Hydrogen sulfide in the experimental models of arterial hypertension

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Abstract

Hydrogen sulfide (H_2S) is the third member of gasotransmitter family together with nitric oxide and carbon monoxide. H_2S is involved in the regulation of blood pressure by controlling vascular tone, sympathetic nervous system activity and renal sodium excretion. Moderate age-dependent hypertension and endothelial dysfunction develop in mice with knockout of cystathionine γ-lyase (CSE), the enzyme involved in H_2S production in the cardiovascular system. Decreased H_2S concentration as well as the expression and activities of H_2S-producing enzymes have been observed in most commonly used animal models of hypertension such as spontaneously hypertensive rats, Dahl salt-sensitive rats, chronic administration of NO synthase inhibitors, angiotensin II infusion and two-kidney one-clip hypertension, the model of renovascular hypertension. Administration of H_2S donors decreases blood pressure in these models but has no major effects on blood pressure in normotensive animals. H_2S donors not only reduce blood pressure but also end-organ injury such as vascular and myocardial hypertrophy and remodeling, hypertension-associated kidney injury or erectile dysfunction. H_2S level and signaling are modulated by some antihypertensive medications as well as natural products with antihypertensive activity such as garlic polysulfides or plant-derived isothiocyanates as well as non-pharmacological interventions. Modifying H_2S signaling is the potential novel therapeutic approach for the management of hypertension, however, more experimental clinical studies about the role of H_2S in hypertension are required.

Key words: hydrogen sulfide, hypertension, vascular tone, myocardial hypertrophy, kidney injury.

1. Introduction

Hydrogen sulfide (H_2S) is a colorless water-soluble flammable gas with a strong odor of rotten eggs. H_2S has been known for a long time as the toxic gas and as such was for interest only for toxicologists. Indeed, H_2S is a potent inhibitor of mitochondrial cytochrome c oxidase. In 1996 Abe and Kimura first demonstrated that endogenously produced H_2S serves as the mediator in the central nervous system [1]. Since that time, H_2S has been demonstrated to be involved in the regulation of many physiological processes in the nervous, cardiovascular, gastrointestinal, immune, endocrine and other systems [2-4]. Nowadays, H_2S is classified as the third, in addition to nitric oxide (NO) and carbon monoxide (CO), member of the family of small inorganic signaling molecules also referred to as "gasotransmitters" [5-7].

H_2S is synthesized enzymatically from L-cysteine or L-homocysteine by three enzymes, cystathionine β-synthase (CBS), cystathionine gamma-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (MPST). CBS and CSE are pyridoxal 5'-phosphate (PLP, vitamin B6)-dependent enzymes of transsulfuration pathway in which homocysteine is converted to cysteine; H_2S synthesis from L-cysteine or L-homocysteine is their additional activity [8]. The third mechanism of H_2S synthesis is initiated by PLP-dependent cysteine aminotransferase which converts L-cysteine to 3-mercaptopyruvate; the substrate for MPST which then produces H_2S and pyruvate in the presence of reducing compounds such as thioredoxin or glutathione [9]. In addition, H_2S synthesis from D-cysteine through the sequential action of diaminoacid oxidase (DAO) and MPST is possible in the central nervous system and the kidney [10], although this pathway seems to play a minor role because D-cysteine is not the endogenous aminoacid and can only be derived from food (Fig. 1).

The main mechanism of H_2S signaling is protein persulfidation, that is conversion of thiol (-SH) to persulfide (-SSH groups) [11, 12]. This modification regulates the activity of multiple

proteins including enzymes, transporters and transcription factors. H_2S itself cannot directly react with –SH groups and persulfidation must be preceded by oxidation of them to, for example, sulfenic group (-SOH) or oxidation of H_2S itself to other reactive sulfur species (RSS) such as inorganic polysulfides (H_2S_n) [12]. Additional mechanisms of H_2S signaling include its reaction with hemoproteins as well as with reactive oxygen and nitrogen species (ROS and RNS, respectively), although the role of these mechanisms is much less recognized [13, 14].

One of the most interesting feature of H_2S is that it may be enzymatically oxidized in mitochondria. H_2S is the first, and actually the only currently known, inorganic substrate for mitochondrial respiratory chain which may provide energy for ATP production. The enzyme sulfide:quinone oxidoreductase (SQR) transfers electrons from H_2S to ubiquinone (coenzyme Q), the electron acceptor for mitochondrial complexes I and II involved in oxidation of organic substrates. At the level of coenzyme Q these electrons enter the canonical electron transport chain consisting of complex III and IV (cytochrome C oxidase). Sulfur produced by SQR is further oxidized by sulfur dioxygenase to sulfite (SO_3^{2-}) and then by sulfite oxidase to sulfate (SO_4^{2-}); the final product of H_2S oxidation [15-18].

High peripheral vascular resistance and abnormal renal sodium handling are the main mechanisms of arterial hypertension. Soon after discovery of H_2S as a gasotransmitter its vasodilating activity was described [19]. In the vascular wall H_2S is produced by CSE in smooth muscle cells and perivascular adipose tissue as well as by CAT and MPST in endothelial cells, and decreases vascular tone by many mechanisms depending on species, vascular preparation, method used to measure vascular tone (wire vs. pressure myography), buffer composition, H_2S donor and the agonist used for vessel precosnstriction. H_2S activates ATP-sensitive K^+ channels (K_{ATP}) in smooth muscle cells to induce cell hyperpolarization [20]. Stimulation of K_{ATP} channels results from persulfidation of Cys^6 and Cys^{26} residues within the extracellular domain of

sulfonylurea receptor (SUR1) subunit [21] and/or persulfidation of SUR2B subunit [22]. In many, but not all vascular preparations vasodilating effect of H_2S donors was inhibited by K_{ATP} channel blocker, glibenclamide. In addition, H_2S produced by smooth muscle cells and perivascular adipocytes activates voltage-sensitive Kv7 (KCNQ) potassium channels in the auto- and paracrine manner, respectively [23, 24]. H_2S may serve as the endothelium-derived hyperpolarizing factor (EDHF). It persulfidates small and intermediate-conductance Ca^{2+}-activated K^+ channels in endothelial cells leading to cell hyperpolarization which then extends to smooth muscle cells directly through the intercellular gap junctions or indirectly, by activating inwardly rectifying K^+ channels and/or Na^+,K^+-ATPase in smooth muscle cells [25-27]. Moreover, large-conductance Ca^{2+}-activated K^+ channels (BK_{Ca}) blockers inhibit H_2S-induced relaxation of mouse coronary arteries [28], human saphenous vein [29] and rat mesenteric arteries [24]. H_2S may stimulate BK_{Ca} channels by inducing Ca^{2+} entry through the transient receptor potential vanilloid type 4 channels [30]. Other mechanisms of H_2S-induced vasorelaxation include stimulation of endothelial NO synthase (eNOS) [31], soluble guanylyl cyclase [32] and protein kinase G [33] as well as inhibition of cGMP-degrading enzyme, phosphodiesterase 5 [34, 35]. Age-dependent hypertension and endothelial dysfunction have been reported in CSE knockout mice [36], although this was not observed in the other study [37]. Plasma H_2S concentration as well as the expression of H_2S-producing enzymes are lower in patients with arterial hypertension than in healthy normotensive subjects [38]. Together with previously appreciated role of "older" gasotransmitters (NO and CO) in the regulation of vascular tone and the role of endothelial NO deficiency in the pathogenesis of arterial hypertension, this raised interest in the role of H_2S in this disease. The aim of this review is to summarize the results of studies focused on the role of H_2S in most commonly used experimental animal models of hypertension as well as to discuss some implications for future research and therapy.

2. Spontaneously hypertensive rat (SHR)

2.1 H_2S deficiency in SHR – vascular aspects

Spontaneously hypertensive rat (SHR) is the commonly used model of essential hypertension. These animals develop hypertension between the age of 5 and 8-10 weeks irrespectively of salt intake. In 2004 Yan et al. [39] first demonstrated that plasma H_2S concentration is more than 50% lower in SHR in comparison to normotensive Wistar-Kyoto rats (WKY). Similarly, H_2S production by thoracic aorta ex vivo as well as CSE expression at the mRNA level in the aortic wall were lower in SHR than in normotensive rats. Administration of H_2S donor, NaHS, at a dose of 56 μmol/kg/day intraperitoneally for 5 weeks reduced systolic blood pressure, increased plasma H_2S concentration and aortic H_2S production and decreased blood pressure in SHR. Importantly, although NaHS increased CSE expression and H_2S production by the aortic wall, it had no effect on blood pressure in WKY. In contrast, CSE inhibitor, propargylglycine (PAG, 37.5 mg/kg/day ip. for 5 weeks), increased systolic blood pressure, decreased CSE expression and H_2S production in WKY rats but had no additional effect in SHR. These results suggested that H_2S deficiency is involved in the pathogenesis of hypertension in this model and that H_2S donors may represent the new therapeutic opportunity.

These findings were later confirmed by other authors. For example, the expression of CBS and CSE in cerebral arteries and arterioles is lower in SHR than in WKY. The difference between both strains is higher for CSE than for CBS and for CSE is higher in vascular smooth muscle cells than in endothelial cells [40].

Interestingly reduced expression of H_2S-producing enzymes and H_2S deficiency may be associated with compensatory increased sensitivity to this gasotransmitter. Indeed, Na_2S-induced relaxation of phenylephrine-preconstricted aortic rings was higher already in young prehypertensive SHR in comparison to WKY [41, 42].

