4). Vascular smooth muscle cells (SMCs) form a layer of contractile cells in the blood vessel wall and are known to be instrumental in maintaining the blood vessel structural integrity and regulating blood pressure and blood flow distribution (5; 6; 7). It is by the coordinated contraction and relaxation of these cells that the blood vessel diameter and stiffness can be modulated, thereby serving as one of the principal clinical control points for cardiovascular physiological parameters. In vascular SMCs, Ca^{2+} signals have been suggested as modulators of cellular functions such as gene transcription, cell proliferation, contraction and phenotypic modulation that occur during vascular disease (5; 8; 9). Cytosolic Ca^{2+} levels are carefully maintained at the hundred nanomolar range and Ca^{2+} signals can be generated through Ca^{2+} mobilization from either the intracellular stores (mainly the sarcoplasmic reticulum; SR) or the extracellular space. By means of Ca^{2+} permeable channels and Ca^{2+} pumps that mediate Ca^{2+} entry and Ca^{2+} extrusion/buffering respectively, SMCs keep intracellular Ca^{2+} levels under tight control. The vascular tone or contractile state of the vessels is regulated through changes in the membrane potential of SMCs whereby membrane depolarization activates Ca^{2+} entry through voltage-activated L-type Ca^{2+} channels leading to activation of contractile proteins resulting in SMC contraction (10; 11). Physiologically, tone and contractility of vascular SMCs are controlled by cellular integration of a plethora of signals in response to vasoactive agonists. It is not unambiguously determined whether vascular reactivity in response to neuronal, humoral and endothelial factors is mediated directly by receptor-generated Ca^{2+} entry or by the indirect activation of L-type Ca^{2+} channels through Na^+-mediated membrane depolarization resulting from receptor activation of non-selective cation channels.

Membrane receptors to vasoactive hormones and neurotransmitters (e.g. norepinephrine, angiotensin II and vasopressin) are typically coupled to G proteins resulting in the activation of isoforms of phospholipase C (PLC). The activation of PLC catalyzes the breakdown of phosphatidylinositol 4,5-bisphosphate (PIP_2) into two intracellular second messengers, Inositol 1,4,5-trisphosphate (IP_3) and Diacylglycerol (DAG) (12). These second messengers play a central role in Ca^{2+} release from intracellular Ca^{2+} stores and Ca^{2+} entry from the extracellular space. Ca^{2+} release from the SR is mediated by the action of IP_3 on its receptor (IP_3R) located at the SR (12). The fall of the Ca^{2+} concentration within the lumen of the SR (store depletion) is functionally coupled to the activation of Ca^{2+} entry from the extracellular space *via* store operated Ca^{2+} (SOC) channels (13; 14). This pathway was originally termed capacitative Ca^{2+} entry (CCE) but is commonly referred to as store-operated Ca^{2+} entry (SOCE) (13; 15; 16). The role of SOCE is to refill the stores and also to signal downstream to the nucleus. In SMCs, SOCE was proposed to meditate contractility as well as cell proliferation and migration (17; 18). The current mediating SOCE was first measured in rat basophilic leukemia (RBL) mast cells and termed Ca^{2+} release-activated Ca^{2+} (CRAC) current (19). CRAC channels exhibit low conductance, strong inward rectification and displays remarkable Ca^{2+} selectivity (13; 19; 20).

In addition to the action of IP_3, the increase in the intracellular Ca^{2+} levels and the concomitant generation of DAG and other downstream metabolites of the phosphoinositide pathway such as Arachidonic Acid (AA) are known to directly mediate the activation of Ca^{2+} entry from the extracellular space *via* Ca^{2+}-permeable store-independent cation channels that are referred to as Receptor-Operated channels (ROC), because their activation does not depend on the state of the stores and requires instead, actions of second messengers produced downstream of receptor activation (1; 13; 21; 22; 23). It is essential to recognize the fundamental distinction between the activation mechanisms and molecular identities of these two Ca^{2+} entry pathways. Although both SOC and ROC channels function downstream of PLC, here we will refer to SOC channels under the strict definition where store depletion is necessary and sufficient for their activation without requirement for actions by Ca^{2+} and other lipid second messengers.

3. CONTRIBUTION OF TRPC CHANNELS TO SMOOTH MUSCLE CALCIUM SIGNALS

The molecular identity of the SOCE pathway in different cell types and in SMCs in particular has been the subject of intense investigations for the past two decades, and remains to this day a highly controversial topic (5; 13; 24). One of the first molecular candidates proposed to encode SOC channels were mammalian transient receptor potential (TRP) channels, particularly members of the canonical family (TRPC), by virtue of their activation downstream of PLC-coupled receptors (25). The discovery of the TRP superfamily of cation channels was initially related to a channelopathy where drosophila flies with mutations in the TRP gene were found to have impaired vision due to the lack of a specific light-induced PLC-dependent Ca^{2+} entry pathway in photoreceptor cells (25; 26; 27; 28; 29). Normally in these cells, excitation by light is maintained and so is depolarization, as long as the stimulus (light) is present. Referring to the specific electric phenotype of mutant flies, where a normal but transient response was present due to failure to maintain depolarization upon light stimulation, this gene was called transient receptor potential or Drosophila TRP (25; 30; 31; 32; 33; 34; 35). The discovery of the drosophila TRP gene eventually led to the identification of a number of TRP homologs in mammals (36). TRPC channels represent one family among the six large families that constitute the TRP superfamily of cation channels, and are termed “classical” or “canonical” because they are structurally the closest to the founding family member, Drosophila TRP (37; 38). The mammalian TRPC family has seven members (TRPC1-TRPC7) out of the 28 members of the human TRP superfamily that have been identified so far. Based on structural homology, functional similarities and direct known interactions, the TRPC family can be divided into four subfamilies: TRPC1, TRPC2, TRPC3/6/7 and TRPC4/5 (or TRPC1 is sometimes included in the TRPC4/5 subfamily) (24; 37; 38). TRPC2, although a pseudogene in humans, is known to encode functional channels in most other mammals. (For a comprehensive review the reader is referred to (39)). The seven mammalian TRPC cation channels share architectural compositions that can be summarized as follows: six transmembrane spanning regions (TM1-6), with a putative pore forming region between TM5 and TM6 (40), and cytoplasmic N- and C-terminus where 3-4 ankyrin-like repeats (ANK1-4) and the invariant TRP signature motif (EWKFAR) are located respectively (38; 41; 42).

Since their discovery, all the TRPCs have been suggested to encode SOC and ROC channels, based on their participation in Ca^{2+} entry routes that were initially shown to be activated downstream of PLC-coupled receptors (24; 38; 41; 42; 43; 44). Ironically, it is now clear that the mechanism by which the Drosophila TRP is activated in its native environment in photoreceptor cells is independent of store depletion (45). Notwithstanding this evolutionary conundrum, a large body of evidence in the past decade supported a role for TRPC channels as SOCs in a variety of mammalian cell types including SMCs and endothelial cells (ECs) from different vascular beds (for reviews (13; 24; 44)). However, a large number of laboratories, including our own showed that TRPCs do not function as SOCs when ectopically expressed in HEK293 cells and that native SOCE in SMCs and ECs functions independently of TRPC channels (14; 18; 24; 46). In fact, the past 4-5 years yielded significant advancements regarding the molecular composition and the activation mechanism of SOC channels and had a remarkable impact in revitalizing the quest for understanding SOC regulation. Using RNA interference (RNAi)-based high throughput screens combined with the SERCA pump blocker thapsigargin to passively deplete the stores, four independent groups clearly identified two conserved genes encoding proteins that are required for SOCE in drosophilae Shneider2 (S2) cells and mammalian cells, STIM1 and Orai1 (*dSTIM* and *dOrai* in drosophilae; mammals have 2 STIMs and 3 Orais encoded by separate genes while drosophilae has one of each) (47; 48; 49; 50; 51). STIM1, a type 1 single-pass transmembrane protein that contains a single low affinity Ca^{2+} binding EF-hand domain and is resident mostly in the endoplasmic reticulum (ER; in some cell types it populates the plasma membrane to a lesser extent) is the long-sought Ca^{2+} sensor that senses the fall of Ca^{2+} concentration within the lumen of the ER (52; 53). It is now well accepted that upon store depletion STIM1 is capable of oligomerization and reorganization into punctuate structures (14; 54; 55), in areas of the ER that are the closest to the plasma membrane, to signal the activation of Orai1, the pore forming subunit of the CRAC/SOC channel. More recent studies have identified a minimal, highly conserved domain of approximately 100-amino acid in STIM1 C-terminus called STIM Orai activating Region (SOAR) or CRAC activating domain (CAD) that binds directly to the N- and C-termini of Orai1 to activate Ca^{2+} entry (56; 57; 58; 59).

One thing is certain, in no circumstance has an ectopically expressed TRPC served to recapitulate the biophysical characteristics of the well-characterized CRAC channel expressed in T lymphocytes, mast cells and other hematopoietic cells (13; 60). In fact, number of studies analyzing the electrophysiological properties of cloned mammalian TRPCs revealed that upon activation, these channels are nonselective and conduct Na^+, K^+ and Ca^{2+} (61; 62). Although it is now clearly established that the archetypical CRAC channel is structurally formed by Orai1 proteins, the involvement of TRPC proteins either in conjunction with Orai1 in making up the CRAC channel or alone in forming a nonselective SOC channel distinct from CRAC and activated in a STIM1-dependent manner remains an open question (36; 63; 64). In fact, a number of "SOC currents" measured in different cell types including vascular SMCs from different vascular beds and species have been reported to be non-selective and to present biophysical properties that differ from those of CRAC channels (13; 65; 66). A number of studies have showed reduced SOCE when TRPC expression is either knocked down or knocked out, suggesting a role of these proteins in the mediation of the non-CRAC nonselective SOC channels (13; 24). Furthermore, a ternary complex between TRPC1, STIM1 and Orai1 has been reported to be essential for the activation of a nonselective channel in response to store depletion in human salivary gland cells (67). On the other hand, an extensive body of literature supports a role for TRPC proteins as receptor operated (ROC) channels rather than store-operated channels (SOC) (68; 69; 70). Recently, DeHaven *et al* presented strong evidence that TRPC channel activation does not depend on STIM1 and that Orai and TRPC channels are located in distinct regions of the plasma membrane and function independently (71). Studies from our laboratory showed that SOCE in human umbilical vein endothelial cells (HUVECs), human pulmonary artery endothelial cells (HPAEC) and primary rat aortic smooth muscle cells is mediated through CRAC channels contributed by STIM1 and Orai1 independently of TRPC proteins and other Orai isoforms (18; 46).

4. TRPC CHANNELS AND VASCULAR SMOOTH MUSCLE PHENOTYPIC MODULATION

Vascular SMCs express a large repertoire of ion channels that are critical to translate physiological stimuli into critical cellular functions such as contraction, migration and proliferation (5; 72). In normal conditions, SMCs within the adult vasculature are characterized by an extremely low rate of proliferation, very low synthetic activity and a unique repertoire of ion channels, contractile proteins and signaling molecules that are all required for their proper function (5; 6; 73). However, it is known that cell type-specific channel profiles exist between smooth muscle cells residing in different anatomical locations, and that this specific channel expression profile is critical when defining the phenotypic identity of the smooth muscle cell (7; 74). Unlike cardiac and skeletal myocytes that are terminally differentiated, vascular SMCs retain remarkable phenotypic plasticity that is responsive to humoral, environmental and pathophysiological cues. Dedifferentiation from the quiescent phenotype to the synthetic one is accompanied by adaptive changes in expression profile of different ion channels, transporters and Ca^{2+} binding proteins that provides the cell with means to support its new proliferative and migratory phenotype. This phenotypic modulation or switching from a contractile to a synthetic phenotype can be seen upon vascular injury and in various vascular disease states such as atherosclerosis and hypertension. Synthetic vascular SMCs downregulate the expression of L-type voltage gated Ca^{2+} channels and concomitantly increase the expression of the low voltage-activated (T-type) Ca^{2+} channels and TRPC channels (5). Recent studies have suggested that Ca^{2+}-responsive pathways are responsible for transcriptionally regulating their own components whereby a Ca^{2+} entry *via* a specific Ca^{2+} channel is capable of activating the transcription of this channel's mRNA as recently described for TRPC6 channels (75). Thus, TRPC channels, which are upregulated in synthetic SMCs, may activate pro-proliferative pro-migratory downstream signaling pathways in vascular SMCs and control the transcriptional regulation of the Ca^{2+} responsive components of these pathways. Evidence for a functional role of TRPC channels in mediating vascular SMC phenotypic modulation in disease will be discussed later in this review.

