Homocysteine and hydrogen sulfide in epigenetic, metabolic and microbiota related renovascular hypertension

Gregory J Weber, **Sathnur B. Pushpakumar**, **Suresh C. Tyagi**, and **Utpal Sen**
Department of Physiology, University of Louisville, School of Medicine, Louisville, KY-40202

Abstract

Over the past several years, hydrogen sulfide (H_2S) has been shown to be an important player in a variety of physiological functions, including neuromodulation, vasodilation, oxidant regulation, inflammation, and angiogenesis. H_2S is synthesized primarily through metabolic processes from the amino acid cysteine and homocysteine in various organ systems including neuronal, cardiovascular, gastrointestinal, and kidney. Derangement of cysteine and homocysteine metabolism and clearance, particularly in the renal vasculature, leads to H_2S biosynthesis deregulation causing or contributing to existing high blood pressure. While a variety of environmental influences, such as diet can have an effect on H_2S regulation and function, genetic factors, and more recently epigenetics, also have a vital role in H_2S regulation and function, and therefore disease initiation and progression. In addition, new research into the role of gut microbiota in the development of hypertension has highlighted the need to further explore these microorganisms and how they influence the levels of H_2S throughout the body and possibly exploiting microbiota for use of hypertension treatment. In this review, we summarize recent advances in the field of hypertension research emphasizing renal contribution and how H_2S physiology can be exploited as a possible therapeutic strategy to ameliorate kidney dysfunction as well as to control blood pressure.

Graphical abstract
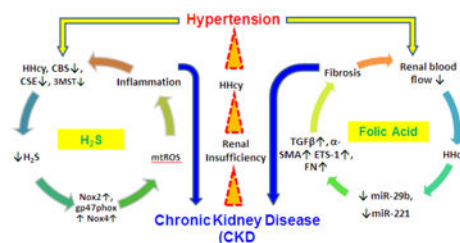
Address correspondence to: Utpal Sen, Ph.D., FAHA, Department of Physiology, University of Louisville School of Medicine, 500 South Preston St, Louisville, KY-40292, Ph: 502-852-2030; Fax: 502-852-6239; u0sen001@louisville.edu.

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Keywords

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1. Introduction

The link between mortality and hypertension is well documented and the prevalence of this disease grows each year with an estimated one-third of the population in the United States having high blood pressure and the rates steadily increase with age, accounting for 41.5% of all deaths [1]. Two of the major outcomes attributed to high blood pressure are increase risk for developing cardiovascular disease (CVD) and stroke, along with decreased life expectancy [1]. There are several risk factors associated with hypertension as the etiology and complexity of the disease is not completely understood. Genetics plays a role in determining high blood pressure and that different sets of genes affect blood pressure regulation at different stages of life [2]. Environmental factors, such as lifestyle preferences and diet can also influence blood pressure. A study in women revealed that body mass index (BMI) was the strongest indicator of predicting high blood pressure leading to hypertension, with a BMI of greater than 24.7 being the most affected group [3]. Diet, such as higher sodium, lack of fruits and vegetables intake, low physical activity, higher alcohol consumption and low folic acid (folate) supplementation were also factors related to development of hypertension [4].

The kidney is one of several organ systems that are adversely affected by hypertension and is one of the leading causes of chronic kidney diseases (CKD) resulting in kidney dysfunction [5, 6]. It is believed that CKD affects over 20 million adults in the US, with approximately 60% of these cases having associated hypertension [7]. A decrease in glomerular filtration rate (GFR) is a key feature of a hypertensive kidney [8]. Blood flow in the renal medulla is important for regulating blood pressure and excreting waste from the kidneys, such as excess sodium, and is often found to be decreased in hypertension [9]. Increased fluid retention and the activation of the renin-angiotensin system (RAS) are key characteristics of the pathophysiology in the hypertensive kidney [10]. The increased expression of angiotensin II (Ang-II) can cause endothelial damage, fibrosis, promote inflammation and mesangial cell proliferation, and vasoconstriction [11-13]. Ang-II can also cause increased reactive oxygen species (ROS), which is linked with essential hypertension, production through mitochondrial dysfunction and subsequent expression and activation of p47^{phox}, a subunit of the NADPH-oxidase complex [14-16]. High salt intake can also stimulate NADPH-oxidase activity and oxidative stress while also decreasing superoxide dismutase scavengers, which further exacerbates renal injury [17].

Kidney, in addition to maintaining normal physiological function, also produces several gaseous molecules such as nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide (H_2S) [18]. Alterations in these gaseous molecules during pathophysiological kidney remodeling impact normal kidney function. The role of NO in normal vascular function including renal vasculature in disease condition is well documented [19, 20]. Much more has

yet to be studied for CO to get a comprehensive role and mechanism of its impact on kidney function. Recently, H_2S has appeared a strong regulatory determinant of maintaining kidney function in normal and disease condition [21, 22]. This is primarily because of the presence of H_2S production enzymes, such as cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3MST) in the kidney and these enzymes are prime targets of stress in the kidney vasculature [23-25]. Diseases like hypertension diminish these enzymes expression, activity and local production of H_2S [26-30]. Genes of these enzymes or their regulatory genes are also partially impacted by epigenetic modification, such as hypermethylation or homocysteinylation [31-33]. Ultimately the normal production of H_2S is essentially diminished in these pathophysiological changes, which in turn affect renal functional outcomes. In this review, in light of current literature we will discuss dysregulation of H_2S production in the kidney during normal and disease states leading to exaggerated renal pathophysiology of hypertension and how H_2S can potentially be used to mitigate hypertension and reduced kidney function.

2. Mechanisms of renovascular hypertension

2.1 Hypertension and kidney

In general, several distinct but overlapping mechanisms of hypertension have been described in the literature involving kidney. This review is primarily focused on renovascular hypertension, however it may be worthwhile introducing a brief overview of all these mechanisms of hypertension affecting or relating to kidney for an easy distinction between these conditions, and overlapping if any, from semantic point of view. These are: 1) Primary hypertension; 2) Secondary hypertension; and 3) Other types of hypertension. Primary hypertension is also called 'essential hypertension' or 'idiopathic hypertension', which accounts 90-95% of patients diagnosed with hypertension [34, 35].There is no known cause of primary hypertension, and therefore diagnosis of this type of hypertension is predominantly made after excluding known causes of secondary hypertension. Interestingly, primary hypertension can in turn lead to 'hypertensive nephrosclerosis', and 'chronic kidney disease'. Unlike primary hypertension, a number of conditions can cause secondary hypertension. The most common conditions which are related to kidney disease include diabetic nephropathy (DN), polycystic kidney diseases, glomerulosclerosis and other glomerular diseases, and renovascular hypertension [36]. Although in typical sense renovascular hypertension is caused by narrowing of one or both renal arteries, in this review article we generalized this term to mean entire renal vascular bed including glomerulus. Other types of hypertension include isolated systolic hypertension, malignant hypertension and resistant hypertension.

Besides these above described mechanisms of hypertension, there is also a topic of hypertension in primary chronic kidney disease (CKD) and its pathogenesis includes sodium retention, renin-angiotensin system (RAS) activation, enhanced activity of the sympathetic nervous system etc. [37]. It is important to note that while hypertension can cause chronic kidney disease, chronic kidney disease in turn can cause hypertension as well [38]. In all of the above-mentioned topics, which reflect the clinical setting, the role of the RAS is variable, in that it is of paramount importance in renovascular hypertension, for example.

But in the other forms involvements of RAS can vary. In fact, ACE inhibitors and Angiotensin II receptor blockers (ARBs) produce an antihypertensive response in 30 to 50 percent of patients [39, 40], and ARBs itself have been found to reduce blood pressure of all ARBs about 48-55% [41]. While these topics are of great clinical importance and discussed extensively in the literature, and perhaps should be a topic of future update, in the next section of this article we focused our discussion on RAS and how H_2S may modulate RAS activation.

