Hydrogen Sulfide: Physiological and Pathophysiological Functions

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Yi Tong Liu and Jin-Song Bian

Abstract

Hydrogen sulfide (H_2S) has been recognized as an endogenous gaseous mediator. The past decade has seen an exponential growth of scientific interest in the physiological and pathological significance of H_2S especially with respect to its roles in the central nervous and the cardiovascular systems. In cardiovascular system, H_2S regulates heart contractile function and may serve as a cardioprotectant for treating ischemic heart diseases and heart failure. Alterations of the endogenous H_2S level have been found in animal models with various pathological conditions such as myocardial ischemia, spontaneous hypertension, and hypoxic pulmonary hypertension. In the central nervous system, H_2S facilitates long-term potentiation and regulates intracellular calcium concentration in brain cells. Intriguingly, H_2S produces antioxidant, anti-inflammatory, and anti-apoptotic effects that may be of relevance to neurodegenerative disorders. Abnormal generation and metabolism of H_2S have been reported in the pathogenesis of ischemic stroke, Alzheimer's disease, Parkinson's disease, and recurrent febrile seizure. Exogenously applied H_2S is demonstrated to be valuable in the treatment against febrile seizure and Parkinson's disease. In addition, H_2S also regulates the physiological and pathological functions of kidney, pancreas and bone. Exogenously applied H_2S may protect against ischemic kidney injuries and osteoporosis. This article surveys the growing recognition of H_2S as an endogenous signaling molecule in mammals and its functions in different biological systems. We will emphasize on its physiological and pathological functions in the cardiovascular, central nervous and renal systems.

Keywords

Gasotransmitter • Hydrogen sulphide • Pharmacology • Physiology • Pathology

Y.T. Liu • J.-S. Bian (✉)
Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, 18 Medical Drive, Singapore 117597, Singapore
e-mail: phcbjs@nus.edu.sg

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Abbreviations

$[Ca^{2+}]_i$	Intracellular Ca^{2+}
$\pm LVdp/dt_{max}$	Maximal/minimal left ventricular pressure development
1-K	Uninephrectomy
2K1C	2-Kidneys-1-clip
3-MST	Mercaptopyruvate sulfurtransferase
6-OHDA	6-Hydroxydopamine
AC	Adenylyl cyclase
ACE	Angiotensin-converting enzyme
AD	Alzheimer's disease
Ang II	Angiotensin II
AOAA	Aminooxyacetic acid
APD	Action potential duration
ApoE	Apolipoprotein E
ATN	Acute tubular necrosis
ATP	Adenosine triphosphate
AVF	Arteriovenous fistula
BACE-1	Beta-site amyloid precursor protein cleaving enzyme 1
BK_{Ca}	Large Conductance Ca^{2+}−activated potassium channels
BP	Blood pressure
cAMP	Cyclic adenosine monophosphate
CAT	Cysteine aminotransferase
CBS	Cystathionine β-synthase
cGMP	Cyclic guanosine monophosphate
CKD	Chronic kidney disease
CNS	Central nervous system
CO	Carbon monoxide
COX-2	Cyclooxygenase 2
CSE	Cystathionine γ-lyase
DA	Dopamine/dopaminergic
DEANO	Diethylamine nitric oxide
ECs	Endothelial cells
EDHF	Endothelium derived hyperpolarizing factor
ER	Endoplasmic reticulum
ERK (MAPK)	Extracellular signal-regulated kinase
FE_{Na}	Fractional excretion of Na^+
FE_K	Fractional excretion of K^+
FF	Filtration rate
FS	Febrile seizures
GABA	Gamma-aminobutyric acid
GFR	Glomerular filtration rate
GLT1	Glial glutamate transporter 1

GSH	Glutathione
GSK-3β	Glycogen synthase kinase-3
GSSG	Oxidized glutationine
H_2S	Hydrogen sulfide
HF	Heart failure
HIF	Hypoxia-inducible factors
HMC1.1	Human mast cell line 1.1
HNO	Nitroxyl anion
Hsp	Heat shock protein
HUVECs	Human umbilical vein endothelial cells
I/R	Ischemia/reperfusion
ICAM-1	Intercellular adhesion molecule 1
IK_{Ca}	Intermediate conductance Ca^{2+}-activated potassium channels
IL	Interleukin
iNOS	Inducible NO synthase
IPreC	Ischemic preconditioning
IRR	Intrarenal resistance
ISO	Isoproterenol
JNK	c-Jun N-terminal kinases
K_{ATP}	ATP-sensitive potassium channel
LCA	Left coronary artery
LDL	Low-density lipoprotein
LPO	Lipid hydroperoxidation
LPS	Lipppolysaccharide
LTCC $I_{Ca,\ L}$	L-type Ca^{2+} channels
MAP	Mean arteriole pressure
MCAO	Middle cerebral artery occlusion
MDA	Malondialdehyde
MEK	ERK Kinase
MI	Myocardial infarction
MMP	Matrix metalloproteinase
MPP^+	1-Methyl-4-phenylpyridine
mPTP	Mitochondrial permeability transition pore
MR	Mental retardation
Na_2S	Sodium sulfide
NADPH	Nicotinamide adenine dinucleotide phosphate
NaHS	Sodium hydrosulfide
NF-E2	Nuclear factor-erythroid-derived 2
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NHE	Na^+/H^+ exchanger
NO	Nitric oxide
NOS	Nitric oxide synthase
NRF-1	Nuclear respiratory factor 1
Nrf2	NF-E2 related factor 2
NSAIDs	Non-steroidal anti-inflammatory drugs

$ONOO^-$	Peroxynitrite
PAG	DL-Propargylglycine
PARP	Poly (ADP-ribose) polymerase
p-CREB	Phosphorlyated cAMP response element-binding protein
PD	Parkinson's disease
pH_i	Intracellular pH
PI3K	Phosphoinositide 3-kinase
PKA	Protein kinase A
PKC	Protein kinase C
PGE	Prostaglandin E2
PKG	Protein kinase G
p-NR1	Phosphorylated N-methyl-D-aspartate receptor 1 subunit
p-NR2A	Phosphorylated N-methyl-D-aspartate receptor 2A subunit
p-NR2B	Phosphorylated N-methyl-D-aspartate receptor 2B subunit
RAS	Renin-angiotensin system
RBF	Renal blood flow
ROS	Reactive oxygen species
SBP	Systolic blood pressure
SHR	Spontaneously hypertensive rats
SIN-1	3-Morpholinosydnonimine
SK_{Ca}	Small conductance Ca^{2+}-activated potassium channels
SMCs	Smooth muscle cells
SNAP	S-Nitroso-N-acetylpenicillamine
SNP	Sodium nitroprusside
SOD	Superoxide dismutase
SPreC	H_2S preconditioning
STAT	Signal transducer and activator of transcription
TH	Tyrosine hydroxylase
TNF	Tumor necrosis factor
TUNEL	Terminal deoxynucleotidyl transferase dUTP Nick end labeling
U.V	Urine flow rate
UCP2	Uncoupling protein 2
$U_K.V$	Urinary K^+ excretion
$U_{Na}.V$	Urinary Na^+ excretion
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor
WT	Wild type

