Oxygen tension, H_2S, and NO bioavailability: Is there an interaction?

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Abstract

Molecular oxygen (O_2) is an essential component for the survival and development of many organisms. Mammalian and other vertebrate systems have evolved to maintain O_2 homeostasis and respond to changes in O_2 concentrations. Variation in O_2 levels leads to changes in molecular signaling and ultimately affects the physiological functions of many organisms. Nitric oxide (NO) and hydrogen sulfide (H_2S) are two gaseous cellular signaling molecules that play key roles in several physiological functions involved in maintaining vascular homeostasis including vasodilation, anti-inflammation, and vascular growth. Apart from the aforementioned functions, NO and H_2S are believed to mediate hypoxic responses and serve as O_2 chemosensors in biological systems. In this literature review, we briefly discuss the roles of NO and H_2S during hypoxia.

Introduction

Oxygen (O_2) is one of the key factors required for cellular respiration, growth, and development of organisms. It serves as a modulator of various cellular signaling and physiological functions. In contrast to ambient O_2 concentrations (21%), O_2 tension in tissues ranges between 0 and 9%, directly relating to the metabolic demand of a given cell type in a given organ (32, 55). The mechanism of cellular response and adaptation to changing O_2 concentrations, such as hypoxia, is a subject of continuous interest to basic scientists and medical professionals alike.

In cells, O_2 acts as the primary electron acceptor in multiple intracellular biochemical reactions, including the generation of ATP by mitochondria for survival. However, low O_2 conditions play an integral role in both pathophysiological and physiological functions, such as embryonic development, including differentiation of embryonic stem cells and progenitor cells (20). A low O_2 environment can result from insufficient blood flow to the tissue leading to inadequate tissue oxygenation, tissue hypoxia, and a reduction of mitochondrial respiration or oxidative metabolism (83). Ultimately, chronic exposure of tissue to hypoxic conditions can lead to necrosis. Cells respond to these ischemic conditions through stimulation of several molecules that regulate various physiological functions including proliferation, migration, vascular regulation, growth, and remodeling (51). Recently two more gases, nitric oxide (NO) and hydrogen sulfide (H_2S), have been studied as potential therapeutic due to a complicated interplay each other and with O_2.

In ancient times Greeks, Egyptians, and Romans regularly bathed in natural sulfur springs as treatments for disease (59). The levels of H_2S in these sulfur springs vary based on the microbiota and O_2 content (74) and have been noted to have several beneficial effects such as anti-inflammatory, anti-microbial and vasodilatory properties (53). Between the well-documented historical reports and the modern day studies of organosulfur compounds, such as garlic having health benefits like lowering blood pressure and cholesterol, it is clear that H_2S can have cardiovascular benefits (5, 6). The use of nitrite (a precursor to NO) has also been documented since ancient times, first showing up as an additive in gunpowder in ancient China. Nitrite has also been

used as a food additive; its documented use as a cardiovascular therapeutic occurred around 1791 when it was used as a treatment for angina (12).

NO and H_2S are two major gaseous signaling molecules that play pivotal roles in the regulation of vascular tone and remodeling, anti-inflammation, and neurological functions. NO is highly reactive and circulating pools of nitrite are typically reported to demonstrate the bioavailability of nitric oxide, plasma levels of nitrite are in the high nanomolar range (68). H_2S is found in blood and tissues at concentrations below 1 μM, however there is contention in the field over this number with much higher values being reported; the conflicting data reflects the lack of a standard measuring technique (39). Recent literature reflects an increased study of the interactions and co-adducts of NO and H_2S (45, 46). Although the individual roles these two gaseous molecules play in both physiological and pathophysiological function is appreciated, consequences of their interactions are less well known. Understanding the interactions between these two molecules will provide a better understanding of their therapeutic effects. The present review focuses primarily on the probable interactions between NO and H_2S on pathophysiological functions under hypoxic conditions.

