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Interplay Between Calcium and Reactive Oxygen/Nitrogen Species: An Essential Paradigm for Vascular Smooth Muscle Signaling

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Abstract

Signaling cascades initiated or regulated by calcium (Ca^{2+}), reactive oxygen (ROS), and nitrogen (RNS) species are essential to diverse physiological and pathological processes in vascular smooth muscle. Stimuli-induced changes in intracellular Ca^{2+} regulate the activity of primary ROS and RNS, producing enzymes including NADPH oxidases (Nox) and nitric oxide synthases (NOS). At the same time, alteration in intracellular ROS and RNS production reciprocates through redox-based post-translational modifications altering Ca^{2+} signaling networks. These may include Ca^{2+} pumps such as sarcoplasmic endoplasmic reticulum Ca^{2+}-ATPase (SERCA), voltage-gated channels, transient receptor potential canonical (TRPC), melastatin2 (TRPM2), and ankyrin1 (TRPA1) channels, store operated Ca^{2+} channels such as Orai1/stromal interaction molecule 1 (STIM1), and Ca^{2+} effectors such as Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). In this review, we summarize and highlight current experimental evidence supporting the idea that cross-talk between Ca^{2+} and ROS/RNS may represent a well-integrated signaling network in vascular smooth muscle. *Antioxid. Redox Signal.* 12, 657–674.

Introduction

Smooth muscle cells are a class of contractile cells with diverse phenotypes that are necessary for the proper function of many organs, including the uterus, bladder, gastrointestinal tract, and systemic and airway vasculature (14). Vascular smooth muscle cells (VSMC), which are characterized by high phenotypic plasticity, provide structural integrity to the vessel wall and ensure precise regulation of vascular tone and blood pressure through their contractile function (121). The voltage-activated L-type calcium (Ca^{2+}) channels at the plasma membrane (PM) play a central role in the function of VSMC by controlling their contraction. VSMC also express voltage-insensitive Ca^{2+} channels at the PM that are typically activated by mechanical stimuli or downstream of phospholipase C (PLC) isoforms in response to stimulation by vasoactive and growth factor receptors. These receptor-activated channels control VSMC contraction, as well as other VSMC functions such as proliferation and migration (76).

Reactive oxygen and nitrogen species (ROS and RNS) represent a large class of molecules regulating many aspects of VSMC biology, including contraction, proliferation, and migration. Primary sources of ROS and RNS in VSMC include the mitochondria, multiple isoforms of NADPH oxidases (NOX), and nitric oxide synthases (NOS), as well as storages pools of nitric oxide (23). Many of the enzymes involved in ROS and RNS synthesis are Ca^{2+}-sensitive and changes in the amplitude and oscillatory patterns of intracellular Ca^{2+} signals in response to mechanical and chemical stimulations allow for rapid modulation of ROS/RNS production. Reciprocally, ROS/RNS regulate VSMC Ca^{2+} signaling through site-specific modifications of amino acid residues such as the oxidation, nitrosation, or nitration of cysteine and tyrosine residues. These alter the molecular components that directly regulate intracellular Ca^{2+} concentration (*i.e.*, ion channels or transporters) or modify specific signaling molecules that are regulated by intracellular Ca^{2+}. In this review, we discuss the evidence indicating crosstalk between Ca^{2+} and ROS/RNS signaling at multiple levels, lending credence to the paradigm that these represent well-integrated signaling systems in vascular smooth muscle (Fig. 1).

Calcium Signaling and Signaling Pathways in Vascular Smooth Muscles

Ca^{2+} and smooth muscle contractility

Unlike cardiac myocytes, VSMC are characterized by relatively slow contractions that are, in some instances, sustained over extended periods of time. This is presumably due to the ability of VSMC to utilize Ca^{2+} gradients at low global intracellular Ca^{2+} concentrations and thus maintain contraction. VSMC generate rhythmical contractions resulting from a

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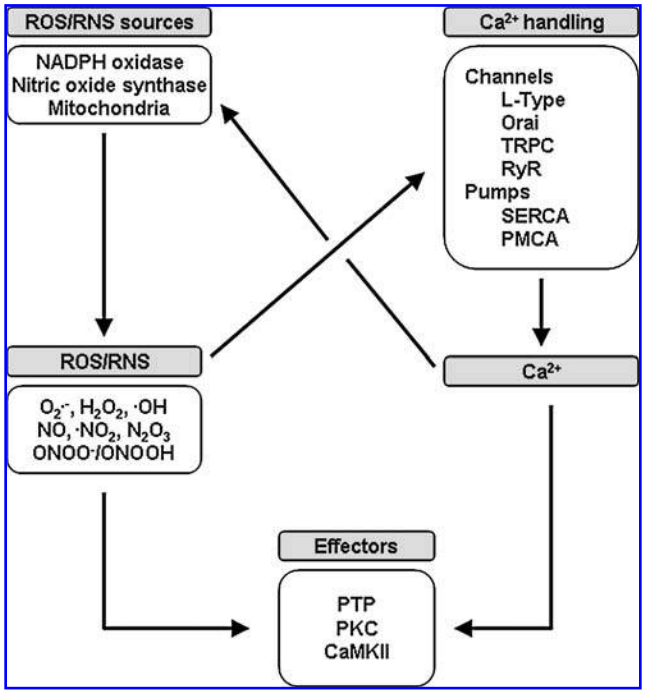

FIG. 1. Interplay between calcium and ROS/RNS signaling in vascular smooth muscle. Changes in intracellular Ca^{2+} concentrations regulate the activity of many sources of reactive oxygen and nitrogen species (ROS/RNS), including NADPH oxidase, nitric oxide synthase, and the mitochondria. ROS and RNS represent a variety of chemicals that are not only formed as products from enzymatic activities but are also derived from secondary reactions with other intermediates such as molecular oxygen. At the same time, ROS/RNS modify, through post-translational modifications, specific amino acid residues in channels and pumps with concomitant changes in Ca^{2+} handling. Both ROS/RNS and Ca^{2+} converge onto effector molecules such as protein tyrosine phosphatase (PTP), protein kinase C (PKC), and Ca^{2+}/calmodulin-dependent protein kinase II to modulate vascular smooth muscle cell function including contraction, migration, and proliferation.

complex interplay between ion channels, pumps, and transporters that increase global intracellular Ca^{2+} concentration in the form of a longitudinally traveling Ca^{2+} wave. The contractility of VSMC *in vivo* is under the control of a plethora of neuronal and humoral agonists that usually act through membrane receptors that couple to phosphoinositide-specific PLC to produce inositol-1,4,5-trisphosphate (IP_3) and cause a series of Ca^{2+} transients due to Ca^{2+} release from the sarcoplasmic reticulum (SR) *via* the IP_3 receptor (IP_3R) (14). These agonist-induced Ca^{2+} transients or oscillations are necessary for the initiation of VSMC contraction. Since internal Ca^{2+} stores are finite, sustained increase in VSMC intracellular Ca^{2+} depends on Ca^{2+} entry through PM channels that are required to replenish the SR after each cycle of Ca^{2+} release. There is a wide variety of PM channels that have been implicated in this process; their relative importance and the extent of their contribution might depend on the VSMC type and the nature of the stimulus involved. Nevertheless, it is clearly established that the L-type high voltage-gated Ca^{2+} channels play an important role in increasing global Ca^{2+} levels during VSMC contraction (14). The PM depolarization necessary for activating L-type Ca^{2+} channels might be achieved through agonist-activated nonselective ion channels, such as isoforms of the transient receptor potential canonical channels (*e.g.*, TRPC6) (157). Alternatively, discrete clusters of persistently active L-type Ca^{2+} channels operating in a high open probability mode, could contribute to steady-state Ca^{2+} entry into VSMC (14, 130). Another class of voltage-activated Ca^{2+} channels called T-type (named for the transient nature of their currents) has been suggested to contribute to global Ca^{2+} entry in airway smooth muscle (14). Ca^{2+} entry through PM channels activates, through the process of Ca^{2+}-induced Ca^{2+} release (CICR), the ryanodine receptors (RyR) at the SR inducing further Ca^{2+} release. Further Ca^{2+} release might be contributed through the IP_3R activated by agonist-induced IP_3 production and by sensitization of the IP3R by Ca^{2+}. These regenerative Ca^{2+} release cycles maintained by Ca^{2+} entry channels will cause global Ca^{2+} rise in the form of a traveling wave along the cell that leads to the initiation of the contractile response through activation of Ca^{2+}/calmodulin-dependent myosin light chain kinase (MLCK). Ca^{2+} can activate Ca^{2+}-dependent chloride (Cl^-) channels, causing further depolarization and activation of L-type Ca^{2+} channels and subsequent Ca^{2+} release from the SR, ensuring the establishment of a positive feedback loop. Through gap junctions, depolarization can reach neighboring cells, activating their L-type Ca^{2+} channels and Ca^{2+} transients and helping synchronize the contractility wave along the vessel (14).

The reversal of VSMC contractility or vasorelaxation is achieved through mechanisms that limit Ca^{2+} entry through the PM in combination with cytoplasmic Ca^{2+} clearance. Discrete subplasmalemmal Ca^{2+} release through RyR, also called Ca^{2+} sparks, reduces Ca^{2+} entry through L-type Ca^{2+} channels by activating Ca^{2+}-activated potassium (K^+) channels and causing hyperpolarization (117). Ca^{2+} clearance from the cytoplasm depends on the action of sarcoplasmic-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) and PM Ca^{2+}-ATPase (PMCA) that pump Ca^{2+} into the SR and outside the cell respectively. Figure 2 depicts the major ion channels and transporters controlling intracellular Ca^{2+} concentration in contractile VSMC.

Smooth muscle receptor-activated channels

Store-operated Ca^{2+} entry (SOCE). VSMC functions are regulated by a wide variety of growth factors and vasoactive compounds that achieve their goal through binding to their specific receptors at the PM. These receptors typically couple to activation of PLC isoforms and production of second messengers IP_3 and diacylglycerol (DAG) upon hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2; Fig. 2). The fall of the Ca^{2+} concentration within the lumen of the ER as a result of the action of IP_3 on the IP_3R activates a ubiquitous entry of Ca^{2+} across the PM originally recognized and termed *capacitative Ca^{2+} entry* (CCE) by Putney (138). The channels mediating this entry are called *store-operated channels* (SOC) (124, 138). The first and best characterized SOC channel is found in hematopoietic cells and conducts a highly Ca^{2+}-selective, nonvoltage-gated, inwardly rectifying current first described in rat basophilic leukemia (RBL) mast cells and termed the Ca^{2+} release activated Ca^{2+} current (CRAC) (75). Major advances have been achieved recently regarding the molecular identity of SOC channels and the mechanisms link-

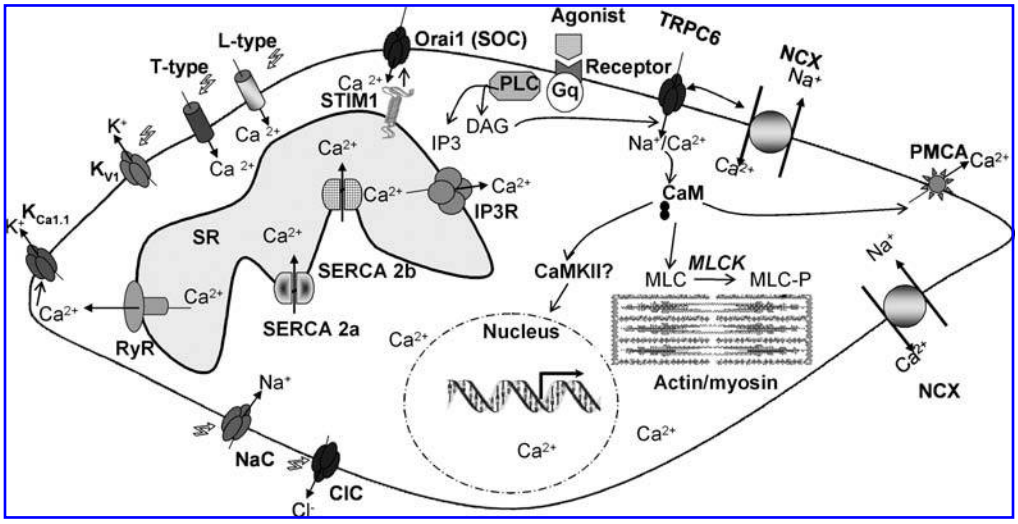

FIG. 2. Ion channels and transporters in vascular smooth muscle. The contractile vascular smooth muscle cell (VSMC) express voltage-activated Ca^{2+} channels of the L-type that are major contributors to Ca^{2+} entry into VSMC; VSMC also express T-type voltage activated Ca^{2+} channels. Major Ca^{2+} release channels in the sarcoplasmic reticulum (SR) of VSMC include ryanodine receptors (RyR) activated by Ca^{2+}, and IP_3 receptors (IP_3R) activated by IP_3. Vasoactive agonists, by binding to their specific receptors, mediate Ca^{2+} release through IP_3 binding to the IP_3R as well as Ca^{2+} entry through store-operated channels (SOC) and second messenger-activated channels such TRPC6 that is activated by diacylglycerol (DAG). SOC channels are encoded by Orai1 and are activated through store depletion-mediated aggregation of the SR Ca^{2+} sensor STIM1 and its direct interaction with Orai1 channels. Global increase of Ca^{2+} levels in the cytoplasm of VSMC are necessary for VSMC contractility and *de novo* gene transcription by activating Ca^{2+}/calmodulin-dependent kinases such as CaMKII and MLCK. Direct coupling between nonselective TRPC channels and Na^+/Ca^{2+} exchanger (NCX) have been proposed. Na^+ entry through TRPC6 channels could lead to Ca^{2+} entry in VSMC through NCX operating in its reverse mode. Voltage-activated chloride (Cl^-) and sodium (Na^+) channels can depolarize VSMC, causing subsequent activation of voltage-activated L-type Ca^{2+} channels. Voltage-activated and calcium-activated potassium (K^+) channels can limit Ca^{2+} entry into VSMC by causing cell hyperpolarization. SERCA and PMCA pumps are responsible for clearing Ca^{2+} from the cytoplasm by pumping Ca^{2+} into the SR and outside the cell respectively; PMCA activity can be enhanced by CaM.

ing internal Ca^{2+} store depletion to this Ca^{2+} entry in non-excitable cells (140). An RNA silencing (siRNA)-based screen identified a Ca^{2+}-binding protein, stromal interaction molecule 1 (STIM1) as the long sought Ca^{2+} sensor in the ER (98, 144) capable of oligomerization and reorganization into punctuate structures in defined ER–PM junctional areas upon store depletion to somehow signal the activation of the SOC channel at the PM (Fig. 3). Subsequently, genome-wide screens revealed that the membrane protein Orai1 is an essential component of SOC channels (45, 183) and that Orai1 is an essential pore forming unit of SOC channels. STIM1 and Orai1 alone can recapitulate most of the biophysical characteristics of CRAC currents. The mechanisms of STIM1/Orai1 coupling are beginning to emerge and appear to involve direct binding of a minimal, highly conserved ~107-amino acid region of STIM1 to the N- and C-termini of Orai1 to open the CRAC channel (114, 125, 199). Nonetheless, it is clear that additional STIM1/Orai1 binding partners are involved (188). STIM1 is a type I transmembrane protein residing primarily in the ER, but can be found to a limited extent in the PM (~10% of total proteins) (68). PM STIM1 is involved in the activation of the arachidonate-activated Ca^{2+} entry pathway (108). STIM1 has a closely related homologue, STIM2 (68), which is active at basal ER Ca^{2+} concentrations and can activate Ca^{2+} influx *via* Orai1 upon smaller decrease in ER Ca^{2+} content, compared to STIM1 (21). Orai1 has two homologs, Orai2 and Orai3, that exhibit distinct pharmacological, biophysical, and ion selectivity properties (32); the role of Orai2 and Orai3 in native Ca^{2+} entry pathways in different cell types including smooth muscle remains unknown. Nonetheless, Orai isoforms were shown to form heteromeric channels when ectopically expressed in HEK293 cells (99), and it is therefore conceivable that such heteromultimers might contribute to the diversity of Ca^{2+} entry pathways in different cell types by providing specific channels tailored to the cells' signaling needs.

