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Hydrogen Sulfide in Renal Physiology and Disease

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Abstract

Significance: Hydrogen sulfide (H₂S) has only recently gained recognition for its physiological effects. It is synthesized widely in the mammalian tissues and regulates several biologic processes ranging from development, angiogenesis, neurotransmission to protein synthesis. **Recent Advances:** The aim of this review is to critically evaluate the evidence for a role for H₂S in kidney function and disease. **Critical Issues:** H₂S regulates fundamental kidney physiologic processes such as glomerular filtration and sodium reabsorption. In kidney disease states H₂S appears to play a complex role in a context-dependent manner. In some disease states such as ischemia-reperfusion and diabetic kidney disease it can serve as an agent that ameliorates kidney injury. In other diseases such as cis-platinum-induced kidney disease it may mediate kidney injury although more investigation is needed. Recent studies have revealed that the actions of nitric oxide and H₂S may be integrated in kidney cells. **Future Directions:** Further studies are needed to understand the full impact of H₂S on kidney physiology. As it is endowed with the properties of regulating blood flow, oxidative stress, and inflammation, H₂S should be investigated for its role in inflammatory and toxic diseases of the kidney. Such in-depth exploration may identify specific kidney diseases in which H₂S may constitute a unique target for therapeutic intervention. *Antioxid. Redox Signal.* 25, 720–731.

Keywords: renal physiology, acute kidney injury, chronic kidney disease, diabetic kidney disease, hypertension

Introduction

AMONG THE PHYSICAL forms of matter, gases are gaining attention as important regulators of cell function. Hydrogen sulfide (H₂S) has only recently received recognition for its physiological effects in joining gases that are active in mammalian homeostasis, including nitric oxide and carbon monoxide. Although it was known for its toxic effects as an environmental hazard, recent findings have highlighted its regulatory effects on fundamental cellular processes in several tissues. Our review will focus on the role of H₂S in kidney physiology and disease.

H₂S Synthesis

In mammalian cells, H₂S is primarily produced in the cytosol by the enzymes of the trans-sulfuration pathway, cystathionine β -synthase (CBS), and cystathionine γ -lyase (CSE) (13, 70, 83). Homocysteine (HCy) is condensed with serine by CBS to generate cystathionine, which is converted into L-cysteine by CSE. L-cysteine can be used as a substrate by both CBS and CSE to produce H₂S (Fig. 1). CSE also catalyzes H₂S production by the conversion of HCy into

homolanthionine (70). Additionally, cysteine is converted by aspartate aminotransferase in the presence of α -ketoglutarate to form 3-mercaptopyruvate and glutamate. Another enzyme, 3-mercaptopyruvate sulfurtransferase (3-MST) contributes to the endogenous H₂S production in the mitochondria in the presence of a reductant using 3-mercaptopyruvate as a substrate (65, 82) (Fig. 1).

It was recently discovered that H₂S can also be produced from D-cysteine involving 3-MST and D-amino acid oxidase (81). In the kidney, more H₂S is said to be produced from D-serine than from L-serine (81) (Fig. 1).

H₂S Signaling Through Sulfhydrylation

Recent studies have shown that H₂S can regulate several signaling pathways that are involved in cell biological processes; these will be reviewed later. Additionally, H₂S can post-translationally modify proteins by forming a covalent link to the SH of cysteine by the process of sulfhydrylation (Fig. 2). A functional role for sulfhydrylation of proteins had been demonstrated for xanthine oxidase (60) and aldehyde oxidase (9), both of which have an active site persulfide that is essential for catalysis. In contrast, sulfhydrylation of rat liver

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FIG. 1. H₂S synthesis. H₂S, hydrogen sulfide; CBS, cystathionine β -synthase; CSE, cystathionine γ -lyase; 3-MST, 3-mercaptopyruvate sulfurtransferase; AAT, aspartate aminotransferase; DAO, D-amino acid oxidase.

tyrosine aminotransferase, which is CSE dependent, leads to enzyme inactivation (29), and it is enhanced in streptozotocin (STZ)-induced diabetes (28). The physiological importance of sulfhydrylation has been recently discovered; a proteomic analysis of persulfidation revealed that ~ 10 –25% of liver proteins harbor this modification (67).

The functional consequences of sulfhydrylation have been characterized for some proteins. TNF α stimulates Sp1-mediated transcriptional activation of CSE, which increases H₂S production and sulfhydrylation of the p65 subunit of the transcription factor NF- κ B, leading to an increase in its DNA binding (76). Sulfhydrylation of the ATP-sensitive K⁺ channel (KATP) activates the channel and causes hyperpolarization and vasorelaxation of rodent mesenteric arteries (68). Sulfhydrylation of protein tyrosine phosphatase, PTP1B, inhibits its activity and potentiates the activation of protein kinase R-like endoplasmic reticulum (ER) kinase (PERK) in response to ER stress (45).

