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CARBON MONOXIDE (CO) AND HYDROGEN SULFIDE (H₂S) IN HYPOXIC SENSING BY THE CAROTID BODY

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Abstract

Carotid bodies are sensory organs for monitoring arterial blood oxygen (O₂) levels, and the ensuing reflexes maintain cardio-respiratory homeostasis during hypoxia. This article provides a brief update of the role of carbon monoxide (CO) and hydrogen sulfide (H₂S) in hypoxic sensing by the carotid body. Glomus cells, the primary site of O₂ sensing in the carotid body express heme oxygenase-2 (HO-2), a CO catalyzing enzyme. HO-2 is a heme containing enzyme and has high affinity for O₂. Hypoxia inhibits HO-2 activity and reduces CO generation. Pharmacological and genetic approaches suggest that CO inhibits carotid body sensory activity. Stimulation of carotid body activity by hypoxia may reflect reduced formation of CO. Glomus cells also express cystathionine γ -lyase (CSE), an H₂S generating enzyme. Exogenous application of H₂S donors, like hypoxia, stimulate the carotid body activity and CSE knockout mice exhibit severely impaired sensory excitation by hypoxia, suggesting that CSE catalyzed H₂S is an excitatory gas messenger. Hypoxia increases H₂S generation in the carotid body, and this response was attenuated or absent in CSE knockout mice. HO inhibitor increased and CO donor inhibited H₂S generation. It is proposed that carotid body response to hypoxia requires interactions between HO-2-CO and CSE-H₂S systems.

Keywords

Oxygen sensing; gas messengers; K⁺ channels; Calcium signaling

1. Introduction

Carotid bodies are the sensory organs for detecting arterial blood O₂ levels. Hypoxemia increases the sensory activity of the carotid body leading to reflex stimulation of breathing and increase in blood pressure (Fitzgerald and Lahiri, 1986). The exquisite sensitivity and rapidity with which carotid body responds to hypoxia makes it a unique sensory organ for monitoring systemic O₂ levels. Carotid bodies are comprised of primarily two cell types: glomus or type I cells, which are of neuronal origin and sustentacular or type II cells, which resemble glial cells of the nervous system. Ever since the discovery of carotid bodies as sensory organs for detecting systemic O₂ levels, much attention has been focused on

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delineating the mechanisms of sensory transduction and transmission. Much of the available evidence suggests that the Type I cells and the opposing afferent nerve endings as the primary site of sensory transduction. Current concepts on transduction and transmission mechanisms of the carotid body are recent reviewed in detail elsewhere (Kumar and Prabhakar, 2012).

Nearly 4 decades ago Lloyd et al. found that brief inhalation of carbon monoxide (CO) inhibits ventilatory stimulation by hypoxia (Lloyd et al. 1968). Because CO has high affinity for hemoglobin, these authors suggested that “*Carotid body might possess a pigment rather like hemoglobin or myoglobin, and might report the extent to which this pigment is in its deoxygenated form*” (Lloyd et al., 1968). As early as in 1922, it was reported that inhalation of hydrogen sulfide (H₂S) stimulates breathing (Haggard et al. 1922), and this effect was subsequently attributed to stimulation of the carotid body (Heymans et al., 1931). A brief historical account of H₂S on breathing can be found in a recent article by Buckler (Buckler, 2012). CO and H₂S, which were once used as tools to understand the carotid body physiology, are now known to be endogenously produced by the glomus tissue (see Prabhakar, 1999; Prabhakar et al., 1995; Kumar and Prabhakar, 2012 for ref). Unlike other chemical messengers (e.g., biogenic amines and peptides), CO and H₂S are not stored in vesicles and once generated in response to a stimulus, they diffuse to neighboring cells and are rapidly inactivated. The purpose of this article is to highlight recent studies addressing the roles of endogenous CO and H₂S in carotid body response to hypoxia.

2. Carbon monoxide (CO)

2.1. Heme oxygenase expression in carotid bodies

CO is endogenously generated during degradation of heme by heme oxygenases (HOs) utilizing NADPH, cytochrome P-450 reductase, and molecular O₂ as co-factors (Maines, 1997). Heme oxygenases are heme containing enzymes, and have high affinity for O₂ with Km values in the range of 30–80 μ M (Migita et al., 1998). Substrates for HO include alpha and beta chains of hemoglobin, denatured myoglobin, met-hemoglobin and proteolytic products of cytochrome. Two HO isoforms have been identified including a constitutively expressed HO-2 and an inducible HO-1 (also called Heat Shock Protein 32 or HSP-32; Maines, 1997). HO-2 is expressed in glomus cells of cat, rat (Prabhakar et al., 1995) and mouse carotid bodies (Ortega-Saenz et al., 2006), but not in the nerve fibers or in type II cells (Prabhakar et al., 1995). Under basal conditions, HO-1 expression is not evident in the carotid body.

