Hypoxia in the renal medulla: Implications for hydrogen sulfide signaling

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Running title: Hypoxia and renal \( \text{H}_2\text{S} \) signaling

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ABBREVIATIONS: 3-MST, 3-mercaptopyruvate sulfurtransferase; CBS, cystathionine \( \beta \)-synthase; CO, carbon monoxide; COX-2, cyclooxygenase-2; CSE, cystathionine \( \gamma \)-lyase; DCT, distal convoluted tubule; ERK, extracellular signal-regulated kinase; GFR, glomerular filtration rate; HIF-1, hypoxia-induced factor; HO-1, heme oxygenase-1; \( \text{H}_2\text{S} \), hydrogen sulfide; ICAM-1, intercellular adhesion molecule-1; iNOS, inducible NO synthase; JNK, c-Jun N-terminal kinase; \( \text{K}_\text{ATP} \), ATP-sensitive potassium channels; MAPK, mitogen-activated...
protein kinase; MMP, metalloproteinase; mTAL, medullary thick ascending limb; NCC – sodium/chloride cotransporter; NKCC, sodium/potassium/chloride cotransporter; NO, nitric oxide; PGE₂, prostaglandin E₂; ROS, reactive oxygen species; SQR, sulfide:quinone oxidoreductase; TNF-α, tumor necrosis factor-alpha

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Abstract

Hydrogen sulfide (H₂S) is enzymatically generated in mammalian tissues from either L-cysteine or L-homocysteine. H₂S possesses multiple biological activities including regulation of vascular tone and blood pressure. Hydrogen sulfide, produced in endothelial cells, vascular smooth muscle cells and perivascular adipose tissue dilates blood vessels by activating ATP-sensitive potassium channels. In addition, H₂S produced locally within the kidney stimulates natriuresis and diuresis by increasing glomerular filtration and inhibiting tubular sodium reabsorption. Because H₂S is oxidized in mitochondria in pO₂-dependent manner and ambient pO₂ is physiologically low in the renal medulla, it is expected that the activity of H₂S is higher in medullary than in cortical region. H₂S, accumulating in increased amounts in the renal medulla under hypoxic conditions, may function as an oxygen sensor which restores O₂ balance by increasing medullary blood flow, reducing energy requirements for tubular transport, and directly inhibiting mitochondrial respiration. Hypoxia is an important pathogenetic factor in many renal diseases such as ischemia/reperfusion- or nephrotoxin-induced acute renal failure, progression of chronic nephropathies, diabetic nephropathy, and arterial hypertension. Deficiency of endogenous H₂S may contribute to the pathogenesis of these pathologies by compromising medullary oxygenation, and administration of H₂S donors may be of therapeutic value in these disorders.
Introduction

Studies performed during the last decade indicate that, apart from nitric oxide (NO) and carbon monoxide (CO), hydrogen sulfide (H$_2$S) is the third “gasotransmitter” involved in the regulation of various physiological functions including vascular tone and blood pressure, inflammatory reaction, neurotransmission and gastrointestinal system function. H$_2$S is enzymatically synthesized in three metabolic pathways (Fig. 1): (1) desulfhydration of L-cysteine or L-homocysteine by cystathionine γ-lyase (CSE, EC 4.4.1.1), (2) desulfhydration of L-cysteine by cystathionine β-synthase (CBS, EC 4.2.1.22), (3) transamination of L-cysteine by cysteine aminotransferase (identical with aspartate aminotransferase) to 3-mercaptoppyruvate, followed by its desulfhydration to pyruvate by 3-mercaptoppyruvate sulfurtransferase (3-MST, EC 2.8.1.2). Hydrogen sulfide activates ATP-sensitive potassium channels ($K_{ATP}$) in various cells, although many other signaling mechanisms have also been described. H$_2$S is inactivated by binding to hemoglobin to form sulfhemoglobin, excretion in exhaled air, and, first of all, oxidation in mitochondria. Many studies addressed the role of H$_2$S in the regulation of vascular tone and blood pressure. Indeed, it is suggested that H$_2$S deficiency may contribute to the pathogenesis of arterial hypertension both in experimental animal models and in humans. Recently, renal synthesis and activity of H$_2$S have been characterized (Xia et al., 2009). Because, apart from vascular tone, renal sodium handling has a prominent role in the long-term regulation of blood pressure, renal effects of this gas are of great interest for cardiovascular pharmacologist. In addition, it is well established that renal medulla is a hypoxic environment and that H$_2$S metabolism is O$_2$-dependent. In this article, I address the possible relationship between renal hypoxia and H$_2$S signaling in physiologic and pathologic conditions.