H₂S has been proposed to modify cysteine residues by redox-dependent sulfhydrylation (38). In the reducing intracellular milieu, cysteines are likely to exist predominantly in

the thiol form that does not react with H₂S. Transient increases in reactive oxygen species (ROS) concentration would increase the proportion of cysteines present in the oxidized form, for example, sulfenic acid and disulfides, both of which are reactive to H₂S (Fig. 2). Hydrogen persulfide, formed by two-electron oxidation of H₂S, has been proposed as a possible cellular sulfhydrylation reagent (69).

Renal Effects of H₂S

The anatomical and physiological unit of the kidney is the nephron. The mammalian nephron consists of a capillary filtration unit called the glomerulus and a tubular system downstream of the glomerulus. The kidney is a highly vascular organ receiving nearly 25% of the cardiac output. The unique feature of the glomerular capillary is that being interposed between two arterioles it is exposed to much higher pressures than capillaries elsewhere. The hydrostatic pressure in the glomerular capillary is maintained at ~ 50 mm Hg, whereas the hydrostatic pressure in other capillaries falls from about 35 mm Hg at the arteriolar end to about 15 mm Hg at the venular end (8, 24).

The glomerulus is connected to a tubule that consists of a series of highly specialized segments that are tasked with elaborate processes for solute and ionic absorption and secretion. The tubules are classified into the proximal tubule, the descending and ascending loops of Henle (the ascending limb has thin and thick segments), the distal convoluted

FIG. 2. Modification of proteins by sulfhydrylation.

tubule, the connecting duct, and the collecting duct. The proximal tubule performs the bulk of transport functions of the nephron. It reabsorbs nearly two thirds of the filtered sodium facilitated by the actions of the Na-H antiporter. Sodium reabsorption in the ascending limb of the loop of Henle is an active process under the control of Na-K-2Cl cotransporter. Sodium reabsorption in the distal convoluted tubule is under the control of Na-Cl cotransporter, whereas that in the connecting duct and the collecting duct is regulated by the epithelial sodium channel (ENaC). In these segments, the functions of transporters governing sodium reabsorption are integrated with the actions of Na⁺,K⁺-ATPase.

The human kidney filters ~150–200 L of plasma at the glomerulus daily; the tubules extensively reabsorb and modify the composition of the glomerular filtrate such that the volume of urine is about 1–2 L/day. The tubules are surrounded by peritubular blood vessels that are derived from the efferent arteriole; these blood vessels participate in returning to the systemic circulation the vast amount of solutes and water that are reabsorbed by the tubules. It is important to note that the tubules have elaborate secretory pathways that add substances from the peritubular blood vessels to the filtrate, including drugs for elimination in the urine. Since so much of the kidney function is under the control of blood vessels, that is, afferent and efferent arterioles, glomerular capillary, and peritubular blood vessels, it is no wonder that gases such as H₂S and nitric oxide (NO), highly vasoactive agents, regulate renal physiology.

H₂S production by the kidney

It has been long known that the kidney generates measurable quantities of H₂S (86). Both CSE and CBS are present in the kidney. CSE is expressed by the endothelial cells, mesangial cells, and the podocytes that together form the majority of the cells in the glomerulus. It is expressed in the proximal and distal tubular epithelial cells and in the peritubular capillaries; nearly 75% of all renal cells express CSE (7, 49). Yamamoto *et al.* did not detect either CBS or CSE in the glomerulus or in the renal blood vessels but found their expression in the proximal tubules (98).

Physiological effects of H₂S in the kidney

Infusion of H₂S in the form of sodium hydrosulfide (NaHS) into the renal artery increases the renal blood flow and augments the glomerular filtration rate, which is an index of the clearance function of the kidney; urinary flow rate was also increased, and these effects were dose dependent (97).

H₂S augments the urinary excretion of sodium and potassium, probably by the inhibition of Na-K-2Cl cotransporter in the ascending limb of the loop of Henle and Na K ATPase (97). Patch clamp studies on *Xenopus* A6 cells have shown that hydrogen peroxide augments phosphatidyl inositol 3 kinase activity to activate the ENaC; this is inhibited by H₂S (103). In addition to the *Xenopus* frog epithelial cells, recent reports have shown that in human lung epithelial cells (H441) H₂S inhibits β -adrenergic agonist-induced ENaC-mediated Na transport; H₂S also inhibits ENaC-dependent lung fluid clearance in rats (1). Additionally, H₂S augments blood flow in the peritubular blood vessels, and NaHS corrects hypoperfusion in diabetic mice (98).