2.2. Effects of hypoxia on CO generation

Hypoxia decreases HO activity in a stimulus-dependent manner in placenta (Appleton et al., 2002) and in cerebral vasculature (Morikawa et al., 2012). Our recent unpublished study (Prabhakar NR and Kumar GK; 2012 unpublished data) showed relatively high levels of CO under basal conditions in rat carotid bodies (12 nmol/min/mg protein), and hypoxia (PO₂ ~ 40 mm Hg) markedly reduced CO generation (4 nmol/min/mg protein). These observations suggest that physiological levels of hypoxia inhibit HO activity and reduce CO generation.

2.3. Studies with hemeoxygenase inhibitors

2.3.1. Effects on carotid body sensory activity—Zinc-protoporphyrin-9 (ZnPP9), a HO inhibitor, increased the carotid body sensory activity in a dose-dependent manner. However, CuPP9, which has negligible effects on HO activity, had no effect on the carotid body activity (Prabhakar et al., 1995). Exogenous application of CO prevented ZnPP9-induced sensory excitation of the carotid body. In anesthetized rats, ZnPP9 augmented the hypoxic ventilatory response, and bilateral sectioning of carotid sinus nerves abolished this

effect (Prabhakar, 1998). These observations suggest that endogenous CO is a physiological inhibitor of the carotid body sensory activity.

2.4. Effects on ion channels and $[Ca^{2+}]_i$ in glomus cells

A major advance in the carotid body research is the demonstration that hypoxia inhibits certain K^+ channels in glomus cells resulting in depolarization, leading to activation of voltage-gated Ca^{2+} channels and Ca^{2+} influx (see Peers and Wyatt, 2007; Kumar and Prabhakar, 2012 for ref). Peers (1990) identified Ca^{2+} -activated K^+ channel (maxi K) as one of the targets of hypoxia in glomus cells. CO enhances maxi K channel activity in glomus cells (Riesco-Fagundo et al., 2001; Williams et al., 2004). On the other hand, disrupting HO-2 activity, like hypoxia, inhibits the maxi K channel activity (Williams et al., 2004). HO inhibitor, ZnPP9 increased $[Ca^{2+}]_i$ in rat glomus cells and exogenous application of CO abolished this response (Prabhakar, 1998). $[Ca^{2+}]_i$ responses to ZnPP9 were absent in Ca^{2+} free medium, suggesting that inhibition of HO leads to Ca^{2+} influx involving activation of Ca^{2+} channels. Glomus cells express a variety of high voltage gated Ca^{2+} channels including L-, P, Q and R-type currents (e Silva and Lewis, 1995; Overholt and Prabhakar, 1997). ZnPP9 enhanced Ca^{2+} currents in glomus cells (Prabhakar, 1998). However, the identity of Ca^{2+} channels activated by HO-inhibitor remains to be investigated. These observations suggest that inhibition of maxi K channels, activation of voltage-gated Ca^{2+} channels and the ensuing Ca^{2+} influx contribute in part to the carotid body sensory excitation evoked by ZnPP9.

2.5. Studies on HO-2 knockout mice

Although studies with pharmacological approaches suggest that endogenous CO is an inhibitory gas messenger, a recent study using HO-2 knockout mice questioned this possibility (Ortega-Saenz et al., 2006). Catecholamine secretion from glomus cells was monitored by carbon-fiber amperometry in carotid body slices from wild type and HO-2 knockout mice (Ortega-Saenz et al., 2006). These authors found that the magnitude of hypoxia-evoked catecholamine secretion was unaltered in HO-2 mice compared to wild type controls. Accordingly, it was concluded that HO-2/CO system is not critical for glomus cell response to hypoxia. We recently recorded carotid body responses to hypoxia in age, and gender matched wild type and HO-2 knockout mice (Prabhakar, NR and Peng Y-J, 2012). Carotid bodies from HO-2 knockout mice exhibited: a) elevated baseline sensory activity, and b) augmented sensory response to hypoxia compared to wild type mice (FIG.1). Thus, there appears to be a discrepancy between the effects of HO-2 deletion on glomus cell secretory *versus* sensory response to hypoxia. Nonetheless, pharmacological and genetic approaches are consistent with the idea that CO generated by HO-2 is a physiological inhibitor of the carotid body sensory activity. Given that hypoxia inhibits HO-2 activity, stimulation of the carotid body activity by low O_2 may reflect, in part, reduced CO formation (Prabhakar, 1999). Recent studies provided interesting insights into the mechanism(s) by which CO exerts its inhibitory influence on carotid body activity (*see below*).

