British Journal of Pharmacology

DOI:10.1111/bph.12900
www.brjpharmacol.org

Themed Section: Chinese Innovation in Cardiovascular Drug Discovery

REVIEW

Emerging role of hydrogen sulfide in hypertension and related cardiovascular diseases

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Received
21 June 2014
Revised
18 August 2014
Accepted
28 August 2014

Hydrogen sulfide (H_2S) has traditionally been viewed as a highly toxic gas; however, recent studies have implicated H_2S as a third member of the gasotransmitter family, exhibiting properties similar to NO and carbon monoxide. Accumulating evidence has suggested that H_2S influences a wide range of physiological and pathological processes, among which blood vessel relaxation, cardioprotection and atherosclerosis have been particularly studied. In the cardiovascular system, H_2S production is predominantly catalyzed by cystathionine γ-lyase (CSE). Decreased endogenous H_2S levels have been found in hypertensive patients and animals, and $CSE^{-/-}$ mice develop hypertension with age, suggesting that a deficiency in H_2S contributes importantly to BP regulation. H_2S supplementation attenuates hypertension in different hypertensive animal models. The mechanism by which H_2S was originally proposed to attenuate hypertension was by virtue of its action on vascular tone, which may be related to effects on different ion channels. Both H_2S and NO cause vasodilatation and there is cross-talk between these two molecules to regulate BP. Suppression of oxidative stress may also contribute to antihypertensive effects of H_2S. This review also summarizes the state of research on H_2S and hypertension in China. A better understanding of the role of H_2S in hypertension and related cardiovascular diseases will allow novel strategies to be devised for their treatment.

LINKED ARTICLES

This article is part of a themed section on Chinese Innovation in Cardiovascular Drug Discovery. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2015.172.issue-23

Abbreviation

2K1C, two-kidney-one-clip; Ang II; angiotensin II; AOA, aminooxyacetic acid; AT_1 receptor, angiotensin II type 1 receptor; CBS, cystathionine-β-synthase; CO, carbon monoxide; CSE, cystathionine-γ-lyase; DBP, diastolic BP; eNOS, endothelial NOS; H_2S, hydrogen sulfide; I/R, ischaemia/reperfusion; L-NAME, N^G-nitro-l-arginine methyl ester; MAP, mean arterial pressure; MPST, 3-mercaptopyruvate sulfurtransferase; MWT, medial wall thickness; PAAT, pulmonary arterial acceleration time; PAG; DL-propargylglycine; PHT, pulmonary hypertension; RVET, right ventricular ejection time; RVH, right ventricular hypertrophy; SBP, systolic BP; SHR, spontaneously hypertensive rat; SMCs, smooth muscle cells; VEGFR-1, soluble fms-like tyrosine kinase 1; VD, vas deferens

Tables of Links

TARGETS	
GPCR[a]	**Catalytic receptors**[d]
AT_1 receptor	VEGFR-1
Muscarinic receptors	**Enzymes**[e]
Thromboxane A_2 receptor	CBS
Ligand-gated ion channels[b]	CSE
Epithelial sodium channels (ENaC)	eNOS
Ion channels[c]	ERK1/2
BK_{Ca} channels	HO1
Ca_V channels	p38MAPK
$Ca_V1.1$-1.4 (L-type Ca) channels	PKG
K_{ATP} channels	PTEN
K_{ir} channels	MPST
K_V channels	
$K_V7.x$ (KCNQ) channels	

LIGANDS	
ACh	L-NAME
Angiotensin II	NaHS
AOA	Nifedipine
Carbachol	Nitric oxide (NO)
Glibenclamide	Noradrenaline
Homocysteine	Phenylephrine
Iberiotoxin	Tetraethylammonium
L-arginine	U46619
L-cysteine	XE991

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 ([a,b,c,d,e]Alexander *et al.*, 2013a,b,c,d,e).

Introduction

In recent years, the 'gasotransmitters' NO, carbon monoxide (CO) and hydrogen sulfide (H_2S) have been the object of intense research (Papapetropoulos *et al.*, 2014). In the last two decades, NO has been extensively studied; and in more recent times, the importance of H_2S in cardiovascular regulation has become increasingly apparent (Polhemus and Lefer, 2014). Until the H_2S content within the brain was first measured in *postmortem* studies in 1989, H_2S was traditionally viewed as a highly toxic gas devoid of beneficial biological or physiological functions (Goodwin *et al.*, 1989). Subsequently, H_2S quickly emerged as an important signalling molecule with widespread physiological functions.

Three enzymatic pathways have been identified which produce H_2S in mammals: cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (MPST). CBS and CSE are believed to be the critical enzymes for H_2S generation in the modulation of neurological and cardiovascular functions, respectively, while expression of MPST has also been found in vascular endothelium (Wang, 2012). Accumulating evidence has confirmed that a wide range of physiological and pathological processes can be mediated by H_2S, especially in the cardiovascular system, including blood vessel relaxation, cardioprotection and atherosclerosis (Wang, 2011; Tang *et al.*, 2013; Yang *et al.*, 2013; Bos *et al.*, 2014; King *et al.*, 2014; Mani *et al.*, 2014). This review will focus on the roles of H_2S in hypertension and related cardiovascular diseases.

