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Redox Regulation of Transient Receptor Potential Channels in the Endothelium

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Abstract

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are important mediators of signaling pathways in the endothelium. Specific members of the transient receptor potential (TRP) superfamily of cation channels act as important Ca^{2+} influx pathways in endothelial cells, and are involved in endothelium-dependent vasodilation, regulation of barrier permeability, and angiogenesis. ROS and RNS can modulate the activity of certain TRP channels mainly by modifying specific cysteine residues or by stimulating the production of second messengers. In this review we highlight the recent literature describing in redox regulation of TRP channel activity in endothelial cells as well as the physiological importance of these pathways and implication for cardiovascular diseases.

Keywords

redox signaling; TRP channels; endothelial cells; Ca^{2+} influx

Introduction

Endothelial cells that collectively form the vascular endothelium are actively involved in the regulation of vascular tone, the exchange of nutrients between tissue and blood, and the formation of semi-permeable barriers to limit blood-borne pathogens and toxic substances from accessing sensitive tissues, such as the brain parenchyma. Ca^{2+} ions are ubiquitous second messengers that act as a unifying mediator in maintenance of endothelial function. Increases in intracellular $[\text{Ca}^{2+}]$ induce endothelium-dependent vasodilation¹, regulate endothelial cell permeability² and promotes the formation and maintenance of the blood-brain barrier³. Ca^{2+} released from intracellular stores following the opening of inositol-triphosphate receptors or Ca^{2+} influx through Ca^{2+} -permeable ion channels located in the plasma membrane increase global or localized intracellular endothelial $[\text{Ca}^{2+}]$ to stimulate

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Ca²⁺-dependent signaling pathways. Endothelial cells are non-excitable cells (i.e., lacking voltage-gated ion channels) and the activation of Ca²⁺-permeable channels occurs by chemical and physical stimuli, such as membrane stretch caused by intraluminal pressure and shear stress⁴⁻⁶, or by soluble substances produced by autocrine or paracrine mechanisms. This review is focused on the control of endothelial cell Ca²⁺ influx by reduction-oxidation reactions (redox) regulation, defined here as reactions in which the oxidation states of atoms are changed. In biological systems, redox regulation is often mediated by reactive oxygen species (ROS) and reactive nitrogen species (RNS).

Redox Signaling

ROS and RNS are small, inorganic and highly reactive compounds with short half-lives in biological systems. Due to their strongly electrophilic nature, ROS and RNS rapidly react with and modify organic molecules, such as nucleic acids, lipids and proteins. Under physiological conditions, ROS and RNS take part in numerous signaling pathways. Tissue oxidative stress occurs when generation of these agents outstrips removal and detoxification, leading to cellular damage and, in the vasculature, impaired endothelial function⁷⁻⁹. ROS and RNS are constantly generated by mitochondrial oxidative metabolism¹⁰, activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOX)¹¹ and coupled or uncoupled activity of other oxidative enzymes, most predominately endothelial nitric oxide synthase (eNOS)¹² (Figure 1). NOX enzymes hydrolyze NADPH into NADP⁺ and transfer an electron to molecular oxygen, generating superoxide (O₂⁻)¹³, which can in turn be dismutated to H₂O₂ and, in the presence of iron, converted to the highly reactive hydroxyl radical (OH⁻) by the Fenton reaction. OH⁻ reacts strongly with proteins, nucleic acids and lipids, the latter generating lipid peroxides compounds¹⁴ which can act as signaling molecules. O₂⁻ is also produced by the ubiquinone in the interface between complexes I, II and III within the electron transport chain pathway of the mitochondria¹⁵, which can then leak to the cytoplasm via voltage-dependent anion channels located in the outer mitochondrial membrane¹⁶. Under physiological conditions, eNOS generates nitric oxide (NO) that can react with amino acids containing thiol moieties, such as cysteine, leading to S-nitrosylation¹⁷. However, under pathological conditions or when the intracellular pool of the co-factor tetrahydrobiopterin is limited, eNOS uses L-arginine to produce O₂⁻¹⁸, a state known as uncoupled NOS.

