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Review

Hydrogen Sulfide in the Cardiovascular System: a Small Molecule with Promising Therapeutic Potential

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Abstract: this review summarizes current knowledge of the hydrogen sulfide role in cardiovascular system, the proposed mechanisms of its action and the prospects for its applicability in the treatment of cardiovascular diseases. Hydrogen sulfide was recently recognized as gasotransmitter – simple signaling molecule which freely penetrates the cell membrane and regulates a number of biological functions. In humans endogenous H_2S is generated via enzymatic and non-enzymatic pathways and its content varies in different tissues and is strictly regulated. In cardiovascular system H_2S is produced by myocardial, vascular and blood cells and regulates a number of vital functions. Numerous experimental data prove that endogenously generated as well as exogenously administered H_2S exerts a wide range of actions in cardiovascular system, including vasodilator/vasoconstrictor effects, regulation of blood pressure, pro-apoptotic and anti-proliferative effects in the vascular smooth muscle cells, influence on angiogenesis and erythropoiesis, myocardial cytoprotection in ischemia-reperfusion injury, oxygen sensing, inhibition of platelet aggregation and blood coagulation, modification of erythrocyte microrheological properties (aggregability and deformability). Understanding of molecular mechanisms of H_2S action and molecular crosstalk between H_2S, NO, and CO is essential for the development of its diagnostic and therapeutic potential.

Keywords: gasotransmitters; hydrogen sulfide; cardiovascular system; circulation; hemostasis; erythrocytes; therapeutic potential.

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1. Introduction

For a long time, hydrogen sulfide was considered to be a useless malodorous gas, rather waste of the body. Nowadays H_2S is known as an endogenously produced gaseous signaling molecule which has been recognized recently as a gasotransmitter, likewise nitric oxide (NO) and carbon monoxide (CO). These three gasotransmitters (NO, CO, and H_2S) were initially simply regarded as toxic molecules, until the discovery that most forms of life - from bacteria to human -produce or scavenge all these gases to accomplish specific functions [1]. The strong toxicity of hydrogen sulfide, comparable to the poisonous effects of carbon monoxide and hydrogen cyanide, has been known for a long time [2], this harmful effect is most pronounced in the central nervous system [3]. H_2S blockades cytochrome c oxidase, inhibiting mitochondrial respiration and this is the main mechanism of its toxicity [4].

Gasotransmitters are a unique class of simple gaseous molecules freely penetrating cell membrane. Their intracellular storage becomes unnecessary due to this ability to easily diffuse into the cell, therefore the release of gasotransmitters is regulated at the level of

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their production. Once produced, gasotransmitters perform a number of specific physiological functions, regulating the activity of vital organs and systems [5].

In humans endogenous H_2S is generated via enzymatic and non-enzymatic pathways and its concentrations vary in different cells and tissues maintaining within a certain range [6], therefore the H_2S levels in various tissues are strictly regulated by balance of its production and consumption [7]. Cystathionine-γ-lyase (CSE) and cystathionine-β-synthase (CBS) are the main enzymes endogenously generating H_2S via direct desulfhydration of cysteine, indirect desulfhydration needs the involvement of reductants and is catalyzed by 3-mercapto-sulfurtransferase (3-MST) [8]. A new enzyme D-amino acid oxidase (DAO) was recently recognized as element of another pathway of H_2S production in presence of 3-MST [9]. It was shown that DAO presents only in brain and kidney, thus the effects of DAO/3-MST pathway are limited, while the significance of this pathway may be related with high H_2S concentration in cerebellum and kidney (up to 80-fold higher than that in other tissues) [10].

It is generally accepted that the main contribution to H_2S homeostasis belongs to the key enzymes (CSE, CBS, and 3-MST) which catalyze enzymatic converting of cysteine or its derivatives to H_2S. The expression of these enzymes differs in various tissues. CBS predominates in the nervous system, H_2S generation in the cardiovascular system is mainly due to CSE activity, 3-MST is responsible for H_2S production in the mitochondria.

Non-enzymatic process of H_2S generation is less understood, it was reported that this pathway involves cysteine and glucose and direct reduction of sulfur and glutathione, as well as endogenous persulfides and polysulfide species [11, 12]. For example, under physiological conditions the production of H_2S from sulfur-containing amino acids (cysteine) via vitamin B_6 and iron has been found in red blood cells and tissues [13]. However, the exact biological roles of this non-enzymatic generation of H_2S have not yet been established [14], and it should be noted that the definite contribution of enzymatic versus non-enzymatic sources to H_2S levels has never been determined in a biological system [15].

Because of H_2S toxicity, its bioavailability needs to be accurately and differentially adjusted in various tissues and organs, matching their specific physiological functions. Biosynthesis of H_2S is the first level of its bioavailability control, the regulation of enzymatic disposal of this potentially toxic molecule is the next important stage [1]. H_2S is a chemically active and labile molecule which can be rapidly synthesized and utilized in many ways after activation of signaling pathways [16]. In living cells H_2S is metabolized by methylation in cytosol or oxidized in mitochondria, in RBC it can be scavenged by methemoglobin forming sulfhemoglobin [17, 18].

Like nitric oxide (NO) and carbon monoxide (CO), H_2S plays important role both in physiological conditions and in the pathogenesis of several diseases [19]. H2S actively participates in regulation of the number of vital functions, its effect was revealed not only for cardiovascular system, it was shown its numerous effects for respiratory and nervous systems, metabolism, kidney, liver and reproduction function, notable anti-inflammatory and antioxidant responses as well as mitochondrial electron transport and cellular bioenergetics [20].

Cardiovascular diseases are considered multifactorial, but the main factors in their pathogenesis are heart and circulation disorders. Numerous investigations of H_2S therapeutic potential in cardiovascular diseases have revealed that hydrogen sulfide at physiological concentrations plays an important role in maintaining the normal functioning and homeostasis of cardiovascular system. Strong evidence has demonstrated a close relation-

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ship between the level of endogenous hydrogen sulfide and the excess risk of unfavorable outcome of cardiovascular diseases [21, 22, 23]. It was shown that and inhibitors of endogenous H_2S production or H_2S donors exert significant effects in cardiovascular diseases, including atherosclerosis, hypertension, ischemic myocardium and heart failure [6].

The expression of main enzymes catalyzing H_2S production such as cystathionine-β-synthase, cystathionine-γ-lyase and 3-mercaptosulfurtransferase within all tissues in cardiovascular system (vasculature, heart and blood cells), as well as detection of H_2S within these tissues strongly evidences that the cardiovascular system is an endogenous source of H_2S production [24, 25, 26]. Since the expression of H_2S-generating enzymes was demonstrated in the cardiovascular system for the first time, numerous experimental studies have been carried out to elucidate the role of hydrogen sulfide in maintaining cardiovascular homeostasis, to vary the content of hydrogen sulfide were used such approaches as overexpression/inhibition of H_2S-synthesizing enzymes or using of exogenous sources of H_2S – its donors [6].