Perivascular adipose tissue (PVAT), which surrounds most of large and medium-sized arteries, plays an important role in the regulation of vascular tone. Under physiological conditions, PVAT reduces vascular contractility by producing several mediators including H_2S [43, 44]. It has been demonstrated that anticontractile effect of PVAT in the isolated mesenteric artery is impaired in 16-week old SHR in comparison to WKY [45]. PAG induced contraction of aortic rings with preserved or removed PVAT in Wistar rats confirming the important role of H_2S in the regulation of vascular tone. However, PAG had no effect on aortic rings with PVAT and induced relaxation of aortic rings without PVAT collected from SHR suggesting impaired contribution of endogenous H_2S to the regulation of vascular tone.

Li et al [46] examined the effect of NaHS on endothelial function in SHR. They demonstrated that endothelium-dependent vasoconstriction (vasoconstrictor response to acetylcholine in the presence of NO synthase inhibitor) was higher whereas endothelium-dependent vasorelaxation (the effect of acetylcholine without NO synthase inhibitor) was impaired in renal artery rings isolated from SHR in comparison to WKY. NaHS treatment (100 μmol/kg/day) administered between 8 and 24 weeks of age reduced endothelium-dependent contraction and improved endothelium-dependent vasorelaxation in SHR but had no effect in WKY. The similar effects were observed when NaHS was applied ex vivo. Interestingly, CSE expression in the renal arteries was lower in SHR than in WKY and was increased by NaHS suggesting the positive feedback between exo- and endogenous H_2S. In addition, NaHS could improve endothelial function by reducing vascular inflammation. Indeed, it has been demonstrated that lipopolysaccharide blocks the effect of NaHS on endothelial function ex vivo [46]. Plasma IL-1β was higher in SHR than in WKY and was reduced in SHR by NaHS treatment. Similarly, the expression of NLRP3 inflammasome, IL-1β and caspase-3 was higher in the renal artery of SHR in comparison to WKY and these markers of inflammation were reduced

by NaHS applied either in vivo or ex vivo. In addition, the expression of NADPH oxidase-2 (NOX2), malonyldialdehyde (MDA) concentration and transcription factor Nrf2 involved in the regulation of antioxidant enzyme expression were higher in the renal artery of SHR and were reduced by NaHS [46].

Vascular endothelial growth factor (VEGF) is involved not only in the regulation of angiogenesis but also increases NO production by the endothelial cells. Zhu et al. [47] examined the role of VEGF in hypotensive effect of H_2S in SHR. They demonstrated that slow-releasing organic H_2S donor, GYY4137, administered at 133 μmol/kg/day for 14 days reduced systolic blood pressure, increased plasma H_2S concentration and improved acetylcholine-induced relaxation of isolated aortic rings of SHR. In addition, GYY4137 increased the expression of VEGF receptor-2 (VEGFR2) in the aorta of normotensive Sprague-Dawley rats in vivo and human umbilical vein endothelial cells in vitro. The effect of GYY4137 on blood pressure in SHR was blocked by VEGFR2 antagonist, ZD6474, but not by VEGFR1 antagonist, TM306416. Lipid peroxidation in the aortic wall was higher and superoxide dismutase (SOD) was lower in SHR than in normotensive rats and GYY4137 normalized these markers of oxidative stress in VEGFR2-dependent manner. GYY4137 increased eNOS expression and activity of its upstream activator enzyme, protein kinase Akt, and this effect was also sensitive to ZD6474. Stimulatory effect of GYY4137 on eNOS-NO pathway in HUVECs was also reversed by VEGFR2 antagonist. Taken together, these data suggest that H_2S increases vascular nitric oxide in SHR by augmenting VEGFR2 signaling [47].

2.2 H_2S and blood pressure regulation by the nervous system in SHR

The role of H_2S in central regulation of blood pressure is controversial since both hyper- and hypotensive effects have been described in normotensive animals. Sikora et al. [48] compared the effect of intracerebroventricularly administered NaHS in normo- and hypertensive

rats and observed the dose-dependent response. In normotensive WKY rats low dose of NaHS reduced mean arterial pressure and heart rate, intermediate doses induced the time-dependent response (decrease followed by increase in blood pressure) whereas high doses induced immediate hypertensive and tachycardic response which was inhibited by ganglionic blocker, hexamethonium. However, in both SHR and Wistar rats made hypertensive by chronic infusion of angiotensin II all doses of NaHS induced hypotensive responses. These data indicate that different response to NaHS results from hypertension irrespectively of its mechanism. It was hypothesized that in hypertensive rats the amount of endogenous H_2S is reduced and even higher doses of exogenous gasotransmitter elicit the dominating hypotensive response. Indeed, Yu et al. [49] have demonstrated that CBS expression in rostral ventrolateral medulla (RVLM) involved in the regulation of sympathetic activity is lower in 16 week old SHR than in WKY although there is no difference in CBS expression in 8 week-old animals. Microinjection of NaHS or S-adenysolmethionine (the CBS activator) into the RVLM decreased mean arterial blood pressure and heart rate suggesting decrease in sympathetic activity whereas CBS inhibitor, hydroxylamine hydrochloride, had the opposite effect [49]. These data suggest that H_2S deficiency could contribute to sympathetic hyperactivity and hypertension.

In contrast, Duan et al. [50] demonstrated that CBS gene transfer into the RVLM of SHR exacerbated hypertension. Interestingly, they also observed reduced CBS expression and decreased H_2S production in RVLM of SHR in comparison to WKY. The suggested mechanism of prohypertensive effect of CBS gene transfer is decrease in the expression of neuronal NO synthase and nitric oxide production by overproduced H_2S. NO is involved in inhibiting sympathetic activity and it has previously been demonstrated that NO production in the RVLM is lower in SHR in comparison to normotensive rats. Therefore, CBS transfer could exacerbate hypertension by augmenting NO deficiency. Authors suggested that downregulation of CBS in

the RVLM of SHR is the beneficial compensatory mechanism aimed at limiting hypertension [50].

Stroke-prone spontaneously hypertensive rats (SPSHR) were derived from SHR by selective mating and are characterized by more severe hypertension as well as high incidence of stroke. Junan et al. [51] have demonstrated that H_2S production, CBS expression at the mRNA and protein levels as well as enzymatic activity are lower in astrocytes isolated from newborn cerebral cortex of SHRSP in comparison to SHR. However, it is unclear if these differences contributes to more severe hypertension in SHRSP.

Reduced baroreceptor reflex sensitivity is a characteristic feature of arterial hypertension. Perfusion of the carotid sinus with NaHS improved baroreceptor reflex sensitivity and decreased sympathetic outflow in SHR. This effect was mediated by activation of ATP-sensitive potassium channels, membrane hyperpolarization and closure of voltage-sensitive L-type Ca^{2+} channels as well as stimulation of $GABA_A$ receptors and chloride influx to the cells [52, 53].

Apart from barorereceptors, arterial chemoreceptors sensitive to oxygen and carbon dioxide pressure are also involved in the regulation of cardiovascular function. Interestingly, the response of carotid body to hypoxia is higher in SHR in comparison to normotensive Sprague-Dawley rats already before blood pressure elevation [54]. The mechanism of this difference was further investigated. The excitability of sensory nerve endings was similar in both strains because chemoreceptor response to the inhibitor of mitochondrial electron transport chain, sodium cyanide, was strain-independent. In addition, the amount of glioma cells in the carotid body was also similar in both strains, although these cells released more catecholamines in response to hypoxia in SHR than in normotensive rats [54]. H_2S is involved in chemoreceptor reflex since hypoxia increases H_2S level in the carotid body glioma cells. Both baseline and hypoxia-stimulated H_2S production in glioma cells was higher in SHR than in normotensive animals [54],

however, the expression of CBS was not different between normotensive and hypertensive rats. Heme oxygenase-derived carbon monoxide reduces CBS activity and is the important regulator of H_2S synthesis in the carotid body. It has been demonstrated that CO production is lower in SHR than in normotensive Sprague-Dawley (SD) rats and that heme oxygenase inhibitor, chromium mesoporphyrin, decreases CO and increases H_2S in normotensive rats and augments their response to hypoxia [54]. In contrast, CO donor, CORM-2, reduced H_2S production by glioma cells and chemoreceptor response to hypoxia in SHR and this effect was mimicked by CBS inhibitor. The expression of heme oxygenase-2 (HO-2), the predominant enzyme isoform in glioma cells, was similar in SD rats and SHR, however, kinetic properties of the enzyme differed significantly. Namely, K_m of purified HO-2 was 13 times higher in SHR than in SD rats whereas maximal enzyme activity was 1,8 times lower in SHR resulting in overall lower CO production at any given substrate concentration. Because sympathetic stimulation is one of the responses triggered by chemoreceptor reflex, increased chemoreceptor sensitivity could contribute to BP elevation in SHR. Indeed, carotid body ablation in 5 week-old animals ameliorated age-dependent increase in blood pressure [54].

2.3 H_2S and renal mechanisms of hypertension in SHR

Although there are a lot of studies about the role of H_2S in the regulation of vascular tone, much less is known about its role in the regulation of renal sodium handling, the second most important mechanism in the regulation of blood pressure. However, it has been demonstrated that H_2S increases glomerular filtration rate, inhibits tubular sodium reabsorption and increases urinary sodium excretion [55]. This effect results from the inhibitory effect of H_2S on Na^+ transport from the tubular fluid to the tubular cells by epithelial sodium channels [56, 57] as well as from reduced activity of basolateral membrane Na^+,K^+-ATPase responsible for active Na^+ reabsorption [58].