2.2. Renin-angiotensin system and H_2S

One of the primary mechanisms involved in renovascular hypertension is the renin-angiotensin system (RAS). The precise control of this system is widely recognized for its importance in regulating several physiological roles, including blood pressure and sodium homeostasis. The protease renin is the first enzyme involved in the pathway and is primarily produced in the kidney. It is responsible for converting angiotensinogen, found primarily in the liver, into angiotensin I (Ang-I), a biologically inactive precursor form to angiotensin II (Ang-II) [42]. Ang-I is converted into Ang-II by the ACE enzyme through hydrolysis and is mainly found in the lungs [43]. While Ang-II can be further converted in to other forms, such as Ang-III, Ang-IV, and Ang-(1-7), Ang-II is believed to be the most biologically active form [42, 44]. Ang-II preferentially targets the AT_1-R, which is expressed in most tissue types in the kidney, and elicits a plethora of effects, including, pro-inflammatory response, release of renin, and sodium reabsorption [45, 46]. Moreover, vasoconstriction of post-glomerular arterioles leads to decreased blood flow and glomerular filtration in the kidney by increasing arteriolar resistance and contraction of mesangial cells [37]. This leads to an overall increase in arterial blood pressure. A second receptor, AT_2-R, also binds Ang-II but is present in a much lower capacity. The response of Ang-II binding to AT_2-R, however, appears to promote the opposite effects of AT_1-R binding, through vasodilation and regulation of sodium regulation [47]. In addition, the homologue ACE2 enzyme has been shown to covert Ang-II to the Ang-(1-7) peptide, which appears to modulate the hypertensive effects elicited by Ang-II [48].

In the recent years, substantial evidences suggest a link between RAS and H_2S in the kidney. In a rat model of two-kidney-one-clip (2K1C) renovascular hypertension, Lu et al demonstrated that H_2S donor significantly diminished plasma renin activity and Ang-II levels compared to vehicle control without affecting plasma ACE activity [49]. Their study concluded that H_2S has potential therapeutic value to treat renovascular hypertension possibly through inhibiting renin activity by decreasing its synthesis and release. In another study, Xue et al demonstrated that high glucose induced AGT, ACE and AT1 receptor and Ang-II concentration in *in vitro* mesangial cells [50]. While they did not measure expression or activity of H_2S producing enzymes in their experiments, they observed attenuated RAS activation following H_2S treatment. Similar results were also observed in a streptozotocin-induced diabetes rat model where increased RAS activity was reversed by H_2S through diminished oxidative stress without affecting glucose levels [50].

2.3 Metabolic syndrome and CKD: role of H_2S

Metabolic syndrome (MetS) is defined as a clustering of cardiovascular risk factors [51]. According to the World Health Organization (WHO), diabetes mellitus, along with two or more other cardiovascular risk factors that include high blood pressure, high levels of triglycerides, low levels of HDL, obesity and increased microalbuminuria are considered as MetS. More specific information about classification of MetS is nicely summarized in a review article referenced in [52]. The association between CKD and all of these above cardiovascular risk factors are well documented in the literature. Therefore, it is obvious to infer that MetS is associated with CKD. In fact several studies have documented higher risks of CKD with increasing traits of MetS [53-55]. The importance of insulin resistance, ROS, inflammation, dyslipidemia and many other factors are proposed in contributing MetS associated CKD; however, the role of H_2S in this disease mechanism is poorly addressed. We have recently shown that in diabetes, H_2S deficiency contributes to unfavorable renovascular remodeling characterized by vascular constriction, increased resistive index, and diminished blood flow, and H_2S treatment improves renal function [56, 57]. Others have shown inhibition of pancreatic H_2S production with daily intraperitoneal injections of CSE inhibitor DL-propargylglycine (PAG) in Zucker diabetic fatty (ZDF) rats increased serum insulin levels and reduced hyperglycemia [58]. In fms-like tyrosine kinase 1-induced hypertensive Sprague-Dawley rats, Holwerda et al demonstrated that proteinuria and glomerular endotheliosis were ameliorated by H_2S treatment and these changes were in part due to increased renal VEGF expression [59]. Further studies are needed to determine association of metabolic diseases and H_2S in CKD, and to evaluate potential H_2S therapy for ramification of renal vascular disorders. Based on the available reports, a proposed mechanism of CKD in H_2S deficiency and high homocysteine levels (hyperhomocysteinemia) associated with hypertension is shown in figure 1. These are two independent, but closely related pathways. More details are explained in the legend.

2.4 Epigenetic mechanism and miRNAs: is H_2S a regulator?

More recent studies have investigated the role of non-coding RNA involvement of hypertension. In particular, microRNAs (miRNAs) have received considerable attention in a variety of kidney diseases [60]. miRNAs are short (~22 nucleotides in length), single-stranded RNA genes that regulate post-transcriptional gene expression. miRNAs target messenger RNAs (mRNAs) by binding to complementary sequences in the 3' untranslated region repressing their translation, and are associated with a broad spectrum of cellular and physiological processes. While there is limited data on the functional roles of miRNAs in the hypertensive kidney, a few studies have yielded promising results. Spontaneously hypertensive rats were shown to have decreased level of miR-29b in the renal cortex compared to normotensive control animals [61]. In addition, the authors reported a significant decrease in miR-29b expression in renal tubular epithelial cells treated with Ang-II. This, in turn, promoted epithelial-mesenchymal transition (EMT) through activation of key genes such as TGF-β and α-SMA, which can lead to renal fibrosis. These effects were reversed upon overexpression of miR-29b, suggesting this microRNA has an inhibitory role in Ang-II induced hypertension [61]. A similar finding was reported in the renal medulla of salt-sensitive rats, where they found miR-29b was expressed at lower levels in these rats compared to the control counterparts [62]. Transfection experiments with miR-29b showed a

decrease in genes known to be involved in collagen formation, leading the authors to conclude that miR-29b plays a protective role in kidney fibrosis by inhibiting extracellular matrix (ECM) remodeling [62]. Ang-II has also been shown to downregulate the expression of miR-221 in mouse kidney and in rat renal interstitial fibroblasts, leading to an increase in the *Ets-1* gene, which had been previously shown to be induced by Ang-II and result in inflammation and reactive oxygen species generation, as well as other kidney diseases [63, 64]. In a study utilizing a more global approach of microarrays, miR-638 and let-7c were found to be downregulated in the renal medulla of male hypertensive patients compared to normotensive individuals [65]. In the renal cortex, a total of 13 miRs were found to be significantly altered, including miR-181a and miR-663 which had lowered expression and led in an increase in renin mRNA expression and hypertensive effects [65]. While all these studies point towards an increasing role of miRs in the development of hypertension in the kidney, there is a lack of knowledge whether H_2S is involved in the regulation of miRs or vice versa in hypertensive kidney. It was however reported that H_2S donor attenuated myocardial injury through upregulation of miR-21 and suppression of inflammasome [66]. Another study reported increased expression of miR-132 and miR-212 in the heart, aortic wall and kidney of Ang-II infused hypertensive rats [67]. More interestingly the same study reported AT1R blockers decreased expression of these two miRs in surplus human mammary arterial tissue obtained from coronary bypass surgery [67]. Taken together, these above reports suggest a clear relationship between miRs and hypertensive disease mechanism. Very recently we have reported downregulation of miR-129 and miR-299b in Ang-II treated mice and normalization of these miRs following GYY4137 (a donor of H_2S) treatment [68]. In contrast, we found upregulated miR-369 in Ang-II treatment which was suppressed in GYY4137 treated mice [68]. To further prove this initial finding and to elucidate the precise mode(s) of H_2S action in regulating miRs in hypertensive kidney, more work needs to be completed. Here we propose a mechanism of Ang-II mediated renal inflammation and dysfunction involving epigenetic modulation of miRs, and how H_2S is predicted to mitigate renal dysfunction (Figure 2).

