ANTIOXIDANTS & REDOX SIGNALING
Volume 15, Number 6, 2011

DOI: 10.1089/ars.2010.3648

FORUM REVIEW ARTICLE

Role of Reactive Oxygen Species and Redox in Regulating the Function of Transient Receptor Potential Channels

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Abstract

Cellular redox status, regulated by production of reactive oxygen species (ROS), greatly contributes to the regulation of vascular smooth muscle cell contraction, migration, proliferation, and apoptosis by modulating the function of transient receptor potential (TRP) channels in the plasma membrane. ROS functionally interact with the channel protein *via* oxidizing the redox-sensitive residues, whereas nitric oxide (NO) regulates TRP channel function by cyclic GMP/protein kinase G-dependent and -independent pathways. Based on the structural differences among different TRP isoforms, the effects of ROS and NO are also different. In addition to regulating TRP channels in the plasma membrane, ROS and NO also modulate Ca^{2+} release channels (*e.g.*, IP_3 and ryanodine receptors) on the sarcoplasmic/endoplasmic reticulum membrane. This review aims at briefly describing (a) the role of TRP channels in receptor-operated and store-operated Ca^{2+} entry, and (b) the role of ROS and redox status in regulating the function and structure of TRP channels. *Antioxid. Redox Signal.* 15, 1549–1565.

Introduction

Reactive oxygen species (ROS) play an important role in the physiology and pathophysiology of the vasculature. Recent research suggests that ROS may mediate the development of hypertension in model organisms such as mice and also in human patients. Cultured vascular smooth muscle cells (VSMC) isolated from arteries of animals (*e.g.*, rats) and patients with hypertension show increased ROS production, abnormal redox signaling, and decreased antioxidant activity (74, 88). Mutant mice deficient in enzymes that generate ROS have lower blood pressure compared to wild-type mice. Treatment with angiotensin II, a vasoconstrictor that increases ROS production in VSMC, in these mutated mice fails to induce hypertension (49). In patients with hypertension, biomarkers of oxidative stress are increased and treatment with antioxidants (*e.g.*, superoxide dismutase mimetics) regresses vascular remodeling and reduces blood pressure (77, 94). These data show the importance of ROS signaling in the physiology and pathophysiology of the vasculature by regulating smooth muscle and endothelial cell functions.

Under pathological or pathophysiological conditions, ROS levels are increased and lead to endothelial dysfunction, VSMC proliferation, and increased contractility (86). These changes may be involved in the pathogenic mechanisms of various cardiovascular diseases (*e.g.*, systemic and pulmonary arterial hypertension). ROS-induced vascular changes occur though redox-sensitive signaling pathways involving mitogen-activated protein kinases, tyrosine kinases, various transcription factors (*e.g.*, NF-κB, AP-1, HIF-1α), and many different steps of the Ca^{2+} signaling cascade (9, 68). Although these are all important signaling mechanisms, for the purposes of this review, we will focus on the role of ROS in regulating Ca^{2+} signaling particularly the effect of ROS on the function of transient receptor potential (TRP) channels. ROS and Ca^{2+} signaling are both relevant to the physiology and pathophysiology of the vasculature, particularly in the function of vascular smooth muscle and endothelial cells. However, we will focus on discussing the effect ROS and redox status on regulating TRPC channels and Ca^{2+} signaling in general rather than on the signaling mechanisms in a specific cell type.

Recent work by many investigators has elucidated much on the effect of redox state on Ca^{2+} signaling. There likely exists an overall trend where increased ROS inhibits Ca^{2+} pumps and opens Ca^{2+}-permeable channels in the SR/ER membrane and plasma membrane, respectively. Inhibition of Ca^{2+} pumps in the plasma membrane attenuates the extrusion of Ca^{2+} out of the cytoplasm, whereas opening of Ca^{2+}-permeable channels on the SR/ER and plasma membranes allows Ca^{2+} to flow down its concentration gradient and into the cytoplasm and increase cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$). Therefore, the net effect of ROS has been demonstrated to increase $[Ca^{2+}]_{cyt}$.

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ROS Chemistry

Oxygen (O_2) is essential for generating the energy needed to live by reducing molecular oxygen to water. During this process, oxygen undergoes a series of one-electron reductions in the mitochondrial electron transport chain to produce superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), and water (H_2O):

$$O_2 \rightarrow O_2^{\bullet -} \rightarrow H_2O_2 \rightarrow HO^{\bullet} \rightarrow H_2O$$
$$O_2 - e \rightarrow O_2^{\bullet -}$$

The superoxide radical ($O_2^{\bullet -}$) is produced from the one electron reduction of oxygen; the major source of superoxide is from oxygen reduction pathway in the electron transport chain of mitochondria and the sarcoplasmic or endoplasmic reticulum. However, superoxide can be produced from NAD(P)H oxidase in the vasculature:

$$NAD(P)H + 2O_2 \rightarrow NAD(P)^+ + H^+ + 2O_2^{\bullet -}$$

Hydrogen peroxide (H_2O_2) is not a radical because it has no unpaired electrons. Hydrogen peroxide, which is relatively stable and can easily pass through cell membranes, is produced from the superoxide radical by superoxide dismutase from microsomes, mitochondria, and phagocytic cells:

$$2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2$$

Most of the effects or damage produced by superoxide and hydrogen peroxide *in vitro* is due to the production of hydroxyl radicals *via* the metal catalyzed Haber-Weiss reaction or the superoxide radical-driven Fenton reaction:

$$O_2^{\bullet -} + H_2O_2 \rightarrow O_2 + HO^- + HO^{\bullet}$$
$$Fe^{3+} + O_2^{\bullet -} \rightarrow Fe^{2+} + O_2 \text{ (Haber - Weiss Reaction)}$$
$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + HO^- \text{ (Fenton Reaction)}$$

In addition, the superoxide can react with nitric oxide (NO) to produce peroxynitrite ($ONOO^-$):

$$O_2^{\bullet -} + H_2O_2 \rightarrow O_2 + HO^{\bullet} + HO^-$$
$$NO + O_2^{\bullet -} \rightarrow ONOO^-$$

The products ($O_2^{\bullet -}$ and $ONOO^-$) of these reactions are very reactive. The cytotoxic effects of the superoxide and hydrogen peroxide also relate to their ability to react with products of other microbicidal systems in cells to generate additional ROS, such as singlet oxygen ($^1O_2$) and ozone (O_3) (86–88).

All these highly reactive molecules ($O_2^{\bullet -}$, $ONOO^-$, OH^-) participate in oxidation–reduction reactions primarily with thiols in the cell. A thiol group (-SH) contains a sulfur hydrogen bond and two thiols can be converted to a disulfide (-S-S-), which is two sulfurs covalently bound together. The redox chemistry of converting thiols to disulfides involves complex intermediates such as sulfinic acid (-SOH), sulfonic acid ($-S(O)_2OH$), or thiolsulfinate (-S(O)-S-) among others. The sum of all the proteins and molecules that can participate in redox chemistry determines the redox status of a cellular compartment. The redox status in a cellular compartment is defined as either reducing or oxidizing. In reduction, an electron acceptor (*e.g.*, two proteins, usually *via* cysteines, connected by a disulfide bond, R-S-S-R) accepts electrons from an electron donor (NADPH or H_2O_2) to become two thiols (2R-SH). In oxidation, the reaction is reversed such that an electron donor (2R-SH) donates electrons to an electron acceptor such as $NADP^+$ or NAD^+ to form a disulfide bridge (R-S-S-R) (Fig. 1). In cells, the protein residue, cysteine, serves an important function in redox chemistry because its thiol functional group can be easily oxidized. The status of disulfide bonds formed between distant cysteines affects the tertiary structure of channel proteins. Therefore, the regulation of redox status affects the activity of various channel proteins and the cellular functions that those channels are involved. In pulmonary VSMC, ROS production and redox

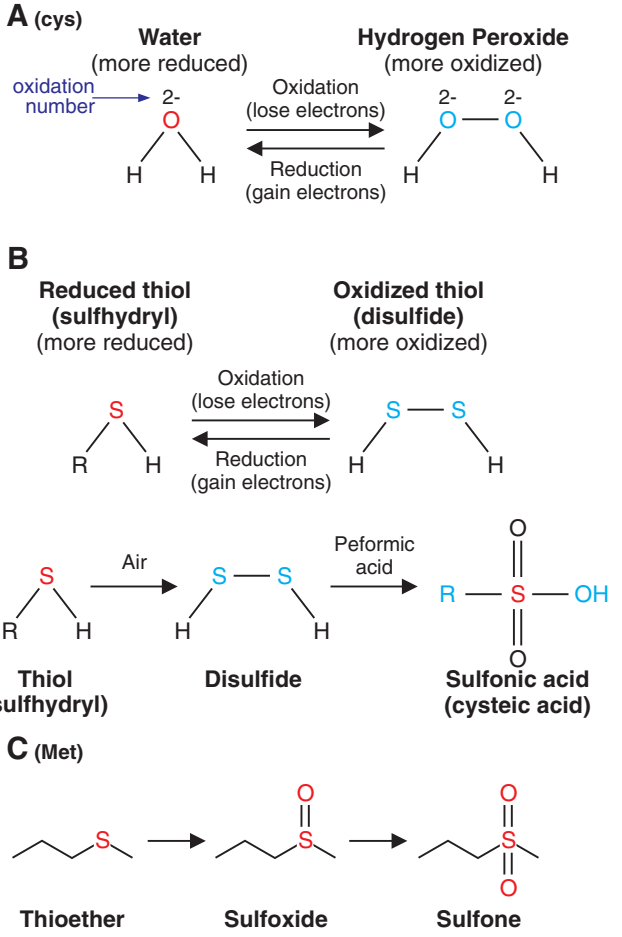

FIG. 1. Oxidation–reduction chemistry occurs through gain or loss of electrons. (A) Water is oxidized to hydrogen peroxide thought the loss of electrons. The reverse reaction, termed reduction, occurs though the gain of electrons by hydrogen peroxide. The change in the sum of oxidation number on all the atoms is the number of electrons gained or lost in the reaction. Cys, cysteine. **(B)** Oxidation–reduction chemistry in the cell often involves the conversion of reduced thiols to oxidized thiols (disulfides). Thiol can be oxidized to disulfide, which can be further oxidized to sulfonic acid. In the cell, the amino acid cysteine (Cys) is oxidized or reduced in this manner. **(C)** The amino acid methionine (Met) can also participate in redox chemistry as a thioester and can be oxidized to a sulfoxide and then to a sulfone. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

status can be altered by hypoxia and agonists (*e.g.*, endothelin-1, serotonin) that cause cell contraction and proliferation (52). It is, however, still controversial whether hypoxia increases or decreases ROS production in pulmonary artery smooth muscle cell (PASMC).

