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Involvement of the Gasotransmitter Hydrogen Sulfide in the Effect of Ozone on the Hemoglobin Oxygen Affinity

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ABSTRACT

This paper presents current data on the blood oxygen transport function and the gasotransmitters system (hydrogen sulfide and nitric oxide) and their interconnection. Hydrogen sulfide contributes to the modification of hemoglobin oxygen affinity, which is achieved through various mechanisms: the formation of sulfhemoglobin, modulation of the intraerythrocytic 2,3-DPG system, the L-arginine-NO system, and also indirectly through systemic mechanisms of hemoglobin functional properties. Based on an analysis of literature and our own data, the role of hydrogen sulfide as a gasotransmitter in the formation of blood oxygen transport function and the development of oxidative damage and hypoxic conditions is discussed.

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Gasotransmitters

Gasotransmitters are a special group of gaseous signaling molecules that regulate various bodily functions both intracellularly and intercellularly. These gasotransmitters include nitric oxide, hydrogen sulfide, and carbon monoxide. Unlike classical messengers, which transmit signals via a cascade mechanism, gasotransmitters modify intracellular proteins. Understanding the molecular targets of gasotransmitters, the structure of their binding sites, and the specific features of their interactions can be crucial in developing pharmacological methods for regulating these signaling system.

Hydrogen Sulfide

The gasotransmitter H2S is produced in various mammalian cells and tissues by three main enzymes: cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfur transferase (3MST). Cystathionine β-synthase and cystathionine γ-lyase are pyridoxal phosphate-dependent enzymes that utilize L-cysteine and homocysteine as substrates, while the substrate for 3-mercaptopyruvate sulfur transferase is 3-mercaptopyruvate, which is produced from L-cysteine via an aminotransferase. An additional enzymatic pathway for H2S production, involving 3-mercaptopyruvate sulfur transferase and D-amino acid oxidase, has been identified in the kidney. It is important to note that cystathionine β-synthase and cystathionine γ-lyase can be inhibited by compounds with varying degrees of selectivity. DL-propargylglycine (PAG) is most commonly used to inhibit cystathionine β-synthase [1].

H2S is a non-synaptic mode of intercellular communication based on the diffusion of inorganic molecules through the intercellular space in all directions and their action on non-synaptic receptors distant from the site of their formation. This gasotransmitter has a wide range of physiological effects on various body systems: the central nervous system, respiration, circulation, and digestion. ATP-dependent potassium channels are the main target of H_2S in the cell. Activation of K^+-ATP channels and inactivation of voltage-dependent L-type Ca^{2+} channels causes a decrease in intracellular Ca^{2+} concentration, leading to hyperpolarization of the vascular smooth muscle cell membrane and relaxation [2]. Hydrogen sulfide is an important regulatory molecule that modulates the function of smooth muscle cells, exerting not only a relaxing effect, but also a constrictor effect due to the activation of the transmembrane ion exchanger Na^+, K^+, 2 Cl— cotransport by low concentrations of H2S.

It was found that glibenclamide does not prevent the increase in erythrocyte deformability under the influence of NaHS, and inhibition of soluble guanylate cyclase completely eliminates the increase in deformability and significantly reduces the aggregation capacity of these cells. Incubation of erythrocytes with the H2S donor sodium hydrosulfide is accompanied by an increase in their deformability and a significant decrease in aggregation [3]. Hydrogen sulfide at low concentrations induces cytoprotective antioxidant reactions, while higher concentrations can lead to mitochondrial inhibition and even cell death. Importantly, while low concentrations of H_2S typically have an anti-inflammatory effect, higher concentrations can stimulate various pro-inflammatory mechanisms [4].

Nitric Oxide and Hydrogen Sulfide

NO and H_2S, as gasotransmitters, share common properties: these gaseous signaling molecules are freely permeable to cell membranes, are generated endogenously and enzymatically, their production is precisely regulated, and they have clearly defined functions at physiologically relevant concentrations. H_2S donors have been shown to increase endothelial NO activity through phosphorylation of Ser1177 and protein kinase B, and therefore increase NO production. H_2S likely interacts with oxidized forms of NO (• NO) or reactive nitrogen species (ONOO–) in the presence of cellular oxidants such as molecular O2, ROS (e.g., H_2O_2), and oxidases, which can generate new molecules—nitrosothiol, nitric oxide, and sulfuric acid. [3].

