Ischemia-reperfusion reduces cystathionine-β-synthase-mediated hydrogen sulfide generation in the kidney

Zhibin Xu,1,3 Gamika Prathapasinghe,1,3 Nan Wu,2,3 Sun-Young Hwang,1,3 Yaw L. Siow,2,3,4 and Karmin O1,2,3

1Department of Animal Science and 2Department of Physiology, University of Manitoba, and 3St. Boniface Hospital Research Centre, Winnipeg, Manitoba; and 4Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada

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Xu Z, Prathapasinghe G, Wu N, Hwang SY, Siow YL, O K. Ischemia-reperfusion reduces cystathionine-β-synthase-mediated hydrogen sulfide generation in the kidney. Am J Physiol Renal Physiol 297: F27–F35, 2009. First published May 13, 2009; doi:10.1152/ajprenal.00096.2009.—Cystathionine-β-synthase (CBS) catalyzes the rate-limiting step in the transsulfuration pathway for the metabolism of homocysteine (Hcy) in the kidney. Our recent study demonstrates that ischemia-reperfusion reduces the activity of CBS leading to Hcy accumulation in the kidney, which in turn contributes to renal injury. CBS is also capable of catalyzing the reaction of cysteine with Hcy to produce hydrogen sulfide (H2S), a gaseous molecule that plays an important role in many physiological and pathological processes. The aim of the present study was to examine the effect of ischemia-reperfusion on CBS-mediated H2S production in the kidney and to determine whether changes in the endogenous H2S generation had any impact on renal ischemia-reperfusion injury. The left kidney of Sprague-Dawley rat was subjected to 45-min ischemia followed by 6-h reperfusion. The ischemia-reperfusion caused lipid peroxidation and cell death in the kidney. The CBS-mediated H2S production was decreased, leading to a significant reduction in the renal H2S level. The activity of cystathionine-γ-lyase, another enzyme responsible for endogenous H2S generation, was not significantly altered in the kidney upon ischemia-reperfusion. Partial restoration of CBS activity by intraperitoneal injection of the nitric oxide scavenger, 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide not only increased renal H2S levels but also alleviated ischemia-reperfusion-induced lipid peroxidation and reduced cell damage in the kidney tissue. Furthermore, administration of an exogenous H2S donor, NaHS (100 μg/kg), improved renal function. Taken together, these results suggest that maintenance of tissue H2S level may offer a renal protective effect against ischemia-reperfusion injury. Hcy is an intermediate amino acid formed during the metabolism of methionine to cysteine. Hcy can be metabolized via two major pathways (Fig. 1A), namely, the remethylation pathway (remethylation of Hcy to methionine) and the transsulfuration pathway (transsulfuration of Hcy to cysteine). It is estimated that the majority of Hcy (78%) in the rat kidney is metabolized through the transsulfuration pathway (12). Cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CGL) catalyze the sequential reactions in the transsulfuration pathway (Fig. 1A). While both CBS and CGL are in the β-family of pyridoxal 5’-phosphate (PLP) dependent enzymes, CGL catalyzes a γ-replacement reaction that is unique among PLP-dependent enzymes (1, 6). CBS is unique in that it is the only known PLP-dependent enzyme that is also heme-dependent (15). Therefore, changes in the redox status of the heme can modulate CBS activity (29). In the standard transsulfuration pathway, the initial reaction is the condensation of Hcy with serine to form cystathionine. The reaction catalyzed by CBS is the rate-limiting step in the transsulfuration pathway. In addition to their role in the standard transsulfuration pathway, both CBS and CGL are responsible for the endogenous synthesis of hydrogen sulfide (H2S), referred to as the desulphydrase pathway (Fig. 1B). CBS catalyzes the synthesis of H2S from cysteine and Hcy by a β-replacement reaction (4). The CGL catalyzes an α,β-disulfide elimination reaction that results in the production of pyruvate and thiocysteine, thiocysteine is then nonenzymatically decomposed to H2S and cysteine (6, 26). Furthermore, a recent study has revealed that both cysteine and Hcy can serve individually or together as substrates for H2S generation catalyzed by CGL (6).

