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Physiology of the Carotid Body: From Molecules to Disease

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Abstract

The carotid body (CB) is an arterial chemoreceptor organ located in the carotid bifurcation and has a well-recognized role in cardiorespiratory regulation. The CB contains neurosecretory sensory cells (glomus cells), which release transmitters in response to hypoxia, hypercapnia, and acidemia to activate afferent sensory fibers terminating in the respiratory and autonomic brainstem centers. Knowledge of the physiology of the CB has progressed enormously in recent years. Herein we review advances concerning the organization and function of the cellular elements of the CB, with emphasis on the molecular mechanisms of acute oxygen sensing by glomus cells. We introduce the modern view of the CB as a multimodal integrated metabolic sensor and describe the properties of the CB stem cell niche, which support CB growth during acclimatization to chronic hypoxia. Finally, we discuss the increasing medical relevance of CB dysfunction and its potential impact on the mechanisms of disease.

INTRODUCTION: ONE HUNDRED YEARS OF CAROTID BODY RESEARCH

CB: carotid body

The carotid body (CB) is a small bilateral organ located in the carotid bifurcation (**Figure 1*a***) that carries out essential functions in cardiorespiratory control, in particular during sojourns at high altitude or in patients with impaired gas exchange in the lungs. The CB was identified around 100 years ago as an arterial chemoreceptor. However, in recent decades the field of CB physiology has progressed enormously. It was Fernando de Castro (1), a young pupil of Santiago Ramón y Cajal, who carried out a detailed investigation of the innervation of the carotid region and suggested that the CB was a sensory organ that "tasted" the chemical composition of the blood. In parallel studies, Corneille Heymans and his group (2) demonstrated that the CB is the origin of respiratory reflexes (hyperventilation) induced by hypoxemia, hypercapnia, and acidosis. A significant milestone among the numerous classical studies that followed this seminal work (see 3) was the development of the in vitro CB/carotid sinus nerve preparation (4), which allowed the CB sensory output (action potential frequency in afferent fibers) to be monitored in response to changes in O_2 and CO_2 tension and of pH via altered composition of the extracellular solution. These studies also demonstrated the release of neurotransmitters, in particular acetylcholine and dopamine, from stimulated CB cells, which was inhibited by the removal of extracellular Ca^{2+} (5). Taken together, these findings suggested a neurosecretory role for CB glomus cells, in agreement with electron microscopy observations revealing the existence of numerous secretory vesicles in these cells (6, 7).

A new era in CB physiology, and in the general field of peripheral chemoreception, started in the mid-1980s thanks to the development of enzymatically dispersed glomus cells and the application of modern techniques (patch clamp, microfluorimetry, and amperometry) to study single-cell physiology. It was shown that, in response to hypoxia, dispersed CB cells secrete catecholamines

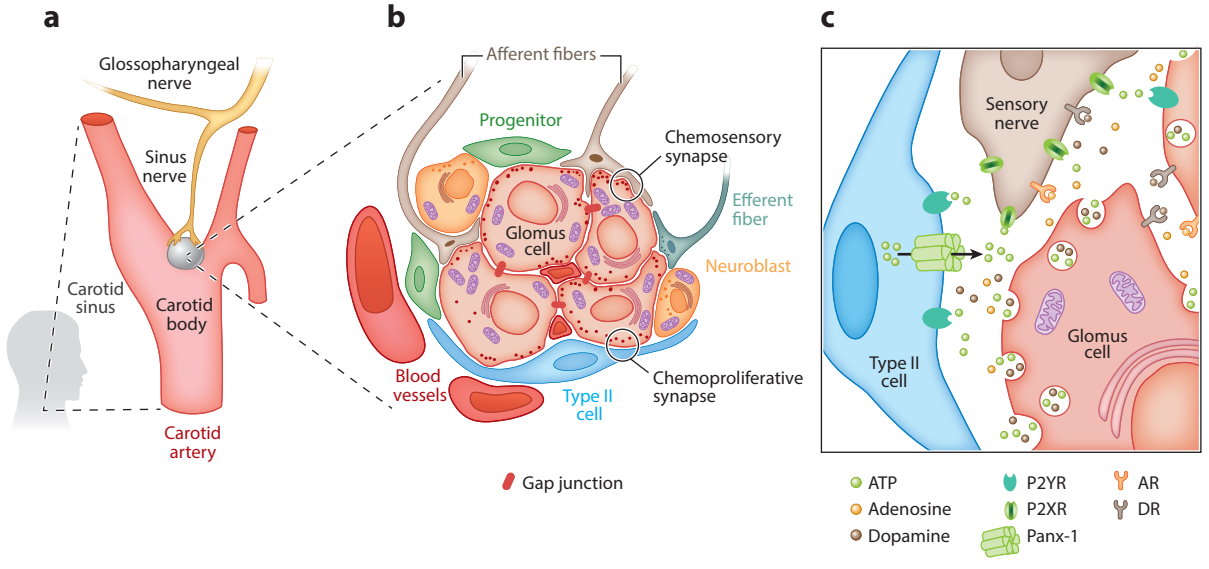

Figure 1

Interactions between cellular elements in the carotid body. (*a*) Carotid bifurcation and location of the carotid body. (*b*) Schematic representation of a carotid body glomerulus. Chemosensory and chemoproliferative synapses are indicated. (*c*) Schematic representation of the tripartite synapse. Abbreviations: AR, adenosine receptor; DR, dopamine receptor; Panx-1, pannexin-1 channel; P2XR, purinergic 2X ionotropic receptor; P2YR, purinergic 2Y metabotropic receptor. Panel *b* adapted with permission from Reference 23. Copyright 2007, Elsevier.

into the culture medium in an external Ca^{2+}-dependent manner (8). Although these very small glomus cells (diameters of ~10 μm) were initially considered to be nonexcitable, patch clamp experiments showed that they possess voltage-dependent Na^+, Ca^{2+}, and K^+ currents typically found in neurons and other excitable cells and therefore were able to generate large, repetitive action potentials (9–11). López-Barneo et al. (10) showed that the K^+ conductance in rabbit glomus cells is reversibly inhibited by hypoxia, thus explaining the O_2-sensing properties of these cells and giving rise to the membrane model of CB chemotransduction. O_2-regulated K^+ channels/currents were found in glomus cells from several species and in various O_2-sensitive tissues, thus forming the homeostatic acute O_2-sensing system (12, 13). An O_2 tension-dependent modulation of other relevant ion channel types, such as L-type Ca^{2+} channels in cardiac or vascular smooth muscle, was also seen (14–16). Microfluorimetric measurements performed on single, Fura 2–loaded glomus cells (17, 18), in conjunction with monitoring of catecholamine vesicle exocytosis by amperometry in dispersed cells and CB slices (18, 19), showed at the single-cell level the presynaptic-like nature of glomus cells. The neurosecretory function of glomus cells was also demonstrated in the in vitro glomus cell–petrosal neuron synapse (20). In parallel with the cellular studies, detailed pharmacological and functional systemic analyses defined the role of the CB in cardiorespiratory and metabolic regulation in humans and other mammals (21, 22).

The field of peripheral chemoreception has grown to such an extent that it is not possible to cover all of its features in a single, comprehensive publication. Noting that several excellent complementary reviews have appeared in recent years, we focus here on current advances in the cellular organization of the CB as well as the molecular mechanisms of acute O_2 sensing. We also discuss the discovery of emerging functions of glomus cells as multimodal and integrated metabolic sensors and the contribution of stem cells to CB plasticity. Finally, we consider the impact of this basic knowledge on our understanding of the mechanisms of disease.