The rapidly expanding published data relating to cardiovascular effects of H_2S evidence that this gaseous molecule mediates various processes in different types of cells and tissues through a number of mechanisms [26]. Some mechanisms and signaling pathways providing the H_2S effect have been established, however, a lot of unknowns on how H_2S influences cardiovascular homeostasis need to be clarified. The major effects and regulatory mechanisms of H_2S in the cardiovascular system, which will be discussed in this review, are schematically shown in Fig.1.

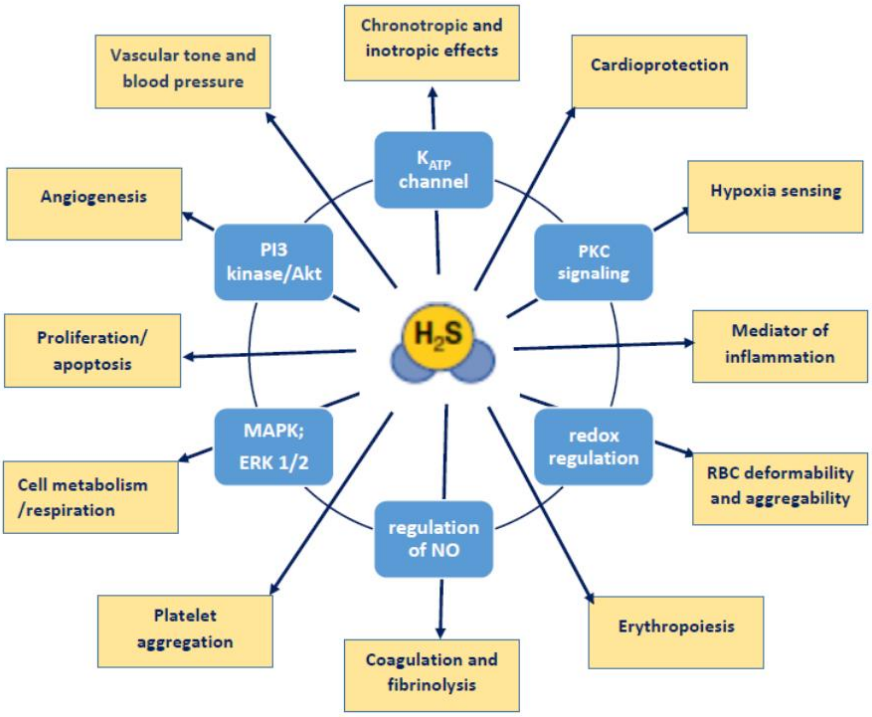

Figure 1. Scheme of the main effects and potential regulatory mechanisms of H_2S in cardiovascular system: Endogenous and exogenously supplied H_2S can regulate many functions in cardiovascular system, including heart functions, circulation and blood properties (outer circle) by means of a number of potential mechanisms that are not yet sufficiently understood (inner circle).

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The growing body of evidence for the biological and clinical importance of hydrogen sulfide in maintaining cardiovascular homeostasis will shed light on the pathogenesis of cardiovascular diseases and provide innovative approaches to their therapy [6].

2. Heart

2.1. Chronotropic and Inotropic Effects of H_2S

The information on the effects of H_2S on heart rates in published data is rather contradictory. A decrease in heart rate in presence of H_2S due to the inhibition of pacemaker cells in SA nodes in rabbits was reported in some studies. This negative chronotropic effect was blocked by inhibitor of K_{ATP} channels glibenclamide (20 μM) proving the involvement of K_{ATP} channels in mediating the chronotropic effect of H_2S [27]. It was shown that NaHS (donor of H_2S) improve arrhythmia associated with I/R injury. Single-channel recording on isolated cardiac myocytes demonstrated that 40 μM NaHS increased the open probability of K_{ATP} channels [28]. On the other hand, heart rate was almost unchanged after administration of H_2S at low concentrations in rats, although their blood pressure was substantially lowered under the same treatment [29]. Action potentials in the pacemaker cells in rabbits did not alter after blockade of endogenous H_2S production by using an inhibitor of endogenous H_2S synthesis propargylglycine (PPG). This suggests that H_2S has no notable chronotropic effect at low concentration [30].

Kohno et al. reported a negative chronotropic effect in rats exposed to 75 ppm H_2S for 1 h [31]. On the contrary, a positive chronotropic effect in rats exposed to 100–200 ppm H_2S for 1 h was registered in another study [32].

In other in vivo and in vitro studies a negative inotropic effect of H_2S was revealed in rats, which may be due to the blocking of voltage-operated Ca^{2+} channels and by inhibiting the adenylate cyclase, producing cyclic AMP, an important secondary messenger, regulating contractility of cardiac myocytes [26]. A decrease in heart rate by H_2S reduces energy consumption and contributes to lowering cardiac work load due to the reduced contract force or the required energy of muscular contractions, therefore the negative chronotropic effect is especially beneficial in case of angina. It was shown that H_2S induces negative inotropic effect in the isolated rat hearts during irreversible ischemia and reperfusion injury (I/R injury), lowering central venous pressure, thereby protecting the heart from damage [33]. In an in vivo model a similar effect was registered in mice receiving 1 mg/kg NaHS at reperfusion [34].

Most researchers have concluded that the opening of K_{ATP} channels in the myocardium plays a key role in the realization of the negative inotropic effect of H_2S, since glibenclamide, a classic blocker of such channels, inhibits this effect of H_2S. This also corresponds to the negative inotropic effect of other activators of K_{ATP} channels, which cause hyperpolarization of the cell membrane [30]. However, a negative inotropic effect of H_2S was not registered in other studies. For example, NaHS did not have a significant effect on the contractility of isolated rat ventricular cardiomyocytes in vitro. In these isolated cardiomyocytes, a negative inotropic effect was recorded for sodium nitroprusside (NO donor) and L-arginine while a positive inotropic effect for isoproterenol (a β-adrenergic receptor agonist). Interestingly, both the negative effect of NO and the positive effect of isoproterenol were attenuated by NaHS. The physiological significance of this role of NaHS in counteracting both positive and negative inotropic effects on the heart need to be clarified [35].

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2.2. Cardioprotective Effect of H_2S

There is numerous experimental evidence that H_2S protects the myocardium from damage during arrhythmia, cardiac hypertrophy, myocardial fibrosis, myocardial infarction, ischemia-reperfusion and heart failure thereby exhibiting a cardioprotective effect [36].

Enhanced H_2S levels in myocardium, whether by increased endogenous H_2S generation or by exogenous H_2S supplementation have been found to prevent ischemic injury protecting the heart. The mechanisms of cardioprotective effect of hydrogen sulfide need to be clarified, however, the molecular mechanisms providing vasodilation, antioxidation, antiapoptosis, anti-inflammation and cellular metabolism alterations have been elucidated [6].

The proving of the cardioprotective role of H_2S, which manifests itself in the reduction of myocardial damage in ischemia/reperfusion, was demonstrated in in vitro and in vivo studies [37]. Under infarction, the blood supply to the heart is disrupted due to coronary arteries damage, causing the necrosis of the myocardium. In a rodent model of myocardial infarction, it was shown that administration of H_2S decreases mortality and reduces the size of the necrosis. Apparently, the vasodilating effect of H_2S causes an increase in coronary blood flow in ischemic diseases and reduces cellular damage. In addition, there is evidence that H_2S stimulates angiogenesis, the formation of new blood vessels, enhancing the migration of endothelial cells, that also has a cardioprotective effect [26].

