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The Role of Macula Densa Nitric Oxide Synthase 1 Beta Splice Variant in Modulating Tubuloglomerular Feedback

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Abstract

Abnormalities in renal electrolyte and water excretion may result in inappropriate salt and water retention, which facilitates the development and maintenance of hypertension, as well as acid-base and electrolyte disorders. A key mechanism by which the kidney regulates renal hemodynamics and electrolyte excretion is via tubuloglomerular feedback (TGF), an intrarenal negative feedback between tubules and arterioles. TGF is initiated by an increase of NaCl delivery at the macula densa cells. The increased NaCl activates luminal Na-K-2Cl cotransporter (NKCC2) of the macula densa cells, which leads to activation of several intracellular processes followed by production of paracrine signals that ultimately result in a constriction of the afferent arteriole and a tonic inhibition of single nephron glomerular filtration rate. Neuronal nitric oxide (NOS1) is highly expressed in the macula densa. NOS1β is the major splice variant and accounts for most of NO generation by the macula densa, which inhibits TGF response. Macula densa NOS1β-mediated modulation of TGF responses play an essential role in control of sodium excretion, volume and electrolyte hemostasis, and blood pressure. In this article, we describe the mechanisms that regulate macula densa-derived NO and their effect on TGF response in physiologic and pathologic conditions.

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INTRODUCTION

Tubuloglomerular feedback (TGF) response is one of the sophisticated and orchestrated mechanisms in the kidney that regulate sodium excretion. TGF is a negative feedback loop between tubules and the glomerular arterioles[1–11]. This feedback loop is initiated by an increase in NaCl delivery to the macula densa, a small cluster of modified thick ascending limb (TAL) cells located near the distal end of the TAL, adjacent to the arterioles of its parent glomerulus. The increase in NaCl delivery to this segment enhances luminal Na-K-2Cl cotransporter (NKCC2) activity of the macula densa cells, consequently raising their intracellular NaCl concentration. This increase in intracellular NaCl leads to several responses including stimulation of basolateral Cl^- efflux, which depolarizes the macula densa cell,[12, 13] and activation of the luminal Na/H exchanger (NHE), thereby alkalinizing the macula densa cell[14], as well as alteration in intracellular calcium[15, 16]. The net effect of the increased NaCl transport mechanisms across the macula densa cells induces release of ATP and/or adenosine from the basolateral membrane of the macula densa,[17] which constrict the afferent arteriole[18–21] and may also dilate the efferent arteriole,[22] thus decreasing GFR and returning tubular flow to normal levels. In this way, TGF response protects against large fluctuations in distal tubular flow and excessive changes in NaCl excretion.

However, the relationship between NaCl delivery and the TGF response cannot be rigid. It must adapt to a number of physiological conditions (*e.g.*, renal growth, pregnancy, volume expansion and depletion), otherwise, it could become detrimental. For instance, volume expansion increases NaCl delivery to the macula densa, thus triggering TGF-induced decreases in glomerular filtration rate (GFR), tubular flow, and NaCl excretion. This response, if unopposed, would cause sodium retention and ultimately volume overload. However, this adverse relationship does not normally occur because the macula densa possesses mechanisms that modulate TGF responsiveness, thus permitting it to adapt to diverse levels of salt intake as well as other physiologic conditions[2, 23]. Indeed, TGF responsiveness is regulated by many factors, including angiotensin II[11, 24, 25], adenosine[26–29], arachidonic acid metabolites[30–33], ATP[18–20, 34], atrial natriuretic factor[35], superoxide (O_2^-)[7, 36, 37] and nitric oxide (NO)[6–8, 14, 38, 39]. Consequently, abnormalities in any of these factors can impair normal adaptation of TGF response to physiologic conditions, and thus lead to impaired NaCl excretion, salt-sensitivity, and/or hypertension.

In this article, we summarize the modulatory effect of macula densa-derived NO on TGF responses in health and disease. The first section provides an overview of macula densa-derived NO during acute TGF responses, whereas the latter sections summarize the role of macula densa-derived NO during different physiologic and pathophysiologic conditions including adaptation to high salt intake, sex differences, salt-sensitive hypertension, glomerular hyperfiltration, and gestational hypertension as described in Figures 1 and 2.

ROLE OF MACULA DENSA-DERIVED NO ON ACUTE TGF RESPONSES

A variety of cells adjacent to the macula densa (particularly endothelial and TAL cells) are capable of generating sufficient NO via NOS3 to potentially alter TGF responsiveness[40, 41].

However, it is the NO generated by the macula densa cells per se via its abundantly expressed NOS1[42, 43] that is likely to be the main modulator of TGF responsiveness under normal physiologic conditions. Indeed, the same increase in NaCl delivery to the macula densa that triggers the TGF response also increases local NO levels.[1, 44] This NO can then either act directly on the macula densa cells by activating cGMP-dependent protein kinase, or diffuse to the afferent arterioles directly blunting the ensuing vasoconstriction, either of which will reduce the magnitude of the TGF response.[8, 14, 36, 45] Indeed, eliciting the TGF response in isolated-perfused juxtaglomerular apparatus (JGA) was accompanied by an increase in NO levels in the macula densa. Blocking NOS1 with 7-nitroindazole (7-NI; a selective inhibitor of NOS1) prevented the increase in NO in the macula densa cells and augmented the magnitude of the TGF response.[1, 44] Since the macula densa is the primary source of NOS1 in the normal renal cortex,[46, 47] the results strongly suggest that the macula densa cells themselves are the primary source of the NO, and that macula densa-derived NO provides an intrinsic feedback mechanism that modulates TGF responsiveness.[1, 44,8, 14, 36, 45, 48–50] Consequently, factors that alter NOS1 activity or NO levels in the macula densa will be quite influential in determining TGF responsiveness.

NOS1 is a constitutively expressed enzyme whose activity is regulated by a variety of pathways, including via calcium-calmodulin mediated mechanisms, posttranslational modifications, and/or protein-protein interactions, which have been thoroughly reviewed by others.[51–54] One mechanism that is triggered by the same stimulus as the TGF response (and is thus of special interest) is cellular alkalinization. Increased NaCl delivery increases the activity of NHE2 and NHE4, on the apical and basolateral sides of the macula densa, respectively[17, 55]. The increased NHE activity in the macula densa cells increases their intracellular pH from 7.0–7.2 to 7.4–7.8,[14, 56, 57] which in turn increases NOS1 activity by up to 5-fold (maximal activation occurs at a *p*H of 8).[58–60] Evidence supporting a key role for NHE-dependent alkalinization of macula densa cells to generate NO is provided by the following. First, directly elevating intracellular pH by using nigericin enhanced NO generation from NOS1 in the macula densa cells[14]. Second, inhibiting apical NHE with amiloride reduced macula densa-derived NO generation and enhanced the TGF response, in a similar manner to 7-NI,[41] but by only 40–60%,[14] thus suggesting that NHE is only partially responsible for the increase in NOS1 activity. The relative contributions of diverse stimuli of NOS1 activity during different physiologic conditions remain incompletely understood.

The Importance of O_2^- in Modulating Macula Densa-Derived NO.

The bioavailability of NO (the amount available to interact with its target) in the macula densa is not only determined by NOS1 activity, but also by increased degradation of NO, which will largely be determined by the levels of reactive oxygen species, in particular superoxide (O_2^-).[61–63,64] O_2^- generation by the macula densa is increased by the same stimuli as the TGF response. Indeed, like the TGF response, NaCl-induced O_2^- generation is prevented by blocking NKCC2 with furosemide. Moreover, it is also blocked by apocynin (a NOX inhibitor), suggesting that activation of NKCC2 stimulates NOX thus increasing O_2^- generation.[65–67]

There are several mechanisms known by which stimulation of the TGF response leads to increased O_2^- generation. The first is investigated by membrane depolarization. Correlation between changes in membrane potential and O_2^- production was first found in human granulocytes[68] and later confirmed in endothelial cells.[69] Macula densa cells behave similarly in response to depolarization. The sequence of events is as follows. Increases in luminal NaCl concentrations activate NKCC2 activity which results in depolarization of the macula densa cells by up to 31 mV (as measured via micro-electrodes).[12] Depolarization of the macula densa cells via increased NKCC2 activity (or independently via valinomycin) leads to translocation of Rac to the apical membrane, and a subsequent increase in macula densa-derived NOX activity and O_2^- generation.[70,65]

The second mechanism that leads to increased NOX activity is similar to that of NOS1, that is, via NHE-induced increases in intracellular *p*H. This is because NOX is highly *p*H sensitive.[71–74] Its activity, in human eosinophils or neutrophils, is directly correlated with intracellular pH between 7.0 to 8.1, above or below which, its activity decreases drastically.[71, 72] Because the intracellular *p*H of macula densa cells fluctuates between these levels depending on TGF activity, O_2^- generation by the macula densa may be dependent on TGF-induced changes in intracellular *p*H. Indeed, the *p*H of macula densa cells during low TGF activity is between 7.0–7.2. Activation of the TGF response immediately increased intracellular *p*H to 7.4–7.8,[14, 56, 57] and O_2^- production by 5-fold (in the presence of the NOS inhibitor *N*-nitro-l-arginine methyl ester), thus demonstrating a correlation between intracellular *p*H and O_2^- production. To determine whether the changes in intracellular pH were causing the changes in O_2^- production, experiments were carried out in the isolated perfused JGA preparation. The delivery of NaCl to the macula densa was fixed, but the intracellular pH was increased by either increasing the pH of the tubular perfusate or clamping it using nigericin, a K^+/H^+ ionophore. The two methods of increasing intracellular pH were equally effective at increasing O_2^- production by the macula densa, despite the absence of changes in NaCl delivery. Tempol and apocynin completely blocked the pH-induced O_2^- production by the macula densa, whereas blocking NHE with dimethyl-amiloride inhibited NaCl-induced O_2^- production by about 40%.[56] It is important to note that the two mechanisms, depolarization and alkalinization of macula densa cells do not act on NOX activity independent of each other. For instance, alkalinization of the macula densa cells only stimulated O_2^- generation when the cells were perfused with 80 mM, rather than 10 mM NaCl.[56,107] The macula densa cells are depolarized when exposed to 80 mM NaCl, but hyperpolarized when perfused with 10 mM NaCl. Together, the above results suggest that increasing NaCl delivery depolarizes the macula densa cells and activate NOX. In addition, it stimulates NHE activity, which in turn increases intracellular pH and further increases NOX activity.

The generated O_2^- does not appear to increase TGF responses directly, rather by counteracting the actions of NO on TGF. Specifically, O_2^- binds to NO, thereby reducing its bioavailability and effect on TGF responses.[1, 8, 36, 37, 56,] Conversely, in the absence of O_2^-, NO will have an unopposed buffering effect on TGF. This concept is supported by the finding that tempol (a stable membrane-permeant superoxide dismutase mimetic) prevented TGF-induced generation of O_2^- and potentiated the buffering effect of NO on TGF[36]. It is noteworthy that the tempol's ability to blunt TGF responses occured only in the presence

of intact NO synthesis, concomitant administration of the NOS1 blocker (7-NI) abolished tempol's effect on TGF.[36] Together, the above studies suggest that the ratio between NO and O_2^- levels determines TGF responsiveness in a variety of conditions.[1, 14, 36, 44, 56, 67]

Under physiological conditions, the balance between NO and O_2^- is heavily tilted towards NO; in fact, O_2^- in the macula densa is largely undetectable when the NOS activity is intact. However, if NOS is inhibited or generation of O_2^- is enhanced (e.g. in conditions associated with hypertension, diabetes, and kidney injury[75–85]) the balance between NO to O_2^- may be shifted in favor of O_2^-. For instance, mice rendered hypertensive by infusing angiotensin II (Ang II) had greatly increased expression and activity of the NAD(P)H oxidase isoforms NOX2 and NOX4 and consequently O_2^- generation in the macula densa.[86, 87,88] This increase in NOX-derived O_2^- production was sufficient to make NO levels in the macula densa undetectable,[89] despite increased macula densa-NOS1 activity[90]. Consequently, this inversion of the NO/O_2^- ratio led to significantly enhanced TGF response.[89, 91] Indeed, the reduction in single nephron GFR in Ang II-treated rats was significantly reduced when they were concomitantly treated with a siRNA against a membrane NOX subunit p22phox.[92] Several studies have demonstrated the importance of the NOX isoforms in regulating glomerular hemodynamics particularly through their actions on TGF, but also via direct vascular effects.[92–99] Importantly, the reaction of NO with O_2^- generates peroxynitrite, which has been demonstrated to modulate NOS2 and NOS3 expression and activity, as well as play an important role in many pathophysiological conditions.[100–102] However, the role of peroxynitrite in the regulation of NOS1 and TGF response has not been investigated.

Because of the significance of O_2^- in modulating TGF, it is important to understand its sources and the regulation of these sources in the kidney. Uncoupling of NOS1 due to decreased availability of its substrate (L-arginine) and/or other cofactors (BH4, NAD, etc.) not only decreases generation of NO, but is also a potential source of O_2^-.[103–106] However, the majority of O_2^- appears to be generated by the NOX isoforms,[7, 37, 107] which are widely expressed in the vasculature and tubules in the cortex and the medulla.[36, 43, 108, 109] Of the five NOX isoforms (NOX1 – NOX5)[110–116] and the two $gp91^{phox}$ isoforms (DUOX1 and DUOX2)[117,118, 119], only NOX1, NOX2 and NOX4 have been found in adult kidneys.[107, 114, 120, 121,113, 116–118] Experiments that combined laser-capture microdissection together with real PCR revealed that the macula densa expresses NOX2 and NOX4[67, 88]. NOX4 was responsible for basal O_2^- production, whereas NOX2 was the main source for NaCl-induced O_2^- generation;[67] both contributed to macula densa-derived O_2^- in Ang II-dependent hypertension.[88,98] Any O_2^- produced in the macula densa cells by these isoforms will be restricted to the cells where it was produced, because O_2^- is not membrane-permeant and is therefore restricted to the compartment where it is generated.[61–63, 122] Likewise, the O_2^- produced by surrounding cells, such as TAL in response to NaCl[123, 124] will not likely affect TGF, at least directly via the macula densa (Fig 1).