3. Involvement of thiol homocysteine and H_2S in renovascular hypertension

3.1 High homocysteine, low H_2S and hypertension

Homocysteine is an amino acid containing sulfur as one of its primary components. As part of the methionine metabolic pathway, it is an intermediate step in the processing of methionine by demethylation. After this initial step, homocysteine can either be further metabolized to cystathionine and eventually to cysteine via the transsulfuration pathway, or can undergo remethylation to once again become methionine [69] (Figure 3). There are several enzymes involved in the transsulfuration pathway, including cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), cysteine aminotransferase (CAT), and 3-mercaptopyruvate sulfurtransferase (3MST). A substantial amount of homocysteine is metabolized in the kidney, making it a critical organ in the regulation of this amino acid as high levels of homocysteine, also known as hyperhomocysteinemia, in the plasma are a risk indicator of renal damage, hypertension and cardiovascular disease [69-72].

There are several adverse effects that are accompanied along with increasing levels of homocysteine, one of which is increasing oxidative stress and resulting in damage to endothelial cells [73]. One mechanism that has been shown is the activation of NADH/NADPH oxidase through the increased production of ceramide by homocysteine, leading to reactive oxygen species (ROS) formation, as evidenced in rat mesangial cells [74]. Homocysteine has also been shown to decrease cellular levels of glutathione peroxidase, which is an important antioxidant enzyme in reducing hydrogen peroxide to water [75, 76]. Moreover, homocysteine can also lead to a decrease in nitric oxide (NO), a potent vasodilator, production through endothelial damage and inhibition of NO through asymmetric dimethylarginine (ADMA) [77-79]. In addition, homocysteine can be oxidized by copper while in circulation and can lead to the production of hydrogen peroxide (H_2O_2) [80]. Similar effects are also observed when H_2S levels are low [81]. In one study where one of the key enzymes (CSE) in H_2S generation was genetically eliminated, it was found that low H_2S levels resulted in decreased availability of NO, resulting in oxidative stress as evidenced by increased lipid peroxidation and protein carbonyl groups [82]. It was further demonstrated that treatment with exogenous H_2S restored NO levels in these animals, leading to less oxidative stress [82]. This finding was also found in a separate study utilizing mice without CSE, where endogenous H_2S levels were found to be low in renal endothelial tissue, and showed increased damage due to ischemia/reperfusion injury (IR/I) in CSE-negative animals, with an increase in ROS and this was reduced in animals receiving exogenous H_2S and in cells with overexpressed CSE [83].

3.2 Angiotensin-II, hyperhomocysteinemia, and CKD: possible role of folate and H_2S to mitigate hypertension

Ang-II induced hypertension is known to cause renal injury by decreasing renal blood flow, glomerular filtration rates, as well as decreasing the levels of CBS and CSE enzymes, leading to elevated levels of homocysteine known as hyperhomocysteinemia [84]. In addition, Ang-II hypertension causes renal injury involving microvascular endothelial damage, which is one of the contributing factors of hypertension [85]. Renal microvascular endothelial cells express enzymes CSE and 3MST, which through the transsulfuration pathway clears homocysteine to produce the gaseous substance, H_2S [86]. Ang-II mediated endothelial damage may affect these enzymes resulting in hyperhomocysteinemia and decreased production of H_2S, a strong antioxidant [87-89] and vasorelaxing molecule [90, 91]. All of these effects may contribute to renovascular remodeling resulting in CKD (Figure 1).

Plasma homocysteine levels are negatively correlated with GFR and the prevalence of hyperhomocysteinemia is 85-100% among ESRD [92]. Although the precise mechanisms of hyperhomocysteinemia in kidney diseases have not been established yet, it is generally accepted that the mechanisms could be multifactorial. While increased homocysteine production or decreased excretion has been ruled out, decreased metabolism in renal or extrarenal sites seem to be the most plausible cause [93]. In fact, it has been demonstrated that folic acid supplementation does not reduce intracellular homocysteine concentration, supporting the hypothesis of negative metabolic effects that could impair intracellular homocysteine metabolism [94]. This is linked to the issue of folate therapy where "folate

resistance" is present in CKD patients [95, 96]. In a recent review article, Perna and Ingrosso have creditably discussed this topic in more details with up-to-date information which are mostly based on clinical findings in patients with renal insufficiency [94]. Besides this, it has also been reported that the correction of multiple remethylation abnormalities with various other drugs in CKD patients, which are not related to folic acid supplementation, removes homocysteine from protein binding sites [97]. This information could add invaluable strategies to decrease homocysteine levels in CKD patients independent of folic acid remethylation-pathway. In this context, it is noteworthy to mention that decreased H_2S and down-regulation of transsulfuration enzymes, CBS and CSE (enzymes responsible for cysteine and homocysteine metabolism) transcript have been reported in ESRD [98]. Similar results were also observed in experimental uraemic rats where malfunction of these transsulfuration enzymes were accompanied with decreased H_2S levels [99]. Further supporting to these findings, in dialysis patients, levels of H_2S were also shown to increase following dialysis suggesting that uraemic toxins are likely the culprits to inhibit H_2S generating enzymes, and thus H_2S levels [100]. Despite the progress of our scientific knowledge with these advanced researches, precise mechanism(s) of high homocysteine and low H_2S levels still remain an open area of future research to discover many more unsolved mysteries of human health related to pathophysiological complexity of CKD.

3.3 Thiol metabolism and H$_2$S production

Both CBS and CSE are cytosolic enzymes and are expressed in the major organs including kidney [101-103]. While CBS produces H_2S from cysteine via β-elimination / β-replacement reaction in which cysteine is condensed with homocysteine [104], CSE produces H_2S via α,β-elimination of cysteine. In hyperhomocysteinemia, α,γ-elimination and γ-replacement reaction of homocysteine also produces H_2S [105]. Two other enzymes, 3MST and CAT also produces H_2S and this pathway known and 3MST/CAT pathway (Figure 4). First, CAT produces 3-mercaptopyruvate from cysteine and α-ketoglutarate, and then 3MST produces H_2S from 3-mercaptopyruvate. Unlike CBS and CSE, 3MST is a mitochondrial enzymes and high concentration of cysteine in the mitochondria leads to 3MST/CAT pathway to produces H_2S [106]. While CBS, CSE and CAT produces H_2S from L-cysteine, another enzyme D-amino acid oxidase (DAO), which is localized in the peroxisome, produces H_2S from D-cysteine [107, 108]. DAO metabolizes D-cysteine to achiral 3MP which further metabolizes to H_2S by 3MST [109-111]. A summary of H_2S production by all these above enzymes are presented in a schematic way in Figures 3 & 4.

3.4 Folic acid supplementation: regulation of blood pressure by homocysteine remethylation

Folic acid is a B-vitamin and is essential in the metabolism of homocysteine to methionine, subsequently lowering plasma levels of homocysteine through the remethylation pathway and thereby lowering oxidative and inflammatory stresses [112]. Through use of the folate cycle and B-12 vitamin, homocysteine can be remethylated back to methionine by methionine synthase and 5-methyltetrahydrofolate reductase (Figure 3). Deficits in either folate or vitamin B-12 have been linked with elevated homocysteine levels and is therefore associated with elevated blood pressure and hypertension [113]. In one study looking at end stage renal disease (ESRD), patients that were given folic acid supplement were found to

have lowered plasma homocysteine levels as well as increased remethylation of homocysteine to methionine [114]. A meta-analysis conducted on individuals with end stage kidney disease (ESKD) found that folic acid treatment reduced plasma homocysteine levels 15-20% [115]. It should be taken into account that several published papers reported higher homocysteine levels without measuring corresponding H_2S levels. Since homocysteine is one of the precursors of H_2S, it is plausible that higher accumulation of homocysteine lowers tissue synthesis, and thus plasma levels of H_2S. Using animal as experimental model, we and others have demonstrated that high levels of homocysteine in fact lower tissue and plasma levels of H_2S [116-119]. However, it was not clearly demonstrated in human and in some cases it was positively related, instead of expected negative relationship. For example, it has been shown in human that in certain pathological condition hyperhomocysteinemia is associated with increased plasma and platelet H_2S levels [120]. This phenomenon is unclear, but possibly linked to H_2S-mediated susceptibility to thrombosis and atherosclerosis in patients with abnormal remethylation pathway [120].