Context-Dependent Role of H₂S in Kidney Injury

Recent investigations have revealed a complex role played by H₂S in the pathogenesis of kidney injury. Whether it ameliorates kidney injury or whether it participates in bringing it about appears to depend on the disease model being studied.

Acute kidney injury

The kidney is vulnerable to injury from changes in the systemic hemodynamics and after the administration of toxic medications. Thus, acute kidney injury is seen in the form of ischemia/reperfusion injury or when nephrotoxic medications such as anticancer agents and antibiotics are administered.

Renal ischemia/reperfusion injury

Ischemia/reperfusion injury is common during kidney transplantation and may affect the survival and function of the graft. In humans, the levels of renal CSE messenger RNA (mRNA) at the time of organ procurement positively correlate with the glomerular filtration rate measured at 2 weeks after transplantation (7), suggesting that H₂S could be protective in the context of renal ischemia. In support of this notion, NaHS has been shown to improve allograft survival and function in rats with bilateral nephrectomy that underwent a subsequent kidney transplant; this was accompanied by a general decrease in the transcription of cellular stress response genes (55).

Ischemia/reperfusion injury that is characterized by oxidative stress has been implicated in delayed function of transplanted kidneys; xanthine oxidoreductase enzyme activation in the form of xanthine oxidase can lead to excessive production of reactive oxygen/nitrogen species and inflammation. Clinically significant changes in the activity of kidney xanthine metabolizing enzymes have been reported in renal transplant patients with delayed graft function when compared with those with early graft function (16). Because H₂S improves ischemia/reperfusion injury in the setting of kidney transplantation, these studies suggest a possible link between the reduced H₂S expression and the activation of xanthine pathway that generates ROS.

That H₂S inhibits ROS generation by the xanthine oxidase pathway was established in the rat model of aspirin-induced gastric ulcers in which oxidative stress plays a major role. Intraperitoneal administration of 2-acetyloxybenzoic acid 4-(3-thioxo-3H-1, 2 dithiol-5-yl) phenyl ester (ACS14, S-aspirin), a form of aspirin that releases H₂S, prevented aspirin-induced gastric ulceration; this protective effect of S-aspirin was reproduced by NaHS establishing that H₂S was responsible for the ameliorative effect (54). The mechanism of H₂S amelioration of aspirin-induced injury involved a decrease in the markers of oxidative stress and a reversal of aspirin-induced increased expression of xanthine oxidase in the gastric mucosa (54).

In animal models of ischemia/reperfusion injury, the renal expression of CBS and CSE and the endogenous production of H₂S are decreased (26). Renal ischemia/reperfusion injury is characterized by increased inflammation, oxidative stress, apoptosis of tubular epithelial cells, and vascular dysfunction (6). Administration of H₂S donors before or after the ischemia/reperfusion insult restores the levels of H₂S-producing enzymes and local H₂S production (26), and it accelerates renal recovery (5, 26, 56). This protective effect is

accompanied by a reduction in oxidative stress (5, 26) and inflammation (56). A role for H₂S deficiency in the pathogenesis of renal ischemia/reperfusion injury was demonstrated by targeting CSE; administration of propargylglycine (PAG), a CSE inhibitor (89), or genetic ablation of CSE (7) significantly worsened both kidney damage and animal mortality.

Recent studies have suggested a protective role for H₂S derived from D-cysteine in ischemia/reperfusion injury in the kidney (81). Furthermore, the ameliorative effects of dietary calorie restriction on ischemia/reperfusion injury to the liver and the kidney are dependent on increased production of H₂S (30).

Obstructive kidney injury

Renal injury after obstruction of the genitourinary tract is a common cause of both acute and chronic kidney injury, particularly in elderly men. In studies on obstructive kidney injury, investigators commonly employ unilateral obstruction to the ureter, which permits the contralateral kidney to serve as a control. Unilateral obstruction by ureteral ligation resulted in kidney injury characterized by inflammation and renal fibrosis in association with reduction in the generation of H₂S in that kidney (85). Reduced H₂S generation in this model was due to a decrease in the expression of CBS. Administration of NaHS significantly reduced the aforementioned manifestations of kidney injury, including inflammation (85). *In vitro* experiments showed that H₂S inhibited transforming growth factor β -induced transformation of fibroblasts to myofibroblasts (85), providing a mechanistic basis for reduction in fibrosis. These data and observations in the ischemic/reperfusion model suggest that depending on the nature of acute kidney injury, CSE or CBS can undergo dynamic regulation in the kidney.

