

Oxygen tension, H₂S, 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 H₂S 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). H₂S 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 H₂S (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 H₂S 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 a₃, and two copper centers (Cu_A and Cu_B). O₂ is reduced at cytochrome a₃ and one copper center

(Cu_B) in the cell, this is the interaction we focus on for figure 1 as NO and H₂S both interact with this reaction. CcO is found in its oxidized (active) form when O₂ is in sufficient supply (figure 1 panel A), but is found mainly in the reduced form as O₂ becomes scarce (91). When CcO is in its reduced state O₂ binding is decreased, yet NO binds to heme in its ferrous state (Fe²⁺). However, when CcO is in its oxidized form, O₂ is bound to the heme while NO binds one of the two copper centers. Both of these binding modifications are reversible. Interestingly, binding of NO to the oxidized CcO results in oxidation of NO to nitrite. As O₂ concentrations decrease, NO is no longer bound to the oxidized CcO and thus is no longer converted to nitrite. The available NO molecules compete with the O₂ molecules, ultimately inhibiting CcO activity (figure 1 panel B). A protective mechanism of NO is then engaged and soluble guanylate cyclase is activated, leading to vasodilation, thereby enhancing O₂ delivery through increased bulk blood flow in an effort to combat the NO competition (91).

Mitochondrial interactions of H₂S are complex and poorly understood. H₂S can act as both an inhibitor and an electron donor for CcO, depending on the concentrations of O₂ and H₂S in the system (61). H₂S concentrations are low (≥10–20nM) in normoxic concentrations, but are increased in hypoxic conditions. At low H₂S concentrations, H₂S is oxidized by sulfide quinone reductase (SQR), which protects CcO from inactivation (18, 30). However, hypoxia leads to increases in H₂S levels that subsequently inhibit CcO (figure 1 panel D). This inhibition of CcO may result in the 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 O₂, the inhibition of CcO by H₂S is noncompetitive with O₂ (18, 30).

At low concentrations and normoxic conditions, H₂S can rapidly reduce Fe³⁺, Cu²⁺, and cytochrome c, the biological reductant of CcO (17, 30). A recent study correlating H₂S to the hibernation of brown bears, *Ursus arctos*, shows that alteration of H₂S 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 O₂ consumption and metabolism. By exploiting this hypometabolic phenomenon, protection from ischemic reperfusion injury could be provided (10, 31). The complex interplay between O₂, NO and H₂S does not end with influencing of CcO; O₂ concentrations alone can directly influence the production of NO and H₂S as well.

Effects of O₂ on H₂S production:

H₂S 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, O₂ influences the production of H₂S 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 (Fe²⁺), 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 O₂ it can be converted from the ferrous to ferric heme state (Fe³⁺), 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 H₂S, whether in the context of steady state *in vivo* concentrations or supplementation via exogenous administration, is dictated by O₂ concentrations. O₂ has an antagonistic effect on H₂S, leading to its oxidation (48) and consequently attenuating its biological actions (85). The spontaneous reaction of H₂S with O₂, while slow, can cause an appreciable decrease in H₂S concentrations; tissues with relatively high O₂ concentrations may have less H₂S compared to tissues with lower O₂ tensions (63). This has implications in pathological states of hypoxia such as ischemia-reperfusion, where the availability and signaling effects of H₂S may be augmented; various studies have reported that H₂S production is enhanced during hypoxia and attenuated in the presence of O₂ (63, 97). Our group has previously demonstrated that O₂ 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% O₂ decreases the stability of sulfide to an approximate level of 70%; at 1% O₂, sulfide increases to >90% stability (80).

Effects of O₂ 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 O₂ 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 O₂ (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 (Fe³⁺) to its ferrous state (Fe²⁺), Fe²⁺ binds with O₂ to form a Fe²⁺-O₂ complex, which then reacts with N^ω-hydroxy-L-arginine to generate Fe³⁺ and NO. However, some of the NO formed by the reaction reacts with heme and forms a more stable Fe²⁺-NO complex. The liberation of NO from the Fe²⁺-NO complex and the regeneration of Fe³⁺ heme in the first step of the reaction requires O₂ (84, 88, 93). Interestingly, the release of NO is governed by the rate constant for the initial reaction of O₂ and Fe²⁺, the rate

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

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

As outlined above, the production of NO and H_2S is dependent on changes in oxidative status and O_2 concentrations in a tissue or cell, an important factor to consider in developing therapeutic treatment. Conditions that affect NO production have also been shown to influence H_2S production; however, NO- H_2S interactions are still poorly understood. Although research studying H_2S and NO interactions has steadily increased in recent years, more studies must be conducted in order to identify 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 H₂S and NO.