ROLE OF MACULA DENSA-DERIVED NO IN MODULATING TGF RESPONSES DURING CHRONIC PHYSIOLOGIC CONDITIONS - THE IMPORTANCE OF THE SPLICE VARIANTS OF NOS1

Chronic Adaptation of NOS1 Activity during High Salt Intake

In the acute setting, TGF-induced decreases in GFR make perfect sense. It prevents large fluctuations in the delivery of NaCl to the distal segments and provides fine tuning of the autoregulatory response. However, if sustained, it becomes maladaptive. An obvious example is that of a high dietary salt intake. This initially would increase the delivery of NaCl to the macula densa, decrease GFR, and facilitate sodium retention. However, resetting or adaption of the TGF response via the interactions between NO and O_2^-, prevent this from occurring. For instance, the TGF response must be reset so that it is reduced during a high NaCl diet. This resetting occurs in a large part due to enhanced NO generation at the macula densa. This notion was first supported by several lines of evidence; 1) early studies found that rodents fed a high salt diet had evidence of enhanced NO generation, including increased plasma levels and renal excretion rates of nitrite and nitrate (NO metabolites),[125–128] as well as increased cGMP levels (a downstream signaling molecule of NO).[125] 2) Increased distal tubular flow enhanced NOS1 activity at the macula densa. 3) Pharmacological inhibition of macula densa NOS1 in vitro augmented TGF responses to a greater extent in animals on a high salt diet, suggesting increased activity of NOS.[3, 129]. 4) Inhibition of NOS had a greater effect on renal blood flow (RBF), GFR and renal vascular resistance in animals fed a high salt diet.[125, 127, 130, 131] 5) In normal and hypertensive humans, a high salt diet was usually associated with an elevation in GFR, RBF, sodium and cGMP excretion compared with low-salt dietary conditions. Moreover, these effects are significantly enhanced by L-arginine administration[132, 133]. 6) Finally, several hypertensive rodent strains, such as Dahl salt-sensitive, as well as Milan and spontaneously hypertensive rats, exhibit impaired NOS1 expression and/or activity.[7, 38, 39] Taken together, these data provide strong evidence that a high NaCl intake increases NOS activity, which in turn influences renal regulation of Na excretion.

Despite this compelling evidence, several well-done studies found a significant discrepancy with the above results. They found that mRNA and protein levels of NOS1 in the renal cortex and macula densa decreased, rather than increased by a high salt diet. In fact, those animals on a low salt diet had a higher NOS1 level.[134–136] A second discrepancy arose when comparing studies that blocked NOS1 using pharmacological vs genetic techniques. Chronic pharmacologic inhibition of NOS1 with 7-NI triggered hypertension in Sprague Dawley rats[6] and caused salt-sensitive hypertension in Dahl salt-resistant rats,[137] again suggesting an important role for NOS1 in regulating blood pressure. However, mice with global NOS1 deletion were not hypertensive, salt-sensitive, or have renal hemodynamic abnormalities.[138–140] Thus, these studies suggested that macula densa NOS1 and NOS1-mediated TGF response did not play an important role in the regulation of sodium excretion, volume homeostasis and blood pressure.[138–140] The reason for the discrepant results between the two approaches was finally resolved with the identification of the NOS1 splice variants in the macula densa cells.[46, 47, 141]

NOS1 Splice Variants in the Macula Densa in Chronic Adaptation to Salt Intake.

One of the unique characteristics of NOS1 is the alternate splicing, which can produce several NOS1 mRNA variants and protein isoforms, while NOS2 and NOS3 do not exhibit this splicing phenomenon. Identified splice variants of NOS1 include α, β, γ, and μ. NOS1μ is only expressed in myocytes. NOS1β exhibits about 80% enzymatic activity of NOS1α, while NOS1γ only has 3% activity of NOS1α.[54, 142–144] NOS1β has been found to be a functional enzyme both in vitro and in vivo studies.[51, 143–147] Splice variants of NOS1 have been found in the kidneys, e.g. Baylis's group reported NOS1α and β proteins in the renal cortex. They showed reduced mRNAs of NOS1α and upregulated NOS1β were found in tubules in a rat model of chronic kidney disease.[146, 148, 149] NOS1β in collecting ducts has been reported by Pollock's laboratory[150, 151]. They demonstrated that the collecting-duct NOS1β played an important role in the control of fluid-electrolyte balance.

The expressions of NOS1 splice variants in the macula densa were examined with laser capture microdissection of the macula densa cells from frozen kidney slices.[46] NOS1α, β, and γ mRNAs were detected in isolated macula densa cells, and the protein isoforms of NOS1α and NOS1β, but not NOS1γ, were detected in renal cortex (mainly from the macula densa).[42, 43, 46] Considering the scarce expression level and low enzymatic activity of NOS1γ, it is unlikely to play an important role in the NO generation, and thus was not further considered. Expression levels of NOS1β mRNA and protein were 30- and 5-fold higher, respectively, than those of NOS1α in the renal cortex of C57BL/6 mice. Furthermore, macula densa NO production was similar in the isolated perfused JGAs from wild-type and NOS1α-knockout mice, whose NOS1β is intact[47]. Finally, NOS1β exhibited a 2–3 fold increase in its levels in the macula densa of rats fed a high salt diet, while NOS1α significantly decreased.[46] These results provided strong evidence that macula densa NOS1β is the major splice variant of NOS1 and accounts for most of the NO generation by the macula densa[46], and is largely responsible for blunting of TGF during salt loading.

The above results also provided a potential answer for the conflicting data about salt-induced changes in NOS1 expression and activity, as well as the disparate results found between the studies that used pharmacological vs. genetic approaches. First, studies that reported decreased NOS1 expression in response to a high salt diet likely used antibodies that targeted the N-terminal of NOS1, and thus identified only the NOS1α splice variant,[54, 142] which decreased during the high salt diet[46]. Second, the global NOS1 KO mouse line used in the previous studies targets exon-2 thus only deletes the NOS1α isoform[152]. NOS1β is still intact and the NO generated by the macula densa is not affected in the NOS1α KO mice.[46, 47, 141] Therefore, no changes in salt sensitivity or blood pressure would be expected. Note that 7-NI blocks the whole enzymic activity of NOS1, thus likely explaining its effects on TGF, salt-sensitivity and blood pressure, albeit an unrecognized non-renal effect of 7-NI cannot yet be ruled out. While the above studies demonstrated that the NOS1α KO mouse line is not a valid strain to study the significance of macula densa NOS1, they do provide strong evidence that NOS1 in the CNS does not play a significant role in control of blood pressure, since NOS1α is the dominant splice variant in the brain.[142, 152, 153]

Sexual Dimorphism in Salt-Sensitive and Ang II-Dependent Hypertension

Experimental and clinical evidence shows that females have a lower risk of developing several forms of hypertension, including salt-sensitive[154–162] and Ang II-induced hypertension.[163–166] However, the underlying mechanisms behind this protection are not elucidated.[154, 167] One potential mechanism may be differences in macula densa NOS1β activity, since its function is central to the pathogenesis of both of these types of hypertension in experimental studies. Indeed, deletion of NOS1β from the macula densa enhances the TGF response, impairs natriuresis, and exacerbates the increase in blood pressure in mice subjected to either a high salt diet or Ang II infusions.[46, 47, 141] Therefore, recent animal studies have examined whether sex-related differences in NOS1β activity exist.[168, 169] Several studies have found no differences in renal cortical NOS1 expression between male and female Sprague-Dawley[148, 170,] and spontaneously hypertensive rats[171] maintained on a normal diet. Likewise, male and female mice maintained on a normal diet also did not exhibit significant differences in NOS1β expression levels.[169] However, this latter study took it a step further and challenged the mice with a high salt diet for 2 weeks. The females had a greater increase in 1) NOS1β expression, 2) phosphorylation of NOS1β at Ser1417 (which increases its activity[172–174]), and 3) NO generation in the macula densa, as compared to males when subjected to the high salt intake.[169] Thus, while they had similar NOS activity under normal conditions, they had a more robust NOS1β response when challenged with high salt, which in turn, conferred greater protection.

Differences in macula densa-NOS1β activity, if functionally important, should translate into differences in TGF responsiveness and GFR regulation between the sexes. As would be expected, male and female mice maintained on normal salt diets (thus having similar expression of NOS1β), had similar TGF responses.[169, 175] However, upon subjecting the animals to a high salt diet, the sex differences became apparent. The increased expression of NOS1β in the females was associated with a greater resetting/blunting of their TGF responsiveness than similarly treated male mice.[169] Deleting the NOS1β gene decreased the sexual dimorphism introduced by the salt loading. The differences in TGF responses and blood pressure to a high salt diet[169] were greatly diminished in the macula densa-NOS1 KO mice, indicating that NOS1β is an important determinant of the sex differences. Interestingly, the sexual dimorphism is also present in the acute setting as well. The rapid increase in RBF, GFR and natriuresis that immediately follows the acute administration of a volume load[176–180] was also accentuated in females.[169] Because blunting of TGF is a key component of this response, it seems likely that acute regulation of TGF responses in females is also enhanced.

Differences in macula densa-NOS response in females are not only limited to changes induced by salt loading. They also have a more robust increase in macula densa-NOS1β when subjected to Ang II, which translates into blunted TGF responses and less hypertension than males. This sexual dimorphism is present during both acute and chronic administration of Ang II. For instance, while an acute infusion of Ang II augmented the sensitivity and magnitude of the TGF response *in vitro* and *in vivo*,[181,182] it did so less in females.[175] Likewise, a chronic infusion of a subpressor dose of Ang II for 2 weeks increased the expression and activity of macula densa NOS1β in male mice, but did so to a

greater extent in the female mice. The changes in macula densa NOS1β were accompanied by accentuated TGF responses in the males.[168] As with the salt loading studies, deletion of macula densa NOS1β abolished the sexual dimorphism in TGF and hypertensive responses during the chronic Ang II infusion.[168] The differences between how males and females regulate macula densa NOS1β and/or TGF when subjected to Ang II, may be in part due to differences in expression of the distinct Ang II receptors, type 1 (AT_1) vs. type 2 (AT_2). As mentioned before, Ang II stimulates NOX activity resulting in increased O_2^- generation, which in turn quenches NO and augments TGF responsiveness. This effect is generally considered to be primarily mediated via the AT_1 receptors[181]. However, it also stimulates macula densa-derived NO production,[183] which is thought to be due to activation of the AT2 receptors, thus raising the possibility that the AT_2 receptors may be implicated in the sex differences to TGF responses during Ang II administration[175]. Indeed, female mice reportedly had a 3-fold higher expression of AT_2 receptor in the kidneys compared to males. Moreover, the sex differences in the Ang II-induced alterations in TGF responses during Ang II administration were abolished in AT_2 receptor knockout mice, suggesting that differences in AT_2-mediated increases in macula densa-NOS1β may play a significant role in the sexual dimorphism observed in TGF responses during conditions associated with elevated Ang II levels.[168, 175]

While the above studies establish an important role for macula densa NOS1β-mediated resetting of TGF in the sexual dimorphism of salt-sensitive and Ang II-induced hypertension, they do not explain all the differences. Other factors may also contribute to the sexual dimorphism of the hypertension, including the diverse sex hormones and the differential activation of their assorted receptors,[184–188,189] as well as alterations in downstream signaling, particularly of the PI3K/Akt and cAMP/PKA pathway which can modulate NOS1 expression and activity.[174, 190–195] In addition, recent studies demonstrated remarkable sex differences in tubular sodium reabsorption along the nephrons[196, 197], which not only contributes to the sex dimorphism in hypertension, but also further complicates the TGF response.

Salt-sensitive Hypertension

Hypertension is a global health problem, with a prevalence of almost 50% in American adults, and is a leading risk factor for cardiovascular morbidity and mortality. About half of hypertensive patients are salt-sensitive,[198–201] suggesting that abnormal renal salt handling may play a role in the pathogenesis of these patients. Increases in GFR following a high salt diet are thought to facilitate the rapid elimination of sodium and restore salt-water balance and a normal blood pressure.[202–205] Conversely, failure of GFR to increase normally in response to a salt loading has been observed in both humans[204, 205] and animal models[202, 203] with salt-sensitive hypertension. Because macula densa-derived NO inhibits TGF response preventing excessive declines in GFR,[8, 43, 44, 206] it is tempting to speculate that abnormalities in the NO-TGF system may be one of the mechanisms that contribute to salt-sensitive hypertension in general.

The initial studies examining the role of renal NOS1 in TGF and blood pressure regulation were described in the previous section. These studies were expanded on to elucidate the

role of the macula densa-derived NO more precisely in the chronic regulation of TGF, salt sensitivity and blood pressure. For this, macula densa specific NOS1 KO mice were generated[47]. NKCC2 Cre mice were crossed with NOS1 floxed mice ($NOS1^{flox/flox}$), which targets exon-6 of NOS1.[143, 150] All splice variants of NOS1 in the macula densa and TAL were deleted in this strain of $NKCC2^{cre}$/ $NOS1^{flox/flox}$. Because the expression of NOS1 in TAL is negligible compared with that in the macula densa[207–209], this model can be considered as a macula densa selective NOS1 KO line (MD-NOS1 KO).

MD-NOS1KO mice exhibited enhanced TGF responsiveness, both in vivo and in vitro. In response to an acute salt loading with saline, the increase in GFR, urinary flow and sodium excretion rate were all significantly blunted in MD-NOS1KO mice as compared to the wildtype controls[47]. Following chronic intake of a high salt diet, the mean arterial pressure (MAP) increased by 10 mmHg in the MD-NOS1KO mice. Chronic infusion of a subpressor dose of Ang II increased the MAP by >30 mmHg in MD-NOS1KO mice fed a high salt diet than the wildtype mice. However, the Ang II infusion increased MAP to the same degree in the MD-NOS1KO and wild-type mice maintained on a low sodium diet.[47] These data suggest that macula densa NOS1-mediated NO release not only blunts TGF responses but prevents salt sensitivity of blood pressure.[47]

A subsequent study further examined the role of NOS1β in the NOS1αKO strain in the control of blood pressure. Similar to the previous studies,[140, 210] a high salt diet did not increase blood pressure in NOS1αKO or wildtype mice,[141] indicating that deletion of NOS1α does not enhance salt-sensitivity of the blood pressure. However, a combination of a high salt diet and 7-NI treatment similarly elevated MAP by about 15 mmHg in both NOS1αKO and wildtype mice.[141] The results from this study demonstrated that pharmacological inhibition of NOS1 with 7-NI enhanced salt sensitivity, possibly mediated by reducing the activity of macula densa NOS1β. These data confirmed that NOS1α does not play a significant functional role in control of sodium excretion, renal hemodynamics and blood pressure, and further supports the notion that macula densa NOS1β-mediated modulation of TGF response is important in the long-term control of sodium and water excretion and salt sensitivity of blood pressure.[47, 141]

Glomerular hyperfiltration in diabetes

Diabetes mellitus prevalence is increasing in most places in the world and has reached pandemic levels. According to the American Diabetes Association (ADA), almost 40 million people in the US are living with diabetes, giving a prevalence of more than 11% of the US population. An adverse renal complication of diabetes is diabetic nephropathy, which is the leading cause of end stage renal disease.[211–213] A risk factor for diabetic nephropathy is an increase in GFR known as glomerular hyperfiltration during early stage of type 1 and type 2 diabetes.[214–219] The prevailing theories explaining the pathophysiology of glomerular hyperfiltration can be divided into vascular and tubular theories. According to the vascular theory, glomerular hyperfiltration results from an imbalance between vasoconstrictive factors and vasodilatory factors.[214, 215, 220] The tubular theory is based on the following sequence of events. Glucose is freely filtered and reabsorbed in the proximal tubule. The vast majority (~97%) is reabsorbed in the S1 and S2 segments via

the sodium-glucose cotransporter 2 (SGLT2), with the remaining 2%–3% is reabsorbed in the S3 segment by sodium-glucose cotransporter 1 (SGLT1).[221] Hyperglycemia leads to increased filtration and thus delivery of glucose to the proximal tubule. This leads to tubular growth and upregulation of SGLT2, resulting in increased proximal tubular Na reabsorption, and reduced NaCl delivery to the macula densa, thereby inhibiting TGF and increasing GFR.[222–224] This proposed mechanism to explain glomerular hyperfiltration in diabetes has been named the SGLT2-NaCl-TGF mechanism.