Cisplatin and doxorubicin nephrotoxicity

Cisplatin is a chemotherapeutic drug that is used for the treatment of lung, ovarian, and cervical cancers. Platinum complexes bind to and cause crosslinking of DNA, which ultimately triggers apoptosis of cancer cells. However, cisplatin is toxic to the kidneys. The nephrotoxicity of cisplatin seems to be related to oxidative stress, since it can be ameliorated by free radical scavenging agents in animal models. In rats, cisplatin causes inflammation of the renal outer medulla that is characterized by the infiltration of macrophages, neutrophils, and T lymphocytes; pretreatment with PAG, an inhibitor of H₂S synthesis, significantly reduces the renal damage induced by cisplatin (14).

In contrast to this report, administration of H₂S donors to rats has been reported to prevent cisplatin-induced renal injury by increasing anti-oxidant mechanisms in kidney cells (2). The conflicting data on the role of H₂S in cisplatin kidney injury emphasize the need for further examination of the issue.

Doxorubicin is another chemotherapeutic agent employed in the treatment of malignant tumors. Doxorubicin may cause congestive heart failure and chronic kidney injury in the form of focal segmental sclerosis of the glomerulus that is associated with marked proteinuria and loss of clearance function of the kidney (51). H₂S has been proposed as a mediator of kidney injury manifesting as inflammation and proteinuria after the administration of doxorubicin (22); however, H₂S donors have been shown to protect the heart from

doxorubicin-induced toxicity (102), suggesting the need for clarification of the role of H₂S in kidney injury induced by that agent. In addition, studies are also needed on the involvement of H₂S in acute kidney injury due to other nephrotoxic agents such as radiocontrast media containing iodine and gadolinium.

Hyperhomocysteinemia

HCy is usually converted into cystathionine by CBS (Fig. 1). Thus, hyperhomocysteinemia (HHCy) occurs either due to decreased expression of CBS or due to inhibition of its activity. HHCy can be caused by deficiencies of vitamins B₆, B₉, and B₁₂ (62). HHCy has been linked to atherosclerosis and thrombosis (11) and to Alzheimer's disease (64). It is also associated with kidney injury, as indicated by the development of albuminuria (34). Increased levels of HCy repress the expression and activity of the H₂S-generating enzymes, CSE, CBS, and 3-MST, leading to a significant decrease in endogenous H₂S production (95). It has been shown that HCy increases the methylation of the CSE promoter by DNA methyltransferase, which leads to the repression of CSE transcription (53). Whether the DNA methylation mechanism affects the expression of CBS and 3-MST is not known.

In addition to its repression of H₂S synthesis, HHCy has profound effects on the vasculature. It increases inflammation (72), oxidative (72) and nitrosative stress (39), and mitophagy (79) in endothelial cells, leading to their dysfunction. Specifically, in the kidney, there appears to be a vicious cycle with regards to HCy metabolism; a decrease in kidney function leads to an elevation of HCy, which, in turn, leads to kidney injury and loss of function. Chronic HHCy in CBS +/- mice is associated with glomerulosclerosis and proteinuria, which are ameliorated by NaHS (77). Augmented apoptosis of cells in the kidney cortex of mice with HHCy was associated with increased activity of matrix metalloproteinase (MMP)-2 and -9; these changes were inhibited by NaHS, leading the authors to the conclusion that activation of MMPs is involved in remodeling of the renal vascular beds in HHCy due to deficiency of renal H₂S content (77).

HHCy in association with decreased H₂S in the kidney is also associated with systemic hypertension, increase in the expression of p47 phox subunit of NADPH oxidase (NOX), glomerular infiltration with macrophages, and type IV collagen accumulation; these changes were also ameliorated by NaHS (78).

HHCy also affects cardiomyocytes directly by the inhibition of H₂S pathway that is associated with increased oxidative stress, mitochondrial dysfunction, and apoptosis (12, 95). In the brain, HHCy induces the disruption of the blood-brain barrier (39), a reduction of the cerebral blood flow (40), an increase in oxidative and nitrosative stress (39), and an increase in cerebral inflammation (39, 40). The fact that all the consequences of HHCy on the vasculature, the myocardium, and the brain are counteracted by H₂S donors (12, 39, 40) suggests that H₂S deficiency is the major mechanism leading to tissue injury in HHCy. This is reinforced by the study showing that a triple gene therapy (CBS-CSE-3-MST) blocks the effects of HHCy on vasorelaxation in an *ex vivo* renal artery culture; this approach also restores the levels of vascular endothelial growth factor (VEGF), CD31 and reduces elevated endostatin levels (79).

