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COMMENTARY

Potential importance of alterations in hydrogen sulphide (H_2S) bioavailability in diabetes

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Despite its long-standing reputation as a foul smelling and toxic gas that is associated with the decay of biological matter, hydrogen sulphide (H_2S) has emerged as an important regulator of cardiovascular homoeostasis. H_2S promotes a number of cellular signals that regulate metabolism, cardiac function and cell survival. Endogenous H_2S bioavailability is regulated by several enzymes involved in the biosynthesis of cysteine. This study by Brancaleone *et al.* in the current issue of the *British Journal of Pharmacology* provides novel insights into the impairment of H_2S biosynthesis in the setting of diabetes mellitus. The authors report that enzymic H_2S biosynthesis is impaired in a murine model of type 1 diabetes and the attenuation in H_2S bioavailability is associated with impaired vascular reactivity. This study has profound implications for the use of pharmacological agents to augment endogenous H_2S synthesis or agents that release H_2S to augment the levels of this gaseous signalling molecule in cardiovascular disease.
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Abbreviations: CBS, cystathionine β-synthase; CGL, cystathionine γ-lyase

Recent basic science studies have highlighted the importance of hydrogen sulphide (H_2S) as a critical regulator of cardiovascular homoeostasis in the normal physiological state (Szabo, 2007). On account of its chemical nature and highly diverse signalling profile, H_2S exerts a number of powerful effects on the heart, blood vessels and circulating blood elements. In this regard, H_2S has been shown to modulate metabolic state (Blackstone *et al.*, 2005), vascular reactivity and systemic blood pressure (Szabo, 2007), leukocyte–endothelial cell interactions (Zanardo, 2006), mitochondrial function, cellular redox status and apoptosis (Szabo, 2007). On account of the profound effects of H_2S on cellular metabolism, early studies were focused on the ability of H_2S to induce a state of suspended animation to promote survival (Blackstone *et al.*, 2005; Szabo, 2007). More recently, investigators have begun to investigate the numerous effects of relatively low levels of H_2S therapy on cardiovascular physiology and pharmacology in a number of *in vitro* and *in vivo* model systems. New studies are emerging at a rapid rate that underscore the importance of this novel, endogenous, gaseous signalling molecule synthesized by the actions of the two endogenous enzymes, cystathionine γ-lyase (CGL or CSE) and cystathionine β-synthase (CBS) (Szabo, 2007). These enzymes are two pyridoxal-5′-phosphate-dependent enzymes that are responsible for metabolizing homocysteine to cysteine in mammals (Szabo, 2007) (Figure 1).

Previous studies have demonstrated that both CBS and CGL are critical for the maintenance of cardiovascular function and that treatment of animals with exogenous forms of hydrogen sulphide have demonstrated very robust protection of various organs during a number of cardiovascular diseases including: ischaemia-reperfusion injury, various forms of shock, stroke, inflammatory disorders and models of ischaemia-induced angiogenesis (Mok, 2004; Kimura *et al.*, 2006; Elrod *et al.*, 2007; Szabo, 2007, Jha *et al.*, 2008). It is important to note that the protective actions of H_2S therapy have been observed primarily at lower dosages or concentrations of H_2S and that higher levels of H_2S are clearly associated with significant toxicity that has been well characterized. Furthermore, the majority of studies evaluating the effects of pharmacological inhibition of CBS or CGL have demonstrated enhanced pathology of various diseases. Studies of gene-targeted mice support the concept that inhibition of H_2S biosynthetic enzymes exacerbates cardiovascular disease whereas genetic overexpression attenuates disease severity (Elrod *et al.*, 2007; Szabo, 2007).

The relatively large body of recent publications demonstrating cytoprotective actions of H_2S therapy in a number of disease conditions provide support for the development of

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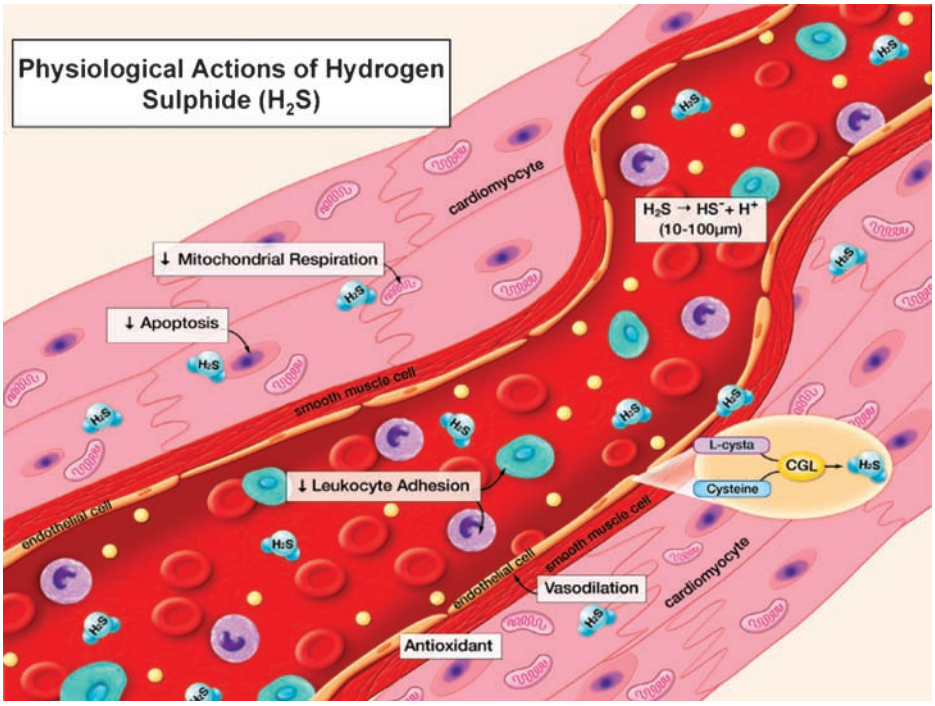

