Available online at www.sciencedirect.com
SCIENCE DIRECT®
Progress in Biophysics and Molecular Biology 91 (2006) 249–286
Progress in Biophysics & Molecular Biology
www.elsevier.com/locate/pbiomolbio

Review

Oxygen sensing in the body

S. Lahiri[a,*,1], A. Roy[a], S.M. Baby[a], T. Hoshi[a], G.L. Semenza[b,c], N.R. Prabhakar[d]

[a]*Department of Physiology, University of Pennsylvania Medical Center, Philadelphia, PA 19104-6085, USA*
[b]*Department of Pediatrics, Institute for Cell Engineering, Medicine, Radiation Oncology, Baltimore, MD 21205, USA*
[c]*Institute of Genetic Medicine, The John Hopkins University School of Medicine, Baltimore, MD 21205, USA*
[d]*Department of Physiology & Biophysics, School of Medicine, Case Western Reserve University, Cleveland, OH 44106-4970, USA*

Available online 15 August 2005

Abstract

This review is divided into three parts: (a) The primary site of oxygen sensing is the carotid body which instantaneously respond to hypoxia without involving new protein synthesis, and is historically known as the first oxygen sensor and is therefore placed in the first section (Lahiri, Roy, Baby and Hoshi). The carotid body senses oxygen in acute hypoxia, and produces appropriate responses such as increases in breathing, replenishing oxygen from air. How this oxygen is sensed at a relatively high level (arterial $P_{O_2} \simeq 50$ Torr) which would not be perceptible by other cells in the body, is a mystery. This response is seen in afferent nerves which are connected synaptically to type I or glomus cells of the carotid body. The major effect of oxygen sensing is the increase in cytosolic calcium, ultimately by influx from extracellular calcium whose concentration is 2×10^4 times greater. There are several contesting hypotheses for this response: one, the mitochondrial hypothesis which states that the electron transport from the substrate to oxygen through the respiratory chain is retarded as the oxygen pressure falls, and the mitochondrial membrane is depolarized leading to the calcium release from the complex of mitochondria–endoplasmic reticulum. This is followed by influx of calcium. Also, the inhibitors of the respiratory chain result in mitochondrial depolarization and calcium release. The other hypothesis (membrane model) states that K^+ channels are suppressed by hypoxia which depolarizes the membrane leading to calcium influx and cytosolic calcium increase. Evidence supports both the hypotheses. Hypoxia also inhibits prolyl hydroxylases which are present in all the cells. This inhibition results in membrane K^+ current suppression which is followed by cell depolarization. The theme of this section covers first what and where the oxygen sensors are; second, what

*Corresponding author. Tel.: +1 215 898 9125; fax: +1 215 573 5851.
E-mail address: lahiri@mail.med.upenn.edu (S. Lahiri).
[1]Invited author.

0079-6107/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.pbiomolbio.2005.07.001

are the effectors; third, what couples oxygen sensors and the effectors. (b) All oxygen consuming cells have a built-in mechanism, the transcription factor HIF-1, the discovery of which has led to the delineation of oxygen-regulated gene expression. This response to chronic hypoxia needs new protein synthesis, and the proteins of these genes mediate the adaptive physiological responses. HIF-1α, which is a part of HIF-1, has come to be known as master regulator for oxygen homeostasis, and is precisely regulated by the cellular oxygen concentration. Thus, the HIF-1 encompasses the chronic responses (gene expression in all cells of the body). The molecular biology of oxygen sensing is reviewed in this section (Semenza). (c) Once oxygen is sensed and Ca^{2+} is released, the neurotransmittesr will be elaborated from the glomus cells of the carotid body. Currently it is believed that hypoxia facilitates release of one or more excitatory transmitters from glomus cells, which by depolarizing the nearby afferent terminals, leads to increases in the sensory discharge. The transmitters expressed in the carotid body can be classified into two major categories: conventional and unconventional. The conventional neurotransmitters include those stored in synaptic vesicles and mediate their action via activation of specific membrane bound receptors often coupled to G-proteins. Unconventional neurotransmitters are those that are not stored in synaptic vesicles, but spontaneously generated by enzymatic reactions and exert their biological responses either by interacting with cytosolic enzymes or by direct modifications of proteins. The gas molecules such as NO and CO belong to this latter category of neurotransmitters and have unique functions. Co-localization and co-release of neurotransmitters have also been described. Often interactions between excitatory and inhibitory messenger molecules also occur. Carotid body contains all kinds of transmitters, and an interplay between them must occur. But very little has come to be known as yet. Glimpses of these interactions are evident in the discussion in the last section (Prabhakar).

© 2005 Elsevier Ltd. All rights reserved.

Keywords: Action spectra; α-ketoglutarate; Chronic hypoxia; Capacitative Ca^{2+} influx; Endogenous gas molecules-NO and CO messenger molecules; ER-mitochondria Ca^{2+} liaison; Glomus cell and nerve junction; Fe^{2+} chelation; HIF-1α; HIF-1β; HIF-1; Heme nature of O_2 sensor; Hydroxylation; Intermittent hypoxia; IP_3-Rs Membrane model; O_2 sensitive K^+ channels; Mitochondrial model; Mitochondria-HIF-1α; Molecular biology of oxygen sensing; Prolyl hydroxylase inhibitors; Neurotransmitters: conventional and unconventional; ROS; Spatial localization of mitochondria; Sustained hypoxia; TEA and 4-AP effects; Ventilation

Contents

1. Introduction

This review of oxygen sensing is divided into three parts: (a) Oxygen sensing in the carotid body is instantaneous and historically the first known oxygen sensor and is placed in the first section. (b) Since hypoxia-inducible factor (HIF-1) is universally expressed in all oxygen consuming cells including the carotid body but is a slower process (chronic hypoxia), oxygen-regulated gene expression is placed in the second section. (c) Once the oxygen is sensed as described in the preceding sections, the neurotransmitters are released, and therefore are placed in the last section.

Section I: Oxygen Sensing in the carotid body (S. Lahiri, A. Roy, S.M. Baby and T. Hoshi)

2. Overview

O_2 is sensed by almost all mammalian cells but it is in the carotid body (CB) that O_2 sensing takes place for global benefit for the organism as a whole. As animals breath, the freshly oxygenated blood returning from the lung-circulation is pumped by the heart to the brain via the carotid artery. The carotid body, a tiny organ situated at the bifurcation of common carotid artery close to the heart, senses P_{O_2} in the blood. Even a small decrease in the blood P_{O_2} (about 1 Torr, increasing the sensitivity at lower P_{O_2}) is sensed almost instantaneously, only involving modification of pre-existing proteins, and the information is carried by the chemoreceptor

afferents to the brain, which in turn initiates respiratory and cardiovascular reflexes to ensure proper oxygenation in the vital organs. This sequence of events is repeated from breath to breath, and life is sustained. How does the carotid body sense O_2? This is a three-part question covering the followings. First, what and where are the O_2 sensors? Second, what are the O_2 effectors? Third, what couples the O_2 sensors and the effectors? These questions have been raised and attempted to answer throughout this review.

$$O_2 \text{ sensors} \longrightarrow \text{couplers} \longrightarrow \text{effectors.}$$

The pivotal role of the carotid body in O_2 sensing was first revealed by Corneille Jean Francois Heymans, a Belgium physiologist. Heymans while working at the University of Ghent in Belgium discovered that injection of cyanide in the carotid body circulation produced hyperventilation in the dog with intact carotid sinus connection with brain but that the hyperventilation disappeared after severance of the sinus nerve, proving that carotid body was responsible for the hypoxic simulation of respiration rate. Heymans was awarded Nobel Prize in 1938 for this discovery of the function of carotid body (see Fitzgerald and Lahiri, 1996; Lahiri, 2000).

Anatomically carotid body consists of two major cell types, sustentacular (type II) and glomus (type I) cells. Glomus cells, primarily responsible for O_2 sensing, are neurosecretory chemoreceptors that fire action potentials and release the neurotransmitters, including dopamine, in a Ca^{2+}-dependent manner. The spike frequency and the transmitter release are greatly enhanced by low blood P_{O_2} or hypoxia ($>40\%$ decrease or $<\sim 60$ mm Hg). Acute O_2 sensing within a glomus cell probably takes place at multiple loci, plasma membrane, cytoplasm and mitochondria. The molecular mechanisms employed by these components may also vary. The exact mechanism of hypoxia sensing may well depend on the experimental preparations; intact carotid bodies, carotid body slices, isolated glomus cells and isolated membrane patches may show hypoxia sensitivity but the underlying mechanisms may be similar. Regardless of the potential diversity in the molecular locus and mechanism of O_2 sensing, these processes together influence the Ca^{2+}-dependent release of the neurotransmitter onto the sensory afferents so that the overall glomus cell response is exquisitely dependent on the blood P_{O_2}.

Hypoxia in blood is often accompanied by alteration of P_{CO_2}. At the organ/cellular level, low O_2 and high CO_2 interact synergistically to stimulate glomus cells (O_2–CO_2 stimulus interaction) (Fitzgerald and Parks, 1971; Lahiri and Delaney, 1975a); the effects of hypoxia and hypercapnia applied simultaneously are greater than the sum of these two stimuli when applied separately to the carotid body. As the P_{O_2} levels decline, the relationship between the sensory afferent nerve activity and P_{CO_2} becomes increasingly steeper (Fig. 1), leading to enhanced ventilatory reflexes (Lahiri and DeLaney, 1975b). A similar stimulus interaction was demonstrated between CO_2 and carbon monoxide (CO) (Osanai et al., 1996) and this interaction is dependent on CO_2–HCO_3^- (Osanai et al., 1997a). Whether these stimulus interactions with CO_2 and CO reflect the intrinsic properties of the O_2 sensor itself or those of the effectors and/or couplers is not clear, but a complete description of the O_2 sensing in carotid body must be able to account for these phenomena. The same phenomenon on interaction at the single model cells can be seen (Peers, 2004). However, this stimulus interaction may not occur at the level of K^+ channels with type I cells (Peers, 2003).

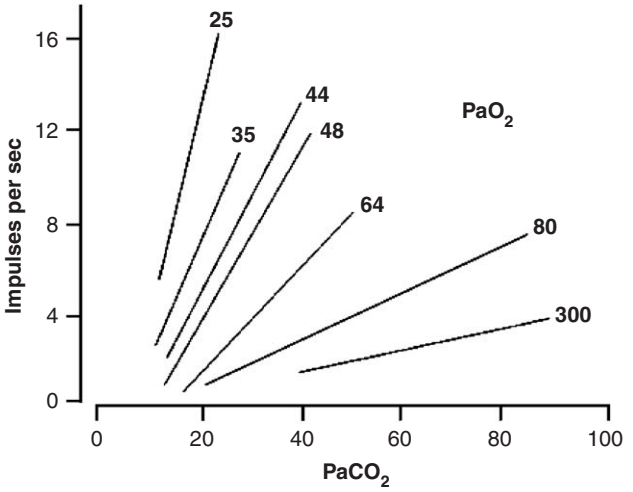


Fig. 1. Plot of the relationship between afferent chemosensory nerve discharge and $PaCO_2$ levels, recorded from the cat carotid body sinus nerve preparation. $PaCO_2$ levels were varied at different background PaO_2 levels, as indicated. Note the increased slope of the relationship as background PaO_2 levels decline. Reproduced with modification from Lahiri and Delaney (1975a, b) and Peers (2004).

3. Cellular oxygen sensing components

3.1. Mitochondrial model

To generate ATP by oxidative phosphorylation, cellular respiration in mitochondria uses oxygen as the final electron acceptor (see Fig. 2). This essential role of oxygen in cellular metabolism led to the idea that changes in mitochondrial functions may serve as an oxygen sensing mechanism (''mitochondrial model''). Historically this concept can be traced back to the ''Pastuer effect'' (see Lahiri, 2000); when oxygen becomes scarce, the cells must abandon oxidative phosphorylation and automatically rely solely on glycolysis for energy production. The switch to anaerobic metabolism is regulated by metabolites of glycolytic enzymes.

Hypoxia is accompanied with numerous changes in mitochondrial function, including changes in the generation of reactive oxygen species (ROS), opening of mitochondrial ion channels, and the decreased activity of the cytochrome oxidase in mitochondrial complex IV. Some of these changes are experimentally mimicked by various pharmacological agents. For example, treatment of glomus cells with oligomycin, which inhibits ATP synthase/oxidative phosphorylation, interferes with the O_2 sensitivity of glomus cells (Mulligan et al., 1981). Which of these events contribute to the acute electrical changes observed in glomus cells is not fully understood but some interesting observations do exist as presented below.

3.2. Effect of mitochondrial inhibitors on Type I cells and sensory discharge

The potential involvement of hypoxia-induced changes in mitochondrial function has been suggested from the results of pharmacological inhibitors of mitochondrial function. Many

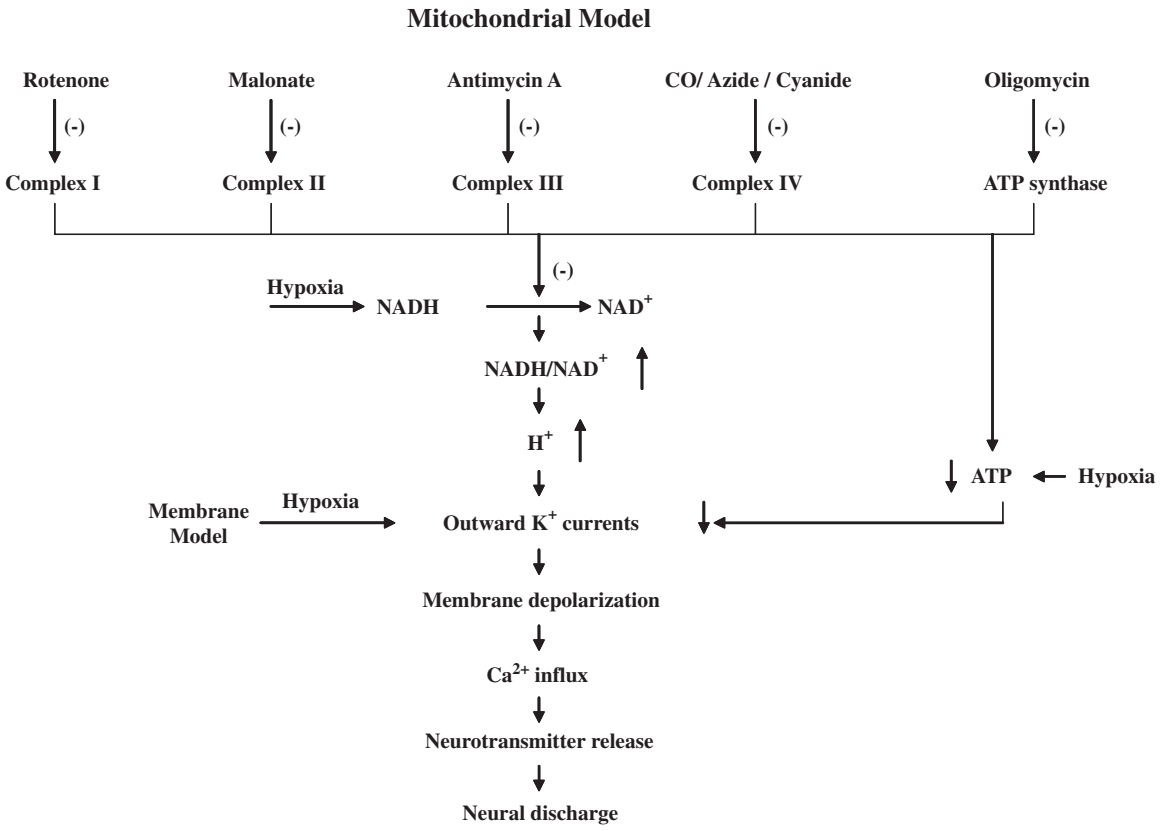


Fig. 2. Mitochondrial and membrane models for oxygen sensing. Sites of complex I, II, III and IV on the inner mitochondrial membrane. Proposed sites of inhibition of the respiratory chain by specific drugs as indicated. This is followed by H^+ increase and then the membrane model comes into the picture.

(but not all) mitochondrial inhibitors at least transiently increase the carotid body activity, mimicking the effect of hypoxia (Anichkov and Belen'kii, 1963) (Note that long-lasting treatment of glomus cells with mitochondria inhibitors actually abolishes the hypoxia sensitivity, as measured by monitoring the sensory discharge (Mulligan and Lahiri, 1982). Rotenone, myxothiazol, antimycin, cyanide and oligomoycin (see Fig. 2) increased $[Ca^{2+}]_c$, which is at least in part mediated by Ca^{2+} entry through voltage-gated Ca^{2+} channels (Wyatt and Buckler, 2004). Uncouplers of oxidative phosphorylation, DNP and FCCP, also produced a similar increase in $[Ca^{2+}]_c$ (Buckler and Vaughan–Jones, 1998). Furthermore, in the presence of these uncouplers, hypoxia caused no further increase in $[Ca^{2+}]_c$ (Duchen and Biscoe,1992; Wyatt and Buckler, 2004). With these selected mitochondrial inhibitors present (Wyatt and Buckler, 2004), the chemosensory response to hypoxia is also lost (Lahiri, 2004; Mulligan et al., 1981). These results suggested that either a common or concurrent transduction mechanism underlie the effects of both hypoxia and mitochondrial inhibitors.

Unfortunately, the mitochondrial involvement may not be straightforward. Rotenone, an inhibitor of the mitochondrial electron transport that preferentially works on complex I, blocks

O_2 sensing in glomus cells as predicted by the mitochondrial model. However, a similar inhibition of O_2 sensing was not observed with different classes of complex I inhibitors with different molecular targets, suggesting that the oxygen sensitivity of glomus cells is not dependent on the mitochondrial electron transport system in a simple way (Ortega-Saenz et al., 2003). Thus it is likely that changes in mitochondrial function do contribute to O_2 sensing but the details remain to be investigated fully.

