

# Gaseous messengers in oxygen sensing

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**Abstract** The carotid body is a sensory organ that detects acute changes in arterial blood oxygen ( $O_2$ ) levels and reflexly mediates systemic cardiac, vascular, and respiratory responses to hypoxia. This article provides a brief update of the roles of gas messengers as well as redox homeostasis by hypoxia-inducible factors (HIFs) in hypoxic sensing by the carotid body. Carbon monoxide (CO) and nitric oxide (NO), generated by heme oxygenase-2 (HO-2) and neuronal nitric oxide synthase (nNOS), respectively, inhibit carotid body activity. Molecular  $O_2$  is a required substrate for the enzymatic activities of HO-2 and nNOS. Stimulation of carotid body activity by hypoxia may reflect reduced formation of CO and NO. Glomus cells, the site of  $O_2$  sensing in the carotid body, express cystathionine  $\gamma$ -lyase (CSE), an  $H_2S$  generating enzyme. *Cth*<sup>-/-</sup> mice, which lack CSE, exhibit severely impaired hypoxia-induced  $H_2S$  generation, sensory excitation, and stimulation of breathing in response to low  $O_2$ . Hypoxia-evoked  $H_2S$  generation in the carotid body requires the interaction of CSE with HO-2, which generates CO. Carotid bodies from *Hif1a*<sup>+/-</sup> mice with partial HIF-1 $\alpha$  deficiency do not respond to hypoxia, whereas carotid bodies from mice with partial HIF-2 $\alpha$  deficiency are hyper-responsive to hypoxia. The opposing roles of HIF-1 $\alpha$  and HIF-2 $\alpha$  in the carotid body have provided novel insight into molecular mechanisms

of redox homeostasis and its role in hypoxia sensing. Heightened carotid body activity has been implicated in the pathogenesis of autonomic morbidities associated with sleep-disordered breathing, congestive heart failure, and essential hypertension. The enzymes that generate gas messengers and redox regulation by HIFs represent potential therapeutic targets for normalizing carotid body function and downstream autonomic output in these disease states.

**Keywords** Blood pressure · Sympathetic nerve activity · Hypoxic adaptations · Recurrent apnea · Periodic breathing

## Introduction

Oxygen ( $O_2$ ) is essential for survival of mammalian cells and hypoxia (reduced  $O_2$  availability) profoundly impacts cells, organs, and physiological systems. The carotid bodies are bilateral sensory organs located at the bifurcation of the common carotid artery that monitor arterial blood  $O_2$  concentrations and relay sensory information to brainstem neurons, which regulate vital functions including breathing, heart rate, and blood pressure [1, 2]. The following features distinguish  $O_2$  sensing by the carotid body from other tissues. First, carotid bodies are exquisitely sensitive to hypoxia and respond to even modest decreases in arterial  $PO_2$  (e.g., from ~100 to 80 mmHg). Second, the sensory response to low  $PO_2$  is fast and occurs within seconds after the onset of hypoxia. The remarkable sensitivity and the speed with which they respond to hypoxia makes the carotid body  $O_2$  sensing unique compared to other tissues [3]. Reflexes arising from carotid bodies are of critical importance in conferring physiological adaptations to high altitude and play central roles in evoking autonomic morbidities under sleep-disordered breathing and congestive heart failure [1].

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Since their discovery as sensory organs, much attention has been focused on understanding how carotid bodies sense and respond to hypoxia [1, 2, 4]. Based on similarities between the stimulus–response of the carotid body and the binding characteristics of O<sub>2</sub> to hemoglobin, it was proposed that oxy→deoxy conformational transition of heme prosthetic group(s) is important for hypoxic sensing by the carotid body [1, 2]. Like O<sub>2</sub>, nitric oxide (NO) and carbon monoxide (CO) are gases that bind to heme [5, 6] and mediate biological effects through the activation of heme-containing proteins (e.g., soluble guanylate cyclase) [7]. Hydrogen sulfide (H<sub>2</sub>S) is emerging as another physiologically important gas messenger. Chemically, H<sub>2</sub>S is a reducing agent and, like hypoxia, generates a reduced cellular milieu. The discovery of the transcriptional activator hypoxia-inducible factor 1 (HIF-1), and subsequent identification of additional HIF family members, has led to important insights into the molecular underpinnings of O<sub>2</sub> and redox homeostasis [8]. Recent studies have revealed that HIFs regulate carotid body responses to hypoxia by determining the levels of reactive oxygen species (ROS), which are metabolites of O<sub>2</sub>. This article reviews the effects of NO, CO, H<sub>2</sub>S, and ROS on hypoxic sensing by the carotid body.

### Gas messengers and carotid body O<sub>2</sub> sensing

#### Nitric oxide (NO)

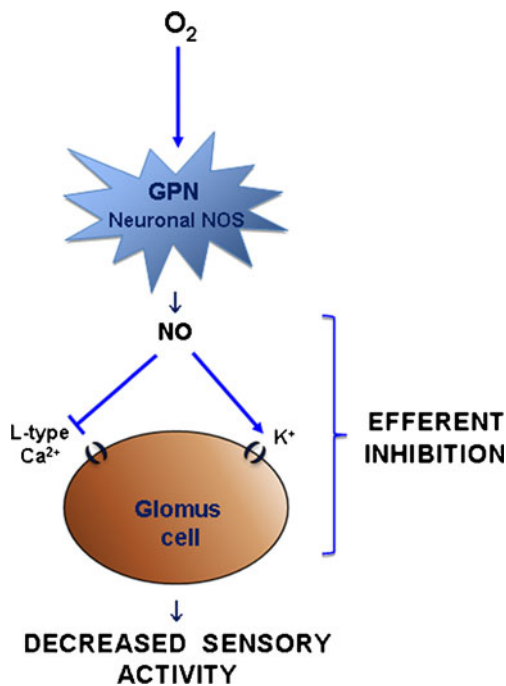
Nitric oxide synthase (NOS) catalyzes the conversion of arginine+O<sub>2</sub> to citrulline+NO [4]. Neuronal (nNOS/ NOS1), endothelial (eNOS/NOS3), and inducible (iNOS/NOS2) isoforms of NOS have been identified. Neuronal and endothelial NOS are constitutively expressed and require Ca<sup>2+</sup> for their activity, whereas iNOS expression is induced in response to a variety of stimuli, including hypoxia, and does not require Ca<sup>2+</sup> for its activity. NOS isoforms contain a heme prosthetic group that binds to O<sub>2</sub>. nNOS has a high K<sub>m</sub> for O<sub>2</sub> relative to the other NOS isoforms, suggesting that even modest reductions in O<sub>2</sub> concentration lead to significant loss of enzyme activity [9]. Indeed, there is a linear relationship between O<sub>2</sub> concentration and nNOS activity over the entire physiological range [10].