Moreover, it has also been demonstrated by the same group that homocysteine is irreversibly degraded to H_2S causing in vivo platelet activation via upregulation of phospholipase A2 and downstream boost of the arachidonate cascade [120, 121]. In this regard, most interventional trials focused on homocysteine lowering by folate administration did not exclude patients routinely taking arachidonate inhibitor, aspirin that could have masked this negative effect associated to H_2S levels in hyperhomocysteinemia [120]. Therefore, the questions: 1) what is the most beneficial dose of folate to see reduced homocysteine levels, and 2) whether other confounding factors are eliminated for a decisive conclusion of folate therapy remains unanswered. These questions warrant future investigation [115].

Homocysteine however contributes to Ang-II mediated renovascular remodeling through NADP(H) and AT_1R, in part, by decreasing vascular density and increasing oxidative, pro-inflammatory, anti-angiogenic and pro-fibrotic factors [84]. We have shown that FA ameliorate the accumulation of oxidized collagen by balancing the matrix metalloproteinase / tissue inhibitor of metalloproteinase (MMP/TIMP) axis, in particular, by decreasing MMP-2 and MMP-9 expression and elevating TIMP-1 and TIMP-2 expression levels [84]. FA supplementation also lowered systolic blood pressure, increased nitric oxide (NO) production, and lowered plasma homocysteine levels [84]. These studies suggest FA treatment can lower plasma levels of homocysteine and mitigate hypertensive effects due to elevated homocysteine through the remethylation pathway of homocysteine to methionine.

3.5 Does homocysteine cause or contribute to hypertension?

Aside from the fact of vascular complications in homocystinuria, the genetic disease where levels of plasma homocysteine are higher and hyperhomocysteinemia is one of the key clinical findings, hypertension is not described [122, 123]. The vast majority of clinical as well as experimental evidences underscore the contribution of homocysteine to hypertension is secondary to its vascular effects rather than primary cause [124, 125]. Recently, we have reported that inhibition of DNA methylation reduced homocysteine levels in CBS+/- mice, an animal model of hyperhomocysteinemia, and mitigated moderate blood pressure implicating epigenetic regulation of vascular fibrotic events involving DNA methylation [31,

126]. This is an experimental evidence of indirect cause and effect relationship between hyperhomocysteinemia and hypertension. Similar to homocystinuria patients, in CKD patients, aged and well-nourished population substantial evidences of hyperhomocysteinemia is established [127-129]. It is still not conclusive whether hyperhomocysteinemia causes hypertension in these patients [130, 131].

Nonetheless, it has been well documented in the literature that hyperhomocysteinemia exacerbates end organ damage in hypertension, if not causing hypertension, especially in ill patients including CKD population [130]. This is in part due to a vicious cycle where diseased kidney fails to properly filter uremic waste causing volume retention which in turn increases plasma homocysteine levels [132-134]. Hyperhomocysteinemia in turn causes cellular damage including endothelial lining and contributes to hypertension in CKD patients [125, 135].

The association between high homocysteine levels and isolated systolic hypertension in some older adults has been reported by Sutton-Tyrell in early nineties where the authors predicted a cause and effect relationship [136]. This association was later concluded as weak, in part, due to inaccurate blood pressure measurement and may have confounded by renal function [137]. Mechanisms that are surfaced in the literature based on animal studies include arterial stiffness, endothelial dysfunction, decreased bioavailability of nitric oxide (NO), increased oxidative stress and peroxynitrite formation [138, 139]. Interestingly, in a cross-sectional study it was estimated that higher plasma homocysteine levels at baseline were associated with an increased, but non-significant risk of incidental hypertension in men [140]. A similar result was reported in older adults where lowering homocysteine levels by B vitamins for a period of two years did not change blood pressure significantly compared to placebo control [141]. On the other hand, in a prospective study the progression of blood pressure on normotensive population over 2-years period Wang et al found that homocysteine is related to hypertension incidence at high levels, and may also increase the risk of hypertension at low levels [142]. Children with early CKD showed blood pressure abnormalities, mostly having a positive correlation with high homocysteine levels along with other factors, in a cross-sectional study [143]. Nevertheless, none of these studies provided strong evidences that the homocysteine causes human hypertension [137, 144]. Only incremental increase in blood pressure was documented in most of these studies. Thus, the contribution of homocysteine to raise blood pressure secondary to its vascular effects is generally accepted, although the direct role of homocysteine in causing hypertension is still not an established fact. Only larger case control studies can finally settle this much debated and controversial issue in future.

3.6 H_2S producing gene manipulation: effect on blood pressure

In 2008, Wang et al reported that knockout of CSE gene elevated blood pressure due to lacking physiological levels of H_2S production in mice [90]. This initial report was further confirmed by many other independent studies around the world using various experimental models [145-147]. In a randomized, double-blind and placebo-controlled trial, Sun et al demonstrated Taurine, a semi-essential sulfur-containing amino acid, supplementation increased plasma H_2S concentration and improved endothelial-dependent and – independent

vasodilation and this was negatively correlated with BP in taurine-treated prehypertensive individuals [148]. The same study also reported that taurine treatment upregulated the expression of H$_2$S synthesizing enzymes, CBS and CSE and reduced agonist-induced vascular tone in human mouse mesenteric arteries and rodent aorta. Finally, they concluded that taurine has potential in intervening vascular tone improvement by targeting H$_2$S-mediated inhibition of transient receptor potential channel subtype 3 (TRPC3)-mediated calcium influx [148].

3.7 H$_2$S on pro-inflammatory molecules regulation in hyperhomocysteinemia, hypertension and macrophage differentiation

The ROS-induced cell injury initiates a pro-inflammatory response leading to further damage. We and others have previously reported that hyperhomocysteinemia can lead to elevated levels of inflammatory response molecules ICAM-1 (intercellular adhesion molecule-1) and VCAM-1 (vascular cell adhesion molecule-1), which further cause endothelial cell injury [149, 150]. It was also reported that not only hyperhomocysteinemia but also homocysteinylated proteins, especially albumin from uraemic patients have been found to increase pro-inflammatory molecules, ICAM-1 and VCAM-1 expression in in vitro endothelial cell culture. These adhesion molecules along with MCP-1 (monocyte chemoattractant protein-1) and its receptor CCR2 (C-C chemokine receptor type -2) expression in the monocyte facilitated chemokines/cytokines-mediated adhesion process and mediators of vascular remodeling including ADAM17 (a disintegrin and metallopeptidase domain 17), MCP-1 and Hsp60 (heat shock protein 60) in the endothelial cells [151]. Additionally, the mature ADAM17 in turn increased TNFα in the culture medium promoting adhesion of monocyte to the endothelial cells [151]. Interestingly, in a separate study the same group has demonstrated that H$_2$S treatment mitigated TNFα-induced monocyte adhesion to endothelial cells by downregulating MCP-1, ADAM17 and related adhesion molecules [152]. Although the authors did not measure homocysteine levels *per se* in this in vitro study, they did measure homocysteine metabolizing and H$_2$S forming enzymes, CSE and 3MST, and found that these enzymes were downregulated [152]. These findings foster the assumption of possible increase in homocysteine levels in these cells. Nevertheless, the findings clearly support the claim that H$_2$S is protective against pro-inflammatory and pro-atherogenic response found in diseases, such as CKD [152].

Further investigation by our group and others have revealed that the exogenous H$_2$S supplementation partially alleviated inflammatory response [153], and improved kidney function in hyperhomocysteinemia [117]. Several other cytokines and chemokines have also been shown to be upregulated in mesangial cells, leading to chronic kidney disease [154, 155]. Of note, macrophage inflammatory protein 2 (MIP-2) was found to be increased in glomerular mesangial cells after treatment with homocysteine while monocyte chemotactic protein 1 (MCP-1) has been shown to be activated in response to increased homocysteine levels through induction via NF-κB pathway [156, 157]. Both of these molecules were found to be reduced after treatment with H$_2$S along with overexpressed CBS and CSE enzymes, concluding that H$_2$S can regulate inflammation in response to renal injury [158].