NaHS administered for 4 weeks at a dose of 56 μmol/kg/day increased glomerular filtration rate (creatinine clearance), urinary sodium excretion and fractional sodium excretion (the ratio between absolute urinary Na^+ excretion and the amount of Na^+ filtered in glomeruli) in SHR. Interestingly, this effect was augmented by superoxide dismutase mimetic, tempol [59] suggesting that increased superoxide contributes to H_2S deficiency in the kidneys of hypertensive rats. Tain et al [60] have demonstrated that total renal H_2S production does not differ between SHR and WKY, however, the expression of CSE and 3-MST at the mRNA level tended to be higher, although not significantly, in SHR than in WKY whereas the expression of CBS was similar. However, the expression of 3-MST measured at the protein level was significantly lower in 12-week old SHR than in WKY. These results suggest that deficiency of 3-MST-derived H_2S in the kidney could contribute to hypertension in SHR by impairing vascular and/or tubular function. In addition, the expression of renin, angiotensinogen and angiotensin AT_1 receptor was higher in the kidney of SHR than in WKY and was normalized by NaHS therapy [60].

Recently it has been demonstrated that renal H_2S production decreases with age in both WKY and SHR, however, this decline is more marked in SHR. In addition, the expression and activity of both CBS and CSE in the kidney was lower in SHR than in WKY [61].

2.4 Effect of H_2S in the prehypertensive stage

Tain et al. [60] have demonstrated that NaHS administered at 14 μmol/kg/day for 4 weeks started in 4-week old and finished in 8-week old SHR reduces systolic and diastolic blood pressure in 12-week old animals. These data indicate that H_2S supplemented at the prehypertensive stage has a persistent hypotensive effect even after cessation of therapy. The mechanism of this effect was not clarified, although NaHS reduced the level of endogenous NOS inhibitor, L-NMMA as well as plasma homocysteine and increased the level of reduced glutathione (GSH) and nitric oxide metabolites.

Similarly, Hsu et al. [62] have demonstrated that either L-cysteine or D-cysteine administered at 8 mmol/kg/day via intragastric gavage in the prehypertensive stage (between 4 and 6 weeks of age) reduced systolic and diastolic blood pressure in 12 week-old SHR additionally receiving 1% NaCl in the drinking water for 8 weeks. In addition, L- or D-cysteine reduced glomerulosclerosis and tubular injury as well as 8-hydroxy-deoxyguanosine (8-OHdG) – the marker of oxidative modification of nucleic acids – in the kidney. The mechanism of this protective effect is unclear. Cysteine supplementation had no effect on the expression of renin, angiotensinogen, angiotensin-converting enzyme and AT_1 receptor as well as on plasma concentration of endogenous NOS inhibitor, ADMA. The effect of D-cysteine resulted probably from its ability to serve as the H_2S donor specifically in the kidney [10].

3. Dahl salt-sensitive rats

Dahl salt-sensitive (Dahl S) rats represent the other commonly used model of genetically determined hypertension. However, in contrast to SHR, Dahl S rats develop hypertension only when fed high salt diet for several weeks. It was first demonstrated in 2006 that transsulfuration pathway, that is homocysteine to cysteine metabolism, is impaired in Dahl salt-sensitive rats even on a low salt diet in comparison to the control Brown Norway SSBN13 rats [63]. This was accompanied by decreased expression of CBS in the kidney at the mRNA and protein levels and hyperhomocysteinemia, however, the implications for H_2S signaling were not examined that time. Later, it has been demonstrated that although plasma H_2S level is higher in Dahl salt-sensitive rats fed a low salt diet than in the control SD rats, high salt feeding (8% NaCl for 8 weeks) decreased its level only in Dahl S rats [64]. Similarly, high salt feeding reduced H_2S concentration in the kidney. Using H_2S-specific fluorescent probe, authors observed that high salt feeding decreases H_2S in the proximal tubular epithelium and endothelial cells of cortical interlobular arteries but not in glomeruli. CBS expression examined by immunohistochemistry

was lower in Dahl S rats in the collecting duct, proximal tubule and endothelium. Interestingly, high salt diet decreased the expression of hypoxia-inducible factor (HIF-1α) only in Dahl S but not in SD rats. In addition, high salt concentration reduced HIF-1α and CBS expression as well as H_2S production in cultured kidney slices ex vivo. The level of HIF-1α protein is regulated by its hydroxylation by prolyl hydroxylases which targets it for proteasomal degradation. In the presence of oxygen, HIF-1α is quickly hydroxylated and directed to proteasomal degradation, however, under hypoxic conditions hydroxylation is compromised and HIF-1α accumulates to trigger the expression of hypoxia-induced genes. . Prolyl hydroxylase-2 (PHD-2) inhibitor, IOX2, reversed the suppressing effect of high salt concentration on CBS and H_2S in kidney slices from Dahl S rats but had no effect on kidney slices from SD rats. However, HIF-1α inhibitor, 2MeOE2, reduced CBS expression and H_2S production in the kidney of SD rats. These data indicate that increased PHD-2 activity depletes HIF-1α leading to the deficiency of CBS and H_2S in high salt-fed Dahl S rats. NaHS therapy (90 μmol/kg/day for 8 weeks) reduced blood pressure in high salt-fed Dahl S rats and ameliorated thickening of thoracic aortic wall. Moreover, renin and angiotensin II concentrations in serum and renal tissue were higher in Dahl S rats and were normalized by NaHS therapy. In normotensive rats, high salt diet increased the expression of HIF-1α in the renal medulla leading to up-regulation of not only CBS but also other gasotransmitter-synthesizing enzymes including NO synthase and heme oxygenase but this mechanism is impaired in salt-sensitive hypertension [64].

Similarly to SHR, H_2S may also be important for the central regulation of blood pressure in Dahl S rats. ROS production and the expression of NOX2 and NOX4 as well as IL-1β concentration in paraventricular nucleus (PVN) are higher in Dahl S rats after 4-week high salt feeding. In contrast, the expression of CBS, H_2S concentration, anti-inflammatory cytokine, IL-10 and SOD1 expression in PVN were lower in hypertensive Dahl S rats than in normotensive

animals [65]. Microinjection of hydroxylamine into the PVN aggravated these effects as well as further increased blood pressure, whereas microinjection of GYY4137 had the opposite effects. These results suggest that H_2S deficiency locally in the PVN may contribute to the pathogenesis of hypertension in this model [65].

Similarly, Liao et al. [66] have demonstrated that 8-week feeding of Dahl S rats with 8% NaCl increased ROS production and MDA concentration but reduced SOD activity in the PVN. In addition, high salt increased the expression of several subunits of pro-inflammatory transcription factor, NF-κB, as well as TNF-α, IL-1β and IL-6 concentrations in the PVN. Either systemic NaHS administration or GYY4137 microinjected into the PVN reduced oxidative stress and markers of inflammation, reduced sympathetic system activity and blood pressure, decreased heart weight/body weight and kidney weight/body weight ratios, decreased plasma norepinephrine concentration, plasma urea and cystatin C concentrations and reduced histological lesions in the kidney [66].

4. Hypertension induced by chronic inhibition of NO synthase

NO continuously produced by vascular endothelial cells stimulated by shear stress plays an important role in the regulation of vascular tone. In addition, NO produced in the kidney inhibits tubular sodium reabsorption. Administration of NO synthase inhibitors induces marked increase in blood pressure and is the well-established model of hypertension.

In 2003, Zhong et al. [67] first examined the role of H_2S in the pathogenesis of hypertension induced by NOS blockade. They demonstrated that administration of synthetic NO synthase inhibitor, L-NAME (50 mg/100 ml drinking water, corresponding to average intake of 70 mg/kg/day) for 6 weeks increased systolic blood pressure by 55%, heart weight/body weight ratio by 23%, decreased plasma concentration of NO metabolites as well as NO production by isolated thoracic aorta and superior mesenteric artery. Plasma H_2S concentration, H_2S production

by isolated thoracic aorta and mesenteric artery and CSE expression in these vessels were lower in L-NAME treated than in control rats by 31%, 53%, 51%, 28% and 23%, respectively. Simultaneous NaHS administration (20 μmol/kg/day ip.) reduced systolic blood pressure and heart weight/body weight ratio by 19% and 12% respectively, although the level of NO metabolites and NO production by the vascular tissue did not change.

In contrast to many studies, the results obtained by Wesseling et al. [68] suggest the unbeneficial role of H_2S in L-NAME-induced hypertension. First, they demonstrated that PAG administered at a dose which very effectively reduced renal H_2S production (37.5 mg/kg/day) had no effect on blood pressure and kidney histology. More importantly, renal H_2S production was reduced by 49% in rats receiving L-NAME for 4 weeks and this effect was augmented by PAG. Surprisingly, however, PAG reduced blood pressure and urinary protein excretion in L-NAME-treated rats. These data suggest that inhibiting rather than increasing H_2S is beneficial. This finding may be explained by vasoconstricting effect of H_2S at low concentrations observed in some experimental studies [69-72] as well as the cross-talk between different gasotransmitters. Specifically, PAG increased the expression of HO-1 as well as CO production in the kidney and CO is well known to improve renal sodium excretion [68]. These data indicate that H_2S inhibits heme oxygenase-dependent CO production in the kidney and that inhibiting H_2S may be beneficial by augmenting vascular and tubular CO signaling.

