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Invited Review

HIGHLIGHTED TOPIC | *Oxygen Sensing in Health and Disease*

Regulation of oxygen sensing by ion channels

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López-Barneo, José, Raquel del Toro, Konstantin L. Levitsky, María D. Chiara, and Patricia Ortega-Sáenz. Regulation of oxygen sensing by ion channels. *J Appl Physiol* 96: 1187–1195, 2004; 10.1152/japplphysiol.00929.2003.—O_2 sensing is of critical importance for cell survival and adaptation of living organisms to changing environments or physiological conditions. O_2-sensitive ion channels are major effectors of the cellular responses to hypoxia. These channels are preferentially found in excitable neurosecretory cells (glomus cells of the carotid body, cells in the neuroepithelial bodies of the lung, and neonatal adrenal chromaffin cells), which mediate fast cardiorespiratory adjustments to hypoxia. O_2-sensitive channels are also expressed in the pulmonary and systemic arterial smooth muscle cells where they participate in the vasomotor responses to low O_2 tension (particularly in hypoxic pulmonary vasoconstriction). The mechanisms underlying O_2 sensing and how the O_2 sensors interact with the ion channels remain unknown. Recent advances in the field give different support to the various current hypotheses. Besides the participation of ion channels in acute O_2 sensing, they also contribute to the gene program developed under chronic hypoxia. Gene expression of T-type calcium channels is upregulated by hypoxia through the same hypoxia-inducible factor-dependent signaling pathway utilized by the classical O_2-regulated genes. Alteration of acute or chronic O_2 sensing by ion channels could participate in the pathophysiology of human diseases, such as sudden infant death syndrome or primary pulmonary hypertension.

electrophysiology; gene expression; hypoxia-inducible factors

OXYGEN SENSING IS OF PARAMOUNT importance for cell survival due to the central role of O_2 as acceptor of the electrons in the mitochondrial respiratory chain, thus making possible the synthesis of adenosine triphosphate (ATP) by oxidative phosphorylation. The provision of sufficient O_2 to the tissues in variable habitats or physiological situations is a homeostatic challenge because even transient localized O_2 deficits can produce irreversible cellular damage. The lack of O_2 participates critically in the pathogenesis of major causes of mortality, such as stroke, myocardial infarction, and chronic lung disease. In mammals, acute hypoxia triggers fast respiratory and cardiovascular counterregulatory adjustments (occurring over a time scale of seconds to minutes) to ensure sufficient O_2 supply to the most critical organs such as the brain or the heart. These acute responses to hypoxia depend on the modulation of O_2-regulated ion channels, which mediate adaptive changes in cell excitability, contractility, and secretory activity (Fig. 1). O_2-regulated ion channels are preferentially expressed in excitable cells of specific tissues such as the arterial and airway chemoreceptors (carotid and neuroepithelial bodies), smooth muscle from the pulmonary and systemic vasculature, and neonatal adrenal medulla (40). Chronic exposure to hypoxia (for hours to days) regulates the expression of numerous genes encoding enzymes, growth factors, or transporters, which induce molecular and histological modifications to reduce the cellular need and dependence on O_2 and increase O_2 supply to the tissues (Fig. 1). Chronic adaptation to hypoxia, observed in almost every cell type, critically depends on transcriptional mechanisms that determine the level of expression of numerous genes (9, 66). Transcriptional activity induced by hypoxia has been shown to rely on O_2-dependent protein hydroxylases, which regulate the activity and nuclear translocation of hypoxia-inducible transcription factors (HIF-1 and isoforms) (42, 66).

In this review, we summarize the participation of ion channels in the cellular and systemic adaptive responses to hypoxia. We discuss the role of ion channels as effectors of the hypoxia signal transduction pathway in cells acutely responding to low P_{O_2}, emphasizing the present hypotheses on the mechanisms underlying O_2 detection. We also stress the growing importance of ion channels as part of the gene expression program triggered by chronic hypoxia. Finally, we comment on the pathophysiological implications of acute and chronic regulation of ion channel function by O_2 tension.