In recent years, H2S has emerged as an important biologically active compound due to its cytotoxic effect and physiological role in health and disease. Administration of H2S donor (14 μmol/kg) results in lung inflammation and increases lung and liver myeloperoxidase activity and plasma TNF-α concentration (16). Exogenous H2S (180 μmol/kg) significantly increases the infarct size after permanent occlusion of the middle cerebral artery (22). Apart from its deleterious effects, increasing evidence suggests that H2S also serves as an essential mediator at physiological concentrations. Its ability to attenuate myocardial infarction injury and enhance ulcer healing has been observed in several animal models. The cytoprotective effect of H2S against myocardial infarction was observed in a murine model (7). The endogenously produced H2S and exogenously injected H2S donors were shown to accelerate the healing of gastric ulcers (33, 43). Recently, Xia et al. (38) reported that H2S participates in the control of renal function under normal conditions via both vascular and tubular actions in the kidney. It becomes increasingly apparent that H2S is

ISCHEMIA-REPERFUSION INJURY is one of the common causes for delayed function of renal allograft and is associated with poor long-term renal function (3). Complete and prolonged interruption of renal arterial blood flow occurs during renal transplantation or surgical procedures such as nephrolithotomy, parenchymal sparing surgery for renal tumors, and renal arterial surgeries. Prolonged renal ischemia can lead to acute renal failure. It has been estimated that ischemic insult, especially during renal transplantation, is responsible for 20–30% primary graft dysfunction (19). Our recent study has demonstrated that homocysteine (Hcy) plays a detrimental role in ischemia-reperfusion injury in the kidney (20).

Address for reprint requests and other correspondence: K. O, Integrative Biology Laboratory, CCARM, St. Boniface Hospital Research Centre, 351 Tache Ave. Winnipeg, Manitoba, Canada R2H 2A6 (e-mail: karmino@sbrc.ca).

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Fig. 1. Metabolism of homocysteine (Hcy) and generation of hydrogen sulfide (H₂S). Cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CGL) involved in the reactions in the standard transsulfuration and remethylation pathway for Hcy metabolism (A) and the desulphydrase pathway for H₂S generation (B) are depicted. ATP, adenosine triphosphate; SAM, S-adenosylmethionine; SAM synthetase, S-adenosylmethionine synthetase; THF, tetrahydrofolate; 5-MTHF, 5-methyl tetrahydrofolate; Met synthase, methionine synthase; SAH, S-adenosylhomocysteine; SAH hydrolase, S-adenosylhomocysteine hydrolase; Met synthase, methionine synthase; THF, tetrahydrofolate; 5-MTHF, 5-methyl tetrahydrofolate.

A. The homocysteine metabolism

- ATP → Methionine
- SAM synthetase → SAM
- SAM → Met synthase → Cystathionine + H₂O
- CGL → L-cysteine + α-ketobutyric acid

B. The desulphydrase pathway

- Hcy + Cystathionine + H₂S → CBS → Cystathionine + H₂O
- CGL → NH₄⁺
- Thiocysteine + pyruvate → L-cysteine + H₂S

synthesized endogenously and plays important roles in body function (23, 45). In rats, the highest concentration of H₂S (300 nmol/g of tissue) is found in the brain, while a substantial amount of H₂S is detected in the liver, kidney, and pancreas (11, 35, 42).

Although CBS is the predominant H₂S-generating enzyme in the brain and the nervous system (8, 35), this enzyme is also highly expressed in the liver and the kidney (26). Our recent study has revealed that the activity of CBS in the standard transsulfuration pathway is markedly reduced in rat kidneys subjected to ischemia-reperfusion (20). Partial restoration of CBS activity in rats treated with an nitric oxide (NO) scavenger not only has reduced Hcy levels in the kidney but also alleviated ischemia-reperfusion-induced renal injury (21). The aim of the present study was to examine the effect of ischemia-reperfusion on CBS-mediated H₂S production in the rat kidney and to determine whether changes in endogenous H₂S generation had any impact on ischemia-reperfusion-induced kidney injury.