Store-independent Ca^{2+} entry pathways. A large number of pathways are known to contribute to the generation of Ca^{2+} signals in cells following receptor activation. The interplay between these different pathways generates a diverse and complex array of Ca^{2+} signals, which are required for VSMC function. The interplay between these Ca^{2+} entry pathways remains largely unknown. In addition to SOC, there are other modes of regulated Ca^{2+} entry across the PM. All of these routes of Ca^{2+} entry into cells that do not depend on the state of filling of internal Ca^{2+} stores are often referred to as noncapacitative or store-independent Ca^{2+} entry pathways (Fig. 3). Products of the PLC pathway (IP_3 and DAG) are both involved in activating PM Ca^{2+} channels. In some instances, DAG in a PKC-independent manner activates PM Ca^{2+} channels (71, 95, 120, 175) and IP_3 is also shown to act directly on IP_3R located at the PM (168). Another lipid second messenger, arachidonic acid (AA), is known to activate PM Ca^{2+} channels (154). Other second messengers such as cyclic ADP-ribose (cADPr), cyclic GMP (cGMP), and Ca^{2+} itself were implicated in a variety of Ca^{2+} entry pathways (7).

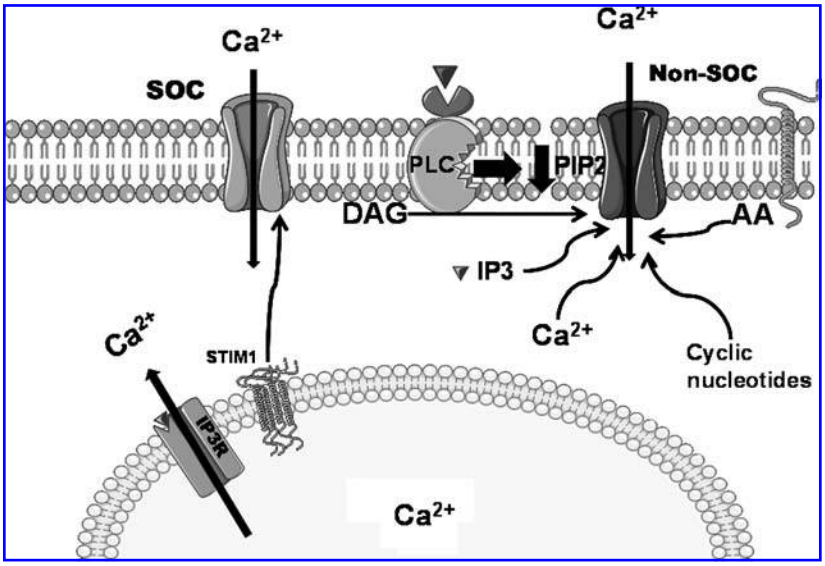

FIG. 3. Store-dependent and store-independent receptor-activated Ca^{2+} channels. The binding of an agonist to phospholipase C (PLC)-coupled receptors is typically followed by hydrolysis of PIP_2 into two second messengers: IP_3 and DAG. The binding of IP_3 to IP_3R in the SR causes SR store depletion followed by STIM1 aggregation into punctuate structures near the plasma membrane, where it binds Orai1 channels and causes their activation. This mode of Ca^{2+} signaling that depends on the state of filling of SR store is called store-operated and Orai1 channels are referred to as store-operated channels (SOC). A plethora of Ca^{2+} entry pathways that do not depend on store depletion are activated upon stimulation of PLC-coupled receptors. These Ca^{2+} entry routes can be activated by second messengers such DAG, IP_3, arachidonic acid, Ca^{2+}, cyclic nucleotides, and PIP2 hydrolysis and are often referred to as receptor-operated channels (ROC) or second messenger operated channels (SMOC).

Shuttleworth and colleagues have described a role of PM-resident STIM1 in activating the AA-regulated Ca^{2+} (ARC) channels to which Orai1 and Orai3 contribute subunits (108, 109). However, the exact signaling mechanisms controlling other store-independent Ca^{2+} channels as well as the molecular identities of these channels are largely unknown (7).

TRPC channels. Canonical TRP (TRPC) channels constitute one of the major subfamily of the larger transient receptor potential (TRP) family of ion channels. To date, 28 mammalian TRPs have been identified that can be divided into six subfamilies: TRPC (Canonical), TRPM (Melastatin), TRPV (Vanilloid), TRPA (Ankyrin), TRPP (Polycystin), and TRPML (Mucolipin). Over the past decade, the seven TRPC members (TRPC1-7) have been the focus of intensive investigations in many cell types, including VSMC as potential candidates for SOC and non-SOC channels, by virtue of their activation by mechanisms downstream of the PLC pathway (156, 174). Patch clamp recordings in VSMC cell lines and primary VSMC cells from different species and different vascular beds have suggested that store depletion activates a nonselective SOC conductance, distinct from CRAC currents (22, 124, 178). In VSMC from rabbit aorta and portal vein, nonselective SOC currents have been described (178). These channels have an estimated conductance of 2–3 pS, were selective for cations but did not discriminate between monovalents and divalents. In particular, evidence from several laboratories suggested that TRPC1 is involved in SOCE in smooth muscle cell lines (22), and in VSMC from arteries and veins isolated from many species (124). A study in human pulmonary artery SMC showed that inhibition of TRPC1 expression using antisense oligonucleotides decreases SOC entry (164). However, the involvement of TRPC proteins in making SOC channels is a highly controversial topic at this time. Simultaneous knockdown using RNA interference of the three isoforms of TRPCs expressed in primary rat aortic VSMC (TRPC1/4/6) failed to affect the magnitude of SOCE (136). Dietrich *et al.* provided evidence that SOC currents in VSMC are intact in TRPC1 knockout mice (34) and internal Ca^{2+} store repletion was found to be normal in TRPC3 knockout mice and TRPC1/4/6 triple knockout mice (67). Furthermore, data from our group demonstrated the functional existence of CRAC currents encoded by STIM1/Orai1 in primary VSMC and the A7r5 cell line (136) as well as in endothelial cells (1), arguing that CRAC is a general mechanism for SOCE in all cells and casting doubt on TRPC channels as candidates for SOC channels. Two previous studies showed a role for STIM1 and Orai1 in thapsigargin-mediated SOCE in airway SMC (128, 129). However, the role of STIM2 and Orai2/3 proteins in VSMC Ca^{2+} signaling remains unknown.

A less controversial hypothesis is that of TRPCs generally encoding store-independent nonselective cation channels. Many groups have showed that TRPC3/6/7 subfamily members are activated by DAG when ectopically expressed in cell lines (176). Inoue *et al*. presented convincing evidence for the involvement of TRPC6 in the endogenous non-SOC DAG-activated cation entry controlled by α1-adrenergic receptors in rabbit portal vein smooth muscle (81). Data from Murayama *et al*. suggested that heteromultimeric TRPC6-TRPC7 channels contribute to vasopressin-activated, nonselective cation channels in A7r5 VSMC (103). Data from Van Breemen and colleagues described functional coupling of the Na^+/Ca^{2+} exchanger (NCX) in its reverse-mode with receptor-activated store-independent TRPC6 in rat aortic smooth muscle cells following ATP stimulation. NCX reversal which contributes to Ca^{2+} entry was enhanced following stimulation with ATP and a DAG analog, consistent with the known properties of TRPC6 (132, 165). Earlier studies by Groschner and co-workers suggested both physical and functional coupling of TRPC3 to the cardiac-type Na^+/Ca^{2+} exchanger (NCX1) in the control of Ca^{2+} homeostasis (145). Upon PLC-coupled receptor stimulation, Na^+ entry through TRPC3 channels triggers Ca^{2+} entry by NCX operating in its reverse mode (39). Xi *et al*. described a store-independent mechanism of

IP_3/IP_3R-dependent activation of TRPC3 channels in constriction of cerebral arteries (189).

Ca^{2+} channel modulation and vascular smooth muscle phenotypes

In response to various environmental stimuli, including growth factors, cytokines, mechanical influences, and various inflammatory mediators, the resident quiescent VSMC undergo transcriptional changes affecting both the downregulation of contractile proteins and concurrent upregulation of proteins supporting a proliferative phenotype (76, 185). This plasticity is believed to have evolved as a mechanism of vascular repair during injury and/or vascular adaptation to increasing demands by enabling VSMC to "switch" to a noncontractile, proliferative, and migratory phenotype. This phenotypic switch can, under certain circumstances, contribute to vascular disease such as atherosclerosis, hypertensive microvessel remodeling, vein graft failure, and restenosis following percutaneous intervention (26, 148). VSMC phenotypic change was shown to correlate with downregulation of voltage-activated L-type Ca^{2+} channels and increase in TRPC1 and TRPC6 mRNA and protein expression (12). Balloon dilatation of isolated human internal mammary artery showed a similar increase in TRPC1 and TRPC6 mRNA expression 48 h post injury (12). TRPC1 is upregulated following experimental vascular injury in mice and pigs, and is associated with the conversion of smooth muscle cells from a quiescent to a proliferative phenotype initiating neointimal formation (89). When TRPC1 function in saphenous vein organ culture was blocked with anti-TRPC1 antibody, neointimal growth in organ culture and VSMC proliferation were significantly inhibited (89). In pulmonary artery SMC, TRPC1 and TRPC6 expression increased following serum stimulation (53, 198). In patients with idiopathic pulmonary arterial hypertension (IPAH), a disease characterized by excessive pulmonary artery SMC proliferation, TRPC3 and TRPC6 were strongly upregulated (45) and siRNA against TRPC6 markedly attenuated pulmonary artery SMC proliferation in culture (197). Berra–Romani *et al.* showed that the protein levels of STIM1 and all Orai isoforms are dramatically increased in proliferative rat mesenteric VSMC, suggesting a role of these proteins in altered Ca^{2+} handling during vascular remodeling (13). Recent data from our group showed a similar increase of STIM1/Orai1 protein levels and SOCE in proliferative migratory rat aortic VSMC. Interestingly, knockdown of either STIM1 or Orai1 attenuated proliferation and migration of these VSMCs (136). More recently, two studies showed increased STIM1 expression in an *in vivo* model of vascular injury and inhibition of neointima formation upon *in vivo* knockdown of STIM1 (4, 60). Furthermore, Giachini *et al.* reported increased expression and activity of STIM1/Orai1 and SOCE in aorta from hypertensive rats (48). All these studies provide evidence that voltage-independent store-operated and store-independent Ca^{2+} channels contribute to vascular disease by controlling VSMC proliferation and migration during phenotypic modulation.

Calcium-Mediated Regulation of Primary Sources of ROS/RNS in Vascular Smooth Muscle

In VSMC, ROS and RNS are derived from multiple sources, which include several isoforms of NADPH oxidases and NO synthases. The biochemistry and physiological implications of Nox and NOS activity in VSMC have been extensively reviewed and we briefly summarize salient biochemical and functional features and highlight the Ca^{2+} requirements for several of these enzymes.

NADPH oxidases

Five NADPH oxidases (Nox) isoforms (Nox1–5) and two homologous oxidases (Duox1 and 2) all share the ability to generate $O_2^{\cdot-}$ and/or H_2O_2 through reduction of molecular oxygen using NADPH as the electron source (91). Nox1, 2, 4, and 5 are found in VSMC and their expression varies between species, vascular beds, and normal and diseased vessels. Nox1 and Nox2 (gp91 phox) are probably the better characterized isoforms. Mainly, they require several cytosolic activators including Rac 1 and 2, p47phox, and p67phox (or Nox1 homologues NOXO1 and NOXOA1) that bind the membrane-associated subunits p22phox and Nox upon stimulation by growth factors and cytokines (92). A number of animal studies point to a role for Nox1 and 2 in cardiovascular diseases including hypertension (36, 104), atherosclerosis (8, 159), and restenosis (95, 172, 188), but their contribution to human diseases is unclear. Another isoform, Nox4, is abundantly expressed in VSMCs (29). Its activity is usually considered to be constitutive and only dependent on protein levels. In contrast to Nox1 and 2, little is known about the regulation of its activity other than interactions with p22phox (85, 102) and polymerase delta interacting protein 2 (Poldip2), (101). Functionally, Nox4 has been implicated in VSMC differentiation (29), polyploidy (105), and response to TGFβ (162), 7-ketocholesterol (127), and TLR-4 signaling (126).

An interesting issue derived from the study of Nox4 is the realization that not all Noxs may in fact produce $O_2^{\cdot-}$. Nox activity is classically believed to be derived from electron transfer from cytosolic NADPH to FAD, the two nonidentical hemes, and finally to molecular oxygen to form $O_2^{\cdot-}$, H_2O_2 being derived from $O_2^{\cdot-}$ dismutation (44). However, recent results obtained from complementary approaches to detect $O_2^{\cdot-}$ and H_2O_2 indicate that the major detectable product of Nox4 is H_2O_2 and not $O_2^{\cdot-}$ (151). Although the underlying mechanism for the distinctive activity of Nox4 among the other Noxs is unknown, it has been rationalized that the interaction of $O_2^{\cdot-}$ at the active site of Nox4 may be stable enough to allow for direct sequential electron transfer to generate H_2O_2 (35).

The subcellular localization of Noxs is essential to rationalize the integration of ROS production to cell signaling, and some of the most detailed studies dealing with this issue have been performed on cytokine-stimulated VSMC. Both TNF-α and IL-1β induce the translocation of Nox1 and 2 into endosomal structures to produce $O_2^{\cdot-}$ into the lumen of the endosome (94, 110). The anion transporter ClC-3 is required to allow charge neutralization of the electron flow produced by Nox1 (110) and superoxide dismutase 1 is recruited to the endosome to facilitate H_2O_2 gradients (66). This results in the spatially segregated production of ROS to control the recruitment of tumor necrosis receptor associated factor (TRAF) molecules to their cognate receptor, leading to downstream activation of the transcription factor NFκB (94). Nox1 also colocalizes with caveolae while Nox4 is associated with focal adhesions in VSMC (70) and in the endoplasmic reticulum in endothelial cells (27).