It has been shown that the administration of exogenous L-cysteine reduces the size of myocardial infarction in ischemic heart disease; it is believed that this protective effect of L-cysteine is due to an increase in endogenous production of H_2S catalyzed by CSE, since inhibition of CSE activity by propargylglycine (PPG) eliminated this effect of L-cysteine [26]. It has been shown that H_2S activates K_{ATP} channels in mitochondria and sarcolemma of cardiomyocytes, which underlies the cardioprotective effect. The H_2S donor NaHS can promote vasodilation of the coronary arteries by increasing the volume of coronary blood flow during ischemia and decreasing cellular damage. The role of H_2S in heart protection is confirmed by the fact that this effect is eliminated by the supplement of PPG [19].

Exogenous administration of H_2S and endogenous overexpression of CSE and modulation of H_2S content proved to be therapeutically justified in ischemic heart failure [38, 39]. Therapy using H_2S has been recognized as successful in models of ischemic lesions. In myocardial ischemia/reperfusion with both preconditioning and postconditioning with compounds releasing free H_2S (NaHS, Na_2S and GYY4137), a decrease in the infarction zone was demonstrated. The mechanism of such protection includes the activation of the antioxidant system with the involvement of anti-apoptotic and anti-inflammatory signaling pathways [40, 41, 42].

3. Blood Vessels and Circulation

3.1. Vascular Tone and Arterial Pressure Regulation

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One of the first revealed beneficial physiological effects of H_2S was its influence on vascular tone and thus blood pressure regulation [30]. Hydrogen sulfide itself and its donors have long been known as substances that promote vascular relaxation, alleviate hypoxic pulmonary hypertension, reduce the adhesion of leukocytes to the vascular wall, reduce vascular restenosis and have an anti-inflammatory effect [43].

Realizing its regulatory action in the arterial vessels, H_2S plays important role in the regulation of blood pressure [44]. The administration of hydrogen sulfide inhalations to patients with arterial hypertension contributed to a decrease in blood pressure [45]. It was found that intravenous bolus administration of hydrogen sulfide solution caused a dose-dependent decrease in blood pressure in rats [18]. In in vitro studies, the donor of hydrogen sulfide (NaHS), which is actively used in experimental practice, also caused relaxation of various vessels: aorta, renal, mesenteric arteries, portal vein, etc. Despite the known role of endothelium in the vascular tone regulation, its removal did not significantly affect the effects of H_2S in vascular smooth muscle cells (VSMC) [18], that indicates a direct effect of hydrogen sulfide on VSMC through their inherent regulatory mechanisms. The relaxing effect of H_2S on VSMC is mainly associated with the opening of K_{ATP} channels [46]. It was proven that both endothelium and vascular smooth muscle cells are able to produce H_2S, while H_2S may exert vasodilator properties that are endothelium-independent [26].

The role of H_2S in the pathogenesis of hypertension in spontaneously hypertensive rats (SHR) has been studied. Hypertension in animals developed spontaneously, with a decrease in H_2S production and CSE expression in the aortic tissues and a decrease in the H_2S content in blood plasma [47]. Administration of NaHS for 5 weeks delayed the development of hypertension in SHR and partially reversed hypertension-induced vascular remodeling and collagen accumulation [48].

One of the ways to prove the physiological significance of endogenous H_2S is the suppression of its endogenous production with the analysis of phenotype changes. In mice with a genetic deletion of CSE, the production of H_2S in the cardiovascular system was substantially (but not completely) blocked. Due to a lack of endogenous hydrogen sulfide in these animals, arterial hypertension was registered at the age of 8 weeks, but it could be successfully prevented by injection of exogenous H_2S. Another important finding in this study was that the development of hypertension in CSE knockout mice was due to severe impairment of endothelial-dependent vasodilation of small resistive arteries. Hydrogen sulfide acts on both endothelial cells and vascular smooth myocytes, causing vascular relaxation. In CSE knockout mice, this chain is disrupted due to lack of CSE [49].

Thus, H_2S is an endogenous gaseous modulator of vascular contractile activity. Unlike vasorelaxation caused by NO and CO, H_2S-induced vasodilatation is not mediated by the involvement of the cGMP signaling pathway. At the same time, like NO and CO, H_2S is able to inhibit the proliferation of vascular smooth myocytes and accelerate apoptosis in vitro [50, 51]. This effect is realized through the activation of MAP kinase and caspase-3. Therefore, H_2S is not only a vasodilator, but also an important regulator of cell growth, capable of reducing the structural remodeling of vascular tissues, which may shed light on the mechanisms of some vascular pathologies and provide new therapeutic approaches [19].

NO is considered as an endothelial-derived relaxing factor (EDRF), however, in many vessels, vasodilation effect is only partially reduced in the presence of NOS inhibitors and upon eNOS knockout. The activity of the EDRF, caused by the action of NO is most

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pronounced in large vessels, such as the aorta, while in resistive vessels that directly regulate blood pressure, the effect of NO is not so obvious. It has been proposed that NO acts as an EDRF for large arteries while H_2S is an EDRF for small resistance arteries [30]. It is believed that H_2S along with NO is an EDRF, causing hyperpolarization of the membrane potential due to the activation of K_{ATP} channels [50]. Based on the observation that physiological action of H_2S is mediated by sulfhydrating and activating the K_{ATP} channel it was hypothesized that H_2S is a major if not predominant mediator of EDRF activity. It was experimentally proven that much if not most EDRF activity involves cGMP-independent hyperpolarization of blood vessels pointing that EDRF is mainly dependent upon an endothelial-derived hyperpolarizing factor (EDHF) whose activity is largely associated with H_2S [52].

It was found that exogenous H_2S demonstrates a biphasic effect on vascular tone: at high concentrations of NaHS (> 400 μM) it exhibits vasodilator properties, and at low concentrations vasoconstriction was noted, it was suggested that this may be due to inhibition of eNOS. However, it was shown that such a biphasic effect is the result of high oxygen tension, since in the physiological range of O_2 tension low concentrations of NaHS causes vascular relaxation [53]. Consequently, the products of H_2S oxidation may be responsible for vasoconstriction under these conditions [54].

It has been hypothesized that tissue concentration of H_2S mainly determines its vascular effect. Based on the observation that endothelial denudation attenuates aortic rings constriction caused by NaHS (10–100 mM), it was suggested that constrictor effect of H_2S on vascular smooth muscle cells is an indirect, possibly it can be mediated by the production of endothelial-derived constrictors such as endothelin or by inhibition of endothelial-derived vasodilators such as NO, [55]. However, vascular relaxation was fixed in the same preparation, applying NaHS at concentrations greater than 100 mM.

3.2. H_2S as Oxygen Sensor

There is evidence that H_2S plays a significant role in hypoxia, however, the mechanisms by which H_2S sense and response to oxygen deficiency are largely unclear. The results of the studies of the electrical and mechanical responses of isolated blood vessels to oxygen deficiency suggest that H_2S acts as an oxygen sensor and provide transducing of the vascular response to hypoxia. Experimental data evidence that the response of blood vessels to hypoxia in vertebrates is attenuated by inhibition of H_2S synthesis, and the level of H_2S in the blood vessel is regulated by the balance between the production of endogenous H_2S and its oxidation by available O_2 [56].