ROS and RNS act on molecular entities that serve as oxidative sensors to stimulate downstream signaling pathways. Proteins containing exposed cysteine residues are the best-characterized ROS and RNS sensors. Cysteine residues are particularly susceptible to redox reactions due to the presence of sulfhydryl radicals that can be converted to thiolate (R-S⁻), a highly nucleophilic radical, at near physiological pH¹⁹. Due to its physicochemical properties, thiolate can easily react with oxidants in the protein microenvironment, either reversibly or irreversibly, including S-nitrosylation and prenylation reactions with farnesyl or geranylgeranyl²⁰. Redox molecules, such as H₂O₂, O₂⁻, lipid peroxides, peroxynitrite and nitric oxide, covalently modify cysteine residues to alter function. Reactive cysteine residues are primarily found in specialized protein domains, including catalytic pockets, regulatory segments, and metal-binding regions²¹. Numerous proteins present in mammalian tissues possess reactive cysteines in their structure, including transcription factors, such as nuclear

factor- κ B (NF- κ B), myofilaments, protein kinases¹⁰ and ion channels²². Modification of cysteine residues by ROS has been shown to alter the activity of voltage-gated Ca^{2+} channels²³, voltage-gated potassium channels²⁴ and, the primary subject of this review, transient receptor potential (TRP) channels²⁵.

Redox-Sensitive TRP Channels in the Endothelium

TRP channels are a superfamily of cation-permeable ion channels first discovered in *Drosophila melanogaster*^{26, 27} that are now recognized as broadly expressed polymodal cellular sensors involved in thermoregulation, mechanotransduction, control of vascular tone¹, nociception, and Ca^{2+} and Mg^{2+} homeostasis, among other functions²⁸. The mammalian TRP superfamily consists of 28 distinct gene products (27 are present in humans²⁹), divided into 6 subfamilies: *canonical* (TRPC), *vanilloid* (TRPV), *ankyrin* (TRPA), *mucolipin* (TRPML), *melastatin* (TRPM) and *polycystin* (TRPP)^{1, 29}. Various reports indicate detection of mRNA or protein of at least 20 TRP channels in cultured and native endothelial cells, including the redox-sensitive channels TRPM2³⁰⁻³², TRPC5^{33, 34}, TRPA1^{35, 36}, TRPV1³⁷, TRPV3^{38, 39}, and TRPV4^{40, 41}. TRPC5, TRPA1, TRPV1, TRPV3, and TRPV4 channels are modulated by covalent modification of cysteine residues by ROS and/or RNS^{42, 43}. TRPML1, a Ca^{2+} -permeable non-selective cation channel that localizes to late endosomes and lysosomes^{44, 45} is also activated by ROS *in vitro* to regulate autophagy⁴⁶. TRPML1 appears to be ubiquitously expressed but is not known to be specifically involved in endothelial function. Regulation of TRPM2 is distinctive and involves direct binding of oxidant-derived second messengers^{47, 48} (Figure 2). TRPA1, TRPV3, and TRPV4 are of particular interest in terms of endothelial cell function because these channels exhibit high Ca^{2+} permeability^{1, 36, 49, 50}.

The remainder of this review will discuss the current literature regarding redox regulation of specific TRP channels and how these pathways influence endothelial function under normal conditions and during cardiovascular disease. Here we attempt to specify if findings were derived from experiments performed in heterologous overexpression system, cultured endothelial cells, or in native tissues. Consideration of these factors is critical because cell culture conditions and overexpression of proteins often leads to phenotypic changes and experimental artifacts. We encourage the reader to carefully consider such issues when interpreting studies that are not corroborated by the use of freshly isolated endothelial cells or *in vivo* methods.

Redox Regulation of TRPM2

TRPM2 was initially described as a non-selective cation channel regulated by the second messenger adenosine diphosphate ribose (ADPR)⁵¹. A later study demonstrating that TRPM2 currents are stimulated by H_2O_2 is the first evidence of redox-dependent TRP channel activation⁵². Subsequent reports linked these two pathways and demonstrated that H_2O_2 indirectly activates TRPM2 by inducing the production of NAD^+ that is promptly converted to poly(ADPR) in the mitochondria⁵³. ADPR binds to a nudix box phosphohydrolase enzymatic domain (NUDT9-H) in the TRPM2 C-terminal tail, consequently activating the channel⁵⁴. Cyclic ADPR indirectly activates TRPM2 channels in

