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Hydrogen Sulfide, a Toxic Gas with Cardiovascular Properties in Uremia: How Harmful Is It?

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Abstract
Hydrogen sulfide (H_2S) is a poisonous gas which can be lethal. However, it is also produced endogenously, thus belonging to the family of gasotransmitters along with nitric oxide and carbon monoxide. H_2S is in fact involved in mediating several signaling and cytoprotective functions, for example in the nervous, cardiovascular, and gastrointestinal systems, such as neuronal transmission, blood pressure regulation and insulin release, among others. When increased, it can mediate inflammation and apoptosis, with a role in shock. When decreased, it can be involved in atherosclerosis, hypertension, myocardial infarction, diabetes, sexual dysfunction, and gastric ulcer; it notably interacts with the other gaseous mediators. Cystathionine γ-lyase, cystathionine β-synthase, and 3-mercaptopyruvate sulfurtransferase are the principal enzymes involved in H_2S production. We have recently studied H_2S metabolism in the plasma of chronic hemodialysis patients and reported that its levels are significantly decreased. The plausible mechanism lies in the transcription inhibition of the cystathionine γ-lyase gene. The finding could be of importance considering that hypertension and high cardiovascular mortality are characteristic in these patients.

Introduction

Hydrogen sulfide (H_2S) is a poisonous and occasionally lethal gas. It is formed in the decomposition of any organic material and represents an industrial occupational safety hazard. Known for its typical stench of rotten eggs, it is now being largely reconsidered relatively to this 'bad guy' role. In fact, it is recognized that the body not only can tolerate this gas in very small amounts, but produces H_2S in a variety of tightly regulated pathways [1–5].

H_2S Formation

It is now believed that H_2S formation (fig. 1) is catalyzed by three enzymes (but nothing hinders that more may be revealed as research grows on the subject; for example, some pharmacological inhibitors of its production

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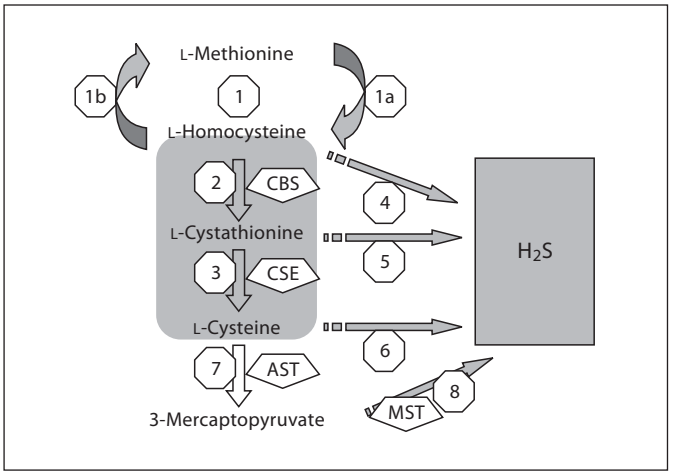

Fig. 1. Metabolism of sulfur amino acids relevant to H_2S production. L-methionine, an essential amino acid, can be converted to L-homocysteine through the methionine-homocysteine cycle (step 1), schematically represented by curved arrows. Step 1a: conversion of methionine into *S*-adenosyl-L-methionine and its demethylation through the action of various methyltransferases, leading to the formation of *S*-adenosyl-L-homocysteine, the direct homocysteine precursor. Step 1b: conversion of homocysteine back to methionine. Reaction 1b is catalyzed by methionine synthase (requiring methyl cobalamine as a coenzyme and N5-methyltetrahydrofolate as a cosubstrate). L-homocysteine is also quantitatively converted into L-cysteine through a two-reaction pathway (transsulfuration; grey area), including step 2 (catalyzed by the rate-limiting CBS) and step 3 (catalyzed by CSE). Vitamin B_6 is required for CBS and CSE activity through its cofactor pyridoxal phosphate. Both CBS and CSE can lead to the formation of H_2S as a side product, CSE being a major H_2S producer. Both L-homocysteine (step 4) and L-cysteine (step 6) can act as H_2S precursors, as well as cystathionine (step 5) [for details see reference 32]. A minor pathway for H_2S production includes the sequential action of aspartate aminotransferase (AST; step 7) and MST (step 8) [see also reference 33].

do not appear to exert hemodynamic effects in normal animals if given acutely): cystathionine γ-lyase (CSE; cystathionase, EC 4.4.1.1), cystathionine β-synthase (CBS; EC 4.2.1.22), and 3-mercaptopyruvate sulfurtransferase (MST; EC 2.8.1.2). In the vascular system, H_2S is synthesized by CSE in vascular smooth muscle cells and endothelial cells, while in the brain its production is attributed to CBS. In the kidney, all three enzymes are active [6–9], with studies suggesting that H_2S is involved in the control of renal function.

