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Review Article

Gaso-transmitter hydrogen sulphide: Potential new target in pharmacotherapy

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Research in the last two decades has transformed the way hydrogen sulphide (H_2S) is perceived from a noxious gas to a gaso-transmitter with a vast potential in pharmacotherapy. H_2S is synthesized in various body-systems using the enzymes cystathionine beta-synthase and cystathionine gamma-lyase; either of these being the predominat enzyme in a particular system. H_2S may be one of the physiological modulators of blood pressure in humans. The gas relaxes the vascular smooth muscle cells by opening up K_{ATP} channels. Moreover, it suppresses the proliferation of vascular smooth muscle cells. H_2S may also be contributing in the protection afforded by ischaemia-preconditioning. Testosterone is thought to be responsible for the higher central nervous system level of H_2S in males. In the central nervous system, H_2S is implicated in Alzheimer's disease, epilepsy, stroke and Down's syndrome. Insulin secretion is associated with a decrease in the H_2S levels. Raised H_2S is detrimental in acute pancreatitis as well as in septic shock. Recently, H_2S-releasing derivatives of certain drugs have shown promise in protection against gastric ulcer and in inflammatory bowel disease. The beneficial effects of certain sulphur containing herbs like ginseng and garlic may be mediated via H_2S. In future, development of specific drugs modulating H_2S levels may prove beneficial in varied disorders.

Keywords: Apoptosis, Cardiovascular system, Gaso-transmitter, Hydrogen sulphide, Nervous system, S-adenosyl-L-methionine

Hydrogen sulphide (H_2S), a gas with the smell of "rotten eggs", is known mostly for its noxious effects. It can cause a gamut of deleterious effects in humans and animals when inhaled in more than safe levels. Even when present in a concentration as low as 0.05 ppm, it makes the perceived air quality unpleasant and causes an increase in the anxiety symptoms[1]. With increasing concentration, it causes irritation of the eyes and the respiratory tract and difficulty in breathing[2]. Long term exposure to low concentrations may result in fatigue, headache, loss of appetite and decline in cognitive functions[2]. Even a few breaths of the gas at the concentration of 1000 ppm can prove to be lethal[3,4]. It is, therefore, not surprising that until two decades ago majority of the studies pertaining to H_2S were concerned mainly about its toxic potential.

H_2S: the third gasotransmitter

Around 1990, however, a turning point came for H_2S. In 1989, endogenous "sulphide" was reported to be present in rat brain tissues and in normal human post-mortem brainstem[5,6]. These findings suggested endogenous production of H_2S in the brain. It is now established that H_2S is produced endogenously in mammals in the brain, blood vessels, liver and kidneys. H_2S is thought to be implicated in various physiological and pathological processes in humans. It is now being regarded as the third "gaso-transmitter" after NO and CO[7]. Being a small and lipid soluble molecule, it has the ability to infiltrate the three-dimensional structure of the receptors and affect their functions[8,9]. This property also makes it easy to pass through biological membranes to exert its effects. Its role is now recognized in various body systems encompassing brain, cardiovascular system, lungs, gastrointestinal tract, liver, kidney and pancreas; and also in inflammation and pain perception[10].

Endogenous regulation of H_2S levels

H_2S production

Endogenous production of H_2S is catalysed by two pyridoxal-5-phosphate-dependent enzymes, cystathionine beta-synthase (CBS) and cystathionine gamma-lyase (CSE) (Fig. 1). In the brain, H_2S production is primarily catalyzed by the enzyme CBS using cysteine and homocysteine as substrates[4].

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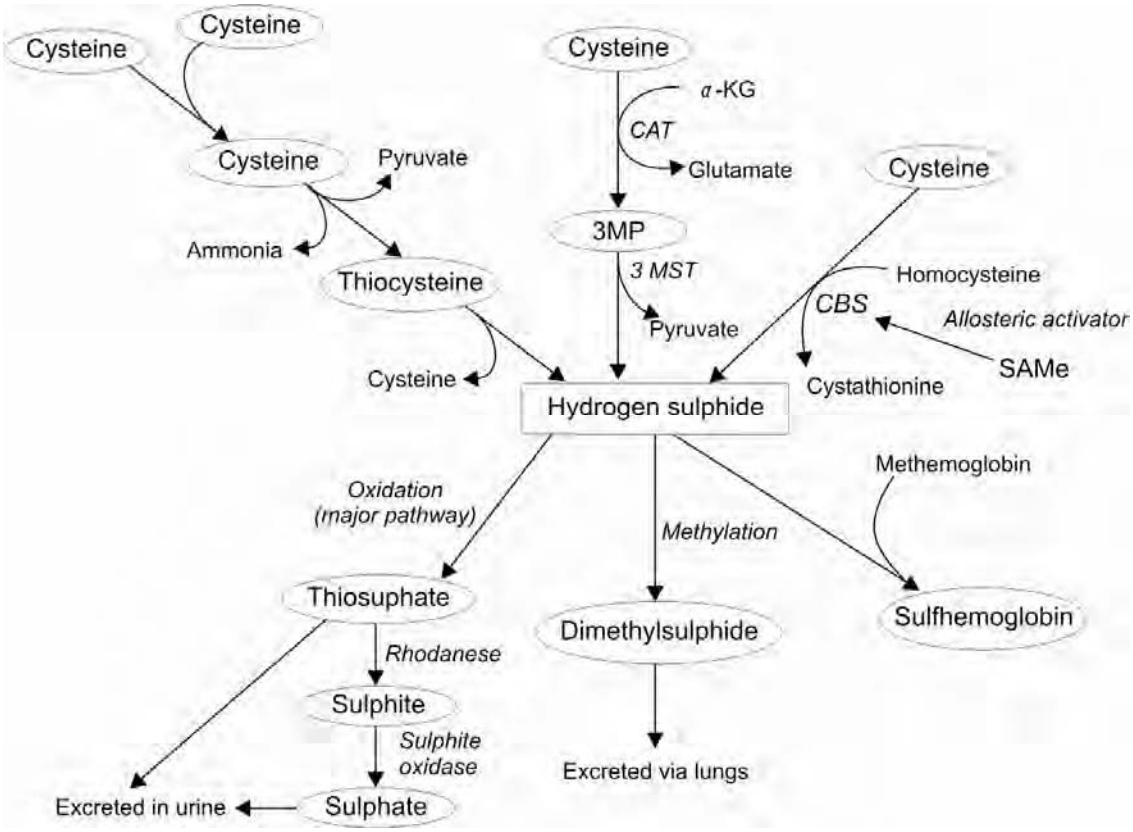