Deficiency of H_2S production and hypertension development

Several studies have shown that H_2S may play important roles in the pathogenesis and development of hypertension in humans. In patients with grade 2 and 3 hypertension, plasma H_2S concentrations were found to be lower than in subjects with normal BP (Sun *et al.*, 2007). In patients with portal hypertension, endogenous H_2S levels have been reported to be lower than in healthy controls; and an inverse relationship was seen between disease severity and lower plasma H_2S levels, the latter being inversely correlated with portal vein diameters and Child-Pugh score (Wang *et al.*, 2014). In patients with pulmonary hypertension (PHT), levels of H_2S and expression of CSE have been found to be reduced; based on evaluation of the receiver-operating characteristic curve, CSE had the most significant sensitivity and specificity to predict dynamic PHT (Sun *et al.*, 2014). H_2S deficiency might be mainly ascribed to reduce CSE activity or expression, although little is known about transcriptional regulation or post-translational modification of CSE.

Genetic modulations of CSE, CBS or MPST levels are effective means to experimentally investigate the cardiovascular actions of H_2S. However, complete genetic deficiency of CBS (as in a homozygote knockout mouse) exhibits a neonatally lethal phenotype because of liver dysfunction (Watanabe *et al.*, 1995). It is possible to circumvent this lethality problem by insertion of a transgene that expresses a cDNA encoding for a human CBS protein that contains the I278T mutation under control of a zinc-inducible metallothionein promoter (Wang *et al.*, 2005). Indeed, expression of this mutant CBS

protein was able to prevent the neonatally lethal phenotype, but failed to normalize the elevated total homocysteine levels (Gupta *et al.*, 2014). Some evidence suggests that, in the context of hyperhomocysteinemia, homocysteine can inactivate CSE by homocysteinylation, a phenomenon seen in hypertension and other cardiovascular diseases (Sen *et al.*, 2010). On the other hand, MPST knockout is not lethal to mice, but there exists a paucity of information regarding any possible effect of genetic deficiency of MPST on cardiovascular disease (Nagahara, 2013). It is worth noting that CSE mutant mice with a C57BL/6J × 129SvEv mixed genetic background develop hypertension with age. Systolic BP (SBP) in the mutant mice has been reported to increase at more than 135 mmHg at 12 weeks of age, which was almost 18 mmHg higher than in control mice. The BP of $CSE^{-/-}$ mice was about 10 mmHg higher than that of $CSE^{-/+}$ mice after 10 weeks of age, all of which suggests that a deficiency of CSE/H_2S may contribute to BP augmentation (Yang *et al.*, 2008). However, it has been controversially reported that C57BL/6J mice with CSE deficiency actually exhibit normal BP (Ishii *et al.*, 2010). This discrepancy may be due to differences in their genetic background. However, it should also be noted that, in the earlier studies, BP was measured using a standard tail-cuff non-invasive measurement system or by intra-arterial catheterization method rather than by telemetric BP recordings, and the latter is much less prone to error than either of the former methods. An augmented level of less than 20 mmHg in $CSE^{-/-}$ mice might also be subjected to error and possible bias. An electronic BP monitor is the only reliable method for analysing systemic BP nowadays. Additionally, there is a limitation that all of the phenotypes are observed in mice with an overall CSE deficiency, not just a conditional knockout in the cardiovascular system. Therefore, the true effect of CSE deficiency on BP remains unclear at present. Lucock *et al.* found that both genetic variants of CSE-G1364T and CBS-844ins68 are biological determinants of H_2S synthesis and are aetiologically important in the regulation of BP (Lucock *et al.*, 2013). In addition, it has been observed that mean arterial pressure (MAP) in rats is not enhanced after treatment with either the CSE inhibitor DL-propargylglycine (PAG) or the CBS inhibitor aminooxyacetic acid (AOA) alone for 4 weeks; however, MAP increased gradually from 99 ± 2 to 130 ± 1 mmHg during 4 weeks of treatment with the combination of PAG and AOA (Roy *et al.*, 2012). The balance of evidence therefore suggests that H_2S plays an important role in regulating BP.

H_2S supplementation attenuates hypertension

It has been reported in many studies that H_2S supplementation decreases BP in different hypertensive models. The protective effects of H_2S in different conditions are discussed in the succeeding text.

Role of H_2S in the spontaneously hypertensive rat (SHR)

