Review Article

Interaction of Hydrogen Sulfide with Oxygen Sensing under Hypoxia

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Based on the discovery of endogenous H2S production, many in depth studies show this gasotransmitter with a variety of physiological and pathological functions. Three enzymes, cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (MST), are involved in enzymatic production of H2S. Emerging evidence has elucidated an important protective role of H2S in hypoxic conditions in many mammalian systems. However, the mechanisms by which H2S senses and responses to hypoxia are largely elusive. Hypoxia-inducible factors (HIFs) function as key regulators of oxygen sensing, activating target genes expression under hypoxia. Recent studies have shown that exogenous H2S regulates HIF action in different patterns. The activation of carotid bodies is a sensitive and prompt response to hypoxia, rapidly enhancing general O2 supply. H2S has been identified as an excitatory mediator of hypoxic sensing in the carotid bodies. This paper presents a brief review of the roles of these two pathways which contribute to hypoxic sensing of H2S.

1. Introduction

H2S had been known mostly due to its unpleasant smell and fierce toxicity before its physiological importance was studied [1]. Production of H2S in mammalian cells had been considered almost as a metabolic waste, until the physiological roles of this small molecular were elucidated in the central nervous system and the cardiovascular system nearly two decades ago [2, 3]. H2S is now identified as a well-known gasotransmitter which plays a critical role in both physiological regulation and pathophysiological processes of different mammalian tissues [4, 5]. Endogenous H2S levels have been reported in different mammalian systems, ranging from 50 to 160 μM [6, 7]. The enzymatic production of endogenous H2S were identified, mostly composed of cystathionine β-synthase (CBS) [8–10], cystathionine γ-lyase (CSE) [11–13], and 3-mercaptopyruvate sulfurtransferase (MST) [14–16].

H2S and oxygen (O2) are mutually exclusive on the Earth. During the Permian period, the depletion of oxygen and accumulation of H2S in the oceans and the air caused great extinctions of more than 90 percent of life species [1, 17]. Physicochemical reactions in living cells require enough molecular oxygen O2 for essential metabolic processes. O2 serves as the terminal electron acceptor in the system of oxidative phosphorylation which produces high-energy phosphate bond in ATP [18, 19]. Insufficient oxygen supply to tissues, organs, or cells constitutes hypoxia. Hypoxia is evoked by several kinds of causes which all lead to oxygen-partial-pressure (PO2) in arterial blood less than 40 mm Hg [19, 20]. Several lines of evidence point to an important protective role of H2S in hypoxic conditions. However, the mechanisms by which H2S senses and responses to hypoxia are largely elusive. A master regulator of hypoxia in mammalian cells is hypoxia-inducible factor-1 (HIF-1), which activates the transcription of >100 target genes under hypoxic contexts [18, 21]. HIF-1 is involved in H2S-mediated angiogenesis under hypoxia [22, 23]. With the remarkable sensitivity and the prompt speed to response to hypoxia, the carotid bodies play a unique role in O2 sensing [24, 25]. It has been shown that H2S is involved in the regulation of
sensory activity in the carotid bodies [26, 27]. In genetically
distinct rat models, variations in endogenous H$_2$S levels have
been reported to be closely correlated with variations of
carotid body O$_2$ sensing in both hypoxia-induced pulmonary
edema and essential hypertension [28]. In this paper, we
review physiological responses of H$_2$S on HIF-1 action
and the O$_2$ sensing role of H$_2$S in the carotid bodies.

2. H$_2$S and HIF-1 under Hypoxia

2.1. Hypoxia and Hypoxia-Inducible Factor-1. HIF-1 was
first identified as the protein responsible for the hypoxia-
evoked transcription of erythropoietin [29, 30]. HIF-1 is a
heterodimer consisting of the inducibly regulated HIF-1$\alpha$
subunits and the constitutively expressed HIF-1$\beta$
subunits [31]. Since the expression of HIF-1$\beta$ is abundant in cells,
HIF-1$\alpha$ determines the protein level and the transcriptional
activity of HIF-1 [21]. The HIF-1 heterodimer combines
with the hypoxia-responsive element in target genes, thus
enhancing target genes transcription [21].