Hypoxia-inducible factors (HIFs) are key regulators of oxygen level, since under hypoxia they activate the expression of target genes. Several recent studies have shown that like NO and CO, H_2S significantly contributes to the regulation of HIF-1 functions under hypoxic contexts. In mammalian cells HIF-1 is the main regulator of hypoxia, which activates the transcription of more than 100 target genes in hypoxic conditions [57]. H_2S-mediated angiogenesis in hypoxia involves activation of HIF-1 [58]. Convincing evidence has been obtained for the protective role of H_2S under hypoxia for many organs in mammals; however, the mechanisms by which H_2S performs the function of a hypoxia sensor and implements a regulatory response are still almost unclear. Resent research has shown that exogenous H_2S regulates HIF in a variety of ways. The carotid bodies activation is a sensitive and quick response to oxygen deficiency, which rapidly restores the overall oxygen supply. A unique role of carotid bodies in the oxygen sensing is due

 doi:10.20944/preprints202101.0193.v1

to their high sensitivity and fast response to hypoxia. It has been proven that H_2S is an excitatory mediator in the sensing of hypoxia by carotid bodies [59, 60]. Upon contact with oxygen H_2S is rapidly converted to polysulfides or hydrogen peroxide. Whether polysulfides are involved in the H_2S-mediated response of carotid bodies to oxygen deficiency or in the HIF functions regulated by H_2S, is not yet clear [60].

3.3. H_2S and Angiogenesis

The proliferation and migration of endothelial cells in response to a stimulus is extremely important in embryogenesis, angiogenesis, wound healing, tissue ischemia, and various inflammatory diseases. While VSMC proliferation is inhibited by hydrogen sulfide, the proliferation and migration of vascular endothelial cells (EC) either in culture or in the blood vessel walls is stimulated by H_2S. Cultured human umbilical vein endothelial cells (HUVECs) and bEnd3 microvascular endothelial cells had a higher proliferative and migratory activity, as well as wound healing ability after treatment with NaHS proving stimulatory effect of H_2S on ECs [61, 62].

Endogenous sulfide production is also important for the endothelial cells migration and growth. CSE knockout in human umbilical vein endotheliocytes and mouse aortic endotheliocytes caused inhibition of the proliferation rate, while CSE overexpression led to its increase [54]. In a model of ischemia of the hind limb in rats, it was demonstrated that four-week administration of NaHS notably increased the collateral vessels growth, enhanced capillary density, and intensified peripheral blood flow in the ischemic limb compared to control [63]. Although a number of studies provide compelling evidence for a regulatory role of sulfur compounds in endothelial proliferation and migration, many key questions remain unanswered.

The study of the cellular response using H_2S-releasing compounds such as NaHS does not guarantee that the biological effect caused by free H_2S is recorded, especially when it comes to hours or days after treatment, since in this case, the proproliferative and promigratory effects of hydrogen sulfide donors may be due to the action of its oxidized metabolites [62].

H_2S-producing enzymes are closely related to basic cellular metabolism, including amino acid synthesis and redox balance, which can also affect cell proliferation and migration, and this should be taken into account when discussing the role of hydrogen sulfide as a gasotransmitter or generator of polysulfides [54].

3.4. Atherosclerosis and H_2S

Atherosclerosis is a complex process that includes, along with other disorders, endothelial dysfunction and vascular inflammation. Numerous studies indicate a considerable role of H_2S in the pathogenesis of atherosclerosis, in particular in its formation and in reducing the consequences of ischemic vascular remodeling and tissue damage during ischemia-reperfusion [64, 30, 65].

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Recently it was demonstrated that H_2S is endogenously produced by macrophages and CSE production of H_2S in macrophages is stimulated by an inflammatory endotoxin lipopolysaccharide (LPS). Moreover, proatherogenic oxidized low-density lipoproteins (ox-LDL) inducing foam cell formation in macrophages were inhibited by NaHS [66].

Knocking out of CSE or CBS, followed by persistent endogenous deficiency of H_2S, accelerates atherosclerosis. It was fixed that in $ApoE^{-/-}CSE^{-/-}$, $ApoE^{-/-}CBS^{-/-}$ and Tg-hCBS $ApoE^{-/-}CBS^{-/-}$ mice early stage of atherosclerosis develops even without diet manipulations. Thus the importance of endogenous H_2S in preventing atherosclerosis was highlighted by these in vivo evidence [67].

Vascular homeostasis and function are largely regulated by blood flow. Shear stress regulates vasodilation, vascular remodeling, and susceptibility to atherosclerotic plaque formation. Disturbed flow in vessel curvatures, branch points, and bifurcations stimulates an atherosusceptible endothelial phenotype with decreased NO production, enhanced oxidant stress, and elevated expression of proinflammatory genes. I was shown that CSE plays an important role in vascular remodeling induced by blood flow. Disturbed flow in conduit vessels stimulates CSE expression and sulfane sulfur production. The enhanced CSE expression correlates with macrophage recruitment to such areas, which is possibly through a NF-κB dependent pathway. CSE knockout mice exhibits a complex change of vascular remodeling under disturbed flow, including reduced medial thickening and inability to narrow lumen size [68, 69].

Recent studies revealed that in native endothelial cells endogenous H_2S is mainly generated by CSE and expression and activity of this enzyme are strictly regulated by shear stress and inflammation [70]. Authors concluded that murine model of atherogenesis could be confirmed in humans, taking into account strong relationship between inactivation of CSE and accelerated disease progression. It was revealed an inverse correlation between circulating L-cystathionine levels, H_2S levels and endothelial function both in mice and humans, pointing L-cystathionine potential applicability as vascular disease biomarker. Moreover, it was shown that CSE expression in situ and in vitro is negatively regulated by blood shear stress, thereby at sites of low or disturbed blood flow the expression of the enzyme is elevated, and these vascular wall sites are considered favorable for the formation of atherosclerotic plaques. It seems evident a protective function of the CSE expression at these sites [70].

It has been shown that exogenous H_2S reduces the expression of the osteopontin gene, thereby reducing vascular calcification, which is usually recorded not only in atherosclerosis, but also in a number of diseases, including diabetes mellitus, hypertension, chronic renal failure, arterial stenosis and aging [71].

4. Blood

4.1. H_2S and Hemostasis

There are few data on the effect of H_2S on platelet functions. Zagli et al. [72] have demonstrated inhibition of platelet aggregation by H_2S, while the concentrations of H_2S used in this research exceeded the physiological values. It was in revealed a number of experimental studies that H_2S exhibits an antithrombotic effect, inhibiting various stages

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of platelet activation (adhesion, secretion, aggregation) and the process of thrombus formation [73]. Other researchers have found a weak inhibitory effect of H_2S at high concentrations on the human platelet aggregation and an insignificant effect on their adhesion [74].

Platelet aggregation caused by various agonists: ADP, arachidonic acid, collagen, adrenaline and thrombin, was dose-dependently reduced by NaHS [72]. Moreover, it was found that the adhesive properties of fibrinogen and collagen are modified by H_2S, and this modification impairs platelet adhesion [75].