The effect of hypoxia on NOS activity in the carotid body was determined by measuring conversion of <sup>3</sup>H-arginine to <sup>3</sup>H-citrulline [11]. Substantial NOS activity was observed under normoxic conditions (P<sub>O<sub>2</sub></sub> ~140 mmHg) and chelation of extracellular Ca<sup>2+</sup> abolished the enzymatic activity, suggesting that it arises from constitutively expressed NOS isoforms [11]. NOS inhibitors (L-NNA or L-NAME) stimulate, and NO donors inhibit, carotid body activity [11–14]. These findings suggest that O<sub>2</sub>-dependent generation of NO exerts an inhibitory influence on the carotid body activity.

Glomus cells are the primary site of O<sub>2</sub> sensing in the carotid body and they are innervated by sensory fibers originating from the petrosal ganglion [2]. Glomus cells do not express nNOS, which is instead produced by nerve fibers as well as neurons of the petrosal ganglion. Besides its sensory innervation, the carotid body receives an efferent nerve supply from the autonomic nervous system. Electrical stimulation of the cut end of the carotid sinus nerve causes depression of carotid body sensory nerve activity [15–17]. This finding implied that the carotid body response to hypoxia is regulated by an efferent inhibitory pathway. Indeed, the carotid body response to hypoxia is augmented in the absence of “efferent inhibition” [18]. Until recently, mechanisms mediating the “efferent inhibition” remained uncertain. The finding that NOS activity increases in response to electrical stimulation of the carotid sinus nerve (as evidenced by elevated <sup>3</sup>H-citrulline production) and that carotid sinus nerve fibers express nNOS led to the suggestion that NO mediates efferent inhibition [14]. Studies by Campanucci and Nurse provided evidence that NO is an important mediator of efferent inhibition of the carotid body [19]. These investigators found that nNOS-expressing nerve fibers originate from neurons located in paraganglia along the glossopharyngeal and carotid sinus nerves (glossopharyngeal neurons or GPNs). It was proposed that NO released from efferent nerves regulates the excitability of glomus cells, resulting in decreased afferent nerve activity [19]. Glomus cells express a variety of K<sup>+</sup> channels and hypoxia inhibits some of these K<sup>+</sup> channels [4, 20]. Ca<sup>2+</sup> influx via voltage gated Ca<sup>2+</sup> channels is an obligatory step in producing sensory excitation by hypoxia [1]. NO donors enhance K<sup>+</sup> conductance [21] and inhibit L-type voltage-activated Ca<sup>2+</sup> channel activity in glomus cells [22] suggesting that NO depresses the glomus cell excitability. Kline et al. [23] reported that nNOS deficient (*Nos1*<sup>−/−</sup>) mice exhibit an enhanced ventilatory response to hypoxia (Table 1), which is a hallmark reflex initiated by the carotid body, and irregular breathing during hypoxia, an effect that was attributed to enhanced carotid body sensitivity to low O<sub>2</sub>. These findings suggest an absence of efferent inhibition of the carotid body in nNOS deficient mice. Figure 1 illustrates the role of nNOS-derived NO in efferent inhibition of carotid body activity.

**Table 1** Carotid body phenotype of mouse mutants

Genotype	Biochemical/molecular defect	Response to hypoxia
<i>Nos1</i> <sup>−/−</sup>	Complete nNOS deficiency	Enhanced
<i>Nos3</i> <sup>−/−</sup>	Complete eNOS deficiency	Blunted
<i>Hmox2</i> <sup>−/−</sup>	Complete HO-2 deficiency	Not determined
<i>Cth</i> <sup>−/−</sup>	Complete CSE deficiency	Impaired
<i>Hif1a</i> <sup>+/-</sup>	Partial HIF-1α deficiency	Absent
<i>Epas1</i> <sup>+/-</sup>	Partial HIF-2α deficiency	Enhanced



**Fig. 1** NO-mediated efferent inhibitory pathway in the carotid body. During normoxia, O<sub>2</sub>-dependent NO generation by neuronal nitric oxide synthase (nNOS) in glossopharyngeal neurons (GPN) mediates efferent inhibition by activating K<sup>+</sup> channels and inhibiting L-type Ca<sup>2+</sup> channels in glomus cells, which results in decreased sensory activity. Under hypoxic conditions, NO production is reduced, which contributes to sensory excitation

Endothelial NOS (eNOS) is expressed in the blood vessels that perfuse the carotid body [24]. eNOS knockout mice exhibit attenuated peripheral chemoreceptor sensitivity, reduced hypoxic ventilatory response, and hypertension [25]. The decreased sensitivity to hypoxia was associated with absence of eNOS expression in carotid body blood vessels. How might NO from eNOS affect the carotid body activity? Carotid body is a highly vascular organ and receives highest blood flow per gram of tissue [1]. NO derived from eNOS is a potent vasodilator and regulate the blood flow to the carotid body. The attenuated hypoxic sensitivity in eNOS knockout mice was attributed to persistent vasoconstriction resulting in reduced blood flow and the ensuing chronic tissue hypoxia of the carotid body. Consistent with this possibility, carotid bodies from eNOS knockout mice displayed hyperplasia of glomus cells [25], similar to that seen with chronic hypoxia [1].