Under normoxic conditions, although continuously tran-
scribed and translated, the protein levels of HIF-1$\alpha$ are
hard to be detected in normal cells due to HIF-1$\alpha$ protein
fast degradation. Tow specific proline residues within the
oxygen-dependent degradation (ODD) domain in HIF-1$\alpha$ are
hydroxylated by the prolyl hydroxylase (PHD) under norm-
oxia. Hydroxylated HIF-1$\alpha$ is then bound by the von Hippel Lindau protein (pVHL). This complex in turn recruits a ubiquitin ligase that targets HIF-1$\alpha$ for its proteasomal
degradation [33] (Figure 1). PHD activity is inhibited under
hypoxic conditions, thus resulting in HIF-1$\alpha$ stabilization.
Other pathways and regulators are also involved in HIF-1$\alpha$
degradation [21].

Compared with the accumulated studies on HIF-1$\alpha$
degradation, translational control of stress-survival protein
HIF-1$\alpha$ under hypoxia is far more beyond our understand-
ing. HIF-1$\alpha$ protein translation under normoxia is depend-
ent on activation of the PtdIns3K-Akt mammalian target
of rapamycin (mTOR) and the mitogen-activated protein
kinases (MAPK) pathways [20, 21]. Under hypoxia-induced
stress, phosphorylation of the translation initiation factor 2$\alpha$
(eIF2$\alpha$) and inhibition of mTORC1 activity have been
suggested to account for HIF-1$\alpha$ protein expression inhibition
[21] (Figure 1). Certain antitumour compounds which inhibit
HIF-1$\alpha$ translation contribute to eIF2$\alpha$ phosphorylation [34,
35]. There is evidence that other factors are involved in HIF-
1$\alpha$ translation regulation, that is, studies on the 5' UTR of HIF-
1$\alpha$ which contains an internal ribosome-entry-site [21].

2.2. Effects of H$_2$S on HIF-1 Accumulation. Nitric oxide (NO),
the first identified gasotransmitter, has been demonstrated
to affect HIF-1 action [36, 37]. An early report showed that NO donors inhibited HIF-1 action under both hypoxia and hypoxia-mimetic conditions [36]. In later studies, by overexpressing the human iNOS to increase endogenous NO production, NO was shown to enhance HIF-1 protein accumulation and its activity [37]. The second gasotransmitter carbon monoxide (CO) has also been shown to regulate HIF-1 protein levels and activity in certain contexts [38]. A series of recent studies showed that H2S, like NO and CO, plays an important role in the regulation of HIF-1 function under hypoxia. Caenorhabditis elegans (C. elegans) is an attractive model for hypoxia relative research [6]. This animal model was used to study the influence of H2S on HIF-1. In C. elegans, the EGG laying defective- (EGL-) 9 is responsible for HIF-1 hydroxylation [39]. HIF-1 was closely correlated with increased tolerance of H2S exposure in C. elegans [39]. Animals with the HIF-1 null mutation did not survive from exposure to only 15 ppm H2S, while wild-type worms survived with high viability even exposure to 50 ppm H2S [39]. Mutations in either EGL-9 or VHL-1 induced an overabundance of HIF-1, which caused animals to tolerate 150 ppm H2S [39]. It is strongly indicated that HIF-1 is required when animals response to H2S. H2S treatment, as well as hypoxia, elevated the levels of HIF-1 protein and its activity [39]. However, H2S and hypoxia caused different expression patterns of a HIF-1 target gene [39].

The effects of H2S on HIF-1 in higher organisms have been investigated. In vascular smooth muscle cells, the interaction between H2S and HIF-1 was tested under CoCl2-induced hypoxia-mimetic conditions [40]. In a concentration of 300 μM, NaHS induced upregulation of HIF-1α mRNA and proteins [40]. HIF-1α transcription activity in the same context was also increased [40]. The authors suggested that H2S upregulates HIF-1α gene transcription. However, HIF-1α synthesis during hypoxia is largely regulated at the level of translation rather than transcription [21]. It is worthy of mentioning that although CoCl2 treatment could induce HIF-1α accumulation to mimetic hypoxic stress, the model itself is not essential hypoxia and may cause other cellular changes [1].