ORGANIZATION AND FUNCTION OF THE CAROTID BODY

The CB derives from neural crest progenitors of the sympathoadrenal lineage, which during embryogenesis migrate from the superior cervical ganglion to the carotid bifurcation (see 23). Besides the various cell types forming the parenchyma, the CB contains abundant vascular, connective, and fat tissues. The size of the CB in adult humans is highly variable, and in a recent study the estimated average volume was ~20 mm^3 without significant differences according to sex or age (24).

Structure of Carotid Body Glomeruli

The CB is anatomically organized in clusters of cells called glomeruli, which are also independent functional sensory units (**Figure 1*a*,*b***). Each glomerulus is composed of approximately 4–8 chemosensitive, neuron-like glomus cells (also called type I or chief cells) of ovoid shape that are in close contact with a profuse network of fenestrated capillaries and richly innervated by afferent sensory fibers from the petrosal ganglion joining the carotid sinus nerve. Autonomic neuronal bodies and efferent fibers terminating on glomus cells and blood vessels are also frequently observed inside CB glomeruli. Glomus cells contain abundant clear and dense-core synaptic vesicles with dopamine and several other neurotransmitters, which allow these cells to be identified with antibodies against tyrosine hydroxylase (TH). Moreover, glomus cells are enveloped by interdigitating processes of less numerous glia-like, type II or sustentacular cells, which can be immunostained with glial fibrillary acidic protein (GFAP) antibodies. Type II cells, or a subpopulation of them, have been identified as multipotent stem cells that support CB growth under conditions of chronic hypoxia (23). Cells that stain positive to nestin (a marker of neural progenitor cells) can be seen in CB glomeruli from humans (24) and other species (25). Although the existence of glomus cell

TH: tyrosine hydroxylase

GFAP: glial fibrillary acidic protein

subclasses was suggested long ago (7, 26), recent studies have identified predifferentiated neuroblasts that express catecholaminergic markers (TH+). Unlike mature glomus cells, which are postmitotic, predifferentiated CB neuroblasts, which are smaller in size and located at the periphery of the glomerulus, can undergo mitotic divisions before final maturation (27). The CB glomerulus has an intricate, organoid-like structural organization that provides the basis for elaborate functional auto- and paracrine interactions among its various cell classes.

Interactions Between Cellular Elements in the Carotid Body Glomerulus

Glomus cells, the sensory elements in the CB, establish reciprocal synapses with their neighbors and chemosensory synapses with afferent sensory fibers. These cells behave as presynaptic-like neurosecretory elements, containing a broad variety of neurotransmitters and neuromodulators (ATP, adenosine, dopamine, acetylcholine, serotonin, GABA, histamine, opioids, substance P, vasoactive intestinal peptide, endothelin-1, and angiotensin II, among others) stored primarily in secretory vesicles that are released by exocytosis (18, 19, 28). It is well established that ATP is the main excitatory neurotransmitter acting on postsynaptic ionotropic (P2X2/3) purinergic receptors in petrosal sensory nerve endings (29–31), although other transmitters and neuromodulators acting pre- or postsynaptically or in both terminals contribute to fine-tuning chemosensory afferent signals (32–36) (**Figure 1*c***). For example, dopamine, one of the most abundant transmitters in glomus cells and known to inhibit ventilation in humans, exerts a negative feedback action by reducing Ca^{2+} current amplitude in glomus cells (37, 38). Dopamine also inhibits a depolarizing cationic current and excitation in the afferent petrosal fibers (34). Similar to dopamine, opioids have a strong inhibitory action on glomus cells (39). In contrast, adenosine, a breakdown product of ATP, is predominantly stimulatory at both pre- and postsynaptic levels (33, 34, 40). Some transmitters, such as ATP, have opposing pre- and postsynaptic effects; in parallel with its excitatory postsynaptic action, ATP binds to metabotropic P2Y receptors in glomus cells to antagonize hypoxia-induced cell depolarization (41, 42). In addition to chemically mediated auto- or paracrine effects, glomus cells appear to be electrically interconnected by gap junctions, although the physiological significance of these is not well understood.

A recent major breakthrough in CB physiology was the realization that type II cells are not simply supportive elements but play a crucial role in chemotransduction and CB plasticity. Electron microscope analyses have shown that secretory granules in glomus cells are not clustered in front of postsynaptic nerve terminals but are uniformly distributed over the entire glomus cell surface. Indeed, the vast majority of vesicles in glomus cells lay facing type II cell membranes, separated by just a small extracellular cleft (43). This morphological arrangement indicates that glomus and type II cells form chemical synapses that are distinct from the glomus cell–nerve ending chemosensory synapse. Type II cells are nonexcitable (i.e., they lack voltage-gated Na^+ and Ca^{2+} channels) and exhibit only a small high-threshold outward K^+ current (9, 11). However, they are activated by ATP released from glomus cells through P2Y metabotropic receptors, which in turn induces Ca^{2+} release from internal stores (44–46). Acetylcholine, serotonin, angiotensin II, and endothelin-1 acting on metabotropic receptors also increase cytosolic Ca^{2+} in type II cells (42, 47). Interestingly, the activation of type II cells by ATP triggers ATP release through pannexin-1 channels, a type of gap junction hemichannel highly expressed in these cells (45, 47). Therefore, it seems that the glomus cell–nerve ending synapse is indeed a tripartite synapse (**Figure 1*c***), in which ATP-induced ATP release from type II cells could contribute to potentiation of the chemosensory response (35, 42). In parallel with this acute action, chemical agents released from glomus cells (e.g., endothelin-1, ATP, or acetylcholine) may also play central roles in the protracted activation of CB neuroblasts or quiescent multipotent CB stem cells (with a type II cell

phenotype) and induce their proliferation and differentiation into mature glomus, smooth muscle, and endothelial cells (25, 27, 43).

Classical light and electron microscopy studies indicated that the CB contains efferent sympathetic and parasympathetic innervation by fibers originating in the superior cervical ganglion, small neuronal clusters in the petrosal ganglion, and by microganglia positioned along the glossopharyngeal and carotid sinus nerve. Parasympathetic neurons can also be found inside the CB (6, 48). In general, the participation of efferent fibers in CB function is poorly known, with the exception of an inhibitory pathway mediated by nitric oxide (NO)–producing neurons (48, 49). NO is released by CB efferent fibers during exposure to hypoxia (50), and this is potentiated by ATP released from glomus cells. In this way, freely diffusible NO exerts a counterregulatory glomus cell inhibition by reducing Ca^{2+} current amplitude and enhancing the maxi-K^+ channel opening (48, 51). In addition to the synaptic and auto- or paracrine interactions within the CB glomerulus, CB chemoreceptor cells are responsive to numerous circulating agents such as leptin, insulin, angiotensin II, or proinflammatory cytokines (see next section).

NO: nitric oxide

HVR: hypoxic ventilatory response

Chemosensory Functions of the Carotid Body: Glomus Cells as Multimodal Sensors

Sensory transduction in the CB is an intrinsic, cell-autonomous process performed by glomus cells, with the organ output modulated by interactions between cellular elements in the glomerulus. The CB has been traditionally associated with the peripheral control of respiration due to its ability to detect changes in blood gas concentrations and pH. However, this view has changed in the last decades, as the CB is now considered a multimodal sensory organ that, in addition to hypoxia, hypercapnia, or acidosis, is also activated by changes in plasma levels of metabolites (e.g., glucose and lactate) and hormones (e.g., insulin and leptin) as well as by modifications in blood temperature, osmolality, or flow (**Figure 2**).