**Role of H$_2$S in the regulation of vascular tone and blood pressure**
Initially, it was suggested that in the blood vessels H$_2$S is produced exclusively by CSE expressed in vascular smooth muscle cells, and activates K$_{ATP}$ channels in these cells leading to membrane hyperpolarization, reduced voltage-dependent Ca$^{2+}$ influx and, ultimately, vasorelaxation (Zhao et al., 2001). More recent studies indicate, however, that the situation is more complex. First, H$_2$S is synthesized also by endothelial cells at least in rodents. In mice aortic endothelial cells H$_2$S is synthesized by CSE and its production is stimulated by cholinergic agonists (Yang et al., 2008). Thus, H$_2$S is suggested to be one of the endothelium-dependent relaxing factors. CSE is not expressed in rat endothelial cells, but H$_2$S is synthesized in these cells in 3-MST-dependent manner (Shibuya et al., 2009). H$_2$S is also produced by perivascular adipose tissue (Fang et al., 2009). Moreover, H$_2$S, especially at low concentrations, may trigger some vasoconstrictor mechanisms such as direct inhibition of endothelial NO synthase or inactivation of NO by binding it to form inactive nitrosothiol (Ali et al., 2006). Nevertheless, arterial hypertension is observed in CSE knockout mice, indicating that the net effect of endogenous H$_2$S is definitely antihypertensive (Yang et al., 2008). Plasma H$_2$S concentration as well as vascular CSE expression and activity are lower in experimental models of hypertension such as spontaneously hypertensive rat and hypertension induced by NO synthase inhibitors (Wagner, 2009). On the other hand, chronic administration of CSE inhibitor, propargylglycine, increases blood pressure in normotensive animals (Yan et al., 2004). Recently, it has been demonstrated that plasma H$_2$S is lower in 25 children with essential hypertension in comparison to 66 normotensive controls (Chen et al., 2007). Taken together, these data indicate that H$_2$S is involved in the regulation of blood pressure and its deficiency may contribute to hypertension.