Many of the cardiovascular changes in the SHR are similar to those in hypertensive human subjects. Levels of H_2S in plasma, urinary and gene expression and activity of CSE in thoracic aorta are all suppressed in SHR (Yan *et al.*, 2004; Ahmad *et al.*, 2014). A profound antihypertensive effect of H_2S in SHR has been identified by several groups. Yan *et al.* and Zhao *et al.* found that exogenous administration of NaHS (56 μmol·kg^{-1} day^{-1}) for 5 weeks attenuated the elevation of pressure and lessened aortic structural remodelling and collagen accumulation during the development of hypertension (Yan *et al.*, 2004; Zhao *et al.*, 2008). Shi *et al.* reported that NaHS treatment for 3 months at doses of 30 and 90 μmol·kg^{-1} day^{-1} reduced SBP, diastolic BP (DBP) and MAP to similar extents in SHR. Moreover, NaHS at 10 μmol·kg^{-1} day^{-1} reduced DBP and MAP, indicating that lower doses of NaHS can reduce BP if treatment if given for a long enough period (Shi *et al.*, 2007). These findings indicate that NaHS at doses between 10 and 90 μmol·kg^{-1} day^{-1} reduce BP in SHR. On the other hand, it is well established that NaHS promotes apoptotic cell death of cultured fibroblasts and smooth muscle cells (SMCs; Baskar *et al.*, 2007) and additionally releases copious amounts of H_2S over a short time frame (s), which does not effectively mimic physiological concentrations of H_2S *in vivo* and might be harmful. The studies have not evaluated specifically for possible toxicity of NaHS; therefore, whether the antihypertensive effect was simply a manifestation of H_2S toxicity remains to be clarified. Because NaHS is not an ideal H_2S donor, several other H_2S donors, including GYY4137 (a slow-releasing H_2S donor), has been synthesized to evaluate the true physiological role of H_2S. GYY4137 did not cause detectable cytotoxicity or alter the cell-cycle profile or p53 expression of cultured rat vascular SMCs; additionally, it releases low amounts of H_2S slowly and persistently and does not trigger signalling pathways leading to cell death. Chronic treatment of conscious animals with GYY4137 at 133 μmol·kg^{-1} reduced SBP in SHR, the fall in BP being apparent after 2 days and persisting after 14 days of treatment. On cessation of drug therapy, the BP of Wistar-Kyoto (WKY) rats returned to pre-injection values within 7 days; while for SHR, the BP was still well controlled at this time point, with BP of all animals returning to pretreatment levels 14 days after cessation of treatment. Despite such lack of toxicity, GYY4137 similarly gave rise to a BP reduction (Li *et al.*, 2008a). In a word, an H_2S supplement is more or less beneficial in reducing BP in SHR.

Role of H_2S in PHT

In tissue bath preparations of small peripheral airways (<5 mm in diameter) from porcine lungs precontracted with the muscarinic ACh receptor agonist carbachol, both H_2S donor NaHS and the precursor L-cysteine causes a large relaxation of the airways (Rashid *et al.*, 2013). Ariyaratnam *et al.* found that 500 μM NaHS causes a reduction in both pulmonary artery and bronchial airway pressures (Ariyaratnam *et al.*, 2013). Moreover, Na_2S, GYY4137 and L-cysteine also cause relaxation of airways (Parkinson *et al.*, 1988; Castro-Piedras and Perez-Zoghbi, 2013).

Collectively, these data suggest that H_2S is a potent dilator of human pulmonary arteries *in vitro*. However, the influence of H_2S on pulmonary blood flow *in vivo* is extremely complex. The notion that H_2S has powerful therapeutic potential for PHT, therefore, needs further confirmation *in vivo*. Rats exposed to 21 days of hypoxia exhibit decreased plasma H_2S concentration and H_2S production in their lungs (Wei *et al.*, 2008). Administration of NaHS (10 μmol·kg^{-1}·day^{-1}) has been

found to reduce the mean pulmonary artery pressure by 31.2%, but this protective effect was largely abolished by PAG, indicating that H_2S might be involved in the development of hypoxia-induced PHT. H_2S supplementation can also attenuate hypoxia-induced hypertension in broilers (Yang *et al.*, 2012). Up-regulating the endogenous H_2S pathway also reduces pulmonary arterial pressure in rats with PHT induced by high pulmonary blood flow (Luo *et al.*, 2013). After aorta-veno cava shunting for 11 weeks, rats exhibit PHT and pulmonary artery collagen remodelling in association with a decrease in lung tissue H_2S content, suggesting that a reduced level of H_2S may contribute to the detrimental effect of shunting (Li *et al.*, 2008b). PHT is also considered as a complication of severe bronchopulmonary dysplasia. Neonatal rats exposed to chronic hyperoxia develop PHT, as demonstrated by a significant decrease in the pulmonary arterial acceleration time/right ventricular ejection time (PAAT/RVET) and an increase in medial wall thickness (MWT) of small pulmonary arteries and right ventricular hypertrophy (RVH); GYY4137 attenuates these functional and structural features of PHT with an increase in mean PAAT/RVET, a decrease in MWT and a reduction in RVH (Vadivel *et al.*, 2014). In human subjects, basal exhaled H_2S is higher than the ambient concentration of H_2S in room air, indicative of endogenous H_2S production in humans; after i.v. administration of Na_2S, a rapid elevation of exhaled H_2S concentration was observed, the amount of exhaled H_2S rapidly decreases after discontinuation of the Na_2S infusion, suggesting that exogenously administered H_2S diffuses to the bronchial tissue (Toombs *et al.*, 2010). Collectively, these findings suggest that H_2S may offer a novel therapeutic target for PHT. However, disagreement exists in H_2S concentration and the possibility of lung injury. Inhalation of 80 ppm H_2S has been reported to ameliorate lung pathology in lipopolysaccharide-induced (Faller *et al.*, 2012) and in ventilator-induced (Faller *et al.*, 2010) lung injury. On the other hand, Francis *et al*. observed that 1 or 5 ppm H_2S did not alter ventilation-induced lung injury, while 60 ppm H_2S worsened it (Francis *et al.*, 2011). It is clear that non-specific toxicity may in itself evoke a decrease in BP. The equipotent inhalation concentration of different H_2S donors by injection has not been evaluated. In other words, further work needs to be done to investigate possible H_2S lung toxicity in relation to dose.