Interleukin-6 (IL-6) has also been found to play a critical role in inflammation associated with hypertension [52, 159]. In a rat model of renal ischemia/reperfusion injury (IR/I), inhibition of H_2S generation by PAG (DL-propargylglycine, an inhibitor of CSE enzyme) activated inflammatory response that include higher expression of IL-6 in renal tissue and promoted IR/I [160]. A similar study also found IR/I attenuated renal CBS and CSE mRNA and protein expression leading to increased pro-inflammatory MCP-1 and IL-6 activation. Further experiments revealed attenuation of cytokines in the renal tubular cells following H_2S donor (NaHS) supplementation [161]. Another study showed Ang-II induced hypertension led to the upregulation of IL-6 in mice and loss of this cytokine drastically reduced renal injury and renal fibrosis [162]. Corroborating with animal studies, in patients with CKD, IL-6 was found to be increased in the kidney of hypertensive individuals [162]. Although the investigators did not measure H_2S levels in these patients, it is possible that H_2S levels may have diminished. In fact, diminished levels of H_2S were confirmed by at least two other studies showing decrease levels of H_2S in coronary heart disease and hypertensive patients as well as in children with hypertension [163].

Macrophages are differentiated monocytes in the damaged tissue which phagocytose foreign body and cellular debris, helps tissue regeneration and development of immunity. When monocytes enter in the injured tissue through endothelial, a process known as leukocyte extravasation, undergo a series of changes to become macrophages. Another process, known as macrophage polarization, occurs when macrophages functionally differentiate into two subtypes depending on their microenvironment signals. While classically activated M1 phenotype is for defense mechanism and can be detrimental when expressed for a long period of time, M2 phenotype is for tissue repair and regeneration. In many CKD diseases, including obstructive nephropathy, IR/I, glomerulosclerosis and diabetic nephropathy macrophage polarization plays a critical role [164, 165]. However, macrophage polarization and their differential role in hypertensive kidney have not yet been fully elucidated. Recently we have demonstrated that Ang-II-induced hypertension caused renal fibrosis by sustained activation of pro-inflammatory M1 macrophage promoting the release of inflammatory cytokines and treatment with H_2S attenuated renal injury and fibrosis by promoting alternate activation of anti-inflammatory M2 macrophage [166].

Interleukin-21, which is a downstream molecule in the IL-6 signaling pathway, was also found to be upregulated in pulmonary-induced hypertension and promoted M2 macrophage polarization, which can lead to fibrosis [167]. In contrast, the anti-inflammatory cytokine interleukin-10 (IL-10) has been shown to have protective effects in response to Ang-II induced hypertension [168]. In IL-10 null mice treated with Ang-II, oxidative stress was increased as evidenced by superoxide and this was reduced in mice with functional IL-10, suggesting a protective function for this cytokine [168]. Despite involvement of interleukin signaling pathways in macrophage polarization and hypertension, the regulatory role of H_2S in these pathways of hypertensive kidney has yet to be defined.

3.8 MMP/TIMP imbalance, renal matrix deposition and role of H₂S in hypertension

Matrix metalloproteinases (MMPs) have a pivotal role in hypertension and extracellular matrix (ECM) remodeling. These zinc-dependent endopeptidases can cleave an array of

substrates such as cell surface receptors, cytokines, chemokines, cell adhesion molecules and other proteases [169]. In addition, they are also responsible for several cellular functions, including maintaining ECM homeostasis, cell proliferation and migration, apoptotic pathways, and epithelial to mesenchymal transition (EMT) and have been associated with several diseases of the kidney, including diabetic nephropathy, glomerulonephritis, and acute renal injury [169, 170]. MMPs are regulated, in part, by a class of inhibitors known as tissue-inhibitors of metalloproteinases (TIMPs). The TIMPs are one type of regulators of MMPs through binding and subsequent inhibition [171]. The improper balance of these proteases can lead to multiple pathological consequences in the kidney. In a study utilizing spontaneous hypertensive rats, MMP-2 and MMP-9 were found to be increased in the renal cortex as well as MMP-9 in the medulla, which led to higher levels of collagen formation in the glomeruli and tubular interstitium. While TIMP-4 was found to be elevated in the medulla, the expression and activity were not sufficient to suppress MMP activity [172]. In another study using the same rodent model, MMP-2 but not MMP-9 was found to be significantly increased in the juxtamedullary cortex along with collagen expression and formation [173]. Inhibitors TIMP-1 and TIMP-2 were also found to be increased in expression, however, the lack of balance between MMPs and TIMPs highlights another example of the effects of hypertension on renal injury [173].

We have shown that in Ang-II induced hypertension, homocysteine contributes to renovascular fibrosis, in part, by disrupting MMP/TIMP balance [84]. This promotes oxidation and accumulation of collagen in the basement membrane of the glomerular capillaries [84]. In another study, we have demonstrated association of MMP-2 and MMP-9 with renal damage through induction of elevated levels of homocysteine in mice heterozygous for CBS enzyme [116]. These animals showed an increase in activity for both MMP-2 and MMP-9 accompanied with elevated superoxide production and apoptosis, but were mitigated by exogenous H_2S supplementation, suggesting H_2S acts as an antioxidant to alleviate and prevent renal damage caused by hypertension [116].

3.9 Polysulfides synthesis

H_2S through sulfuration is stored in the tissue as bound sulfane sulfur [108, 174]. This may occur from endogenously generated H_2S as well as exogenously supplied sources [175]. Endogenously bound sulfane sulfur has been reported by many workers using several tissues including brain and liver [174, 175]. However, to date it is unclear whether bound sulfane sulfur is protein specific and whether release of bound H_2S under physiological condition requires a specific signal.

Very recently Bradley et al published a comprehensive review article summarizing the importance of garlic derived polysulfide synthesis and their possible role in cardiovascular protection through H_2S and NO [176]. According to their hypothesis, garlic-derived polysulfides, such as, diallyl sulfide, diallyl disulfide and diallyl trisulfide are potent H_2S donors that increase NO bioavailability through eNOS phosphorylation resulting in cardioprotection. Whether similar renal protection is possible in CKD by polysulfides or garlic derived H_2S warrants investigation.

4. Other metabolic disorders: H$_2$S is a modulator of renal dysfunction

4.1 Diabetes and H$_2$S

Diabetes mellitus is linked with hypertension and is prevalent in individuals suffering from both type 1 and type 2, with the latter having a 60-80% rate of incidence [177]. Many of the hypertensive risk factors may also apply to the development of diabetes, including poor eating habits, higher salt intake and retention, lack of physical activity, arterial stiffening, and endothelial dysfunction [178]. Individuals who also are diagnosed with diabetes are at greater risk of developing congestive heart failure, stroke, myocardial infarction, periphery arterial disease and nephropathy [179]. Interestingly, accumulating evidence indicate that H$_2$S plays a critical role in the pathogenesis of CKD including diabetic nephropathy. In streptozotocin-induced diabetic rat model, Zhou et al demonstrated plasma and renal tissue levels were diminished and intraperitoneal injection of NaHS (donor of H$_2$S) were able to normalize H$_2$S levels in plasma and kidney [180]. Increased glomerular basement membrane thickening, mesangial matrix deposition and renal interstitial fibrosis were evidenced in these diabetic rats resulting in declined renal function. H$_2$S was able to alleviate diabetic nephropathy in part by attenuating matrix deposition and inflammation [180]. Similar to their study, we also reported that in genetic diabetic Akita mice, diminished tissue and plasma H$_2$S content was associated with matrix deterioration and diminished renal function [56, 57]. Increased matrix metalloproteinse-9 (MMP-9) expression and activity was noticed in Akita diabetic kidney which was normalized by H$_2$S supplementation. Finally, we concluded that renovascular remodeling and dysfunction in Akita kidney was in part due to oxidative stress-mediated MMP-9 activation leading to imbalance in gap junction molecules and renal functional impairment. H$_2$S treatments attenuated oxidative stress and MMP-9 activity, and therefore ameliorated kidney ECM remodeling and function in Akita [56, 57].