Fig. 1—Major putative pathways of H_2S metabolism [CSE: cystathionine γ-lyase, CBS: cystathionine β-synthase, SAMe: S-adenosyl-L-methionine]

CBS is highly expressed in the brain while CSE mRNA levels are undetectable in the brain using northern blot analysis. Also, S-adenosyl-L-methionine (SAMe), the allosteric activator of the enzyme CBS, increases while CBS inhibitors hydroxylamine and amino-oxyacetate decrease the brain H_2S levels. On the other hand, CSE inhibitors D,L-propargylglycine and β-cyano-L-alanine had no effect on the brain H_2S levels[11]. In the cardiovascular system, however, CSE is the major enzyme responsible for H_2S production, catalyzing the conversion of cystine to cysteine, H_2S, NH_3 and pyruvate[12,13]. Recently, another enzymatic pathway of H_2S synthesis has been identified. 3-Mercaptopyruvate sulphur transferase (3MST) in conjunction with cysteine aminotransferase (CAT) can produce H_2S from cysteine in the presence of α-ketoglutarate. 3MST has been found to be localized to neurons as well as to vascular endothelium and smooth muscle cells[14]. However, the physiological significance of this pathway is not yet completely known[15].

Regulation of H_2S production

There appear to be at least three ways in which the endogenous production of H_2S is regulated[16]. The first is neuronal excitation which is responsible for the "fast regulation" of H_2S. This fast regulation is mediated via Ca^{2+}/Calmodulin. In presence of Ca^{2+} and calmodulin, the rate of production of H_2S by CBS is increased 3.5-times than that in their absence. Also, trifluoroperazine and W-13, two inhibitors of calmodulin, result in suppression of H_2S production[17]. A "slower" pathway regulating the endogenous H_2S levels was found to be controlled by testosterone. The idea that sex hormones could play a role in H_2S production came into being after the finding that endogenous H_2S levels in the brains of male mice were higher as compared to that of the females[16]. To test the hypothesis, Eto *et al.*[17] conducted a series of experiments. After a single administration of testosterone, the brain H_2S levels of female mice almost reached the levels that of male mice. The results were further consolidated when castration of male mice resulted in a decrease in the testosterone

levels accompanied by a decrease in brain H_2S levels. In both the experiments, there were no significant changes in the brain CBS levels suggesting that testosterone increases the H_2S levels in the brain by increasing only the "activity" of the enzyme CBS. This increased activity of the enzyme was subsequently found to be mediated via SAMe. Thus, it could be concluded that SAMe is the downstream effector responsible for the changes in brain H_2S levels induced by testosterone[16]. However, it was observed that the difference in the brain H_2S levels was not as great as the difference in the brain testosterone levels. Also, the age-related changes in the testosterone levels did not correlate well with the brain H_2S levels. This suggested that there could be some additional pathway(s) that control the basal H_2S levels. It is hypothesized that glucocorticoids might regulate this third pathway as they have been found to regulate SAMe synthesis in the liver[16].

Catabolism of H_2S

H_2S does not accumulate in the body and is rapidly metabolized. Sulphate is the major end product and is excreted in urine. Thiosulphate, another by-product of H_2S metabolism, is also excreted in the urine and although present in low concentration, it serves as the biomarker of H_2S metabolism[18].

Role of H_2S in the cardio-vascular system

Hypotensive effect

The role of H_2S in the cardiovascular system has been studied in relative detail. It is thought to be implicated in an array of pathophysiological conditions. Interestingly, the hypotensive effects of garlic and other sulphur-containing natural substances, like onions and mushrooms, may be mediated via H_2S[12]. Similar to the other two gaso-transmitters, namely, NO and CO, H_2S also relaxes the vascular smooth muscle cells. H_2S, however, differs from NO and CO, in that the vasorelaxant effect is not mediated via cGMP. H_2S is thought to act by opening up of K_{ATP} channels present on the vascular smooth muscle cells[19-21]. This causes membrane hyperpolarization with resultant relaxation of vascular smooth muscle cells[13]. The probable role of H_2S in hypertension has been studied in animal model of spontaneous hypertension. Exogenous administration of H_2S donor sodium hydrogen sulphide (NaHS) in spontaneously hypertensive rats could alleviate hypertension and help recover the suppressed plasma H_2S levels and CSE activity[22]. The effect of H_2S on the vascular resistance is not limited to its vasorelaxant property. H_2S is also thought to prevent the development of hypertension by suppressing the proliferation of vascular smooth muscle cells and by accelerating their apoptosis via mitogen-activated protein (MAP) kinase pathway[23,24]. These findings suggest that H_2S may be one of the mediators in the regulation of blood-pressure.