**Renal effect of H$_2$S**

In addition to vascular tone, blood pressure is regulated by renal sodium handling. Although H$_2$S-generating enzymes are abundantly expressed in the kidney and H$_2$S formation
by cysteine desulfhydration in the kidney was first described almost three decades ago (Stipanuk and Beck, 1982), renal effect of this gas was, until recently, completely ignored. In the recent issue of JPET, Xia et al. (2009) have reported that H₂S is produced in the kidney and that exogenous H₂S donor, sodium hydrosulfide (NaHS), exerts a significant diuretic, natriuretic and kaliuretic effects. These data suggest that H₂S is antihypertensive not only by inducing vasodilation but also by affecting renal sodium handling. Importantly, diuresis, natriuresis and kaliuresis are significantly reduced in animals treated with a mixture of CBS and CSE inhibitors, indicating that endogenous H₂S regulates renal function under baseline conditions. Natriuretic effect of H₂S results from both the increase in glomerular filtration rate (GFR) and the inhibition of tubular sodium reabsorption, as evidenced by the increase in fractional Na⁺ excretion after administration of NaHS (Xia et al., 2009). Furthermore, tubular effect of H₂S is accounted for, at least in part, by the inhibition of Na⁺,K⁺-ATPase – the major driving force for active Na⁺ reabsorption along the nephron. Indeed, NaHS-derived H₂S reduces sodium pump activity in isolated basolateral membranes of tubular cells. In addition, Xia et al. (2009) aimed to elucidate the possible effects of H₂S on Na⁺⁺/K⁺/2Cl⁻ cotransporter (NKCC) and Na⁺/Cl⁻ cotransporter (NCC), the major apical Na⁺ transporters in the medullary thick ascending limb (mTAL) and distal convoluted tubule (DCT), respectively, by studying the interaction between H₂S donor and specific inhibitors of these transporters, furosemide (NKCC inhibitor) and hydrochlorothiazide (NCC inhibitor). It has been demonstrated that natriuretic effect of NaHS is impaired in rats receiving furosemide but not in those treated with hydrochlorothiazide. From these data, authors concluded that H₂S inhibits NKCC but not NCC (Xia et al., 2009). However, the effect of H₂S on these cotransporters was not studied directly. Although this conclusion may be plausible, the alternative explanation should also be considered, i.e. that the interaction between H₂S and diuretics is determined by specific hypoxic environment of the renal medulla.
Hypoxia in the renal medulla and its role in local H$_2$S signaling

H$_2$S is oxidized in mitochondria by the sequential action of three enzymes: sulfide:quinone oxidoreductase (SQR), sulfur dioxygenase, and sulfite oxidase (Hildebrandt and Grieshaber, 2008). SQR transfers electrons from H$_2$S to quinone where they enter the mitochondrial respiratory chain (Fig. 2). Indeed, H$_2$S is the first and the only currently known inorganic substrate for mitochondria of mammalian cells, and its oxidation may provide energy for ATP synthesis. Mitochondrial H$_2$S metabolism is highly dependent on oxygen tension. Indeed, several studies have demonstrated that the net H$_2$S production by various tissues is pO$_2$-dependent, which is explained by variable degree of mitochondrial oxidation. Net H$_2$S production can be demonstrated under hypoxic but not under normoxic conditions because at physiological pO$_2$ most of H$_2$S is rapidly oxidized (Olson et al., 2006; Whitfield et al., 2008).

It is well established that renal medulla is a hypoxic environment. Indeed, both in the isolated perfused rat kidney and in the rat kidney in situ pO$_2$ in the renal cortex is close to that in the renal vein (70 mmHg) but steeply decreases when the electrode is advanced to the renal medulla (Leichtweiss et al., 1969). Oxygen tension in the renal medulla, where mTAL are localized, is between 5 and 15 mmHg (Epstein, 1997). In most cells in the body cytochrome c oxidase is almost completely oxidized under physiological conditions because its K$_m$ for oxygen is attained at only 1-2 mmHg in isolated mitochondria and at about 10 mmHg in the whole cells, which is much less than ambient pO$_2$ in these tissues. However, in medullary tubular cells 20-40% of cytochrome c oxidase exists in the reduced state, indicating that local pO$_2$ is close to critical at which mitochondrial respiration might be compromised (Epstein et al., 1982). Due to low pO$_2$, the expression of a major molecular target of hypoxia, hypoxia-induced factor-1 (HIF-1) and its target genes such as inducible NO synthase (iNOS), cyclooxygenase-2 (COX-2) and heme oxygenase-1 (HO-1) is much higher in medulla than in
the cortex. In addition, medullary cells possess high capacity for anaerobic glycolysis to survive in this hypoxic environment.

