Low hydrogen sulphide and chronic kidney disease: a dangerous liaison

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Abstract

Hydrogen sulphide, H₂S, is a gaseous compound involved in a number of biological responses, e.g. blood pressure, vascular function and energy metabolism. In particular, H₂S is able to lower blood pressure, protect from injury in models of ischaemia–reperfusion and induce a hypometabolic state. In chronic kidney disease (CKD), low plasma hydrogen sulphide levels have been established in humans and in animal models. The enzymes involved in its production are cystathionine β-synthase, cystathionine γ-lyase and 3-mercaptopyruvate sulphurtransferase. The mechanisms for H₂S decrease in CKD are related to the reduced gene expression (demonstrated in uraemic patient blood cells) and decreased protein levels (in tissues such as liver, kidney, brain in a CKD rat model). In the present Nephrol Dial Transplant issue, in fact, Aminzadeh and Vaziri document that the alterations in this pathway complicate the uraemic state and are linked to CKD progression. They furnish a time frame in CKD and record enzyme tissue distribution. It remains to be established if low H₂S is causally linked to CKD progression and if interventions aimed to restore the status quo ante are able to modify this picture.

Keywords: chronic kidney disease; chronic kidney failure; cysteine; homocysteine; hydrogen sulphide

Introduction

Aminzadeh and Vaziri have utilized a rat model of chronic kidney disease (CKD) to study H₂S generation at 6 and 12 weeks of age, by analysing the protein expression of relevant enzymes in brain, liver and kidney and the H₂S-producing capacity by these tissues. A reduction in plasma concentration of H₂S at 6 weeks and a significant reduction at 12 weeks were observed. Liver and kidney H₂S-producing capacity were reduced as well. Protein expression of the H₂S-producing enzymes in the kidney was significantly reduced. In the liver, two of the three relevant enzymes were significantly reduced. These findings are in accordance with the concept that plasma H₂S deficiency is systemic in origin and with the postulated presence of (a) uraemic toxin(s) inhibiting these enzymes, while not excluding the presence of a post-translational modification.

Hydrogen sulphide: what it is and how it’s formed

Hydrogen sulphide (or sulfidric acid, H₂S) is a poisonous gas known for its typical odour of rotten eggs, to which the human nose is extremely sensitive and with reason. In fact, H₂S can be deadly, for example by appearing in toxic amounts where decaying organic matter is present (such as sea weed on a beach) or in refineries and oil gas fields where it represents a major safety hazard. However, oddly enough, life itself started with the ability to tolerate and even to produce H₂S, so the prevalent theory affirms. During the Permian extinction, when ambient oxygen was very low, green sulphur bacteria survived by consuming and generating H₂S. The species that thrived thereafter were those able to generate H₂S in small amounts. In particular, a class of vertebrates, which evolved much later, mammals, retained this capacity [1, 2].

H₂S represents the third gasotransmitter, whose existence has been known for many centuries, as opposed to nitric oxide and carbon monoxide; however, a steep rise in the appreciation of its properties in physiology and in pathologic states stemmed from the seminal observations of Yang et al. [3]. These scientists have proven that in mice carrying the deletion of cystathionase, (cystathionase; EC 4.4.1.1), an enzyme capable of generating H₂S, the latter is consistently decreased in plasma and tissues, resulting in age-dependent hypertension, along with altered endothelium-dependent vasorelaxation.

H₂S is a weak acid, which is soluble five times more in lipophilic solvents than in water and is able to cross membranes by simple diffusion, with no need for membrane receptors as facilitators [4]. In aqueous solution, it exists in the equilibrium: H₂S ⇌ HS⁻ + H⁺ ⇌ S²⁻ + 2H⁺. Its pkₐ₁ is ~7.0 and its pkₐ₂ is >17. In plasma and in the extracellular fluids, it is present as H₂S in <20% of total and ~80% as the hydrosulphide anion, HS⁻. The undissociated form, H₂S, is volatile.
Three enzymes (Figure 1) catalyse the formation of H\(_2\)S: cystathionine \(\beta\)-synthase (EC 4.2.1.22), cystathionine \(\gamma\)-lyase and 3-mercaptoppyruvate sulphurtransferase (EC 2.8.1.2).