Mitochondrial Respiration/Cytochrome C Oxidase:

Cells generate ATP through the electron transport chain. The final enzyme in the respiratory electron transport chain is cytochrome c oxidase (CcO) or Complex IV (91). CcO is a large transmembrane protein complex that is found in bacteria and eukaryotic mitochondria. It contains two heme centers, cytochrome a, cytochrome a_3, and two copper centers (Cu_A and Cu_B). O_2 is reduced at cytochrome a_3 and one copper center

66 (Cu_B) in the cell, this is the interaction we focus on for figure 1 as NO and H_2S both
67 interact with this reaction. CcO is found in its oxidized (active) form when O_2 is in
68 sufficient supply (figure 1 panel A), but is found mainly in the reduced form as O_2
69 becomes scarce (91). When CcO is in its reduced state O_2 binding is decreased, yet
70 NO binds to heme in its ferrous state (Fe^{2+}). However, when CcO is in its oxidized form,
71 O_2 is bound to the heme while NO binds one of the two copper centers. Both of these
72 binding modifications are reversible. Interestingly, binding of NO to the oxidized CcO
73 results in oxidation of NO to nitrite. As O_2 concentrations decrease, NO is no longer
74 bound to the oxidized CcO and thus is no longer converted to nitrite. The available NO
75 molecules compete with the O_2 molecules, ultimately inhibiting CcO activity (figure 1
76 panel B). A protective mechanism of NO is then engaged and soluble guanylate
77 cyclase is activated, leading to vasodilation, thereby enhancing O_2 delivery through
78 increased bulk blood flow in an effort to combat the NO competition (91).

79 Mitochondrial interactions of H_2S are complex and poorly understood. H_2S can
80 act as both an inhibitor and an electron donor for CcO, depending on the
81 concentrations of O_2 and H_2S in the system (61). H_2S concentrations are low (≥10–
82 20 nM) in normoxic concentrations, but are increased in hypoxic conditions. At low H_2S
83 concentrations, H_2S is oxidized by sulfide quinone reductase (SQR), which protects
84 CcO from inactivation (18, 30). However, hypoxia leads to increases in H_2S levels that
85 subsequently inhibit CcO (figure 1 panel D). This inhibition of CcO may result in the
86 generation of mitochondrial reactive oxygen species as observed under hypoxic

conditions. In contrast to the competitive inhibition of CcO by the binding of NO and O_2, the inhibition of CcO by H_2S is noncompetitive with O_2 (18, 30).

At low concentrations and normoxic conditions, H_2S can rapidly reduce Fe^{3+}, Cu^{2+}, and cytochrome *c*, the biological reductant of CcO (17, 30). A recent study correlating H_2S to the hibernation of brown bears, *Ursus arctos*, shows that alteration of H_2S metabolism and intracellular GSH leads to aerobic metabolic suppression during hibernation (76). Other recent studies have demonstrated that mitochondrial inhibition may lead to a suspended animation-like state (9) with decreased O_2 consumption and metabolism. By exploiting this hypometabolic phenomenon, protection from ischemic reperfusion injury could be provided (10, 31). The complex interplay between O_2, NO and H_2S does not end with influencing of CcO; O_2 concentrations alone can directly influence the production of NO and H_2S as well.

Effects of O_2 on H_2S production:

H_2S can be generated endogenously through multiple pathways, including: L-cysteine by pyridoxal-5′-phosphate (PLP) dependent enzymes, cystathionine γ-lyase (CSE), cystathionine β-synthase (CBS) and from 3-mercaptopyruvate by 3-mercaptopyruvate sulfurtransferase (3-MST) with cysteine aminotransferase (CAT) (44). These various biosynthetic mechanisms have previously been described thoroughly in the literature. Interestingly, through direct and indirect interactions, O_2 influences the production of H_2S and the aforementioned mechanisms. Previously it was reported that the CBS enzyme has a regulatory heme cofactor that acts as a redox-dependent gas sensor (36). In its ferrous form (Fe^{2+}), the heme moiety of CBS can bind with

gaseous molecules such as CO and NO, leading to the inhibition of CBS catalytic activity (36). However, in the presence of O_2 it can be converted from the ferrous to ferric heme state (Fe^{3+}), thereby leading to a recovery of CBS enzymatic activity (36). Under hypoxic conditions the activity of CBS is increased through diminished Fe-CO interactions; an apparent result of this hypoxia-induced activity of CBS is the inhibition of the CO producing enzyme, hemoxygenase-2 (HO-2) (56).