Among the organs of the homeostatic acute O_2 sensing system (13), the CB is considered the main and prototypical O_2 sensor, as it is required for immediate survival in conditions causing hypoxemia. Bilateral CB resection in humans and most mammals results in practical abolition of the hypoxic ventilatory response (HVR) (21, 52, 53). In addition, mice lacking CB due to developmental atrophy do not hyperventilate and cannot survive more than a few days in hypoxic environments (54, 55). The contribution of central respiratory neurons and glia to the HVR is a matter of debate (56). It is widely accepted that acute O_2 sensing in the CB depends on the presence in glomus cells of O_2-regulated K^+ channels whose open probability decreases during hypoxia, thus leading to Ca^{2+} influx and exocytotic transmitter release (57). Glomus cells express several subtypes of O_2-regulated K^+ channels and low- and high-threshold Ca^{2+} channels. The expression of Na^+ channels in glomus cells varies among different species and even among cells in a single animal (12). In the membrane model of hypoxic chemotransduction, glomus cell depolarization and Ca^{2+} channel opening form a common final pathway shared by other stimuli (see below) (**Figure 2*a***). The sensitivity of single glomus cells to hypoxia, as evidenced by changes in cytosolic Ca^{2+} or exocytotic transmitter release as a function of environmental PO_2, follows a hyperbolic function similar to the curve relating ventilation and arterial O_2 tension in whole animals (57).

Hypercapnia and, secondarily, acidemia occur in lung diseases that compromise gas exchange, although these parameters can appear dissociated in cases of metabolic acidosis. Both stimuli depolarize the glomus cell membrane and activate transmitter release in an extracellular Ca^{2+}-dependent manner (58, 59) (**Figure 2*b***). Glomus cell activation by high CO_2 tension is due mainly to intracellular acidification, a process catalyzed by carbonic anhydrase. Several isoforms of this

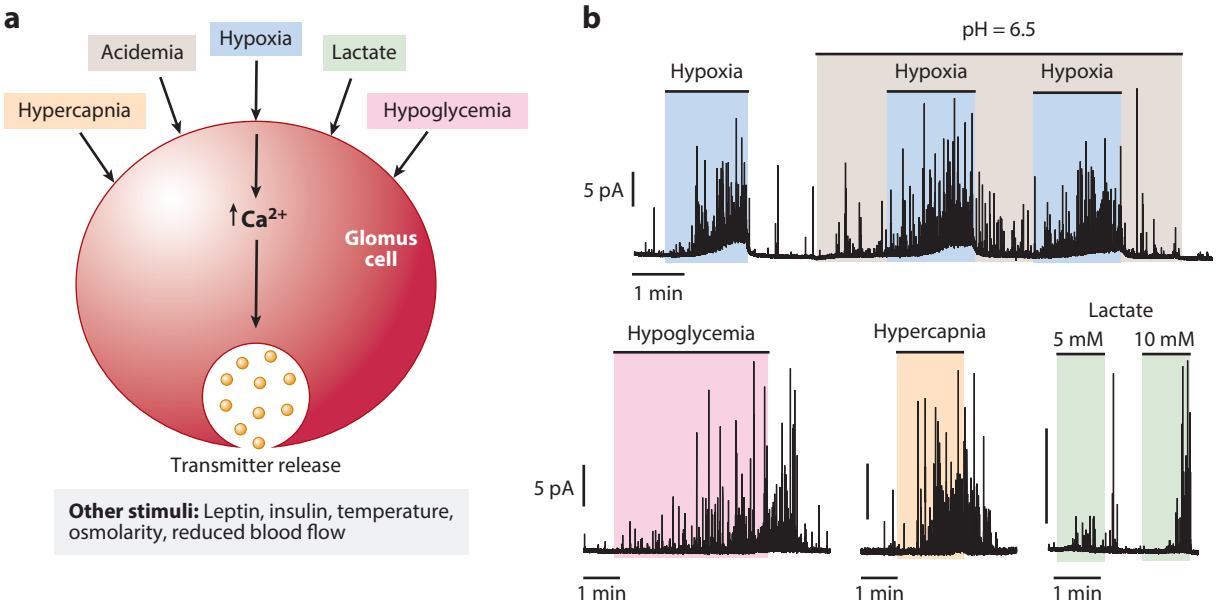

Figure 2

Multimodal sensory function of neurosecretory glomus cells. (*a*) Activation of glomus cells by several independent stimuli with elevation of intracellular Ca^{2+} as the common final pathway. (*b*, *top*) Neurosecretory (catecholamine release) response of a glomus cell to low pH (switching from pH 7.4 to 6.5) and hypoxia monitored by amperometry using a carbon-fiber electrode placed near a glomerulus in a carotid body slice. Note the potentiation by low pH of the response to hypoxia. (*b*, *bottom*) Neurosecretory response of glomus cells to hypoglycemia, hypercapnia, and lactate. Panel *b* adapted with permission from Reference 65. Copyright 2010, Rockefeller Univ. Press.

enzyme are localized in glomus cells (60). It was shown that pharmacological carbonic anhydrase inhibition reduces hypercapnic cellular responses (59, 61) and systemic ventilatory activation (62). Decreases in intracellular pH inhibit the activity of several classes of voltage-dependent and background K^+ channels, which can contribute to cell depolarization to variable degrees (59). It was reported that CO_2 might also increase L-type Ca^{2+} currents in glomus cells independently from changes in internal pH, although the underlying mechanisms are unknown (63). Although reductions in extracellular pH lead to similar changes in glomus cell intracellular pH, the transduction of extracellular acidosis also involves direct inhibition of background TASK-like (64, 65), maxi-K^+ (66), and voltage-gated (67) K^+ channels as well as activation of acid-activated cationic channels (68). An inwardly rectifying chloride current is also activated by extracellular acidification in glomus cells (69). Taken together, these data indicate that CO_2/pH sensing by CB glomus cells results from the redundant modulation of several ion channel subtypes. This may explain why the abolition of genes encoding specific channel types may have little functional cellular or organismal effect. For example, mice lacking TASK1 and TASK3 channels, which have been postulated to play a major role in CO_2 and acid sensing, have a normal ventilatory response and glomus cell responsiveness to hypercapnia (65). Normal ventilatory responses to CO_2 have also been reported recently in mice lacking acid-activated cationic channels (70).