Regarding their tissue distribution, these enzymes are represented in the various organs and systems to different degrees. In the liver, kidney, enterocytes, vascular smooth muscle cells and endothelial cells, H\(_2\)S is synthesized by cystathionine \(\gamma\)-lyase, while in the brain, its production is mainly attributed to cystathionine \(\beta\)-synthase. In addition, 3-mercaptoppyruvate sulphurtransferase is operative at cardiac, kidney and brain levels and in the vascular endothelium. However, its contribution to the overall H\(_2\)S production is more limited, also because the presence of a reductant is necessary [7]. In general, H\(_2\)S in the cardiovascular system is mainly produced by cystathionine \(\gamma\)-lyase [8, 9].

Cystathionine \(\beta\)-synthase catalyses the formation of cystathionine and water by condensing serine and homocysteine, a key irreversible reaction in the transsulfuration pathway (Figure 1). This pathway is connected to the methionine–homocysteine cycle through homocysteine. However, it has been also demonstrated that cystathionine \(\beta\)-synthase can catalyse the formation of cystathionine and H\(_2\)S through condensation of cysteine and homocysteine as alternative substrates [10]. An allosteric modulator of cystathionine \(\beta\)-synthase is \(\text{S}-\text{adenosylmethionine}\), the universal methyl donor in methylation reactions.

Cystathionine \(\gamma\)-lyase catalyses the conversion of cystathionine to cysteine in the transsulfuration pathway. In addition, it catalyses the formation of H\(_2\)S in a reaction utilizing cysteine, a common and spontaneously occurring cysteine oxidation product, producing pyruvate, ammonia and thiocysteine, which in turn decomposes to cysteine and H\(_2\)S [8]. Other alternative reactions have also been reported [11]. Its activity is regulated by calcium calmodulin [3].

The bile acid-activated nuclear receptor farnesoid X receptor is a positive regulator of the cystathionine \(\gamma\)-lyase gene transcription, which testifies that members of the nuclear receptor superfamily are involved in the regulation of H\(_2\)S, along with the already known endogenous nitric oxide synthase transcription regulation [12].

Vitamin B₆ is required for cystathionine \(\beta\)-synthase and cystathionine \(\gamma\)-lyase activity in the form of its cofactor pyridoxal phosphate.

3-Mercaptoppyruvate sulphurtransferase catalyses the formation of H\(_2\)S from 3-mercaptoppyruvate, a cysteine metabolite, or it can transfer its sulphur atom to sulphite, which forms thiosulphate. Cysteine formed by cystathionine \(\gamma\)-lyase can then act as an acceptor of the sulphur transferred from 3-mercaptoppyruvate by 3-mercaptoppyruvate sulphurtransferase [13, 14].

H\(_2\)S is also formed non-enzymatically from elemental sulphur, inorganic polysulphides and organic polysulphides.

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**Fig. 1.** H\(_2\)S biosynthesis and its relationships with sulphur amino acid metabolism. Three enzymes are involved in H\(_2\)S biosynthesis: cystathionine-\(\beta\)-synthase (CBS), cystathionine-\(\gamma\)-lyase (CSE) and 3-mercaptoppyruvate sulphurtransferase (MST). CBS and CSE catalyse the first and the second reactions in the transsulfuration pathway, for homocysteine metabolic disposal and cysteine biosynthesis. They have been shown to be quite versatile in the use of homocysteine and/or cysteine for H\(_2\)S synthesis in various tissues and organs [5]. Cysteine in turn can be utilized for protein (not shown) or glutathione biosynthesis or can be used as one of the substrates of either CBS or CSE for H\(_2\)S biosynthesis. H\(_2\)S can be also formed by the sequential integrated activities of cysteine aminotransferase and MST, yielding pyruvate and thiosulphate, which is finally reduced to H\(_2\)S in the presence of a reducing agent such as glutathione [6].
These compounds are contained for example in garlic, possibly accounting for the latter’s blood pressure-lowering effects [15].

H2S half-life in plasma is <30 min [16]. As for its catabolism, H2S, aside from being exhaled and also bound in circulation to sulphaemoglobin, is also intracellularly oxidized to thiosulphate (S2O32−), then sulphite (SO32−) and sulphate (SO42−). It is also methylated to methanethiol and dimethylsulphide, while in the gut, a rhodanase catalyses the formation of thiocyanate. Being a reducing agent, H2S reacts with peroxynitrite, superoxide and other oxidant species [16].