ACUTE RESPONSES TO HYPOXIA MEDIATED BY ION CHANNELS

Changes of local O_2 tension can induce modifications in the electrical activity of numerous cell types, including neurons; however, neurosecretory cells located in chemoreceptor organs and smooth muscle cells in pulmonary and systemic arteries are

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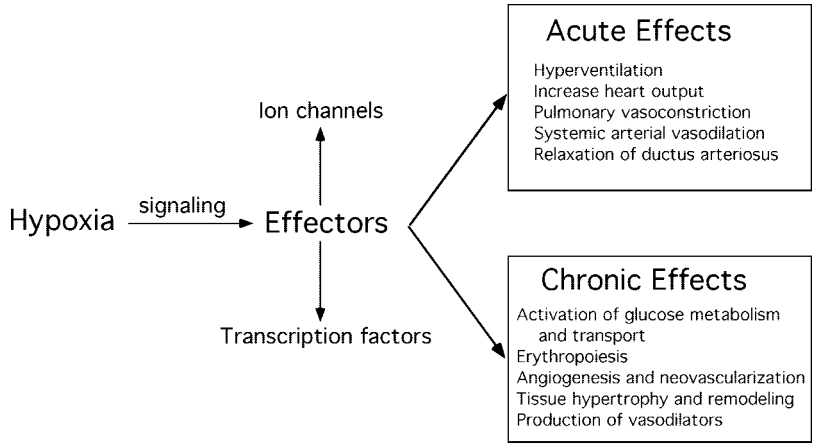


Fig. 1. Hypoxia signaling pathway with indication of the major adaptive responses to acute and chronic hypoxia.

those directly involved in the major systemic adjustments to acute hypoxia.

O_2-Sensitive Neurosecretory Cells

The carotid and aortic bodies or the neuroepithelial bodies of the lung, which are organs capable of sensing global O_2 tension, participate in the compensatory cardiorespiratory adjustments to hypoxia. These organs contain O_2-sensitive neurosecretory cells, which release transmitters on exposure to environmental low P_{O_2} (<50 to 60 Torr) (40). The classical O_2-sensing chemoreceptors are the carotid bodies, composed of clusters of sensory (glomus) cells innervated by afferent nerve fibers. These sensory fibers convey chemosensory discharges to the brain stem respiratory center to evoke hyperventilation during hypoxemia. Similar clusters of excitable neurosecretory cells have also been described in the neuroepithelial bodies of the lung and in adrenal chromaffin cells from the neonate where they detect P_{O_2} changes in the inspired air and in blood, respectively. Excitation of chemoreceptor cells by hypoxia mainly depends on the presence of membrane channels whose activity is modulated by low P_{O_2}. The "O_2-sensitive" channels studied in more detail are K^+ channels, initially described in glomus cells of the carotid body (7, 15, 23, 39, 53, 71) and found in all the hypoxia-responsive neurosecretory cells studied so far (61, 75, 87, 92). Nevertheless, the kind of O_2-sensitive K^+ channel appears to change among the various chemoreceptor cells or among cells in different animal species. Some K^+-channel types proposed to participate in acute O_2 sensing are voltage-dependent channels of the voltage-gated K^+ (Kv) or *Shaker* family, Ca^{2+}-activated K^+ (K_{Ca}) channels, and TASK-like background K^+ channels (see Refs. 38 and 40 and references therein). Recordings illustrating the reversible inhibition by hypoxia of whole cell K^+ currents in several chemoreceptor cell types are shown in Fig. 2*A*, and the "membrane model" of chemosensory transduction is summarized by a scheme in Fig. 2*B*. At least in the case of carotid body glomus cells, all of the indicated steps involved in stimulus-secretion coupling have strong experimental support. Transmitter release induced by hypoxia in isolated cells is *1*) mimicked by depolarization with high extracellular K^+ or application of K^+ channels blockers (51, 78), *2*) paralleled by an increase in cytosolic Ca^{2+} concentration ($[Ca^{2+}]$) (44, 78), and *3*) abolished by removal of extracellular Ca^{2+} or blockade of voltage-gated Ca^{2+} channels (44, 78). In addition, it has directly been shown in patch-clamped cells that hypoxia or K^+-channel blockers produce glomus cell depolarization (8, 85) and an increase of action potential firing frequency (8, 41, 44). The rise of cytosolic $[Ca^{2+}]$ induced by hypoxia is prevented in voltage-clamped cells held at negative membrane potentials (8). Altogether, these data indicate that chemosen-