5. ACTIVATION MECHANISMS OF TRPC CHANNELS IN SMOOTH MUSCLE

TRP channels are expressed in almost every tissue and cell type, where they play unique roles as cellular sensors and signal integrators of a plethora of Ca^{2+}-mediated cellular functions (76; 77). In the vasculature, all seven members of the TRPC family of cation channels are expressed. TRPC1, and TRPC3 through TRPC6 channels are widely expressed in human vessels of all calibers, from the largest conduit vessels to medium size coronary arteries, cerebral arteries, smaller size resistance arteries and vaso vasorum, where they were proposed to mediate physiological and pathophysiological cellular responses (78). With the exception of a study reporting a role of a channel formed by heteromultimeric association between TRPC6 and TRPC7 that is activated by vasopressin in A7r5 smooth muscle cell line (79), the expression of TRPC7 has been found in endothelial cells but not in vascular SMCs. The founding member of the canonical TRP family is TRPC1, which was the first mammalian TRP member to be cloned (41; 80; 81). TRPC1, which is found in vascular SMCs of several species, is widely but not uniformly expressed in all types of vascular beds (78; 82; 83). The proposed physiological roles of TRPC1 include contributions to critical functions such as vascular SMC contraction and proliferation (36; 83; 84; 85; 86). The discovery of TRPC1 in the vasculature led to the hypothesis that this channel was the long sought vascular SOC channel. Subsequently, many researchers have proposed that TRPC1 contributes to SOCE in vascular SMCs from many vascular beds in several species such as human, dog, mouse, rabbit and rat (82; 83; 87; 88; 89; 90). A great part of the accumulated knowledge on the functional properties of TRPC1 has been acquired from studies in which the function of the endogenous protein was impaired by treatment with an antibody against an extracellular loop of the putative pore forming region (82; 91) or by the use of antisense DNA and RNAi targeting TRPC1 mRNA (89; 90; 92). Interestingly, the outcomes of all the studies when focusing on vascular SMCs converge in that these treatments were able to only marginally inhibit SOCE activated by thapsigargin or cyclopiazonic acid (CPA). For example, Xu *et al* showed that using an antibody targeting the putative pore forming region of TRPC1 inhibited SOCE by ~15% (82). An exception is the study by Takahashi *et al* which reported the abrogation by ~60% of SOCE in response to thapsigargin in coronary artery SMCs treated with RNAi against TRPC1, as compared to control (88). In a concurrent study, these authors reported that mediation of SOCE by TRPC1 occurs in a STIM1-dependent manner in human coronary artery smooth muscle cells (93). However, the contribution of membrane depolarization, Ca^{2+}-activated channels and voltage-gated channels to the overall Ca^{2+} signal in these cells is unclear. In fact, a general observation in most of the studies suggesting a role for TRPC channels in SOCE is the lack of current recordings in the presence of strong buffering to rule out contributions from Ca^{2+}-activated currents. At the very least, Ca^{2+} measurements under voltage clamp conditions or the use of protocols with voltage-gated channel inhibitors are necessary to support the Ca^{2+} imaging measurements (54). Another complication of Ca^{2+} measurements is the potential generation of recordings artifacts by the use of SERCA blockers such as thapsigargin and CPA, which by compromising the buffering capacity of the ER/SR might exaggerate the constitutive -not regulated- activity of Ca^{2+} entry through a TRPC channel (94) (discussed in detail in (37)). Despite the large body of evidence supporting a role of TRPC1 (and other TRPC) channels in SOCE, an equal amount of studies from many independent investigators failed to detect any role for TRPC proteins in SOCE. Briefly, studies by Dietrich *et al* have showed that smooth muscle cells isolated from aorta and cerebral arteries of TRPC1 knockout mice possess SOCE currents that were comparable to those recorded in cells from wild type mice (69). Recently, DeHaven *et al* clearly demonstrated that the function of TRPC1, TRPC3, TRPC5, TRPC6 and TRPC7 does not depend on STIM1 (71). Another limitation in studies investigating the role of TRPC1 is the discrepancy between results from different groups when TRPC1 was ectopically expressed in cell lines (60). Although some laboratories reported functional TRPC1 channels at the plasma membrane following TRPC1 ectopic expression, other groups have demonstrated the need of co-expression with other TRPC isoforms for the proper trafficking of TRPC1 to the plasma membrane. A rigorous study by Hofmann *et al* have showed that interactions of TRPC1 with TRPC4 and TRPC5 appear to be necessary to translocate TRPC1 to the plasma membrane, as assessed by four independent experimental approaches (95). Additionally, the interactions of TRPC1 with other TRPC members provide these heterotetrameric channels with unique biophysical properties distinct from channels formed as homotetramers (96). The difficulty in reconciling TRPC channel properties with SOCE has been critically evaluated elsewhere (97), and in general, a less contentious topic is that physiological TRPC1 activation is achieved downstream of PLC activation by still a yet unknown mechanism.

It is well accepted that under physiological conditions, TRPC4/5 channels are activated downstream of PLC-coupled receptors, are insensitive to DAG and IP_3 but show clear requirement of PLC activation (98). The mechanism of activation of TRPC4/5 *via* PLC-coupled receptors is unclear and seems to require complex actions of polyphosphoinositides, G proteins and Ca^{2+} (99; 100;

101; 102). TRPC5 is expressed in a variety of SMC types (86; 103). An additional mechanism has been reported for the activation of TRPC5 channels and involves rapid translocation to the plasma membrane upon growth factor-mediated receptor stimulation (104). TRPC4 has been shown to be widely expressed in the endothelium where it is proposed to coordinate endothelium-dependent vascular smooth muscle regulation (105; 106), but its expression is also found in a great variety of SMCs from different vascular beds (86) (Table 1). The contribution of TRPC4 and TRPC5 to the SOCE pathway also remains uncertain. In a manner similar to TRPC1, interactions of STIM1 with TRPC4/5 channels have been reported in ectopic expression systems in HEK293 cells and proposed to determine the function of TRPC4/5 channels as SOCs (107; 108; 109; 110). Knockdown of TRPC4 using RNAi in pulmonary artery smooth muscle cells inhibited cyclopiazonic acid-activated Ca^{2+} entry as measured with Fura2 imaging (111). Xu *et al* showed that an antibody (T5E3) targeting the putative pore-forming region of TRPC5 was able to inhibit SOCE in arterioles (112). However, other studies on channels formed by TRPC4 and TRPC5 have shown receptor-activated rather than store-operated regulation (100; 101; 102) (for review see (113)). Ulloa *et al* recently showed that human myometrium expresses TRPC4, TRPC1 and TRPC6 mRNAs and demonstrated a store-independent contribution of TRPC4 channels to receptor-activated Ca^{2+} entry (in response to oxytocin, ATP and PGF2α) in PHM1-41 cells and primary human uterine SMCs (114). More recently, non-selective receptor-operated store-independent TRPC4 cation conductances were reported in response to acetylcholine-mediated muscarinic receptor activation in gastrointestinal SMCs (115).

Like all TRPC members, the TRPC3/6/7 subfamily forms Ca^{2+}-permeable non-selective cation channels that are activated through PLC-coupled receptors and display both, inward and outward rectification with reversal potentials around 0mV. The cation permeability ratios p_{Ca}/p_{Na} for TRPC3/6/7 range from 3 to 6, indicating nonselective behavior (37; 38; 116). It is widely accepted that TRPC3/6/7 are activated by DAG analogs in a PKC-independent manner providing a plausible mechanism for their activation through PLC-coupled receptors (61; 68; 117; 118). Furthermore, studies from our laboratory showed that endogenous DAG is sufficient to activate TRPC3 channels independently of IP_3, IP_3R, G proteins and store depletion (68). Treatment of cells with phorbol esters inhibited the DAG analog 1-oleyl-2-acetyl-*sn*-glycerol (OAG)-mediated activation of the TRPC3/6/7 subfamily suggesting negative regulation rather than signal mediation *via* PKC (68; 117; 118). This negative regulation exerted by PKC occurs *via* serine712 phosphorylation on TRPC3 channels (119). While it is clearly established that diacylglycerol (DAG) produced through Phospholipase C-coupled receptor stimulation and structural analogs such as OAG activate TRPC3/6/7, the exact mechanisms of activation of these channels by DAG remains unknown. Furthermore, it appears that TRPC3/6/7 channels require PIP_2 for their proper activation by DAG analogs (120). TRPC6 is the major TRPC expressed in vascular SMC and the most widely studied. TRPC6 is the only TRPC channel that has not been described as SOC; when ectopically expressed, both human and mouse isoforms of TRPC6 behave as a non-selective cation channels whose activation downstream of PLC is independent of intracellular Ca^{2+} store depletion (61; 116; 121). Kim and Saffen showed that an equivalent residue to the serine 712 identified in TRPC3 was present in rat TRPC6 and was implicated in the PKC-mediated phosphorylation and negative regulation of TRPC6 channels (122). As will be discussed below, under physiological conditions TRPC6 channels appear to mediate the effects of vasoactive compounds in vascular SMCs (121; 123; 124).

6. TRPC CHANNELS IN VASCULAR PHYSIOLOGY

Blood flow regulation is mainly achieved by the integration of signals conveyed by vasoactive compounds such as norepinephrine, vasopressin, endothelin-1 and angiotensin II, which upon stimulation of vascular SMC membrane receptors regulate the vascular tone. Many studies have suggested a role for TRPC channels as components of this physiologically relevant pathway (124). A good amount of evidence suggests TRPC1 contribution in mediating the vascular action of vasoactive peptides, hormones and neurotransmitters. Saleh *et al* reported that in freshly isolated rabbit mesenteric artery smooth muscle cells, low and high concentrations of angiotensin II are capable of activating two conductances that were inhibited by an AT1 receptor inhibitor and by antibodies against TRPC1 and TRPC6 (125). Moreover, Bergdahl *et al* have shown that treatment of caudal arteries with a TRPC1 antibody inhibited endothelin-1-induced vasoreactivity and vascular SMC contraction (126). In studies focusing on a canine model of cerebral vasospasm after subarachnoid hemorrhage (SAH), a novel mechanism involving endothelin-1-mediated acute elevations in intracellular Ca^{2+} and severe basilar artery constriction have been described (127). Treatments of SAH arteries with antibodies targeting either TRPC1 or TRPC4 were capable of inhibiting endothelin-1-induced Ca^{2+} entry and vasoconstriction (127). In rat aortic SMCs, the Ca^{2+} signal elicited by endothelin-1 was also inhibited by RNAi targeting TRPC1 (128). Although TRPC4 is found widely expressed in vascular SMCs and endothelial cells from human vascular beds and different size arteries, its contribution to SMC physiology is not well defined (86; 103). Studies in $TRPC4^{-/-}$ mice have shown deficiencies in endothelial-dependent SMC relaxation but interestingly its contribution to the SMC contractile response is unclear (105). TRPC4, along with TRPC6, have been proposed however to play an *in vivo* role in gastrointestinal motility through control of SMC contraction (115); nonselective cationic currents contributed by TRPC4 and TRPC6 channels were shown to be activated through muscarinic receptor stimulation in intestine SMCs. It was suggested that the acetylcholine-activated nonselective TRPC currents thus generated would cause depolarization of intestine SMCs with subsequent L-type Ca^{2+} channel activation and contraction (115). Similarly, it was reported by Walker *et al* that membrane depolarizing currents, causing Ca^{2+} entry through voltage-gated Ca^{2+} channels, had a similar current-voltage