Any change in mitochondrial function induced by hypoxia in glomus cells must ultimately lead to changes in the Ca^{2+}-dependent release of the transmitter dopamine, for example, controlled in part by the plasma membrane voltage. The identity of this coupling mechanism is not known. One possible scenario for the effects of the mitochondrial inhibitors that also inhibit the carotid body function is that these inhibitors increase the NADH/NAD concentrations, leading to an increase in the mitochondrial matrix H^+ concentration in cells (Kaplin et al., 1996). The H^+ would leak to the cytoplasm, which would then block the K^+ currents in the plasma membrane, resulting in depolarization (Peers et al., 2003). This depolarization opens the voltage-gated Ca^{2+} channels, enhancing Ca^{2+} influx and neurotransmitter release. Another scenario is that the inhibitors reduce the supply of ATP (Mulligan et al., 1981), which would depolarize mitochondria and subsequently the plasma membrane (Williams and Buckler, 2004). Also, hypoxic and hypercapnic stimulation of glomus cells lead to co-release of ATP and acetylcholine which together cause cellular excitation (Buettigieg and Nurse, 2003; Zhang and Nurse, 2004) (see also Section 3.3).

Now the mitochondrial model also includes an oxygen sensitive TASK-like background leak K^+ channel in the plasma membrane of the glomus cells of the carotid body which requires cytosolic mediaters. Also, inhibitors of oxidative phosphorylation abolish the hypoxic response, completely overlapping it. Thus the leak K^+ current seems to be an established component of the oxygen sensitive K^+ channel family which plays an important role in oxygen sensing (e.g., Wyatt and Buckler, 2004). However, how the mitochondria connect with the plasma membrane has not been answered, and remained an intruiging problem (Roy et al., 2002).

3.3. *Action-spectrum of the chemosensory afferents: Evidence for mitochondrial involvement*

Mitochondrial respiratory chain was found to respond to CO in normoxia in a light-sensitive manner. The light wavelength dependence of the CO sensitivity closely resembles that of cytochrome oxidases in the yeast (Warburg and Negelein, 1928). In the carotid body system, CO increases the afferent activity and this is fully antagonized by light. This finding was taken as the evidence for mitochondrial involvement in the overall chemosensory response (Wilson et al., 1994; Lahiri et al., 1999; Barbe et al., 2002). One corollary of this postulate is that the light sensitivity of the carotid body system is in fact mediated by glomus cells themselves. However, the available evidence fails to support this assertion because the electrical properties of glomus cells are not light sensitive (Li et al., 2002; Barbe et al., 2002). It points to afferent nerve terminal as the site of action CO for chemosensory stimulation (see Lahiri et al., 1999).

3.4. *Mitochondria and HIF-1α*

One of the coupling factors that link the hypoxia-induced changes in mitochondria to the membrane depolarization and/or Ca^{2+}-dependent neurotransmitter release may be the

transcription factor HIF-1 albeit it may not represent the fastest-acting coupling mechanism. HIF-1 orchestrates a variety of genomic responses to hypoxia and often regarded as a master regulator of oxygen homeostasis. As such, HIF-1 may also relay the information regarding hypoxia-induced changes in mitochondrial function to various effectors.

Prolyl hydroxylases (PH) are key enzymes which upon sensing chronic and acute hypoxia oxidatively modifies HIF-1α. During hypoxia the reaction is retarded. It is also retarded by chelation of Fe^{2+} and by analog of 2-oxogluterate, resulting in HIF-1α accumulation. Lack of oxidative modification by preventing insertion of molecular oxygen (see Lehninger, 1975; Cardinale et al., 1971) in the HIF-1α under normoxia leads to an increased $[Ca^{2+}]_i$ and chemosensory response (Roy et al., 2004a).

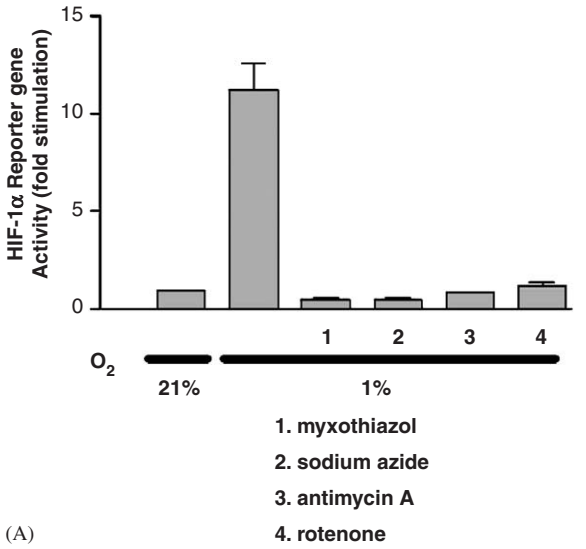
HIF-1 is a heterodimeric protein consisting of two subunits, HIF-1α and HIF-1β· HIF-1β is constitutively expressed and unaffected by changes in cellular P_{O_2} (see for review Bunn and Poyton, 1996; Semenza, 2000; see also Semenza in this review). HIF-1α is continuously synthesized and destroyed in normoxia as a result of its ubiquitination and subsequent degradation by the proteasomal system after hydroxylation, a process which is irreversible and is inhibited during hypoxia, resulting in the protein accumulation and dimerization with the β-subunit to form HIF-1. This is then translocated into the nucleus (Wang and Semenza, 1995a). The oxygen dependent domain (ODD) of HIF-1α interacts with the von Hippel-Lindau (VHL) tumor suppressor protein, a component of E3 ubiquitin ligase complex. Disruption of VHL gene results in a constitutive accumulation of HIF-1α in normoxia (Ohh et al., 2000; Ivan et al., 2001; Jaakkola et al., 2001; Salceda and Caro, 1997) (see Semenza in this review).

Accumulation of HIF-1α during hypoxia may require a functional mitochondrial respiratory chain (Schroedl et al., 2002; Agani et al., 2002; Hagen et al., 2003). This idea suggests that mitochondrial inhibitors should decrease the HIF-1α content of the cells during hypoxia (Fig. 3).

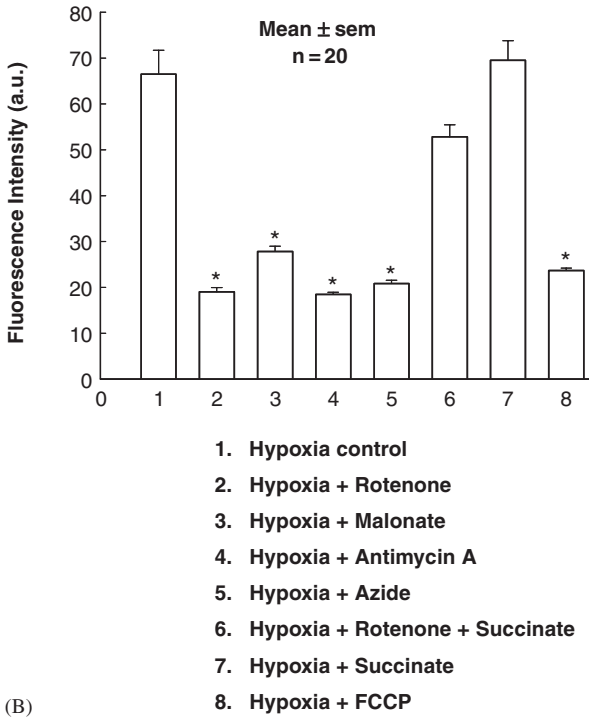
It is also known that mitochondrial inhibitors not only mimic hypoxia but they also abolish the oxygen sensitivity in the glomus cells and carotid body (Mulligan et al., 1981; Wyatt and Buckler, 2004). Thus, HIF-1α is equated to the oxygen sensitivity of the carotid body (Kline et al., 2002; Baby et al., 2005).

4. Membrane model

The final consequence of hypoxic-modulation of the neurosecretroy glomus cell function is a change in the Ca^{2+}-depenent neurotransmitter release. The increase in the intracellular $[Ca^{2+}]$ required for the release is in part mediated by plasma membrane voltage-gated Ca^{2+} channels (Lopez-Barneo et al., 2001) opened by depolarization. The membrane potential is readily regulated by K^+ channels and a variety of K^+ channels are present in glomus cells (Lopez-Barneo et al., 2001; Patel and Honore, 2001). Therefore K^+ channels have been proposed as an important class of hypoxia-effectors in glomus cells as proposed by Lopez-Barneo et al. (1988). The available evidence suggests that multiple types of K^+ channels are indeed inhibited by hypoxia but their relative importance are not yet clear (Lopez-Barneo et al., 2001). Studies also suggest that the hypoxia sensitivities of these K^+ channels may be subject to developmental regulation (Lopez-Lopez et al., 1997) and may vary depending on the experimental preparations and configurations (Lopez-Barneo et al., 1998), further illustrating the complexity.



(A)



(B)

Fig. 3. (A) HIF-1α activity in HEK 293 cells at 21% and 1% O_2 in the presence of mitochondrial inhibitors (modified from Hagen et al., 2003). With 1% O_2 HIF-1α activity was increased while the mitochondrial inhibitors diminished the HIF-1α activity. (B) Quantitative expression of HIF-1α immunofluorescence intensity (arbitrary units, a.u.) in glomus cells exposed to hypoxia (Hx) (P_{O_2} ~7 Torr). Significant induction of HIF-1α protein in Hx alone. Rotenone, malonate, antimycin A and FCCP all significantly suppressed the protein expression during hypoxia. The suppressive effect of rotenone was significantly reversed in presence of succinate which bypassed the inhibitory effect of rotenone (Baby et al., 2005).

Among the K^+ channels present in glomus cells, a class of K^+ channels termed large-conductance Ca^{2+}-activated K^+ channels, which are also known as BK, K_{Ca}, KCa1.1, MaxiK, or Slo1 (Vergara et al., 1998) have been suggested to play an important role in mediating the hypoxic response of gloumus cells (Peers, 1990). Membrane depolarization and/or increasing intracellular Ca^{2+} concentrations allosterically open BK channels to decrease the cellular electrical excitability (Magleby, 2003). Because of the voltage dependence conferred by the allosteric gating mechanism, these channels display a significant open probability even near the negative resting membrane potential (Horrigan et al., 1999), making the channels highly suited to control the frequency of action potentials.

When P_{O_2} was lowered from normoxia ($P_{O_2} \approx 100$ Torr), BK channels in glomus cells recorded in the whole-cell configuration of the patch-clamp method were rapidly and reversibly inhibited (Lopez-Barneo et al., 1988; Peers, 1990). A similar inhibition of the channels was observed with the BK channel blockers TEA, 4-AP and charybdotoxin (CTX) (Wyatt and Peers, 1995). As expected from the idea that hypoxia inhibits BK channels leading to depolarization, one study did report that the BK channel blocker CTX depolarized glomus cells (Wyatt and Peers, 1995). However, others reported that neither TEA nor 4-AP caused depolarization in glomus cells (Buckler, 1997) or that neither these agents nor CTX changed the sensory discharge of the carotid body (Doyle and Donnelly, 1994; Osanai et al. 1997b; Lahiri et al., 1998). Furthermore, glomus cells continue to release catetcholamines after BK channels are blocked by the BK channel toxin iberiotoxin (IbTX) (Pardal et al., 2000). Thus multiple mechanisms appear to be in place to ensure that glomus cells initiate the appropriate respiratory and cardiovascular reflexes.

The experimentally measured hypoxia sensitivity of BK channels depends on the patch-clamp configuration, providing an important clue about the molecular mechanism involved. The channels are inhibited by hypoxia in the whole-cell configuration and also in the perforated configuration where the cytoplasmic environment is relatively intact. However, in the outside-out configuration where the cytoplasmic milieu is severely disrupted, hypoxia had no appreciable effect on the BK channels (Wyatt and Peers, 1995) (but see (Ganfornina and Lopez-Barneo, 1992a, b). An essentially similar observation regarding the configuration-sensitivity of BK channels to hypoxia was made using neocortical neurons (Liu et al., 1999). At least in some inside-out configuration experiments, the hypoxia sensitivity remains intact (Lewis et al., 2002; Williams et al., 2004). These results suggested that some factor, which is often lost in the outside-out configuration, is required to maintain the hypoxia sensitivity of the glomus cell BK channel.

Additional mechanistic clues about the hypoxia sensitivity of BK channels were provided by the observation that BK channels heterologously expressed in HEK cells were also inhibited by hypoxia (Lewis et al., 2002). HEK cells, like glomus cells, contain the all cellular machineries necessary to confer robust hypoxia sensitivity to the channels or the channels themselves are hypoxia sensitive. A proteomic analysis showed that the BK channels expressed in HEK cells are associated with a variety of other proteins (indirectly or directly). Among them, hemoygenase-2 (HO-2) may be sufficient to confer the BK channel hypoxia sensitivity (Williams et al., 2004). Membrane-bound HO-2 is one of the two major types of hemoxygenase that oxidatively breaks down heme to iron, biliverdin and CO using O_2 as a cofactor. CO was known to be a stimulatory factor of BK channels (Wang and Wu, 1997; Xi et al., 2004). Thus, Williams et al. (2004) proposed that in normoxia HO-2, using CO as the gaseous messenger, exerts an excitatory influence on nearby BK channels and that hypoxia diminishes this excitatory influence. Gene

silencing of HO-2 by siRNA inhibited the hypoxia sensitivity of the heterologously expressed BK channels in HEK cells (Williams et al., 2004). Furthermore, native BK channels in glomus cells are regulated by the cofactors CO and/or NADPH essentially in the same manner as the recombinant channels, suggesting that the HO-2/CO mechanism may be operative in glomus cells (Williams et al., 2004). The HO-2/CO hypothesis is attractive but many aspects of the hypothesis require further investigation (Hoshi and Lahiri, 2004). It is noteworthy that the HO-2/CO hypothesis is reminiscent of one of the standing models of oxygen sensing: heme protein model. The model postulated that unbinding of oxygen from heme-containing proteins is involved in the hypoxia-sensitivity but the direct supporting evidence is sparse (Lopez-Barneo et al., 2001).

BK channels are not the only hypoxia-sensitive K^+ channels in glomus cells. Slowly inactivating voltage-gated K^+ channels (Ganfornina and Lopez-Barneo, 1991, 1992a, b; Lopez-Lopez et al., 1997), most likely a member of the Kv4 family (Perez-Garcia et al., 2000), appear to contribute to the hypoxic response of glomus cells. Hypoxia decreases the peak open probability and accelerates the inactivation kinetics of the Kv4-type K^+ channels and this hypoxia sensitivity may be observed in the excised configurations (Ganfornina and Lopez-Barneo, 1992a, b). The physiological importance of these Kv channels in the glomus cell function was demonstrated using a dominant-negative construct designed to specifically interfere with the formation of Kv4 channels (Perez-Garcia et al., 2000). Adenovirus-mediated gene transfer of the Kv4 dominant-negative subunit abolished the hypoxia-mediated depolarization in glomus cells recorded in the whole-cell configuration (Perez-Garcia et al., 2001). Using a more physiological carotid-body slice preparation, Pardal et al. (2000) recorded robust TEA-sensitive depolarization caused by hypoxia even in the presence of the BK channel blocker IbTX, confirming that these Kv-type channels do contribute to the hypoxic response.

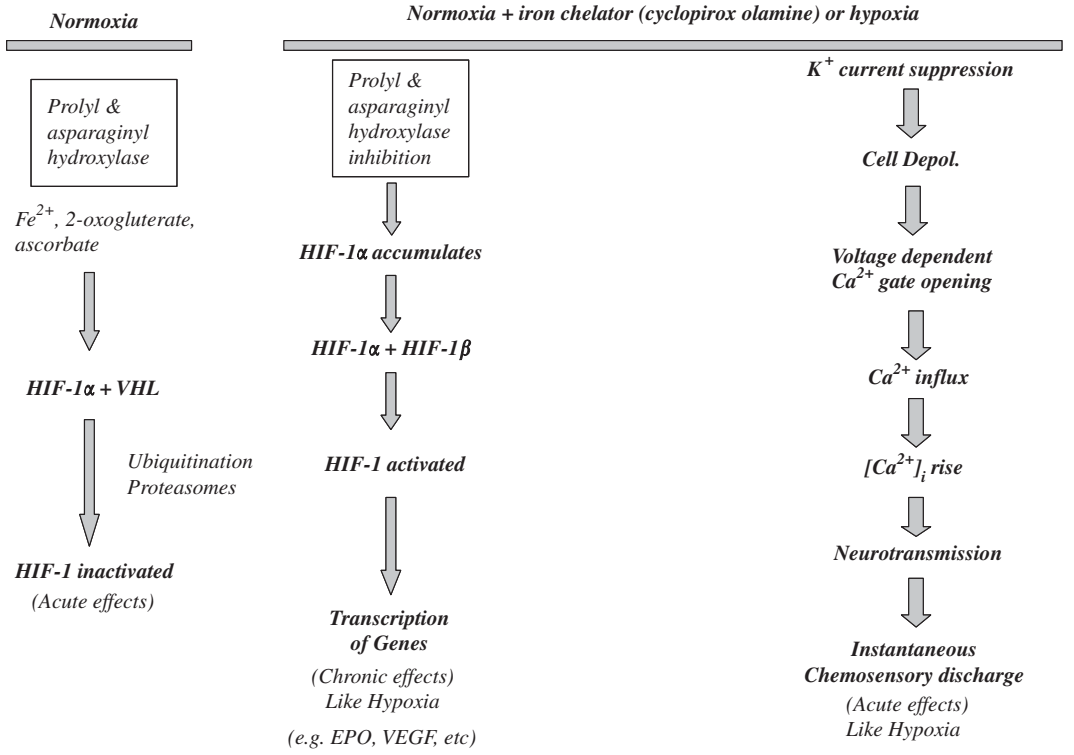
In addition to the aforementioned BK and Kv channels, yet another class of K^+ channels were reported to contribute to the hypoxia sensitivity of glomus cells: two-pore domain "leak" K^+ channels (K_{2P}) (Buckler, 1997; Buckler et al., 2000). These K^+ channels lack voltage sensor and their open probability is largely voltage independent and they are thought to contribute to the background K^+ currents that clamp the membrane potential hyperpolarized near E_K (Lesage and Lazdunski, 2000). When recorded in the whole-cell or the cell-attached configuration, hypoxia ($P_{O_2} = 5$ Torr), the background K^+ current likely mediated by TASK channels typically decreased by 35% and the hypoxia sensitivity was lost in the excised inside-out configuration, suggesting that some cytoplasmic factors may be required for the response (Buckler et al., 2000). That these K^+ channels have a high open probability near resting potentials makes them particularly well suited to mediate the hypoxia-mediated depolarization. Other members of the K_{2P} channel family may be similarly modulated by hypoxia and several other factors including selected lipids and pH (Franks and Honore, 2004; Kemp et al., 2003). The lipid and pH sensitivity of these channels, however, may turn out to be a serious confounding variable in study of their hypoxia sensitivity (Buckler and Honore, 2005; Caley et al., 2005).

