Oxygen sensing by the carotid body: mechanisms and role in adaptation to hypoxia

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López-Barneo J, González-Rodríguez P, Gao L, Fernández-Agüera MC, Pardal R, Ortega-Sáenz P. Oxygen sensing by the carotid body: mechanisms and role in adaptation to hypoxia. Am J Physiol Cell Physiol 310: C629–C642, 2016; doi:10.1152/ajpcell.00265.2015.—Oxygen (O_2) is fundamental for cell and whole-body homeostasis. Our understanding of the adaptive processes that take place in response to a lack of O_2 (hypoxia) has progressed significantly in recent years. The carotid body (CB) is the main arterial chemoreceptor that mediates the acute cardiorespiratory reflexes (hyperventilation and sympathetic activation) triggered by hypoxia. The CB is composed of clusters of cells (glomeruli) in close contact with blood vessels and nerve fibers. Glomus cells, the O_2-sensitive elements in the CB, are neuron-like cells that contain O_2-sensitive K^+ channels, which are inhibited by hypoxia. This leads to cell depolarization, Ca^{2+} entry, and the release of transmitters to activate sensory fibers terminating at the respiratory center. The mechanism whereby O_2 modulates K^+ channels has remained elusive, although several appealing hypotheses have been postulated. Recent data suggest that mitochondria complex I signaling to membrane K^+ channels plays a fundamental role in acute O_2 sensing. CB activation during exposure to low PO_2 is also necessary for acclimatization to chronic hypoxia. CB growth during sustained hypoxia depends on the activation of a resident population of stem cells, which are also activated by transmitters released from the O_2-sensitive glomus cells. These advances should foster further studies on the role of CB dysfunction in the pathogenesis of highly prevalent human diseases.

carotid body; oxygen sensing; ion channels; mitochondria; mitochondrial complex I signaling; adaptation to hypoxia; stem cells

The provision of sufficient oxygen (O_2) to the tissues is a fundamental physiological challenge. This is particularly the case in mammals, as O_2 is an absolute requirement for cellular homeostasis and the lack of O_2 is critical in the pathogenesis of a number of major causes of morbidity and mortality in the human population (11, 88). Adaptation to protracted (chronic) hypoxia (lasting hours or days) depends on the O_2-sensitive prolyl hydroxylase-hypoxia inducible transcription factor (HIF-1α and HIF-2α) signaling pathway, which modulates the level of expression of a broad array of genes that encode transporters, enzymes, cytokines, and growth factors. These effectors drive molecular and histological modifications to reduce the cellular need and requirement for O_2, as well as to increase blood O_2 transport and supply to the tissues (60, 141, 151, 173). However, the survival of higher animals upon exposure to hypoxia requires acute respiratory and cardiovascular adjustments (e.g., hyperventilation and sympathetic activation) that, during the course of seconds or minutes, ensure O_2 delivery to the most critical organs such as the brain and heart. Sustained activation of these hypoxia-induced reflexes is also necessary for acclimatization to hypoxemia (or low O_2 tension—PO_2—in the blood), a frequent condition in the human population as millions of people live at or travel to high altitudes and are therefore exposed to low atmospheric air pressure and decreased O_2 diffusion into the blood (59, 175). In addition, there are highly prevalent medical disorders, such as chronic obstructive pulmonary disease, which present with severe hypoxemia due to a reduction in the O_2 exchange capacity between the air and the pulmonary capillaries (149, 156). Among the organs of the homeostatic O_2-sensitive system (172), the carotid body (CB), a small arterial chemoreceptor located at the bifurcation of the carotid artery, is of particular importance as it assumes the main responsibility for the detection of changes in blood O_2 levels and activation of the respiratory center to elicit the proper adaptive ventilatory response. Although research on the physiology of the CB and other peripheral chemoreceptors has progressed significantly over the past years, several fundamental aspects of acute O_2 sensing remain poorly understood. In this review, we summarize current data available on the structural organization and chemosensory functions of CB cells, with emphasis on the existing theories regarding the mechanisms of acute O_2 sens-
ing. We also discuss the interactions among the various cellular elements in the CB and how they contribute to CB adaptation to chronic hypoxia.

ORGANIZATION AND CHEMOSENSORY PROPERTIES OF CAROTID BODY GLOMUS CELLS

Structural Organization of the Carotid Body

The CB is located at the carotid bifurcation although its precise location varies between mammalian species and even among different individuals of the same species (Fig. 1A). The size of the CB in humans shows large variations (5, 33, 53), although a recent study has reported an estimated average volume of \( \approx 20 \text{ mm}^3 \), with no significant difference among individuals of different age or sex (114). The CB has been classically considered to derive from precursor cells that in embryonic life migrate from the superior cervical ganglion to the wall of the carotid artery (54, 61). This has been confirmed by lineage tracing experiments demonstrating that cells of the CB parenchyma (type I and type II cells) originate from neural crest progenitor cells (121). The CB is composed of functional units called glomeruli, which are clusters of cells separated by a profuse network of small capillaries and connective tissue (Fig. 1, B and C). Each glomerulus contains neuron-like glomus (or type I) cells, which are highly dopaminergic and can therefore be immunostained with antibodies against tyrosine hydroxylase (TH). Glomus cells are enveloped by processes of sustentacular (type II) cells that are positive for antibodies against glial fibrillary acidic protein (GFAP) and other glial markers. Type I and type II cells can be easily distinguished by electron microscopy due to their distinctive characteristics, the most obvious being the abundance of mitochondria and the presence of numerous small dense-core secretory vesicles in glomus cells (Fig. 1C). The CB of mammals, including humans, also contains nestin-positive (nestin\(^+\)) cells, which are progenitors that can generate new mature glomus cells (114) (Fig. 1, B and D). It has been shown that type II cells, or a subpopulation of them, are quiescent stem cells that are activated under hypoxia to proliferate and differentiate into glomus and other cell types (121). In vitro, type II (GFAP\(^+\)) cells rapidly evolve into nestin\(^+\) transit amplifying