Chronic kidney disease of diverse etiology is associated with abnormalities in H₂S metabolism. Reduced expression of CBS, CSE, and H₂S content in circulating leukocytes has been observed in end-stage kidney disease patients on maintenance hemodialysis (71). Removal of most of the functioning nephrons by 5/6 nephrectomy results in chronic kidney disease. The production rate of H₂S and the expression of CBS, CSE, and 3-MST were selectively reduced in the kidney and the liver in rats at 12 weeks after 5/6 nephrectomy (4). These changes were associated with an increase in the markers of oxidative stress, including the expression of NADPH oxidase 4 (NOX4) in the kidney, suggesting that deficiency of H₂S could contribute to the pathogenesis of chronic kidney disease by promoting oxidative stress (4).

Hypertension

Hypertension affects ~20–25% of the adult population in the Western world. In primary hypertension, genetic factors are believed to interact with environmental factors in order to elevate the blood pressure. Mice lacking CSE display H₂S deficiency and hypertension due to reduced endothelium-mediated vasodilation (100); administration of NaHS lowers the blood pressure in both the hypertensive CSE knockout mice (100) and the normotensive mice (87). Recent studies have identified H₂S as the endothelium-derived hyperpolarizing factor (68, 90) that can directly induce vasorelaxation. Further studies have shown that H₂S induces vasorelaxation through activation of the cAMP/PKA signaling pathway, activation of calcium signaling (104), and activation of ATP-dependent K⁺ (KATP) channels and low-threshold voltage-gated K⁺ (KCNQ) channels (44).

In rats, dexamethasone-induced hypertension is accompanied by a reduction of the H₂S-producing enzymes CBS and CSE in the vasculature (mesenteric arterial bed and carotid artery) (15), implying that the reduction of endogenous production of H₂S could contribute to the hypertension in this model. In spontaneously hypertensive rats (SHR), a high salt diet causes hypertension that is accompanied by a reduction of renal CBS protein expression and endogenous H₂S production; administration of NaHS inhibits salt-induced hypertension and activation of the intra-renal renin-angiotensin system (33).

Systemic infusion of angiotensin II in mice causes hypertension that is accompanied by vascular oxidative stress, reduction in NO bioavailability, and reduced aortic endothelium function; these effects were worsened by a CSE inhibitor and reversed by an H₂S donor (3).

H₂S/NO crosstalk

In rats, inhibition of endogenous NO production by NO synthase (NOS) inhibitors, such as N ω -nitro-L-arginine methyl ester (L-NAME), causes hypertension that can be rescued by the administration of NaHS, which also restores NO bioavailability (37, 105). These data suggest the existence of an H₂S/NO crosstalk in the control of blood pressure. In support of this hypothesis, administration of H₂S to normotensive rats lowers blood pressure, an effect that is blocked by an NOS inhibitor, and the hypotensive response to NO donor sodium nitroprusside is abrogated by a CSE inhibitor (91). Interestingly, the effects of H₂S donor and

NO donor are independent of one another in the SHR rats (91), suggesting that the H₂S-NO crosstalk may vary with the model of hypertension.

Pre-eclampsia. Pre-eclampsia is a disorder of pregnancy that is characterized by high blood pressure and proteinuria (19). Circulating H₂S levels are reduced in women with pre-eclampsia (94). Studies conducted in rodents have suggested that excess circulating soluble fms-like tyrosine kinase 1 (sFlt1), a truncated VEGF receptor, contributes to the pathogenesis of pre-eclampsia (61). In placental explants, inhibition of CSE activity or genetic ablation of CSE significantly increased the expression and secretion of sFlt1 (94), and in a mouse model of pre-eclampsia, administration of a slow-releasing H₂S donor reduced circulating (sFlt1) and restored fetal growth (94).

Adenovirus-mediated sFlt1 overexpression in rats leads to hypertension and proteinuria that are similar to pre-eclampsia; administration of NaHS to these rats reduces circulating sFlt1, blood pressure, and proteinuria in association with restoration of circulating VEGF levels, in part due to stimulation of VEGF synthesis by the kidney podocytes (31).

These data suggest that endogenous H₂S is required for a healthy placental vasculature and that a decrease in endogenous H₂S production could contribute to the pathogenesis of pre-eclampsia. In support of this hypothesis, a single nucleotide polymorphism in the CSE gene has been found in association with pre-eclampsia (66).

Diabetes

Diabetic kidney disease

Diabetes is associated with clinically significant kidney injury in about a third of the patients. Kidney injury in diabetes is characterized by kidney growth due to hypertrophy; accumulation of extracellular matrix proteins leading to fibrosis of glomerular and tubulointerstitial compartments; excessive urinary loss of proteins, particularly albumin (proteinuria, albuminuria); and loss of waste clearance function over time. In fact, diabetes is the most common cause of end-stage kidney disease in the United States.