Of the three K^+ channels, BK, Kv4 and K_{2P} channels, it is not completely clear which represents the most important hypoxia-sensitive channel type in vivo. Mice with the pore forming subunit of the BK channel (Slo1) gene disrupted are available (Meredith et al., 2004; Sausbier et al., 2004) but whether they show any abnormal carotid body function has not been reported. Further investigation is required to answer the fundamental standing questions, which K^+ channels are inhibited by what mechanism.

4.1. Iron-chelation and K^+ channel inhibition

Since the discovery of the HIF-1, it has been established that HIF-1α is degraded under normoxia for which Fe^{2+} is required and chelation of intracellular Fe^{2+} stabilizes HIF-1α, mimicking hypoxia (Maxwell et al., 1993; Wang and Semenza, 1993). That HIF-1α occurs in carotid body glomus cells and that Fe^{2+} chelation significantly increased it during normoxia (Baby et al., 2003), and that activation of HIF-1α mRNA also occurred in glomus cells during Fe^{2+} chelation and during hypoxia (Roy et al., 2004b).

While chemosensory discharge are initiated and continue to increase to steady state of hypoxia, the HIF-1α formation would begin to increase in conformity with observations of Jewell et al. (2001). The acute effects do not need new protein synthesis, and the response is instantaneous, like the acute effects of hypoxia. This is formulated as follows.



But these effects occur during inhibition of prolyl hydroxylase (PH) by Fe^{2+} chelation in normoxia. Our question is: how Fe^{2+} chelation produce these hypoxia-like effects in normoxia. The answer is not there but both the effects are very fast: chemosensory effects are instantaneous (e.g., Lahiri et al., 1981; Roy et al., 2004a) and HIF-1α responses do not lag behind (Jewell et al., 2003). There is parallelism of chemosensory and HIF-1α responses. Speed of measurements for the two are different: sensory responses are electrical and the HIF-1α is histological and biochemical. This reaction requires presence of oxygen, reduced form of iron, ascorbic acid and

2-oxogluterate. The inhibition of this PH reaction prevented incorporation of oxygen molecules, thereby producing a lack of oxygen effect even in the presence of normoxia.

Since chemosensory discharge and HIF-1α expression as a result of acute hypoxia are correlated, we could examine what are the other correspondence between these two reactions exist, and are summarized below:

1. The hydroxylation of PH inhibition results in almost simultaneous effects on chemosensory discharge and HIF-1α accumulation like hypoxia effects.
2. Both chemosensory discharge (Mulligan et al., 1982) and HIF-1α (Baby et al., 2005) are inhibited by metabolic inhibitors:
3. The effects of high CO (~300 Torr) in normoxia cause an increase chemosensory discharge and HIF-1α accumulation in the dark. Both the responses are eliminated by bright light exposure (unpublished).
4. Monochromatic light of 430 and 590 nm elicit similar decreases of chemosensory discharge and HIF-1α expressions (unpublished).
5. Hypoxic sensory response is inhibited by high CO (Lahiri et al., 1993; Lopez-Lopez and Gonzalez, 1992). Also, hypoxic HIF-1α activation is inhibited by CO (Huang et al., 1999).

Oxygen sensor in both the instances appears to be the same.

4.2. Further effects of CO

High CO tension (PCO > 300 Torr) always stimulates the chemosensory discharge in vitro in the absence of light. Shinning bright light silenced the chemosensory discharge (Lahiri, 1994). The response was dependent on Ca^{2+} (Riesco-Fagundo et al., 2001).

The light effect was further analyzed by using various wavelengths. At 430 and 590 nm, the sensory discharges were most affected (Wilson et al., 1994), and these effects corresponded to the similar effects of cytochrome oxidase (Warburg and Negelein, 1928). Thus the primary oxygen sensor was identified as the cytochrome oxidase (Fig. 4).

Using the same CO as a tool, Lahiri et al. (1993) found hypoxic chemosensory discharge was inhibited by high tesion of CO which was light sensitive, i.e., light suppressed the response (Fig. 5). However, glomus cell K^+ current was suppressed by hypoxia (5% O_2) which was reversed by 10% CO (Lopez-Lopez and Gonzalez, 1992) (Fig. 6). They therefore concluded that the glomus

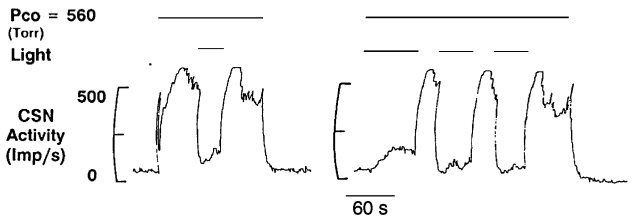


Fig. 4. Photoreversible effect of PCO on chemosensory excitation of cat carotid body perfused with tyrode solution. Carotid chemosensory activity (CSN) is plotted against time (Lahiri, 1994).

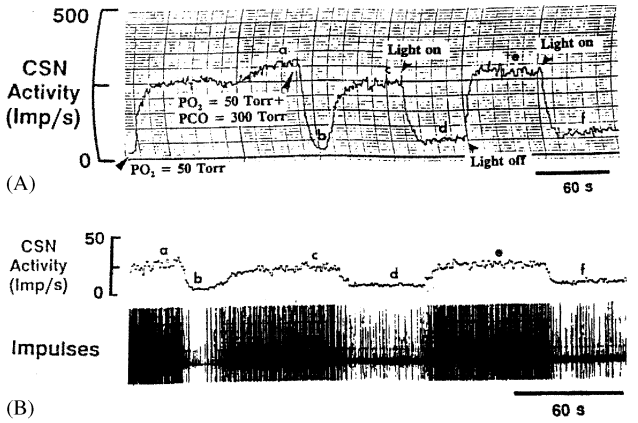


Fig. 5. (A) PCO of 300 Torr prevented hypoxic chemosensory response in presence of light. Hypoxia stimulated activity at point (a). Then PCO of 300 Torr plus P_{O_2} 50 Torr was infused in absence of light. Immediate effect was a decrease of activity near baseline (b). This was followed by a rise to a stable activity (c). Shining light decreased activity to near baseline (d) despite hypoxia. Subsequent absence (e) and presence (f) of light, respectively, increased and decreased activity in a reproducible way. Excitation in absence of light resulted from CO complex formation, and its attenuation was due to photodissociation. Near absence of activity even in presene of hypoxia during illumination is presumably due to formation of CO complex with putative membrane heme and inhibition of activity. (B) Activities of a few fibers at the points (a–f) are shown corresponding to those in (A) (Lahiri, 1994).

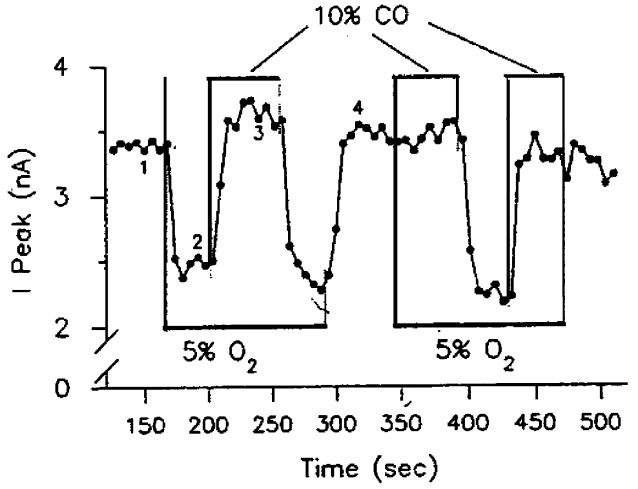


Fig. 6. Effects of carbon monoxide on the inhibition of the K^+ current by low P_{O_2}. (Lopez-Lopez and Gonzalez, 1992).

cell membrane was the sit of oxygen sensing. But the chemosensory excitation of CO in normoxia (Fig. 4) was not accounted for. This is due to cytosolic cytochrome oxidase.

The membrane current of CO treated cells was not sensitive to light (Barbe et al., 2002; Li et al., 2002) unlike the chemosensory discharge. The conclusion was that nerve terminal was the sit of response. A similar speculation was reached earlier by Lahiri and Acker (1999) (see Section 3.3).

It was interesting to see the findings of the groups led by Bunn (Huang et al., 1999) and Moncada (Hagen et al., 2003) in this context. They found that CO and NO inhibited the HIF-1α, apparently in the absence of light. Both CO and NO specifically targeted the internal oxygen dependent degradation domain of HIF-1α, and also repressed the C-terminal transactivation domain of HIF-1α. This was without participation of mitochondrial respiration (Huang et al., 1999). On the other hand, CO, NO and other mitochondrial inhibitors prevented stabilization of HIF-1α during hypoxia (Hagen et al., 2003). Further investigation is required to understand various implications of the effects of CO and NO.

4.3. ATP release in the afferent nerve terminals

More recently, evidence has been found that afferent terminals surrounding individual glomus cells are responsible for the ATP release and sensory excitation (Rong et al., 2003).On the basis of cumulative evidence Spyer's group formulated a unifying hypothesis that ATP-mediated purinergic signaling have a potential role in chemosensory control of the respiratory function (Varas et al., 2003; Spyer et al., 2004).

Hypoxia evoked an increase in extracellular ATP that was inhibited by L-type Ca^{2+} channel blockers. Additionally IbTX, a blocker of O_2-sensitive Ca^{2+}-dependent K^+ (BK) channels stimulated ATP release (Buttigieg and Nurse, 2001). In an important contribution, Zhang and Nurse (2004) found co-release of ACh and ATP in CO_2/pH chemosensory signaling in co-cultures of rat carotid body receptors and petrosal neurons and is consistent with those of Mokashi et al. (2003) on the effects of small concentration of extracellular ATP on chemosensory excitation.

4.4. Intracellular [Ca^{2+}] rise as a result of hypoxia

Based on the mitochondrial model, Duchen and Biscoe (1992) suggested that endoplasmic reticulum (ER)–mitochondria related intracellular [Ca^{2+}] stores are crucial in hypoxic response and release of calcium during hypoxic stimulation, enhancing CSN activity (see also Lopez-Lopez and Gonzalez, 1992; Riesco-Fagundo et al., 2001). Similar observation related to the role of intracellular calcium release during hypoxia was reported with the use of thapsigargin, a blocker of Ca^{2+}-ATPase (Lahiri et al., 1995). But the significance of intracellular store was at variance with those of Wyatt and Buckler (2004) and Vicario et al. (2000), and they stressed the crucial role of Ca^{2+} influx through cellular membrane, in global intracellular Ca^{2+} rise in hypoxia. Also, earlier Lopez-Barneo et al. (1988) had proposed that plasma membrane depolarization in hypoxia due to reversible inhibition of oxygen sensitive K^+ channels, and it enhanced extracellular [Ca^{2+}] influx causing neurochemical release and increased CSN activity. However, there is an increasing amount of evidence that [Ca^{2+}] store associated with ER–mitochondrial complex can play an important role (Rizzuto et al., 1994; Rutter and Rizzuto, 2000; Parekh, 2003).

4.5. IP_3-receptor mediated response: membrane vs. mitochondria

Inositol 1,4,5-triphosphate (IP_3) and 1,2-diacylglycerol (DAG) are generated from the hydrolysis of PIP_2 by phospholipase C which is a component of plasma membrane. IP_3 acts as a messenger to link with receptors (IP_3-Rs) which are located on intracellular Ca^{2+} stores, such as

the ER (Belousov et al., 1995; Berridge, 1993). The ER also often shares the same domain of the mitochondria which have a low affinity, high-capacity for Ca^{2+} uptake mechanisms (Meldolesi and Pozzan, 1998). Thus, IP_3-Rs are an ideal candidate for Ca^{2+}-related cellular functions. Rizzuto et al. 1994, 1998) have shown that $[Ca^{2+}]_m$ increases rapidly and transiently upon stimulation with agonists coupled to IP_3-Rs generation. This leads to high $[Ca^{2+}]_i$ response close to IP_3-Rs and sensed by mitochondria and ER-complex (Lahiri et al., 2003a).

Inhibitors and blockers have been used successfully as tools to explore the biological functions (Wyatt and Buckler, 2004; Ortega-Saenz et al., 2003). We used cell permeant 2-APB (2-amino ethoxydiphenyl borate) to block Ca^{2+} release from the IP_3-Rs pathway in the ER–mitochondria complex, and examine the effects on global $[Ca^{2+}]$ in glomus cells, and sensory responses of the rat carotid body. Hypoxic response was obliterated leaving the hypercapnic response intact (Lahiri, 2004). The hypercapnic Ca^{2+} is not dependent on IP_3-Rs. The sensory response corresponded to the $[Ca^{2+}]_c$ response. The IP_3 receptor plays an important part in $[Ca^{2+}]_c$ regulation, and the subsequent sensory response (Lahiri, 2004). In the context, the same phenomenon has been observed after treatment of oligomycin to glomus cells (Lahiri, 2004) and carotid body (Mulligan et al., 1981), implicating a similar mechanism.

5. Oxygen sensing and coupling: a possible mechanism

The results presented above collectively show that selected K^+ channels are inhibited by hypoxia and this hypoxic inhibition may involve multiple components, fast direct inhibition of the channels and somewhat slower responses potentially involving Ca^{2+}, $Fe^{2+/3+}$ and HIF. However, the primary oxygen sensor that mediates the first step in oxygen sensing in the carotid body is not known. As suggested by the existence of multiple hypoxia-sensitive ion channel types, it is quite plausible that multiple oxygen sensors may operative such that disruption of one may initiate compensatory responses and may not necessarily lead to sustained dysfunction of the carotid body.

Studies from simpler organisms, such as bacteria, show that reactive metals including $Fe^{2+/3+}$ and Cu^{2+} play important roles in gas sensitivity of many proteins. The reversible redox states of $Fe^{2+/3+}$ may be particularly important. Furthermore, many bacterial hemoproteins with reactive iron groups are gas-regulated, rendering them potential oxygen sensors. In mammalian carotid body type I cells, studies suggest that the primary oxygen sensing elements are proteins containing reactive metals, such as hemoproteins. The likely candidate proteins are those in the mitochondrial respiratory chain component (Wilson et al., 1994), non-mitochondrial proteins such as the NAPDH oxidase (Cross et al., 1990) or K^+ channel associated proteins (Lopez-Barneo et al., 1999, 2004; Williams and Buckler, 2004).

5.1. Molecular nature of the oxygen sensor

Lloyd et al. (1968) first observed that a burst of CO during inspiration of a hypoxic mixture caused a transient depression of ventilation in man. They proposed that the carotid body contains a hemoglobin or myglobin-like fragment that detected CO or O_2 levels. Lahiri and DeLaney (1975a) showed by comparing the dissociation curves of HbO_2 and carotid sinus nerve responses to arterial P_{O_2}, that a pigment with a P_{50} of around 40 mm Hg is responsible for the hypoxic

response. Later Goldberg et al. (1988), working with erythropoietin producing cell line showed that hemoprotein could be an oxygen sensor. The same notion was supported by Lopez-Lopez and Gonzalez (1992) showing that O_2-sensitive K^+ current in isolated glomus cells was prevented or reversed by CO. This finding was confirmed by Lahiri et al. (1993) using an intact carotid body preparation. They also showed that CO-induced excitation of chemosensory discharge was sensitive to various wavelengths of light mimicking the action spectrum of mitochondrial respiratory chain. This confirmed the heme nature of oxygen sensing (Wilson et al., 1994; Lahiri and Acker, 1999; Streller et al., 2002). All these observations suggested that O_2 sensor was a hemoprotein, as envisioned by Tang et al. (2003).

Consistent with the observations listed above, one possible oxygen sensor that is relatively directly coupled to inhibition of K^+ channels may be hemoxygenase-2 (HO-2) (Williams et al., 2004). HO-2, a plasma membrane associated protein, oxidatively breakdowns heme into CO, iron and billiverdin. This reaction requires NADPH/Cytochrome P450 reductase and O_2 to proceed. Without O_2, the heme catabolism catalyzed by HO-2 slows down, leading to a decrease in CO production. What is noteworthy is that CO, a gaseous messenger akin to nitric oxide (NO), increases the BK channel activity in different systems (Perez-Garcia et al., 2000; Wang and Wu, 1997; Xi et al., 2004). Thus, in normoxia, CO produced by the HO-2 mediated catabolism of heme occurring near the plasma membrane normally exerts and excitatory influence on the BK channel and hypoxia decreases the CO production, leading to an inhibition of the channel. Furthermore, the proteomics analysis by Williams et al. (2004) suggests that the pore forming subunit of the BK channel complex (Slo1, KCNMA1) may be closely associated with HO-2, at least in a heterologous expression system. Thus, the CO signaling between HO-2 and the nearby BK channel complex is gaseous and local in nature. The idea that HO-2 is a candidate oxygen sensor coupled to BK channels is supported by a variety of experiments performed in an expression system, however, the evidence is more limited in native glomus cells (Williams et al., 2004). Nonetheless, the hypothesis that HO-2 is an oxygen sensor and that the gaseous messenger CO is the coupling mechanism is an exciting one and further investigation is warranted (see Hoshi and Lahiri, 2004). Undoubtedly, HO-2 is not the only sensor and other mechanisms need to be considered.