From the literature it is clear that both NO and H₂S regulate various pathophysiological conditions and are capable of influencing corresponding signaling mechanisms that are related in this process. Additionally, O₂ concentrations play an important role in production of NO and H₂S as discussed in the previous section. The following sections will discuss the regulation and status of NO and H₂S under varied O₂ levels during transport of oxygen, vasoregulation, and in cardiovascular and cerebral pathophysiology.

Hemoglobin

Hemoglobin (Hb) has a prominent role in the circulatory system, O₂ transport in blood. The transport of O₂ by hemoglobin is tightly regulated and the loading and unloading of O₂ is sensitive to pH, temperature, O₂ concentration, and several other physical factors, including H₂S. H₂S binds to Hb in red blood cells to form sulfhemoglobin, which decreases the affinity of hemoglobin for O₂ and thereby inhibits O₂ transport (7, 15). A reduction in the O₂ transport capacity of Hb then sets off a chain of reactions, adversely affecting electron flow and mitochondrial ATP formation as H₂S and HS⁻ ligate the heme a₃ of CcO (62), which can then activate the K_{ATP} channels (16). H₂S further affects hemoglobin under certain conditions, such as a significant decrease in Hb saturation (arterial O₂ saturation) during hypoxia (86, 87) as shown by Stein et. al.

H₂S further decreased Hb saturation under hypoxic conditions, decreasing O₂ transport capacity and thereby inducing a state of hypometabolism or suspended animation.

Reduction of the ferric (Fe³⁺) center to ferrous (Fe²⁺) in hemes appears to be a common reaction for all heme proteins that generate the highly reactive HS⁻ molecule, eventually producing protein persulfides or inorganic polysulfides (60). A recent study by the Banerjee lab showed that Hb plays an interesting role in facilitating oxidation of H₂S (94). In its ferric state, Hb in red blood cells (RBCs) catalyze the oxidation of H₂S, which then produces thiosulfate and hydropolysulfides. This study also demonstrated that H₂S produced in RBCs is generated via the 3-mercaptopyruvate sulfurtransferase (MST) pathway that facilitates the oxidation of H₂S in the presence of hemoglobin. The methemoglobin-dependent sulfide oxidation cycle is completed by NADPH/flavoprotein/methemoglobin reductase, which restores hemoglobin back to its oxy-Hb state (94).

H₂S can also modify hemes in myoglobin and hemoglobin by reacting with the oxyhemoglobin to generate sulfhemoglobin, a dangerous complex that disrupts O₂ loading in the blood (72). 'Sulfhemoglobinemia' is a medical condition (67) in which heme is modified to form a sulfheme derivative (77). Sulfheme is likely irreversible and impairs the O₂ binding capacity of the metal centers, leading to potentially lethal cyanosis. On the other hand, reversible sulfide binding to Hb could be an area of scientific interest as manipulating reversible sulfide binding could potentially regulate the levels of free H₂S and maintain reserve pools in the circulation and decrease toxic 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 O₂ 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 H₂S/NO interactions. Furthermore, hemoglobin is extremely sensitive to shifting O₂ 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 H₂S hold immense potential for creating a better understanding of O₂ depletion-related pathologies, the applicable

cardioprotective properties of H₂S and NO, and novel therapeutic strategies. For example, areas of blocked blood flow might benefit from delivery of extra H₂S 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 H₂S have been recently studied (33, 90). H₂S 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 O₂ concentrations and H₂S on vessel regulation and varied O₂ levels should also be considered; reports indicate that H₂S induced vasorelaxation at physiological O₂ levels is further potentiated at low O₂ conditions (43, 63).

However, with higher than normal O₂ levels, H₂S has the tendency to induce vasoconstriction (43), which could be due to oxidation of sulfide to sulfite. Possible H₂S interactions with NO and variations in nitrosothiols may also explain the differential effects of H₂S (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 H₂S - 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 H₂S - NO mechanisms.

Cardiovascular disease and I/R injury:

The formation of atherosclerotic plaques deprives the circulatory system of O₂, 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 H₂S is a consequence of reduced expression of the enzyme cystathionine-γ-lyase (CSE) (52, 71, 98). Extensive studies have demonstrated the cytoprotective effects of H₂S 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).