Recently, a second tubular mechanism was introduced and dubbed the SGLT1-NOS1-TGF pathway.[225, 226] This new mechanism emphasizes the importance of SGLT1 (rather than SGLT2) as a key determinant of GFR. It is based on the finding that SGLT1 is not only present on the S3 segment of the proximal tubular cells, but also on the apical membrane of macula densa cells in rodents[227, 228] and in humans.[225] The progression of events that explains this model is as follows. The luminal glucose concentration at the macula densa is usually negligible under normoglycemic conditions. However, it will raise when the amount of filtered glucose exceeds the maximal capacity of reabsorption by the proximal tubules in hyperglycemic states. This increase in luminal glucose at the macula densa activates SGLT1 and enhances NOS1-dependent NO formation, thereby inhibiting TGF responsiveness and promoting glomerular hyperfiltration in diabetes. This pathway is supported by several lines of evidence.

It has been well recognized that GFR increases in response to intravenous infusions of glucose in humans[229–231] and experimental animals.[225, 232, 233] Inhibition of the TGF mechanism has been found to play an essential role in hyperglycemia-induced hyperfiltration.[224] TGF responses have been shown to be inhibited or reset in both type 1 and type 2 diabetic animal models.[216, 222, 223, 234, 235] In these studies, the TGF responsiveness was evaluated by measurements of proximal tubular stop flow pressure (P_{SF}), proximal-distal differences for single nephron GFR, or free flow perturbation analysis of TGF efficiency at the natural operating point in db/db mice or streptozotocin-induced diabetes in rats. In non-diabetic mice, acute hyperglycemia enhanced macula densa NOS1 expression and NO generation, inhibited TGF responses in vivo and in vitro, and quickly increased GFR[225]. Only D-glucose, but not L-glucose nor mannitol exhibited these effects on NO, TGF and GFR. In addition, only the rise of glucose concentration at the apical rather than the basolateral side of the macula densa influenced NO production and TGF response[225]. All these studies indicate that TGF plays a central role in diabetes-induced hyperfiltration.

The possibility that the SGLT1-NOS1-TGF pathway plays an important role in diabetes-induced glomerular hyperfiltration originated, when SGLT1 was detected on the apical membrane of the macula densa cells using single-cell RNA-sequencing profile of mouse kidneys.[236] In this study, tubular glucose transporters in the macula densa were identified by the co-expression of NOS1 and NKCC2. SGLT1 was found to be the glucose transporter with the highest transcriptional level. Thus, studies examining the functional role of SGLT1 on NOS1 and TGF were then undertaken. In the presence of the selective SGLT1 inhibitor KGA-2727, the glucose-induced macula densa NO generation and TGF inhibition were blocked.[225] In addition, the glucose-induced effects on TGF response and GFR were

absent in mice with macula densa specific NOS1 deletion (i.e. MD-NOS1KO).[225] While other mechanisms are also likely to contribute to hyperglycemia-induced hyperfiltration, including SGLT2-mediated sodium-glucose reabsorption,[221, 223, 237] vasodilation of the afferent arteriole via GLUT1[238] and GPR91[239], and insulin,[240, 241] the study described[225] provides strong evidence that glomerular hyperfiltration induced by acute hyperglycemia is dependent on SGLT1-NOS1-TGF pathway.

Gestational Hypertension and Preeclampsia

Maternal adaption to normal pregnancy is characterized by systemic vasodilation, in which RBF and GFR increase by 45–50 %[242, 243] and blood pressure decreases by 5–10 mmHg[244, 245]. Inappropriate or inadequate cardiorenal adaptations during pregnancy may lead to serious pathological consequences, such as gestational hypertension and preeclampsia, the latter being a leading cause of maternal and fetal morbidity and mortality.[246–248] Gestational hypertension and preeclampsia are characterized by new-onset hypertension, and increased renal vascular resistance causing reduced RBF and GFR by 20–40% in preeclampsia compared to normal pregnancies[246–248]. These changes may impair sodium excretion, thereby facilitating the development and progression of gestational hypertension.[249–252] The physiological mechanisms underlying maternal adaptions in normal pregnancy and the pathophysiological mechanisms contributing to the development of preeclampsia are complex and involve many different components.[253] Among them, NO has been found to play a crucial role in control of hemodynamics during pregnancy.[242, 254] NO generation was increased in normal pregnancies[255, 256] and reduced NO bioavailability has been demonstrated in clinical studies of preeclampsia.[257, 258] Indeed, inhibition of NOS with L-NAME prevented normal pregnancy-induced elevations of GFR and RBF, and induces hypertension[259, 260]. However, mice with deletion of any of the three NOS isoforms (NOS1, NOS2, or NOS3) had normal pregnancies with appropriate adaptions in RBF, GFR and blood pressure.[261, 262] These findings indicate a key role for NOS1β in gestational hypertension and and/or preeclampsia (the global NOS1 KO mice is a NOS1α KO strain with intact NOS1β).[152]

The role of macula densa-NOS1β-mediated TGF modulation in the development of gestational hypertension was evaluated in a recent study.[263] Deficient trophoblast invasion and/or spiral artery remodeling, as well as insufficient blood supply to the fetus are considered as causal factors for development of preeclampsia.[264, 265] For instance, surgical reduction of uteroplacental perfusion (RUPP) has been shown to induce gestational hypertension and/or preeclampsia in several mammals.[266, 267,268] Mice subjected to RUUP not only had hypertension, but also exhibited reduced expression of macula densa-NOS1β and less NO generation, suggesting that macula densa-NOS1β may be implicated in the abnormal renal hemodynamics and hypertension in this model.[263] Interestingly, macula densa-NOS1β was also reduced in African Green Monkeys who spontaneously developed preeclampsia, but was increased in those who had a normal pregnancy.[263] Evidence indicating that these alterations in macula densa-NOS1β expression play a causal role in gestational hypertension and/or preeclampsia is provided by experiments, in which macula densa-NOS1β was genetically deleted. Mice lacking NOS1β specifically in the macula densa, exhibited impaired adaptation to pregnancy, while they had a normal phenotype

under non-stressed conditions. The pregnant MD-NOS1KO mice did not have the same pregnancy-induced changes in renal parameters as the wildtypes; that is, 1) TGF responses were not as attenuated, 2) GFR was not as elevated, 3) renal sodium excretion was impaired, and 4) blood pressure was increased, rather than decreased.[263] Hence, one can conclude that impaired function of the macula densa NOS1β is significantly contributing to the development of gestational renal dysfunction and hypertension in these animals. Because animals subjected to RUPP may have additional mechanisms contributing to the gestational hypertension (in addition to decreased macula densa-NOS1β), a RUPP procedure was applied to the macula densa-NOS1KO mice. Subjecting pregnant, MD-NOS1KO mice with RUPP surgery only caused a modest further alteration in TGF, GFR and blood pressure. The lack of additive effects suggested that they were largely working via the same mechanism. Taken together, these experimental results suggest that macula densa NOS1β-mediated modulation of TGF and renal hemodynamic responses plays a vital role in the development of hypertension in preeclampsia.[263] One could speculate that this pathway might also be implicated in gestational diabetes although this has never been tested.

Conclusions and Perspectives

Much progress has been made regarding the mechanisms that regulate TGF responses and their functional significance, especially with regards to their role in the various physiologic and pathologic conditions reviewed in this manuscript. The discovery of NOS1 splice variants and the subsequent description of their different regulation and function have led to novel understandings of how the kidney adapts to chronic homeostatic challenges (such as changes in response to elevated Ang II, high salt intake, or pregnancy), and helps explain sex dimorphisms. Moreover, the discovery that the glucose transporter SGLT1 is present in macula densa cells and interacts with NOS1 has shed new light on how glucosuria can lead to glomerular hyperfiltration during diabetes via the SGLT1-NOS1-TGF pathway. The identification of the mechanisms that lead to the abnormal regulation of macula densa-NOS1β and/or abnormal downstream signaling of this pathway, may lead to the identification of novel therapeutic targets.

However, there are several limitations and challenges when studying the functional significance of TGF response. One of them is the difficulty of dissecting the specific role and contribution of the TGF response from other factors or signaling pathways in vivo. The current best available approach is to use transgenic animal models with genetically edited macula densa cells. However, due to the difficulties accessing the macula densa cells, genomic profiling of these cells is incomplete. The development of single cell RNA sequencing technique provides a unique approach for quantitatively analyzing the macula densa gene profiles and characteristics under physiological and pathological conditions. In addition, due to the lack of a reliable macula densa cell line, it is challenging to examine the molecular mechanisms that regulate gene and protein expressions and functions in the macula densa. The duration of micropuncture and microperfusion experiments are usually limited to 3–4 hours. We believe that with the availability of these techniques and approaches, the knowledge from basic and translational research on TGF regulation and function will increase significantly.

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REFERENCES:

1. Liu R, Pittner J and Persson AE. Changes of cell volume and nitric oxide concentration in macula densa cells caused by changes in luminal NaCl concentration. J Am Soc Nephrol. 2002;13:2688–96. [PubMed: 12397038]
2. Schnermann J and Briggs JP. The macula densa is worth its salt. J Clin Invest. 1999;104:1007–1009. [PubMed: 10525035]
3. Wilcox CS and Welch WJ. TGF and nitric oxide: effects of salt intake and salt-sensitive hypertension. Kidney Int. 1996;49 (Suppl 55):S-9–S-13.
4. Navar LG, Inscho EW, Majid DS, Imig JD, Harrison-Bernard LM and Mitchell KD. Paracrine regulation of the renal microcirculation. Physiol Rev. 1996;76:425–536. [PubMed: 8618962]
5. Ito S and Carretero OA. Macula densa control of glomerular hemodynamics. Kidney Int Suppl. 1991;32:S83–S85. [PubMed: 1881058]
6. Ollerstam A, Pittner J, Persson AEG and Thorup C. Increased blood pressure in rats after long-term inhibition of the neuronal isoform of nitric oxide synthase. J Clin Invest. 1997;99:2212–2218. [PubMed: 9151793]
7. Welch WJ, Tojo A and Wilcox CS. Roles of NO and oxygen radicals in tubuloglomerular feedback in SHR. Am J Physiol Renal Physiol. 2000;278:F769–F776. [PubMed: 10807588]
8. Ren Y, Garvin JL and Carretero OA. Role of macula densa nitric oxide and cGMP in the regulation of tubuloglomerular feedback. Kidney Int. 2000;58:2053–2060. [PubMed: 11044225]
9. Briggs JP and Schnermann J. Macula densa control of renin secretion and glomerular vascular tone: evidence for common cellular mechanisms. Renal Physiol (Basel). 1986;9:193–203.
10. Schnermann J and Briggs JP. Function of the juxtaglomerular apparatus. Control of glomerular hemodynamics and renin secretion. In: Seldin DW and Giebisch G, eds. The Kidney: Physiology and Pathology. 2 ed. New York: Raven Press; 1992: 1249–1289.
11. Welch WJ and Wilcox CS. Feedback responses during sequential inhibition of angiotensin and thromboxane. Am J Physiol. 1990;258:F457–F466. [PubMed: 2316660]
12. Schlatter E, Salomonsson M, Persson AEG and Greger R. Macula densa cells sense luminal NaCl concentration via furosemide sensitive $Na^+2Cl^-K^+$ cotransport. Pflugers Arch. 1989;414:286–290. [PubMed: 2780213]
13. Bell PD, Lapointe JY and Peti-Peterdi J. Macula densa cell signaling. Annu Rev Physiol. 2003;65:32.1–32.20.
14. Liu R, Carretero OA, Ren Y and Garvin JL. Increased intracellular pH at the macula densa activates nNOS during tubuloglomerular feedback. Kidney Int. 2004;67:1837–1843.
15. Liu R and Persson AE. Simultaneous changes of cell volume and cytosolic calcium concentration in macula densa cells caused by alterations of luminal NaCl concentration. J Physiol. 2005;563:895–901. [PubMed: 15661823]
16. Peti-Peterdi J and Bell PD. Cytosolic $[Ca^{2+}]$ signaling pathway in macula densa cells. Am J Physiol. 1999;277:F472–F476. [PubMed: 10484531]
17. Bell PD, Lapointe J-Y, Sabirov R, Hayashi S, Peti-Peterdi J, Manabe K, Kovacs G and Okada Y. Macula densa cell signaling involves ATP release through a maxi anion channel. Proc Natl Acad Sci USA. 2003;100:4322–4327. [PubMed: 12655045]
18. Ren Y, Garvin JL, Liu R and Carretero OA. Role of macula densa adenosine triphosphate (ATP) in tubuloglomerular feedback. Kidney Int. 2004;66:1479–85. [PubMed: 15458441]
19. Inscho EW, Ohishi K and Navar LG. Effects of ATP on pre- and postglomerular juxtamedullary microvasculature. Am J Physiol. 1992;263:F886–F893. [PubMed: 1443177]
20. Nishiyama A and Navar LG. ATP mediates tubuloglomerular feedback. Am J Physiol Regul Integr Comp Physiol. 2002;283:R273–R275. [PubMed: 12069954]

