TRP channels as sensors of oxygen availability

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Abstract

An ability to adapt to changes in oxygen availability is essential for survival in both prokaryotic and eukaryotic organisms. Recently, cation channels encoded by the *transient receptor potential* (*trp*) gene superfamily have been recognized as multimodal sensors of a wide variety of factors inside the cells and in the extracellular environment, and also as transducers of electrical and chemical signals mediated by ions such as Ca^{2+}. The functional features of TRP channels enable the body to react and adapt to different forms of environmental changes, including oxygen levels. A sub-class of TRP channels regulate various cellular processes in response to fluctuations in oxygen. In this article, we describe the physiological and pathological significance of the oxygen-sensitive TRP channels, which are heterogeneous in the cellular responses to acute changes in oxygen, by contrasting their oxygen-monitoring function with that of other ion channels, transporters and enzymes. We also discuss physiological relevance of oxygen-sensitive TRP channels as a novel class of target proteins for pharmaceutical therapeutics.

Keywords: TRP channels; oxygen; cysteine; TRPA1; Ca^{2+}

Introduction

Living organisms, from bacteria to humans, possess mechanisms for oxygen (O_2) homeostasis. Cellular O_2 concentrations must be tightly regulated within a narrow physiological range for organisms to flourish. In aerobic organisms, both O_2 deprivation and excess can be deleterious: hypoxia stifles ATP generation and results in breakdown of essential cellular functions, whereas hyperoxia drives the generation of reactive oxygen intermediates that can cause lethal damage to membranes and DNA.

There are a variety of tissues that sense the O_2 level, including Type I cells of the carotid body, neuroepithelial bodies in the lungs, chromaffin cells in the fetal adrenal medulla and smooth muscle cells in the pulmonary, fetoplacental and systemic arteries and the ductus arteriosus. The O_2 sensitivity of these tissues varies depending on their anatomical location, and together they constitute a homeostatic O_2-sensing system (73, 113).

Cellular responses to changes in O_2 availability can be acute or chronic (60). Acute responses rely mainly on O_2-regulated ion channels, which mediate adaptive changes in cell excitability, contractility and secretory activity (32, 52, 60, 73, 112). Ion channels are well suited for detecting acute and rapid changes in the environment owing to their fast kinetics and plasma membrane localization, as a direct interface with the extracellular environment. There are two major ways in which ion channels sense O_2: firstly through direct alternation of the redox status of channel proteins themselves *via* modification of thiol-rich molecules (direct pathways) and secondly through interaction with complexes with other proteins carrying O_2 sensor domains (indirect pathway). Most of the channels sense O_2 through the indirect pathways. Ryanodine-sensitive Ca^{2+} release channel (ryanodine receptor; RyR) and TRPA1 channel are the representative

ion channels that take the direct pathway (57, 102). On the other hand, chronic responses depend on the modulation of transcription factors such as hypoxia-inducible factor (HIF), which determines the expression of numerous genes encoding growth factors, enzymes and transporters (92, 93, 111).

Drosophila melanogaster transient receptor potential (*trp*) protein and its homologs are putative six-transmembrane polypeptide subunits that assemble into tetramers to form channels. In mammalian systems, TRP channels comprise six related protein subfamilies: TRPC, TRPV, TRPM, TRPA, TRPP and TRPML (29). These channels are activated by sensing diverse stimuli, including receptor stimulation, heat, osmotic pressure and mechanical and oxidative stress from the extracellular and intracellular milieu (29, 78, 109). Recently, a class of TRP channels was found to be modulated by hypoxia and hyperoxia and to control various cellular processes in response. This review explores the physiological and pathological significance of the O_2-sensitive TRP channels (Fig. 1), and compare their O_2-monitoring function with that of other ion channels, transporters and enzymes to demonstrate the heterogeneity in cellular responses specifically to acute changes in O_2. Their responses to chronic changes in O_2 have been excellently reviewed elsewhere (30, 35, 53, 89, 90, 92, 94, 112, 115) and will not be covered in detail here.