Fig. 1. Structure and organization of the carotid body (CB). A: photograph of the human carotid bifurcation after cleaning the connective and fat tissue surrounding the CB (arrow) region. Scale bar, 1 cm. Male, 42 yr old. [Modified from Ortega-Saenz et al. (114) with permission from John Wiley and Sons.] B: schematic representation of the cellular components in the CB glomerulus. v, Blood vessel. Nestin\(^+\) progenitor cells appear in green. [Modified from Pardal et al. (121), with permission from Elsevier.] C: electron microscope photograph illustrating the ultrastructure and anatomical relations of the two main cellular elements in the mouse CB; type I cells (red) enveloped by type II cells (dark blue). The inset shows the abundance of mitochondria and dense core secretory vesicles in type I cells. D: histological section of a human CB immunostained with antibodies against dopamine decarboxylase (DDC), a glomus cell marker, and against nestin, a progenitor cell marker. Note the proximity between progenitor cells and glomus cells within the CB glomeruli. Scale bar, 20 \( \mu \text{m} \). [Modified from Ortega-Saenz et al. (114), with permission from John Wiley and Sons.] E: representative histological sections of three different CB neurospheres cultured (9 days) in floating conditions immunostained with antibodies against tyrosine hydroxylase (TH) and nestin. c, Neurosphere core; b, blebs of differentiated TH\(^+\) cells. Scale bar for the three panels, 50 \( \mu \text{m} \).
progenitors, which form clonal colonies (neurospheres) that give rise to TH⁺ glomus cells (Fig. 1E). The CB is highly innervated by afferent nerve fibers that, together with glomus cells, form chemical synapses. It also contains numerous autonomic efferent fibers that terminate in the parenchyma and near blood vessels. CB glomeruli are “organoid”-like structures with a sophisticated organization; they support a myriad of complex functional interactions among their different cellular elements, the nature of which is only beginning to be understood (108, 133, 134, 161).

Physiology of Carotid Body Glomus Cells. O₂-Sensitive Ion Channels

Although early research on the CB firmly established its role as a major arterial chemoreceptor (41), the mechanism of CB O₂ sensing remained obscure for several decades until technological advances facilitated physiological measurements in the small glomus cells. Early neurochemical studies in dispersed glomus cells demonstrated dopamine release in response to hypoxia in an extracellular Ca²⁺-dependent manner (40). Using the patch-clamp technique it was shown that glomus cells, which were thought to be nonexcitable, contain voltage-dependent Na⁺, Ca²⁺, and K⁺ currents and can therefore generate action potentials (37, 85). It was found that K⁺ channel open probability in glomus cells is inhibited within a few seconds of exposure to low O₂ tension (30, 48, 85, 124, 154), thereby providing the basis for a mechanism of CB chemotransduction (Fig. 2A). These observations, complemented by experiments in which membrane potential, cytosolic Ca²⁺ concentration ([Ca²⁺]), and catecholamine secretion were monitored and controlled in single cells (16, 84, 103, 163) (Fig. 2, B and C), supported the current “membrane model” in which glomus cells function as O₂-sensitive presynaptic elements. In this scheme, blockade of K⁺ channels, which are open at the cell’s resting potential, leads to glomus cell depolarization, Ca²⁺ entry, and transmitter release, which activate the afferent fibers of the sinus nerve (88) (Fig. 2D). As discussed below, this “membrane model” of chemotransduction is not in opposition with the potential role played by cell metabolism in O₂ sensing and signaling to membrane ion channels during hypoxia. The

![Fig. 2. Responses to hypoxia in single rodent glomus cells.](Image)

A: whole cell membrane currents from a glomus cell recorded with the perforated patch technique during the application of a depolarizing ramp. Note the reversible inhibition of the holding and voltage-dependent K⁺ currents during repeated (h1 and h2) exposures to hypoxia (P_0₂ = 10 mmHg). Control (c) and recovery (r) recordings were obtained in normoxic conditions (P_0₂ = 145 mmHg). Reversal of the current was displaced by 8 mV to depolarized membrane potentials during exposure to hypoxia. B and C: cytosolic Ca²⁺ (B) and catecholamine secretion (C) of rodent glomus cells as a function of O₂ tension. Inset in B illustrates the rise of cytosolic [Ca²⁺] in response to hypoxia and the abolition of this signal upon removal of external Ca²⁺. Inset in C is the secretory response to hypoxia of a cell in CB slices as monitored by amperometry. [Data in B from Urena et al. (163); A and C, modified from Montoro et al. (103) and Ortega-Saenz et al. (115), with permission from the Rockefeller University Press.] D: membrane model of acute O₂ sensing by carotid body glomus cells. See text for further explanation. [Modified from Lopez-Barneo et al. (82), with permission from Elsevier.]
so-called “metabolic hypothesis” (105, 110) is a concept adopted to stress the involvement of mitochondria in O2 sensing before it was revealed that glomus cells are excitable and contain O2-sensitive membrane ion channels. Since their discovery in CB glomus cells, O2-sensitive ion channels (K+, Na+, Ca2+, Cl−, or nonselective channels) have been found ubiquitously distributed in a variety of mammalian cell types, including systemic arterial smooth muscle, heart muscle, and neurons (87, 88, 107, 152). O2-sensitive K+ channels similar to those of the CB exist in other cells, such as PC12 cells (187), chromaffin cells of the adrenal medulla (76, 102, 147, 159), and cells in the neuroepithelial bodies of the lung (109, 180), to induce transmitter release under hypoxia, as well as in pulmonary arterial smooth muscle to produce hypoxic vasoconstriction (135, 183).