Production of ROS in the Vasculature

In the vascular cells (including adventitial, smooth muscle, and endothelial cells), ROS are produced primarily *via* an NADPH oxidase (NOX) and the mitochondrial electron transport chain (Fig. 2A) (11). Other mechanisms, including xanthine oxidase, cytochrome P-450, cyclooxygenase, and NO synthase (NOS), also contribute to ROS level (8, 21). NADPH catalyzes superoxide ($O_2^{\bullet -}$) production by donating an electron to molecular oxygen (O_2). Superoxide can then react with H^+ to produce hydrogen peroxide (OH^-).

The NOX family is composed of NOX1-5 and Duox1-2. All five NOX isoforms are predominantly localized on the plasma membrane. NOX2 produces large amounts of ROS in phagocytes in a process termed "respiratory burst" for the purpose of mediating host defense against invading microorganisms. NOX 1, 2, 4, and 5 are important in the physiology and pathophysiology of the cardiovascular, pulmonary, and renal system. NOX3 is involved in the function of the vestibular system. NOX5 is expressed in spleen, testis, and vascular tissues. NOX5 has been found in both the smooth muscle and the endothelial cells of the vasculature (12, 26, 89).

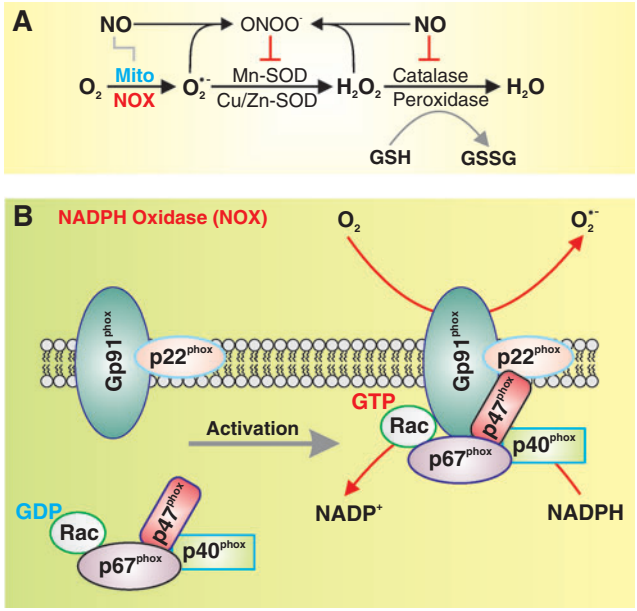

FIG. 2. Structure of NADPH oxidase (NOX) and organization of NOX isoforms in the plasma membrane. (A) The conversion of nitric oxide (NO) to $ONOO^-$ is involved in the regulation of redox status by regulating the conversion of O_2 to superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and water (H_2O). **(B)** The NOX comprises a membrane-bound $gp91^{phox}/p22^{phox}$ heterodimer, a $p67^{phox}$ subunit, a $p47^{phox}$ subunit, a $p40^{phox}$ subunit, and Rac. The NADPH-binding domain is on one side of the membrane, whereas $O_2^{\bullet -}$ generation on the other. The NOX is located on both the plasma membrane and intracellular membranes. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

Interestingly, coronary artery disease has been correlated with the increased expression of NOX5 in vascular cells. The vascular NOX, which is constitutively active and a major source of vascular superoxide production, is comprised of two cytochrome b_{558} subunits, $p22^{phox}$ and $gp91^{phox}$ (Fig. 2B), which have been demonstrated to be important for electron transport and the reduction of molecular oxygen to superoxide. Interestingly, the voltage-gated K^+ (Kv) channel β subunit, a cytoplasmic regulatory subunit that interacts with the pore-forming α subunit to form functional Kv channels, has >60% homology to the NOX, implying that (a) the Kv channel β subunits may have NOX activity and/or (b) the vascular NOX can function as a regulatory β subunit for certain Kv channels (34, 35).

Regulation of NOX

In the vasculature, NOX is regulated by chemical means such as cytokines, growth factors, and vasoactive agents as well as physical means such as stretch, strain, pressure, and shear stress. These different factors tightly control the level of ROS at a low level such that it can function as a signaling molecule to regulate endothelial function and vasorelaxation and vasocontraction. Various vasoconstrictors and mitogens such as angiotensin II, serotonin, endothelin-1, platelet-derived growth factor, and tumor necrosis factor-α stimulate the production of superoxide (27, 48, 80).

Not only do ROS regulate Ca^{2+} signaling mechanisms, but Ca^{2+} can also regulate the activity of NOX. NOX5, for instance, is distinguished from NOX1-4 by four Ca^{2+} binding EF hand domains on an extended intracellular N-terminal domain. It has been shown that elevation of $[Ca^{2+}]_{cyt}$ promotes the binding of Ca^{2+} to the NOX5 EF hand domains, changing the conformation of NOX5 and enabling the protein to generate superoxide (27). That is, a rise in $[Ca^{2+}]_{cyt}$ is an important trigger for production of ROS. This positive "chain reaction" circle of ROS and $[Ca^{2+}]_{cyt}$ increase in VSMC may lead to sustained vasoconstriction and excessive cell proliferation and migration *via* Ca^{2+}-sensitive signaling cascades (*e.g.*, MLCK, CaMK, and MAPK) and transcription factors (*e.g.*, AP-1, NF-κB, CREB, and NFAT).

Regulation of Redox Status by Thioredoxin and Glutathione Systems

Redox status in cells is not solely determined by the concentration of ROS. The major determinants of redox status are the thioredoxin (Trx) and glutathione (GSH) systems (41). These two regulatory systems of redox status are the primary control mechanism of the status of thiols in the cell and are therefore very important in the regulation of cellular functions. These two systems are present in the cytosol and mitochondria, which are the main sources of ROS in the cell. In the cytosol, $NADP^+$ is reduced in the pentose phosphate pathway to NADPH, and in mitochondria NADPH is generated through oxidative phosphorylation. NADPH functions as a cofactor in the Trx and GSH systems. The Trx and GSH systems are regulated by hydrogen peroxide through peroxiredoxins (Prx) and GSH peroxidases, respectively (Fig. 3). Under hypoxic or hyperoxic conditions, redox status may change in different directions in systemic and pulmonary vascular smooth muscle and endothelial cells.

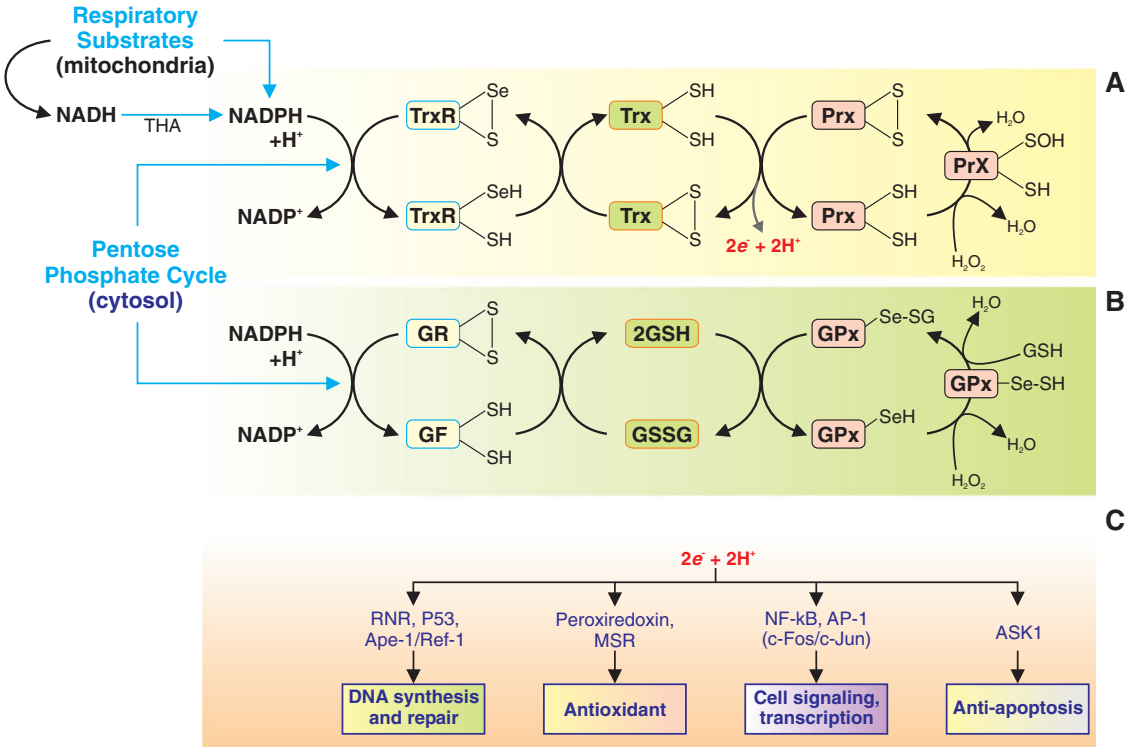

FIG. 3. The thioredoxin (Trx) and glutathione (GSH) systems regulate redox status in the cell. (A) Trx system regulates redox status in the cell. Oxidation and reduction of thiols and selenocysteines on Trx, thioredoxin reductases (TrxR), and peroxiredoxins (Prx) control the redox status of a cellular compartment. The redox state of these proteins determines the concentration of H_2O_2 and reducing equivalents $2e^- + H^+$. **(B)** Glutathione system regulates redox status in the cell. Oxidation and reduction of thiols and selenocysteines on GSH, GSH reductases, and glutathione peroxidase (GPx) also control the redox status of a cellular compartment. The redox state of these proteins determines the concentration of H_2O_2 and reducing equivalents $2e^- + H^+$. **(C)** The redox status of the cell affects many cellular processes such as DNA synthesis and repair, cell signaling and transcription, and apoptosis through various intracellular enzymes, transcription factors, and intracellular kinases. RNR, ribonucleotide-diphosphate reductase; Ape-1, apurinic/apyrimidinic (AP) endonuclease (also known as HAP 1 or Ref-1); MSR, methionine sulfoxide reductase; NF-κB, nuclear factor κB; AP-1, activator protein 1; and ASK1, apoptosis signal-regulating kinase 1, which is the reduced form of glutathions. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