Distinctive sensing function of TRP channels

The TRPC homologs are receptor-activated Ca^{2+}-permeable cation channels (RACCs) that are activated by sensing metabotoropic changes upon receptor stimulation, which activates and induce phospholipase C (PLC) to hydrolyze phosphatidylinositol-4,5-bisphosphate into inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) (77, 106). TRPV Ca^{2+}-permeable channels can be functionally defined as thermosensors (10, 29, 55, 106, 109), but they are in fact sensors for diverse stimuli. TRPV5 and TRPV6 are distinct from other TRPVs because they are the only highly Ca^{2+}-selective channels and tightly regulated by intracellular Ca^{2+} ($[Ca^{2+}]_i$) in the TRP family (76, 78). The TRPM subfamily is named after melastatin (TRPM1), a tumor suppressor protein isolated in a screen for genes whose level of expression was inversely correlated with the severity of metastatic potential in a melanoma cell line (21). TRPM8 channels, in contrast to TRPV, are activated by low temperatures (<25 °C) and menthol (63, 84). Regarding the temperature sensitivity, the principle of temperature-dependent gating in cold-sensitive TRPM8 and heat-sensitive TRPV1 channels have recently been shown (107, 108). The sole member of the TRPA subfamily, TRPA1, has a large amino-(*N*-) terminal domain with 14-19 ankyrin repeats (AR) (28, 75), and is activated by pungent compounds (e.g. allyl isothiocyanate found in mustard oil) and noxious cold stimuli (<17 °C) (47, 75, 97). Thus, TRP channels serve as sensors for a variety of environmental factors, and the Ca^{2+} influx communicated *via* these TRP channels regulates a diverse array of cellular processes.

O_2-sensing by TRPA1 channels in vagal and sensory neurons

Sensory and vagal afferent neurons, which project nerve endings throughout the body, have been reported to detect hypoxia in organs such as the airway, lungs and heart after ischemia and other conditions of low O_2 supply (19, 34, 42, 59). However, hypoxia detection by sensory and vagal neurons was controversial and elusive (59). In terms of hyperoxia, *Caenorhabditis elegans* appears to be adept at evading hyperoxia owing to detection mechanisms in sensory neurons (33). Furthermore, insects breathe discontinuously to avoid O_2 toxicity during hyperoxia (41). However, the physiological relevance of hyperoxia detection through sensory/autonomic systems is less clear in vertebrates, and the delineation of these vertebrate neuronal hyperoxia-sensing molecular processes remains an exciting area of research.

In mammals, enhanced discharges in vagal afferents induce respiratory, cardiac and vascular responses (50, 59, 65) and chemicals encountered in the airway are detected by airway vagal C fibers (50). Recently, TRPA1 has been shown to sense environmental irritants, thus initiating defensive reflexes such as coughing and respiratory depression in the C fibers (10, 11, 72). Notably, our group has shown that TRPA1 in vagal and sensory neurons detects changes in O_2 levels (102) and has a reverse bell-shaped O_2 dependency with minimum activity at a partial O_2 pressure (PO_2) of 137 mmHg, slightly below the atmospheric PO_2 of 159 mmHg (106) (Fig. 2). Corroborating this finding is the observation that disruption of the *Trpa1* gene in mice abolishes hyperoxia- and mild hypoxia (15% O_2)-induced cationic currents in vagal and sensory neurons and thereby impedes enhancement of *in vivo* vagal discharges induced by hypoxia (102). These results suggest that TRPA1 has a previously unidentified role as an O_2 sensor.

Molecular mechanisms underlying O_2-sensing by TRPA1

TRPA1 is characterized by a large *N*-terminal domain with 17 predicted AR (28), and have a tetrameric assembly with a compact transmembrane domain and a basket-like cytoplasmic domain structure (18). TRPA1 is activated by a variety of electrophilic compounds, including endogenous inflammatory mediators, products of oxidative stress and exogenous pungent chemicals. These compounds are potentially susceptible to nucleophilic attack by the sulfhydryl groups of cysteine (Cys) residues (7, 62).

Sensing of hypoxia by TRPA1 is based on proline (Pro) hydroxylation by Pro hydroxylases (PHDs). In normoxia, PHDs hydroxylate the conserved Pro394 residue in the 10th AR of human TRPA1, inhibiting its activity. Hypoxia impairs PHD activity, relieving TRPA1 from the inhibitory action of Pro hydroxylation and leading to an increase in its activity, achieved either by insertion of fresh, unmodified TRPA1 proteins into the plasma membrane or by dehydroxylation of modified proteins through an unidentified molecular mechanism (102).

Sensing of hyperoxia by TRPA1 is mediated by Cys modification. In order to evaluate the oxidation sensitivity of TRP channels quantitatively, we systematically compared the responses of redox-sensitive TRP channels (TRPA1, TRPV1, TRPV2, TRPV3, TRPV4 and TRPC5) with a congeneric series of reactive disulfides, which show differential electron acceptor (oxidation) abilities indicated as redox potentials obtained using rotating disc-electrode voltammetry (102, 117). Strikingly, among the TRPs tested, only TRPA1 responded to inert electrophiles with a redox potential of –2,950 mV. The redox potential of O_2 (–2,765 mV) is less negative than the threshold redox potential for TRPA1 (approximately –3,400 mV) but is more negative than that