Some later studies provided further understanding of the influence of H2S on HIF-1 in mammalian cells. It is reported that 1 mM NaHS (H2S donor) decreased hypoxia-induced HIF-1α protein accumulation and HIF-1 target gene expression in mammalian cell lines [22]. However, H2S had no similar effect on HIF-1 in hypoxia-mimetic conditions [22]. In contrast, another study showed that NaHS at relatively low concentrations (10–100 μM) treatment decreased HIF-1α protein levels in HEK293T, Hep3B, and EA.hy926 cells under both hypoxia (1% O2) and hypoxia-mimetic conditions [23]. The controversial observations will be considered in subsequent sections.

2.3. Mechanisms of H2S Effects on HIF-1 Accumulation. In the C. elegans study, H2S-induced HIF-1 accumulation was dependent on EGL-9, indicating that H2S may prevent HIF-1 degradation [39]. In the same study, H2S-evoked HIF-1 increase was independent of VHL-1, which implies that C. elegans may have a special HIF-1 degradation pathway. However, hypoxia-mediated HIF-1 accumulation in mammals is largely dependent on VHL-1. It has been shown that VHL-independent degradation pathways play important roles in controlling HIF-1 levels [21]. CYSL-1 is homologous to CBS which is a key enzyme for H2S production in mammalian cells [41]. CYSL-1 identified from genetic screens negatively regulated EGL-9 and thus enhanced HIF-1 stability [41]. It was proposed that H2S promoted the interaction of EGL-9 and CYSL-1 to cause HIF-1 accumulation in C. elegans under hypoxia [41].

Kai and colleagues showed that H2S did not affect HIF-1 levels in EB8 cells under hypoxia, compared with control cells [22]. The fact that EB8 cells have no mtDNA suggested that mitochondria was involved in H2S-inhibited HIF-1 activation [22]. The authors found that 1 mM NaHS inhibited mitochondrial oxygen consumption and thus increased the oxygen level in hypoxic cells. H2S did not inhibit the stabilization of HIF-1α protein under hypoxia-mimetic conditions [22]. The authors concluded that H2S promoted HIF-1α degradation under hypoxia, due to NaHS-induced mitochondrial oxygen consumption inhibition (Figure 1). However, they did not provide the direct evidence that the speed of HIF-1α degradation was altered in the presence of H2S. On the other hand, it is worthy of mentioning that most of the key observations in their study were obtained with NaHS at 1 mM. This high concentration of H2S is clearly not within the physiological range of endogenous H2S and may have toxic effects on cells for long-time treatments [1, 42].

NaHS at 10–100 μM has been used in different studies to reflect physiologically relevant concentrations of H2S in vivo [43, 44]. In a study using NaHS at 10–100 μM, NaHS significantly lowered HIF-1α protein levels under both hypoxia (1% O2) and hypoxia-mimetic conditions (DFX or CoCl2) [23]. Although inducing HIF-1α accumulation, both DFX and CoCl2 have no effect on oxygen partial pressure in the culture medium [23]. It is suggested that oxygen partial pressure change or the alteration of mitochondrial respiration may not be involved in H2S-induced HIF-1α downregulation under hypoxia. Both HIF-1α degradation and HIF-1α ubiquitination were not changed by NaHS treatment [23]. In contrast, cycloheximide (CHX), a translation inhibitor, blocked the effect of NaHS on HIF-1α protein levels [23], suggesting that H2S mediates HIF-1α translation suppression. The authors further demonstrated that the key mechanism for H2S-induced HIF-1α downregulation was H2S-evoked repression of HIF-1α protein translation, rather than an effect on the ubiquitin proteosomal degradation pathway (Figure 1). Eukaryotic translation initiation factor 2α (eIF2α) is a critical regulatory molecule for eukaryotic initiation of translation [21]. eIF2α is responsible for the transformation of GDP to GTP, an essential step for translation start. Phosphorylation of eIF2α at Ser51 prevents the reformation of the eIF2 ternary complex and thus inhibits protein translation [21]. Certain antitumour compounds which essentially decrease HIF-1α translation contribute to eIF2α phosphorylation [34, 35]. In eIF2α knockdown cells exposed to hypoxia, H2S-induced reduction of HIF-1α was partially reversed [23]. Therefore, HIF-1α translational suppression is associated with
H₂S-induced eIF2α phosphorylation in hypoxic contexts (Figure 1).