Plasma levels of H₂S in patients with type 2 diabetes are lower than in normoglycemic humans (35), and they negatively correlate with markers of adiposity (96). Among chronic dialysis patients, plasma H₂S levels have been reported to be lower in diabetic patients compared with those with nondiabetic cause of end-stage kidney disease; plasma H₂S levels significantly correlated with indices of uremic atherosclerotic disease, suggesting that reduced H₂S may contribute to the latter (52).

Recent investigations have shown that the renal cortical expression of H₂S-producing enzymes CBS and CSE is reduced in rodents with type 1 or type 2 diabetes (49, 101, 106). Administration of NaHS inhibits diabetes-induced increase in the expression of transforming growth factor- β , the pro-fibrogenic cytokine, glomerular and tubulointerstitial deposition of extracellular matrix proteins, oxidative stress, and proteinuria, indicating that H₂S deficiency contributes to the development of kidney injury in diabetes (101, 106).

H₂S promotes vasodilation and reduces blood pressure in CSE knockout mice with systemic hypertension (100). Changes in renal blood flow and intraglomerular

hemodynamics constitute an important contributory mechanism of injury in diabetic kidney disease. In view of its vasoactivity, it is likely that H₂S participates in the regulation on renal microcirculation in diabetes. In the initial stages, the renal blood flow and glomerular filtration rate are increased in diabetic kidney disease; whether a transient local increase in glomerular microvascular H₂S generation contributes to this phenomenon is not known and needs to be studied.

In established diabetic kidney injury in the Akita mice with type 1 genetic diabetes, Kundu *et al.* have employed multiple mutually confirmatory approaches, including renal resistive index, to show a decrease in renal cortical blood flow that was associated with reduced vascular density; these changes were improved by the administration of NaHS (46).

Changes in renal microcirculation could include changes in glomerular and/or peritubular capillary systems in diabetic mice. Fibrosis of the tubulo-interstitial compartment in the kidney positively correlates with poor outcome in diabetic kidney disease (63). However, changes in the structure and function of peritubular capillaries in diabetic kidney disease have not been well understood. Renal resistance index ap-

pears to correlate inversely with peritubular capillary density in kidney biopsy in patients with diabetic kidney disease (42); this is probably an indirect correlation, since vascular resistance is a function of arterial blood vessels. Peritubular capillary diameter and blood flow are reported to be reduced in type 1 diabetic mice; administration of NaHS reversed these changes.

Matrix remodeling by metalloproteinases may be involved in reduced H₂S production in the kidney in diabetes. MMP-9 expression and activity are increased in the renal parenchyma in Akita mice with type 1 diabetes due to oxidative stress. Using MMP9 knockout mice in their studies, Kundu *et al.* have shown that MMP-9 is involved in the reduced renal expression of CBS and CSE, leading to a decrease in H₂S generation in diabetic mice (47). These investigators have proposed a link between MMP-9 activation, reduced H₂S generation, and activation of N-methyl-D-aspartate receptor, leading to abnormal regulation of the gap junction proteins, connexins, that may be involved in renovascular remodeling (47). H₂S inhibits diabetes-induced kidney injury by several other mechanisms, including inhibition of signaling mechanisms that govern protein synthesis.

FIG. 3. Signaling pathways involved in H₂S amelioration of high glucose-induced protein synthesis. NaHS, sodium H₂S; CaMKK β , Ca(2+)/calmodulin-dependent protein kinase kinase β ; LKB1, liver kinase B1; AMPK, AMP-activated protein kinase; mTOR, mechanistic target of rapamycin; eIF4E, eukaryotic initiation factor 4E; 4E-BP1, eIF4E-binding protein 1; eIF4A, eukaryotic initiation factor 4A; eIF4G, eukaryotic initiation factor 4G; PDCD4, programmed cell death protein 4; p70S6K, p70 S6 kinase; eEF2, eukaryotic elongation factor 2; eEF2K, eEF2 kinase.

Kidney hypertrophy and an increase in matrix protein deposition require stimulation of protein synthesis. mRNA translation is a rate-limiting step in the control of protein synthesis (32), and it is under the direct control of the mechanistic target of rapamycin (mTOR). Studies from our group have shown that the phosphatidylinositol 3 kinase-Akt-mTORC1 signaling pathway is activated, coinciding with renal hypertrophy and onset of matrix protein deposition in rodent models of diabetes (21, 50, 57, 59, 74). In fact, rapamycin, an inhibitor of mTOR, ameliorates kidney injury in early stages of diabetes (74). Recent studies have also shown that reduction in the activity of kidney AMP-activated protein kinase (AMPK) in diabetes permits stimulation of mTOR; thus, stimulation of AMPK ameliorates diabetes-induced kidney injury (18, 50, 80).