The biological actions of NO are mediated by multiple pathways, which include activation of heme-containing guanylate cyclase and subsequent elevation of cyclic guanosine monophosphate (cGMP) levels [7]. NOS inhibitors reduce, and NO donors increase, cGMP levels in the carotid body [14]. Besides the cGMP pathway, effects of NO also involve the formation of *S*-nitrosothiols, whose actions depend on the redox environment of the cell [26, 27]. Summers et al. [22]

reported that NO-induced inhibition of L-type Ca<sup>2+</sup> currents in rabbit glomus cells is prevented by *N*-ethylmaleimide, which blocks nitrosylation of proteins by NO, implicating *S*-nitrosothiol signaling. These studies, albeit limited, indicate that both cGMP-dependent and cGMP-independent signaling pathways mediate the effects of NO in glomus cells.

#### Carbon monoxide (CO)

CO was thought to be a physiologically inert gas like N<sub>2</sub> and H<sub>2</sub>. Over the decades, respiratory physiologists employed CO as a tool to understand the mechanisms of O<sub>2</sub> transport and the control of breathing by hypoxia. For instance, at the beginning of this century, Haldane and Krogh used CO to test whether O<sub>2</sub> passively diffuses or is actively secreted from the lungs [28]. In the late 1960s, Lloyd, Cunningham, and co-workers [29] reported that brief inhalation of CO gas for several breaths eliminated hypoxia-evoked hyperventilation in human subjects. Because of the high affinity of CO for hemoglobin, they proposed that the deoxy conformation of a heme prosthetic group triggered hypoxic signaling by the carotid body [29].

Sjostrand [30] was one of the first to suggest that CO is formed during the breakdown of heme. It is now well established that CO is generated in mammalian cells during heme degradation by the enzyme heme oxygenase (HO), with NADPH and cytochrome P-450 reductase as co-factors [31]. Enzymatic generation of CO requires molecular oxygen. The affinity of the heme–HO complex for O<sub>2</sub> is 30–90-fold greater than myoglobin [6]. Substrates for HO include the  $\alpha$  and  $\beta$  chains of hemoglobin, denatured myoglobin, met-hemoglobin, and proteolytic products of cytochrome *c*; in contrast, oxy-hemoglobin and intact myoglobin or cytochrome *b* are not substrates for HO [32].

Inducible (HO-1) and constitutively expressed (HO-2) isoforms of HO have been identified [31], and the latter isoform is predominantly expressed in neuronal cells [33]. Carotid body glomus (type I) cells express HO-2 and its expression is not evident in either nerve fibers or type II supporting cells [34]. The *K<sub>m</sub>* of HO-1 and HO-2 for O<sub>2</sub> are in the range of 30–80  $\mu$ M [6].

Zinc-protoporphyrin-9 (ZnPP9), a potent HO inhibitor, stimulated carotid body activity in a dose-dependent manner, whereas CuPP9, which has negligible effects on HO activity, had no effect on carotid body activity [34]. Exogenous administration of CO reversed ZnPP9-induced sensory excitation of the carotid body. Furthermore, administration of an HO inhibitor augmented the hypoxic ventilatory response, and bilateral sectioning of carotid sinus nerves or exogenous CO abolished this effect [33]. These observations provided evidence that CO generated by HO-2 is an important gas messenger contributing to O<sub>2</sub> sensing by the carotid body.

Once generated, CO might act back on the same glomus cell (autocrine action) and/or nearby glomus cells (paracrine

action) and/or afferent nerve endings. Since elevation of cytosolic  $\text{Ca}^{2+}$  in glomus cells is an obligatory step in evoking sensory excitation in response to hypoxia [2], the effects of HO inhibitors on  $\text{Ca}^{2+}$  responses of glomus cells were tested. ZnPP9 elevated  $[\text{Ca}^{2+}]_i$  in glomus cells and this response was eliminated by exogenous administration of CO [35]. ZnPP9-evoked elevation of  $[\text{Ca}^{2+}]_i$  was absent after removing extracellular  $\text{Ca}^{2+}$ , suggesting that this response requires activation of  $\text{Ca}^{2+}$  channels. Indeed, ZnPP9 augmented  $\text{Ca}^{2+}$  currents [35].

A recent study reported that hypoxia-evoked glomus cell secretion of catecholamines was not altered in *Hmox2*<sup>-/-</sup> mice, which lack HO-2 expression [36]. Previous studies reported that glomus cell secretory response often does not coincide with sensory excitation of the carotid body by hypoxia [37]. Therefore, further studies are needed to determine whether the carotid body sensory response to hypoxia is altered in *Hmox2*<sup>-/-</sup> mice. Notwithstanding this limitation, pharmacological studies in intact rats suggest that endogenously generated CO inhibits carotid body sensory activity. Because HO-2 requires  $\text{O}_2$  for its activity, stimulation of carotid body activity by hypoxia may reflect, in part, reduced formation of CO [38]. Recent studies have provided interesting insights into the mechanism(s) by which CO exerts its inhibitory influence on carotid body activity (see below).

## Hydrogen sulfide ( $\text{H}_2\text{S}$ )

Cystathionine  $\gamma$ -lyase (CSE) and cystathionine  $\beta$ -synthase (CBS) are two major enzymes that catalyze the formation of  $\text{H}_2\text{S}$ . While CBS is more abundant in the central nervous system, CSE is the predominant enzyme in peripheral tissues [39]. CSE immunoreactivity was seen in glomus cells of rat and mouse carotid bodies as demonstrated by colocalization with tyrosine hydroxylase, an established marker of glomus cells [40]. CBS expression was also reported in murine and cat carotid bodies [41, 42].