H2S detection in blood is currently performed either with the spectrophotometric measurement of methylene blue formation or with electrochemical methods. It has been argued that these assays actually measure free H2S plus its protein-bound and acid-labile moieties. Because of these methodological problems, the normal plasma concentration of H2S is currently debated [17]. In fact, wide variations can be found in the literature. In addition, since H2S adheres to glass and rubber, special significance is attributed to the use of plastic tubes. In a recent article, Shen et al. [18] propose a more sensitive method devoid of the above-stated drawbacks.

Mechanisms of action

It is entirely possible that the main effects of H2S (in what follows, in general we refer to the total amount of H2S, both dissociated and undissociated) are due to protein S-sulphydrylation, mediated through HS−, which occurs at the level of cysteine residues, leading to the formation of persulphides (-SSH groups, [19]). In fact, the sulfhydration of glyceraldehyde-3-phosphate dehydrogenase, among other proteins, is able to dramatically improve its activity. Also, H2S enhances actin polymerization [19].

However, it is also possible that since very high free levels of H2S are found in mouse aortic tissue compared to the low levels occurring in other tissues, free H2S mediates its vasoactive functions, while in other tissues, a receptor-like mechanism modulates its activity [20]. It can be speculated that this mechanism may involve protein S-sulphydrylation. It has also been reported that H2S actions in the cardiovascular and other systems recognize both cyclic guanosine monophosphate and cyclic adenosine monophosphate as second messengers [21].

H2S and oxidation, apoptosis and inflammation

H2S is involved in general as a protective agent in oxidation, inflammation and apoptosis, all events paving the way to acute and chronic diseases such as ischaemia–reperfusion injury (I/R), pulmonary hypertension, atherosclerosis, CKD progression and complications [1, 2, 14].

For example, H2S exerts anti-atherosclerotic effects through the inhibition of vascular smooth muscle cell proliferation and promotion of the proliferation of endothelial cells [22]. In apolipoprotein E knockout mice [23], treatment with NaHS, an H2S donor, is able to reduce plaque size, an effect probably mediated by the reduced concentration of intracellular adhesion molecule-1 (ICAM-1) in circulation and on the endothelial cells. H2S is an antioxidant, in fact it increases reduced glutathione in neurons [24], but it also directly scavenges superoxide anions, hydrogen peroxide [25] and peroxynitrite [26] to suppress oxidative stress. Recently, Yang et al. [27] investigated the cytoprotection of H2S in HaCaT cells treated with cobalt chloride (CoCl2), a well-known mimetic agent of hypoxia/ischaemia, which induces oxidative stress and inflammation. HaCaT cells are derived from spontaneous transformation of human adult keratinocytes. They showed that H2S protects HaCaT cells against CoCl2-induced injury and inflammatory response by inhibiting the reactive oxygen species (ROS)-activated NF-κB/COX-2 pathway.

Regarding inflammation, conflicting reports appeared in the literature showing both pro- or anti-inflammatory actions, which is explained probably by differences in H2S concentrations and models utilized. Recently, Pan et al. [28] have shown that in human umbilical vein endothelial cells (HUVECs), H2S is able to suppress tumour necrosis factor-α-induced ICAM-1 and vascular cell adhesion molecule 1 expression and U937 monocyte adhesion, through a mechanism involving the cytoprotective enzyme haem oxygenase-1 up-regulation.

A recent study showed that NaHS (10–1000 μM) treatment for 20 min could protect HUVECs and fibroblasts (3T3s) against I/R-induced apoptosis [29].

Cardiovascular effects with special focus on blood pressure

H2S exerts cardioprotective actions in several models of cardiac injury, such as I/R injury and heart failure [30–32]. H2S is also able to reduce blood pressure in rats and to induce vasodilation of isolated blood vessels [32, 33]. Its actions are mediated by the opening of potassium ATP-dependent channels in vascular smooth muscle cells, independently of membrane receptors and partially through a K+ conductance in endothelial cells [33]. Other channels are also involved, making H2S a multichannel agent [34]. For example, H2S also activates large-conductance Ca2+-activated potassium channels [35].

