Recent Advances in the Regulation of Nitric Oxide in the Kidney

Marcela Herrera, Jeffrey L. Garvin

Abstract—Nitric oxide (NO) plays important roles in the regulation of renal function and the long-term control of blood pressure. New roles of NO have been proposed recently in diabetes, nephrotoxicity, and pregnancy. NO derived from all 3 NOS isoforms contributes to the overall regulation of kidney function, and recent advances in our understanding of their regulation have been made lately. In this regard, substrate and cofactor availability play important roles in regulating nitric oxide synthase (NOS) activity not only by limiting enzyme activity but also by influencing the coupling of NOS with its cofactors, tetrahydrobiopterin and NADPH. Protein–protein interactions are now recognized to be important negative and positive regulators of NOS. Phosphorylation is another component of the mechanism whereby NOS is activated or deactivated. Increased NOS expression can also influence enzyme activity; however, the degree of expression does not always correlate with enzyme activity because increased NO levels can result in inhibition of NOS. Finally, other potential regulators of NOS such as endogenous L-arginine analogs may also be important. In this article, we summarize recent advances in the regulation of activity and expression of the NOS isoforms within the kidney. **(*Hypertension*. 2005;45:1062-1067.)**

Key Words: hemodynamics ■ nitric oxide ■ nitric oxide synthase ■ sodium

Nitric oxide (NO) plays an important role in the control of renal function and long-term regulation of blood pressure.[1–4] This is best evidenced by the fact that inhibiting intrarenal NO production increased blood pressure.[5] In addition, reduced NO has been identified as a common denominator of many hypertensive models.[6–9] The effects of NO on blood pressure via actions in the kidney occur through multiple mechanisms. These include increasing renal blood flow caused by vasodilatation,[10] increasing glomerular filtration,[11] inhibiting sodium transport along the nephron,[12–14] and regulating release of renin.[15] NO produced by each of the 3 nitric oxide synthases (NOS), NOS 1, NOS 2, and NOS 3, reportedly contributes to the regulation of renal function. Inhibition of NOS activity within the kidney is known to lead to sodium retention and hypertension. This review addresses recent advances in our understanding of the role played by renal NO in various physiological and pathophysiological conditions, as well as how NO production is regulated.

Roles for Renal NO

Historically, NO produced by the kidney has been thought of primarily as a factor that regulates urinary volume and sodium excretion. The physiological effects of NO can be mediated via changes in renal hemodynamics and/or salt and water absorption by the nephron. NO reduces renal vascular tone in part by dilating the afferent arteriole.[16] It also increases glomerular filtration rate.[11] NO modulates renin secretion by the juxtaglomerular apparatus[15] and tubuloglomerular feedback.[3] Finally, NO regulates transport in various nephron segments as reviewed recently by Ortiz and Garvin.[12]

More recently it has been recognized that in a number of pathophysiological conditions, the actions of NO on renal hemodynamics and/or nephron transport are altered. In early diabetes, NO appears to play a more pronounced role in the maintenance of blood pressure. NOS inhibition results in hypertension in diabetic but not in control rats,[17] showing that acute NOS 1 inhibition reduces glomerular filtration rate only in diabetic rats, and therefore suggesting that NO plays an enhanced role in regulating kidney function during diabetes.[18] NO also plays a protective role within the kidney. Augmenting NO by means of NO donors decreases nephrotoxicity caused by acetaminophen.[19] NOS 3 polymorphisms associated with decreased NO production correlate with end-stage renal disease in humans.[20] These data suggest that lack of renal NO may be important in advanced nephropathy and renal damage.[21] In addition, promoting NO production by administering L-arginine is known to attenuate pregnancy-induced hypertension.[22] This may be caused by effects on the kidney, because large increases in NO that enhance renal blood flow occur during normal pregnancy.[23–26] However, the precise role of each of the 3 NOS isoforms is unclear in these pathological as well as physiological circumstances.

Received December 22, 2004; first decision January 7, 2005; revision accepted February 8, 2005.

From the Division of Hypertension and Vascular Research (M.H., J.L.G.), Henry Ford Hospital and Department of Physiology (J.L.G.), Wayne State University, Detroit, Mich.