The kidneys receive about 20% of cardiac output and high renal blood flow is mandatory for glomerular filtration. Thus, the whole kidney is well oxygenated and total renal oxygen extraction is much lower than in other tissues. However, most of blood delivered to the kidney flows through the renal cortex, whereas medullary blood flow is only about 10% of total renal perfusion. In addition, medullary descending and ascending vasa recta are arranged in the countercurrent manner which is essential for maintaining high osmolality in the renal medulla. This countercurrent arrangement allows oxygen to shunt from descending to ascending vasa recta thus bypassing the inner zone of the renal medulla. Finally, oxygen balance of the renal medulla is compromised by high O2 consumption by the thick ascending limb which utilizes large amounts of oxygen for active Na+,K+-ATPase-dependent sodium reabsorption. It is estimated that 60% of renal O2 consumption is accounted for by active Na+ transport in the mTAL. Furosemide, which inhibits Na+ reabsorption in mTAL, reduces oxygen requirement and increase local medullary pO2. In healthy human volunteers, rapid infusion of 20 mg furosemide increased renal medullary oxygenation measured by blood oxygen level-dependent magnetic resonance imaging by 25%, whereas cortical oxygenation increased only by 10% (Epstein and Prasad, 2000). Interestingly, furosemide improves medullary oxygenation while having no effect or even decreasing medullary blood flow, indicating that increase in pO2 results from the inhibition of Na+ reabsorption in the mTAL. By improving local oxygenation, furosemide reduces HIF-1 expression in the outer and inner medulla (Zou et al., 2001). In the isolated perfused rat kidney, furosemide and bumetanide (the other NKCC inhibitor) reduced the amount of cytochrome c oxidase existing in the reduced state from 40 to 20% (Epstein et al., 1982). Similarly, in the intact anesthetized rat, furosemide increased medullary pO2 from 16 to 35 mmHg (Brezis et al., 1994). In contrast,
factors which inhibit proximal tubular reabsorption such as acetazolamide, inhibitors of gluconeogenesis or fatty acid oxidation, had no effect on the oxygenation of the renal cortex. In addition, the effect of furosemide was not observed in kidneys perfused with hyperoncotic solutions to reduce GFR and Na⁺ load available for reabsorption. These data indicate that the rate of active Na⁺ reabsorption in the mTAL is critical for local medullary oxygen balance.

Xia et al. (2009) did not measure H₂S production separately in the cortex and medulla. However, taking into account the above considerations, it is likely that local H₂S concentration is greater in hypoxic medullary microenvironment. In addition, H₂S generated from exogenous NaHS could be more slowly oxidized in the renal medulla and exert more marked effects on medullary nephron segments. It might be hypothesized that furosemide, by inhibiting Na⁺ reabsorption in the mTAL, reduces O₂ consumption and increases local O₂ availability, thus accelerating H₂S oxidation. Consequently, impaired natriuretic effect of NaHS in rats receiving furosemide could have resulted not only from the fact that, as suggested by the authors, both H₂S and furosemide inhibit NKCC (pharmacodynamic interaction) but also from lower local H₂S concentration in furosemide-treated animals (pharmacokinetic interaction). On the contrary, NCC is localized in the distal convoluted tubule in the renal cortex, which has a much better oxygen supply. Consequently, although thiazides inhibit Na⁺ transport and O₂ consumption in this segment, they have negligible effect on cortical pO₂. Thus, equally potent effect of NaHS in rats treated and not treated with hydrochlorothiazide may result from comparable degrees of H₂S oxidation and local levels of the gasotransmitter in the distal tubules of both groups.

H₂S as the oxygen sensor in the kidney?

It is suggested that H₂S functions as an “oxygen sensor” both in the blood vessels (Olson and Whitfield, 2010) and in arterial chemoreceptors (Li et al., 2010). Hypoxia impairs H₂S metabolism and increases its concentration leading to vasodilation and stimulation of
chemoreceptor afferent neurons, respectively. Indeed, vascular effect of H₂S closely resembles the effect of hypoxia (e.g. both induce systemic vasorelaxation and pulmonary vasoconstriction), and the response of blood vessels and arterial chemoreceptors to hypoxia is abolished by the inhibitors of H₂S synthesis. Thus, H₂S seems to mediate some effects of hypoxia on the cardiovascular system.