Peng et al. [40] examined the contribution of CSE to the carotid body response to hypoxia using genetic and pharmacological approaches. In *Cth*<sup>-/-</sup> mice, which lack CSE, basal  $\text{H}_2\text{S}$  levels in the carotid bodies were reduced by one half compared to wild-type mice. The residual  $\text{H}_2\text{S}$  must arise from sources other than CSE, presumably CBS. *Cth*<sup>-/-</sup> mice exhibited a severely impaired hypoxic sensory response (Table 1). A similar absence of the hypoxic sensory response was also seen in rats treated with DL-propargylglycine (PAG), an inhibitor of CSE [43, 44]. In contrast, the carotid body response to  $\text{CO}_2$  was unaffected in *Cth*<sup>-/-</sup> mice and was even augmented in rats treated with PAG, suggesting that impaired CSE function selectively affects the hypoxic sensory response. The ventilatory response to hypoxia was also severely impaired in *Cth*<sup>-/-</sup> mice and in PAG-treated rats. These findings demonstrate that CSE contributes to hypoxic sensing by catalyzing  $\text{H}_2\text{S}$  formation.

Li et al. [41] reported that amino oxyacetic acid (AOAA) and hydroxylamine, which are inhibitors of CBS, impaired the mouse carotid body response to hypoxia and ventilatory stimulation by hypoxia. These results suggest that CBS-catalyzed  $\text{H}_2\text{S}$  production may also play a role in sensory excitation by hypoxia.

Exogenous application of the  $\text{H}_2\text{S}$  donor NaHS stimulates carotid body sensory activity in mice and rats in a concentration-dependent manner [40, 41]. NaHS-evoked sensory excitation, like hypoxia, was rapid in onset, occurred within seconds after its application, and returned to baseline after termination of the stimulus [40, 41]. NaHS also stimulated carotid body sensory activity in *Cth*<sup>-/-</sup> mice, suggesting that  $\text{H}_2\text{S}$  is a downstream signaling molecule. It has been proposed that  $\text{H}_2\text{S}$  also mediates  $\text{O}_2$  sensing by trout gill chemoreceptors, indicating that it is a conserved system across vertebrate phyla [45].

$\text{K}_{\text{ATP}}$  channels are the major targets of  $\text{H}_2\text{S}$  in blood vessels [46].  $\text{K}_{\text{ATP}}$  channels are expressed in glomus cells [47]. However, glibenclamide, a potent inhibitor of  $\text{K}_{\text{ATP}}$  channels, was ineffective in preventing carotid body stimulation by NaHS or hypoxia [40]. The excitatory effects of  $\text{H}_2\text{S}$  on carotid body activity seem to require  $\text{Ca}^{2+}$  [40, 41] and may involve enhanced excitability of glomus cells resulting from inhibition of  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  currents [41, 48].

Acetylcholine (ACh) and adenosine triphosphate (ATP) are putative excitatory transmitters implicated in the sensory transmission of the hypoxic stimulus at the carotid body [49]. Li et al. [41] reported that pyridoxal phosphate-6-azophenyl-2',4'-disulfonic acid, which is an inhibitor of purinergic receptors, and hexamethonium, a blocker of nicotinic cholinergic receptors, prevented NaHS-evoked sensory excitation of the mouse carotid body. Based on these observations, Li et al. [41] suggested that  $\text{H}_2\text{S}$ -induced sensory excitation is coupled to the release of ATP/ACh from glomus cells. However, Fitzgerald et al. [42] reported that  $\text{Na}_2\text{S}$ , which is another  $\text{H}_2\text{S}$  donor, inhibited ACh release and had variable effects on ATP release from cat carotid bodies. Pinacidil, an opener of  $\text{K}_{\text{ATP}}$  channels, mimicked the effects of  $\text{Na}_2\text{S}$  on ACh and ATP release [42]. These findings are intriguing because  $\text{H}_2\text{S}$  donors stimulate carotid body sensory activity, yet they seem to inhibit the release of ACh and ATP, two neurotransmitters that are implicated in sensory excitation by hypoxia.

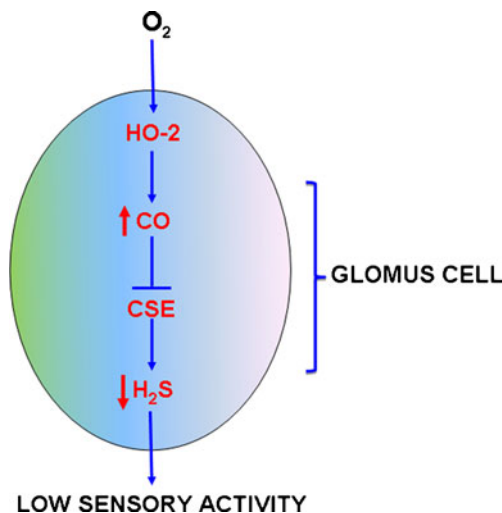
Hypoxia increases  $\text{H}_2\text{S}$  generation: evidence for interaction of CSE with HO-2