Cardioprotective effect

H_2S has also been reported to possess cardio-protective effect[19,21,25]. Ischaemia-preconditioning of heart (subjecting the tissue to multiple brief periods of ischaemia) protects against myocardial damage caused by actual ischaemia[26]. This protective effect was observed to be abolished by treatment with CSE inhibitors DL-propargylglycine or β-cyano-L-alanine. The protection is restored when the H_2S donor NaHS is co-administered. Thus, H_2S seems to be responsible for the protection achieved by ischaemia preconditioning. This was further substantiated by the fact that ischaemia causes a significant decrease in the H_2S levels in cultured cardiac myocytes, an effect which is attenuated by ischaemia-preconditioning and pretreatment with NaHS[19]. In addition, NaHS has been shown to offer protection against myocardial infarction in an animal model[27,28]. Recently, the protective effect of H_2S against regional myocardial ischaemia/reperfusion injury was shown to be due to the gas's anti-apoptotic and anti-inflammatory effects[29]. H_2S is also thought to be involved in several other cardiovascular pathologies like hypoxia-induced pulmonary hypertension[30], high blood flow induced pulmonary hypertension[31], cystathioninuria[27] and septic and endotoxic shock[32].

Role of H_2S in the central nervous system

Neuromodulator role

Following reports that high levels of H_2S are present in the brain[5,6,33], the physiological function of H_2S was explored. It was observed that H_2S facilitates the long-term potentiation (LTP) in the hippocampus. This induction of LTP was not seen in the presence of 2-amino-S-phosphonovalerate, an NMDA receptor antagonist indicating that H_2S requires activation of NMDA receptors for the induction of hippocampal LTP[11]. This is in contrast to CO and NO that can induce LTP even when the NMDA receptors are blocked[34]. Also, H_2S could facilitate LTP only in

active synapses suggesting its role in associative learning and as a neuromodulator[11]. Further investigating the role of H_2S in synaptic activity, Eto *et al.*[17] found that absence of H_2S in CBS knock-out mice altered the LTP in hippocampal slices.

H_2S has been shown to reduce the potassium-stimulated release of corticotrophin releasing hormone by rat hypothalamus. Also, H_2S attenuates the hypothermia-induced glucocorticoid release, implicating the role of H_2S in the negative feedback of the hypothalamo-pituitary-adrenal axis[35]. These observations imply an important role of H_2S in neuromodulation.

Role in Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disease of increasing public health importance. Although several risk factors have been found to be associated with an increased incidence of AD, the exact mechanism of disease causation is still to be unravelled. Increased oxidative stress due to free radicals has been proposed as one of the potential mechanisms for the development of AD[36]. In fact, oxidative stress has been found to be linked to "neurofibrillary tangle", a major pathological finding in AD[37].

Interestingly, the levels of H_2S in AD brains have been found to be significantly lower than in the normal post-mortem brains. This is associated with low levels of SAMe, thus suggesting decreased activity of the enzyme CBS in AD brains[38]. It has been shown that decreased levels of H_2S result in inefficient scavenging of oxidising species like peroxynitrite and hypochlorous acid resulting in neuronal damage, intracellular protein oxidation and lipid peroxidation thereby contributing to the pathogenesis of AD[39].

Of late, disturbance in the cerebral microvasculature, resulting in cerebral ischaemia, has also been linked to the pathogenesis of AD. H_2S may be involved in this pathway as well, considering its role in vascular relaxation[38]. Finally, the role of H_2S as a facilitator of long term potentiation in the hippocampus may also be implicated in the cognitive compromise encountered in AD[10].

Role in epilepsy

Human studies have found hippocampal damage to be an important feature of recurrent febrile seizures[40]. Following the reports of high expression of CBS in the hippocampus[11] and potential neuromodulatory role of H_2S[11,17], Han *et al.*[8] studied the role of H_2S in animal model of recurrent febrile seizures using sodium hydrogen sulphide as a donor of H_2S. High levels of H_2S could inhibit synaptic transmission in the hippocampus preventing the neurons from excitatory toxicity. Also, H_2S could down-regulate c-fos during the development of febrile seizures and prevents mossy fibre sprouting, indicating neuronal protection[8].

American ginseng root is known to contain 0.15 % sulphur[41]. The effect of a closely related ginseng — *Panax ginseng* was studied in pentylenetetrazole-induced kindling in rats[42]. *Panax ginseng* group showed significantly higher protection as compared to the vehicle group. Though the exact mechanism of antiepileptic activity of *Panax ginseng* has not been elucidated, partial involvement of H_2S cannot be ruled out considering the sulphur content of the herb. Further research in this direction is warranted.

Dahiya *et al.*[43] also suggested a protective role of SAMe against pentylenetetrazole (PTZ)-induced seizures in rats. Adult male Wistar weighing 180-240 g were divided into three groups of six each. PTZ was administered to each group in a dose of 60 mg/kg, ip to induce seizures. All the animals in group 1 (negative control) experienced generalized tonic-clonic (GTC) seizures. Group 2 (positive control) animals were pretreated with valproic acid (300 mg/kg, ip) 30 min prior to PTZ administration. None of the animals in this group experienced seizures. Animals in group 3 (SAMe) were administered SAMe (400 mg/kg/day, po) for three days before PTZ administration. PTZ was administered on the third day, 90 min after the last dose of SAMe. Three out six animals in this group did not experience GTC seizures. The other three animals who did experience seizures had significantly increased latency to GTC, indicating protective effect of SAMe against seizures induced by PTZ[43]. SAMe is known to increase the levels of H_2S by increasing the activity of the enzyme CBS[35]. Eto and Kimura[16] reported that administration of SAMe once a day for three days causes brain H_2S levels to be significantly increased. Conversely, decreased activity of SAMe has been related to decreased levels of H_2S[38]. Additionally, H_2S donor sodium hydrogen sulphide has been shown to be protective against hippocampal damage produced by recurrent febrile seizures in rats by raising brain H_2S levels[8]. Thus, it is possible that

the protection afforded against seizures[43] may be due to raised brain H_2S levels after the administration of SAMe. Further research, aimed at estimation of brain H_2S levels and their correlation with seizure-protection is, however, needed to consolidate this hypothesis.