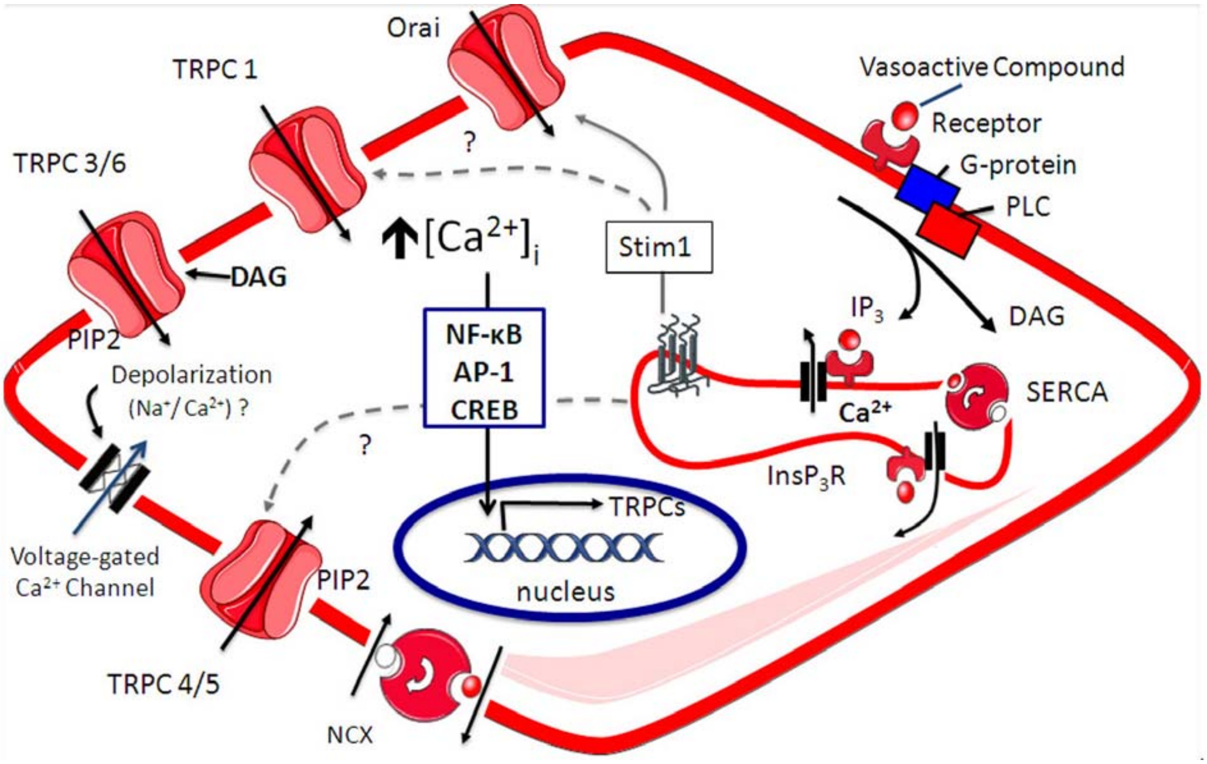

Figure 1. TRPC-mediated signaling in smooth muscle cells. The engagement of a vasoactive compound/growth factor receptor in vascular smooth muscle cells leads to the activation of phospholipase C (PLC) which catalyzes the breakdown of phosphatidylinositol 4,5-bisphosphate (PIP_2) into two intracellular second messengers, the Inositol 1,4,5-trisphosphate (IP_3) and Diacylglycerol (DAG). IP_3-mediated Ca^{2+} store depletion activates store-operated Orai1 channels in a mechanism dependent on STIM1 aggregation and translocation into areas of close SR-PM contacts. The role of TRPC channels in mediating SOC channels remains to this day a highly contentious issue. All TRPC are activated by mechanisms downstream of PLC; TRPC3/6/7 have been shown to be activated by DAG in a PKC independent manner while TRPC1/4/5 exact mechanisms of activation *via* membrane receptors is still unclear and seems to involve PIP_2 breakdown and Ca^{2+}. Na^+ entry through nonselective TRPC channels has been proposed to couple to activation of Ca^{2+} entry either through the Na^+/Ca^{2+} exchanger (NCX) or *via* depolarization and subsequent activation of L-type Ca^{2+} channels. Increasing evidence supports a signaling paradigm in which Ca^{2+} signals mediated by specific TRPC isoforms are able to activate transcription factors in smooth muscle that act to increase the corresponding TRPC channel expression.

relationship to those observed for heterologously expressed TRPC4 (129; 130). Xi *et al* proposed that IP_3-induced vasoconstriction of cerebral arteries occurs as a result of IP_3 receptor-dependent nonselective cationic current activation that depended on TRPC3 channels. The resulting membrane depolarization is proposed to activate voltage-dependent Ca^{2+} channels and subsequent SMC vasoconstriction (131). Poburko *et al* demonstrated NCX-mediated Ca^{2+} entry in aortic SMCs by localized Na^+ transients generated by agonist-mediated activation of channels to which TRPC6 contributes subunits (132). Whether TRPC channels mediate their vasoactive effects in SMCs directly through Ca^{2+} or by Na^+-dependent membrane depolarization remains an open question. Nevertheless, observations from the above mentioned studies support the prevailing idea that nonselective TRPCs mediate their contractile function in SMCs mainly through Na^+ entry either by causing membrane depolarization and subsequent activation of voltage gated Ca^{2+} channels or by coupling, as will be discussed below, to the Na^+/Ca^{2+} exchanger (NCX) functioning in its reverse mode (133; 134; 135; 136) (Figure 1). Despite efforts aimed at elucidating the mechanisms of regulation and activation of TRPC5, little is known about its physiological relevance in SMCs. The involvement of TRPC5 in the control of vascular SMC motility through cellular sensing of sphingosine 1-phosphate has been proposed (137). TRPC5 appears to form a functional channel in arteriolar smooth muscle cells, where Xu *et al* characterized a TRPC1/TRPC5-like heteromultimeric currents activated by store depletion and inhibited by an antibody targeting TRPC5 (112). Moreover, another study has identified a TRPC5-like current upon activation of muscarinic receptors in SMCs from the stomach and suggested TRPC5 as the non-selective cation channel activated by agonists such as acetylcholine (138).

TRPC3 mRNA expression pattern suggests that this nonselective cation channel is mostly expressed in embryonic brain and cardiac tissues (81; 139). While TRPC3 expression has been found in vascular SMCs, no clear physiological function has been assigned or correlated with its expression (85). It is now appreciated that TRPC3 has substantial constitutive activity (140), that may confer to this channel the ability to modulate basal SMC contractility through control of membrane potential and regulation of the activity of L-type Ca^{2+} channels. Along those lines, antisense DNA targeting TRPC3 mRNA inhibited depolarization and vasoconstriction of intact cerebral arteries induced by uridine 5'-trisphosphate (UTP). Treatment with antisense DNA targeting TRPC3 also inhibits UTP-evoked whole cell currents when measured in isolated SMCs (141). Compared to TRPC6, TRPC3 displays higher spontaneous activity and thus might play a prominent role in smooth muscle tonicity (140). The ability of TRPC3 to form heteromultimers with other TRPC channels might generate a higher capacity of tonic cation entry and chronic smooth muscle contraction that could contribute to vascular pathologies such as hypertension (142). Further insights into the role of TRPC3 in vascular SMC physiology were gained from studies with knockout mice. TRPC6 knockout ($TRPC6^{-/-}$) mice showed compensatory increase in TRPC3 expression in SMCs from aorta and cerebral artery causing vascular hypercontractility and elevated blood pressure (143). Vascular SMCs from these $TRPC6^{-/-}$ mice showed a more depolarized membrane potential accompanied by an enhanced spontaneous and agonist-induced Ca^{2+} entry and contraction (143). The constitutive nature of TRPC3 activity suggests that physiologically, this channel might be responsible for basal smooth muscle tone regulation. The physiological relevance of TRPC6 channel was apparent when Inoue *et al* reported convincing biophysical and pharmacological similarities between ectopically expressed TRPC6 in HEK293 cells and the native non-selective cation conductance activated upon α1-adrenoreceptor stimulation in rabbit portal vein smooth muscle cells (121; 144). In addition, vasopressin stimulation in the aortic SMC line A7r5 activated membrane conductances that depended on TRPC6 (79; 133; 145; 146). Subsequently, other studies have suggested that TRPC6 is activated in response to other physiologically relevant vasoactive peptides such as angiotensin II. Saleh *et al* reported TRPC6 activation upon stimulation with angiotensin II of vascular SMC isolated from rabbit mesenteric artery (125). In afferent arterioles, Ca^{2+} entry thought to elicit arteriolar contraction in response to treatment with angiotensin II was dependent on TRPC6 and reverse mode function of NCX (134). It was proposed that the arterial myogenic response known as Bayliss effect, or the inherent capacity of vessel constriction to avoid hemodynamic changes following elevated intravascular pressure, is in part TRPC6-dependent (147). This function of TRPC6 was proposed to be mediated indirectly through depolarization and activation of Ca^{2+} influx *via* voltage-gated Ca^{2+} channels. Finally, a member of the larger TRPM family, TRPM4 was also proposed to contribute in a similar manner to the contractile response of vascular SMCs (148), but the precise function of TRPM4 channels in SMCs requires further investigation. Cellular growth and proliferation is one of the many cellular functions that are regulated by TRPC channels. In pulmonary artery SMCs, PDGF-mediated cellular proliferation is associated with c-jun/STAT3-mediated transcription and up-regulation of TRPC6 expression (149).

7. IMPLICATIONS OF TRPC CHANNELS IN VASCULAR DISEASE

The phenotypic change of vascular SMC from quiescent to synthetic is thought to be an integral part of the pathophysiological response of SMCs and is of paramount importance in the development of vascular disease. For instance, upon vascular injury the expression of TRPC channels is upregulated and is believed to take part in the definition of the proliferative migratory state of synthetic vascular SMCs (5; 123; 150). Specifically, TRPC1 has been implicated in mediating several SMC pathologies such restenosis, pulmonary hypertension and atherosclerosis (5; 85). The pathophysiological relevance of TRPC1 upregulation was assessed in a human saphenous vein organ culture where intimal structures containing SMCs expressed higher levels of TRPC1 compared to medial layer cells (91). In this study, the use of an antibody targeting the putative pore-forming region of TRPC1 was able to significantly inhibit the extent of neointima formation, Ca^{2+} entry and vascular SMC proliferation (91). Similarly, upon vascular injury by balloon dilatation in the internal mammary artery TRPC1 expression was enhanced (123). Golovina *et al* have reported that in proliferative human pulmonary artery smooth muscle cells, TRPC1 protein expression as well as SOCE was increased as compared to non-proliferative cells (87). Unpublished results from our laboratory showed that rat aortic synthetic SMCs have upregulated levels of TRPC1 and TRPC6 compared to quiescent freshly isolated SMCs. Takahashi *et al* showed that in cultured coronary artery SMCs, TRPC1 expression increased upon angiotensin II stimulation while that of TRPC3/4/5/6 was not affected and suggested that angiotensin II-induced vascular SMC hypertrophy, which is one of the major events leading to atherosclerosis, is mediated through NF-κB-induced increase in TRPC1 and subsequent Ca^{2+} entry (88). Here we should point out that the correlative increase in SOCE and TRPC expression reported in proliferative SMCs by the studies mentioned above can be equally explained by increased expression in synthetic SMCs of the newly discovered SOCE machinery (STIM1 and Orai1 proteins) reported by our group and others (18; 150). Indeed, studies from our laboratory showed that protein levels of STIM1 and Orai1 are significantly increased in synthetic SMCs compared to quiescent cells (18) as well as in neointimal SMCs from rat carotids subjected to balloon angioplasty (Unpublished results). Furthermore, we showed that the increase in SOCE in synthetic SMCs was inhibited upon either STIM1 or Orai1 protein knockdown, while individual or combined protein knockdown of TRPC1/4/6 did not affect the extent of SOCE activation (18). We also showed that protein knockdown of STIM1 and Orai1 inhibited synthetic SMC migration and proliferation while protein knockdown of STIM2, Orai2 and Orai3 were without effect, suggesting a selective role of STIM1/Orai1 in SMC proliferation and migration. The *in vivo* relevance of STIM1 in vascular disease was recently demonstrated in two studies showing that *in vivo* knockdown of STIM1 using viral particles encoding STIM1-targeted shRNA in rat balloon-injured vessels inhibited neointima formation *(*151; 152).