a similar manner⁵⁵. Interestingly, the NUDT9-H domain of TRPM2 channels has slow hydrolytic kinetics, thus it is likely involved in ADPR binding and channel gating, rather than ADPR hydrolysis^{51, 56, 57}, as opposed to the homologous mitochondrial pyrophosphatase NUDIT9⁵⁷. ADPR-induced gating of TRPM2 requires co-binding of Ca^{2+} ⁵⁸, suggesting that the channel acts as a co-incidence detector of upstream signaling events involving increases in intracellular $[\text{Ca}^{2+}]$ and generation of ADPR. TRPM2 currents are difficult to isolate and study in native cells and the *in vivo* setting because the current-voltage relationship is linear and no selective pharmacological inhibitors are currently available.

Several recent studies show that activation of TRPM2 channels by ROS in the endothelium could be involved in vascular disease. For example, H_2O_2 induced TRPM2-like currents and transient Ca^{2+} entry in “ Ca^{2+} add-back experiments” (similar to store operated Ca^{2+} entry protocols) in cultured human pulmonary artery endothelial cells. This study also linked TRPM2 activity to a reduction in transendothelial electrical resistance in cultured monolayers treated with H_2O_2 , suggesting a role for the channel in the failure of tight junctions forming endothelial barriers³⁰. This mechanism could potentially be involved in edema formation^{59, 60} and breakdown of the blood-brain barrier and neurotoxicity during oxidative stress⁶¹, although this has not been demonstrated experimentally. Another study links sustained activation of TRPM2 to endothelial cell apoptosis³¹. Apoptotic mechanisms may underlie cerebral capillary rarefaction often observed in chronic vascular diseases associated with oxidative stress, such as hypertension⁹, but this process has not yet been associated with TRPM2 activity. Recently, TRPM2 was shown to be involved in β -amyloid-induced neurovascular dysfunction, an important pathology contributing to Alzheimer’s dementia³². This study suggests that β -amyloid binds to CD36, which activates NOX and ultimately leads to TRPM2 activation by increasing the intracellular pool of ADPR³². Increased TRPM2 activity was linked to endothelial dysfunction and impairment in neurovascular coupling caused by β -amyloid, and this response was not observed in *TRPM2*^{-/-} mice³².

The role of TRPM2 on the pathophysiology of ischemic strokes has been investigated *in vivo*. TRPM2 mRNA is increased in the brain of rats after 1 and 4 weeks following transient middle cerebral artery occlusion⁶², a well-accepted model of cerebral ischemia/reperfusion injury⁶³. Increased expression appears to occur primarily in microglial cells. Clotrimazole, a purported TRPM2 blocker, successfully reduced cerebral infarct in male mice after transient focal ischemia, possibly a consequence of improved neuronal survival after oxygen-glucose deprivation⁶⁴. Clotrimazole did not reduce ischemic damage in the brain of female mice⁶⁴. A sexual dimorphic response was also observed after global cerebral ischemia caused by cardiac arrest in mice. In this study, inhibition of TRPM2 with clotrimazole was protective in males but not in females⁶⁵. Interpretation of *in vivo* experiments using this compound is difficult. The sexual dimorphic nature of the effects of clotrimazole on stroke is claimed to be caused by androgen-dependent production of ADPR after an ischemic stroke⁶⁶, although simply administering androgens to females did not cause a TRPM2-dependent increase in cerebral infarct⁶⁷. It should be noted here that clotrimazole is used medically as an anti-fungal compound and is not a selective TRPM2 blocker. Clotrimazole impedes TRPM2 activity but has many additional effects including inhibition of cytochrome P450 enzymes⁶⁸,

block of intermediate conductance Ca^{2+} activated K^+ channels⁶⁹, L-type voltage gated Ca^{2+} channels⁷⁰, ATP-gated K^+ channels⁷¹, and TRPM8⁷² channels, and activation of TRPV1 and TRPA1 channels⁷². A more convincing study using *TRPM2*^{-/-} mice showed reduced infarct after ischemia/reperfusion injury, which was a consequence of reduced neutrophil infiltration⁷³. Interestingly, transplantation of wild type bone marrow into *TRPM2*^{-/-} mice reversed the protective effect of TRPM2 deletion, possibly due to increased neutrophil infiltration⁷³. Recently, a novel peptide inhibitor of TRPM2, tat-M2NX, was shown to reduce infarct size in male mice, even when administered 3 hours after reperfusion⁷⁴. These findings identify TRPM2 as a possible therapeutic target to reduce ischemic damage, although direct involvement of the endothelium was not considered in these studies.