Hypoxia increased  $\text{H}_2\text{S}$  levels in both mouse and rat carotid bodies in a stimulus-dependent manner and this response was absent in *Cth*<sup>-/-</sup> mice as well as in rats treated with PAG [40]. These findings suggest that CSE is a major source of  $\text{H}_2\text{S}$  generation in response to hypoxia. How might hypoxia increase  $\text{H}_2\text{S}$  generation? HO-2 catalyzes the formation of CO,

which inhibits carotid body activity [34]. Since HO-2 generation of CO is O<sub>2</sub> dependent, it is likely that the low levels of H<sub>2</sub>S under normoxia are due to the inhibitory influence of CO on CSE. This hypothesis is supported by the finding that an HO-2 inhibitor markedly elevated basal H<sub>2</sub>S levels in the carotid body under normoxia and increased baseline sensory activity in wild-type mice. Remarkably, these effects were absent in *Cth*<sup>-/-</sup> mice [40]. Furthermore, a CO donor inhibited hypoxia-evoked H<sub>2</sub>S generation in the carotid body [40]. These findings suggest that CO physiologically inhibits the CSE-dependent generation of H<sub>2</sub>S and that hypoxia reduces HO-2 activity to reverse the inhibition and augment H<sub>2</sub>S formation. It was proposed that interacting proteins working in concert as a “chemosome” mediate hypoxic sensing by the carotid body [3]. It is likely that interactions between HO-2 and CSE constitute an important component of the “chemosome”. The potential interaction between HO-2/CO and CSE/H<sub>2</sub>S and their contribution to the carotid body response to hypoxia are illustrated in Fig. 2. A recent study by Morikawa et al [50] reported that interaction between HO2/CO and CBS/H<sub>2</sub>S is critical for hypoxia evoked cerebral vasodilatation. However, unlike CBS, CSE is not a heme containing enzyme. Therefore, further studies are needed to delineate the precise molecular mechanisms by which CO inhibits H<sub>2</sub>S generation from CSE in the carotid body.

Role of hypoxia-inducible factors in O<sub>2</sub> sensing by the carotid body

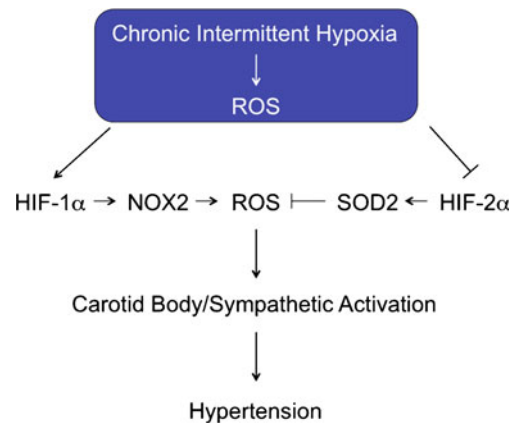
Hypoxia-inducible factors (HIFs) play essential roles as master regulators of O<sub>2</sub> and redox homeostasis [8]. HIF-1



**Fig. 2** Potential interaction between heme oxygenase-2 (HO-2) and cystathionine γ-lyase (CSE) in glomus cells of the carotid body. O<sub>2</sub>-dependent CO generation from HO-2 inhibits CSE activity resulting in low levels of H<sub>2</sub>S and sensory activity. During hypoxia CO generation from HO-2 is reduced, resulting in removal of CO inhibition on CSE, leading to elevated H<sub>2</sub>S levels, which contribute to sensory excitation

and HIF-2 are heterodimeric proteins, which consist of a constitutively expressed HIF-1β subunit and an O<sub>2</sub>-regulated HIF-1α or HIF-2α subunit, respectively. *Hif1a*<sup>-/-</sup> mice, which have a complete deficiency of HIF-1α, die at mid-gestation with defects in the heart, blood vessels, and red blood cell production, indicating that all three components of the circulatory system are dependent upon HIF-1 for their normal development [51–53]. *Hif1a*<sup>+/-</sup> mice, which have a partial deficiency of HIF-1α, develop normally but have impaired responses to hypoxia and ischemia [54–63].

Glomus cells express low levels of HIF-1α and relatively high levels of HIF-2α [1]. Carotid bodies from *Hif1a*<sup>+/-</sup> mice appear histologically normal but do not respond to hypoxia [59] (Table 1). In contrast to *Hif1a*<sup>+/-</sup> mice, carotid bodies from *Epas1*<sup>+/-</sup> mice, which have a partial deficiency of HIF-2α, show augmented responses to hypoxia (Table 1) and *Epas1*<sup>+/-</sup> mice manifest hypertension and respiratory instability, two hallmarks of carotid body hyperactivity [64]. HIF-2α is required for the production of anti-oxidant enzymes [60] and the expression of SOD2 mRNA, which encodes manganese superoxide dismutase, is significantly reduced in the carotid bodies of *Epas1*<sup>+/-</sup> mice [64]. Daily treatment of *Epas1*<sup>+/-</sup> mice for 2 weeks with manganese (III) tetrakis (1-methyl-4-pyridyl) porphyrin pentachloride, a potent membrane permeable anti-oxidant, corrected the hyperactive carotid body response to hypoxia, hypertension, and respiratory instability [64]. In contrast, *Hif1a*<sup>+/-</sup> mice have impaired carotid body expression of NOX2 mRNA, which encodes NADPH oxidase, a pro-oxidant enzyme that generates superoxide [65]. Taken together, these data



**Fig. 3** Pathogenesis of hypertension in patients with obstructive sleep apnea. Airway obstruction results in hypoxemia, awakening, airway clearing, and reoxygenation. This cycle of intermittent hypoxia is repeated dozens of times per night. In the carotid body, chronic intermittent hypoxia results in increased ROS levels, which induce increased synthesis and decreased degradation of HIF-1α as well as increased degradation of HIF-2α. Increased HIF-1α-dependent NOX2 expression and decreased HIF-2α-dependent SOD2 expression result in further increases in ROS levels, which trigger glomus cell depolarization, leading to sympathetic activation and systemic hypertension

suggest that a balance between HIF-1 $\alpha$  and HIF-2 $\alpha$  is required for redox homeostasis in the carotid body, which in turn is required for the maintenance of respiratory and cardiovascular homeostasis.