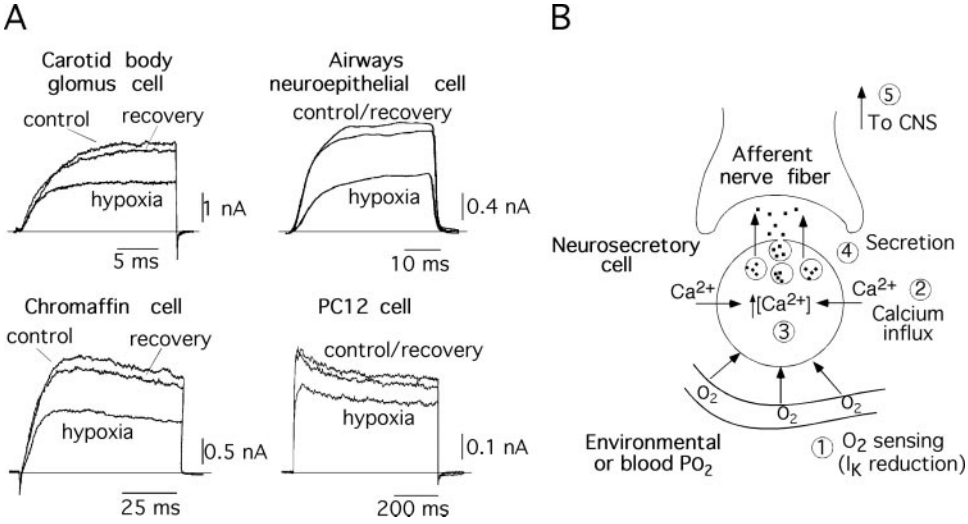


Fig. 2. *A*: reversible reduction of macroscopic K^+ currents by hypoxia in 4 representative O_2-sensitive neurosecretory cells. *B*: scheme of the membrane model of O_2 sensing in neurosecretory cells. $[Ca^{2+}]$, Ca^{2+} concentration; CNS, central nervous system; I_K, K^+ current.

sory transduction is initiated by the closure of K^+ channels by low P_{O_2}, which leads to membrane depolarization and/or increase of action potential firing frequency, extracellular Ca^{2+} influx through voltage-gated channels, and transmitter release to the extracellular milieu (38).

Vascular Smooth Muscle Cells

Vascular smooth muscle cells (VSMCs) control blood flow and tone, and their contraction is directly influenced by blood O_2 tension. The acute vascular responses to hypoxia studied in more detail are pulmonary vasoconstriction and dilation of systemic vessels (82, 91).

Hypoxia-induced vasoconstriction (pulmonary vasculature). Hypoxic pulmonary vasoconstriction (HPV), which occurs predominantly in small resistance arteries, is essential for fetal life because it helps to maintain the high pulmonary vascular resistance that diverts blood through the ductus arteriosus. In adults, HPV reduces blood flow through poorly ventilated alveoli, thus contributing to matching perfusion to ventilation and preventing systemic hypoxemia when atelectasis is present. Although the adaptive changes of pulmonary myocytes to low P_{O_2} are complex, these cells respond, similar to the O_2-sensitive neurosecretory cells, with reduction in amplitude of the macroscopic voltage-dependent K^+ currents (49, 56, 89, 90). Inhibition of one or several types of K^+ channels by hypoxia leads to membrane depolarization, opening of voltage-gated Ca^{2+} channels, and myocyte contraction (82, 89).

Hypoxia-induced vasodilation. Hypoxic vasodilation is another fast response to hypoxia of VSMCs, particularly well manifested in coronary and cerebral vessels, that helps to increase the perfusion of blood to the O_2-deprived tissues. A major component of hypoxic vasodilation is mediated by ATP-sensitive K^+ (K_{ATP}) channels of vascular myocytes, which open in response to hypoxia due to decreased ATP production (13). However, there are other O_2-sensitive ionic mechanisms causing myocyte relaxation because it occurs with P_{O_2} levels that do not compromise energy metabolism. K_{Ca} channels potentiated by low P_{O_2} have been described in isolated cerebral resistance myocytes (24), and a somewhat similar mechanism (inhibition of K^+ channels by normoxia) has been proposed to induce contraction of the ductus arteriosus at birth once the blood in the newborn is oxygenated (77). Moreover, there is considerable evidence indicating that, in arterial myocytes, transmembrane Ca^{2+} influx is also directly inhibited by low P_{O_2}. Relaxation by hypoxia is produced in arteries precontracted with K^+ (a condition that prevents repolarization by opening of K_{ATP} or K_{Ca} channels), and in isolated myocytes the elevation of cytosolic [Ca^{2+}] induced by high K^+ concentration is reversibly reduced by low P_{O_2} (20, 79). Inhibition of L-type Ca^{2+} channels by hypoxia has been described in patch-clamped systemic arterial myocytes (20, 21, 68).