Morel et al. [75] demonstrated inhibition of the generation of O_2 in platelets by NaHS in vitro, and the most pronounced inhibitory effect was noted for thrombin-activated platelets. The results obtained in vitro indicate the anticoagulation activity of H_2S. Therefore, H_2S administration was proposed as a potential treatment for preventing thrombosis in pathologies with high procoagulant plasma activity. Hydrogen sulfide is able to modify the main proteins of the hemostatic system (such as fibrinogen, thrombin and plasminogen) causing the notable changes both in the process of coagulation and fibrinolysis [75].

The mechanisms by which H_2S can affect the functional properties of platelets are not clear. It was found that the revealed inhibitory effect of hydrogen sulfide does not depend on either NO synthesis or involvement of K_{ATP} channels or activation of adenylate cyclase or guanylate cyclase [72]. It has been suggested that the alteration of platelet functions may be due to thiol-disulfide reactions [76], and an alternative mechanism of H_2S action on platelets may be associated with presence of thiol group. Recent studies have demonstrated that sulfhydration of platelet proteins is stimulated by the H_2S donor GYY4137, the expression of adhesion molecules is dose-dependently inhibited, and platelet activation morphological signs are reduced.

In murine model, GYY4137 also significantly increased the time of venular thrombus formation; the authors concluded that H_2S has antithrombotic properties and proposed GYY4137 ability to regulate thrombogenesis by influencing the processes of platelet activation, adhesion, and aggregation [77]. In a continuation of these studies evaluating the effect of GYY4137 on thrombus stability and microvascular thrombolysis, a significant acceleration of arteriolar and venular thrombolysis by GYY4137 in comparison with control (DMSO) was shown, thrombus stability was reduced by GYY4137 decreasing platelet-leukocyte aggregation and contributing to endogenous thrombolysis in mice [78].

Blood clotting time was prolonged in presence of NaHS (0.01–100 μM), the fibrin polymerization velocity was decreased and the fibrinolysis was stimulated in human plasma. These results point the potential anticoagulant properties of H_2S in in vitro study and suggest the ability of H_2S to be a powerful agent for thrombosis prevention in cases of pathology with enhanced procoagulant plasma activity. However, the exact mechanisms of hydrogen sulfide influence on process of hemostasis and thrombosis need to be clarified. One of the possible mechanisms may be the involvement of H_2S in the plasma proteins S-sulfhydration. Particular sensitivity to H_2S action was revealed for fibrinogen which function in clotting cascade is leading among other plasma proteins. Due to the complex nature of hemostasis the effects of H_2S on various elements of coagulation system seems to be manifold because of its pleiotropic character [73].

4.2. Erythropoiesis and Hemorrhage

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Both H_2S-generating enzymes CBS and CSE were found to be active in the blood. The endogenous source of hydrogen sulfide are endothelial cells that secreted these enzymes [79]. Another source of hydrogen sulfide in the blood are erythrocytes, which are able to generate it non-enzymatically from elemental sulfur or inorganic polysulfides. This way of H_2S production is stimulated by hyperglycemia and increased oxidative stress [5, 24, 25]. The needed amounts of reducible sulfur as well as other essential components of this non-enzymatic pathway are present in blood in vivo. It was proven by the presence of sulfur in millimolar concentration in blood circulation in humans and mice [30]. Besides non-enzymatic pathway enzymatic synthesis of hydrogen sulfide was recorded in erythrocytes, the key enzyme of endogenous H_2S generation in rat erythrocytes is 3-mercaptopyruvate sulfurtransferase (MPST), H_2S production from erythrocyte by L-cysteine pathway is markedly lower [80].

In experiments in vitro it was demonstrated that the use of H_2S has a positive effect on the renal erythropoietin (EPO) generating during hypoxia, but not normoxia. Then in murine model it was elucidated the effect of H_2S on in vivo EPO production by the kidneys. Apparently, H_2S has a significant effect on erythropoiesis and the production of erythropoietin by the kidneys. The important role of H_2S in oxygen sensing during erythropoiesis as well as the involvement of the HIF pathway in regulation of EPO production by H_2S was stressed by these findings. Significant lowering of hemoglobin, EPO, CBS, and NFκB-p65 levels during hypoxia was registered under knocking out one of the three major H_2S-generating enzymes compared to wild-type mice. This effect was reversible and attenuated upon supplementation of exogenous H_2S. This phenomenon was also reversed during normoxia by the upregulation of hemoglobin and a variety of HIF-regulated genes in comparison with wild-type mice [81, 82].

Clinical studies have shown that in patients with chronic renal failure and anemia, the content of thiosulfate in the urine was significantly lower than in non-anemic patients with chronic renal failure, which indirectly confirmed the role of H_2S in maintaining the normal count of erythrocytes [81].

Published data concerning the role of hydrogen sulfide in blood loss are contradictory. There is experimental evidence that H_2S is able to reversibly reduce the metabolic requirements of tissues under massive blood loss causing insufficient oxygen supply. In rodent model with controlled hemorrhage (60% of the total blood volume), the 24 h survival rate in bled animals after hemorrhage was no more than 23%, while it was increased up to 75% after administration of exogenous H_2S by inhalation of gaseous H_2S or intravenous infusion of NaHS under the same other experimental conditions. These animals survived after H_2S supplementation demonstrated normal behavior and their respiration analysis confirmed stable metabolism during and after hemorrhage [83]. Conversely, in another study, it was demonstrated that in rats with hemorrhagic shock, heart rate and blood pressure recovered faster, tissue damage was minimized in the presence of PPG, which inhibits H_2S synthesis, thereby indicating a negative role of hydrogen sulfide in this process [84].

4.3. H_2S and Erythrocyte Microrheological Properties

Blood rheology is a key determinant of blood flow and tissue perfusion. Red blood cells (RBC) flowing through narrow capillaries which lumen is comparable or smaller than cellular diameter, need to be deformable to supply oxygen to the tissue. Thus, RBCs are highly deformable in norm, and this rheological property significantly contributes to

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providing blood flow in the microcirculation [85]. Another one important cellular determinant of apparent blood viscosity is RBCs aggregability – the tendency of erythrocytes to join forming reversible aggregates (so called "rouleaux" because of their similarity to a stack of coins) under low shear flow or in stasis. In norm such aggregates are dispersed by enhancing shear forces, while RBC may reunite under flow lowering or in stasis, thus the size of RBC aggregates is inversely proportional to the magnitude of shear forces. Therefore, the RBC aggregation affects the in vivo fluidity of blood, obstructing low-shear microvascular blood flow [86]. Unfavorable changes in RBC microrheological properties (deformability and aggregability) in pathology may affect blood viscosity and oxygen supply to the tissues, which in turn may impact blood flow and disease progression [87]. Based on the fact that RBC are actively involved in the metabolism and scavenge of hydrogen sulfide [5, 24, 25, 80], it can be assumed that the functional properties of erythrocytes may be influenced by the alterations of this gasotransmitter content.