3. Hydrogen Sulfide (H_2S)

3.1. H_2S generating enzymes in the carotid body

Cystathionine γ -lyase (CSE) and cystathionine β -synthase (CBS) are the two major enzymes, which catalyze endogenous H_2S formation. Rat carotid body expresses mRNAs encoding CBS and CSE (Telezhkin et al., 2010). CBS protein is localized to glomus cells in mouse (Li et al., 2010), and cat (Fitzgerald et al., 2011) carotid bodies. CSE is also expressed in glomus cells from mouse and rat carotid bodies as evidenced by co-localization with tyrosine hydroxylase, a marker of this cell type (Peng et al., 2010). Genetic deletion of

CSE reduced basal H₂S levels in the carotid bodies approximately by one-half compared to wild type controls (Peng et al. 2010). The residual H₂S must arise from sources other than CSE, presumably CBS.

3.1.2. Effects of disrupting CBS function on the carotid body activity—Mouse treated with aminooxyacetic acid (AOAA), a putative inhibitor of CBS, exhibit impaired carotid body and ventilatory responses to hypoxia (Li et al., 2010). These observations led to the suggestion that CBS-catalyzed H₂S mediates carotid body sensory response to hypoxia (Li et al., 2010). However, AOAA is a general inhibitor of pyridoxal phosphate (PLP)-dependent enzymes including 4-aminobutyrate aminotransferase (GABA-T; Beeler and Churchich, 1976). AOAA is also known to increase GABA levels in tissues (Wallach, 1961) and disrupts mitochondrial function (Kauppinen et al., 1987). GABA inhibits carotid body sensory response to hypoxia (Zhang et al., 2009). It is also known that inhibition of mitochondrial function affects carotid body response to hypoxia (see Kumar and Prabhakar, 2012 for ref). Therefore, whether the reduced carotid body response to hypoxia by AOAA is due to inhibition of H₂S generation or secondary to changes in GABA levels and/or mitochondrial function remain to be established.

3.1.3. Effects of disrupting CSE function on carotid body activity—Peng et al. (2010) employed both genetic and pharmacological interventions to assess the role of CSE catalyzed H₂S in the carotid body response to hypoxia. Carotid bodies from CSE knockout mice showed absence of CSE in glomus cells and exhibited severely impaired sensory response to hypoxia and markedly attenuated ventilatory stimulation by low O₂ (Peng et al., 2010). Rat carotid bodies treated with DL-propargylglycine (PAG), an inhibitor of CSE (Abeles and Walsh, 1973; Washtien and Abeles, 1977) also showed a similar impairment of carotid body response to hypoxia (Peng et al. 2010). In contrast, the carotid body response to CO₂ was unaffected in CSE knockout mice and was even augmented in rats treated with PAG, suggesting that disruption of CSE function selectively affects the carotid body response to hypoxia. A recent study reported that hypoxia increased H₂S levels in isolated glomus cells from rat carotid bodies, and this response was prevented by PAG in a dose-dependent manner. Hypoxia evoked catecholamine secretion as well as [Ca²⁺]_i elevations were markedly attenuated in PAG treated glomus cells as well as cells from CSE knockout mice (Makarenko et al. 2012). These observations taken together suggest that H₂S generated by CSE is a physiologic mediator of carotid body response to hypoxia.

3.2. Effects of exogenous application of H₂S donors on carotid body and glomus cell responses to hypoxia

NaHS, a H₂S donor, increased the sensory discharge of the carotid bodies from mice, and rats with a time course and magnitude similar to that evoked by hypoxia (Li et al., 2010; Peng et al., 2010). K_{ATP} channels are the major targets of H₂S in blood vessels (Zhao et al., 2001). However, glibenclamide, an inhibitor of K_{ATP} channels, had no effect on carotid body response to NaHS and hypoxia (Peng et al., 2010). However, in the presence of Ca²⁺-free medium, carotid body responses to NaHS as well as hypoxia were completely abolished (Li et al., 2010; Peng et al., 2010), suggesting that the effects of NaHS and hypoxia require Ca²⁺ influx from extracellular sources.

Recent studies showed that NaHS mimics many of the glomus cell responses to hypoxia. For instance, like hypoxia, NaHS inhibits background K⁺ current (TASK; Buckler, 2012) and Ca²⁺ activated K⁺ currents (Li et al., 2010; Telezhkin et al., 2010) in glomus cells. NaHS resulted in prompt and reversible increase in [Ca²⁺]_i in rat glomus cells (Buckler, 2012; Makarenko et al. 2012). Voltage clamping at the resting membrane potential abolished NaHS-evoked elevation in [Ca²⁺]_i, suggesting that this effect requires Ca²⁺ entry via

voltage gated Ca^{2+} channels (Buckler, 2012). Activation of L-type Ca^{2+} channels seem to mediate $[\text{Ca}^{2+}]_i$ elevation by NaHS (Makarenko et al. 2012). These studies suggest that H_2S excites glomus cells through inhibition of TASK and/or maxi K^+ channels leading to membrane depolarization and voltage gated Ca^{2+} entry. In addition, Buckler (2012) also showed that exogenous application of H_2S inhibits mitochondrial function in rat glomus cells. Li et al. (2010) found that blockade of purinergic and nicotinic cholinergic receptors abolish sensory excitation of the mouse carotid body by NaHS. However, Fitzgerald et al. (Fitzgerald et al., 2011) reported that Na_2S , which is another H_2S donor, inhibited ACh release and had variable effects on ATP release from cat carotid bodies.