Low H2S generation has been demonstrated in the vasculature of spontaneously hypertensive rats, and H2S administration lowers their blood pressure, while chronic administration of a cystathionine γ-lyase inhibitor induces arterial hypertension [32, 33]. As mentioned above, in cystathionine γ-lyase knockout mice, H2S is markedly reduced in serum and many tissues; pronounced age-dependent hypertension and reduced endothelium-dependent vasorelaxation are distinct features [3]. Mutant mice display hyperhomocysteinaemia and low cysteine levels, as expected as a consequence of their metabolic block. In addition, in this model, hypertension does not depend on brain or kidney alterations, which were found unaltered. An observational study in coronary heart disease patients, hypertensives and smokers, has also shown that plasma H2S is lower compared to normal subjects [36]. Low H2S was found in hypertensive children as well [37].

H2S and the kidney

An intertwined web is being unraveled on the effects of H2S on the kidney as well as on the contribution of the renal
metabolic machinery to H$_2$S biosynthesis, as schematically depicted in Figure 2. These connections appear more and more complex, as further data on this issue have been accumulating in recent years.

H$_2$S is produced in the kidney by combined actions of cystathionine $\beta$-synthase and cystathionine $\gamma$-lyase, present mainly in the proximal tubule, and 3-mercaptopyruvate sulphurtransferase is also present. H$_2$S affects both the renal tubule and vasculature. Acute simultaneous administration of aminooxyacetic acid, an inhibitor of cystathionine $\beta$-synthase, and propargylglycine, an inhibitor of cystathionine $\gamma$-lyase, but neither independently, decreases the glomerular filtration rate and sodium and potassium excretion, whereas these are increased by infusion of either H$_2$S or cysteine [41]. In fact, H$_2$S injected in the renal artery increases renal blood flow and glomerular filtration rate, and it is therefore diuraetic, natriuraetic and kaliuraetic. These data on the renal plasma flow and glomerular filtration rate indicate that H$_2$S produces greater vasodilatation in pre-glomerular arterioles than in post-glomerular arterioles.

Fig. 2. The interplay between renal metabolism and H$_2$S production in various models. Schematic representation of the lines of evidence regarding the extent of the involvement of kidney functions in sulphur amino acid metabolism and H$_2$S production. The animal or the human silhouette portrayed in each panel denotes the model (mouse, rat or human) in which the depicted enzymatic or compound alterations were found. (A) In murine-derived models: H$_2$S production, in kidney extracts, decreased by 15% in the presence of both cysteine and homocysteine substrates (see also Figure 1) and 40% when homocysteine is used alone, if propargylglycine (PPG; a cystathionine-$\gamma$-lyase inhibitor) was present. A significant reduction in H$_2$S production when homocysteine alone was used as a substrate and in the presence of PPG could also be detected in the brain (not shown) [38]. (B) Decreased H$_2$S, accompanied by increased cysteine and decreased sulphaemoglobin, and transcriptional down-regulation of both transsulfuration enzymes in end-stage renal disease [39]. (C) A significant H$_2$S decrease is also observed in uraemic rats and is accompanied by a malfunction of both transsulfuration enzymes, responsible for H$_2$S production [40]. Hyperhomocysteinaemia has been constantly acknowledged in the uraemic rat model in previous reports, although not specifically investigated by these authors.
In another study [42], however, rats chronically treated with both aminooxyacetic acid and propargylglycine for 4 weeks showed an increase in blood pressure, but glomerular filtration rate was not altered, while renal blood flow was lowered. Urine flow and sodium excretion were also increased. Cortical blood flow and medullary blood flow were reduced. These experiments indicate that there were proportionate reductions in vascular resistances in both afferent and efferent arterioles. Therefore, chronic H2S deficiency seems to cause an increase in vascular resistance leading to renal vasoconstriction and systemic hypertension.

It has been suggested that since H2S is oxidized in mitochondria in a pO2-dependent manner and ambient pO2 is physiologically low in the renal medulla, H2S is an oxygen sensor restoring O2 balance by increasing medullary blood flow, reducing energy requirements for tubular transport and inhibiting mitochondrial respiration [43]. As suggested by Aminzadeh and Vaziri [40], since kidney disease progression could be mediated in part by medullary hypoxia, low H2S could contribute in this way to progression. However, the hypertensive cystathionine γ-lyase–/– mice [3] do not develop renal damage at least from the histology studies made at 10–12 weeks speaking against this gas being involved in renal diseases. In any case, hypertension is not due to kidney damage in this model. Nevertheless, this does not rule out that H2S could be causally involved in kidney disease.