for the other channels investigated (Fig. 3), suggesting that TRPA1 is activated by O_2 (a weak oxidant) to function as a hyperoxia sensor (102). In this paradigm, O_2 activates TPRA1 by oxidizing Cys633 and/or Cys856, located within the 17th AR and the intracellular linker region between S4 and S5, respectively. TRPA1 can assume at least two oxidized forms during hyperoxia: a relatively unstable oxidized state (State 1) readily reversed by glutathione, and a relatively stable oxidized state (State 2). It is conceivable that sulfhydryl groups on the key Cys residues (Cys633 and Cys856) are modified to sulfenic acid (S-OH) in State 1, and form disulfide bonds (S-S) in State 2 (102). This oxidation mechanism activates TRPA1 by overriding the inhibition exerted by Pro hydroxylation. To illustrate this phenomenon, single-channel currents were significantly enhanced by hyperoxic solution but not by hypoxic solution applied from the intracellular side of cell-free excised inside-out patches. These results imply that TRPA1 is activated by a direct action of O_2 in hyperoxia, and by intracellular O_2-sensing mediators in hypoxia (102). In physiological conditions, glutathione and thioredoxin systems can regulate the redox balance for TRPA1 activation.

Regulation *via* Cys oxidation by oxidants, reductants and electrophiles is not unique to TRPA1 among ion channels. A form of O_2 sensing has also been proposed involving the effects of PO_2 on the number of Cys residues present in the RyR. In skeletal muscle cells, RyR is localized on the sarcoplasmic reticulum (SR) and interacts directly with voltage-gated Ca^{2+} channels to initiate muscle contraction. RyR has 100 Cys residues per subunit, ≈20 of which are free for redox modifications (4, 99, 110). A number of redox-sensitive Cys residues have been identified in both the open and closed states of the channel and appear to be distributed across the primary structure of the cytoplasmic region (4, 110). Several of these sites, including Cys36 and Cys315, have

been mapped to the clamp domains (3, 36, 57, 58) involved in inducing a major conformational changes to open and close the channel (54), whereas Cys3635 is located in subdomain 3 of the calmodulin binding site in rabbit RyR (99). The *S*-nitrosylation of Cys3635 appears to occur only at physiological tissue O_2 tension (PO_2 ≈10 mm Hg) and facilitates muscle contraction (23). Recently, a combination of fluorescence detection and mass spectrometry of RyR1 identified two redox-sensitive Cys residues (Cys1040 and Cys1303) that were labeled in the presence of reduced and oxidized glutathione (87). A [^{3}H]-based ryanodine ligand binding assay also revealed that point substitution of each of 18 Cys residues with serine (Ser) or alanine modulated the RyR activity of each mutant in response to reduced and oxidized glutathione. Three single-site RyR1 mutants (Cys1781Ser, Cys2436Ser and Cys2606Ser) exhibited a reduced redox response compared with wildtype RyR1 (87), suggesting that multiple Cys residues contribute to the redox state and activity of RyR1. These Cys modifications occur *via* coupling of RyR1 to nicotinamide adenine dinucleotide phosphate oxidase (NOX) 4. In skeletal muscle, an increase in local O_2 tension proportionally increases reactive oxygen species (ROS) produced by NOX4 located on the SR (101). This local increase in ROS at the SR causes oxidation of multiple Cys residues on RyR1 and leads to its activation (101).

Anoxia-sensing mediated by TRPM7 and TRPM2 channels in the brain

Acute neuronal damage after injury involves a complex combination of processes including excitotoxicity, inflammation, necrosis and apoptosis in the brain. The theory that activation of *N*-methyl-D-aspartate (NMDA) excitatory glutamate receptors mediates excessive Ca^{2+} entry and precipitates critical excitotoxic events that result in

neuronal death has been enthusiastically embraced given the prospect of using NMDA receptor antagonists to prevent the associated brain injury. Unfortunately, these receptor antagonists fail to act effectively as an anti-excitotoxicity therapy (AET) for human stroke (12, 43). In addressing this problem, Aarts *et al*. have revealed that the TRPM7 channel, termed ‘chanzyme’, a channel equipped with an α-kinase domain (70, 91), is activated by oxygen-glucose deprivation through the production of ROS and reactive nitrogen species (RNS), facilitating Ca^{2+} uptake that further stimulates ROS production and TRPM7 activation in heterologous expression systems and rat cortical neurons (1). Suppressing TRPM7 expression in rat cortical neurons prevents anoxic neuronal death even in the absence of AET, indicating that TRPM7 is an essential mediator of anoxic death. It is possible that patients enrolled in failed trials studying the use of AET for stroke or traumatic brain injury were selected to have severe injuries (5, 67) or that these disorders in humans, by their nature, induce severe ischemia. TRPM7 may be activated in these rigorous ischemic conditions. Therefore, future treatment of such disorders may also need to inhibit TRPM7. Indeed, it has been shown that suppression of hippocampal TRPM7 by intrahippocampal injections of viral vectors bearing short hairpin RNA specific for TRPM7 makes neurons resistant to ischemic death after brain ischemia and preserves neuronal morphology and function in rats (98). TRPM7 suppression also prevents ischemia-induced deficits in long-term potentiation and preserves performance in fear-associated and spatial-navigational memory tasks (104). Thus, regional suppression of TRPM7 is feasible and well tolerated, and inhibits delayed neuronal death *in vivo* in an animal model.