The interconnectivity of diabetes and hypertension is substantial, including inflammation (IL-6, ICAM-1, VCAM-1, CRP), oxidative stress, vascular remodeling and endothelial dysfunction, all of which are induced by Ang-II [179]. Inhibition of Ang-II can lead to a decrease in proteinuria, a key feature of diabetes in urine, and slow the progression of end-stage renal disease [181]. It was reported that in streptozotocin-induced diabetic kidney angiotensin converting enzyme (ACE), AT1R and Ang-II was upregulated, and H$_2$S in plasma and kidney tissue were diminished [180]. Direct correlation of diminished H$_2$S and increased Ang-II was established by the fact that H$_2$S treatment partially normalized all the above three effectors / inducers of hypertension in diabetic kidney [180]. Although this study in particular did not measure proteinuria and kidney function per se, we reported improved kidney function in Akita kidney [57]. Another study reported diuretic effect of H$_2$S in spontaneously hypertensive diabetic rats in addition to reduction of blood pressure, restoration of H$_2$S and plasma sodium levels compared to non-H$_2$S treated diabetic rats [182]. In a post-hoc analysis study it was found that urinary sulfate (sulfate in the urine is the metabolic end product of H$_2$S) was negatively associated with albuminuria suggesting the authors to conclude higher urinary sulfate concentration is associated with reduced renal risks in type 2 diabetic nephropathy [183]. Similar results were reported from another independent post-hoc prospective, randomized, controlled trial in which it was noticed that high urinary sulfate excretion was associated with slower decline of assessed GFR in

diabetic nephropathy patients, and this was independent of known end-stage renal disease progression promoters [184]. All these above reports clearly establish a link between diabetes, hypertension and kidney dysfunction where H_2S plays a key role to modulate hypertension and kidney dysfunction in diabetes.

4.2 Gut microbiota, CKD and H_2S

An area of biomedical research receiving more attention in the recent years is the role of gut microbiota in the development of hypertension. Two main species of microbes predominately live in the gut, *Firmicutes* and *Bacterioidetes*, and the amount of these microbes can fluctuate depending on diet and physical fitness [185]. Increased population of these microbes has been shown in hypertensive animal models, including spontaneously-hypertensive rats, salt-induced models, as well as Ang-II induced hypertension [186]. Genetics and epigenetics can also influence gut microbiota. One study showed that the expression of renal olfactory receptor 78 (Olfr78) responded to propionate, a short chain fatty acid derived from gut microbiota, and regulated the amount of renin produced in the kidney. The knockout of this Olfr78 receptor, coupled with antibiotics that reduce the amount of microbiota in the gut led to an increase in blood pressure [187].

Microbiota, like many mammalian cells and tissues, also produces H_2S. They exploit this gaseous molecule as an antioxidant defense mechanism, energy production, and cell cycle regulation [188-190]. In CKD patients, a close relationship between gut microbiota and kidney has often been highlighted. Deranged microbiota in the gut produces endotoxin which enters into the bloodstream and translocates to distant organs, including kidney, causing uremic pathology [191]. Due to damaged intestinal epithelial barrier it is plausible that live bacteria may even translocate to organs through the bloodstream. Therefore, it is not surprising that microbiota in the gut and/or translocated microbiome in the organ system can influence overall H_2S levels in mammalian body under pathological dysbiosis condition. It was estimated that 50% of fecal H_2S is derived from bacteria, since germ free mice only synthesize 50% which was observed in colonized mice [192]. Thus total plasma H_2S pool varies depending on individual s health condition associated with microbiota milieu in the gut. Similarly, it is also possible that individual organs may harbor specific bacterial strain that further influence H_2S synthesis and local release causing microenvironment change in microbiota-related disease condition.

To this end, currently it is not known whether microbial settlement in the kidney *per se* influences local H_2S synthesis and release, and thus overall renal health in CKD. It is however reported that a number of uremic solutes are more prominent in plasma from dialysis patients with normal colon compared with colectomy patients [193]. Of these, five uremic toxins namely α-phenylacetyl-l-glutamine, 5-hydroxyindole, indoxyl glucuronide, *p*-cresol sulfate, and indoxyl sulfate were identified as colon derived uremic toxins. These important findings strongly suggest that colonic microbes produce a notable portion of uremic solutes, which otherwise absent or significantly lower in patients without colons [193]. Few years later of this aforementioned report, a similar study found even more uremic solutes in plasma of hemodialysis patients adding 48 newly identified solutes to the list of previously reported uremic toxins totaling altogether 270 [194]. Analyzing plasma from

colectomy patients and normal subjects with hemodialysis the authors found that 9 solutes were colon derived, of which 6 were not previously been reported. Although they concluded their findings that many of these polyphenols are plant derived and can escape gut linings, a portion of these compounds are produced by gut microbiota [194]. In a recent review article, Vanholder and Glorieux nicely integrated how the composition of intestinal microbiota impacts overall uremic solute retention, and how these uremic status affects intestinal microbiome causing generation of more uremic solutes and pouring to the plasma [195]. It was also reported that in uremic condition colon bacteria trend to migrate or translocate to the distal parts of the body where normally they are absent including jejunum, ileum, lymph nodes, liver and spleen [196-198]. Bacterial DNAs were detected in the blood of non-dialyzed ESRD patients corroborating their specific genera overgrown in the guts [199]. Patients with bacterial DNA in the blood showed higher inflammatory marker which was indicative of direct correlation of bacterial translocation with inflammation in ESRD [199]. Using 5/6 kidney ablated rat model same research group has reported gut microbiome dysbiosis promotes bacterial translocation to the lymph and systemic circulation contributing microinflammation in experimental uremia [200]. Since gut microbiota produces considerable amount of H_2S [201], it is highly possible that during dysbiosis in CKD patients translocated microbiota may also produce and add up to the local pool of H_2S in their destined organ including kidney, and may affect kidney function. Research in this direction is highly warranted and in the near future we may witness some developments in this relatively unexplored area of human health.

5. Future research

At elevated levels homocysteine converts to homocysteine-thiolactone as a result of error-editing function of some aminoacyl-tRNA synthetases [202-204]. Homocysteine-thiolactone is a reactive metabolite that causes protein N-homocysteinylation through formation of amide bonds with protein lysine residues [204], which alters or impairs protein function [203]. Together, diminished CBS, CSE and 3MST enzymes in hypertension elevates homocysteine levels and reduces H_2S production. Furthermore, hyperhomocysteinemia through protein homocysteinylation may contribute to renal remodeling. Whether these mechanisms are involved in hypertensive CKD, and whether H_2S can modulate protein homocysteinylation, and therefore improves renal function is currently not known. Future investigation may provide some lights into these possibilities.

6. Summary and conclusion

Cardiorenovascular morbidity and mortality still remains the leading cause of death worldwide despite huge improvements in healthcare. Abnormalities in metabolic processes, such as carbohydrate, lipid and protein metabolism contribute to conditions related to vasculopathies including hypertension. While the cause of idiopathic hypertension remains to be elucidated for future therapeutic strategy, the known cause of hypertension related to dysregulated amino acid metabolism could easily be prevented or treated with available tools. H_2S is such a tool that may be further exploited mechanistically either alone or in combination with available drugs to prevent or even to treat high homocysteine associated hypertensive vascular complications in patients with metabolic disorders and CKD. Towards

this end, several clinical trials are ongoing to delineate safety and efficacy of H_2S treatment to mitigate cardiorenovascular complications. We anticipate that the outcomes will provide us insightful information how H_2S can be utilized as a future drug in treating hypertension associated with metabolic, cardiovascular and CKD.