Although the role of the CB as a glucose sensor has been the subject of much debate over the last two decades (53, 71), there is mounting evidence that adult CB glomus cells (dispersed or in CB slices) from several species, including humans, can be activated by lowering glucose concentration in the physiological range to release neurotransmitters in an external Ca^{2+}-dependent manner (24, 72–74) (**Figure 2*b***). Low glucose induces K^+ channel inhibition and activation of an

Na^+-dependent inward current, which results in depolarization and the opening of voltage-gated Ca^{2+} channels in glomus cells (72, 75). Activation by low glucose has been confirmed by experiments on the glomus cell–petrosal neuron synapse, in which it has also been demonstrated that, as seen with hypoxia, ATP and acetylcholine are the main postsynaptic excitatory neurotransmitters released by glomus cells (73). Hypoxia and hypoglycemia are stimuli that potentiate each other and therefore have a synergistic effect on glomus cell cytosolic Ca^{2+} and neurotransmitter release (72). Although they have a common final pathway, these two stimuli operate via different signaling mechanisms. Acute O_2 sensing depends on mitochondrial activity, such that abolition of mitochondrial complex I (MCI) activity with either rotenone (75, 76) or genetic mutations (77) abolishes responsiveness to hypoxia (see the section titled An Integrated Mitochondria-to-Membrane Signaling Model of Acute Oxygen Sensing). Under these conditions, however, sensitivity to low glucose is maintained or even augmented (75, 77). In parallel with in vitro observations, animal studies have also demonstrated an association between the CB and glucose homeostasis. CB activity is inhibited by the intravascular administration of glucose, while CB denervation impairs the counterregulatory response to hypoglycemia (78). Participation of the CB in the control of glycemia has also been recently suggested by experiments in humans (22, 79). Hyperoxia (which blunts CB activity) inhibits the hypoglycemia-induced increase in counterregulatory hormones (80), and this effect is lost in CB-resected individuals (81). CB inhibition or resection also attenuates the neuroendocrine response to exercise in dogs (82) and humans (22). It was long ago suspected that lactate, the plasma levels of which increase during exposure to hypoxia, activates the CB given that blood lactate, independently from acidosis, can elicit hyperventilation (83). Lactate, which was classically considered to be a metabolic waste product, is now believed to play major roles in intercellular and interorgan fuel distribution. Recently, the direct activation of single CB glomus cells by extracellular lactate was demonstrated (84) (**Figure 2*b***). In this way, the paracrine activation of glomus cells by lactate released from neighboring glomus or type II cells might potentiate the response to hypoxia (36).

MCI: mitochondrial complex I

Insulin is known to increase sympathetic tone through the activation of hypothalamic neurons. In addition to this central function, recent evidence in animal models (85) and humans (86) suggests that insulin may also directly activate the CB independently on the level of glycemia. Sympathetic outflow secondary to CB excitation causes insulin resistance, thereby contributing to metabolic syndrome and type II diabetes. In this regard, leptin, a hormone generated by adipose tissue, may also have a fundamental pathogenic role, as it seems to contribute to increased sympathetic activity in obese individuals. CB cells contain leptin receptors, and leptin administration induces phosphorylation of signal transducer and activator of transcription 3 and immediate early gene *c-fos* protein (87–89). Leptin also facilitates the HVR (90). Recent experiments have provided compelling data indicating that leptin induces direct CB activation and increases ventilation (91, 92).

It is known that the CB is modulated by blood osmolality, although the available data are incomplete (22). It was shown that dispersed chemoreceptor cells are selectively depolarized by hypoosmotic solutions due to activation of chloride channels, which leads to extracellular Ca^{2+} influx (93). Carotid sinus nerve activity increases when the CB is exposed to high temperature, which suggests that the CB contributes to hyperthermia-induced hyperventilation. In agreement with this notion, the hyperventilatory response to hyperthermia in humans is reduced when breathing hyperoxic air (94) or after administration of dopamine (22), both of which are treatments that inhibit CB activity. Interestingly, the CB also appears to be sensitive to blood flow, as inhibition of glomus cell K^+ currents by hypoxia and the CB-dependent chemoreflex are enhanced in animals with chronic heart failure. Nevertheless, the link between reduced CB flow and altered CB signaling remains to be identified (95).

ACUTE OXYGEN-SENSING MECHANISMS

ETC: electron transport chain

Although it has been more than 30 years since the membrane model of glomus cell chemotransduction was enunciated, the mechanisms whereby O_2 regulates ion channel activity in these and other hypoxia-responsive cells have remained elusive (57, 96, 97). Acute O_2 sensing was originally thought to be a property of specialized K^+ channels, which were directly or indirectly modulated by O_2 through an O_2 sensor closely associated with the channel subunits (98). However, this possibility is now deemed unlikely, as various subtypes of O_2-regulated K^+ channels, which include voltage-gated K^+ channels, Ca^{2+}-activated channels, and background K^+ channels, have been described (12). In addition, the modulation of ion channel activity during hypoxia is a broadly observed phenomenon affecting a variety of ion channel classes in numerous tissues (12, 15, 16). In this regard, a critical observation is that ablation of genes coding TASK1 and TASK3 channel subunits, the main O_2-sensitive background K^+ channels in rodents, does not significantly alter glomus cell responsiveness to hypoxia. Moreover, responsiveness to hypoxia is maintained in TASK1-/TASK3-deficient cells in the presence of iberiotoxin (a selective blocker of maxi-K^+ channels) or tetraethylammonium (a nonselective blocker of maxi-K^+ and several voltage-gated K^+ channels) (65). These data suggest that hypoxia triggers a promiscuous signaling pathway in glomus cells, which can redundantly modulate different types of ion channels to induce the cell depolarization and Ca^{2+} influx necessary for neurotransmitter release. Several O_2-sensing mechanisms have been postulated over the past 20 years or so, including the modulation of NADPH oxidase, activation of AMP kinase, production of gasotransmitters (carbon monoxide and hydrogen sulfide), and activation of a lactate-sensitive atypical olfactory receptor (Olfr78) expressed in glomus cells (57). However, none of these putative mechanisms appear to be essential for acute O_2 sensing, as mice with ablation of genes coding for the relevant enzymes or receptors exhibit normal CB responses to hypoxia (84, 99–103). As these hypotheses of CB acute O_2 sensing have been discussed in recent reviews (57, 97), we focus here on the mitochondrial-to-membrane signaling model, which is, in our view, the proposal that currently provides the strongest experimental evidence describing CB acute O_2 sensing.

An Integrated Mitochondria-to-Membrane Signaling Model of Acute Oxygen Sensing

Mitochondria have been classically thought to participate in CB O_2 sensing, given that cyanide and other mitochondrial inhibitors are potent CB activators. Several decades ago, Mills & Jöbsis (104) postulated the existence of a low-affinity cytochrome oxidase in the CB, although they hypothetically placed the enzyme in type II cells rather than in glomus cells. This and subsequent work, suggesting that hypoxia depresses mitochondrial metabolism and decreases ATP levels, was the foundation for the metabolic hypothesis of CB O_2 sensing. In support of these ideas, Duchen & Biscoe (105) showed that glomus cell mitochondrial membrane potential and NADH levels are highly sensitive to changes in O_2 tension. However, these authors suggested that Ca^{2+} release from the mitochondria was the predominant signal to trigger transmitter release during hypoxia, a mechanism incompatible with the absolute requirement of membrane depolarization and extracellular Ca^{2+} influx for hypoxic glomus cell activation (17, 18) (**Figure 3*a***). A first hint of the solution to the conflict between the membrane and metabolic hypotheses of acute O_2 sensing came from experiments in PC12 cells (a catecholaminergic cell line with sensitivity to hypoxia) (106) and CB slices (76). These showed that, similar to hypoxia, the mitochondrial electron transport chain (ETC) inhibitors (comprising cyanide, antimycin A, mixothiazol, or rotenone) induce transmitter release in an external Ca^{2+}-dependent manner. In parallel experiments on dispersed glomus cells it was shown that, similar to hypoxia, the ETC blockers inhibit background K^+ channels and

