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**REVIEW**

# The Role of Hydrogen Sulphide in Blood Pressure Regulation

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**Summary**

Cardiovascular studies have confirmed that hydrogen sulphide ($H_2S$) is involved in various signaling pathways in both physiological and pathological conditions, including hypertension. In contrast to nitric oxide (NO), which has a clear vasorelaxant action, $H_2S$ has both vasorelaxing and vasoconstricting effects on the cardiovascular system. $H_2S$ is an important antihypertensive agent, and the reduced production of $H_2S$ and the alterations in its functions are involved in the initiation of spontaneous hypertension. Moreover, cross-talk between $H_2S$ and NO has been reported. NO-$H_2S$ interactions include reactions between the molecules themselves, and each has been shown to regulate the endogenous production of the other. In addition, NO and $H_2S$ can interact to form a nitrosothiol/s complex, which has original properties and represents a novel nitroso-sulphide signaling pathway. Furthermore, recent results have shown that the interaction between $H_2S$ and NO could be involved in the endothelium-regulated compensatory mechanisms that are observed in juvenile spontaneously hypertensive rats. The present review is devoted to role of $H_2S$ in vascular tone regulation. We primarily focus on the mechanisms of $H_2S$-NO interactions and on the role of $H_2S$ in blood pressure regulation in normotensive and spontaneously hypertensive rats.

**Key words**

Hydrogen sulphide • Nitric oxide • Vascular tone • Hypertension • Nitroso-sulphide

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## Introduction

Hydrogen sulphide ($H_2S$) is a simple gaseous molecule that participates as a transmitter in the regulation of vascular reactivity. Until the last two decades of the 20th century, all known chemical transmitters were liquids. Furchgott and Zawadzki (1980) demonstrated that the relaxation of rabbit aorta following acetylcholine administration is dependent on the endothelium, and the substance responsible for the vascular relaxation was determined to be an endothelium-derived relaxing factor. Palmer *et al.* (1987) proved that this substance is pharmacologically identical to nitric oxide (NO). NO was then determined to be one of the most important signaling molecules in biological control systems. Moreover, NO was the first gaseous molecule that fulfilled the criteria of a transmitter. Specifically, gaseous transmitters must be 1) freely membrane permeable; 2) endogenously and enzymatically generated and regulated; 3) have defined functions at physiological concentrations; and 4) have specific cellular and molecular targets, although second messengers are not needed (Wang 2002). Marks *et al.* (1991) discovered that another simple gaseous molecule, carbon monoxide (CO), operates as a transmitter in the mediation of vasoactivity. Abe and Kimura (1996), who studied neuronal activity, identified a third gaseous transmitter, namely, $H_2S$. The vasoactivity of this compound was revealed by Hosoki *et al.* (1997).

The gaseous transmitters in the cardiovascular system differ in terms of their physiological concentrations. In arterial blood, the concentration of NO in physiological conditions is about 150 nmol/l (Gerová

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*et al.* 1996). This value was measured with a porphyrinic biosensor in the blood stream close to endothelial cells in the femoral artery of a normotensive dog. The non-pathological CO production of the human body is 20 µmol/h (Durante *et al.* 2006). Data on the concentration of $H_2S$ in the cardiovascular system varies between 10 nmol/l and 300 µmol/l (discussed in more detail below). Unfortunately, the published data on the concentration of gaseous transmitters often depend on the methods used.

As all three gaseous transmitters have vasoactive effects, it is likely that they participate in blood pressure regulation. The vascular tone is determined by the interactions between various neurohumoral factors and mechanical forces in cooperation with vasorelaxant and vasoconstrictor substances released by the vascular wall. In physiological conditions, the final effect of these factors is shifted towards vasorelaxation. In pathological conditions, e.g. hypertension, the balance is disturbed due to enhanced vasoconstricting effects and increased vascular tone. $H_2S$, in contrast to NO, which has a clear vasorelaxant action, has both vasorelaxing and vasoconstricting effects on the arterial system. It is difficult to study the contribution of $H_2S$ to the regulation of vascular tone in physiological and/or pathological conditions, and these effects have been unsatisfactorily explored in the literature. NO and CO act *via* binding to the heme moiety at the active site of guanylate cyclase. $H_2S$ acts in part by opening ATP-sensitive potassium channels ($K_{ATP}$) on the vascular smooth muscle cells (Zhao *et al.* 2001, Zhao and Wang 2002, Drobna *et al.* 2015) and in part by stimulating endothelium-derived NO production (Zhao and Wang 2002). Although gaseous transmitters operate in distinct ways, studies have revealed that they can act cooperatively. In the present review, we focus our attention primarily on the physiological effects of $H_2S$ in the cardiovascular system.

## Biochemical properties, biosynthesis and breakdown of $H_2S$

The synthesis of $H_2S$, similarly to NO and CO, occurs endogenously by means of various enzymes, and this compound has been proven to be involved in many pathological processes, including vascular relaxation, hypertension, cellular proliferation, gene expression, cardioprotection, neuroprotection, intestinal secretion, diabetes, apoptosis, atherosclerosis and inflammation. The molecular background of the effects of $H_2S$ and its signaling at the cellular level are currently unknown. However, it is possible that one of the key mechanisms could be a modification of cysteine SH groups to SSH groups during the generation of S-sulph-hydrated proteins. S-sulph-hydration, evoked by endogenously produced $H_2S$, can occur on various proteins and modifies their physiological properties. This posttranslational modification is similar to S-nitrosylation, which is induced by NO, and could be an important signaling mechanism with various effects on the cardiovascular system.

$H_2S$ dissolved in water is a weak acid and dissociates into $H^+$, $HS^-$, and $S^{2-}$. At physiological pH (7.4), such as in the blood and other physiological solutions, approximately 14 % of the free sulphides are present as gaseous $H_2S$, more than 80 % is present as $HS^-$, and the rest is $S^{2-}$. It is still undetermined which form is biologically active. An important property of gaseous $H_2S$ is its lipophilicity. Similarly to $O_2$ and $CO_2$, $H_2S$ easily penetrates the cell membrane (Wang *et al.* 2012). Due to its vaporous quality, $H_2S$ easily leaves the blood into the lung and/or from the incubating medium to the air (Liu *et al.* 2012). Some studies state that the concentration of free $H_2S$ in blood and tissues is only 14-15 nmol/l (Doeller *et al*. 2005, Furne *et al.* 2008). The concentration of free sulphides in the blood and other tissues/physiological solutions of mammals is very low (<100 nmol/l), but it can be increased in the parts of the body where increased concentrations of $H_2S$ synthesizing enzymes are present (Whitfield *et al.* 2008). In specific intracellular spaces (microspaces), the concentration of free $H_2S$ can be increased several fold, whereupon it immediately diffuses, binds or oxidizes. For example, a much higher concentration of $H_2S$ (1 µmol/l) is observed in the aorta of mice. This concentration is 20-200 times higher in comparison with other tissues (Levitt *et al.* 2011). It is suggested that endogenously produced $H_2S$ is rapidly oxidized to sulphates or incorporated into proteins. In this form, free $H_2S$ can be released after some physiological stimuli and temporally achieve increased concentrations (Ishigami *et al.* 2009). Under the *in vitro* conditions (e.g. organ bath, cell culture), a concentration of $H_2S$ lower than 100 µmol/l is proposed to be physiologically relevant. The *in vivo* experiments indicate that the mode of $H_2S$ application (i.e. intravenous, intraperitoneal, hypodermic) is important given that this choice can modulate $H_2S$ bioavailability. In the context of oral administration, a high percentage of $H_2S$ is metabolized in the gastrointestinal tract and the liver before it reaches the target organ.