Recently, TRPM2 has also been implicated in anoxic neuronal cell death (64, 104). TRPM2, which is widely distributed in the central nervous system (49, 79, 116), can be

activated, directly or indirectly, by several factors that are present in excess in ischemia, including H_2O_2, ROS and RNS (39, 64). Moreover, TRPM2 is gated by cytosolic adenosine diphosphate ribose (ADPR). Raising $[Ca^{2+}]_i$ lowers the half-maximal effector concentration for channel activation by ADPR (86). In ischemia, the influx of Na^+ and Ca^{2+} through TRPM2 channels, which promotes membrane depolarization, mitochondrial membrane disruption and increase in the $[Ca^{2+}]_i$, may participate in a positive feedback loop in which cytosolic ADPR, ROS and RNS aggravate damages of neurons (104). Importantly, inhibiting TRPM2 by RNA interference reduces TRPM2-like currents in hippocampal neurons (79) and protects the cell against H_2O_2 toxicity in HEK cells (49), cultured neurons, and brain tissue (46), suggesting that TRPM7 and TRPM2 channels are potential therapeutic targets for reducing the ischemic cell damage and excitotoxicity that follow stroke.

Hypoxia-sensing by TRPC6 channels in pulmonary smooth muscle cells

Hypoxic pulmonary vasoconstriction (HPV) is an essential mechanism in the lung for matching blood perfusion to ventilation during local alveolar hypoxia and functions to optimize pulmonary gas exchange (114). Disturbances in HPV can cause life-threatening hypoxemia, whereas chronic hypoxia triggers vascular remodeling in the lungs and pulmonary hypertension (71, 114, 115). TRPC6 appears to be a key regulator of acute HPV. In studying the signaling cascades that comprise this vitally important mechanism, Weissmann *et al.* illustrated that although recombinantly expressed TRPC6 cannot be activated by hypoxia, severe hypoxia (1% O_2)-induced cation influx and currents in smooth muscle cells are largely absent in precapillary pulmonary arteries in *Trpc6*-knockout mice, whereas the pulmonary vasoconstrictor

response to the thromboxane mimetic, U46619, is unchanged (115). Hypoxia-induced TRPC6 activation in smooth muscle cells is mediated by DAG accumulation, probably downstream of activated PLCs. Accordingly, induction of regional hypoventilation results in severe arterial hypoxemia in *Trpc6* knockout mice, but not in wild-type mice. Notably, though, chronic hypoxia-induced pulmonary hypertension is independent of TRPC6 activity. Thus, TRPC6 plays a unique and indispensable role in acute HPV. Manipulation of TRPC6 function may thus offer a therapeutic strategy for the control of pulmonary hemodynamics and gas exchange.

Hypoxia-sensing by TRPC1-STIM1-Orai1 complex in pulmonary smooth muscle cells

Stromal interacting molecule 1 (STIM1), a 90-kDa transmembrane Ca^{2+}-binding protein found in endoplasmic reticulum and plasma membrane, has been reported to play a pivotal role in regulation by hypoxia of store-operated Ca^{2+} channels (SOCC) composed of TRPCs and Orai channels (82) in pulmonary arterial smooth muscle cells (PASMCs) (61, 74). The response of SOCC to acute hypoxia shows a initial transient followed by a sustained rise in $[Ca^{2+}]_i$ and induces colocalizations of TRPC1-STIM1 and Orai1-STIM1 complexes in PASMCs (61). Suppressing the expression of either TRPC1, STIM1 or Orai1 with small interfering RNA (siRNA) in PASMCs significantly inhibits both hypoxia-induced transient and sustained component of capacitative Ca^{2+} entry (CCE), suggesting a ternary complex formed by these proteins to mediate CCE (61). One possible mechanism could be that acute hypoxia triggers the production of ROS and modifies the Cys residue on STIM1 to alter its functional and physical interaction with TRPC1 and Orai1 (40, 74). Interestingly, SOCs

may be differentially assembled by a combination of TRPC channels, STIM1, and/or Orai proteins (82, 105). TRPC1, TRPC6, TRPC4, TRPC3 and TRPC5 are also expressed in PASMCs (61) and these TRPC channels may also form a complex with STIM1 and/or Orai1.