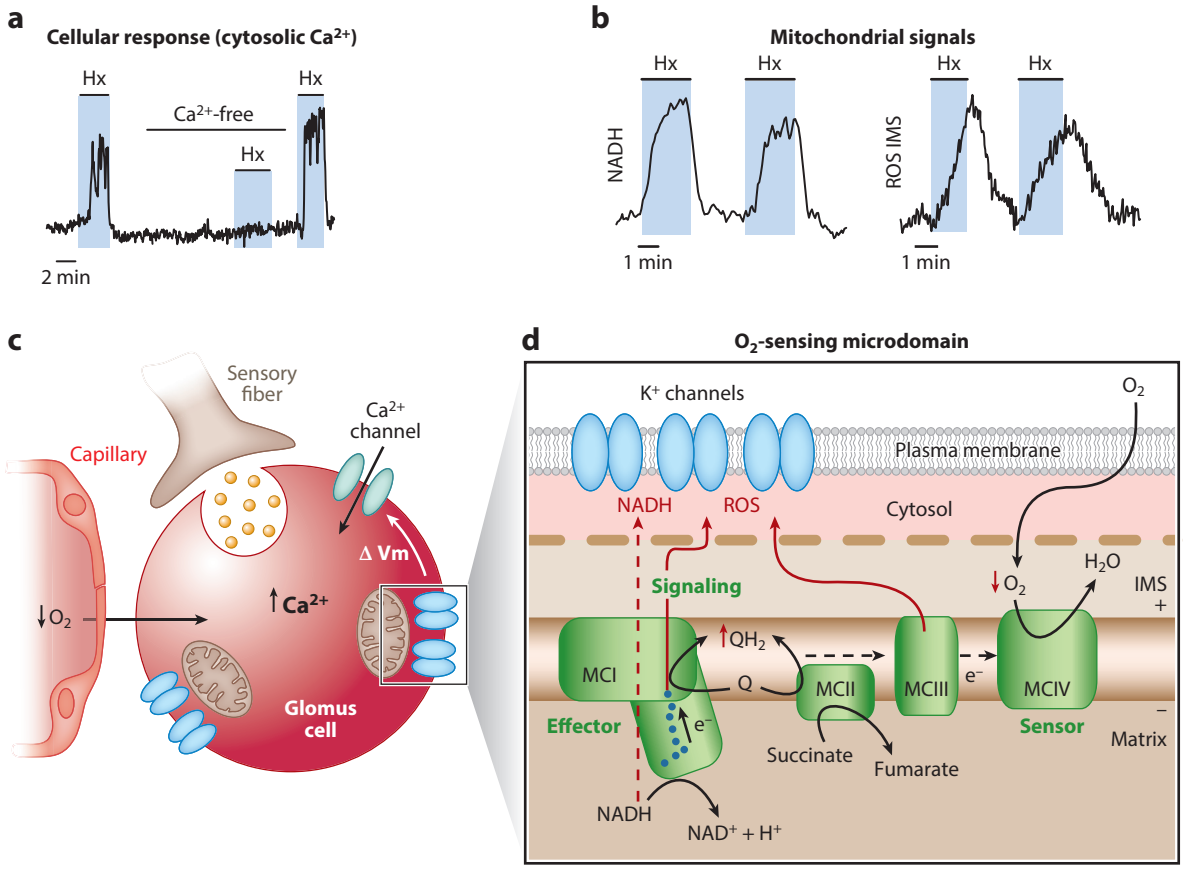

Figure 3

Mitochondria-to-membrane signaling model of acute O_2 sensing by glomus cells. (*a*) Increase in cytosolic Ca^{2+} in glomus cells in response to hypoxia depends on extracellular Ca^{2+} influx. (*b*) Increase in mitochondrial signals, NAD(P)H autofluorescence, and ROS from the IMS during exposure of glomus cells to hypoxia. (*c*,*d*) Model of mitochondrial signaling to membrane ion channels during acute hypoxia. Hypoxia produces an increase in the reduced state of MCIV, which causes a backlog of electrons along the electron transport chain, thus leading to an increase in QH_2. Increased level of QH_2 results in slow down (or even reversion) of MCI with production of ROS and accumulation of NADH. These agents regulate membrane ion channels. In carotid body glomus cells, mitochondria may be near membrane ion channels forming O_2 sensing microdomains. Abbreviations: ΔVm, membrane depolarization; IMS, mitochondrial intermembrane space; MCI–IV, mitochondrial complexes I–IV; Q, ubiquinone; QH_2, reduced ubiquinone; ROS, reactive oxygen species. Panel *a* adapted with permission from Reference 3. Copyright 2016, Elsevier. Panels *b* and *c* adapted with permission from Reference 77. Copyright 2015, Elsevier.

induce cell depolarization and extracellular Ca^{2+} influx (107). Ortega-Sáenz et al. (76) observed that rotenone was particularly efficient in occluding sensitivity to hypoxia compared to other ETC inhibitors, for which it was suggested that a rotenone-binding site could be critically involved in CB acute O_2 sensing.

This idea has been confirmed in recent years by using mice with conditional ablation of the gene coding NDUFS2, one of the three essential MCI subunits that contribute to the ubiquinone-binding site in MCI (77). Rotenone binds with high affinity to this site and prevents ubiquinone reduction. The HVR of *Ndufs2* knockout mice is practically abolished, while *Ndufs2*-deficient glomus cells are unresponsive to hypoxia, although they show normal responses to other stimuli such as hypercapnia or low glucose (77). Among the mitochondrial signals generated by hypoxia are

NADH and reactive oxygen species (ROS) (**Figure 3*b***). Hypoxia induces a dose-dependent increase in NADH in glomus cells over a range of O_2 tension values compatible with their sensory function (77, 105, 108, 109), and this signal is strongly reduced in *Ndufs2*-deficient glomus cells (77, 109). On the other hand, while mitochondrial ROS have been suggested to play a role in acute O_2 sensing in pulmonary arteries, whether ROS production increases or decreases during hypoxia has been a matter of intense debate (13, 110). Recently, rapid and reversible compartmentalized changes in mitochondrial ROS induced by hypoxia have been recorded in glomus cells. Hypoxia elicits a rise in ROS at the intermembrane space or cytosol and a decrease in the matrix. The generation of hypoxia-induced intermembrane space ROS is inhibited by rotenone and markedly decreased in *Ndufs2*-deficient glomus cells, whereas changes in matrix ROS are unaltered. Therefore, the hypoxic increase in intermembrane space ROS generated in MCI is a signal associated with acute O_2 sensing (109). In line with these concepts, the intracellular application of H_2O_2 produces an inhibition of the background K^+ conductance and a marked increase in electrical resistance in glomus cells (77). In addition to NADH and ROS, changes in mitochondrial ATP during hypoxia could also modulate ion channel activity (107). Although experimental data available do not support a signaling role of ATP (84, 109), a definitive answer to this will only be forthcoming once direct monitoring of changes in ATP levels can be performed during exposure of glomus cells to physiological hypoxia.

ROS: reactive oxygen species

Q/QH$_2$: quinone/reduced quinone

HIF: hypoxia-inducible factor

Taken together, these observations suggest a model of acute O_2 sensing in which MCIV acts as an O_2 sensor, and MCI and possibly MCIII act as the effector (**Figure 3*c*,*d***). In CB mitochondria, with high O_2 consumption, the rate of oxidation of the catalytic site in cytochrome oxidase (heme a3/CuB center) is highly sensitive to decreases in O_2 tension due to the expression of tissue-specific mitochondrial subunits and a particularly high oxidative metabolism. Under these conditions, hypoxia causes a backlog of electrons along the ETC and the accumulation of reduced ubiquinone (QH_2). This leads to a slow down or even reversal of MCI (the main effector of hypoxic mitochondria) and the production of signaling molecules (NADH and ROS) that modulate ion channels (77, 109). Further support for this model (**Figure 3*d***), which integrates the membrane and metabolic hypotheses of acute O_2 sensing, comes from experiments indicating that modifications in the mitochondrial Q/QH_2 ratio, resulting from the activation or inhibition of succinate dehydrogenase activity, lead to an increase or decrease, respectively, of glomus cell responsiveness to hypoxia (109). It is conceivable that glomus cell mitochondria located in close proximity to the cell membrane form O_2-sensing microdomains in which the signaling molecules (NADH and ROS) reach the local concentration required for ion channel modulation (109) (**Figure 3*c*,*d***). These O_2 sensing microdomains could explain the hypoxic modulation of ion channel activity recorded in excised membrane patches, which may contain attached cytosolic organelles. Disarrangement of the O_2 sensing microdomains in cells exposed to enzymatic or mechanical stress could also result in the loss of acute cellular activation during hypoxia.