Pulmonary hypertension refers to an increased blood pressure in the pulmonary circulation and can be triggered either by decreased in cardiac function or by exposure to hypoxic conditions. Exposure of the pulmonary vasculature to low levels of oxygen evokes a physiological response whereby pulmonary vasculature constriction orchestrates the optimization of blood oxygenation. Hypoxic pulmonary vasoconstriction is characterized by chronic episodes of alveolar hypoxia whereby hypoxic episodes promote acute constriction of the pulmonary vasculature, to minimize ventilation-perfusion mismatch and optimize oxygenation and gas exchange in the lung (153; 154). However, prolonged exposure to hypoxia evokes a series of arterial structural changes that subsequently elevate the pulmonary vascular resistance leading to development of pulmonary hypertension and ultimately, right heart failure (155). One of the hallmarks of severe pulmonary artery hypertension is the arterial hypertrophy that arises due to excessive pulmonary artery smooth muscle cell proliferation. The excessive vascular remodeling observed in hypoxic pulmonary hypertension is accompanied by distortional Ca^{2+} homeostasis in pulmonary artery SMCs believed to play a central role in the development of the disease (92; 156; 157; 158). Studies with isolated proliferative pulmonary artery SMCs treated with antisense oligonucleotides targeting TRPC1 mRNA were able to decrease Ca^{2+} entry and SMC proliferation (87; 90). These findings suggest that TRPC1 might be a potential target for therapy of pulmonary hypertension. In pulmonary artery SMCs isolated from rats exposed to chronic hypoxic conditions for three weeks, the levels of TRPC1 and TRPC6 expression as well as Ca^{2+} entry in response to either passive store depletion or agonits was increased (92; 159). In a rat model of hypoxia-induced pulmonary hypertension TRPC1 and TRPC6 upregulation was shown to be mediated by hypoxia inducible factor 1 (HIF-1) and exposure of mice heterozygous for HIF-1 to hypoxic conditions failed to increase TRPC1 expression (157). The upregulated expression of TRPC1 and TRPC6 observed in this animal model of hypoxic pulmonary hypertension is accompanied by increased basal and agonist-induced Ca^{2+} entry in pulmonary SMCs (156; 157). Similarly, Lin *et al* showed that TRPC6 expression was upregulated in pulmonary artery SMCs isolated from rats with hypoxic pulmonary hypertension (92). In this study, OAG-induced cation entry recorded in pulmonary artery SMCs from hypoxic rats was significantly increased when compared to cells isolated from control normoxic animals (92). Zhang *et al* suggested that a low-dose of ATP exerts part of its mitogenic effect in human pulmonary artery SMCs through CREB-mediated upregulation of TRPC4 channel expression and subsequent increase in Ca^{2+} influx. In this study treatment with ATP markedly increased TRPC4 expression through CREB phosphorylation, suggesting a possible role of TRPC4 in vascular remodeling during pathophysiological responses and its contribution to development of pulmonary hypertension (111). In pulmonary artery endothelial cells, exposure to hypoxia causes increase in TRPC4 expression and the transcription factor AP-1 binding activity (160). These authors proposed that hypoxia increases AP-1 binding activity by enhancing Ca^{2+} influx through TRPC4 channels in human pulmonary endothelial cells and that Ca^{2+}-mediated increase in AP-1 binding may upregulate expression of growth factors that would, in turn, stimulate pulmonary vascular remodeling in patients with hypoxia-induced pulmonary hypertension. Therefore, TRPC4 contribution to vascular pathophysiology might be more complex involving changes in endothelium-dependent SMC signaling.

The role of TRPC5 in the development of vascular disease has been less defined and little is known about its exact contribution. Nonetheless, it has been shown that TRPC5 homomultimers as well as TRPC1/5 heteromultimers are activated in response to sphingosine-1-phosphate, a signaling phospholipid that accumulates in atherosclerotic lesions (137). In this study, sphingosine-1-phosphate was found to stimulate motility of SMCs isolated from human saphenous vein and this action was inhibited by pre-treatment of cells with the E3-targeted anti-TRPC5 antibody or by disrupting the normal function of the channel by the use of a TRPC5 pore mutant (137). Pulmonary artery SMCs from patients suffering from idiopathic pulmonary arterial hypertension (IPAH) are characterized by hyperproliferative behavior and show upregulation of TRPC isoforms: TRPC3 and TRPC6 (161; 162). In these cells, proliferation and TRPC6 expression were strongly attenuated by the use of RNAi specifically targeting TRPC6 (161). Moreover, it has been reported that the endothelin receptor blocker bosentan, an antiproliferative agent currently used for treatment of IPAH, significantly downregulate TRPC6 expression likely through a mechanism independent of endothelin receptor blockade (163). In a follow up study, this group identified a single-nucleotide polymorphism (SNP) 254 (C→G) in the TRPC6 gene promoter that created a binding sequence for the inflammatory transcription factor NF-κB and suggested that the 254 (C→G) SNP may predispose individuals to an increased risk of IPAH by linking abnormal TRPC6 transcription to nuclear NF-κB. The 254 (C→G) SNP enhanced nuclear NF-κB-mediated promoter activity and stimulated TRPC6 expression in pulmonary artery SMCs while inhibition of nuclear NF-κB activity attenuated TRPC6 expression and decreased agonist-activated Ca^{2+} influx in pulmonary artery SMCs from IPAH patients harboring the 254G allele (164)

The *in vivo* relevance of TRPC isoforms extends to resistance arteries where they are implicated in the pathology of secondary hypertension. In deoxycosticosterone acetate (DOCA)-salt hypertensive rats, hypertension is thought to be developed due to an increased in agonist-mediated vascular SMC contractility that leads to chronic elevation of blood pressures (165). Studies on mesenteric arteries isolated from DOCA-salt sensitive rats display enhanced serotonin and norepinephrine-induced cation currents that are absent in control normotensive rats. This increased in cation current activity correlated with concomitant increase in TRPC6 expression; the expression of TRPC1/3 channels was not affected (166). Recently, Pulina *et al* reported increased TRPC1 and TRPC6 expression in arterial SMCs from ouabain hypertensive rats, in addition to the ouabain-

Table 1. Expression patterns, mechanisms of activation and pathological implications of smooth muscle TRPC channels

TRPC1	TRPC3	TRPC4	TRPC5	TRPC6
SMC Type				
Coronary Artery (88)	Cerebral Artery (141; 143)	Pulmonary Artery (111)	Arteriolar (112; 171)	Aortic (143; 145)
Aortic (128)	Aortic (143)	Gastrointestinal (115; 172)	Gastrointestinal (115)	Portal Vein (121; 173)
Cerebral Artery (127)	Pulmonary Artery (161; 162) Airway (114)	Cerebral Artery (127) Uterine (174)	Stomach (138)	Mesenteric (125)
Pulmonary Artery (87)			Saphenous Vein (137)	Arteriolar (134) Pulmonary artery (161; 164)
Portal Vein (175)				Gastrointestinal (115)
Saphenous Vein (91)				
Internal Mammary Artery (123) Airway (114)				
Activating Signal				
Store depletion (87; 88; 123; 175; 176)	OAG (61) DAG (68)	Store depletion (38; 111; 160)	Store depletion (171)	OAG (61)
Angiotensin II (125)	UTP (141)	ATP (111; 174) Oxytocin (174)	Sphingosine-1-phosphate (137)	Angiotensin II (125)
Endothelin-1 (126; 127; 128)		Endothelin-1 (127)	Lanthanum (177)	Vasopressin (145)
		Acetylcholine (Ach) (115)	Carbachol (CCh) (138)	Serotonin (178)
			Acetylcholine (Ach) (138)	Phenylephrine (121)
				Acetylcholine (115)
Pathology				
Pulmonary Artery Hypertension (PAH) (158)	Pulmonary Artery Hypertension (PAH) (142; 143)	Pulmonary Artery Hypertension (PAH) (157)	Atherosclerosis (137)	Hypoxic Pulmonary Vasoconstriction (179)
Restenosis (88; 91)	Hypertension (161; 162)	Subarachnoid Hemorrhage (SAH) (127)		Hypertension (162) Idiopathic pulmonary artery hypertension (161; 164)
Atherosclerosis (137)	Asthma (114)			Remodeling (75)
Subarachnoid Hemorrhage (127)				
Asthma (114)				

sensitive α2 Na^+ pumps and the Na^+/Ca^{2+} exchanger-1 (NCX1) (167). Liu *et al* showed that TRPC3 mRNA and protein are increased in vascular SMCs and aortic rings from spontaneously hypertensive rats compared to normotensive Wistar Kyoto rats. Angiotensin II-induced Ca^{2+} increase was significantly enhanced in vascular SMCs from spontaneously hypertensive rats compared with normotensive rats. Furthermore, knockdown of TRPC3 gene expression by RNAi reduced the angiotensin II-induced Ca^{2+} entry by ~30%, and TRPC3 overexpression increased this Ca^{2+} entry by ~ 55% (168). Xiao *et al* recently showed that TRPC1 and TRPC3 proteins and mRNAs were expressed in freshly isolated airway smooth muscle tissues. Using blocking antibodies and RNAi against TRPC1 and TRPC3 they proposed TRPC3 as an important component of native nonselective cationic channels in airway smooth muscle. TRPC3 blockade inhibited the nonselective cationic currents and caused membrane hyperpolarization in airway SMCs. In the same study, increased TRPC3 expression appears to mediate membrane depolarization and hyperresponsiveness in an animal model of asthma where airway SMCs are sensitized by ovalbumin; TRPC1 channels were also proposed to contribute to nonselective cationic currents in ovalbumin-sensitized/challenged airway SMCs (114). To date, a potential pathophysiological role for TRPC7 within the vasculature remains unknown. TRPC7 involvement in apoptosis has been reported in two different cell systems (169; 170), but whether TRPC7 plays a role in SMC hyperplasia characteristic of vascular disease remains to be investigated.

8. CONCLUSION

The proposed mechanisms of activations of TRPC channels are depicted in Figure 1. Table 1 summarizes tissue distributions and SMC pathologies where TRPC channels are involved. It is clear from the studies discussed above that TRPC channels have a far-reaching role in both physiological and pathophysiological functions of SMCs in the pulmonary and systemic cardiovascular system. Additional roles for TRPC channels in SMCs from other organs such as the gastrointestinal tract, uterus and bladder are beginning to emerge. The upregulation of TRPC channels in SMCs, especially that of TRPC1 and TRPC6, in conditions of systemic and pulmonary hypertension and vascular remodeling suggests a major role of these proteins in the abnormal SMC proliferation and contractility characteristic of these diseases. Future TRPC channels blockers are likely to be beneficial in the therapeutic control of SMC function during various vascular pathologies.

9. ACKNOWLEDGMENTS

Research in this laboratory is supported by an NIH early career grant K22ES014729 to Mohamed Trebak.

