From primordial gas to the medicine cabinet

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Hydrogen sulfide (H$_2$S) is a signalling gasotransmitter which plays important roles in regulating the functions of cardiovascular and respiratory systems, metabolism and liver function, kidney and reproduction function, neuronal activity and cognitive function, antioxidants and anti-inflammatory responses and mitochondrial bioenergetics, among others (Wallace & Wang, 2015; Wang, 2002). The biological and physiological effects of H$_2$S are produced through an array of interactions with its numerous molecular targets. Well-known examples of these targets include the activation of K$_{ATP}$ channels to hyperpolarize membrane potential (Zhao, Zhang, Lu, & Wang, 2001), activation of eNOS to produce more NO (Altaay, Jü, Yang, & Wang, 2014), inhibition of PDEs to increase cGMP levels (Bucci et al., 2012) and induction of S-sulphydrylation to alter the functions of the targeted proteins. This special issue of BJPh has examined some of the existing and newly discovered mechanisms that underlie the cellular effects of H$_2$S.

Although protein S-sulphydrylation has been established for 10 years (Mustafa et al., 2009), Kimura has provided an updated review on this mechanism with a focus on the comparison between hydrogen sulfide and polysulfides (2020). This comparison is important and relevant since endogenous H$_2$S is produced via the enzymatic actions of cystathionine $\beta$-synthase (CSE), cystathionine $\gamma$-lyase (CBS) and 3-mercaptoypyruvate sulfurtransferase (MST) and H$_2$S can also be further oxidized to polysulfides by MST. Both H$_2$S and polysulfides have the potential to induce S-sulphydrylation but the enabling conditions for them are not identical.

Mitidieri et al. (2020) reported the effects of L-serine on vascular tone, especially its correlation with the functionality of the reverse trans-sulphuration pathway in vascular endothelium. This is a novel mechanism considering that conversion of L-cysteine to L-serine and H$_2$S occurs in the presence of CBS. The condensation of L-serine with palmitoyl-CoA leads to de novo sphingolipid biosynthesis. Sphingosine-1-phosphate (S1P) is a major sphingolipid and it can activate eNOS to increase NO production. Mitidieri et al. linked the reverse trans-sulphuration pathway to S1P/NO axis through L-serine. They report that the addition of exogenous L-serine to the organ bath caused relaxation of mouse aorta rings in an endothelium-dependent manner, similar to the effects of L-cysteine. This vasorelaxant effect of L-serine was suppressed after application of an S1P receptor antagonist. Whether stimulation of CBS increases endogenous L-serine levels and whether an increase in endogenous L-serine levels results in vasorelaxation remain unknown.

Demonstrating the role of H$_2$S as a gasotransmitter requires proof that the physiological effects of endogenous H$_2$S can be mimicked by exogenous H$_2$S donors (Wang, 2002). H$_2$S donors may be ideal agents for the treatment of human diseases related to deficiency of endogenous H$_2$S, but only if they can be delivered at appropriate concentrations/rates to the desired sites of action. The most widely used H$_2$S donors for animal and in vitro studies are NaHS and Na$_2$S. These simple sulfur salts provide fast but short-lived H$_2$S release, generally with rapid uptake by the targeted cells. Of course, for many studies, a more controlled and generally slower release of H$_2$S is desired. This was first achieved with the compound of GYY4137 (Li et al., 2008). Over the last decade, numerous other slow-releasing H$_2$S donors have been developed and characterized, including sulfur-hybrid molecules (Szabo & Papapetrou, 2017; Wallace & Wang, 2015) and H$_2$S produgs (Polhemus et al., 2015). Beyond the rate of release, the targeted delivery of H$_2$S has also become a hot area for the design of novel H$_2$S donors. The mitochondrion-targeted H$_2$S donor, AP39, is a case-in-point (Szczesny et al., 2014).