Acknowledgments

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Abbreviations

3MST	3-mercaptopyruvate sulfurtransferase
ACE2	angiotensin-converting enzyme 2
AGT	angiotensinogen
Ang-II	angiotensin II
AT1/T2-R	angiotensin type-1 / type-2 receptor
BMI	body mass index
BP	blood pressure
CAT	cysteine aminotransferase
CBS	cystathionine β-synthase
CKD	chronic kidney diseases
CO	carbon monoxide
CRP	C-reactive protein
CSE	cystathionine γ-lyase
CVD	cardiovascular disease
DAO	D-amino acid oxidase
ECM	extracellular matrix
EMT	epithelial-mesenchymal transition
eNOS	endothelial nitric oxide synthase
FA	folic acid
GFR	glomerular filtration rate
H_2S	hydrogen sulfide
HDL	high-density lipoproteins
ICAM-1	intercellular adhesion molecule-1
IL	interleukin
IR/I	ischemia-reperfusion injury
MCP-1	monocyte chemotactic protein 1

MetS	Metabolic syndrome
MIP-2	macrophage inflammatory protein 2
miR	microRNA
MMP	matrix metalloproteinase
NADPH	nicotinamide adenine dinucleotide phosphate
NO	nitric oxide
PAG	DL-propargylglycine
RAS	renin-angiotensin system
ROS	reactive oxygen species
TGF-β	transforming growth factor-β
TIMP	tissue inhibitor of metalloproteinase
VCAM-1	vascular cell adhesion molecule-1
VEGF	vascular endothelial growth factor
WHO	World Health Organization
ZDF	Zucker diabetic fatty
α-SMA	α smooth muscle actin

Highlights

H$_2$S attenuates RAS activity in the kidney, and therefore has potential to treat renovascular hypertension.

In metabolic and epigenetic hypermethylation diseases, H$_2$S deficiency exacerbates hypertension.

High homocysteine, and low H$_2$S is associated with CKD-mediated renovascular hypertension