#### Perspective, physiological and clinical implications

Reflexes from the carotid bodies are important for physiological adaptations to chronic hypoxia. For instance, ventilatory acclimatization to hypoxia (VAH), a hallmark adaptation to high altitude, is manifested by progressive increase in ventilation. VAH is absent in carotid body denervated animals and in mice deficient in HIF-1 $\alpha$  [1]. Chronic hypoxia lasting several days to months lead to desensitization of the carotid body response to hypoxia and blunted hypoxic ventilatory response, which was attributed to upregulation of iNOS expression and the resulting increase in NO [1]. The roles of gaseous messengers other than NO in the chemoreflex function during chronic hypoxia have not been investigated.

Carotid body reflexes are implicated in the pathogenesis of a variety of cardiovascular diseases associated with autonomic dysfunction [66]. For instance, a heightened carotid body reflex may underlie the increased sympathetic nervous system activity seen in patients with sleep-disordered breathing due to obstructive sleep apnea, congestive heart failure (CHF), and neurogenic hypertension. It is likely that altered levels of gas messengers as well as alterations in redox homeostasis by HIFs contribute to heightened carotid body reflexes under these conditions. Recent studies involving an experimental model of CHF lend partial support to this hypothesis. Rabbits with CHF exhibit enhanced carotid body sensitivity to hypoxia and augmented sympathetic activity, and these effects were associated with reduced nNOS expression in the carotid body [21]. Overexpression of nNOS in the carotid body using adenoviral vectors partially normalized the carotid body response to hypoxia and sympathetic nerve activity in rabbits with CHF [21]. In a mouse model of obstructive sleep apnea, increased expression of HIF-1 $\alpha$  and decreased expression of HIF-2 $\alpha$  leading to increased ROS levels (Fig. 3) has been shown to play a critical role in the dysregulation of autonomic control of blood pressure and breathing [61, 67]. These findings suggest that enzymes generating gas messengers and redox regulation by HIFs represent potential therapeutic targets in CHF, sleep-disordered breathing, and neurogenic hypertension.

Besides carotid bodies, aortic bodies located at the aortic arch represent another O<sub>2</sub>-sensing chemo-sensory organs [1, 2]. However, compared to carotid body, the role of O<sub>2</sub> sensing by the aortic body in health and disease is less well established. Also, little is known on the cellular mechanisms and the role of gaseous messengers in O<sub>2</sub> sensing by the aortic bodies.

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#### References

- Kumar P, Prabhakar NR (2012) Peripheral chemoreceptors: function and plasticity of the carotid body. *Compr Physiol* 2:141–219
- Prabhakar NR (2000) Oxygen sensing by the carotid body chemoreceptors. *J Appl Physiol* 88:2287–2295
- Prabhakar NR (2006) O<sub>2</sub> sensing at the mammalian carotid body: why multiple O<sub>2</sub> sensors and multiple transmitters? *Exp Physiol* 91:17–23
- Weir EK, Lopez-Barneo J, Buckler KJ, Archer SL (2005) Acute oxygen-sensing mechanisms. *N Engl J Med* 353:2042–2055
- Abu-Soud HM, Rousseau DL, Stuehr DJ (1996) Nitric oxide binding to the heme of neuronal nitric-oxide synthase links its activity to changes in oxygen tension. *J Biol Chem* 271:32515–32518
- Migita CT, Matera KM, Ikeda-Saito M, Olson JS, Fujii H, Yoshimura T, Zhou H, Yoshida T (1998) The oxygen and carbon monoxide reactions of heme oxygenase. *J Biol Chem* 273:945–949
- Snyder SH (1992) Nitric oxide: first in a new class of neurotransmitters. *Science* 257:494–496
- Semenza GL (2011) Oxygen sensing, homeostasis, and disease. *N Engl J Med* 365:537–547
- Stuehr DJ, Santolini J, Wang ZQ, Wei CC, Adak S (2004) Update on mechanism and catalytic regulation in the NO synthases. *J Biol Chem* 279:36167–36170
- Elayan IM, Axley MJ, Prasad PV, Ahlers ST, Aufer CR (2000) Effect of hyperbaric oxygen treatment on nitric oxide and oxygen free radicals in rat brain. *J Neurophysiol* 83:2022–2029
- Prabhakar NR, Kumar GK, Chang CH, Agani FH, Haxhiu MA (1993) Nitric oxide in the sensory function of the carotid body. *Brain Res* 625:16–22
- Chugh DK, Katayama M, Mokashi A, Bebout DE, Ray DK, Lahiri S (1994) Nitric oxide-related inhibition of carotid chemosensory nerve activity in the cat. *Respir Physiol* 97:147–156
- Trzebski A, Sato Y, Suzuki A, Sato A (1995) Inhibition of nitric oxide synthesis potentiates the responsiveness of carotid chemoreceptors to systemic hypoxia in the rat. *Neurosci Lett* 190:29–32
- Wang ZZ, Stensaas LJ, Dinger BG, Fidone SJ (1995) Nitric oxide mediates chemoreceptor inhibition in the cat carotid body. *Neuroscience* 65:217–229
- Fidone SJ, Sato A (1970) Efferent inhibition and antidromic depression of chemoreceptor A-fibers from the cat carotid body. *Brain Res* 22:181–193
- Neil E, O'Regan RG (1971) Efferent and afferent impulse activity recorded from few-fibre preparations of otherwise intact sinus and aortic nerves. *J Physiol* 215:33–47
- Neil E, O'Regan RG (1971) The effects of electrical stimulation of the distal end of the cut sinus and aortic nerves on peripheral arterial chemoreceptor activity in the cat. *J Physiol* 215:15–32
- Lahiri S, Smatresk N, Pokorski M, Barnard P, Mokashi A (1983) Efferent inhibition of carotid body chemoreception in chronically hypoxic cats. *Am J Physiol* 245:R678–R683
- Campanucci VA, Nurse CA (2007) Autonomic innervation of the carotid body: role in efferent inhibition. *Respir Physiol Neurobiol* 157:83–92
- Peers C, Wyatt CN, Evans AM (2010) Mechanisms for acute oxygen sensing in the carotid body. *Respir Physiol Neurobiol* 174:292–298
- Schultz HD, Li YL (2007) Carotid body function in heart failure. *Respir Physiol Neurobiol* 157:171–185