The bioavailability of H_2S, whether in the context of steady state *in vivo* concentrations or supplementation via exogenous administration, is dictated by O_2 concentrations. O_2 has an antagonistic effect on H_2S, leading to its oxidation (48) and consequently attenuating its biological actions (85). The spontaneous reaction of H_2S with O_2, while slow, can cause an appreciable decrease in H_2S concentrations; tissues with relatively high O_2 concentrations may have less H_2S compared to tissues with lower O_2 tensions (63). This has implications in pathological states of hypoxia such as ischemia-reperfusion, where the availability and signaling effects of H_2S may be augmented; various studies have reported that H_2S production is enhanced during hypoxia and attenuated in the presence of O_2 (63, 97). Our group has previously demonstrated that O_2 concentration affects sulfide stability and its measurements from biological samples, apart from pH (79, 80). At a given pH of 9.5, the presence of 21% O_2 decreases the stability of sulfide to an approximate level of 70%; at 1% O_2, sulfide increases to >90% stability (80).

Effects of O_2 on NOS and NO production:

NO is an uncharged, small and membrane-permeable molecule that participates in cellular events either by directly modifying proteins via S-nitrosylation or by activating specific signaling pathways. The synthesis of cellular NO is enzyme driven and requires L-arginine and O_2 as substrates. In addition, cofactors such as tetrahydrobiopterin (BH4), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and reducing equivalents donated by NADPH are essential for NO production. NO is synthesized by the enzyme NO synthase (NOS) that exists in three isomeric forms, namely endothelial NOS (eNOS), inducible NOS (iNOS), and neuronal NOS (nNOS). Although the three isoforms generate NO, each enzyme maintains different binding affinities for substrates and differential cell type-specific expressions (29). The substrate L-arginine is hydroxylated enzymatically to N^{ω}-hydroxy-L-arginine, which then converts into L-citrulline and NO in a process requiring two molecules of O_2 (92). Each of the NOS enzymes are heme containing flavoproteins that produce NO in a calcium-dependent manner.

The catalysis of NOS, leading to the biosynthesis of NO, depends on the oxidation state of iron (Fe) in heme. Briefly, upon reduction of iron from its ferric state (Fe^{3+}) to its ferrous state (Fe^{2+}), Fe^{2+} binds with O_2 to form a Fe^{2+}-O_2 complex, which then reacts with N^{ω}-hydroxy-L-arginine to generate Fe^{3+} and NO. However, some of the NO formed by the reaction reacts with heme and forms a more stable Fe^{2+}-NO complex. The liberation of NO from the Fe^{2+}-NO complex and the regeneration of Fe^{3+} heme in the first step of the reaction requires O_2 (84, 88, 93). Interestingly, the release of NO is governed by the rate constant for the initial reaction of O_2 and Fe^{2+}, the rate

153 constant for NO dissociation, and the rate constant for reduction of heme (88). Each of
154 the NOS isoforms have different K_mO_2 values; in terms of enzymatic activity, K_mO_2 of
155 NOS is the amount of O_2 that is required to drive NOS catalysis to half of its maximal
156 velocity (93). The isoform with the highest rate constant is nNOS at 350 μM, followed
157 by iNOS with a value of 135 μM (21), and eNOS with the lowest rate constant at 23 μM.
158 The varying K_mO_2 values for the different NOS isoforms suggest that there is a
159 difference in NO production, dependent on the partial pressure of O_2 (29).

160 It is important to note that O_2 tension varies from tissue to tissue, resulting in
161 varying levels of NO production from each different NOS isoform. Based on their
162 respective K_mO_2 values, nNOS is the most sensitive and eNOS is the least sensitive to
163 changes in physiological O_2 tension levels (93). Using stop-flow experiments, Abu-Soud
164 et al. showed that the release of NO, trapped as Fe^{2+}-NO, through nNOS activity is
165 dependent on O_2 concentration; O_2 was demonstrated to be an important rate-limiting
166 factor in NO bioavailability (84).