6.1 Introduction

The physiologic importance of H_2S was first reported by Abe and Kimura in 1996, when H_2S was found to act as a novel neuromodulator (Abe and Kimura 1996). H_2S is now commonly regarded as the third 'gasotransmitter' subsequent to nitric oxide (NO) and carbon monoxide (CO) (Wang 2002). Similar to NO and CO, H_2S can be endogenously synthesized by several enzymes. It has also been well demonstrated to influence a wide range of physiological and pathological processes. In the heart, H_2S has been recognized to induce protective effects (Johansen et al. 2006; Elrod et al. 2007). In vascular tissues, H_2S induces both blood vessel relaxation (Hosoki et al. 1997; Zhao et al. 2001; Zhao and Wang 2002; Cheng et al. 2004; Ali et al. 2006; Kiss et al. 2008; Webb et al. 2008; Yang et al. 2008) as well as constriction (Ali et al. 2006; Kiss et al. 2008; Lim et al. 2008; Webb et al. 2008), depending on the concentration of H_2S administered and the type of vessels involved. In the nervous system, H_2S has been found to mediate neurotransmission (Abe and Kimura 1996) and induces both neuroprotection and neurotoxicity (Hu et al. 2010; Kida et al. 2010). H_2S has also been reported to regulate inflammation (Li et al. 2006a; Hu et al. 2007) and insulin release. In this chapter, we present current knowledge of H_2S to facilitate better understanding of its biological functions in both health and disease, with a special emphasis on its protective effects in cardiovascular, central nervous and renal systems.

Under physiological conditions, H_2S is present in plasma and organ systems as ~14 % H_2S, 86 % HS^- and a trace of S^{2-} (Giggenbach 1971; Hvitved-Jacobsen 2002; Dombkowski et al. 2004). Since these species coexist in aqueous solution together, it is difficult to identify the biologically active species that underlie the effects observed. Hence, the terminology "H_2S" refers to the sum of H_2S, HS^- and S^{2-}in the context of this chapter unless otherwise specified. Till date, most researchers utilize NaHS or Na_2S (or their hydrous forms) as exogenous sources of H_2S. In aqueous solution, both release rapid bolus of H_2S which triggers downstream mechanisms. More recently, a handful of slow-releasing H_2S compounds have been developed (Li et al. 2007, 2008; Sidhapuriwala et al. 2007; Lee et al. 2010b; Xie et al. 2013). The effects of these exogenous H_2S donors in different systems are discussed in this chapter.

6.2 Physiological and Pathological Functions of H_2S in the Cardiovascular System

6.2.1 H_2S Biosynthesis in the Cardiovascular System

The most important mammalian enzymes that are responsible for the synthesis of H_2S are cystathionine β-synthase (CBS, EC 4.2.1.22), cystathionine γ-lyase (cystathionase, CSE, EC 4.4.1.1), mercaptopyruvate sulfurtransferase (3-MST, EC 2.8.1.2) and cysteine aminotransferase (CAT, EC 2.6.1.3). Recently, a novel H_2S

biosynthetic pathway from D-cysteine involving 3-MST and D-amino acid oxidase has been unveiled (Shibuya et al. 2013).

Among these enzymes, CSE is the main H_2S-generating enzyme that is expressed in the cardiovascular system (Zhao et al. 2001; Bian et al. 2006) and various vascular tissues (Chen et al. 1999; Zhao et al. 2001). CSE mRNA expression has been detected in the myocardium (Geng et al. 2004b), endothelial cells (ECs) (Yang et al. 2008) and smooth muscle cells (SMCs) (Zhao et al. 2001), and the intensity rank of CSE mRNA expression in various vascular tissues is as follows:

Pulmonary artery > aorta > tail artery > mesenteric artery (Zhao et al. 2001).

6.2.2 Physiological Functions of H_2S in the Cardiovascular System

6.2.2.1 Physiological Functions of H_2S in the Heart

H_2S may markedly reduce action potential duration (APD) and decelerate sinus rhythm, while having no significant effect on the amplitude of action potential and resting potential (Sun et al. 2008). HERG/I_{kr} and KvLQT1/I_{ks} are two important potassium channels that control APD. Till date, H_2S has not been reported to affect the function of these channels in the heart. Therefore, the effect of H_2S on APD is probably attributed to the opening of K_{ATP} channels (Abramochkin et al. 2009). H_2S is capable of opening K_{ATP} channels directly, first demonstrated by Wang and coworkers (Tang et al. 2005; Jiang et al. 2010). Furthermore, H_2S may also activate K_{ATP} channels indirectly by inducing intracellular acidosis (Cuevas et al. 1991; Koyano et al. 1993; Bethell et al. 1998; Lee et al. 2007) and other potassium channels (Martelli et al. 2013). However, the involvement of these channel activations towards shortening of APD is yet to be clearly understood and warrants further research.

H_2S produces negative inotropic effect in rat hearts. In isolated rat ventricular myocytes, H_2S decreased the amplitudes of myocyte twitch and electrically-induced calcium transients upon stimulation of β_1-adrenergic receptors with isoproterenol (ISO) (Yong et al. 2008b). Using isolated heart, perfusion with H_2S inhibited maximal/minimal left ventricular pressure development ($\pm LVdp/dt_{max}$) (Geng et al. 2004b). H_2S perfusion in vivo via femoral vein produced a similar effect on the cardiodynamics of anesthetized rats (Geng et al. 2004b). However, H_2S at concentration up to 100 μM NaHS had no significant effect on heart rate in isolated rat hearts (Zhong et al. 2003; Minamishima et al. 2009).

Different mechanisms have been implicated in the inhibitory effect of H_2S on heart contractility. Firstly, H_2S opens K_{ATP} channels. Secondly, H_2S may inhibit adenylyl cyclase (AC)/ cyclic adenosine monophosphate (cAMP) pathway to suppress β-adrenoceptor system, thereby producing negative inotropic effects (Yong et al. 2008b). Thirdly, H_2S reduced peak current of L-type Ca^{2+} channels (LTCC; $I_{Ca,\ L}$) which is important in controlling heart contractility and cardiac rhythm (Sun et al. 2008). Interestingly, in various brain cell types, H_2S (100–300 μM) has been reported to increase $[Ca^{2+}]_i$ via opening (instead of closing)

LTCC (Nagai et al. 2004; Garcia-Bereguiain et al. 2008; Yong et al. 2010a). It is intriguing that H_2S directly blocks LTCC in cardiomyocytes, but opens the same channels in neurons. One possibility accounting for such phenomenon is that the effect of H_2S on LTCC may be secondary to other signaling pathways. For instance, reduction of Ca^{2+} current through LTCC could have resulted from hyperpolarization caused by opening of K_{ATP} channels (Tang et al. 2005; Jiang et al. 2010) or the suppression of cAMP/PKA pathway (Yong et al. 2008b). More evidence, including single channel recording, is needed to conclude whether H_2S is a direct LTCC blocker.

It should be noted, however, that the significance of findings mentioned above may require further valiadation since H_2S concentrations administered in those experiments are much higher than physiological, which is now generally regarded to be less than 0.1–1 μM (Whitfield et al. 2008; Levitt et al. 2011). Nonetheless, sulfides may bind to proteins in plasma and tissues, and is released in response to stimuli (Liu et al. 2012b). For instance the concentration of acid-labile sulfur in the heart was reported to be about 300 μM (Levitt et al. 2011). Free and bound sulfide originates from the action of enzymes that synthesize H_2S. It is therefore unclear if H_2S administered at concentrations between 100 and 500 μM plays physiological roles in heart functions.