Among the 5 Noxs, Nox5 is the only isoform that is directly Ca^{2+} sensitive. It is not expressed in rodents but has been found

in human VSMC and its expression is increased in VSMC in human atherosclerotic coronary arteries (61). There is little known regarding the functional implications of Nox5. In ovarian VSMC of *Drosophila melanogaster*, Nox5 participates in Ca^{2+} signal transduction linked to muscle contraction and ovulation (142). Nox5 also positively regulates PDGF-induced proliferation in human aortic smooth muscle cells through stimulation of the JAK/STAT pathway (83). Unlike the other Noxs, Nox5 does not require p22phox or the cytosolic subunits to function but instead contains four canonical EF-hands on its N-terminal end that provide direct sensitivity to Ca^{2+} (6). A binding site for calmodulin is located in the NADPH-binding domain (169), and PKC-mediated phosphorylation of Ser/Thr residues in the FAD-binding domain may confer increase Ca^{2+} sensitivity to Nox5 (82). Similarly, Duox1 and 2 contain EF-hands that may provide regulation by Ca^{2+} but their expression and function in VSMC have not been characterized (91).

In addition to its direct interaction with catalytic subunits, Ca^{2+} may also control Nox activity by regulating cytosolic components of Nox1 and 2. For example, in VSMC from human small arteries, serine phosphorylation of p47phox is required for ROS production by angiotensin II (173). One of the kinases responsible for p47phox phosphorylation is protein kinase C, although the specific isozyme responsible has not been identified. Similarly, in rat aortic VSMC, angiotensin II stimulation of Nox1 is partly regulated by a PKC (152). These observations—although incomplete—suggest that conventional PKC isozymes (α, βI, βII, and γ) which require Ca^{2+} for activation might participate in Nox activation in VSMC.

Nitric oxide synthase

Mammalian cells synthesize NO through the five-electron oxidation of one of the two-guanidino nitrogen of L-arginine. Three classes of nitric oxide synthases (NOS) have been described: neuronal-NOS (nNOS, type I), inducible-NOS (iNOS, type II), and endothelial-NOS (eNOS, type III) (112). All three isoforms are expressed in the vasculature and all require oxygen, NADPH, 5,6,7,8-tetrahydrobiopterin, and Ca^{+2}/calmodulin as cofactors. In contrast to eNOS and nNOS, the Ca^{2+}-independence of iNOS activity is due to calmodulin tightly bound to the enzyme (146).

All three NOS may generate other reaction products than NO itself, including nitroxyl (NO^-) (150), $O_2^{\cdot -}$, and peroxynitrite ($ONOO^-$/ONOOH) (181). The latter two products may be formed when electron transfer within the active site of NOS becomes independent of L-arginine oxidation. Decreased bioavailability of L-arginine and H_4B contributes to this uncoupling of NOS activity, which has been implicated in a variety of vascular disorders such as hypertension and atherosclerosis (111).

In the healthy vessel, the endothelium serves as the main source of NO through eNOS activity to maintain vascular tone, and regulate platelet aggregation and leukocyte adhesion (87, 112, 139). The activation of soluble guanylate cyclase by diffusible NO in VSMC to produce the second messenger cGMP is the primary transduction pathway responsible for eNOS-dependent vasorelaxation. Cyclic GMP acts directly or indirectly—via activation of cGMP-dependent protein kinase (PKG)—on many effector proteins to lower intracellular Ca^{2+} levels (97). Effector proteins include the type 1 IP_3R, and phospholamban in the SR, the smooth muscle Ca^{2+}-activated K^+ channels, and L-type Ca^{2+} channels. Another potential mechanism is through the PKG-dependent phosphorylation of threonine and serine residues on TRPC proteins including TRPC3 and possibly TRPC6 and 7 (90, 195). As a consequence, the NO-cGMP pathway reduces the activity of these channels providing an additional feedback mechanism to regulate intracellular Ca^{2+} (195).

Neuronal NOS is expressed in VSMC upon stimulation by growth factors (115), statins (116), and shear stress (122). The upregulation of nNOS contributes to the compensatory mechanism associated with adaptive chronic hypoxia (186) and nNOS VSMC is vasculoprotective (113) and atheroprotective (88). In a fashion similar to eNOS in the endothelium, the generation of NO by VSMC nNOS is coupled to Ca^{2+} because nNOS requires Ca^{2+}/calmodulin for maximum activity (112). Based on overexpression studies, the PDZ domain of nNOS has been found to interact with the C-terminal end of the human plasma membrane Ca^{2+} ATPase 4 (hPMCA4), resulting in a decrease in NO production due to a local decrease in Ca^{2+} concentration (55). The significance of these findings to VSMC function is unclear but studies by Gros *et al.* indicate that PMCA-dependent regulation of nNOS activity may regulate smooth muscle vascular reactivity (55).

Modalidities of Signaling Through ROS/RNS

Proteins may be modified by ROS and RNS through a plethora of reactions. Examples of resultant modifications include the carbonylation and deamination of amino acid side chains, the oxidation of methione residues to sulfoxides, the nitration of tyrosine residues, and the oxidation and nitrosation of cysteine residues. Functionally, these result in increase susceptibility of proteins to degradation, increase protein fragmentation, or alteration in protein function.

Reactive oxygen species (ROS)

For Nox-derived ROS, it is generally assumed that H_2O_2 regulates signaling pathways through reactions with amino acid residues in proteins, principally cysteine. This residue is one of the most reactive and carries out disulfide formation, metal-binding, electron donation, hydrolysis, and redox catalysis (49). The oxidative modifications include disulfide bridges, or the formation of oxidation products of increasing oxidation states, including sufenic (Cys-SOH), sulfinic (Cys-SO_2H), and sulfonic (Cys-SO_3H) acid (Fig. 4). The protein tyrosine phosphatase (PTP) family contains a canonical CXXXXXR sequence, in which the conserved cysteine residue acts as a nucleophile to form a thiol phosphate intermediate during catalysis (33). The local environment of the active site contributes to the low pK_a (4.5–5.5) of the cysteine such that the thiolate anion (RS^-) is the dominant form. This increases the susceptibility of cysteine to reversible oxidation by H_2O_2 to sulfenic acid with the consequential reversible inhibition of phosphatase activity (106). As such, PTP by H_2O_2 is one of the most common mechanisms for the effect of H_2O_2 on signaling networks (141). Oxidation of the thiolate to higher oxidation state products may also occur, and in the case of the H_2O_2 scavenging enzyme peroxiredoxin, this may provides a mean to regulate intracellular H_2O_2 concentrations (188).

Rather than a direct reaction of H_2O_2 with active sites of proteins containing cysteine residues, it is possible that specificity might depend on intermediate protein transducers such as thiol peroxidases. In this case, a specific thiol peroxidase would reversibly oxidize cysteine residues in binding

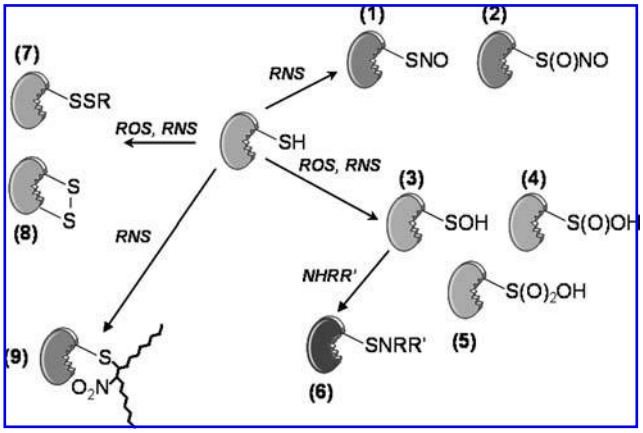

FIG. 4. Cysteine-based RNS and ROS modifications. Biochemical analyses have revealed an important role for cysteine residues in ROS and RNS-mediated signaling. This occurs through many different reactions, which lead to a wide range of redox based modifications. Reactive nitrogen species derived from nitric oxide nitrosate and nitrate cysteine residues to form nitroso (**1**) and nitro (**2**) thiols, respectively. Alternatively, RNS such as nitrogen dioxide (.NO2) or peroxynitrite ($ONOO^-$/ONOOH) oxidize sulhydryl groups to sulfenic acid (**3**), sulfinic acid (**4**), or sulfonic acid (**5**). Sulfenic acid may be subsequently converted to sulphenyl-amide (**6**) if a proximal nitrogen is available. Intermediates such as sulfenic acid may rapidly react to yield inter (**7**) or intra (**8**) molecular disulfides. Recently, Batthyany *et al.* have shown that nitroalkene derived from the reaction of RNS with unsaturated fatty acids reacts with nucleophilic cysteine residues to form nitroalkylated proteins (**9**). In many of these reactions, deprotonated thiols (RS-) rather than the protonated form are usually the reactive species. In the same fashion, ROS such as hydrogen peroxide oxidize cysteine residues in proteins such as protein tyrosine phosphatase (PTP) to intermediates such as sulfenic acid (**3**).

protein partners and would serve to signal and propagate local changes in ROS gradients (31). For example, peroxiredoxin 2 (PRX2) associates with the PDGF receptor in human and mouse VSMCs to regulate the phosphorylation of specific tyrosine residues on the receptor in response to an endogenous source of H_2O_2 (28).

Reactive nitrogen species (RNS)

Reactive nitrogen species (RNS) represent a variety of chemical intermediates among which the prototypical molecule is NO. In general, RNS may be formed through secondary reactions of NO with ROS or as products of enzymatic activities such as peroxidases and the three NOS isozymes (54). The detection of nitrated tyrosine residues has been used in many studies as evidence for RNS formation because the reaction requires peroxynitrite derived from the diffusion-limited reaction of NO with $O_2^{\cdot -}$ (10) or the oxidation of nitrite (NO_2^-) to nitrogen dioxide ($^{\cdot}NO_2^{\cdot}$) by peroxidases (40). Increase immunostaining for nitrotyrosine has been determined in many cardiovascular conditions, including atherosclerosis and restenosis, and in some cases has been localized to smooth muscle cells (11, 123). The daunting task for many of these studies is to understand the functional significance of such modifications under conditions where multiple oxidative, nitrative, and nitrosative hits are likely to occur on the same pool of macromolecules. This is further complicated by the steady increase in the number of modifications that are being characterized, which now include also nitroalkylated and N-nitrosated proteins (Fig. 4) (9, 23).

The S-nitrosation of cysteine residues in proteins is a post-translational modification with important implications regarding cell signaling (160). The term nitrosation is often used interchangeably with "nitrosylation" in reference to other post-translational modifications such as phosphorylation. In many cases, a role for cysteine nitrosation has been demonstrated by mutation of the relevant cysteine residue and evidence of coincidental decrease in nitrosation and protein function or activity. However, absolute quantitation of nitrosated protein levels relative to activity is usually difficult to assess. Most of all, the direct mutation of a cysteine residue does not dissociate the effect of nitrosation from other alternate roles of the residue (electron donation, hydrolysis, or others). To this end, decoupling of the effect of nitrosation may be achieved through mutational modifications of the immediate environment of the cysteine residue rather than the nitrosated cysteine itself (17).

Nitric oxide alone reacts only very slowly with cysteine residues and proposed mechanisms of protein nitrosation include the transnitrosation of cysteine with low molecular weight S-nitrosothiols, the reaction of thiols with dinitrosyl iron or iron–nitrosyl complexes, direct reaction of thiols with NO in the presence of an electron acceptor, copper-catalyzed nitrosation, reaction of thiols with either peroxynitrite, or autoxidation products of NO (59). The reaction of NO with thiols in oxygenated environments does not lead exclusively to the formation of S-nitrosothiols but a large fraction of products may rather represent oxidized forms of cysteine including glutathionylated residues and disulfide bridges (84, 147). Alternatively, RNS and ROS-modified cysteine residues may only serve as transient intermediates as in the case of the regulation of small GTPase proteins including p21(Ras) (140). Finally, the importance of molecular oxygen in promoting nitrosation has been recently challenged by new studies showing that intracellular S-nitrosation is independent of oxygen (19) and that oxygen in fact promotes inactivation of NO through dioxygenation to nitrate (NO_3^-) (62).

Ca^{2+} Signaling Through ROS/RNS

Although little is known concerning the role of ROS/RNS in VSMC Ca^{2+} signaling, an emerging paradigm for the action of ROS/RNS on Ca^{2+} signaling from a variety of cell types seems to involve the inhibition of Ca^{2+} pumps and activation of Ca^{2+} release and entry channels thus increasing the overall cytoplasmic Ca^{2+} concentration (69, 172).

SERCA and PMCA pumps

Through ATP hydrolysis, SERCA and PMCA actively clear Ca^{2+} from the cytoplasm and thus play a key role by maintaining cytoplasmic Ca^{2+} homeostasis. Quiescent VSMC express two splice variants of the SERCA2 gene, 2a and 2b and multiple isoforms of PMCA1 and 4 (3, 76, 170). The role of ROS/RNS in modulating SERCA activity remains controversial. SERCA inhibition *in vitro* through modification of sulfhydryl groups either by ROS or RNS has been reported in several cell types (69). Treatment of coronary arteries denuded of endothelium with peroxynitrite leads to inhibited

contractile responses to angiotensin II or SERCA pump inhibitors such as thapsigargin (57, 58). Treatment of SR vesicles with NO donors and peroxynitrite induces S-nitrosylation of cysteines on SERCA (69). However, earlier reports suggested that NO-mediated SERCA activation in VSMC increases Ca^{2+} uptake into the SR, thus inhibiting store-operated Ca^{2+} entry and causing vasorelaxation (30, 177). An explanation for this apparent discrepancy might be that small concentrations of ROS/RNS are stimulatory while elevated concentrations cause inhibition. Similarly, the PMCA pump has been reported to be reversibly inhibited by H_2O_2, $O_2^{\cdot-}$, $^{\cdot}OH$, and $ONOO^-$/ONOOH (69).

Ca^{2+} release channels: IP_3R and RyR

Several studies reported stimulation of IP_3R-mediated Ca^{2+} release from internal stores in response to ROS (69). The modification of cysteine residues in the IP_3R by the thiol reagent, thimerosal, causes Ca^{2+} spikes through increase IP_3R sensitivity to resting levels of IP_3 (18). Subsequent reports showed that physiological levels of cellular ROS produced through membrane-associated NOXs could increase the sensitivity of the IP_3R to IP_3 (79). Another major Ca^{2+} release channel, RyR, has been shown to be modified by compounds affecting sulfhydryl groups with consequences on channel function (38, 69, 190). RyR isoforms can be activated by S-nitrosylation (163, 192). Exogenous addition of redox compounds as well as endogenous production of ROS/RNS modify RyR on cysteines residues and cause their activation. Experimental evidence in skeletal muscle suggested that Cys3635 is involved in RyR1 redox sensing; the modification of this residue cause RyR1 activation and protection from calmodulin binding-mediated inhibition at high Ca^{2+} concentrations (133, 200). Furthermore, S-nitrosylation of Cys3635-containing the calmodulin binding domain is responsible for RyR1 activation by NO at physiologically relevant oxygen tensions (163). Other studies suggested that endogenous ROS produced *via* NOX enzymes activates Ca^{2+} release through RyR (42).