Deleterious effect of H_2S on central nervous system

There is some evidence of deleterious effects of H_2S in the CNS too. CNS levels of H_2S have been found to be increased in Down's syndrome (trisomy of chromosome 21). Intriguingly, the gene encoding CBS is located on chromosome 21 at the position 22.3[44]. Excess of H_2S in Down's syndrome is thought to cause neuronal damage via inhibition of cytochrome c oxidase or overstimulation of NMDA receptors[45]. Apart from this, detrimental effect of H_2S has been seen in middle cerebral artery occlusion model of stroke[46]. It was found that administration of NaHS, an H_2S donor, before permanent occlusion of the middle cerebral artery, increased the infarct size. On the other hand, various inhibitors of H_2S production decreased the infarct volume. The inhibitors of the enzyme CBS (amino-oxyacetate and hydroxylamine) were found to have a greater protective effect than the inhibitors of CSE (β-cyano-L-alanine and DL-propargylglycine) which is in concordance with the fact that CBS is the major enzyme catalyzing the formation of H_2S in the brain. It was also noted that the protective effect was directly proportional to the potency of the inhibitors to decrease H_2S production *in vitro*[46].

Other roles of H_2S

Systemic and local inflammation

The vasodilator action of H_2S suggests its potential role in inflammation. This was proved in several studies in which inflammation was induced locally or systemically in animals. H_2S levels were found to increase in both septic and endotoxic shock along with an increase in nitric oxide level. The levels of H_2S were found to be negatively correlated with the blood pressure and cardiac function[47]. It was found that H_2S is increased in experimental model of pancreatitis[48,49]. That H_2S was not just an accompanying feature of pancreatitis but was involved in its causation was inferred from the fact that administration of DL-propargylglycine not just inhibited H_2S production but also attenuated the severity of pancreatic inflammation and the lung injury that accompanies severe pancreatitis. Though the pancreatic-inflammation and the associated oedema could be explained by the vasodilator property of H_2S, acinar-cell necrosis could not. It was proposed that H_2S produced locally could activate the zymogens initiating the autodigestive process[48].

Insulin release

Insulin release from pancreatic β-cells is thought to be regulated via K_{ATP} channel. When the extracellular level of glucose is low, K_{ATP} channels are open keeping the β cell hyperpolarized. With increasing concentration of glucose, a series of events takes place causing inhibition of K_{ATP} channel. This causes increase in membrane electric potential resulting in opening up of voltage-sensitive Ca^{2+} channels. Raised intracellular Ca^{2+} finally results in release of insulin from storage granules. Sulfonylureas, like glucose, cause insulin secretion by blocking K_{ATP} channel[50].

Considering the fact that H_2S causes relaxation of vascular smooth muscle cells via opening up of K_{ATP} channels, it is logical to envisage that the gas might as well affect K_{ATP} channels in pancreatic β cells, although molecular composition of K_{ATP} channels is different among different cell types. To test this possibility, the effect of H_2S was studied on INS-1E cells derived from insulinoma pancreatic β-cell line. INS-1E cells were found to produce significant amounts of H_2S, mainly via CSE enzyme. At low concentration of extracellular glucose, H_2S level is high, keeping the β cell hyperpolarized. Increasing the level of extracellular glucose could inhibit H_2S production causing membrane depolarization resulting in insulin release. The fact that H_2S production is regulated by extracellular glucose concentration implies that H_2S could be one of the physiological regulators of insulin secretion in humans[51].

Renal ischaemia-reperfusion injury

Analogous to its protective effect against ischemia-reperfusion (I/R) injury to the heart, H_2S has shown to improve the outcome of I/R in porcine model of non-heart-beating donor kidneys[52]. In brief, the kidneys were subjected to 25 min of warm ischaemia followed by 18 h of cold storage. They were then reperfused *ex vivo* with autologous blood. In one group, H_2S donor sodium hydrogen sulphide was administered 10 min before and after reperfusion, while in the other group (control), no such treatment was given. It was observed that, H_2S caused significant improvement in

the markers of kidney function. Renal blood flow improved significantly as compared to the control group attributable to the vasodilation caused by H_2S. This was associated with reduced oxidative damage, lower serum creatinine levels, greater creatinine clearance and higher urine output in the H_2S-treated group. The finding, thus, indicates potential role of H_2S in kidney transplant recipients[52].

Apoptosis

Apoptosis is a process of programmed cell death in response to appropriate trigger. The mitogen-activated protein kinases (MAPK) superfamily, consisting of extracellular signal-regulated kinase (ERK), p38 MAPK and c-Jun N-terminal kinase (JNK), plays an important role in cell survival and apoptosis[53]. Both pro-apoptotic and anti-apoptotic actions of H_2S have been identified in different situations. Yang *et al.*[24] demonstrated that physiological concentrations of H_2S induce apoptosis of human aorta smooth muscle cells. They further proved that this action of H_2S was via the activation of ERK and was associated with increased concentrations of caspase-3, an important executioner of apoptosis[24]. On the other hand, in ischaemia/reperfusion model, sodium hydrogen sulphide, a donor of H_2S, was found to oppose apoptosis by preventing the activation caspase-9. Sodium hydrogen sulphide also ameliorated the decreased expression of Bcl-2, an anti-apoptotic protein, caused by ischaemia/reperfusion. It was shown that these anti-apoptotic effects of sodium hydrogen sulphide could be mediated via opening up of putative mitochondrial K_{ATP} channels[29]. In another study, sodium hydrogen sulphide concentration-dependently suppressed rotenone-induced apoptosis in human-derived dopaminergic neuroblastoma cell line. This finding has implications in the therapy of neurodegenerative diseases like Parkinson's disease[54]. Recently, sodium hydrogen sulphide has shown to protect the H9c2 embryonic rat cardiac cells against cobalt chloride-induced injury. The effect was attributed to the ability of H_2S to attenuate inflammation and suppress caspase-3 activation, thereby preventing apoptosis[55].