CSE in the transsulfuration pathway catalyzes the conversion of cystathionine to cysteine. It also catalyzes H_2S formation in a reaction utilizing cystine, a cysteine oxidation product, producing pyruvate, ammonia and thiocysteine, which in turn forms cysteine and H_2S. CBS catalyzes the formation of cystathionine and water by condensing serine and homocysteine, a key irreversible reaction in the transsulfuration pathway. Homocysteine lies at a crucial metabolic branch point between transsulfuration and the methionine-homocysteine cycle. It has been also demonstrated that CBS can, in an alternate reaction, catalyze the formation of cystathionine and H_2S through condensation of cysteine and homocysteine as substrates [10]. Vitamin B_6 is required for CBS and CSE activity through its cofactor pyridoxal phosphate. MST catalyzes the formation of H_2S from 3-mercaptopyruvate, a cysteine metabolite, or it can transfer its sulfur atom to sulfite, which forms thiosulfate. Cysteine formed by CSE can then act as an acceptor of the sulfur transferred from 3-mercaptopyruvate by MST [11].

In addition, H_2S is formed nonenzymatically from elemental sulfur, inorganic polysulfides and organic polysulfides, contained for example in garlic. Therefore, garlic's apparent health benefits may be related to H_2S [12]. Garlic extracts are effective for example in slowing the progression of subclinical atherosclerosis, due to H_2S generation from S-allylcysteine and S-allylmercaptocysteine [13].

H_2S Properties

H_2S is a weak acid, more soluble in lipophilic solvents than in water. There are no known membrane receptors: H_2S seems to cross membranes through simple diffusion. In aqueous solution, it exists in the equilibrium: $H_2S \leftrightarrows HS^- + H^+ \leftrightarrows S^{2-} + 2H^+$. In plasma and in the extracellular fluid, H_2S is present in its undissociated acid form, in less than 20%, and about 80% as the hydrosulfide anion HS^-. The undissociated form H_2S is volatile, while HS^- is not.

Biological Effects

H_2S is entitled to belong to the gasotransmitter family, along with nitric oxide (NO) and carbon monoxide (CO). In fact, H_2S exerts cardioprotective actions in several models of cardiac injury, such as ischemia/reperfusion injury and heart failure, and plays a role in pulmonary hypertension induced by hypoxia. H_2S is able to reduce blood pressure in rats, and to induce vasodilation of isolated blood vessels. Low H_2S generation has been demonstrated in the vasculature of spontaneously hypertensive rats, and chronic administration of a CSE inhibitor in-

duces arterial hypertension. Its actions are mediated by the opening of potassium ATP-dependent channels in vascular smooth muscle cells, independently of membrane receptors, and partially through K^+ conductance in endothelial cells. H_2S is produced by vascular smooth muscle cells, and by endothelial cells through CSE. Importantly, Yang et al. [14] demonstrated that in CSE knockout mice, H_2S is markedly reduced in serum and many tissues; pronounced age-dependent hypertension and reduced endothelium-dependent vasorelaxation are present. Mutant mice display hyperhomocysteinemia and low cysteine levels, as expected as a consequence of their metabolic block. H_2S is consistently reduced in plasma and tissues [14]. An observational study in coronary heart disease patients, hypertensives, and smokers, has also shown that plasma H_2S is lower compared to normal subjects. Low H_2S was found in hypertensive children as well [see 15 for review on this topic]. In this respect, it has recently been shown that H_2S inhibits plasma renin activity by decreasing synthesis and release of renin in a rat model of renovascular hypertension [16].

In addition, H_2S exerts antiatherosclerotic effects, for example in apolipoprotein E knockout mice [17]. In these mice, treatment with NaHS, an H_2S donor, is able to reduce plaque size, which is probably mediated by reduced intracellular adhesion molecule-1 in circulation and in endothelial cells. H_2S deficiency could be also involved in sexual dysfunction [18].

It has been shown by Wu et al. [19] that this gas could be important in the pathogenesis of diabetes mellitus; in fact, it is produced in β-cells. In animals with type 1 diabetes, streptozotocin-induced H_2S production is increased; the authors argue that this excess implies two consequences: one the one hand, it destroys a great number of β-cells, leaving in site too few cells to deal with the necessities of the body. On the other hand, it inhibits insulin release from the remaining cells. Other authors [20, 21] show instead that this is a biphasic effect, in the sense that at low concentrations insulin release is inhibited through a K_{ATP}-dependent/Ca^{2+}-independent mechanism, while higher levels induce β-cell death through a pathway dependent on endoplasmic reticulum stress. Similarly, in nonobese diabetic mice, H_2S levels and the response of vascular tissue to endothelium-dependent vasodilators, such as acetylcholine, are reduced, and aortic H_2S synthesis is progressively reduced with the progression of diabetic disease [22]. In type 2 diabetes patients, plasma H_2S levels are significantly reduced, with respect to lean control patients. In the latter study, adiposity revealed itself as an important determinant of H_2S levels [23]. In this context, it is relevant that in uninephrectomized mice, characterized by CBS deficiency, proteinuria and kidney function indexes get significantly worse when compared to wild-type mice [24]. In addition, the chronic administration of propargylglycine, a CSE inhibitor, induces nephropathy. Jain et al. [25] have recently shown that H_2S is lower in the blood of type 2 diabetic patients, confirming the data by Whiteman et al. [23], and that in human monocytes treated with glucose, H_2S or cysteine supplementation prevents interleukin-8 and monocyte chemoattractant protein-1 secretion [25]. In addition, it is interesting to note that cysteine administration inhibits insulin release by pancreatic β-cells [26].