H₂S 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 O₂ consumption. In a myocardial infarction model using cardiac-specific CSE^{-/-} and CSE overexpressed mice, Lefer and colleagues demonstrated that CSE interactions with H₂S increase the survival of mice through reduced oxidative stress and enhanced

cardiac function (13, 22, 42). However, H₂S 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 (NO₂⁻) ions and can undergo further oxidation to form nitrate (NO₃⁻) 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 H₂S in cardiovascular disease and I/R injury are not completely known and further investigation of the interactions between NO and H₂S in the context of O₂ 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

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

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

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

H₂S producing enzymes, such as CSE and CBS, which delay cerebral ischemic injury and improve neurological function (27, 54). Production of H₂S 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 H₂S can lead to severe cerebral damage. A study in a rat cerebral ischemia model showed that increased CBS expression and corresponding H₂S levels resulted in a damaged cortex region with an increased infarct volume. However, upon administration of CBS/CSE inhibitors that reduced cortical H₂S production, a correlating reduction in infarct size was observed (73). Similarly, in a global cerebral ischemia model, abnormally high concentrations of H₂S treatments enhanced neuronal injury, while low concentrations attenuated damage (75). This biphasic response should be further researched to discover the precise role of H₂S in cerebral I/R disease and the corresponding concentrations of H₂S 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 H₂S and NO have been found to have a biphasic relationship in the brain. Early production of NO and low levels of H₂S are found to have a beneficial, protective result, while late production (excess NO) and high levels of H₂S have been found to be detrimental. While this provides an opportunity for the therapeutic delivery of both of these gaseous molecules, possible interactions of H₂S and NO must be taken into consideration while designing potential therapy. If H₂S 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.

H₂S 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 H₂S in I/R injury, oxidative stress, and apoptosis. However, the mechanisms that mediate H₂S-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 H₂S in mammalian systems. Therapeutic potential of H₂S has been exploited for treating multiple defects including cardiovascular dysfunction, inflammation, ischemia-reperfusion injury and shock (89, 95). There are many H₂S-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

369 of H₂S on pathophysiological functions, focusing on the hypoxic/ischemic setting.
370 However, a comprehensive mechanism of H₂S-mediated effects and its interactions
371 with NO under varied O₂ conditions has yet to be studied. Research on the interactions
372 of NO and H₂S under varied O₂ conditions would prove immensely beneficial in
373 developing novel therapeutic strategies.

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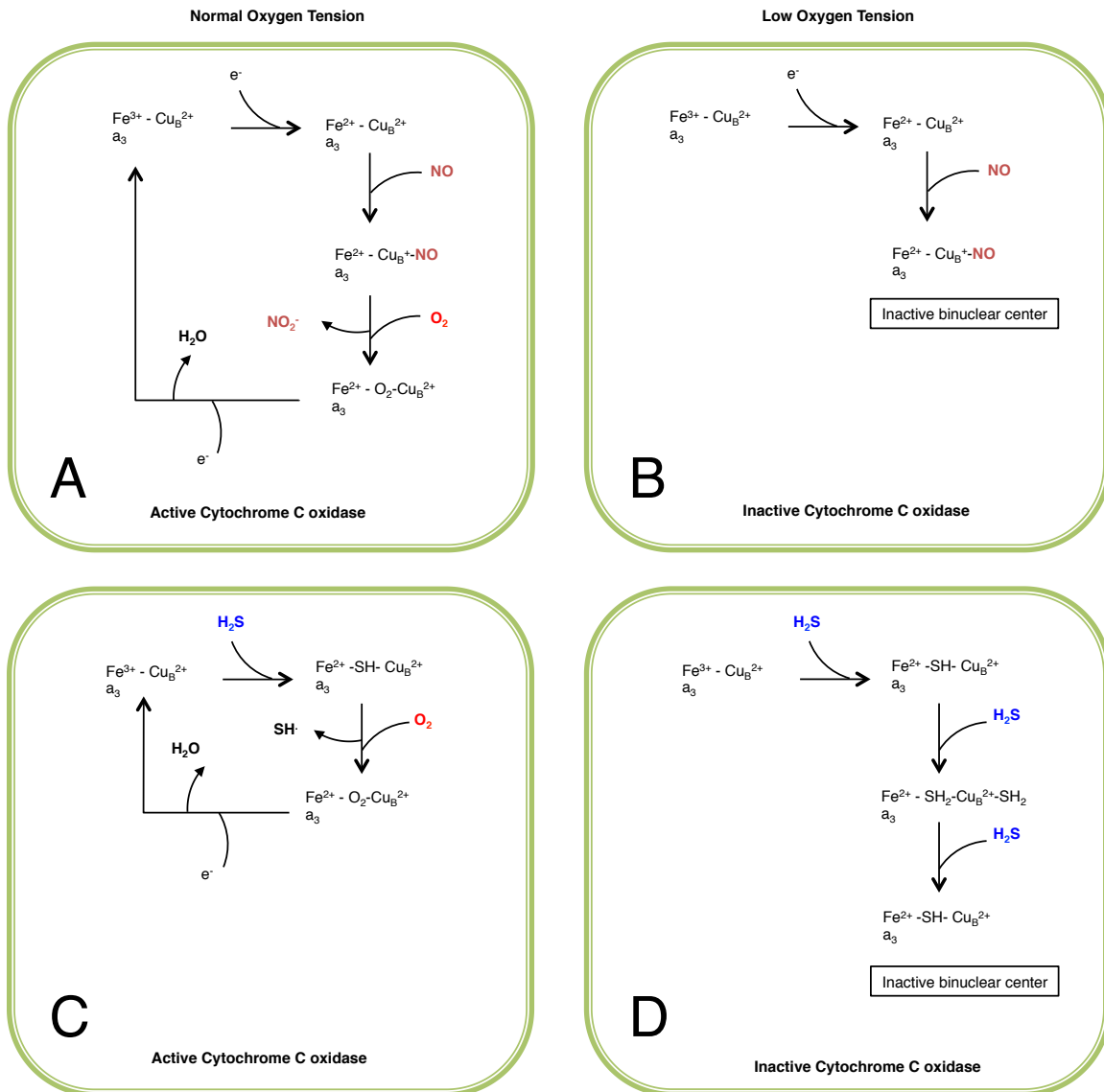