10. REFERENCES

1. G.J. Barritt: Receptor-activated Ca2+ inflow in animal cells: a variety of pathways tailored to meet different

intracellular Ca2+ signalling requirements. *Biochem J* 337(2), 153-69 (1999)

2. M.J. Berridge: Smooth muscle cell calcium activation mechanisms. *J Physiol* 586, 5047-61 (2008)

3. M.J. Berridge, P. Lipp, and M.D. Bootman: The versatility and universality of calcium signalling. *Nat Rev Mol Cell Biol* 1, 11-21 (2000)

4. M.J. Berridge: Calcium microdomains: organization and function. *Cell Calcium* 40, 405-12 (2006)

5. S.J. House, M. Potier, J. Bisaillon, H.A. Singer, and M. Trebak: The non-excitable smooth muscle: calcium signaling and phenotypic switching during vascular disease. *Pflugers Arch* 456, 769-85 (2008)

6. G.K. Owens, M.S. Kumar, and B.R. Wamhoff: Molecular regulation of vascular smooth muscle cell differentiation in development and disease. *Physiol Rev* 84, 767-801 (2004)

7. T. Yoshida, and G.K. Owens: Molecular determinants of vascular smooth muscle cell diversity. *Circ Res* 96, 280-91 (2005)

8. D.E. Clapham: Calcium signaling. *Cell* 131, 1047-58 (2007)

9. O.H. Petersen, M. Michalak, and A. Verkhratsky: Calcium signalling: past, present and future. *Cell Calcium* 38, 161-9 (2005)

10. J.E. Brayden, S. Earley, M.T. Nelson, and S. Reading: Transient receptor potential (TRP) channels, vascular tone and autoregulation of cerebral blood flow. *Clin Exp Pharmacol Physiol* 35, 1116-20 (2008)

11. S. Wray, T. Burdyga, and K. Noble: Calcium signalling in smooth muscle. *Cell Calcium* 38, 397-407 (2005)

12. M.J. Berridge: Inositol trisphosphate and calcium signalling. *Nature* 361, 315-25 (1993)

13. A.B. Parekh, and J.W. Putney, Jr.: Store-operated calcium channels. *Physiol Rev* 85, 757-810 (2005)

14. M. Potier, and M. Trebak: New developments in the signaling mechanisms of the store-operated calcium entry pathway. *Pflugers Arch* 457, 405-15 (2008)

15. J.W. Putney, Jr.: A model for receptor-regulated calcium entry. *Cell Calcium* 7, 1-12 (1986)

16. J.W. Putney, Jr.: Capacitative calcium entry revisited. *Cell Calcium* 11, 611-24 (1990)

17. A.P. Albert, and W.A. Large: Store-operated Ca2+-permeable non-selective cation channels in smooth muscle cells. *Cell Calcium* 33, 345-56 (2003)

18. M. Potier, J.C. Gonzalez, R.K. Motiani, I.F. Abdullaev, J.M. Bisaillon, H.A. Singer, and M. Trebak: Evidence for STIM1- and Orai1-dependent store-operated calcium influx through ICRAC in vascular smooth muscle cells: role in proliferation and migration. *Faseb J* 23(8), 2425-37 (2009)

19. M. Hoth, and R. Penner: Depletion of intracellular calcium stores activates a calcium current in mast cells. *Nature* 355, 353-6 (1992)

20. A.B. Parekh, and R. Penner: Store depletion and calcium influx. *Physiol Rev* 77, 901-30 (1997)

21. G.S. Bird, O. Aziz, J.P. Lievremont, B.J. Wedel, M. Trebak, G. Vazquez, and J.W. Putney, Jr.: Mechanisms of phospholipase C-regulated calcium entry. *Curr Mol Med* 4, 291-301 (2004)

22. T.J. Shuttleworth: Arachidonic acid, ARC channels, and Orai proteins. *Cell Calcium* 45(6), 602-10 (2009)

23. T.J. Shuttleworth, J.L. Thompson, and O. Mignen: ARC channels: a novel pathway for receptor-activated calcium entry. *Physiology* (Bethesda) 19, 355-61 (2004)

24. M. Trebak, L. Lemonnier, J.T. Smyth, G. Vazquez, and J.W. Putney, Jr.: Phospholipase C-coupled receptors and activation of TRPC channels. *Handb Exp Pharmacol*, 593-614 (2007)

25. R.C. Hardie, and B. Minke: Novel Ca2+ channels underlying transduction in Drosophila photoreceptors: implications for phosphoinositide-mediated Ca2+ mobilization. *Trends Neurosci* 16, 371-6 (1993)

26. R.C. Hardie: TRP channels and lipids: from Drosophila to mammalian physiology. *J Physiol* 578, 9-24 (2007)

27. B. Minke, and B. Cook: TRP channel proteins and signal transduction. *Physiol Rev* 82, 429-72 (2002)

28. C. Montell: Drosophila TRP channels. *Pflugers Arch* 451, 19-28 (2005)

29. B. Nilius: TRP channels in disease. *Biochim Biophys Acta* 1772, 805-12 (2007)

30. D.J. Cosens, and A. Manning: Abnormal electroretinogram from a Drosophila mutant. *Nature* 224, 285-7 (1969)

31. R.C. Hardie, and B. Minke: The trp gene is essential for a light-activated Ca2+ channel in Drosophila photoreceptors. *Neuron* 8, 643-51 (1992)

32. R.C. Hardie, and B. Minke: Phosphoinositide-mediated phototransduction in Drosophila photoreceptors: the role of Ca2+ and trp. *Cell Calcium* 18, 256-74 (1995)

33. B. Minke: Light-induced reduction in excitation efficiency in the trp mutant of Drosophila. *J Gen Physiol* 79, 361-85 (1982)

34. B. Minke, and Z. Selinger: The roles of trp and calcium in regulating photoreceptor function in Drosophila. *Curr Opin Neurobiol* 6, 459-66 (1996)

35. C. Montell, and G.M. Rubin: Molecular characterization of the Drosophila trp locus: a putative integral membrane protein required for phototransduction. *Neuron* 2, 1313-23 (1989)

36. J. Abramowitz, and L. Birnbaumer: Physiology and pathophysiology of canonical transient receptor potential channels. *Faseb J* 23, 297-328 (2009)

37. M. Trebak, G. Vazquez, G.S. Bird, and J.W. Putney, Jr.: The TRPC3/6/7 subfamily of cation channels. *Cell Calcium* 33, 451-61 (2003)

38. G. Vazquez, B.J. Wedel, O. Aziz, M. Trebak, and J.W. Putney, Jr.: The mammalian TRPC cation channels. *Biochim Biophys Acta* 1742, 21-36 (2004)

39. E. Yildirim, and L. Birnbaumer: TRPC2: molecular biology and functional importance. *Handb Exp Pharmacol*, 53-75 (2007)

40. B. Vannier, X. Zhu, D. Brown, and L. Birnbaumer: The membrane topology of human transient receptor potential 3 as inferred from glycosylation-scanning mutagenesis and epitope immunocytochemistry. *J Biol Chem* 273, 8675-9 (1998)

41. S.F. Pedersen, G. Owsianik, and B. Nilius: TRP channels: an overview. *Cell Calcium* 38, 233-52 (2005)

42. R. Vennekens, T. Voets, R.J. Bindels, G. Droogmans, and B. Nilius: Current understanding of mammalian TRP homologues. *Cell Calcium* 31, 253-64 (2002)

43. L. Birnbaumer: The TRPC class of ion channels: a critical review of their roles in slow, sustained increases in intracellular Ca (2+) concentrations. *Annu Rev Pharmacol Toxicol* 49, 395-426 (2009)

44. J.T. Smyth, W.I. Dehaven, B.F. Jones, J.C. Mercer, M. Trebak, G. Vazquez, and J.W. Putney, Jr.: Emerging perspectives in store-operated Ca2+ entry: roles of Orai, Stim and TRP. *Biochim Biophys Acta* 1763, 1147-60 (2006)

45. R.C. Hardie: Regulation of TRP channels via lipid second messengers. *Annu Rev Physiol* 65, 735-59 (2003)

46. I.F. Abdullaev, J.M. Bisaillon, M. Potier, J.C. Gonzalez, R.K. Motiani, and M. Trebak: Stim1 and Orai1 mediate CRAC currents and store-operated calcium entry important for endothelial cell proliferation. *Circ Res* 103, 1289-99 (2008)

47. S. Feske, Y. Gwack, M. Prakriya, S. Srikanth, S.H. Puppel, B. Tanasa, P.G. Hogan, R.S. Lewis, M. Daly, and A. Rao: A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature* 441, 179-85 (2006)

48. J. Liou, M.L. Kim, W.D. Heo, J.T. Jones, J.W. Myers, J.E. Ferrell, Jr., and T. Meyer: STIM is a Ca2+ sensor essential for Ca2+-store-depletion-triggered Ca2+ influx. *Curr Biol* 15, 1235-41 (2005)

49. J. Roos, P.J. DiGregorio, A.V. Yeromin, K. Ohlsen, M. Lioudyno, S. Zhang, O. Safrina, J.A. Kozak, S.L. Wagner, M.D. Cahalan, G. Velicelebi, and K.A. Stauderman: STIM1, an essential and conserved component of store-operated Ca2+ channel function. *J Cell Biol* 169, 435-45 (2005)

50. M. Vig, C. Peinelt, A. Beck, D.L. Koomoa, D. Rabah, M. Koblan-Huberson, S. Kraft, H. Turner, A. Fleig, R. Penner, and J.P. Kinet: CRACM1 is a plasma membrane protein essential for store-operated Ca2+ entry. *Science* 312, 1220-3 (2006)

51. S.L. Zhang, A.V. Yeromin, X.H. Zhang, Y. Yu, O. Safrina, A. Penna, J. Roos, K.A. Stauderman, and M.D. Cahalan: Genome-wide RNAi screen of Ca (2+) influx identifies genes that regulate Ca (2+) release-activated Ca (2+) channel activity. *Proc Natl Acad Sci U S A* 103, 9357-62 (2006)

52. S.S. Manji, N.J. Parker, R.T. Williams, L. van Stekelenburg, R.B. Pearson, M. Dziadek, and P.J. Smith: STIM1: a novel phosphoprotein located at the cell surface. *Biochim Biophys Acta* 1481, 147-55 (2000)

53. R.T. Williams, P.V. Senior, L. Van Stekelenburg, J.E. Layton, P.J. Smith, and M.A. Dziadek: Stromal interaction molecule 1 (STIM1), a transmembrane protein with growth suppressor activity, contains an extracellular SAM domain modified by N-linked glycosylation. *Biochim Biophys Acta* 1596, 131-7 (2002)

54. G.S. Bird, W.I. DeHaven, J.T. Smyth, and J.W. Putney, Jr.: Methods for studying store-operated calcium entry. *Methods* 46, 204-12 (2008)

55. P. Draber, and L. Draberova: Lifting the fog in store-operated Ca2+ entry. *Trends Immunol* 26, 621-4 (2005)

56. T. Kawasaki, I. Lange, and S. Feske: A minimal regulatory domain in the C terminus of STIM1 binds to and activates ORAI1 CRAC channels. *Biochem Biophys Res Commun* 385(1), 49-54 (2009)

57. M. Muik, M. Fahrner, I. Derler, R. Schindl, J. Bergsmann, I. Frischauf, K. Groschner, and C. Romanin: A cytosolic homomerization and a modulatory domain within STIM1 C-terminus determine coupling to ORAI1 channels. *J Biol Chem* 284(13), 8421-6 (2009)

58. C.Y. Park, P.J. Hoover, F.M. Mullins, P. Bachhawat, E.D. Covington, S. Raunser, T. Walz, K.C. Garcia, R.E. Dolmetsch, and R.S. Lewis: STIM1 Clusters and Activates

CRAC Channels via Direct Binding of a Cytosolic Domain to Orai1. *Cell* 136(5), 876-90 (2009)

59. J.P. Yuan, W. Zeng, M.R. Dorwart, Y.J. Choi, P.F. Worley, and S. Muallem: SOAR and the polybasic STIM1 domains gate and regulate Orai channels. *Nat Cell Biol* 11, 337-43 (2009)

60. J.W. Putney: Physiological mechanisms of TRPC activation. *Pflugers Arch* 451, 29-34 (2005)

61. T. Hofmann, A.G. Obukhov, M. Schaefer, C. Harteneck, T. Gudermann, and G. Schultz: Direct activation of human TRPC6 and TRPC3 channels by diacylglycerol. *Nature* 397, 259-63 (1999)