22. Summers BA, Overholt JL, Prabhakar NR (1999) Nitric oxide inhibits L-type Ca<sup>2+</sup> current in glomus cells of the rabbit carotid body via a cGMP-independent mechanism. *J Neurophysiol* 81:1449–1457
23. Kline DD, Yang T, Huang PL, Prabhakar NR (1998) Altered respiratory responses to hypoxia in mutant mice deficient in neuronal nitric oxide synthase. *J Physiol* 511(Pt 1):273–287
24. Wang ZZ, Stensaaen LJ, Bredt DS, Dinger B, Fidone SJ (1994) Localization and actions of nitric oxide in the cat carotid body. *Neuroscience* 60:275–286
25. Kline DD, Yang T, Premkumar DR, Thomas AJ, Prabhakar NR (2000) Blunted respiratory responses to hypoxia in mutant mice deficient in nitric oxide synthase-3. *J Appl Physiol* 88:1496–1508
26. Campbell DL, Stamler JS, Strauss HC (1996) Redox modulation of L-type calcium channels in ferret ventricular myocytes. Dual mechanism regulation by nitric oxide and S-nitrosothiols. *J Gen Physiol* 108:277–293
27. Stamler JS (1994) Redox signaling: nitrosylation and related target interactions of nitric oxide. *Cell* 78:931–936
28. Torrance RW (1996) Carbon monoxide excretion, not oxygen secretion? *Adv Exp Med Biol* 410:335–339
29. Lloyd BB, Cunningham DJC, Goode RC (1968) Depression of hypoxic hyperventilation in man by sudden inspiration of carbon monoxide. In: Torrance RW (ed) *Arterial Chemoreceptors* Oxford, Blackwell, pp 145–150
30. Sjostrand T (1949) Endogenous formation of carbon monoxide in man. *Nature* 164:580
31. Maines MD (1997) The heme oxygenase system: a regulator of second messenger gases. *Annu Rev Pharmacol Toxicol* 37:517–554
32. Kutty RK, Maines MD (1981) Purification and characterization of biliverdin reductase from rat liver. *J Biol Chem* 256:3956–3962
33. Prabhakar NR (1998) Endogenous carbon monoxide in control of respiration. *Respir Physiol* 114:57–64
34. Prabhakar NR, Dinerman JL, Agani FH, Snyder SH (1995) Carbon monoxide: a role in carotid body chemoreception. *Proc Natl Acad Sci U S A* 92:1994–1997
35. Overholt JL, Bright GR, Prabhakar NR (1996) Carbon monoxide and carotid body chemoreception. *Adv Exp Med Biol* 410:341–344
36. Ortega-Saenz P, Pascual A, Gomez-Diaz R, Lopez-Barneo J (2006) Acute oxygen sensing in heme oxygenase-2 null mice. *J Gen Physiol* 128:405–411
37. Donnelly DF (1996) Chemoreceptor nerve excitation may not be proportional to catecholamine secretion. *J Appl Physiol* 81:657–664
38. Prabhakar NR (1999) NO and CO as second messengers in oxygen sensing in the carotid body. *Respir Physiol* 115:161–168
39. Gadalla MM, Snyder SH (2010) Hydrogen sulfide as a gaso-transmitter. *J Neurochem* 113:14–26
40. Peng YJ, Nanduri J, Raghuraman G, Souvannakitti D, Gadalla MM, Kumar GK, Snyder SH, Prabhakar NR (2010) H<sub>2</sub>S mediates O<sub>2</sub> sensing in the carotid body. *Proc Natl Acad Sci U S A* 107:10719–10724
41. Li Q, Sun B, Wang X, Jin Z, Zhou Y, Dong L, Jiang LH, Rong W (2010) A crucial role for hydrogen sulfide in oxygen sensing via modulating large conductance calcium-activated potassium channels. *Antioxid Redox Signal* 12:1179–1189
42. Fitzgerald RS, Shirahata M, Chang I, Kostuk E, Kiihl S (2011) The impact of hydrogen sulfide (H<sub>2</sub>S) on neurotransmitter release from the cat carotid body. *Respir Physiol Neurobiol* 176:80–89
43. Abeles RH, Walsh CT (1973) Acetylenic enzyme inactivators. Inactivation of gamma-cystathionase, in vitro and in vivo, by propargylglycine. *J Am Chem Soc* 95:6124–6125
44. Washtien W, Abeles RH (1977) Mechanism of inactivation of gamma-cystathionase by the acetylenic substrate analogue propargylglycine. *Biochemistry* 16:2485–2491
45. Olson KR, Healy MJ, Qin Z, Skovgaard N, Vulesevic B, Duff DW, Whitfield NL, Yang G, Wang R, Perry SF (2008) Hydrogen sulfide as an oxygen sensor in trout gill chemoreceptors. *Am J Physiol Regul Integr Comp Physiol* 295:R669–R680
46. Zhao W, Zhang J, Lu Y, Wang R (2001) The vasorelaxant effect of H<sub>2</sub>S as a novel endogenous gaseous K(ATP) channel opener. *EMBO J* 20:6008–6016
47. Kim D, Kim I, Papreck JR, Donnelly DF, Carroll JL (2011) Characterization of an ATP-sensitive K(+) channel in rat carotid body glomus cells. *Respir Physiol Neurobiol* 177:247–255
48. Telezhkin V, Brazier SP, Cayzac SH, Wilkinson WJ, Riccardi D, Kemp PJ (2010) Mechanism of inhibition by hydrogen sulfide of native and recombinant BKCa channels. *Respir Physiol Neurobiol* 172:169–178
49. Nurse CA (2010) Neurotransmitter and neuromodulatory mechanisms at peripheral arterial chemoreceptors. *Exp Physiol* 95:657–667
50. Morikawa T, Kajimura M, Nakamura T, Hishiki T, Nakanishi T, Yukutake Y, Nagahata Y, Ishikawa M, Hattori K, Takenouchi T et al (2012) Hypoxic regulation of the cerebral microcirculation is mediated by a carbon monoxide-sensitive hydrogen sulfide pathway. *Proc Natl Acad Sci U S A*. doi:10.1073/pnas.1119658109
51. Compennolle 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-1alpha. *Cardiovasc Res* 60:569–579
52. Iyer NV, Kotch LE, Agani F, Leung SW, Laughner E, Wenger RH, Gassmann M, Gearhart JD, Lawler AM, Yu AY et al (1998) Cellular and developmental control of O<sub>2</sub> homeostasis by hypoxia-inducible factor 1 alpha. *Genes Dev* 12:149–162
53. Yoon D, Pastore YD, Divoky V, Liu E, Mlodnicka AE, Rainey K, Ponka P, Semenza GL, Schumacher A, Prchal JT (2006) Hypoxia-inducible factor-1 deficiency results in dysregulated erythropoiesis signaling and iron homeostasis in mouse development. *J Biol Chem* 281:25703–25711
54. Bosch-Marce M, Okuyama H, Wesley JB, Sarkar K, Kimura H, Liu YV, Zhang H, Strazza M, Rey S, Savino L et al (2007) Effects of aging and hypoxia-inducible factor-1 activity on angiogenic cell mobilization and recovery of perfusion after limb ischemia. *Circ Res* 101:1310–1318
55. Cai Z, Manalo DJ, Wei G, Rodriguez ER, Fox-Talbot K, Lu H, Zweier JL, Semenza GL (2003) Hearts from rodents exposed to intermittent hypoxia or erythropoietin are protected against ischemia-reperfusion injury. *Circulation* 108:79–85
56. Cai Z, Zhong H, Bosch-Marce M, Fox-Talbot K, Wang L, Wei C, Trush MA, Semenza GL (2008) Complete loss of ischaemic preconditioning-induced cardioprotection in mice with partial deficiency of HIF-1 alpha. *Cardiovasc Res* 77:463–470
57. Feinman R, Deitch EA, Watkins AC, Abungu B, Colorado I, Kannan KB, Sheth SU, Caputo FJ, Lu Q, Ramanathan M et al (2010) HIF-1 mediates pathogenic inflammatory responses to intestinal ischemia-reperfusion injury. *Am J Physiol Gastrointest Liver Physiol* 299:G833–G843
58. Kannan KB, Colorado I, Reino D, Palange D, Lu Q, Qin X, Abungu B, Watkins A, Caputo FJ, Xu DZ et al (2011) Hypoxia-inducible factor plays a gut-injurious role in intestinal ischemia reperfusion injury. *Am J Physiol Gastrointest Liver Physiol* 300:G853–G861
59. Kline DD, Peng YJ, Manalo DJ, Semenza GL, Prabhakar NR (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 U S A* 99:821–826
60. Li J, Bosch-Marce M, Nanayakkara A, Savransky V, Fried SK, Semenza GL, Polotsky VY (2006) Altered metabolic responses to intermittent hypoxia in mice with partial deficiency of hypoxia-inducible factor-1alpha. *Physiol Genomics* 25:450–457
61. Peng YJ, Yuan G, Ramakrishnan D, Sharma SD, Bosch-Marce M, Kumar GK, Semenza GL, Prabhakar NR (2006) Heterozygous