The overall importance of TRPM2 as a redox-regulated Ca^{2+} entry channel in native endothelial cells remains undetermined. TRPM2 is a non-selective cation channel that is reported more permeable to Na^+ versus Ca^{2+} ions ($P_{\text{Ca}}:P_{\text{Na}} \sim 0.6$)^{47, 51}, although a single report suggests that the relative Ca^{2+} to Na^+ is permeability is $\sim 6:1$ in HEK293 cells at physiological temperatures ($>35^\circ\text{C}$)⁷⁵. Regardless of the $P_{\text{Ca}}:P_{\text{Na}}$, the Ca^{2+} fraction of the mixed cation current conducted by TRPM2 is expected to be very small because the channel appears to be equally permeable to K^+ and Na^+ ⁷⁶ leading to a localized depolarization-induced reduction in the driving force for Ca^{2+} entry in these non-excitable cells⁷⁷. In contrast, TRPV4 channels, which support nonselective cation currents with a high Ca^{2+} fraction⁷⁸, are more permeable for K^+ versus Na^+ ⁷⁷. On this basis, we suggest that the biophysical properties of TRPM2 channels are inconsistent with a potential role as an oxidant-dependent Ca^{2+} influx pathway in the endothelium. However, it is conceivable that Na^+ influx through TRPM2 could drive the $\text{Na}^+/\text{Ca}^{2+}$ exchanger into reverse mode to initiate Ca^{2+} influx⁷⁹, but this has yet to be demonstrated experimentally. It is also possible that oxidative stress-dependent activation of TRPM2 underlies pathological loss of endothelial cell homeostasis by destabilizing of the membrane potential, thereby contributing to loss of barrier function and other vascular pathologies.

Regulation of TRPC5 Activity by S-nitrosylation

TRPC5 is a receptor-operated channel responsive to stimulation of $\text{G}_{\text{q/11}}$ -protein coupled receptors upstream of PLC β and receptor tyrosine kinases linked to PLC γ ^{80, 81}. Activity of the channel is potentiated by increases in intracellular Ca^{2+} ⁸². TRPC5 is a non-selective cation channel permeable to Ca^{2+} , Na^+ and K^+ , with reports of relative permeability Ca^{2+} to Na^+ permeability ranging from $P_{\text{Ca}}:P_{\text{Na}} \sim 2$ ^{83, 84} to $P_{\text{Ca}}:P_{\text{Na}} \sim 14$ ⁸⁵. The extracellular linker domain between the transmembrane domain S5 and S6 contains two cysteine residues (Cys⁵⁵³ and Cys⁵⁵⁸) that are potential targets for NO-dependent S-nitrosylation⁴³. It has been suggested that when these residues are S-nitrosylated a disulfide bond forms in the linker region stabilizing the channel in the open configuration⁸⁶. This NO-dependent mechanism could potentially have important physiological consequences in the endothelium. In cultured bovine aortic endothelial cells, TRPC5 activation was linked to inhibition of endothelial cell migration⁸⁷ as a consequence of a Ca^{2+} influx-dependent rearrangement of the cytoskeleton⁸⁸. These findings could have implications in the context of angiogenesis and recovery of endothelial cell lesions in arteries of patients with atherosclerosis. However, activation of TRPC5 by S-nitrosylation remains controversial and no *in vivo* studies have

demonstrated involvement of TRPC5 in endothelial function under normal conditions or in cardiovascular disease models.