62. R.S. Hurst, X. Zhu, G. Boulay, L. Birnbaumer, and E. Stefani: Ionic currents underlying HTRP3 mediated agonist-dependent Ca2+ influx in stably transfected HEK293 cells. *FEBS Lett* 422, 333-8 (1998)

63. I.S. Ambudkar: TRPC1: a core component of store-operated calcium channels. *Biochem Soc Trans* 35, 96-100 (2007)

64. J.P. Yuan, M.S. Kim, W. Zeng, D.M. Shin, G. Huang, P.F. Worley, and S. Muallem: TRPC channels as STIM1-regulated SOCs. *Channels* (Austin) 3, 221-5 (2009)

65. A.P. Albert, and W.A. Large: A Ca2+-permeable non-selective cation channel activated by depletion of internal Ca2+ stores in single rabbit portal vein myocytes*. J Physiol* 538, 717-28 (2002)

66. E.S. Trepakova, M. Gericke, Y. Hirakawa, R.M. Weisbrod, R.A. Cohen, and V.M. Bolotina: Properties of a native cation channel activated by Ca2+ store depletion in vascular smooth muscle cells. *J Biol Chem* 276, 7782-90 (2001)

67. H.L. Ong, K.T. Cheng, X. Liu, B.C. Bandyopadhyay, B.C. Paria, J. Soboloff, B. Pani, Y. Gwack, S. Srikanth, B.B. Singh, D. Gill, and I.S. Ambudkar: Dynamic assembly of TRPC1-STIM1-Orai1 ternary complex is involved in store-operated calcium influx. Evidence for similarities in store-operated and calcium release-activated calcium channel components. *J Biol Chem* 282, 9105-16 (2007)

68. M. Trebak, J.B.G. St, R.R. McKay, L. Birnbaumer, and J.W. Putney, Jr.: Signaling mechanism for receptor-activated canonical transient receptor potential 3 (TRPC3) channels. *J Biol Chem* 278, 16244-52 (2003)

69. A. Dietrich, H. Kalwa, U. Storch, M. Mederos y Schnitzler, B. Salanova, O. Pinkenburg, G. Dubrovska, K. Essin, M. Gollasch, L. Birnbaumer, and T. Gudermann: Pressure-induced and store-operated cation influx in vascular smooth muscle cells is independent of TRPC1. *Pflugers Arch* 455, 465-77 (2007)

70. D. Varga-Szabo, K.S. Authi, A. Braun, M. Bender, A. Ambily, S.R. Hassock, T. Gudermann, A. Dietrich, and B. Nieswandt: Store-operated Ca (2+) entry in platelets occurs independently of transient receptor potential (TRP) C1. *Pflugers Arch* 457(2), 377-87 (2008)

71. W. Dehaven, B. Jones, J. Petranka, J. Smyth, T. Tomita, G. Bird, and J. Putney: TRPC channels function independently of STIM1 and Orai1. *J Physiol* 587(10), 2275-98 (2009)

72. G. Isenberg: Nonselective cation channels in cardiac and smooth muscle cells. *Exs* 66, 247-60 (1993)

73. A.P. Somlyo, and A.V. Somlyo: Ca2+ sensitivity of smooth muscle and nonmuscle myosin II: modulated by G proteins, kinases, and myosin phosphatase. *Physiol Rev* 83, 1325-58 (2003)

74. G.K. Owens: Regulation of differentiation of vascular smooth muscle cells. *Physiol Rev* 75, 487-517 (1995)

75. K. Kuwahara, Y. Wang, J. McAnally, J.A. Richardson, R. Bassel-Duby, J.A. Hill, and E.N. Olson: TRPC6 fulfills a calcineurin signaling circuit during pathologic cardiac remodeling. *J Clin Invest* 116, 3114-26 (2006)

76. B. Nilius: Transient receptor potential (TRP) cation channels: rewarding unique proteins. *Bull Mem Acad R Med Belg* 162, 244-53 (2007)

77. B. Nilius, and T. Voets: Diversity of TRP channel activation. *Novartis Found Symp* 258, 140-9; discussion 149-59, 263-6 (2004)

78. R. Inoue, L.J. Jensen, J. Shi, H. Morita, M. Nishida, A. Honda, and Y. Ito: Transient receptor potential channels in cardiovascular function and disease. *Circ Res* 99, 119-31 (2006)

79. Y. Maruyama, Y. Nakanishi, E.J. Walsh, D.P. Wilson, D.G. Welsh, and W.C. Cole: Heteromultimeric TRPC6-TRPC7 channels contribute to arginine vasopressin-induced cation current of A7r5 vascular smooth muscle cells. *Circ Res* 98, 1520-7 (2006)

80. P.D. Wes, J. Chevesich, A. Jeromin, C. Rosenberg, G. Stetten, and C. Montell: TRPC1, a human homolog of a Drosophila store-operated channel. *Proc Natl Acad Sci U S A* 92, 9652-6 (1995)

81. X. Zhu, M. Jiang, M. Peyton, G. Boulay, R. Hurst, E. Stefani, and L. Birnbaumer: trp, a novel mammalian gene family essential for agonist-activated capacitative Ca2+ entry. *Cell* 85, 661-71 (1996)

82. S.Z. Xu, and D.J. Beech: TrpC1 is a membrane-spanning subunit of store-operated Ca (2+) channels in native vascular smooth muscle cells. *Circ Res* 88, 84-7 (2001)

83. D.J. Beech: TRPC1: store-operated channel and more. *Pflugers Arch* 451, 53-60 (2005)

84. M. Trebak: Canonical transient receptor potential channels in disease: targets for novel drug therapy? *Drug Discov Today* 11, 924-30 (2006)

85. A. Dietrich, V. Chubanov, H. Kalwa, B.R. Rost, and T. Gudermann: Cation channels of the transient receptor potential superfamily: their role in physiological and pathophysiological processes of smooth muscle cells. *Pharmacol Ther* 112, 744-60 (2006)

86. H. Watanabe, M. Murakami, T. Ohba, Y. Takahashi, and H. Ito: TRP channel and cardiovascular disease. *Pharmacol Ther* 118, 337-51 (2008)

87. V.A. Golovina, O. Platoshyn, C.L. Bailey, J. Wang, A. Limsuwan, M. Sweeney, L.J. Rubin, and J.X. Yuan: Upregulated TRP and enhanced capacitative Ca (2+) entry in human pulmonary artery myocytes during proliferation. *Am J Physiol Heart Circ Physiol* 280, H746-55 (2001)

88. Y. Takahashi, H. Watanabe, M. Murakami, T. Ohba, M. Radovanovic, K. Ono, T. Iijima, and H. Ito: Involvement of transient receptor potential canonical 1 (TRPC1) in angiotensin II-induced vascular smooth muscle cell hypertrophy. *Atherosclerosis* 195, 287-96 (2007)

89. L.I. Brueggemann, D.R. Markun, K.K. Henderson, L.L. Cribbs, and K.L. Byron: Pharmacological and electrophysiological characterization of store-operated currents and capacitative Ca (2+) entry in vascular smooth muscle cells. *J Pharmacol Exp Ther* 317, 488-99 (2006)

90. M. Sweeney, Y. Yu, O. Platoshyn, S. Zhang, S.S. McDaniel, and J.X. Yuan: Inhibition of endogenous TRP1 decreases capacitative Ca2+ entry and attenuates pulmonary artery smooth muscle cell proliferation. *Am J Physiol Lung Cell Mol Physiol* 283, L144-55 (2002)

91. B. Kumar, K. Dreja, S.S. Shah, A. Cheong, S.Z. Xu, P. Sukumar, J. Naylor, A. Forte, M. Cipollaro, D. McHugh, P.A. Kingston, A.M. Heagerty, C.M. Munsch, A. Bergdahl, A. Hultgardh-Nilsson, M.F. Gomez, K.E. Porter, P. Hellstrand, and D.J. Beech: Upregulated TRPC1 channel in vascular injury *in vivo* and its role in human neointimal hyperplasia. *Circ Res* 98, 557-63 (2006)

92. M.J. Lin, G.P. Leung, W.M. Zhang, X.R. Yang, K.P. Yip, C.M. Tse, and J.S. Sham: Chronic hypoxia-induced upregulation of store-operated and receptor-operated Ca2+ channels in pulmonary arterial smooth muscle cells: a novel mechanism of hypoxic pulmonary hypertension. *Circ Res* 95, 496-505 (2004)

93. Y. Takahashi, H. Watanabe, M. Murakami, K. Ono, Y. Munehisa, T. Koyama, K. Nobori, T. Iijima, and H. Ito: Functional role of stromal interaction molecule 1 (STIM1) in vascular smooth muscle cells. *Biochem Biophys Res Commun* 361, 934-40 (2007)

94. M. Trebak, G.S. Bird, R.R. McKay, and J.W. Putney, Jr.: Comparison of human TRPC3 channels in receptor-activated and store-operated modes. Differential sensitivity to channel blockers suggests fundamental differences in channel composition. *J Biol Chem* 277, 21617-23 (2002)

95. T. Hofmann, M. Schaefer, G. Schultz, and T. Gudermann: Subunit composition of mammalian transient receptor potential channels in living cells. *Proc Natl Acad Sci U S A* 99, 7461-6 (2002)

96. C. Strubing, G. Krapivinsky, L. Krapivinsky, and D.E. Clapham: TRPC1 and TRPC5 form a novel cation channel in mammalian brain. *Neuron* 29, 645-55 (2001)

97. M. Trebak: STIM1/Orai1, ICRAC, and endothelial SOC. *Circ Res* 104, e56-7 (2009)

98. M. Schaefer, T.D. Plant, A.G. Obukhov, T. Hofmann, T. Gudermann, and G. Schultz: Receptor-mediated regulation of the nonselective cation channels TRPC4 and TRPC5. *J Biol Chem* 275, 17517-26 (2000)

99. H. Kanki, M. Kinoshita, A. Akaike, M. Satoh, Y. Mori, and S. Kaneko: Activation of inositol 1,4,5-trisphosphate receptor is essential for the opening of mouse TRP5 channels. *Mol Pharmacol* 60, 989-98 (2001)

100. M. Trebak, L. Lemonnier, W.I. Dehaven, B.J. Wedel, G.S. Bird, and J.W. Putney, Jr.: Complex functions of phosphatidylinositol 4,5-bisphosphate in regulation of TRPC5 cation channels. *Pflugers Arch* 457, 757-69 (2009)

101. K. Otsuguro, J. Tang, Y. Tang, R. Xiao, M. Freichel, V. Tsvilovskyy, S. Ito, V. Flockerzi, M.X. Zhu, and A.V. Zholos: Isoform-specific inhibition of TRPC4 channel by phosphatidylinositol 4,5-bisphosphate. *J Biol Chem* 283, 10026-36 (2008)

102. S.A. Gross, G.A. Guzman, U. Wissenbach, S.E. Philipp, M.X. Zhu, D. Bruns, and A. Cavalie: TRPC5 is a Ca2+ -activated channel functionally coupled to Ca2+ -selective ion channels. *J Biol Chem* 284(49), 34423-32 (2009)

103. H. Yip, W.Y. Chan, P.C. Leung, H.Y. Kwan, C. Liu, Y. Huang, V. Michel, D.T. Yew, and X. Yao: Expression of TRPC homologs in endothelial cells and smooth muscle layers of human arteries. *Histochem Cell Biol* 122, 553-61 (2004)

104. V.J. Bezzerides, I.S. Ramsey, S. Kotecha, A. Greka, and D.E. Clapham: Rapid vesicular translocation and insertion of TRP channels. *Nat Cell Biol* 6, 709-20 (2004)

105. M. Freichel, S.H. Suh, A. Pfeifer, U. Schweig, C. Trost, P. Weissgerber, M. Biel, S. Philipp, D. Freise, G. Droogmans, F. Hofmann, V. Flockerzi, and B. Nilius: Lack of an endothelial store-operated Ca2+ current impairs agonist-dependent vasorelaxation in TRP4-/- mice. *Nat Cell Biol* 3, 121-7 (2001)