Gut dysbiosis contributes to the total plasma H$_2$S pool, and therefore affects overall individual s health related to H$_2$S patho/physiology.
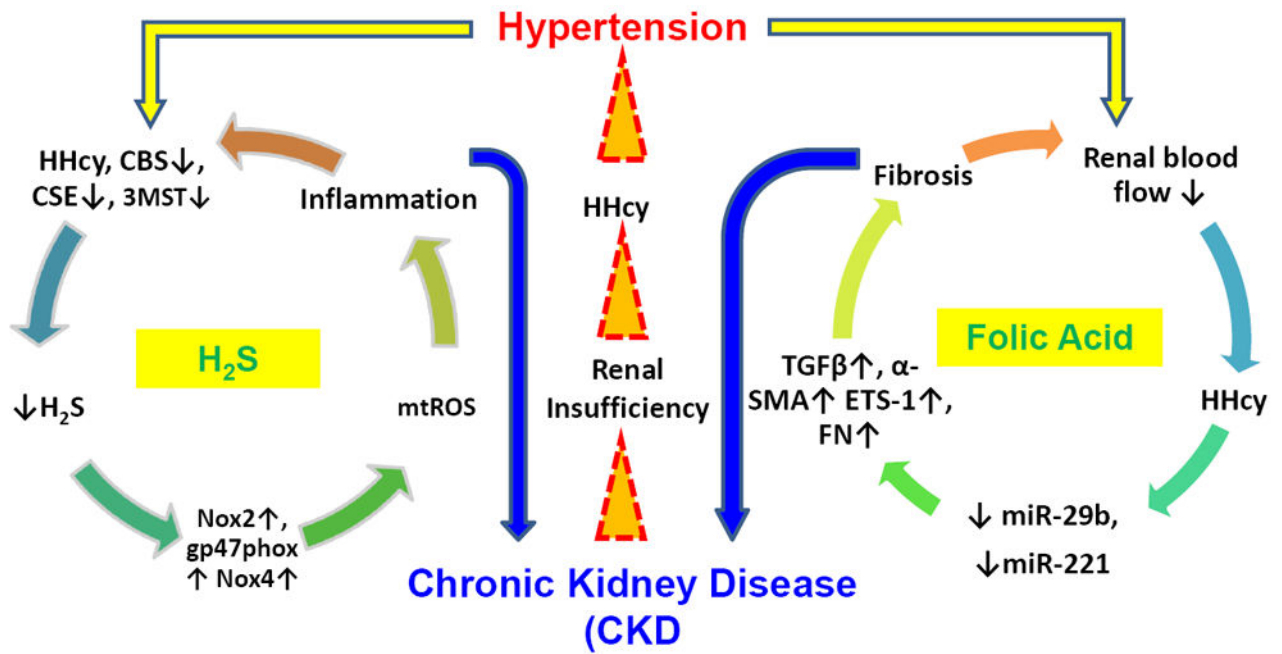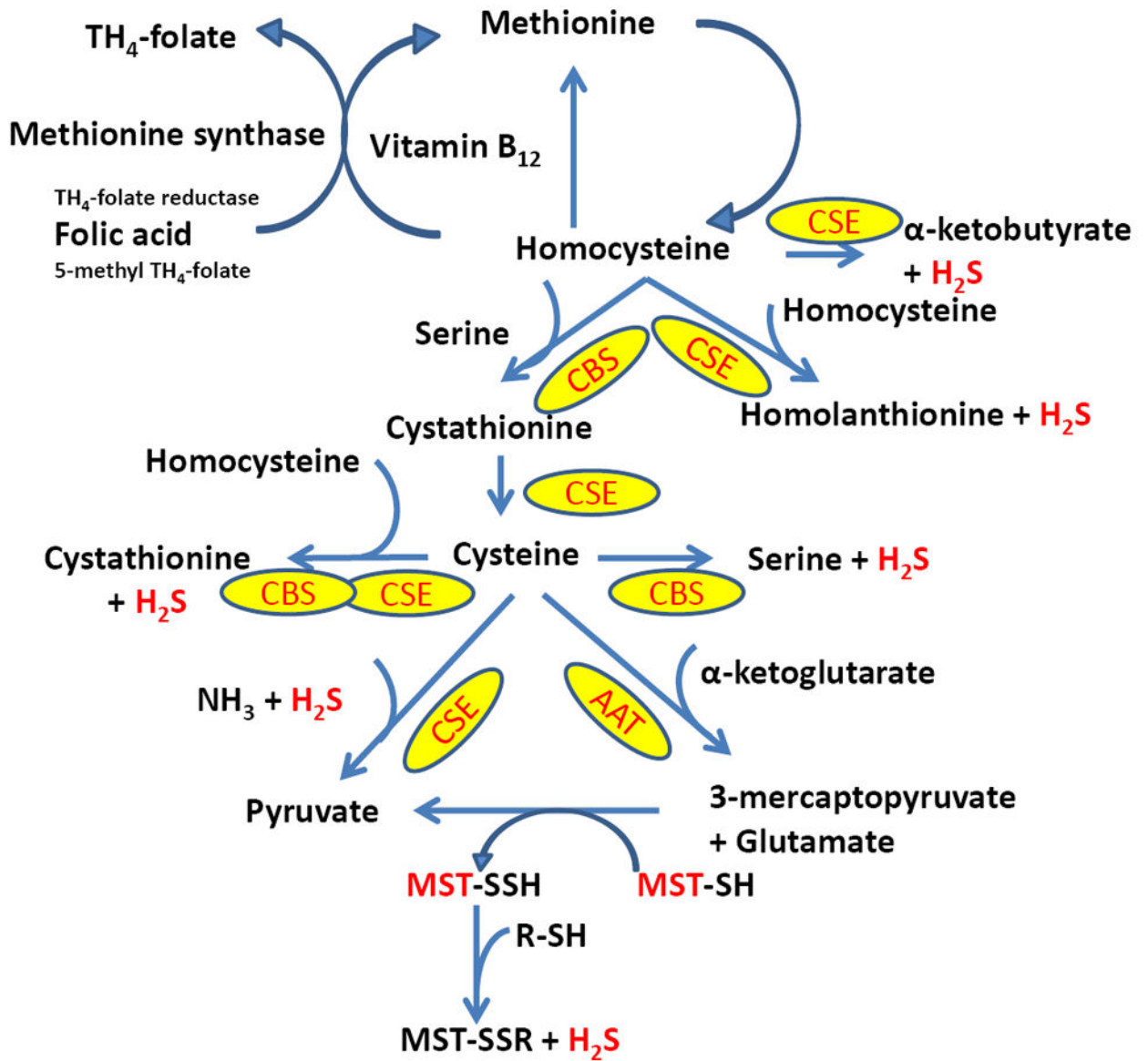
Figure 1.
Schematic of CKD mechanism in hypertension depicting important roles of Hyperhomocysteinemia and H_2S. Hypertension causes renal impairment of H_2S producing enzymes leading to H_2S deficiency and increase mitochondrial generated oxidative inflammation (on the left side). Through a different but related mechanism of H_2S deficiency (since homocysteine is a precursor of H_2S, homocysteine accumulation, *i.e*, Hyperhomocysteinemia diminishes H_2S production), hypertension increases resistive index and reduces blood flow in the renal vasculature (on the right). As a result kidney impairment occurs and homocysteine clearance impairs. High levels of homocysteine in turn activate miRNAs leading to pro-fibrotic gene activation and fibrosis. These above described two vicious cycles worsen CKD in hypertension.
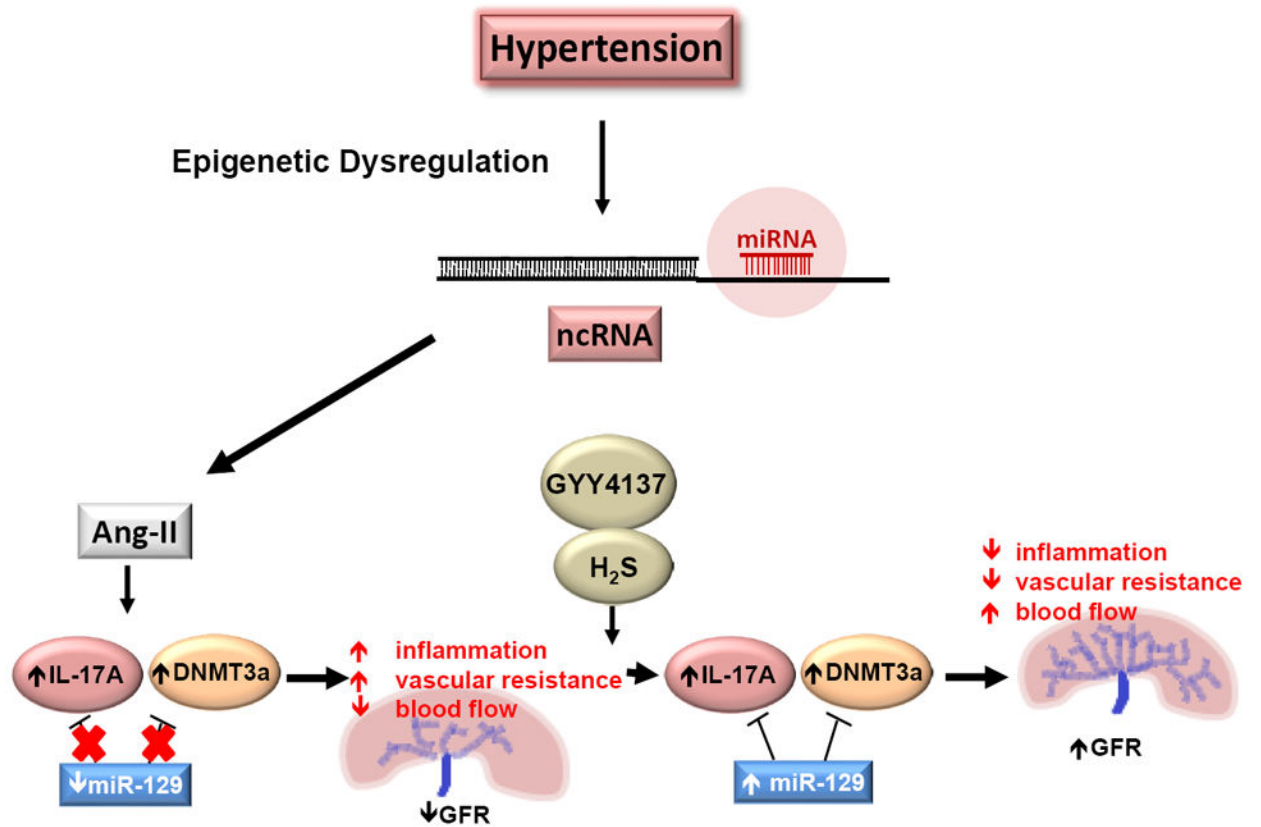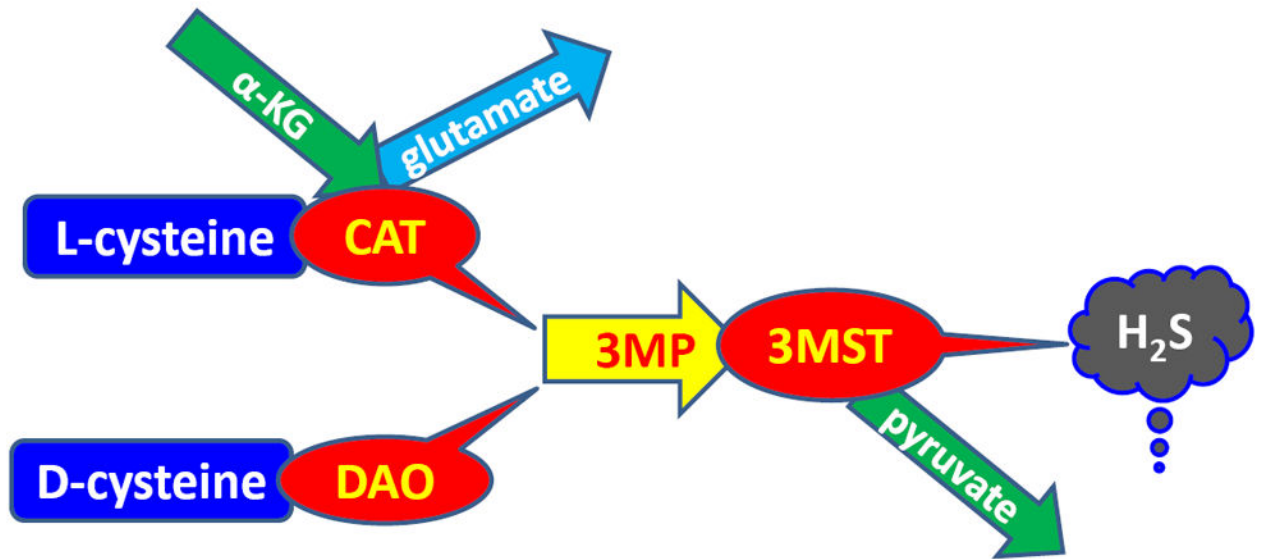
Figure 2.
Schematic of hypertension-induced epigenetic mechanism of kidney dysfunction. Ang-II causes disruption of epigenetic mechanisms (methylation, histone modification, non-coding RNAs) leading to inflammation and increased vascular resistance causing kidney dysfunction in hypertension. GYY4137 alleviates these effects and improves kidney function. DNA methyltransferase DNMT3a and pro-inflammatory cytokine IL-17 are increased in the hypertensive kidney while miR-129 is suppressed [68]. The miR-129 is predicted to target and regulate both DNMT3a and IL-17 and its expression is increased with H$_2$S donor GYY4137 treatment, leading to a decrease in IL-17 and DNMT3a expression [68].

Figure 3.
Schematic of Homocysteine metabolism and H2S biosynthesis. H2S; hydrogen sulfide; TH4-folate, tetrahydrofolate; CBS, cystathionine β-synthase; CSE, cystathionine γ-lyase; 3MST, 3-mercaptopyruvate sulfurtransferase; AAT, aspartate aminotransferase.

Figure 4.
Schematic of H$_2$S biosynthesis form cysteine. Metabolism of L-cysteine by cysteine aminotransferase (CAT) and D-cysteine by D-amino acid oxidase (DAO) produces 3-mercaptopyruvate (3MP). 3MP then metabolizes to produce H$_2$S by 3-mercaptopyruvate sulfurtransferase (3MST).