Several studies demonstrated that endogenous sulfur dioxide (SO_2) serves as the other sulfur-containing gasotransmitter in the cardiovascular system [73]. The recent study [74] suggests that SO_2 may be the backup vasodilating mechanism which is up-regulated in experimental hypertension induced by chronic NO synthase blockade. SO_2 concentration in the aortic wall was by 89% higher in mice chronically treated with L-NAME (100 mg/kg/day by osmotic minipump for 8 weeks). This effect was accompanied by higher activity of the rate-

limiting enzyme of SO_2 synthesis, aspartate aminotransferase)AAT), in the aortic wall, although the expression of AAT1 protein was not changed. Aortic media thickness as well as collage type I and type III content in the aortic wall were higher in L-NAME-treated than in control mice. Administration of AAT inhibitor, HDX, decreased SO_2 concentration by 82%, increased systolic blood pressure by 14% and increased aortic media thickness and collagen content, suggesting that endogenous SO_2 plays an important role in the regulation of blood pressure and inhibiting vascular remodeling. Interestingly, co-administration of HDX with L-NAME augmented blood pressure elevation and aortic remodeling. The similar effects were observed in small arterioles isolated from brown adipose tissue.

As expected, knockdown of eNOS in cultured aortic endothelial cells increased collagen production by co-cultured smooth muscle cells which was further aggravated by HDX. eNOS deficiency increased SO_2 production by about 50%. In contrast, the NO donor, sodium nitropruside, decreased SO_2 production by vascular smooth muscle cells and AAT1 activity but did not modify the amount of AAT1 protein. Sodium nitroprusside also reduced the activity of isolated AAT, presumably, by nitrosylating thiol group of cysteine 192 residue. Altogether, these results suggest that SO_2 synthesis is inhibited by physiological NO level and is a compensatory mechanism up-regulated in hypertension induced by NO deficiency. It remains to be established if SO_2 system in the vasculature is modified also in other models of hypertension [74].

5. Angiotensin II-induced hypertension

Angiotensin II is a potent vasoconstrictor which increases blood pressure not only by activating Ca^{2+} influx into vascular smooth muscle cells but also by stimulating vascular NADPH oxidase-dependent superoxide anion production and oxidative stress. Excess of superoxide induces NO deficiency by scavenging it to form peroxynitrite ($ONOO^-$) – a very reactive

oxygen/nitrogen species. Chronic administration of angiotensin II is a well-established model of hypertension. However, the data about the role of H_2S in this model are controversial.

Oosterhuis et al. [75] demonstrated that CSE inhibitor, PAG (37,5 mg/kg/day) reduced blood pressure in male Sprague-Dawley rats made hypertensive by 3-week angiotensin II infusion through the osmotic minipump at a dose of 435 ng/kg/min. In addition, PAG decreased serum creatinine level and reduced proteinuria and histological features of renal damage. Importantly, PAG did not modify plasma concentration and urinary excretion of NO metabolites in angiotensin II-treated rats although it decreased NO production in normotensive rats not receiving angiotensin II. In addition, PAG decreased renal fractional excretion of sodium in angiotensin II-treated rats indicating that improved sodium excretion is not involved in antihypertensive effect of this CSE inhibitor. Irrespectively of the mechanism, these results indicate that endogenous H_2S plays a detrimental role in this model of hypertension, similarly as previously described in L-NAME-induced hypertension.

In contrast, NaHS (10 μmol/kg/day) not only reduced blood pressure but also improved acetylcholine-induced relaxation of isolated aortic rings , increased NO availability, improved sodium nitroprusside-induced relaxation and decreased NADPH oxidase-dependent superoxide anion production in C57BL/6J mice treated with angiotensin II for 2 weeks at a dose of 0.7 mg/kg/day [76]. H_2S production by isolated aorta was almost negligible in angiotensin II-treated mice. L-cysteine-induced relaxation of isolated aortic rings, presumably resulting from its conversion to H_2S, was impaired in angiotensin II-treated mice whereas NaHS improved this effect. Co-administration of PAG together with angiotensin II had no additional effect on blood pressure and endothelial dysfunction although aggravated H_2S deficiency and oxidative stress in the aortic wall. H_2S may inhibit angiotensin II signaling by reducing disulfide bonds in the extracellular domain of AT_1 receptor which are important for hormone binding [76].

Gut microbiota digest alimentary fibers to produce short-chain fatty acids (SCFA) which are utilized as the energy substrate by host cells but also have important signaling function. SCFA activate olfactory receptor-78 (Olfr78) in the kidney to stimulate renin production but also vascular G protein-coupled receptor-41 (Gpr41) to directly induce vasorelaxation. Weber at al. [77] demonstrated that the composition of gut microbiota was not altered in mice treated with angiotensin II (1 μmol/kg/min) for 4 weeks, however, the expression of Olfr78 was higher whereas the expression of Gpr41 and Gpr43 was lower in the kidney of angiotensin II-treated than in control mice. GYY4137 administered at 133 μmol/kg/day by intraperitoneal injections reduced the amount of *Formicates* and increased the amount of *Bacteroitides* in gut microbiome, reduced the expression of Olfr78 and increased the expression of Gpr41 and 43 in the kidney of angiotensin II-treated mice [77].

Retinoid acid-related orphan receptors γ2 and γ3 (RORγ2 and RORγ3) are transcription factors involved in the development of IL-17-producing Th17 lymphocytes and IL-17 have been demonstrated to be involved in hypertension. Angiotensin II increased the expression of RORγ2 and RORγ3, the amount of Th17 cells and IL-17 and IL-6 concentrations in the kidney; all these effects were attenuated or abolished by GYY4137 [77]. The expression of long-chain fatty acid receptor with anti-inflammatory activity, Gpr120, was reduced in the kidney of angiotensin II-treated mice but was stimulated by GYY4137.

The expression of Olfr78 and Gpr41 is inhibited by microRNA miR-129 and miR-329, respectively. Angiotensin II infusion reduced the expression of miR-129 and increased the expression of miR-329 whereas simultaneous treatment with the H_2S donor normalized their levels. These data indicate that angiotensin II and H_2S modify the expression of fatty acid receptors by regulating their regulatory microRNAs [77].

Zonulin is the intestinal structural protein involved in the regulation of intestinal barrier integrity. Impaired barrier integrity results in partial absorption of bacteria-derived molecules such as LPS leading to chronic low-grade inflammation of the remote organs. Angiotensin II-induced hypertension is associated with impaired intestinal barrier integrity as evidenced by the increased amount of bacterial rRNA in the kidney and higher haptoglobin (the zonulin precursor) level in the blood [62]. On the other hand, GYY4137 reduced circulating haptoglobin and bacterial rRNA in the kidney.

Chi et al. [78] have demonstrated that histone deacetylase (HDAC) IIb inhibitor, tubostatin A, alleviates angiotensin II-induced hypertension by inhibiting CSE degradation. HDACIIb is the subgroup of HDAC family which primarily deacetylate non-histone proteins. Tubostatin A (50 mg/kg/day) reduced blood pressure and improved endothelial function in mice with angiotensin II-induced hypertension. Ex vivo, angiotensin II reduced H_2S production by isolated aortic rings and human aortic smooth muscle cells whereas tubostatin A had the opposite effects. Angiotensin II increased the expression of HDAC6 and knockdown of this enzyme, but not of other HDACs, attenuated the effect of angiotensin II on CSE expression. HDAC6 deacetylates CSE at lysine^{73} residue and reduces enzyme stability by directing it to proteasomal and, to a lesser extent, lysosomal degradation, whereas HDAC6 inhibitors increases CSE acetylation level and stability even in cells incubated with angiotensin II [78].

One of the mechanisms through which H_2S exerts beneficial effects in angiotensin II-induced hypertension may be amelioration of oxidative stress and inflammation. Indeed, Li et al. [46] have demonstrated that angiotensin II increases ROS production, decreases the expression of SOD1 and catalase, increases the expression of NLRP3 inflammasome, caspase-1 and IL-1β in cultured human umbilical vein endothelial cells whereas NaHS attenuated these effects. The beneficial effect of NaHS was ameliorated by Nrf2 knockdown suggesting that stimulation of this

transcription factor is involved in the antioxidant and anti-inflammatory effect of H_2S in endothelial cells.

6. Renovascular hypertension

Renovascular hypertension is one of the most common causes of secondary hypertension in humans. This type of hypertension results from hypoperfusion of the kidney and increased renin secretion by juxtaglomerular apparatus. Two-kidney one clip (2K1C) is a commonly used model of renovascular hypertension in animals.

Lu et al. [79] first demonstrated that NaHS administered at 10-100 μmol/kg/day starting on the 3rd day after clipping the renal artery attenuated blood pressure elevation in a dose-dependent manner. Moreover, NaHS therapy started 8 days after the surgery was effective in the treatment of already hypertension in this model. Plasma renin activity and angiotensin II concentration as well as angiotensin-converting enzyme activity in the aortic wall were markedly higher in 2K1C rats and were markedly reduced by NaHS. In addition, renin mRNA and protein levels in the clipped kidney were higher than in contralateral unclipped kidney but were normalized by NaHS. Importantly, NaHS had no effect on plasma renin in control rats as well as in rats with one kidney one-clip (1K1C) hypertension. Increased renin production in the 1K1C model is involved in blood pressure elevation only in the first phase whereas later hypertension becomes renin-independent and is mediated by factors such as high sympathetic activity or sodium retention.