The proapoptotic effect of H_2S indicates its potential antineoplastic action. In fact, H_2S, at physiological concentration, has been shown to decrease the viability of colon cancer cells[56]. The anti-neoplastic effects of garlic have been attributed to its sulphur content[57]. In various animal studies, consumption of garlic has been shown to have a protective effect against tumours of the gastrointestinal tract, mammary glands, skin and liver[58-62]. However, there is also some evidence suggesting a mutagenic potential of H_2S[63]. Further studies are required to explain the controversy.

H_2S: future prospects and unresolved issues

In concordance with the vasodilator property of H_2S, NaHS, a precursor of H_2S, was shown to improve penile length and cavernous pressure in primates. DL-propargylglycine, on the other hand, had an opposite effect by inhibiting H_2S formation[64]. Thus, in future, therapy of erectile dysfunction may include modulation of H_2S release.

Present modulators of H_2S levels are non-specific[65] and thus their use is limited to research only. However, a few drugs, chemically modified to release H_2S, are being tested for their additional benefit[66]. "H_2S-releasing derivatives" of non-steroidal anti-inflammatory drugs (NSAIDs) diclofenac and indomethacin have been found to cause significantly less gastric hemorrhagic lesions and leucocyte adherence than the "original drugs"[67]. Leucocyte adherence to the vascular endothelium of the mesenteric circulation was linked with the NSAIDs-induced gastric damage[67]. In addition, H_2S-releasing derivative of mesalamine has been shown to decrease the inflammation associated with inflammatory bowel disease[68]. H_2S has also shown analgesic effect on visceral pain which is a troublesome feature of inflammatory bowel disease[69]. There is evidence that this anti-nociceptive effect is mediated via K^+_{ATP} channels[70].

The role of H_2S in nociception is, however, not well-defined. H_2S has been shown to increase the neutrophil migration at the site of inflammation. This is thought to cause increased nociception by increasing PGE_2 production[71]. On the other hand, exogenously increasing the H_2S in colorectal distension-induced nociception model in rats has shown anti-nociceptive property via activation of K^+_{ATP} channels[69,70]. These controversial results could be because of the difference in the H_2S concentration achieved by endogenous and exogenous routes[72].

Keeping in view the wide ranging effects of H_2S, (Fig. 2) discovery of suitable modulators of its levels in future may open new avenues of treatment options in a variety of diseases like hypertension, Alzheimer's disease, Down's syndrome, inflammatory conditions

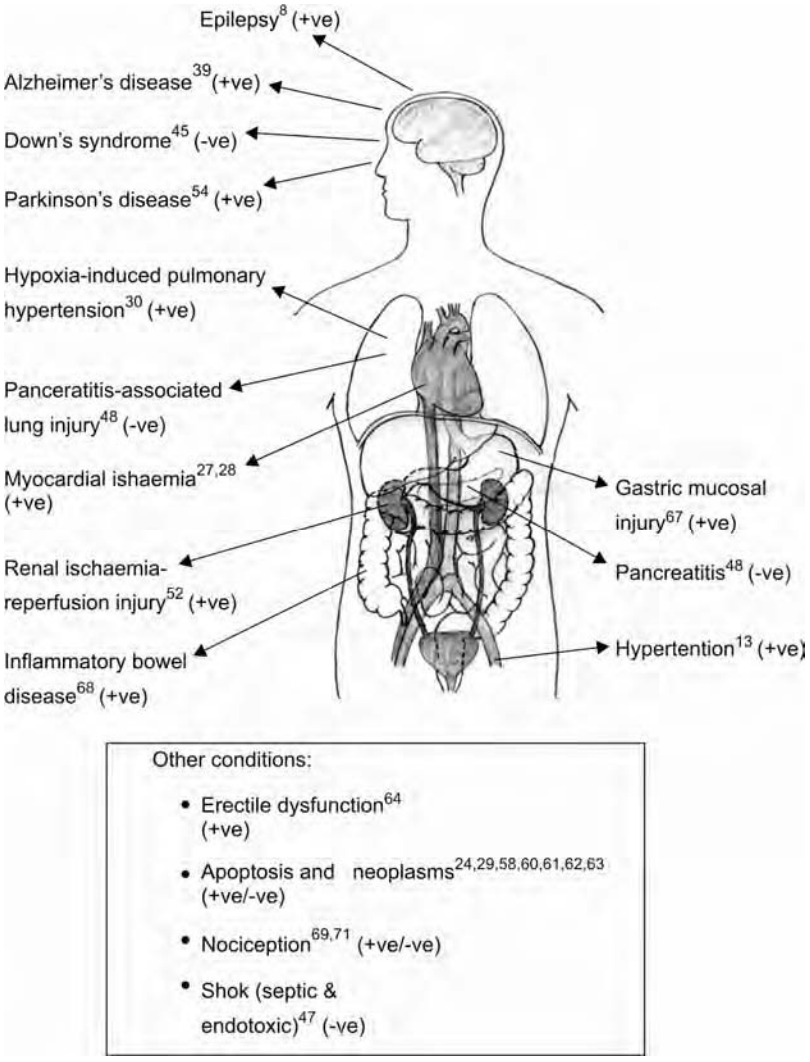

Fig. 2—Major pathological conditions in which increase in the H_2S levels may have a beneficial (+ve) or detrimental (-ve) effect

like inflammatory bowel disease, septic shock and acute pancreatitis, various neoplasms and erectile dysfunction. Also, the effects of various sulphur-containing herbs like garlic and ginseng may be mediated via H_2S. Further research is thus required to fully realize the potential of H_2S.