Glomus cells contain several transmitters (mainly acetylcholine, dopamine, serotonin, ATP) and neuropeptides (substance P, endothelins, enkephalins, among others) and are particularly rich in small dense-core secretory granules. One matter of debate has been the nature of the agent or agents that postsynaptically activate the afferent fiber in the glomus cell-afferent nerve synapse. An important technical advance in this regard was the development of the in vitro chemosensory synapse preparation using co-cultures of dispersed glomus cells and petrosal sensory neurons (186). Among the transmitter substances released from glomus cells in response to hypoxia, acetylcholine and ATP are likely to be responsible for activation of sensory nerve fibers (42, 185). However, other substances may have a modulatory role. For example, opioids and dopamine have been shown to exert auto- or paracrine control of CB activation by inhibiting the Ca2+ current and thereby decreasing the secretory activity of type I cells (8, 69).

Although the most important stimulus for CB activation is a decrease in blood PO2, other potent stimuli are hypercapnia and a decrease in extracellular pH. As in the case of hypoxia, both low pH and hypercapnia produce modulation of membrane channels due to direct interaction of external or internal protons with several classes of K+ and nonselective cationic channels in the glomus cell membrane (14, 89). The net result is Ca2+ influx, an increase in cytosolic Ca2+, and transmitter release. In addition to these classical stimuli, other variables, such as blood temperature and osmolarity, also seem to be detected by glomus cells; however, these sensory responses and their physiological impact have not been studied in detail. Interestingly, the CB may be sensitive to blood flow as the CB-dependent chemoreflex and sympathetic outflow are increased in several models of chronic heart failure. Inhibition of glomus cell K+ currents by hypoxia is enhanced in animals with chronic heart failure (80), probably as a result of impaired angiotensin II function and redox regulation; nonetheless the link between reduced CB flow and altered CB signaling remains to be identified (150). Glomus cells can also depolarize and release transmitters when the extracellular glucose concentration is reduced (42, 49, 119, 184). This has led to the proposal that the CB is a combined glucose and O2 sensor (82). These observations confirm previous work at the systemic level on non-primate mammals, suggesting that the CB participates in glucose homeostasis (2, 72). Although the role of the CB in the regulation of plasma glucose has been the subject of debate (9, 168), studies in humans have yielded results compatible with CB involvement in the counter-regulatory response to hypoglycemia (171). In line with these observations, we have recently shown that the chemosensory responses of glomus cells to hypoxia and hypoglycemia, which to date have only been studied in animal models, particularly in rodents and cats, are preserved in humans even at advanced ages (114).

**MECHANISMS OF CAROTID BODY ACUTE O2-SENSING BY MEMBRANE CHANNELS**

Despite the identification of numerous types of O2-sensitive ion channels in different tissues (87, 88, 152), the mechanism or mechanisms underlying the O2-dependent modulation of ion channel function have remained unknown. A detailed discussion of the broad variety of acute O2-sensing mechanisms that have been proposed in various cells is beyond the scope of the current review. Instead, we will focus on the available mechanistic data that are relevant for the understanding of CB O2 sensing and will also emphasize the theories that in our view are most appealing and experimentally robust (Table 1). Initially, it was thought that, as in other sensory systems, an O2 sensor could directly or indirectly regulate the function of a specific type of glomus cell K+ channel (83). However, this possibility is now deemed unlikely, as numerous studies have shown the existence of many classes of O2-sensitive K+ channels in glomus cells, including several subtypes of voltage-dependent channels (24, 47, 90), maxi-K+ channels (144, 174, 178), and background K+ channels (13, 30, 66, 117, 162). Although these channels operate in different ranges of membrane potential, it is probable that all of them contribute to the sensitivity of CB cells to hypoxia. Indeed, although background K+ channels, open at negative membrane potentials, constitute the main K+ conductance in resting glomus cells, and are therefore probably responsible for the initial depolarization induced by hypoxia (66, 162), it is known that ibotenic acid and tetraethylammonium (TEA+), which are blockers of maxi-K+ and voltage-dependent K+ channels, can induce secretion in glomus cells (118). Therefore, these channels could contribute to the potentiation of cell depolarization and action potential duration upon exposure to hypoxia, and even support the hypoxic response in cells with reduced expression of background K+ channels. In line with these notions, responsiveness to hypoxia is maintained in cells after pharmacological blockade of voltage-dependent K+ channels with TEA+ and 4-aminopyridine or after selective blockade of maxi-K+ channels with iberiotoxin (13, 74, 112, 145). Moreover, genetic ablation of Task1 and Task3 genes, which encode the ion channel subunits most likely to contribute to the O2-sensitive background K+ conductance (66), does not prevent the generation of a powerful secretory response to hypoxia in glomus cells (112). Our current view is that CB O2 sensing may be based on a promiscuous signaling mechanism that impinges on several different ion channel classes (see discussion in refs. 39, 112). Another difficulty inherent to CB O2 sensing is that the responsiveness of membrane ion channels to hypoxia is a labile phenomenon that for as yet unknown reasons can be lost in apparently healthy cells. This has led us to develop the thin CB slice, a preparation that does not require the use of enzymes or vigorous mechanical treatments and therefore permits highly reproducible monitoring of secretory responses to hypoxia by amperometry in intact single cells from adult animals (118, 120). Adaptation of this technique to mice has allowed us to
analyze the potential contribution of several molecules to $O_2$ sensing using genetically modified models (39, 112, 115, 116).