6.2.2.2 Physiological Functions of H_2S in the Vascular System

Regulation of Vascular Tone

The biological function of H_2S on vascular tissue is biphasic. H_2S induces vasorelaxation at a higher concentration range (NaHS 100–1,600 μM), but causes concentration-dependent constriction at lower concentrations (NaHS 10–100 μM) (Ali et al. 2006; Kubo et al. 2007; Lim et al. 2008). H_2S induced vasodilation has been reported in thoracic aorta, mesenteric arteries, pulmonary artery, tail artery and other types of vascular tissues (Hosoki et al. 1997; Zhao et al. 2001). H_2S-induced vasorelaxation is mainly underlied by opening of K_{ATP} channels (Zhao et al. 2001; Cheng et al. 2004; Kubo et al. 2007) and partially mediated by endothelium-dependent mechanism(s) (Zhao et al. 2001). Other signaling mechanisms involved includes intracellular acidosis (Lee et al. 2007), depletion of intracellular ATP levels (Szabo 2007; Kiss et al. 2008; Webb et al. 2008) and elevations in cyclic guanosine monophosphate (cGMP)/protein kinase G (PKG) (Bucci et al. 2010). More recent studies refer H_2S as an endothelium derived hyperpolarizing factor (EDHF) (Mustafa et al. 2011). This is supported by findings that intermediate and small conductance Ca^{2+}-activated potassium channels (IK_{Ca}/SK_{Ca}) channels underlie H_2S effect, and IK_{Ca}, but not K_{ATP} and large conductance Ca^{2+}-activated potassium channels (BK_{Ca}) channels, mediate H_2S-induced hyperpolarization in cultured human aortic ECs (Mustafa et al. 2011). Taken together, these studies are suggestive that H_2S plays important roles in mediating vascular responses of small and intermediate resistance vessels.

H_2S-induced vasoconstrictive effects are also mediated by multiple mechanisms. It has been found that H_2S may reduce NO synthesis in endothelium (Kubo et al. 2007), or interact with NO to form a nitrosothiol compound, which itself has no effect

on vascular activity (Ali et al. 2006). However, H_2S-induced vasoconstriction is not completely abolished in the presence of NO synthase (NOS) inhibitor or removal of endothelium, suggesting that other NO-independent mechanisms might be implicated. One possibility is the downregulation of cAMP level in VSMCs (Lim et al. 2008), which then upregulates the activation of myosin light chain kinase to induce vasoconstriction.

Angiogenesis

Current evidence suggests that H_2S promotes angiogenesis and cell growth. H_2S enhances cell migration, growth and proliferation in endothelial cells (Cai et al. 2007; Papapetropoulos et al. 2009). Under hypoxic conditions, H_2S-induced angiogenesis is probably hypoxia-inducible factors (HIF)-1α/ vascular endothelial growth factor (VEGF)-dependent (Liu et al. 2010).

H_2S also promotes vascular network formation under pathological situations. A hindlimb ischemic model was established in rats that were subjected to unilateral femoral artery ligation. NaHS at 50 μmol/kg/day, but not (200 μmol/kg/day), promoted collateral vessel growth in ischemic hindlimbs, along with increased regional blood flow and increased capillary density (Wang et al. 2010). This implies that H_2S may promote vascular network formation in vivo at near physiological concentrations. The signaling mechanisms for the angiogenic effect of H_2S involve activation of Akt (Cai et al. 2007), extracellular signal-regulated (ERK)-kinase (MEK) (Papapetropoulos et al. 2009) and heat shock protein (Hsp)-27 (Papapetropoulos et al. 2009).

6.2.2.3 Interaction Among Gasotransmitters in the Cardiovascular System

Under physiological conditions, gaseous mediators (i.e. H_2S, NO and CO) might be present at the same time, and accumulating evidence now suggests that the interaction among gaseous mediators may influence or alter overall biological effects, in contrast to their individual effects (Kashiba et al. 2002; Fukuto and Collins 2007; Li et al. 2009; Olson and Donald 2009; Kajimura et al. 2010). Interaction between H_2S and NO may also regulate heart function. Yong et al. first reported that a mixture of NO donor and H_2S (100 μM) produces positive isotropic effect in the heart whereas H_2S and NO alone produces opposite effect. The effect of interaction could be abolished by thiols, suggesting that a new molecule that is thiol sensitive could have been formed. Nitroxyl (HNO) was proposed to be the product (Yong et al. 2010b) due to the strong reducing capability of H_2S (Warenycia et al. 1989b; Wang 2002; Szabo 2007) and the structural and pharmacological similarities with HNO (Yong et al. 2010b). The formation of HNO as an end-product of H_2S and NO donor (sodium nitroprusside; SNP) interaction was further supported by Filipovic et al. under physiological cellular conditions and in isolated mouse heart (Filipovic et al. 2013). Filipovic et al. proposed that the interaction is independent of NO released from SNP, but rather a direct effect between H_2S and SNP. This is in contrast with Yong et al.'s observations in which various types of NO donors such as L-arginine (NOS substrate) or diethylamine NO (DEANO) were also used and

similar effect to that of SNP was found (Yong et al. 2010b, 2011). Nevertheless, the formation of HNO as a result of H_2S and NO or SNP interaction warrants further in depth studies to be fully resolved.

In the vascular system, interaction between NO and H_2S is controversial. Hosoki et al. first reported that NO and H_2S act synergistically in vasorelaxations (Hosoki et al. 1997). On the contrary, later studies reported that H_2S pretreatment inhibited SNP-induced vasorelaxations (Zhao and Wang 2002). Ali et al. showed that mixing NO donors (SNP, SIN-1 or SNAP) with NaHS (100 μM) reduced the extent of vasorelaxation compared to the relaxation with NO donors alone, further indicating inactivation of NO by H_2S (Ali et al. 2006). The authors ascribed these observations to formation of a nitrosothiol compound (Ali et al. 2006), which is still unidentified till date. It is highly likely that this new compound is HNO, as mentioned above, instead of a nitrosothiol (Yong et al. 2010b, 2011; Filipovic et al. 2013).

6.2.3 Pathological Functions of H_2S in the Cardiovascular System

6.2.3.1 Acute Ischemic Heart Diseases

Endogenous H_2S Level Under Ischemic Conditions

Accumulating evidence now suggests that under ischemic conditions, endogenous H_2S production in the heart is reduced. In ventricular myocytes, for example, treatment with ischemic solution reduced endogenous H_2S level (Bian et al. 2006). Under ischemic conditions, both in vivo and in vitro studies showed that CSE activity (Yong et al. 2008a) and mRNA gene expression (Zhu et al. 2007) were downregulated.

In an in vivo animal study, rats that were injected with ISO to produce "infarct-like" myocardial necrosis were found to have reduced H_2S levels in myocardium (Rona et al. 1959). Geng et al. further confirmed that plasma H_2S level dropped by 66 % (from 60 to 20 μM) in an ISO-induced myocardial ischemic rat model (Geng et al. 2004a). Consistent with this, a clinical observational study showed that plasma H_2S concentration in patients with coronary diseases is significantly lower compared with control subjects (26 μM vs. 52 μM), suggesting that the decreased plasma H_2S levels may correlate with severity of coronary diseases (Jiang et al. 2005). These observations suggest that plasma H_2S level has the potential to be used as a biomarker for ischemic heart diseases.

Ischemia/reperfusion (I/R)-induced arrhythmias may develop as a result of free radical species (ROS) production and accumulation in the myocardium during reperfusion. Since H_2S production is markedly decreased during ischemia (Geng et al. 2004a; Jiang et al. 2005; Bian et al. 2006; Yong et al. 2008a, b), ROS may therefore be increased. Excessive free radicals may react with proteins, lipids and nucleic acids, thereby disrupting myocardium structure and functions.