MATERIALS AND METHODS

Kidney ischemia-reperfusion. Male Sprague-Dawley rats weighing 250–300 g were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg). Rats were kept on a heat pad and the rectal temperature was maintained at 37°C throughout the experimental procedure. Renal ischemia was induced by clamping the left renal artery for 45 min with a nontraumatic vascular clamp (20, 21, 46). Rats were then subjected to 6-h reperfusion after ischemia. A sham-operated group of rats were subjected to the same surgical procedure. Renal ischemia was induced by clamping the left renal artery for 45 min with a nontraumatic vascular clamp (20, 21, 46). Rats were then subjected to 6-h reperfusion after ischemia. A sham-operated group of rats were subjected to the same surgical procedure. Renal ischemia was induced by clamping the left renal artery for 45 min with a nontraumatic vascular clamp (20, 21, 46).

Measurement of plasma H₂S. The initial reaction mixture contained 50 µl plasma and 200 µl of 15:1 (vol/vol) of 1% zinc acetate and 12% NaOH. After incubation at 37°C for 10 min, 1.75 ml of H₂O, 200 µl of 20 mM N,N,dimethyl-p-phenylenediamine sulfate dissolved in 7.2 M HCl, and 200 µl of 30 mM FeCl₃ dissolved in 1.2 M HCl were added into the reaction mixture. The reaction was allowed to proceed for 10 min in a dark box, and the absorbance of the resulting reaction mixture was measured at 670 nm. The NaHS with the concentrations of 2.5, 25, 50, 100, and 200 µM were used as the standard.

Determination of CBS activity and the H₂S level in the kidney. The assay for CBS activity in the H₂S production pathway was based on a method described by Stipanuk and Beck (26). Kidney tissue was homogenized in 50 mM potassium phosphate buffer (pH 6.9, 5%, wt/vol) followed by centrifugation at 15,000 × g for 30 min at 4°C. The supernatant was collected, and the CBS activity was measured in a reaction mixture containing 0.3 ml supernatant, 10 mM l-cysteine, 10 mM DL-Hcy, 2 mM pyridoxal-5'-phosphate, and 0.05 mM S-adenosylmethionine prepared in 100 mM potassium phosphate buffer (pH 7.4). The reaction was carried out in a 25-ml Erlenmeyer flask covered with a septum stopper containing a plastic center well. A folded 2 cm × 2.5 cm piece of filter paper was soaked in 0.5 ml 15:1 (vol/vol) mixture of 1% (wt/vol) zinc acetate and 12% (wt/vol) NaOH in a 2-ml volume tube. The tube was placed in the flask and N₂ was blown into the flask. The flask was covered immediately and placed in a water bath at 37°C for 30 min. The reaction was stopped by injecting 0.5 ml of 30% (wt/vol) TCA into the flask. After further incubation for 60 min at 37°C, the filter paper was transferred to a test tube containing 3.5 ml water. Then 0.4 ml of 20 mM N,N,dimethyl-p-phenylenediamine sulfate dissolved in 7.2 M HCl and 0.4 ml of 30 mM FeCl₃ dissolved in 1.2 M HCl were added into the reaction mixture. The reaction was allowed to proceed for 10 min in a dark box and the absorbance of the resulting solution was measured at 670 nm. The NaHS with the concentrations of 2.5, 25, 50, 100, and 200 µM were used as the standard. The tissue H₂S level was determined in the same way with the exception that 30% TCA (0.5 ml) was added before the incubation period.

Lipid peroxidation assay. The degree of lipid peroxidation in the kidney tissue was determined by measuring the malondialdehyde (MDA) level with thiobarbituric acid reactive substances assay (18, 27). A 10% (wt/vol) kidney homogenate was prepared in 100 mM...
Molecular Biochemical, Quebec, Canada) followed by counterstaining with Mayer’s hematoxylin. TUNEL-positive cells were recognized by focal nuclear staining. As a positive control, a section of sham-operated kidney was pretreated with DNase to differentiate the appearance of apoptotic cells from nonapoptotic cells. The number of TUNEL-positive cells in the renal cortex and medulla were counted in a blinded manner in 10 microscopic fields under a light microscope at a magnification of ×400.