Role of H_2S in other types of hypertension

Pre-eclampsia is a hypertensive syndrome that affects 4–7% of all pregnancies and is a major contributor to maternal and fetal morbidity and mortality worldwide. Plasma H_2S levels and CSE mRNA expression in the pre-eclamptic placenta have been found to be reduced in pre-eclampsia compared with normotensive controls (Wang *et al.*, 2013). After PAG treatment of pregnant C57Bl6/J mice from E8.5 to E17.5, a dose-dependent decrease in circulating H_2S levels was observed; importantly, MAP increased in response to PAG in a dose-dependent manner, an effect which was attenuated by co-administration of 0.25 $mg \cdot kg^{-1}$ GYY4137 (Wang *et al.*, 2013). On the other hand, Holwerda *et al*. observed that placental CBS mRNA expression decreased in the early-onset pre-eclampsia, whereas CSE mRNA in placenta was unchanged in severe pre-eclampsia (Holwerda *et al.*, 2012). Cindrova-Davies *et al*. reported CSE level to be reduced in placentas from pregnancies with severe early-onset growth restriction and pre-eclampsia displaying abnormal umbilical artery Doppler waveforms, compared with both pre-eclamptic placentas with normal waveforms and controls (Cindrova-Davies *et al.*, 2013). These contradictory findings regarding placental expression of H_2S-synthesizing enzymes may be explained by a lack of significant results because of the small sample sizes in these studies (Patel *et al.*, 2009; Holwerda *et al.*, 2012; Cindrova-Davies *et al.*, 2013) and highlight the need for larger studies to be performed. Soluble fms-related tyrosine kinase 1 (VEGFR-1), a circulating anti-angiogenic protein, contributes to the development of pre-eclampsia; treatment with NaHS (50 $\mu mol \cdot kg^{-1}$, twice daily) for 8 days reduced VEGFR-1-induced hypertension by up-regulating VEGF expression in rats, although whether this effect is sustained at later time points is presently unknown (Holwerda *et al.*, 2014). Collectively, these data suggest that endogenous H_2S is required for healthy placental vascular function and that a decrease in CSE/H_2S activity may contribute to the pathogenesis of pre-eclampsia.

Following induction of hypertension in Wistar rats by oral administration of the L-arginine analogue N^G-nitro-l-arginine methyl ester (L-NAME) in drinking water for 6 weeks, NaHS treatment decreased in SBP by 19%; furthermore, the observed inhibition of H_2S generation and CSE activity in these rats was also greatly attenuated by NaHS treatment (Zhong *et al.*, 2003). Li *et al*. also found that acute i.v. pre-injection of GYY4137 (133 $\mu mol \cdot kg^{-1}$), but not of NaHS (2.5 $\mu mol \cdot kg^{-1}$) or saline, reduced the L-NAME-mediated hypertension (Li *et al.*, 2008a). This suggests that there is likely to be an optimal balance between H_2S and NO to maintain a dynamic equilibrium and that, if the balance is disturbed for example by L-NAME administration, H_2S supplementation can redress the disturbance in BP.

Tan *et al*. reported that CCl_4 reduces serum H_2S levels, hepatic H_2S production and CSE expression in rats; exogenous NaHS was found to attenuate CCl_4-induced hepatotoxicity, liver cirrhosis and portal hypertension, indicating that targeting H_2S may present a promising approach, particularly in relation to prevention, against portal hypertension (Tan *et al.*, 2011). Rats with cirrhosis induced by bile duct ligation for 4 weeks were treated daily with NaHS for 5 days, then isolated livers were perfused first with NaHS for 20 min followed by noradrenaline (NA). It was found that bile duct ligation resulted in down-regulation of CSE mRNA/protein levels and activity, indicating that a reduction of CSE expression in the liver with cirrhosis contributes to the development of increased intrahepatic resistance and portal hypertension. NA administration resulted in a dose-dependent increase of portal pressure and this effect was restored by H_2S treatment (Fiorucci *et al.*, 2005). However, inhibition of CSE prevents acute inflammatory liver failure by augmenting thiosulfate levels and up-regulating antioxidant and anti-apoptotic defence in the liver (Shirozu *et al.*, 2014). It is possible that excess or even an exogenous supplement of H_2S might result in liver injury. That is, there is a possibility that the attenuating effect of H_2S on portal hypertension is not due to its pharmacological characteristics but is due merely to non-specific toxic effects. A more accurate conclusion can only be made on the precondition that the H_2S supplement is not harmful to the liver.