Due to the conflicting needs to simultaneously maintain medullary hyperosmolality for urine concentration and to provide sufficient O₂ for tubular transport, renal medullary blood flow must be carefully regulated. Too high medullary perfusion will wash-out the corticomedullary osmotic gradient and disrupt the kidneys’ ability to concentrate urine, whereas insufficient blood flow might reduce local oxygenation below critical level leading to the tubular damage. Medullary blood flow is provided by efferent arterioles of juxtamedullary nephrons, which proceed toward the medulla as descending vasa recta. Vasa recta are surrounded by pericytes – the smooth muscle-like cells which regulate medullary perfusion by responding to many vasoactive agents. Interestingly, pericytes respond relatively weakly to vasoconstrictors such as angiotensin II, endothelin-1 and vasopressin, but strongly to vasodilators including NO, CO, prostaglandin E₂ or adenosine (Pallone et al., 2003). Interestingly, these vasodilators are generated in great amounts in the renal medulla, partially due to high local expression of enzymes involved in their formation, NO synthases, COX-2 and HO-1. Continuous action of these vasodilators is essential to maintain medullary blood flow and local oxygen balance, and inhibition of their synthesis with, for example, NO synthase or COX inhibitors, easily compromises medullary oxygenation and may induce tubular damage. Medullary blood flow must not only be maintained at the precise level, but also has to be adjusted to match variable tubular oxygen requirements. Oxygen demand of the mTAL depends on solute load delivered to this segment, which may vary depending on GFR and the fraction of filtrate reabsorbed in the proximal tubule. It is suggested that the “cross-
“talk” between tubular cells and vascular pericytes adjusts local blood flow to oxygen demand. Indeed, formation of vasa recta vasodilators increases under hypoxic conditions. Nitric oxide is scavenged by reactive oxygen species, in particular superoxide anion radical, which is generated by tubular cells’ NADPH oxidase in pO₂-dependent manner. Thus, the level of NO increases at low pO₂. Adenosine is produced from ATP in increased amounts under hypoxic conditions. In addition, prolonged hypoxia stimulates the expression of iNOS, COX-2 and HO-1 by increasing transcriptional activity of HIF-1. NO, PGE₂, CO and adenosine improve medullary pO₂ not only by matching blood flow to the rate of tubular transport but also reduce oxygen consumption by inhibiting Na⁺ reabsorption in the mTAL.

H₂S emerges as a candidate for a novel oxygen sensor and mediator of tubulovascular cross-talk in the renal medulla. Its level is directly dependent on oxygen tension, and it both inhibits Na⁺ transport in the mTAL (Xia et al., 2009) and induces vasodilation. Although the effect of H₂S on vasa recta has not been studied so far, H₂S relaxes various blood vessels in the experimental studies (Zhao et al., 2001), and Kₐ₅P channels, the main vascular target for H₂S, are expressed in descending vasa recta pericytes (Cao et al., 2005). Kₐ₅P channel opener, pinacidil, dilates, whereas their blocker, glibenclamide, constricts descending vasa recta. Because H₂S stimulates Kₐ₅P channels directly, not through any intermediate signaling mechanisms, it is very likely that locally generated H₂S regulates pericycle tone. Apart from increasing blood flow and inhibiting tubular Na⁺ reabsorption, H₂S may improve medullary oxygen status by directly inhibiting mitochondrial respiration (Fig. 2). Indeed, H₂S is a potent reversible inhibitor of cytochrome c oxidase. Cytochrome c oxidase is also competitively inhibited by NO in the renal medulla (Palm et al., 2009). However, H₂S seems to posses several potential advantages over NO as an oxygen sensor. Formation of NO is dependent on many factors such as expression of various NOS isoforms, availability of its substrate, L-arginine, its cofactor, tetrahydrobiopterin, and its inhibitor, asymmetric dimethylarginine, and...
is compromised due to one or more of these mechanisms in many disease states. In addition, NO is rapidly scavenged by ROS even under physiological conditions and this process is enhanced when oxidative stress exists (Palm et al., 2009). The regulation of H$_2$S-generating enzymes is less understood but it is likely that its level is subjected to less bias by factors other than hypoxia, or at least is affected by factors other than that which regulate NO, making the global amount of both gases more directly related to pO$_2$ than either of them alone.