Hypoxia-sensing by TRP channels in the central nervous system

The brain is particularly sensitive to hypoxia and oxidative stress (2). This vulnerability is mainly due to the extraordinary metabolic requirements of neurons to function. Vital neuronal processes such as neurotransmission and ion homeostasis are critically dependent on a continuous supply of O_2 and glucose (2). During stroke and hypoxia, cranial blood vessel occlusion starves the brain of O_2 and glucose. Some TRP channels clearly function as O_2-sensitive channels in the central nervous system and cardiac system. For instance, TRPV4, a Ca^{2+}-permeable cationic channel that is gated by various stimuli such as temperature and cell swelling, is involved in ischemia-induced Ca^{2+} entry in reactive astrocytes and participates in the pathogenic mechanisms of astroglial reactivity following ischemia. After induction of cerebral hypoxia or ischemia by bilateral occlusion of the carotids combined with hypoxic conditions and followed by reperfusion, cell swelling and cytokines or growth factor up-regulates TRPV4 expression and activity in rat hippocampal astrocytes (14). In cardiac spinal sympathetic sensory fibers and myocytes, TRPV1 and TRPC3 act as sensors of tissue ischemia (81, 95). These TRP channels have other non-sensory functions including release of substance P, neurokinin A and calcitonin gene-related peptide from peripheral nerve terminals in the epicardial surface of the cardiac ventricle and around the coronary blood vessels. The neuropeptides induce vasodilation and

negative inotropic and chronotropic effects to mitigate the effects of ischemia and reperfusion injury (76). Thus, TRP channels that indirectly sense O_2 also have significant therapeutic potential as targets for pharmacological therapy in ischemia reperfusion and stroke.

Oxygen sensing by other ion channels and transporters

K^+ channel

The most recognizable O_2-regulated ion channels are K^+ channels in the carotid body. O_2-regulated K^+ channels were initially studied in Type I cells of the carotid body (13, 20, 27, 52, 83, 96), but similar observations were later reported in the neuroepithelial body (80, 118), adrenal chromaffin cells (16, 103, 119), smooth muscle cells of the pulmonary resistance arteries (88), aortic body (45), fetoplacental arteries (38) and thoracic aorta (45). O_2-sensitive K^+ currents can be either inhibited or potentiated by hypoxia, and this dual regulation is observed even in the same channel type expressed in different cells. For example, maxi calcium-sensitive potassium (K_{Ca}) channels are inhibited by hypoxia in rat glomus cells and fetal ovine pulmonary vascular smooth muscle, but are potentiated in cerebral arterial myocytes (60). Developmental changes affect the type and expression level of O_2-sensitive K_V and TASK-1 channels (60). Nine families of K_V channels α-subunits are recognized from cloning studies (K_V1–9). These channels often display differences in voltage sensitivity, current kinetics and steady-state activation and inactivation. Based on patch-clamp recordings with specific K_V antibodies, studies in expression systems or the use of reverse transcription polymerase chain reaction, the potential candidate K_V channel α-subunits that could form O_2-sensitive channels are K_V1.2, K_V1.5, K_V2.1, K_V3.1 and

$K_V9.3$ (113). On the other hand, the members of "two-pore domain" potassium (K_{2P}) channels were divided into six subfamilies (TWIK, TREK, TASK, TALK, THIK and TRESK) on the basis of sequence similarity and functional resemblance (22). The K_{2P} channels are regulated by a wide variety of voltage-independent factors, such as pH, temperature and membrane stretch. The pharmacological profile of O_2-sensitive background K^+ current, together with the strong mRNA expression, suggests that TASK channels play the primary role in sensing acidosis and hypoxia in glomus cells (22). In the embryo and neonate, it seems that membrane potential is largely controlled by K_{Ca} channels that are inhibited by hypoxia in smooth muscle cells. However, upon maturation the control of membrane potential shifts to K_V and TASK-1 channels. An identical developmental shift from K_{Ca} to K_V and TASK-like channels is seen in Type I cells of the carotid body. The O_2 content of fetal blood is much higher than that of adult blood (68), and it seems likely that K_{Ca} channels contribute to the control of membrane potential at higher levels of O_2, whereas K_V and TASK channels act at lower O_2 levels.