Correspondence to Jeffrey L. Garvin, Division of Hypertension and Vascular Research, Henry Ford Hospital, 2799 West Grand Blvd, Detroit, MI 48202. E-mail jgarvin1@hfhs.org

Hypertension **is available at http://www.hypertensionaha.org** **DOI: 10.1161/01.HYP.0000159760.88697.1e**

The use of selective NOS inhibitors and knockout mice has allowed us in some instances to investigate the individual roles of the 3 NOS isoforms in regulating renal function. However, we have not made a great deal of progress in identifying the role of NO produced by a given isoform in a given cell type. This is caused by a variety of factors, including: (1) the multiplicity of NOS isoforms, which are activated by different stimuli; (2) varying expression of NOS isoforms in different cell types; (3) differences in chronic regulation of expression and activity of the various NOS isoforms; and (4) the complex anatomy of the kidney, such that NO produced in one cell type can act in a different cell type.

As a first step in addressing this problem, our laboratory developed a technique to restore the function of a single NOS isoform in a single nephron segment, as in endothelial cells.[27] NOS 3 is responsible for autocrine inhibition of NaCl absorption in the thick ascending limb.[28] We showed that NOS 3 function could be restored selectively to this segment of NOS 3 knockout mice using an adenovirus with a tissue-specific promoter.[13] Because we were able to transduce ≈75% to 95% of thick ascending limbs in vivo, we are now studying the specific role of the medullary thick ascending limb NOS 3 in the regulation of salt and water excretion in vivo. In theory, this same approach could be used for the other NOS isoforms in other cell types, provided that one has the appropriate tissue-specific promoter.

Regulation of NOS Activity

NOS 1[29] and NOS 3[30] have been thought to be regulated primarily by increases in intracellular calcium and NOS 2 by changes in expression of the enzyme.[31] However, these views have recently been challenged by studies in a number of renal and other cell types. Changes in substrate and cofactor availability, protein–protein interactions and phosphorylation state have all come to light as significant regulators of NOS activity.

Availability of substrate and cofactors as a limiting factor of NOS activity has been primarily attributed to pathophysiological situations. However, cofactor availability may also be a physiological regulator of NOS activity. Increasing NaCl concentration in the lumen of the macula densa activates NOS 1[29] and also raises intracellular pH. Wang et al[32] first reported that the increase in intracellular pH caused by increasing luminal NaCl may activate macula densa NOS 1. Blocking luminal Na^+/H^+ exchange (which prevents alkalinization of macula densa cells) augmented tubuloglomerular feedback similarly to selective NOS 1 inhibition. More recently, Liu et al[33] found that the increase in NO production caused by elevated NaCl was blunted when the increase in intracellular pH was blocked, and that raising intracellular pH without increasing luminal NaCl was sufficient to induce NO production. A similar effect of pH on NOS 2 activity was also reported in mesangial cells.[34] In these cells, reducing extracellular pH, and presumably intracellular pH, decreased NOS 2 activity by 80%.

The mechanism by which pH alters NOS 1 and 2 activity appears to involve a combination of direct effects of protons per se on the enzyme and availability of the cofactor NADPH. Decreased NOS 1 activity at low pH has been shown to be caused by "uncoupling" of NADPH oxidation, resulting in increased formation of H_2O_2.[35] Mesangial cells exposed to low pH showed an increase in oxidized nicotinamide adenine dinucleotide phosphate/citrulline ratio.[34] The authors concluded that at low intracellular pH, there is less NADPH to accept electrons from NOS 2 during production of NO, so that NADPH is "uncoupled" from NO production. Interestingly, when this occurs, NOS would be predicted to produce superoxide, which could scavenge any NO produced. However, at present we know of no reports regarding NOS uncoupling in other structures within the kidney, such as vasculature and nephron segments.

The ability of changes in pH to regulate NOS 3 has not been investigated to our knowledge. However, it would be surprising if it did not control NO production by NOS 3 because this parameter modulates both NOS 1 and NOS 2 activity. Because virtually all cells have transporters to regulate intracellular pH, this mechanism may play an important role in all renal cells. Furthermore, regulation of NOS activity by intracellular pH may link NO production with acid/base balance and superoxide generation. The latter has recently been shown to depend on Na^+/H^+ exchange activity in the thick ascending limb.[36]