**Energy Metabolism**

Mitochondria have classically been thought to participate in acute $O_2$ sensing by CB chemoreceptors due to their exquisite sensitivity to cyanide and other inhibitors of the mitochondrial electron transport chain (ETC) (101, 105). In addition, mitochondrial parameters (e.g., mitochondrial membrane potential or NAD(P)H autofluorescence) in chemoreceptor cells are highly sensitive to $O_2$ levels (15, 35, 36). In early studies it was proposed that Ca$^{2+}$ release from these organelles is the signal that triggers secretion during exposure to hypoxia (36). However, it was soon demonstrated that, as described in the preceding section, the rise of cytosolic [Ca$^{2+}$] observed in glomus cells under hypoxia requires membrane depolarization and is generated by extracellular Ca$^{2+}$ influx through membrane channels. However, mitochondria use $O_2$ and could therefore detect changes in $O_2$ availability and signal the membrane channels in response to hypoxia. Indeed, it has long been proposed that changes in reactive oxygen species (ROS) of mitochondrial origin signal membrane K$^+$ channels during hypoxia in pulmonary myocytes (see below) (3, 169) (Table 1). It has also been suggested that glomus cell mitochondria possess a special isofrom of cytochrome-c oxidase with low $O_2$ affinity (101) and that a decrease in $O_2$ due to mitochondrial inhibition during hypoxia is the signal that mediates the closing of background K$^+$ channels, thereby leading to glomus cell depolarization (176) (Table 1). A different version of the “metabolic hypothesis” of $O_2$ sensing is based on the activation of AMP-activated protein kinase due to an increase in the AMP-to-ATP ratio during hypoxia and the subsequent phosphorylation of amino acids that regulate ion channel function (38, 177) (Table 1). Although these are appealing proposals, they have not reached broad acceptance due to variability in the observations made by different groups (67). Moreover, responsiveness to hypoxia is seen in patch-camped glomus cells dialyzed with ATP (85) and ATP levels are maintained in the CB (164) or $O_2$-sensitive pulmonary arterial myocytes (169) exposed to hypoxia.

**Redox Signaling**

As indicated above, a “redox hypothesis” of $O_2$ sensing has been postulated to explain the function of $O_2$-sensitive K$^+$ channels in pulmonary arterial myocytes (Table 1). However, whether hypoxia increases or decreases the cytosolic levels of mitochondrial ROS has been a matter of debate (50, 55, 100). Recently, it was proposed that the Fe/S Rieske protein in mitochondrial complex III (MCIII) is an $O_2$ sensor that mediates the production of ROS during hypoxia. Ablation of the gene encoding this protein abolishes the rise of ROS and ATP during hypoxia (3, 169) (Table 1). Although these are appealing proposals, they have not reached broad acceptance due to variability in the observations made by different groups (67). Moreover, responsiveness to hypoxia is seen in patch-camped glomus cells dialyzed with ATP (85) and ATP levels are maintained in the CB (164) or $O_2$-sensitive pulmonary arterial myocytes (169) exposed to hypoxia.

**References**

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**Table 1. Proposed mechanistic models of $O_2$ sensing by ion channels in carotid body glomus cells**

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*Whether reactive oxygen species (ROS) increase or decrease during hypoxia in pulmonary myocytes and other cell types is under debate. †Based on data in pulmonary arterial myocytes. For carotid body (CB) data, see refs. 39, 150. CSE, cystathionine-$\gamma$-lyase.
Produced (NADPH) oxidase (NOX) (25) (Table 1). NOX, histo-
chemically localized in CB cells, can transduce O₂ levels by
changing the rate of production of superoxide anion, which,
after conversion to hydrogen peroxide, can oxidize ion chan-
nels and thereby regulate their function. The best-studied
enzyme commonly found in neutrophils (NOX-2 isoform) is an
oligomer composed of a membrane-attached catalytic complex
(forming by gp91 phox and p22phox), a cytochrome, and
several cytosolic regulatory subunits (p47phox among others).
Although several reports using gp91phox-null mutant mice
have suggested the direct involvement of NOX-2 in O₂ sensing
by airway chemoreceptor cells, which also contain O₂-regu-
lated K⁺ channels (18, 45, 180), these findings have not been
reproduced in the CB (51, 52, 146) or pulmonary artery (4).
Moreover, deletion of the gene encoding the p47phox subunit
can modulate the activity of CB chemoreceptor cells but does
not prevent their basic O₂-sensing properties (52, 148). In
addition to NOX-2, other NADPH isoforms are broadly dis-
tributed among tissues (75) and have been proposed to partici-
patate in O₂ sensing. In particular, it has been suggested that
NOX-4, a protein that shares ~40% sequence identity with
gp91-nox-2, mediates the O₂ sensitivity of Task-1 channels
(77). Furthermore, using heterologous expression (in HEK
cells) of recombinant proteins it has been reported that p22 (a
NOX-4 subunit) is necessary for the interaction of NOX-4 and
Task-1 (122). As discussed above, ablation of the Task1 gene
leaves the secretory responses to hypoxia of glomus cells
virtually unaltered (116). Therefore, the actual role of the
NOXs in CB oxygen sensing remains unresolved.