167 As outlined above, the production of NO and H_2S is dependent on changes in
168 oxidative status and O_2 concentrations in a tissue or cell, an important factor to
169 consider in developing therapeutic treatment. Conditions that affect NO production
170 have also been shown to influence H_2S production; however, NO-H_2S interactions are
171 still poorly understood. Although research studying H_2S and NO interactions has
172 steadily increased in recent years, more studies must be conducted in order to identify
173 some of these complex interaction; a deeper understanding of the interactions

between the two gases would enable the scientific community to better explore potential therapeutic applications of H_2S and NO.

From the literature it is clear that both NO and H_2S regulate various pathophysiological conditions and are capable of influencing corresponding signaling mechanisms that are related in this process. Additionally, O_2 concentrations play an important role in production of NO and H_2S as discussed in the previous section. The following sections will discuss the regulation and status of NO and H_2S under varied O_2 levels during transport of oxygen, vasoregulation, and in cardiovascular and cerebral pathophysiology.

Hemoglobin

Hemoglobin (Hb) has a prominent role in the circulatory system, O_2 transport in blood. The transport of O_2 by hemoglobin is tightly regulated and the loading and unloading of O_2 is sensitive to pH, temperature, O_2 concentration, and several other physical factors, including H_2S. H_2S binds to Hb in red blood cells to form sulfhemoglobin, which decreases the affinity of hemoglobin for O_2 and thereby inhibits O_2 transport (7, 15). A reduction in the O_2 transport capacity of Hb then sets off a chain of reactions, adversely affecting electron flow and mitochondrial ATP formation as H_2S and HS^- ligate the heme a_3 of CcO (62), which can then activate the K_{ATP} channels (16). H_2S further affects hemoglobin under certain conditions, such as a significant decrease in Hb saturation (arterial O_2 saturation) during hypoxia (86, 87) as shown by Stein et. al.

195 H_2S further decreased Hb saturation under hypoxic conditions, decreasing O_2 transport
196 capacity and thereby inducing a state of hypometabolism or suspended animation.

197 Reduction of the ferric (Fe^{3+}) center to ferrous (Fe^{2+}) in hemes appears to be a
198 common reaction for all heme proteins that generate the highly reactive HS^- molecule,
199 eventually producing protein persulfides or inorganic polysulfides (60). A recent study
200 by the Banerjee lab showed that Hb plays an interesting role in facilitating oxidation of
201 H_2S (94). In its ferric state, Hb in red blood cells (RBCs) catalyze the oxidation of H_2S,
202 which then produces thiosulfate and hydropolysulfides. This study also demonstrated
203 that H_2S produced in RBCs is generated via the 3-mercaptopyruvate sulfurtransferase
204 (MST) pathway that facilitates the oxidation of H_2S in the presence of hemoglobin. The
205 methemoglobin-dependent sulfide oxidation cycle is completed by
206 NADPH/flavoprotein/methemoglobin reductase, which restores hemoglobin back to its
207 oxy-Hb state (94).

208 H_2S can also modify hemes in myoglobin and hemoglobin by reacting with the
209 oxyhemoglobin to generate sulfhemoglobin, a dangerous complex that disrupts O_2
210 loading in the blood (72). 'Sulfhemoglobinemia' is a medical condition (67) in which
211 heme is modified to form a sulfheme derivative (77). Sulfheme is likely irreversible and
212 impairs the O_2 binding capacity of the metal centers, leading to potentially lethal
213 cyanosis. On the other hand, reversible sulfide binding to Hb could be an area of
214 scientific interest as manipulating reversible sulfide binding could potentially regulate
215 the levels of free H_2S and maintain reserve pools in the circulation and decrease toxic
216 levels of sulfide. However, the role of hemoglobin–sulfide interactions and their kinetics

is still unclear. More studies focused on understanding heme-sulfide interaction and sulfide oxidation under physiological conditions must be performed in order to facilitate a more accurate understanding of the complex mechanisms that regulate O_2 transport.