References

1 Fiedler N, Kipen H, Ohman-Strickland P, Zhang J, Weisel C & Laumbach R, Sensory and cognitive effects of acute exposure to hydrogen sulfide, Environ Health Perspect, 116 (2008) 78.

2 Agency for toxic substances and disease registry: Hydrogen sulphide [Online], (1999) [Cited 2009 May 23], Available from: URL:http://www.msdshazcom.com/ATSDR/DOCS/WCD00000/WCD000EA.PDF

3 Kobayashi K & Fukushima H, Suicidal poisoning due to hydrogen sulfide produced by mixing a liquid bath essence containing sulfur and a toilet bowl cleaner containing hydrochloric acid, *Chudoku Kenkyu*, 21 (2008) 183.

4 Fiorucci S, Distrutti E, Cirino G & Wallace JL, The emerging roles of hydrogen sulfide in the gastrointestinal tract and liver, *Gastroenterology,* 131 (2006) 259.

5 Warenycia MW, Goodwin LR, Benishin CG, Reiffenstein RJ, Francom DM, Taylor JD & Dieken FP, Acute hydrogen sulphide poisoning: Demonstration of selective uptake of sulphide by the brainstem by measurement of brain sulphide levels, *Biochem Pharmacol,* 38 (1989) 973.

6 Goodwin LR, Francom D, Dieken FP, Taylor JD, Warenycia MW, Reiffenstein RJ & Dowling G, Determination of sulfide in brain tissue by gas dialysis/ion chromatography: Post-mortem studies and two case reports, *J Anal Toxicol*, 13 (1989) 105.

7 Wang R, Two's company, three's a crowd: Can H_2S be the third endogenous gaseous transmitter? *FASEB J*, 16 (2002) 1792.

8 Han Y, Qin J, Chang X, Yang Z, Tang X & Du J, Hydrogen sulfide may improve the hippocampal damage induced by

recurrent febrile seizures in rats, *Biochem Biophys Res Commun*, 327 (2005) 431.

9 Zhao W & Wang R, H_2S-induced vasorelaxation and underlying cellular and molecular mechanisms, *Am J Physiol Heart Circ Physiol,* 283 (2002) H474.

10 Lowicka E & Beltowski J, Hydrogen sulphide - the third gas of interest for pharmacologists, *Pharmacol Rep,* 59 (2007) 4.

11 Abe K & Kimura H, The possible role of hydrogen sulfide as an endogenous neuromodulator, *J Neurosci*, 16 (1996) 1066.

12 Jacob C, Anwar A & Burkholz T, Perspective on recent developments on sulfur-containing agents and hydrogen sulfide signaling, *Planta Med*, 74 (2008) 1580.

13 Zhao W, Zhang J, Lu Y & Wang R, The vasorelaxant effect of H2S as a novel endogenous gaseous K_{ATP} channel opener, *EMBO J,* 20 (2001) 6008.

14 Kimura H, Hydrogen sulfide: From brain to gut, *Antioxid Redox Signal*, 12 (2010) 1111.

15 Wang R, Hydrogen sulfide: The third gasotransmitter in biology and medicine, *Antioxid Redox Signal*, 12(9) (2010) 1061.

16 Eto K & Kimura H, The production of hydrogen sulfide is regulated by testosterone and S-adenosyl-L-methionine in mouse brain, *J Neurochem*, 83 (2002) 80.

17 Eto K, Ogasawara M, Umemura K, Nagai Y & Kimura H, Hydrogen sulfide is produced in response to neuronal excitation, *J Neurosci,* 22 (2002) 3386.

18 Belardinelli MC, Chabli A, Chadefaux-Vekemans B & Kamoun P, Urinary sulfur compounds in Down syndrome, *Clin Chem,* 47 (2001) 1500.

19 Bian JS, Yong QC, Pan TT, Feng ZN, Ali MY, Zhou S & Moore PK, Role of hydrogen sulfide in the cardioprotection caused by ischemic preconditioning in the rat heart and cardiac myocytes, *J Pharmacol Exp Ther*, 316 (2006) 670.

20 Bannenberg GL & Vieira HL, Therapeutic applications of the gaseous mediators carbon monoxide and hydrogen sulfide, *Expert Opin Ther Pat*, 19 (2009) 663.

21 Calvert JW, Coetzee WA & Lefer DJ, Novel insights into hydrogen sulfide–mediated cytoprotection, *Antioxid Redox Signal*, 12 (2010) 1203.

22 Yan H, Du JB & Tang CS, The possible role of hydrogen sulfide on the pathogenesis of spontaneous hypertension in rats, *Biochem Biophys Res Commun*, 313 (2004) 22.

23 Du J, Hui Y, Cheung Y, Bin G, Jiang H, Chen X & Tang C, The possible role of hydrogen sulfide as a smooth muscle cell proliferation inhibitor in rat cultured cells, *Heart Vessels,* 19 (2004) 75.

24 Yang G, Sun X & Wang R, Hydrogen sulfide-induced apoptosis of human aorta smooth muscle cells via the activation of mitogen-activated protein kinases and caspase-3, *FASEB J,* 18 (2004) 1782.

25 Johansen D, Ytrehus K & Baxter GF. Exogenous hydrogen sulfide (H_2S) protects against regional myocardial ischemia-reperfusion injury: Evidence for a role of K_{ATP} channels, *Basic Res Cardiol*, 101 (2006) 53.

26 Murry CE, Jennings RB & Reimer KA, Preconditioning with ischemia: A delay of lethal cell injury in ischemic myocardium, *Circulation,* 74 (1986) 1124.

27 Chen C, Xin H & Zhu Y, Hydrogen sulfide: Third gaseous transmitter, but with great pharmacological potential, *Acta Pharmacol Sin*, 28(11) (2007) 1709.

