Hypoxia in the renal medulla: Implications for hydrogen sulfide signaling

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ABBREVIATIONS: 3-MST, 3-mercaptopyruvate sulfurtransferase; CBS, cystathionine β-synthase; CO, carbon monoxide; COX-2, cyclooxygenase-2; CSE, cystathionine γ-lyase; DCT, distal convoluted tubule; ERK, extracellular signal-regulated kinase; GFR, glomerular filtration rate; HIF-1, hypoxia-induced factor; HO-1, heme oxygenase-1; H_2S, hydrogen sulfide; ICAM-1, intercellular adhesion molecule-1; iNOS, inducible NO synthase; JNK, c-Jun N-terminal kinase; K_{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; PGE_2, prostaglandin E_2; ROS, reactive oxygen species; SQR, sulfide:quinone

oxidoreductase; TNF-α, tumor necrosis factor-alpha

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Abstract

Hydrogen sulfide (H_2S) is enzymatically generated in mammalian tissues from either L-cysteine or L-homocysteine. H_2S 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, H_2S produced locally within the kidney stimulates natriuresis and diuresis by increasing glomerular filtration and inhibiting tubular sodium reabsorption. Because H_2S is oxidized in mitochondria in pO_2-dependent manner and ambient pO_2 is physiologically low in the renal medulla, it is expected that the activity of H_2S is higher in medullary than in cortical region. H_2S, accumulating in increased amounts in the renal medulla under hypoxic conditions, may function as an oxygen sensor which restores O_2 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 H_2S may contribute to the pathogenesis of these pathologies by compromising medullary oxygenation, and administration of H_2S 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_2S) 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_2S 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-mercaptopyruvate, followed by its desulfhydration to pyruvate by 3-mercaptopyruvate 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_2S 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_2S in the regulation of vascular tone and blood pressure. Indeed, it is suggested that H_2S deficiency may contribute to the pathogenesis of arterial hypertension both in experimental animal models and in humans. Recently, renal synthesis and activity of H_2S 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_2S metabolism is O_2-dependent. In this article, I address the possible relationship between renal hypoxia and H_2S signaling in physiologic and pathologic conditions.

Role of H_2S in the regulation of vascular tone and blood pressure

Initially, it was suggested that in the blood vessels H_2S 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_2S is synthesized also by endothelial cells at least in rodents. In mice aortic endothelial cells H_2S is synthesized by CSE and its production is stimulated by cholinergic agonists (Yang et al., 2008). Thus, H_2S is suggested to be one of the endothelium-dependent relaxing factors. CSE is not expressed in rat endothelial cells, but H_2S is synthesized in these cells in 3-MST-dependent manner (Shibuya et al., 2009). H_2S is also produced by perivascular adipose tissue (Fang et al., 2009). Moreover, H_2S, 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_2S is definitely antihypertensive (Yang et al., 2008). Plasma H_2S 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_2S 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_2S is involved in the regulation of blood pressure and its deficiency may contribute to hypertension.

Renal effect of H_2S

In addition to vascular tone, blood pressure is regulated by renal sodium handling. Although H_2S-generating enzymes are abundantly expressed in the kidney and H_2S 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 H_2S is produced in the kidney

and that exogenous H_2S donor, sodium hydrosulfide (NaHS), exerts a significant diuretic,

natriuretic and kaliuretic effects. These data suggest that H_2S 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 H_2S regulates renal function under baseline

conditions. Natriuretic effect of H_2S 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 H_2S 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 H_2S

reduces sodium pump activity in isolated basolateral membranes of tubular cells. In addition,

Xia et al. (2009) aimed to elucidate the possible effects of H_2S 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 H_2S 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 H_2S inhibits NKCC but not

NCC (Xia et al., 2009). However, the effect of H_2S 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 H_2S and diuretics is determined by specific

hypoxic environment of the renal medulla.