Figure 1: NO interaction with CcO under normoxic conditions (A), and low oxygen conditions (B). H_2S is shown reacting with CcO under normoxic (C), and hypoxic (D) conditions. NO, nitric oxide; H_2S , hydrogen sulfide; Fe, iron; Fe^{3+} (oxidized), Fe^{2+} (reduced); Cu, copper; e^- , electron; SH, sulfhydryl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

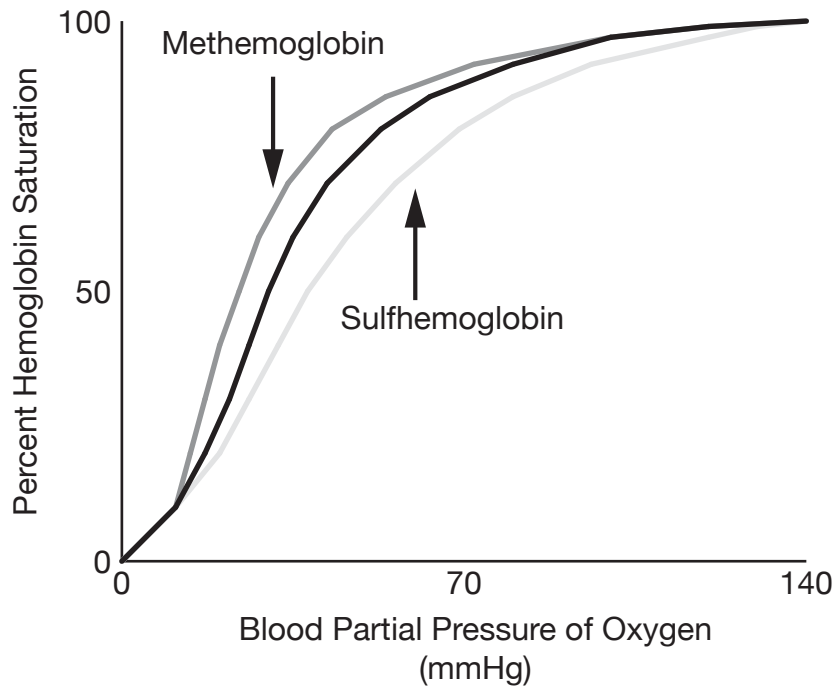


Figure 2: Hydrogen sulfide interaction with hemoglobin (sulfhemoglobin) causes a rightward shift in the oxyhemoglobin dissociation curve, however NO interaction with hemoglobin (methemoglobin) causes a leftward shift.