Therapeutic Effects of H_2S Against Ischemic Heart Diseases

Exogenously applied H_2S may reduce myocardial infarction (MI) size in rats (Johansen et al. 2006; Zhu et al. 2007; Pan et al. 2009), mice (Elrod et al. 2007) and pigs (Sodha et al. 2008; Osipov et al. 2009; Sodha et al. 2009). Treatment with H_2S also significantly protected heart against I/R-induced arrhythmias (Bian et al. 2006; Zhang et al. 2007) and improved myocardial contractile function in ISO-induced ischemic rat heart (Geng et al. 2004a) and I/R-induced ischemic porcine heart (Sodha et al. 2008). Endogenous H_2S level is vital to protect heart against ischemic injuries. Inhibition of endogenous H_2S production significantly increased infarct size (Sivarajah et al. 2006; Bliksoen et al. 2008), whereas stimulation of endogenously produced H_2S by overexpression of CSE reduced infarct size (Elrod et al. 2007).

H_2S inhibits the progression of apoptosis subsequent to I/R injury. H_2S treatment suppressed the activation of caspase-3, poly (ADP-ribose) polymerase (PARP) and/or terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive nuclei in mice (Elrod et al. 2007) and swine (Sodha et al. 2008). It also suppressed the expression of pro-apoptotic proteins via caspase-independent cell death through phosphorylation of glycogen synthase kinase-3 (GSK-3β) (Osipov et al. 2009). Similarly, Yao et al. also demonstrated that H_2S increased phosphorylation of GSK-3β (Ser9) and thus inhibited the opening of mitochondrial permeability transition pore (mPTP) (Yao et al. 2010). H_2S also improved cardiac ATP pools (Szabo et al. 2011) and reduced mitochondrial oxygen consumption (Elrod et al. 2007). It preserves mitochondrial function by increasing complex I and II efficiency (Alves et al. 2011) and inhibiting respiration and thus limiting the generation of ROS (Elrod et al. 2007). Therefore, the cardioprotective effects of H_2S also involve its anti-oxidative function (Sojitra et al. 2011; Szabo et al. 2011).

Anti-inflammatory effect of H_2S also contributes to its cardioprotection. H_2S decreased the number of leukocytes within the ischemic zone by inhibition of leukocyte-EC interactions (Elrod et al. 2007). It also decreased myocardial interleukin (IL)-1β (Elrod et al. 2007), TNF-α, IL-6 and IL-8 levels (Sodha et al. 2009). Therefore, inhibition of leukocyte transmigration and inhibition of cytokine release are possible mechanisms for the anti-inflammatory and cardioprotective effects of H_2S. Other cardioprotective mechanisms of H_2S may include suppression of β-adrenergic function (Yong et al. 2008b), inhibition of Na^+/H^+ exchanger (NHE) activity (Hu et al. 2011a), opening of K_{ATP} channels (Johansen et al. 2006), blockade of LTCC (Sun et al. 2008), attenuation of endoplasmic reticulum (ER) stress (Wei et al. 2010) and preservation of endothelial function (Szabo et al. 2011) etc.

Ischemic preconditioning (IPreC) is a powerful natural cardioprotective mechanism. H_2S preconditioning (SPreC) produces cardioprotective effects (Bian et al. 2006; Pan et al. 2006, 2008, 2009; Hu et al. 2008a, b; Calvert et al. 2009). Interestingly, SPreC produces stronger effect than post-ischemic H_2S treatment (Pan et al. 2009). The protective effects of direct H_2S treatment may rely mainly on the ability of sulfide to reduce inflammatory responses (Zanardo et al. 2006) and to neutralize cytotoxic ROS such as peroxynitrite ($ONOO^-$) (Whiteman et al. 2004),

which may relieve oxidative stress partly, but not enough to salvage infarcted myocardium. SPreC is more likely to protect the heart by switching it to a defensive mode against ischemic insult.

SPreC may trigger a series of signaling proteins including opening K_{ATP} channels (Pan et al. 2006) activation of Protein Kinase C (PKC, especially ε-isoform) (Pan et al. 2008), ERK1/2-MAPK (Hu et al. 2008b) and PI3K/Akt pathways (Hu et al. 2008b). By activating pro-survival pathways, SPreC may stimulate cells to counteract stressful conditions. These pathways result in the production of various molecules (e.g. HSPs, GSH, and bilirubin) endowed with antioxidant and antiapoptotic activities (Calvert et al. 2009). SPreC also activates signal transducer and activator of transcription (STAT)-3, which prevents cleavage of caspase-3, inhibits translocation of cytochrome C and reduces the number of TUNEL-positive nuclei (Calvert et al. 2009). The anti-apoptotic actions are found to be, at least partially, mediated by inhibition of pro-apoptotic factor Bad, upregulation of pro-survival factors Bcl-2 and Bcl-xL, and an upregulation of Hsps.

In addition, COX-2/PGE2 pathway (Hu et al. 2008a; Sojitra et al. 2011), prevention of intracellular calcium overload and hypercontracture (Pan et al. 2008), NO (Pan et al. 2006) and nuclear factor-erythroid-derived 2 (NF-E2) related factor 2 (Nrf2)/anti-oxidative stress (Calvert et al. 2009) have all been implicated in SPreC-induced cardioprotection (Liu et al. 2012b). These results suggest that H_2S therapy may enhance endogenous antioxidant defense of myocytes and create an environmental resistance to the oxidative stress associated with myocardial I/R injury, as evidenced by the preservation of redox state and a reduction in lipid peroxidation.

6.2.3.2 Hypertrophy, Cardiomyopathy and Heart Failure (HF)

Myocardial infarction (MI) is the leading cause of HF. Cardiac hypertrophy as a result of sustained overload can lead to progression of HF. Plasma H_2S level was found to be decreased in MI-induced (Wang et al. 2011), and arteriovenous fistula (AVF)-induced CHF model (Sen et al. 2008; Mishra et al. 2010). In addition, endogenous H_2S synthesis in the heart was also found to be lowered in adriamycin -induced cardiomyopathy model (Su et al. 2009). This was further supported by transgenic mice overexpressing CSE as excessive H_2S production protected against CHF injuries in both permanent LCA ligation model as well as LCA I/R model (Calvert et al. 2010).

H_2S pretreatment prevented cardiomyocyte hypertrophy by lowering intracellular ROS, upregulating microRNA-133a and suppressing microRNA-21 in rat primary cultures (Liu et al. 2011a). Overexpression of CSE reduces left ventricle dilation and cardiac hypertrophy (Calvert et al. 2010). Exogenous application of H_2S attenuated the development of hypertrophy in spontaneously hypertensive rats (SHR) (Shi et al. 2007). H_2S also attenuated development of adriamycin-induced cardiomyopathy (Su et al. 2009).

Anti-oxidative effect of H_2S is the main mechanism for its therapeutic effect on CHF. Application with H_2S inhibited lipid hydroperoxidation (LPO) and increased superoxide dismutase (SOD) and GSH peroxidase activities. Therefore, treatment

with H_2S stimulated the activity of anti-oxidant enzymes (Zhang et al. 2011b). H_2S also reduced LPO and protected heart against HF injury via stimulation of Akt and nuclear localization of nuclear respiratory factor 1 (NRF-1) and NF-E2 related factor 2 (Nrf2) (Calvert et al. 2010). H_2S also decreased the number of apoptotic cells through promoting the expression of anti-apoptotic factor Bcl-2 while suppressing expressions of pro-apoptotic factors Bax and caspase-3. The release of cytochrome c from mitochondria was reduced. These anti-apoptotic effects therefore mediated the cardioprotective effects of H_2S (Wang et al. 2011). In addition, H_2S may also protect against HF via promoting angiogenesis (Mishra et al. 2010; Givvimani et al. 2011).