5.2. *Possible role of ROS*

Recently, several workers have elegantly shown such insertion of O_2 molecule directly in HIF-1α by PH, making participation of ROS unlikely (Epstein et al., 2001; Ivan et al., 2001; Jaakkola et al., 2001). It has been known for some time that the two oxygen atoms of molecular oxygen are directly inserted into the substrate in hydroxylation reaction as it has been shown with the use of the ^{18}O isotope as tracer (Lehninger, 1975; Cardinale et al., 1971). Such lack of introduction of O_2 molecule would lead to excitation of chemoreception. But see Section II for further effects of ROS, and Schumacker (2002).

6. NO and CO effects

NO and CO are two gas molecules that are derived endogenously. These are dealt in details in Section III by Prabhakar. Briefly, they are both inhibitory and excitatory to the carotid body

sensory discharge (Itturiaga et al., 2000). NOS—positive innervations of the carotid body appears to be on the blood vessels (Grimes et al., 1995; Wang et al., 1993). Inhibition of NOS activity augmented the chemosensory activity, implying that endogenous NO is inhibitory to the carotid body (Chugh et al., 1994; Grimes et al., 1995; Prabhakar et al., 1993a, b; Wang et al., 1993).

CO is produced endogenously by the enzyme hemoxygenase I and II which catalyze the formation of CO, and molecular oxygen is required for CO synthesis just like NO. HO-II is constitutive and is present predominantly in neural tissues. HO-II inhibition by Zn-protoporphrin-IX augmented the chemosensory responses in dose levels of 3–10 μM. The CO produced caused inhibition of the chemosensory discharge and the inhibition of endogenous CO augmented the chemosensory activity (Prabhakar et al., 1993a, b). This is consistent with the inhibition of chemosensory activity during hypoxia in presence of low level of CO (Lahiri et al., 2003b). And now HO-II has been claimed as an oxygen sensor (Williams et al., 2004).

Both NO and CO inhibited HIF-1 DNA binding by abrogating hypoxia induced accumulation of HIF-1α protein (Huang et al., 1999). Similarly, Snyder et al. (1998) asserted that NO and CO show parallel roles as a neural messenger as mediators of blood vessel relaxation. Like NO effects on sensory discharge show efferent inhibition (Wang et al., 1993), dopaminergic inhibition of the sensory discharge in chronic hypoxia is augmented (Lahiri et al., 1980). However, the NO effects are much more complicated (Huang et al., 1999).

7. Effects of chronic hypoxia

7.1. Intermittent vs. sustained hypoxia

Molecular mechanisms associated with sustained hypoxia (SH) have been extensively studied (Wang and Semenza, 1993; for review see Bunn and Poyton, 1996). Intermittent hypoxia (IH) occurs in human subjects during obstructive and central apnea particularly during sleep at high altitude (e.g. Lahiri et al., 1983, 2002; Prabhakar, 2001). There are however important differences between intermittent and SH. Baby et al. (2004) found that HIF-1α protein expression in the carotid body glomus cells required greater than 3 days of IH as compared with the SH which is consistent with those of Prabhakar (2001). Hui et al. (2003) showed that it is during sustained hypoxia the response develop at a faster rate and was stronger. Gozal et al. (2005) found that IH induced a reduced site specific tyrosine hydroxylase (TH) activity in the rat brain compared to SH. No doubt that a response would be found in IH as it is found in SH. But a different paradigm of hypoxia would make a difference. For example, Yuan et al. (2004, 2005) demonstrated that for a given duration and intensity, IH is more potent in activating HIF-1 as well as *c-fos* mediated transcription than SH. Furthermore, IH-induced activation of *c-fos* and downstream gene activation are coupled to increased generation of ROS involving inhibition of mitochondrial complex I of the electron transport chain (Yuan et al., 2004). Similarly, HIF-1 activation by IH critically depends on phosphorylation of co-activator p300 by CaMKII, a mechanism which is distinct from SH (Yuan et al., 2005). This field of research is in early phase of infancy.

Chronic hypoxia initiates multitude of responses that tend to mitigate adverse effects of hypoxia (Dinger et al., 2003; Wilson et al., 2005). Glomus cells underwent hypertrophy

(McGregor et al., 1984; Pequignot et al., 1984), carotid body became more excitable (Bernard et al., 1987; He et al., 2005; Nielson et al., 1988; Vizek et al., 1987) due to a possible reduced K^+ current amplitude of glomus cells (Nurse and Vollmer, 1993).

Also, after chronic hypoxia the secretory responses of PC-12 cells to acute hypoxia is augmented (Taylor et al., 1999). After birth hypoxic responses of the carotid body are reset as a result of oxygen exposure (Carroll, 2003; Kumar, 2003). The mechanism of resetting of the carotid body that occurs soon after birth is not known.

8. Clinical implications

A loss of carotid body function results in a loss of hypoxic ventilatory response. Some of these diseases can occur at sea level. At high altitude with low P_{O_2}, these become more common (Lahiri and Milledge, 1998).

HIF-1 is a key element in chronic hypoxia. This transcription factor turns up in the activity of a variety of genes at a low oxygen pressure. When P_{O_2} becomes low, the cells are switched off (e.g., oxidative enzymes and PH) or on (e.g., glycolytic enzymes). The master regulator is switched on in the case of ischemic heart disease and strokes. It functions as a growth factor and produces the repair work. In cancers which carry elevated levels of proteins, this factor contributes to the growth and spread of the cancer. In tumors, new blood vessels are formed. Sometimes, inhibition of growth is an useful treatment. Some of these implications are described well elsewhere by Semenza in this review (see Section II of this review).

Section II: Oxygen-regulated expression of hypoxia-inducible factor 1 (HIF-1) (G.L. Semenza)

9. Hypoxia-inducible factor 1 (HIF-1)

Hypoxia induces both acute and chronic physiological responses. Acute responses occur on a time scale of seconds or less and involve the modification of pre-existing proteins. Chronic responses occur on a time scale of minutes or more and involve changes in gene expression leading to new protein synthesis. Whereas the analysis of carotid body physiology primarily has focused on understanding acute responses leading to depolarization of glomus cells, the discovery of the transcription factor HIF-1 has led to the delineation of molecular mechanisms of O_2-regulated gene expression that are operative in all cells including those within the carotid body. Over 70 genes are known to be directly regulated by HIF-1 in response to hypoxia (Semenza, 2003). The proteins encoded by these genes mediate adaptive physiological responses, such as angiogenesis, erythropoiesis, and glycolysis that either serve to increase O_2 delivery or allow metabolic adaptation to reduced O_2 availability. HIF-1 is present in simple invertebrates such as the round worm *Caenorhabditis elegans*, which consists of $\sim 10^3$ cells and relies on simple diffusion for O_2 transport, more complex invertebrates such as the fruitfly *Drosophila melanogaster*, in which O_2 is distributed through the body via a set of specialized tracheal tubes, and in complex vertebrates such as *Homosapiens,* which consist of $>10^{13}$ cells that are supplied with O_2 via the combined functioning of highly complex and specialized circulatory and respiratory systems. Complete HIF-1 deficiency in mice results in embryonic lethality at midgestation due to failure to establish a functioning circulatory system (Compernolle et al., 2003; Iyer et al., 1998;

Ryan et al., 1998), which is required because by this stage the size of the growing embryo no longer allows all cells to receive adequate O_2 by simple diffusion.

HIF-1 is a heterodimeric protein that is composed of an O_2-regulated HIF-1α subunit and a constitutively expressed HIF-1β subunit (Wang et al., 1995; Wang and Semenza, 1995; Jiang et al., 1996). HIF-1 DNA-binding activity and HIF-1α protein levels were analyzed in HeLa cells exposed to a range of O_2 concentrations from 0% to 20% for 4 h either in the presence or absence of 1 mM KCN to eliminate O_2 consumption and intracellular O_2 gradients (Jiang et al., 1996). In both cases, the cells responded to hypoxia in a graded manner, with a half-maximal response at 1.5–2% O_2 and a maximal response at 0.5% O_2. O_2-regulated expression of HIF-1α was also demonstrated in isolated perfused and ventilated ferret lung preparations (Yu et al., 1998).

HIF-1α is hydroxylated on proline residues 402 and/or 564 by the enzymes prolyl hydroxylase (PHD) 1, 2, and 3 (Bruick and McKnight, 2001; Epstein et al., 2001; Ivan et al., 2002). These PH have a K_m for O_2 that makes it the rate limiting reaction substrate under physiological conditions (Epstein et al., 2001; Hirsila et al., 2003). In addition to O_2, 2-oxoglutarate (α-ketoglutarate) and Fe^{2+} are also required for the hydroxylation reaction. The use of a Krebs cycle intermediate in this reaction represents an intriguing link between O_2 and energy homeostasis that requires further investigation. Finally, ascorbate appears to function as a co-factor to return the oxidized Fe(III) form of the enzyme to its reduced Fe(II) state, which represents a nexus between O_2 sensing and cellular redox status the significance of which will also require further investigation.

Prolyl hydroxylation of HIF-1α is required for binding of the VHL protein, which is the recognition component of an E3 ubiquitin-protein ligase (Hon et al., 2002; Ivan et al., 2001; Jaakkola et al., 2001; Kamura et al., 2000; Min et al., 2002; Yu et al. 2001). Binding of VHL targets HIF-1α for ubiquitination and degradation by the 26*S* proteasome (Huang et al., 1998; Kallio et al., 1999; Maxwell et al., 1999; Salceda and Caro, 1997; Sutter et al., 2000; Tanimoto et al., 2000). Under hypoxic conditions, the hydroxylation of HIF-1α is inhibited resulting in accumulation of the protein, which translocates to the nucleus, dimerizes with HIF-1β, and binds to specific DNA sequences in target genes that contain the core 5′-RCGTG-3′ (C, cytosine; G, guanine; R, adenine or guanine; T, thymidine).

HIF-1α is also hydroxylated on asparagine-803 under normoxic conditions by FIH-1 (factor inhibiting HIF-1) and this modification prevents HIF-1α from interacting with the coactivators CBP and P300 (Dann et al., 2002; Elkins et al., 2003; Hewitson et al., 2002; Kallio et al., 1998; Lando et al., 2002a, b; Mahon et al., 2001; McNeill et al., 2002). The coactivators function as a bridge linking HIF-1 to RNA polymerase II and other general transcription factors within the initiation complex. In addition, CBP and P300 have histone acetyltransferase activity that is required for Pol II to associate with the DNA template and transcribe it into mRNA. Under hypoxic conditions, O_2 becomes rate limiting for FIH-1 activity and unhydroxylated HIF-1α is available to associate with CBP and P300 (Dames et al., 2002; Freedman et al., 2002; Koivunen et al., 2004). Remarkably, FIH-1 also interacts with VHL, establishing a link between the regulation of protein stability and transcriptional activity (Mahon et al., 2001).

Thus, both the half-life and specific activity of HIF-1α are precisely regulated by the cellular O_2 concentration. As a result, changes in gene expression can occur that are both rapid and graded with respect to the duration and severity of the hypoxic stimulus. Thus, the HIF-1 pathway encompasses both acute responses (O_2-dependent hydroxylation of HIF-1α) and chronic responses (changes in HIF-1 target gene expression). Furthermore, the ability of the carotid

body to sense O_2 is also highly dependent upon HIF-1 activity (Kline et al., 2002), providing a connection to the acute responses that are described in Sections I and III.

When wild-type mice are exposed to 10% O_2 for 3 days and returned to room air, they manifest an augmented ventilatory response to a subsequent acute hypoxic challenge, indicating a physiological adaptation to hypoxia. *Hif1a*$^{+/-}$ mice, which are heterozygous for a knockout allele at the locus encoding HIF-1α, manifest normal ventilatory responses to acute hypoxia but manifest no ventilatory adaptation in response to chronic hypoxia (Kline et al., 2002). Ventilatory adaptation to chronic hypoxia is mediated by the carotid body in wild-type mice, suggesting that abnormal carotid body function in *Hif1a*$^{+/-}$ mice. When carotid bodies isolated from wild-type mice were exposed to either cyanide or hypoxia, a marked increase in carotid sinus nerve activity was recorded. Remarkably, carotid bodies from *Hif1a*$^{+/-}$ mice depolarized in response to cyanide but showed no neural activity in response to hypoxia. This profound but selective physiological defect was not associated with any histological abnormality of carotid body size or glomus cell number.

In *Hif1a*$^{+/-}$ mice, the ventilatory responses to acute hypoxia were mediated by other chemoreceptors, such as the aortic bodies, that utilize vagal afferents to signal to the central nervous system. Whereas vagotomy had little effect on ventilatory responses to acute hypoxia in wild-type littermates, it significantly impaired these responses in *Hif1a*$^{+/-}$ mice. This reliance on chemoreceptors other than the carotid body is similar to the adaptation that occurs in normal animals following carotid denervation. Thus partial HIF-1α deficiency has a dramatic effect on carotid body neural activity and ventilatory adaptation that occurs in normal animals following carotid denervation. Thus partial HIF-1α deficiency has a dramatic effect on carotid body neural activity and ventilatory adaptation to chronic hypoxia. Furthermore, these studies indicate differences in the regulation of carotid and non-carotid body chemoreceptor function as reflected in the variable effects of partial HIF-1α deficiency.

The profound effects of partial HIF-1α deficiency may reflect the fact that HIF-1 coordinates physiological responses by regulating the expression of multiple genes encoding proteins that are required for these responses, resulting in a synergistic effect of partial loss-of-function for multiple system components. An alternative and not mutually exclusive possibility is based upon the demonstration that HIF-1α expression increases exponentially as O_2 concentrations decline, providing a mechanism for a graded transcriptional response to hypoxia. The decreased levels of HIF-1α expressed at any given level of hypoxia in *Hif1a*$^{+/-}$ mice would thus result in a suboptimal response to the stimulus. The virtually complete failure of *Hif1a*$^{+/-}$ carotid body glomus cells to depolarize in response to hypoxia suggests that a threshold level of HIF-1α may be necessary for some physiological responses to occur, although it is possible that an underlying defect in carotid body development that is not apparent on histological examination may be responsible. Further analysis of *Hif1a*$^{+/-}$ mice may reveal important clues regarding the mechanisms of O_2 sensing (Section I) and signal transduction (Section II) in the carotid body.

Section III: Oxygen sensing and neurotransmitter release from carotid body (N.R. Prabhakar)

10. Sensory transmission in the carotid body

It is fairly certain that transmitters are critical for the transmission of the hypoxic signal from the glomus cell to the afferent nerve ending. Morphological and physiological studies suggest that

glomus cells are secretory cells and satisfy several criteria for pre-synaptic cells (see Gonzalez et al., 1994, Prabhakar, 2000, Kumar et al., 2003 for references). Currently it is believed that hypoxia facilitates release of one or more excitatory transmitters from the glomus cells, which by depolarizing the nearby afferent nerve terminals, leads to increase in the sensory discharge. The following section summarizes the recent progress in the sensory transmission and the role neurotransmitters in the carotid body.

11. Transmitters expressed in the glomus cells

The carotid bodies express as many types of neurotransmitters as the mammalian brain (see Kumar et al., 2003 for references). The transmitters expressed in the carotid body can be classified into two major categories; conventional and unconventional. The *conventional* neurotransmitters include those stored in synaptic vesicles and mediate their actions via activation of specific membrane bound receptors often coupled to G-proteins. *Unconventional* neurotransmitters are those that are not stored in synaptic vesicles, but spontaneously generated by enzymatic reactions and exert their biological actions either by interacting with cytosolic enzymes or by direct modification of proteins. Recently identified gas molecules such as nitric oxide (NO) and carbon monoxide (CO) belong to an unconventional class of neurotransmitters.

12. Conventional transmitters

12.1. Acetylcholine (ACh)

Glomus cells express cholineacetyltransferase (CAT), the enzyme associated with ACh synthesis, and acetylcholinesterase (AChE), the enzyme that terminates the actions of ACh (Nurse and Zhang, 1999) indicating the enzymatic machinery for the generation and inactivation of ACh. Hypoxia results in a modest release of ACh in cat carotid bodies, whereas a greater release was noted when hypoxic stimulus (4% O_2) was applied in combination with 2% CO_2 (see Fig. 3 in Fitzgerald et al., 1999). In rat and rabbit carotid bodies, however, hypoxia inhibits the basal release of ACh, and this inhibitory effect of hypoxia can be relieved after blockade of muscuranic receptors (Kim et al., 2004). These results are similar to earlier studies on central and peripheral nervous system that showed inhibition of ACh release by hypoxia (Chleide and Ishikawa, 1990; Gibson and Peterson, 1982).

Several studies documented the excitatory actions of ACh in cat and rat carotid bodies (see Fitzgerald, 2000; Gonzalez et al., 1994 for references). However, in rabbit ACh inhibits the sensory activity (see Fidone and Gonzalez, 1986; Prabhakar, 1994 for references). The excitatory effects of ACh in the carotid body are mediated by nicotinic acetylcholine receptors (nAChR). Afferent nerve terminals appear to express nAChR that contains $\alpha 7$ subunits (Shirahata et al., 1998). On the other hand, muscarinic cholinergic receptors (mAChRs) seem to be coupled to inhibition of the sensory activity (Dinger et al., 1986). The relative abundance of nAChR and mAChR in the carotid body varies among species (Hirano et al., 1992) that may explain the

species dependent inhibition or excitation of the sensory discharge by ACh. Cholinergic antagonists that block the actions of exogenous ACh have little influence on the chemosensory response to hypoxia (see Fidone and Gonzalez, 1986; Gonzalez et al., 1994; Prabhakar, 2000 for references). However, a cocktail of cholinergic blockers are needed to block or to attenuate the sensory response to acute hypoxia (Fitzgerald, 2000). Recently, Zahng et al. (2000) reported that hexamethonium or mecamylamine in combination with suramin, a non-selective purinergic receptor antagonist, prevents the sensory excitation by hypoxia in co-cultures of rat glomus cells with petrosal neurons, suggesting that ACh, in concert with ATP, mediates the sensory excitation by hypoxia. Interestingly, combined application of cholinergic blockers and suramin also attenuates the sensory response of the carotid body to hypercapnia (Zahng et al., 2000). Although the evidence thus far suggests that ACh can augment the sensory activity, but the inability of cholinergic blockers to prevent the sensory excitation by hypoxia indicates that the hypoxic sensory transmission requires in addition to ACh, other neurotransmitters as well.