Deoxygenated red blood cells are able to reduce nitrite to form NO, however oxygenated red blood cells oxidize nitrite to nitrate (43). During this reaction methemoglobin is formed. During periods of redox imbalance methemoglobin can also be formed by direct oxidation of hemoglobin by NO (28). The ferrous center of heme is oxidized to the ferric form and unable to bind oxygen during the formation of methemoglobin. Figure 2 represents the anticipated oxygen hemoglobin dissociation curve for a patient presenting with either mild methemoglobinemia or mild sulfhemoglobinemia. Sulfhemoglobin is unable to carry oxygen however high levels of sulfhemoglobin can be still be well tolerated due to the rightward shift (figure 2) of the oxygen hemoglobin dissociation curve (promoting oxygen unloading for tissues) (3). In contrast to sulfhemoglobin, methemoglobin causes a leftward shift (decreasing oxygen release) of the oxygen hemoglobin dissociation curve (figure 2), high levels will result in severe tissue oxygen deprivation (3).

The presence of an iron center in hemoglobin makes it an ideal candidate to study H_2S/NO interactions. Furthermore, hemoglobin is extremely sensitive to shifting O_2 concentrations in the blood milieu due to conditions such as ischemia, cellular metabolic demand, and hypoxia. More studies on the interactions between hemoglobin and the cellular signaling molecules NO and H_2S hold immense potential for creating a better understanding of O_2 depletion-related pathologies, the applicable

cardioprotective properties of H_2S and NO, and novel therapeutic strategies. For example, areas of blocked blood flow might benefit from delivery of extra H_2S and/or NO to the location, stimulating vasodilation and increasing the amount of blood delivered. This more effective delivery system could be utilized therapeutically.

Vasoregulation

The vasoregulatory effects of H_2S have been recently studied (33, 90). H_2S acts as a hyperpolarizing factor on blood vessels via the regulation of K^+ channel activity and elevation of cGMP, a second messenger molecule that relaxes smooth muscle cells and thereby increases blood flow (96). The effects of physiological O_2 concentrations and H_2S on vessel regulation and varied O_2 levels should also be considered; reports indicate that H_2S induced vasorelaxation at physiological O_2 levels is further potentiated at low O_2 conditions (43, 63).

However, with higher than normal O_2 levels, H_2S has the tendency to induce vasoconstriction (43), which could be due to oxidation of sulfide to sulfite. Possible H_2S interactions with NO and variations in nitrosothiols may also explain the differential effects of H_2S (2, 19, 23). It was shown that nitrosothiol formation causes hypoxic vasodilation, often mediated by red blood cells (19). However, a few studies report that the formation of nitrosothiol may cause vasoconstriction (2). Future studies that focus on H_2S - NO interactions with vasodilation should be performed in order to reconcile such discrepancies in the literature. Further elucidation of interactions between

physiological regulators of blood pressure and vasodilation would open numerous possibilities of therapeutic delivery and agents through H_2S - NO mechanisms.

Cardiovascular disease and I/R injury:

The formation of atherosclerotic plaques deprives the circulatory system of O_2, leading to reduced tissue perfusion and ischemia. Progression of this condition leads to severe vascular dysfunctions such as peripheral vascular disease (PVD), coronary artery disease (CAD), and myocardial injury. Models of atherosclerosis and cardiovascular dysfunction in the literature suggest that a decrease in bioavailable H_2S is a consequence of reduced expression of the enzyme cystathionine-γ-lyase (CSE) (52, 71, 98). Extensive studies have demonstrated the cytoprotective effects of H_2S under ischemic reperfusion (I/R) injury in the heart (13, 14, 22, 42, 64, 66, 81, 82). Sulfide-based therapies have been shown to ameliorate the metabolic changes that contribute to cardiovascular disease and these protective effects have been demonstrated in various animal species (13, 14, 22, 42, 64, 66, 81, 82).

H_2S therapy improves multiple cardiac functions such as collateral formation, improved left ventricular (LV) pressures, suppression of leucocyte infiltration, attenuation of fibroblast hyperplasia, and the preservation of mitochondrial O_2 consumption. In a myocardial infarction model using cardiac-specific $CSE^{-/-}$ and CSE overexpressed mice, Lefer and colleagues demonstrated that CSE interactions with H_2S increase the survival of mice through reduced oxidative stress and enhanced

cardiac function (13, 22, 42). However, H_2S as a treatment for chronic and end stage cardiovascular diseases requires more elaborate research.