In summary of this section, it is likely that two key factors, the extent of hypoxia together with the concentration of H₂S in the same condition, determine the final result of the action of H₂S on HIF-1. H₂S-induced upregulation of HIF-1 was observed in a *C. elegans* model. *C. elegans* naturally lives where O₂ level is lower than that in the air [6, 7]. The animals have no circulatory system. O₂ delivery in this species is dependent on diffusion. *C. elegans* are able to survive from O₂ depletion, due to an anoxia-induced suspended animation state [6, 7]. In contrast, models in which H₂S-triggered downregulation of HIF-1 come from high organisms which are highly oxygen-demanded. The differences in both species and oxygen demand may explain the opposite regulational patterns in the action of H₂S on HIF-1. On the other hand, the effect of H₂S on HIF-1 regulation may be dose-dependent. Differences in NaHS concentrations may be correlated with various observations in abovementioned studies. Thus, further studies on the role of endogenous H₂S may provide further understanding of the interaction between H₂S and HIF-1.

Emerging evidence indicates that VHL-independent HIF-1α degradation has an important role in controlling HIF-1α levels. Although this kind of pathways seems to be less dependent on oxygen levels in cells, that is, receptor of activated protein kinase C- (RACK1) mediated HIF-1α degradation [21], and further research is still required to solve whether these pathways are involved in the effect of H₂S on HIF-1 in hypoxic contexts. On the other hand, translational regulation of HIF-1α under hypoxia is largely elusive. Therefore, whether H₂S targets other factors which potentially regulate HIF-1α translation is another challenge. Moreover, it is not yet clear how H₂S-mediated HIF-1α regulation contributes to the protective role of H₂S under hypoxia.

### 3. H₂S and Hypoxic Sensing in the Carotid Body

#### 3.1. O₂ Sensing in the Carotid Body

With the remarkable sensitivity and the fast speed to hypoxic response, the carotid body plays a unique role in O₂ sensing [19]. Carotid bodies are small sensory organs located at the bifurcation of the common carotid artery [14, 19]. Changes in O₂ levels of arterial blood rapidly activate the carotid bodies, which in turn transduce sensory information to brainstem neurons [14]. The final response in the central nervous system regulates vital functions including breathing, heart rate, and blood pressure to increase ventilation and systemic delivery of oxygen [15]. Carotid bodies are primarily composed of two cell types: glomus cells (also called type I cells) and sustentacular cells (also called type II cells) [15]. Accumulated evidence suggests that type I cells are the primary site of hypoxic sensing in carotid bodies. NO and CO inhibited carotid body activity [15]. Neuronal nitric oxide synthase- (nNOS-) generated NO has been proposed as an important mediator of efferent inhibition of the carotid body [25, 45]. Heme oxygenase-2 (HO-2), a key enzyme for CO production, is expressed in glomus cells of many mammalian carotid bodies [24, 46]. Exogenous application of CO at low concentrations and HO inhibitors inhibited and stimulated the carotid body activity, respectively [16, 24].

#### 3.2. Effects of CSE/H₂S on Carotid Body Activity

Emerging evidence demonstrated that H₂S is involved in sensing and response to hypoxia in many tissues that possess the ability to sense hypoxia. H₂S stimulated catecholamine secretion from chromaffin cells in trout [30]. H₂S regulated the O₂-sensing signal in trout chemoreceptors [47]. It was proposed that the balance between endogenous H₂S production and its oxidation by available O₂ contributed to H₂S-mediated O₂ sensing [47]. Recent studies showed that H₂S, like NO and CO, is another gas regulator for hypoxic sensing in carotid bodies. Both CSE [26] and CBS proteins [27] are expressed in glomus cells. Rat carotid body expresses mRNAs for both CBS and CSE [48]. Hypoxia increased H₂S generation in both mouse and rat carotid bodies [26]. Hypoxia-induced H₂S production and secretion of catecholamine were significantly decreased in CSE knockout mice or in wide-type mice treated with CSE inhibitors DL-propargylglycine (PPG) [26]. CSE knockout mice exhibited severely impaired function of carotid body sensing to hypoxia. The similar observation was shown in rats treated with PPG [26]. Exogenous application of H₂S donor, NaHS, increased the sensory excitation of the carotid bodies from mice and rats [26, 27]. The patterns of NaHS action on carotid bodies are similar to that of hypoxia. H₂S produced by CSE may function as an excitatory mediator for the sensory excitation by hypoxia [26] (Figure 2).