Trx System

Trx is ubiquitously found in organisms from prokaryotes to mammals. They act as an antioxidant by reducing disulfide bonds to thiols (4, 51, 69). Trx is a 12-kDa enzyme that itself contains a active site composed of two cysteines that are capable of redox chemistry and are integral in the enzymes ability to reduce other proteins such as ribonuclease, chorionadotropins, coagulation factors, glucocorticoid receptor, and insulin (50, 55). Thioredoxin reductase (TrxR) regulates the redox status of Trx in a NADPH/$NADP^+$-dependent manner. The active site of mammalian TrxR includes a selenocysteine (-Cys-SeCys-), which participates in redox chemistry. TrxR, which include TrxR1 (cytosol and nucleus) and TrxR2 (mitochondria), catalyze the oxidation–reduction of Trx. TrxR's own redox state is mediated by the interconversion of NADPH and $NADP^+$. TrxR act as electron donors to a number of enzymes, including Prx. Prx can act as an antioxidant to catalyze the conversion of hydrogen peroxide to water. Trx, TrxR, and Prx form a system of enzymes that regulate the redox state in cells (Fig. 3A) and regulate cell proliferation and apoptosis, as well as intracellular signaling cascades by modulating various signal transduction proteins, transcription factors, and intracellular enzymes (Fig. 3C).

Glutathione System

Glutathione is the main nonprotein thiol in cells and exists at a high concentration of up to 5 m*M* (19). Glutathione is a tripeptide with a reactive thiol group that allows it to exist as reduced glutathione (GSH) and oxidized glutathione (GSSG) states. In the reduced state, GSH is able to convert a disulfide bond to two thiols. In addition, after donating an electron, GSH readily reacts with other reactive GSHs to from a disulfide, which is the reduced form of glutathione. The enzyme GSH reductase regulates the redox status of GSH analogous to how TrxR regulates Trx. GSH can transfer its reducing equivalents to GSH peroxidases, which catalyze the conversion of hydrogen peroxide to water. An increased GSSG/GSH ratio is considered indicative of oxidative stress (Fig. 3B) (9). The changes in redox status mediated by GSH system also contribute to regulating DNA synthesis and repair, cell signaling, cell proliferation, and apoptosis (Fig. 3C).

Importance of Intracellular Ca^{2+}

The presence and regulation of the divalent cation calcium (Ca^{2+}) is fundamentally important to the functions of that constitute life at the cellular level. Much energy is expended in

extruding Ca^{2+} from the cytoplasm and sequestering Ca^{2+} into the sarcoplasmic (or endoplasmic) reticulum, mitochondria, and extracellular space (to maintain a low concentration of Ca^{2+} in the cytosol). Ca^{2+} gradients across the plasma membranes allow for the function of metabolism, nerve propagation, muscle contraction, and other important processes. Ca^{2+} is also fundamentally important in the regulation of transcription, translation, cellular division, and apoptosis (23, 28). It is then unsurprising that the regulation of intracellular Ca^{2+} levels in the cytosol and organelles is fundamentally involved in many forms of pathophysiology, including diseases involving the vasculature (23, 72, 76).

The concentration of Ca^{2+} in different intracellular compartments and the flux of Ca^{2+} between compartments are of great importance in the function of smooth muscle cells and endothelial cells. Since smooth muscle and endothelial cells are the principle cells in determining vascular tone and blood vessel diameter, the regulation of cytosolic Ca^{2+} is of great importance to the physiological function of the vasculature. Abnormalities in intracellular Ca^{2+} regulation and chronic elevation of resting $[Ca^{2+}]_{cyt}$ have been linked to pulmonary and systemic arterial hypertension. Therefore, understanding the function and regulation of the ion channels and pumps, which mediate Ca^{2+} transportation across the plasma membrane and intracellular organelle membranes, is of critical importance.

Intracellular Ca^{2+} Homeostasis

The lipid membrane is impermeable to Ca^{2+}. Transportation of Ca^{2+} across the plasma membrane (and the SR/ER membrane) takes place through various Ca^{2+} channels, pumps, and exchangers. The Ca^{2+}-permeable channels allow Ca^{2+} to flux through the membrane based on its electrochemical gradient, the Ca^{2+} pumps (*e.g.*, Ca^{2+}/Mg^{2+} ATPase or Ca^{2+} pump) transport Ca^{2+} against its concentration gradient using energy produced by ATP, and the Ca^{2+} exchangers (*e.g.*, Na^+/Ca^{2+} exchanger, NCX) can transport Ca^{2+} across the membrane using the transmembrane electrochemical gradients of other ions (*e.g.*, the transmembrane Na^+ gradient for NCX) (Fig. 4).

The extracellular Ca^{2+} concentration is 1.6–1.8 m*M*, whereas the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) is ~100 n*M*. Various Ca^{2+} transporters are functionally involved in maintaining the concentration gradient of Ca^{2+} across membranes and the low resting $[Ca^{2+}]_{cyt}$ (29). The Ca^{2+} concentration in the intracellular stores, such as SR/ER, can be as high as 1 m*M* because of the active Ca^{2+} pumps on the SR/ER membrane (*i.e.*, sarcoplamic endoplasmic reticulum Ca^{2+} ATPase [SERCA]), which uses the energy produced by ATP to constantly transport (or uptake) Ca^{2+} from the cytosol to the SR/ER (Fig. 4). In addition, intracellular Ca^{2+} level is also regulated by many other Ca^{2+} transporters, such as Na^+/Ca^{2+} exchangers (NCX) and H^+/Ca^{2+} exchangers in the plasma membrane and SR/ER membrane, and Ca^{2+} uniporters on the mitochondrial membrane (29).

$[Ca^{2+}]_{cyt}$ can be increased by Ca^{2+} release from the SR/ER through inositol-1,4,5 phosphate receptors (IP_3R) and ryanodine receptors (RyR) on the SE/ER membrane, and Ca^{2+} influx through different Ca^{2+} channels on the plasma membrane. There are three major Ca^{2+}-permeable channels

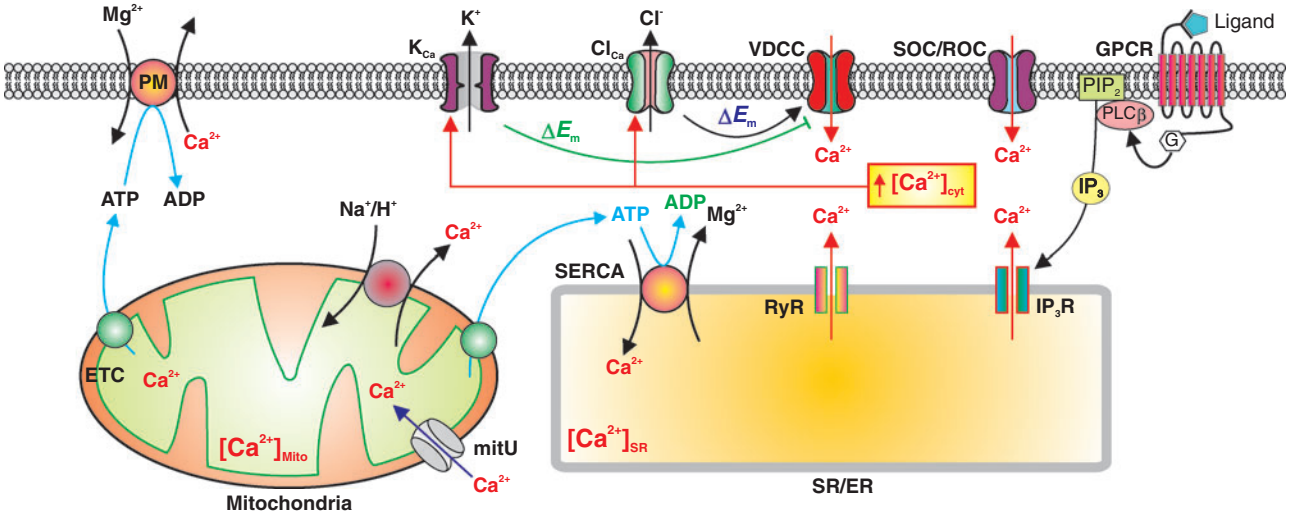

FIG. 4. The concentration of cytosolic Ca^{2+} ($[Ca^{2+}]_{cyt}$) is tightly regulated by various Ca^{2+} channels and transporters. Activation of membrane receptors, such as G protein-coupled receptors (GPCR), stimulates IP_3 synthesis, induces Ca^{2+} release from the SR/ER through IP_3R, and increases $[Ca^{2+}]_{cyt}$. The IP_3-induced store depletion opens store-operated Ca^{2+} channels (SOC), induces store-operated Ca^{2+} entry (SOCE), and further increases $[Ca^{2+}]_{cyt}$. Further, membrane depolarization (induced by decreased activity of K^+ channels and Na^+/K^+ ATPase) opens voltage-dependent Ca^{2+} channels (VDCC), increases Ca^{2+} influx, and increases $[Ca^{2+}]_{cyt}$, whereas membrane depolarization can also activate ryanodine receptors (RyR) and induce Ca^{2+} mobilization from the SR/ER to the cytosol. $[Ca^{2+}]_{cyt}$ can be decreased by Ca^{2+} sequestration into the SR/ER and mitochondria by SERCA and the mitochondrial Ca^{2+} uniporter (mitU), respectively, and by Ca^{2+} extrusion *via* the Ca^{2+}/Mg^{2+} ATPase (Ca^{2+} pump, PM) on the plasma membrane. $[Ca^{2+}]_{cyt}$ can be increased not only by Ca^{2+} release from the SR/ER through RyR/IP_3R, but also from the mitochondria Na^+/Ca^{2+} exchanger. A rise in $[Ca^{2+}]_{cyt}$ can (i) open Ca^{2+}-activated K^+ (K_{Ca}) channels and cause membrane hyperpolarization or repolarization, which closes VDCC, and (ii) open Ca^{2+}-activated Cl^- (Cl_{Ca}) channels and cause membrane depolarization, which activates VDCC and causes further increase in $[Ca^{2+}]_{cyt}$. IP_3R, inositol-1,4,5 phosphate receptors; SERCA, sarcoplamic endoplasmic reticulum Ca^{2+} ATPase; PLCβ, phospholipase C *β*. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

in the plasma membrane that are functionally expressed in VSMC: (a) voltage-dependent Ca^{2+} channels (VDCC) that are opened by membrane depolarization, (b) receptor-operated Ca^{2+} channels (ROC) that are opened by diacylglycerol (DAG) synthesized *via* PIP_2 when membrane receptors are activated by ligands, and (c) store-operated Ca^{2+} channels (SOC) that are opened when Ca^{2+} in the intracellular Ca^{2+} stores (*e.g.*, SR/ER) is depleted by, for example, activation of IP_3R/RyR and inhibition of SERCA on the SR/ER membrane (Fig. 4).