Propidium iodide staining. Propidium iodide is membrane impermeable and generally excluded by viable cells. Propidium iodide fluorescence staining is used for identifying dead cells and used as an index for necrotic cells (31). Briefly, a portion of frozen kidney tissue was cut in Shandon Cryotome apparatus (model Microm HM525; Thermo Scientific). The slides containing sliced kidney sections (10 μm) were fixed in 10% neutral-buffered formaldehyde for 30 min and in 80% ethanol for 2 h. The sections were incubated with 1 μM propidium iodide in PBS buffer containing 100 μg/ml DNase-free RNase for 30 min in a dark box. The number of propidium iodide-stained positive cells (red) in the cortex and medulla were counted in a blinded manner in 10 microscopic fields under a fluorescence microscope at a magnification of ×400.

Statistical analysis. Results were analyzed using Student’s t-test and ANOVA followed by Newman-Keuls multiple comparison test as appropriate. Data were presented as means ± SE. A P value ≤ 0.05 was considered significant.

**RESULTS**

Reduction of H₂S levels in the kidney and in the plasma during renal ischemia-reperfusion. The H₂S was quantified in kidneys and in the plasma after 45-min ischemia followed by

![Fig. 2. Measurement of H₂S concentration in the kidney and plasma.](image)

KCl solution containing 3 mM EDTA followed by centrifugation at 600 g for 15 min at 4°C. An aliquot of supernatant was added to the reaction mixture containing 8.1% SDS, 20% acetic acid, 0.8% thio-barbituric acid, and water. After incubation at 95°C for 60 min, the reaction mixture was cooled down and centrifuged at 2,000 g for 15 min at 4°C. The absorbance for MDA formed in the reaction mixture was measured by spectrophotometer at 532 nm. MDA was used as the standard, and the amount of MDA correlated with the amount of lipid peroxides in the tissue.

**Determination of CGL activity.** A 50% (wt/vol) kidney homogenate was prepared in 50 mM potassium phosphate buffer (pH 6.9) followed by centrifugation at 15,000 g for 30 min at 4°C. The resulting supernatant was used for the CGL activity assay carried out in a 96-well microplate. The reaction mixture, in a total volume of 200 μl per well, contained 100 mM potassium phosphate buffer (pH 7.4), 4.0 mM L-cystathionine, 0.125 mM pyridoxal-5′-phosphate, 0.32 mM NADH, 1.5 units of lactate dehydrogenase, and 5 μl kidney homogenate (25). The decrease in optical density at absorbance of 340 nm was monitored with a microplate reader (Spectramax-5; Molecular Devices, Sunnyvale, CA) at 37°C for 30 min. Blank reactions were performed using the same reaction; however, L-cystathionine was omitted. Maximum velocities were calculated from the linear portion of the graphs.

**Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling.** Renal ischemia-reperfusion in animal models leads to both necrotic and apoptotic forms of cell death (20). DNA fragmentation and terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL staining) were performed in renal sections as an index of cell injury (9, 46). Briefly, a portion of kidney was fixed in 10% neutral-buffered formaldehyde overnight and then embedded in paraffin. DNA fragments in the sliced sections were labeled with 3 μM biotin-conjugated dUTP and 0.3 U/μl TdT (Roche

![Fig. 3. Effect of NaHS and DTT administration on plasma creatinine levels.](image)
6-h reperfusion. The H₂S levels were significantly lower in kidneys subjected to ischemia-reperfusion than in those of the sham-operated group (Fig. 2A). In accordance, plasma H₂S levels were significantly decreased in rats subjected to renal ischemia-reperfusion (Fig. 2B). There was a significant increase in plasma creatinine levels in rats that were subjected to renal ischemia-reperfusion, indicating that renal function was impaired in this group of rats (Fig. 3A).
Effect of exogenous H$_2$S administration on ischemia-reperfusion-induced renal injury. To investigate whether a reduction of H$_2$S level played any role in impaired renal function, exogenous H$_2$S donor (NaHS) was given to rats that were subjected to ischemia-reperfusion. First, administration of NaHS to those rats significantly reduced plasma creatinine levels (Fig. 3A). Second, the effect of NaHS administration on ischemia-reperfusion-induced oxidative stress in the kidney was investigated by measuring the level of MDA in the kidney tissue. Ischemia-reperfusion caused a significant increase in the level of MDA in the kidney (Fig. 3B), indicating that lipid peroxidation was increased. The MDA level in the kidney was significantly reduced in rats that received NaHS treatment prior to renal ischemia-reperfusion (Fig. 3B). To determine whether the protective effect is specific for NaHS, rats were intraperitoneally administered 50 µg/kg of DTT, another thiol-containing compound. Treatment with DTT had a similar effect as that of NaHS injection on the levels of plasma creatinine and renal lipid peroxides (Fig. 3).