The interaction of NO and H_2S-mediated signaling systems can occur through nitrosylation of free SH-groups in cystothionine-γ-lyase, as well as through the activation of protein kinase G. The interaction of NO and H2S is realized through various mechanisms, namely, NO increases: the expression of the enzyme cystothionine-γ-lyase, but inhibits its activity, cellular release of cysteine, leading to the formation of new S-nitrosothiols molecules, and H2S in acidosis induces the formation of NO from nitrites and other NO derivatives [5].

The interaction between NO and H_2S is important for the development of oxidative stress. H2S is known to reduce oxidative damage during endothelial dysfunction by acting as a superoxide anion scavenger and reducing its production by NADPH oxidase [6]. In the regulation of nitrosative DNA damage that induces activation of the SoxRS regulon, NO and H_2S molecules can function in a coordinated, complementary manner. H_2S reacts with peroxynitrite to form HSNO and sulfinyl nitrite, thereby reducing the negative effects of peroxynitrite and achieving a cytoprotective effect against various types of oxidative stress. [7].

It has been shown that NO or H_2S can also influence the concentration of each other. In this regard, along with studying the effects that NO and H_2S exhibit in isolation, the "cross-interaction" between these molecules is of particular interest. Gasotransmitters (NO, CO and H2S) interact, affecting bioavailability and reactivity of these gases. NO and H_2S can affect the formation of enzymes necessary for the synthesis of gasotransmitters, and also interact at the metabolite level. Thus, nitric oxide synthases (NOSs) substrates or NO donors can enhance the expression of enzymes that produce H_2S: sodium nitroprusside increases the activity of cystathionine β-synthase in a suspension of rat brain cells; L-arginine enhances the expression of mRNA for cystathionine γ-lyase and its activity in lung tissue; and incubation with NO donors increases H2S production in blood vessels [8].

NO inhibits the activity of cystathionine β-synthase by binding to its heme group. Sodium hydrosulfide (NaSH) has been shown to directly inhibit a recombinant form of bovine endothelial NOS, presumably through the following mechanism: the enzymatic activity of endothelial NOS is inhibited by a potential interaction between NaSH and endothelial NOS cofactors, such as NADP or tetrahydrobiopterin [9].

H2S and NO can not only inhibit each other's synthesis but also activate them. NO increases endogenous H2S production by increasing the expression of cystathionine γ-lyase and cystathionine β-synthase in vascular smooth muscle cells [10]. The hydrogen sulfide donor NaSH enhances NO synthesis by activating interleukin-1β-induced expression of inducible NOS [11]. It's clear that these gasotransmitters influence each other's synthesis and can also directly interact to form new molecular entities, which in turn modulate their physiological effects and bioavailability. The interaction of sodium nitroprusside and H2S leads to the formation of nitrosothiol-like species, which exhibit the same physiological effects as NO.

These gaseous signaling molecules, interacting with each other, form a complex system that controls the body's integrative processes. Many of H_2S physiological effects are due to its interaction with other gasotransmitters (NO, CO), which occurs both at the level of regulating the enzymes that synthesize them and at the level of their targets. Therefore, it is proposed to consider gasotransmitters not individually, but as a combination of molecules that regulate cellular processes.

Hydrogen Sulfide and the Blood Oxygen Binding Properties

Hemoglobin plays a key role in the facultative oxidation of H2S, resulting in the formation of thiosulfate and hydrosulfide. Methemoglobin also mediates the oxidation of H2S [12]. Together with NO derivatives of hemoglobin, sulfhemoglobin can alter the position of the oxyhemoglobin dissociation curve (ODC). Methemoglobin and nitrosohemoglobin shift the ODC to the left, while nitrosylhemoglobin and sulfhemoglobin shift it to the right. The interaction of NO and H2S may be important for the body's oxygen supply. Hydrogen sulfide metabolism is part of the oxygen-sensing mechanism: under normal conditions, H2S is oxidized in the mitochondria to sulfite; under hypoxia, its utilization decreases, and therefore its contribution to the hypoxic response increases. [13]. Undoubtedly, the gasotransmitter hydrogen sulfide is involved in the modification of the blood oxygen binding properties, which is achieved through various mechanisms: the formation of various hemoglobin derivatives (sulfhemoglobin), modulation of the intraerythrocytic 2,3-DPG system, the L-arginine-NO system, and also indirectly through systemic mechanisms of the functional Hemoglobin properties formation (Figure 1) [14,15].