Gaseuxtransmitters

Several gas compounds, such as nitric oxide (NO), carbon
monoxide (CO), and hydrogen sulfide (H₂S), are known to be
involved in numerous cell functions and have also been sug-
gested to participate in O₂ sensing by CB cells (139). NO is
known to modulate Ca²⁺ and K⁺ channels present in glomus
cells (138). In addition, NO production by efferent fibers
terminating in the CB or by local microganglionic neurons could
have a modulatory role on glomus cell excitability (46, 81,
167). However, the direct involvement of NO on acute CB
oxygen sensing has not been demonstrated. Mutant mice defi-
cient in NO synthase 1 show ventilatory responses to hypoxia
(70). In addition, the secretory responses to hypoxia of glomus
cells in CB slices remain unchanged after their incubation with
N⁶-nitro-L-arginine methyl ester, an inhibitor of NO synthesis
(111).

It has been suggested that hemoxygenase (HO)-2, an anti-
oxidant enzyme expressed in numerous cell types, is an O₂
sensor in CB glomus cells (Table 1). This enzyme uses O₂ for
oxidative degradation of heme to biliverdin and CO. As CO is
a maxi-K⁺ channel activator (144, 166), it has been proposed
that, during hypoxia, the decrease in CO leads to inhibition of
 maxi-K⁺ channel activity in glomus cells (174). Although this
model of O₂ sensing is attractive, it has been challenged by
experiments on HO-2 knockout animals, which develop nor-
mally without signs of respiratory distress and show normal
CB responsiveness to hypoxia (115). Analysis of CB gene
expression in HO-2-null animals have demonstrated upregula-
tion of TH mRNA, the rate limiting enzyme in the synthesis of
dopamine in glomus cells, and downregulation of Slo1 mRNA,
Mitochondrial Complex I Signaling

Another perspective on the role of mitochondria as O$_2$ sensors resulted from our experiments showing that rotenone and 1-methyl-4-phenylpyridinium (MPP$^+$), which are distal inhibitors of mitochondrial complex I (MCI) that prevent ubiquinone binding, suppress responsiveness to hypoxia in CB glomus cells without altering the responses to hypoglycemia. In contrast, hypoxia responsiveness is virtually unaffected by MCI blockers acting outside the ubiquinone site (49, 113). Inhibitors that bind downstream in the ETC (at complexes II–IV), activate, as rotenone, glomus cells in an external Ca$^{2+}$-dependent manner but appear to be less efficient in preventing a further effect of hypoxia (113). Although experiments using pharmacological agents must be interpreted with caution given that high concentrations of mitochondrial inhibitors are known to inhibit membrane channels independent of their effects on mitochondria (86), these results, confirmed in O$_2$-sensitive adrenal medulla chromaffin cells (64, 158), led us to postulate that a rotenone-binding molecule is involved in CB O$_2$ sensing. To test this hypothesis, we generated genetically modified mice with conditional ablation of the Ndufs2 gene, which encodes a 49-kDa protein that contributes to the ubiquinone-binding site of MCI (6, 39, 63). The transfer of electrons from the Fe/S clusters to ubiquinone, the last step in the NADH/ubiquinone oxidoreductase reaction, takes place at this site, which is located near the distal Fe/S cluster (N2 center) in MCI. We have recently shown that biallelic deletion of Ndufs2 restricted to catecholaminergic (TH$^+$) cells results in animals insensitive to changes in P$_{O_2}$, despite exhibiting full responsiveness to other stimuli (high K$^+$, hypercapnia, and hypoglycemia) (Fig. 3, A and B). Modulation of background and voltage-dependent K$^+$ currents by P$_{O_2}$ is also abolished in Ndufs2-deficient glomus cells (39). Extensive functional and biochemical studies performed on several mouse models with mutations in MCI Ndufs2 and Ndufs4 genes (39) suggest that, in O$_2$-sensing cells of the peripheral chemoreceptor organs (CB and adrenal medulla), hypoxia induces a MCI state characterized by the elevated production of ROS and reduced pyridine nucleotides (Fig. 3, C and D). This could result in inhibition of neighboring membrane K$^+$ channels located near mitochondria, thereby forming “O$_2$ sensing microdomains”. The “hypoxic” state of MCI is favored by the slowing down of the ETC during hypoxia and the accumulation of reduced quinone due to a particularly high succinate metabolism, which seems to be a characteristic feature of glomus cells (39) (Fig. 4). Besides the special metabolic adaptations, which permit glomus cells to survive without a functional MCI, the unusual sensitivity of glomus cell mitochondria to reductions in P$_{O_2}$ could be, as suggested previously (101), a consequence of the existence in these cells of a special cytochrome-c oxidase with low O$_2$ affinity. However, it is also possible that although maintaining a normal cytochrome-c oxidase, CB cells have highly uncoupled mitochondria that make them behave as “O$_2$ sinks,” with an accelerated ETC and therefore highly sensitive to reductions in O$_2$ levels. Indeed, it has long been recognized that the CB has an extraordinarily high blood flow (7), O$_2$ consumption, and metabolic rate (29, 153). The model of acute O$_2$ sensing based on MCI signaling provides an explanation for most of the experimental data available on CB glomus cells. For example, it explains the high levels of glial cell-derived neu-