While the concentration of Ca^{2+} and proportion of Ca^{2+} bound to calmodulin (CaM) in the nucleus are important in regulating various transcription factors involved in VSMC physiology and pathophysiology, the concentration of Ca^{2+} in the nuclear compartment is unregulated and roughly equal to the cytosolic compartment due to free flow though large nuclear pores. By sequestering Ca^{2+} into the SR/ER and, to a lesser extent, into mitochondria, and by extruding Ca^{2+} from the cytosol to the extracellular space, the concentration of Ca^{2+} in the cytoplasm is maintained at a low level such that influx of Ca^{2+} from outside the cell or release of Ca^{2+} from intracellular stores requires no energy and functions as a signaling event for cell contraction, migration, and proliferation. In smooth muscle cells, a rise in $[Ca^{2+}]_{cyt}$ leads to cell contraction, increases cell migration, and stimulates cell proliferation. Therefore, an acute rise in $[Ca^{2+}]_{cyt}$ can cause vasoconstriction, whereas chronically elevated $[Ca^{2+}]_{cyt}$ leads to thickening of the vessel wall (due to its stimulating effect on cell proliferation and migration), thus decreasing the lumen of the vessel and the ability of the vessel to expand to meet increased flow.

Plasma Membrane Ca^{2+} ATPase and ROS

Both the Ca^{2+}/Mg^{2+} ATPase (or Ca^{2+} pump) in the plasma membrane and CaM in the cytoplasm are regulated by redox state. ROS inactivates the Ca^{2+} pump in the plasma membrane (PMCA), which leads to less pumping of Ca^{2+} out of the cytoplasm into the extracellular space and an increase in Ca^{2+} concentration in the cytoplasm. While the specific amino acids that mediate inactivation of PMCA by ROS have not been determined experimentally, an *in silico* analysis suggests that residues Tyr589, Met622, and Met831 are responsible (54). CaM, which is also regulated by oxidative modification, binds to PMCA and induces the dissociation of the autoinhibitory domain from the active site of the enzyme and causes a several fold increase in PMCA's Ca^{2+} transportation rate. Oxidation of CaM methionine residues 144 and 145 by ROS may lead to the inhibition of PMCA and increased $[Ca^{2+}]_{cyt}$ (3, 13).

SERCA and ROS

The Ca^{2+} pump on the SR/ER membrane (SERCA) sequesters Ca^{2+} from the cytoplasm to the SR/ER in many cell types, including vascular smooth muscle and endothelial cells. It has been reported that SERCA is inhibited by ROS or reactive nitrogen species ROC receptor-operated channel (RNS). Early reports suggested that NO activated SERCA in VSMC, thus leading to decreased $[Ca^{2+}]_{cyt}$ and vasorelaxation (2). While this finding conflicts with the general trend that ROS inhibits Ca^{2+} pumps, other studies have shown that ROS inhibit SERCA activity by modifying cysteine residues (6, 33, 97). The different SERCA isoforms contain between 22 and 28 cysteine residues, and it is currently believed that the redox state of these residues is crucial for SERCA function. In fact, in atherosclerotic arteries, the intracellular ROS is increased and up to 9 SERCA cysteine residues are irreversibly sulfonylated (2).

IP_3R and RyR and ROS

Activation of IP_3R and RyR on the SR/ER membrane allows Ca^{2+} release from the SR/ER to the cytosol through the Ca^{2+} release channels and, upon stimulation or activation of receptors in the plasma membrane, lead to an increase in $[Ca^{2+}]_{cyt}$ and vasoconstriction. In accordance with the trend of ROS elevating $[Ca^{2+}]_{cyt}$, ROS stimulate the activity of IP_3R and RyR. Early reports from several groups demonstrated that oxidation of thiol groups by endogenous redox agents, such as superoxide and GSSG, promotes IP_3R-mediated Ca^{2+} release in different cell types as well as from sarcoplasmic reticulum isolated from VSMC (10, 58, 79, 82). The thiol reagent thimerosal caused elevation of $[Ca^{2+}]_{cyt}$ by increasing the sensitivity of IP_3R to the resting levels of IP_3 (38). Further, endogenous hydrogen peroxide could also increase the sensitivity of IP_3R to IP_3, demonstrating the physiological relevance of the effect of ROS on IP_3R activity in human aortic endothelial cells (43). ROS modify RyR activity by targeting hyperreactive cysteine thiol residues (36, 37, 65). Skeletal muscle primarily contains the RyR1 isoform, cardiac muscle the RyR2 isoform, and brain and smooth muscle the RyR3 isoform although small amounts of the other two isoforms are also expressed in VSMC. Modification of RyR cysteine residues by ROS results in the activation of RyR and Ca^{2+} mobilization from the SR/ER to the cytoplasm. It is believed that Cys3635 is involved in RyR1 redox sensing (65).

VDCC and ROS

The membrane potential of VSMCs is critical in controlling $[Ca^{2+}]_{cyt}$. L-type VDCC on the plasma membrane are opened by membrane depolarization and allows the influx of Ca^{2+} to the cytoplasm. This increase of $[Ca^{2+}]_{cyt}$ activates Ca^{2+}/CaM-dependent myosin light chain kinase (MLCK) and ultimately causes smooth muscle contraction. In isolated arteries, high K^+ solution causes a sustained contraction, which is mainly due to membrane depolarization and opening of VDCC in VSMC. In addition to activating MLCK and causing contraction, a localized rise in cytosolic $[Ca^{2+}]$ due to Ca^{2+} release through RyR on the SR/ER membrane leads to Ca^{2+} sparks, which subsequently opens Ca^{2+}-activated K^+ (K_{Ca}) channels in the plasma membrane, induces membrane hyperpolarization, and causes relaxation of VSMCs. Further, extrusion of Ca^{2+} by the PMCA or reuptake of Ca^{2+} by SERCA can also decrease $[Ca^{2+}]_{cyt}$ and lead to vasorelaxation.

VDCC on the plasma membrane are affected by exogenous and endogenous redox compounds (42). However, there is controversy regarding the effect of oxidation on L-type VDCC activity. A study in isolated guinea pig ventricular myocytes demonstrated that oxidation of thiol groups in L-type VDCC inhibited Ca^{2+} entry (47). Further, exogenous reagents inhibited Ca^{2+} currents though these channels when expressed in HEK293 cells and DTT reversed this inhibition (14). In contrast to these findings, however, thiol reducing agents decreased basal L-type Ca^{2+} currents in ventricular myocytes and oxi-

dizing agents increased these currents independent of cAMP and G-protein, suggesting the involvement of thiol groups (99). Also, transient exposure of cardiac myocytes to hydrogen peroxide increased basal L-type channel activity (93).

ROC and SOC

$[Ca^{2+}]_{cyt}$ is also affected by influx of Ca^{2+} through ROC and SOC on the plasma membrane (Fig. 5). Activation of membrane receptors, for example, G protein-coupled receptors (GPCR) and tyrosine kinase receptors, leads to increased synthesis of DAG and IP_3. DAG opens ROC, promotes receptor-operated Ca^{2+} entry, and increases $[Ca^{2+}]_{cyt}$ (Fig. 5A). IP_3 activates IP_3R on the SR/ER membrane, mediates Ca^{2+} release or mobilization from the intracellular stores to the cytosol, and increases $[Ca^{2+}]_{cyt}$. Depletion of Ca^{2+} in the intracellular stores (*e.g.*, SR/ER) by IP_3-mediated Ca^{2+} mobilization opens SOC on the plasma membrane and induces store-operated Ca^{2+} entry (SOCE), which is also referred to as capacitative Ca^{2+} entry (Fig. 5B). In addition to active depletion of Ca^{2+} from the intracellular stores *via* IP_3-mediated Ca^{2+} release, passive depletion of Ca^{2+} from the SR/ER by inhibition of SERCA with cyclopiazonic acid or thapsygargin can also activate ROC and induce receptor-operated Ca^{2+} entry in vascular smooth muscle and endothelial cells. It has been demonstrated that functional ROC and SOC are formed by TRP channel subunits.