21. Schnermann J and Levine DZ. Paracrine factors in tubuloglomerular feedback: adenosine, ATP, and nitric oxide. Annu Rev Physiol. 2003;65:501–529. [PubMed: 12208992]
22. Ren Y, Garvin JL, Liu R and Carretero OA. Possible mechanism of efferent arteriole (Ef-Art) tubuloglomerular feedback. Kidney Int. 2007;71:861–6. [PubMed: 17342182]
23. Vallon V and Schnermann J. Tubuloglomerular feedback. Methods Mol Med. 2003;86:429–441. [PubMed: 12886786]
24. Ren Y, Garvin JL and Carretero OA. Vasodilator action of angiotensin-(1–7) on isolated rabbit afferent arterioles. Hypertension. 2002;39:799–802. [PubMed: 11897767]
25. Vallon V, Richter K, Huang DY, Rieg T and Schnermann J. Functional consequences at the single-nephron level of the lack of adenosine A1 receptors and tubuloglomerular feedback in mice. Pflugers Arch. 2004;448:214–221. [PubMed: 14767772]
26. Li L, Mizel D, Huang Y, Eisner C, Hoerl M, Thiel M and Schnermann J. Tubuloglomerular feedback and renal function in mice with targeted deletion of the type 1 equilibrative nucleoside transporter. Am J Physiol Renal Physiol. 2013;304:F382–F389. [PubMed: 23269643]
27. Sallstrom J, Eriksson T, Fredholm BB, Persson AE and Palm F. Inhibition of sodium-linked glucose reabsorption normalizes diabetes-induced glomerular hyperfiltration in conscious adenosine A -receptor deficient mice. Acta Physiol (Oxf). 2013.
28. Sun D, Samuelson LC, Yang T, Huang Y, Paliege A, Saunders T, Briggs J and Schnermann J. Mediation of tubuloglomerular feedback by adenosine: evidence from mice lacking adenosine 1 receptors. Proc Natl Acad Sci USA. 2001;98:9983–9988. [PubMed: 11504952]
29. Ren Y, Arima S, Carretero OA and Ito S. Possible role of adenosine in macula densa control of glomerular hemodynamics. Kidney Int. 2002;61:169–176. [PubMed: 11786098]
30. Zou A-P, Imig JD, Ortiz de Montellano PR, Sui Z, Falck JR and Roman RJ. Effect of P-450 omega-hydroxylase metabolites of arachidonic acid on tubuloglomerular feedback. Am J Physiol. 1994;266:F934–F941. [PubMed: 8023972]
31. Zou AP, Imig JD, Kaldunski M, Ortiz de Montellano PR, Sui Z and Roman RJ. Inhibition of renal vascular 20-HETE production impairs autoregulation of renal blood flow. Am J Physiol. 1994;266:F275–F282. [PubMed: 8141328]
32. Imig JD. Eicosanoid regulation of the renal vasculature. Am J Physiol Renal Physiol. 2000;279:F965–F981. [PubMed: 11097615]
33. Imig JD, Zou AP, Stec DE, Harder DR, Falck JR and Roman RJ. Formation and actions of 20-hydroxyeicosatetraenoic acid in rat renal arterioles. Am J Physiol. 1996;270:R217–R227. [PubMed: 8769805]
34. Nishiyama A, Majid DSA, Walker M III, Miyatake A and Navar LG. Renal interstitial ATP responses to changes in arterial pressure during alterations in tubuloglomerular feedback activity. Hypertension. 2001;37:753–759. [PubMed: 11230369]
35. Huang CL and Cogan MG. Atrial natriuretic factor inhibits maximal tubuloglomerular feedback response. Am J Physiol. 1987;252:F825–F828. [PubMed: 2953251]
36. Liu R, Ren Y, Garvin JL and Carretero OA. Superoxide enhances tubuloglomerular feedback by constricting the afferent arteriole. Kidney Int. 2004;66:268–74. [PubMed: 15200433]
37. Ren Y, Carretero OA and Garvin JL. Mechanism by which superoxide potentiates tubuloglomerular feedback. Hypertension. 2002;39:624–628. [PubMed: 11882620]
38. Ichihara A, Inscho EW, Imig JD and Navar LG. Neuronal nitric oxide synthase modulates rat renal microvascular function. Am J Physiol. 1998;274:F516–F524. [PubMed: 9530268]
39. Ichihara A and Navar LG. Neuronal NOS contributes to biphasic autoregulatory response during enhanced TGF activity. Am J Physiol. 1999;277:F113–F120. [PubMed: 10409304]
40. Wang H, Carretero OA and Garvin JL. Nitric oxide produced by THAL nitric oxide synthase inhibits TGF. Hypertension. 2002;39:662–666. [PubMed: 11882627]
41. Wang H, Carretero OA and Garvin JL. Inhibition of apical Na^+/H^+ exchangers on the macula densa cells augments tubuloglomerular feedback. Hypertension. 2003;41:688–691. [PubMed: 12623980]
42. Wilcox CS, Welch WJ, Murad F, Gross SS, Taylor G, Levi R and Schmidt HHW. Nitric oxide synthase in macula densa regulates glomerular capillary pressure. Proc Natl Acad Sci USA. 1992;89:11993–11997. [PubMed: 1281548]

43. Mundel P, Bachmann S, Bader M, Fischer A, Kummer W, Mayer B and Kriz W. Expression of nitric oxide synthase in kidney macula densa cells. Kidney Int. 1992;42:1017–1019. [PubMed: 1280698]
44. Kovacs G, Komlosi P, Fuson A, Peti-Peterdi J, Rosivall L and Bell PD. Neuronal nitric oxide synthase: Its role and regulation in macula densa cells. Journal of the American Society of Nephrology. 2003;14:2475–2483. [PubMed: 14514725]
45. Ito S and Ren Y. Evidence for the role of nitric oxide in macula densa control of glomerular hemodynamics. J Clin Invest. 1993;92:1093–1098. [PubMed: 8349792]
46. Lu D, Fu Y, Lopez-Ruiz A, Zhang R, Juncos R, Liu H, Manning RD Jr., Juncos LA and Liu R. Salt-sensitive splice variant of nNOS expressed in the macula densa cells. Am J Physiol Renal Physiol. 2010;298:F1465–71. [PubMed: 20335319]
47. Lu Y, Wei J, Stec DE, Roman RJ, Ge Y, Cheng L, Liu EY, Zhang J, Hansen PB, Fan F, Juncos LA, Wang L, Pollock J, Huang PL, Fu Y, Wang S and Liu R. Macula Densa Nitric Oxide Synthase 1beta Protects against Salt-Sensitive Hypertension. J Am Soc Nephrol. 2016;27:2346–56. [PubMed: 26647426]
48. Thorup C and Persson AEG. Inhibition of locally produced nitric oxide resets tubuloglomerular feedback mechanism. Am J Physiol. 1994;267:F606–F611. [PubMed: 7524359]
49. Thorup C and Persson AEG. Macula densa derived nitric oxide in regulation of glomerular capillary pressure. Kidney Int. 1996;49:430–436. [PubMed: 8821827]
50. Wagner C, Godecke A, Ford M, Schnermann J, Schrader J and Kurtz A. Regulation of renin gene expression in kidneys of eNOS- and nNOS-deficient mice. Pflugers Arch. 2000;439:567–72. [PubMed: 10764216]
51. Eliasson MJ, Blackshaw S, Schell MJ and Snyder SH. Neuronal nitric oxide synthase alternatively spliced forms: prominent functional localizations in the brain. Proc Natl Acad Sci U S A. 1997;94:3396–3401. [PubMed: 9096405]
52. Alderton WK, Cooper CE and Knowles RG. Nitric oxide synthases: structure, function and inhibition. Biochem J. 2001;357:593–615. [PubMed: 11463332]
53. Bredt DS, Hwang PM and Snyder SH. Localization of nitric oxide synthase indicating a neural role for nitric oxide. Nature. 1990;347:768–70. [PubMed: 1700301]
54. Jaffrey SR and Snyder SH. PIN: an associated protein inhibitor of neuronal nitric oxide synthase. Science. 1996;274:774–777. [PubMed: 8864115]
55. Peti-Peterdi J, Chambrey R, Bebok Z, Biemesderfer D, St.John PL, Abrahamson DR, Warnock DG and Bell PD. Macula densa Na^+/H^+ exchange activities mediated by apical NHE2 and basolateral NHE4 isoforms. Am J Physiol Renal Physiol. 2000;278:F452–F463. [PubMed: 10710550]
56. Liu R, Carretero OA, Ren Y, Wang H and Garvin JL. Intracellular pH regulates superoxide production by the macula densa. Am J Physiol Renal Physiol. 2008;295:F851–6. [PubMed: 18667487]
57. Fowler BC, Chang Y-S, Laamarti A, Higdon M, Lapointe J-Y and Bell PD. Evidence for apical sodium proton exchange in macula densa cells. Kidney Int. 1995;47:746–751. [PubMed: 7752573]
58. Wilcox CS. L-arginine-nitric oxide pathway. In: Seldin DW and Giebisch G, eds. The Kidney: Physiology and Pathophysiology. 3rd ed. New York: Raven Press; 2000: 849–871.
59. Gorren ACF, Schrammel A, Schmidt K and Mayer B. Effects of pH on the structure and function of neuronal nitric oxide synthase. Biochem J. 1998;331:801–807. [PubMed: 9560307]
60. Yaqoob M, Edelstein CL, Wieder ED, Alkhunaizi AM, Gengaro PE, Nemenoff RA and Schrier RW. Nitric oxide kinetics during hypoxia in proximal tubules: effects of acidosis and glycine. Kidney Int. 1996;49:1314–1319. [PubMed: 8731096]
61. Schnackenberg CG. Physiological and pathophysiological roles of oxygen radicals in the renal microvasculature. Am J Physiol Regul Integr Comp Physiol. 2002;282:R335–R342. [PubMed: 11792641]
62. Halliwell B. Reactive oxygen species in living systems: source, biochemistry, and role in human disease. Am J Med. 1991;91 (Suppl 3C):3C-14S–3C-22S.
63. Knight JA. Free radicals: their history and current status in aging and disease. Ann Clin Lab Sci. 1998;28:331–346. [PubMed: 9846200]

64. Pierini D and Bryan NS. Nitric oxide availability as a marker of oxidative stress. Methods Mol Biol. 2015;1208:63–71. [PubMed: 25323499]

65. Liu R and Juncos LA. GTPase-Rac enhances depolarization-induced superoxide production by the macula densa during tubuloglomerular feedback. Am J Physiol Regul Integr Comp Physiol. 2010;298:R453–8. [PubMed: 20007513]

66. Fu YL, Zhang R, Lu DY, Liu HF, Chandrashekar K, Juncos LA and Liu RS. NOX2 is the primary source of angiotensin II-induced superoxide in the macula densa. Am J Physiol-Reg I. 2010;298:R707–R712.

67. Zhang R, Harding P, Garvin JL, Juncos R, Peterson E, Juncos LA and Liu R. Isoforms and Functions of NADPH Oxidase at the Macula Densa. Hypertension. 2009;53:556–563. [PubMed: 19204183]

68. W JC, C CE, S ER, C ME and C HJ. Correlation between membrane potential changes and superoxide production in human granulocytes stimulated by phorbol myristate acetate. Evidence for defective activation in chronic granulomatous disease. J Biol Chem. 1980;255:1874–1878. [PubMed: 6243652]

69. Sohn H-Y, Keller M, Gloe T, Morawietz H, Rueckschloss U and Pohl U. The small G-protein Rac mediates depolarization-induced superoxide formation in human endothelial cells. J Biol Chem. 2000;275:18745–18750. [PubMed: 10764736]

70. Liu R, Garvin JL, Ren Y, Pagano PJ and Carretero OA. Depolarization of the macula densa induces superoxide production via NAD(P)H oxidase. Am J Physiol Renal Physiol. 2007;292:F1867–72. [PubMed: 17344185]

71. Suzuki Y and Lehrer RI. NAD(P)H oxidase activity in human neutrophils stimulated by phorbol myristate acetate. J Clin Invest. 1980;66:1409–1418. [PubMed: 6255012]

72. Morgan D, Cherny VV, M R, K BZ and DeCoursey TE. The pH dependence of NADPH oxidase in human eosinophils. J Physiol. 2005;569:419–431. [PubMed: 16195320]

73. G TG, B SI and B BM. Effects of oxygen tension and pH on the respiratory burst of human neutrophils. Blood. 1979;53:1133–1139. [PubMed: 36182]

74. Simchowitz L. Intracellular pH modulates the generation of superoxide radicals by human neutrophils. J Clin Invest. 1985;76:1079–1089. [PubMed: 2995444]

75. Higashi Y, Sasaki S, Nakagawa K, Matsuura H, Oshima T and Chayama K. Endothelial dysfunction and oxidative stress in renovascular hypertension. N Engl J Med. 2002;346:1954–1962. [PubMed: 12075056]

76. Lee VM, Quinn PA, Jennings SC and Ng LL. Neutrophil activation and production of reactive oxygen species in pre-eclampsia. J Hypertens. 2003;21:395–402. [PubMed: 12569271]

77. Lip GY, Edmunds E, Nuttall SL, Landray MJ, Blann AD and Beevers DG. Oxidative stress in malignant and non-malignant phase hypertension. J Hum Hypertens. 2002;16:333–336. [PubMed: 12082494]

78. Ward NC, Hodgson JM, Puddey IB, Mori TA, Beilin LJ and Croft KD. Oxidative stress in human hypertension: association with antihypertensive treatment, gender, nutrition, and lifestyle. Free Radic Biol Med. 2004;36:226–232. [PubMed: 14744634]

79. Dobrian AD, Schriver SD, Khraibi AA and Prewitt RL. Pioglitazone prevents hypertension and reduces oxidative stress in diet-induced obesity. Hypertension. 2004;43:48–56. [PubMed: 14638618]

80. Fujii S, Zhang L, Igarashi J and Kosaka H. L-arginine reverses p47phox and gp91phox expression induced by high salt in Dahl rats. Hypertension. 2003;42:1014–1020. [PubMed: 14504257]

81. Kishi T, Hirooka Y, Kimura Y, Ito K, Shimokawa H and Takeshita A. Increased reactive oxygen species in rostral ventrolateral medulla contribute to neural mechanisms of hypertension in stroke-prone spontaneously hypertensive rats. Circulation. 2004;109:2357–2362. [PubMed: 15117836]

82. Landmesser U, Cai H, Dikalov S, McCann L, Hwang J, Jo H, Holland SM and Harrison DG. Role of p47(phox) in vascular oxidative stress and hypertension caused by angiotensin II. Hypertension. 2002;40:511–515. [PubMed: 12364355]

83. Nishiyama A, Yao L, Nagai Y, Miyata K, Yoshizumi M, Kagami S, Kondo S, Kiyomoto H, Shokoji T, Kimura S, Kohno M and Abe Y. Possible contributions of reactive oxygen species

and mitogen-activated protein kinase to renal injury in alsosterone/salt-induced hypertensive rats. Hypertension. 2004;24:841–848.