Renin secretion and synthesis by juxtaglomerular cells are stimulated by cAMP. In primary culture of renin-producing cells, NaHS alleviated forskolin (the adenylyl cyclase activator)-stimulated renin secretion [79].

Later, it was confirmed that plasma H_2S is lower in rats with 2K1C model of hypertension [80]. Vasoconstricting effect of angiotensin II was enhanced whereas endothelium-dependent

acetylcholine-induced relaxation of aortic rings was impaired in 2K1C rats and these abnormalities of vascular response were normalized by NaHS therapy. Clipping the renal artery increased MDA concentration and the expression of Nox2 and Nox4, reduced SOD activity and SOD1 and SOD2 expression in the aortic wall whereas NaHS administration had the opposite effects.

Bone morphogenetic protein-4 (BMP-4) is a member of TGF-β family of growth factors. Chronic infusion of BMP4 induces hypertension due to up-regulation of NADPH oxidases and cyclooxygenase-2 which produces vasoconstricting prostaglandins such as PGF2α [81]. Xiao et al. [82] have demonstrated that 2K1C hypertension in the rat is associated with reduced renal CSE expression, up-regulation of BMP4 in the renal cortex and medulla, higher expression of $p67^{phox}$, Nox2 and Nox4, high concentration of 3-nitrotyrosine and phosphorylation level of p38 MAPK which is the regulator of BMP4 expression. In addition, COX-2 expression in the renal artery was higher in the clipped kidney which was accompanied by augmented endothelium-dependent vasoconstriction and impaired endothelium-dependent relaxation of renal artery rings. NaHS reduced blood pressure, decreased BMP4 expression, normalized vascular responses and decreased the expression of oxidative stress-associated proteins and COX-2 as well as p38 MAPK phosphorylation. These data indicate that BMP4 is involved and 2K1C hypertension and the protective effect of H_2S is mediated, in part, by decreasing the expression of this protein.

Similarly to other models of hypertension, baroreceptor sensitivity is impaired in renovascular hypertension. Indeed, decrease in heart rate per unit of mean blood pressure increase after phenylephrine administration, the marker of baroreceptor sensitivity, was significantly compromised 4 weeks after clipping the renal artery in the rat whereas NaHS therapy improved it [83]. The expression of all 3 H_2S-producing enzymes in the nodose ganglion and nucleus tractus solitari, the centers involved in baroreceptor reflex, was lower in 2K1C than in normal rats.

Microinjection of NaHS or L-cysteine into the nodose ganglion decreased blood pressure in both control normotensive and 2K1C hypertensive rats, although the effect in 2K1C rats was impaired. The doses of NaHS and L-cysteine inducing half-maximal responses were 4 and 3 times, respectively, higher in rats with renovascular hypertension in comparison to control rats. The K_{ATP} channel blocker, glibenclamide, reduced the effect of NaHS on blood pressure in rats with renovascular hypertension and partially attenuated the effect of NaHS on baroreceptor sensitivity. Interestingly, NaHS increased the expression of Kir6.2 and SUR1/2 – subunits of K_{ATP} channels – in nucleus tractus solitary and nodose ganglion. In addition to activating K_{ATP} channels, H_2S may improve baroreceptor reflex sensitivity by reducing protein kinase A expression and decreasing phosphorylation of NMDA receptors at Ser^{897} [83].

Chronic infusion of angiotensin II (200 ng/kg/min) increased microglia activity in the paraventricular nucleus [84]. Pro-inflammatory signaling in the PVN increases sympathetic output in various models of hypertension. Chronic intracerebroventricular infusion of NaHS reduced PVN inflammation, decreased sympathetic system activity and mean arterial pressure in rats treated chronically with angiotensin II [84].

7. H_2S and end-organ injury in hypertension

7.1 Vascular remodeling

Thickening of vascular wall is the important consequence of hypertension. In the coronary microcirculation, medial thickening contributes to vascular stenosis, myocardial ischemia and dysfunction. Shi et al. [85] observed that thickness of coronary arteriole wall was higher in adult SHR than in Wistar rats, which was accompanied by perivascular fibrosis, interstitial fibrosis and increased myocardial ROS production whereas systolic and diastolic function of the left ventricle were still normal. Thus, the model represents the early phase of myocardial remodeling without functional consequences. NaHS treatment (10-90 μmol/kg/day ip.) started at the age of 4 weeks

(at the prehypertensive stage) attenuated these abnormalities in SHR. The ratio between left ventricle weight to body weight (the marker of myocardial hypertrophy) was higher in SHR than in WKY but was not reduced by NaHS. The effect of NaHS on blood pressure and coronary arterioles was abolished by concomitant administration of K_{ATP} channel blocker, glibenclamide (5 mg/kg/day p.o). Interestingly, hydralazine administered at 10 mg/kg/day normalized blood pressure in SHR but had no effect on coronary arterioles, interstitial fibrosis and myocardial ROS indicating that protective effect of NaHS did not result from blood pressure normalization. The left ventricular myocardium of SHR, apart from increased ROS production, exhibited other features of oxidative stress such as increased concentration of conjugated dienes and reduced concentration of reduced –SH groups. It was concluded that H_2S inhibits remodeling of coronary arterioles by ameliorating oxidative stress [85].

Zhao et al. [86] demonstrated increased fibrosis of the thoracic aorta as evidenced by higher concentration of hydroxyproline and collagen type I content in adult SHR in comparison to WKY. NaHS treatment started at the age of 4 weeks and continued for consecutive 5 weeks reduced these markers of vascular fibrosis, however, hydralazine did not. These authors also observed that stimulatory effect of angiotensin II on the proliferation of vascular smooth muscle cells in vitro was stronger in SHR than in WKY and that NaHS inhibits proliferation as well as fibrosis more potently in SHR than in normotensive rats due to reduced affinity of angiotensin AT_1 receptor to angiotensin II after NaHS treatment.

Thoracic aorta of 18-week old SHR exhibited significant structural alterations characteristic for vascular wall hypertrophy. Interestingly, the expression of K_{ATP} channels in the thoracic aorta was lower in SHR than in WKY [87]. NaHS administration started at this age (90 μmol/kg/day) and continued for 2 months not only reduced systolic blood pressure but also increased the expression of K_{ATP} channel Kir6.1 and SUR2B subunits and normalized aortic wall

structure. Similar results were observed in small resistance vessels including mesenteric arteries, tail arteries as well as microvessels of the left ventricle and scapular muscle. Pinacidil (the K_{ATP} channel opener)-induced relaxation of aortic rings with removed endothelium was compromised in SHR and was improved by NaHS. In cultured vascular smooth muscle cell line as well as in primary rat aortic smooth muscle cells, NaHS had no effect on baseline expression of K_{ATP} channels, however, reduced the inhibitory effect of endothelin-1 on Kir6.1 and SUR2B expression at the mRNA and protein levels. The effect of NaHS was mediated by stimulating nuclear translocation of FOXO1 and FOXO3 – transcription factors involved in the regulation of K_{ATP} channel expression [87].

NaHS not only reduced blood pressure but also reduced vascular hypertrophy and remodeling as evidenced by the decrease in aortic weight, media thickens and elastin content, decreased oxidative stress, the expression of interferon-γ and vascular cells adhesion-molecule-1 (VCAM-1) as well as infiltration of Th lymphocytes in the aortic wall in L-NAME-treated rats [88]. All these markers of vascular remodeling were significantly higher in L-NAME-treated than in control animals.

Vascular effects of H_2S in the regulation of vascular tone and remodeling relevant for hypertension are summarized on Fig. 1 and Table 1

7.2 Myocardial hypertrophy and remodeling

Myocardial hypertrophy and remodeling are the important complication of hypertension ultimately leading to heart failure if blood pressure is not managed accordingly. The important role in this process is played by transforming growth factors (TGFs)β-1, -2 and -3 which signal via phosphorylation of Smad proteins. Phosphorylated Smad proteins then translocate to the nucleus and stimulate collagen synthesis as well as reduce the expression of matrix metalloproteases (MMPs) involved in the turnover of extracellular matrix proteins. Sun et al. [89]

have demonstrated that NaHS administered at 90 μmol/kg/day for 9 weeks and starting at the age of 9 weeks improved myocardial ultrastructure in SHR toward normal picture found in the control WKY rats. SHR exhibited myocardial hypertrophy as evidenced by higher left ventricle weight/body weight ratio but this was not affected by NaHS. The expression of TGF-β1 and its receptors, phosphorylation of Smad2 and Smad3, collagen type I, collagen type III and tissue inhibitor of metalloproteinase (TIMP-1) in the heart was higher in SHR than in WKY whereas the expression of MMP-13 was lower in SHR. NaHS treatment normalized these markers of myocardial fibrosis except MMP-13 and TIMP-1 which were not altered by therapy. In addition, in the myocardial fibroblast cell culture, NaHS reduced TGF-β1-induced phosphorylation of TGF-β receptor, phosphorylation of Smad2 and Smad3 and collagen type I and type III expression. These data indicate that H_2S inhibits myocardial fibrosis while having no effect on cardiomyocyte hypertrophy. Importantly, hydralazine had no effect on myocardial fibrosis in SHR although it reduced blood pressure as effectively as NaHS suggesting that anti-fibrotic effect of NaHS is independent of its effect on blood pressure.