To date, few data are available on the effect of H_2S on renal hypertension, a type of secondary hypertension. Renal hypertension can be induced with two-kidney-one-clip (2K1C, a clip constricting one renal artery) in animals. One group found that NaHS treatment (5.6 $mg \cdot kg^{-1} \cdot day^{-1}$) over 4 weeks reversed the BP elevation in 2K1C rats but not in one-kidney-one-clip rats, suggesting that the antihypertensive effect of H_2S may be greater in hypertension associated with higher plasma renin activity (Lu *et al.*, 2010). Zhang *et al.* found that H_2S also prevented H_2O_2-induced activation of epithelial sodium channels, through which sodium can be reabsorbed in the distal renal tubules to regulate salt-sensitive hypertension, through a phosphatase and tensins homologue (PTEN; previously known as phosphatidylinositol 3,4,5-trisphosphate-dependent) pathway (Zhang *et al.*, 2013). Further investigation of the effect of H_2S on epithelial sodium channel activity in animal models may therefore be relevant to the clinical management of salt-sensitive hypertension. Recently, it has been reported that exogenous administration of an H_2S donor attenuates angiotensin II (Ang II)-induced hypertension (Snijder *et al.*, 2014). Although Zhao *et al.* found that NaHS decreases the binding affinity of the angiotensin II type 1 (AT_1) receptor and attenuates AT_1 receptor activation (Zhao *et al.*, 2008), the mechanism by which H_2S regulates Ang II-induced hypertension is not clear because of the involvement of complex interacting networks including renal sympathetic nerve activity and the cardiac sympathetic afferent reflex.

Principal possible mechanisms of the antihypertensive effect of H_2S

Relaxation of vascular smooth muscle

One of the earliest proposed beneficial physiological effects of H_2S was its action on vascular tone. The endothelium-dependent vasorelaxation induced by H_2S shares many common mechanistic traits with that of endothelium-derived hyperpolarizing factor (Edwards *et al.*, 2012). Deficiency in CSE expression diminishes endothelium-dependent relaxation of resistance arteries (Yang *et al.*, 2008). Tang *et al.* also found that CSE-knockout mice exhibit elevated resting membrane potential of SMCs, and lack a methacholine-induced endothelium-dependent relaxation of mesenteric arteries, whereas that of aorta is preserved; methacholine caused hyperpolarization of SMC in endothelium-intact mesenteric arteries from wild-type mice, but this effect was abolished in CSE-knockout mice, and treatment with exogenous H_2S hyperpolarized vascular SMCs and endothelial cells from both wild-type and CSE-knockout mice, suggesting that H_2S is indeed an endothelium-derived hyperpolarizing factor (Tang *et al.*, 2013). Loss of endothelium attenuates the relaxation of rat aortic tissues induced by H_2S and shifts the H_2S concentration-response curve to the right (Zhao and Wang, 2002). The endothelium dependence of the H_2S effect is more pronounced in isolated and perfused rat mesenteric artery bed, such that removal of functional endothelium reduced H_2S-induced relaxation of rat mesenteric artery bed by about sevenfold, with an increase in EC_{50} of H_2S from 25 to 161 μM (Cheng *et al.*, 2004). This tissue-selective endothelium-dependent effect of H_2S is similar to that of endothelium-derived hyperpolarizing factor. However, NaHS (0.1–3.0 mM) has been found to elicit concentration-dependent relaxation of rat middle cerebral arteries, which is unaffected by endothelium removal (Streeter *et al.*, 2012). NaHS relaxes coronary arteries precontracted by U46619 (a thromboxane A_2 agonist), this relaxation is similarly unaffected by endothelium removal (Cheang *et al.*, 2010). Expression of CSE and CBS protein has been observed in vascular endothelial cells (Wen *et al.*, 2013). However, it remains unclear why the endothelium dependence of H_2S-mediated relaxation appears to vary from one blood vessel type to another. Specific targets in the endothelium are still not available nowadays.

A large part of H_2S-induced vasorelaxation appears to be dependent on the activation of ATP-sensitive K^+ channel (KATP) in vascular smooth muscle (Liu *et al.*, 2011; Wang, 2011) by increasing whole-cell K_{ATP} currents to hyperpolarize membrane potentials and improving single-channel activity by enhancing permeability of single K_{ATP} channels (Tang *et al.*, 2005). Using the whole-cell and single-channel patch-clamp technique, direct evidence was obtained that exogenous H_2S activates K_{ATP} channels and hyperpolarizes cell membrane of rat aorta and mesenteric artery SMCs, and that inhibition of endogenous H_2S production with PAG reduces whole-cell K_{ATP} currents (Zhao *et al.*, 2001). In the concentration range 100 nM–100 μM, GYY4137 elicits a concentration-dependent relaxation of phenylephrine-induced contraction in isolated posterior ciliary arteries, which is attenuated by the K_{ATP} channel blocker glibenclamide, suggesting that vascular smooth muscle relaxation induced by H_2S is mediated, at least in part, by K_{ATP} channels (Chitnis *et al.*, 2013). Reduced expression of CSE and increased miR-21 in placentas are also associated with increased vascular resistance. Perfusion of normal placentas with NaHS, after preconstriction with a thromboxane mimetic, results in a dose-dependent vasorelaxation, which can be partially blocked by glibenclamide (Cindrova-Davies *et al.*, 2013). Nevertheless, the detailed mechanism by which H_2S activates K_{ATP} channels remains to be elucidated. After treatment of mouse aortic rings with NaHS, cGMP-dependent PKG activation and NaHS-stimulated relaxation were evoked in a time-dependent manner, which could be attenuated by DT-2 (a PKG1 inhibitor), although interestingly, vasodilator responses to a slow-releasing H_2S donor (GYY4137) were unaffected by DT-2, suggesting that this donor dilates mouse aorta through PKG-independent pathways. Dilator responses to NaHS were reduced in vessels of $PKG^{-/-}$ mice, and moreover, glibenclamide inhibited NaHS-induced vasorelaxation in vessels from wild-type animals, but not PKG-$I^{-/-}$ mice, suggesting that there is cross-talk between K_{ATP} and PKG (Bucci *et al.*, 2012). Besides PKG, there may also be other as yet undiscovered H_2S-induced signal-transduction pathways mediating K_{ATP} channel activation.