**H$_2$S and renal ischemia-reperfusion injury**

Ischemia-reperfusion injury is the most common mechanism of acute renal failure. Due to low pO$_2$ even under physiological conditions, mTAL is particularly sensitive to the hypoxic injury and is the segment most commonly affected by ischemic necrosis. Furosemide, as well as other factors which inhibit Na$^+$ reabsorption in the mTAL such as ouabain or GFR reduction, diminish hypoxic mTAL damage in the hypoperfused kidney (Brezis and Rosen, 1995; Rosenberger et al., 2006). Classically, acute tubular necrosis is classified into that caused by ischemic and toxic insults. However, more recent studies indicate that hypoxia contributes significantly to the tubular damage induced also by various nephrotoxins, such as radiocontrast agents, myoglobin, hemoglobin, amphotericin B, and non-steroidal antiinflammatory drugs (Rosenberger et al., 2006). Inhibitors of mTAL reabsorption reduce tubular damage at least in some of these disorders by improving medullary O$_2$ balance (Heyman et al., 1989).

It is well established that hydrogen sulfide protects from ischemia-reperfusion injury of myocardium, liver, small intestine, neurons and the lung. Several recent studies indicate that H$_2$S is protective also in renal ischemia-reperfusion. H$_2$S applied in the breathing air increased survival of mice subjected to bilateral renal ischemia-reperfusion, attenuated renal dysfunction (reduced plasma urea and creatinine concentrations), reduced morphological
indices of acute tubular necrosis, decreased elevated caspase-3 activity, and attenuated the expression of proapoptotic protein Bax (Bos et al., 2009). In addition, H₂S reduced renal infiltration with monocytes/macrophages and granulocytes. Similarly, NaHS improved renal function, ameliorated oxidative stress and reduced cell damage in the rat and mouse models of renal ischemia-reperfusion (Xu et al. 2009; Tripatara et al., 2009). In contrast, CSE inhibitor, propargylglycine, impaired the recovery of renal function after reperfusion and worsened morphological tubular damage (Tripatara et al., 2008). Various mechanisms may contribute to the protective effect of H₂S in renal ischemia such as amelioration of tubular cell apoptosis (Bos et al. 2009), antiinflammatory effect (Tripatara et al., 2008; Bos et al. 2009), reduction of oxidative stress (Xu et al., 2009), and inhibition of proapoptotic protein kinases: p38 MAPK, ERK and JNK (Tripatara et al., 2008). Reduction of oxygen consumption and improvement of medullary oxygenation may also play a prominent role in the protective effect of H₂S against renal ischemic damage. It should be noted that in ischemic acute renal failure hypoxia is not confined to the renal medulla and mTAL, but renal cortex and other tubular segments such as S3 segment of the proximal tubule experience a severe hypoxia as well. It is likely that H₂S concentration increases also in other regions apart from the mTAL and is involved in their protection from the ischemic damage.

It is unclear if renal ischemia-reperfusion affects the level of endogenous H₂S in the kidney. In the rat kidney, ischemia-reperfusion decreased CBS activity resulting in the reduction of CBS-dependent H₂S production and accumulation of homocysteine in the kidney (Xu et al., 2009). Decrease in CBS activity results from both acidosis (an optimal pH for CBS is above 7) and overproduction of NO, which inactivates CBS by binding to its heme iron. In contrast to CBS, CSE activity was not changed. In contrast, in the mouse model of in vivo renal ischemia-reperfusion, CSE expression and H₂S production in the kidney are increased (Tripatara et al., 2009). It is possible that intrarenal H₂S deficiency due to other factors such
as nephrotoxic agents may predispose the kidney to the subsequent ischemic insult. For example, since H$_2$S avidly binds to heme-containing proteins, its deficiency is likely to occur in hemoglobin- or myoglobin-induced nephropathy.