84. Virdis A, Neves MF, Amiri F, Viel E, Touyz RM and Schiffrin EL. Spironolactone improves angiotensin-induced vascular changes and oxidative stress. Hypertension. 2002;40:504–510. [PubMed: 12364354]
85. Wallwork CJ, Parks DA and Schmid-Schonbein GW. Xanthine oxidase activity in the dexamethasone-induced hypertensive rat. Microvasc Res. 2003;66:30–37. [PubMed: 12826072]
86. Wilcox CS. Oxidative stress and nitric oxide deficiency in the kidney: a critical link to hypertension? Am J Physiol Regul Integr Comp Physiol. 2005;289:R913–R935. [PubMed: 16183628]
87. Gill PS and Wilcox CS. NADPH oxidases in the kidney. Antioxid Redox Signal. 2006;8:1597–1607. [PubMed: 16987014]
88. Zhang J, Chandrashekar K, Lu Y, Duan Y, Qu P, Wei J, Juncos LA and Liu R. Enhanced expression and activity of Nox2 and Nox4 in the macula densa in ANG II-induced hypertensive mice. Am J Physiol Renal Physiol. 2014;306:F344–50. [PubMed: 24285500]
89. Song J, Lu Y, Lai EY, Wei J, Wang L, Chandrashekar K, Wang S, Shen C, Juncos LA and Liu R. Oxidative status in the macula densa modulates tubuloglomerular feedback responsiveness in angiotensin II-induced hypertension. Acta Physiol (Oxf). 2015;213:249–58. [PubMed: 25089004]
90. Liu R and Persson AE. Angiotensin II stimulates calcium and nitric oxide release from macula densa cells through AT1 receptors. Hypertension. 2004;43:649–653. [PubMed: 14744924]
91. Araujo M and Welch WJ. Tubuloglomerular feedback is decreased in COX-1 knockout mice after chronic angiotensin II infusion. Am J Physiol Renal Physiol. 2010;298:F1059–F1063. [PubMed: 20107114]
92. Nouri P, Gill P, Li M, Wilcox CS and Welch WJ. p22phox in the macula densa regulates single nephron GFR during angiotensin II infusion in rats. Am J Physiol Heart Circ Physiol. 2007;292:H1685–H1689. [PubMed: 17220186]
93. Welch WJ, Chabrashvili T, Solis G, Chen Y, Gill PS, Aslam S, Wang X, Ji H, Sandberg K, Jose P and Wilcox CS. Role of extracellular superoxide dismutase in the mouse angiotensin slow pressor response. Hypertension. 2006;48:934–941. [PubMed: 17015770]
94. Wilcox CS and Welch WJ. Interaction between nitric oxide and oxygen radicals in regulation of tubuloglomerular feedback. Acta Physiol Scand. 2000;168:119–124. [PubMed: 10691789]
95. Lai EY, Solis G, Luo Z, Carlstrom M, Sandberg K, Holland S, Wellstein A, Welch WJ and Wilcox CS. p47(phox) is required for afferent arteriolar contractile responses to angiotensin II and perfusion pressure in mice. Hypertension. 2012;59:415–420. [PubMed: 22184329]
96. Wang D, Luo Z, Wang X, Jose PA, Falck JR, Welch WJ, Aslam S, Teerlink T and Wilcox CS. Impaired endothelial function and microvascular asymmetrical dimethylarginine in angiotensin II-infused rats: effects of tempol. Hypertension. 2010;56:950–955. [PubMed: 20837884]
97. Gao X, Patzak A, Sendeski M, Scheffer PG, Teerlink T, Sallstrom J, Fredholm BB, Persson AE and Carlstrom M. Adenosine A(1)-receptor deficiency diminishes afferent arteriolar and blood pressure responses during nitric oxide inhibition and angiotensin II treatment. Am J Physiol Regul Integr Comp Physiol. 2011;301:R1669–R1681. [PubMed: 21975649]
98. Carlstrom M, Lai EY, Ma Z, Patzak A, Brown RD and Persson AE. Role of NOX2 in the regulation of afferent arteriole responsiveness. Am J Physiol Regul Integr Comp Physiol. 2009;296:R72–R79. [PubMed: 18987286]
99. Carlstrom M, Lai EY, Ma Z, Steege A, Patzak A, Eriksson UJ, Lundberg JO, Wilcox CS and Persson AE. Superoxide dismutase 1 limits renal microvascular remodeling and attenuates arteriole and blood pressure responses to angiotensin II via modulation of nitric oxide bioavailability. Hypertension. 2010;56:907–913. [PubMed: 20876452]
100. Pacher P, Beckman JS and Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiol Rev. 2007;87:315–424. [PubMed: 17237348]
101. Chen W, Druhan LJ, Chen CA, Hemann C, Chen YR, Berka V, Tsai AL and Zweier JL. Peroxynitrite induces destruction of the tetrahydrobiopterin and heme in endothelial nitric oxide synthase: transition from reversible to irreversible enzyme inhibition. Biochemistry. 2010;49:3129–37. [PubMed: 20184376]

102. Miller MJS, Thompson JH, Zhang X-J, Sadowska-Krowicka H, Kakkis JL, Munshi UP, Sandoval M, Rossi JL, Eloby-Childress S, Beckman JS, Ye YZ, Rodi CP, Manning PT, Currie MG and Clark DA. Role of inducible nitric oxide synthase expression and peroxynitrite formation in guinea pig ileitis. Gastroenterology. 1995;109:1475–1483. [PubMed: 7557128]
103. Pou S, Keaton L, Surichamorn W and Rosen GM. Mechanism of superoxide generation by neuronal nitric-oxide synthase. J Biol Chem. 1999;274:9573–9580. [PubMed: 10092643]
104. Costa ED, Rezende BA, Cortes SF and Lemos VS. Neuronal Nitric Oxide Synthase in Vascular Physiology and Diseases. Front Physiol. 2016;7:206. [PubMed: 27313545]
105. Gebhart V, Reiss K, Kollau A, Mayer B and Gorren ACF. Site and mechanism of uncoupling of nitric-oxide synthase: Uncoupling by monomerization and other misconceptions. Nitric Oxide. 2019;89:14–21. [PubMed: 31022534]
106. Sanchez A, Contreras C, Martinez MP, Climent B, Benedito S, Garcia-Sacristan A, Hernandez M and Prieto D. Role of neural NO synthase (nNOS) uncoupling in the dysfunctional nitrergic vasorelaxation of penile arteries from insulin-resistant obese Zucker rats. PLoS One. 2012;7:e36027. [PubMed: 22540017]
107. Chabrashvili T, Tojo A, Onozato ML, Kitiyakara C, Quinn MT, Fujita T, Welch WJ and Wilcox CS. Expression and cellular localization of classic NADPH oxidase subunits in the spontaneously hypertensive rat kidney. Hypertension. 2002;39:269–274. [PubMed: 11847196]
108. Mitchell KD and Navar LG. Enhanced tubuloglomerular feedback during peritubular infusions of angiotensins I and II. Am J Physiol. 1988;255:F383–F390. [PubMed: 3414799]
109. Modlinger P, Chabrashvili T, Gill PS, Mendonca M, Harrison DG, Griendling KK, Li M, Raggio J, Wellstein A, Chen Y, Welch WJ and Wilcox CS. RNA silencing in vivo reveals role of $p22^{phox}$ in rat angiotensin slow pressor response. Hypertension. 2006;47:238–244. [PubMed: 16391171]
110. Suh Y-A, Arnold RS, Lassegue B, Shi J, Xu X, Sorescu D, Chung AB, Griendling KK and Lambeth JD. Cell transformation by the superoxide-generating oxidase Mox1 [letter]. Nature. 1999;401:79–82. [PubMed: 10485709]
111. Banfi B, Maturana A, Jaconi S, Arnaudeau S, Laforge T, Sinha B, Ligeti E, Demaurex N and Krause KH. A mammalian H+ channel generated through alternative splicing of the NADPH oxidase homolog NOH-1. Science. 2000;287:138–142. [PubMed: 10615049]
112. Touyz RM, Chen X, Tabet F, Yao G, He G, Quinn MT, Pagano PJ and Schiffrin EL. Expression of a functionally active gp91phox-containing neutrophil-type NAD(P)H oxidase in smooth muscle cells from human resistance arteries. Regulation by angiotensin II. Circ Res. 2002;90:1205–1213. [PubMed: 12065324]
113. Kikuchi H, Hikage M, Miyashita H and Fukumoto M. NADPH oxidase subunit, 6p91(phox) homologue, preferentially expressed in human colon epithelial cells. Gene. 2000;254:237–243. [PubMed: 10974555]
114. Geiszt M, Kopp JB, Várnai P and Leto TL. Identification of Renox, an NAD(P)H oxidase in kidney. Proc Natl Acad Sci USA. 2000;97:8010–8014. [PubMed: 10869423]
115. Shiose A, Kuroda J, Tsuruya K, Hirai M, Hirakata H, Naito S, Hattori M, Sakaki Y and Sumimoto H. A novel superoxide-producing NAD(P)H oxidase in kidney. J Biol Chem. 2001;276:1417–1423. [PubMed: 11032835]
116. Banfi B, Molnar G, Maturana A, Steger K, Hegedus B, Demaurex N and Krause KH. A Ca(2+)-activated NADPH oxidase in testis, spleen, and lymph nodes. J Biol Chem. 2001;276:37594–37601. [PubMed: 11483596]
117. De Deken X, Wang D, Many MC, Costagliola S, Libert F, Vassart G, Dumont JE and Miot F. Cloning of two human thyroid cDNAs encoding new members of the NADPH oxidase family. J Biol Chem. 2000;275:23227–23233. [PubMed: 10806195]
118. Dupuy C, Ohayon R, Valent A, Noel-Hudson MS, Deme D and Virion A. Purification of a novel flavoprotein involved in the thyroid NADPH oxidase. Cloning of the porcine and human cDNAs. J Biol Chem. 1999;274:37265–37269. [PubMed: 10601291]
119. Edens WA, Sharling L, Cheng G, Shapira R, Kinkade JM, Lee T, Edens HA, Tang X, Sullards C, Flaherty DB, Benian GM and Lambeth JD. Tyrosine cross-linking of extracellular matrix is catalyzed by Duox, a multidomain oxidase/peroxidase with homology to the phagocyte oxidase subunit gp91phox. J Cell Biol. 2001;154:879–891. [PubMed: 11514595]

120. Zou AP, Li N and Cowley AW Jr., Production and actions of superoxide in the renal medulla. Hypertension. 2001;37:547–553. [PubMed: 11230333]

121. Jha JC, Banal C, Okabe J, Gray SP, Hettige T, Chow BSM, Thallas-Bonke V, De Vos L, Holterman CE, Coughlan MT, Power DA, Skene A, Ekinci EI, Cooper ME, Touyz RM, Kennedy CR and Jandeleit-Dahm K. NADPH Oxidase Nox5 Accelerates Renal Injury in Diabetic Nephropathy. Diabetes. 2017;66:2691–2703. [PubMed: 28747378]

122. Terada LS. Specificity in reactive oxidant signaling: think globally, act locally. J Cell Biol. 2006;174:615–623. [PubMed: 16923830]

123. Herrera M, Silva G and Garvin JL. A high-salt diet dissociates NO synthase-3 expression and NO production by the thick ascending limb. Hypertension. 2006;47:95–101. [PubMed: 16344378]

124. Mori T and Cowley AW Jr., Renal oxidative stress in medullary thick ascending limbs produced by elevated NaCl and glucose. Hypertension. 2004;43:341–346. [PubMed: 14718354]

125. Shultz PJ and Tolins JP. Adaptation to increased dietary salt intake in the rat. Role of endogenous nitric oxide. J Clin Invest. 1993;91:642–650. [PubMed: 7679414]

126. Ritthaler T, Scholz H, Ackermann M, Riegger G, Kurtz A and Kramer BK. Effects of endothelins on renin secretion from isolated mouse renal juxtaglomerular cells. Am J Physiol. 1995;268:F39–F45. [PubMed: 7840246]

127. Tolins JP and Shultz PJ. Endogenous nitric oxide synthesis determines sensitivity to the pressor effect of salt. Kidney Int. 1994;46:230–236. [PubMed: 7523754]

128. Griffin KA, Picken M and Bidani AK. Radiotelemetric BP monitoring, antihypertensives and glomeruloprotection in remnant kidney model. Kidney Int. 1994;46:1010–1018. [PubMed: 7861695]

129. Welch WJ and Wilcox CS. Role of nitric oxide in tubuloglomerular feedback: effects of dietary salt. Clin Exp Pharmacol Physiol. 1997;24:582–586. [PubMed: 9269531]

130. Deng X, Welch WJ and Wilcox CS. Renal vasodilation with L-arginine. Effects of dietary salt. Hypertension. 1995;26:256–262. [PubMed: 7635532]

131. Wilcox CS, Deng X and Welch WJ. NO generation and action during changes in salt intake: roles of nNOS and macula densa. Am J Physiol. 1998;274:R1588–R1593. [PubMed: 9608012]

132. Barri YM and Wilcox CS. Salt intake determines the renal response to L-arginine infusion in normal human subjects. Kidney Int. 1998;53:1299–1304. [PubMed: 9573545]

133. Parmer RJ, Stone RA and Cervenka JH. Renal hemodynamics in essential hypertension. Racial differences in responses to changes in dietary sodium. Hypertension. 1994;24:752–757. [PubMed: 7995633]

134. Bosse HM, Bohm R, Resch S and Bachmann S. Parallel regulation of constitutive NO synthase and renin at JGA of rat kidney under various stimuli. Am J Physiol. 1995;269:F793–F805. [PubMed: 8594873]

135. Tojo A, Kimoto M and Wilcox CS. Renal expression of constitutive NOS and DDAH: separate effects of salt intake and angiotensin. Kidney Int. 2000;58:2075–2083. [PubMed: 11044228]