In addition to NADPH, the availability of tetrahydrobiopterin and arginine may also control NOS activity. A decrease in the ratio of reduced tetrahydrobiopterin to oxidized dihydrobiopterin in the renal medulla has been shown to blunt NO production, and has been proposed to contribute to salt-sensitive hypertension.[37] Oral L-arginine supplementation reverses p47 phox and gp91 phox expression induced by high salt in the renal cortex of Dahl rats,[38] suggesting that substrate supplementation can restore the imbalance between NO and reactive oxygen species, presumably by increasing NOS-derived NO. In addition, L-arginine transport has been shown to affect NOS activity and NO production in the renal medulla.[39] Arginine transport by the y+ transporter may be especially significant in angiotensin-dependent forms of hypertension, because y+ activity and expression are regulated by angiotensin.[40]

Activity of all 3 NOS isoforms is modulated by protein–protein interactions.[41] Protein inhibitor of neuronal NOS (PIN),[42,43] caveolin-1,[44] caveolin-3,[45] and several proteins bearing PDZ domains[46] that influence targeting to discrete membrane domains of excitable tissues regulate NOS 1 activity. Although some of these proteins have been localized to the kidney,[47] we know of no studies investigating the role of these proteins in renal NOS 1 activity.

Several proteins have been identified that directly interact with NOS 2. Kalirin and NOS-associated protein-110 have been shown to interact with NOS 2 and inhibit its activity.[48] In addition, Kuncewicz et al[49] found that Rac1 and Rac2, members of the Rho GTPase family, both interact with NOS 2. These authors also demonstrated that the point of interaction for Rac2 is the NOS 2 oxygenase domain and that overexpression of Rac2 augments NO production in immune-activated murine macrophages. Because Rac is important for assembly and activation of NADPH oxidase, this finding suggests coordinated regulation of NADPH oxidase and NOS

2-derived NO production. However, these interactions have not been shown to occur in renal cells to our knowledge.

Since the original publications showing that NOS 3 activity is inhibited by caveolin-1[50] and enhanced by heat shock protein 90[51] in endothelial cells, several other protein–protein interactions have been defined, including discovery of the NOS 3 inhibitory proteins NOSIP[52] and NOSTRIN.[53] It is likely that these regulatory proteins alter NOS 3 activity in all endothelial cells, including those in the kidney. However, their significance in the regulation of NOS 3 activity and/or expression in renal epithelial and interstitial cells has not been thoroughly investigated except for the interaction of NOS 3 and heat shock protein 90. Recently, activation and translocation of NOS 3 in the thick ascending limb have been reported to require heat shock protein 90 ATPase activity.[54]

Phosphorylation of NOS 3 by protein kinase A was first reported in 2001.[55] However, not until NOS 3 activation by shear stress was shown to be mediated by phosphorylation of serine 1179 in endothelial cells[56] were the potential consequences appreciated. Flow-induced activation of NO production occurs in afferent arterioles,[16] inner medullary collecting ducts,[57] and thick ascending limbs.[58] In thick ascending limbs, activation of NOS 3 by flow is caused by phosphorylation of serine 1179, as it is in endothelial cells. It is unclear whether this is also true for renal vessels, because flow-induced activation of NOS 3 in the vasa recta does not appear to be caused by phosphorylation at serine 1179, but rather simply an increase in intracellular calcium.[30] In addition to serine 1179, at least 4 other phosphorylation sites on NOS 3 are known.[59] Insulin has also been suggested to enhance NOS 3 activity in the renal medulla by dephosphorylating threonine 495 in diabetic rats.[60] Phosphorylation of NOS 3 at this amino acid may be significant because threonine 495 has been proposed to be a "switch" that determines whether NOS 3 produces NO or superoxide.[61] Thus, measurements of bioavailable NO are crucial to determine the physiological significance of increased NOS phosphorylation at this amino acid.

Compared with our understanding of the role of phosphorylation of specific amino acids in NOS 3, our knowledge of NOS 1 and 2 is minimal. NOS 1 is known to be constitutively phosphorylated at serine 741, and dephosphorylation at this amino acid increases enzyme activity.[62] In addition, phosphorylation at serine 847 can attenuate the catalytic activity of the enzyme in neuronal cells.[63] Presumably, homologous amino acids in the different NOS isoforms have the potential to be phosphorylated; however, the effect of phosphorylating such putative sites on NOS 1 and NOS 2 within the kidney has not been studied to our knowledge.