H_2S was also found recently to prevent HF progression via attenuating mast cell accumulation and degranulation in response to toxic cardiomyopathy (Liu et al. 2013). The inhibition of mast cell number increments is probably due to downregulation of leukotriene A4 hydrolase protein expression and leukotriene B4 level, which acts as chemoattractant in the recruitment of mast cell uptake into tissue. In addition, H_2S treatment inhibited forskolin-induced renin degranulation mast cell line (HMC1.1) via lowering of intracellular cAMP level (Liu et al. 2013).

6.2.3.3 Atherosclerosis

H_2S level was found to be significantly reduced in either vascular beds or plasma during the development of atherosclerosis. This is probably due to the inhibition of CSE expression and activity (Wu et al. 2006; Meng et al. 2007). In apolipoprotein E knockout (apoE$^{-/-}$) mice, plasma H_2S and aortic H_2S synthesis were decreased. However, CSE mRNA in aorta was found to be elevated, probably due to the existence of a positive compensatory feedback mechanism (Wang et al. 2009).

Exogenously administered H_2S suppressed the development of neointima hyperplasia (Meng et al. 2007), decreased vascular calcium content, calcium overload and alkaline phosphatase activity in calcified vessels (Wu et al. 2006) and reduced atherosclerotic plaque size and improved aortic ultrastructure (Wang et al. 2009). The anti-atherosclerotic effects involve anti-inflammatory (Wang et al. 2009) and anti-apoptotic (Yang et al. 2006) effects on SMCs, cytoprotective effects in ECs (Jeney et al. 2009) and inhibition of LDL modifications and oxidation (Laggner et al. 2007b; Jeney et al. 2009).

6.2.3.4 Hypertension

The role of endogenous H_2S in blood pressure (BP) regulation is still controversial. Pharmacological blockade of endogenous H_2S production with hydroxylamine hydrochloride, a non-specific inhibitor of both CSE and CBS, for 4 weeks failed to influence systolic BP in rats (Lu et al. 2010b). In contrast, Yan et al. found that administration of PAG, an inhibitor of CSE, to rats for 5 weeks significantly elevated blood pressure (Yan et al. 2004). The discrepancy was also observed in CSE-knockout mice. Yang et al. reported that CSE knockouts exhibited pronounced hypertension (Yang et al. 2008), whereas Ishii et al. did not find hypertension in these mice (Ishii et al. 2010).

Plasma level of H_2S and the expression of CSE mRNA was significantly lowered in spontaneously hypertensive rats (SHR) (Yan et al. 2004) and hypoxic pulmonary hypertensive rats (Zhang et al. 2003). These findings suggest that the hypertension in SHR involves a reduction in the production and function of H_2S (Yan et al. 2004).

Treatment with H_2S can significantly lower BP in different hypertensive animal models, such as SHR (Yan et al. 2004), renovascular hypertension (Lu et al. 2010b) and pulmonary hypertension (Zhang et al. 2003). The mechanisms for its anti-hypertensive effects probably implicate the inhibition of renin-angiotensin system (RAS) (Lu et al. 2011), attenuation of vascular remodeling (Zhao et al. 2008) and activation of K_{ATP} channels (Li et al. 2008).

6.3 Physiological and Pathological Function of H_2S in the Central Nervous System

Accumulating evidence establishes that H_2S is a neuromodulator in CNS and regulation of H_2S synthetic system may be a promising therapeutic approach for CNS diseases. Therefore H_2S confers pathophysiological regulatory functions in brain, instead of being a 'mere' environmental toxin.

6.3.1 H_2S Biosynthesis in Brain

Earlier reports from various groups showed high concentrations of H_2S in brain (ranging 50–160 μM) in a variety of mammalian species including rat, bovine, mouse and human (Goodwin et al. 1989; Warenycia et al. 1989a; Savage and Gould 1990). Recent works suggest that the concentration of H_2S in brain may be in the nanomolar range (Furne et al. 2008; Ishigami et al. 2009). Determination of H_2S in biological samples is often influenced by a number of factors such as its instability, high volatility, great susceptibility to oxidation, and release of sulfide out of the commonly used reagent dithiothreitol. Therefore, without a reliable and well-validated method with high sensitivity at the nanomolar range, it is difficult to determine the actual value of H_2S level in the brain.

All the three H_2S biosynthesis enzymes, namely, CBS, CSE and 3-MST, are expressed in the brain. CBS is the primary physiologic source of H_2S in the CNS (Abe and Kimura 1996). CBS protein is predominantly localized in most areas of the brain, especially in hippocampus and cerebellum (Robert et al. 2003). It was found to be preferentially expressed in astrocytes rather than in neurons (Enokido et al. 2005; Lee et al. 2009). CSE is expressed in brain and was found to be predominantly present in neurons. It is critical for maintaining GSH homeostasis in brain (Diwakar and Ravindranath 2007). Furthermore, an intact transsulfuration pathway in the brain mediated by both CBS and CSE links to GSH homeostasis, which greatly contributes to the redox-buffering capacity in brain (Vitvitsky et al. 2006). 3-MST in combination with CAT produces H_2S from L-cysteine (Shibuya et al. 2009). 3-MST is localized to mitochondria and nerve endings. However, the

contributions of CBS and 3-MST with respect to H_2S generation under different physiological and pathological conditions are still not clearly understood. Detailed biosynthesis and metabolism of H_2S in CNS was described in a previous publication (Hu et al. 2011b). A novel pathway for the production of H_2S from D-cysteine was recently reported in mammalian cells (Shibuya et al. 2013). Unlike the L-cysteine pathway, this D-cysteine-dependent pathway operates predominantly in the cerebellum. This study presents a novel pathway of H_2S production and provides a new therapeutic approach to deliver H_2S.

6.3.2 Physiological Function of H_2S in Brain

H_2S may serve as a neuromodulator based on the following evidence. H_2S modulates LTP in active synapses. It facilitates the induction of LTP in the presence of a weak tetanic stimulation (Abe and Kimura 1996) and reversibly inhibits both fast and slow synaptic responses in dorsal raphe serotonergic neurons (Kombian et al. 1993). As H_2S upregulates the expression of γ-aminobutyric acid (GABA) B receptor (Han et al. 2005a), it is therefore critical in maintaining the excitatory/inhibitory balance. H_2S also induces astrocytic glutamate uptake (Lu et al. 2008), which removes excessive glutamate from synaptic clefts and maintains normal neurotransmission between neurons. These observations indicate that H_2S plays an important modulatory role in CNS.

Intracellular calcium ($[Ca^{2+}]_i$) is vital in regulating various brain functions. H_2S increases $[Ca^{2+}]_i$ in neurons, astrocytes and microglia (Nagai et al. 2004; Lee et al. 2006; Yong et al. 2010a), therefore plays important regulatory roles in synaptic activity and plasticity, as well as signal transmission between neuron and glial cells. H_2S also regulates intracellular pH (pH_i) in microglia and astrocytes (Lu et al. 2010a). Taken together, these findings suggest that H_2S modulates cell function via changes in ion channel conductance, synaptic transmission as well as gap junctions.