106. C. Tiruppathi, M. Freichel, S.M. Vogel, B.C. Paria, D. Mehta, V. Flockerzi, and A.B. Malik: Impairment of store-operated Ca2+ entry in TRPC4 (-/-) mice interferes with

increase in lung microvascular permeability. *Circ Res* 91, 70-6 (2002)

107. J.P. Yuan, W. Zeng, G.N. Huang, P.F. Worley, and S. Muallem: STIM1 heteromultimerizes TRPC channels to determine their function as store-operated channels. *Nat Cell Biol* 9, 636-45 (2007)

108. L.C. Ng, M.D. McCormack, J.A. Airey, C.A. Singer, P.S. Keller, X.M. Shen, and J.R. Hume: TRPC1 and STIM1 mediate capacitative Ca2+ entry in mouse pulmonary arterial smooth muscle cells. *J Physiol* 587, 2429-42 (2009)

109. P.F. Worley, W. Zeng, G.N. Huang, J.P. Yuan, J.Y. Kim, M.G. Lee, and S. Muallem: TRPC channels as STIM1-regulated store-operated channels. *Cell Calcium* 42, 205-11 (2007)

110. W. Zeng, J.P. Yuan, M.S. Kim, Y.J. Choi, G.N. Huang, P.F. Worley, and S. Muallem: STIM1 gates TRPC channels, but not Orai1, by electrostatic interaction. *Mol Cell* 32, 439-48 (2008)

111. S. Zhang, C.V. Remillard, I. Fantozzi, and J.X. Yuan: ATP-induced mitogenesis is mediated by cyclic AMP response element-binding protein-enhanced TRPC4 expression and activity in human pulmonary artery smooth muscle cells. *Am J Physiol Cell Physiol* 287, C1192-201 (2004)

112. S.Z. Xu, G. Boulay, R. Flemming, and D.J. Beech: E3-targeted anti-TRPC5 antibody inhibits store-operated calcium entry in freshly isolated pial arterioles. *Am J Physiol Heart Circ Physiol* 291, H2653-9 (2006)

113. T.D. Plant, and M. Schaefer: Receptor-operated cation channels formed by TRPC4 and TRPC5. *Naunyn Schmiedebergs Arch Pharmacol* 371, 266-76 (2005)

114. J.H. Xiao, Y.M. Zheng, B. Liao, and Y.X. Wang: Functional role of TRPC1 and TRPC3 in normal and asthmatic airway smooth muscle. *Am J Respir Cell Mol Biol*, Epub ahead of print. (2009)

115. V.V. Tsvilovskyy, A.V. Zholos, T. Aberle, S.E. Philipp, A. Dietrich, M.X. Zhu, L. Birnbaumer, M. Freichel, and V. Flockerzi: Deletion of TRPC4 and TRPC6 in mice impairs smooth muscle contraction and intestinal motility *in vivo*. *Gastroenterology* 137, 1415-24 (2009)

116. T. Gudermann, T. Hofmann, M. Mederos y Schnitzler, and A. Dietrich: Activation, subunit composition and physiological relevance of DAG-sensitive TRPC proteins. *Novartis Found Symp* 258, 103-18; discussion 118-22, 155-9, 263-6 (2004)

117. T. Okada, R. Inoue, K. Yamazaki, A. Maeda, T. Kurosaki, T. Yamakuni, I. Tanaka, S. Shimizu, K. Ikenaka, K. Imoto, and Y. Mori: Molecular and functional characterization of a novel mouse transient receptor potential protein homologue TRP7. Ca (2+)-permeable cation channel that is constitutively activated and enhanced by stimulation of G protein-coupled receptor. *J Biol Chem* 274, 27359-70 (1999)

118. L. Zhang, and D. Saffen: Muscarinic Acetylcholine Receptor Regulation of TRP6 Ca2+ Channel Isoforms. Molecular Structures And Functional Characterization. *J Biol Chem* 276, 13331-13339 (2001)

119. M. Trebak, N. Hempel, B.J. Wedel, J.T. Smyth, G.S. Bird, and J.W. Putney, Jr.: Negative regulation of TRPC3 channels by protein kinase C-mediated phosphorylation of serine 712. *Mol Pharmacol* 67, 558-63 (2005)

120. L. Lemonnier, M. Trebak, and J.W. Putney, Jr.: Complex regulation of the TRPC3, 6 and 7 channel subfamily by diacylglycerol and phosphatidylinositol-4,5-bisphosphate. *Cell Calcium* 43, 506-14 (2008)

121. R. Inoue, T. Okada, H. Onoue, Y. Hara, S. Shimizu, S. Naitoh, Y. Ito, and Y. Mori: The transient receptor potential protein homologue TRP6 is the essential component of vascular alpha (1)-adrenoceptor-activated Ca (2+)-permeable cation channel. *Circ Res* 88, 325-32 (2001)

122. J.Y. Kim, and D. Saffen: Activation of M1 Muscarinic Acetylcholine Receptors Stimulates the Formation of a Multiprotein Complex Centered on TRPC6 Channels. *J Biol Chem* 280(36), 32035-47 (2005)

123. A. Bergdahl, M.F. Gomez, A.K. Wihlborg, D. Erlinge, A. Eyjolfson, S.Z. Xu, D.J. Beech, K. Dreja, and P. Hellstrand: Plasticity of TRPC expression in arterial smooth muscle: correlation with store-operated Ca2+ entry. *Am J Physiol Cell Physiol* 288, C872-80 (2005)

124. A. Dietrich, H. Kalwa, B. Fuchs, F. Grimminger, N. Weissmann, and T. Gudermann: *In vivo* TRPC functions in the cardiopulmonary vasculature. *Cell Calcium* 42, 233-44 (2007)

125. S.N. Saleh, A.P. Albert, C.M. Peppiatt, and W.A. Large: Angiotensin II activates two cation conductances with distinct TRPC1 and TRPC6 channel properties in rabbit mesenteric artery myocytes. *J Physiol* 577, 479-95 (2006)

126. A. Bergdahl, M.F. Gomez, K. Dreja, S.Z. Xu, M. Adner, D.J. Beech, J. Broman, P. Hellstrand, and K. Sward: Cholesterol depletion impairs vascular reactivity to endothelin-1 by reducing store-operated Ca2+ entry dependent on TRPC1. *Circ Res* 93, 839-47 (2003)

127. A. Xie, Y. Aihara, V.A. Bouryi, E. Nikitina, B.S. Jahromi, Z.D. Zhang, M. Takahashi, and R.L. Macdonald: Novel mechanism of endothelin-1-induced vasospasm after subarachnoid hemorrhage. *J Cereb Blood Flow Metab* 27, 1692-701 (2007)

128. K. Tai, M.C. Hamaide, H. Debaix, P. Gailly, M. Wibo, and N. Morel: Agonist-evoked calcium entry in vascular

smooth muscle cells requires IP3 receptor-mediated activation of TRPC1. *Eur J Pharmacol* 583, 135-47 (2008)

129. R.L. Walker, S.D. Koh, G.P. Sergeant, K.M. Sanders, and B. Horowitz: TRPC4 currents have properties similar to the pacemaker current in interstitial cells of Cajal. *Am J Physiol Cell Physiol* 283, C1637-45 (2002)

130. S. Torihashi, T. Fujimoto, C. Trost, and S. Nakayama: Calcium oscillation linked to pacemaking of interstitial cells of Cajal: requirement of calcium influx and localization of TRP4 in caveolae. *J Biol Chem* 277, 19191-7 (2002)

131. Q. Xi, A. Adebiyi, G. Zhao, K.E. Chapman, C.M. Waters, A. Hassid, and J.H. Jaggar: IP3 constricts cerebral arteries via IP3 receptor-mediated TRPC3 channel activation and independently of sarcoplasmic reticulum Ca2+ release. *Circ Res* 102, 1118-26 (2008)

132. D. Poburko, C.H. Liao, V.S. Lemos, E. Lin, Y. Maruyama, W.C. Cole, and C. van Breemen: Transient receptor potential channel 6-mediated, localized cytosolic Na+. transients drive Na+/Ca2+ exchanger-mediated Ca2+ entry in purinergically stimulated aorta smooth muscle cells. *Circ Res* 101, 1030-8 (2007)

133. J. Soboloff, M. Spassova, W. Xu, L.P. He, N. Cuesta, and D.L. Gill: Role of endogenous TRPC6 channels in Ca2+ signal generation in A7r5 smooth muscle cells. *J Biol Chem* 280, 39786-94 (2005)

134. S.K. Fellner, and W.J. Arendshorst: Angiotensin II-stimulated Ca2+ entry mechanisms in afferent arterioles: role of transient receptor potential canonical channels and reverse Na+/Ca2+ exchange. *Am J Physiol Renal Physiol* 294, F212-9 (2008)

135. P. Eder, M. Poteser, C. Romanin, and K. Groschner: Na (+) entry and modulation of Na (+)/Ca (2+) exchange as a key mechanism of TRPC signaling. *Pflugers Arch* 451, 99-104 (2005)

136. M. Trebak: The puzzling role of TRPC3 channels in motor coordination. *Pflugers Arch* 459(3), 369-75 (2009)

137. S.Z. Xu, K. Muraki, F. Zeng, J. Li, P. Sukumar, S. Shah, A.M. Dedman, P.K. Flemming, D. McHugh, J. Naylor, A. Cheong, A.N. Bateson, C.M. Munsch, K.E. Porter, and D.J. Beech: A sphingosine-1-phosphate-activated calcium channel controlling vascular smooth muscle cell motility. *Circ Res* 98, 1381-9 (2006)

138. Y.M. Lee, B.J. Kim, H.J. Kim, D.K. Yang, M.H. Zhu, K.P. Lee, I. So, and K.W. Kim: TRPC5 as a candidate for the nonselective cation channel activated by muscarinic stimulation in murine stomach. *Am J Physiol Gastrointest Liver Physiol* 284, G604-16 (2003)

139. A. Riccio, A.D. Medhurst, C. Mattei, R.E. Kelsell, A.R. Calver, A.D. Randall, C.D. Benham, and M.N. Pangalos: mRNA distribution analysis of human TRPC family in CNS and peripheral tissues. *Brain Res Mol Brain Res* 109, 95-104 (2002)

140. A. Dietrich, M. Mederos y Schnitzler, J. Emmel, H. Kalwa, T. Hofmann, and T. Gudermann: N-linked protein glycosylation is a major determinant for basal TRPC3 and TRPC6 channel activity. *J Biol Chem* 278, 47842-52 (2003)

141. S.A. Reading, S. Earley, B.J. Waldron, D.G. Welsh, and J.E. Brayden: TRPC3 mediates pyrimidine receptor-induced depolarization of cerebral arteries. *Am J Physiol Heart Circ Physiol* 288, H2055-61 (2005)

142. D.J. Beech: Emerging functions of 10 types of TRP cationic channel in vascular smooth muscle. *Clin Exp Pharmacol Physiol* 32, 597-603 (2005)

143. A. Dietrich, Y.S.M. Mederos, M. Gollasch, V. Gross, U. Storch, G. Dubrovska, M. Obst, E. Yildirim, B. Salanova, H. Kalwa, K. Essin, O. Pinkenburg, F.C. Luft, T. Gudermann, and L. Birnbaumer: Increased vascular smooth muscle contractility in TRPC6-/- mice. *Mol Cell Biol* 25, 6980-9 (2005)

144. A.J. Hill, J.M. Hinton, H. Cheng, Z. Gao, D.O. Bates, J.C. Hancox, P.D. Langton, and A.F. James: A TRPC-like non-selective cation current activated by alpha 1-adrenoceptors in rat mesenteric artery smooth muscle cells. *Cell Calcium* 40, 29-40 (2006)

145. S. Jung, R. Strotmann, G. Schultz, and T.D. Plant: TRPC6 is a candidate channel involved in receptor-stimulated cation currents in A7r5 smooth muscle cells. *Am J Physiol Cell Physiol* 282, C347-59 (2002)