Ca^{2+} entry channels

While several reports have shown that oxidation increases the activity of voltage-gated Ca^{2+} channels, others reported inhibition of channel activity by oxidizing agents (69). This might be explained by the concentrations of ROS/RNS involved. Alternatively, different ROS/RNS might have different effects on ion transport mechanisms or different cell types might display different sensitivity to various modifiers. In addition to their effects on Ca^{2+} release channels, ROS such as H_2O_2 and $O_2^{\cdot-}$ were shown to stimulate Ca^{2+} entry through L-type and T-type voltage-gated channels in VSMC (167). Other groups reported increased L-type channel activity by ROS (80), and a role of H_2O_2-activated L-type Ca^{2+} channels in mitochondria-derived superoxide production (184). In smooth muscle and cardiac myocytes, NO seems to have an inhibitory effect on L-type Ca^{2+} channels through S-nitrosylation (25, 135). Hool reported that hypoxia in cardiac myocytes can inhibit the basal L-type Ca^{2+} current and increases the sensitivity of this current to beta-adrenergic receptor stimulation (72). The effects of hypoxia could be mimicked by decreasing cellular H_2O_2 with catalase, arguing for a role for ROS in the sensitization of L-type Ca^{2+} channels (73). However, Hudasek *et al.* found a role of endogenous H_2O_2 production in activating L-type Ca^{2+} channels, but failed to detect an effect of either hydrogen peroxide or NOX in the hypoxia-dependent regulation of the these channels (80).

While pharmacological compounds revealed that H_2O_2 induced Ca^{2+} mobilization in pulmonary artery smooth muscle through multiple pathways involving release from the internal stores and entry from the outside (96), information regarding the involvement of ROS/RNS in modulating voltage-independent Ca^{2+} channels such as store-operated and receptor-operated Ca^{2+} channels in different cell types in general and VSMC in particular remains scarce. Studies on TRPC3 and TRPC4 cation channels expressed in HEK293 cells showed they formed redox-sensitive ion channels that could be activated by exogenous application of hydrogen peroxide (5, 56). Whether TRPC channels are directly modified by ROS remains unknown but redox activation of TRPC3 was proposed to occur through ROS-induced tyrosine phosphorylation and activation of PLC (56). More recently, the same group presented evidence for existence of redox-sensitive TRPC3/TRPC4 heteromeric channels in endothelial cells (134). In this study, TRPC3/4 heteromeric channels were activated by cholesterol oxidase, suggesting that ROS activation of TRPC3/4 channels might be mediated by cholesterol oxidation. NO-mediated cysteine nitrosylation have been shown to cause activation of several members of the wider TRP family, TRPC1, TRPC4, TRPC5, TRPV1, TRPV3, and TRPV4 (196). A cytoplasmic Cys553 along with Cys558 in TRPC5 are nitrosylated and native TRPC5 is nitrosylated in response to G-protein coupled receptor stimulation, thus eliciting Ca^{2+} influx in endothelial cells (196).

Hydrogen peroxide was shown to evoke Ca^{2+} entry through activation of the widely expressed melastatin-related TRP channel, TRPM2 (65). Yamamoto *et al.* showed that TRPM2 activation controls ROS-induced chemokine production in monocytes. Hydrogen peroxide-activated Ca^{2+} influx through TRPM2 was essential for downstream signaling and production of interleukin-8. Monocytes from TRPM2-deficient mice showed impaired hydrogen peroxide-activated Ca^{2+} influx and production of the mouse functional homolog of interleukin-8 (193). Based on studies on a splice variant of TRPM2 with a deletion in its C-terminus, which is sensitive to hydrogen peroxide but not to ADP-ribose, Wehage *et al.* proposed that oxidative stress-induced gating of TRPM2 is distinct from that of ADP-ribose (187). However, Perraud *et al.* suggested that oxidative and nitrosative stress-induced TRPM2 activation occurs through stimulation of ADP-ribose production by mitochondria. ADP-ribose interaction within a binding cleft in the C-terminal NUDT9-H domain of TRPM2 would activate the channel (131). Another TRP channel, TRPA1, can be activated by thiol reactive compounds of diverse structure via reversible covalent modification of cysteine residues within the cytoplasmic N terminus of the channel (179). TRPA1 channels were shown to be strongly activated by hypochlorite and H_2O_2 in primary sensory neurons of the airway, suggesting that TRAP1 acts as an oxidant sensor in the airway (15). A recent report demonstrated a role for TRPA1 in mediating airway inflammation and hyperreactivity in asthma (24). Other ion transport mechanisms not discussed here are also targets of ROS/RNS, such Na^+/Ca^{2+} and Na^+/H^+ exchangers, Na^+, Cl^- and K^+ channels, ion co-transporters and other pumps such as the Na^+, K^+ ATPase (69, 90). Modification of the newly discovered components of

the SOC pathway (STIM and Orai) by ROS/RNS has not been reported. Nevertheless, STIM1 proteins possess several cysteines residues that could be targets for modification. Similarly, all three Orai isoforms possess predicted extracellular and intracellular cysteines.

ROS and RNS Signaling Through Regulation of Ca^{+2}-Dependent Effectors: The Example of Ca^{2+}/Calmodulin-Dependent Protein Kinase II

As described in the previous sections, the reciprocal regulation of intracellular Ca^{2+} and ROS/RNS concentrations is an essential integration point of Ca^{2+} and ROS/RNS signaling. It is also evident that many Ca^{2+}-dependent effectors are themselves potential effector molecules for ROS and RNS. This has been already discussed in the context of phosphatases and we argued earlier for an important role for protein kinase C in redox-dependent cytokine signaling in VSMC. Other examples in the context of smooth muscle include ion channels and transporters, transcriptional regulators, and kinases. In this regard, Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) is of particular interest because of its known roles in mediating VSMC signaling pathways and its ability to mediate transcriptional events. Recent evidence would indicate that it is also a direct effector of ROS and RNS. Below, we summarize CaMKII signaling in vascular smooth muscle and detail its regulation by ROS and RNS as a prototypical example of Ca^{2+}-dependent kinase, which activity is also directly impacted by ROS and RNS.

Four CaMKII isoforms (α, β, γ, and δ) have been identified with each isoform having multiple splice variants (171). These isoforms are capable of forming homo-or heteromultimers (155). Recent structural information suggests that CaMKII exists intracellularly as a dodecamer containing two rings of six CaMKII subunits CaMKIIγ and CaMKIIδ are the predominant CaMKII isoforms expressed in VSMC. Along with the dynamic changes in Ca^{2+} handling that occur as VSMC undergo phenotypic change, the content of CaMKII in VSMC also changes. CaMKIIγ is most abundantly expressed in differentiated, contractile VSMC with at least six splice variants having been identified (47, 155). In the synthetic VSMC, which are proliferative and migratory, CaMKIIδ_2 is the major CaMKII isoform expressed. CaMKIIδ_3, a splice variant of CaMKIIδ_2 that localizes to the nucleus has also been identified in synthetic VSMC (149) albeit at much lower expression levels than CaMKIIδ_2. Recently, we reported that the dramatic shift in expression from CaMKIIγ in differentiated VSM to CaMKIIδ in synthetic VSM occurs not only *in vitro* when VSMC are removed from intact blood vessels and cultured but also in response to vascular injury (77). VSMC taken from the neointima of injured carotid arteries were shown to primarily express CaMKIIδ and suppression of CaMKIIδ with shRNA prevented neointimal formation (77). In synthetic VSMC, CaMKIIδ_2 is an important mediator of cell proliferation (75) and migration (107), suggesting that the shift in expression from CaMKIIγ to CaMKIIδ is an important component of a blood vessel's response to injury. Additional functions of CaMKIIδ in VSMC include regulation of

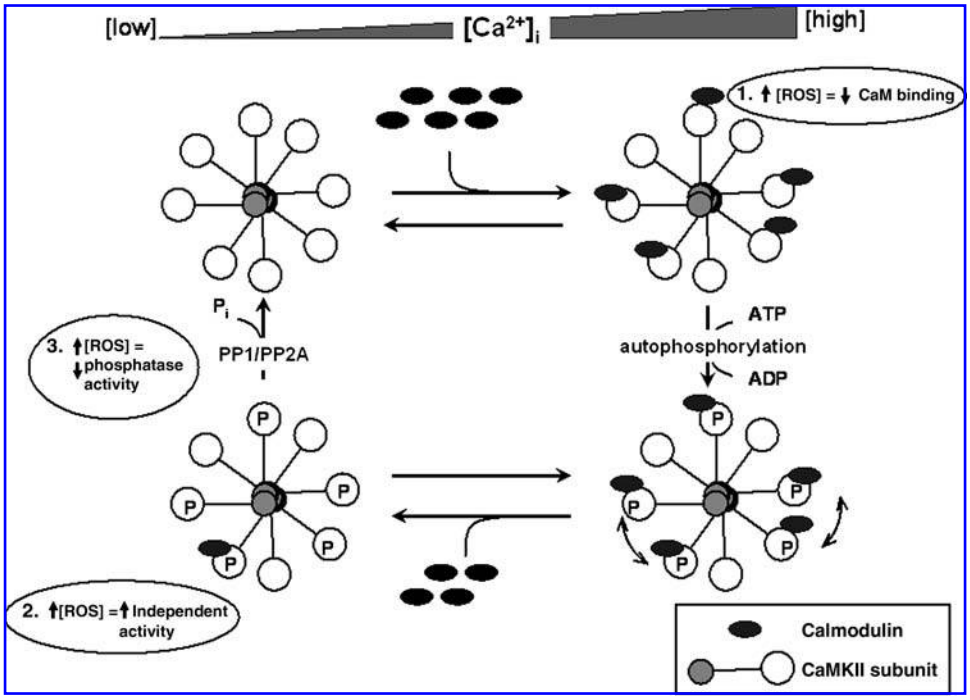

FIG. 5. ROS-dependent regulation of CaMKII activity. Activation of CaMKII is a multistep process involving the binding of Ca^{2+}/CaM that allows for autophosphorylation and intersubunit phosphorylation events. These phosphorylation events are required for the maintenance of CaMKII activity when intracellular Ca^{2+} concentration decreases (independent activity). Phosphatase-dependent dephosphorylation of the kinase results in the inactivation of CaMKII. There are at least three points in CaMKII's activation cycle that are potentially ROS-sensitive. 1. ROS may decrease CaM's affinity for Ca^{2+} which reduces the overall amount of activatable CaMKII. 2. ROS may help maintain CaMKII independent activity by modifying CaMKII's regulatory domain 3. ROS result in the inactivation of PP1/PP2A resulting in sustained CaMKII activity after stimulation or basal activation of CaMKII in the absence of stimulation.

Ca^{2+}-dependent tyrosine kinase activity (52) and G protein coupled receptor (GPCR) - and adhesion-dependent activation of ERK (2, 51, 100).

The activation of CaMKII is multifaceted involving several steps (Fig. 5). Upon association with Ca^{2+}/CaM, CaMKII's autoinhibition is relieved, allowing full catalytic activation. This is followed rapidly by intersubunit, intraholoenzyme autophosphorylation of Threonine 286/287 which markedly increases CaM binding affinity (63) and also enables the retention of kinase activity in the absence of high intracellular Ca^{2+}(64). The multimeric structure of CaMKII allows for inter- and intrasubunit phosphorylations which further contributes to the means that CaMKII can be regulated (64). Serine/threonine phosphatases, most notably PP2A and PP1 (20, 161), are also important components in the regulation of CaMKII activity. Both PP2A and PP1 have the capability to dephosphorylate CaMKII and suppress its activity (161). There is increasing evidence that these phosphatases physically interact with CaMKII and mediate both basal and stimulated CaMKII activity (161). The specific phosphatase that mediates CaMKII may be CaMKII isozyme and cell type dependent.

While it is well-established that regulation of CaMKII activity is dependent upon intracellular Ca^{2+}, there is some evidence that CaMKII activity is also mediated by ROS such as H_2O_2 (118) and NO (158) (Fig. 5). Recent evidence strongly suggests that increases of CaMKII activity can be measured in endothelial cells (118) and cardiomyocytes (191) in response to H_2O_2 and result in CAMKII-dependent activation of p38MAPK, cytoskeletal rearrangements, and abnormal heart function. The mechanisms by which CaMKII is activated in response to increases in ROS such as H_2O_2 have not been completely elucidated. Nor is it clear if ROS other than H_2O_2 are capable of modulating CaMKII. More detailed studies have revealed that CaMKII can be modulated in a direct manner by ROS or indirectly through ROS-dependent regulation of CaM (16) or phosphatase activity (194).

CaMKIIδ contains two methionine residues in its regulatory domain. A study performed in cardiomyocytes demonstrated that these methionine residues are oxidized in response to angiotensin II, and this oxidation enables CaMKIIδ to retain kinase activity in the absence of Ca^{2+}/CaM (41). Interestingly, this study also showed that oxidation of cysteine residues on CaMKII has no impact on CaMKIIδ activity. In rat pituitary tumour GH3 cells, nNOS-dependent increases in NO resulted in the S-nitrosylation of cysteine residues on CaMKIIα suppressing CaMKIIα's activity and function (158). A study done with mouse synaptosomes revealed that CaMKII is oxidized under ischemic conditions and that this oxidation attenuated CaMKII activity by inducing disulfide- and nondisulfide-dependent aggregations (153). Taken together, these reports indicate that CaMKII is susceptible to direct oxidation that has both positive and negative consequences. These studies also indicate that whether methionine or cysteine residues are oxidized and/or which CaMKII isozyme is being affected is important in determining the overall effects of ROS-dependent modification of CaMKII. Because of the paucity of studies examining the consequences of direct oxidation of CaMKII, it is premature to make any generalizations as to its physiological relevance.

CaMKII can also be modulated in an indirect manner in response to increasing ROS concentrations. One interesting way is through the oxidation of CaM (46), a mechanism that is potentially important for ion channels and pumps that are regulated by CaM such as voltage-gated Ca^{2+} channels and TRP channels. The oxidation of methionine residues reduces CaM's ability to bind Ca^{2+} and to interact with its target proteins. Robison *et al.* showed that oxidation of CaM's methionine residues resulted in a decrease of CaMKII activity, reduced CaMKII's ability to interact with cellular receptors, and attenuated CaM's ability to mediate CaMKII's association with cytoskeletal proteins (143). It is interesting to note that while methionine oxidation of CaM may reduce CaMKII activity, methionine oxidation of CaMKII itself enhances CaMKII activity (41). This apparent contradiction further emphasizes the need to more thoroughly examine the relationship between increasing ROS levels and CaMKII activation. Another mean by which ROS may mediate CaMKII is through regulation of phosphatase activity. In Jurkat cells, stimulation with PMA, a potent PKC activator, resulted in the activation of CaMKII without increasing intracellular Ca^{2+}. Catalase, an H_2O_2 scavenger, was able to attenuate this PMA-induced CaMKII activity. Further analysis revealed that stimulation with PMA also resulted in the inactivation of the serine/threonine phosphatase PP2A, leading the authors to conclude that stimulation with PMA increased ROS concentration that resulted in the induced loss of PP2A activity and resultant gain in CaMKII activity (78). Although this is the only report showing that CaMKII may be activated in this manner, several studies have shown that ROS mediate PKC activity through regulation of phosphatase activity (50).