The distribution and access of various H$_2$S donors to mammalian cells, which are encompassed by a lipid bilayer, constitutes an important consideration when evaluating the rapidity and efficacy of H$_2$S release. In this issue, Durham et al. (2020) described the cellular uptake mechanism for ammonium tetrahydrourate (ATTT) and ammonium tetrahydromolybdate (ATTM), two representatives of the thiomellitate slow-releasing sulfide donors. Sulfide release by ATTT and ATTM is a thiol-dependent process. Interestingly, pretreatment of intact human red blood cells with the inhibitor for anion exchange-1 (AE-1) decreased ATTT-induced formation of intracellular sulfhaemoglobin, an indicator of the elevation of intracellular H$_2$S level. The role of AE-1 in mediating cellular uptake of thiomellitates was also confirmed by direct measurement of the intracellular and extracellular concentrations of thiomellitates in the presence and absence of AE-1 inhibitor. On the other hand, the cellular uptake of NaHS,
again reflected by the formation of intracellular sulfhaemoglobin, was not affected by AE-1 inhibition. These observations support the notion that the trans-membrane movement of H$_2$S is not limited by the plasma membrane and that thiomylates require the presence of AE-1 to gain access to the intracellular milieu. AE-1 is a plasma membrane anion transporter, present in various types of mammalian cells, including cardiomyocytes, endothelial cells and intercelated cells of the kidney. The requirement of AE-1 for the cellular uptake of selective H$_2$S donors may offer an option for target-selective delivery of exogenous H$_2$S.

Manipulation of H$_2$S levels to achieve positive therapeutic effects has been used in various disease models, such as lipid metabolism-associated liver disease. Loisel, Yang, and Wu (2020) reviewed the critical importance of H$_2$S in hepatic lipid metabolism, which has significant implications for liver disease establishment and progression. The authors suggest that increasing endogenous H$_2$S production is a valuable strategy for prevention and treatment of liver disease. The insulin-sensitizing drug, metformin, and lipid-lowering statins both increased endogenous H$_2$S levels in rodents, contributing to the amelioration of fatty liver, steatosis and non-alcoholic fatty liver disease (NASHLD). Other examples for impacting liver lipid metabolism and H$_2$S production include garlic oil and sulfonaphane (contained in broccoli sprouts), which decreased lipid accumulation and liver damage in animal models of both NASHLD and alcohol-induced fatty liver. These treatment strategies remain to be tested in humans. As the authors pointed out, “understanding of the molecular mechanisms through which the H$_2$S metabolic pathway regulates hepatic lipid levels, as well as the functioning of other organs, will be key for the development of effective therapeutic options” (Loisel et al., 2020).

Advances in fundamental research on the biomedical effects of H$_2$S over the past two decades and the development of a range of novel H$_2$S donors, have not been matched with consequential clinical applications. Clinical trials with the aim of H$_2$S supplementation for different human diseases have been limited (Wallace & Wang, 2015). However, a phase 2B, double-blind clinical trial of the gastrointestinal safety of a H$_2$S-releasing drug reported in this issue of BJH (Wallace et al., 2020) is promising and encouraging. In this 2-week, double-blind clinical trial, 244 healthy volunteers received ATB-346 or naproxen and had their upper gastrointestinal (GI) ulceration examined endoscopically. Naproxen is one of the most widely used non-steroidal anti-inflammatory drugs (NSAIDs) for reducing pain, fever and inflammation. However, the adverse effects of naproxen and other NSAIDs, particularly GI ulceration and bleeding, have been the reasons for serious concerns about the safety of this class of drugs. The key mechanism underlying the ability of NSAIDs to cause gastro-duodenal ulceration is inhibition of cyclo-oxygenase (COX) enzymes (Vane, 1971). ATB-346 is an anti-inflammatory and analgesic H$_2$S-releasing derivative of naproxen. Administration of ATB-346 to healthy subjects resulted in a significant elevation of plasma H$_2$S. ATB-346 and naproxen were equally effective in inhibiting COX activity (>94%). However, the incidence of at least one ulcer in the upper GI tract was 14-times greater in the subjects receiving naproxen than in the subjects receiving ATB-346 (P < .001).

This Themed Issue features two reports on H$_2$S and biomineralization. Castelblanco and colleagues analysed the existing literature on the distinct roles of H$_2$S in physiological (bone and teeth) versus pathological calcification. In the context of physiological calcification, H$_2$S promotes the differentiation of mesenchymal stem cells into osteoblasts, odontoblasts and chondrocytes at the growth plate and enhances the expression of calcifying genes, while inhibiting mesenchymal-induced osteoclast precursor differentiation. In contrast, during pathological calcification that occurs in the blood vessels, the cartilage, or the kidney, H$_2$S exerts a protective role by decreasing oxidative stress, inhibiting inflammatory cytokine production, and inhibiting trans-differentiation of non-calcifying cells to a calcifying phenotype.