6.3.3 Pathological Functions of H_2S in the Central Nervous System

6.3.3.1 Neuroprotective Effects of H_2S

At micromolar range, H_2S may produce neuroprotective effects via its anti-inflammatory, anti-apoptotic and anti-oxidative actions.

Microglia cells are the resident macrophages of the brain, and thus act as the first and main form of active immune defense in CNS. H_2S inhibits production and release of NO and TNF-α in microglia and astrocytes when these cells are treated with lipopolysaccharide (LPS) (Hu et al. 2007). This is further confirmed by different groups of scientists with different H_2S-releasing compounds (Lee et al. 2010a; Yin et al. 2013). H_2S may exert anti-neuroinflammatory actions via inhibiting the production of pro-inflammatory factors and enhancing the production of anti-inflammatory cytokines. Inhibition of p38/JNK MAPK and NF-κB

signalling pathways are recognized as possible mechanisms by which H_2S restrains the extent of neuroinflammation and thereby limits the extent of neuronal injury.

H_2S, by itself, may act as a poor reductant (Kabil and Banerjee 2010). Physiological relevance of the antioxidant properties of H_2S probably rely more on other mechanisms. H_2S stimulates glutamate uptake in astrocytes by enhancing the trafficking of glial glutamate transporter GLT-1 (Lu et al. 2008). The enhanced glutamate uptake lowers extracellular glutamate and relieves the inhibition by glutamate on cystine transportation. This produces the driving force for cystine/glutamate antiporter Xc^- which transports cystine into cells, thereby an increase in intracellular L-cysteine followed by an increase in intracellular GSH. Moreover, H_2S may also increase GSH levels both directly or indirectly (Kimura and Kimura 2004; Whiteman et al. 2005; Umemura and Kimura 2007). These findings are strongly suggestive of the powerful anti-oxidative actions of H_2S in CNS.

H_2S has anti-apoptotic property in neuronal cells. H_2S protects hippocampal neurons against vascular dementia-induced cell apoptosis (Zhang et al. 2009), and inhibits apoptosis of neuronal cells induced by various toxins that are commonly used in establishing in vivo and in vitro models for PD and AD. These toxins include 1-methyl-4-phenylpyridine (MPP^+), 6-hydroxydopamine (6-OHDA), rotenone and β amyloid (Tang et al. 2008; Yin et al. 2009; Tiong et al. 2010). Preservation of mitochondrial integrity is the main mechanism for the anti-apoptotic effects of H_2S (Hu et al. 2009; Yin et al. 2009). H_2S prevents formation and opening of mitochondrial permeability transition pore, the subsequent release of cytochrome c from mitochondria to cytosol and the activation of caspase cascades. H_2S exerts these effects via opening of mitochondrial K_{ATP} channels and suppression of p38-MAPK (Hu et al. 2009).

6.3.3.2 H_2S in CNS Diseases

H_2S at normal level is important in brain physiology. Abnormal H_2S biosynthesis may contribute towards the progression of CNS diseases. Deficiency of CBS in humans, for example, results in higher plasma levels of homocysteine and methionine along with decreased level of L-cysteine. Patients with Alzheimer's Disease (AD) or Parkinson's Disease (PD) commonly show significantly increased homocysteine level in their cerebrospinal fluid (Isobe et al. 2005). This indicates that alterations of H_2S level in brain may contributes to pathophysiology of CNS diseases.

Alzheimer's Disease (AD)

The role of H_2S in AD development is incompletely understood. The level of S-adenosylmethionine, a CBS activator, is largely reduced in the brain of AD patients (Morrison et al. 1996). Furthermore, the serum level of homocysteine, a precursor of L-cysteine, is elevated in AD patients (Clarke et al. 1998). One possible explanation is that the transsulfuration pathway linking homocysteine and GSH metabolism, mediated by CBS and CSE, is disrupted.

There are various pieces of evidence that suggest H_2S treatment is capable of eliciting neuroprotective effects against pathological progression of AD. For example,

H_2S may decrease **β**-site amyloid precursor protein cleaving enzyme 1 (BACE-1) mRNA and protein expression and Aβ1-42 release in PC-12 neuronal cells (Zhang et al. 2011a). In addition, H_2S ameliorates β amyloid-induced damage in microglial (Liu and Bian 2010) and neuronal cells (Tang et al. 2008). Furthermore, H_2S attenuated LPS-induced cognitive impairment through reducing the overproduction of pro-inflammatory mediators via inhibition of NF-κB pathways in rats (Gong et al. 2010). These data imply that H_2S would be beneficial for AD treatment. However, more direct evidence for the potential benefits of H_2S or its donors in AD animal models is lacking at present.

Parkinson's Disease (PD)

The therapeutic effect of H_2S on PD has been well studied by several groups. Endogenous H_2S levels in substantia nigra and striatum were found to be reduced in PD animal models created by 6-OHDA or rotenone (Hu et al. 2010). This suggests that endogenous H_2S is likely to play a role in the development of PD. H_2S treatment was found to inhibit microglial activation in the substantia nigra and inflammation in the striatum. Since neuroinflammation is considered to be a critical factor in the pathogenesis of PD, these findings may suggest a therapeutic effect of H_2S. In separate animal models, H_2S treatment has been shown to inhibit loss of tyrosine hydroxylase positive (TH^+)-neurons in substantia nigra, and progression of movement dysfunction in these PD models was attenuated (Hu et al. 2010; Kida et al. 2010; Lu et al. 2012).

The mechanisms underlying therapeutic effects of H_2S on PD include anti-oxidative stress (Hu et al. 2010; Kida et al. 2010), anti-inflammation (Hu et al. 2010), anti-apoptosis (Hu et al. 2009) and anti-ER stress (Xie et al. 2012). Interestingly, Lu et al. reported that H_2S induced protection in dopaminergic (DA) neurons against neurodegeneration is independent of K_{ATP} activation (Lu et al. 2012), but mediated through a uncoupling protein 2 (UCP2) dependent mechanism. A very recent study demonstrated that H_2S may also induce S-sulfhydration of neuroprotective ubiquitin E3 ligase, parkin, to enhance its catalytic activity. Moreover, Parkin sulfhydration is markedly depleted in the brains of PD patients (Vandiver et al. 2013).

Taken together, these data further confirm that H_2S donors may be of high therapeutic value in the treatment of PD. ACS84 is a hydrogen sulfide-releasing-L-Dopa derivative compound. ACS84 has been found to prevent neurodegeneration via an anti-oxidative mechanism, and shown to have potential therapeutic values against PD (Xie et al. 2013).

Ischemic Stroke

High plasma level of L-cysteine correlates with poor clinical outcome 3 months post stroke in acute stroke patients (Wong et al. 2006). L-cysteine loading increases infarct volume in rats after middle cerebral artery occlusion (MCAO), and this effect can be reversed by inhibition of H_2S synthesis (Wong et al. 2006) and mimicked by exogenous application of H_2S (Qu et al. 2006).

However, under in vitro conditions, H_2S protects neurons against hypoxic injury (Tay et al. 2010; Li et al. 2011; Yin et al. 2013). The protective effects were mediated by anti-inflammatory, anti-oxidative and anti-apoptotic properties of H_2S. The discrepancy in observations could have resulted from differing concentrations of H_2S used in these studies. It is highly likely that physiological level of H_2S exerts a protective effect on cells against insults, such as hypoxia. During stroke, however, over-production of H_2S may facilitate cell death through enhancing excitotoxicity induced by excessive accumulation of extracellular glutamate.

