Editors' view

Human pharmacology of hydrogen sulfide, putative gaseous mediator

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Gaseous mediators

Nitric oxide (NO), the first low molecular weight inorganic gas to be established as a biological mediator, had previously been regarded merely as a toxic pollutant. It was joined, similarly implausibly to the minds of a generation brought up with the hazards of coal gas as domestic heating, by carbon monoxide (CO) – potentially lethal as an exhaust gas, but also formed in mammalian tissues together with biliverdin by inducible and/or constitutive forms of haem oxygenase, and implicated subsequently as a signalling molecule, not only in the central nervous system (especially olfactory pathways) and cardiovascular system but also in respiratory, gastrointestinal, endocrine and reproductive functions [1].

Hydrogen sulfide (H₂S) was known to generations of schoolboys in one biological context – as the source of the odour of rotten eggs – but even so, the proposal in the early 2000s [2] that it too is a gaseous mediator was met with some scepticism. The toxicology of H₂S had previously been studied [3], and actions on enzymes including monoamine oxidase and carbonic anhydrase identified, but more recent work has also shown a rich and diverse pharmacology consistent with functions as a signalling molecule under physiological conditions. It has also been implicated both as a harmful and also as a protective factor in the pathophysiology of a range of experimental models of disease [4], and its therapeutic potential has attracted comment [5].

There are striking similarities between these three gaseous mediators, as well as contrasts, that underlie complex and imperfectly understood functional interactions between them. All three are highly diffusible labile molecules that are rapidly eliminated from the body: NO as nitrite and nitrate in urine as well as NO in exhaled air; CO in exhaled air; H₂S as thiosulfate, sulfite and sulfate in urine (Figure 1) as well as (it emerges, see below) in exhaled breath. All three react with haemoglobin, yielding methae-

moglobin or nitrosylhaemoglobin (respectively inactive and active metabolites) from distinct reactions of haem and of globin with NO; carboxyhaemoglobin or sulfhaemoglobin from CO or H₂S. All three affect cellular energetics via actions on cytochrome c oxidase. All three have vasodilator effects, and all have anti-inflammatory and cytoprotective effects at low concentrations in contrast to causing cellular injury at higher concentrations – consistent with Paracelsus's aphorism that the distinction between drugs and poisons is determined exclusively by the dose.

H₂S: biosynthesis, tissue concentrations and actions

Endogenous H₂S is produced from L-cysteine by the actions of two pyridoxal 5' phosphate-dependent enzymes cystathionine γ -lyase (EC4.4.1.1 also known as cystathionase or CSE) and cystathionine β -synthase (CBS). Large amounts of CBS occur in mammalian brain (especially hippocampus and cerebellar Purkinje cells), whereas CSE activity is greatest in liver, kidney and media of blood vessels. These enzymes are under regulatory control (e.g. by lipopolysaccharide and by TNF α) and their expression is altered in experimental diseases (including pancreatitis and diabetes mellitus). Pharmacological inhibitors of their synthesis (e.g. DL-propargylglycine, a CSE inhibitor, and amino-oxyacetic acid, a CBS inhibitor) are so far only of modest potency and specificity and so have been much less useful in delineating any physiological role of H₂S than have L-N^G-monomethyl arginine (LNMMA) and related compounds in defining the physiology and pathophysiology of the L-arginine/NO pathway. Several assays of H₂S in biological fluids actually measure total sulfide (only some of which is biologically available) rather than H₂S *per se*, and in any event H₂S is so evanescent and reactive that it has been suggested that attempts to measure it in plasma