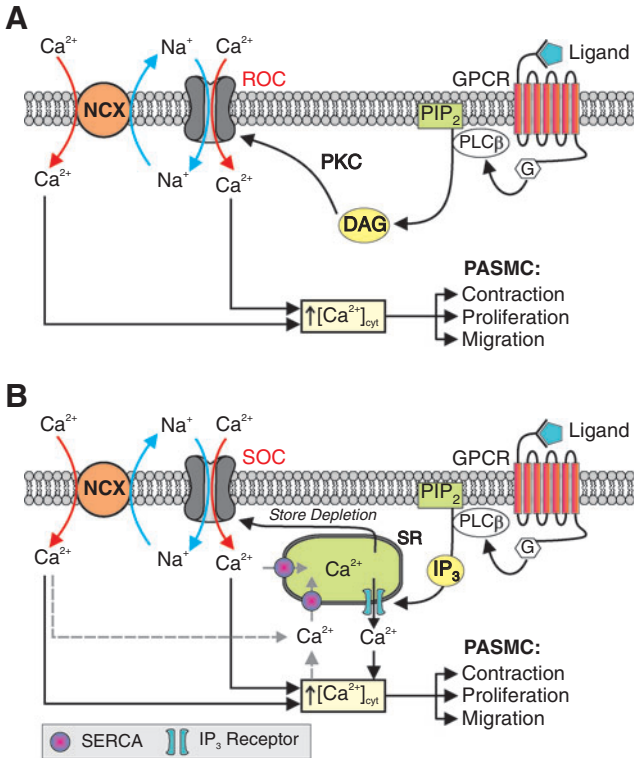

FIG. 5. ROCE and SOCE occurs through ROC or SOC. (A) GPCR signal though second messengers diacylglycerol (DAG), which opens ROC on the plasma membrane. Ca^{2+} influx though ROC (termed ROCE) increases $[Ca^{2+}]_{cyt}$ and leads to VSMC contraction, proliferation, and migration. **(B)** Alternatively, second messenger IP_3 activates IP_3R on the SR/ER membrane and causes Ca^{2+} release or mobilization from the SR/ER to the cytosol. The subsequent depletion of Ca^{2+} from the stores opens SOC on the plasma membrane and leads to SOCE. Further, increased cytosolic $[Na^+]$ due to Na^+ influx through ROC and SOC can activate the reverse mode of Na^+/Ca^{2+} exchange and cause inward transportation of Ca^{2+}. ROC, receptor-operated Ca^{2+} channels; ROCE, receptor-operated Ca^{2+} entry. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

TRP Channels

TRP channels are cation channels that mainly mediate the influx of Na^+ and Ca^{2+} across the plasma membrane and into the cytoplasm (7). TRP channels were first discovered due to a mutation in *Drosophila* photoreceptor, which resulted in inhibited Ca^{2+} permeability and sensitivity to light (70). The influx of cations into the cytoplasm depolarizes cells and is necessary for action potentials in excitable cells such as neurons (85). In nonexcitable cells, membrane depolarization by TRP channels stimulates voltage-dependent channels (Ca^{2+}, K^+, Cl^-) and influences many cellular events such as transcription and translation (15), whereas increased Ca^{2+} entry through TRP channels is involved in ligand-mediated cell contraction, migration, and proliferation. TRP channels and their regulation are thus fundamentally important in cellular function and disease (7).

TRP genes are expressed in organisms from archaea to plants to animals (18). In animals, TRP is expressed in brain, heart, lung, and other tissues (18). The expression profile of TRP proteins in vascular tissues is shown in Table 1. Mammalian TRP channels possess a high degree of sequence homology particularly in the putative functional regions (59). The *TRP* superfamily of genes is categorized into two groups based on sequence and topological similarities (59). Group 1 includes *TRPC*, *TRPV*, *TRPM*, *TRPA*, and *TRPN*. Group 2 includes *TRPP* and *TRPML* (Fig. 6).

Structure, Function, and Regulation of TRP Channels

TRP proteins form cation channels with varying selectivity to different cations (63). TRP proteins are transmembrane proteins with six transmembrane domains with a pore domain wedged between the fifth and sixth transmembrane domains (Fig. 6). The N- and C-terminal domains are intracellular and believed to be involved in regulation of TRP channel function and in channel assembly. It is believed that TRP channels are homo- or hetero-tetramers of TRP proteins with each subunit contributing to selectivity of the ion-conducting pore (17). Allosteric interactions between subunits are thought to contribute to gating of TRP channels; however, the location and structure of these gates are unknown. Amino acid sequences flanking the pore-forming regions of TRP proteins are strongly conserved across the various TRP channel families highlighting their importance in pore formation and/or pore gating (61).

TRP channel function is regulated by four basic mechanisms: (a) plasma membrane receptor activation, (b) ligand activation, (c) direct activation, and (d) indirect activation (71). GPCR and receptor tyrosine kinase act through DAG (as well as IP_3-mediated store depletion) to activate TRP channels on the plasma membrane (39, 64). Further, activation of GPCRs and receptor tyrosine kinases also increase synthesis of IP_3, depletes Ca^{2+} from the intracellular Ca^{2+} stores (*i.e.*, SR/ER)

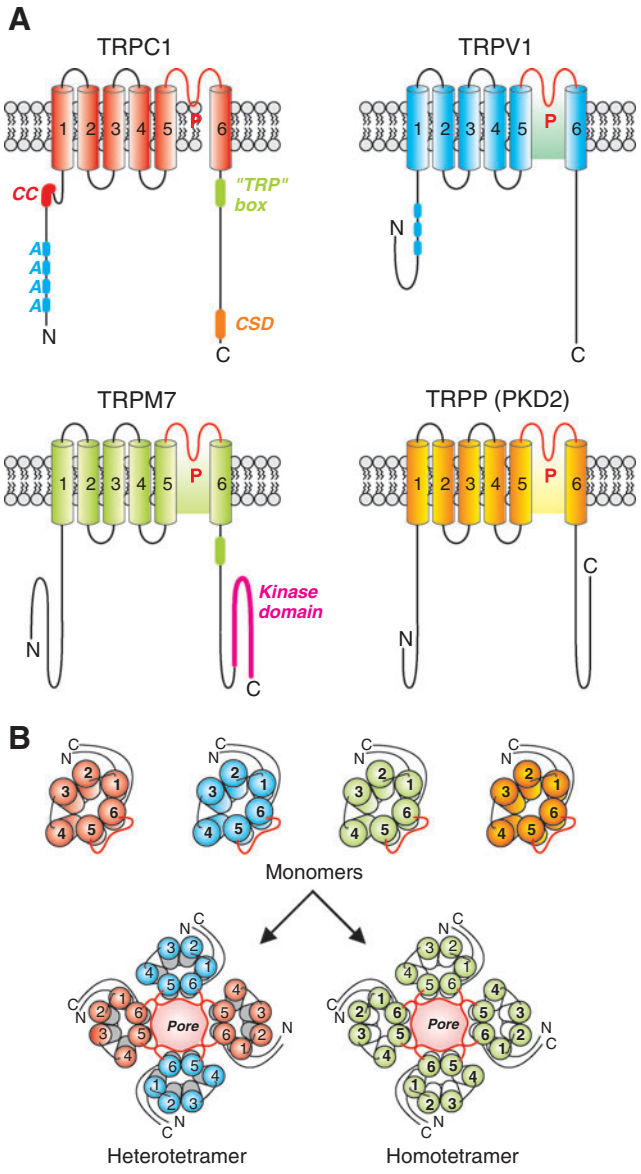

FIG. 6. Structure and topology of TRP channels. (A) TRPC, TRPV, TRPM, and TRPP classes of TRP channels are six-transmembrane domain ion channels in the plasma membrane. The pore forming domain (P) lies between the S5 and S6 transmembrane domains. TRPC1 contains a coiled-coil domain and many ankyrin repeats near its N-terminus. Near the C-terminal domain of TRPC1 is a TRP box domain, which is a conserved region following the last transmembrane domain, and a caveolin-1 scaffolding domain (CSD). TRPV1 also contains several ankyrin repeats. TRPM7 contains a kinase domain near its C-terminus. **(B)** Functional TRP channels are hetero or homo-tetramers formed by various TRP monomers. TRP, transient receptor potential. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

and leads to SOCE through TRP channels on the plasma membrane (95). The TRP channels that are activated by DAG are termed ROC, whereas the TRP channels that are activated by IP_3-mediated store depletion are termed SOC. Most of TRP channel subunits can form both ROC and SOC in many cell types, including vascular smooth muscle and endothelial cells.

Various ligands can activate TRP channels. Ligand activation includes activation by (a) exogenous small molecules such as capsaicin, icilin, 2-APB; (b) endogenous lipids such as DAG, phosphoinosidides, eicosanoids, purine nucleotides; (c) ions such as Ca^{2+} and Mg^{2+}; and (d) the Ca^{2+}/CaM complex (92, 105, 107). TRP channel activity can be stimulated through direct activation as well. Examples of direct activation of TRP channels include temperature change (16), mechanical stimulation (56), and conformational coupling with other proteins such as stromal interacting molecule 1 (STIM1) or IP_3R. Indirect activation refers to transcriptional control or insertion of vesicles containing TRP proteins into the plasma membrane.

Over 10 TRP isoforms are expressed in the vasculature. Expression of mRNA, protein, or both of TRPC1-7, TRPV1-4, TRPP2, TRPM2-4, -7, -8, has been implicated in different vascular smooth muscle and endothelial cells. Of the different TRP isoforms expressed in the vasculature, much work has been done on the canonical class of TRP channels, or TRPC channels, and this review will focus on this class of proteins.

TRPC

TRPC1-7 are categorized into three categories based on sequence and functional characteristics (18). TRPC1, 4, and 5 form one group. TRPC1 was the first mammalian TRP protein discovered. It is widely expressed in many tissues and thought to form heteromeric channels with TRPC4 and TRPC5 (96). TRPC4 and TRPC5 are believed to form homomeric channels. When expressed together, TRPC1, 4, and 5 form nonselective cation channels that are activated by G_q signaling through a phospholipase C β1 pathway (66). Growth factor stimulates rapid translocation of TRPC5 into the plasma membrane from vesicles located near the plasma membrane (44).

TRPC3, *6*, and *7* have roughly 75% sequence homology and when co-expressed reconstitute nonselective, inward and outward rectifying, cation channels (71). These channels are activated by receptor-mediated pathway involving DAG and are believed to be important in vascular and airway smooth muscle (40). Channels formed by TRPC3 or TRPC6 are also regulated by N-linked glycosylation and Ca/CaM (20). TRPC3 is activated by phosphorylation by protein kinase G (PKG) (45), whereas TRPC6 can be phosphorylated by the Src family of tyrosine kinases (91). *TRPC2* shares roughly 30% sequence homology with *TRPC3/6/7* (90). *TRPC2* full-length mRNA is expressed in mouse and rat tissues (53). However, *TRPC2* is a pseudogene in humans (71).