- HIF-1alpha deficiency impairs carotid body-mediated systemic responses and reactive oxygen species generation in mice exposed to intermittent hypoxia. *J Physiol* 577:705–716
62. Yu AY, Shimoda LA, Iyer NV, Huso DL, Sun X, McWilliams R, Beaty T, Sham JS, Wiener CM, Sylvester JT et al (1999) Impaired physiological responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1alpha. *J Clin Invest* 103:691–696
63. Zhang X, Liu L, Wei X, Tan YS, Tong L, Chang R, Ghanamah MS, Reinblatt M, Marti GP, Harmon JW et al (2010) Impaired angiogenesis and mobilization of circulating angiogenic cells in HIF-1alpha heterozygous-null mice after burn wounding. *Wound Repair Regen* 18:193–201
64. Peng YJ, Nanduri J, Khan SA, Yuan G, Wang N, Kinsman B, Vaddi DR, Kumar GK, Garcia JA, Semenza GL et al (2011) Hypoxia-inducible factor 2alpha (HIF-2alpha) heterozygous-null mice exhibit exaggerated carotid body sensitivity to hypoxia, breathing instability, and hypertension. *Proc Natl Acad Sci U S A* 108:3065–3070
65. Yuan G, Khan SA, Luo W, Nanduri J, Semenza GL, Prabhakar NR (2011) Hypoxia-inducible factor 1 mediates increased expression of NADPH oxidase-2 in response to intermittent hypoxia. *J Cell Physiol* 226:2925–2933
66. Prabhakar NR, Peng YJ (2004) Peripheral chemoreceptors in health and disease. *J Appl Physiol* 96:359–366
67. Nanduri J, Wang N, Yuan G, Khan SA, Souvannakitti D, Peng YJ, Kumar GK, Garcia JA, Prabhakar NR (2009) Intermittent hypoxia degrades HIF-2alpha via calpains resulting in oxidative stress: implications for recurrent apnea-induced morbidities. *Proc Natl Acad Sci U S A* 106:1199–1204