Both free and bounded sulphides are produced by the enzymes that synthesized $H_2S$. Three enzymes can convert the amino acid L-cysteine to $H_2S$: cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) and cysteine-aminotransferase (CAT) in conjunction with mercaptopyruvate-sulphurtransferase (3-MST). The gene expression of CBS and CSE has been detected in various cell types, including the liver, kidney, lymphatic system, vascular wall, cardiomyocytes, and fibroblasts. While these enzymes contribute equally to the local production of $H_2S$ in the liver and kidney (Xia *et al.* 2009), one of the enzymes could be dominant in other contexts. There is a prevalence of CSE in cardiovascular system, although CSE expression is 24 % higher in the myocardium in comparison to the thoracic aorta (Geng *et al.* 2004). Relatively high concentration of CSE is observed in arteries, and $H_2S$ is produced by both endothelial cells (Yang *et al.* 2008) and smooth muscle cells of the vessel wall (Zhao *et al.* 2001). The expression of CAT and 3-MST was also observed in the endothelium (Shibuya *et al.* 2009). The key enzyme for $H_2S$ synthesis in the central and peripheral nervous system is CBS (Abe and Kimura 1996). The source of $H_2S$ in brain could also be the CAT/3-MST complex (Ram 1988).

The sources of $H_2S$ are the amino acids cysteine and methionine, which are present in food. Nearly all synthesized $H_2S$ is immediately oxidized and incorporated into the structure of other liver proteins before entering into hepatic veins and the *vena cava inferior* (Furne *et al.* 2008). Most endogenously synthesized $H_2S$ is oxidized to sulphates, which are then excreted by the kidney. Although every cell is able to oxidize $H_2S$, it is primary degraded in liver (Furne *et al.* 2001, 2008). Mitochondria are very active in sulphide oxidation. $H_2S$ molecules are oxidized to thiosulphate, which is ultimately converted to sulphide and sulphate by sulphate oxidase (Furne *et al.* 2008). Sulphates are then excreted in the urine in the free or conjugated form. $H_2S$ is also trapped by hemoglobin or by molecules with metal or disulphide groups (e.g. oxidized glutathione). Hemoglobin generally decreases the level of all three gaseous transmitters (CO, NO, and $H_2S$). Due to their strong affinity to oxygen, this binding can mediate a great deal of the toxic effects of these molecules. The lung also participates in $H_2S$ excretion in the case of increased $H_2S$ production, e.g. during hemorrhagic conditions, septic shock or pancreatitis. In normal conditions, the amount of $H_2S$ excreted by expiration is negligible (Liu *et al.* 2012).

## Vasoactive effects of $H_2S$

The level of $H_2S$ in the body depends on the presence of pathological conditions, including hypertension (Chen *et al.* 2007). Nevertheless, it is still an open question whether the concentration of $H_2S$ depends on pathological conditions or *vice versa*. In addition, the mechanisms of $H_2S$ action are not fully elucidated. In general, $H_2S$ has been shown to have dual effects on the tone of the vascular wall.

Perfusion of the mesenteric system with 1 mmol/l cysteine (precursor of $H_2S$) resulted in an increase of endogenous $H_2S$ production and a dilation of the mesenteric circulation (Cheng *et al.* 2004). Sodium hydrosulphide (NaHS) at concentrations over 100 μmol/l evoked the relaxation of precontracted isolated rat arteries (Ali *et al.* 2006, Hosoki *et al.* 1997, Zhao *et al.* 2001). On the other hand, some observations revealed an opposite effect of $H_2S$ on smooth muscle cells of the arterial wall. The application of the same doses on isolated precontracted arterial segments evoked vasoconstriction (Lim *et al.* 2008, Liu and Bian 2010). The vasoactive response of vessels to $H_2S$ differs in dependence on several factors, for example, the type of vessel (conduit arteries, resistance arteries) the presence of an endothelium, the substance used for precontraction, and the concentration of $H_2S$ applied. Higher concentrations of $H_2S$ (sodium disulphide (NaHS): 2.8 and 14 μmol/kg; 0.1-1 mmol/l) evoked decrease of blood pressure or vasorelaxation in some types of isolated vessels (Zhao *et al.* 2001, Zhao and Wang 2002). Lower concentrations of $H_2S$ ($Na_2S$: 3 μmol/kg; 10-100 μmol/l) resulted in blood pressure increase and vasoconstriction of the same vessels (Kubo *et al.* 2007, Lim *et al.* 2008, Drobna *et al.* 2015).

Published data indicate numerous possible mechanisms of $H_2S$-induced vasoconstriction. One possible mechanisms of $H_2S$-induced vasoconstriction is decreased levels of cyclic adenosine monophosphate (cAMP) in smooth muscle cells. Li *et al.* (2015) showed on the rat cerebral artery that $H_2S$ evoked a decrease of cAMP levels, an effect that was associated with the promotion of an interaction between actin and myosin. The $H_2S$-mediated decrease in cAMP concentrations stimulated the activation of myosin light chain kinase, an enzyme that mediates the interaction between actin and myosin (Lim *et al.* 2008). Li *et al.* (2015) also proved that

$H_2S$ did not directly influence cAMP levels but significantly reduced forskolin-stimulated adenylyl cyclase activity in human brain vascular smooth muscle cells. This result demonstrated that $H_2S$-induced vasoconstriction was due to the inhibition of the cAMP/adenylyl cyclase pathway. It was also shown that the administration of low concentrations of $H_2S$ (5-100 µmol/l) inhibited forskolin-induced cAMP accumulation in aortic smooth muscle. Moreover, NaHS was observed to inhibit vasorelaxing effects *via* β-adrenergic vasodilators and to induce vasoconstricting effects *via* adenylate cyclase and cAMP inhibition (Coletta *et al.* 2012). Ping *et al.* (2015) found that prostanoids could be involved in NaHS-induced vasoconstriction because the vasoconstriction evoked by $H_2S$ was markedly attenuated in the presence of a cyclooxygenase inhibitor (indomethacin, 10 µmol/l). It was concluded by the same authors that the contractile effect of $H_2S$ was mediated by an influx of extracellular $Ca^{2+}$ because the effect was totally inhibited in a $Ca^{2+}$-free solution and following incubation with the $Ca^{2+}$ influx blocker nifedipine.

$H_2S$-induced vascular smooth muscle relaxation is predominantly induced through the activation of potassium channels leading to membrane hyper-polarization. The participation of several additional signaling pathways and mechanisms was also confirmed, including changes in intracellular pH or ATP levels as well as endothelium-derived mechanisms (Liu *et al.* 2012).