Fig. 3. Selective abolition of responsiveness to hypoxia in glomus cells from Ndufs2-deficient mice (NDUFS2-KO). A: secretory response of CB cells in slices to direct membrane depolarization with high (40 mM) extracellular K$^+$. No quantitative differences in the secretion rate were observed between the two experimental conditions. B: secretory responses to hypoxia (P$_{O_2}$, $\approx$ 10 mmHg) and hypercapnia (20% CO$_2$). C: changes in NADP(H) autofluorescence in dispersed glomus cells under hypoxia. D: changes in cytosolic levels of reactive oxygen species (ROS) in glomus cells transfected with a roGFP probe targeted to the cytosol. [Modified from Fernández-Agüera et al. (39), with permission from Elsevier.]
rotrophic factor in the CB (114, 165) necessary to upregulate the genes encoding antioxidant enzymes and prosurvival factors in cells that are continuously exposed to oxidative stress (27). However, definitive support for this model requires additional research.

**Lactate Receptor**

Recently, a G protein-coupled olfactory receptor that is highly expressed in CB glomus cells compared with chromaffin cells has been reported to mediate O₂ sensing in the CB by acting primarily as a receptor for lactate secreted from neighboring cells during hypoxia (22). This interesting proposal is compatible with the model of MCI signaling in acute O₂ sensing, as lactate dehydrogenase could reduce pyruvate to lactate using the NADH accumulated during hypoxia in glomus cells. However, this “paracrine lactate model” does not explain numerous developmental and functional observations in glomus cells, in particular the responsiveness to hypoxia of single, isolated, glomus cells placed in a chamber with continuous flow of solution, which should immediately dilute any lactate secreted from the cells. The effects of intra- and/or extracellular lactate on glomus cells are not known in detail and remain to be studied. Nonetheless, we cannot rule out the possibility that arterial chemoreceptors may use multiple O₂ sensors, possibly with different but overlapping PO₂ sensitivities, which create a redundancy or safety margin that ensures a proper physiological response to acute hypoxia.

**ROLE OF CAROTID BODY IN ADAPTATION TO HYPOXIA**

**Carotid Body Growth Under Sustained Hypoxia. Carotid Body Stem Cells**

In addition to its role as an acute O₂ sensor, the CB also contributes to the physiological acclimatization to chronic hypoxia. The CB is a highly plastic organ that, in high-altitude residents or in patients suffering cardiopulmonary diseases who present with hypoxemia, can grow to several fold its normal size due to angiogenesis and enlargement of the neural parenchyma. This response, unusual for a neuronal organ, leads to augmentation of the excitatory electrical signals that act on the brainstem respiratory center to produce hyperventilation (5, 59, 98). Although some TH⁺ CB cells can undergo mitotic divisions, experiments performed in the past few years have shown that the organ contains a resident population of adult neural stem cells (NSCs) that contribute to its growth during acclimatization to hypoxia (121). Multipotent NSCs that give rise to neurons and glia throughout life are found in the subventricular zone (SVZ) and the hippocampus of the mammalian brain (73). NSCs of neural crest origin are also found in the adult peripheral nervous system, although, with the exception of the CB stem cells, their functional role in vivo is poorly understood (58). CB NSCs can be identified in vitro by their ability to form clonal spherical colonies (neurospheres) when they are cultured in floating conditions (see Fig. 1E). Neurosphere assays based on dispersed adult CBs have demonstrated their differentiation into glomus cells, mesenchymal-like smooth muscle actin-positive cells (a typical neural-crest derivative), and endothelial cells. In adult rats and mice, CB stem cells represent approximately 0.5% to 1% of the total number of cells obtained by enzymatic dispersion (121). Several lines of evidence have suggested that CB stem cells are the sustentacular GFAP⁺ type II cells, or a subpopulation of them. Cell fate mapping experiments in vivo have shown that during exposure to chronic hypoxia, X-gal-marked GFAP⁺ cells are converted to TH⁺ glomus cells. When the GFAP⁺/X-gal⁺ cells are cultured, they form colonies in which all the progenitors and their derivatives (glomerus cells or smooth muscle) maintain the X-gal deposits, indicating a common lineage for the entire cell population (121). Moreover, animal models with genetically induced mitochondrial damage to GFAP⁺ cells display marked brain atrophy but a normal peripheral nervous system, with the exception of the CB, which also undergoes significant postnatal developmental inhibition (34). These observations further support the glial (GFAP⁺) origin and singularity of CB stem cells. Taken together, these data suggest that the CB behaves in a similar manner to the neurogenic niches in the brain, with glia-like stem cells that can be converted to intermediate nestin⁺ progenitors, which in turn can differentiate into glomus and other cell types (71). Newly generated stem cell-derived TH⁺ cells, like normal glomus cells, contain voltage-dependent Na⁺, Ca²⁺, and K⁺ channels and can also release transmitters in response to acute hypoxia and other stimuli. Therefore, it seems that these cells have a clear functional role contributing to the CB growth necessary for acclimatization to chronic hypoxia (121). As mentioned earlier, the neurogenic niche is found fully developed in the human CB even at advanced ages (114).