Figure 1

Synthesis, sites of action and disposition of H₂S. Endogenous biosynthesis from sulfur-containing amino acids (methionine, cysteine) via actions of the regulated enzymes methionine cystathionine γ -lyase (CSE) and cystathionine β -synthase (CBS) is shown; pharmacological H₂S donors (red-rimmed box) may be administered exogenously. Most H₂S is probably renally excreted as sulfate (yellow-rimmed box). Some is eliminated in exhaled air (green-rimmed box). Some molecular targets of H₂S are indicated in the orange-rimmed box

or tissue fluids may be 'nonsensical', with figures from rat plasma and brain homogenate of 50–100 $\mu\text{mol.L}^{-1}$ implying absurdly that the body is 'awash with H₂S'¹ [4]. This critical point is well made and confounds the interpretation of reports of altered plasma concentrations of H₂S in disease models, but may not apply to the measurement of H₂S in the gas phase in exhaled air (see below). Thiosulfate excretion (Fig. 1) may represent a better analytical target than plasma sulfide when the issue is total body turnover of H₂S; sulfite and sulfate (to which thiosulfate is converted) are not satisfactory, as their production from other sources of sulfur swamps the H₂S signal.

H₂S: pharmacological effects and therapeutic potential

H₂S has potent pharmacological effects in the cardiovascular system, including vasorelaxation secondary to activation of vascular smooth muscle K_{ATP} channels, in models of inflammation and in the central nervous system [4,5]. Endocrine effects include inhibition of glucose-stimulated insulin secretion whereas DL-propargylglycine, a CSE inhibitor, increases insulin release from rat insulinoma cells [4], so actions on K_{ATP} channels may be important here also. One of the most striking effects of H₂S is to induce a state

¹ Reminiscent of the early days of prostacyclin when assays of poor specificity, together with the capacity of vascular tissue to synthesise large amounts of this prostanoid when injured during blood sampling, resulted in the erroneous hypothesis that prostacyclin functions as a circulating hormone rather than as a local mediator.

of suspended animation, described first in nematode worms but then also in rodents, together with hypothermia [6]. Subsequently, a whole range of cytotoxic (high concentration) and cytoprotective (low concentration) effects of H₂S and H₂S donors have been described in a wide variety of cell types including primary hippocampal astrocytes, human aortic smooth muscle, intestinal crypt cells, kidney cells, macrophages, colon cancer cells, cortical neurons, myoblasts, human blood neutrophils eosinophils and lymphocytes, pulmonary fibroblasts, and hepatocytes from various species [summarised in 5]. These findings provided a rationale for studies of effects of H₂S donors in experimental disease models [also summarised in 5] as diverse as pulmonary vasoconstriction, NSAID-induced gastropathy, ischaemia-reperfusion in rat heart, the mouse air-pouch model, carrageenan-induced hindpaw oedema, bleomycin-induced pulmonary fibrosis, and cerebral artery occlusive stroke. The results have been sufficiently encouraging to provide a rationale for studying H₂S donors in man. Several sulfide-releasing derivatives (e.g. based on diclofenac and on mesalazine) are in pre-clinical development, but in the current issue of the Journal we publish an account of the pharmacokinetics of an intravenously administered formulation of the simple inorganic salt sodium sulfide versus placebo control in healthy human volunteers [7].

Intravenous infusion of sodium sulfide in healthy humans

There were no significant changes in blood pressure or other vital signs up to the highest dose administered (0.2 mg kg⁻¹ administered over 1 minute), but blood sulfide and thiosulfate concentrations increased from baseline. Basal exhaled H₂S was higher than in ambient air, and this rapidly increased during infusion of sodium sulfide and rapidly returned to baseline when the infusion finished. There was a dose-related increase in the proportion of subjects reporting an odour of rotten eggs. The authors conclude that exhaled H₂S represents a detectable route of elimination after parenteral administration of sodium sulfide. Since the study was performed in awake subjects with intact cardiovascular reflexes, the lack of significant effects on heart rate and blood pressure do not exclude effects on vascular tone, and future studies could address this in various ways (e.g. by measuring total systemic vascular resistance during intravenous infusion, or by measuring forearm blood flow during brachial artery administration). Measurement of exhaled NO has yielded important insights and provides a means of assessing airways inflammation, for example in asthmatic patients. The paper by Christopher Toombs and his colleagues [7] will, we hope, open up a similarly rich window on the *in vivo* physiology and pathophysiology of H₂S in healthy and diseased humans.

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