Several types of potassium channels have been reported to be major molecular targets of vasorelaxant $H_2S$ effects. $H_2S$ was able to induce hyperpolarization by stimulating $K_{ATP}$, $K_V$ and KCNQ potassium channels in a tissue-dependent manner. Zhao *et al.* (2001) confirmed an important role of $K_{ATP}$ channels in high-dose $H_2S$-induced vasorelaxation in isolated rat aortas. The acute administration of glibenclamide ($K_{ATP}$ channel inhibitor) significantly inhibited the relaxant effects of $H_2S$. Consistent with the role of $K_{ATP}$ channels in mediating the effects of $H_2S$, reduced endogenous synthesis of $H_2S$ decreased $K_{ATP}$ channel activity. Moreover, exogenous $H_2S$ administration activated $K_{ATP}$ channels and hyperpolarized the membrane of vascular smooth muscle cells isolated from rat mesenteric arteries (Tang *et al.* 2005). However, Cheang *et al.* (2010) showed that $K_{ATP}$ channels were not involved in mediating effects of $H_2S$ in rat coronary arteries. These authors suggested voltage-dependent potassium ($K_V$) channels as possible mediators of NaHS-evoked vasorelaxation. This conclusion was reached because specific inhibition of $K_V$ channels with 4-aminopyridine reduced $H_2S$-induced relaxation of deendothelized rat coronary arteries. Schleifenbaum *et al.* (2010) proposed $H_2S$ as a vasorelaxing factor released from perivascular adipose tissue and acting *via* the stimulation of special Kv type channels – KNCQ channels. Additionally, small, intermediate, and large conductance calcium-dependent potassium channels ($SK_{Ca}$, $IK_{Ca}$ and $BK_{Ca}$) have also been demonstrated as possible mediators of $H_2S$ vasodilator effects in resistance vessels (Mustafa *et al.* 2011, Jackson-Weaver *et al.* 2013). An $H_2S$-evoked increase in cyclic guanosine monophosphate (cGMP) levels could also be involved in $H_2S$-induced vasorelaxation of smooth muscle cells. Bucci *et al.* (2010) confirmed that $H_2S$ results in vasorelaxation by non-selectively inhibiting endogenous phosphodiesterase (PDE). This effect would increase tissue levels of cyclic nucleotides, such as cGMP. Changes in the intracellular acid-base balance also influence the vasoactivity of vascular smooth muscle cells. Generally, acidification has a vasorelaxant effect, whereas the alkalinization of the intracellular environment causes vasoconstriction in most of the vascular bed. According to data published by Lee *et al.* (2007), $H_2S$ could modify the pH equilibrium in cells by activating the $Cl^-/HCO_3^-$ exchanger and thereby induce acidification. This signaling pathway is also associated with the stimulation of $K_{ATP}$ channels and so is involved in cell membrane hyperpolarization and vasorelaxation. The above-mentioned mechanisms of $H_2S$ that are involved in the regulation of vascular smooth muscle tone represent only a small portion of possible $H_2S$ signaling pathways. Indeed, one of the most important mechanisms of $H_2S$ is its involvement in the regulation of vessel wall activity by influencing NO synthesis and bioactivity.

## Interactions between the NO and $H_2S$ signaling pathways

$H_2S$ is an important component of the NO signaling pathway. Cross-talk between NO and $H_2S$ has been suggested but has not been fully characterized. NO-$H_2S$ interactions and their effects on vascular tone control are the subjects of extensive research, and it has been confirmed that these interactions occur at different levels, i.e. at the molecular level as well as in the context of their respective synthetic pathways.

Contradictory results regarding the synergistic and antagonistic effects of these gases have been published in recent years. Hosoki *et al.* (1997) reported the synergistic effect between NO and $H_2S$. Pretreatment with 30 μmol/l of the $H_2S$ donor NaHS alone did not show any relaxation effect on the thoracic aorta but significantly enhanced (by several fold) smooth muscle relaxation induced by exogenous NO donors (sodium nitroprusside and 3-morpholinosydnonimine). NaHS also shifted the dose-response curve of both NO donors to much lower concentrations. The synergistic effect with NO on smooth muscles was specific for $H_2S$ given that other thiols, including endogenous substances (e.g. cysteine and glutathione), did not induce any relaxation effect alone or in synergy with NO. Coletta *et al.* (2012) also showed that NO and $H_2S$ are mutually required for the physiological control of vascular function. The authors confirmed that pretreatment with a low concentration of NaHS (30 μmol/l, 15 min) potentiated the vasorelaxant response of the thoracic aorta to acetylcholine and to NO donor diethylammonium salt (2-(N,N-diethylamino)-diazenolate-2-oxide, DEA/NO). Moreover, this pretreatment significantly increased cGMP levels in response to DEA/NO. In addition, CSE silencing resulted in a significant inhibition of the vasodilator responses of vascular rings to both vasodilators. In our previous study, we reported that NaHS induced NO release from nitrosothiols, namely, S-nitrosoglutathione (GSNO), S-nitroso-N-acetyl-DL-penicillamine and from the metal nitrosyl complex nitroprusside (Ondrias *et al.* 2008). These results were obtained using electron paramagnetic resonance spectroscopy and by measuring the NO oxidation product (nitrite) using the Griess reaction. We also showed that pretreatment with NaHS (30 μmol/l, 2-3 min) potentiated the relaxation effect of GSNO on precontracted aortas at 7.5 pH. The guanylate cyclase pathway was involved in this effect given that a selective inhibitor of soluble guanylate cyclase ODQ (1H-[1,2,4]oxadiazolo[4,3-a] quinoxalin-1-one) inhibited the NaHS-potentiated relaxation. Exogenously applied NaHS or endogenously produced $H_2S$ form a mixture of $H_2S$, $HS^-$ and traces of $S^{2-}$; however, it is not known which form is biologically active. The proportion of $H_2S$ decreases with increasing pH, but the proportions of $HS^-$ and $S^{2-}$ increase. The actual concentration of $S^{2-}$ is very low but increases 100-fold by changing the pH from 6.0 to 8.0. As the NO release that was induced by '$H_2S$' increased only five- to seven-fold by increasing the pH from 6.0 to 8.0 in our study, we assumed that $S^{2-}$ was not the active form of '$H_2S$'. As the observed NO-releasing effect was more pronounced at pH 8.0 than pH 6.0 and was correlated with the proportion of $HS^-$ in the buffer at pH 6.0, 7.4, and 8.0, we hypothesized that $HS^-$, rather than $H_2S$, was responsible for the observed NO release. This model would also explain the pronounced relaxation effect of NaHS at pH 7.5 after the pretreatment of aorta and the minor relaxation effect at pH 6.3. The pH-dependent effect of NO release from NaHS may be important in the context of both physiological and pathological processes in which pH plays significant role.