Several other studies have found that H_2S may also induce vasodilatation by affecting other ion channels besides K_{ATP}. Suppression of L-type calcium channels with nifedipine or inhibiting potassium conductance with 50 mM K^+ reduce the maximum relaxation elicited by NaHS in rat middle cerebral arteries; however, selective blockers of K_{ATP}, calcium-sensitive (K_{Ca}), voltage-dependent (K_V) or inward rectifier (K_{ir}) potassium channels alone or in combination did not affect the

response to NaHS, indicating that H_2S-mediated relaxation is partly mediated by inhibition of $Ca_V1.1$-1.4 (L-type) calcium channels, with an additional contribution by K^+ channels which are not of the K_{ATP}, K_{Ca}, K_V or K_{ir} subtypes (Streeter *et al.*, 2012). NaHS elicits concentration-dependent vasorelaxation in mesenteric arteries and aortas, which can be blocked by the $K_V7.x$ channel (KCNQ) inhibitor XE991, and the vasodilator capacity of the KCNQ channel opener retigabine is preserved following inhibition of H_2S generation (Schleifenbaum *et al.*, 2010). Li *et al.* reported that CBS and CSE are functionally expressed in vas deferens (VD) and that H_2S mediates VD smooth muscle relaxation; transient receptor potential and K_{ATP} channels do not appear to contribute to the NaHS-induced relaxant effect, whereas the large-conductance Ca^{2+}-activated potassium (BK_{Ca}) channel blockers iberiotoxin or tetraethylammonium largely reverse the relaxant effect, suggesting that H_2S may target BK_{Ca} channels in VD smooth muscle (Li *et al.*, 2012). NaHS-induced relaxation and membrane hyperpolarization in coronary arteries were found to be reduced by 4-aminopyridine but unaffected by glibenclamide (Cheang *et al.*, 2010). NaHS has been reported to dilate cerebral arteries from Sprague-Dawley rats with the same potency following precontraction by either 5-hydroxytryptamine or 60 mmol·L^{-1} KCl, which were unaffected by several K^+ channel blockers. Patch clamp recordings showed that NaHS reduced the amplitude of L-type Ca^{2+} currents in single myocytes isolated enzymatically from the cerebral artery, indicating that NaHS relaxes cerebral arteries primarily through inhibiting Ca^{2+} influx via Ca^{2+} channels (Tian *et al.*, 2012). These data suggest that different ion channels may be responsible for mediating vasorelaxation in different vascular beds. How they may interact with each other, and whether they play different roles in the vasodilator response, needs further investigation in future studies.

Taken together, the results show that the effects of H_2S on ion channels varies in different physiological or pathological situations. K_{ATP} channels and other ion channels play different roles in the relaxation of vascular smooth muscle. But is there any specific contributions caused by H_2S? If this is true, which ion channel plays the crucial role? Is the relaxant effect on vascular smooth muscle just an artifact or a complicated response? More precise mechanisms need to be studied in the future.

Interaction with NO and CO

Although H_2S and NO exhibit independent signalling, and H_2S but not NO targets K_{ATP} channels, both of these gasotransmitters mediate vasodilatation. Previous studies suggest that a cross-talk exists between these two molecules. H_2S content in lung tissue is increased by L-arginine treatment after aorta-veno cava shunting, whereas mean pulmonary artery pressure and relative median area of pulmonary arteries are attenuated (Yanfei *et al.*, 2006). Several lines of evidence have reported that H_2S therapy results in cardioprotection following transverse aortic constriction via up-regulation of endothelial NOS (eNOS) and NO bioavailability (Kondo *et al.*, 2013; Polhemus *et al.*, 2013). We have found that GYY4137 partially restores aortic endothelium-dependent relaxation in $apoE^{-/-}$ mice, which may be related to increased phosphorylation of eNOS in aorta (Liu *et al.*, 2013). Altaany *et al.* reported that incubation of HUVECs with NaHS stimulates the phosphorylation of eNOS and enhanced NO production; blockade of NO production by eNOS-specific siRNA or L-NAME is seen to reverse, and eNOS overexpression to potentiate, the proliferative effect of H_2S on HUVECs (Altaany *et al.*, 2013). Mice lacking CSE exhibit dysfunctional eNOS, diminished NO levels and exacerbated myocardial ischaemia/reperfusion (I/R) injury; acute H_2S therapy restores eNOS function and NO bioavailability and attenuates I/R injury, however, does not protect against I/R injury in eNOS phospho-mutant mice (King *et al.*, 2014). Coletta *et al.* showed that inhibition of eNOS attenuates H_2S-stimulated vasorelaxation (Coletta *et al.*, 2012). These data demonstrate that that H_2S-mediated cytoprotective signalling is dependent largely on eNOS activation and NO generation. However, there is no direct evidence that NO is involved in the antihypertensive effect of H_2S. In the future, this possibility could be investigated by examining the effects of H_2S in an animal model with both NO deficiency and hypertension, for example the eNOS knockout mouse.