Voltage-dependent Na^+ channel

Voltage-dependent Na^+ channels play a key role in excitotoxic damage in ischemia. Blockers of Na^+ channels inhibit depolarization, thereby reducing Ca^{2+} influx through voltage-dependent Ca^{2+} and NMDA receptor channels and prevent the reversal of the Ca^{2+}/Na^+ exchanger (15). These improve the ionic homeostasis and cellular energy levels, and prevent ischemia-hypoxia induced neuronal injury and neuronal damage mediated by Ca^{2+} overload. Anoxia and metabolic inhibition produce a large negative shift in the steady-state inactivation curve for the voltage-dependent Na^+ current, that results in reduced Na^+ current amplitude in human neocortical pyramidal

neurons and promotes neuronal survival during periods of O_2 deprivation (17). However, after several minutes of hypoxia, a persistently elevated, non-inactivating Na^+ current was observed as the primary cause of an increase in $[Ca^{2+}]_i$ (37). These mechanisms may contribute both directly and indirectly to the sequence of events, including elevated $[Ca^{2+}]_i$, that follow hypoxia and culminate in cell death.

Voltage-dependent Ca^+ channels

Increased $[Ca^{2+}]_i$ is necessary to elicit hypoxic constriction of the pulmonary resistance arteries. In PASMCs, $[Ca^{2+}]_i$ is increased by Ca^{2+} influx through Ca^{2+}-permeable channels, such as TRPC6 channel (see above), and/or by Ca^{2+} mobilization from intracellular Ca^{2+} stores. Under hypoxic conditions, the influx of extracellular Ca^{2+} across the cell membrane is perhaps the predominant mechanism for increasing the $[Ca^{2+}]_i$ in PASMCs (115), and employs L-type voltage-dependent Ca^{2+} channels under the regulatory control of the resting membrane potential. In freshly dispersed rabbit arterial myocytes, hypoxia selectively and reversibly inhibits L-type Ca^{2+} channel activity, whereas T-type Ca^{2+} channel-mediated current is unaltered (25, 26). Hypoxia similarly inhibits recombinantly expressed L-type channels (24).

A single type of ion channel can often have bidirectional effects, activating a physiological function in one tissue but inhibiting the same function in another. L-type Ca^{2+} channels are present in both conduit (proximal) and resistance (distal) myocytes, but the density of these channels is approximately double in the resistance myocytes. Although the L-type channels are structurally and functionally homogeneous, influx of Ca^{2+} into the smooth muscle cells of the resistance arteries is enhanced by hypoxia, while in the conduit smooth muscle cells it is inhibited (25). In addition, in pacemaker

neurons of the rostral ventrolateral medulla, hypoxia activates Ca^{2+} current (100). These variable responses of the L-type Ca^{2+} channel to hypoxia are probably due to spatial or quantitative differences in expression that have not yet been characterized, or to unidentified cell-specific O_2-sensitive factors that modulate the L-type Ca^{2+} channel in these locations.

Ion transporters

Some ion transporters are also sensitive to changes in O_2 level. Multiple O_2-sensitive ion transporters play roles in ion homeostasis within red blood cells (RBCs) to control cellular volume and integrity (31). These transporters include the Na^+-K^+-Cl^--cotransporter (NKCC), K^+-Cl^--cotransporter (KCC) and Na^+/H^+ exchanger (NHE), each of which responds differently to changes in O_2.

NKCC is inhibited by hyperoxia and stimulated by hypoxia, as observed in a number of avian species (31). In turkey RBCs, hypoxia is the major physiological stimulus for NKCC activity with a PO_2 for half-maximal activation of ≈41 mmHg (69). Interestingly, O_2 levels also regulate the effect of other stimuli on the activity of NKCC. At normal O_2 levels, NKCC is activated by β-adrenergic stimulation and cell volume shrinkage, but hypoxia causes the transporter to become unresponsive to these stimuli (69).

The response of KCC to change in O_2 depends on presence or absence of the stimuli such as urea, pH, β-adrenergic agonist and cell swelling. For example, KCC is inhibited by hypoxia and potentiated by raising PO_2 (60). KCC is activated by cell swelling, acidification and urea but in hypoxia, the transporter is inactivated and becomes refractory to activation by these other stimuli (31). It is possible that this

regulatory mechanism fully activates KCC in arterial blood under hyperoxia, to prevent RBC shrinkage but inactivates KCC in kidney medulla and muscle beds under hypoxia. In some cases, hypoxia stimulates KCC, and may even be a prerequisite for transporter function (9, 31). In other cases, O_2 has no effect. In several teleosts (e.g. trout, sea bream) and in frog RBCs, hypoxia increases the response to β-adrenergic agonists in lamprey RBCs and intracellular acidification. However, in these species, hypoxia is not a prerequisite for transporter function, but rather amplifies the response to the agonist (9, 31).

Like NKCC, NHE activity in RBCs is often increased by hypoxia (48, 66). The NHE is controlled by multiple stimuli including cell shrinkage, intracellular acidification and β-adrenergic stimulation, interacting with a number of different stimuli, some of which are O_2-sensitive, others that are O_2-independent. The diversity of mechanisms by which O_2 modulates NHE may reflect the multiple roles of NHE in different species and transduction pathways.