Physiological Redox Signaling Regulates TRPA1 Activity

The sole member of the ankyrin subfamily of TRP channels, TRPA1, is a large-conductance (~100 pS) cation channel that is more permeable to Ca^{2+} compared with Na^{+} ions ($P_{\text{Ca}}:P_{\text{Na}} \sim 7.9$)^{1, 89}. TRPA1 conducts the highest fractional Ca^{2+} current of all TRP channels with the exception of the epithelial cell Ca^{2+} -selective channels TRPV5 and TRPV6⁹⁰. TRPA1 is activated by a host of electrophilic compounds including the dietary molecules allyl isothiocyanate (from mustard), cinnamaldehyde (from cinnamon), and allicin (from garlic)¹. Trevisani *et al.* reported the first evidence of a redox-dependent regulation when they showed that TRPA1 currents can be elicited by an endogenous-produced lipid peroxidation product, 4-hydroxy-2-nonenal (4-HNE), in an HEK293 expression system and dorsal root ganglia neurons⁹¹. Interestingly, a recent study shows that stimulation of TRPA1 activity with 4-HNE translated to an increase in release of the algescic neuropeptides substance P and calcitonin-gene related peptide (CGRP) from nerve terminals in the spinal cord and pain behavior in rodents⁹¹. Adding complexity to redox regulation of TRPA1 channels, a recent report showed that activation of TRPA1 with cinnamaldehyde causes neurogenic vasodilation in the mouse ear by activation of perivascular CGRP and nitrergic nerve terminals⁹². Interestingly, TRPA1 activation was upstream of ROS and RNS generation, and the resulting vasodilation was likely mediated by an increase in peroxynitrite⁹², which has been shown to induce smooth muscle relaxation by stimulation of cGMP production^{93, 94} and activation of K_{ATP} channels^{94, 95}.

A recent report by Sullivan *et al.* showed that ROS generated by NOX2 activity activates TRPA1 in endothelial cells of cerebral arteries in an autocrine manner, leading to vasodilation³⁵. This study showed that TRPA1 and NOX2 co-localize within myendothelial projections in cerebral arteries, likely forming signaling complexes, and dissected a pathway in which NOX activity generates O_2^- that, through a series of reactions, leads to production of 4-HNE. Administration of NADPH, the substrate for NOX, to primary endothelial cells increased TRPA1 elemental activity, leading to a localized influx of Ca^{2+} , recorded as TRPA1 sparklets, that activates nearby intermediate conductance Ca^{2+} -activated K^{+} (IK) channels causing vasodilation of isolated cerebral arteries³⁵. NADPH and exogenous 4-HNE failed to cause dilation of cerebral arteries pre-incubated with the TRPA1 inhibitor HC-030031 and cerebral arteries from endothelial cell-specific *TRPA1*^{-/-} mice, demonstrating that NOX-derived ROS activates TRPA1 in endothelial cells³⁵. Interestingly, TRPA1 seems to have selective expression in different vascular beds, as TRPA1 is found in cerebral artery endothelial cells, but not in mesenteric, coronary or renal arteries³⁵. This unique distribution pattern may have important implications for therapeutic interventions in ischemic stroke and chronic cerebral hypoperfusion.

Two studies investigated the hypothesis that TRPA1 channels, which are present in sensory and vagal afferent neurons in the lungs and the heart, are cellular sensors of local oxygen concentration^{96, 97}. Measuring Ca^{2+} influx in an HEK expression system, Takahashi *et al.* showed that during “normoxic” conditions, defined by the authors as a partial pressure of O_2

(pO_2) of ~150 mmHg, TRPA1 activity was undetectable. In contrast, when pO_2 was ~100 mmHg, TRPA1 activity was present and at a pO_2 of 80 mmHg (defined by the authors as hypoxia), TRPA1-mediated Ca^{2+} influx was nearly maximal⁹⁶. These data suggest that a shift in pO_2 from 150 mmHg to 80 mmHg strongly stimulates TRPA1-mediated Ca^{2+} influx, which the authors interpreted as hypoxia-induced activation. However, at sea level, pO_2 within the lungs is ~100 mmHg, dropping to ~80 mmHg at 1500 m (the elevation of our laboratory and many other cities in Western North America). Hemoglobin saturation is ~100% when the pO_2 of inspired air is 80 mmHg and as such, this condition does not stimulate hypoxic adaptation in healthy individuals. Furthermore, under normal physiological conditions in healthy humans, pO_2 of arterial blood is ~80-100 mmHg within the aorta, ~30-70 mmHg in capillaries and ~20-40 mmHg in the venous system⁹⁸. Thus, under normoxic conditions at sea level, physiological pO_2 levels vary between ~100 mmHg within the lungs and ~20-40 mmHg in venous blood. Tissue pO_2 levels are typically reported to be in the range of venous blood, or lower⁹⁸. If the model proposed by Takahashi *et al.* is correct, these facts suggest that TRPA1 channels are tonically active in all tissues all of the time. This possibility is incompatible with our observations and the majority of the literature on this topic. Thus, rather than being reflective of hypoxia-induced activation, the phenomena reported by Takahashi *et al.* may represent oxidant-induced activation or sensitization of TRPA1 during recovery from hyperoxic conditions.