#### 3.3. Potential Effects of CBS/H₂S on O₂ Sensing in Carotid Body

It is worthy of mentioning that, in CSE knockout mice, the basal levels of H₂S in the carotid bodies were half reduced compared to those of control wild-type animals [26]. It is likely that CBS may also be responsible for H₂S production in the carotid bodies. Aminoxyacetic acid (AOA) and hydroxylamine (HA), two inhibitors of CBS, suppressed carotid body and ventilatory responses to hypoxia [27]. This study suggested that CBS-catalyzed H₂S also plays a role in excitatory carotid bodies sensing under hypoxia (Figure 2). Compared to CSE inhibitor PPG, AOA and HA are nonspecific inhibitors [1]. HA inhibits many heme-containing enzymes [49, 50], while AOA generally targets aminotransferase [51, 52]. Given the pharmacologic nonspecific inhibition, further studies need to elucidate the effects of endogenous CBS on O₂ sensing in carotid bodies. In addition, the relative importance of H₂S/CBS and H₂S/CBS on carotid bodies hypoxic sensing needs to be established.

The role of H₂S/CBS in O₂ sensing in tissues, other than carotid bodies, has been revealed in recent studies. CBS is a nuclear encoding heme protein [8]. The levels of CBS proteins in liver mitochondria are regulated in a Lon protease-mediated O₂-dependent pathway [8]. Under normoxic conditions, a low level of CBS proteins was shown in mitochondria [8]. Hypoxia inhibited Lon protease activity and thus increased the accumulation of CBS in mitochondria...
Figure 2: Potential interaction of heme oxygenase-2- (HO-2-) generated CO with cystathionine γ-lyase (CSE) and cystathionine β-synthase (CBS) in glomus cells of the carotid body. (a) Under normoxia, HO-2-generated CO is relatively high, resulting in inhibition of both CSE and CBS activity. H₂S production is relatively low, contributing to low sensory activity in glomus cells. (b) Under hypoxia, CO generation from HO-2 is reduced, resulting in removal of CO inhibition on CSE and CBS activity. H₂S levels are increased due to enhanced CSE and CBS activities. Increased H₂S may activate Ca²⁺ channels due to membrane depolarization via the inhibition of K⁺ channels, which in turn excites sensory responses in glomus cells.
Reoxygenation recovered Lon protease activity and thus accelerated the degradation of CBS proteins [8]. This study indicates that CBS accumulated in mitochondria is oxygen-sensitive. CBS serves as a CO-sensitive modulator of H$_2$S in liver [53]. Studies using recombinant CBS indicated that CO bound to the prosthetic heme in CBS and thus specifically inhibited CBS activity [53]. In livers of heterozygous CBS knockout mice, overproducing CO had no effect on H$_2$S generation. Another further study showed that CBS-catalyzed H$_2$S functioned as a vasodilator in the cerebral circulation [54]. CO produced by HO-2 was O$_2$-dependent in the brain. Endogenous CO negatively regulated CBS activity under normoxia, while, under hypoxia, CBS activity was increased due to the decrease of CO production [54]. However, it is not clear whether the interaction between CO and CBS affects the sensory action of carotid body.