Metabolic Specifications in Acute Oxygen-Sensing Cells

Two recent independent gene expression studies provided relevant cues for understanding glomus cell acute O_2 sensing. Single glomus cell sequencing from neonatal mice highlighted the elevated expression of numerous genes coding molecules involved in G protein–coupled receptor signaling (111); this finding is consistent with the profuse auto- and paracrine modulation of glomus cells by metabotropic ligands present in the CB glomeruli. In the same study, several ion channels that included TASK1, the low-threshold Ca^{2+} channel α1H subunit, and TRPC5, hypoxia-inducible factor (HIF)2α and two atypical mitochondrial subunits (NDUFA4l2 and COX4I2) were among the most specifically expressed genes identified in the glomus cells. In a parallel microarray analysis, comparative gene expression profiles of three adult neural tissues (CB, adrenal medulla, and

superior cervical ganglion) possessing the same neural crest embryological origin but variable O_2 sensitivity (CB>adrenal medulla>superior cervical ganglion) were studied (112). This work demonstrated a set of genes, including some of those reported in the single-cell sequencing analysis (111), which are highly expressed in glomus cells, and to a lesser extent in adrenal medulla chromaffin cells compared with superior cervical ganglion neurons. The most relevant genes coded for HIF2α, three atypical mitochondrial subunits (NDUFA4L2, COX4I2, and COX8B), pyruvate carboxylase, and four types of ion channels (TASK1, TASK3, the α1H Ca^{2+} channel subunit, and TRPC5). In addition, the gene coding prolyl hydroxylase 3 (PHD3) appeared to be strongly downregulated in glomus and chromaffin cells. The signature metabolic profile represented by these genes is probably responsible for the acute O_2-sensing properties of CB glomus cells (112).

PHD: prolyl hydroxylase

CSH: chronic sustained hypoxia

COX4I2 and COX8B are atypical isoforms of the more broadly distributed COX4I1 and COX8A subunits forming part of the catalytic core of MCIV. These subunits contain adjacent transmembrane helices running in parallel at the periphery of MCIV (113). On the other hand, NDUFA4L2 is an isoform of the most frequently expressed NDUFA4 subunit, which is also associated with MCIV. Therefore, it is most likely that the combined expression of these subunits confers glomus cells with cytochrome oxidase O_2 sensitivity in the physiological range (112). Indeed, COX4I2-null mice exhibit strong inhibition of hypoxic pulmonary vasoconstriction (114), an acute response to hypoxia that, similar to hypoxic glomus cell activation, depends on the production of ROS by mitochondria and the modulation of O_2-sensitive K^+ channels (13, 110). In addition to their particularly high MCIV sensitivity to O_2, glomus cells are known to have high O_2 consumption and an active oxidative metabolism, which are metabolic features compatible with the presence of elevated levels of pyruvate carboxylase and its cofactor biotin (115). Pyruvate carboxylase is an anaplerotic enzyme required for the generation of oxaloacetate, thereby replenishing the pool of tricarboxylic acid cycle intermediates and increasing oxidative phosphorylation. This observation is in agreement with the high levels of succinate in the CB and explains the postulated accumulation of QH_2 during hypoxia (77) as well as the inhibition of responsiveness to acute hypoxia after genetic (112) or pharmacological (109) blockade of succinate dehydrogenase in glomus cells. In addition to specific ETC subunits, highly active oxidative mitochondria are therefore probably required for the O_2-sensing properties of glomus cells.

CAROTID BODY PLASTICITY AND STEM CELLS

A notable property of the CB is its extraordinary plasticity at the cellular and whole-organ levels, which is critical for physiological adaptation to changing environments and pathological conditions. A highly relevant example is represented by ventilatory acclimatization to sustained hypoxia (e.g., when moving from low to high altitude), a process that requires an increased sensitivity of the CB to the reduction in O_2 tension and one that results in a constant hyperventilatory response to avoid an overt decrease in arterial O_2 level (116, 117). In the first instance, plasticity of the CB depends on functional changes occurring in the expression of ion channels and/or the availability of neurotransmitters and peptides that modulate cellular interactions within the CB glomeruli (118). However, during protracted/sustained activation, the adult CB exhibits a remarkable structural plasticity because, unlike other neural organs, it can increase in size several fold and thus increase afferent inputs to the respiratory center (116).

Functional Plasticity

Several studies have indicated that chronic sustained hypoxia (CSH) induces the expression of Na^+ and/or Ca^{2+} channels and reduces K^+ current amplitude (118), resulting in increased glomus cell excitability. Cultured neonatal glomus cells exposed to hypoxia present normal levels of TASK1

channels, although hypoxic inhibition of the background K^+ currents in these cells is enhanced after 48 h of CSH (119). In parallel to changes in ion channel expression, CSH induces complex modifications in the synthesis/release of several neurochemicals and their receptors involved in CB functions, which are not yet well understood (117, 118). Hypoxia strongly induces TH, the rate-limiting enzyme in the synthesis of dopamine, as well as NO synthase. In parallel, hypoxia downregulates the expression of D2 dopamine receptors. On the other hand, nicotinic cholinergic receptors are induced in CB postsynaptic afferent fibers during CSH, thereby enhancing the excitatory effect of acetylcholine (117). Although hypoxia does not seem to significantly alter the expression of P2X receptors in afferent chemosensory fibers, it has been shown that exposure of rats to CSH for 4–5 days leads to the upregulation of surface-located ectonucleotidases, which catalyze the conversion of ATP to adenosine (120), an excitatory agent acting pre- and postsynaptically in the glomus cell–afferent fiber synapse (33, 34, 40). Endothelin-1, a vasoactive peptide that is induced by CSH in CB cells (121), seems to be involved in the proliferation of CB cells during hypoxia (see the section titled Structural Plasticity: Carotid Body Growth in Chronic Hypoxia). However, endothelin-1 and its receptors also participate in the increase of CB responsiveness induced by CSH probably via upregulation of the Ca^{2+} current amplitude in glomus cells (121, 122). Taken together, these data suggest that CSH induces a balanced change in excitatory and inhibitory neurotransmitters to increase CB activation while simultaneously preventing excessive CB stimulation.