MECHANISMS OF O_2 SENSING BY ION CHANNELS

O_2 Sensor in Close Contact with the Ion Channels

Although the role of ion channels as effectors in the acute cellular responses to hypoxia is well established, the identity of the O_2 sensor molecules and the signaling pathways linking the sensors to the effectors remain, however, enigmatic. Because some channels retain the hypoxia responsiveness in excised membrane patches, it has been suggested that the O_2 sensor is closely associated with the channel oligomer, either attached to the pore-forming α-subunit or as part of an auxiliary subunit (23, 32, 37, 59). Switching of the sensor between oxi- and deoxi-conformations would result in alteration of the channel gating owing to direct allosteric interactions. Some specific types of K^+ channel α- and/or β-subunits are expressed in O_2-sensitive cells (4, 10, 14, 25, 30, 62). The levels of protein expression of Kvα1.5 and Kvα3.1 as well as Kvβ1.1 subunits are reported to be higher in pulmonary resistance arterial myocytes than in other arterial VSMCs (10). It has been shown that mice lacking the Kvα1.5 subunits have impaired HPV and reduced sensitivity of whole cell voltage-gated K^+ currents to hypoxia (2) and that antibodies against Kv2.1 diminish O_2-sensitive currents in rat pulmonary myocytes (30). However, whether Kv1.5, Kv2.1, or any other K^+ channel α-subunit acts as an O_2 sensor or whether they are simply effectors in the hypoxia signaling cascade of pulmonary VSMCs is not known. Some recombinant subunits of K^+ and Ca^{2+} channels expressed in heterologous cells have been shown to be O_2 sensitive (18, 31, 35, 36, 50, 52). These studies lead us to expect rapid progress in the identification of the O_2 sensors; however, advances produced so far have been relatively minor, and the data available are inconclusive. The O_2 sensitivity of α or combinations of α and β K^+ and Ca^{2+} channel subunits changes, depending on the experimental conditions used in the various laboratories and on the cell types used for heterologous protein expression (see Ref. 40 for a detailed discussion). Therefore, it seems that O_2 sensitivity is not absolutely intrinsic to the ion channels but requires the interaction between the O_2-sensing signaling molecules and the pore-forming channel subunits.

Redox Model of Acute O_2 Sensing

An alternative view to the existence of an O_2 sensor attached to the ion channels is the redox model of O_2 sensing based on the conversion of O_2 into reactive oxygen species (ROS), which would then alter the cellular redox status and the function of the ion channels (which contain numerous residues susceptible to redox modification) (1, 11). The two ROS-producing systems postulated as O_2 sensors are the NADPH oxidase and mitochondria.

NADPH oxidase as possible O_2 sensor. NADPH oxidase has been proposed to transduce O_2 levels by changing the rate of superoxide anion (O_2^-) production, which after conversion to H_2O_2 oxidizes ion channels (11). Although mice lacking the gp91 catalytic subunit of the neutrophil's oxidase have impaired O_2 sensitivity of airway chemoreceptor cells (22), the hypoxia responsiveness of carotid bodies (60), neonatal adrenal medulla (74), and pulmonary VSMCs (3) remains unaltered. Furthermore, the histological appearance of glomus cells and the modulation of the O_2-sensitive K^+ current by P_{O_2} are also unchanged in the gp91 mutant mice (27). Surprisingly, this same group reported that genetic suppression of another component of the neutrophil's oxidase ($p47^{phox}$) results in mutant mice with increased basal activity in the carotid sinus nerve and exacerbated ventilatory response to hypoxia (63). Whether this phenotype, reflecting overexcitability of the glomus cell-afferent fiber synapse, is a nonspecific side effect of the $p47^{phox}$ deletion or whether it is due to selective alteration of the carotid body O_2-sensing machinery is presently unknown. Although these studies may suggest that the

phagocytic NADPH oxidase is not a general O_2 sensor, other isoforms, existing in numerous tissues (see Ref. 38), could participate in O_2 sensing.