**H$_2$S, hypoxia, and chronic kidney disease**

Many studies suggest that aggravated medullary hypoxia exists in patients with chronic kidney disease and is involved in the progression of renal damage. Several factors contribute to aggravation of medullary hypoxia in these patients, including reduction of peritubular capillary density, decrease in medullary blood flow due to glomerular hypoperfusion, altered regulation of vascular tone of the descending vasa recta due to ROS-mediated NO deficiency, anemia, increased oxygen demand of the surviving nephrons because of both the increase in single nephron GFR and reabsorption and degradation of filtered proteins (Heyman et al., 2008). Hypoxia promotes progression of renal disease by stimulating extracellular matrix accumulation, endothelial damage, tubulointerstitial injury, transdifferentiation of proximal tubule cells to myofibroblasts, and stimulation of tumor necrosis factor alpha (TNF-α) and adhesion molecule ICAM-1 in tubular cells (Tanaka and Nangaku, 2010). Of note, treatment strategies which reduce tubular oxygen consumption or improve renal oxygenation such as low salt and low-protein diet, erythropoietin, or renin-angiotensin system inhibitors retard the progression of kidney disease.

Chronic kidney disease is associated with hyperhomocysteinemia due to impaired metabolism of this aminoacid in the kidney through the transsulfuration pathway. Several lines of evidence suggest that H$_2$S deficiency may be involved in the pathogenesis of chronic kidney disease. First, renal dysfunction and proteinuria are aggravated in CBS deficient uninephrectomized mice in comparison to wild-type uninephrectomized mice (Sen et al., 2009). Second, NaHS normalizes augmented expression of MMP-2 and MMP-9, reduces glomerular cell apoptosis, decreases the expression of podocyte injury marker, pendrin,
corrects deficiency of glomerular slit diaphragm protein, nephrin, and reduces proteinuria in these mice. Third, chronic administration of CSE inhibitor, propargylglycine, induces nephropathy. Finally, blood H$_2$S and sulfhemoglobin levels as well as CSE expression in blood mononuclear cells are reduced in end-stage renal disease patients in comparison to healthy controls, indicating that chronic renal failure is a state of H$_2$S deficiency (Perna et al., 2009).

**H$_2$S and renal oxygenation in hypertension**

Abnormal renal oxygenation is also observed in experimental models of hypertension. Oxygen tension in the proximal and distal tubules as well as in the superficial cortical tissue is lower in spontaneously hypertensive than in normotensive Wistar-Kyoto rat (Welch et al., 2001). Hypoxia in the hypertensive kidney is attributed to oxidative stress-mediated NO deficiency and the resulting abnormalities of vascular tone and tubular Na$^+$ transport. In addition, NO, by inhibiting mitochondrial oxygen consumption, increases O$_2$ efficiency of tubular transport, i.e. reduces the amount of O$_2$ consumed per unit of reabsorbed Na$^+$. Reduced transport efficiency is observed in hypertensive rats and is improved by superoxide scavenger, tempol, which increases renal NO availability (Adler et al., 2002; Welch 2006).

Arterial hypertension is observed in H$_2$S-deficient CSE$^{-/-}$ mice (Yang et al., 2008). Although blood pressure elevation in these animals is attributed to abnormal regulation of vascular tone, it cannot be excluded that impaired H$_2$S effects in the kidney, including its effects on renal oxygenation, contribute to the observed phenotype. Although renal H$_2$S production was not measured in other models of hypertension, CBS expression and activity is markedly reduced in the kidney of Dahl salt-sensitive hypertensive rats (Li et al., 2006). It should be noted that deficiency of other vasodilators in the renal medulla induced by local infusion of NO synthase, cyclooxygenase, or HO-1 inhibitors induces hypertension in experimental animals by increasing tubular Na$^+$ reabsorption.
Conclusions