NO, which is synthesized by three isoforms of NO-synthase (NOS), binds to the thiol group of different thiols, such as glutathione, cysteine and albumin, altering their function (Miersch and Mutus 2005). Endogenous nitrosothiols (e.g. GSNO) may act as intermediates in the storage and/or transport of NO to places where it is utilized in smooth muscle cells (Stamler *et al.* 2001, Ng *et al.* 2007). Thus, nitroso-compounds serve as stores and carriers of NO as part of the nitroso-signaling pathway (Zhang and Hogg 2005). Similarly to NO, it has been found that $H_2S$ can act either directly in a paracrine fashion or be bound to proteins and thus be transported within the organism and released at the target location (Kimura 2010). Bound sulphane-sulphur compounds serve as stores and carriers of $H_2S$ (Ishigami *et al.* 2009). Both high and low pH and other unknown mechanisms can release $H_2S$ that is bound to a protein. This process of $H_2S$ transport and function is referred to as sulphide-signaling. As mentioned above, we demonstrated that sulphide-signaling may be directly associated with nitroso-signaling and that protein-bound $H_2S$ (or more precisely, protein-bound sulphur) can induce NO release from endogenous NO donors to act *in situ* (Ondrias *et al.* 2008). Therefore, sulphide-nitroso signaling occurs, but it is not known whether S-compounds directly release NO from nitroso-compounds or whether $H_2S$ released from S-compounds induces NO release. Our subsequent finding was that NaHS (100 μmol/l) induced NO release from several nitroso-compounds (nitroso-cysteine, nitroso-N-acetylcysteine, nitroso-bovine serum albumin) in a concentration-dependent manner, similarly as we previously described for GSNO. Moreover, we observed that $H_2S$ led to the generation of a novel modified compound, "unknown interface of nitroso-sulphide signaling pathway" in the vessel wall. This conclusion was reached since preincubation of the vessel wall with low $H_2S$ concentrations subsequently led, in the absence of $H_2S$, to heightened relaxation induced by GSNO-derived NO (Bertova *et al.* 2010). We also showed that the addition of low $H_2S$ concentrations (having a slight contractile effect), resulted in the potentiation of NO release from a nitroso-protein (nitroso-bovine serum albumin) thus leading to the opposite effect, i.e. vasorelaxation. Therefore, NO and $H_2S$ interacted in the tissue to form an unknown complex of nitrosothiols. This complex has physiological properties that are different from the effects of both NO and $H_2S$. We assumed that NO release from nitroso-compounds, either directly by $H_2S$ or indirectly by $H_2S$-induced sulphur-bound compounds, represents a coupled sulphide-nitroso signaling pathway. Moreover, several authors demonstrated that the mixture of both gases before their application can lead to the generation of a novel compound and eliminate the individual effects of NO and $H_2S$. Ali *et al.* (2006) confirmed that a 1-min mixing of subthreshold concentrations of NaHS (100 µmol/l) with NO donors (sodium nitroprusside, nitrosoacetyl-penicillamine, 3-morpholinosydnonimine) resulted in a markedly diminished vasorelaxant effect of each NO donor, providing direct evidence that $H_2S$ can quench and thereby inactivate NO *in vitro*. The finding that $H_2S$ reduces the relaxant effect of three chemically very distinct NO donor molecules pointed to a direct chemical interaction between $H_2S$ (derived from NaHS) and NO (derived from NO donors). NO and $H_2S$ react in aqueous solutions to form a novel, as yet unidentified, nitrosothiol molecule. The direct vasodilator effect of $H_2S$ is unstable, transient and primarily observed in *in vitro* organ bath studies at NaHS concentrations above 100 µmol/l. However, plasma concentrations of this gas in both humans and animals are generally in the range of 30-100 µmol/l (Richardson *et al.* 2000). These low $H_2S$ concentrations are consistent with its ability to interact with NO. It has therefore been suggested that a principal physiological role of $H_2S$, released from the vasculature, may be to regulate local concentrations of NO rather than to directly dilate blood vessels. Yong *et al.* (2010) also found that a mixture of NO and $H_2S$ produced an opposite effect compared with either gas alone. These authors have shown by measuring myocyte contractility that 50 mmol/l NaHS had a negligible effect, whereas NO donors produced negative inotropic effects in cardiomyocytes. Unexpectedly, when these two types of donors were mixed, a marked increase in myocyte contractility accompanied by augmented velocities of myocyte contraction and relaxation were observed. $H_2S$ might interact with NO to form a thiol-sensitive molecule that produces positive inotropic and lusitropic effects. The nitroxyl anion (HNO) is a potential candidate, but several other compounds have been suggested to mediate the bioactivity of the interaction between S-nitrosothiols and $H_2S$. For example, thionitrous acid (HSNO) has been proposed (Filipovic *et al.* 2012), but the provided evidence appeared to be inconsistent with the known chemical properties of HSNO. Moreover, HSNO would be expected to rapidly react with excess sulphide to form other species (e.g. HNO and hydrogen disulphide); thus, its biological effect would be very short-lived. Cortese-Krott *et al.* (2014) observed that sulphide reacted with S-nitrosothiols to form multiple bioactive products and proposed that nitrosopersulphide ($SSNO^-$) could account for some of the longer-lived effects of the interaction between S-nitrosothiols and $H_2S$. $SSNO^-$ generated both NO and polysulphides on decomposition, resulting in a sustained potentiation of nitrosothiol-induced soluble guanylate cyclase stimulation. Cortese-Krott *et al.* (2015) reported that NO and sulphide form a network of cascading chemical reactions that generate radical intermediates as well as anionic and uncharged solutes, with accumulation of three major products: $SSNO^−$, and dinitrososulfite [N-nitrosohydroxylamine-N-sulfonate (SULFI/NO)], and polysulphides, each with a distinct chemical biology and *in vitro* and *in vivo* bioactivity. $SSNO^−$ efficiently donates both sulphane sulphur and NO, and potently lowers blood pressure. SULFI/NO is a weak combined NO/nitroxyl donor that releases mainly nitrous oxide ($N_2O$) on decomposition, although it affects blood pressure only mildly, it markedly increases cardiac contractility, and formation of its precursor sulphite likely contributes to NO scavenging (Cortese-Krott *et al.* 2015). Polysulphides have recently been shown to exert potent biological effects on a number of targets and may explain, at least in part, some of the effects of endogenously produced $H_2S$ and those observed with pharmacological sources of $H_2S$ (Greiner *et al.* 2013, Kimura 2015). We investigated the vascular effects of the longer-lived products of the $H_2S$-GSNO interaction (Berenyiova *et al.* 2015). To prepare the reaction products, a 10:1 molar excess of $Na_2S$ over GSNO was obtained by mixing equal volumes of $Na_2S$ (20 mmol/l) with GSNO (2 mmol/l). We showed that the products of this reaction (100 nmol/l) relaxed phenylephrine-precontracted isolated rings from the rat thoracic aorta and mesenteric artery with a more than twofold potency compared with GSNO (100 nmol/l) alone. In contrast,