136. Singh I, Grams M, Wang W-H, Yang T, Killen P, Smart A, Schnermann J and Briggs JP. Coordinate regulation of renal expression of nitric oxide synthase, renin, and angiotensinogen mRNA by dietary salt. Am J Physiol. 1996;270:F1027–F1037. [PubMed: 8764322]

137. Tan DY, Meng S and Manning RD Jr., Role of neuronal nitric oxide synthase in Dahl salt-sensitive hypertension. Hypertension. 1999;33:456–461. [PubMed: 9931147]

138. Hannan RL, John MC, Kouretas PC, Hack BD, Matherne GP and Laubach VE. Deletion of endothelial nitric oxide synthase exacerbates myocardial stunning in an isolated mouse heart model. J Surg Res. 2000;93:127–132. [PubMed: 10945953]

139. Barouch LA, Harrison RW, Skaf MW, Rosas GO, Cappola TP, Kobeissi ZA, Hobai IA, Lemmon CA, Burnett AL, O'Rourke B, Rodriguez ER, Huang PL, Lima JAC, Berkowitz DE and Hare JM. Nitric oxide regulates the heart by spatial confinement of nitric oxide synthase isoforms. Nature. 2002;416:337–340. [PubMed: 11907582]

140. Sallstrom J, Carlstrom M, Jensen BL, Skott O, Brown RD and Persson AE. Neuronal nitric oxide synthase-deficient mice have impaired renin release but normal blood pressure. Am J Hypertens. 2008;21:111–116. [PubMed: 18091753]

141. Wang X, Chandrashekar K, Wang L, Lai EY, Wei J, Zhang G, Wang S, Zhang J, Juncos LA and Liu R. Inhibition of Nitric Oxide Synthase 1 Induces Salt-Sensitive Hypertension in Nitric Oxide Synthase 1alpha Knockout and Wild-Type Mice. Hypertension. 2016;67:792–9. [PubMed: 26883268]

142. Brenman JE, Chao DS, Gee SH, McGee AW, Craven SE, Santillano DR, Wu Z, Huang F, Xia H, Peters MF, Froehner SC and Bredt DS. Interaction of nitric oxide synthase with the postsynaptic density protein PSD-95 and a1-syntrophin mediated by PDZ domains. Cell. 1996;84:757–767. [PubMed: 8625413]

143. Gyurko R, Leupen S and Huang PL. Deletion of exon 6 of the neuronal nitric oxide synthase gene in mice results in hypogonadism and infertility. Endocrinology. 2002;143:2767–2774. [PubMed: 12072412]

144. Tranguch S and Huet-Hudson Y. Decreased viability of nitric oxide synthase double knockout mice. Mol Reprod Dev. 2003;65:175–179. [PubMed: 12704728]

145. Brenman JE, Xia H, Chao DS, Black SM and Bredt DS. Regulation of neuronal nitric oxide synthase through alternative transcripts. Dev Neurosci. 1997;19:224–231. [PubMed: 9208206]

146. Smith C, Merchant M, Fekete A, Nyugen HL, Oh P, Tain YL, Klein JB and Baylis C. Splice variants of neuronal nitric oxide synthase are present in the rat kidney. Nephrol Dial Transplant. 2009;24:1422–1428. [PubMed: 19073653]

147. Hurt KJ, Sezen SF, Champion HC, Crone JK, Palese MA, Huang PL, Sawa A, Luo X, Musicki B, Snyder SH and Burnett AL. Alternatively spliced neuronal nitric oxide synthase mediates penile erection. Proc Natl Acad Sci U S A. 2006;103:3440–3443. [PubMed: 16488973]

148. Erdely A, Greenfeld Z, Wagner L and Baylis C. Sexual dimorphism in the aging kidney: Effects on injury and nitric oxide system. Kidney Int. 2003;63:1021–1026. [PubMed: 12631083]

149. Moningka NC, Sindler AL, Muller-Delp JM and Baylis C. Twelve weeks of treadmill exercise does not alter age-dependent chronic kidney disease in the Fisher 344 male rat. J Physiol. 2011;589:6129–38. [PubMed: 21969451]

150. Hyndman KA, Boesen EI, Elmarakby AA, Brands MW, Huang P, Kohan DE, Pollock DM and Pollock JS. Renal collecting duct NOS1 maintains fluid-electrolyte homeostasis and blood pressure. Hypertension. 2013;62:91–98. [PubMed: 23608660]

151. Hyndman KA, Bugaj V, Mironova E, Stockand JD and Pollock JS. NOS1-dependent negative feedback regulation of the epithelial sodium channel in the collecting duct. Am J Physiol Renal Physiol. 2015;308:F244–F251. [PubMed: 25391901]

152. Huang PL, Dawson TM, Bredt DS, Snyder SH and Fishman MC. Targeted disruption of the neuronal nitric oxide synthase gene. Cell. 1993;75:1273–1286. [PubMed: 7505721]

153. Christopherson KS, Hillier BJ, Lim WA and Bredt DS. PSD-95 assembles a ternary complex with the N-methyl-D-aspartic acid receptor and a bivalent neuronal NO synthase PDZ domain. J Biol Chem. 1999;274:27467–27473. [PubMed: 10488080]

154. Reckelhoff JF. Gender differences in the regulation of blood pressure. Hypertension. 2001;37:1199–1208. [PubMed: 11358929]

155. Burt VL, Whelton P, Roccella EJ, Brown C, Cutler JA, Higgins M, Horan MJ and Labarthe D. Prevalence of hypertension in the US adult population. Results from the Third National Health and Nutrition Examination Survey, 1988–1991. Hypertension. 1995;25:305–313. [PubMed: 7875754]

156. Wiinberg N, Hoegholm A, Christensen HR, Bang LE, Mikkelsen KL, Nielsen PE, Svendsen TL, Kampmann JP, Madsen NH and Bentzon MW. 24-h ambulatory blood pressure in 352 normal Danish subjects, related to age and gender. Am J Hypertens. 1995;8:978–86. [PubMed: 8845079]

157. Khoury S, Yarows SA, O'Brien TK and Sowers JR. Ambulatory blood pressure monitoring in a nonacademic setting. Effects of age and sex. Am J Hypertens. 1992;5:616–23. [PubMed: 1418850]

158. Dahl LK, Knudsen KD, Ohanian EV, Muirhead M and Tuthill R. Role of the gonads in hypertension-prone rats. J Exp Med. 1975;142:748–759. [PubMed: 1165474]

159. Xue B, Pamidimukkala J and Hay M. Sex differences in the development of angiotensin II-induced hypertension in conscious mice. Am J Physiol Heart Circ Physiol. 2005;288:H2177–H2184. [PubMed: 15626687]

160. Sartori-Valinotti JC, Iliescu R, Yanes LL, Dorsett-Martin W and Reckelhoff JF. Sex differences in the pressor response to angiotensin II when the endogenous renin-angiotensin system is blocked. Hypertension. 2008;51:1170–1176. [PubMed: 18259017]

161. Bayorh MA, Socci RR, Eatman D, Wang M and Thierry-Palmer M. The role of gender in salt-induced hypertension. Clinical and Experimental Hypertension. 2001;23:241–255. [PubMed: 11339690]

162. Ouchi Y, Share L, Crofton JT, Iitake K and Brooks DP. Sex difference in the development of deoxycorticosterone-salt hypertension in the rat. Hypertension. 1987;9:172–177. [PubMed: 3818014]

163. Lee SH, Lee YH, Jung SW, Kim DJ, Park SH, Song SJ, Jeong KH, Moon JY, Ihm CG, Lee TW, Kim JS, Sohn I, Lee SY, Kim DO and Kim YG. Sex-related differences in the intratubular renin-angiotensin system in two-kidney, one-clip hypertensive rats. Am J Physiol-Renal. 2019;317:E670–E682.

164. Ramirez LA, Gillis EE, Musall JB, Mohamed R, Snyder E, El-Marakby A and Sullivan JC. Hypertensive female Sprague-Dawley rats require an intact nitric oxide synthase system for compensatory increases in renal regulatory T cells. Am J Physiol-Renal. 2020;319:F192–F201.

165. Wolf E, Diaz EJ, Hollis AN, Hoang TA, Azad HA, Bendt KM, Griffiths RC and Sparks MA. Vascular type 1 angiotensin receptors control blood pressure by augmenting peripheral vascular resistance in female mice. Am J Physiol Renal Physiol. 2018;315:F997–F1005. [PubMed: 29897266]

166. Pai AV, West CA, de Souza AMA, Kadam PS, Pollner EJ, West DA, Li J, Ji H, Wu X, Zhu MJ, Baylis C and Sandberg K. Renal T cell infiltration occurs despite attenuation of development of hypertension with hydralazine in Envigo's female Dahl rat maintained on a low-Na+ diet. Am J Physiol-Renal. 2019;317:E572–E583.

167. Maranon R and Reckelhoff JF. Sex and gender differences in control of blood pressure. Clin Sci (Lond). 2013;125:311–318. [PubMed: 23746374]

168. Zhang J, Qu L, Wei J, Jiang S, Xu L, Wang L, Cheng F, Jiang K, Buggs J and Liu R. A new mechanism for the sex differences in angiotensin II-induced hypertension: the role of macula densa NOS1beta-mediated tubuloglomerular feedback. Am J Physiol Renal Physiol. 2020;319:F908–F919. [PubMed: 33044868]

169. Zhang J, Zhu J, Wei J, Jiang S, Xu L, Qu L, Yang K, Wang L, Buggs J, Cheng F, Tan X and Liu R. New Mechanism for the Sex Differences in Salt-Sensitive Hypertension: The Role of Macula Densa NOS1beta-Mediated Tubuloglomerular Feedback. Hypertension. 2020;75:449–457. [PubMed: 31865794]

170. Ji H, Pesce C, Zheng W, Kim J, Zhang YH, Menini S, Haywood JR and Sandberg K. Sex differences in renal injury and nitric oxide production in renal wrap hypertension. Am J Physiol-Heart C. 2005;288:H43–H47.

171. Sullivan JC, Pardieck JL, Hyndman KA and Pollock JS. Renal NOS activity, expression, and localization in male and female spontaneously hypertensive rats. Am J Physiol-Reg I. 2010;298:R61–R69.

172. Bredt DS, Ferris CD and Snyder SH. Nitric-Oxide Synthase Regulatory Sites - Phosphorylation by Cyclic Amp-Dependent Protein-Kinase, Protein-Kinase-C, and Calcium Calmodulin Protein-Kinase - Identification of Flavin and Calmodulin Binding-Sites. Journal of Biological Chemistry. 1992;267:10976–10981. [PubMed: 1375933]

173. Adak S, Santolini J, Tikunova S, Wang Q, Johnson JD and Stuehr DJ. Neuronal nitric-oxide synthase mutant (Ser-1412 -> Asp) demonstrates surprising connections between heme reduction, NO complex formation, and catalysis. Journal of Biological Chemistry. 2001;276:1244–1252. [PubMed: 11038355]

174. Hurt KJ, Sezen SF, Lagoda GF, Musicki B, Rameau GA, Snyder SH and Burnett AL. Cyclic AMP-dependent phosphorylation of neuronal nitric oxide synthase mediates penile erection. Proc Natl Acad Sci U S A. 2012;109:16624–16629. [PubMed: 23012472]

175. Brown RD, Hilliard LM, Head GA, Jones ES, Widdop RE and Denton KM. Sex differences in the pressor and tubuloglomerular feedback response to angiotensin II. Hypertension. 2012;59:129–135. [PubMed: 22124434]

176. Chiolero A, Maillard M, Nussberger J, Brunner HR and Burnier M. Proximal sodium reabsorption: An independent determinant of blood pressure response to salt. Hypertension. 2000;36:631–637. [PubMed: 11040249]

177. Visser FW, Krikken JA, Muntinga JH, Dierckx RA and Navis GJ. Rise in extracellular fluid volume during high sodium depends on BMI in healthy men. Obesity (Silver Spring). 2009;17:1684–1688. [PubMed: 19282825]

178. Cannon PJ, Svahn DS and Demartini FE. The influence of hypertonic saline infusions upon the fractional reabsorption of urate and other ions in normal and hypertensive man. Circulation. 1970;41:97–108. [PubMed: 5420637]

179. Pechere-Bertschi A, Maillard M, Stalder H, Bischof P, Fathi M, Brunner HR and Burnier M. Renal hemodynamic and tubular responses to salt in women using oral contraceptives. Kidney Int. 2003;64:1374–1380. [PubMed: 12969156]

180. Rasmussen MS, Simonsen JA, Sandgaard NC, Hoilund-Carlsen PF and Bie P. Mechanisms of acute natriuresis in normal humans on low sodium diet. J Physiol. 2003;546:591–603. [PubMed: 12527745]

181. Wang H, Garvin JL and Carretero OA. Angiotensin II enhances tubuloglomerular feedback *via* luminal AT_1 receptors on the macula densa. Kidney Int. 2001;60:1851–1857. [PubMed: 11703603]

182. Schnermann J and Briggs JP. Restoration of Tubuloglomerular Feedback in Volume-Expanded Rats by Angiotensin-Ii. American Journal of Physiology. 1990;259:F565–F572. [PubMed: 2221094]

183. Liu RS and Persson AEG. Angiotensin II stimulates calcium and nitric oxide release from macula densa cells through AT(1) receptors. Hypertension. 2004;43:649–653. [PubMed: 14744924]

184. Crofton JT, Share L and Brooks DP. Gonadectomy abolishes the sexual dimorphism in DOC-salt hypertension in the rat. Clin Exp Hypertens [A]. 1989;11:1249–1261.

185. Crofton JT and Share L. Gonadal hormones modulate deoxycorticosterone-salt hypertension in male and female rats. Hypertension. 1997;29:494–9. [PubMed: 9039148]

186. Hinojosa-Laborde C, Lange DL and Haywood JR. Role of female sex hormones in the development and reversal of dahl hypertension. Hypertension. 2000;35:484–9. [PubMed: 10642346]

187. Rowland NE and Fregly MJ. Role of gonadal hormones in hypertension in the Dahl salt-sensitive rat. Clin Exp Hypertens. 1992;A14:367–375.