Synthetic slow-releasing H_2S donor, GYY4137, administered at a dose of 50 mg/kg/day for 4 weeks in adult SHR reduced myocardial hypertrophy as well as the expression of ANP (the marker of remodeling) in ventricular myocytes [90]. The effect was partially mediated by down-regulating the pro-hypertrophic transcription factor, KLF5. Indeed, the expression of KLF5 was higher in the left ventricle of SHR than in normotensive rats and was reduced by GYY4137. In cultured rat neonatal cardiomyocytes, GYY4137 reduced KLF5 expression by sulfurating its regulatory transcription factor SP-1 at Cys^{664}. Knockdown of KLF5 attenuated the inhibitory effect of GYY4137 on cardiomyocyte hypertrophy and ANP expression [90]. In addition, GYY4137 reduced myocardial fibrosis as demonstrated by histological collagen staining, reduced

collagen-specific hydroxyproline content and decreased concentration of cross-linked insoluble collagen in the heart [91]

Huang et al. [92] have demonstrated that H_2S production by the myocardium is impaired in high salt-fed Dahl S rats which is associated with reduced expression of CBS at both mRNA and protein levels. CSE expression in the heart was reduced only at the mRNA but not at the protein level whereas MPST expression was normal. High salt fed Dahl S rats were characterized by myocardial hypertrophy as evidenced by increased heart weight/body weight ratio, cardiomyocyte cross-sectional area, reduced expression of adult-type myosin heavy chain α-isoform and increased expression of fetal-type MHC-β. These effects were accompanied by myocardial oxidative stress, that is increase in MDA concentration and decrease in reduced glutathione (GSH), total antioxidant capacity, catalase, glutathione peroxidase and superoxide dismutase activities as well as decreased expression of SOD1 and SOD2 proteins. NaHS administered at 90 μmol/kg/day reduced heart weight/body weight ratio and cardiomyocyte cross-sectional area, normalized the profile of MHC subunits, reduced MDA content and increased the expression and activity of antioxidant enzymes. These data suggest that local H_2S deficiency contributes to oxidative stress and myocardial hypertrophy/remodeling in Dahl S rats. Interestingly, CBS inhibitor, hydroxylamine, had no effect on myocardial mass in control Sprague-Dawley rats fed normal salt diet but induced myocardial hypertrophy if these animals were fed high-salt diet [92].

NaHS improved myocardial contractility, reduced heart weight/body weight ratio and left ventricle weight/body weight ratio and decreased myocardial interstitial collagen content in rats treated with L-NAME for 5 weeks [83]. NaHS increased plasma concentration of NO metabolites, NOS activity in the left ventricle and both eNOS and its upstream activator, Akt,

phosphorylation. These data indicate that H_2S may improve NO synthesis in the heart despite administration of NO synthase inhibitor.

Nguyen et al. [94] have demonstrated that sodium thiosulfate administered at 2 g/kg/day in the drinking water for 3 weeks reduced blood pressure and improved cardiac performance as evidenced by the increase in ejection fraction, stroke volume and cardiac index, decreased left ventricular posterior wall thickness and interventricular septum thickness and decreased myocardial fibrosis in rats receiving NO synthase inhibitor, L-NNA (40 mg/kg/day) for 3 weeks. Thiosulfate may be converted in vivo to H_2S in vivo, however, it is unclear to what extent the effect of sodium thiosulfate in that study was mediated by H_2S. Similarly, NaHS or sodium thiosulfate reduced heart weight/body weight ratio, cardiomyocyte size and atrial natriuretic peptide (ANP) expression, cardiac collagen content, the expression of pro-fibrotic proteins, fibronectin and galectin-3 in the heart of rats with angiotensin II-induced hypertension [95].

The main effects of H_2S in the hypertensive heart are presented on Fig. 2 and Table 2

7.3 Kidney injury

Huang et al. [96] have demonstrated that NaHS administered at a dose of 90 μmol/kg/day improved renal function as evidenced by increase in creatinine clearance, reduction of proteinuria and renal interstitial fibrosis in Dahl S rats fed the high-salt diet. Moreover, renal H_2S production was impaired in high salt-fed Dahl S rats. Oxidative stress markers including H_2O_2, MDA, oxidized glutathione (GSSG) and myeloperoxidase activity were elevated in the kidney of high salt-fed Dahl S rats which was accompanied by the decrease in total antioxidant capacity, reduced glutathione (GSH), catalase, GPx and SOD activities as well as reduced expression of SOD1 and SOD2 proteins whereas NaHS treatment normalized these markers. In contrast, CBS inhibitor, hydroxylamine, induced oxidative stress in the kidney of normotensive Dahl S rats fed low salt diet. These results suggest that local H_2S deficiency in the kidney may contribute to kidney injury

in hypertensive Dahl S rats, at least in part by inducing oxidative stress, whereas administration of exogenous H_2S donor is protective.

Taurine is the non-protein aminoacid produced from L-cysteine by the concerted action of cysteine dioxygenase (CDO) and cysteine sulfenic acid decarboxylase (CSAD). Taurine supplementation decreases blood pressure in both experimental and clinical studies [97]. Huang et al. [98] have demonstrated that CDO and CSAD expression in the kidneys is reduced in high salt-fed Dahl S rats which results in taurine deficiency. Taurine supplementation in the drinking water (2% for 6 weeks) reduced blood pressure, increased creatinine clearance, decreased proteinuria and plasma urea concentration and increased CBS, CSE and MPST expression in the kidney. In contrast, taurine had no effect on blood pressure, renal CBS and H_2S production in normotensive SS-13BN rats fed the high salt diet which were used as the control. In addition, taurine reduced oxidative stress in the kidney of high salt-fed Dahl S rats as well as reduced renin, angiotensin II and aldosterone concentrations [81]. Incubation of renal tissue slices of Dahl S rats fed the regular diet with increased NaCl concentration ex vivo resulted in the down-regulation CBS expression and H_2S production as well as oxidative stress whereas taurine reversed these effects. However, the effects of taurine on oxidative stress and renin-angiotensin system ex vivo was blocked by hydroxylamine. It was concluded that in Dahl S rats high salt diet downregulates taurine—producing enzymes and induces taurine depletion leading to the inhibition of CBS-H_2S pathway, oxidative stress and activation of renin-angiotensin system in the kidney. Similarly, high salt medium reduced CBS expression and H_2S production in cultured endothelial cells obtained from Dahl S rats and increased the expression of several subunits of NADPH oxidase as well as renin expression. Addition of taurine to the culture medium reversed these effects of high-salt conditions [98].

Sodium thiosulfate administered in the drinking water improved urinary sodium excretion, decreased systolic blood pressure and plasma urea concentration and increased creatinine clearance and renal plasma flow in rats treated with L-NNA for 3 weeks. Interestingly, L-NNA treated rats exhibited impaired efficacy of tubular sodium transport as evidenced by decrease in the ratio between the amount of sodium reabsorbed and oxygen consumption and this was improved by sodium thiosulfate [99]. As stated before, it is unclear how much of the effect of thiosulfate is mediated by H_2S.

Similarly, sodium thiosulfate decreased systolic and diastolic blood pressure, renin expression in the kidney, urinary protein excretion, plasma urea concentration, the expression of kidney injury molecule-1 (KIM-1), the marker of tubular damage and desmin, the marker of glomerular injury, reduced macrophage infiltration into the renal interstitium and increased creatinine clearance in rats with angiotensin II-induced hypertension. In addition, sodium thiosulfate reduced the expression of Nox2, TGF-β and α-smooth muscle actin (a marker of myofibroblast transformation) as well as urinary MDA excretion in angiotensin II-treated rats [100]. NaHS had the effects very similar to sodium thiosulfate suggesting that the effect of the latter could be mediated by H_2S. Angiotensin II infusion reduced the expression of CBS, CSE and MPST in the kidney whereas either NaHS or thiosulfate improved it. Importantly, NaHS added to the perfusion fluid reduced intrarenal pressure in the perfused kidney whereas thiosulfate did not, indirectly suggesting that renoprotective effect of thiosulfate was not mediated by lowering blood pressure [100].

Fig. 3 and Table 3 present summariy of data about the effects of H_2S relevant for hypertension-associated kidney dysfunction and injury.

7.4 Erectile dysfunction

Erectile dysfunction, one of the consequences of endothelial dysfunction, is commonly observed in patients with hypertension. Yilmaz et al. have demonstrated that NaHS increased the ratio of intracavernosal pressure to mean arterial pressure, the marker of erectile performance, in L-NAME treated rats [101]. In addition, both acetylcholine- and electric field-induced relaxation of phenylephrine-pre-constricted penile tissue strips were impaired in L-NAME-treated rats but not in rats treated with both L-NAME and NaHS. Neither L-NAME nor NaHS had any effects on phenylephrine-induced constriction and sodium nitroprusside-induced relaxation of the cavernous tissue strips. The expression of both eNOS and nNOS as well as the ratio of smooth muscle cells to collagen content were lower in the penile tissue of L-NAME-treated rats which was accompanied by reduced expression of CSE and H_2S production and increased expression of NF-κB. NaHS administration increased eNOS, nNOS and smooth muscle cell/collage ratio but reduced NF-κB expression. This study [101] demonstrates that H_2S deficiency contributes to erectile dysfunction in rats with chronic NO deficiency.