Information on the possible effect of gaseous molecules on the functional properties of erythrocytes (including their microrheological characteristics) in published data is very scarce. While the assessment of the effect of NO on blood rheology was presented in published data to some extent [88, 89, 90], the studies of the hydrogen sulfide effect on the microrheological properties of erythrocytes and the blood flowing properties were undertaken recently and are presented in few publications. Our research group has demonstrated that in vitro in presence of H_2S as well as after NO treatment human's RBC microrheological properties were positively changed: extent of aggregation was notably reduced and deformability was moderately but statistically increased. A dose-dependent effect of NO and H_2S donors (sodium nitroprusside and NaHS) on the microrheological properties of the separated by age erythrocytes was registered. The extent of RBC aggregation was reduced in the presence of both gasotransmitters, and this decrease was most notable for "old" cells [91]. Further study of possible mechanisms of the hydrogen sulfide effect on the aggregability and deformability of erythrocytes suggests that there are a direct (cGMP-independent) pathway of gasotransmitters action on the membrane viscoelastic properties of red blood cells [92] and a cross-talk between NO and H_2S during their combined supplementation is realized [93]. Revealed in in vitro study positive effect of the exogenous H_2S on the microrheological characteristics of human erythrocytes was more considerable in healthy control compared to patients with type 2 diabetes mellitus [94].

5. Regulatory Mechanisms of Hydrogen Sulfide

5.1. Signaling Pathways Involved in the Realization of the H_2S Effect

A number of various cellular and molecular signals are involved in signaling pathways mediating the realization of hydrogen sulfide effects on the cardiovascular system. In the cell, the targets of H_2S action can be ion channels, membrane and intracellular enzymes, various proteins, etc. It was found that the protein modification is one of the major mechanisms of H_2S action, H_2S is a strong reducing agent and can reduce double disulfide bonds. Another mechanism is the attaching of an additional sulfur atom to the thiol group. Such chemical modification of proteins leads to a change in their conformation and functional activity [30].

Like NO, H_2S binds with high affinity to heme; however, under physiological conditions, cyclic guanylate cyclase is not stimulated [46]. Guanylate cyclase inhibitors do not affect the ability of H_2S to relax blood vessels; therefore, the effect of H_2S does not depend on this enzyme [95].

 doi:10.20944/preprints202101.0193.v1

The generally accepted concept of intercellular communication has undergone dramatic changes after the identification of mechanisms of gasotransmitters action. For instance, there are no intracellular vesicular stores for gasotransmitters, therefore these gaseous molecules must be synthesized as needed. It means that regulation of gasotransmitters action should be realized not by controlling the releasing of a gaseous molecule from its storage, but by alteration of their synthesizing enzymes activity. To reach intracellular targets gasotransmitters do not require specific binding to plasma membrane receptors, they simply diffuse into the cells through its membrane.

Because of their high chemical reactivity both gasotransmitters (NO and H_2S) must be inactivated after random diffusion throughout cells. For this capture and neutralization, a suitable compound (such as glutathione) must be present in the cell in abundant concentrations. Nitric oxide typically realizes its targeted action by binding various forms of NO synthase generating NO with target proteins. Probably a similar mechanism of directed action of hydrogen sulfide on its specific targets also exists, but this is still unknown.

Apparently the signaling molecular mechanisms are the most unique feature of gasotransmitters. The molecule of classic messenger acts through an amplifying signal cascades, including long sequence of molecular interactions. Gasotransmitters act immediately, chemically modifying intracellular proteins, and thus affecting cellular metabolism in a faster and direct way [96].

It was shown that H_2S realizes its effects through mechanisms similar to nitrosylation, forming covalent bonds with the SH-group of cysteines; this process was called sulfhydration. Compared to nitrosylation sulfhydration is much more common. While usually up to 5% of most proteins are nitrosylated, 10-25% of actin and β-tubulin are mainly sulfhydrated. Sulfhydration can affect the function of proteins differently from nitrosylation. During nitrosylation, the active SH groups of cysteines are covered, which usually leads to inactivation of proteins, although sometimes an activating effect was also recorded. Sulfhydration converts the SH-group into SSH-group, which is more chemically reactive and faster interacts with the cellular environment. For example, activity of glyceraldehyde-3-phosphate dehydrogenase increases up to 700% by its sulfhydration [52].

5.2. Polysulfides

Since today there are certain technical problems with methods for accurately measuring the content of H_2S and its metabolites, it is not entirely clear whether the observed regulatory effects are related to the action of free hydrogen sulfide or whether it is a "merit" of its polysulfide derivatives. Therefore, the statement that only the hydrogen sulfide molecule as such is capable to realize all the signaling and biological effects described in the literature can be considered too simplified [97]. Although H_2S is a short-lived molecule, numerous studies demonstrate its prolonged effect in mammals, which allows to hypothesize the physiological significance of hydrogen sulfide metabolites such as polysulfides, persulfides and other active forms of sulfur (RSS). In addition to the exogenous formation of inorganic polysulfides in a NaHS solution, the existence of endogenous inorganic polysulfides was also recorded [68, 98].

The concept of gasotransmitters considers the specific properties of these signaling molecules of gases, including their good permeability through cell membranes, interaction

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with hemoproteins, and their ability to regulate biological processes by activating certain signaling mechanisms. However, this concept does not take into account the fact that the metabolic products of these gases (for example, oxidation products) are often mediators of many biological functions to a greater extent than the molecules of these gases themselves. Oxidation of H_2S in real biological systems is an inevitable process, as a result of which polysulfides and persulfides are formed, which exhibit the same effects as H_2S [68]. It was demonstrated that, along with H_2S, polysulfides and persulfides are equally capable to regulate various endothelial functions [65]. The cardioprotective role of sulfide and polysulfide was revealed on different models of cardiovascular diseases with tissue damage [99]. For instance, the risk reduction of cardiovascular disease associated with the consumption of garlic can be attributed to the protective effect of H_2S produced in erythrocytes from organic polysulfides of garlic [25].

5.3 Crosstalk between Gasotransmitters

The chemical and biological properties of the currently known gasotransmitters H_2S, NO, and CO are similar, all of them have common molecular targets and demonstrate similar cellular effects. They complete each other as well as compete with each other in the regulation of biological functions. For instance, vasodilation is a common effect of these three gases at the tissue level, inhibition is typical for all three gases at the cellular level, H_2S and NO act on cytochrome c oxidase, all of them bind to hemoglobin [5].

The effects of gasotransmitters can be mediated by their interaction with each other, which has been confirmed in recent years. The interaction takes place both at the level of regulation of synthesis enzymes and the targets of their action. Hydrogen sulfide, for example, inhibits the activity of enzymes that synthesize NO, an endothelial factor of relaxation of the aorta and other large vessels [100], and the NO donor, sodium nitroprusside, enhances the expression of cystathionine-γ-lyase and cystathionine-β-synthase [29]. If the vasodilation effect of NO is realized in the aorta, the relaxation of the mesenteric arteries, which are related to resistive vessels and are more significant for the regulation of peripheral blood pressure, is mainly associated with H_2S. In addition, the mechanisms of action of H_2S and NO in the vessels are different. The effects of NO are mediated through the soluble form of guanylate cyclase and modulation of the K_{Ca}-channels, and H_2S through hyperpolarization, which is provided by the activity of the K_{ATP}-channels [101]. NO, CO, and H_2S can activate high conductivity K_{Ca}-channels by means of various chemical modifications of the channel proteins. NO modifies sulfhydryl groups, CO modifies histidine residues, and H_2S reduces disulfide bonds [5].