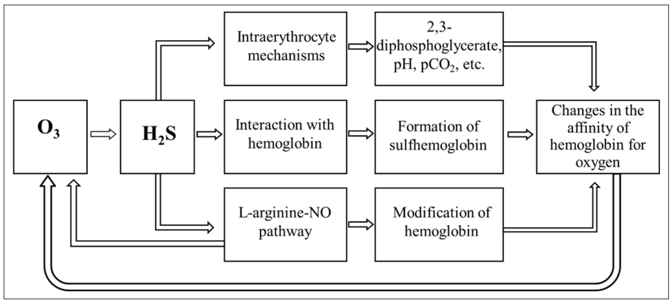

Figure 1: The main mechanisms of the ozone effect on the hemoglobin oxygen affinity with hydrogen sulfide participation

Ozone and Hydrogen Sulfide

Ozone effect on the NO-generating system is realized both through its direct impact on hydrogen sulfide synthesis mechanisms and the L-arginine-NO system activity, and through modification of the functional properties of hemoglobin. NO is an allosteric effector of the NO-generating system. The methemoglobin and nitrosohemoglobin formed during ozone interaction with hemoprotein increase hemoglobin oxygen affinity, while nitrosylhemoglobin decreases it. According our data nitroglycerin usage affecting NO-generating system leads to an enhanced effect of ozone [14].

The gasotransmitter hydrogen sulfide is involved in the effect of ozone on the blood oxygen binding properties (Figure 2). H2S is known to participate in the oxidative modification of protein molecules through sulfhydration via the H2S-mediated oxidation of cysteine to persulfides, facilitating their chemical reorganization [16]. In addition, H_2S promotes the activation of disphosphoglycerate mutase, which stimulates the synthesis of 2,3-DPG and reduces the hemoglobin oxygen affinity [17]. The interaction of hemoglobin with this gas additionally leads to the formation of sulfhemoglobin, which also reduces hemoglobin oxygen affinity. H_2S, in combination with an NO donor (nitroglycerin), enhances the effect of ozone on blood oxygen transport parameters [14]. It has been shown that combined treatment with NO and H_2S donors ensures the necessary NO-signaling activity through the formation of dinitrosyl iron complexes with persulfide ligands in the cell. The NO molecule interacts with cysteine, transforming its spatial structure and increasing the energy of chemical bonds. In our study, propargylglycine reduces nitrate/nitrite levels under ozone exposure, while sodium hydrosulfide leads to their increase [14]. NaHS and nitroglycerin, when combined, promote an increase in plasma H_2S. NaHS increases H_2S levels by 64.5% ($p < 0.05$). The mechanism of this phenomenon is based on increased phosphorylation of the constitutive isoforms of NO synthase, followed by increased NO production [18]. The effect of ozone on the blood oxygen binding properties was revealed when applied directly to the erythrocyte suspension, which is manifested in an increase in pO_2, SO_2 [19]. A shift in the oxyhemoglobin dissociation curve to the right is observed. The addition of a nitric oxide donor (nitroglycerin) enhances the effect of this gas on the oxygen transport parameters of the red blood cell suspension.

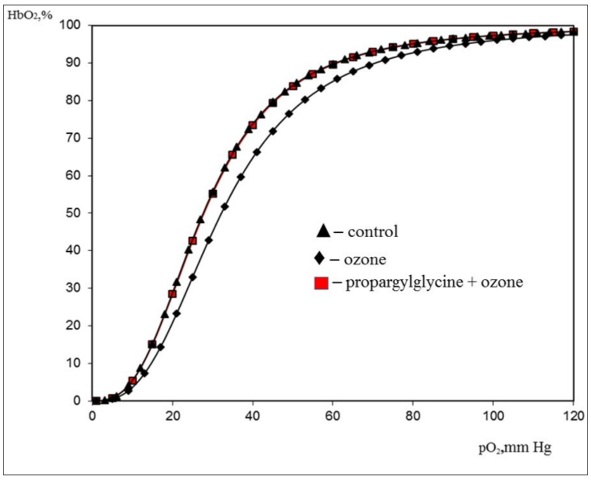

Figure 2: The effect of ozone on the position of the oxyhemoglobin dissociation curve at real pH and PCO_2 values under conditions of the cysteine/cystine system activity changes