146. M. Li, J. Zacharia, X. Sun, and W.G. Wier: Effects of siRNA knock-down of TRPC6 and InsP (3)R1 in vasopressin-induced Ca (2+) oscillations of A7r5 vascular smooth muscle cells. *Pharmacol Res* 58, 308-15 (2008)

147. D.G. Welsh, A.D. Morielli, M.T. Nelson, and J.E. Brayden: Transient receptor potential channels regulate myogenic tone of resistance arteries. *Circ Res* 90, 248-50 (2002)

148. S. Earley, B.J. Waldron, and J.E. Brayden: Critical role for transient receptor potential channel TRPM4 in myogenic constriction of cerebral arteries. *Circ Res* 95, 922-9 (2004)

149. Y. Yu, M. Sweeney, S. Zhang, O. Platoshyn, J. Landsberg, A. Rothman, and J.X. Yuan: PDGF stimulates pulmonary vascular smooth muscle cell proliferation by upregulating TRPC6 expression. *Am J Physiol Cell Physiol* 284, C316-30 (2003)

150. R. Berra-Romani, A. Mazzocco-Spezzia, M.V. Pulina, and V.A. Golovina: Ca2+ handling is altered when arterial myocytes progress from a contractile to a proliferative phenotype in culture. *Am J Physiol Cell Physiol* 295, C779-90 (2008)

151. R.W. Guo, H. Wang, P. Gao, M.Q. Li, C.Y. Zeng, Y. Yu, J.F. Chen, M.B. Song, Y.K. Shi, and L. Huang: An Essential Role for STIM1 in Neointima Formation Following Arterial Injury. *Cardiovasc Res* 81(4), 660-8 (2008)

152. F.C. Aubart, Y. Sassi, A. Coulombe, N. Mougenot, C. Vrignaud, P. Leprince, P. Lechat, A.M. Lompre, and J.S. Hulot: RNA Interference Targeting STIM1 Suppresses Vascular Smooth Muscle Cell Proliferation and Neointima Formation in the Rat. *Mol Ther* 17(3), 455-62 (2008)

153. N. Weissmann, F. Grimminger, and W. Seeger: Hypoxia in lung vascular biology and disease. *Cardiovasc Res* 71, 618-9 (2006)

154. N. Weissmann, N. Sommer, R.T. Schermuly, H.A. Ghofrani, W. Seeger, and F. Grimminger: Oxygen sensors in hypoxic pulmonary vasoconstriction. *Cardiovasc Res* 71, 620-9 (2006)

155. L.J. Rubin: Primary pulmonary hypertension. *N Engl J Med* 336, 111-7 (1997)

156. J. Wang, L.A. Shimoda, L. Weigand, W. Wang, D. Sun, and J.T. Sylvester: Acute hypoxia increases intracellular Ca2+. in pulmonary arterial smooth muscle by enhancing capacitative Ca2+ entry. *Am J Physiol Lung Cell Mol Physiol* 288, L1059-69 (2005)

157. J. Wang, L. Weigand, W. Lu, J.T. Sylvester, G.L. Semenza, and L.A. Shimoda: Hypoxia inducible factor 1 mediates hypoxia-induced TRPC expression and elevated intracellular Ca2+ in pulmonary arterial smooth muscle cells. *Circ Res* 98, 1528-37 (2006)

158. P.I. Aaronson: TRPC Channel upregulation in chronically hypoxic pulmonary arteries: the HIF-1 bandwagon gathers steam. *Circ Res* 98, 1465-7 (2006)

159. J.W. Landsberg, and J.X. Yuan: Calcium and TRP channels in pulmonary vascular smooth muscle cell proliferation. *News Physiol Sci* 19, 44-50 (2004)

160. I. Fantozzi, S. Zhang, O. Platoshyn, C.V. Remillard, R.T. Cowling, and J.X. Yuan: Hypoxia increases AP-1 binding activity by enhancing capacitative Ca2+ entry in human pulmonary artery endothelial cells. *Am J Physiol Lung Cell Mol Physiol* 285, L1233-45 (2003)

161. Y. Yu, I. Fantozzi, C.V. Remillard, J.W. Landsberg, N. Kunichika, O. Platoshyn, D.D. Tigno, P.A. Thistlethwaite, L.J. Rubin, and J.X. Yuan: Enhanced expression of transient receptor potential channels in idiopathic pulmonary arterial hypertension. *Proc Natl Acad Sci U S A* 101, 13861-6 (2004)

162. A.L. Firth, C.V. Remillard, and J.X. Yuan: TRP channels in hypertension. *Biochim Biophys Acta* 1772, 895-906 (2007)

163. N. Kunichika, J.W. Landsberg, Y. Yu, H. Kunichika, P.A. Thistlethwaite, L.J. Rubin, and J.X. Yuan: Bosentan inhibits transient receptor potential channel expression in pulmonary vascular myocytes. *Am J Respir Crit Care Med* 170, 1101-7 (2004)

164. J. Yu, S.H. Keller, C.V. Remillard, O. Safrina, A. Nicholson, S.L. Zhang, J. W., N. Vangala, J.W. Landsberg, J.Y. Wang, P.A.C. Thistlethwaite, R. N., I.M. Robbins, J.E. Loyd, H.A. Ghofrani, F. Grimminger, R.T. Schermuly, M.D. Cahalan, L.J. Rubin, and J.X. Yuan: A functional single-nucleotide polymorphism in the TRPC6 gene promoter associated with idiopathic pulmonary arterial hypertension. *Circulation* 119, 2313-2322 (2009)

165. Y.M. Pinto, M. Paul, and D. Ganten: Lessons from rat models of hypertension: from Goldblatt to genetic engineering. *Cardiovasc Res* 39, 77-88 (1998)

166. Y.M. Bae, A. Kim, Y.J. Lee, W. Lim, Y.H. Noh, E.J. Kim, J. Kim, T.K. Kim, S.W. Park, B. Kim, S.I. Cho, D.K. Kim, and W.K. Ho: Enhancement of receptor-operated cation current and TRPC6 expression in arterial smooth muscle cells of deoxycorticosterone acetate-salt hypertensive rats. *J Hypertens* 25, 809-17 (2007)

167. M.V. Pulina, A. Zulian, R. Berra-Romani, O. Beskina, A. Mazzocco-Spezzia, S.G. Baryshnikov, I. Papparella, J.M. Hamlyn, M.P. Blaustein, and V.A. Golovina: Up-regulation of Na+ and Ca2+ transporters in arterial smooth muscle from ouabain hypertensive rats. *Am J Physiol Heart Circ Physiol* 298(1), H263-74 (2009)

168. D. Liu, D. Yang, H. He, X. Chen, T. Cao, X. Feng, L. Ma, Z. Luo, L. Wang, Z. Yan, Z. Zhu, and M. Tepel: Increased transient receptor potential canonical type 3 channels in vasculature from hypertensive rats. *Hypertension* 53, 70-6 (2009)

169. M. Foller, R.S. Kasinathan, C. Duranton, T. Wieder, S.M. Huber, and F. Lang: PGE2-induced apoptotic cell death in K562 human leukaemia cells. *Cell Physiol Biochem* 17, 201-10 (2006)

170. S. Satoh, H. Tanaka, Y. Ueda, J. Oyama, M. Sugano, H. Sumimoto, Y. Mori, and N. Makino: Transient receptor potential (TRP) protein 7 acts as a G protein-activated Ca2+ channel mediating angiotensin II-induced myocardial apoptosis. *Mol Cell Biochem* 294, 205-15 (2007)

171. S.Z. Xu, F. Zeng, G. Boulay, C. Grimm, C. Harteneck, and D.J. Beech: Block of TRPC5 channels by 2-aminoethoxydiphenyl borate: a differential, extracellular and voltage-dependent effect. Br J Pharmacol 145, 405-14 (2005)

172. K.P. Lee, J.Y. Jun, I.Y. Chang, S.H. Suh, I. So, and K.W. Kim: TRPC4 is an essential component of the nonselective cation channel activated by muscarinic stimulation in mouse visceral smooth muscle cells. *Mol Cells* 20, 435-41 (2005)

173. A.P. Albert, and W.A. Large: Synergism between inositol phosphates and diacylglycerol on native TRPC6-like channels in rabbit portal vein myocytes. *J Physiol* 552, 789-95 (2003)

174. A. Ulloa, A.L. Gonzales, M. Zhong, Y.S. Kim, J. Cantlon, C. Clay, C.Y. Ku, S. Earley, and B.M. Sanborn: Reduction in TRPC4 expression specifically attenuates G-protein coupled receptor-stimulated increases in intracelular calcium in human myometrial cells. *Cell Calcium* 46, 73-84 (2009)

175. S.N. Saleh, A.P. Albert, C.M. Peppiatt-Wildman, and W.A. Large: Diverse properties of store-operated TRPC channels activated by protein kinase C in vascular myocytes. *J Physiol* 586, 2463-76 (2008)

176. D.J. Beech, S.Z. Xu, D. McHugh, and R. Flemming: TRPC1 store-operated cationic channel subunit. *Cell Calcium* 33, 433-40 (2003)

177. S. Jung, A. Muhle, M. Schaefer, R. Strotmann, G. Schultz, and T.D. Plant: Lanthanides potentiate TRPC5 currents by an action at extracellular sites close to the pore mouth. *J Biol Chem* 278, 3562-71 (2003)

178. Y.M. Bae, D.J. Sung, H.J. Noh, J. Kim, S.W. Park, B. Kim, and S.I. Cho: Serotonin-induced ion channel modulations in mesenteric artery myocytes from normotensive and DOCA-salt hypertensive rats. *J Smooth Muscle Res* 43, 85-97 (2007)

179. N. Weissmann, A. Dietrich, B. Fuchs, H. Kalwa, M. Ay, R. Dumitrascu, A. Olschewski, U. Storch, M. Mederos y Schnitzler, H.A. Ghofrani, R.T. Schermuly, O. Pinkenburg, W. Seeger, F. Grimminger, and T. Gudermann: Classical transient receptor potential channel 6 (TRPC6) is essential for hypoxic pulmonary vasoconstriction and alveolar gas exchange. *Proc Natl Acad Sci U S A* 103, 19093-8 (2006)

Abbreviations: AA: Arachidonate, Arachidonic Acid, AP-1: Apetala 1 Transcription Factor, CAD: CRAC Activating Domain, CPA: Cyclopiazonic Acid, CRAC: Calcium Release Activated Calcium current, CREB: cAMP Response Element Binding Protein, DAG: Diacylglycerol, DOCA: Deoxycosticosterone Acetate, ET-1: Endothelin-1, HIF-1: Hypoxia Inducible Factor 1, IP3: Inositol 1,4,5-trisphosphate , IP3R: IP3 Receptor, IPAH: Idiopathic Pulmonary Artery Hypertension, L-type: High Voltage Voltage-gated Ca2+ Channel, NCX: Na+/Ca2+ exchanger, OAG: 1-oleyl-2-acetyl-sn-glycerol, PIP2: Phosphatidylinositol 4,5-bisphosphate, PLC: phospholipase C, ROC: Receptor-Operated Channels, S1P: Sphingosine 1-phosphate, SMC: Smooth Muscle Cell, SOAR: STIM Orai Activating Region, SOCE: Store-operated Ca2+ entry, SOC: Store-Operated Channels, STIM: Stromal Interaction Molecule , TM5-TM6: Transmembrane Spanning Region 5/6, TRP: Transient Receptor Potential, TRPC: Transient Receptor Potential Canonical

Key Words: Transient Receptor Potential Canonical, Calcium Channels, Proliferation, Smooth Muscle, Vascular Disease, Hypertension, Vascular Remodeling, Review

Send correspondence to: Mohamed Trebak,. Center for cardiovascular Sciences , MC8, 47 New Scotland Ave, Albany NY 12208, Tel: 518-262-4682, Fax: 518-262-8101, E-mail: trebakm@mail.amc.edu

http://www.bioscience.org/current/vol15.htm