Aside from cardiovascular and metabolic effects, H_2S has been shown to act as a neuromodulator, agonist of the N-methyl-D-aspartate receptor at the cerebral level. H_2S is also involved in inflammation, apoptosis, and antioxidation [for review see 1–5]; it may influence longevity, and it is possible that it can be employed in eliciting suspended animation, therefore finding a role in transplantation and space travel. Indeed, it is difficult to find, like its gaseous companions NO and CO, a molecule with more pleiotropic properties.

Mechanisms of Action

The mechanisms through which H_2S acts on the various systems and functions are direct or through its HS^- form. It is also entirely possible that all the main effects of H_2S are due to protein S-sulfhydration, mediated through HS^-, which occurs at the level of cysteine residues, leading to the formation of persulfides (-SSH groups) [27]. In fact, the sulfhydration of glyceraldehyde-3-phosphate dehydrogenase, among other proteins, is able to dramatically improve its activity. Also, H_2S enhances actin polymerization [27].

H_2S in Hemodialysis Patients

Chronic kidney disease (CKD), especially in its terminal stage, is characterized by high cardiovascular mortality. While in the general population cardiovascular risk decreased steadily in the last few decades, this did not happen in these patients. More reliable biomarkers for predicting cardiovascular risk in CKD patients are under investigation. Thiol metabolites, such as homocysteine, cysteine, and S-adenosylhomocysteine, are increased in

CKD patients, reaching their highest levels in hemodialysis patients [28]. Homocysteine and cysteine, or their direct derivatives, are utilized as substrates by the key enzymes involved in H_2S biosynthesis, namely, CBS, CSE, and MST.

Contrary to the expectation, however, H_2S is decreased in the plasma of hemodialysis patients [29]. We have in fact shown that not only H_2S is lower, but also red cell sulfhemoglobin (a putative marker of chronic H_2S exposure), while high plasma homocysteine and cysteine are present, with a significant negative correlation between cysteine and H_2S. The cofactor of CBS and CSE, vitamin B_6, is not different in patients with respect to controls. The methylenetetrahydrofolate reductase gene polymorphism does not influence H_2S blood levels. Gene expression of CBS, CSE and MST was measured in nucleated blood cells. Expression of CBS was not present in blood, while that of CSE is significantly lower [29].

In light of these findings, it can be hypothesized that the mechanism of hyperhomocysteinemia in uremia, an issue still not resolved, could find a plausible explanation in this genetic derangement in CSE function. As seen in the CSE knockout mice, where CSE function is not present, hyperhomocysteinemia is part of the picture. In addition to this, the lower H_2S production can be explained by CSE gene downregulation.

MST expression was found to be significantly increased. MST is mainly devoted to the detoxification of cyanide, which is transformed into thiocyanate. Interestingly, cyanide and thiocyanide levels are higher in blood of hemodialysis patients [29]. We can therefore speculate that this enzyme's gene expression is increased because of the necessity to dispose of excess plasma cyanide, typical of these patients. However, since CSE is quantitatively more important in H_2S production, the end result is lower blood H_2S in these patients. Interestingly, in a model of uninephrectomized hyperhomocysteinemic mice, H_2S is reduced in blood, and H_2S supplementation prevents the hyperhomocysteinemia-associated renal damage [24].

In conclusion, H_2S deficiency due to reduced gene expression of CSE can be considered one of the manifold manifestations of the uremic toxicity syndrome, and can in turn cause some of its characteristics. However, it is not to be expected that such a complex disease condition could be easily represented, for what related to deranged vascular homeostasis, by a relatively simple model, i.e. using inhibitors of H_2S-producing enzymes (particularly CSE) to mimic the molecular scenario underlying the generation of hypertension in chronic uremia. Nevertheless, a better knowledge of H_2S and its functions in this patient population could have high therapeutic value [30, 31]. This is especially true if we consider that H_2S may indeed regulate blood pressure through its interactions with NO [30], and in disease states such as the one we are evaluating, it may regulate vascular tone also in more subtle ways [30].

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