$Na_2S$ and exogenous polysulphides had little effect at 1-5 µmol/l. Moreover, the onset of vasorelaxation of the reaction products was 7-10 times faster compared with GSNO. We also demonstrated that GSNO-induced relaxation (100-500 nmol/l) was blocked by an inhibitor of soluble guanylyl cyclase (ODQ, 0.1 and 10 µmol/l) and by the NO scavenger cPTIO (100 µmol/l). However, the effect was inhibited to a lesser degree by prior acidification (pH 2-4) and was unaffected by the HNO scavengers N-acetylcysteine (1 mmol/l) and methemoglobin (20 µmol/l). The relaxation induced by $H_2S$-GSNO reaction products (100-500 nmol/l) was inhibited by ODQ, slightly decreased by cPTIO, markedly inhibited by N-acetylcysteine and methemoglobin, and abolished by acidification of the reactants before addition to the organ bath. Therefore, while GSNO and the product(s) of its chemical interaction with $H_2S$ both act *via* stimulation of soluble guanylate cyclase, their relaxation profiles were differentially modulated by NO scavengers, HNO scavengers and pH. These results strongly suggest the involvement of more than one product (in the reaction mixture) in mediating cGMP activation and vasorelaxation. While NO was clearly demonstrated (using EPR spectra) to be involved in vasorelaxation, a significant portion of the relaxation induced by the $H_2S$-GSNO reaction products was mediated by a free NO-independent mechanism that directly activated soluble guanylate cyclase. Because these effects are reminiscent of those of the HNO donor Angeli's salt (Bobko *et al.* 2014), we suggested that HNO, as another reactive intermediate, may be involved in the relaxation effects induced by the mixture of both gases. Moreover, as the products of the $H_2S$-GSNO interaction were applied 3 min after mixing, at which time no further absorbance changes were seen based on EPR measurements, relatively long-lived reaction product(s) must also be involved in the observed relaxation. These products may include polysulphides and $SSNO^-$. Given that polysulphides, at the concentrations expected to prevail in the reaction mixture, did not induce vasorelaxation under comparable conditions, $SSNO^-$ appeared to account for the remaining relaxation effects of the reaction products. Nevertheless, whether any of these compounds contribute to the biological cross-talk between sulphide and NO in the cardiovascular system warrants further investigation.

The literature suggests that $H_2S$ and NO can also react in the context of their endogenous production. Ali *et al.* (2006) showed that an i.v. infusion of NaHS (25 mmol/kg/min) lowered blood pressure in rats, whereas an infusion of a low dose of NaHS (10 mmol//kg/min) increased blood pressure. The effect was relatively small (10-15 mm Hg) but was statistically significant and was maintained for several minutes. The vasopressor activity of NaHS was abolished in animals that were pretreated with L-NAME (25 mg/kg i.v.) to inhibit endogenous NO biosynthesis. This suggests that $H_2S$ (derived from NaHS) quenched endogenous endothelium-derived NO, leading to a loss of NO-derived vasodilator tone and increased blood pressure. Similarly, Kubo *et al.* (2007) showed that NaHS induced the inhibition of eNOS activity in the arterial walls of rat and mouse aortas, and this effect was associated with an increase in arterial tension. Following L-NAME pretreatment, no vasoconstricting effect was observed. They also compared the effect of cumulative doses of NaHS (1-300 µmol/l) on endothelium-dependent and -independent vasorelaxant responses in the thoracic aorta. Their data showed that pretreatment with a $H_2S$ donor significantly inhibited acetylcholine-induced vasorelaxation but did not affect the vasorelaxation effects of sodium nitroprusside. The authors suggested that low $H_2S$ concentrations inhibited eNOS activity in the presence of endogenously produced NO, possibly *via* an interaction between $H_2S$ and NOS cofactors, such as NADPH or tetrahydrobiopterin. On the other hand, Zhao *et al.* (2001, 2003) demonstrated that the NO donor sodium nitroprusside upregulated $H_2S$ production by increasing CSE expression and activity in rat vascular tissues in a concentration-dependent manner. Geng *et al.* (2007) showed that NaHS inhibited NO generation in cultured aortic tissue and that low NaHS doses downregulated the L-arginine/NO pathway 1) by inhibiting endothelial NOS expression and L-arginine transporter and/or 2) by decreasing NOS activity. According to these findings, it appears that the interaction between the endogenous production pathways of both transmitters ensures the maintenance of a dynamic balance. This interaction consists of a negative feedback loop in which NO stimulates $H_2S$ production and increased $H_2S$ levels inhibit endogenous NO production and activity.

Among the data on the opposing effects of $H_2S$ are results indicating the participation of NO/NOS in $H_2S$-induced vasorelaxation and the potentiation of NO pathway by $H_2S$. Zhao *et al.* (2001) demonstrated that $H_2S$ relaxed rat aortic tissues *in vitro* in a $K_{ATP}$ channel-dependent manner. Nevertheless, a small portion of the vasorelaxant effect of $H_2S$ was potentiated by the endothelium, indicating that $H_2S$ might act as an endothelium-dependent hyperpolarizing factor (EDHF). Zhao and Wang (2002) demonstrated that vasorelaxant potency of $H_2S$ (0.01-1 mmol/l) was attenuated by the removal of the endothelium and by blocking NO synthesis after L-NAME addition. Contrary to the findings of Hosoki *et al.* (1997), these authors showed that pretreatment of rat aortic tissues with 60 μmol/l $H_2S$ shifted the concentration-response curve for sodium nitroprusside to the right, revealing that $H_2S$ inhibited the vasorelaxant effect of the NO donor. The discrepancy between these results could result from different experimental conditions, i.e. the tissue preparation procedure and/or the level of vascular tone after precontraction. Nevertheless, this study indicated that both the endothelium and vascular smooth muscle may serve as targets of $H_2S$. As denervation does not alter the effects of $H_2S$ effect and the molecule can still significantly relax vascular tissue after endothelium removal, it has been proposed that the vasorelaxant effects of $H_2S$ are primarily due to its direct interaction with smooth muscle cells. Moreover, $H_2S$ can relax vascular tissue independent of the activation of cGMP pathway but requires calcium handling. Therefore, by acting on the endothelium, $H_2S$ may facilitate the release of vasorelaxant factors, including NO and EDHF, and by acting directly on vascular smooth muscle cells, $H_2S$ may reduce extracellular calcium entry and relax vascular tissues (Zhao and Wang 2002). Coletta *et al.* (2012) showed that the inhibition of endothelial isoform of NOS attenuated $H_2S$-stimulated vasorelaxation, demonstrating the requirement of NO in vascular $H_2S$ signaling. Conversely, silencing the $H_2S$-producing enzyme CSE abolished NO-stimulated cGMP accumulation and attenuated acetylcholine-induced vasorelaxation, indicating a partial requirement of $H_2S$ in the vascular activity of NO. The actions of $H_2S$ and NO converged at cGMP because $H_2S$ maintained a tonic inhibitory effect on phosphodiesterase type 5 (PDE-5), thereby delaying cGMP degradation. It has also been confirmed in chronic experiments that NO and $H_2S$ are mutually required for the physiological control of vascular function. Zhao *et al.* (2003) demonstrated dysfunction of the vascular $H_2S$ synthesis/$H_2S$ pathway in L-NAME-induced hypertensive rats. They showed that a 6-week administration of L-NAME to Wistar rats induced the downregulation of CSE gene expression followed by decreased CSE activity. This treatment also reduced $H_2S$ generation in the thoracic aorta and superior mesenteric artery as well as $H_2S$ plasma levels. Moreover, exogenous $H_2S$ effectively prevented the development of L-NAME-induced hypertension. These findings suggest that $H_2S$ synthesis and the $H_2S$ pathway participated in NO deficiency-induced hypertension.