The mechanism underlying activation of CB growth in response to hypoxia has remained obscure even though this is a well-known process that is central to understanding the physiology of acclimatization to hypoxia (5, 59, 98). The CB niche provides an ideal model in which to study activity-dependent neurogenesis and to explore the mechanisms whereby NSCs switch from dormancy to cycling. As hypoxia can modulate the behavior of stem cells, it was originally thought that CB stem cells could enter directly into a proliferative state in response to hypoxia (71, 121). However, recent experiments have shown that the growth of CB neurospheres is...
unaffected by changes in O2 tension between 1% and 21% (approximately 7 and 150 mmHg, respectively) (133). In this regard, CB stem cells behave in a similar manner to central NSCs, which are also insensitive to hypoxia (28). In addition, systemic application of dimethyloxalylglycine (DMOG), a nonselective inhibitor of prolylhydroxylases that causes “pharmacological hypoxia,” does not produce CB growth in rats, despite the fact that this treatment upregulates HIF-dependent genes (10, 133). These observations, indicating that CB stem cells are not directly activated by hypoxia, led us to suggest that CB growth in response to hypoxia could be a niche property that is probably dependent on the activation of the O2-sensitive glomus cells. Indeed, the detailed analysis of the localization of secretory vesicles in glomus cells indicates that only a relatively small number of vesicles are confronted with a postsynaptic nerve membrane (“chemosensory synapse”) to activate afferent sensory fibers that act upon the respiratory center. Unexpectedly, the vast majority of vesicles are facing the membrane of type II cell processes that envelop glomus cells (see Fig. 1C). Therefore, when activated by hypoxia, transmitters released from glomus cells could act on type II cells to induce their proliferation. Experimental evidence supporting the existence of robust glomus cell-stem cell “chemoproliferative synapses” has recently been obtained (133). Endothelin 1, a peptide known to be present in glomus cells (23, 99), is one of the agents identified as a potential mediator of the “chemoproliferative synapses,” Endothelin 1 acts on G protein-coupled receptors (mainly of the B subtype) that are found in practically all type II cells and induces a pro-proliferative cascade possibly involving MAP kinases and c-myc (133). Taken together, these studies indicate that the CB glomerulus is a highly sophisticated structure with complex interactions among its different cellular components (108, 161). The O2-sensitive glomus cells are the central elements that orchestrate the responses of the CB to hypoxia through the “chemosensory” and “chemoproliferative” synapses. As the neurotransmitters, cell targets, and intracellular pathways are different in the two types of O2-sensitive synapses, the development of a selective pharmacological strategy could have potential therapeutic application.

Changes in Carotid Body Function Induced by Chronic Intermittent Hypoxia

In addition to its role in adaptation to chronic sustained hypoxia, a growing body of evidence suggests that the CB may also play a critical role in the pathogenesis of comorbidities associated with medical conditions characterized by chronic intermittent hypoxemia (IH). Recurrent apneas with transient hemoglobin desaturation can frequently occur in neonatal respiratory disorders, asthmatic patients, and, particularly, in obstructive sleep apnea (OSA) syndrome. OSA syndrome is a highly prevalent disease that affects ~4% of the adult population in developed countries and is an independent risk factor for hypertension and nonfatal and fatal cardiac events (97, 132, 179). Intermittent obstruction of the airways in OSA patients leads to numerous pathophysiological alterations, which include repeated wakening and sleep fragmentation. However, it is believed that stimulation of CB chemoreceptors by intermittent hypoxemia and hypercapnia, leading to strong activation of cardiovascular sympathetic reflexes, is the most important factor responsible for the development of hypertension (26, 32, 57, 106). Studies on rodent models performed more than two decades ago showed that denervation of the CB prevents the development of hypertension in animals exposed to IH (44). More recently, it has been demonstrated that chronic IH enhances the CB chemoreflex, the ventilatory response to hypoxia, and selectively induces long-term facilitation of the CB, a form of plasticity that results in increased CB excitation during acute episodes of IH (96, 128, 129, 142). In the past few years, CB overactivation has been recognized to contribute to the enhanced sympathetic outflow present in OSA syndrome and possibly other chronic diseases (130, 143, 150). Therefore, CB denervation has been proposed for the treatment of refractory neurogenic hypertension in humans (92, 110). The molecular mechanisms underlying CB plasticity during IH are as yet poorly known. In contrast to sustained chronic hypoxia, CB from animals subjected to IH do not show obvious morphometric changes, although they exhibit abnormal ROS production and decreased MCI activity (128). ROS-mediated alterations in transmitter signaling (in particular endothelin 1, angiotensin II, and NO), and proinflammatory cytokines have been proposed to mediate the IH-induced changes in CB chemosensory function (57, 150). In addition, hypoxic inhibition of TASK-like K+ channels in glomus cells is increased in animals treated with chronic IH (117).