It is known that inhibition of any of the H_2S-producing enzymes (CSE, CBS, or 3-MST) decreases the phosphorylation of eNOS at Ser1177 in response to shear stress, which indicates the fundamental role of H2S metabolism in endothelial activation caused by shear [102].

The data on the mutual influence of production and release of H_2S and NO are rather contradictory. Along with the data indicating that H_2S stimulates the production of NO by the endothelium [29], other studies have suggested that H_2S inhibits the activity of eNOS and blocks the effect of SNP (sodium nitroprusside) [95]. It was found that H_2S is able to modify the activity of phosphodiesterases, thereby affecting the level of cyclic nucleotides [103]. It was shown that NO has the ability to increase the expression and activity of CSE and to bind to the CSE circulating in the blood [95], and possibly to CBS

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[104]. At the same time, L-NAME can decrease the H_2S content, decreasing the activation and expression of CSE [105].

In addition to the effect on enzymes, it was found that NO and H_2S are capable of forming nitrosothiol compounds with a certain physiological role, S-nitroso compounds, inhibiting the synthesis of thromboxane TxA_2, directly leading to cGMP-independent blockade of platelet activation [106]. Also, NO can be reduced by H_2S to nitroxyl (HNO), which presumably can independently act on cAMP and cGMP, possibly through the activation of SER-CA; however, at present it is technically impossible to measure the HNO content, and, consequently, to evaluate its role [107].

An increased content of free hydrogen sulfide in blood plasma may be a compensatory response to endothelial dysfunction and dysregulation of NO bioavailability [108], Recent studies have shown that H_2S can affect the expression and functional activity of eNOS, promoting the reduction of the nitrite anion to NO, acting as an alternative pathway for regulating the bioavailability of NO [62]. NO can influence the level of H_2S in vascular tissues through two mechanisms. It was found that NO increases the activity of CSE in vascular tissues [95]. The homogenate of aortic tissues was incubated with NO donor for 90 minutes, resulting in a notable dose-dependent H_2S generation rise. This elevation of hydrogen sulfide production may be due to the stimulation of the H_2S-generating enzyme CSE by cGMP-dependent protein kinase which activity was increased by NO. In addition, NO can directly affect the CSE activity. The CSE protein in mammalian consists of 12 cysteines. It is not yet established if there are specific cysteine residues that can interact with NO, however, it is quite possible that NO is able to nitrosylate certain free SH- groups of CSE.

The regulation of CSE expression is the second mechanism of NO-induced H_2S generation. Incubation of a cultured vascular smooth muscle cells with a NO donor for 6 hours considerably increases the expression of CSE [95]. Other studies have also shown that the NO donor S-nitroso-N-acetylpenicillamine (SNAP) increases the CSE expression, while another NO donor (SNP) enhances the CSE activity [109]. It was found that a number of physiological and pathological processes are mediated by H_2S, polysulfides and their interaction with NO. The importance of interaction of H_2S and NO was highlighted in regulation of vascular tone and cardioprotection [110].

The interaction of CO and H_2S is not well understood; however, it was shown that CO is able to bind to both CBS and CSE, blocking their activity, and the affinity of CO binding to CBS is higher than that of NO [111].

On the whole, the current evidence suggests that there is a system of interaction between gasotransmitters, which allows to regulate body functions at low concentrations of these gases due to their synergistic effect, when they are combined, because the total effect of interacting gaseous significantly exceeds the simple sum of their separate effects [74].

5.4 Biphasic Effects of H_2S

Apparently, H_2S participates in numerous physiological and pathophysiological activities in cardiovascular system. However, numerous studies often provide conflicting data on the H_2S effect. This may be due to both different experimental conditions and various concentrations of hydrogen sulfide used to assess its effect.

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Recently it was established that H_2S has a bell-shaped curve of dose-response, indicating its biphasic effect that means that lower concentrations of hydrogen sulfide exert notably different effects in comparison with the effects of H_2S seen at higher concentrations, moreover these effects sometimes are opposite [15, 112].

One of the most compelling examples of dual effects is the effect of H_2S on mitochondrial functions. A variety of effects of hydrogen sulfide in the mitochondria is well known. The direct donation of electrons into the electron transport chain of mitochondria is one of the effects of H_2S at low concentrations, another one is the supporting mitochondrial functions by inhibiting its cAMP phosphodiesterases. H_2S can also exert mitochondrial antioxidant effects and promote mitochondrial DNA repair directly interacting with its DNA repair enzymes. The activity of mitochondrial ATP synthase can be directly stimulated by H_2S through sulfhydration. At the same time, cellular respiration is blocked by high concentrations of H_2S due to the inhibition of cytochrome c oxidase, that is an essential element of the oxidative phosphorylation process within the cell normally binding oxygen. Inhibition of this enzyme impairs mitochondrial electron transport and ATP generation [15]. Other dual or uncertain effects of H_2S in cardiovascular system are summarized in Table 1.

Active substance	Concentration	Object	Effect	References
NaHS	10 mM	platelet aggregation	inhibition	Zagli G. et al., 2007
NaHS	1, 5 and 10 mM	activated partial thromboplastin time (APTT); thrombin time (TT); INR; fibrin lysis	no effect	Olas B. et al., 2019
NaHS	1, 5 and 10 mM	platelet aggregation fibrinogen	inhibition modification	Morel A. et al., 2012
NaHS	0.01–100 μM	activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT); fibrin polymerization in whole plasma; fibrinolysis	prolonged reduced stimulated	Olas B., Kontek B., 2014
NaHS	10 μM 100 μM 1000 μM	plasma lipid peroxidation	reduced no effect increased	Olas B., Kontek B., 2015
H_2S	0.1-1 μM	mitochondrial	stimulation	Módis K et al., 2013

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	3-30 μM	electron transport and cellular bioenergetics	inhibition	
NaHS	600 μM	rat vascular (aortic) tissue	relaxation	Zhao W., Wang R., 2002
NaHS	10–100 μM	aortic rings	constriction	Ali MY et al., 2006
NaHS	30 μM	smooth muscle relaxation effect of NO	enhanced	Hosoki R et al., 1997
H_2S	60 μM	relaxant effect of NO in the rat aorta	inhibited	Zhao W, Wang R, 2002
NaHS	50, 100, 200 μM	velocity of diastolic depolarization (VDD) and rate of pacemaker firing (RPF) in normal pacemaker cells in SA nodes (in rabbit)	decreased (in a concentration-dependent manner)	Xu M. et al., 2008
H_2S	2.8 and 14 μM/kg	heart rate in rats	no effect	Zhao W., Wang R., 2002
H_2S	75 ppm (60 min)	heart rate in rats	decreased	Kohno M. et al., 1991
H_2S	100-200 ppm (60 min)	heart rate in rats	increased	Higuchi Y., 1977
NaHS	50 or 100 μM	isolated rat ventricular myocytes	no effect	Yong QC et al., 2010

6. Diagnostic and Therapeutic Potential of H_2S

The therapeutic relevance of hydrogen sulfide in treatment of cardiovascular diseases is implied from its ability to regulate the peripheral blood circulation and heart functions disorders. It became evident that the state of so called "relative deficiency of H_2S" in certain cells, tissues and organs is linked to pathogenesis and progress of cardiovascular diseases [11]. Preclinical and clinical examination of various cardiovascular diseases, particularly myocardial ischemia/reperfusion injury and heart failure, have revealed that endogenous H_2S production is blunted in these pathological states and that this insufficient production of H_2S contributes to the progression of disease. With this discovery, the enthusiasm for the development of sulfide-based therapies has grown [113].