The regulation of eNOS phosphorylation is complex. We have previously found that association of globular actin with eNOS plays an essential role in agonist-induced eNOS activation through enabling its phosphorylation by Akt at serine residue 1177 (Mi *et al.*, 2011). Several agents, such as pyridoxine (Xie *et al.*, 2012) and 17β-estradiol (Han *et al.*, 2012), regulate eNOS activity through effects on its phosphorylation. Several lines of evidence suggest that H_2S activates PI3K/Akt, thereby increasing eNOS phosphorylation at Ser1177 and enhancing vascular endothelial NO production (Predmore *et al.*, 2011; Coletta *et al.*, 2012). However, one study found that NaHS induced phosphorylation of eNOS at the phosphoserine residue Ser1179 in a manner not affected by PI3K/Akt inhibition in endothelial cells (Kida *et al.*, 2013). In addition, silencing of the CSE abolishes NO-stimulated cGMP accumulation and attenuates the ACh-induced vasorelaxation, indicating a partial requirement of H_2S in the vascular activity of NO (Coletta *et al.*, 2012). Homocysteine also induces inducible NOS, reduces eNOS in endothelial cells and reduces bioavailability of NO through the formation of nitrotyrosine, which may exacerbate hyperhomocysteinemia-associated hypertension (Sen *et al.*, 2010). Collectively, these results suggest that NO and H_2S may play mutually complementary roles in the physiological control of vascular tone. Although sulfhydration of some proteins by H_2S appears to be a physiological determinant of transcriptional activity (Sen *et al.*, 2012), no data currently exist on possible sulfhydration of eNOS after H_2S treatment.

Treatment with a slow H_2S releasing donor (named as ADTOH) has been shown to inhibit oxidative stress in retinal ganglion cells and increase haem oxygenase 1 (HO1), the main enzyme responsible for endogenous CO generation (Majid *et al.*, 2013; Polhemus and Lefer, 2014). Constitutively, produced CO inhibits CBS physiologically (Kajimura *et al.*, 2010) and hypoxia diminishes CO generation and thereby leads to increasing H_2S which mediate the vasodilatation of precapillary arterioles, suggesting that hypoxic regulation of the cerebral microcirculation is mediated by a CO-sensitive H_2S pathway (Morikawa *et al.*, 2012). Reducing CO levels in Brown-Norway rats increases H_2S generation, restores O_2 sensing and prevents hypoxia-induced pulmonary oedema. Increasing CO levels in SHR has been found to enhance

carotid H_2S generation, prevent hypersensitivity to hypoxia and control hypertension in SHR (Peng *et al.*, 2014). H_2S has also been demonstrated to be an effective and specific novel therapy for acute CO poisoning (Yu *et al.*, 2011). Collectively, these data provide evidence for H_2S-CO cross-talk. Nevertheless, the vast majority of biologically produced CO is exhaled via the lungs, and inhalation of H_2S is very difficult to achieve in practice, therefore, an in-depth study of their interaction is problematic.

Suppression of oxidative stress

The application of H_2S may trigger a number of protective actions through its antioxidative effects. Moreover, redox-sensitive signalling pathways play an important role in hypertension (Shao *et al.*, 2012). NaHS can elicit vasoprotection by both scavenging O_2^- and reducing vascular NADPH oxidase-derived O_2^- production, in an acute oxidative stress model with xanthine oxidase or with the O_2^- generator pyrogallol (Al-Magableh *et al.*, 2014). H_2S has also been found to inhibit H_2O_2-mediated mitochondrial dysfunction in human endothelial cells by preserving the activities and protein expression levels of the antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase (Wen *et al.*, 2013). The protective effect of H_2S on endothelial cells in the presence of high glucose may also involve an antioxidative stress mechanism (Guan *et al.*, 2012). In cultured H9C2 myoblasts, exogenous H_2S exerts a protective effect against H_2O_2-induced or high glucose-induced cell injury by inhibiting activation of the p38 MAPK and ERK1/2 pathways and preventing oxidative stress (Szabo *et al.*, 2011; Xu *et al.*, 2013). As a strong reducing agent, the above-mentioned antioxidative effects of H_2S *in vitro* are to a large extent predictable. Additionally, *in vivo* studies have found that H_2S acts as an antioxidant in the context of oxidative stress associated with hypoxic PHT and the mechanism appears to be partly through attenuating cellular content of oxidized glutathione (Wei *et al.*, 2007). Treatment with NaHS decreases BP and oxidative stress in SHR, and combined NaHS and tempol therapy in SHR decreases BP to a greater extent (Ahmad *et al.*, 2014). GYY4137 decreases superoxide generation in aorta of high-fat-fed apoE$^{-/-}$ mice, while CSE-knockout mice fed with atherogenic diet exhibit increased lesional oxidative stress (Liu *et al.*, 2013; Mani *et al.*, 2013). Zhou *et al.* found that H_2S protects against chronic alcohol-induced left ventricular remodelling via attenuating oxidative stress (Zhou *et al.*, 2013). Another group showed that chronic NaHS treatment for 3 months prevents hypertrophy of intramyocardial arterioles and ventricular fibrosis, as well as decreases myocardial reactive oxygen species in SHR (Shi *et al.*, 2007). These findings collectively suggest that H_2S decreases BP and suppresses oxidative stress; however, whether the antioxidative ability of H_2S in itself is important in reducing the BP in concert with other BP-lowering actions needs to be further investigated.