The signaling mechanism of NaHS amelioration of high glucose-induced kidney injury involves stimulation of AMPK, which, in turn, inhibits high glucose-induced mTORC1 activity, leading to a reduction in kidney cell hypertrophy and increased matrix protein expression (48, 49). AMPK activity is dependent on Thr172 phosphorylation of the α subunit of the kinase, which is under the control of either LKB1 or calcium calmodulin kinase kinase- β . Employing siRNA against these enzymes, Lee *et al.* showed that H₂S-induced AMPK phosphorylation is dependent on calcium calmodulin kinase kinase- β (49) (Fig. 3).

mRNA translation, a crucial step in peptide synthesis, consists of initiation, elongation, and termination phases (41). mTORC1 activates mRNA translation, which is required for high glucose induction of matrix protein synthesis (50, 58, 74). In the resting state, the eukaryotic initiation factor 4E (eIF4E) and eIF4A are held inactive by their binding partners, 4E-BP1 and Programmed Cell Death protein 4 (PDCD4), respectively (41). Phosphorylation of 4E-BP1 by mTORC1 releases eIF4E, which binds to eIF4A and eIF4G to form eIF4F complex that promotes mRNA translation (84).

In kidney epithelial cells exposed to high glucose, mTORC1 activity was stimulated as indicated by the increase in phosphorylation of its direct substrates 4E-BP1 and p70S6 kinase that was abolished by NaHS (Fig. 3). High glucose reduced the expression of PDCD4, which was inhibited by NaHS (49). Additionally, high glucose promoted the dissociation of eIF4A from PDCD4 and increased its association with eIF4G; these reactions were also abrogated by NaHS (49).

The elongation phase of translation is facilitated by the eukaryotic elongation factor 2 (eEF2), which is active when dephosphorylated (41). Phosphorylation of eEF2 is under the control of eEF2 kinase; p70S6 kinase, the mTORC1 substrate, phosphorylates eEF2 kinase and inhibits its activity (41). Thus, mTORC1 also regulates the elongation phase of mRNA translation. High glucose promoted the phosphorylation of eEF2 kinase and the dephosphorylation of eEF2; NaHS inhibited these changes (Fig. 3). These observations showed that H₂S abrogates high glucose-stimulated initiation and elongation phases of mRNA translation by inhibiting mTORC1 in diverse renal cells.

A fascinating new development in this area is that the ameliorative effect of H₂S in kidney cells that are exposed to high glucose involves NO. Diabetes-induced kidney injury is dependent on the production of ROS, mainly by the activation of NOX4 (25). Thus, diabetes-induced kidney hypertrophy, albuminuria, and extracellular matrix protein accumulation are inhibited in NOX4 knockout mice and by

the administration of NOX4 inhibitors (25, 36, 75, 92). H₂S ameliorates oxidative stress by inhibiting mitochondrial ROS generation (88), increasing GSH production (43), and promoting the transcription of antioxidant genes via the activation of Nrf2 transcription factor (10, 23, 27). H₂S has also been reported to inhibit Hcy-induced NOX4 expression in the brain endothelial cells (40). Whether H₂S regulation of NOX4 involves NO is not known.

We studied high glucose-induced regulation of NOX4 by H₂S in the kidney proximal tubular epithelial cells (unpublished). High glucose-induced increase in NOX4 expression, ROS generation, and matrix protein laminin γ 1 expression was inhibited by H₂S by the activation of AMPK. H₂S inhibition of high glucose-induced NOX4 expression was abrogated by L-NAME, an inhibitor of NOS. L-NAME abolished the inhibitory effect of H₂S on high glucose-induced laminin γ 1 expression. In exploring the type of NOS involved, we found that H₂S did not affect the expression of eNOS but increased the expression of inducible nitric oxide synthase (iNOS). siRNA against iNOS abolished the ameliorative effects of H₂S on high glucose-induced NOX4 expression. These data suggest that H₂S augments iNOS expression to increase NO production, which inhibits high glucose-induced NOX4, ROS, and the downstream consequence of matrix increment (Fig. 4A).

Since NaHS is unacceptable to humans because of its foul smell, we investigated whether phosphodiesterase-5 (PDE-5) inhibitors can promote H₂S generation in kidney cells, the rationale being that the protective effect of PDE5 inhibitors on cardiac ischemia reperfusion injury is H₂S dependent

FIG. 4. Signaling pathways activated by H₂S in renal cells. (A) Signaling pathways involved in H₂S amelioration of high glucose-induced renal extracellular matrix increment in kidney cells. (B) Signaling pathways involved in tadalafil (a phosphodiesterase-5 inhibitor)-induced amelioration of high glucose-induced renal extracellular matrix increment in kidney cells. iNOS, inducible nitric oxide synthase; NO, nitric oxide; Nox4, NADPH oxidase 4; mTORC1, mechanistic target of rapamycin complex 1.