Hypoxia in the renal medulla and its role in local H_2S signaling

H2S 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_2S to quinone where they enter the

mitochondrial respiratory chain (Fig. 2). Indeed, H_2S 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_2S metabolism is highly dependent on oxygen

tension. Indeed, several studies have demonstrated that the net H_2S production by various

tissues is pO_2-dependent, which is explained by variable degree of mitochondrial oxidation.

Net H_2S production can be demonstrated under hypoxic but not under normoxic conditions

because at physiological pO_2 most of H_2S 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 posses high capacity for anerobic 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 O_2 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 O_2 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 pO_2. 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 pO_2 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 pO_2 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 H_2S production separately in the cortex and medulla.

However, taking into account the above considerations, it is likely that local H_2S

concentration is greater in hypoxic medullary microenvironment. In addition, H_2S 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 O_2 consumption and increases local O_2

availability, thus accelerating H_2S 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 H_2S and furosemide inhibit NKCC (pharmacodynamic

interaction) but also from lower local H_2S 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 O_2 consumption in this segment, they have negligible

effect on cortical pO_2. Thus, equally potent effect of NaHS in rats treated and not treated with

hydrochlorothiazide may result from comparable degrees of H_2S oxidation and local levels of

the gasotransmitter in the distal tubules of both groups.

H_2S as the oxygen sensor in the kidney?

It is suggested that H_2S functions as an "oxygen sensor" both in the blood vessels

(Olson and Whitfield, 2010) and in arterial chemoreceptors (Li et al., 2010). Hypoxia impairs

H_2S metabolism and increases its concentration leading to vasodilation and stimulation of

chemoreceptor afferent neurons, respectively. Indeed, vascular effect of H_2S 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 H_2S synthesis. Thus, H_2S 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 O_2 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 E_2 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 pO_2-dependent manner. Thus, the level of NO

increases at low pO_2. 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, PGE_2, CO and adenosine improve

medullary pO_2 not only by matching blood flow to the rate of tubular transport but also reduce

oxygen consumption by inhibiting Na^+ reabsorption in the mTAL.

H_2S 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 H_2S on vasa recta has not been studied so far, H_2S relaxes various blood vessels in

the experimental studies (Zhao et al., 2001), and K_{ATP} channels, the main vascular target for

H_2S, are expressed in descending vasa recta pericytes (Cao et al., 2005). K_{ATP} channel opener,

pinacidil, dilates, whereas their blocker, glibenclamide, constricts descending vasa recta.

Because H_2S stimulates K_{ATP} channels directly, not through any intermediate signaling

mechanisms, it is very likely that locally generated H_2S regulates pericyte tone. Apart from

increasing blood flow and inhibiting tubular Na^+ reabsorption, H_2S may improve medullary

oxygen status by directly inhibiting mitochondrial respiration (Fig. 2). Indeed, H_2S 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, H_2S 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_2S-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_2S 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_2S is protective also in renal ischemia-reperfusion. H_2S 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, H_2S 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 H_2S 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 H_2S 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 H_2S 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 H_2S in the

kidney. In the rat kidney, ischemia-reperfusion decreased CBS activity resulting in the

reduction of CBS-dependent H_2S 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 H_2S production in the kidney are increased

(Tripatara et al., 2009). It is possible that intrarenal H_2S deficiency due to other factors such

as nephrotoxic agents may predispose the kidney to the subsequent ischemic insult. For example, since H_2S avidly binds to heme-containing proteins, its deficiency is likely to occur in hemoglobin- or myoglobin-induced nephropathy.

H_2S, 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_2S 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_2S 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_2S deficiency (Perna et al., 2009).