## $H_2S$ in hypertension

The effects of $H_2S$ on blood pressure are characterized by considerable heterogeneity due to the concentration-dependent effects of $H_2S$. It was reported that acute intravenous addition of high concentrations of exogenous $H_2S$-donors (>10 μmol/kg) reduce blood pressure (Zhao *et al.* 2001), while low concentrations of NaHS (<10 μmol/kg) induce a significant increase (Ali *et al.* 2006). Slow $H_2S$-donor AP39 decreased and consequently increased blood pressure at 0.2-1.0 μmol/kg (Tomasova *et al.* 2015). Our *in vivo* experiments showed that intravenously injected $Na_2S$ (3 μmol/kg) had vasopressor effects only; however, at higher $Na_2S$ doses (8-30 μmol/kg), we demonstrated a transient biphasic effect on blood pressure (Drobna *et al.* 2015). The $K_{ATP}$ channel has been reported to be a major molecular target of the vasorelaxant and vasodepressor effects of $H_2S$ (Zhao *et al.* 2001). We confirmed this finding using glibenclamide, a $K_{ATP}$ channel inhibitor. Specifically, this inhibitor blocked the vasorelaxation induced by higher doses of $H_2S$ (Drobna *et al.* 2015). Given that 1) acute pretreatment with glibenclamide did not affect low-dose $H_2S$-induced contractile responses of isolated thoracic aortas and 2) the increase in blood pressure was observed at lower $H_2S$ concentrations than the biphasic effects, we concluded that $K_{ATP}$ channels were not involved in the transient blood pressure increase. The increased phase of biphasic blood pressure response to $H_2S$ could be associated with a sympathetic reflex response. This hypothesis is consistent with reports on several vasoactive substances, such as endothelin, urotensin and apelin (King *et al.* 1990, Gardiner *et al.* 2004, Charles *et al.* 2006). Gines *et al.* (1994) also suggested that the sympathetic reflex vasopressor response observed after intravenous acetylcholine injection resulted from pressure receptor stimulation following the detection of arterial hypotension. Moreover, some baroreceptors are membrane channels, which are influenced by $H_2S$. Therefore, it could be hypothesized that $H_2S$ influences baroreceptors through its action on membrane channels (Malekova *et al.* 2009, Peers *et al.* 2012).

Commonly used $H_2S$ donors (NaHS and $Na_2S$) release a large amount of $H_2S$ in a few seconds. Hence, the relevant tissue comes into the contact with high concentrations of $H_2S$, the effect of which is time-limited and non-physiological. Therefore, under the physiological conditions, when the concentration of $H_2S$ within the bloodstream is in the nanomolar range (<20 nmol/l), $H_2S$ likely evokes vasoconstricting and hypertensive effects. Nevertheless, the exogenous administration of $H_2S$ to adult spontaneously hypertensive rats (SHR) partially inhibited both the development of hypertension and aortic structural remodeling (Yan *et al.* 2004)). They also demonstrated significantly lower plasma $H_2S$ levels and a partially modified synthesis of $H_2S$. This latter effect was caused by changes in gene expression and the inhibition of CSE expression in this strain. Moreover, chronic treatment with NaHS as well as with a slow-releasing $H_2S$ donor (GYY4137) induced a significant decrease in blood pressure (Shi *et al.* 2007, Li *et al.* 2008). All of these findings suggest that $H_2S$ is engaged in the etiopathogenesis of hypertension.

Lu *et al.* (2010) showed that the intraperitoneal administration of NaHS significantly reduced the development of hypertension in two-kidney-one-clip (2K1C) rats, which represent a model of renovascular hypertension. This animal model is characterized by increased production of renin, and plasma levels of angiotensin II are 5-fold higher than in normal rats. The authors confirmed that NaHS inhibited plasma renin activity in these rats and that the $H_2S$-induced reduction of degranulation and renin release was mediated by the inhibition of adenylate cyclase activity and cAMP synthesis. In contrast, NaHS did not affect blood pressure or plasma renin activity in normal or one-kidney-one-clip (1K1C) rats, both of which exhibited normal plasma renin activity (Lu *et al.* 2012). Moreover, $H_2S$ can react with metal ions (i.e. Cu, Fe, Zn) in metalloproteins. The angiotensin-converting enzyme (ACE), which is responsible for vasoconstriction, is a zinc-containing enzyme. Laggner *et al.* (2007) proved that $H_2S$ directly inhibited ACE activity in monolayers of cultured human umbilical vein endothelial cells by interfering with the zinc atom in the active centre of ACE. $H_2S$ thereby reduced angiotensin II production and inhibited bradykinin degradation. NaHS also negatively influenced the binding of angiotensin II to its $AT_1$ receptor by reducing the affinity of the reaction. NaHS also inhibited oxidative stress signaling pathways, an effect that was associated with an inhibitory effect on smooth muscle proliferation and collagen generation (Zhao *et al.* 2008). These data suggest that the inhibition of different components of the renin-angiotensin system (RAS) could play a crucial role in the antihypertensive effect of $H_2S$. RAS plays a key role in the development of essential hypertension, and its interaction with $H_2S$ appears to be a possible mechanism of $H_2S$ involvement in the etiopathogenesis of hypertension. Our recent findings also support the above-mentioned hypothesis. We observed that a bolus administration of captopril (an angiotensin-converting enzyme inhibitor) *in vivo* reduced the $H_2S$ donor-induced decrease in blood pressure. This result suggests that captopril might inhibit the mechanism responsible for the depressor effect of $H_2S$ (Drobna *et al.* 2015). We suggested that captopril disabled the inhibitory effect of $H_2S$ on RAS, thereby masking the depressor effects of $H_2S$. As acetylcholine decreased blood pressure to the same extent as before captopril treatment, we assumed that a change in the blood pressure baseline after captopril administration was not responsible for the weaker $H_2S$ effect. Nevertheless, as the effect of $H_2S$ was reduced but not blocked by captopril, we suggested that the RAS was only partially involved. Moreover, the associations between $H_2S$, the baroreflex mechanism and the autonomic nervous system should also be taken into account. Increases in angiotensin II levels in the central nervous system have been shown to affect arterial baroreflex control and to increase the sympathetic outflow (Gao *et al.* 2005). Because the administration of captopril inhibited angiotensin II synthesis, this compound may have masked the partial baroreflex-mediated effects of $H_2S$. Moreover, Grman *et al.* (2013) and Drobna *et al.* (2015) observed that captopril inhibited $H_2S$-induced NO release from low molecular thiols, such as cysteine, N-acetylcysteine, glutathione and GSNO. As captopril contains a thiol moiety, it is possible that captopril interfered with NO signaling *via* this pathway. We previously showed that 1) $H_2S$ caused NO release from NO donors, increasing their vasorelaxant effects (Ondrias *et al.* 2008, Bertova *et al.* 2010), and 2) $Na_2S$-induced blood pressure decrease at transient $Na_2S$ blood concentrations can be triggered *via* NO release from GSNO (Drobna *et al.* 2015). If we assume that the transient presence of $H_2S$ led to the release of NO from endogenous NO-donors, then inhibiting NO release from these sources after captopril addition should also be considered. Using the Griess assay and UV-VIS spectrometry, we confirmed that captopril decreased $H_2S$-induced NO release from GSNO at pH 7.4 *in vitro*. We therefore hypothesized that this effect could contribute to the attenuated blood pressure decrease. This