Calcification of cardiovascular tissues (typically, large and medium size arteries and valves) is a chronic disease, associated with increased risk of cardiovascular morbidity and mortality (Yuzet et al., 2014). It occurs more frequently in patients with diabetes and chronic kidney disease and in the elderly. As detailed in the review by Castelblanco, Nasl, Pasch, So, and Busso (2020), H$_2$S donors reduce vascular smooth muscle calcification. Sikura et al. (2020) extended these observations to heart valves and unravelled the molecular mechanisms that H$_2$S employs to protect valve calcification. The authors isolated valvular interstitial cells (VIC) from human aortic valves and tested the ability of a series of H$_2$S donors to modify VIC mineralization/osteoblastic trans-differentiation. They found that several H$_2$S donors (NaHS, Na$_2$S, GYY4137, AP67 and AP72) all inhibited calcium content of VIC grown in calcification medium. They proposed that the anti-calcifying effect of exogenous H$_2$S resulted from reduced phosphate uptake that limited runt-related transcription factor 2 (RUNX2) nuclear translocation and activation. This in turn limited alkaline phosphatase expression, reduced osteocalcin secretion and limited calcium deposition in the extracellular matrix. The inhibitory effect of H$_2$S on Pi uptake was postulated, but not proven, to be due to sulfhydration of the phosphate transporter Pit. H$_2$S also increased the expression of Ankyrin G1 (ANK1) and ectonucleotide pyrophosphatase/FDE member 2 (ENPP2), leading to generation of pyrophosphate that inhibits hydroxyapatite formation. This latter pathway is targeted by endogenously generated H$_2$S through CSE and CBS. Silencing CSE and CBS simultaneously reduced ANK1 and ENPP2 expression exacerbating calcification. Interestingly, aortic valves with calcification expressed more CSE but produced less H$_2$S. Although not directly tested in the study, it is possible that CSE in calcified tissue is hyperphosphorylated on S377, leading to reduced activity. A similar finding has been recently reported for CSE in atheromas (Bibi et al., 2019). Based on their findings, the authors proposed that H$_2$S donors might be useful in the treatment of valve calcification. Interestingly, sodium thiosulfate, a degradation product of H$_2$S that has the ability to be converted to H$_2$S, has been successfully used in patients with calciphylaxis (vascular calcification; Nigwekar et al., 2015).

Unlike the plethora of information available for the roles of H$_2$S in smooth muscle function (Wang, 2012), only limited information about its production and biological activities in skeletal muscle is
available. Malignant hyperthermia (MH) is a rare condition in which affected individuals exhibit uncontrolled release of calcium in striated muscle after exposure to general anaesthetics, depolarizing muscle agents (succinylcholine) or during extreme physical activity in hot environments (Hopkins, 2011). The authors had previously shown enhanced CBS expression and H₂S levels in skeletal muscle of patients susceptible to MH that contributed to hypercontractility (Velleco et al., 2016). They have now unravelled the molecular alterations through which H₂S leads to the hypercontractility characteristic of MH (Velleco et al., 2020). They demonstrated that the Kv 7.4 channel in human biopsies from MH-susceptible individuals is persulfidated and that the Kv7 activator retigabine exhibits an anomalous pharmacological profile, triggering depolarization rather than hyperpolarization. Moreover, in biopsies from non-susceptible individuals, prior exposure to H₂S led to a contractile response after stimulation with retigabine. These findings not only provide clues for the molecular mechanism underlying MH but also suggest that H₂S levels and Kv7.4 persulfidation could help identify individuals likely to manifest MH using a less invasive needle biopsy, compared to the currently used in vitro contracture test.

Based on the fact that H₂S exerts a variety of protective effects in the cardiovascular system and that H₂S levels are depleted in cardiovascular disease (Wang et al., 2015), H₂S donors have been proposed as putative therapeutic modalities (Szabo & Papapetropoulos, 2017). Although most of the commonly used H₂S donors are synthetic, a few naturally occurring H₂S-releasing molecules have been described. Among them, the garlic constituents diallyl disulfide and diallyl trisulfide are the best characterized (Szabo & Papapetropoulos, 2017). In this issue, Martell and collaborators have studied the vasodilatory and anti-hypertensive properties of erucin, an H₂S-releasing molecule found in edible cruciferous plants. They report that incubation of vascular smooth muscle cells with erucin raises intracellular H₂S levels and hyperpolarizes their membrane. Moreover, erucin triggered vasorelaxation in an endothelium-independent manner. In line with the known synergistic actions of H₂S and NO (Bucci et al., 2012; Coletta et al., 2012), the effects of erucin were potentiated by NO release from the endothelium. The authors also demonstrated that erucin increased coronary blood flow of preconstricted coronary arteries and reduced systolic blood pressure in hypertensive rats. Based on the above-mentioned results, erucin can now be added to the list of H₂S donors with favourable vascular actions.