In the two-kidney one-clip rat model of renal vascular hypertension, exogenous H2S decreases blood pressure, plasma renin activity and angiotensin II concentration (but does not affect plasma angiotensin-converting enzyme activity), and it inhibits up-regulation of renin messenger RNA [44].

Zavaczki et al. [45] have shown that H2S inhibits calcification and osteoblastic differentiation of vascular smooth muscle cells, a process involved in progression and cardiovascular mortality of CKD patients.

Sen et al. utilized a genetic model of hyperhomocysteinemia (heterozygous CBS+/− mice) subjected to uninephrectomy, that is a model reproducing CKD, and showed that the animals were proteinuric, and plasma H2S was decreased. Increased activity of matrix metalloproteinase-2 and -9 and apoptosis, increased desmin and down-regulation of nephrin were also present in renal cortical tissue, as well as increased superoxide production and decreased reduced to oxidized glutathione ratio. H2S supplementation prevented proteinuria, matrix metalloproteinase-2 activity is attenuated, apoptosis decreases as well as the reduced to oxidized glutathione ratio and ROS production [46].

These results demonstrate that hyperhomocysteinemia-associated renal damage is related to decreased endogenous H2S generation in the body. Therefore, this work provides evidence that H2S supplementation (obtained by NaHS administration in the rat) prevents hyperhomocysteinemia-associated renal damage, in part, through its antioxidant properties.

In this same model, Sen et al. [47] demonstrated that H2S up-regulates adhesion molecules and inflammatory mediators and reduces macrophage infiltration, interstitial fibrosis and glomerulosclerosis.

Sen et al. have also shown that cystathionine β-synthase and cystathionine γ-lyase double gene transfer ameliorate homocysteine-mediated mesangial cell inflammation. Hyperhomocysteinaemia causes up-regulation of inflammatory molecule monocyte chemotactic protein-1 (MCP-1) and macrophage inflammatory protein 2 (MIP-2) in mesangial cells through attenuated H2S generation. Over-expression of the two enzymes mitigates the response by these two inflammatory molecules by increasing H2S generation through extracellular signal-regulated kinase 1/2 (ERK1/2) and c-Jun NH2-terminal kinase (JNK1/2)-dependent pathways [48].

CKD progression and complications and H2S

CKD is characterized by high cardiovascular mortality. While in the general population cardiovascular risk decreased in the last decades, this did not happen in these patients. In addition, halting the rate of progression of the disease still eludes even the most careful team of caregivers. New biomarkers for preventing risk and progression in CKD patients are constantly being sought. Among these, we can find various sulphur-containing metabolites, such as the thiol amino acids homocysteine and cysteine and the thioether S-adenosylhomocysteine. These are all consistently increased in the majority of CKD patients, reaching their highest levels in haemodialysis patients [39, 49–51]. As for the cause of hyperhomocysteinaemia in uraemia, while increased homocysteine production or decreased excretion have been ruled out, decreased metabolism in renal or extrarenal sites seem to be the most plausible cause. In this context, homocysteine and cysteine, or their direct derivatives, are utilized as substrates for H2S biosynthesis, by the key enzymes cystathionine β-synthase, cystathionine γ-lyase and 3-mercaptopyruvate sulphurtransferase.

H2S is decreased in the plasma of haemodialysis patients, as we have recently shown [39]. This finding is confirmed by low red cell sulphamoglobin (a putative marker of chronic H2S exposure) levels, present in this patient population, and is accompanied by high plasma homocysteine and cysteine, with a significant negative correlation between cysteine and H2S. Gene expression of cystathionine γ-lyase in blood mononuclear cells is significantly lower, realizing a condition where a transcriptional down-regulation of the gene encoding for a key H2S-producing enzyme is present [39].

3-Mercaptopyruvate sulphurtransferase expression was found instead to be significantly increased. Functionally, 3-mercaptopyruvate sulphurtransferase is devoted to the detoxification of cyanide, which is transformed into thiocyanate. Interestingly, high cyanide and thiocyanide levels are found in the blood of haemodialysis patients [52]. We can therefore speculate that this enzyme’s gene expression levels are possibly increased as a readjusting mechanism to the increased demand to dispose of excess plasma cyanide, typical of CKD.