Furthermore, the effect of NaHS administration on ischemia-reperfusion-induced cell death in the kidney was examined. TUNEL-positive cells were present in the cortex and medulla of the rat kidney upon ischemia-reperfusion, indicating cell death due to apoptosis and necrosis (Fig. 4, A and B). Administration of NaHS to rats subjected to renal ischemia-reperfusion significantly reduced the number of TUNEL-positive cells in the kidney tissue (Fig. 4, A and B). To further distinguish apoptotic cells and necrotic cells, propidium iodide staining was performed. Propidium iodide positive cells (representing necrotic cells) were detected in the cortex and medulla of kidneys subjected to ischemia-reperfusion (Fig. 4, C and D). Injection of NaHS to rats subjected to renal ischemia-reperfusion also significantly reduced the number of propidium iodide-positive cells in the kidney tissue (Fig. 4, C and D). These results suggested that treatment with exogenous H$_2$S donor could offer a renal protective effect against ischemia-reperfusion.

Effect of ischemia-reperfusion on the activities of CBS and CGL in the kidney. To investigate the mechanism by which H$_2$S metabolism was impaired in the kidney during ischemia-reperfusion, the activities of two enzymes (CBS and CGL) responsible for generating endogenous H$_2$S in the kidney were determined. First, the generation of H$_2$S in the kidney tissue homogenate (Table 1). The combined effect of PAG and HA was also determined (Table 1). These results suggested that Hcy and cysteine, individually or both, could serve as substrate for H$_2$S generation in the kidney.

The enzyme activities of CBS and CGL were also determined in kidneys subjected to sham operation or ischemia-reperfusion. CBS activity in the H$_2$S production pathway was markedly decreased in kidneys subjected to ischemia-reperfusion (Fig. 5A), while CGL activity was not significantly changed (Fig. 5B). To further examine the role of CBS in H$_2$S generation in the kidney during ischemia-reperfusion in vivo, HA (50 mg/kg) was administered intraperitoneally to rats. After administration of HA, there was a significant decrease in CBS activity in the H$_2$S production pathway (Fig. 5A) and a reduction of total H$_2$S level (Fig. 5C) in the kidney. Injection of HA resulted in an increase in the plasma creatinine level (Fig. 5D) and lipid peroxidation (Fig. 5E) in the kidney tissue. In another set of experiments, a CGL inhibitor PAG (30 mg/kg) was administered intraperitoneally to rats. Administration of PAG markedly reduced CGL activity in the kidney (Fig. 5B). However, such a treatment did not affect renal H$_2$S level, lipid peroxidation, and plasma creatinine levels (Fig. 5, C–E).

Table 1. Measurement of hydrogen sulfide (H$_2$S) production in the kidney in the presence of various substrates and inhibitors

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Control</th>
<th>PAG</th>
<th>HA</th>
<th>HA+PAG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hcy</td>
<td>0.29±0.05</td>
<td>0.04±0.03*</td>
<td>0.23±0.01</td>
<td>0.04±0.02*</td>
</tr>
<tr>
<td>Cys</td>
<td>1.62±0.13</td>
<td>1.43±0.33</td>
<td>0.11±0.03*</td>
<td>0.13±0.03*</td>
</tr>
<tr>
<td>Hcy+Cys</td>
<td>2.83±0.49</td>
<td>2.85±0.34</td>
<td>0.16±0.10*</td>
<td>0.14±0.10*</td>
</tr>
</tbody>
</table>

Data are mean ± SE (nmol·min$^{-1}$·mg protein$^{-1}$), n = 6. Kidney tissue homogenate (5% wt/vol) was prepared in 50 mM potassium phosphate buffer (pH 6.9). The H$_2$S generation was measured in the kidney homogenate in the presence of 20 mM homocysteine (Hcy), 20 mM cysteine (Cys), and 10 mM Hcy + 10 mM Cys, which were used as substrates individually. Assays were also conducted in the presence of PAG (2 mM) and HA (2 mM). The assays conducted in the absence of inhibitors were used as a control. *P ≤ 0.05 when compared with the value obtained from the corresponding control group.