Concluding Remarks

It is evident (and has been known for a long time for NOS) that Ca^{+2} activates various Nox and NOS. An important issue that still needs to be addressed is the molecular identity of the Ca^{+2}-mobilizing stimuli in VSMC that regulate Nox and NOS. In addition, in spite of constant progresses in the identification of specific ROS/RNS intermediates through electron paramagnetic resonance or other analytical approaches, the technology to resolve spatially and temporally intracellular ROS and RNS concentration is still inadequate. This is clearly lagging when compared to the resolution attained with regard to Ca^{+2} imaging of cellular microdomains and sophisticated patch clamp protocols for direct measurements of ion channel conductances. Advancements in ROS/RNS measurements are bound to improve with the development of a new generation of redox sensitive protein-based detectors (37).

As illustrated in this review, recent studies have focused on oxidative and nitrosative modifications of amino acid residues in proteins as a mean to regulate Ca^{2+} signaling. It is important to note that the oxidation of polyunsaturated fatty acids may also represent an important process by which Ca^{2+} signaling may be regulated by ROS. This is because many lipid messengers such as phosphoinositides (PIP, PIP_2, PIP_3, etc.), DAG, and arachidonid acid are important regulators of Ca^{2+} channels and transporters, such that lipid peroxidation may indirectly impact the activity of these proteins. Although the implications of ROS-mediated lipid peroxidation in the context of tissue injury have been documented for some time now (119), evidence for a role for lipid peroxidation in Ca^{2+} signaling remains scarce.

Overall, the mechanism by which specificity is determined through ROS/RNS signaling is still poorly understood, even more so in the context of human diseases. Deregulation of

Ca^{2+}, ROS, and RNS homeostasis are hallmarks of many cardiovascular diseases in which VSMC proliferation is a burden. As such, the processes that result in atherosclerotic and restenosis lesions involve dramatic increases in NOX and NOS activities and changes in Ca^{2+} handling and effectors in smooth muscle. We have illustrated potential direct effects of ROS/RNS on Ca^{2+} release and entry channels, transporters, and effectors such as CaMKII. An intriguing possibility is the role that ROS and RNS may play in processes of neointimal formation that depend on increased Ca^{2+} concentrations and activation of effector molecules controlling VSMC proliferation and migration. Although not yet reported, it is certainly reasonable to think that in these ROS/RNS rich environments, certain ion channels along with downstream effectors such as CaMKII might be enriched and where their activity and function may be dramatically affected.

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References

1. Abdullaev IF, Bisaillon JM, Potier M, Gonzalez JC, Motiani RK, and Trebak M. Stim1 and Orai1 mediate CRAC currents and store-operated calcium entry important for endothelial cell proliferation. *Circ Res* 103: 1289–1299, 2008.
2. Abraham ST, Benscoter HA, Schworer CM, and Singer HA. A role for CaM kinase II in the MAP kinase signaling cascade in cultured rat aortic vascular smooth muscle cells. *Circ Res* 81: 575–584, 1997.
3. Abramowitz J, Ydemir–Koksoy A, Helgason T, Jemelka S, Odebunmi T, Seidel CL, and Allen JC. Expression of plasma membrane calcium ATPases in phenotypically distinct canine vascular smooth muscle cells. *J Mol Cell Cardio* 32: 777–789, 2000.
4. Aubart FC, Sassi Y, Coulombe A, Mougenot N, Vrignaud C, Leprince P, Lechat P, Lompre AM, and Hulot JS. RNA interference targeting STIM1 suppresses vascular smooth muscle cell proliferation and neointima formation in the rat. *Mol Ther* 17: 455–462, 2009.
5. Balzer M, Lintschinger B, and Groschner K. Evidence for a role of Trp proteins in the oxidative stress-induced membrane conductances of porcine aortic endothelial cells. *Cardiovasc Res* 42: 543–549, 1999.
6. Banfi B, Tirone F, Durussel I, Knisz J, Moskwa P, Molnar GZ, Krause KH, and Cox JA. Mechanism of Ca2+ activation of the NADPH oxidase 5 (NOX5). *J Biol Chem* 279: 18583–18591, 2004.
7. Barritt GJ. Receptor-activated Ca2+ inflow in animal cells: A variety of pathways tailored to meet different intracellular Ca2+ signalling requirements. *Biochem J* 337: 153–169, 1999.
8. Barry-Lane PA, Patterson C, van der Merwe M, Hu Z, Holland SM, Yeh ETH, and Runge MS. p47phox is required for atherosclerotic lesion progression in ApoE−/− mice. *J Clin Invest* 108: 1513–1522, 2001.
9. Batthyany C, Schopfer FJ, Baker PR, Duran R, Baker LM, Huang Y, Cervenansky C, Branchaud BP, and Freeman BA. Reversible post-translational modification of proteins by nitrated fatty acids *in vivo*. *J Biol Chem* 281: 20450–20463, 2006.
10. Beckman JS and Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: The good, the bad, and the ugly. *Am J Physiol* 271: C1424–C1437, 1996.
11. Beller CJ, Radovits T, Kosse J, Gero D, Szabo C, and Szabo G. Activation of the peroxynitrite-poly(adenosine diphosphate-ribose) polymerase pathway during neointima proliferation: A new target to prevent restenosis after endarterectomy. *J Vasc Surg* 43: 824–830, 2006.
12. Bergdahl A, Gomez MF, Wihlborg AK, Erlinge D, Eyjolfson A, Xu SZ, Beech DJ, Dreja K, and Hellstrand P. Plasticity of TRPC expression in arterial smooth muscle: Correlation with store-operated Ca2+ entry. *Am J Physiol* 288: C872–C880, 2005.
13. Berra-Romani R, Mazzocco-Spezzia A, Pulina MV, and Golovina VA. Ca2+ handling is altered when arterial myocytes progress from a contractile to a proliferative phenotype in culture. *Am J of Physiol* 295: C779–C790, 2008.
14. Berridge MJ. Smooth muscle cell calcium activation mechanisms. *J Physiol* 586: 5047–5061, 2008.
15. Bessac BF, Sivula M, von Hehn CA, Escalera J, Cohn L, and Jordt SE. TRPA1 is a major oxidant sensor in murine airway sensory neurons. *J Clin Invest* 118: 1899–1910, 2008.
16. Bigelow DJ and Squier TC. Redox modulation of cellular signaling and metabolism through reversible oxidation of methionine sensors in calcium regulatory proteins. *Biochim Biophys Acta* 1703: 121–134, 2005.
17. Blackinton J, Lakshminarasimhan M, Thomas KJ, Ahmad R, Greggio E, Raza AS, Cookson MR, and Wilson MA. Formation of a stabilized cysteine sulfinic acid is critical for the mitochondrial function of the parkinsonism protein DJ-1. *J Biol Chem* 284: 6476–6485, 2009.
18. Bootman MD, Taylor CW, and Berridge MJ. The thiol reagent, thimerosal, evokes Ca2+ spikes in HeLa cells by sensitizing the inositol 1,4,5-trisphosphate receptor. *J Biol Chem* 267: 25113–25119, 1992.
19. Bosworth CA, Toledo JC, Jr., Zmijewski JW, Li Q, and Lancaster JR, Jr. Dinitrosyliron complexes and the mechanism(s) of cellular protein nitrosothiol formation from nitric oxide. *Proc Natl Acad Sci USA* 106: 4671–4676, 2009.
20. Bradshaw JM, Kubota Y, Meyer T, and Schulman H. An ultrasensitive Ca2+/calmodulin-dependent protein kinase II-protein phosphatase 1 switch facilitates specificity in postsynaptic calcium signaling. *Proc Natl Acad Sci USA* 100: 10512–10517, 2003.
21. Brandman O, Liou J, Park WS, and Meyer T. STIM2 is a feedback regulator that stabilizes basal cytosolic and endoplasmic reticulum Ca2+ levels. *Cell* 131: 1327–1339, 2007.
22. Brueggemann LI, Markun DR, Henderson KK, Cribbs LL, and Byron KL. Pharmacological and electrophysiological characterization of store-operated currents and capacitative Ca(2+) entry in vascular smooth muscle cells. *J Pharm Exp Ther* 317: 488–499, 2006.
23. Bryan NS, Rassaf T, Maloney RE, Rodriguez CM, Saijo F, Rodriguez JR, and Feelisch M. Cellular targets and mechanisms of nitros(yl)ation: An insight into their nature and kinetics *in vivo*. *Proc Natl Acad Sci USA* 101: 4308–4313, 2004.
24. Caceres AI, Brackmann M, Elia MD, Bessac BF, Del CD, D'Amours M, Witek JS, Fanger CM, Chong JA, Hayward NJ, Homer RJ, Cohn L, Huang X, Moran MM, and Jordt SE. A sensory neuronal ion channel essential for airway inflammation and hyperreactivity in asthma. *Pro Natl Acad Sci USA* 106: 9099–9104, 2009.

25. Campbell DL, Stamler JS, and Strauss HC. Redox modulation of L-type calcium channels in ferret ventricular myocytes. Dual mechanism regulation by nitric oxide and S-nitrosothiols. *J Gen Physiol* 108: 277–293, 1996.
26. Chamley-Campbell J, Campbell GR, and Ross R. The smooth muscle cell in culture. *Physiol Rev* 59: 1–61, 1979.
27. Chen K, Kirber MT, Xiao H, Yang Y, and Keaney JF, Jr. Regulation of ROS signal transduction by NADPH oxidase 4 localization. *J Cell Biol* 181: 1129–1139, 2008.
28. Choi MH, Lee IK, Kim GW, Kim BU, Han YH, Yu DY, Park HS, Kim KY, Lee JS, Choi C, Bae YS, Lee BI, Rhee SG, and Kang SW. Regulation of PDGF signalling and vascular remodelling by peroxiredoxin II. *Nature* 435: 347–353, 2005.
29. Clempus RE, Sorescu D, Dikalova AE, Pounkova L, Jo P, Sorescu GP, Lassegue B, and Griendling KK. Nox4 is required for maintenance of the differentiated vascular smooth muscle cell phenotype. *Arterioscler Thromb Vasc Biol* 27: 42–48, 2007.
30. Cohen RA, Weisbrod RM, Gericke M, Yaghoubi M, Bierl C, and Bolotina VM. Mechanism of nitric oxide-induced vasodilatation: Refilling of intracellular stores by sarcoplasmic reticulum Ca2+ ATPase and inhibition of store-operated Ca2+ influx. *Circ Res* 84: 210–219, 1999.
31. D'Autreaux B and Toledano MB. ROS as signalling molecules: Mechanisms that generate specificity in ROS homeostasis. *Nat Rev Mol Cell Biol* 8: 813–824, 2007.
32. DeHaven WI, Smyth JT, Boyles RR, and Putney JW, Jr. Calcium inhibition and calcium potentiation of Orai1, Orai2, and Orai3 calcium release-activated calcium channels. *J Biol Chem* 282: 17548–17556, 2007.
33. Denu JM and Dixon JE. Protein tyrosine phosphatases: Mechanisms of catalysis and regulation. *Curr Opin Chem Biol* 2: 633–641, 1998.
34. Dietrich A, Kalwa H, Storch U, Schnitzler M, Salanova B, Pinkenburg O, Dubrovska G, Essin K, Gollasch M, Birnbaumer L, and Gudermann T. Pressure-induced and store-operated cation influx in vascular smooth muscle cells is independent of TRPC1. *Pflugers Arch* 455: 465–477, 2007.
35. Dikalov SI, Dikalova AE, Bikineyeva AT, Schmidt HHHW, Harrison DG, and Griendling KK. Distinct roles of Nox1 and Nox4 in basal and angiotensin II-stimulated superoxide and hydrogen peroxide production. *Free Radic Biol Med* 45: 1340–1351, 2008.
36. Dikalova A, Clempus R, Lassegue B, Cheng G, McCoy J, Dikalov S, San MA, Lyle A, Weber DS, Weiss D, Taylor WR, Schmidt HH, Owens GK, Lambeth JD, and Griendling KK. Nox1 overexpression potentiates angiotensin II-induced hypertension and vascular smooth muscle hypertrophy in transgenic mice. *Circulation* 112: 2668–2676, 2005.
37. Dooley CT, Dore TM, Hanson GT, Jackson WC, Remington SJ, and Tsien RY. Imaging dynamic redox changes in mammalian cells with green fluorescent protein indicators. *J Biol Chem* 279: 22284–22293, 2004.
38. Du W, Frazier M, McMahon TJ, and Eu JP. Redox activation of intracellular calcium release channels (ryanodine receptors) in the sustained phase of hypoxia-induced pulmonary vasoconstriction. *Chest* 128: 556S–558S, 2005.
39. Eder P, Probst D, Rosker C, Poteser M, Wolinski H, Kohlwein SD, Romanin C, and Groschner K. Phospholipase C-dependent control of cardiac calcium homeostasis involves a TRPC3-NCX1 signaling complex. *Cardiovasc Res* 73: 111–119, 2007.
40. Eiserich JP, Baldus S, Brennan ML, Ma W, Zhang C, Tousson A, Castro L, Lusis AJ, Nauseef WM, White CR, and Freeman BA. Myeloperoxidase, a leukocyte-derived vascular NO oxidase. *Science* 296: 2391–2394, 2002.
41. Erickson JR, Joiner ML, Guan X, Kutschke W, Yang J, Oddis CV, Bartlett RK, Lowe JS, O'Donnell SE, Aykin-Burns N, Zimmerman MC, Zimmerman K, Ham AJ, Weiss RM, Spitz DR, Shea MA, Colbran RJ, Mohler PJ, and Anderson ME. A dynamic pathway for calcium-independent activation of CaMKII by methionine oxidation. *Cell* 133: 462–474, 2008.
42. Espinosa A, Leiva A, Pena M, Muller M, Debandi A, Hidalgo C, Carrasco MA, and Jaimovich E. Myotube depolarization generates reactive oxygen species through NAD(P)H oxidase; ROS-elicited Ca2+ stimulates ERK, CREB, early genes. *J Cell Physiol* 209: 379–388, 2006.
43. Feske S, Gwack Y, Prakriya M, Srikanth S, Puppel SH, Tanasa B, Hogan PG, Lewis RS, Daly M, and Rao A. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature* 441: 179–185, 2006.
44. Finegold AA, Shatwell KP, Segal AW, Klausner RD, and Dancis A. Intramembrane bis-heme motif for transmembrane electron transport conserved in a yeast iron reductase and the human NADPH oxidase. *J Biol Chem* 271: 31021–31024, 1996.
45. Firth AL, Remillard CV, and Yuan JX. TRP channels in hypertension. *Biochim Biophys Acta* 1772: 895–906, 2007.
46. Franklin RA, Rodriguez–Mora OG, Lahair MM, and McCubrey JA. Activation of the calcium/calmodulin-dependent protein kinases as a consequence of oxidative stress. *Antioxid Redox Signal* 8: 1807–1817, 2006.
47. Gangopadhyay SS, Barber AL, Gallant C, Grabarek Z, Smith JL, and Morgan KG. Differential functional properties of calmodulin-dependent protein kinase IIgamma variants isolated from smooth muscle. *Biochem J* 372: 347–357, 2003.
48. Giachini FR, Chiao CW, Carneiro FS, Lima VV, Carneiro ZN, Dorrance AM, Tostes RC, and Webb RC. Increased activation of stromal interaction molecule-1/Orai-1 in aorta from hypertensive rats: A novel insight into vascular dysfunction. *Hypertension* 53: 409–416, 2009.
49. Giles NM, Giles GI, and Jacob C. Multiple roles of cysteine in biocatalysis. *Biochem Biophys Res Commun* 300: 1–4, 2003.
50. Ginnan R, Guikema BJ, Halligan KE, Singer HA, and Jourd'heuil D. Regulation of smooth muscle by inducible nitric oxide synthase and NADPH oxidase in vascular proliferative diseases. *Free Radic Biol Med* 44: 1232–1245, 2008.
51. Ginnan R, Pfleiderer PJ, Pumglia K, and Singer HA. PKCδ and CaMKIIδ mediate ATP-dependent activation of ERK1/2 in vascular smooth muscle. *Am J Physiol* 286: C1281–C1289, 2004.
52. Ginnan R and Singer HA. CaM kinase II-dependent activation of tyrosine kinases and ERK1/2 in vascular smooth muscle. *Am J Physiol* 282: C754–C761, 2002.
53. Golovina VA, Platoshyn O, Bailey CL, Wang J, Limsuwan A, Sweeney M, Rubin LJ, and Yuan JX. Upregulated TRP and enhanced capacitative Ca(2+) entry in human pulmonary artery myocytes during proliferation. *Am J Physiol* 280: H746–H755, 2001.
54. Grisham MB, Jourd'heuil D, and Wink DA. Nitric oxide. I. Physiological chemistry of nitric oxide and its metaolites: implication in inflammation. *Am J Physiol* 39: G315–G321, 1999.