12.2. Catecholamines

It is fairly established that carotid bodies express catecholamines such as dopamine and norepinephrine. There is no convincing evidence for epinephrine expression in the carotid body. Type I cells from a variety of species express TH and dopamine β hydroxylase (DBH) the enzymes responsible for the synthesis of dopamine (DA) and norepinephrine (NE), respectively (Fidone and Gonzalez, 1986; Gonzalez et al., 1994; Wang et al., 1992). Nerve fibers (of sensory as well as autonomic origin) and ganglion cells also show TH immunoreactivity (Wang et al., 1992). Evidence for DA and/or NE transporters in the carotid body is lacking.

Hypoxia releases DA from cat, rabbit and rat carotid bodies (Bairam et al., 2001; Fidone et al., 1982; Gomez-Nino et al., 1990; Gonzalez-Guerrero et al., 1993; Obeso et al., 1992; Pardal et al., 2000; Vicario et al., 2000). Recent studies with chronoamperometry on carotid body slices have provided evidence that much of the DA released during hypoxia comes from glomus cells (Pardal et al., 2000). DA release by hypoxia is Ca^{2+} dependent and involves at least voltage-gated L-type Ca^{2+} channels (Obeso et al., 1992). Acidosis (pH = 7.2–6.6) as well as high CO_2 (20% CO_2, pH 7.4) also stimulates DA release from the rabbit carotid body in a Ca^{2+}-dependent manner (Rigual et al., 1991). Although type I cells express NE, hypoxia preferentially releases DA more than NE (Gonzalez et al., 1994; Vicario et al., 2000).

DA is inhibitory to carotid body activity in a number of species, and blockade of dopaminergic receptors augments the baseline activity and potentiates the response to hypoxia. Recent studies suggest that NE, under physiological conditions, functions as an inhibitory messenger, similar to DA (Kou et al., 1991). The inhibitory actions of NE are coupled to α_2-adrenergic receptors. Blockade of α_2-receptors stimulates the baseline activity and further augments the sensory response to hypoxia (Kou et al., 1991). The actions of α_2-receptors are coupled to inhibition of Ca^{2+} current via G-protein coupled voltage gated Ca^{2+} channels in the glomus cells (Overholt and Prabhakar, 1999). The sensory excitation seen with pharmacological doses of NE is conceivably due to vasoconstriction of carotid body vessels mediated by β-adrenergic receptors (see Fidone and Gonzalez 1986 for references).

12.3. 5-Hydroxytryptamine (5-HT)

5-HT-like immunoreactivity has been reported in type I cells of rat (Zhang and Nurse, 2000) and mouse (Oomori et al., 1994) carotid bodies. 5-HT results in brief excitation followed by inhibition of carotid body activity in cat (Nishi, 1975) and dogs (see Prabhakar, 1994 for references). In cat, the initial excitatory phase is mediated by 5-HT3 receptors, whereas the delayed excitation is coupled to 5-HT2 receptors (Kirby and McQueen, 1984). In rats, in addition to the biphasic response, 5-HT also causes a delayed slow increase in the sensory discharge, which appears secondary to cardiovascular changes. 5-HT produces spontaneous rhythmic activation of isolated rat glomus cell clusters, and this effect is prevented by the 5-HT2 receptor antagonist, ketansarin (Zhang and Nurse, 2000). Wang et al. (2000) reported 5-HT5a receptors in rat glomus cells as well as in the neurons of the petrosal ganglion. However, the significance of 5-HT5a receptors remains to be studied.

Although 5-HT has marked effects on the sensory discharge, its significance in sensory transmission during hypoxia is less clear. Neither 5-HT-2 nor 5-HT-3 receptor blockers had any significant influence on the sensory response to hypoxia (Kirby and McQueen, 1984) and the release of 5-HT during hypoxia has not been yet demonstrated.

12.4. Neuropeptides

Carotid bodies express a variety of neuropeptides that serve as transmitters or modulators elsewhere in the nervous system.

12.5. Substance P (SP)

SP-like immunoreactivity is expressed in glomus cells as well as nerve fibers of sensory and autonomic origin in the carotid body (Chen et al., 1986; Gauda et al., 1998; Kusakabe et al., 1994; Prabhakar et al., 1989a). Glomus cells express carboxypeptidase-E (CPE), an enzyme necessary for processing of SP (Gonzalez-Guerrero et al., 1993; Kumar, 1996), and the enzyme neutral endopeptidase (NEP), responsible for terminating the actions of SP, is also found in the interstial spaces surrounding the type I cells and the nerve terminals (Kumar, 1997).

Kim et al. (2001a, b) reported that hypoxia releases SP from rabbit carotid bodies in a stimulus dependent manner. Hypoxia-evoked SP release requires extra-cellular Ca^{2+} and activation of N and L-type Ca^{2+} channels. On the other hand, hypercapnia has no effect on the release of SP. Exogenous administration of SP augments the sensory discharge of the carotid body in a number of species (Cragg et al., 1994; Kumar et al., 2000; McQueen 1980; Monti-Bloch and Eyzaguirre, 1985; Prabhakar et al., 1989b) with the exception of goats (Pizarro et al., 1995). SP potentiates the hypoxic sensory response (Prabhakar et al., 1987b), and whereas SP receptor antagonists, when given in nanomolar concentration, prevent the excitatory effects of SP, and abolish the sensory response to hypoxia while leaving the sensory response to hypercapnia unaffected in cats (Prabhakar et al., 1984, 1987a, 1993a) as well as in rats (Cragg et al., 1994).

Autoradiographic analysis has also revealed SP-binding sites in the cat carotid body (see Prabhakar, 1994 for references). Blockade of NK-1 receptors in the cat carotid body prevented hypoxia-evoked sensory excitation (Prabhakar et al., 1993a) suggesting that SP, acting on NK-1

receptors, participates in the sensory transmission in this species. In rats, although SP stimulates the carotid body activity (Cragg et al., 1994), there is no evidence for NK-1 receptor mRNA either in glomus cells or in the petrosal neurons (Gauda et al., 1998). In rats, the actions of SP therefore may be mediated by other neurokinin receptors (for example, NK-2 or NK-3). SP may directly influence mitochondrial metabolism by acting as a protonophore (Prabhakar et al., 1989a). Therefore, in rats, a dual mechanism, one involving receptor activation (via NK-2 or NK-3) and the other via acting on mitochondria perhaps may mediate the sensory excitation by SP. Thus, SP-like peptide(s) may participate in the sensory transmission during hypoxia by mechanisms not strictly adherent to the criteria proposed for conventional synaptic transmission.

12.6. Endothelin-1 (ET-1)

Under normoxic conditions ET-1 expression is either low or undetectable in type I cells. However, chronic SH markedly increases ET-1 expression (He et al., 1996). ET-1 stimulates carotid body activity by acting on ET-A receptors (Chen et al., 2000). While ET-1 has no effect on basal $[Ca^{2+}]_i$ in glomus cells, hypoxia-evoked increase in $[Ca^{2+}]_i$ is augmented. ET-1 also potentiated hypoxia-induced elevation in cAMP in the carotid body. It has been suggested that ET, by promoting the phosphorylation of Ca^{2+} channel proteins, leads to potentiation of the sensory response to hypoxia. These studies suggest that ET is a potent modulator of the sensory response to hypoxia.

12.7. Enkephalins (ENK)

Nearly 98% of glomus cells express ENK-like immunoreactivity (Fidone and Gonzalez, 1986; Wang et al., 1992). Hanson et al. (1986) reported a decrease in met-ENK content following 30 min of hypoxia in rabbit carotid bodies indicating release of ENK by hypoxia. ENK inhibits the carotid body activity, and naloxone and delta-opiate receptor antagonist augment the baseline carotid body activity as well as the sensory response to hypoxia (Pokorski and Lahiri, 1981).

12.8. Other peptides

Type I cells also express atrial natriuretic peptide (ANP; Wang et al., 1991). Wang et al. (1991) showed that ANP inhibits the carotid body activity and its actions are mediated by cGMP. Galanin, neuropeptide Y (NPY) and calcitonin-gene-related peptide (CGRP)-like immunoreactivities were reported in the nerve fibers innervating the glomus tissue (Ichikawa and Helke, 1993; Kirby and McQueen, 1986; see Prabhakar, 1994 for references). However, the significance of these peptides in the sensory transmission has not been established.

12.9. ATP, adenosine and purinergic receptors

ATP is stored along with other transmitters in secretory vesicles and participates as a co-transmitter by acting on purinergic receptors. ATP causes prompt excitation followed by inhibition of the sensory activity (see Spergel and Lahiri, 1993 for earlier references). ATP increases cytosolic calcium in glomus cells (Mokashi et al., 2003). Prasad et al., (2000)

demonstrated P2 × 2−P2 × 3 receptors in glomus cells as well as in the petrosal neurons that make synaptic contact with glomus cells (Prasad et al., 2000). Suramin, a non-selective purinergic blocker given in combination with hexamethonium or mecamylamine (nicotinic receptor antagonist) prevented the sensory response to hypoxia and hypercapnia in co-cultures of glomus cell and petrosal neuronal preparations in vitro (Prasad et al., 2000). Studies by Lahiri and DeLaney (1975a) have shown that O_2 and CO_2 interact synergistically leading to a greater sensory response than either stimulus alone. Prasad et al. (2000) suggested that purinergic receptors contribute to O_2–CO_2 interaction at the rat carotid body. Rong et al. (2003) recently reported markedly attenuated hypoxic sensory response in mutant mice lacking P2 × 2 receptors. Therefore, it is likely that ATP alone or in co-operation with other transmitters might play an important role in the sensory transmission during hypoxia (Buttigieg and Nurse, 2004).

Adenosine, a metabolite of ATP stimulates carotid (McQueen and Ribeiro, 1986; Runold et al., 1990a) as well as the aortic chemoreceptors (Runold et al., 1990b). Type I cells express A2a adenosine receptors (Gauda et al., 1998; Kobayashi et al., 2000); whereas A1 adenosine receptor expression seems confined to neurons of the petrosal ganglion (Gauda et al., 1998). Selective A2a receptor antagonist prevents adenosine evoked inhibition of voltage gated Ca^{2+} currents and modulatory effects of adenosine on hypoxia-evoked elevation of $[Ca^{2+}]_i$ in glomus cells (Kobayashi et al., 2000). The functional significance of A1 receptors, however, is unclear.

12.10. Amino acid transmitters

Glutamate-like immunoreactivity is associated with type I cells and to a lesser extent with type II cells or afferent axon (Torrealba et al., 1996). Many glomus cells express GABA-like immunoreactivity in the mouse carotid body (Oomori et al., 1994). Whether the amino acid transmitters are released in response to hypoxia and, if so participate in the sensory transmission remain to be investigated.

12.11. Unconventional neurotransmitters

12.11.1. Nitric oxide (NO)

Of the three NO synthase (NOS) isoforms, NOS-1 and NOS-3 are constitutively expressed in nerve fibers of sensory and autonomic origin and vascular endothelial cells of the carotid body, respectively (Prabhakar, 1994; Chugh et al., 1994; Grimes et al., 1995; Wang et al., 1993). There is no evidence for the expression of inducible NOS isoform in the carotid body. NOS activity is markedly inhibited in carotid body extracts exposed to hypoxia, suggesting that hypoxia might decrease the NO level in the carotid body (Prabhakar et al., 1993b). However, Itturiaga et al. (2000) reported no alterations in NO levels during hypoxia in an ex vivo cat carotid body preparation. Fung et al. (2001) observed a dramatic increase in NO levels during hypoxia. The increases in NO levels during hypoxia reported by Fung et al. (2001) are difficult to reconcile with the biochemical studies showing inhibition of NOS activity by hypoxia. Therefore, whether hypoxia increases or decreases NO levels in the carotid body remains unclear.

Endogenous NO exerts a tonic inhibitory influence on carotid body activity. Evidence includes: (1) NOS inhibitors augment baseline activity and potentiate the sensory response to hypoxia; and (2) NO donors inhibit the sensory activity (see Prabhakar, 1999 for references). Furthermore,

targeted deletion of the NOS-1 isoform leads to an enhanced peripheral chemoreceptor sensitivity to hypoxia (Kline et al., 1998) and this is further confirmed by direct measurements of carotid body activity (Kline et al., 2000). On the other hand, hypoxic sensitivity of the carotid body seems to be depressed in NOS-3 deficient mice (Kline et al., 2000). It has been suggested that chronic vasoconstriction in NOS-3 deficient mice may render the carotid body insensitive to hypoxia. Reported cellular mechanisms associated with the actions of NO include: (a) elevation of cGMP; (b) nitrosylation of Ca^{2+} channel proteins leading to altered Ca^{2+} homeostasis; and (c) influence of NO on mitochondrial function (Prabhakar, 1999; Summers et al., 1999). In addition, NO has been shown to modulate the actions of other neurotransmitters elsewhere in the nervous system (Prabhakar, 1999) and recent studies suggest that NO inhibits hypoxia-evoked SP release from the carotid bodies (Kim et al., 2001c).

12.11.2. Carbon monoxide (CO)

Heme oxygenase 1 and 2 (HO-1 and HO-2) catalyze the formation of CO and molecular oxygen is required for CO synthesis (Prabhakar, 1999). HO-2 is constitutively expressed in type I cells and inhibition of HO-2 by Zinc protoporphyrin-9 augments the sensory activity of the carotid body (Prabhakar et al., 1995). These observations indicate that type I cells of the carotid bodies are capable of producing CO and it exerts inhibitory influence on the carotid body sensory activity. Consistent with this notion are the findings of Lahiri and Acker (1999), who reported that low doses of CO inhibit chemosensory activity of the isolated rat carotid bodies. In a series of elegant studies, Lahiri and his co-workers tested the involvement of heme proteins in hypoxic sensing at the carotid body using CO as a tool (Lahiri et al., 1993; Wilson et al., 1994; Lahiri et al., 1997; Osanai et al., 1997a). These investigators found that physiological saline equilibrated with high concentrations of CO mimicked the effects of hypoxia on the sensory discharge and provided the evidence for involvement of heme proteins in sensory excitation by hypoxia and CO.

12.12. Interactions amongst the transmitters; push-pull mechanism

Co-localization and co-release: Most of the neurotransmitters are often co-localized in the carotid body. Examples include co-localization of SP/TH, SP/ENK, SP/NKA, SP/galanin, SP/5-HT, galanin/CGRP, TH/NOS-1, TH/DBH, and TH/HO-2 (Wang et al., 1992). There is also evidence for co-release of transmitters during hypoxia. For example, hypoxia evokes simultaneous release of DA and NE (Rigual et al., 1991) and SP and DA (Kim et al., 2001a, b) from rabbit carotid bodies. Pharmacological studies suggest co-release of ACh and ATP during hypoxia (Prasad et al., 2000). It is likely that once a transmitter is released by hypoxia it may modulate the release of others by acting in an autocrine or paracrine fashion. Interaction of the transmitters at the level of the glomus cells may be more critical to the sensory transmission than hitherto appreciated.

It is clear from the above studies that carotid bodies express a large number of inhibitory transmitters along with excitatory ones and co-released during hypoxia. What could be the significance of the inhibitory transmitters? It has been established that carotid bodies are slowly adapting type of sensory receptor in that the increase in sensory discharge evoked by hypoxia is maintained more or less during the entire duration of the stimulus. If an excitatory transmitter alone participates in the sensory transmission, then one would expect only a brief excitation

followed by prompt return to base line discharge, despite maintaining the hypoxic stimulus. In other words, sensory excitation will no longer be maintained during the entire period of hypoxia. On the other hand, co-release of inhibitory messengers aid in producing sustained excitation by preventing the over excitation caused by excitatory transmitter. Thus, excitatory and inhibitory messengers act in concert like a ''push-pull'' mechanism (Prabhakar, 1992). Indeed, many biological processes are regulated by such a ''push-pull'' mechanism involving interactions between excitatory and inhibitory messenger molecules. Therefore, it is conceivable that initiation and maintenance of the carotid body sensory response to hypoxia depends on complex interplay between excitatory and inhibitory neurochemicals as suggested earlier (Prabhakar, 1992).

Acknowledgments

In Section I the authors regret that many contributions to the field could not be cited including their own due to focus and brevity of this review. This work is supported by grants to SL (R-37-HL43413, RO1-HL50180 and US Navy, N00014-01-1-0948 and to TH (R01 GM57654) and American Heart Association.

In Section II work in the author's laboratory is supported by grants from NCI, NIDDK, and NHLBI.

In Section III the work is supported by grants from the National Institutes of Health, Heart, Lung and Blood Institute HL-25830, HL-46462 and HL-66448.

References

Agani, F.H., Pichiule, P., Carlos Chavez, J., LaManna, J.C., 2002. Inhibitors of mitochondrial complex I attenuate the accumulation of hypoxia-inducible factor-1 during hypoxia in Hep3B cells. Comp. Biochem. Physiol. 132, 107–109.

Anichkov, S.V., Belen'kii, M.L., 1963. Pharmacology of the Carotid Body Chemoreceptors. Pergamon Press, Oxford, UK.

Baby, S.M., Roy, A., Mokashi, A.M., Lahiri, S., 2003. Effects of hypoxia and intracellular iron chelation on hypoxia-inducible factor-1alpha and -1beta in the rat carotid body and glomus cells. Histochem. Cell Biol. 120, 343–352.

Baby, S.M., Roy, A., Mokashi, A., Lahiri, S., 2004. Hypoxia inducible factor-1α protein in the carotid body glomus cells of rats requires chronic intermittent hypoxia greater than 3-days. FASEB J. Part I A 725.

Baby, S.M., Roy, A., Lahiri, S., 2005. Role of mitochondria in the regulation of hypoxia-inducible factor-1α in the rat carotid body glomus cells (communicated).