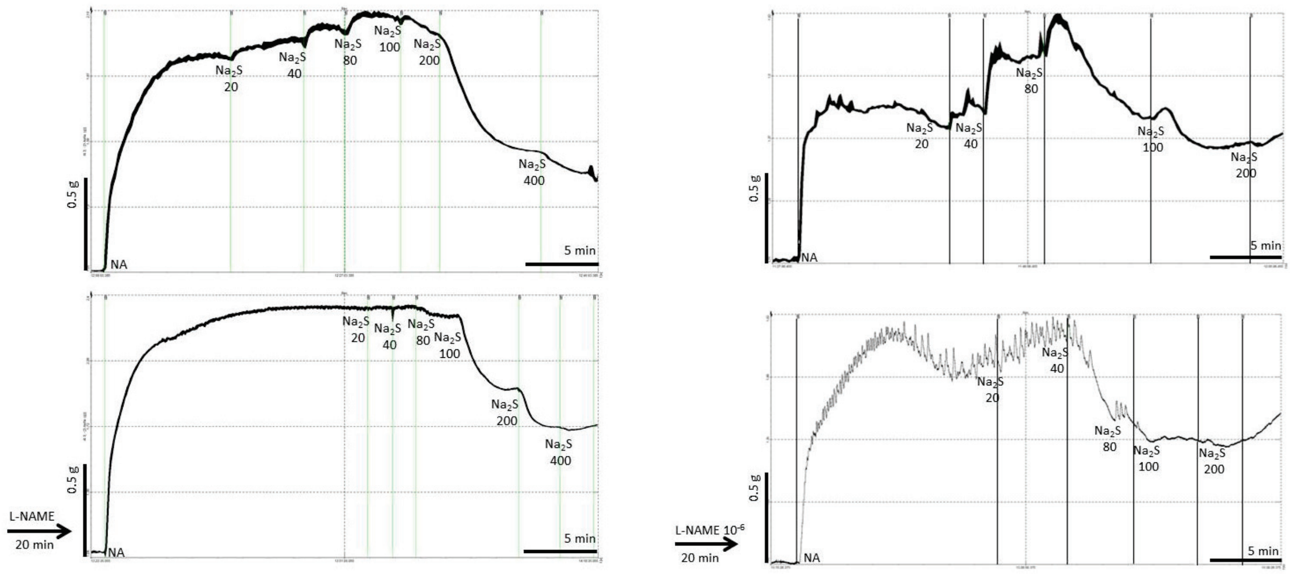


**Fig. 1.** The original record of changes in noradrenaline (NA, 1 µmol/l)-increased arterial tone which were induced by cumulative concentrations of $Na_2S$ (20-200 µmol/l) in Wistar rats **(left panels)** and SHR **(right panels)** before **(upper panels)** and after **(lower panels)** acute addition of NO-synthase inhibitor L-NAME (1 µmol/l).

idea is also supported by the observation that captopril interferes with the NO pathway and that the captopril thiol group was found to be important in preventing spontaneous hypertension (Pecháňová *et al.* 2007).

As mentioned, $H_2S$ induces a biphasic vasoactive effect. Specifically, while low concentrations evoke contractile responses, high concentrations induce relaxation of the arterial wall. However, the information on the role of $H_2S$ and its possible interactions with NO in the developmental stage of spontaneous hypertension has not been published. We compared the vasoactive effect of $Na_2S$ (applied at concentrations of 20, 40, 80, 100, 200 and 400 µmol/l) on the responses of isolated thoracic aortas in 4-week-old normotensive rats and SHRs. We showed that in 4-week-old Wistar rats, $Na_2S$ concentrations of 20-80 µmol/l induced vasoconstriction, whereas 100-400 µmol/l led to vasorelaxation. On the other hand, in young SHRs, contractile responses were obtained at concentrations of 20-40 µmol/l and vasorelaxation at 80 µmol/l. This dose-dependent shift confirmed that $H_2S$ regulates arterial tone in favor of vasorelaxation in young prehypertensive rats (Berenyiova *et al.* 2013). We also evaluated the effect of the acute inhibition of endogenous NO production on $H_2S$-induced vasoactive responses. Pretreatment with L-NAME diminished the contractile component of vasoactive effects of $H_2S$ and increased the relaxant component in young normotensive rats as well as prehypertensive SHRs (Fig. 1). These results are in agreement with findings that NO-independent pathways, predominantly $K_{ATP}$ activation, are responsible for the vasorelaxing effects of $H_2S$ (Zhao *et al.* 2001, Drobna *et al.* 2015). On the other hand, we suppose that the $H_2S$ induced vasoconstriction in our experiment is very probably associated with inhibitory action of $H_2S$ on endogenously produced NO. Kubo *et al.* (2007) observed similar results in 7- to 9-week-old Wistar rats, reporting that pretreatment with NaHS led to enhanced phenylephrine-induced contraction in endothelium-preserved thoracic aortas. Moreover, this NaHS-evoked enhancement was significantly decreased by pretreatment with L-NAME. As mentioned above, other experiments have shown that the vasoconstricting effects of $H_2S$ may depend on the presence of endogenously synthesized NO (Ali *et al.* 2006). Several authors have confirmed in both cultured and isolated vascular tissues of normotensive animals that $H_2S$ donors downregulate the L-arginine/NO pathway *via* several mechanisms (Geng *et al*. 2007, Kubo *et al.* 2007). In our experiment, we suggested that acute pretreatment with L-NAME disabled the inhibitory effect of $H_2S$ on NO production, masking the contractile effects of $H_2S$ not only in Wistar rats but also in SHRs. Moreover, our results confirmed that this effect was stronger in SHRs. In young prehypertensive rats, $H_2S$ regulated the arterial tone towards of vasorelaxant phase, and this effect was accentuated after the inhibition of endogenous NO. We showed that the pretreatment with L-NAME changed the contractile response to the relaxation in both strains. This switch-over was shifted to lower $Na_2S$ concentration in SHR (40 µmol/l) compared to Wistar rats (80 µmol/l).

These effects could be a part of the compensatory mechanisms triggered in SHRs to counter-regulate the increased vascular tone. Indeed, recent studies have shown that SHRs very likely have a unique genetic program that has compensatory and adaptive effects during the later developmental stage of hypertension. Increased activity of NO system is thought to be one of the compensatory mechanisms during increased blood pressure and arterial tonus. This hypothesis was confirmed by a study of Zhao *et al.* (2012), who observed reduced contractile responses of thoracic aortas in SHRs compared to normotensive rats. Only in SHRs was inhibited vasoconstriction associated with the ability of endothelial cells to release a vasoconstriction-reducing compound. The authors also showed that the endothelium-released substance that reduced arterial tone was NO that was not synthesized by NO synthase. Other experiments demonstrated that compared to normotensive rats, SHRs generated higher level of nitrites and nitrates, which represent NOS-independent NO sources (Wu and Yen 1999). These molecules can substitute for bioactive nitrous oxide, including NO. Zhao *et al.* (2012) confirmed that the alternative production of NO from nitrites and nitrates represents a compensatory effect of the arterial wall to overcome insufficient synthesis of NO by endothelial NOS during hypertension. Moreover, our previous findings confirmed that the arterial wall produces physiologically active NO not only by endothelial but also by smooth muscle cells, where the expression of two NOS isoforms was confirmed (Buchwalow *et al.* 2008, Cacanyiova *et al.* 2013). These results showed that non-endothelial NO production could represent an additional compensatory mechanism to ensure vasorelaxant responses when NO produced by endothelial sources is eliminated by the increased production of free radicals (Cacanyiova *et al.* 2013). Consistent with this hypothesis, Boulanger *et al.* (1998) demonstrated in the carotid artery of SHRs that the neuronal NOS isoform was activated in vascular smooth muscle cells upon stimulation by angiotensin and could compensate for a weakened endothelial response; this was not observed in normotensive animals. Our finding of increased arterial sensitivity to $H_2S$ in favor of vasorelaxation in young SHRs compared to normotensive rats is consistent with the above-mentioned findings and confirms that endothelium-regulated compensatory mechanisms have already been triggered in the crucial juvenile stage of hypertension progression.