H_2S 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_2S-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_2S effects in the kidney, including its effects on renal oxygenation, contribute to the observed phenotype. Although renal H_2S 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_2S 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_2S in the kidney. In addition, H_2S 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_2S may exert a significant protective effect by improving medullary oxygenation in these states. Currently available H_2S donors such as NaHS are not suitable for therapy because release H_2S rapidly and in high amounts. However, attempts are being made to obtain more appropriate H_2S precursors for therapeutic purposes. Recently, the first water-soluble nontoxic H_2S donor, GYY4137, which releases H_2S 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_2S-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_2S 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_2S-releasing derivatives may be a useful alternative for the renal patients.

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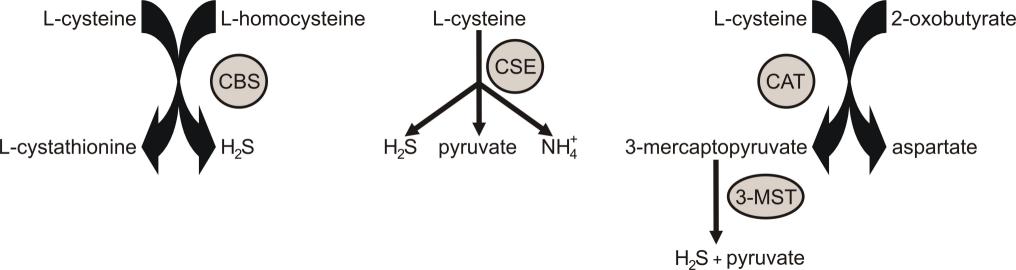
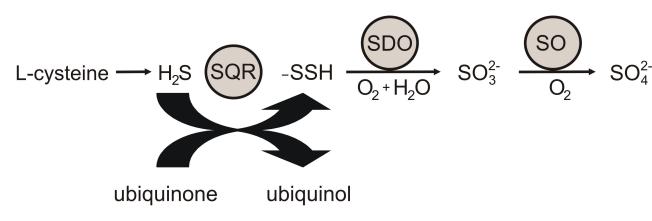Zou AP, Yang ZZ, Li PL, Cowley AW JR (2001) Oxygen-dependent expression of hypoxia-

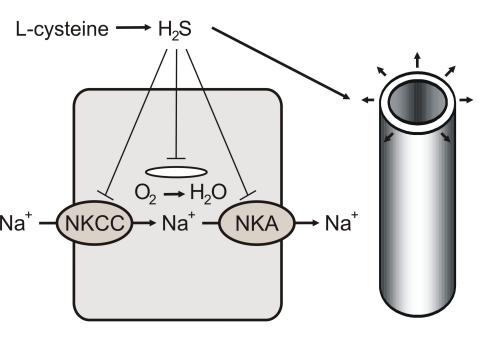inducible factor-1α in renal medullary cells of rats. *Physiol Genomics* **6**:159-168.

Figure legends

Fig. 1. Three pathways of enzymatic H_2S formation: synthesis of L-cystathionine and H_2S from L-cysteine and L-homocysteine by cystathionine β-synthase (CBS), desulfhydration of L-cysteine by cystathionine γ-lyase (CSE), and transamination of L-cysteine to 3-mercaptopyruvate by cysteine aminotransferase (CAT), followed by its desulfhydration to pyruvate by 3-mercaptopyruvate sulfurtransferase (3-MST).

Fig. 2. Hypothetical role of H_2S as the oxygen sensor in the kidney. Under normoxic conditions (top) H_2S 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_2S oxidation is impaired and its level increases; H_2S 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_2S 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 $\longrightarrow$ H_2S (SQR) $-SSH$ $\xrightarrow[O_2 + H_2O]{\text{(SDO)}}$ SO_3^{2-} $\xrightarrow[O_2]{\text{(SO)}}$ SO_4^{2-}

ubiquinone   ubiquinol

L-cysteine $\longrightarrow$ H_2S

$O_2 \longrightarrow H_2O$

Na^+ — (NKCC) $\longrightarrow$ Na^+ — (NKA) $\longrightarrow$ Na^+

mTAL cell

DVR