188. Otsuka K, Suzuki H, Sasaki T, Ishii N, Itoh H and Saruta T. Blunted pressure natriuresis in ovariectomized Dahl-Iwai salt-sensitive rats. Hypertension. 1996;27:119–24. [PubMed: 8591873]

189. Fu Y, Lu Y, Liu EY, Zhu X, Mahajan GJ, Lu D, Roman RJ and Liu R. Testosterone enhances tubuloglomerular feedback by increasing superoxide production in the macula densa. Am J Physiol Regul Integr Comp Physiol. 2013;304:R726–33. [PubMed: 23467324]

190. Hinchee-Rodriguez K, Garg N, Venkatakrishnan P, Roman MG, Adamo ML, Masters BS and Roman LJ. Neuronal nitric oxide synthase is phosphorylated in response to insulin stimulation in skeletal muscle. Biochem Biophys Res Commun. 2013;435:501–505. [PubMed: 23680665]

191. Tanaka S, Hosogi S, Sawabe Y, Shimamoto C, Matsumura H, Inui T, Marunaka Y and Nakahari T. PPARalpha induced NOS1 phosphorylation via PI3K/Akt in guinea pig antral mucous cells: NO-enhancement in Ca(2+)-regulated exocytosis. Biomed Res. 2016;37:167–178. [PubMed: 27356604]

192. Lonze BE and Ginty DD. Function and regulation of CREB family transcription factors in the nervous system. Neuron. 2002;35:605–623. [PubMed: 12194863]

193. Sasaki M, Gonzalez-Zulueta M, Huang H, Herring WJ, Ahn SY, Ginty DD, Dawson VL and Dawson TM. Dynamic regulation of neuronal NO synthase transcription by calcium influx through a CREB family transcription factor-dependent mechanism. Proceedings of the National Academy of Sciences of the United States of America. 2000;97:8617–8622. [PubMed: 10900019]

194. Boissel JP, Bros M, Schrock A, Godtel-Armbrust U and Forstermann U. Cyclic AMP-mediated upregulation of the expression of neuronal NO synthase in human A673 neuroepithelioma

cells results in a decrease in the level of bioactive NO production: analysis of the signaling mechanisms that are involved. Biochemistry. 2004;43:7197–7206. [PubMed: 15170357]

195. Yen DHT, Chen LC, Shen YC, Chiu YC, Ho IC, Lou YJ, Chen IC and Yen JC. Protein kinase A-dependent Neuronal Nitric Oxide Synthase Activation Mediates the Enhancement of Baroreflex Response by Adrenomedullin in the Nucleus Tractus Solitarii of Rats. J Biomed Sci. 2011;18. [PubMed: 21324201]
196. Gohar EY, De Miguel C, Obi IE, Daugherty EM, Hyndman KA, Becker BK, Jin C, Sedaka R, Johnston JG, Liu P, Speed JS, Mitchell T, Kriegel AJ, Pollock JS and Pollock DM. Acclimation to a High-Salt Diet Is Sex Dependent. J Am Heart Assoc. 2022;11:e020450. [PubMed: 35191321]
197. Veiras LC, Girardi ACC, Curry J, Pei L, Ralph DL, Tran A, Castelo-Branco RC, Pastor-Soler N, Arranz CT, Yu ASL and McDonough AA. Sexual Dimorphic Pattern of Renal Transporters and Electrolyte Homeostasis. J Am Soc Nephrol. 2017;28:3504–3517. [PubMed: 28774999]
198. Fields LE, Burt VL, Cutler JA, Hughes J, Roccella EJ and Sorlie P. The burden of adult hypertension in the United States 1999 to 2000: a rising tide. Hypertension. 2004;44:398–404. [PubMed: 15326093]
199. Vasan RS, Beiser A, Seshadri S, Larson MG, Kannel WB, D'Agostino RB and Levy D. Residual lifetime risk for developing hypertension in middle-aged women and men. The Framingham Heart Study. JAMA. 2002;287:1003–1010. [PubMed: 11866648]
200. Weinberger MH. Salt sensitivity of blood pressure in humans. Hypertension. 1996;27:481–490. [PubMed: 8613190]
201. Luft FC. Salt and hypertension: recent advances and perspectives. J Lab Clin Med. 1989;114:215–221. [PubMed: 2671214]
202. Hua J, Kaskel FJ, Juno CJ, Moore LC and Mccaughran JA. Salt Intake and Renal Hemodynamics in Immature and Mature Dahl Salt-Sensitive (Ds/Jr) and Salt-Resistant (Dr/Jr) Rats. American Journal of Hypertension. 1990;3:268–273. [PubMed: 2346632]
203. Simchon S, Manger WM, Carlin RD, Peeters LL, Rodriguez J, Batista D, Brown T, Merchant NB, Jan KM and Chien S. Salt-Induced Hypertension in Dahl Salt-Sensitive Rats - Hemodynamics and Renal Responses. Hypertension. 1989;13:612–621. [PubMed: 2525523]
204. Campese VM, Parise M, Karubian F and Bigazzi R. Abnormal renal hemodynamics in black salt-sensitive patients with hypertension. Hypertension. 1991;18:805–812. [PubMed: 1743761]
205. Stadler P, Pusterla C and Berettapiccoli C. Renal Tubular Handling of Sodium and Familial Predisposition to Essential-Hypertension. Journal of Hypertension. 1987;5:727–732. [PubMed: 3429871]
206. Wilcox CS, Welch WJ, Murad F, Gross SS, Taylor G, Levi R and Schmidt HH. Nitric oxide synthase in macula densa regulates glomerular capillary pressure. Proc Natl Acad Sci U S A. 1992;89:11993–7. [PubMed: 1281548]
207. Tojo A, Madsen KM and Wilcox CS. Expression of immunoreactive nitric oxide synthase isoforms in rat kidney. Effects of dietary salt and losartan. Jpn Heart J. 1995;36:389–398. [PubMed: 7544416]
208. Tojo A, Gross SS, Zhang L, Tisher CC, Schmidt HHHW, Wilcox CS and Madsen KM. Immunocytochemical localization of distinct isoforms of nitric oxide synthase in the juxtaglomerular apparatus of the kidney. J Am Soc Nephrol. 1994;4:1438–1447. [PubMed: 7512831]
209. Bachmann S, Bosse HM and Mundel P. Topography of nitric oxide synthesis by localizing constitutive NO synthases in mammalian kidney. Am J Physiol. 1995;268:F885–F898. [PubMed: 7539586]
210. Brown RD, Thoren P, Steege A, Mrowka R, Sallstrom J, Skott O, Fredholm BB and Persson AE. Influence of the adenosine A1 receptor on blood pressure regulation and renin release. Am J Physiol Regul Integr Comp Physiol. 2006;290:R1324–R1329. [PubMed: 16357099]
211. Selby JV, FitzSimmons SC, Newman JM, Katz PP, Sepe S and Showstack J. The natural history and epidemiology of diabetic nephropathy. Implications for prevention and control. JAMA. 1990;263:1954–60. [PubMed: 2179596]

212. Gross JL, de Azevedo MJ, Silveiro SP, Canani LH, Caramori ML and Zelmanovitz T. Diabetic nephropathy: diagnosis, prevention, and treatment. Diabetes Care. 2005;28:164–76. [PubMed: 15616252]

213. Tervaert TW, Mooyaart AL, Amann K, Cohen AH, Cook HT, Drachenberg CB, Ferrario F, Fogo AB, Haas M, de Heer E, Joh K, Noel LH, Radhakrishnan J, Seshan SV, Bajema IM, Bruijn JA and Renal Pathology S. Pathologic classification of diabetic nephropathy. J Am Soc Nephrol. 2010;21:556–63. [PubMed: 20167701]

214. Bank N. Mechanisms of diabetic hyperfiltration. Kidney Int. 1991;40:792–807. [PubMed: 1745032]

215. Levine DZ. Can rodent models of diabetic kidney disease clarify the significance of early hyperfiltration?: recognizing clinical and experimental uncertainties. Clin Sci (Lond). 2008;114:109–118. [PubMed: 18062776]

216. Levine DZ, Iacovitti M and Robertson SJ. Modulation of single-nephron GFR in the db/db mouse model of type 2 diabetes mellitus. II. Effects of renal mass reduction. Am J Physiol Regul Integr Comp Physiol. 2008;294:R1840–R1846. [PubMed: 18417648]

217. Vora JP, Dolben J, Dean JD, Thomas D, Williams JD, Owens DR and Peters JR. Renal hemodynamics in newly presenting non-insulin dependent diabetes mellitus. Kidney Int. 1992;41:829–835. [PubMed: 1513105]

218. Nelson RG, Bennett PH, Beck GJ, Tan M, Knowler WC, Mitch WE, Hirschman GH and Myers BD. Development and progression of renal disease in Pima Indians with non-insulin-dependent diabetes mellitus. Diabetic Renal Disease Study Group. N Engl J Med. 1996;335:1636–1642. [PubMed: 8929360]

219. Keller CK, Bergis KH, Fliser D and Ritz E. Renal findings in patients with short-term type 2 diabetes. J Am Soc Nephrol. 1996;7:2627–2635. [PubMed: 8989741]

220. Anderson S and Vora JP. Current concepts of renal hemodynamics in diabetes. J Diabetes Complications. 1995;9:304–307. [PubMed: 8573753]

221. Vallon V and Thomson SC. Targeting renal glucose reabsorption to treat hyperglycaemia: the pleiotropic effects of SGLT2 inhibition. Diabetologia. 2017;60:215–225. [PubMed: 27878313]

222. Thomson SC, Deng A, Bao D, Satriano J, Blantz RC and Vallon V. Ornithine decarboxylase, kidney size, and the tubular hypothesis of glomerular hyperfiltration in experimental diabetes. J Clin Invest. 2001;107:217–224. [PubMed: 11160138]

223. Vallon V, Richter K, Blantz RC, Thomson S and Osswald H. Glomerular hyperfiltration in experimental diabetes mellitus: potential role of tubular reabsorption. J Am Soc Nephrol. 1999;10:2569–2576. [PubMed: 10589696]

224. Vallon V and Thomson SC. Renal function in diabetic disease models: the tubular system in the pathophysiology of the diabetic kidney. Annu Rev Physiol. 2012;74:351–375. [PubMed: 22335797]

225. Zhang J, Wei J, Jiang S, Xu L, Wang L, Cheng F, Buggs J, Koepsell H, Vallon V and Liu R. Macula Densa SGLT1-NOS1-Tubuloglomerular Feedback Pathway, a New Mechanism for Glomerular Hyperfiltration during Hyperglycemia. J Am Soc Nephrol. 2019;30:578–593. [PubMed: 30867247]

226. Carlstrom M. The Other Glucose Transporter, SGLT1 - Also a Potential Trouble Maker in Diabetes? J Am Soc Nephrol. 2019;30:519–521. [PubMed: 30867245]

227. Madunic IV, Breljak D, Karaica D, Koepsell H and Sabolic I. Expression profiling and immunolocalization of Na+-d-glucose-cotransporter 1 in mice employing knockout mice as specificity control indicate novel locations and differences between mice and rats. Pflug Arch Eur J Phy. 2017;469:1545–1565.

228. Balen D, Ljubojevic M, Breljak D, Brzica H, Zlender V, Koepsell H and Sabolic I. Revised immunolocalization of the Na+-D-glucose cotransporter SGLT1 in rat organs with an improved antibody. AM J Physiol Cell Physiol. 2008;295:C475–C489. [PubMed: 18524944]

229. Christiansen JS, Frandsen M and Parving HH. Effect of Intravenous Glucose-Infusion on Renal-Function in Normal Man and in Insulin-Dependent Diabetics. Diabetologia. 1981;21:368–373. [PubMed: 7286497]

230. BOUNOUS G and SHUMACKER HB Jr., Influence of blood sugar levels upon renal blood flow. Ann Surg. 1960;151:441–452. [PubMed: 13803279]

231. Fox M, Rosenberg LE, Segal S and Thier S. Impaired Renal Tubular Function Induced by Sugar Infusion in Man. J Clin Endocr Metab. 1964;24:1318–+. [PubMed: 14243176]

232. Noonan WT, Shapiro VM and Banks RO. Renal glucose reabsorption during hypertonic glucose infusion in female streptozotocin-induced diabetic rats. Life Sci. 2001;68:2967–2977. [PubMed: 11411796]

233. Woods LL, Mizelle HL and Hall JE. Control of Renal Hemodynamics in Hyperglycemia - Possible Role of Tubuloglomerular Feedback. American Journal of Physiology. 1987;252:F65–F73. [PubMed: 3812702]

234. Vallon V and Thomson S. Inhibition of local nitric oxide synthase increases homeostatic efficiency of tubuloglomerular feedback. Am J Physiol. 1995;269:F892–F899. [PubMed: 8594885]

235. Levine DZ. Hyperfiltration, nitric oxide, and diabetic nephropathy. Curr Hypertens Rep. 2006;8:153–157. [PubMed: 16672149]

236. Park J, Shrestha R, Qiu CX, Kondo A, Huang SZ, Werth M, Li MY, Barasch J and Susztak K. Single-cell transcriptomics of the mouse kidney reveals potential cellular targets of kidney disease. Science. 2018;360:758–763. [PubMed: 29622724]

237. Thomson SC, Rieg T, Miracle C, Mansoury H, Whaley J, Vallon V and Singh P. Acute and chronic effects of SGLT2 blockade on glomerular and tubular function in the early diabetic rat. Am J Physiol Regul Integr Comp Physiol. 2012;302:R75–R83. [PubMed: 21940401]

238. Zhang J, Jiang S, Wei J, Yip KP, Wang L, Lai EY and Liu RS. Glucose dilates renal afferent arterioles via glucose transporter-1. Am J Physiol-Renal. 2018;315:F123–F129.

239. Toma I, Kang JJ, Sipos A, Vargas S, Bansal E, Hanner F, Meer E and Peti-Peterdi J. Succinate receptor GPR91 provides a direct link between high glucose bevels and renin release in murine and rabbit kidney. Journal of Clinical Investigation. 2008;118:2526–2534. [PubMed: 18535668]

240. Hall JE, Coleman TG, Mizelle HL and Smith MJ. Chronic Hyperinsulinemia and Blood-Pressure Regulation. American Journal of Physiology. 1990;258:F722–F731. [PubMed: 2180321]

241. Cohen AJ, Mccarthy DM and Stoff JS. Direct Hemodynamic-Effect of Insulin in the Isolated Perfused Kidney. American Journal of Physiology. 1989;257:F580–F585. [PubMed: 2679144]

242. Odutayo A and Hladunewich M. Obstetric nephrology: renal hemodynamic and metabolic physiology in normal pregnancy. Clin J Am Soc Nephrol. 2012;7:2073–2080. [PubMed: 22879432]

243. Cheung KL and Lafayette RA. Renal physiology of pregnancy. Adv Chronic Kidney Dis. 2013;20:209–214. [PubMed: 23928384]

244. Mahendru AA, Everett TR, Wilkinson IB, Lees CC and McEniery CM. A longitudinal study of maternal cardiovascular function from preconception to the postpartum period. J Hypertens. 2014;32:849–856. [PubMed: 24406777]

245. Chapman AB. The structure-function relationship in preeclampsia. Kidney Int. 1998;54:1394–1395. [PubMed: 9773683]

246. Phipps E, Prasanna D, Brima W and Jim B. Preeclampsia: Updates in Pathogenesis, Definitions, and Guidelines. Clin J Am Soc Nephro. 2016;11:1102–1113.