4. Mechanisms of increased H_2S generation by hypoxia

Hypoxia increased H_2S levels in both mouse and rat carotid bodies in a stimulus-dependent manner and this response was absent in CSE knockout mice and in rats treated with PAG (Peng et al., 2010). These findings suggest that CSE is a major enzyme that contributes to hypoxia-evoked H_2S generation. How might hypoxia increase H_2S generation? Given that CO inhibits carotid body sensory activity we reasoned that it inhibits H_2S generation by CSE. Indeed, HO inhibitor markedly increased basal H_2S levels in the carotid body under normoxia and increased baseline sensory activity in wild type mice, and these effects were absent in CSE knock out mice (Peng et al., 2010). On the other hand, CORM-2, a CO donor, inhibited hypoxia-evoked H_2S generation in a dose-dependent manner (Peng et al., 2010). These observations suggest that CO is a physiological inhibitor of CSE-dependent H_2S generation. Based on these observations, it was proposed that low sensory activity of the carotid body under normoxia is due to inhibition of H_2S generation by CO, and that hypoxia reduces HO-2 activity to reverse the inhibition and augment H_2S formation (Peng et al. 2010). A recent study reported that hypoxia-induced cerebral vasodilation also require interaction between HO-2/CO and CBS/ H_2S (Morikawa et al., 2012). Further studies are needed to delineate the molecular mechanisms by which CO inhibits CSE activity and reduce H_2S generation. The interactions between HO-2/CO and CSE/ H_2S and their contribution to the carotid body response to hypoxia are illustrated in Figure 2.

5. Summary and Perspective

Although Lloyd et al (1968) proposed that hypoxic sensory response of the carotid body involves deoxygenated form of a heme protein the identity of this protein(s) remained elusive. The following observations suggest that HO-2 satisfies some of the criterion of the heme protein that initiates the hypoxic sensory response of the carotid body. First, HO-2 is expressed in the glomus cells, the primary site of O_2 sensing in the carotid body. Second, HO-2 has the high affinity for O_2 , suggesting that even modest reductions in O_2 leads to substantial loss of its activity. Indeed, physiological levels of hypoxia ($\text{PO}_2 \sim 40$ mmHg) inhibited HO-2 enzyme activity. The mechanism by which deoxygenated HO-2 participates in carotid body response to hypoxia appears complex. Inhibition of HO-2 activity by hypoxia results in reduced generation of CO, an inhibitory gas messenger, which by removing inhibition on CSE increases H_2S generation, an excitatory gas messenger. Available evidence suggests that H_2S by affecting glomus cell excitability contributes in part to the sensory excitation. Whether H_2S also directly acts on the sensory ending, however, remain to be investigated. It was proposed that protein-protein interactions working in concert as a “chemosome” mediate carotid body response to hypoxia (Prabhakar, 2006). It is likely that interactions between HO-2-CO and CSE- H_2S systems in concert with K^+ channels constitute major components of the “chemosome”. It was proposed that H_2S functions as an “ O_2 sensor” in trout gill chemoreceptors, and vascular smooth muscle (Olson et al 2008). However, in the carotid body, H_2S production by CSE is regulated by O_2 –

dependent HO-2-CO system. Consequently, endogenous H₂S seem to function as an important “mediator” of the carotid body response to hypoxia rather than an “O₂ sensor”.

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Figure 1.

Example illustrating the effect of hypoxia (Hx; PO₂ ~36 mmHg) on carotid body ex vivo sensory activity in a 8 week old, male wild type (HO-2^{+/+}) and age and gender matched heme oxygenase-2 (HO-2) knockout mice (HO-2^{-/-}). Black bar represents the duration of the hypoxic challenge. Inset represents the single unit action potential from which the data are derived. Note the elevated baseline activity and augmented sensory response to hypoxia in HO-2 knockout carotid body.

Figure 2.
Schematic presentation of interactions between heme oxygenase-2 (HO-2) catalyzed carbon monoxide (CO) and hydrogen sulfide (H₂S) catalyzed by cystathionine-γ-lyase (CSE).