Mitochondria as possible O_2 sensor. Mitochondria have also been considered by some authors to be the site for acute O_2 sensing because, similar to hypoxia, inhibitors of the electron transport chain (ETC) and metabolic poisons stimulate the carotid body. The concept behind the "mitochondrial hypothesis" of O_2 sensing is that the lack of O_2 would reduce the activity of cytochrome *c* oxidase in complex IV, thus resulting in mitochondrial depolarization and Ca^{2+} release (72). This form of mitochondria involvement in O_2 sensing lost support after the discovery that cell responsiveness to low P_{O_2} requires membrane depolarization and Ca^{2+} entry through plasmalemmal voltage-gated channels (see Ref. 37). Nevertheless, the interest in mitochondria has resurged in the past years owing to studies testing the redox model of acute O_2 sensing (1, 34, 43, 81). Mitochondria consume almost all available O_2 and are major sources of O_2^- due to inefficient transfer of electrons along the respiratory chain. Although there is no general agreement on whether hypoxia decreases or increases cell ROS production, it has recently been proposed for pulmonary arterial myocytes that hypoxia is sensed by the decrease in the velocity of electron transfer from cytochrome *c* to O_2, thus leading to accumulation of ETC intermediates in the reduced state and the production of ROS. It is thought that radicals are preferentially generated at the semiubiquinone site, where an electron can leak out to produce O_2^- (34, 79). This view contrasts with earlier observations indicating that hypoxia shifts pulmonary VSMCs to a more reduced state (1, 84). Although participation of mitochondria in the response to hypoxia of arterial pulmonary myocytes cannot be discarded (1, 34, 43, 81), the assumption that these organelles have a general role in O_2 sensing is questioned by numerous experimental findings. O_2-sensitive maxi-K^+ channels of rat glomus cells respond to P_{O_2} changes independently of redox modification (59), and reduction of K^+ currents by hypoxia is maintained in airway chemoreceptor cells devoid of mitochondria or after mitochondrial inhibition (65). In whole carotid bodies, the reduced (GSH)-to-oxidized (GSSG) glutathione ratio remains unchanged during exposure to hypoxia despite the fact that this quotient increases after incubation of carotid bodies with *N*-acetylcysteine, a precursor to GSH and ROS scavenger (64). In addition, it has been shown that hypoxia responsiveness of intact glomus cells is unaffected by the complete blockade of the mitochondrial electron flow with saturating concentrations of ETC inhibitors acting at the different mitochondrial complexes. Interestingly, rotenone selectively occludes responsiveness to hypoxia, an effect not mimicked by other complex I inhibitors and unaltered by feeding electrons through complex II with succinate (48). Therefore, it seems, that although a rotenone-inhibited molecule is essential for carotid body O_2 sensing, this phenomenon is independent of mitochondrial electron flow. Discrete rotenone binding sites outside mitochondria have not been reported, but the existence of cytosolic aggregates of preassembled complex I proteins of unknown function has been documented (see Ref. 48). Rotenone has a relatively high affinity (in the nM range) for the carotid body O_2-sensing machinery; therefore, it could be used as a probe to investigate its location and nature in glomus cells.

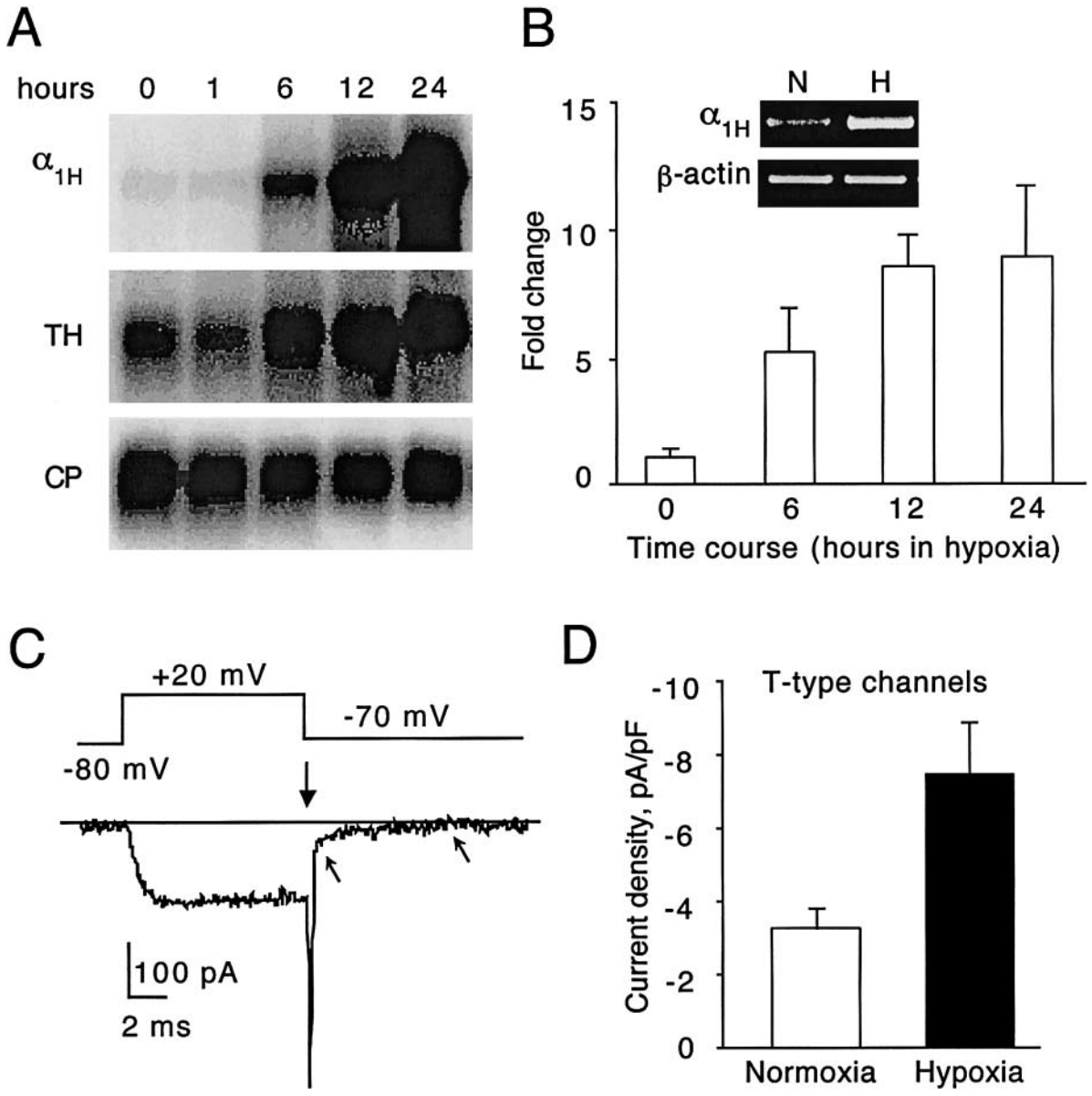
O_2-Dependent Hydroxylases, HIF, and Acute O_2 Sensing