Although it seems unlikely that hypoxia *per se* regulates TRPA1 activity, one study suggests that TRPA1 gene expression may be regulated by hypoxia. Using a bioinformatic approach, Hatano *et al.* identified ten consensus hypoxia-responsive elements in the promoter region for the human TRPA1 gene. The authors used chromatin immunoprecipitation to show that the transcription factor hypoxia inducible factor-1 α (HIF-1 α) binds to these sites in synovial cells. Further, a luciferase reporter assay identified that one of the reverse hypoxia-responsive elements within the promoter is sufficient to activate transcription⁹⁹. However, to date the effects of hypoxia on TRPA1 expression levels in the endothelium have not been reported.

S-nitrosylation of TRPV3 channels

TRPV3 has the highest unitary conductance in the vanilloid subfamily (~200 pS) and is more permeable to Ca^{2+} ions compared with Na^+ ions ($P_{Ca}:P_{Na} \sim 10$)⁴⁹. TRPV3 is activated by innocuous heat (>30°C)¹⁰⁰, suggesting a role in thermosensation and tonic activity at physiological temperatures. Plant-derived monoterpenoids, such as carvacrol (from *Origanum vulgare*) and camphor (from *Cinnamomum camphora*) are chemical activators of TRPV3^{101, 102}. It has been postulated that intermediate metabolites of the mevalonate biosynthetic pathway involved in cholesterol biosynthesis are endogenous TRPV3 agonists¹⁰³. Farnesyl pyrophosphate was shown to activate TRPV3 in heterologous expression systems¹⁰⁴, whereas its precursor, isopentenyl pyrophosphate, inhibits the channel^{39, 105}. Interestingly, one report indicates that TRPV3 subunits can form active heteromultimeric channels with TRPV1 subunits in HEK293 cells expressing a cDNA construct encoding a fused TRPV1/TRPV3 protein¹⁰⁶. The resulting channels exhibit a unitary conductance and temperature sensitivity that is intermediate compared to the respective homomeric channels and increased sensitivity to capsaicin compared to TRPV1

homomeric channels¹⁰⁶. It is not known if TRPV3 forms heteromultimeric channels with TRPV1 or other TRPV channels *in vivo*.

TRPV3 is present in the cerebral endothelium and activation by carvacrol results in endothelium-dependent vasodilation of cerebral pial arteries and parenchymal arterioles^{38, 39, 107} by a Ca²⁺-dependent signaling pathway requiring the activity of IK and small conductance Ca²⁺-activated K⁺ (SK) channel³⁸. Interestingly, although TRPV3 might be expected to be constitutively active at physiological temperatures in mammals based on thermosensitivity¹⁰⁰, does not seem to be involved in maintenance of basal myogenic tone in cerebral parenchymal arterioles, as inhibition with isopentenyl pyrophosphate had no effect in pressure myograph experiments³⁹. However, it is possible that during disease states, TRPV3 activity may be altered as result of possible excessive production of farnesyl pyrophosphate and isopentenyl pyrophosphate, that may occur when cholesterol biosynthesis is increased, such as during obesity¹⁰⁸. TRPV3 activity has been shown to be modulated by RNS. NO causes S-nitrosylation of cysteine residues near the pore-forming region of TRPV3 expressed in HEK293 cells, leading to channel opening and Ca²⁺ influx⁴³. H₂O₂ failed to induce TRPV3 activation in HEK293 cells, suggesting a NO specific mechanism⁴³. NO-dependent activation of TRPV3 channel could have important implications for vascular function and cardiovascular disease, but this mode of regulation has yet to be demonstrated in native endothelial cells.