TRPC1 is expressed in the vasculature and is linked to Ca^{2+} entry as an ROC and an SOC. Expression and knockdown data support the theory that TRPC1 forms heteromultimeric channels with TRPC3-5 and TRPP2 in the vasculature. Further, TRPC1 may be the key isoform responsible for SOCE that causes pulmonary vasoconstriction and stimulates PASMC proliferation (14, 17, 18). Our lab has previously demonstrated that siRNA-mediated knockdown of TRPC1 in PASMC mitigates the SOCE phenomenon, thus lending credence to the theory that TRPCs can function as SOCs as well as ROCs (13, 19).

TRPC3 and 6 are expressed in vascular tissues and are believed to function primarily as ROCs, although TRPC6 may also form heterotetrameric channels with other TRP isoforms that can be activated by store depletion in pulmonary vas-

cular smooth muscle and endothelial cells. TRPC6 is expressed in VSMCs in the systemic and pulmonary vasculature. Knockout of TRPC6 abrogates the hypoxic pulmonary vasoconstriction and hypoxia-induced cation influx. TRPC4 is predominantly expressed in the endothelium and it is important in regulating lung microvascular permeablilty (44), agonist-dependent vasorelaxation (43), and gene transcription (45). While TRPC4 is expressed at a lower level in VSMC, it may play a role in regulating contraction and proliferation in both store and receptor-mediated manners. TRPC5 expression in VSMC is unclear. Some researchers describe TRPC5 protein and transcript expression in PASMCs and pulmonary artery endothelial cells, whereas other researchers show conflicting data. TRPC7 is thought to contribute to both ROC and SOC formation.

TRPC Channels and Contraction and Proliferation of SMCs

TRPC channels are important in the regulation of vascular tone since they mediate the Ca^{2+} influx that mediates agonist-induced vasoconstriction and mitogen-mediated smooth muscle cell proliferation. Further, the ability of TRPC channels to alter $[Ca^{2+}]_{cyt}$ with a change in membrane potential lends them the ability to modulate vasoconstriction and vasorelaxation through a voltage-independent mechanism. Agonist- and hypoxia-induced pulmonary vasoconstriction is believed to be, at least in part, mediated though Ca^{2+} influx through TRPC1 and TRPC6 channels. Upregulated TRPC channel expression, enhanced SOCE, and increased $[Ca^{2+}]_{cyt}$ are associated with enhanced proliferation of PASMCs isolated from patients with idiopathic pulmonary arterial hypertension (102).

TRPC and ROS

TRPC3 and TRPC4 expressed in HEK293 cells formed redox-sensitive cation channels that were activated by hydrogen peroxide (32). It is, however, unknown if the activation of TRPC3 and TRPC4 is mediated through the oxidation–reduction of cysteine thiol groups. It has been suggested that hydrogen peroxide may indirectly activates TRPC3 and TRPC4 channels though activation of phospholipase C. In endothelial cells, TRPC3 and TRPC4 are believed to be able to form heteromeric channels that are redox sensitive. In these cells, tert-butylhydroperoxide leads to membrane depolarization with currents that resembled TRP currents in terms of cation selectivity, La^{3+} sensitivity, and a lack of voltage dependence. Further, expression of the N-terminal fragment of human TRPC3, but not the C-terminal fragment, abolished the oxidant-induced cation current. These data suggest that TRPC3 forms channels that are activated by ROS in porcine aortic endothelial cells (31). Later, the same group of investigators showed that TRPC3 and TRPC4 associate to form heteromeric channels in porcine aortic endothelial cells and HEK293 cells (67). These TRPC3/4 heterometic channels were activated by cholesterol oxidase, suggesting that they are regulated by redox state. However, no direct evidence links TRPC4 itself to redox regulation. TRPC5 homotetrameric channels and TRPC5/TRPC1 heterotetrameric channels, expressed in HEK293 cells, are activated by extracellular Trx. Reduced Trx breaks a disulfide bond in the extracelluar loop adjacent to the ion selectivity filter of TRPC5 and increase the ion conductivity of the channel (Fig. 7) (98).

TRPC and NO

NO is a cell signaling molecule involved in a number of physiological and pathophysiological processes. In the nervous system, NO is a neural transmitter, whereas in the vascular system, NO is mainly an endothelium-derived relaxing and hyperpolarizing factor. NO is synthesized by NOS, which include at least three isoforms: endothelial NOS, inducible NOS, and neuronal NOS. Each of the enzymes is a homodimer requiring three co-substrates (L-arginine, NADPH, and O_2) and five cofactors (FAD, FMN, Ca^{2+}-CaM, heme, and tetrahydrobiopterin) for activity. The redox biochemistry of NO involves the action of three redox species: NO^+ (nitrosonium), NO^- (nitroxyl anion), and NO• (the free radical gas). NO can react with oxygen (O_2), superoxide anion ($O_2^{\bullet -}$), redox metals, and other radical species. The effects of NO can be mediated through the formation of reactive nitrogen oxide species from reactions of NO with either oxygen or superoxide anion. There are several mechanisms by which NO affects the function of living cells: (a) oxidation of iron-containing proteins, (b) ADP ribosylation of proteins, (c) nitrosylation of protein sulfhydryl group, (d) activation of iron regulatory factors, (e) nitration of amino acid residues in proteins, and (f) activation of soluble guanylate cyclase. In other words, NO can directly cause nitrosative reaction by adding the equivalent of NO^+ to an amino,

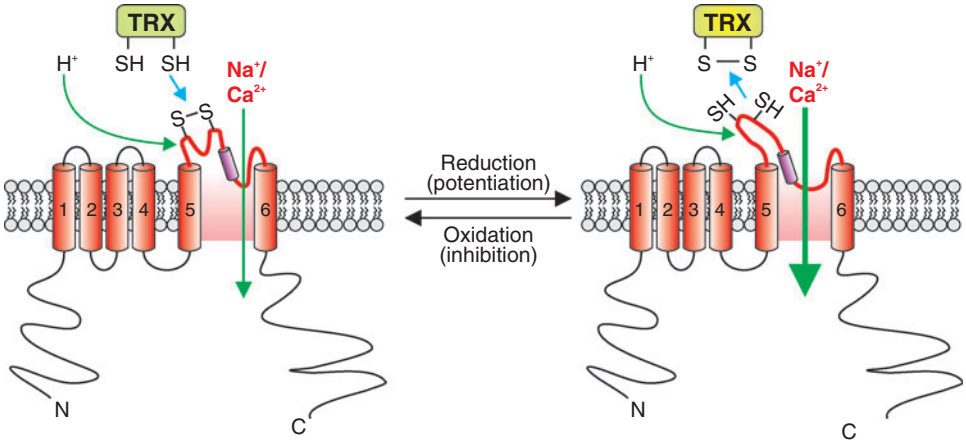

FIG. 7. Oxidation and reduction of disulfides can affect TRP channel function. Oxidation of disulfide bonds near the pore region of TRP channels are thought to allow for increased Ca^{2+} influx, whereas reduction of thiols to disulfides is thought to inhibit the channel. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

thiol, or hydroxyl aromatic group, oxidative reaction by removal of 1 or 2 electrons from a substrate, or nitration reactions by adding an equivalent of an NO_2^+ to an aromatic group.

In VSMC, NO mainly acts through the stimulation of the soluble guanylate cyclase, which is a heterodimeric enzyme with subsequent formation of cyclic GMP (cGMP). cGMP activates PKG, which causes phosphorylation of myosin light chain phosphatase and therefore inactivation of MLCK and leads ultimately to the dephosphorylation of the myosin light chair, causing smooth muscle relaxation. In addition to phosphorylation of myosin light chain phosphatase, cGMP/PKG also causes phosphorylation of other proteins, such as TRPC6 channels (84). Indeed, NO/cGMP/PKG-mediated phosphorylation of threonine (Thr69) in the N-terminal of TRPC6 protein significantly attenuates receptor-mediated Ca^{2+} entry through TRPC6 channels (Fig. 8A, B) (84). These observations imply that PKG-mediated phosphorylation of specific amino acid residues (*e.g.*, threonine and serine residues) in the TRPC6 protein causes inhibition of the channel activity. In contrast, NO-mediated cGMP-independent S-nitrosylation of certain cysteine residues in the TRPC1/4/5 and TRPV1/3/4 proteins significantly enhances Ca^{2+} influx through these channels (25, 101). Specifically, for example, Cys553 and Cys558 of TRPC5 are localized in the cytoplasm and mediate the channel's sensitivity to NO/S-nitrosylation in endothelial cells (Fig. 8B–D) (25, 101). S-nitrosylation of TRPC5 in endothelial cells upon G protein-coupled ATP receptor stimulation elicits Ca^{2+} entry. These observations imply that cGMP/PKG-dependent phosphorylation and cGMP/PKG-independent S-nitrosylation are two different pathways for NO-mediated effect on TRP channels. Whether NO-mediated effect is to activate or inhibit the channels depends on (a) which pathway (PKG-dependent phosphorylation *vs.* PKG-independent S-nitrosylation) occurs faster to reach the target protein (or which pathway is predominant in a given cell), (b) which TRP channel subtype (*e.g.*, TRPC5 *vs.* TRPC6) is predominantly expressed in a given cell as the target, and (c) whether the target amino acids (*e.g.*, threonine and serine for phosphorylation, and cysteine residues for S-nitrosylation) for phosphorylation or S-nitrosylation are present in the predominant TRP channels in a given cell.

SOCE

As mentioned earlier, SOCE is the phenomenon through which depletion of intracellular Ca^{2+} stores opens SOC on the plasma membrane. Upon depletion of Ca^{2+} in the SR/ER, this Ca^{2+} deficiency is sensed and transmitted to channels on the plasma membrane to allow Ca^{2+} to flow into the cytoplasm where it is then sequestered into the SR/ER by SERCA, thus replenishing Ca^{2+} stores. The SOCE is necessary for long-term regulation of Ca^{2+} signaling. Without SOCE, SR/ER Ca^{2+} stores would become depleted when cells are stimulated with ligands and the membrane receptors are activated.