CIH: chronic intermittent hypoxia

CB functional plasticity is also induced by chronic intermittent hypoxia (CIH), a common phenomenon in the human population that is associated with recurrent obstructive apneas occurring in some individuals during sleep (sleep apnea syndrome). CIH enhances the HVR due to long-term facilitation of the CB chemoreflex, a form of plasticity that results in increased CB excitation during acute episodes of hypoxia (123–125). The changes in CB function induced by CIH probably depend on ROS-mediated alterations in neurotransmitter signaling (in particular endothelin-1, angiotensin II, and NO) and proinflammatory cytokine concentration (123, 125, 126). In addition, hypoxic inhibition of TASK-like K^+ channel activity in glomus cells is increased in animals exposed to CIH (126). It has been suggested that a mitochondrial ROS-mediated imbalance in the HIF1α and HIF2α signaling pathways is responsible for the CIH-induced alterations in CB glomus cells (127). In addition to hypoxia, sustained CB activation by other stimuli can also produce plastic changes in glomus cell responsiveness and CB overactivation. For example, an increased CB chemoreflex is observed in obese mice and mice fed chronically with a high-fat diet (85). Although detailed mechanisms underlying these responses are not known, they seem to be related to modifications in CB cell reactivity induced by insulin, leptin, and other agents (88, 90, 91). Similarly, a considerable body of evidence suggests that increased CB chemosensitivity is involved in the autonomic imbalance conferring a poor prognosis on patients with chronic heart failure (95, 128). This form of CB overactivation was suggested to be secondary to the elevated mitochondrial production of superoxide anion and angiotensin II-mediated changes in glomus cells, which are generated by sustained reductions in blood flow and downregulation of downstream signaling molecules (129). An experimentally imposed reduction of carotid blood flow in rabbits decreases the expression of Krüppel-like factor 2, a mechanosensitive transcription factor induced by shear stress in the vasculature that controls the transcription of genes associated with antioxidant defense and angiotensin metabolism (130).

Structural Plasticity: Carotid Body Growth in Chronic Hypoxia

Exposure of experimental animals and humans for several days to CSH induces dramatic structural changes in the CB, with a two- to threefold increase in size due to angiogenesis and the

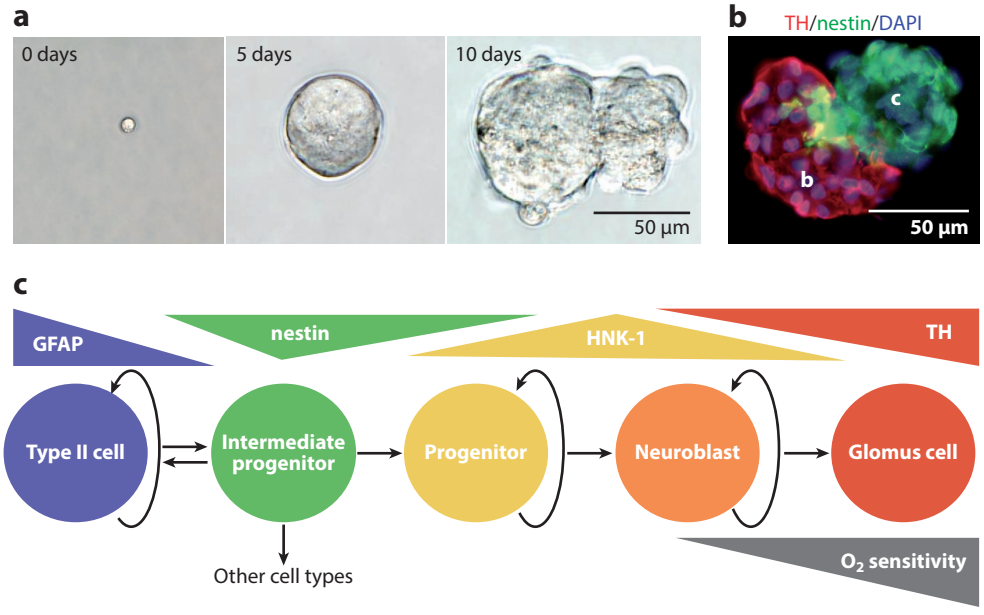

Figure 4

Proliferation and differentiation of carotid body stem cells. (*a*) Single-cell-derived carotid body neurosphere in vitro. Days of culture are indicated. (*b*) Histological section of a carotid body-derived neurosphere stained with the indicated markers: c, neurosphere core; b, bleb of TH+ and O_2-sensitive cells. (*c*) Model of carotid body stem cell proliferation and differentiation, with changes shown in the expression of indicated markers and in sensitivity to acute hypoxia. Abbreviations: DAPI, 4′, 6-diamidino-2-phenylindole; GFAP, glial fibrillary acidic protein; HNK-1, human natural killer-1; TH, tyrosine hydroxylase. Panels *a* and *b* adapted with permission from Reference 23. Copyright 2007, Elsevier.

generation of new glomeruli. Although this response was described long ago (116, 131–133), the underlying mechanisms have remained largely unstudied until recently (23, 121). CB structural plasticity depends on the existence in this organ of a population of multipotent adult neural crest–derived stem cells, which are quiescent in normoxia and activated during hypoxia to proliferate and differentiate into new glomus cells, as well as smooth muscle and endothelial cells (23, 25, 54). Cell fate mapping experiments have shown that CB stem cells are sustentacular or type II cells that lose their characteristic GFAP+ phenotype upon activation and are converted into nestin+ proliferating progenitors (23) (**Figure 4*a–c***). The changes induced by hypoxia in the CB stem cell niche can be reproduced in vitro using CB dispersed cells. A small percentage of these cells (~1% in the rat CB) generate clonal cell colonies (named neurospheres) that normally derive from a single stem cell (**Figure 4*a***). CB neurospheres are composed of a core of nestin+ proliferating progenitors surrounded by buds of differentiating TH+ cells forming blebs attached to the neurosphere core, which grow in size with time in culture (**Figure 4*b***). As mature glomus cells develop in situ, the TH+ cells generated in the neurospheres contain voltage-gated Ca^{2+} and K^+ channels, secrete catecholamines in response to hypoxia or hypoglycemia, and express glial cell line–derived neurotrophic factor (GDNF) (23). Proliferation of CB progenitors in vitro is independent of hypoxia, or pharmacological stabilization of HIF with DMOG, although lowering O_2 tension favors the appearance and growth of blebs of TH+ cells (43). These findings suggest that CB stem cells are not intrinsically sensitive to hypoxia and that their activation in vivo depends on the presence of the O_2-sensitive glomus cells. Mature glomus cells and stem (type II) cells

GDNF: glial cell line–derived neurotrophic factor

establish numerous chemical synapses (denoted chemoproliferative synapses) that form the basis for stem cell activation during hypoxia. Among the several substances possibly involved in these synapses is endothelin-1. In vitro and in vivo studies have shown that CB progenitor cells are induced to proliferate by endothelin-1 released from glomus cells and that type II cells contain endothelin-1 receptors (43, 121). Although HIF induction in progenitor cells is not sufficient to trigger their proliferation under hypoxia, the PHD–HIF pathway is required for CB plasticity. Transgenic overexpression of HIF2α elicits CB hypertrophy (54), and a lack of HIF2α during embryogenesis results in CB atrophy (55). On the other hand, inducible downregulation of HIF2α in adult mice leads to an inhibition of CB cell proliferation during CSH (134).

OIRD: opioid-induced respiratory depression

CB growth induced by CSH requires exposure to hypoxia for several days; however, it is known that some TH+ cells undergo mitosis during the first 24–48 h in hypoxia (23, 135, 136). A recent study reported the existence of predifferentiated CB neuroblasts that express catecholaminergic markers (TH+) and voltage-dependent ion channels but are unresponsive to hypoxia (27) (**Figure 4*c***). Unlike mature glomus cells, CB neuroblasts can undergo mitotic divisions before their final maturation, which is induced by hypoxia as well as by neurotransmitters, in particular acetylcholine and ATP, released from glomus cells. This phenotypic change in the CB TH+ cell population (shifting from non-O_2-sensitive to O_2-sensitive) could have an important pathogenic role in CB oversensitivity observed in conditions, such as sleep apnea or CIH, without evident CB hypertrophy. Interestingly, the number of CB neuroblasts is smaller in the mouse than in the rat (27). In addition, the regenerative potential of cultured CB stem cells, although qualitatively similar in several species, is also less potent in mouse and human samples than in the rat (24).