Fig. 4. Terminal deoxyribonucleotide transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) staining and propidium iodide (PI) staining of the kidney tissue. Ischemia-reperfusion-induced cell death in the kidney was examined. TUNEL-positive cells were present in the cortex and medulla of the rat kidney upon ischemia-reperfusion, indicating cell death due to apoptosis and necrosis (Fig. 4, A and B).
These results further indicated that CBS played an important role in the generation of H$_2$S in rat kidney.

We previously reported that injection of the NO scavenger, 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (PTIO) to rats could partially restore CBS activity in the standard trans-sulfuration pathway in the kidneys that were subjected to ischaemia-reperfusion (2). In the present study, PTIO treatment not only partially restored CBS-mediated H$_2$S generation in the kidney (Fig. 6A) but also significantly reduced lipid peroxidation in the kidney tissue (Fig. 6B). Such a treatment also reduced the plasma creatinine levels in rats subjected to renal ischaemia-reperfusion (Fig. 6C).

DISCUSSION

The novel findings of the present study are 1) ischaemia-reperfusion causes a marked reduction in H$_2$S levels in the kidney; 2) impaired CBS activity is responsible for a decrease in renal H$_2$S synthesis. Such a reduction in CBS-mediated H$_2$S synthesis correlates to renal injury as indicated by increased lipid peroxidation and cell death; 3) partial restoration of CBS activity by an NO scavenger cannot only increase renal H$_2$S levels but also improve renal function; 4) administration of exogenous NaHS is able to alleviate ischaemia-reperfusion-induced kidney injury and improve kidney function. These results suggest that restoration of CBS-mediated H$_2$S synthesis may exert a renal protective effect against ischaemia-reperfusion injury.

Both CBS and CGL are involved in the generation of H$_2$S in the kidney (38). Our results indicate that CBS-mediated but not CGL-mediated H$_2$S generation is impaired in the kidney upon ischaemia-reperfusion. In accordance with the data reported in the literature (13), both Hcy and cysteine are utilized as substrates in the CBS-mediated H$_2$S generation in rat kidney. Several lines of evidence obtained from the present study suggest that impaired CBS-mediated H$_2$S synthesis contributed to renal ischaemia-reperfusion injury. First, the production of H$_2$S in the kidney tissue homogenate was determined in vitro. Inhibition of CBS caused a significant decrease in H$_2$S pro-
activity could lead to a decrease in H2S generation in the function. These results suggested that a reduction of CBS inhibited CBS activity but also caused an impairment of kidney experiments, injection of a CBS inhibitor into rats not only in the kidney and reduced renal function. In another set of kidney injury was evident as indicated by increased cell death of H2S levels in the kidney tissue. Upon ischemia-reperfusion, that were subjected to ischemia-reperfusion. A decrease in the production. Second, the activity of CBS was decreased in kidneys upon ischemia-reperfusion. The kidney CBS injection of 250 μl of 1 mM 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (I-45 min/R-6h+PTIO) at the onset of reperfusion. The kidney CBS activity (A) and lipid peroxides (B) were determined. Plasma creatinine levels (C) were measured. MDA, malondialdehyde. Results are expressed as means ± SE (n = 6). *P ≤ 0.05 when compared with the sham-operated group; #P ≤ 0.05 when compared with the I-45 min/R-6 h group.