Another possible form of acute O_2 sensing that has recently been explored involves O_2-dependent prolyl and asparaginyl hydroxylases, which are known to regulate the activity of hypoxia-inducible transcription factors (mainly HIF isoforms 1α and 2α) (42). Protein hydroxylation does not seem, however, to participate directly on acute O_2 sensing, as incubation of carotid body slices with dimethyloxalylglycine, a membrane-permeant competitive inhibitor of oxoglutarate that completely inhibits hydroxylases and induces the expression of O_2-sensitive genes (16), does not alter the responsiveness of glomus cells to hypoxia (47). However, HIFs appear to be necessary for setting the appropriate level of expression of the O_2-sensing machinery in carotid body cells and pulmonary myocytes. Heterozygous HIF-$1\alpha^{+/-}$ mice, with apparently normal carotid body histology, have impaired responses to low P_{O_2} and adaptability to chronic hypoxia (33). Similarly, in HIF-$1\alpha^{+/-}$ mice, the changes of pulmonary arterial myocyte membrane potential and K^+ channel density induced by chronic hypoxia are blunted (67).

EFFECTS OF CHRONIC HYPOXIA ON ION CHANNEL GENE EXPRESSION

Despite the progress in the understanding of the role of ion channels in the acute cellular responses to lowering O_2 tension, the participation of these proteins in the adaptive long-term changes induced by chronic hypoxia has not been studied in detail. It is known that prolonged hypoxia downregulates various Kv channel genes in pulmonary artery smooth muscle cells (55, 69, 80). Chronic hypoxia also reduces K^+ current amplitude (26, 86) but increases the density of Na^+ and Ca^{2+} channels in carotid body glomus cells (29, 70). Recently, detailed molecular biology and electrophysiological studies have shown that T-type Ca^{2+} channels are upregulated by hypoxia in PC12 cells and possibly in other tissues (16). A five- to eightfold increase in the level of the T-type subunit α1H mRNA is found in PC12 cells when exposed to hypoxia (~20 Torr), and mRNA induction is paralleled by an increase in the density of T-type Ca^{2+} currents (Fig. 3, *A–D*). These

Fig. 3. Upregulation by chronic hypoxia of T-type Ca^{2+} channel gene (α_{1H}). *A*: Northern blot analysis of α_{1H} mRNAs from cells exposed to either normoxia (21% oxygen, *time 0*) or hypoxia (3% oxygen) for the indicated periods of time. The levels of the cyclophilin (CP) and tyrosine hydroxilase (TH) mRNAs were analyzed to normalize the amount of RNA in each line and to check for the ability of PC12 cells to induce the expression of an O_2-sensitive gene. *B*: average fold induction of α_{1H} mRNA levels expressed as -fold change ± SE in the hypoxic samples compared with the normoxic sample (*time 0*). *Inset*: example of a PCR experiment (30 cycles) where β-actin was used to normalize the amount of RNA in each line. N, normoxic; H, hypoxic. *C* and *D*: electrophysiological identification of slowly deactivating T-type channels in PC12 cells. Current density due to T-type Ca^{2+} channel activity increased 2.3-fold on exposure to hypoxia (3% oxygen) for 18–24 h. *E*: alignment of the nucleotide sequences of the rat, mouse, and human α_{1H} 5′-flanking region containing 6 putative hypoxia-inducible factor (HIF) consensus DNA binding sites. The core motifs are in bold face and upperlined by arrows to indicate the plus (rightward arrow) or minus (leftward arrow) DNA strand location. Note that 2 putative HIF binding sites (indicated by letters of larger size) are conserved among the 3 species. (Modified from Ref. 16.)