Research on H_2S and hypertension in China

Professor Chaoshu Tang in Peking University was the first to study the effects of H_2S on hypertension in China. His group were the first to find, in 2003, that a lack of endogenous H_2S was involved in the pathogenesis of hypoxic PHT and that exogenous H_2S could exert a beneficial effect in this context (Chunyu *et al.*, 2003). Subsequently, other groups found that exogenous H_2S effectively prevents the development of different forms of hypertension, such as L-NAME-induced hypertension, SHR, high blood flow-induced PHT and portal hypertension (Zhong *et al.*, 2003; Yan *et al.*, 2004; Yanfei *et al.*, 2006; Shi *et al.*, 2007; Tan *et al.*, 2011). They proposed several possible mechanisms, such as vasorelaxation, induction of apoptosis of pulmonary artery SMCs, attenuation of Ang II-induced AT_1 receptor activation and inhibition of oxidative stress (Shi *et al.*, 2007; Zhao *et al.*, 2008; Fang *et al.*, 2009; Li *et al.*, 2009). An important breakthrough occurred with the finding of Zhang *et al.* that H_2S also prevents H_2O_2-induced activation of epithelial sodium channels, through which sodium can be reabsorbed in the distal nephron to regulate salt-sensitive hypertension through a PTEN(phosphatidylinositol 3,4,5-trisphosphate)-dependent pathway (Zhang *et al.*, 2013). Their findings raise the possibility that the effect of H_2S on epithelial sodium channel activity seen in animal models may further be exploited clinically for the management of salt-sensitive hypertension. A major focus of future work on H_2S in China will be on discovery of novel drugs targeting the H_2S pathway and on subsequent clinical translation.

Concluding remarks and future perspectives

Over the last few decades, significant progress has been achieved in delineating the antihypertensive effect and molecular mechanisms underlying the actions of H_2S (Figure 1). However, several questions remain to be answered. The precise mechanisms underpinning H_2S-induced vasodilatation need to be better delineated. Moreover, some studies suggest that H_2S can in fact exert vasoconstrictor effects (Koenitzer *et al.*, 2007; Polhemus and Lefer, 2014). This apparent discrepancy needs further investigation to specifically determine whether this depends on factors such as concentration of H_2S, vascular bed or the oxygen tension. The precise biological roles of H_2S in amelioration of oxidative stress also remain unclear. Moreover, the vasodilator actions of H_2S may be a result, at least in part, of eNOS-generated NO promoted by H_2S signalling. However, the exact manner of cross-talk between H_2S and NO is incompletely understood and deserves to be better elucidated; an improved understanding of how these two molecules cooperate will allow better design of clinically useful therapies. Finally, precisely how CSE activity is regulated at the post-translational level needs to be better defined.

Clinically, it remains to be determined whether the research findings described earlier can be translated to practice for the management of hypertension and other related cardiovascular diseases; and most importantly, whether H_2S releasing agents and CSE/H_2S activators will prevent or treat hypertension and its complications. As well as such efficacy considerations, the safety of such agents needs to be rigorously confirmed, as well as the dose relationship of any toxic effects.

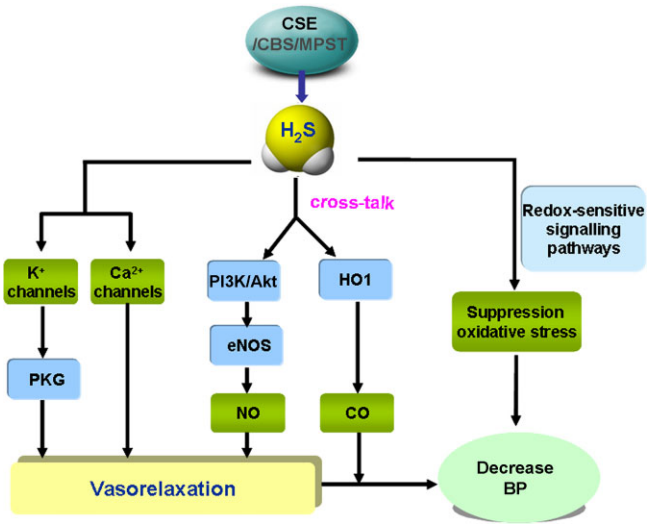

Figure 1

Schematic illustration of possible mechanisms that may underlie H_2S-induced BP lowering. H_2S lowers BP via vasodilatation by activation of vascular K_{ATP} channels and/or inhibiting Ca^{2+} influx via Ca^{2+} channels. NO and H_2S share cross-talk regulatory roles in vasorelaxation via the PI3K/Akt-eNOS-NO pathway. H_2S increase haem oxygenase 1 (HO1), which is the main enzyme for CO generation. H_2S inhibits reactive oxygen species (ROS) production through redox-sensitive signalling pathways.

Acknowledgements

This work was supported by grants from the National Basic Research Program of China (973) (grant no 2012CB517503), the National Natural Science Foundation of China (grant nos. 81170083, 81330004, 31371156), the Collaborative Innovation Center for Cardiovascular Disease Translational Medicine and PAPD.

Conflict of interest

The authors declare no conflict of interest.

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