Hydrogen sulfide is produced in the kidney by both CBS and CSE, and stimulates diuresis and natriuresis by both increasing glomerular filtration and inhibiting active tubular reabsorption. Because H$_2$S is oxidized in mitochondrial respiratory chain in pO$_2$-dependent manner and ambient pO$_2$ is much lower in the renal medulla than in the renal cortex, it is likely that renal medulla is a principal target tissue for H$_2$S in the kidney. In addition, H$_2$S may function as the oxygen sensor which maintains local oxygen balance under hypoxic conditions by increasing medullary blood flow and reducing tubular O$_2$ consumption. Hypoxia plays an important role in the pathogenesis of renal ischemia-reperfusion injury, chronic renal disease and arterial hypertension, and both endo- and exogenous H$_2$S may exert a significant protective effect by improving medullary oxygenation in these states. Currently available H$_2$S donors such as NaHS are not suitable for therapy because release H$_2$S rapidly and in high amounts. However, attempts are being made to obtain more appropriate H$_2$S precursors for therapeutic purposes. Recently, the first water-soluble nontoxic H$_2$S donor, GYY4137, which releases H$_2$S slowly in vitro and in vivo has been characterized. Such compounds may become useful in the future treatment of renal diseases. In addition, several H$_2$S-releasing derivatives of non-steroidal antiinflammatory drugs have been synthesized which are less toxic for the gastrointestinal system than their parent compounds (Wallace, 2007). Given the possible role of H$_2$S in the regulation of renal oxygenation, as well as compromising effect of classic non-steroidal antiinflammatory drugs on renal medullary circulation and their nephrotoxicity, H$_2$S-releasing derivatives may be a useful alternative for the renal patients.
References


Figure legends

Fig. 1. Three pathways of enzymatic H$_2$S formation: synthesis of L-cystathionine and H$_2$S from L-cysteine and L-homocysteine by cystathionine $\beta$-synthase (CBS), desulphhydration of L-cysteine by cystathionine $\gamma$-lyase (CSE), and transamination of L-cysteine to 3-mercaptoppyruvate by cysteine aminotransferase (CAT), followed by its desulphhydration to pyruvate by 3-mercaptoppyruvate sulfurtransferase (3-MST).

Fig. 2. Hypothetical role of H$_2$S as the oxygen sensor in the kidney. Under normoxic conditions (top) H$_2$S is rapidly oxidized in mitochondria by the sequential action of sulfide:quinine oxidoreductase (SQR), sulfur dioxygenase (SDO) and sulfite oxidase (SO). Under hypoxic conditions (bottom), H$_2$S oxidation is impaired and its level increases; H$_2$S inhibits active Na$^+$ reabsorption in the medullary thick ascending limb (mTAL) by reducing the activity of Na$^+$/K$^+$/2Cl$^-$ cotransporter (NKCC) in the apical membrane and Na$^+$,K$^+$-ATPase (NKA) in the basolateral membrane and thus reduces oxygen consumption. In addition, H$_2$S decreases O$_2$ consumption by directly inhibiting mitochondrial respiration, and increases O$_2$ supply by dilating descending vasa recta (DVR). Decrease in O$_2$ consumption and increase in O$_2$ delivery improve oxygenation status of the renal medulla.
L-cysteine \rightarrow L\text{-}homocysteine

\text{CBS}

L\text{-}cystathionine \rightarrow H_2S

\text{L-cysteine} \rightarrow \text{H}_2\text{S}, \text{pyruvate} \rightarrow \text{NH}_4^+ \rightarrow 3\text{-mercaptopyruvate} \rightarrow \text{aspartate}

\text{CSE}

\text{CAT}

2\text{-oxobutyrate} \rightarrow \text{H}_2\text{S} + \text{pyruvate}