Bairam, A., Neji, H., Kinkead, R., Marchal, F., 2001. Carbachol effect on carotid body dopamine in vitro release in response to hypoxia in adult and pup rabbit. Neurosci. Res. 40, 183–188.

Barbe, C., Al-Hashem, F., Conway, A.F., Dubuis, E., Vandier, C., Kumar, P., 2002. A possible dual site of action for carbon monoxide-mediated chemoexcitation in the rat carotid body. J. Physiol. 543, 933–945.

Belousov, A., Godfraind, J.M., Krnjevic, K., 1995. Internal Ca^{2+} stores involved in anoxic responses of rat hippocampal neurons. J. Physiol. 486, 547–556.

Bernard, P., Andronikou, S., Pokorski, M., Smartresk, N., Mokashi, A., Lahiri, S., 1987. Time-dependent effect of hypoxia on carotid body chemosensory function. J. Appl. Physiol. 63, 685–691.

Berridge, M.J., 1993. Inositol triphosphate and calcium signaling. Nature 361, 315–325.

Bruick, R.K., McKnight, S.L., 2001. A conserved family of prolyl-4-hydroxylases that modify HIF. Science 294, 1337–1340.

Buckler, K.J., 1997. A novel oxygen-sensitive potassium current in rat carotid body type I cells. J. Physiol. 498, 649–662.

Buckler, K.J., Honore, E., 2005. The lipid-activated two-pore domain K + channel TREK-1 is resistant to hypoxia: implication for ischaemic neuroprotection. J. Physiol. 562, 213–222.

Buckler, K.J., Vaughan-Jones, R.D., 1998. Effects of mitochondrial uncouplers on intracellular calcium, pH and membrane potential in rat carotid body type I cells. J. Physiol. 513, 819–833.

Buckler, K.J., Williams, B.A., Honore, E., 2000. An oxygen-, acid- and anaesthetic-sensitive TASK-like background potassium channel in rat arterial chemoreceptor cells. J. Physiol. 525, 135–142.

Bunn, H.F., Poyton, R.O., 1996. Oxygen sensing and molecular adaptation to hypoxia. Physiol. Rev. 76, 839–885.

Buttigieg, J., Nurse, C.A., 2004. Detection of hypoxia-evoked ATP release from chemoreceptor cells of the rat carotid body. Biochem. Biophys. Res. Commun. 322, 82–87.

Caley, A.J., Gruss, M., Franks, N.P., 2005. The effects of hypoxia on the modulation of human TREK-1 potassium channels. J. Physiol. 562, 205–212.

Cardinale, G.J., Rhoads, R.E., Udenfriend, S., 1971. Simultaneous incorporation of ^{18}O into succinate and hyproxyproline catalyzed by collagen praline hydroxylase. Biochem. Biophys. Res. Commun. 43, 537–543.

Carroll, J.L., 2003. Post natal maturation of the carotid chemoreceptor O_2 sensitivity at the cellular level. In: Lahiri, S., Semenza, S., Prabhakar, N.R. (Eds.), Oxygen Sensing: Responses and Adaptation to Hypoxia, vol. 175, pp. 251–272.

Chen, I.L., Yates, R.D., Hansen, J.T., 1986. Substance P-like immunoreactivity in rat and cat carotid bodies: light and electron microscopic studies. Histol. Histopathol. 1, 203–212.

Chen, J., He, L., Dinger, B., Fidone, S., 2000. Cellular mechanisms involved in rabbit carotid body excitation elicited by endothelin peptides. Respir. Physiol. 121, 13–23.

Chleide, E., Ishikawa, K., 1990. Hypoxia-induced decrease of brain acetylcholine release detected by microdialysis. Neuroreport 1, 197–199.

Chugh, D.K., Katayama, M., Mokashi, A., Bebaut, D.E., Ray, D.K., Lahiri, S., 1994. Nitric oxide-related inhibition of carotid chemosensory nerve activity in the cat. Respir. Physiol. 97, 147–156.

Compernolle, V., Brusselmans, K., Franco, D., Moorman, A., Dewerchin, M., Collen, D., Carmeliet, P., 2003. Cardia bifida, defective heart development and abnormal neural crest migration in embryos lacking hypoxia-inducible factor-1α. Cardiovasc. Res. 60, 569–579.

Cragg, P.A., Runold, M., Kou, Y.R., Prabhakar, N.R., 1994. Tachykinin antagonists in carotid body responses to hypoxia and substance P in the rat. Respir. Physiol. 95, 295–310.

Cross, A.R., Henderson, L., Jones, O.T., Delpiano, Hentschel, L., Acker, H., 1990. Involvement of an NAD(P)H oxidase as a pO_2 sensor protein in the rat carotid body. Biochem. J. 272, 743–747.

Dames, S.A., Martinez-Yamout, M., De Guzman, R.N., Dyson, H.J., Wright, P.E., 2002. Structural basis for HIF-1α/CBP recognition in the cellular hypoxic response. Proc. Natl. Acad. Sci. USA 99, 5271–5276.

Dann III, C.E., Bruick, R.K., Deisenhofer, J., 2002. Structure of factor inhibiting hypoxia-inducible factor 1: an asparaginyl hydroxylase involved in the hypoxic response pathway. Proc. Natl. Acad. Sci. USA 99, 15351–15356.

Dinger, B., Hirano, T., Fidone, S.J., 1986. Autoradiographic localization of muscarinic receptors in rabbit carotid body. Brain Res. 367, 328–331.

Dinger, B., He, L., Chen, J., Stensaas, L.J., Fidone, S.J., 2003. Mechanisms of morphological and functional plasticity in the chronically hypoxic carotid body. In: Lahiri, S., Semenza, S., Prabhakar, N.R. (Eds.), Oxygen Sensing: Responses and Adaptation to Hypoxia, vol. 175, pp. 439–459.

Doyle, T.P., Donnelly, D.F., 1994. Effect of Na^+ and K^+ channel blockade on baseline and anoxia-induced catecholamine release from rat carotid body. J. Appl. Physiol. 77, 2606–2611.

Duchen, M.R., Biscoe, T.J., 1992. Relative mitochondrial membrane potential and $[Ca^{2+}]_i$ in type I cells isolated from the rabbit carotid body. J. Physiol. 450, 33–61.

Elkins, J.M., Hewitson, K.S., McNeill, L.A., Seibel, J.F., Schlemminger, I., Pugh, C.W., Ratcliffe, P.J., Schofield, C.J., 2003. Structure of factor inhibiting hypoxia-inducible factor (HIF) reveals mechanism of oxidative modification of HIF-1α. J. Biol. Chem. 278, 1802–1806.

Epstein, A.C., Gleadle, J.M., McNeill, L.A., Hewitson, K.S., O'Rourke, J., Mole, D.R., Mukherji, M., Metzen, E., Wilson, M.I., Dhanda, A., Tian, Y.M., Masson, N., Hamilton, D.L., Jaakkola, P., Barstead, R., Hodgkin, J.,

Maxwell, P.H., Pugh, C.W., Schofield, C.J., Ratcliffe, P.J., 2001. *C. elegans* EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. Cell 107, 43–54.

Fidone, S., Gonzalez, C., 1986. Initiation and control of chemoreceptor activity in the carotid body. In: Cherniack, N.S., Widdicombe, J.G. (Eds.), Handbook of Physiology. Section 3: The Respiratory System, Bethesda, vol. II. American Physiological Society, pp. 267–312.

Fidone, S., Gonzalez, C., Yoshizaki, K., 1982. Effects of low oxygen on the release of dopamine from the rabbit carotid body in vitro. J. Physiol. (Lond.) 333, 93–110.

Fitzgerald, R.S., 2000. Oxygen and carotid body chemotransduction: the cholinergic hypothesis—a brief history and new evaluation. Respir. Physiol. 120, 89–104.

Fitzgerald, R.S., Lahiri, S., 1996. The history of chemoreception. In: West, J.B. (Ed.), Respiratory Physiology: People and Ideas. Oxford University Press, New York, pp. 289–318.

Fitzgerald, R.S., Parks, D.C., 1971. Effect of hypoxia on carotid chemoreceptor response to carbon dioxide in cats. Respir. Physiol. 12, 218–229.

Fitzgerald, R.S., Shirahata, M., Wang, H.Y., 1999. Acetylcholine release from cat carotid bodies. Brain Res. 841, 53–61.

Franks, N.P., Honore, E., 2004. The TREK K_{2P} channels and their role in general anaesthesia and neuroprotection. Trends Pharmacol. Sci. 25, 601–608.

Freedman, S.J., Sun, Z.Y., Poy, F., Kung, A.L., Livingston, D.M., Wagner, G., Eck, M.J., 2002. Structural basis for recruitment of CBP/p300 by hypoxia-inducible factor-1α. Proc. Natl. Acad. Sci. USA 99, 5367–5372.

Fung, M.L., Ye, J.S., Fung, P.C., 2001. Acute hypoxia elevates nitric oxide generation in rat carotid body in vitro. Pflugers Arch. 442, 903–909.

Ganfornina, M.D., Lopez-Barneo, J., 1991. Single K^+ channels in membrane patches of arterial chemoreceptor cells are modulated by O_2 tension. Proc. Natl. Acad. Sci. 88, 2927–2930.

Ganfornina, M.D., Lopez-Barneo, J., 1992a. Gating of O2-sensitive K^+ channels of arterial chemoreceptor cells and kinetic modifications induced by low PO_2. J. Gen. Physiol. 100, 427–455.

Ganfornina, M.D., Lopez-Barneo, J., 1992b. Potassium channel types in arterial chemoreceptor cells and their selective modulation by oxygen. J. Gen. Physiol. 100, 401–426.

Gauda, E.B., Bamford, O.S., Northington, F.J., 1998. Lack of induction of substance P gene expression by hypoxia and absence of neurokinin 1-receptor mRNA in the rat carotid body. J. Auton. Nerv. Syst. 74, 100–108.

Gibson, G.E., Peterson, C., 1982. Decreases in the release of acetylcholine in vitro with low oxygen. Biochem. Pharmacol. 31, 111–115.

Goldberg, M.A., Dunning, S.P., Bunn, H.F., 1988. Regulation of the erythropoietin gene: evidence that the oxygen sensor is a heme protein. Science, 1412–1414.

Gomez-Nino, A., Dinger, B., Gonzalez, C., Fidone, S., 1990. Differential stimulus coupling to dopamine and norepinephrine stores in rabbit carotid body type I cells. Brain Res. 525, 160–164.

Gonzalez, C., Almaraz, L., Obeso, A., Rigual, R., 1994. Carotid body chemoreceptors: from natural stimuli to sensory discharges. Physiol. Rev. 74, 829–898.

Gonzalez-Guerrero, P.R., Rigual, R., Gonzalez, C., 1993. Opioid peptides in the rabbit carotid body: identification and evidence for co-utilization and interactions with dopamine. J. Neurochem. 60, 1762–1768.

Gozal, E., Shah, Z.A., Pequignot, J.M., Pequignot, J., Sachleben, L.R., Czyzyk-Krzeska, M.F., Li, R.C., Guo, S.Z., Gozal, D., 2005. Tyrosine Hydroxylase Expression and Activity in the Rat Brain: Differential Regulation Following Long-term Intermittent or Sustained Hypoxia. J. Appl. Physiol. (in press).

Grimes, P.A., Mokashi, A., Stone, R.A., Lahiri, S., 1995. Nitric oxide synthase in autonomic innervation of the cat carotid body. J. Auton. Nerv. Syst. 54, 80–86.

Hagen, T., Taylor, C.T., Lam, F., Moncada, S., 2003. Redistribution of intracellualr oxygen in hypoxia by nitric oxide: effect on HIF1α. Science 302, 1975–1978.

Hanson, G., Jones, L., Fidone, S., 1986. Physiological chemoreceptor stimulation decreases enkephalin and substance P in the carotid body. Peptides 7, 767–769.

He, L., Chen, J., Dinger, B., Stensaas, L., Fidone, S., 1996. Endothelin modulates chemoreceptor cell function in mammalian carotid body. Adv. Exp. Med. Biol. 410, 305–311.

He, L., Dinger, B., Fidone, S., 2005. Effect of chronic hypoxia on cholinergic chemotransmission in rat carotid body. J. Appl. Physiol. 98, 614–619.

Hewitson, K.S., McNeill, L.A., Riordan, M.V., Tian, Y.M., Bullock, A.N., Welford, R.W., Elkins, J.M., Oldham, N.J., Bhattacharya, S., Gleadle, J.M., Ratcliffe, P.J., Pugh, C.W., Schofield, C.J., 2002. Hypoxia-inducible factor asparaginyl hydroxylase is identical to factor inhibiting HIF-1 (FIH-1) and is related to the cupin structural family. J. Biol. Chem. 277, 26351–26355.

Hirano, T., Dinger, B., Yoshizaki, K., Gonzalez, C., Fidone, S., 1992. Nicotinic versus muscarinic binding sites in cat and rabbit carotid body, Biol. Signals 1, 143–149.

Hirsila, M., Koivunen, P., Gunzler, V., Kivirikko, K.I., Myllyharju, J., 2003. Characterization of the human prolyl 4-hydroxylases that modify the hypoxia-inducible factor. J. Biol. Chem. 278, 30772–30780.

Hon, W.C., Wilson, M.I., Harlos, K., Claridge, T.D., Schofield, C.J., Pugh, C.W., Maxwell, P.H., Ratcliffe, P.J., Stuart, D.I., Jones, E.Y., 2002. Structural basis for the recognition of hydroxyproline in HIF-1α by pVHL. Nature 417, 975–978.

Horrigan, F.T., Cui, J., Aldrich, R.W., 1999. Allosteric voltage gating of potassium channels I. mslo ionic currents in the absence of Ca^{2+}. J. Gen. Physiol. 114, 277–304.

Hoshi, T., Lahiri, S., 2004. Cell biology. Oxygen sensing: it's a gas!. Science 306, 2050–2051.

Huang, L.E., Gu, J., Schau, M., Bunn, H.F., 1998. Regulation of hypoxia-inducible factor 1α is mediated by an O_2-dependent degradation domain via the ubiquitin-proteasome pathway. Proc. Natl. Acad. Sci. USA 95, 7987–7992.

Huang, L.E., Willmore, W.G., Gu, J., Goldberg, M.A., Bunn, H.F., 1999. Inhibition of hypoxia-inducible factor 1 activation by carbon monoxide and nitric oxide. J. Biol. Chem. 274, 9038–9044.

Hui, A.S., Striet, J.B., Gudelsky, G., Soukhova, G.K., Gozal, E., Beitner-Johnson, D., Guo, S-Z., Sachleben, L.R., Haycock, J.W., Gozal, D., Czyzyk-Krzeska, M.F., 2003. Regulation of catecholamine by sustained and intermittent hypoxia in neuroendocrine and sympathetic neurons. Hypertension 42, 1–7.

Ichikawa, H., Helke, C.J., 1993. Distribution, origin and plasticity of galanin immunoreactivity in the rat carotid body. Neuroscience 52, 757–767.

Itturiaga, R., Villanova, S., Mosqueivar, M., 2000. Dual effects of nitric oxide on cat carotid body chemoreception. J. Appl. Physiol. 89, 1005–1012.

Ivan, M., Kondo, K., Yang, H., Kim, W., Valiando, J., Ohh, M., Salic, A., Asara, J.M., Lane, W.S., Kaelin Jr., W.G., 2001. HIF-1α targeted for VHL-mediated destruction by proline hydroxylation, implications for O_2 sensing. Science 292, 464–468.

Ivan, M., Haberberger, T., Gervasi, D.C., Michelson, K.S., Gunzler, V., Kondo, K., Yang, H., Sorokina, I., Conaway, R.C., Conaway, J.W., Kaelin Jr., W.G., 2002. Biochemical purification and pharmacological inhibition of a mammalian prolyl hydroxylase acting on hypoxia-inducible factor. Proc. Natl. Acad. Sci. USA 99, 13459–13464.

Iyer, N.V., Kotch, L.E., Agani, F., Leung, S.W., Laughner, E., Wenger, R.H., Gassmann, M., Gearhart, J.D., Lawler, A.M., Yu, A.Y., Semenza, G.L., 1998. Cellular and developmental control of O_2 homeostasis by hypoxia-inducible factor 1α. Genes Dev. 12, 149–162.

Jaakkola, P., Mole, D.R., Tian, Y.M., Wilson, M.I., Gielbert, J., Gaskell, S.J., Kriegsheim, A., Hebestreit, H.F., Mukherji, M., Schofield, C.J., Maxwell, P.H., Pugh, C.W., Ratcliffe, P.J., 2001. Targeting of HIF-α to the von Hippel-Lindau ubiquitylation complex by O_2-regulated prolyl hydroxylation. Science 292, 468–472.

Jewell, U.R., Kvietikova, I., Scheid, A., Bauer, C., Wenger, R.H., Gassmann, M., 2001. Induction of HIF-1α in response to hypoxia is instantaneous. FASEB J. 15, 1312–1324.

Jiang, B.H., Semenza, G.L., Bauer, C., Marti, H.H., 1996. Hypoxia-inducible factor 1 levels vary exponentially over a physiologically relevant range of O_2 tension. Am. J. Physiol. 271, C1172–C1180.

Kallio, P.J., Okamoto, K., O'Brien, S., Carrero, P., Makino, Y., Tanaka, H., Poellinger, L., 1998. Signal transduction in hypoxic cells: inducible nuclear translocation and recruitment of the CBP/p300 coactivator by the hypoxia-inducible factor-1α. EMBO J. 17, 6573–6586.

Kallio, P.J., Wilson, W.J., O'Brien, S., Makino, Y., Poellinger, L., 1999. Regulation of the hypoxia-inducible transcription factor 1α by the ubiquitin-proteasome pathway. J. Biol. Chem. 274, 6519–6525.

Kamura, T., Sato, S., Iwai, K., Czyzyk-Krzeska, M., Conaway, R.C., Conaway, J.W., 2000. Activation of HIF1α ubiquitination by a reconstituted von Hippel-Lindau (VHL) tumor suppressor complex. Proc. Natl. Acad. Sci. USA 97, 10430–10435.