Oxidant-dependent regulation of TRPV3 was demonstrated by a study showing that severe prolonged hypoxia (24 hrs., 0.1% O₂) potentiated 2-aminoethoxydiphenyl borate induced currents in HEK293 cells expressing recombinant TRPV3 channels¹⁰⁹. This study also showed that the enzyme Factor Inhibiting HIF (FIH), a 2-oxoglutarate-dependent dioxygenase, constitutively hydroxylates asparagine residues within the ankyrin repeat domain of TRPV3, thereby reducing channel activity. Low cellular O₂ reduces the activity of FIH, releasing the inhibition and increasing TRPV3 current density upon activation¹⁰⁹. TRPV3 channels are also activated by intracellular protons H⁺¹¹⁰, which could contribute to activation of TRPV3 under hypoxic conditions channels *in vivo*. The effects of hypoxia on TRPV3 activity in the endothelium have not been reported.

TRPV4: Regulated by and Regulator of Redox Signaling

TRPV4 channels are present in the smooth muscle and endothelial layer of arterial wall in many vascular beds^{111, 112}. TRPV4 has a single channel conductance of approximately 60 pS at negative membrane potentials⁵⁰, and a higher permeability for Ca²⁺ versus Na⁺ ions (P_{Ca}:P_{Na} ~6)^{50, 113}. TRPV4 channels were initially described as osmotically regulated channels acting as mechanosensors¹¹⁴. Subsequent studies suggest that TRPV4 is not inherently mechanosensitive, but can be indirectly activated by force-dependent signaling pathways⁵. For example, in cultured endothelial cells a complex formed between TRPV4 and integrins responds to mechanical strain¹¹⁵. TRPV4 is activated by epoxyeicosatrienoic acids and anandamide^{111, 116, 117}, which may be endogenous agonists of the channel, and activity is initially potentiated¹¹⁸ and subsequently inhibited by increases in intracellular Ca²⁺¹¹⁹.

TRPV4 activity has been linked to endothelium-dependent arterial dilation in different vascular beds. Activation of TRPV4 was shown to induce dilation of mesenteric arteries^{112, 120}, cerebral arteries^{41, 111} and coronary arterioles¹²¹. In mesenteric arteries, TRPV4 activation with the highly selective pharmacological activator GSK1016790A leads to vasodilation associated with elemental Ca^{2+} entry through TRPV4 channels, recorded as TRPV4 sparklets. Interestingly, stimulation of the muscarinic receptor with acetylcholine also increased TRPV4 sparklet activity in endothelial cells of mesenteric arteries, suggesting that downstream phospholipase C and protein kinase C signaling pathways influence TRPV4 activity¹²⁰.

A few studies suggest that TRPV4 activity may be regulated by ROS and RNS. NO promotes S-nitrosylation of cysteines close to the pore-forming domain of TRPV4 monomers, which may increase activity⁴³. In contrast, a study by Lee *et al.* showed that S-nitrosylation of the Cys⁸⁵³ residue, located on the intracellular C-terminal tail, prevented channel activation by 4 α -phorbol-12,13-didecanoate in HEK293 cells¹²². Desensitization appears to be a consequence of reduced interaction between TRPV4 and calmodulin¹²², a previously described Ca^{2+} -dependent mechanism of TRPV4 inactivation¹¹⁸. In cultured pulmonary endothelial cells, H_2O_2 induces Ca^{2+} influx through a mechanism that is dependent of TRPV4 activity¹²³. Although not directly evaluated in the study, it is possible that in the short-term (1 hour of exposure) H_2O_2 may act directly on the TRPV4 channel, whereas on the long-term (24 hours of exposure to H_2O_2) TRPV4 activity is regulated by the Src kinase Fyn¹²³. Interestingly, Ca^{2+} entry through TRPV4 channels may elevate ROS production in the coronary endothelium, providing a positive feedback mechanism to stimulate flow-induced dilation¹²¹. This study shows that in human coronary endothelial cells, TRPV4-mediated Ca^{2+} influx stimulated with 4 α -phorbol-12,13-didecanoate induced O_2^- and H_2O_2 production in the mitochondria of these cells. This response and flow-induced dilation of coronary arteries were blocked by TRPV4 inhibition, linking the ROS and TRPV4 pathways¹²¹.