Figure 1 Physiological actions of hydrogen sulphide (H_2S). H_2S is synthesized in the vasculature through the action of cystathionine γ-lyase (CGL), converting cysteine to H_2S under normal physiological conditions. Normal circulating blood concentrations of H_2S are believed to be in a range between 10–100 μM. H_2S exerts a number of actions on the heart and circulation. (Modified from Lefer, 2007, with permission).

novel H_2S-based therapeutic agents. There appear to be several options for H_2S therapy in cardiovascular disease including H_2S gas, H_2S donors or releasing compounds, and H_2S pro-drugs that activate H_2S-generating enzymes to increase circulating and tissue levels of H_2S. During acute cardiovascular diseases (that is, shock states or acute myocardial infarction) rapidly acting compounds with shorter half-lives may be more appropriate. Under these conditions, H_2S gas or H_2S donors would be ideal candidates to evaluate. For more chronic cardiovascular disease states and chronic inflammatory states, the H_2S pro-drugs such as S-allylcysteine, a compound derived from garlic, would be more suitable. It will also be important to design H_2S therapeutics that can be administered by a number of routes including oral, intravenous and inhalation routes. One of the major challenges in the development of H_2S therapy is the extremely short half-life of H_2S in the circulation as it is very rapidly metabolized to inactive metabolites. Furthermore, there are also a number of challenges related to the accurate measurement of both circulating and tissue H_2S levels. Finally, because of the extensive history of H_2S as a highly toxic environmental hazard, extensive investigations into the potential toxicity of H_2S are required (Szabo, 2007). The majority of information regarding H_2S toxicity is derived from studies of sulphide gas inhalation. Future studies will be aimed at the investigation of H_2S therapy administered through the oral route or following injection of H_2S donors or pro-drugs.

To date, the vast majority of studies related to the molecular, biochemical or biological actions of H_2S have been performed in healthy animals or tissues derived from normal animals. This is somewhat problematic in that persons suffering from cardiovascular disease typically present with a number of pre-existing risk factors, including hypertension, dyslipidaemia, and diabetes mellitus. It is well appreciated that these prevalent cardiovascular risk factors attenuate endothelial cell generation of another gaseous signalling molecule, nitric oxide, and result in a state referred to as 'endothelial dysfunction' (Cai and Harrison, 2000). At present, very little is known regarding H_2S bioavailability and vascular reactivity to H_2S in the setting of established cardiovascular risk factors. The report by Brancaleone *et al*. (2008) in this issue of the *British Journal of Pharmacology* investigates the state of H_2S biosynthesis in non-obese diabetic mice and takes an important first step towards defining the effects of diabetes on H_2S synthesis. This elegant study by Brancaleone *et al*. clearly demonstrates that vascular reactivity, plasma H_2S levels, and vascular H_2S production all decline progressively as the severity of diabetes increases over time in the non-obese diabetic mouse model. These data support earlier observations that plasma levels are significantly reduced in patients with coronary heart disease and in hypertensive rats. The authors investigated three distinct stages of diabetes mellitus (mild to severe), as determined by both blood glucose and urine glucose levels, and found that vasorelaxation of isolated aortic segments was attenuated by up to 75–80% in the most severe diabetic state. Interestingly, the authors found that the diabetic vessels displayed normal or enhanced reactivity to exogenous H_2S donors suggesting that H_2S donor therapy may be efficacious in diabetic animals and patients. Clearly, additional studies investigating endogenous H_2S biosynthesis and bioavailability in a number

of cardiovascular disease states including hypertension, obesity and metabolic syndrome, diabetes and hyperlipidaemia are warranted. Furthermore, it is also important to examine the vascular reactivity of various vascular beds both *in vitro* and *in vivo* in the setting of cardiovascular disease in the future. The study by Brancaleone *et al*. (2008) provides an excellent starting point for future investigations into H_2S biochemistry and physiology in the diabetic state as well as in other important diseases. It appears that investigation of hydrogen sulphide will provide exciting research opportunities for many years to come.

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