Kaplin, A.I., Snyder, S.H., Linden, D.J., 1996. Reduced nicotinamide adenine dinucleotide selective stimulation of inositol 1,4,5-triphospahte receptors mediates hypoxic mobilization of calcium. J. Neurosci. 16, 2001–2002.

Kemp, P.J., Peers, C., Miller, P., Lewis, A., 2003. Oxygen sensing by human recombinant tandem-P domain potassium channels. Adv. Exp. Med. Biol. 536, 201–208.

Kim, D., Oh, E.K., Summers, B.A., Prabhakar, N.R., Kumar, G.K., 2001a. Release of substance P by low oxygen in the rabbit carotid body: evidence for the involvement of calcium channels. Brain Res. 892, 359–369.

Kim, D., Summers, B.A., Prabhakar, N.R., Kumar, G.K., 2001b. Neurotransmitter release from the rabbit carotid body: differential effects of hypoxia on substance P and acetylcholine release. Adv. Exp. Med. Biol. 499, 39–43.

Kim, D., Summers, B.A., Prabhakar, N.R., Kumar, G.K., 2001c. Nitric oxide modulates substance P release in the carotid body. FASEB J. 15, A150.

Kim, D.K., Prabhakar, N.R., Kumar, G.K., 2004. Ach release from the carotid body by hypoxia: evidence for the involvement of autoinhibitory receptors. J. Appl. Physiol. 96, 376–383.

Kirby, G.C., McQueen, D.S., 1984. Effects of the antagonist MDL 72222 and ketanserin on responses of cat carotid body chemoreceptors to 5-hydroxytryptamine. Br. J. Pharmacol. 83, 259–269.

Kirby, G.C., McQueen, D.S., 1986. Characterization of opioid receptors in the cat carotid body involved in chemosensory depression in vivo. Br. J. Pharmacol. 88, 889–898.

Kline, D.D., Yang, T., Huang, P.L., Prabhakar, N.R., 1998. Altered respiratory responses to hypoxia in mutant mice deficient in neuronal nitric oxide synthase. J. Physiol. 511, 273–287.

Kline, D.D., Yang, T., Premkumar, D.R., Thomas, A.J., Prabhakar, N.R., 2000. Blunted respiratory responses to hypoxia in mutant mice deficient in nitric oxide synthase-3. J. Appl. Physiol. 88, 1496–1508.

Kline, D.D., Peng, Y.J., Manalo, D.J., Semenza, G.L., Prabhakar, N.R., 2002. Defective carotid body function and impaired ventilatory responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1 alpha. Proc. Natl. Acad. Sci. 99, 821–826.

Kobayashi, S., Conforti, L., Millhorn, D.E., 2000. Gene expression and function of adenosine A(2A) receptor in the rat carotid body. Am. J. Physiol. Lung Cell Mol. Physiol. 279, L273–L282.

Koivunen, P., Hirsila, M., Gunzler, V., Kivirikko, K.I., Myllyharju, J., 2004. Catalytic properties of the asparaginyl hydroxylase (FIH) in the oxygen sensing pathway are distinct from those of its prolyl-4-hydroxylases. J. Biol. Chem. 279, 9899–9904.

Kou, Y.R., Ernsberger, P., Cragg, P.A., Cherniack, N.S., Prabhakar, N.R., 1991. Role of α_2-adrenergic receptors in the carotid body response to isocapnic hypoxia. Respir. Physiol. 83, 353–364.

Kumar, G.K., 1996. Neuropeptide processing enzymes of the carotid body. Biochemical and immunological characterization of carboxypeptidase activity. Adv. Exp. Med. Biol. 410, 319–323.

Kumar, G.K., 1997. Peptidases of the peripheral chemoreceptors: biochemical, immunological, in vitro hydrolytic studies and electron microscopic analysis of neutral endopeptidase-like activity of the carotid body. Brain Res. 748, 39–50.

Kumar, G.K., Kou, Y.R., Overholt, J.L., Prabhakar, N.R., 2000. Involvement of substance P in neutral endopeptidase modulation of carotid body sensory responses to hypoxia. J. Appl. Physiol. 88, 195–202.

Kumar, G.K., Overholt, J.L., Prabhakar, N.R., 2003. Multiple roles of neurotransmitters in the carotid body: Involvement in sensory transmission and adaptation to hypoxia. In: Lahiri, S., Semenza, G.L., Prabhakar, N.R. (Eds.), Oxygen Sensing: Responses and adaptations to hypoxia. Lung Biology in Health Disease, vol. 175, pp. 421–438.

Kusakabe, T., Kawakami, T., Tanabe, Y., Fujii, S., Takenaka, T., 1994. Distribution of substance P-containing and catecholaminergic nerve fibers in the rabbit carotid body: an immunohistochemical study in combination with catecholamine fluorescent histochemistry. Arch. Histol. Cytol. 57, 193–199.

Lahiri, S., 1994. Chromophores in O_2 chemoreception: the carotid body model. NIPS 9, 161–165.

Lahiri, S., 2000. Historical perspectives of cellular oxygen sensingand responses to hypoxia. J. Appl. Physiol. 88, 1467–1473.

Lahiri, S., 2004. Oxygen sensing in health and disease. J. Appl. Physiol. 96, 1170–1172.

Lahiri, S., Acker, H., 1999. Redox-dependent binding of CO to heme protein controls PO2-sensitive chemoreceptor discharge of the rat carotid body. Respir. Physiol. 115, 169–177.

Lahiri, S., DeLaney, R.G., 1975a. Stimulus interaction in the responses of carotid body chemoreceptor single afferent fibers. Respir. Physiol. 24, 249–266.

Lahiri, S., DeLaney, R.G., 1975b. Relationship between carotid chemoreceptor activity and ventilation in the cat. Respir. Physiol. 24, 267–286.

Lahiri, S., Milledge, J.S., 1998. Pulmonary adaptation and clinical disorders related to high altitude. In: Alfred, P.F., Jack A.E., Jay A.F., Michael A.G., Larry R.K., Robert M.S. (Eds.), Fishman's Pulmonary Diseases and Disorder. 3rd ed., vol. 1, McGraw-Hill, US, pp. 965–977.

Lahiri, S., Nishino, T., Mokashi, A., Mulligan, E., 1980. Interaction of dopiamine and haloperidol with O_2 and CO_2 chemoreception in carotid body. J. Appl. Physiol. 49, 45–51.

Lahiri, S., Maret, K., Sherpa, K.G., 1983. Dependence of high altitude sleep apnea on ventilatory sensitivity to hypoxia. Respir. Physiol. 52, 281–301.

Lahiri, S., Iturriaga, R., Mokashi, A., Ray, D.K., Chugh, D., 1993. CO reveals dual mechanisms of O_2 chemoreception in the cat carotid body. Respir. Physiol. 94, 227–240.

Lahiri, S., Buerk, D.G., Chugh, D., Osanai, S., Mokashi, A., 1995. Reciprocal photolabile O_2 consumption and chemoreceptor excitation by carbon monoxide in the cat carotid body: evidence for cytochrome a_3 as the primary O_2 sensor. Brain Res. 684, 194–200.

Lahiri, S., Buerk, D.G., Osanai, S., Mokashi, A., Chugh, D.K., 1997. Effect of CO on VO2 of carotid body and chemoreception with and without Ca^{2+}. J. Auton. Nerv. Syst. 66, 1–6.

Lahiri, S., Roy, A., Rozanov, C., Mokashi, A., 1998. K^+-current modulated by PO_2 in type I cells in rat carotid body is not a chemosensor. Brain Res. 794 (1), 162–165 (May 25).

Lahiri, S., Ehleben, W., Acker, H., 1999. Chemoreceptor discharges and cytochrome redox changes of the rat carotid body: role of heme ligands. Proc. Natl. Acad. of Sci. 96, 9427–9432.

Lahiri, S., DiGiulio, C., Roy, A., 2002. Lessons from chronic intermittent and sustained hypoxia at high altitudes. Respir. Physiol. Neurobiol. 130, 223–233.

Lahiri, S., Roy, A., Li, J., Mokashi, A., Baby, S.M., 2003a. Ca^{2+} responses to hypoxia are mediated by IP_3-R on Ca^{2+} store depletion. Adv. Exp. Med. Biol. 536, 25–32.

Lahiri, S., Roy, A., Mokashi, A., Daudu, P.A., Li, J., Baby, S.M., Buerk, D.G., 2003b. Further evidence that oxygen sensing in the carotid body involves iron and heme. In: Lahiri, S., Semenza, S., Prabhakar, N.R. (Eds.), Oxygen Sensing: Responses and Adaptation to Hypoxia, vol. 175, pp. 289–297.

Lando, D., Peet, D.J., Gorman, J.J., Whelan, D.A., Whitelaw, M.L., Bruick, R.K., 2002a. FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. Genes Dev. 16, 1466–1471.

Lando, D., Peet, D.J., Whelan, D.A., Gorman, J.J., Whitelaw, M.L., 2002b. Asparagine hydroxylation of the HIF transactivation domain a hypoxic switch. Science 295, 858–861.

Lehninger, A.L., 1975. Biochemistry (2nd edition): the Molecular Basis of Cell Structure and Function, p. 501.

Lesage, F., Lazdunski, M., 2000. Molecular and functional properties of two-pore-domain potassium channels. Am. J. Physiol. Renal Physiol. 279, F793–F801.

Lewis, A., Peers, C., Ashford, M.L., Kemp, P.J., 2002. Hypoxia inhibits human recombinant large conductance, Ca^{2+}-activated K^+ (maxi-K) channels by a mechanism which is membrane delimited and Ca^{2+} sensitive. J. Physiol. 540, 771–780.

Li, J., Roy, A., Mokashi, A., Lahiri, S., 2002. CO-induced K^+ currents in rat glomus cells are insensitive to light unlike carotid body neural discharge and VO_2. Respir. Physiol. Neurobiol. 131, 285–290.

Liu, H., Moczydlowski, E., Haddad, G.G., 1999. O_2 deprivation inhibits Ca^{2+}-activated K^+ channels via cytosolic factors in mice neocortical neurons. J. Clin. Invest. 104, 577–588.

Lloyd, B.B., Cunningham, D.J.C., Goode, R.C., 1968. Depression of hypoxic hyperventilation in man by sudden inspiration of carbon monoxide. In: Torrence, R.W. (Ed.), Arterial Chemorecptors. Blackwell, Oxford, UK, pp. 145–148.

Lopez-Barneo, J., Lopez-Lopez, J.R., Urena, J., Gonzalez, C., 1988. Chemotransduction in the carotid body: K+ current modulated by PO2 in type I chemoreceptor cells. Science 241, 580–582.

Lopez-Barneo, J., Montoro, R., Ortega-Saenz, P., Urena, J., 1998. Oxygen-regulated ion channels: functional roles and mechanisms. In: Lopez-Barneo, J., Weir, E.K. (Eds.), Oxygen Regulation of Ion Channels and Gene Expression. Futura Publishing Company, Armonk, NY, pp. 127–144.

Lopez-Barneo, J., Pardal, J., Montoro, R.J., Smani, T., Garcia-Hirschfeld, J., Urena, J., 1999. K^+ and Ca^{2+} channel activity and cytosolic [Ca^{2+}] oxygen-sensing tissues. Respir. Physiol. 115, 215–227.

Lopez-Barneo, J., Pardal, R., Ortega-Saenz, P., 2001. Cellular mechanisms of oxygen sensing. Ann. Rev. Physiol. 63, 259–287.

Lopez-Barneo, J., del Toro, R., Konstantin, L., Chiara, M.D., Ortega-Saenz, P., 2004. Regulation of oxygen sensing by ion channels. J. Appl. Physiol. 96, 1187–1195.

Lopez-Lopez, J.R., Gonzalez, C., 1992. Time course of K^+ current inhibition by low oxygen in chemoreceptor cells of adult rabbit carotid body: effects of carbon monoxide. FEBS Lett. 299, 251–254.

Lopez-Lopez, J.R., Gonzalez, C., Perez-Garcia, M.T., 1997. Properties of ionic currents from isolated adult rat carotid body chemoreceptor cells: effect of hypoxia. J. Physiol. 499, 429–441.

Magleby, K.L., 2003. Gating mechanism of BK (Slo1) channels: so near, yet so far. J. Gen. Physiol. 121, 81–96.

Mahon, P.C., Hirota, K., Semenza, G.L., 2001. FIH-1: a novel protein that interacts with HIF-1α and VHL to mediate repression of HIF-1 transcriptional activity. Genes Dev. 15, 2675–2686.

Maxwell, P.H., Wiesener, M.S., Chang, G.W., Clifford, S.C., Vaux, E.C., Cockman, M.E., Wykoff, C.C., Pugh, C.W., Maher, E.R., Ratcliffe, P.J., 1999. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature 399, 271–275.

McGregor, K.H., Gill, J., Lahiri, S., 1984. A morphometric study of the carotid body in chronically hypoxic rats. J. Appl. Physiol. 57, 1430–1438.

McNeill, L.A., Hewitson, K.A., Claridge, T.D., Seibel, J.F., Horsfall, L.E., Schofield, C.J., 2002. Hypoxia-inducible factor asparaginyl hydroxylase (FIH-1) catalyzes hydroxylation at the beta-carbon of asparagine-803. Biochem. J. 367, 571–575.

McQueen, D.S., 1980. Effects of substance P on carotid chemoreceptor activity in the cat. J. Physiol. (Lond.). 302, 31–47.

McQueen, D.S., Ribeiro, J.A., 1986. Pharmacological characterization of the receptor involved in chemoexcitation induced by adenosine. Br. J. Pharmacol. 88, 615–620.

Meldolesi, J., Pozzan, T., 1998. The endoplasmic reticulum Ca^{2+} store: a view from the lumen. Trends Biochem. Sci. 23, 10–14.

Meredith, A.L., Thorneloe, K.S., Werner, M.E., Nelson, M.T., Aldrich, R.W., 2004. Overactive bladder and incontinence in the absence of the BK large conductance Ca^{2+}-activated K^+ channel. J. Biol Chem. 279, 36746–36752.

Min, J.H., Yang, H., Ivan, M., Gertler, F., Kaelin Jr., W.G., Pavletich, N.P., 2002. Structure of a HIF-1α-pVHL complex: hydroxyproline recognition in signaling. Science 296, 1886–1889.

Mokashi, A., Rozanov, C., Roy, A., Lahiri, S., 2003. ATP induces $[Ca^{2+}]_I$ rise in rat carotid body cultured glomus cells. Respir. Physiol. Neurobiol. 138, 1–18.

Monti-Bloch, L., Eyzaguirre, C., 1985. Effects of methionine and substance P on the chemosensory discharge of the cat carotid body. Brain Res. 338, 297–307.

Mulligan, E., Lahiri, S., 1982. Separation odf carotid body chemosensory responses to O_2 and CO_2 by oligomycin and antimycin A. Am. J. Physiol. 242, C200–C206.

Mulligan, E., Lahiri, S., Storey, B.T., 1981. Carotid body O_2 chemoreception and mitochondrial oxidative phosphorylation. J. Appl. Physiol. 51, 438–446.

Nielson, A.M., Bisgard, G.E., Vidruk, E.H., 1988. Carotid chemoreceptor activity during acute and sustained hypoxia in goats. J. Appl. Physiol. 65, 1796–1802.

Nishi, K., 1975. The action of 5-hydroxytryptamine on chemoreceptor discharges of the cat's carotid body. Br. J. Pharmacol. 55, 27–40.

Nurse, C., Vollmer, C., 1993. Effects of hypoxia on cultured chemoreceptors of the rat carotid body: DNA synthesis and mitotic activity in glomus cells. Adv. Exp. Med. Biol. 337, 79–84.

Nurse, C.A., Zhang, M., 1999. Acetylcholine contributes to hypoxic chemotransmission in co-cultures of rat type I cells and petrosal neurons. Respir. Physiol. 115, 189–199.

Obeso, A., Rocher, A., Fidone, S., Gonzalez, C., 1992. The role of dihydropyridine-sensitive Ca^{2+} channels in stimulus-evoked catecholamine release from chemoreceptor cells of the carotid body. Neuroscience 47, 463–472.

Ohh, M., Park, C.W., Ivan, M., Hoffman, M.A., Kim, T.Y., Huang, L.E., Pavletich, N., Chau, V., Kalein, W.G., 2000. Ubiquitination of hypoxia-inducible factor requires direct binding to the β-domain of the von Hippel-Lindau protein. Nat. Cell. Biol. 2, 423–427.

Oomori, Y., Nakaya, K., Tanaka, H., Iuchi, H., Ishikawa, K., Satoh, Y., Ono, K., 1994. Immunocytochemical and histochemical evidence for the presence of noradrenaline, serotonin and gamma-aminobutyric acid in chief cells of the mouse carotid body. Cell Tissue Res. 278, 249–254.

Ortega-Saenz, P., Garcia-Fernandez, M., Pardal, R., Alvarez, E., Lopez-Barneo, J., 2003. Studies on glomus cell sensitivity to hypoxia in carotid body slices. Adv. Exp. Med. Biol. 536, 65–73.

Osanai, S., Chugh, D.K., Mokashi, A., Lahiri, S., 1996. Stimulus interaction between CO and CO_2 in the cat carotid body chemoreception. Brain Res. 711, 56–63.

Osanai, S., Rozanov, C., Mokashi, A., Buerk, D.G., Lahiri, S., 1997a. CO interact with intracellular [H+] with and without CO_2-HCO_3- in the cat carotid chemosensory discharge. Brain Res. 764, 221–224.

Osanai, S., Buerk, D.G., Mokashi, A., Chugh, D.K., Lahiri, S., 1997b. Cat carotid body chemosensory discharge (in vitro) is insensitive to charybdotoxin. Brain Res. 747, 324–327.

Overholt, J.L., Prabhakar, N.R., 1999. Norepinephrine inhibits a toxin resistant Ca^{2+} current in carotid body glomus cells: Evidence for a direct G protein mechanism. J. Neurophysiol. 81, 225–233.