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rat     5'-AATTGGTGTCGCGTCGCGCATCGCGCCCCACGTCGCCCACGCGCTCCAG-G-ACACTGGC
mouse   5'-AATTGGTGTCGCGTCGCGCAACGCGCCCCACGTCGCCCACGCGCCCCAAAG-ACACTGGC
human   5'-------------GCCGTAAAGCACACCCCCGCCCGCCCCCGCGCCCCATCACACACGCGC

rat     GCCCCGGCACACCCAAGACA--CACGCGGTGGGGGAAGGGCACGC-GCCTCGCGGA---C
mouse   GCCCCGGCACACCCAAGACA--CACGCGGTGGGGGAAGGGCACGC-GCCTCGCGGG---C
human   GCGCGGGCACACCCGGGACACACACGCGGCGGGGAAGGGGCACGCAGCCTCTCGCCTTCC

rat     ATGGTCACGGCGTGCCT---CCCACGCCTGCCGAGCCCCGCCCCCTAGC----------3'
mouse   ATGGTCACGGCGTGCCT---CCCACGCCTGCTGAGCCCCGCCCCCTAGC----------3'
human   ACGCCCGCCCGGTCCCCGCCCCTCCGCCGACCCGGGCCCGCGTCGTAGTGGGGGTGGGGG-3'
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observations have suggested that upregulation of T-type Ca^{2+} channels by hypoxia may contribute to cellular functions susceptible of modulation by low O_2 concentration, such as cellular excitability, differentiation, growth, and proliferation. T-type Ca^{2+} channel gene induction is also stimulated by desferroxamine, cobalt, or dimethyloxalylglycine. These compounds mimic hypoxia by inhibiting oxygen-, Fe^{2+}-, and oxoglutarate-dependent dioxygenases that under normoxic conditions hydroxylate specific proline and asparagine residues in HIF before its degradation (42). In addition, it has been shown that stabilization of HIF or induction of α1H mRNA by hypoxia is blocked when the cells are incubated with HIF antisense oligonucleotides (16). The involvement of HIF in the hypoxic upregulation of the α1H Ca^{2+} channel suggested by these experiments is further supported by the presence of hypoxia responsive elements (HIF to DNA binding sites) in the 5′-flanking region of the α1H gene (Fig. 3*E*). This promoter region is highly conserved among mammals, with more than 71% similarity between rodents and humans and 93% similarity between rats and mice. These results indicate that T-type Ca^{2+} channels, and possibly other ion channels, are part of the gene program developed under chronic hypoxia. The data summarized in Fig. 3 represent the first example of an ion channel gene whose expression, similar to erythropoietin and other classical O_2-sensitive genes, is regulated by the O_2-sensitive hydroxylase-HIF pathway (9, 42, 66).

PATHOPHYSIOLOGY ASSOCIATED WITH ION CHANNEL-DEPENDENT O_2 SENSING

Primary Alterations of Acute O_2 Sensing

There are several human diseases that seem to be related to primary alterations of the acutely responding O_2-sensitive cells. Some cases of congenital central hypoventilation syndrome (CCHS) appear without alterations in central respiratory centers but with marked decrease in the number of glomus cells and hypoplasia of carotid bodies despite a two- to threefold increase of sustentacular cells (12). In this same study, compensatory hyperplasia of the neuroepithelial bodies of the lung was also observed. In ~10–20% of patients, CCHS is associated with Hirschsprung disease, thus raising the possibility that the RET protooncogene, altered in Hirschsprung disease, participates in the mechanisms of O_2 sensing. Interestingly, RET is part of the multicomponent receptor complex of the glial cell line-derived neurotrophic factor (GDNF); in addition, both RET and GDNF are highly expressed in adult carotid bodies (76). Therefore, GDNF activation of RET is probably required for the maintenance of the O_2 sensitivity of glomus cells. Increased sustentacular cell number (28) and decreased carotid body size (45) have also been reported for some cases of sudden infant death syndrome (SIDS). Unexpected sudden death has been reported after bilateral carotid body denervation in humans and animals (17, 73), and infants prone to apnea have altered responses to mild hypoxia (6). Carotid body dysfunction in these syndromes could be the result of a primary alteration of either the O_2 sensor or the ion channels acting as effectors. Chronic exposure to hypoxia or application of chemostimulants induce glomus cell overexcitability, increased Ca^{2+} entry, and carotid body hyperplasia (29, 48, 86). Thus glomus cell hypoexcitability could underlie the hypoplasia observed in CCHS and SIDS. Perrin et al. (54) reported in patients affected by SIDS the presence of higher carotid body dopamine content than in normal children. This could also be the cause of carotid body hypoexcitability, as it is known that dopamine inhibits Ca^{2+} currents in glomus cells (5).