247. Brown MA. Pregnancy-induced hypertension: current concepts. Anaesth Intensive Care. 1989;17:185–197. [PubMed: 2655493]

248. Krane NK and Hamrahian M. Pregnancy: kidney diseases and hypertension. Am J Kidney Dis. 2007;49:336–45. [PubMed: 17261438]

249. Chesley LC and Duffus GM. Preeclampsia, posture and renal function. Obstet Gynecol. 1971;38:1–5. [PubMed: 5561082]

250. Moran P, Lindheimer MD and Davison JM. The renal response to preeclampsia. Semin Nephrol. 2004;24:588–595. [PubMed: 15529294]

251. Irons DW, Baylis PH, Butler TJ and Davison JM. Atrial natriuretic peptide in preeclampsia: metabolic clearance, sodium excretion and renal hemodynamics. Am J Physiol-Renal. 1997;273:F483–F487.

252. Maynard SE, Min JY, Merchan J, Lim KH, Li JY, Mondal S, Libermann TA, Morgan LP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP and Karumanchi SA. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfimction, hypertension, and proteinuria in preeclampsia. Journal of Clinical Investigation. 2003;111:649–658. [PubMed: 12618519]

253. Chaiworapongsa T, Chaemsaithong P, Yeo L and Romero R. Pre-eclampsia part 1: current understanding of its pathophysiology. Nat Rev Nephrol. 2014;10:466–80. [PubMed: 25003615]

254. Sladek SM, Magness RR and Conrad KP. Nitric oxide and pregnancy. Am J Physiol. 1997;272:R441–R463. [PubMed: 9124465]

255. Choi JW, Im MW and Pai SH. Nitric oxide production increases during normal pregnancy and decreases in preeclampsia. Ann Clin Lab Sci. 2002;32:257–263. [PubMed: 12175088]

256. Vaisanen-Tommiska M, Nuutila M, Aittomaki K, Hiilesmaa V and Ylikorkala O. Nitric oxide metabolites in cervical fluid during pregnancy: Further evidence for the role of cervical nitric oxide in cervical ripening. Am J Obstet Gynecol. 2003;188:779–785. [PubMed: 12634657]

257. Sandrim VC, Montenegro MF, Palei AC, Metzger IF, Sertorio JT, Cavalli RC and Tanus-Santos JE. Increased circulating cell-free hemoglobin levels reduce nitric oxide bioavailability in preeclampsia. Free Radic Biol Med. 2010;49:493–500. [PubMed: 20510352]

258. McCann Haworth SM, Zhuge Z, Nihlen C, Von Rosen MF, Weitzberg E, Lundberg JO, Krmar RT, Nasiell J and Carlstrom M. Red blood cells from patients with pre-eclampsia induce endothelial dysfunction. J Hypertens. 2021;39:1628–1641. [PubMed: 33657586]

259. Kassab S, Miller MT, Hester R, Novak J and Granger JP. Systemic hemodynamics and regional blood flow during chronic nitric oxide synthesis inhibition in pregnant rats. Hypertension. 1998;31:315–320. [PubMed: 9453322]

260. Cadnapaphornchai MA, Ohara M, Morris KG Jr., Knotek M, Rogachev B, Ladtkow T, Carter EP and Schrier RW. Chronic NOS inhibition reverses systemic vasodilation and glomerular hyperfiltration in pregnancy. Am J Physiol Renal Physiol. 2001;280:F592–F598. [PubMed: 11249850]

261. Kulandavelu S, Qu DW and Adamson SL. Cardiovascular function in mice during normal pregnancy and in the absence of endothelial NO synthase. Hypertension. 2006;47:1175–1182. [PubMed: 16636199]

262. Shesely EG, Gilbert C, Granderson G, Carretero CD, Carretero OA and Beierwaltes WH. Nitric oxide synthase gene knockout mice do not become hypertensive during pregnancy. Am J Obstet Gynecol. 2001;185:1198–1203. [PubMed: 11717657]

263. Wei J, Zhang J, Jiang S, Xu L, Qu L, Pang B, Jiang K, Wang L, Intapad S, Buggs J, Cheng F, Mohapatra S, Juncos LA, Osborn JL, Granger JP and Liu R. Macula Densa NOS1beta Modulates Renal Hemodynamics and Blood Pressure during Pregnancy: Role in Gestational Hypertension. J Am Soc Nephrol. 2021;32:2485–2500. [PubMed: 34127535]

264. Furuya M, Ishida J, Aoki I and Fukamizu A. Pathophysiology of placentation abnormalities in pregnancy-induced hypertension. Vasc Health Risk Manag. 2008;4:1301–13. [PubMed: 19337544]

265. Pijnenborg R, Anthony J, Davey DA, Rees A, Tiltman A, Vercruysse L and van Assche A. Placental bed spiral arteries in the hypertensive disorders of pregnancy. Br J Obstet Gynaecol. 1991;98:648–55. [PubMed: 1883787]

266. Makris A, Yeung KR, Lim SM, Sunderland N, Heffernan S, Thompson JF, Iliopoulos J, Killingsworth MC, Yong J, Xu B, Ogle RF, Thadhani R, Karumanchi SA and Hennessy A. Placental Growth Factor Reduces Blood Pressure in a Uteroplacental Ischemia Model of Preeclampsia in Nonhuman Primates. Hypertension. 2016;67:1263–72. [PubMed: 27091894]

267. Cavanagh D, Rao PS, Knuppel RA, Desai U and Balis JU. Pregnancy-Induced Hypertension - Development of a Model in the Pregnant Primate (Papio-Anubis). Am J Obstet Gynecol. 1985;151:987–999. [PubMed: 3885739]

268. Intapad S, Warrington JP, Spradley FT, Palei AC, Drummond HA, Ryan MJ, Granger JP and Alexander BT. Reduced uterine perfusion pressure induces hypertension in the pregnant mouse. Am J Physiol Regul Integr Comp Physiol. 2014;307:R1353–7. [PubMed: 25298513]

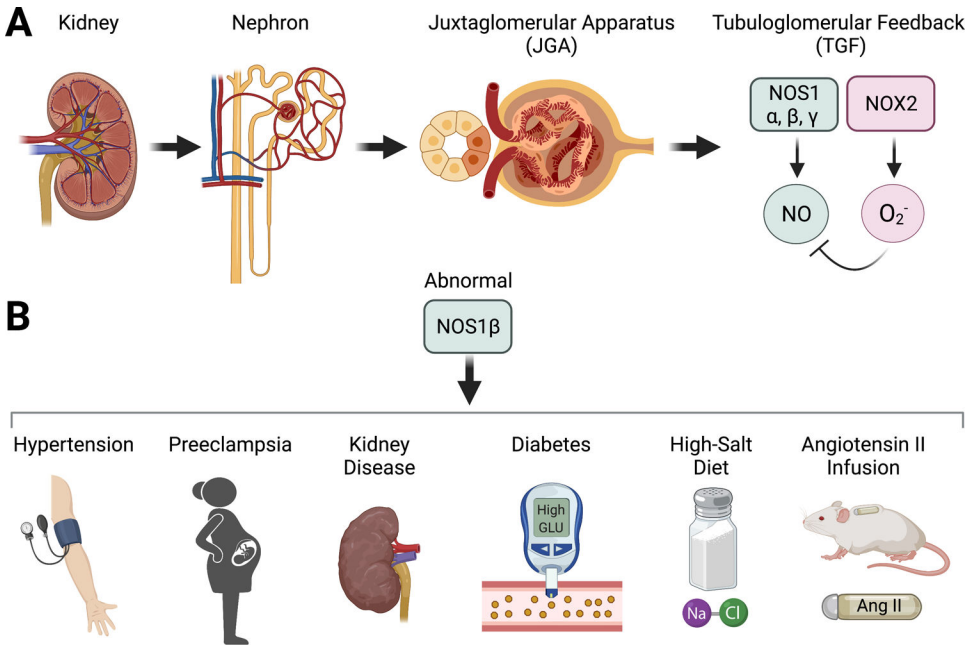

Figure 1.

A. The kidney via its functional units, the nephrons, importantly contribute to maintain body fluid and electrolyte homeostasis. Tubuloglomerular feedback (TGF) is operating within the juxtaglomerular apparatus (JGA), which consists of specialized structures/cells in the region between the thick ascending limb (TAL) of the loop of Henle and the distal convoluted tubule, near the afferent arteriole. TGF is initiated via mechanisms located to the macula densa sense and reacts in response to changes in tubular lumen NaCl. At high NaCl load, a paracrine signal is generated and transferred from macula densa in the TAL to the adjacent endothelial and vascular smooth muscle cells of the afferent arteriole. Tuning of the TGF is modulated by the activity of the enzyme isoforms nitric oxide synthase 1 (NOS1) and NAD(P)H oxidase 2 (NOX). The former enzyme exists in three different splice variants, *i.e.* alfa (α), beta (β) and gamma (γ), which all are known to generate nitric oxide (NO). Bioavailability and signaling of NOS1-derived NO is dampened, via scavenging, by NOX2-derived reactive oxygen species (ROS) including superoxide (O_2^-). Conditions with oxidative stress (*i.e.*, increased ROS production, reduced antioxidant capacity and/or decreased NO bioactivity) can sensitize TGF whereas states with increased NO formation can attenuate the TGF response. In the JGA, particularly in the macula densa cells, the NOS1β plays an important role in the regulation of TGF. **B**. Abnormal expression and function of this splice variant of NOS1 has been associated with several cardiovascular, renal, and metabolic disorders (*e.g.*, hypertension, preeclampsia, kidney disease and diabetes). Much knowledge regarding the regulation of NOS1β expression and its interaction with other enzyme systems in the JGA (*e.g.*, NOX2), as well as downstream signaling, have been obtained from experimental animal models with chronic high-salt diet treatment and infusion of subpressor doses of angiotensin II (Ang II) using osmotic minipumps. *Figure was created with* BioRender.com

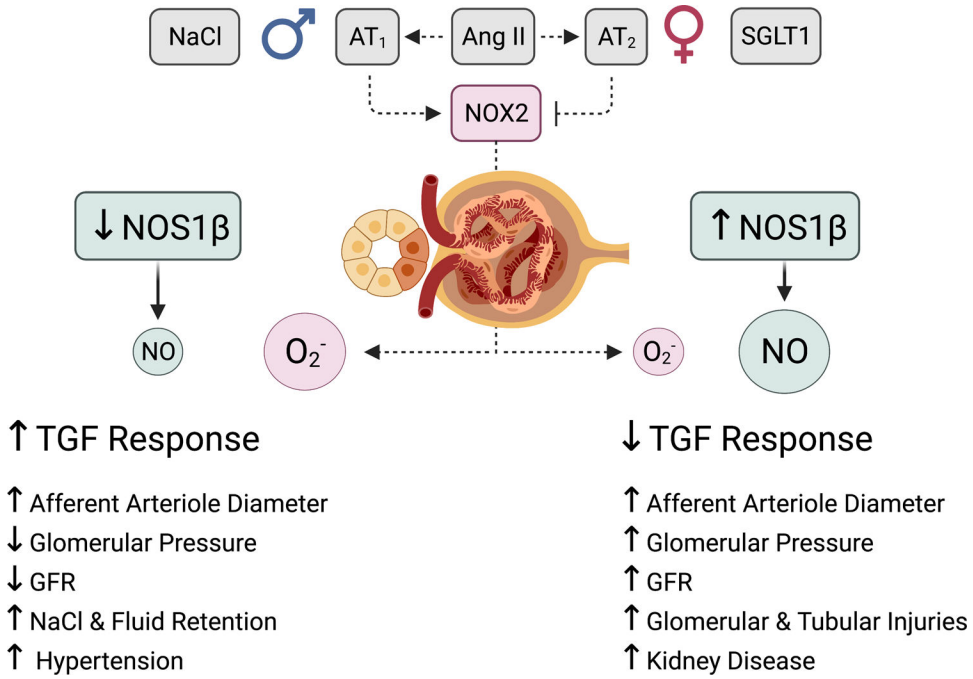

Figure 2.

The balance between nitric oxide synthase 1 (NOS1)-derived nitric oxide NO) and NADPH oxidase 2 (NOX2)-derived superoxide (O_2^-) formation importantly influence the responsiveness of the tubuloglomerular feedback (TGF). Emerging evidence have demonstrated that among the different splice variants of NOS1, the β-version expressed in macula densa cells is of particular importance in the regulation of TGF. Numerous factors and conditions have been associated altered function and expression of NOS1β and NADPH oxidase 2 (NOX2). **A**. Chronic dietary high-salt (NaCl) intake, as well as high levels of angiotensin II (Ang II) and hypertension has been associated with increased NOX2, which to some extent can be balanced by increased NOS1β expression. Ang II, via activation of its type 1 receptor (AT_1) stimulates NOX2, whereas activation of its type 2 receptor (AT_2) inhibits NOX2 and may also activate NOS1β. Altered balance between AT_1 and AT_2 receptor expression/activation, in favor of the latter, has been suggested to protect young-to-middle aged females from Ang II-induced pathophysiological events. Moreover, in chronic conditions with hyperglycemia (diabetes mellitus), the sodium-glucose transporter-2 and −1 (SGLT2 and SGLT1) in the proximal tubules may be saturated, leading to activation of SGLT1 in the macula densa cells in the thick ascending limb. Here, SGLT1 activation has been associated with increased expression and activity of the NOS1β splice variant. **B**. Reduced expression of NOS1β and/or high production of O_2^- is associated with decreased NO bioactivity and increased TGF response, which leads to contraction of the afferent arteriole and reduction of glomerular pressure. In pathological conditions this has been associated with reduced kidney function (glomerular filtration rate, GFR), retention of salt and fluid and hence elevated blood pressure (hypertension). **C**. Increased expression of NOS1β and/or low production of O_2^- is associated with increased NO bioactivity and decreased TGF response, which leads to dilatation of the afferent arteriole and increase of glomerular pressure. In pathological conditions this has been associated with increased GFR

(hyperfiltration), development of glomerular and tubular injuries and kidney disease. *Figure was created with* BioRender.com