Other CNS Diseases

Down syndrome is the most common chromosomal abnormality in humans. It is typically associated with a delay in cognitive ability (mental retardation, or MR) and physical growth, and a particular set of facial characteristics. High level of thiosulfate – a catabolite of H_2S, was found in the urine of Down syndrome patients (Belardinelli et al. 2001). Overproduction of endogenous H_2S was also found in Down syndrome patients and thus established a correlation between Down syndrome and chronic H_2S poisoning. Excessive H_2S may account for many clinical features of Down syndrome such as MR (Kamoun 2001).

There is an interaction between CBS and Huntington disease. Deficiency of CBS causes homocystinuria, as homocysteine is a substrate of CBS. The plasma homocysteine levels are also reported to be higher in patients with Huntington disease (Boutell et al. 1998; Andrich et al. 2004). Homocysteine is metabolized to homocysteate and homocysteine sulphinate, both are known to be powerful excitotoxic amino acids. It has been suggested that Huntington disease involves the action of excitotoxic amino acids and this interaction with CBS may suggest a mechanism for H_2S in this disorder.

Recurrent febrile seizures (FS) is the most common seizure type in children, often causing hippocampal damage. H_2S treatment may alleviate hippocampal damage induced by recurrent FS whereas inhibition of H_2S synthesis aggravates this damage (Han et al. 2005b). However, in a rat models of recurrent FS, the plasma level of H_2S and expressions of CBS in hippocampus were dramatically increased (Han et al. 2005b, 2006). As a result, the elevated H_2S concentration and CBS expression during recurrent FS may be a compensatory response to suppress neuronal hyperexcitability and thus alleviate neuronal damage in hippocampus.

Repeated exposure to opioids leads to development of addiction dependence, which can be assessed by observing emergence of withdrawal syndromes subsequent to discontinuation of chronic opioid administration or the administration of a competitive opioid antagonist such as naloxone (Maldonado and Koob 1993). Withdrawal-induced symptoms are the main cause to keep drug-dependent individuals craving continued opioids. It was found that exogenous administration of H_2S alleviates morphine and heroin withdrawal symptoms. This was mediated by suppression of supersensitivity of AC/cAMP/p-CREB pathway and modification of the levels of p-NR1, p-NR2A and p-NR2B levels (Jiang et al. 2012; Yang et al. 2013).

6.4 Physiological and Pathological Function of H_2S in the Kidneys

6.4.1 H_2S Synthesis in Kidneys

Endogenous H_2S plays an important role in mediating both glomerular and tubular functions of the kidneys. H_2S synthesizing enzymes are highly expressed in renal tissues, especially in proximal tubules (House et al. 2003; Ishii et al. 2004; Li et al. 2006b). This results in high amount of H_2S production when renal tissues are incubated with L-cysteine, the H_2S synthesizing enzyme substrate. Moreover, blockade of endogenous H_2S production with PAG (a CSE inhibitor) and aminooxyacetic acid (AOAA, a CBS inhibitor) reduced H_2S synthesis completely (Xia et al. 2009), suggesting that both enzymes contribute towards H_2S production in kidneys (Stipanuk and Beck 1982; House et al. 2003). The involvement of 3-MST and CAT in H_2S synthesis has yet to be characterized in renal tissues, hence warrants future research to understand the full picture of H_2S generation in kidneys.

6.4.2 Physiology Function of H_2S in Kidney

When H_2S is exogenously infused into renal artery, vascular activity of kidney such as renal blood flow (RBF), glomerular filtration rate (GFR) and filtration rate (FF) are significantly increased. However, there is no change in mean arteriole blood pressure (MAP), suggesting that H_2S may produce greater vasodilation in preglomerular arterioles than in postglomerular arterioles (Xia et al. 2009). H_2S also increased urine flow rate (U.V), urinary Na^+ and K^+ excretion ($U_{Na}.V$; $U_K.V$), fractional excretion of Na^+ and K^+ (FE_{Na}, FE_K) (Xia et al. 2009), suggesting that H_2S infusion altered renal tubular function (Xia et al. 2009).

Consistent effects on renal hemodynamics and excretory functions were observed when L-cysteine was infused into renal artery. These effects could be abolished by a combination of PAG and AOAA, but not by either of these alone. These observations bespeak physiological importance of endogenous H_2S, produced by CBS and CSE, in the basal regulation of renal filtration and tubular functions (Xia et al. 2009).

6.4.3 H_2S and Renal Ischemic Injury

6.4.3.1 Endogenous H_2S Production in Renal I/R

The effect of I/R on the level of endogenous H_2S is unclear due to controversies in research findings. Xu et al. reported that renal and plasma H_2S level in rats subjected to unilateral renal occlusion were significantly decreased (Xu et al. 2009), an effect due to reduced CBS activity in the ischemic kidney (Prathapasinghe et al. 2008;

Xu et al. 2009). Wu et al. also noticed a significant decrease in CBS enzyme activity during renal I/R, and they postulated that this effect was underlied by a decrease in Sp1 transcriptional activity (Wu et al. 2010).

CSE activity in the kidneys, however, was reported to be unaffected by Xu et al. (2009). This is in direct contrast to Tripatara et al.'s findings which suggest that renal H_2S production rate and plasma H_2S concentration were markedly elevated in mice subjected to bilateral renal occlusion due to an upregulation of CSE expression (Tripatara et al. 2009).

Nevertheless, both groups believed that endogenous H_2S protects against I/R injury. Xu et al. postulated that H_2S production by CBS is compromised in the kidneys during renal I/R, and the resultant reduction in H_2S leads to renal injuries (Xu et al. 2009). Tripatara et al., on the other hand, proposed that the elevated CSE activity and endogenous H_2S level act as a defensive mechanism against I/R induced injuries (Tripatara et al. 2008; Liu et al. 2011b).

6.4.3.2 Protective Effects of H_2S Against Renal Ischemia Injury

H_2S protects against I/R-induced renal injury, reperfusion injury, glomerular dysfunction and tubular dysfunction (Tripatara et al. 2009; Xu et al. 2009). H_2S administration also decreased elevated FE_{Na} during I/R, but had no significant effect on urine flow (Tripatara et al. 2009). In a large animal model of non-heart beating donor kidneys, H_2S protected the kidneys against I/R injuries, probably through improvements in RBF and decrease in intrarenal resistance (IRR) of kidneys (Hosgood and Nicholson 2010).

Renal I/R leads to both necrotic and apoptotic forms of cell death (Prathapasinghe et al. 2007). Rats that were subjected to I/R displayed severe acute tubular damage. Treatment with H_2S markedly reduced these histological signs and histological score for acute tubular necrosis (ATN), indicating that H_2S protects against I/R induced structural injuries (Tripatara et al. 2008).

The protective effects of H_2S involve its anti-oxidant effects. H_2S significantly reduce urinary 8-isoprostane, indicative of reduced extent of lipid peroxidation (Hosgood and Nicholson 2010). H_2S also reduces MDA level in the kidney as compared to I/R model rats (Xu et al. 2009), indicating that H_2S provides protective effect against IR-induced lipid peroxidation.