(73). In renal podocytes exposed to high glucose, tadalafil, a long-acting PDE-5 inhibitor, increased the CSE expression, H₂S generation, and AMPK stimulation, leading to inhibition of mTOR and a reduction in cell hypertrophy and matrix protein expression. The mechanism of tadalafil regulation of CSE involved not only an increase in cGMP, a well-known effect of that agent due to PDE5 inhibition, but also stimulation of iNOS expression (48) (Fig. 4B).

Together, these observations suggest that H₂S recruits NO to ameliorate high glucose-induced kidney cell injury, whereas tadalafil is employed as NO upstream of H₂S. These data show a high degree of integration of activities of the two physiological gases, NO and H₂S, in the kidney. The surprising development is the ameliorative role played by iNOS in the aforementioned experiments on high glucose-induced kidney cell injury; iNOS is generally believed to mediate tissue injury. These observations emphasize the fact that whether iNOS mediates or ameliorates tissue injury is highly context dependent. PDE-5 inhibitors have been reported to ameliorate diabetic kidney injury in rodent models (20); however, whether H₂S mediates the salutary effect of PDE-5 inhibitor was not examined in those studies.

Type 1 diabetes

Type 1 diabetes mellitus results from the autoimmune destruction of the insulin-producing β cells in the islets of Langerhans in the pancreas, resulting in severe insulin deficiency. In mice with STZ-induced type 1 diabetes, STZ directly activates CSE in the pancreatic β cells to increase the local H₂S production, leading to cell death. STZ-induced β cell death is attenuated in mice lacking CSE. Furthermore, NaHS significantly reduces insulin secretion and glucose tolerance but does not increase blood glucose in normoglycemic mice (99). Although intra-islet levels of H₂S are increased in mice with type 1 diabetes, plasma levels of H₂S are lower in mice with type 1 diabetes than in normoglycemic control mice (17). Further studies are needed to understand the role of H₂S in the destruction of insulin-secreting cells of the pancreas in spontaneous models of diabetes as distinct from the cytotoxicity induced by STZ.

Conclusion

H₂S is a bioactive gas with far-reaching actions that are comparable to those of NO and carbon monoxide. Its regulation of cellular processes is highly cell specific; even in individual cells, its role as a mediator or as an ameliorator of cell injury is highly context specific. In-depth investigations are urgently needed to define its role in individual physiological and pathological states and its evaluation as a potential therapeutic target. For example, inflammatory diseases of the kidney prominently involve glomerular endothelial cells that produce H₂S. It is possible that kidney H₂S metabolism in dysregulated in inflammatory diseases such as systemic lupus erythematosus.

Several agents have been identified as therapeutic sources of H₂S for interventional use, and systematic evaluation is in progress in a variety of nonrenal diseases (93). Following a more complete understanding of the role of H₂S in individual acute and chronic kidney diseases due to inflammatory or toxic causes such treatment approaches may prove to be useful in kidney diseases as well.

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Abbreviations Used

3-MST = 3-mercaptopyruvate sulfurtransferase
 AAT = aspartate aminotransferase
 AMPK = AMP-activated protein kinase
 CaMKK β = calcium calmodulin kinase kinase β
 CBS = cystathionine β -synthase
 CSE = cystathionine γ -lyase
 DAO = D-amino acid oxidase
 eEF2 = eukaryotic elongation factor 2
 eEF2K = eEF2 kinase.
 eIF4E = eukaryotic initiation factor 4E
 ENaC = epithelial sodium channel
 ER = endoplasmic reticulum
 H₂S = hydrogen sulfide
 Hcy = homocysteine
 HHcy = hyperhomocysteinemia
 iNOS = inducible nitric oxide synthase
 LKB1 = liver kinase B1
 L-NAME = N ω -nitro-L-argininemethylester
 MMP = matrix metalloproteinase
 mRNA = messenger RNA
 mTOR = mechanistic target of rapamycin
 NaHS = sodium H₂S
 NO = nitric oxide
 NOS = NO synthase
 NOX = NADPH oxidase
 Nox4 = NADPH oxidase 4
 p70S6K = p70 S6 kinase
 PAG = propargylglycine
 PDCD4 = programmed cell death protein 4
 PDE-5 = phosphodiesterase-5
 ROS = reactive oxygen species
 SHR = spontaneously hypertensive rats
 STZ = streptozotocin
 VEGF = vascular endothelial growth factor