8. H_2S and the immune mechanisms of hypertension

Both experimental and clinical studies demonstrated that hypertension is associated infiltration of $CD4^+$ and $CD8^+$ lymphocytes into the perivascular region of blood vessels and the kidney. Cytokines produced by T lymphocytes contribute to vascular remodeling and hypertension-associated kidney injury. On the other hand, H_2S inhibits proliferation and activation of lymphocytes. Ni et al. [102] reported that the amount of lymphocytes in the carotid artery wall and renal interstitium as well as the amount of $CD4^+$ T cells in the blood was higher whereas the amount of $CD8^+$ cells as well regulatory T cells was lower in SHR than in WKY. Concentration of pro-inflammatory cytokines, IL-2 and IL-6, was higher whereas anti-inflammatory cytokine, IL-10, was lower in SHR. The expression of connexin 40 (Cx40) was higher in $CD4^+$ and $CD8^+$ cells of SHR. NaHS administered at 56 μmol/kg/day ip. for 9 weeks

started in 9 week-old animals reduced lymphocyte infiltration in the carotid artery wall and the kidney, normalized blood T cell profiles, cytokine levels and Cx40 in SHR [102].

Recently, Cui et al. [103] have demonstrated that CSE expression by lymphocytes and H_2S production by these cells is lower in patients with untreated hypertension than in normotensive control subjects. However, there is no difference in CSE expression and H_2S production between control subjects and patients with hypertension with well-controlled blood pressure. CBS expression in lymphocytes tended to be lower in hypertensive patients but the difference was not significant. Similarly, CSE-H_2S system in lymphocytes of SHR was suppressed in comparison to normotensive rats but was improved by NaHS supplementation at doses which normalized blood pressure. These results indicate that CSE-H_2S system in lymphocytes is sensitive to blood pressure changes. In addition, NaHS increase serum IL-10 concentration (the marker of regulatory T cell activity) but reduced the expression of IL-17 (the marker of Th17 cell activity), IL-4 (the marker of Th2 activity) and IFN-γ (the marker of Th1 cell activity). The expression of transcription factor RORC (Th17 marker) was higher in lymphocytes isolated from SHR whereas the expression of Foxp3 (Treg marker) was lower in hypertensive rats. NaHS reduced RORC and increased Foxp3 in lymphocytes of SHR but had no effect in normotensive rats. Moreover, the amount of Treg cells in the blood and kidneys was lower in SHR in comparison to normotensive rats but was partially improved by NaHS. The angiotensin AT_1 receptor antagonist, irbesartan, had the similar effect. The amount of Treg cells in lymph nodes, thymus and spleen was also lower in SHR. NaHS treatment increased the amount of Treg cells in lymph nodes and thymus but not in the spleen whereas irbesartan has no effect on Treg cells in these tissues. These data indicate that H_2S promotes Treg differentiation in lymph nodes and thymus. IL-10 concentration in the kidney was lower in SHR than in control rats and was improved by NaHS therapy. Male and female mice with conditional knockout of CSE specifically in T lymphocytes exhibit blood

pressure elevation by about 5 mmHg and 8 mmHg, respectively. Angiotensin II infused by osmotic minipumps at 500 ng/kg/min for 4 weeks increased blood pressure in mice lacking CSE in lymphocytes to a greater extent than in wild-type animals. Angiotensin II infusion reduced the amount of Treg cells in the blood and the kidney but not in the spleen, lymph nodes and thymus. Angiotensin II infusion induced infiltration with $CD4^+$ and $CD8^+$ lymphocytes in the kidney and perivascular adipose tissue and this effect was augmented in mice lacking CSE in lymphocytes. Transfer of $CD4^+$ lymphocytes lacking CSE by tail vein injection into the CD4 null mice augmented pro-hypertensive effect of angiotensin II, reduced Treg cells in the blood and kidney, impaired endothelium-dependent vasorelaxation, augmented inflammatory cell infiltration in the kidney and perivascular adipose tissue. Taken together, these results indicate that H_2S produced by T lymphocytes stimulates their differentiation to Treg cells and that hypertension is associated with Treg deficiency leading to inflammatory reaction in the kidney, blood vessels and perivascular adipose tissue which then contributes to blood pressure elevation.

9. Summary and future perspectives

The results of experimental studies indicate that various models of hypertension (SHR, Dahl S rats, NO synthase inhibitor-induced, angiotensin II-induced and 2K1C-induced) are associated with vascular and/or renal H_2S deficiency and that administration of H_2S donors not only decrease blood pressure but also reduce complications such as vascular hypertrophy, myocardial remodeling and fibrosis, kidney injury and erectile dysfunction. The mechanism of antihypertensive effect of H_2S may include vasodilation, inhibiting inflammation and oxidative stress, improvement of VEGF signaling, increase in natriuresis, reduction of sympathetic activity, decrease in BMP4, modulation of gut microbiota, intestinal barrier integrity and the expression of the receptors for microbiota-derived metabolites in peripheral tissues and, last but not least, modification of immune cell differentiation and activity. Several drugs known to reduce blood

pressure such as angiotensin-converting enzyme inhibitors [104] or statins [105] improve H_2S signaling. H_2S may also be involved in the mechanism of action of non-pharmacological interventions such as physical exercise [106] or caloric restriction [107-109]. Several plant-derived products such as garlic polysulfides [110, 111] or isothiocyanates [112-114] decrease blood pressure at least partially by releasing H_2S.

However, many questions regarding the role of H_2S in hypertension still remain open. In most studies plasma concentration of H_2S was measured by the colorimetric methylene blue method which is non-specific and measures various reactive sulfur species and, consequently, overestimates H_2S concentration. Second, most studies were performed in male animals. There are huge sex-differences in the pathophysiology of hypertension and both estradiol [115, 116] and testosterone [116] regulate vascular H_2S system. More studies performed in both sexes are required Third, in most studies which demonstrated protective effect of H_2S donors on hypertension-induced end organ injury other blood pressure-lowering medications were not used for comparison and it is unclear if, and to what extent, protective effect of H_2S is dependent on normalization of blood pressure. Fourth, inorganic sulfide salts (particularly NaHS) were mainly used as the H_2S donors. These salts are easily oxidized to other sulfur species such as organic polysulfides which may have overlapping but also divergent activity than H_2S itself. On the other hand, propargylglycine was commonly used as the CSE inhibitor. Although PAG is commonly used in various aspects of H_2S research, it has important limitations. First, by inhibiting CSE, PAG can suppress homocysteine to cysteine transformation and increase homocysteine concentration. Second, commercially available racemic mixture (D,L-PAG) is usually used. Only L-PAG inhibits CSE whereas D-PAG is metabolized by DAO to nephrotoxic compounds. Finally, PAG has limited membrane permeability and is not specific for CSE but can also inhibit other PLP-dependent enzymes [118] The mechanism of H_2S deficiency in hypertension is also

controversial since different data about the expression of H_2S-producing enzymes, even in the same model of hypertension, have been reported. Finally, many mechanisms of antihypertensive effect of H_2S were described but most of them only in one experimental model; it is unclear if these mechanisms are common or specific only to one type of hypertension. This issue is especially important if using H_2S donors in specific types of hypertension in humans is considered.

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Figure legends

Fig. 1. Synthesis, signaling and metabolism of H_2S. H_2S is synthesized from L-cysteine (L-Cys) by cystathionine β-synthase (CBS) or cystathionine γ-lyase (CSE). Alternatively, cysteine is transformed into 3-mercaptopyruvate (3-MP) by cysteine aminotransferase (CAT). 3-MP serves as the substrate for 3-mercaptopyruvate sulfurtransferase (MPST) which produces H_2S. In addition, 3-MP may be synthesized from alimentary D-cysteine by D-aminoacid oxidase (DAO) in some tissues. H_2S signals through persulfidation of protein cysteine thiol (Pr-SH) to persulfide (Pr-SSH) groups. H_2S is enzymatically oxidized in mitochondria. First, sulfide:quinone oxidoreductase (SQR) transfers electrons from H_2S to ubiquinone oxidizing it to sulfane sulfur (Pr-SSH) which is then oxidized to sulfite (SO_2^{2-}) and finally sulfate (SO_3^{2-}) by sulfur dioxygenase (SDO) and sulfite oxidase (SO), respectively.

Fig. 2. Antihypertensive effects of H_2S in the blood vessels. H_2S induces vasorelaxation by activating ATP-sensitive K^+ channels (K_{ATP}) and voltage-sensitive Kv7 channels in smooth muscle cells. In addition, H_2S augments nitric oxide (NO) signaling by activating endothelial NO synthase (eNOS), soluble guanylyl cyclase (sGC) and cGMP-sensitive protein kinase G (PKG) as well as by inhibiting phosphodiesterase-5 (PDE5). H_2S also stimulates vascular endothelial growth factor receptor-2 (VEGFR2) signaling. Treatment of hypertensive animals with H_2S donors inhibits NADPH oxidase (NOX)-dependent formation of reactive oxygen species and inhibits angiotensin II (AngII) formation by suppressing angiotensin-converting enzyme (ACE) as well as AngII signaling by AT_1 receptors. Finally, H_2S inhibits inflammatory reaction (NLRP-3 inflammasome) and stimulates regulatory T lymphocytes (Treg cells). Consequently, H_2S augments endothelium-dependent vasorelaxation as well as inhibits endothelium-dependent contraction and, in the long run, suppresses vascular fibrosis.