duction. Second, the activity of CBS was decreased in kidneys that were subjected to ischemia-reperfusion. A decrease in the activity of CBS was also accompanied by a marked reduction of H2S levels in the kidney tissue. Upon ischemia-reperfusion, kidney injury was evident as indicated by increased cell death in the kidney and reduced renal function. In another set of experiments, injection of a CBS inhibitor into rats not only inhibited CBS activity but also caused an impairment of kidney function. These results suggested that a reduction of CBS activity could lead to a decrease in H2S generation in the kidney. Third, partial restoration of CBS activity significantly increased the level of H2S as well as minimized ischemia-reperfusion-induced lipid peroxidation and cell death in the kidney tissue. Our recent study has identified that increased inducible NO synthase-mediated NO synthesis during the reperfusion period contributes to the reduced CBS activity in the kidney (21). Administration of a NO scavenger (PTIO) to the kidney at the onset of reperfusion results in a significant recovery of CBS activity in the standard transsulfuration pathway (21). In the present study, administration of PTIO to rats also increased CBS activity in the desulphhydration pathway and hence, the H2S levels were increased in kidneys that were subjected to ischemia-reperfusion. Moreover, in PTIO-treated rats that had been subjected to ischemia-reperfusion, there was a significant reduction in lipid peroxide formation and cell death induced by ischemia-reperfusion. The kidney function was significantly improved in PTIO-treated group. Finally, when the exogenous sulfide donor NaHS was administered to rats prior to the induction of ischemia, the degree of lipid oxidation resulting from ischemia-reperfusion was markedly reduced and renal function was improved. Although ischemia-reperfusion caused a decrease in CBS activity in the kidney, it did not seem to affect the activity of CGL. Furthermore, administration of CGL inhibitor markedly reduced the activity of CGL but did not affect the H2S level in the kidney. It was recently reported that deletion of the CGL gene in mice reduced the level of H2S in the serum and in organs, such as heart and aorta (41). However, the histological analysis did not reveal any detectable structural abnormality in kidneys in mice with a genetic deletion of CGL (41). Taken together, the CBS-mediated H2S production appeared to be responsible for a marked decrease in the levels of H2S in kidneys upon ischemia-reperfusion. The regulation of H2S homeostasis might have a renal protective effect against ischemia-reperfusion injury.

Increasing evidence has suggested that H2S plays an important role in many physiological and pathological processes. It is generally believed that H2S, at physiological concentrations, exerts beneficial effects, while at the elevated concentrations it has cytotoxic effects (32). In the brain, H2S acts as a neuro-modulator by enhancing the NMDA receptor-mediated response and inducing the long-term hippocampal potential (8). In the heart, exogenous H2S donors are shown to have a protective effect against myocardial ischemia-reperfusion injury (14, 24). H2S also plays a role in the regulation of vascular function (34). For example, it mimics the vasodilatory properties of other gaseous mediators such as NO and carbon monoxide (28), relaxes the precontracted rat aorta in vitro (2), and lowers blood pressure in rats (5, 44). A recent study indicates that both CBS and CGL are responsible for generation of H2S that participates in regulating renal function (38). In the present study, CBS activity was reduced in kidneys upon ischemia-reperfusion, while no significant change in CGL activity was observed. CGL might be more resistant to ischemia-reperfusion and therefore play an important role in preventing further decline in renal H2S levels. In spontaneously hypertensive rats, plasma H2S levels are much lower than that of the normotensive counterparts (39). The cytoprotective effect of H2S may also be related to its ability to neutralize a variety of reactive molecules including superoxide radical anion (17), hydrogen peroxide (10), peroxynitrite (36), and hypochlorite (37). Additionally, it has been shown that H2S can reduce Hcy-induced free radical generation in vascular smooth muscle cells (40). In accordance with these findings, the present study showed that injection of DTT, a strong reducing agent (30), into rats was able to reduce ischemia-reperfusion-induced oxidative stress and improve renal function. Results from our previous studies...
have shown that a decrease in CBS activity in the kidney upon ischemia-reperfusion impairs Hcy metabolism via the transsulfuration pathway, leading to Hcy accumulation in the kidney (20, 21). The Hcy at elevated levels contributes, in part, to ischemia-reperfusion-induced injury in the kidney (20, 21). It is plausible that a reduction in H2S level combined with an elevation of Hcy level in the kidney upon ischemia-reperfusion may contribute synergistically to kidney injury.

To the best of our knowledge, this is the first study to demonstrate that impaired CBS activity is responsible for a decrease in H2S level in the kidney upon ischemia-reperfusion. Partial restoration of the CBS-mediated H2S synthesis by a NO scavenger (PTIO) or administration of an exogenous sulfide donor (NaHS) can alleviate ischemia-reperfusion-induced oxidative stress and reduce cell death in the kidney. These results suggest that regulation of H2S homeostasis may have therapeutic potential to protect against renal ischemia-reperfusion injury.

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