Glucose transporters

The initial step in the metabolism of glucose is its transport across the plasma membrane, a step that is "rate-limiting" in the vast majority of cells and tissues. In hypoxia, glucose transport is important in tissues such as skeletal and cardiac muscle and brain that have immediate energy demands. Hypoxia mediates the enhancement of glucose transport by firstly inducing the translocation of GLUT-1 and GLUT-4 glucose transporters from intracellular vesicles to the plasma membrane, and secondly by activating preexisting GLUT-1 in the membrane (8).

O_2-sensitive factors coupled to ion channels

NADPH oxidases

NOX contain heme, flavin adenine dinucleotide, and transmembrane domains, and transfer electrons from nicotinamide adenine dinucleotide phosphate (NADPH) to O_2 to generate superoxide anion (O_2^-) that participates in intracellular signaling (6). In hypoxia, human PASMC produce transforming growth factor-β1 (TGF-β1) that induces the expression of insulin-like growth factor binding protein-3 (IGFBP-3) *via* the PI3K/Akt pathway. This IGFBP-3 increases NOX4 gene expression, resulting in cell proliferation (44). In hyperoxia, NOX4 mRNA and protein expression are upregulated and are involved in ROS production in human pulmonary artery endothelial cells. In addition, hyperoxia-induced cell migration and capillary tube formation are regulated by ROS generated *via* a NOX4-dependent pathway (85). Thus, NOX4 can be an O_2 sensor. As mentioned above, ROS are generated in proportion to PO_2 by NOX4 in the SR and the consequent oxidation of a small set of RyR1 Cys-thiols results firstly in increased RyR1 activity and Ca^{2+} release in isolated SR and in cultured myofibers, and secondly in enhanced contractility of intact muscle (101). Another molecule proposed to be downstream of O_2-dependent NOX4 activation is TASK-1. Hypoxia inhibits TASK-1 activity, a response that is abolished by NOX4 siRNA and NOX inhibitors (56). Further studies are needed to determine if NOX4 has any regulatory role in these fundamental O_2-dependence cellular processes.

Mitochondria

An obvious site where O_2 sensing is vital is the mitochondrion because this organelle consumes O_2 in the final step of the electron transport chain. Historically, a

model of O_2 sensing based on the "mitochondrial model" has been advocated (51). The potential involvement of hypoxia-induced changes in mitochondrial function has been suggested from the results of experiments investigating the effects of pharmacological inhibitors on mitochondrial function. However, the role of ion channels in sensing O_2 in mitochondria may not be straightforward. For example, O_2 sensing in glomus cells involves inhibition of O_2-sensitive K^+ channels that are modulated by O_2 tensions higher than those that depress cellular metabolic function. Mitochondrial respiration is not limited by O_2 supply until extracellular PO_2 falls below 2–3 mmHg, whereas ion channels are regulated by PO_2 values below 80 mmHg (60). Therefore, the involvement of some components of the mitochondrial electron transport chain in cellular O_2 sensing cannot be discounted but much remains to be elucidated about their identity and mode of interaction with the effector ion channels.

Conclusion

O_2-sensitive ion channels are ubiquitous and widely distributed, and participate in many O_2-dependent cellular functions. TRP channels can respond to multiple activation triggers and therefore serve as O_2 detectors both in a direct and indirect manner, as part of a complex signaling system that maintains the O_2 supply to the tissues and protects cells against damage. For example, in sensory and vagal neurons, TRPA1 translates O_2 signals into electrical signals that enhance *in vivo* hypoxia-induced vagal discharges. Considering that TRPA1 activation exhibits an inverted bell-shaped O_2-dependency curve, this bidirectional regulation of channel activity may be advantageous because it adds versatility to the biophysical mechanisms that cells use to adapt or respond to environmental triggers.

Despite recent advances in the molecular characterization of O_2-regulated ion channels and enzymes, many important questions still remain unanswered regarding the identity of the molecules and mechanisms involved in O_2 sensing. Identification of the O_2-sensing molecules provides insights into how organisms detect changes in O_2 level. Indeed, TRPA1 activation during hypoxia is mediated by Pro hydroxylation, as is the modulation of the well-known O_2-sensitive transcription factor, HIF. To further our understanding of O_2-sensing TRP channels, identification of redox-sensitive factors that interact with TRP channels (as seen with K_{Ca} channels and RyR1) will be important. In addition, O_2-dependent translocation between intracellular compartments and the plasma membrane, as seen for the GLUT1 transporter, may also be at play in TRP-dependent O_2 sensing mechanisms.