Fig. 3. Effects of H_2S in hypertensive heart. H_2S inhibits myocardial hypertrophy and remodeling as evidenced by normalization of myosin heavy chain (MHC) expression profile and reduced expression of atrial natriuretic peptide (ANP) in ventricular myocytes. In addition, H_2S inhibits oxidative stress and reduces myocardial fibrosis (collagen content) by inhibiting synthesis of transforming growth factor-β (TGF- β) and TGF- β signaling mediated by phosphorylated Smad proteins. H_2S also inhibits collagen turnover by reducing the expression/activity of matrix metalloproteases (MMP) and stimulating tissue inhibitors of metalloproteases (TIMP).

Fig. 4. Effects of H_2S in hypertensive kidney. H_2S increases glomerular filtration rate and inhibits tubular sodium reabsorption by reducing the activity of epithelial sodium channel (ENaC) in the luminal and Na^+,K^+-ATPase in the basolateral membrane. In addition, H_2S inhibits oxidative stress, fibrosis and inflammation, reduces the infiltration with IL-17-producing Th17 cells and increases the amount of regulatory T cells, reduces the expression of renin and angiotensin AT_1 receptor as well as renin-stimulating Olfr78 receptor and increases the expression of vasodilating Gpr41, Gpr43 and antinflammatory Gpr120.

Manuscript
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Hydrogen sulfide in the experimental models of arterial hypertension

Credit author statement.

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Literature search and analysis, Writing - original draft. Visualization.

Table 1. Vascular effects of H_2S in experimental models of hypertension.

Effect	Vascular preparation	Experimental model	H_2S donor	Reference
↑ endothelium-dependent vasorelaxation	renal artery	SHR	NaHS	[46]
	aortic rings	SHR	GYY4137	[47]
	aortic rings	AngII-infused mice	NaHS	[76]
	aortic rings	2K1C (rat)	NaHS	[80]
	renal artery	2K1C (rat)	NaHS	[82]
↓ NLRP3 inflammasome and IL-1β	renal artery	SHR	NaHS	[46]
↓ VCAM-1 and IFN-γ	aorta	L-NAME-treated rats	NaHS	[88]
↓ oxidative stress	renal artery	SHR	NaHS	[46]
	aorta	SHR	GYY4137	[47]
	aorta	AngII-infused mice	NaHS	[76]
	aorta	L-NAME-treated rats	NaHS	[88]
↓ vascular wall thickening	thoracic aorta	SHR	NaHS	[87]
	coronary arterioles	SHR	NaHS	[85]
	thoracic aorta	Dahl S rats	NaHS	[64]
	aorta	L-NAME treated rats	NaHS	[88]
↓ ACE expression	renal artery	2K1C (rat)	NaHS	[79]
	aorta	2K1C (rat)	NaHS	[80]
↓ fibrosis of the vascular wall	thoracic aorta	SHR	NaHS	[86]

	aorta	L-NAME treated rats	NaHS	[88]

Table 2. Cardiac effects of H_2S in experimental models of hypertension

Effect	Experimental model	H_2S donor	Reference
↓ myocardial hypertrophy	SHR	GYY4137	[90]
	Dahl S rats	NaHS	[92]
	L-NAME-treated rats	NaHS	[67]
	L-NAME-treated rats	NaHS	[93]
	L-NNA-treated rats	sodium thiosulfate	[94]
	AngII-infused rat	NaHS	[95]
↓ fibrosis	SHR	NaHS	[85]
	SHR	NaHS	[89]
	SHR	GYY4137	[91]
	L-NAME treated rats	NaHS	[93]
	L-NNA induced hypertension (rat)	sodium thiosulfate	[94]
	AngII-infused rat	NaHS	[95]
↓ oxidative stress	SHR	NaHS	[85]
	Dahl S rats	NaHS	[92]

Table 3. Renal effects of H_2S in experimental models of hypertension

Effect	Experimental model	H_2S donor	Reference
↓ renin expression	SHR	NaHS	[61]
	Dahl S rats	NaHS	[60]
	2K1C (rat)	NaHS	[79]
	AngII-infused rat	sodium thiosulfate	[100]
↓ glomerulosclerosis	SHR	L-Cys	[62]
		D-Cys	[62]
↓ oxidative stress	SHR	L-Cys D-Cys	[62]
	Dahl S rats	NaHS	[96]
	AngII-infused rat	Sodium thiosulfate	[100]
	AngII-infused rat	NaHS	[100]
↓ interstitial fibrosis	Dahl S rats	NaHS	[96]
	AngII-infused rat	Sodium thiosulfate	[100]
	AngII-infused rat	NaHS	[100]

Figure 1

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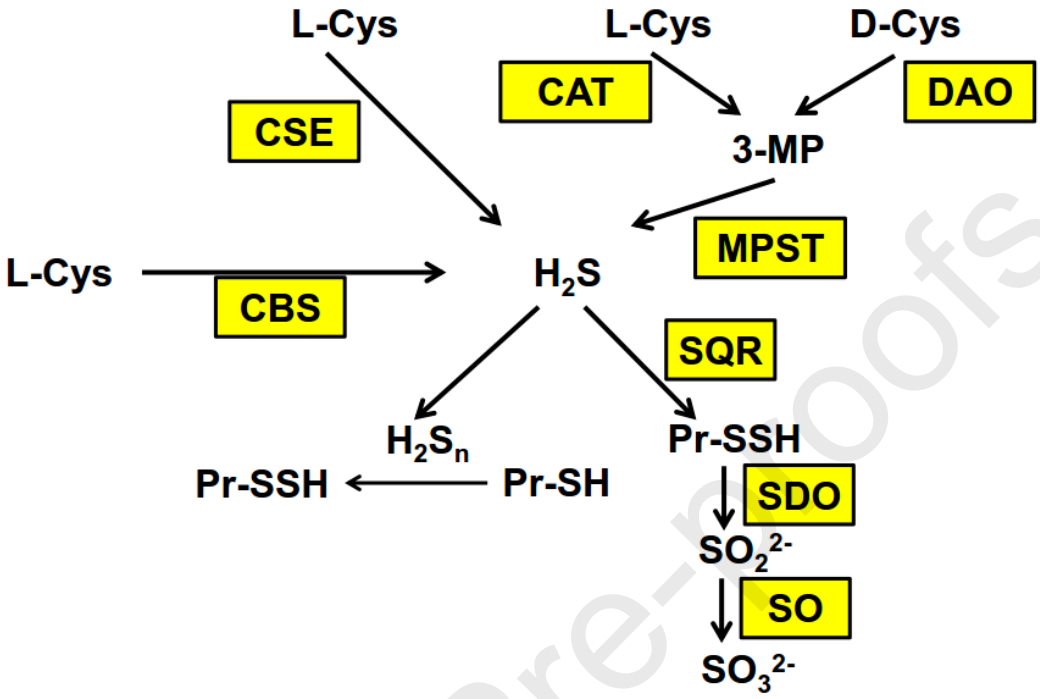

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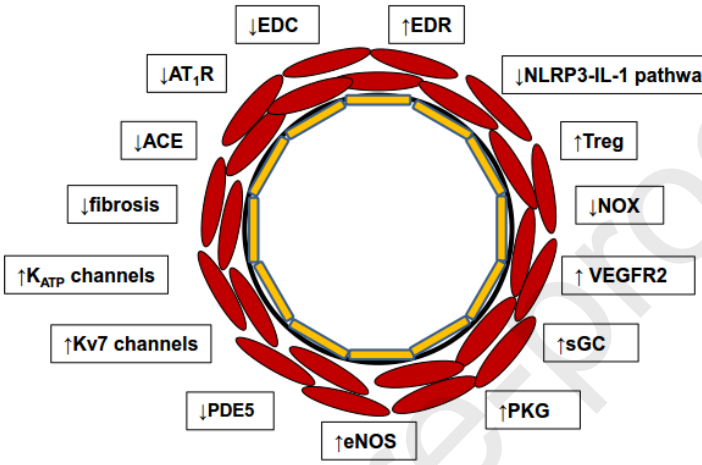

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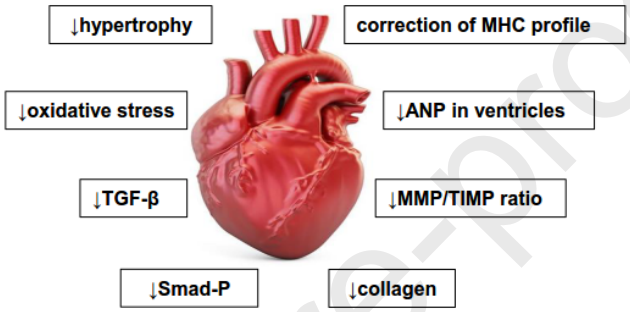

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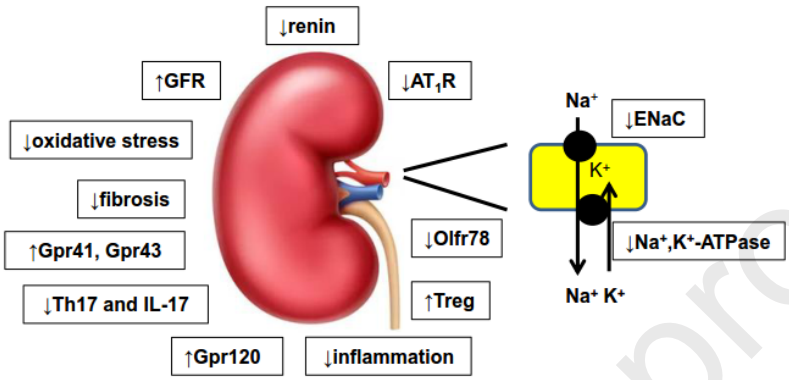

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