In models of ischemia reperfusion, NO bioavailability is reduced due to many factors, such as oxidation, which results in a poor prognosis. Investigators have shown in cardiac ischemia reperfusion models that a healthy tissue phenotype can be restored by supplementing NO prodrugs (38). In murine models of peripheral artery disease, nitrite therapy augmented angiogenesis in a NO dependent manner (8, 40, 47, 68, 69). During myocardial ischemia reperfusion, eNOS derived-NO production is important in the attenuation of neutrophil recruitment and decreased infarct sizes (35).

NO can be found in the circulation in many different forms and can change forms at different sites in the body. In the plasma, the oxidation of NO forms nitrite (NO_2^-) ions and can undergo further oxidation to form nitrate (NO_3^-) ions. NO also reacts very rapidly with superoxide to form the potent peroxynitrite vasoconstrictor ($ONOO^-$), which is responsible for loss of NO bioavailability (78). The exact mechanisms of NO and H_2S in cardiovascular disease and I/R injury are not completely known and further investigation of the interactions between NO and H_2S in the context of O_2 is warranted.

Cerebral I/R injury:

Ischemic cerebrovascular disease is a serious health complication with high morbidity. Multiple studies have demonstrated that severe neurological conditions, such as stroke and Alzheimer's disease, are the result of a variety of vascular abnormalities (26). Several factors, including impairment of neurovascular coupling and

303 blood-brain barrier leakage, are responsible for the neurodegeneration that may lead to
304 chronic cerebral ischemia, thereby causing cognitive decline and behavioral changes
305 (4, 70).

306 Abe and Kimura were the first to demonstrate the function of H_2S as a
307 neuromodulator, serving as a potential physiological signal regulator at low
308 concentrations and a toxic gas at high concentrations (1). Normal regulation of
309 neuronal and cerebrovascular functions is dependent on H_2S (41)**.** The effect of H_2S on
310 the brain varies depending on its concentration and the extent of hypoxia/ischemia-
311 induced injuries. Levels of S-adenosylmethionine, a molecule made from ATP which
312 plays an integral role in anabolic reactions, are reduced in patients with Alzheimer's
313 disease, possibly due to reduced CBS activity and H_2S production (50,58). CBS is
314 linked to neurodegenerative diseases caused by genetic defects such as
315 Homocystinuria, Down Syndrome, and Huntington's Disease (11, 37). These
316 observations suggest that neuronal dysfunction is directly related to the abnormal
317 regulation of H_2S production.

318 A variety of protective effects of H_2S are mediated by endogenous and
319 exogenous concentrations of H_2S. H_2S may function as a neuromodulator by
320 enhancing the N-methyl-D-aspartate (NMDA) receptor-mediated responses and
321 subsequent hippocampal long-term potentiation (LTP) (1). Studies demonstrate that
322 H_2S reduces infarct size, inflammation and apoptosis, as well as, improves neurological
323 function by reducing hippocampal damage in cerebral occlusion models (24, 49, 75,
324 99). Additional protective effects have also been demonstrated in the overexpression of

H_2S producing enzymes, such as CSE and CBS, which delay cerebral ischemic injury and improve neurological function (27, 54). Production of H_2S by CSE causes post-ischemic cerebral vasodilation and plays a significant role in early disruption of the blood brain barrier following cerebral ischemia (34).

Several studies have demonstrated that an excess production of H_2S can lead to severe cerebral damage. A study in a rat cerebral ischemia model showed that increased CBS expression and corresponding H_2S levels resulted in a damaged cortex region with an increased infarct volume. However, upon administration of CBS/CSE inhibitors that reduced cortical H_2S production, a correlating reduction in infarct size was observed (73). Similarly, in a global cerebral ischemia model, abnormally high concentrations of H_2S treatments enhanced neuronal injury, while low concentrations attenuated damage (75). This biphasic response should be further researched to discover the precise role of H_2S in cerebral I/R disease and the corresponding concentrations of H_2S during ischemic events.

Shortly following cerebral ischemia, typically caused by a stroke, eNOS releases NO locally leading to vasodilation as a protective mechanism (57). However, in long-term stroke-induced ischemia an overproduction of NO by nNOS and iNOS leads to exacerbated injury (57). The biphasic nature of NO release has led to a variety of therapeutic strategies during stroke-induced ischemia. The ideal time to administer NO-releasing drugs has been determined to be during the initial or protective phase (25), which would then ideally followed be by an inhibition of NO to prevent damage during the second or detrimental phase.