H_2S also significantly reduced the number of TUNEL-positive cells in renal tissues subjected to I/R (Xu et al. 2009). Furthermore, H_2S injection reduced the number of propidium iodide-positive cells, an index for necrotic cells, in the kidney tissues of rats subjected to I/R (Xu et al. 2009). Administration of H_2S into kidneys subjected to renal I/R also prevented caspase-3 activation (Bos et al. 2009). Tripatara et al. showed that NaHS administration attenuated I/R-induced Bid translocation and activation, which prevented I/R induced decrease in Bcl-2 protein levels (Tripatara et al. 2008). On the contrary, Bos et al. failed to observe change in Bcl-2 mRNA expression among treatment groups. Instead, they showed that H_2S

pretreatment decreased IR-induced elevation of Bax (Bos et al. 2009). These data suggest that H_2S produces anti-apoptotic effects to against ischemic injury.

In addition, H_2S also produced anti-inflammatory effect. H_2S lowered total NO level, a marker for tubular cell inflammation, in urine of pigs subjected to I/R (Hosgood and Nicholson 2010). Triparata et al. reported that H_2S attenuates NF-κB activation and expression of its dependent proteins, iNOS, COX-2 and ICAM-1 in the kidneys. These results clearly demonstrated the anti-inflammatory effects of H_2S in renal I/R model (Tripatara et al. 2008). Immunohistochemical staining of inflammatory components were assessed by Bos et al. H_2S pretreatment, but not post-treatment, significantly reduced the influx of Mac-1 (present on macrophages, monocytes, granulocytes and natural killer cells) and Ly-6G-positive cells (expressed on mature granulocytes) (Bos et al. 2009).

6.4.4 Role of H_2S in Other Renal Diseases

6.4.4.1 Renovascular Hypertension

In a renovascular hypertensive model established by 2-kidneys-1-clip (2K1C), Lu et al. found that H_2S exerted antihypertensive effects via inhibition of plasma renin activity and Ang II production in plasma (Lu et al. 2010b). This effect was underlied by downregulation of elevated cAMP by H_2S in kidney tissue (Lu et al. 2010b). In fact, using primary cultured renin-rich kidney cells, H_2S has also been proven to inhibit renin release by decreasing intracellular cAMP levels. Interestingly, H_2S was reported to inhibit angiotensin-converting enzyme (ACE) activity in human umbilical vein endothelial cells (HUVECs) (Laggner et al. 2007a). Lu and colleagues, however, observed no such inhibitory effect of H_2S on ACE activity of rat aortic endothelial cells (Lu et al. 2010b).

6.4.4.2 Chronic Kidney Disease (CKD)

In an experiment conducted on humans, Perna et al. found that endogenous production of H_2S was lowered in uraemic patients due to downregulation of CSE (Perna et al. 2009). Interestingly, 3-MST was found to be upregulated despite an overall decrease in plasma H_2S concentration, suggesting a predominant role of CSE in producing H_2S.

CBS heterozygous (CBS +/−) mice and/or uninephrectomy (1-K) were used as models of HHcy-associated end stage renal failure (Sen et al. 2009). H_2S supplementation prevents apoptosis of glomerular cells, macrophage infiltration, excessive superoxide production and decrease in glutathione (GSH) -to-oxidized glutationine (GSSG) ratio of CBS (+/−) 2-K, 1-K mice and WT 1-K mice. H_2S treatment also rectifies the expressions of desmin, nephrin, pro- and active forms of matrix metalloproteinase (MMP) −2 and −9, collagen IV, NAD(P)H oxidase p47 ^{phox} subunit, inflammatory molecules ICAM-1 and VCAM-1 (Sen et al. 2009, 2010).

6.5 Physiological and Pathological Functions of H_2S in Other Systems

6.5.1 Gastrointestinal System

In an in vivo model of hepatic I/R injury, exogenously applied H_2S significantly reduced elevations in serum alanine aminotransferase (Jha et al. 2008), suggesting that H_2S protects against liver injury.

H_2S also protects gastric mucosa against injury caused by NSAIDs or ischemic injury. This effect was mediated by suppression of leukocyte adherence (Fiorucci et al. 2005) and stimulation of production of antioxidant enzymes like SOD-1 and GSH (Liu et al. 2012a; Cui et al. 2013). In addition, H_2S may also improve blood flow to the injured gastric mucosal (Fiorucci et al. 2005; Henderson et al. 2010; Liu et al. 2012a).

6.5.2 Lungs

In a pulmonary I/R model, pretreatment of isolated rat lung with H_2S attenuated I/R injury, indicated by improvements in lung histological change, perfusion flow rate, ratio of lung wet weight to dry weight and lung compliance (Fu et al. 2008). In separate experiments, perfusion of lungs with PAG, a CSE inhibitor, showed aggravated lung I/R injury (Fu et al. 2008). Therefore, it is likely that endogenous H_2S is involved in the pathogenesis of lung I/R injury, whereas exogenous H_2S may be of clinical benefit to lung I/R injury.

6.5.3 Bones

Using an in vitro osteoblastic cell system, Xu et al. demonstrated that H_2S may be of potential therapeutic value for treatment against osteoporosis (Xu et al. 2011). In osteoblastic cells treated with H_2O_2, H_2S treatment stimulated osteoblast proliferation by enhancing both transcription and activity of alkaline phosphatase, and stimulated the transcriptional level of osteocalcin, the main bone matrix protein, and protein expression of collagen, a major constituent of bone tissue. These effects were mediated by antioxidant and anti-inflammatory effects of H_2S via a MAPK (p38 and ERK1/2)-dependent mechanism (Xu et al. 2011).

6.5.4 Diabetes

CSE/H_2S system plays an important role in regulating β-cell functions (Yang et al. 2011). H_2S can promote glucose uptake and produce insulin-sensitizing effects in type 2 diabetes (Xue et al. 2013). In vitro experiments demonstrated that H_2S enhances glucose uptake in both myotubes and adipocytes. These effects are

mediated by upregulated phosphorylation of insulin receptors, PI3K and Akt (Xue et al. 2013). In Goto-Kakizaki diabetic rats, chronic H_2S treatment decreased fasting blood glucose, increased insulin sensitivity, and increased glucose tolerance with increased phosphorylation of PI3K and Akt in muscles (Xue et al. 2013). Henceforth, H_2S holds promise as a new therapeutic drug against insulin resistance. The role of H_2S in the pathogenesis of diabetes mellitus has been extensively discussed in a recent review (Szabo 2012).

6.6 Concluding Remarks

In summary, H_2S acts as a gaseous modulator in many mammalian tissues and is likely to be involved in the pathogenesis of many diseases. Knowledge of H_2S biology in mammalian systems raises the possibility of manipulating H_2S system for therapeutic benefits to patients suffering from various diseases. The narrow therapeutic window of H_2S, however, continues to pose as a major challenge for its widespread utilization as a therapeutic drug despite its potent and beneficial effects seen in many systems and diseased conditions. Slow-releasing H_2S donors that mimic endogenous H_2S synthesis release are therefore urgently sought. Research efforts in recent years has focused on the development of a variety of such novel donors, including GYY4137, S-diclofenac and S-dopa (Li et al. 2007, 2008; Xie et al. 2013). Moreover, currently available H_2S biosynthesis inhibitors, such as AOAA and hydroxylamine, are nonspecific for their actions. More potent and specific inhibitors are deemed to be developed for us to have a better understanding of endogenous H_2S functions.

At present, most of these slow-releasing H_2S donors and H_2S biosynthesis inhibitors have poor solubility in physiological medium, and the adverse side effects of most novel compounds yet to be fully explored. There is still a long way to go before H_2S-releasing compound can be exploited for clinical usage; the biology of H_2S still beholds lots of mysteries and excitements waiting to be unveiled.

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