Previous studies have demonstrated the ability of H₂S to ameliorate the vascular and cardiac complications of diabetes (Wang et al., 2015). In this issue, Sun et al. (2020) unravelled the role of MuRF1, an E3 ubiquitin ligase, in the protective effects of H₂S in diabetic cardiomyopathy. After demonstrating that H₂S levels and CSE expression were reduced in myocardial tissue from db/db mice, the authors used NaHS to “replenish” H₂S. Interestingly, treatment with an exogenous source of H₂S up-regulated endogenous production of H₂S. This response has been seen in several different experimental settings (Li et al., 2016; Wu et al., 2017) and is contrary to the general principle of agonist-induced down-regulation of receptors and effectors. NaHS administration restored cardiac function and rescued sarcomere degradation in the hearts of db/db mice. The improvement seen following NaHS treatment was associated with reduced muscle RING finger-1 (MuRF1) expression and enhanced sulphydrylation of MuRF1 on Cys44 that inhibited its function, leading to reduced ubiquitination and prevention of degradation of myosin heavy chain 6 and myosin light chain 2.

H₂S also plays a range of important roles on the external surfaces of organisms, including the skin and in the GI tract. Indeed, some of the earliest applications of H₂S as a therapeutic include the still very popular use of thermal sulfur baths. Coayoy-Sanchez, Costa, and Muscara (2020) have comprehensively reviewed the current and potential future use of H₂S donors for treating conditions such as psoriasis, atopic dermatitis, wounds, urticaria, rosacea, acne, diabetic ulcers and melanoma. Based on laboratory studies, there appears to be great promise of utilizing H₂S donors for many of these conditions. As mentioned above, release of H₂S within the GI tract has been shown to accelerate the healing of ulcers (Wallace, Dicay, McKnight, & Martin, 2007), as well as preventing the formation of ulcers in response to use of NSAIDs such as naproxen (Wallace et al., 2020). One of the most remarkable elements of the biology of H₂S is the capacity for it to drive generation of ATP, particularly in the GI epithelial cells (Gobeur, Andriamihaja, Nübel, Blachier, & Boulliau, 2007). It has long been known that NSAIDs can uncouple oxidative phosphorylation in the GI tract and that this contributes to ulcer formation. Administration of H₂S donors has been shown to prevent these effects (Gobeur et al., 2007; Wallace et al., 2007). Moreover, H₂S released from microbiota within the intestine contribute significantly to maintenance of the integrity of the intestinal lining, including promoting healing and resolution of inflammation (Motta et al., 2015).

Continuing on the theme of healing, Modis et al. (this issue) examined newly identified regulatory roles for the enzyme 3-MST, particularly in angiogenesis and in metabolic switching by endothelial cells. The suggested “a wider role of 3-MST activity” in the metabolome of endothelial cells, beyond sulfur metabolism. They further suggested that pharmacological inhibitors of 3-MST may be exploited as anti-angiogenic therapeutics.

For many years, there has been strong interest in the possible use of H₂S donors to prevent various forms of cancer. De Cicco et al. (2020) performed a series of elegant experiments focused on modulation of myeloid suppressor cells (MDSC) in vivo. The growth of tumours in melanoma-bearing mice could be significantly reduced by treating the mice with H₂S donors (e.g. diallyl trisulfide). These effects appeared to be related to a reduction of the immune suppressive effects of MDSCs, leading to a restoration of T cell proliferation.

This issue of BJP highlights the substantial breadth of research related to H₂S. Like NO and carbon monoxide, H₂S plays very important roles in a wide range of physiological and pathophysiological processes. Moreover, significant progress has been made in recent years towards targeting H₂S in drug design, with translation to human applications on the horizon.

**CONFLICT OF INTEREST**

The authors declare no conflicts of interest.
REFERENCES


malignant hyperthermia syndrome unmasks a key role for \( \text{H}_2\text{S} \) and persulfidation in skeletal muscle. *British Journal of Pharmacology*, 177 (4), 810–823.