3.4. Mechanisms of Regulating Carotid Body Activity by H$_2$S. Recent studies showed that hypoxia induces the opening of voltage-dependent Ca$^{2+}$ channels (VDCC) through inhibition of K$^+$ channels in carotid bodies [14, 15]. VDCC activation increases intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]) and thus enhances the release of neurotransmitters under hypoxia [15, 19] (Figure 2). NaHS inhibited big-conductance Ca$^{2+}$-sensitive K$^+$ (BK$_{Ca}$) channels [27, 48], background K$^+$ current (TASK) [55], and elevated [Ca$^{2+}$], in glomus cells [55]. NaHS-induced elevation of [Ca$^{2+}$] was abolished in the absence of extracellular Ca$^{2+}$ [55, 56] or in case that the depolarization was prevented by voltage clamping at the resting membrane potential [55]. In cultured rat glomus cells, NaHS-induced elevation of [Ca$^{2+}$], was blocked by nifedipine, a L-type VDCC blocker [56]. Similar finding was observed in both astrocytes [57] and rat neurons [58]. These findings support the view that L-type VDCC may be involved in H$_2$S-induced elevation of [Ca$^{2+}$], in carotid bodies [56] (Figure 2). A series of studies demonstrated that H$_2$S was also involved in the activation of T-type VDCC [59–61]. More recently, it is suggested that Ca$_{v}$3.2 T-type VDCC contributed to H$_2$S-mediated carotid body response to hypoxia [62]. Ca$_{v}$3.2 was the major T-type VDCC isoform expressed in the carotid body [62] (Figure 2). In brief, all abovementioned observations indicate that H$_2$S may function as a physiological excitatory mediator of the carotid body sensory response to hypoxia (Figure 2). Moreover ion channels are closely correlated with this regulation (Figure 2).

In addition, exogenous application of H$_2$S inhibited mitochondrial function in rat glomus cells [55]. The author proposed that the inhibitory effect of H$_2$S on TASK was due to inhibition of oxidative phosphorylation. Given that mitochondrial reactive oxygen species is speculated to mediate carotid body action [63, 64], whether mitochondria is directly involved in H$_2$S-mediated excitatory sensing in carotid bodies needs to be demonstrated. In addition, it is reported that neurotransmitters were involved in H$_2$S-induced sensory excitation of carotid bodies. Acetylcholine (ACh) and adenosine triphosphate (ATP) are two excitatory neurotransmitters in the carotid bodies of both cats and rats [27, 65, 66]. Inhibition of purinergic receptors using pyridoxal phosphate-6-azophenyl-2′,4′-disulfonic acid, or application of hexamethonium, a blocker of nicotinic cholinergic receptors, prevented NaHS-evoked sensory excitation of the mouse carotid body [27]. This study indicates that the release of ATP/ACh from glomus cells is correlated with H$_2$S-induced sensory excitation. However, Na$_2$S (another H$_2$S donors) significantly reduced the release of both ACh and ATP in the cat carotid body [65]. The effect of H$_2$S on neurotransmitters release needs to be further established. Whether neurotransmitters release is associated with H$_2$S-regulated carotid body activity is not clear.

3.5. Hypoxia-Induced H$_2$S Increase: Evidence for Interaction of CSE with HO-2. In CSE knockout mice and rats treated with PPG, hypoxia failed to increase H$_2$S generation in both mouse and rat carotid bodies [26]. These observations suggest that hypoxia-induced H$_2$S production may be due to increased CSE activity. Hemeoxygenase-2 (HO-2) converts heme to CO [67]. HO-2 is constitutively expressed in type I cells of mammals [16]. It has been demonstrated that CO is an inhibitory mediator for carotid bodies hypoxia sensing [16] (Figure 2). Under normoxia, pharmacological inhibition of HO-2 greatly increased H$_2$S generation [26]. In contrast, a CO donor inhibited the hypoxia-evoked H$_2$S production in the carotid body [26]. These couple of findings suggested that CO may be a negative regulator for H$_2$S production in the carotid body (Figure 2). In CSE knockout mice, the HO-2 inhibitor had no effect on H$_2$S generation. Under hypoxia, reduced CO generation may improve H$_2$S generation and the sensory action of the carotid body [26]. Taken together, it was proposed that H$_2$S generation is negatively controlled by CO-inhibited CSE activity [26] (Figure 2). Given that CSE does not contain a heme group, this enzyme is unlikely to be bound by CO [1, 14]. To date, the mechanisms by which CO inhibits CSE activity are still unknown.