In addition, both H_2S and NO have been found to have a biphasic relationship in the brain. Early production of NO and low levels of H_2S are found to have a beneficial, protective result, while late production (excess NO) and high levels of H_2S have been found to be detrimental. While this provides an opportunity for the therapeutic delivery of both of these gaseous molecules, possible interactions of H_2S and NO must be taken into consideration while designing potential therapy. If H_2S and NO influence one another either in production or in chemical interaction, therapeutic doses of one without considering the effects of the other could result in a non-therapeutic outcome.

H_2S protection and recovery from an I/R mediated injury and oxidative stress is a well-studied phenomenon. Several studies have been carried out using genetic or pharmacological approaches in multiple organs such as the heart and brain to elucidate the role of H_2S in I/R injury, oxidative stress, and apoptosis. However, the mechanisms that mediate H_2S-induced protection, specifically via interactions with NO, remain unknown and require further study.

Conclusion

Over the past decade, there have been several studies that demonstrate the physiological effects of H_2S in mammalian systems. Therapeutic potential of H_2S has been exploited for treating multiple defects including cardiovascular dysfunction, inflammation, ischemia-reperfusion injury and shock (89, 95). There are many H_2S-producing compounds that regulate various biological functions and likely interact with NO (39). In this review we have discussed both the endogenous and exogenous effects

of H_2S on pathophysiological functions, focusing on the hypoxic/ischemic setting. However, a comprehensive mechanism of H_2S-mediated effects and its interactions with NO under varied O_2 conditions has yet to be studied. Research on the interactions of NO and H_2S under varied O_2 conditions would prove immensely beneficial in developing novel therapeutic strategies.

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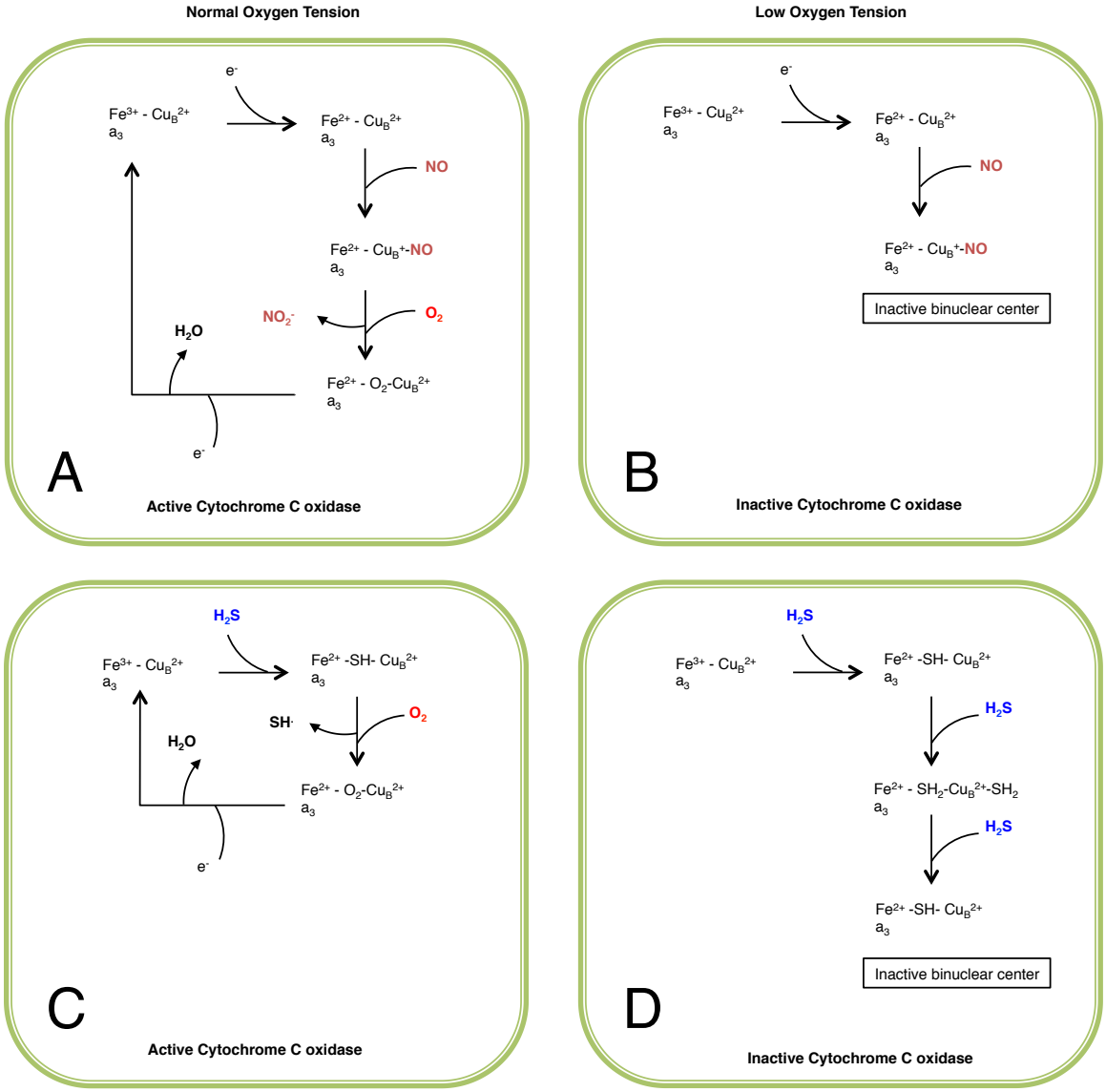