55. Gros R, Afroze T, You XM, Kabir G, Van WR, Kalair W, Hoque AE, Mungrue IN, and Husain M. Plasma membrane calcium ATPase overexpression in arterial smooth muscle increases vasomotor responsiveness and blood pressure. *Circ Res* 93: 614–621, 2003.
56. Groschner K, Rosker C, and Lukas M. Role of TRP channels in oxidative stress. *Novartis Found Symp* 258: 222–230, 2004.
57. Grover AK, Kwan CY, and Samson SE. Effects of peroxynitrite on sarco/endoplasmic reticulum Ca2+ pump isoforms SERCA2b and SERCA3a. *Am J Physiol* 285: C1537–C1543, 2003.
58. Grover AK, Samson SE, Robinson S, and Kwan CY. Effects of peroxynitrite on sarcoplasmic reticulum Ca2+ pump in pig coronary artery smooth muscle. *Am J Physiol* 284: C294–C301, 2003.
59. Guikema B, Lu Q, and Jourd'heuil D. Chemical considerations and biological selectivity of protein nitrosation: Implications for NO-mediated signal transduction. *Antioxid Redox Signal* 7: 593–606, 2005.
60. Guo RW, Wang H, Gao P, Li MQ, Zeng CY, Yu Y, Chen JF, Song MB, Shi YK, and Huang L. An essential role for STIM1 in neointima formation following arterial injury. *Cardiovasc Res* 81: 660–668, 2009.
61. Guzik TJ, Chen W, Gongora MC, Guzik B, Lob HE, Mangalat D, Hoch N, Dikalov S, Rudzinski P, Kapelak B, Sadowski J, and Harrison DG. Calcium-dependent NOX5 nicotinamide adenine dinucleotide phosphate oxidase contributes to vascular oxidative stress in human coronary artery disease. *J Am Coll Cardiol* 52: 1803–1809, 2008.
62. Halligan KE, Jourd'heuil FL, and Jourd'heuil D. Cytoglobin is expressed in the vasculature and regulates cell respiration and proliferation via nitric oxide dioxygenation. *J Biol Chem* 284: 8539–8547, 2009.
63. Hanson PI, Kapiloff MS, Lou LL, Rosenfield MG, and Schulman H. Expression of a multifunctional Ca^{2+}/calmodulin-dependent protein kinase and mutational analysis of its autoregulation. *Neuron* 3: 59–70, 1989.
64. Hanson PI, Meyer T, Stryer L, and Schulman H. Dual role of calmodulin in autophosphorylation of multifunctional CaM kinase may underlie decoding of calcium signals. *Neuron* 12: 943–956, 1994.
65. Hara Y, Wakamori M, Ishii M, Maeno E, Nishida M, Yoshida T, Yamada H, Shimizu S, Mori E, Kudoh J, Shimizu N, Kurose H, Okada Y, Imoto K, and Mori Y. LTRPC2 Ca2+-permeable channel activated by changes in redox status confers susceptibility to cell death. *Mol Cell* 9: 163–173, 2002.
66. Harraz MM, Marden JJ, Zhou W, Zhang Y, Williams A, Sharov VS, Nelson K, Luo M, Paulson H, Schoneich C, and Engelhardt J. SOD1 mutations disrupt redox-sensitive Rac regulation of NADPH oxidase in a familial ALS model. *J Clin Invest* 118: 659–670, 2008.
67. Hartmann J, Dragicevic E, Adelsberger H, Henning HA, Sumser M, Abramowitz J, Blum R, Dietrich A, Freichel M, Flockerzi V, Birnbaumer L, and Konnerth A. TRPC3 channels are required for synaptic transmission and motor coordination. *Neuron* 59: 392–398, 2008.
68. Hewavitharana T, Deng X, Soboloff J, and Gill DL. Role of STIM and Orai proteins in the store-operated calcium signaling pathway. *Cell Calcium* 42: 173–182, 2007.
69. Hidalgo C and Donoso P. Crosstalk between calcium and redox signaling: From molecular mechanisms to health implications. *Antioxid Redox Signal* 10: 1275–1312, 2008.
70. Hilenski LL, Clempus RE, Quinn MT, Lambeth JD, and Griendling KK. Distinct subcellular localizations of Nox1 and Nox4 in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 24: 677–683, 2004.
71. Hofmann T, Obukhov AG, Schaefer M, Harteneck C, Gudermann T, and Schultz G. Direct activation of human TRPC6 and TRPC3 channels by diacylglycerol. *Nature* 397: 259–263, 1999.
72. Hool LC. Hypoxia increases the sensitivity of the L-type Ca(2+) current to beta-adrenergic receptor stimulation via a C2 region-containing protein kinase C isoform. *Circ Res* 87: 1164–1171, 2000.
73. Hool LC and Arthur PG. Decreasing cellular hydrogen peroxide with catalase mimics the effects of hypoxia on the sensitivity of the L-type Ca2+ channel to beta-adrenergic receptor stimulation in cardiac myocytes. *Circ Res* 91: 601–609, 2002.
74. Hoth M and Penner R. Depletion of intracellular calcium stores activates a calcium current in mast cells. *Nature* 355: 353–356, 1992.
75. House SJ, Ginnan RG, Armstrong SE, and Singer HA. Calcium/calmodulin-dependent protein kinase II-delta isoform regulation of vascular smooth muscle cell proliferation. *Am J Physiol* 292: C2276–C2287, 2007.
76. House SJ, Potier M, Bisaillon J, Singer HA, and Trebak M. The non-excitable smooth muscle: calcium signaling and phenotypic switching during vascular disease. *Pflugers Arch* 456: 769–785, 2008.
77. House SJ and Singer HA. CaMKII-delta isoform regulation of neointima formation after vascular injury. *Arterioscler Thromb Vasc Biol* 28: 441–447, 2008.
78. Howe CJ, Lahair MM, McCubrey JA, and Franklin RA. Redox regulation of the calcium/calmodulin-dependent protein kinases. *J Biol Chem* 279: 44573–44581, 2004.
79. Hu Q, Zheng G, Zweier JL, Deshpande S, Irani K, and Ziegelstein RC. NADPH oxidase activation increases the sensitivity of intracellular Ca2+ stores to inositol 1,4,5-trisphosphate in human endothelial cells. *J Biol Chem* 275: 15749–15757, 2000.
80. Hudasek K, Brown ST, and Fearon IM. H2O2 regulates recombinant Ca2+ channel alpha1C subunits but does not mediate their sensitivity to acute hypoxia. *Biochem Biophys Res Comm* 318: 135–141, 2004.
81. Inoue R, Okada T, Onoue H, Hara Y, Shimizu S, Naitoh S, Ito Y, and Mori Y. 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: 325–332, 2001.
82. Jagnandan D, Church JE, Banfi B, Stuehr DJ, Marrero MB, and Fulton DJ. Novel mechanism of activation of NADPH oxidase 5. calcium sensitization via phosphorylation. *J Biol Chem* 282: 6494–6507, 2007.
83. Jay DB, Papaharalambus CA, Seidel-Rogol B, Dikalova AE, Lassegue B, and Griendling KK. Nox5 mediates PDGF-induced proliferation in human aortic smooth muscle cells. *Free Radic Biol Med* 45: 329–335, 2008.
84. Jourd'heuil D, Jourd'heuil FL, and Feelisch M. Oxidation and nitrosation of thiols at low micromolar exposure to nitric oxide. Evidence for a free radical mechanism. *J Biol Chem* 278: 15720–15726, 2003.
85. Kawahara T, Ritsick D, Cheng G, and Lambeth JD. Point mutations in the proline-rich region of p22phox are dominant inhibitors of Nox1- and Nox2-dependent reactive oxygen generation. *J Biol Chem* 280: 31859–31869, 2005.

86. Kourie JI. Interaction of reactive oxygen species with ion transport mechanisms. *Am J Physiol* 275: C1–24, 1998.
87. Kubes P, Suzuki M, and Granger DN. Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proc Natl Acad Sci USA* 88: 4651–4655, 1991.
88. Kuhlencordt PJ, Hotten S, Schodel J, Rutzel S, Hu K, Widder J, Marx A, Huang PL, and Ertl G. Atheroprotective effects of neuronal nitric oxide synthase in apolipoprotein e knockout mice. *Arterioscler Thromb Vasc Biol* 26: 1539–1544, 2006.
89. Kumar B, Dreja K, Shah SS, Cheong A, Xu SZ, Sukumar P, Naylor J, Forte A, Cipollaro M, McHugh D, Kingston PA, Heagerty AM, Munsch CM, Bergdahl A, Hultgardh–Nilsson A, Gomez MF, Porter KE, Hellstrand P, and Beech DJ. Upregulated TRPC1 channel in vascular injury *in vivo* and its role in human neointimal hyperplasia. *Circ Res* 98: 557–563, 2006.
90. Kwan HY, Huang Y, and Yao X. Regulation of canonical transient receptor potential isoform 3 (TRPC3) channel by protein kinase G. *Proc Natl Acad Sci USA* 101: 2625–2630, 2004.
91. Lambeth JD. NOX enzymes and the biology of reactive oxygen. *Nat Rev Immunol* 4: 181–189, 2004.
92. Lambeth JD, Kawahara T, and Diebold B. Regulation of Nox and Duox enzymatic activity and expression. *Free Radic Biol Med* 43: 319–331, 2007.
93. Lee MY, Martin AS, Mehta PK, Dikalova AE, Garrido AM, Datla SR, Lyons E, Krause KH, Banfi B, Lambeth JD, Lassegue B, and Griendling KK. Mechanisms of vascular smooth muscle NADPH oxidase 1 (Nox1) contribution to injury-induced neointimal formation. *Arterioscler Thromb Vasc Biol* 29: 480–487, 2009.
94. Li Q, Harraz MM, Zhou W, Zhang LN, Ding W, Zhang Y, Eggleston T, Yeaman C, Banfi B, and Engelhardt JF. Nox2 and Rac1 regulate H2O2-dependent recruitment of TRAF6 to endosomal interleukin-1 receptor complexes. *Mol Cell Biol* 26: 140–154, 2006.
95. Lievremont JP, Bird GS, and Putney JW, Jr. Mechanism of inhibition of TRPC cation channels by 2-aminoethoxydiphenylborane. *Mol Pharmacol* 68: 758–762, 2005.
96. Lin MJ, Yang XR, Cao YN, and Sham JS. Hydrogen peroxide-induced Ca2+ mobilization in pulmonary arterial smooth muscle cells. *Am J Physiol* 292: L1598–L1608, 2007.
97. Lincoln TM, Dey N, and Sellak H. Signal transduction in smooth muscle: Invited Review: cGMP-dependent protein kinase signaling mechanisms in smooth muscle: From the regulation of tone to gene expression. *J Appl Physiol* 91: 1421–1430, 2001.
98. Liou J, Kim ML, Heo WD, Jones JT, Myers JW, Ferrell JE, Jr., and Meyer T. STIM is a Ca2+ sensor essential for Ca2+-store-depletion-triggered Ca2+ influx. *Curr Biol* 15: 1235–1241, 2005.
99. Lis A, Peinelt C, Beck A, Parvez S, Monteilh-Zoller M, Fleig A, and Penner R. CRACM1, CRACM2, and CRACM3 are store-operated Ca2+ channels with distinct functional properties. *Curr Biol* 17: 794–800, 2007.
100. Lu KK, Armstrong SE, Ginnan R, and Singer HA. Adhesion-dependent activation of CaMKII and regulation of ERK activation in vascular smooth muscle. *Am J Physiol* 289: C1343–C1350, 2005.
101. Lyle AN, Deshpande NN, Taniyama Y, Seidel-Rogol B, Pounkova L, Du P, Papaharalambus C, Lassegue B, and Griendling KK. Poldip2, a novel regulator of Nox4 and cytoskeletal integrity in vascular smooth muscle cells. *Circ Res* 105: 249–259, 2009.
102. Martyn KD, Frederick LM, von Loehneysen K, Dinauer MC, and Knaus UG. Functional analysis of Nox4 reveals unique characteristics compared to other NADPH oxidases. *Cell Sig* 18: 69–82, 2006.
103. Maruyama Y, Nakanishi Y, Walsh EJ, Wilson DP, Welsh DG, and Cole WC. Heteromultimeric TRPC6-TRPC7 channels contribute to arginine vasopressin-induced cation current of A7r5 vascular smooth muscle cells. *Circ Res* 98: 1520–1527, 2006.
104. Matsuno K, Yamada H, Iwata K, Jin D, Katsuyama M, Matsuki M, Takai S, Yamanishi K, Miyazaki M, Matsubara H, and Yabe-Nishimura C. Nox1 is involved in angiotensin II-mediated hypertension: A study in Nox1-deficient mice. *Circulation* 112: 2677–2685, 2005.
105. McCrann DJ, Yang D, Chen H, Carroll S, and Ravid K. Upregulation of Nox4 in the aging vasculature and its association with smooth muscle cell polyploidy. *Cell Cycle* 8: 902–908, 2009.
106. Meng TC, Fukada T, and Tonks NK. Reversible oxidation and inactivation of protein tyrosine phosphatases *in vivo*. *Mol Cell* 9: 387–399, 2002.
107. Mercure MZ, Ginnan R, and Singer HA. CaM kinase II delta2-dependent regulation of vascular smooth muscle cell polarization and migration. *Am J Physiol* 294: C1465–C1475, 2008.
108. Mignen O, Thompson JL, and Shuttleworth TJ. STIM1 regulates Ca2+ entry via arachidonate-regulated Ca2+-selective (ARC) channels without store depletion or translocation to the plasma membrane. *J Physiol* 579: 703–715, 2007.
109. Mignen O, Thompson JL and Shuttleworth TJ. Both Orai1 and Orai3 are essential components of the arachidonate-regulated Ca2+-selective (ARC) channels. *J Physiol* 586: 185–195, 2008.
110. Miller FJ Jr., Filali M, Huss GJ, Stanic B, Chamseddine A, Barna TJ, and Lamb FS. Cytokine activation of nuclear factor {kappa}B in vascular smooth muscle cells requires signaling endosomes containing Nox1 and ClC-3. *Circ Res* 101: 663–671, 2007.
111. Moens AL and Kass DA. Tetrahydrobiopterin and cardiovascular disease. *Arterioscler Thromb Vasc Biol* 26: 2439–2444, 2006.
112. Moncada S, Palmer RMJ, and Higgs EA. Nitric oxide: Physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 43: 109–142, 1991.
113. Morishita T, Tsutsui M, Shimokawa H, Horiuchi M, Tanimoto A, Suda O, Tasaki H, Huang PL, Sasaguri Y, Yanagihara N, and Nakashima Y. Vasculoprotective roles of neuronal nitric oxide synthase. *FASEB J* 02: 0155, 2002.
114. Muik M, Fahrner M, Derler I, Schindl R, Bergsmann J, Frischauf I, Groschner K, and Romanin C. A cytosolic homomerization and a modulatory domain within STIM1 C-terminus determine coupling to ORAI1 channels. *J Biol Chem* 284: 8421–8426, 2009.
115. Nakata S, Tsutsui M, Shimokawa H, Tamura M, Tasaki H, Morishita T, Suda O, Ueno S, Toyohira Y, Nakashima Y, and Yanagihara N. Vascular neuronal NO synthase is selectively upregulated by platelet-derived growth factor: Involvement of the MEK/ERK pathway. *Arterioscler Thromb Vasc Biol* 25: 2502–2508, 2005.