Pardal, H., Ludewig, U., Garcia-Hirschfeld, J., Lopez-Barneo, J., 2000. Secretory responses of intact glomus cells in thin slices of rat carotid body to hypoxia and tetraethylammonium. Proc. Natl. Acad. Sci. 97, 2361–2366.

Parekh, A.B., 2003. Store-operated Ca^{2+} entry: dynamic interplay between endoplasmic reticulum, mitochondria and plasma membrane. J. Physiol. 547, 333–348.

Patel, A.J., Honore, E., 2001. Molecular physiology of oxygen-sensitive potassium channels. Eur. Respir. J. 18, 221–227.

Peers, C., 1990. Hypoxic suppression of K+ currents in type I carotid body cells: selective effect on the Ca^{2+}-activated K^+ current. Neurosci. Lett. 119, 253–256.

Peers, C., 2004. Interaction of chemostimuli at the single cell level: studies in a model system. Exp. Physiol. 89, 60–65.

Pequignot, J.M., Hellstrom, S., Johansson, C., 1984. Intact and sympathectomied carotid bodies of long-term hypoxic rats: a morphometric ultrastructural study. J. Neurochem. 13, 481–493.

Perez-Garcia, M.T., Lopez-Lppez, J.R., Riesco, A.M., Hoppe, U.C., Marban, E., Gonzalez, C., Johns, D.C., 2000. Viral gene transfer of dominant-negative Kv4 construct suppresses an O2-sensitive K+ current in chemoreceptor cells. J. Neurosci. 20, 5689–5695.

Pizarro, J., Ryan, M.L., Hedrick, M.S., Xue, D.H., Keith, I.M., Bisgard, G.E., 1995. Intracarotid substance P infusion inhibits ventilation in the goat. Respir. Physiol. 101, 11–22.

Pokorski, M., Lahiri, S., 1981. Effects of naloxone on carotid body chemoreception and ventilation in the cat. J. Appl. Physiol. 51, 1533–1538.

Prabhakar, N.R., 1992. Significance of excitatory and inhibitory neurochemicals in hypoxic chemotransmission of the carotid body. In: Honda, Y., Miyamoto, Y., Konno, K., Widdicombe, J.G. (Eds.), Control of Breathing and its Modeling Perspect ive. Plenum Press, New York, pp. 205–212.

Prabhakar, N.R., 1994. Neurotransmitters in the carotid body. Adv. Exp. Med. Biol. 360, 57–69.

Prabhakar, N.R., 1999. NO and CO as second messengers in oxygen sensing in the carotid body. Respir. Physiol. 115, 161–168.

Prabhakar, N.R., 2000. Oxygen sensing by the carotid body chemoreceptors. J. Appl. Physiol. 88, 2287–2295.

Prabhakar, N.R., 2001. Oxygen sensing during intermittent hypoxia: cellular and molecular mechanisms. J. Appl. Physiol. 90, 1986–1994.

Prabhakar, N.R., Runold, M., Yamamoto, Y., Lagercrantz, H., von Euler, C., 1984. Effect of substance P antagonist on the hypoxia-induced carotid chemoreceptor activity. Acta Physiol. Scand. 121, 301–303.

Prabhakar, N.R., Mitra, J., Cherniack, N.S., 1987a. Role of substance P in hypercapnic excitation of carotid chemoreceptors. J. Appl. Physiol. 63, 2418–2425.

Prabhakar, N.R., Runold, M., Yamamoto, Y., Lagercrantz, H., Cherniack, N.S., von Euler, C., 1987b. Role of the vagal afferents in substance P-induced respiratory responses in anaesthetized rabbits. Acta Physiol. Scand. 131, 63–71.

Prabhakar, N.R., Landis, S.C., Kumar, G.K., Mullikin-Kilpatrick, D., Cherniack, N.S., Leeman, S., 1989a. Substance P and neurokinin A in the cat carotid body: localization, exogenous effects and changes in content in response to arterial PO_2. Brain Res. 481, 205–214.

Prabhakar, N.R., Runold, M., Kumar, G.K., Cherniack, N.S., Scarpa, A., 1989b. Substance P and mitochondrial oxygen consumption: Evidence for a direct intracellular role for the peptide. Peptides 10, 1003–1006.

Prabhakar, N.R., Cao, H., Lowe, J.A.D., Snider, R.M., 1993a. Selective inhibition of the carotid body sensory response to hypoxia by the substance P receptor antagonist CP-96,345. Proc. Natl. Acad. Sci. USA 90, 10041–10045.

Prabhakar, N.R., Kumar, G.K., Chang, C.H., Agani, F.A., Haxhiu, M.A., 1993b. Nitric oxide in the sensory function of the carotid body. Brain Res. 625, 16–22.

Prabhakar, N.R., Dinerman, J.L., Agani, F.H., Snyder, S.H., 1995. Carbon monoxide: a role in carotid body chemoreception. Proc. Natl. Acad. Sci. USA 92, 1994–1997.

Prasad, M., Fearon, I.M., Zhang, M., Laing, M., Vollmer, C., Nurse, C.A., 2000. Expression of $P2\times2$ and $P2\times3$ receptor subunits in rat carotid body afferent neurones: role in chemosensory signaling. J. Physiol. 537 (3), 667–677.

Riesco-Fagundo, A.M., Perez-Garcia, M.T., Gonzalez, C., Lopez-Lopez, J.R., 2001. O_2 modulates large-conductance Ca^{2+}-dependent K^+ channels of rat chemoreceptor cells by a membrane-restricted and CO-sensitive mechanism. Circ. Res. 89, 430–436.

Rigual, R., Lopez-Lopez, J.R., Gonzalez, C., 1991. Release of dopamine and chemoreceptor discharge induced by low pH and high PCO_2 stimulation of the cat carotid body. J. Physiol. 433, 519–531.

Rizzuto, R., Bastianutto, C., Brini, M., Murgia, M., Pozzan, T., 1994. Mitochondrial Ca^{2+} homeostasis in intact cells. J. Cell Biol. 126, 1183–1194.

Rizzuto, R., Penton, P., Carrington, W., Fay, F.S., Fogarty, K.E., Lifshitz, L.M., Tuft, R.A., Pozzan, T., 1998. Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca^{2+} response. Science 280, 1763–1766.

Rong, W., Gourine, A.V., Cockayne, D.A., Xiang, Z., Ford, A.P., Spyer, K.M., Burnstock, G., 2003. Pivotal role of nucleotide $P2\times2$ receptor subunit of the ATP-gated ion channel mediating ventilatory response to hypoxia. J. Neurosci. 23, 11315–11321.

Roy, A., Li, J., Al-Mehdi, A.B., Mokashi, A., Lahiri, S., 2002. Effect of acute hypoxia on glomus cell E_m and ψ_m as measured by fluorescence imaging. J. Appl. Physiol. 93, 1987–1998.

Roy, A., Li, J., Baby, S.M., Mokashi, A., Buerk, D.G., Lahiri, S., 2004a. Effects of iron-chelators on ion- channels and HIF-1α in the carotid body. Respir. Physiol. Neurobiol. 141, 115–123.

Roy, A., Volgin, D.V., Baby, S.M., Mokashi, A., Kubin, L., Lahiri, S., 2004b. Activation of HIF-1α mRNA by hypoxia and chelator in isolated rat carotid body. Neurosci. Lett. 363, 229–232.

Runold, M., Cherniack, N.S., Prabhakar, N.R., 1990a. Effect of adenosine on chemosensory activity of the cat carotid body. Respir. Physiol. 80, 299–306.

Runold, M., Cherniack, N.S., Prabhakar, N.R., 1990b. Effect of adenosine on isolated and superfused cat carotid body activity. Neurosci. Lett. 113, 111–114.

Rutter, G.A., Rizzuto, R., 2000. Regulation of mitochondrial metabolism by ER Ca^{2+} release: an intimate connection. TIBS 25, 215–220.

Ryan, H.E., Lo, J., Johnson, R.S., 1998. HIF-1α is required for solid tumor formation and embryonic vascularization. EMBO J. 17, 3005–3015.

Salceda, S., Caro, J., 1997. Hypoxia-inducible factor 1α (HIF-1α) protein is rapidly degraded by the ubiquitin-proteasome system under normoxic conditions. Its stabilization by hypoxia depends on redox-induced changes. J. Biol. Chem. 272, 22642–22647.

Sausbier, M., Hu, H., Arntz, C., Feil, S., Kamm, S., Adelsberger, H., Sausbier, U., Sailer, C.A., Feil, R., Hofmann, F., Korth, M., Shipston, M.J., Knaus, H.G., Wolfer, D.P., Pedroarena, C.M., Storm, J.F., Ruth., P., 2004. Cerebellar ataxia and Purkinje cell dysfunction caused by Ca^{2+}-activated K^+ channel deficiency. Proc. Natl. Acad. Sci. USA 101, 9474–9478.

Schroedl, C., McClintock, D.S., Budinger, G.R., Chandel, N.S., 2002. Hypoxic but not anoxic stabilization of HIF-1α requires mitochondrial reactive oxygen species. Am. J. Physiol. 283, L922–L931.

Schumacker, P.T., 2002. Hypoxia, anoxia and O_2 sensing: the search continues. Am J. Physiol. 283, L918–L921.
Semenza, G.L., 2000. HIF-1: mediator of physiological and pathophysiological responses to hypoxia. J. Appl. Physiol. 88, 1474–1480.
Semenza, G.L., 2003. Targeting HIF-1 for cancer therapy. Nat. Rev. Cancer 3, 721–732.
Shirahata, M., Ishizawa, Y., Rudisill, M., Schofield, B., Fitzgerald, R.S., 1998. Presence of nicotinic acetylcholine receptors in cat carotid body afferent system. Brain Res. 814, 213–217.
Snyder, S.H., Jaffery, S.R., Zakary, R., 1998. Nitric oxide and carbon monoxide: parallel roles as neural messengers. Brain Res. 26, 167–175.
Spergel, D., Lahiri, S., 1993. Differential modulation by extracellular ATP of carotid chemosensory responses. J. Appl. Physiol. 74, 3052–3056.
Spyer, K.M., Dale, N., Gourine, A.V., 2004. ATP is a key mediator of central and peripheral chemosensory transduction. Exp. Physiol. 89, 53–59.
Streller, T., Huckstrof, C., Pfeiffer, C., Acker, H., 2002. Unusual cytochrome a_{592} with low Po_2 affinity correlates as putative oxygen sensor with rat carotid body chemoreceptor discharge. FASEB J. 16, 1277–1279.
Summers, B.A., Overholt, J.L., Prabhakar, N.R., 1999. Nitric oxide inhibits L-type Ca^{2+} current in glomus cells of the rabbit carotid body via a cGMP-independent mechanism. J. Neurophysiol. 81, 1449–1457.
Sutter, C.H., Laughner, E., Semenza, G.L., 2000. Hypoxia-inducible factor 1α protein expression is controlled by oxygen-regulated ubiquitination that is disrupted by deletions and missense mutations. Proc. Natl. Acad. Sci. USA 97, 4748–4753.
Tang, X.D., Xu, R., Reynolds, M.F., Garcia, M.L., Heinemann, S.H., Hosi, T., 2003. Haem can bind to and inhibit mammalian calcium-dependent Slo1 BK channels. Nature 425, 531–535.
Tanimoto, K., Makino, Y., Pereira, T., Poellinger, L., 2000. Mechanism of regulation of the hypoxia-inducible factor-1α by the von Hippel-Lindau tumor suppressor protein. EMBO J. 19, 4298–4309.
Taylor, S.C., Robets, M.L., Peers, C., 1999. Acid-evoked quamtal catecholamine secretion from rat pheochromocytoma cells and its interaction with hypoxia secretion. J. Physiol. 519, 765–774.
Torrealba, F., Bustos, G., Montero, V.M., 1996. Glutamate in the glomus cells of the cat carotid body: immunocytochemistry and in vitro release. Neurochem. Int. 28, 625–631.
Varas, R., Alcayaga, J., Iturriaga, R., 2003. Ach and ATP mediate excitatory transmission in cat carotid body identified chemoreceptor units in vitro. Brain Res. 988, 154–163.
Vergara, C.R., Latorre, N.V., Marrion, J.P., Adelman, 1998. Calcium-activated potassium channels. Curr. Opin. Neurobiol. 8, 321–329.
Vicario, I., Obeso, A., Rocher, A., Lopez-Barneo, J., Gonzalez, C., 2000. Intracellular Ca^{2+} stores in chemoreceptor cells of the rabbit carotid body: significance for chemoreception. Am. J. Physiol. 279, C51–C61.
Vizek, M., Pickett, C.K., Weil, J.V., 1987. Increased carotid bnody hypoxic sensitivity during acclimatization to hypobaric hypoxia. J. Appl. Physiol. 63, 2403–2410.
Wang, G.L., Semenza, G.L., 1993. Desferrioxamine induces erythropoietin gene expression and hypoxia-inducible factor 1 DNA-binding activity: implications for models of hypoxia signal transduction. Blood 82, 3610–3615.
Wang, G.L., Semenza, G.L., 1995. Purification and characterization of hypoxia-inducible factor 1. J. Biol. Chem. 270, 1230–1237.
Wang, R., Wu, L., 1997. The chemical modification of KCa channels by carbon monoxide in vascular smooth muscle cells. J. Biol. Chem. 272, 8222–8226.
Wang, Z.Z., He, L., Stensaas, L.J., Dinger, B.G., Fidone, S.J., 1991. Localization and in vitro actions of atrial natriuretic peptide in the cat carotid body. J. Appl. Physiol. 70, 942–946.
Wang, Z.Z., Stensaas, L.J., Dinger, B., Fidone, S.J., 1992. The co-existence of biogenic amines and neuropeptides in the type I cells of the cat carotid body. Neuroscience 47, 473–480.
Wang, Z.Z., Bredt, D.S., Fidone, S.J., Stensaas, L.J., 1993. Neurons synthesizing nitric oxide innervate the mammalian carotid body. J. Comp. Neurol. 336, 419–432.
Wang, G.L., Jiang, B.H., Rue, E.A., Semenza, G.L., 1995. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O_2 tension. Proc. Natl. Acad. Sci. USA 92, 5510–5514.
Wang, Z.Y., Keith, I.M., Beckman, M.J., Brownfield, M.S., Vidruk, E.H., Bisgard, G.E., 2000. 5-HT_{5a} receptors in the carotid body chemoreception pathway of rat. Neurosci. Lett. 278, 9–12.

Warburg, O., Negelein, E., 1928. Über die photochemische Dissoziation bei intermittierender Bleichuterung und das absolute Absorptionssperktrum des Atmungsferments. Biochem. J. 202, 202–228.

Williams, B.A., Buckler, K.J., 2004. Biophysical properties and metabolic regulation of a TASK-like potassium channel in rat carotid body type 1 cells. Am. J. Physiol. 286, L221–L230.

Williams, S.E., Wootton, P., Mason, H.S., Bould, J., Iles, D.E., Riccardi, D., Peers, C., Kemp, P.J., 2004. Hemoxygenase-2 is an oxygen sensor for a calcium-sensitive potassium channel. Science 306, 2093–2097.

Wilson, D.F., Mokashi, A., Chugh, D., Vinogradov, S., Osanai, S., Lahiri, S., 1994. The primary oxygen sensor of the cat carotid body is cytochrome *a*3 of the mitochondrial respiratory chain. FEBS Lett. 351, 370–374.

Wilson, D.F., Roy, A., Lahiri, S., 2005. Short and long-term responses of the carotid body to high altitude 6, 97–111.

Wyatt, C.N., Buckler, K.J., 2004. The effect of mitochondrial inhibitors on membrane currents in isolated neonatal rat carotid body type I cells. J. Physiol. 566, 175–191.

Wyatt, C.N., Peers, C., 1995. Ca^{2+}-activated K^+ channels in isolated type I cells of the neonatal rat carotid body. J. Physiol. 483, 559–565.

Xi, Q., Tcheranova, D., Parfenova, H., Horowitz, B., Leffler, C.W., Jaggar, J.H., 2004. Carbon monoxide activates K_{Ca} channels in newborn arteriole smooth muscle cells by increasing apparent Ca^{2+} sensitivity of *a*-subunits. Am. J. Physiol. Heart Circ. Physiol. 286, H610–H618.

Yu, A.Y., Frid, M.G., Shimoda, L.A., Wiener, C.M., Stenmark, K., Semenza, G.L., 1998. Temporal, spatial, and oxygen-regulated expression of hypoxia-inducible factor-1 in the lung. Am. J. Physiol. 275, L818–L826.

Yu, F., White, S.B., Zhao, Q., Lee, F.S., 2001. HIF-1α binding to VHL is regulated by stimulus-sensitive proline hydroxylation. Proc. Natl. Acad. Sci. USA 98, 9630–9635.

Yuan, G., Adhikary, G., McCormick, A.A., Holcroft, J.J., Kumar, G.K., Prabhakar, N.R., 2004. Role of oxidative stress in intermittent hypoxia-induced immediate early gene activation in rat PC12 cells. J. Physiol. 557, 773–783.

Yuan, G., Nanduri, J., Bhasker, C.R., Semenza, G.L., Prabhakar, N.R., 2005. Ca^{2+}/calmodulin kinase-dependent activation of hypoxia inducible factor 1 transcriptional activity in cells subjected to intermittent hypoxia. J. Biol. Chem. 280, 4321–4328.

Zhang, M., Nurse, C.A., 2000. Does endogenous 5-HT mediate spontaneous rhythmic activity in chemoreceptor clusters of rat carotid body? Brain Res. 872, 199–203.

Zhang, M., Nurse, C.A., 2004. CO_2/pH chemosensory signaling in co-cultures of rat carotid body receptors and petrosal neurons: role of ATP and ACh. J. Neurophysiol. 92, 3433–3445.

Zhang, M., Zhong, H., Vollmer, C., Nurse, C.A., 2000. Co-release of ATP and ACh mediates hypoxic signaling at rat carotid body chemoreceptors. J. Physiol. 525 (1), 143–158.