The reduction in H2S production due to reduced gene expression of cystathionine γ-lyase can thus be considered one of the manifold manifestations of the uraemic toxicity syndrome and can be in turn responsible for some of its features.
It is possible that a uraemic toxin is able to down-regulate H$_2$S-producing enzymes, and one possibility is that homocysteine itself functions as an inhibitor [53]. Chang et al. [53] have shown that this applies to cystathionine γ-lyase. For these authors, rats rendered hyperhomocysteinaemic through homocysteine injection displayed low myocardial H$_2$S levels and decreased cystathionine γ-lyase activity. In the rat model utilized by Sen et al. [46], both ureaemia (one kidney wild-type animals) and hyperhomocysteinaemia (one kidney and two kidney heterozygous cystathionine β-synthase $+/-$ rats) present reduced plasma H$_2$S levels. This is consistent with Chiku et al. and Singh et al. who pointed out in their carefully performed enzyme kinetic data that high homocysteine shifts the H$_2$S formation from cystathionine β-synthase to cystathionine γ-lyase [5, 13, 54]. These data, obtained with the recombinant purified enzymes or tissue extracts, showed that when homocysteine is utilized alone, H$_2$S production generally diminishes. Another possible agent, calcitriol, is active on cystathionine β-synthase [38]. In an osteoblastic cell model, calcitriol induced cystathionine β-synthase expression and activity. A functional vitamin D response element in the second intron of cystathionine β-synthase was demonstrated. The effects of low calcitriol levels, as it occurs in uraemia, in this respect are yet to be explored.

Aminzadeh and Vaziri [40] have utilized the classical 5/6 nephrectomy rat model of CKD to study the protein expression of cystathionine β-synthase, cystathionine γ-lyase and 3-mercaptopyruvate sulphurtransferase in brain, liver and kidney and H$_2$S-producing capacity by these tissues at 6 and 12 weeks of age. The CKD group was heavily proteinuric and hypertensive at 12 weeks and showed manifest oxidative stress (as measured by several markers). In particular, the authors evaluated the levels of advanced lipoxidation end products including malondialdehide and other thiobarbituric acid-reactive substances. They could detect an increase of lipid oxidation markers both in plasma and in the kidney, mirrored by an increase of plasma-oxidized glutathione, while a decrease in reduced glutathione was also observed. A significant up-regulation of the superoxide-generating enzyme, NOX4, and down-regulation of the glutamate–cysteine ligase, the enzyme catalysing the first step in glutathione biosynthesis, was present.

This model is associated with a reduction in plasma concentration of H$_2$S at 6 weeks and a significant reduction at 12 weeks. H$_2$S-producing capacity by the brain did not differ from control; however, liver and kidney H$_2$S-producing capacity were reduced at 6 weeks and more at 12 weeks. Protein expression of the H$_2$S-producing enzymes, cystathionine β-synthase, cystathionine γ-lyase and 3-mercaptopyruvate sulphurtransferase in the kidney, were, at 6 weeks, decreasing, although only for cystathionine β-synthase was this effect statistically relevant; while at 12 weeks, all enzymes were significantly reduced. It is clear from the blots that the most quantitatively important enzyme in the normal and CKD kidney is cystathionine γ-lyase (~10 times with respect to cystathionine β-synthase). This was confirmed by other recent studies in mice [5], where cystathionine γ-lyase is ~20 times higher than cystathionine β-synthase. However, it must be considered that when the contribution of these enzymes to H$_2$S generation under saturating substrate concentration is considered, cystathionine β-synthase is the major source of H$_2$S in the kidney [5]. In fact, in the paper by Aminzadeh and Vaziri, cystathionine β-synthase is hit much earlier when CKD ensues with respect to cystathionine γ-lyase.

In the liver, all three enzymes are represented, but in CKD rats, 3-mercaptopyruvate sulphurtransferase does not differ from control, while cystathionine β-synthase and cystathionine γ-lyase are significantly reduced both at 6 and 12 weeks. The paper therefore confirms and integrates previous findings in humans [39], which were limited to the gene expression levels, while Aminzadeh and Vaziri explore enzyme protein expression and H$_2$S capacity (Figure 2). In addition, they contribute new information about the differential tissue patterns present in CKD and their time dependency. For example, they show that in the kidney, these enzymes are all reduced, starting with cystathionine β-synthase, while in the liver, this is true only for cystathionine β-synthase and cystathionine γ-lyase (Figure 2). 3-Mercaptopyruvate sulphurtransferase is probably not reduced because of the need to get rid of the excess cyanide (given the known increase in cyanide present in uraemia) and the cyanide detoxifying properties of this enzyme.