Concluding remarks

Controlled production of reactive oxidant radicals as cellular signaling molecules is now an accepted paradigm, whereas excessive generation of these molecules is associated with numerous pathological conditions. This topic is of considerable interest in the context of chronic cardiovascular diseases, where oxidative stress is a strong predictive marker¹²⁴, but antioxidant therapy has no demonstrable protective effects¹²⁵. The literature reviewed here shows that ROS and RNS can stimulate the activity of several different TRP channels present in the endothelium to locally or globally increase in intracellular $[\text{Ca}^{2+}]$ and stimulate Ca^{2+} -dependent signaling pathways. In healthy tissues, the outcomes of these redox pathways can be adaptive, such as endothelium-dependent dilation and increased tissue perfusion, whereas during oxidative stress, elevated and/or prolonged activation of Ca^{2+} signaling pathways can have detrimental effects including loss of barrier function and apoptosis. Failure of antioxidant therapy to improve cardiovascular disease outcomes could be a consequence of indiscriminately inhibiting both positive and negative aspects of redox-dependent signaling networks. The studies reviewed here provide a foundation for future integrative *in vivo* experiments exploring new therapeutic avenues for vascular diseases

related to oxidative stress that target specific redox-sensitive TRP channels in the endothelium rather than blanket suppression of oxidant radical production.

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List of abbreviations

4-HNE	4-hydroxynonenal
ADPR	adenosine diphosphate ribose
CGRP	calcitonin-gene related peptide
eNOS	endothelial nitric oxide synthase
FIH	Factor Inhibiting HIF
H₂O₂	hydrogen peroxide
HIF-1α	hypoxia inducible factor-1 α
IK	intermediate conductance Ca ²⁺ -activated K ⁺ channel
K_{ATP}	ATP-sensitive K ⁺ channel
NADPH	nicotinamide adenine dinucleotide phosphate
NO	nitric oxide
NOX	nicotinamide adenine dinucleotide phosphate oxidases
O₂⁻	superoxide anion
ROS	reactive oxygen species
RNS	reactive nitrogen species
SK	small conductance Ca ²⁺ -activated K ⁺ channel
TRP	transient receptor potential channel
TRPA	transient receptor potential ankyrin
TRPC	transient receptor potential canonical
TRPM	transient receptor potential melastatin
TRPML	transient receptor potential mucolipin
TRPV	transient receptor potential vanilloid

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Figure 1. Major pathways generating ROS and RNS in endothelial cells

A) The enzyme NADPH oxidase (left) catalyzes the transfer of an electron from NADPH to molecular oxygen, generating superoxide (O₂⁻) in the extracellular environment. The enzyme endothelial nitric oxide synthase (eNOS, middle) generates nitric oxide in the presence of the co-factor tetrahydrobiopterin (BH₄, coupled eNOS) or O₂⁻ in the absence of BH₄ (uncoupled eNOS). O₂⁻ is constantly produced in the mitochondria (right) by members of the oxidative phosphorylation complexes in the inner mitochondrial membrane. O₂⁻ located in the intermembrane space can then leak to the cytoplasm through voltage-dependent anion channels immersed in the outer mitochondrial membrane. **B)** Ubiquinone in the mitochondrial respiratory chain can be reduced by electron leak from Complex I or II, generating ubiquinol (UQ), and transports electrons to Complex III. Electron leak from ubiquinol and Complex III can interact with O₂ present in the intermembrane space and generate O₂⁻ that flows to the cytoplasm. Most of the O₂⁻ generated in the mitochondrial matrix is inactivated by antioxidant defenses.

Figure 2. Redox modifications of TRP channel activity

ROS generated by NADPH oxidase activity generate the lipid peroxide 4-hydroxynonenal (4-HNE), which activated the TRPA1 channel. Further, many reactive cysteine radicals (red circles) are present in the ankyrin repeat domains of the N-terminal tail of the TRPA1 channel, and they have the potential to alter TRPA1 function. Nitric oxide (NO) mediated S-nitrosylation of reactive cysteines located near the pore-forming has also been shown to activate TRPV3, TRPV4 and TRPC5. However, S-nitrosylation of a cysteine in the C-terminal tail of TRPV4 inhibits the channel. TRPM2 is activated by ADP-ribose, which binds to the NUDIX motif in the C-terminal tail of the channel. Oxidative stress, such as the generated by accumulation of β -amyloid, increase ADP-ribose in cells, leading to prolonged TRPM2 activation.