Nitroglycerin is a potent mitochondrial regulator, reducing the oxygen affinity of cytochrome c oxidase, the terminal electron acceptor of the mitochondrial electron transport chain [20]. Erythrocytes are involved in maintaining the oxygen-dependent balance between the formation and oxidation of H2S due to their ability to generate this gasotransmitter [21]. Ozone significantly improves microcirculation by increasing the functional activity of capillaries, changing the physicochemical characteristics of the blood and, in particular, the blood oxygen binding properties [22]. Exposure to ozone under hypoxic conditions leads to an increase in NO_2-/NO_2-and H2S levels, while the addition of nitroglycerin and sodium hydrosulfide increases these levels. NO facilitates the body adaptation to changes in pO_2 by influencing the mechanisms that adjust the functional status of red blood cells. [23]. In addition, this gasotransmitter is the main regulator of vascular tone and angioprotector, which determines its effect on local microcirculation [24]. Complex relationships between O_3 and the group of NO synthase enzymes that participate in NO generation are described [25]. O_3 can activate the inducible isoform of NOS, which leads to an increase in the concentration of the latter, and also restores NO_2– at low pH values [26]. Another gasotransmitter, hydrogen sulfide, is also involved in these mechanisms. Red blood cells produce endogenous H2S using 3-mercaptopyruvate as a substrate [27]. H2S production in red blood cells depends on the serum NO level [28].

The mechanisms of intraerythrocytic regulation of oxygen hemoglobin affinity are realized at various levels: changes in the structural organization of erythrocytes, simulating the action of allosteric effectors on the hemoglobin molecule. In response to ozone, erythrocytes alter NO and H_2S formation, which directly influences both the modification of hemoglobin properties and indirectly, through hemoglobin-independent mechanisms, changes the structural organization of the erythrocyte membrane. Our studies demonstrate the effect of ozone on blood oxygen transport at the erythrocyte level, which is realized through modification of the intraerythrocytic hemoglobin oxygen affinity regulation system, thereby meeting the needs of aerobic metabolism at the regional and systemic levels. Modification of hemoglobin oxygen affinity is achieved through changes in the membrane organization of erythrocytes (Band-3), changes in their metabolism, ionic composition, the action of a number of modulators (2,3-DPG, glutathione, etc.), as well as the Bohr and Haldane effects. According to our data, detailed above, the gasotransmitter system participates in adaptive changes in the functional properties of hemoglobin under the influence of O_3 under conditions of varying pO_2 and pCO_2 values, meeting local tissue oxygen needs.

Conclusion

Analysis of literature data, our own experimental and clinical research results indicate that the gasotransmitter hydrogen sulfide acts as an allosteric effector on hemoglobin, altering its affinity for oxygen and determining the state of the blood oxygen transport function. It is hypothesized that hydrogen sulfide plays a role in adjusting the functional properties of hemoglobin by modifying its affinity for oxygen through systemic and regional, intraerythrocytic regulatory mechanisms. This is important in the pathogenesis of hypoxic conditions, endothelial dysfunction, and oxidative stress, and especially for their correction.

The efficiency of the oxygen transport system is determined by the functional state of the blood, its ability to bind the required amount of oxygen in the lungs and release it into tissue capillaries. The development of hypoxia leads to complex changes at all stages of oxygen transport processes. Blood oxygen transport mechanisms are the most important targets for the effects of NO and H2S. Impaired gasotransmitter function leads to a decrease of oxygen supply to the tissue, the development of oxidative damage, and hypoxic conditions. The involvement of H2S gas in the formation of blood oxygen transport function is important not only for oxygen delivery to tissues but also for maintaining the prooxidant-antioxidant balance.

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