The lipid composition of biological membranes is crucial for many aspects of organelle function. Fatty acids are a major energy source and are important constituents of membrane lipids, serving as cellular signaling molecules. $H_2S$ is soluble in lipids and readily crosses membranes. We found that lipids and fatty acids can affect the modulator effect of $H_2S$ on NO release from nitroso-compounds, and this effect depends on the particular lipid or fatty acid used. Unsaturated fatty acid, linoleic acid, and lipids with unsaturated fatty acids (asolectin) depressed NaHS-induced NO release from GSNO. Alternatively, the depressive effects of myristic acid (a saturated fatty acid) and lipids with saturated fatty acids were less pronounced (Tomaskova *et al.* 2009). This result may indicate an important role of the composition of membrane lipids in the environment in which $H_2S$ is produced. We propose that the products of NaHS, $H_2S$, $HS^-$ and/or $S^{2-}$ may chemically interact with the unsaturated bonds of fatty acids and thereby decrease the effective $H_2S$ concentration that can interact with GSNO. This effect could inhibit the $H_2S$-induced potentiation of vasorelaxation. Moreover, Muellner *et al.* (2009) showed that $H_2S$ may act as an antiatherogenic agent by reducing highly reactive lipid hydroperoxides in oxidized LDL, thereby abrogating their pathological activity. Therefore, the quenching of $H_2S$ by unsaturated fatty acids could inhibit the attenuation of lipid hydroperoxide formation. Moreover, the primary step in sulphide reactions is the electron transfer from $H_2S$/HS species to a suitable acceptor, e.g. $O_2$, thereby producing $HS^\bullet$ and $S^{\bullet-}$ radicals. Stasko *et al.* (2009) and Lykakis *et al.* (2007) demonstrated the potential of $HS^\bullet$ and $S^{\bullet-}$ radicals derived from $H_2S$ to access hydrophobic fatty acid chains and attack the double bonds, isomerising the double bonds in cell membrane lipids and leading to their instability. An altered lipid membrane composition as a result of disordered lipid metabolism and altered fatty acid metabolism is connected to several diseases, such as obesity, hypertension, diabetes mellitus and others (Das 2006).

Whiteman *et al.* (2010) confirmed that the concentration of $H_2S$ in human plasma was reduced in patients with diabetes mellitus type II and that adiposity, obesity and overweight were determinants of this effect. We performed pilot experiments on renal arteries isolated from humans suffering from arterial hypertension, showing that a mixture of exogenous NO and $H_2S$ induced a vasorelaxant effect. This effect not only differed from that observed in isolated rat thoracic aortas (Berenyiova *et al.* 2015) but was also modulated by a patient´s metabolic malfunction (e.g. diabetes mellitus or hypercholesterolemia) or obesity. Obesity, which is an important risk factor in the development of hypertension, is characterized by excessive and abnormal adipose tissue accumulation, including perivascular adipose tissue. Perivascular adipose tissue is a local deposit of adipose tissue that surrounds the vasculature. This tissue is metabolically active and secretes a wide array of bioactive substances, termed adipokines. $H_2S$ was identified as an adipocyte-derived relaxing factor (Schleifenbaum *et al.* 2010), and adipokines produced by perivascular adipose tissue may affect the endothelial function of arteries (Ma *et al.* 2016). Human studies showed that this tissue is physiologically active and produces beneficial compounds. Nevertheless, it appears that the balance among lipid metabolism, which involves adipose deposits in the arterial wall, and $H_2S$ signaling pathways and endothelial function, could be injured in pathological conditions. However, further studies are required to substantiate the precise relationship between these factors and their roles in various pathologies.

## Conclusions

$H_2S$ and NO interact on different levels, acting on both arterial smooth muscle cells and endothelial cells and modulating the chemical structure of endogenous proteins. Some of the affected proteins include the enzymes responsible for the endogenous production of these signaling molecules, which regulate and maintain vascular homeostasis and dynamic balance. Imbalances in this network may contribute to the pathogenesis of cardiovascular diseases. Research into the regulation of the interaction between these gases will likely reveal novel avenues for understanding the pathological mechanisms of cardiovascular diseases and for the development of novel prevention and treatment strategies.

## Conflict of Interest

There is no conflict of interest.

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## Abbreviations

1K1C rats – one-kidney-one-clip rats
2K1C rats – two-kidney-one-clip rats
3-MST – mercaptopyruvate-sulphurtransferase
ACE – angiotensin-converting enzyme
$AT_1$ – angiotensine II receptor type 1
ATP – adenosine triphosphate
$BK_{Ca}$ – big conductance $Ca^{2+}$-activated $K^+$ channel
cAMP – cyclic adenosine monophosphate
CAT – cysteine-aminotransferase
CBS – cystathionine β-synthase
cGMP – cyclic guanosine monophosphate
$Cl^-/HCO_3^-$ – bicarbonate transporter protein
CO – carbon monoxide
$CO_2$ – carbon dioxide
cPTIO – 2-(4-Carboxyphenyl)-4,4,5,5-tetramethyl-imidazoline-1-oxyl-3-oxide
CSE – cystathionine γ-lyase
DEA/NO – 2-(N,N-diethylamino)-diazenolate-2-oxide
EDHF – endothelium-dependent hyperpolarizing factor
eNOS – endothelial NO-synthase
EPR – electron paramagnetic resonance
GSNO – S-nitrosogluthatione
$H_2S$ – hydrogen sulphide
HNO – nitroxyl anion
$HS^-$ – hydrosulfide ion
$HS^{\cdot}$ – hydrosulfide radical
HSNO – thionitrous acid
$IK_{Ca}$ – intermediate conductance $Ca^{2+}$-activated $K^+$ channel
$K_{ATP}$ – ATP-sensitive $K^+$ channel
KCNQ – subfamily of voltage-gated $K^+$ channels
$K_v$ – voltage-gated $K^+$ channel
LDL – low density lipoprotein
L-NAME – $N^G$-nitro-L-arginine-methylester
$N_2O$ – nitrous oxide
$Na_2S$ – sodium sulphide
NADPH – nicotinamide adenine dinucleotide phosphate
NaHS – sodium hydrosulphide
NO – nitric oxide
NOS – NO-synthase
$O_2$ – diatomic oxygen
ODQ – 1H-[1,2,4]oxadiazolo[4,3- a]quinoxalin-1-one
PDE – phosphodiesterase
PDE-5 – phosphodiesterase type 5
RAS – renin-angiotensin system
$S^{2-}$ – sulphide dianion
SHR – spontaneously hypertensive rats
$SK_{Ca}$ – small conductance $Ca^{2+}$-activated $K^+$ channel
$SSNO^-$ – nitrosopersulphide
SULFI/NO – N-nitrosohydroxylamine-N-sulfonate

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