28 Zhu YZ, Wang ZJ, Ho P, Loke YY, Zhu YC, Huang XW, Tan CS, Whiteman M, Lu J & Moore PK, Hydrogen sulfide and its cardioprotective effects in myocardial ischemia in experimental rats, *J Appl Physiol,* 102 (2007) 261.

29 Sivarajah A, Collino M, Yasin M, Benetti E, Gallicchio M, Mazzon E, Cuzzocrea S, Fantozzi R & Thiemermann C, Anti-apoptotic and anti-inflammatory effects of hydrogen sulfide in a rat model of regional myocardial I/R, *Shock*, 31 (2009) 267.

30 Zhang C, Du J, Bu D, Yan H, Tang X, Si Q & Tang C, The regulatory effect of endogenous hydrogen sulfide on hypoxic pulmonary hypertension, *Beijing Da Xue Bao,* 35 (2003) 488.

31 Wang YF, Shi L, Du JB & Tang CS, Impact of L-arginine on hydrogen sulfide/cystathionine-γ-lyase pathway in rats with high blood flow-induced pulmonary hypertension, *Biochem Biophys Res Commun,* 345 (2006) 851.

32 Yan H, Du JB, Tang CS, Geng B & Jiang HF, Changes in arterial hydrogen sulfide (H_2S) content during septic shock and endotoxin shock in rats, *J Infect,* 47 (2003) 155.

33 Savage JC & Gould DH, Determination of sulfides in brain tissue and rumen fluid by ion-interaction reversed-phase high-performance liquid chromatography, *J Chromatogr,* 526 (1990) 540.

34 Zhuo M, Small SA, Kandel ER & Hawkins RD, Nitric oxide and carbon monoxide produce activity-dependent long-term synaptic enhancement in hippocampus, *Science,* 260 (5116) (1993) 1946.

35 Dello Russo C, Tringali G, Ragazzoni E, Maggiano N, Menini E, Vairano M, Preziosi P & Navarra P, Evidence that hydrogen sulphide can modulate hypothalamo-pituitary-adrenal axis function: *In vitro* and *in vivo* studies in the rat, *J Neuroendocrinol,* 12 (2000) 225.

36 Zhu X, Smith MA, Honda K, Aliev G, Moreira PI, Nunomura A, Casadesus G, Harris PLR, Siedlak SL & Perry G, Vascular oxidative stress in Alzheimer disease, *J Neurol Sci,* 257(1-2) (2007) 240.

37 Good PF, Werner P, Hsu A, Olanow & Perl DP, Evidence of neural oxidative damage in Alzheimer's disease, *Am J Pathol*, 149 (1996) 21.

38 Eto K, Asada T, Arima K, Makifuchi T & Kimura H, Brain hydrogen sulfide is severely decreased in Alzheimer's disease, *Biochem Biophys Res Commun,* 293 (2002) 1485.

39 Whiteman M, Cheung NS, Zhu Y, Chu SH Siau JL, Wong BS, Armstrong JS & Moore PK, Hydrogen sulphide: A novel inhibitor of hypochlorous acid-mediated oxidative damage in the brain? *Biochem Biophys Res Commun,* 326(4) (2005) 794.

40 Cendes F, Andermann F, Dubeau F, Gloor P, Evans A, Jones-Gotman M, Olivier A, Andermann E, Robitaille Y, Lopes-Cendes I, Peters T & Melanson D, Early childhood prolonged febrile convulsions, atrophy and sclerosis of mesial structures, and temporal lobe epilepsy: An MRI volumetric study, *Neurology*, 43 (1993) 1083.

41 Flora: Herb and supplement encyclopedia: American ginseng root [Online], [Cited 2009 July 06], Available from: URL:http://www.florahealth.com/flora/home/Canada/HealthInformation/Encyclopedias/AmericanGinsengRoot.htm

42 Gupta YK, Sharma M & Chaudhary G, Antiepileptic activity of *Panax ginseng* against pentylenetetrazole induced kindling in rats, *Indian J Physiol Pharmacol*, 45(4) (2001) 502.

43 Dahiya AK, Reeta KH, Kumar G & Gupta YK, Evaluation of the antiepileptic potential of S-adenosylmethionine in pentylenetetrazole-induced seizures in rats (abstract), *Indian J Pharmacol,* 41(Suppl 1) (2009) S58, Abstract no. PP-138.

44 Kraus JP, Janosik M, Kozich V, Mandell R, Shih V, Sperandeo MP, Sebastio G, de Franchis R, Andria G, Kluijtmans LA, Blom H, Boers GH, Gordon RB, Kamoun P, Tsai MY, Kruger WD, Koch HG, Ohura T & Gaustadnes M, Cystathionine beta-synthase mutations in homocystinuria, *Hum Mutat*, 13(5) (1999) 362.

45 Kamoun P, Mental retardation in Down's syndrome: A hydrogen sulfide hypothesis. *Med Hypothesis*, 57 (2001) 389.

46 Qu K, Chen CP, Halliwell B, Moore PK & Wong PT, Hydrogen sulfide is a mediator of cerebral ischemic damage, *Stroke*, 37 (2006) 889.

47 Hui Y, Du J, Tang C, Bin G & Jiang H, Changes in arterial hydrogen sulfide (H_2S) content during septic shock and endotoxic shock in rats, *J Infect*, 47 (2003) 155.

48 Bhatia M, Wong FL, Fu D, Lau HY, Moochhala SM & Moore PK, Role of hydrogen sulfide in acute pancreatitis and associated lung injury, *FASEB J*, 19 (2005) 623.

49 Bhatia M, Hydrogen sulfide and substance P in inflammation, *Antioxid Redox Signal*, 12 (2010) 1191.

50 Davis SN, Insulin, oral hypoglycemic agents, and the pharmacology of the endocrine pancreas, in: *Goodman & Gilman's*: *The pharmacological basis of therapeutics*, 11th edition, edited by Brunton LL, Lazo JS and Parker KL (The McGraw-Hill Companies, United States of America) 2005, 1613.