These findings give new breath to the concept that plasma H$_2$S deficiency is systemic in origin and are in accordance with the postulated presence of one or more uraemic toxins inhibiting both cystathionine β-synthase and cystathionine γ-lyase expression. Still, it cannot be excluded that post-translational modifications are present as well [45].

By the way, under conditions of moderate and severe hyperhomocysteinaemia, cystathionine γ-lyase is predicted to become an increasingly important contributor to the general H$_2$S generation [54]. However, this is true only when renal function is normal. If renal function is compromised, the relevant enzymes are down-regulated and/or inhibited. As noted above, hyperhomocysteinaemia can transfer from cystathionine β-synthase to cystathionine γ-lyase the enzyme activity relative to H$_2$S formation [5, 13, 54], but in this case, it does not matter which enzyme activity is influenced by homocysteine levels because low H$_2$S levels are the end result.

Even if it has been expertly suggested [5] that relying on cysteine alone as a substrate will bias data towards cystathionine γ-lyase-dependent H$_2$S production, as in the model of Aminzadeh and Vaziri, here we must expect that hyper-homocysteinaemia would be present as well due to renal failure. Therefore, the second substrate, homocysteine, would be furnished endogenously, in the in vivo situation, and the data would be reliable in assessing the contribution of the enzymes in terms of H$_2$S-producing capacity.

In general, the work of Drs Vaziri and Aminzadeh further strengthens the thesis that the protective levels of H$_2$S are decreased in renal failure. Their findings help us to better understand the various pathological mechanisms involved in kidney failure, both in establishing it (progression) and in sustaining it (complications).

However, before the importance of H$_2$S in ameliorating this dysfunction can be openly pursued, let’s not forget that not all birds sing to the same tune. In fact, it has been reported that treatment with propargylglycine, a cystathionine γ-lyase inhibitor, was able to reduce cisplatin-induced...
renal damage [55], and the same results were seen with adriamycin injection [56]. These contradicting results could, however, reflect the pleiotropic effects of propargylglycine itself, such as for example the inhibition exerted by propargylglycine on other enzymes; pleiotropic effects of alterations of H2S concentration may also come into play. On the line of evidence of Della Coletta Francescato et al. [56] and Francescato et al. [56], Wu et al. [57] have shown that during kidney I/R, cystathionine β-synthase gene expression is reduced. A significant decrease in cystathionine β-synthase enzyme activity, causing an elevation in homo-
cysteine and a decline of H2S levels in the kidney, was observed. The authors suggest that this is a protective measure to limit damage. However, it should be remarked, in this respect, that cystathionine β-synthase is a highly regulated enzyme since it includes a haem sensor, it is activated by S-adenosylmethionine and it is also strongly dependent on pyridoxal phosphate, all variables which may be important under the circumstances. On the other hand, for Tripatara et al., generation of H2S by cystathionine γ-lyase is able to limit renal I/R injury and dysfunction [58] and cystathionine γ-lyase expression is increased after I/R injury. They suggest that this is one of the many endogenous mechanisms limiting injury itself [59]. Bos et al. [60] have also shown in mice that H2S-induced hypometabolism prevents renal I/R injury.

Finally, we have recently shown that after dialysis, H2S levels increase significantly, in a group of 131 patients, possibly lending credit to the idea that dialysis eliminates a uraemic toxin inhibiting the H2S-generating enzymes [61].

Next to the fact that one reasonably can accept that in CKD there are several mechanisms lowering H2S, the question also could be raised whether in turn H2S harms the kidney, therefore leading to CKD. This possibility is unanswered and only hinted at by the experiments of Aminzadeh and Vaziri.

All in all, these considerations bring us back to the notion that the line between harm and benefit is very thin and still much remains to be done in this field. It is especially difficult to understand which modification is a noxious effect of the pathological process and which one is a protective means in cell and organ defense; in other words, uraemia seems to be the cause of the enzymatic derange-
ments leading to low H2S but is low H2S involved in the progression of renal failure?

Conflict of interest statement. None declared.


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