116. Nakata S, Tsutsui M, Shimokawa H, Yamashita T, Tanimoto A, Tasaki H, Ozumi K, Sabanai K, Morishita T, Suda O, Hirano H, Sasaguri Y, Nakashima Y, and Yanagihara N. Statin treatment upregulates vascular neuronal nitric oxide synthase through Akt/NF-kappaB pathway. *Arterioscler Thromb Vasc Biol* 27: 92–98, 2007.
117. Nelson MT, Cheng H, Rubart M, Santana LF, Bonev AD, Knot HJ, and Lederer WJ. Relaxation of arterial smooth muscle by calcium sparks. *Science* 270: 633–637, 1995.
118. Nguyen A, Chen P and Cai H. Role of CaMKII in hydrogen peroxide activation of ERK1/2, p38 MAPK, HSP27 and actin reorganization in endothelial cells. *FEBS Lett* 572: 307–313, 2004.
119. O'Donnell VB and Freeman BA. Interactions between nitric oxide and lipid oxidation pathways: Implications for vascular disease. *Circ Res* 88: 12–21, 2001.
120. Okada T, Inoue R, Yamazaki K, Maeda A, Kurosaki T, Yamakuni T, Tanaka I, Shimizu S, Ikenaka K, Imoto K, and Mori Y. 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: 27359–27370, 1999.
121. Owens GK. Molecular control of vascular smooth muscle cell differentiation and phenotypic plasticity. *Novartis Found Symp* 283: 174–191, 2007.
122. Papadaki M, Tilton RG, Eskin SG, and McIntire LV. Nitric oxide production by cultured human aortic smooth muscle cells: Stimulation by fluid flow. *Am J Physiol* 274: H616–H626, 1998.
123. Parastatidis I, Thomson L, Fries DM, Moore RE, Tohyama J, Fu X, Hazen SL, Heijnen HF, Dennehy MK, Liebler DC, Rader DJ, and Ischiropoulos H. Increased protein nitration burden in the atherosclerotic lesions and plasma of apolipoprotein A-I deficient mice. *Circ Res* 101: 368–376, 2007.
124. Parekh AB and Putney JW, Jr. Store-operated calcium channels. *Physiol Rev* 85: 757–810, 2005.
125. Park CY, Hoover PJ, Mullins FM, Bachhawat P, Covington ED, Raunser S, Walz T, Garcia KC, Dolmetsch RE, and Lewis RS. STIM1 clusters and activates CRAC channels via direct binding of a cytosolic domain to Orai1. *Cell* 136: 876–890, 2009.
126. Patel DN, Bailey SR, Gresham JK, Schuchman DB, Shelhamer JH, Goldstein BJ, Foxwell BM, Stemerman MB, Maranchie JK, and Valente AJ. TLR4-NOX4-AP-1 signaling mediates lipopolysaccharide-induced CXCR6 expression in human aortic smooth muscle cells. *Biochem Biophys Res Commun* 347: 1113–1120, 2006.
127. Pedruzzi E, Guichard C, Ollivier V, Driss F, Fay M, Prunet C, Marie JC, Pouzet C, Samadi M, Elbim C, O'Dowd Y, Bens M, Vandewalle A, Gougerot-Pocidalo MA, Lizard G, and Ogier-Denis E. NAD(P)H oxidase Nox-4 mediates 7-ketocholesterol-induced endoplasmic reticulum stress and apoptosis in human aortic smooth muscle cells. *Mol Cell Biol* 24: 10703–10717, 2004.
128. Peel SE, Liu B, and Hall IP. A key role for STIM1 in store operated calcium channel activation in airway smooth muscle. *Respir Res* 7: 119, 2006.
129. Peel SE, Liu B, and Hall IP. ORAI and store-operated calcium influx in human airway smooth muscle cells. *Am J Resp Cell and Mol Biology* 38: 744–749, 2008.
130. Perez JF and Sanderson MJ. The frequency of calcium oscillations induced by 5-HT, ACH, and KCl determine the contraction of smooth muscle cells of intrapulmonary bronchioles. *J Gen Physiol* 125: 535–553, 2005.
131. Perraud AL, Takanishi CL, Shen B, Kang S, Smith MK, Schmitz C, Knowles HM, Ferraris D, Li W, Zhang J, Stoddard BL, and Scharenberg AM. Accumulation of free ADP-ribose from mitochondria mediates oxidative stress-induced gating of TRPM2 cation channels. *J Biol Chem* 280: 6138–6148, 2005.
132. Poburko D, Liao CH, Lemos VS, Lin E, Maruyama Y, Cole WC, and van Breemen C. 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: 1030–1038, 2007.
133. Porter Moore C, Zhang JZ, and Hamilton SL. A role for cysteine 3635 of RYR1 in redox modulation and calmodulin binding. *J Biol Chem* 274: 36831–36834, 1999.
134. Poteser M, Graziani A, Rosker C, Eder P, Derler I, Kahr H, Zhu MX, Romanin C, and Groschner K. TRPC3 and TRPC4 associate to form a redox-sensitive cation channel. Evidence for expression of native TRPC3-TRPC4 heteromeric channels in endothelial cells. *J Biol Chem* 281: 13588–13595, 2006.
135. Poteser M, Romanin C, Schreibmayer W, Mayer B, and Groschner K. S-nitrosation controls gating and conductance of the alpha 1 subunit of class C L-type Ca(2+) channels. *J Biol Chem* 276: 14797–14803, 2001.
136. Potier M, Gonzalez JC, Motiani RK, Abdullaev IF, Bisaillon JM, Singer HA, and Trebak M. 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: 2425–2437, 2009.
137. Potier M and Trebak M. New developments in the signaling mechanisms of the store-operated calcium entry pathway. *Pflugers Arch* 457: 405–415, 2008.
138. Putney JW Jr. A model for receptor-regulated calcium entry. *Cell Calcium* 7: 1–12, 1986.
139. Radomski MW, Palmer RMJ, and Moncada S. An L-arginine/nitric oxide pathway present in human platelets regulates aggregation. *Proc Natl Acad Sci USA* 87: 5193–5197, 1990.
140. Raines KW, Bonini MG, and Campbell SL. Nitric oxide cell signaling: S-nitrosation of Ras superfamily GTPases. *Cardiovasc Res* 75: 229–239, 2007.
141. Rhee SG. Cell signaling. H2O2, a necessary evil for cell signaling. *Science* 312: 1882–1883, 2006.
142. Ritsick DR, Edens WA, Finnerty V, and Lambeth JD. Nox regulation of smooth muscle contraction. *Free Radic Biol Med* 43: 31–38, 2007.
143. Robison AJ, Winder DG, Colbran RJ, and Bartlett RK. Oxidation of calmodulin alters activation and regulation of CaMKII. *Biochem Biophys Res Commun* 356: 97–101, 2007.
144. Roos J, DiGregorio PJ, Yeromin AV, Ohlsen K, Lioudyno M, Zhang S, Safrina O, Kozak JA, Wagner SL, Cahalan MD, Velicelebi G, and Stauderman KA. STIM1, an essential and conserved component of store-operated Ca2+ channel function. *J Cell Biol* 169: 435–445, 2005.
145. Rosker C, Graziani A, Lukas M, Eder P, Zhu MX, Romanin C, and Groschner K. Ca(2+) signaling by TRPC3 involves Na(+) entry and local coupling to the Na(+)/Ca(2+) exchanger. *J Biol Chem* 279: 13696–13704, 2004.
146. Ruan J, Xie Q, Hutchinson N, Cho H, Wolfe GC, and Nathan C. Inducible nitric oxide synthase requires both the canonical calmodulin-binding domain and additional

sequences in order to bind calmodulin and produce nitric oxide in the absence of free Ca2+. *J Biol Chem* 271: 22679–22686, 1996.
147. Schrammel A, Gorren AC, Schmidt K, Pfeiffer S, and Mayer B. S-nitrosation of glutathione by nitric oxide, peroxynitrite, and NO/O_2^-. *Free Radic Biol Med* 34: 1078–1088, 2003.
148. Schwartz SM, Campbell GR, and Campbell JH. Replication of smooth muscle cells in vascular disease. *Circ Res* 58: 427–444, 1986.
149. Schworer CM, Rothblum LI, Thekkumkara TJ, and Singer HA. Identification of novel isoforms of the δ-subunit of Ca^{2+}/calmodulin-dependent protein kinase II: Differential expression in rat brain and aorta. *J Biol Chem* 268: 14443–14449, 1993.
150. Scmidt HHH, Hofman H, Schindler U, Shutenko ZS, Cunningham DD, and Feelisch M. No NO from NO synthase. *Proc Natl Acad Sci USA* 93: 14492–14497, 1996.
151. Serrander L, Cartier L, Bedard K, Banfi B, Lardy B, Plastre O, Sienkiewicz A, Forro L, Schlegel W, and Krause KH. NOX4 activity is determined by mRNA levels and reveals a unique pattern of ROS generation. *Biochem J* 406: 105–114, 2007.
152. Seshiah PN, Weber DS, Rocic P, Valppu L, Taniyama Y, and Griendling KK. Angiotensin II stimulation of NAD(P)H oxidase activity: Upstream mediators. *Circ Res* 91: 406–413, 2002.
153. Shetty PK, Huang FL, and Huang KP. Ischemia-elicited oxidative modulation of Ca2+/calmodulin-dependent protein kinase II. *J Biol Chem* 283: 5389–5401, 2008.
154. Shuttleworth TJ, Thompson JL, and Mignen O. STIM1 and the noncapacitative ARC channels. *Cell Calcium* 42: 183–191, 2007.
155. Singer HA, Benscoter HA, and Schworer CM. Novel Ca^{2+}/calmodulin-dependent protein kinase II g-subunit variants expressed in vascular smooth muscle, brain and cardiomyocytes. *J Biol Chem* 272: 9393–9400, 1997.
156. Smyth JT, DeHaven WI, Jones BF, Mercer JC, Trebak M, Vazquez G, and Putney JW, Jr. Emerging perspectives in store-operated Ca2+ entry: Roles of Orai, Stim and TRP. *Biochimica Biophysica Acta* 1763: 1147–1160, 2006.
157. Soboloff J, Spassova M, Xu W, He LP, Cuesta N, and Gill DL. Role of endogenous TRPC6 channels in Ca2+ signal generation in A7r5 smooth muscle cells. *J Biol Chem* 280: 39786–39794, 2005.
158. Song T, Hatano N, Kambe T, Miyamoto Y, Ihara H, Yamamoto H, Sugimoto K, Kume K, Yamaguchi F, Tokuda M, and Watanabe Y. Nitric oxide-mediated modulation of calcium/calmodulin-dependent protein kinase II. *Biochem J* 412: 223–231, 2008.
159. Sorescu D, Weiss D, Lassegue B, Clempus RE, Szocs K, Sorescu GP, Valppu L, Quinn MT, Lambeth JD, Vega JD, Taylor WR, and Griendling KK. Superoxide production and expression of nox family proteins in human atherosclerosis. *Circulation* 105: 1429–1435, 2002.
160. Stamler JS, Toone EJ, Lipton SA, and Sucher NJ. (S)NO signals: Translocation, regulation, and a consensus motif. *Neuron* 18: 691–696, 1997.
161. Strack S, Barban MA, Wadzinski BE and Colbran RJ. Differential inactivation of postsynaptic density-associated and soluble Ca2+/calmodulin-dependent protein kinase II by protein phosphatases 1 and 2A. *J Neurochem* 68: 2119–2128, 1997.
162. Sturrock A, Huecksteadt TP, Norman K, Sanders K, Murphy TM, Chitano P, Wilson K, Hoidal JR, and Kennedy TP. Nox4 mediates TGF-beta1-induced retinoblastoma protein phosphorylation, proliferation, and hypertrophy in human airway smooth muscle cells. *Am J Physiol* 292: L1543–L1555, 2007.
163. Sun J, Xin C, Eu JP, Stamler JS, and Meissner G. Cysteine-3635 is responsible for skeletal muscle ryanodine receptor modulation by NO. *Proc Natl Acad Sci USA* 98: 11158–11162, 2001.
164. Sweeney M, Yu Y, Platoshyn O, Zhang S, McDaniel SS, and Yuan JX. Inhibition of endogenous TRP1 decreases capacitative Ca2+ entry and attenuates pulmonary artery smooth muscle cell proliferation. *Am J Physiol* 283: L144–L155, 2002.
165. Syyong HT, Poburko D, Fameli N, and van Breemen C. ATP promotes NCX-reversal in aortic smooth muscle cells by DAG-activated Na+entry. *Biochem Biophys Res Comm* 357: 1177–1182, 2007.
166. Szöcs K, Lassègue B, Sorescu D, Hilenski LL, Valppu L, Cousse TL, Wilcox JN, Quinn MT, and Griendling KK. Upregulation of Nox-based NAD(P)H oxidases in restenosis after carotid injury. *Arterioscler Thromb Vasc Biol* 22: 21–27, 2002.
167. Tabet F, Savoia C, Schiffrin EL, and Touyz RM. Differential calcium regulation by hydrogen peroxide and superoxide in vascular smooth muscle cells from spontaneously hypertensive rats. *J Cardiovas Pharmacol* 44: 200–208, 2004.
168. Taylor CW and Dellis O. Plasma membrane IP3 receptors. *Biochem Soc Transac* 34: 910–912, 2006.
169. Tirone F and Cox JA. NADPH oxidase 5 (NOX5) interacts with and is regulated by calmodulin. *FEBS Lett* 581: 1202–1208, 2007.
170. Tiwari S, Zhang Y, Heller J, Abernethy DR, and Soldatov NM. Atherosclerosis-related molecular alteration of the human CaV1.2 calcium channel alpha1C subunit. *Proc Natl Acad Sci USA* 103: 17024–17029, 2006.
171. Tobimatsu T and Fujisawa H. Tissue-specific expression of four types of rat calmodulin-dependent protein kinase II mRNAs. *J Biol Chem* 264: 17907–17912, 1989.
172. Touyz RM. Reactive oxygen species as mediators of calcium signaling by angiotensin II: Implications in vascular physiology and pathophysiology. *Antioxid Redox Signal* 7: 1302–1314, 2005.
173. Touyz RM, Yao G, and Schiffrin EL. c-Src induces phosphorylation and translocation of p47phox: Role in superoxide generation by angiotensin II in human vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 23: 981–987, 2003.
174. Trebak M, Lemonnier L, Smyth JT, Vazquez G, and Putney JW, Jr. Phospholipase C-coupled receptors and activation of TRPC channels. *Handb Exp Pharmacol* 593–614, 2007.
175. Trebak M, St JB, McKay RR, Birnbaumer L, and Putney JW, Jr. Signaling mechanism for receptor-activated canonical transient receptor potential 3 (TRPC3) channels. *J Biol Chem* 278: 16244–16252, 2003.
176. Trebak M, Vazquez G, Bird GS, and Putney JW, Jr. The TRPC3/6/7 subfamily of cation channels. *Cell Calcium* 33: 451–461, 2003.
177. Trepakova ES, Cohen RA, and Bolotina VM. Nitric oxide inhibits capacitative cation influx in human platelets by promoting sarcoplasmic/endoplasmic reticulum Ca2+-ATPase-dependent refilling of Ca2+ stores. *Circ Res* 84: 201–209, 1999.