51 Yang W, Yang G, Jia X, Wu L & Wang R, Activation of K_{ATP} channels by H_2S in rat insulin-secreting cells and the underlying mechanisms, *J Physiol*, 569 (2005) 519.

52 Hosgood SA & Nicholson ML, Hydrogen sulphide ameliorates ischaemia-reperfusion injury in an experimental model of non-heart-beating donor kidney transplantation, *Br J Surg*, 97 (2010) 202.

53 Xia Z, Dickens M, Raingeaud J, Davis RJ & Greenberg ME, Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis, *Science*, 270 (1995) 1326.

54 Hu LF, Lu M, Wu ZY, Wong PT & Bian JS, Hydrogen sulfide inhibits rotenone-induced apoptosis via preservation of mitochondrial function, *Mol Pharmacol*, 75 (2009) 27.

55 Chen SL, Yang CT, Yang ZL, Guo RX, Meng JL, Cui Y, Lan AP, Chen PX & Feng JQ, Hydrogen sulphide protects H9c2 cells against chemical hypoxia-induced injury, *Clin Exp Pharmacol Physiol*, 37 (2010) 316.

56 Cao Q, Zhang L, Yang G, Xu C & Wang R, Butyrate-stimulated H_2S production in colon cancer cells, *Antioxid Redox Signal,* 12 (2010) 1101.

57 Milner JA, Garlic: Its anti-carcinogenic and anti-tumorigenic properties, *Nutr Rev*, 54 (1996) S82.

58 Wargovich MJ, Diallyl sulfide, a flavor component of garlic (*Allium sativum*), inhibits dimethylhydrazine-induced colon cancer, *Carcinogenesis*, 8 (1987) 487.

59 Wargovich MJ, Woods C, Eng VW, Stephens LC & Gray K, Chemoprevention of *N*-nitrosomethylbenzylamine-induced esophageal cancer in rats by the naturally occurring thieother, diallyl sulfide, *Cancer Res*, 48 (1988) 6872.

60 Schaffer EM, Liu JZ, Green J, Dangler DA & Milner JA, Garlic and associated allyl sulfur components inhibit *N*-methyl-*N*-nitrosourea induced rat mammary carcinogenesis, *Cancer Lett*, 102 (1996) 199.

61 Sadhana AS, Rao AR, Kucheria K & Bijani V, Inhibitory action of garlic oil on the initiation of benzo[a]pyrene-induced skin carcinogenesis in mice, *Cancer Lett*, 40 (1988) 193.

62 Hayes MA, Rushmore TH & Goldberg MT, Inhibition of hepatocarcinogenic responses to 1,2-dimethylhydrazine by diallyl sulfide, a component of garlic oil, *Carcinogenesis*, 8 (1987) 1155.

63 Attene-Ramos MS, Wagner ED, Plewa MJ & Gaskins HR, Evidence that hydrogen sulfide is a genotoxic agent, *Mol Cancer Res*, 4(1) (2006) 9.

64 Srilatha B, Adaikan PG & Moore PK, Possible role for the novel gasotransmitter hydrogen sulphide in erectile dysfunction — a pilot study, *Eur J Pharmacol*, 535 (1-3) (2006) 280.

65 Linden DR, Levitt MD, Farrugia G & Szurszewski JH, Endogenous production of H_2S in the gastrointestinal tract: Still in search of a physiological function, *Antioxid Redox Signal*, 12 (2010) 1135.

66 Mustafa AK, Gadalla MM & Snyder SH, Signalling by gasotransmitters, *Sci Signal*, 2 (2009) re2.

67 Wallace JL, Hydrogen sulfide-releasing anti-inflammatory drugs, *Trends Pharmacol Sci*, 28(10) (2007) 501.

68 Fiorucci S, Orlandi S, Mencarelli A, Caliendo G, Santagada V, Distrutti E, Santucci L, Cirino G & Wallace JL, Enhanced activity of a hydrogen sulphide-releasing derivative of mesalamine (ATB-429) in a mouse model of colitis, *Br J Pharmacol*, 150 (2007) 996.

69 Distrutti E, Sediari L, Mencarelli A, Renga B, Orlandi S, Russo G, Caliendo G, Santagada V, Cirino G, Wallace JL & Fiorucci S, 5-Amino-2-hydroxybenzoic acid 4-(5-thioxo-5H-[1,2]dithiol-3yl)-phenyl ester (ATB-429), a hydrogen sulfide-releasing derivative of mesalamine, exerts antinociceptive effects in a model of postinflammatory hypersensitivity, *J Pharmacol Exp Ther,* 319 (2006) 447.

70 Distrutti E, Sediari L, Mencarelli A, Renga B, Orlandi S, Antonelli E, Roviezzo F, Morelli A, Cirino G, Wallace JL & Fiorucci S, Evidence that hydrogen sulfide exerts antinociceptive effects in the gastrointestinal tract by activating K_{ATP} channels, *J Pharmacol Exp Ther,* 316 (2006) 325.

71 Cunha TM, Verri WA Jr., Schivo IR, Napimoga MH, Parada CA, Poole S, Teixeira MM, Ferreira SH & Cunha FQ, Crucial role of neutrophils in the development of mechanical inflammatory hypernociception, *J Leukoc Biol* 83 (2008) 824.

72 Cunha TM, Dal-Secco D, Verri WA Jr., Guerroro AT, Souza GR, Vieira SM, Lotufo CM, Neto AF, Ferreira SH & Cunha FQ, Dual role of hydrogen sulfide in mechanical inflammatory hypernociception, *Eur J Pharmacol*, 590 (1-3) (2008) 127.