3.6. H$_2$S-Mediated Sensory Activity of Carotid Bodies in Diseases. More recently, inherent variations in CO-mediated H$_2$S have been shown to contribute to reflex variation of carotid body O$_2$ sensing in three genetically distinct rat strains, including Sprague-Dawley (SD) rats, Brown-Norway (BN) rats, and spontaneous hypertensive (SH) rats [28]. In comparison with SD rats, BN rats display a profoundly reduced ventilatory response to hypoxia and also develop pulmonary edema when challenged with hypoxia [28, 68]. SH rats, compared with SD rats, exhibit an exaggerated response to hypoxia [69] and this alteration is vital for the development of essential hypertension [70]. Variability in kinetic properties of HO-2 was shown in liver microsomes from SD, BN, and SH rats, consistent with changes in CO levels from these three rat strains [28]. The authors proposed that variability of HO-2 activity of the liver was similar to that of the carotid bodies, based on the observation that hypoxic responses of the liver microsomes paralleled those of carotid bodies in the respective strain [28]. Impaired hypoxic sensitivity in BN rats was associated with augmented basal levels of CO and reduced H$_2$S [28]. In contrast, the exaggerated hypoxic response in SH rats was correlated with
lower CO and higher basal H\textsubscript{2}S [28]. Different substrate affinities for HO-2 resulted in various basal levels of CO and H\textsubscript{2}S generation [28]. Taken together, this series of findings further demonstrates that CO-H\textsubscript{2}S-mediated O\textsubscript{2} sensing is a fundamental mechanism in the carotid body chemosensory reflex [28].

4. Summary and Perspective

An abundant and continuous supply of O\textsubscript{2} is essential for survival of all mammalian cells. Reduced O\textsubscript{2} availability or hypoxia evokes many important physiological responses and changes, for the sake of increasing oxygen delivery. The activation of carotid bodies is a sensitive and prompt response to hypoxia, rapidly enhancing general O\textsubscript{2} supply. The family of HIFs, master regulators of cellular O\textsubscript{2}, induces an elegant series of delayed changes through activating target genes expression under hypoxia.

Emerging evidence shows that H\textsubscript{2}S plays a crucial role in O\textsubscript{2} sensing, through regulating carotid body activity and HIF-1 action. The regulation of HIF-1 accumulation via exogenous H\textsubscript{2}S has been shown, but the mechanisms for this effect are largely elusive and controversial. The patterns for H\textsubscript{2}S-regulated HIF-1 may be dose-dependent. Further studies need to identify the role of endogenous H\textsubscript{2}S on HIF-1 action. Compared with many studies on the role of H\textsubscript{2}S on HIF-\textsubscript{1a}, few have been done to elucidate the interaction between H\textsubscript{2}S and HIF-2a. Glomus cells had higher expression of HIF-2\textalpha than that of HIF-1\textalpha [71]. Heterozygous HIF-1\textalpha gene-knockout mice had no carotid bodies responses to hypoxia [72]. Carotid bodies from mice with heterozygous knockout of HIF-2\textalpha gene exhibited exaggerated responses to hypoxia [73]. It was proposed that a balance between HIF-1\textalpha and HIF-2\textalpha contributed to changes of redox homeostasis in the carotid body [15]. Based on this hypothesis, would H\textsubscript{2}S affect HIF-1\textalpha and/or HIF-2\textalpha in the carotid body? Endogenously generated H\textsubscript{2}S has been shown as a stimulator of carotid body activity under hypoxia. Although CO has been shown to negatively regulate CSE-catalyzed H\textsubscript{2}S production, the accurate molecular mechanisms by which CO affects CSE activity are still unknown. Moreover, the effect of CBS/H\textsubscript{2}S on carotid body action is elusive.

One of the most recently identified mechanisms for H\textsubscript{2}S effect is S-sulphydrlation [74–76]. The potential targets of S-sulphydrlation in H\textsubscript{2}S-mediated O\textsubscript{2} sensing remain to be determined. In addition, a novel study showed that H\textsubscript{2}S activated protein kinase G (PKG) \textalpha oxidation by inducing disulfide formation [77]. The key mediator in that event is polysulfides. H\textsubscript{2}S rapidly converts to polysulfides when H\textsubscript{2}S contacts O\textsubscript{2} or hydrogen peroxide [1, 77]. It is not yet clear whether polysulfides are involved in H\textsubscript{2}S-mediated carotid body response to hypoxia or H\textsubscript{2}S-regulated HIF functions.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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