Studies of O_2-sensitive TRP channels have now matured from the functional characterization of single molecules to the analysis and integration of complex molecular systems controlled by TRPs. Ultimately, the delineation of these TRP-dependent O_2 sensory networks could lead to the development of novel pharmacological tools for use in a broad spectrum of pathophysiological conditions that have O_2 imbalance as part of their etiology.

Figure legends

Fig. 1. Oxygen-sensitive TRP channels. TRPA1, TRPM2, TRPM7, TRPC1, TRPC3, TRPC6, TRPV1 and TRPV4 indirectly sense O_2 under hypoxia (indicated in green). TRPA1 directly sense O_2 under hyperoxia (indicated in red).

Fig. 2. Bidirectional regulation of TRPA1 channel activity by O_2. Single-channel traces in cell-attached mode illustrate step-wise opening and closing, which keeps the membrane potential at approximately –60 mV. Proline hydroxylases (PHDs) hydroxylate specific Pro residues in the *N*-terminal AR domain of TRPA1 protein in hyperoxia and normoxia, whereas a decrease in O_2 concentration reduces PHD activity and relieves TRPA1 from this hydroxylation, leading to channel activation. In hyperoxia, O_2 oxidizes specific Cys residues, thereby activating TRPA1. The sulfhydryl group(s) of these key Cys residues may be modified to sulfenic acid in the non-oxidized state of TRPA1, but may form a disulfide bond(s) when the channel is oxidized. These oxidation mechanisms over-ride the inhibition by Pro hydroxylation to activate TRPA1. These activation mechanisms are advantageous because they add versatility to the biophysical mechanisms that the cells use to adapt or respond to environmental changes.

Fig. 3. Oxidation sensitivity of TRP channels. Range of redox potentials for activation of respective TRP channels. The redox potential of O_2 (–2,765 mV) is less negative than the threshold redox potential for TRPA1.

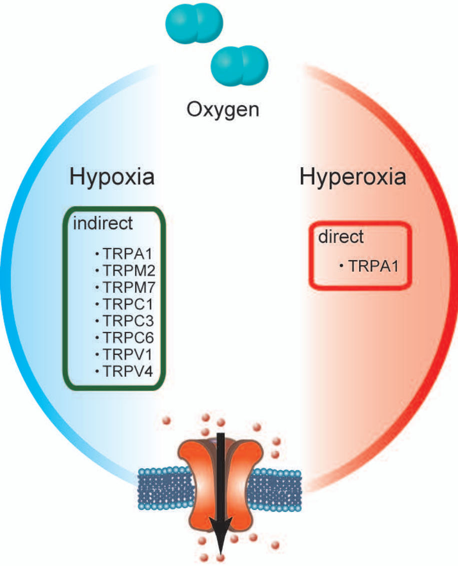

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Figure 1

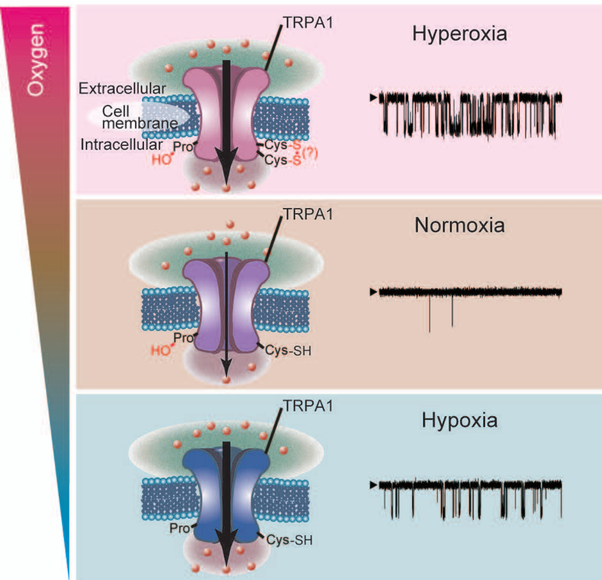

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Figure 2

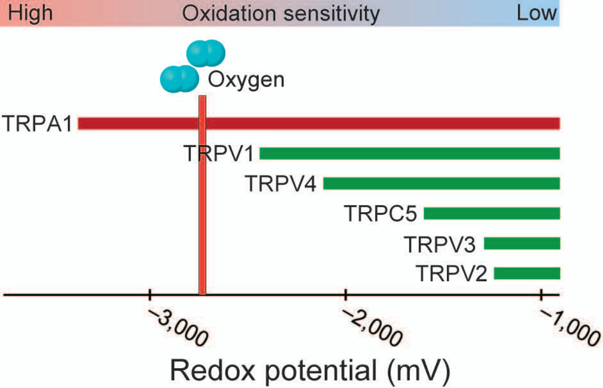

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Figure 3

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