CAROTID BODY AND DISEASE

In recent years the CB has gained medical attention because in addition to its essential physiological role as a multimodal chemoreceptor, it is also involved in the pathogenesis of numerous diseases, some of them with high prevalence in the human population. Below we present a brief summary of the most relevant aspects of CB translational research.

Carotid Body Inhibition

Because the CB is the first line of defense against hypoxic challenges during wakefulness and sleep, CB inhibition may therefore have fatal consequences. Maturation of the CB chemoreflex seems to be critical for adaptation of the newborn to extrauterine life, with developmental defects in the CB suggested to contribute to respiratory disorders such as sudden infant death or central congenital hyperventilation syndromes (3, 13). However, the most frequent cases of CB inhibition are secondary to surgical removal or the administration of drugs. Patients with bilateral CB resection, most commonly due to neck tumors or asthma treatment, have a practically abolished HVR (21, 52) and the counterregulatory response to hypoglycemia (53, 80, 81). Although these patients appear to live unaffected in normoxic conditions, disturbances during sleep and unexplained cases of death have been reported. On the other hand, CB inhibition is a side effect of several anesthetics, sedatives, and analgesic drugs, as many of these agents are K^+ channel activators and/or Ca^{2+} channel inhibitors and interfere with the glomus cell neurosecretory response (see 3). A special case is represented by opioid-induced respiratory depression (OIRD), a common and potentially life-threatening complication associated with opioid treatment or abuse. Although analgesic doses of morphine depress ventilation in humans via central and peripheral mechanisms (39, 137), the precise role of the CB in this process has not been fully elucidated. Intracarotid administration of methionine-enkephalin and morphine to a lesser extent depresses CB chemoafferent discharges (39, 138). Moreover, these agents can upregulate K^+ channels and inhibit Ca^{2+}

currents in chemoreceptor cells (3). It was suggested that reliable tests of ventilatory sensitivity to hypoxia or hypercapnia should be included in clinical protocols to assess susceptibility to OIRD (139). Despite these observations, morphine-induced respiratory depression in conscious rats is greatly enhanced after bilateral CB denervation, suggesting a protective rather than causative role of the CB in OIRD (140). Thus, activation of peripheral chemoreceptors with K^+ channel inhibitors is a promising strategy to alleviate OIRD in humans (3, 140).

SDHD: succinate dehydrogenase subunit D

Carotid Body Overactivation

As discussed above, CB overactivation has been identified as a major cause of the exaggerated sympathetic outflow characteristic of some highly prevalent disorders such as hypertension, sleep apnea, and chronic heart failure as well as obesity and metabolic syndrome (see 3). Although the molecular bases for these processes of CB maladaptive plasticity remain poorly elucidated, several groups have shown that bilateral CB ablation or denervation results in a decreased sympathetic tone and improvement of most of the associated cardiovascular and metabolic alterations (85, 141–143). However, the translation of these procedures to the clinical setting must proceed with caution because CB ablation as seen in hypertensive rats, for example, may potentially trigger cardiovascular events, particularly during episodes of hypoxia and hypercapnia (144). In a pilot clinical trial, bilateral CB ablation improved the autonomic imbalance in chronic heart failure patients, but also increased the occurrence of nocturnal hypoxia, particularly in patients with concomitant sleep apnea (145). In addition to potential surgical complications, the abolition of CB function may also result in severe side effects, such as a reduced ability to acclimatize to high altitudes or altered glucose regulation. The development of pharmacological therapies to selectively modulate CB chemosensory activity and plasticity is an optimal alternative to surgery that could favor the translation of CB research to a clinical reality (146, 147).

Tumorigenesis

CB tumors (chemodectomas) are mostly benign and belong to the group of paragangliomas affecting the peripheral nervous system. The histological features of chemodectomas resemble those of hypertrophied CBs from individuals subjected to chronic hypoxia (131, 132), and their incidence increases in persons living at high altitude (148). Hence, a question under debate is whether CB tumorigenesis is related to the proliferative potential of the CB neurogenic niche. Mutations in the succinate dehydrogenase subunit D (SDHD) are the most common cause of hereditary CB paraganglioma (149), and it has been suggested that succinate accumulation, PHD inhibition, and HIF stabilization could be critical events triggering the appearance of paragangliomas (150, 151). Indeed, PHD2 inactivation induces a HIF2α-dependent glomus cell hyperplasia and structural CB changes resembling a paraganglioma-like phenotype (152). However, CB tumors are not triggered in mouse models with mono or biallelic deletions of SDHD (153). In contrast, the loss of SDHD leads to extensive cell death in the CB and other central and peripheral catecholaminergic tissues (153). These data suggest that the mechanisms of CB hypertrophy and tumorigenesis are not necessarily interrelated. In addition to the CB, paragangliomas can frequently appear in tissues such as the adrenal medulla without a hypertrophic response to hypoxia.

Cell Therapy in Parkinson's Disease

Because the CB has particularly high dopamine content, the intrastriatal transplantation of CB tissue has been tested for cell replacement therapy in Parkinson's disease. Successful preclinical

studies carried out in rat and monkey models (154, 155) were followed by pilot clinical trials in Parkinson's patients (156). However, these studies also suggested that the beneficial effect of CB transplants was due to a trophic action mediated by the abundant expression of GDNF in the transplanted cells rather than as a consequence of exogenous dopamine delivery (157). Indeed, the endogenous production of GDNF by striatal GABAergic interneurons is necessary for maintenance of the nigrostriatal pathway and activation of endogenous GDNF production has been proposed as a potential therapeutic approach to Parkinson's disease (158). The physiological role of GDNF produced by glomus cells (157, 159) is unknown, although it could be related to the auto- or paracrine induction of antioxidant defense enzymes, which in the CB setting may be necessary to inactivate mitochondrial ROS produced by acute hypoxia signaling in these cells (77, 109).

SUMMARY POINTS

1. The CB is an integral arterial chemoreceptor essential for the regulation of respiration by blood gas (O_2 and CO_2) levels. It can also detect changes in blood-borne metabolic signals (glucose, lactate, or pH), hormones (insulin and leptin), and blood flow.
2. Glomus cells are multimodal sensory elements in the CB and prototypical acute O_2 sensors in the body. O_2 sensing is performed by specialized mitochondria, which signal membrane ion channels.
3. The CB is organized in clusters (glomeruli) of glomus and other cell types. Autocrine and paracrine interactions between these cells mediate CB plasticity.
4. Carotid body growth, a classical adaptive response to hypoxia, is mediated by glia-like, multipotent adult stem cells.
5. Carotid body dysfunction influences the pathogenesis of highly prevalent diseases (hypertension, diabetes, or chronic heart failure) as well as susceptibility to respiratory depression.

FUTURE ISSUES

1. The molecular mechanisms underlying acute O_2 sensing and responsiveness of glomus cells to other stimuli (hypoglycemia, lactate, or changes in blood flow) should be clarified.
2. Researchers should develop the pharmacological tools to safely and selectively modulate chemosensory signals as well as CB overactivation.
3. Extrapolating the CB physiology will help facilitate our understanding of other chemosensitive responses in the body (e.g., hypoxic vasodilation or hypoxic pulmonary vasoconstriction).

DISCLOSURE STATEMENT

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