Role of the O2-Sensitive Prolyl Hydroxylase-Hypoxia-Inducible Factor Pathway in Carotid Body-Mediated Changes During Adaptation to Chronic Hypoxia

HIFs modulated by O2-sensitive prolyl and asparaginyl hydroxylases are well-established key effector molecules in the genetic program developed during cell adaptation to hypoxia (60, 152). Incubation of CB cells with DMOG, a nonselective inhibitor of prolyl hydroxylases, does not prevent a powerful secretory response to hypoxia (111), and cells in CB slices obtained from Hif1α−/− mice show normal sensitivity to variations in O2 tension (116). However, HIF isoforms (HIF1α and HIF2α) play a fundamental role in CB homeostasis and adaptation to chronic sustained or intermittent hypoxia (140). HIF2α is necessary for the correct development and maintenance of the peripheral catecholaminergic nervous system and is highly expressed in CB cells (160). Genetic overexpression of HIF2α (but not HIF1α) produces CB hypertrophy (91), whereas downregulation of HIF2α in catecholaminergic cells results in a depressed hypoxic ventilatory response and blunting of CB growth in chronic hypoxia (56). Chronic hypoxia is known to modulate the expression of ion channels, which modify the excitability of glomus (21, 155) and adrenal medulla (AM) (19, 31, 78) cells. Interestingly, prolyl (157) or asparaginyl (62) hydroxylations has been reported to inhibit the activity of transient receptor potential (TRP) A1 and V3 channels, respectively, in normoxic conditions. Some of these channels (particularly TRPA1) are also very sensitive to cysteine oxidation (157). TRP channel families are expressed in the CB chemosensory synapse (17), and CB cells have a background cationic current, which contributes to the setting of the membrane potential and therefore responsiveness to hypoxia (20, 49). It is possible that the function, expression, and/or membrane distribution of TRP channels is modulated by O2-dependent hydroxylation or ROS levels, thereby allowing them...
to participate in the adaptation to hypoxia/ischemia in the CB and other body tissues (104).

Abnormal expression of HIF isoforms seems to be involved in the exaggerated CB function and increased sympathetic tone induced by intermittent hypoxia. HIF1α is upregulated in animals exposed to IH, and this effect is prevented in animals pretreated with antioxidants. Moreover, CB-mediated cardiorespiratory changes are markedly inhibited in heterozygous HIF1α mice (131). It has been shown that IH-mediated ROS production of mitochondrial origin upregulates HIF1α, which in turn could favor the maintenance of ROS production (128, 131). On the other hand, heterozygous HIF2α mice exhibit augmented CB sensitivity to hypoxia, hypertension, and elevated plasma levels of norepinephrine, which are associated with increased ROS and decreased expression of antioxidant enzymes in the AM (126). It has been suggested that a redox balance determined by mutual antagonism between HIF-α isoforms is required for proper hypoxic responses by the CB-AM axis to maintain cardio-respiratory homeostasis (181).

CONCLUSIONS AND FUTURE DIRECTIONS

The specialized O2-sensitive glomus cells are the main arterial chemoreceptors that mediate the reflex hypoxic hypertensive ventilatory response characteristic of most mammalian species. Glomus cells are excitable neuron-like cells that contain several classes of K+ channels, which are inhibited under hypoxia. Closure of the K+ channels leads to depolarization, Ca2+ influx, and exocytotic transmitter release to activate afferent nerve fibers that terminate at the brainstem respiratory center. Although this membrane model of chemosensory transduction is broadly accepted, the mechanisms underlying O2 sensing have remained elusive. Several gasotransmitters released within the CB (NO, CO, and H2S) seem to have a direct or indirect action on K+ channels in glomus cells and could therefore modulate the responsiveness of the cells to hypoxia (139, 182). However, it seems that mitochondria are the most likely candidates for sensing changes in O2 tension and signaling the membrane K+ channels in glomus cells. Recent work in animals with genetic disruption of the ubiquinone-binding site in MCI has shown that CB glomus cells can survive well without a functional MCI although they are insensitive to hypoxia. Lack of responsiveness to hypoxia is maintained even in cells incubated with succinate to support respiration through MCII. Nonetheless, these cells (lacking MCI function) respond normally to all other stimuli tested (direct membrane depolarization with K+, hypercapnia, and hypoglycemia) (39). This work has suggested that, under hypoxia, ROS and reduced pyridine nucleotides generated in MCI signal membrane K+ channels. In addition, lactate released from hypoxic glomus cells has been proposed to activate neighboring cells (22). Although these data provide new insight into the mechanism of acute O2 sensing by CB cells, future investigations must clarify whether a single process fully explains all of the O2-dependent acute responses to hypoxia in glomus cells and whether this mechanism is present in other organs of the homeostatic O2-sensing system. It is critical that, to avoid any bias or misinterpretation, the different proposals on acute O2 sensing are tested in different laboratories. In addition, the special metabolic properties of glomus cells must be studied in detail to elucidate their role in responsiveness to hypoxia. The CB is the only organ in the peripheral nervous system that contains an adult stem cell niche with a known physiological role, which is to support the growth of the organ under sustained hypoxia (121). Glomus cells, stem cells (type II cells), and other elements in the niche are organized in clusters named glomeruli, which are highly specialized structures with complex paracrine interactions among their constituents. For example, glomus cells establish abundant chemical synapses with stem cells to induce their growth in response to hypoxia (133). A better understanding of the functional interaction among the different cellular elements in the CB glomusculus should provide a new perspective on CB physiology and its participation in the mechanisms of disease. CB pathophysiology is an exciting translational chapter of CB research, which has been expanding rapidly in recent years. Defective CB chemoreceptor function may be the cause of several hypoventilatory syndromes or the depression of ventilation observed with anesthetics and opioid drugs (136). In contrast, CB overactivation and a subsequent increase in the sympathetic tone in patients with cardiac failure or recurrent apneas seems to underline the appearance of co-morbidities (hypertension and insulin resistance among others) associated with these diseases (95, 123, 130). As human CB function can now be studied (114), it is expected that future research will provide a more precise understanding of the involvement of the CB in disease mechanism.

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Themes

ACUTE OXYGEN SENSING

showed glomus cell [Ca^{2+}]i and respiratory responses to hypoxia. 