Figure 1: NO interaction with CcO under normoxic conditions (A), and low oxygen conditions (B). H_2S is shown reacting with CcO under normoxic (C), and hypoxic (D) conditions. NO, nitric oxide; H_2S, hydrogen sulfide; Fe, iron; Fe^{3+} (oxidized), Fe^{2+} (reduced); Cu, copper; e^-, electron; SH, sulfhydryl group.

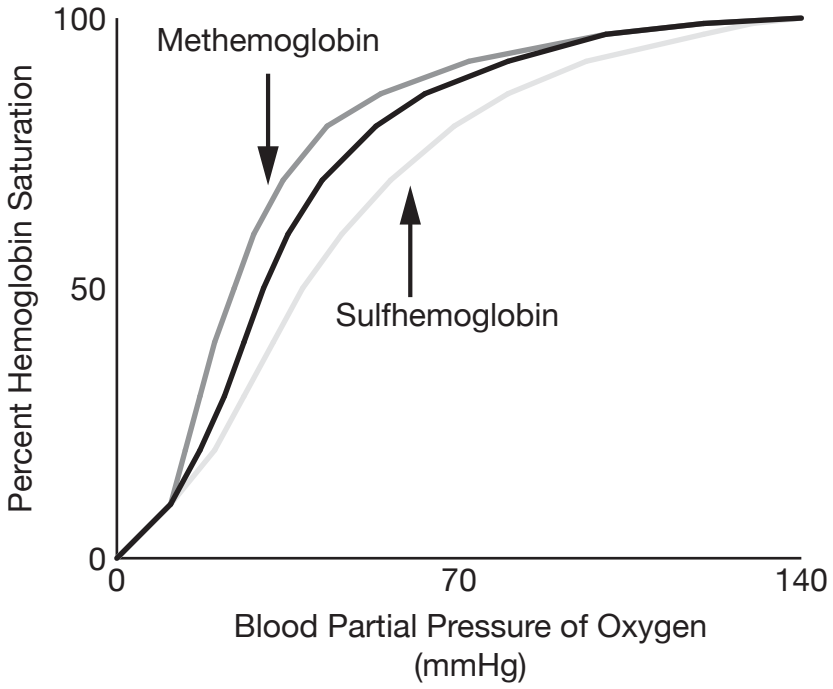

Figure 2: Hydrogen sulfide interaction with hemoglobin (sulfhemoglobin) causes a rightward shift in the oxyhemoglobin dissociation curve, however NO interaction with hemoglobin (methemoglobin) causes a leftward shift.