One of the most accessible and informative indicators of this deficiency can be the H_2S content in the blood plasma. It was proven that this indicator is dramatically decreased in cardiovascular diseases. Studies on humans have shown that in the cohort of people with normal blood pressure, the concentration of H2S in the blood plasma was 34 μM, while in patients with arterial hypertension its content was reduced to 20 μM [45]. In patients with coronary heart disease a lowering of hydrogen sulfide concentration in plasma from 50 μM to 25 μM was revealed. However, it should be noted that there is

 doi:10.20944/preprints202101.0193.v1

still an unresolved technical problem of accurate determination of hydrogen sulfide level, which leads to the marked variability in the baseline values reported by various research groups because the absolute levels are highly dependent on the method used [4, 15]. Therefore, the more accurate and reliable indicators should be preferably used for diagnostics. As mentioned above, circulating L-cystathionine levels have recently been suggested as a potential biomarker of vascular disease in both mice and humans, based on its inverse correlation with H2S levels and endothelial function [70].

To achieve desired therapeutic effects manipulation of H_2S levels has been tested in various disease models demonstrating the high efficacy of this approach. However, pharmacological profile of H_2S is quite complex (often bell-shaped) which creates certain difficulties in its clinical application [113]. The using of precursors for endogenous H_2S synthesis (cysteine and homocysteine for CSE and CBS, 3-mercaptopyruvate for 3-MST, and a-ketoglutarate for CAT) is the most obvious method to rise H_2S levels in vivo. These three substrates provide elevation of H_2S levels and cause physiologic effects corresponding to increase of H_2S generating, such as organ protection [26], vascular smooth muscle relaxation, cell proliferation and angiogenesis [62], and bell-shaped effects on mitochondrial function [114, 15]. It was found that the chronic cardiovascular pathologies are associated with downregulation of these three H_2S-generating enzymes responsible for H_2S synthesis, however the precise effects of these enzymes and their impact in cardiovascular homeostasis need to be clarified [6]. To develop effective therapeutics on base of this approach a greater depth of knowledge is required, direct gene therapy or localized drug delivery will become real if the features of location and activity of these enzymes in particular disease and responsiveness of certain tissues to H_2S therapy are understood [66].

Another promising approach in therapeutic of cardiovascular disease is using of H_2S donors. If cardiovascular disease is associated with deficiency of endogenous H_2S, the ideal treatment may be administration of H_2S donors. The main problem in this case is how to deliver this donor at appropriate concentrations and/or rates to the desired point of application [16].

Many H_2S-releasing compounds of various chemical composition and pharmacological properties have been proposed as potential candidates for therapeutics. The peculiarity of hydrogen sulfide as a signaling molecule is the absence of definite effect, because its action depends on its concentration, the kind of target cells and disease type. H_2S is short-living molecules, this creates certain problems in the use of donors, as well as instant uncontrolled release of hydrogen sulfide by existing H_2S donors. Therefore, the immediate tasks of translational medicine are the creation of compounds with desired properties, and the most important of them is the sustained controlled release of H_2S [3].

The first inorganic compounds used as donors of hydrogen sulfide in the studies of the H_2S role in cardiovascular system were sodium sulfide (Na_2S) and sodium hydrosulfide (NaHS). Although these early simple H_2S donors provided an excellent foundation to elucidate the importance of H_2S in physiological processes and disease, there are considerable limitations to using these donors as potential therapeutics. Administration of sulfide salts in vivo results in a rapid and largely uncontrollable surge in H_2S concentration in the circulation and tissue followed by a rapid decline. This pharmacokinetic profile is unsuitable for the treatment of chronic cardiovascular diseases, such as hypertension or heart failure, and may result in untoward or toxic effects [58].

Recently, the development of new H_2S donors has become a rapidly growing industry and a number of new donor types have been reported. Along with the general property of directly producing H_2S, these compounds are classified by the way of stimulation of hydrogen sulfide releasing. Release of H_2S may be triggered by various stimuli such as

 doi:10.20944/preprints202101.0193.v1

light, water, enzyme action, or other ones [6, 8, 66, 115, 116, 117]. The application of novel conjugated compounds in a clinical setting proved to be effective for therapy of concomitant diseases, one of them H_2S releasing aspirin (ACS14) demonstrated not only protective effects within the cardiovascular system but provided decreased gastrointestinal side effects compared to native aspirin [74]. Continuous original innovation in donor's synthesis and a deeper understanding of the physiology of H_2S may ultimately pave the way to the clinic for H_2S therapeutics [8].

However, despite significant advances in basic research on the biomedical effects of H_2S over the past decades and the development of a number of new H_2S donors, this approach has not yet found wide clinical application. Clinical trials to supplement H_2S in various human diseases have been limited [20].

To elucidate the molecular mechanisms involved in action of endogenous or exogenous H_2S is the aim of future investigations. The synthesis of novel stable donors with controlled H_2S release is one of the main directions of development of the gasotransmitters pharmacology. As the biological benefit of H_2S is deeper understood, its therapeutic effects in cardiovascular system can be utilized more efficiently in clinic [16].

7. Conclusion

Hydrogen sulfide is a small gaseous signaling molecule that plays an important role in regulation of vital physiological functions and in pathogenesis and progression of many diseases. Research on hydrogen sulfide role in the cardiovascular system is growing at the fastest rate in the field of gasotransmitters, as more and more evidence demonstrates its powerful protective effect on the cardiovascular system. Numerous studies have shed light on the multifaceted ability of H_2S to affect the cardiovascular system through various signaling mechanisms. Over the past decade, H_2S has been the subject of intense research aimed at understanding its physiological role in health and cardiovascular disease, and using its biological effects to achieve therapeutic benefits. Due to the promising cardiovascular effects of H_2S, a number of drugs have been designed on the basis of this gasoransmitter, while to develop effective therapeutics a greater depth of knowledge is required.

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Abbreviations

CSE - cystathionine-γ-lyase

CBS - cystathionine-β-synthase

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3-MST - 3-mercapto-sulfurtransferase

DAO - D-amino acid oxidase

SA – sinoatrial

VSMC – vascular smooth muscle cells

EC - endothelial cells

RBC – red blood cells

I/R – ischemia/reperfusion

NOS – NO synthase

EDRF - endothelial-derived relaxing factor

EDHF - endothelial-derived hyperpolarizing factor

HIF - hypoxia-inducible factor

LPS – lipopolysaccharide

oxLDL - oxidized low-density lipoprotein

EPO – erythropoietin

SER-CA - Ca2+ -ATPase of the sarcoplasmic reticulum

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