A mutation in Orai1, an important molecular component of SOCE, has been demonstrated to be associated with severe combined immune deficiency in humans. Further, upregulation or downregulation of SOCE can chronically increase or decrease the rate and magnitude of the other intracellular Ca^{2+} signaling mechanisms and have a chronic effect on cellular biology (29). Much evidence links SOCE to altered proliferation and contraction in PASMCs, which are intimately involved in the function and pathophysiology of the pulmonary circulation. Our lab has demonstrated that SOCE can increase resting $[Ca^{2+}]_{cyt}$ in PASMC independent of receptor- or voltage- dependent mechanisms. Inhibition of SOCE decreases $[Ca^{2+}]_{cyt}$ and attenuates proliferation in PASMC. Further, PASMC isolated from patients with idiopathic pulmonary arterial hypertension demonstrate higher resting $[Ca^{2+}]_{cyt}$ and greater SOCE than cells isolated from normal subjects and normotensive control patients (102, 103).

Mechanism of SOCE: STIM1 as the Ca^{2+} Sensor

Recently, much has been discovered on the mechanism of SOCE. Through genome-wide RNAi screening, investigators have identified the sensor that detects $[Ca^{2+}]$ in the SR/ER ($[Ca^{2+}]_{SR/ER}$) as STIM1 (1). STIM1 is a 685-amino acid-long single transmembrane protein that is expressed on the SR/ER membrane and plasma membrane. Near the N-terminus of STIM1 is an EF-hand domain that senses $[Ca^{2+}]_{SR/ER}$ (which is in the range of 0.5–2 m*M*). When Ca^{2+} is not bound to the EF-hand domain when, for example, the SR/ER Ca^{2+} store is depleted, STIM1 undergoes a conformational change, which allows it to multimerize, translocates to the SR/ER-plasma membrane junction (or puncta), binds with Orai1 tetramers on the plasma membrane, activates SOC, and induces SOCE (Fig. 9) (78). Mutagenesis of the EF-hand domain (mimicking low Ca^{2+}) leads to STIM1 multimerizing in distinct regions of the plasma membrane, called puncta, in the absence of store depletion and to activating SOC. Examination by total internal reflection (TIRE) microscopy shows that store depletion-induced puncta, which contains STIM1, localizes 10–20 nm from the plasma membrane, allowing for SR/ER STIM1 to interact with SOC in the plasma membrane (60).

SOC: Orai1

Strong evidence suggests that Orai1 interacts with STIM1 and functions as a SOC. Orai1 was discovered through genome-wide RNAi screening. A mutation in Orai1 in patients with severe combined immune deficiency eliminates the Ca^{2+} release-activated Ca^{2+} currents (I_{CRAC}), which can then be reconstituted by expressing wild-type Orai1 (22, 57). Orai1 spans the plasma membrane four times with both the C- and N-terminus in the cytoplasm. Co-expression of STIM1 with Orai1 in HEK293 cells resulted in large increases (roughly 25–30 times control) in SOCE and I_{CRAC} compared to vector control cells (81). Co-immunoprecipitation data show that STIM1 and Orai1 interact, and store depletion significantly increases the amount to interaction (100). Further, other isoforms of Orai (*e.g.*, Orai2 and Orai3) are also thought to play a role in SOCE (57). It is unclear, however, what the role of these Orai isoforms are in the function of smooth muscle cells.

TRPC as SOC

As mentioned earlier, another category of channel subunits that have been proposed as store operated are the TRP channels (71). Of the TRPC's, TRPC1, 3, 4, 6, and 7 have shown promise as SOC. In fact, our lab has demonstrated that inhibition of endogenous TRPC1 in PASMC decreases SOCE (83). Researchers also suggest that TRPC1, STIM1, and Orai1 form a ternary complex, which constitutes an active SOC (104).

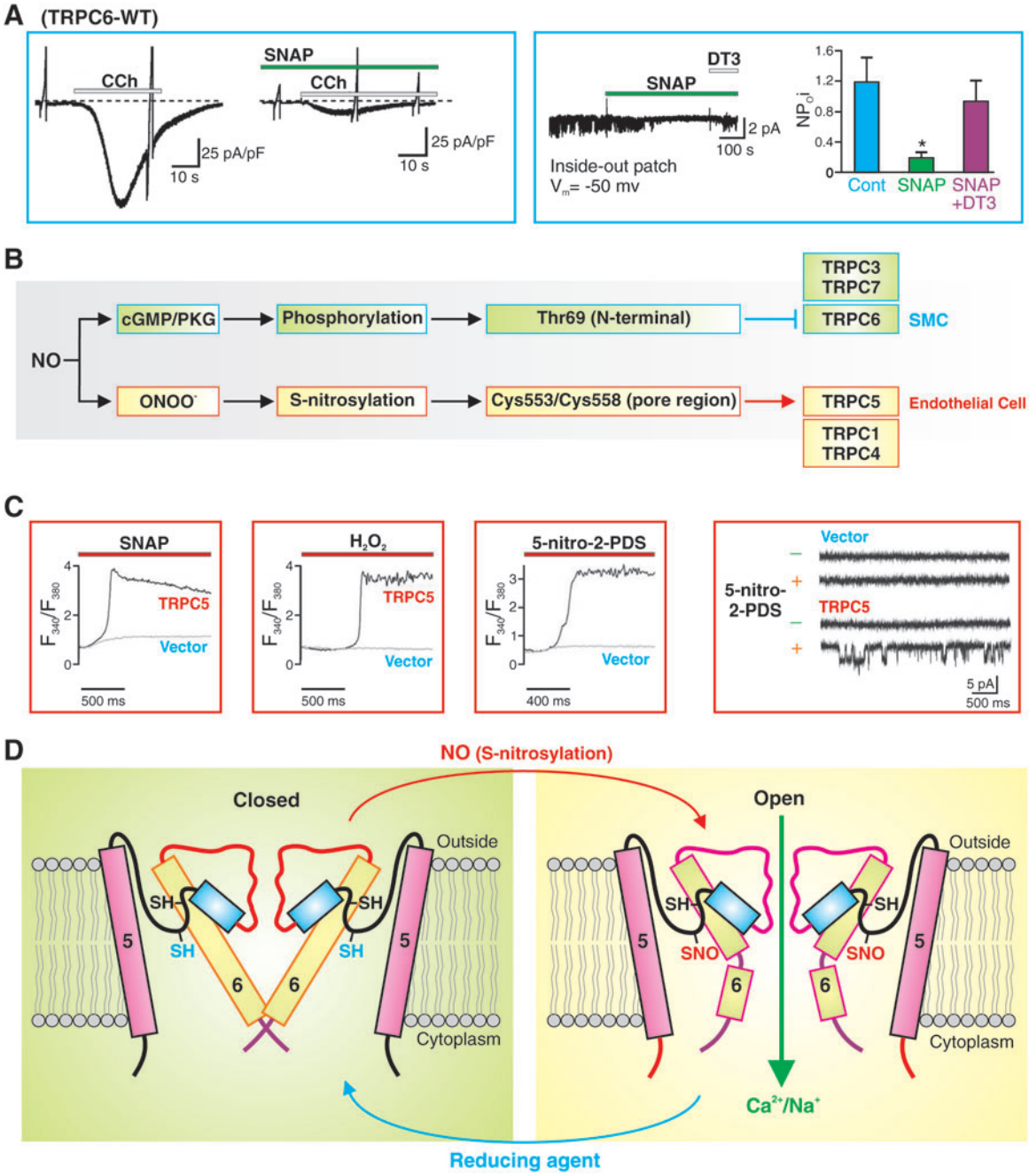

FIG. 8. NO inhibits TRPC6 channels in SMC by cGMP/PKG-dependent phosphorylation and activates TRPC5 in endothelial cells by s-nitrosylation. (A) NO donor SNAP attenuates carbocal (CCh)-induced whole-cell (left panel) and single-channel (right panel) Ca^{2+} currents through TRPC6 channels by stimulating cGMP production and PKG activation. DT3 specifically inhibits cGMP thus blocking the effect of SNAP on single-channel Ca^{2+} currents in cells overexpressed with TRPC6. **(B)** NO divergently affects TRPC6 and TRPC5 *via* different pathways. NO inhibits TRPC6 channels in SMC by stimulation of cGMP and activation of PKG. The PKG-mediated phosphorylation of Thr69 of TRPC6 channels inhibits the channel function. NO activates TRPC5 in endothelial cells by $ONOO^-$- mediated S-nitrosylation. $ONOO^-$, a reactive nitrogen species, nitrosylates the two cysteines (cys553/cys558) in the pore region of TRPC5 channels and opens the channels. **(C)** SNAP (NO donor), H_2O_2, and 5-nitro-2-PDS all significantly increase $[Ca^{2+}]_{cyt}$ in cells overexpressed TRPC6 (left panels). In TRPC6-transfected cells, 5-nitro-2-PDS also increases single-channel open probability of TRPC5 (right panel). **(D)** Schematic diagram showing that S-nitrosylation of Cys553/Cys558 in the pore region of TRPC5 channels opens the pore allowing Ca^{2+} influx. Reproduced from Refs. 88 and 89. PKG, protein kinase G; cGMP, cyclic GMP. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