Alteration of O_2-sensitive K^+ channels could also participate in the pathophysiology of primary pulmonary hypertension, a condition characterized by increased resistance of the fine branches of the pulmonary artery. VSMCs taken from small pulmonary arteries of patients with primary pulmonary hypertension appear to be depolarized and to have higher cytosolic [Ca^{2+}] levels relative to cells from patients with secondary pulmonary hypertension (88). In addition, several anorexic drugs (aminorex, fenfluramine, and others), known to produce pulmonary hypertension, have been shown to inhibit macroscopic K^+ currents in pulmonary arterial smooth muscle (83). A direct link between maxi-K^+ channels and pulmonary hypertension has been demonstrated in newborn lambs (46). Interestingly, it has recently been reported that in vivo transfer of Kv1.5 channels reduces pulmonary hypertension and restores HPV in chronically hypoxic rats (57).

Chronic Hypoxia and Modifications of Ion Channel Gene Expression

Different forms of chronic hypoxia (sustained or intermittent) cause alterations of various O_2-sensitive tissues (58). Maintained reductions of O_2 tension (either in high altitude or in cages for experimental animals) induce a marked carotid body hypertrophia and blunted response to low P_{O_2}. It has been reported that glomus cells from chronically hypoxic carotid bodies are more excitable, due to the overexpression of Na^+ and Ca^{2+} channels, but they also have reduced voltage-dependent K^+ current amplitude (26, 29, 70, 86). Intermittent hypoxia is known to cause hypertension, secondarily to activation of arterial chemoreceptors and subsequent sympathetic stimulation (19). However, it is also possible that hypoxia causes alteration in the expression of channels that regulate smooth muscle excitability in the systemic vasculature. In the pulmonary arterial tree, chronic hypoxia reduces the amplitude of macroscopic K^+ currents (69) and downregulates various voltage-gated K^+ channels (55, 80). As described in the preceding section, the expression of T-type Ca^{2+} channel genes is increased by hypoxia in PC12 and other cell types (16). Given the broad distribution of these channels, they probably have a major role in cell adaptation to chronic hypoxia.

FUTURE RESEARCH DIRECTIONS

Over the past decade, we have witnessed a rapid development and maturation of the field of O_2 sensing due to the identification and characterization of effector molecules, particularly ion channels and transcription factors. A major recent advance has been the discovery of the O_2-dependent hydroxylases that regulate the stabilization and transcriptional activity of HIF (42). Regarding the role of ion channels in O_2 sensing, a significant contribution has been the demonstration that, as the classical O_2-sensitive genes, they are also regulated by the HIF signaling pathway and therefore are part of the gene program developed under chronic hypoxia (16, 66). The study of ion channel-encoding O_2-sensitive genes will surely receive higher attention in the near future. Although the present experimental work further supports the notion that O_2-regulated

ion channels participate in the acute cellular responses to hypoxia, there are several unresolved pivotal questions pertaining to the nature of the O_2 sensors and the mechanisms of interaction of the sensors with the ion channels. We have summarized in this review recent work that, in our view, helps to distinguish between the mechanisms that need to be explored further and those that lack experimental support. It is possible that acute O_2 sensing does not utilize a single mechanism but that different O_2 sensors are expressed in the various hypoxia responsive cells. Nevertheless, new experimental work is urgently needed to fully characterize the involvement of ion channels in the hypoxia signaling pathway. The combination of biophysical, molecular biology, and pharmacological techniques applied to in vitro preparations and animal models are experimental approaches that are already yielding important results. These techniques are helping to clarify the critical role of ion channels in hypoxic pulmonary hypertension (57, 80, 83, 88). Similarly, rotenone, a drug that selectively occludes sensitivity to hypoxia of carotid body glomus cells (48), could be a useful tool to search for the location and nature of the carotid body O_2 sensor. Finally, it can be expected that this basic knowledge will have a medical impact. Ion channels and other O_2-sensitive effectors are involved in vasomotor and cardiorespiratory control, and localized lacks of O_2 are critical in the pathogenesis of major causes of mortality. Therefore, amplification or inhibition of adaptive responses to hypoxia is a promising pharmacological strategy that may result in effective therapies for human diseases.

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