178. Trepakova ES, Gericke M, Hirakawa Y, Weisbrod RM, Cohen RA, and Bolotina VM. Properties of a native cation channel activated by Ca2+ store depletion in vascular smooth muscle cells. *J Biol Chem* 276: 7782–7790, 2001.
179. Trevisani M, Siemens J, Materazzi S, Bautista DM, Nassini R, Campi B, Imamachi N, Andre E, Patacchini R, Cottrell GS, Gatti R, Basbaum AI, Bunnett NW, Julius D, and Geppetti P. 4-Hydroxynonenal, an endogenous aldehyde, causes pain and neurogenic inflammation through activation of the irritant receptor TRPA1. *Proc Natl Acad Sci USA* 104: 13519–13524, 2007.
180. Varnai P, Toth B, Toth DJ, Hunyady L, and Balla T. Visualization and manipulation of plasma membrane-endoplasmic reticulum contact sites indicates the presence of additional molecular components within the STIM1-Orai1 complex. *J Biol Chem* 282: 29678–29690, 2007.
181. Vasquez-Vivar J, Kalyanaraman B, Martasek P, Hogg N, Masters BS, Karoui H, Tordo P, and Pritchard KA, Jr. Superoxide generation by endothelial nitric oxide synthase: The influence of cofactors. *Pro Natl Acad Sci USA* 95: 9220–9225, 1998.
182. Vendrov AE, Madamanchi NR, Hakim ZS, Rojas M, and Runge MS. Thrombin and NAD(P)H oxidase-mediated regulation of CD44 and BMP4-Id pathway in VSMC, restenosis, and atherosclerosis. *Circ Res* 98: 1254–1263, 2006.
183. Vig M, Peinelt C, Beck A, Koomoa DL, Rabah D, Koblan–Huberson M, Kraft S, Turner H, Fleig A, Penner R, and Kinet JP. CRACM1 is a plasma membrane protein essential for store-operated Ca2+ entry. *Science* 312: 1220–1223, 2006.
184. Viola HM, Arthur PG, and Hool LC. Transient exposure to hydrogen peroxide causes an increase in mitochondria-derived superoxide as a result of sustained alteration in L-type Ca2+ channel function in the absence of apoptosis in ventricular myocytes. *Circ Res* 100: 1036–1044, 2007.
185. Wamhoff BR, Bowles DK, and Owens GK. Excitation-transcription coupling in arterial smooth muscle. *Circ Res* 98: 868–878, 2006.
186. Ward ME, Toporsian M, Scott JA, Teoh H, Govindaraju V, Quan A, Wener AD, Wang G, Bevan SC, Newton DC, and Marsden PA. Hypoxia induces a functionally significant and translationally efficient neuronal NO synthase mRNA variant. *J Clin Invest* 115: 3128–3139, 2005.
187. Wehage E, Eisfeld J, Heiner I, Jungling E, Zitt C, and Luckhoff A. Activation of the cation channel long transient receptor potential channel 2 (LTRPC2) by hydrogen peroxide. A splice variant reveals a mode of activation independent of ADP-ribose. *J Biol Chem* 277: 23150–23156, 2002.
188. Woo HA, Jeong W, Chang TS, Park KJ, Park SJ, Yang JS, and Rhee SG. Reduction of cysteine sulfinic acid by sulfiredoxin is specific to 2-Cys peroxiredoxins. *J Biol Chem* 280: 3125–3128, 2005.
189. Xi Q, Adebiyi A, Zhao G, Chapman KE, Waters CM, Hassid A, and Jaggar JH. IP3 constricts cerebral arteries via IP3 receptor-mediated TRPC3 channel activation and independently of sarcoplasmic reticulum Ca2+ release. *Circ Res* 102: 1118–1126, 2008.
190. Xi Q, Cheranov SY, and Jaggar JH. Mitochondria-derived reactive oxygen species dilate cerebral arteries by activating Ca2+ sparks. *Circ Res* 97: 354–362, 2005.
191. Xie LH, Chen F, Karagueuzian HS, and Weiss JN. Oxidative stress-induced after depolarizations and calmodulin kinase II signaling. *Circ Res* 104: 79–86, 2009.
192. Xu L, Eu JP, Meissner G, and Stamler JS. Activation of the cardiac calcium release channel (ryanodine receptor) by poly-S-nitrosylation. *Science* 279: 234–237, 1998.
193. Yamamoto S, Shimizu S, Kiyonaka S, Takahashi N, Wajima T, Hara Y, Negoro T, Hiroi T, Kiuchi Y, Okada T, Kaneko S, Lange I, Fleig A, Penner R, Nishi M, Takeshima H, and Mori Y. TRPM2-mediated Ca2+ influx induces chemokine production in monocytes that aggravates inflammatory neutrophil infiltration. *Nature Med* 14: 738–747, 2008.
194. Yang M and Kahn AM. Insulin-inhibited and stimulated cultured vascular smooth muscle cell migration are related to divergent effects on protein phosphatase-2A and autonomous calcium/calmodulin-dependent protein kinase II. *Atherosclerosis* 196: 227–233, 2008.
195. Yao X. TRPC, cGMP-dependent protein kinases and cytosolic Ca2+. *Handb Exp Pharmacol* 527–540, 2007.
196. Yoshida T, Inoue R, Morii T, Takahashi N, Yamamoto S, Hara Y, Tominaga M, Shimizu S, Sato Y, and Mori Y. Nitric oxide activates TRP channels by cysteine S-nitrosylation. *Nature Chem Biol* 2: 596–607, 2006.
197. Yu Y, Fantozzi I, Remillard CV, Landsberg JW, Kunichika N, Platoshyn O, Tigno DD, Thistlethwaite PA, Rubin LJ, and Yuan JX. Enhanced expression of transient receptor potential channels in idiopathic pulmonary arterial hypertension. *Proc Natl Acad Sci USA*101: 13861–13866, 2004.
198. Yu Y, Sweeney M, Zhang S, Platoshyn O, Landsberg J, Rothman A, and Yuan JX. PDGF stimulates pulmonary vascular smooth muscle cell proliferation by upregulating TRPC6 expression. *Am J Physiol* 284: C316–C330, 2003.
199. Yuan JP, Zeng W, Dorwart MR, Choi YJ, Worley PF, and Muallem S. SOAR and the polybasic STIM1 domains gate and regulate Orai channels. *Nature Cell Biol* 11: 337–343, 2009.
200. Zhang JZ, Wu Y, Williams BY, Rodney G, Mandel F, Strasburg GM, and Hamilton SL. Oxidation of the skeletal muscle Ca2+ release channel alters calmodulin binding. *Am J Physiol* 276: C46–C53, 1999.

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Abbreviations Used

Ang II = angiotensin II
Ca^{2+} = calcium
CaM = calmodulin
CaMKII = Ca^{2+}/CaM-dependent protein kinase II
cGMP = cyclic guanosine monophosphate
ClC-3 = chloride channel-3
CRAC = calcium release activated calcium
DAG = diacylglycerol
Duox = dual oxidase
ERK = extracellular regulated kinase
GPCR = G protein coupled receptor
H_2O_2 = hydrogen peroxide
hPMCA4 = human plasma membrane Ca^{+2} ATPase 4
IL-1β = interleukin-1β
IP3 = inositol 1,4,5, trisphosphate
IP_3R = IP_3 receptor
JAK/STAT = Janus kinase/signal transducer and activator of transcription
NADPH = nicotinamide adenine dinucleotide phosphate
NF-κB = nuclear factor κB
NO = nitric oxide
NO^- = nitroxyl
$^{\bullet}NO_2$ = nitrogen dioxide
NO_2^- = nitrite
NO_3^- = nitrate
NOS = nitric oxide synthase
Nox = NADPH oxidase
NOXO1 = NADPH oxidase organizer 1
NOXOA1 = NADPH oxidase activator 1
O_2^- = superoxide
$^{\bullet}OH$ = hydroxyl radical
$ONOO^-$/ONOOH = peroxynitrite
Phox = phagocytic oxidase
PIP2 = phosphatidylinositol 4,5 bisphosphate
PKC = protein kinase C
PKG = cGMP-dependent protein kinase
PLC = phospholipase C
PP1 = protein phosphatase 1
PP2A = protein phosphatase 2A
RNS = reactive nitrogen species
ROS = reactive oxygen species
RyR = ryanodine receptor
shRNA = short hairpin RNA interference
SOC = store-operated channels
TGFβ = transforming growth factor β
TNF-α = tumor necrosis factor-α
TRAF = tumor necrosis receptor associated factor
TRPC = transient receptor potential canonical
VSMC = vascular smooth muscle cell

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2. Tiziana Anelli , Leda Bergamelli , Eva Margittai , Alessandro Rimessi , Claudio Fagioli , Antonio Malgaroli , Paolo Pinton , Maddalena Ripamonti , Rosario Rizzuto , Roberto Sitia . 2012. Ero1# Regulates Ca2+ Fluxes at the Endoplasmic Reticulum–Mitochondria Interface (MAM). *Antioxidants & Redox Signaling* **16**:10, 1077-1087. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links] [Supplemental material]
3. Mathieu Da Silva, Grayson K. Jaggers, Sandra V. Verstraeten, Alejandra G. Erlejman, Cesar G. Fraga, Patricia I. Oteiza. 2012. Large procyanidins prevent bile-acid-induced oxidant production and membrane-initiated ERK1/2, p38, and Akt activation in Caco-2 cells. *Free Radical Biology and Medicine* **52**:1, 151-159. [CrossRef]
4. Rhian M. Touyz, Ernesto L. SchiffrinArterial Hypertension 1311-1319. [CrossRef]
5. Theodor Burdyga, Richard J. PaulCalcium Homeostasis and Signaling in Smooth Muscle 1155-1171. [CrossRef]
6. Ivan Bogeski, Reinhard Kappl, Carsten Kummerow, Rubin Gulaboski, Markus Hoth, Barbara A. Niemeyer. 2011. Redox regulation of calcium ion channels: Chemical and physiological aspects. *Cell Calcium* . [CrossRef]
7. Nina Queisser, Nicole Schupp, Helga Stopper, Reinhard Schinzel, Patricia I. Oteiza. 2011. Aldosterone increases kidney tubule cell oxidants through calcium-mediated activation of NADPH oxidase and nitric oxide synthase. *Free Radical Biology and Medicine* . [CrossRef]
8. Jong-Hau Hsu, Jiunn-Ren Wu, Shu-Fen Liou, Huai-Min Chen, Zen-Kong Dai, Ing-Jun Chen, Jwu-Lai Yeh. 2011. Labedipinedilol-A prevents lysophosphatidylcholine-induced vascular smooth muscle cell death through reducing reactive oxygen species production and anti-apoptosis. *Atherosclerosis* **217**:2, 379-386. [CrossRef]
9. Vidhi P. Shah, Hesum A. Chegini, Susan R. Vishneski, Ross V. Weatherman, Peter F. Blackmore, Yuliya Dobrydneva. 2011. Tamoxifen promotes superoxide production in platelets by activation of PI3-Kinase and NADPH oxidase pathways. *Thrombosis Research* . [CrossRef]
10. Michel Félétou. 2011. The Endothelium, Part I: Multiple Functions of the Endothelial Cells -- Focus on Endothelium-Derived Vasoactive Mediators. *Colloquium Series on Integrated Systems Physiology: From Molecule to Function* **3**:4, 1-306. [CrossRef]
11. Michel Félétou. 2011. The Endothelium, Part II: EDHF-Mediated Responses "The Classical Pathway". *Colloquium Series on Integrated Systems Physiology: From Molecule to Function* **3**:4, 1-306. [CrossRef]
12. Andrea U. Steinbicker, Heling Liu, Kim Jiramongkolchai, Rajeev Malhotra, Elizabeth Y. Choe, Cornelius J. Busch, Amanda R. Graveline, Sonya M. Kao, Yasuko Nagasaka, Fumito Ichinose, Emmanuel S. Buys, Peter Brouckaert, Warren M. Zapol, Kenneth D. Bloch. 2011. Nitric oxide regulates pulmonary vascular smooth muscle cell expression of the inducible cAMP early repressor gene. *Nitric Oxide* . [CrossRef]
13. Lijuan Wang, Yingxian Sun, Michio Asahi, Kinya Otsu. 2011. Acrolein, an Environmental Toxin, Induces Cardiomyocyte Apoptosis via Elevated Intracellular Calcium and Free Radicals. *Cell Biochemistry and Biophysics* . [CrossRef]
14. Rakhee S. Gupte , Hirotaka Ata , Dhawjbahadur Rawat , Madoka Abe , Mark S. Taylor , Rikuo Ochi , Sachin A. Gupte . 2011. Glucose-6-Phosphate Dehydrogenase Is a Regulator of Vascular Smooth Muscle Contraction. *Antioxidants & Redox Signaling* **14**:4, 543-558. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links] [Supplemental material]
15. Peter F. Blackmore. 2011. Biphasic effects of nitric oxide on calcium influx in human platelets. *Thrombosis Research* **127**:1, e8-e14. [CrossRef]
16. Michelle J. Connolly, Philip I. Aaronson. 2010. Cell redox state and hypoxic pulmonary vasoconstriction: Recent evidence and possible mechanisms#. *Respiratory Physiology & Neurobiology* **174**:3, 165-174. [CrossRef]
17. David Jourd'heuil . 2010. Redox Control of Vascular Smooth Muscle Function. *Antioxidants & Redox Signaling* **12**:5, 579-581. [Citation] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]