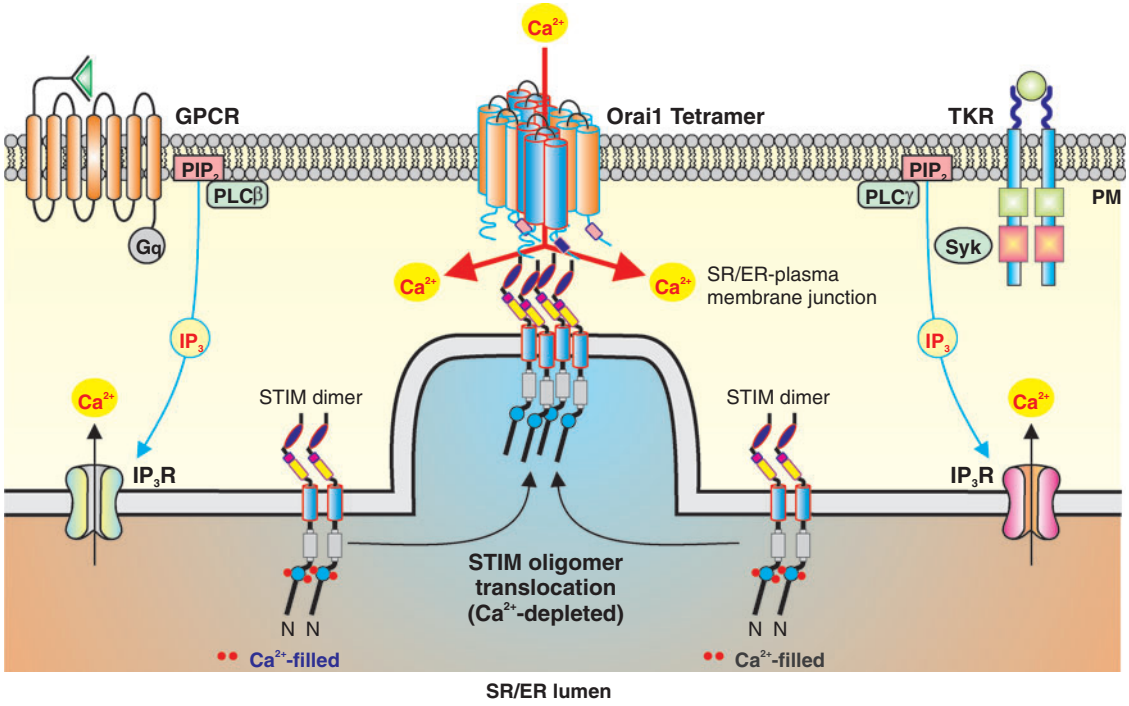

FIG. 9. Mechanism of SOCE. Stimulation of GPCR or tyrosine kinase receptors on the plasma membrane increases IP_3 synthesis, activates IP_3R on the SR/ER membrane, induces Ca^{2+} mobilization from the SR/ER to the cytosol, and leads to depletion of the SR/ER Ca^{2+} stores. The store depletion activates STIM causing them to oligomerize and translocate to regions of the SR/ER membrane near the plasma membrane (or SR/ER-plasma membrane junction). The N-terminal region of STIM is located in the SR/ER lumen and contains a Ca^{2+} binding EF-hand domain. Depletion of Ca^{2+} in the SR/ER leads to uncoupling of Ca^{2+} with the ER-hand domain leading to activation of STIM. Translocated STIM oligomers can then activate Orai1, which forms SOC as tetramers, and trigger Ca^{2+} influx (or SOCE). STIM, stromal interacting molecule. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

Table 1. TRP Expression in Vascular Tissues

	Aorta/A7r5	*Caudal artery*	*Cereb. arteriole*	*Cereb. artery*	*CA*	*MA*	*PA*	*RA*	*VEC*	*PAEC*
Species	*R, M*	*R*	*Rabbit*	*M, H, R*	*H*	*M, R, F, Rabbit*	*R, H, M*	*R, C*	*M, H, R*	*H, R, B*
TRPC										
1	m/p	m	m	m/p	p	m/p	m/p	m/p	m/p	m
2	m	–	–	m	–	–	m	–	–	m
3	m/p	m	m	m/p	p	m	m/p	m/p	m/p	m
4	m/p	–	m	m/p	p	m/p	m/p	m/p	m/p	m/p
5	m/p	–	m	m/p	p	m	m	m/p	m/p	m
6	m/p	m	m	m/p	p	m/p	m/p	m/p	m/p	m
7	m	–	–	m/p	–	–	m	m	m/p	m
TRPV										
1	m/p	–	–	–	–	m	m/p	–	–	m
2	m.p	–	–	m/p	–	m/p	m	–	–	m
3	m	–	–	–	–	–	m	–	–	–
4	m/p	–	–	m/p	–	–	m/p	–	m/p	m
TRPP2	m/p	–	–	m	–	–	–	–	–	–
TRPM										
1	–	–	–	–	–	–	–	–	–	m
2	m/p	–	–	–	–	–	m/p	–	–	–
3	m	–	–	–	–	–	m	–	–	m
4	m	–	–	m	–	–	m	–	–	m
5	–	–	–	–	–	–	–	–	–	–
6	–	–	–	–	–	m/p	–	–	–	m
7	m	–	–	–	–	m/p	m	–	–	m
8	m	–	–	–	–	–	m/p	–	–	m

Cereb., cerebral; CA, coronary artery; MA, mesenteric artery; PA, pulmonary artery; RA, renal artery; VEC, vascular endothelial cells (isolated from the middle cerebral arteriole, coronary and mesenteric artery, and aorta); PAEC, pulmonary artery endothelial cell; R, rat; M, mouse; H, human; C, canine; B, bovine; F, frog.; m, mRNA identified only; p, protein identified only; m/p, both mRNA and protein identified; –, no data available.

However, while SOCE is a fundamental process in PASMC (30, 52, 75, 83, 103, 106), pulmonary artery endothelial cells (23, 46), and intestinal epithelial cells (62, 73), it is still unclear how TRP, Orai, and STIM interact precisely to form SOC and sense store depletion in vascular smooth muscle and endothelial cells.

SOCE and ROS

Not much is known on the effect of redox state on SOCE. In thyroid cells, hydrogen peroxide seems to inhibit SOCE. However, this is likely due to activation of protein kinase C and not modification of proteins directly involved in SOCE such as the STIM, Orai, or TRP families of proteins. Both STIM and Orai proteins contain cysteine residues and therefore may in fact be regulated by redox state. TRPM2 channel is believed to be activated by hydrogen peroxide to secrete insulin in pancreatic B cells; however, this may be due to the effect of hydrogen peroxide on mitochondria (24). TRPA1 channels in peripheral nociceptive neurons are activated by covalent modification of cysteine thiol residues (5). As discussed earlier, ROS and NO regulate TRPC channels, as well as other molecular components of SOCE (*e.g.*, Orai and STIM), through different pathways, such as direct oxidation, nitration, and S-nitrosylation of the cysteine thiols and indirect phosphorylation of the threonine and serine residues mediated.

Conclusion

The regulation of intracellular $[Ca^{2+}]$ in different cellular compartments is of integral importance in the physiology and pathophysiology of the vasculature. Changes in $[Ca^{2+}]_{cyt}$, $[Ca^{2+}]_{SR/ER}$, and $[Ca^{2+}]_{Mit}$ all play important roles in regulating cell contraction, proliferation, migration, and apoptosis. The concentration of Ca^{2+} in these cellular compartments is tightly regulated by Ca^{2+} channels and transporters functionally expressed in the plasma membrane and intracellular organelle membranes. TRP channels are a family of proteins that participate in forming functional ROC and SOC in vascular smooth muscle and endothelial cells, and thus play a critical role in the regulation of vascular tone, remodeling, and injury. ROS and redox state, in general, has emerged as an important system for regulating Ca^{2+} signaling, partially through their modulatory effect on TRP channels as well as ROC and SOC. More and more studies have been focused on examining whether and how ROS and redox status regulate these channels' function, kinetics, expression, and trafficking in vascular smooth muscle and endothelial cells. Regulation of intracellular Ca^{2+} homeostasis by ROS/RNS offers explanations to the important role of ROS/RNS signaling in the vasculature physiology and pathophysiology. Further, the fact that many proteins and enzymes involved in regulating the redox status of cells are actually regulated, *per se*, by Ca^{2+} and Ca^{2+}/CaM complex makes for a complicated and interesting intracellular regulatory system; derangement of this precisely controlled system would lead to the development of cardiovascular diseases.

Much work still needs to be done to elucidate the relationship between ROS and Ca^{2+} regulation. TRPC channels have been known to be important in regulating $[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]_{SR/ER}$ in the vasculature. While researchers have made some headway in determining how ROS affect TRPC channels, more investigation needs to be conducted to, for example, (a) define the effect of ROS and RNS on STIM and Orai, two important families of proteins that mediate SOCE, (b) specify the domains and amino acid residues in TRP proteins that are differentially regulated by ROS-mediated oxidation, nitration, S-nitrosylation, and protein kinase-mediated phosphorylation, and (c) compare the ROS/redox status-mediated regulation of TRP channels, Orai and STIM, between normal and diseased tissues and cells.

Acknowledgments

This work was supported in part by grants from the National Institutes of Health (HL054043 and HL066012 to J.X.-J.Y., and DK083506 to A.M.). M.Y.S. is supported by an NIH training grant (T32 DK007202).

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Date of first submission to ARS Central, September 30, 2010; date of acceptance, December 2, 2010.

Abbreviations Used

AP-1 = activator protein 1
ASK1 = apoptosis signal-regulating kinase 1
CaM = calmodulin
cGMP = cyclic GMP
CSD = caveolin-1 scaffolding domain
DAG = diacylglycerol
GPCR = G protein-coupled receptors
GPx = glutathione peroxidase
GSH = glutathione
IP_3R = inositol-1,4,5 phosphate receptors
Kv = voltage gated potassium channel
MLCK = myosin light chain kinase
MSR = methionine sulfoxide reductase
NF-κB = nuclear factor κB
NO = nitric oxide
NOS = nitric oxide synthase
NOX = NADPH oxidase
PAEC = pulmonary artery endothelial cell
PASMC = pulmonary artery smooth muscle cell
PKG = protein kinase G
PLCβ = phospholipase C β
PMCA = plasma membrane Ca^{2+} ATPase
Prx = peroxiredoxins
RNR = ribonucleotide-diphosphate reductase
RNS = reactive nitrogen species ROC receptor-operated channel
ROC = receptor-operated Ca^{2+} channels
ROCE = receptor-operated Ca^{2+} entry
ROS = reactive oxygen species
RyR = ryanodine receptor
SERCA = sarcoplamic endoplasmic reticulum Ca^{2+} ATPase
SOC = store-operated Ca^{2+} channel
SOCE = store-operated Ca^{2+} entry
STIM = stromal interacting molecule
TRP = transient receptor potential
Trx = thioredoxin
TrxR = Thioredoxin reductase
VDCC = voltage-dependent Ca^{2+} channels
VSMC = vascular smooth muscle cells