Hormonal Regulation of NOS

Renal NOS activity recently has been shown to be acutely regulated by several humoral factors. Arima et al[64] reported that aldosterone can activate NOS 3 in renal arterioles, leading to NO production; however, the mechanism involved was not investigated. Taylor et al[65] found that in ET_B receptor-deficient rats, medullary NOS activity and renal endothelin production are decreased, indicating that renal endothelin regulates NOS 3 activity as reported for the thick ascending limb.[66] Mori et al[67] found that vasopressin stimulated a rapid increase in intracellular NO via increased intracellular calcium levels in the inner medullary collecting duct. Although they did not assess which NOS isoform was activated, NOS 3 is likely responsible because of its high expression in the collecting duct. Finally, angiotensin II acutely regulates NO production by NOS 1 in the macula densa, apparently because of increased intracellular calcium.[29] However, the physiological significance of this finding is unclear, because angiotensin II enhances tubuloglomerular feedback, whereas NO inhibits it.

Regulation of Expression

Chronic changes in NOS expression may be important in conditions such as high salt intake and diabetes. High salt increases the expression of all 3 NOS isoforms in the medulla.[68] The mechanisms by which this occurs have not been worked out for NOS 1 and 2, but have been defined for NOS 3 in medullary thick ascending limbs.[69] High salt increased outer medullary osmolality and hyperosmolality enhanced NOS 3 expression in primary thick ascending limb cultures, and an ET_B receptor antagonist could block this effect. Hyperosmolality also enhanced endothelin-1 release. Finally, in vivo a dual ET_A/ET_B antagonist blocked the effects of high salt on NOS 3 expression.[69] In addition, a low-sodium diet causes chaperone heat shock protein 90 to relocate from the apical to the basolateral side of the thick ascending limb.[70] Because NOS 3 is known to interact with heat shock protein 90, leading to increased enzyme activity,[51,71] heat shock protein 90 may play a role in regulation of NOS 3 expression by salt intake.

Exposure to endothelin-1 alone also augments NOS 3 expression in the thick ascending limb.[72] Similarly, endothelin-1 stimulates NOS 3 expression in inner medullary collecting ducts.[73] However, unlike the thick ascending limb where the effect was mediated only by ET_B receptors,[72] in the inner medullary collecting duct both ET_A and ET_B were involved. Osmotic stimuli have also been shown to increase NOS 3 expression in inner medullary collecting ducts in culture.[74] This may be important for regulation of function in the renal medulla, where osmolality is extremely variable and dependent on salt and water intake. Given that similar factors regulate NOS 3 expression in the inner medullary collecting duct and thick ascending limb, high salt may induce expression by similar mechanisms in both cell types.

Changes in NOS expression may also be important in diabetes. High glucose increases NOS 2 mRNA and protein expression in mesangial cells in the presence of cytokines.[75] The increase was mediated by protein kinase C. Transcriptional/translational regulation of NOS 2 in mesangial cells by glucose may also involve JAK2, p38 MAPK, and nuclear factor $\kappa\beta$, which have been shown to regulate NOS 2 expression in renal epithelial cells.[76]

Repression of NOS 2 transcription may be just as important as induction in controlling the final amount of NOS 2 protein. Yu and Kone[77] demonstrated that treating mesangial cells with DNA methylation inhibitors augmented cytokine induction of endogenous NO production and NOS 2 protein. In vitro methylation of the NOS 2 promoter blunted its

activity, whereas inhibition of DNA methyltransferase increased NOS 2 promoter activity and nitrate production. Moreover, in vitro methylation inhibited binding of nuclear factor κB to the NOS 2 enhancer element. These results suggest that cytosine methylation is an important repressor of NOS 2 transcription in these cells. Whether NOS 1 and 3 expressions are regulated in diabetes is unclear and the mechanisms involved have not been extensively studied.

Other Regulators of NOS Activity

In addition to the regulators described, several other compounds may modulate NOS activity in the kidney. The most important of these in terms of hypertension may be the endogenous L-arginine analogues such as asymmetrical dimethylarginine. This compound inhibits NOS activity,[78–80] and circulating concentrations are elevated in hypertension[81,82] and by high salt intake.[83] Carbon monoxide produced by heme oxygenase has recently been recognized as a NOS regulator,[84,85] although the nature of this interaction is still unclear. Several drugs may have a marked impact on NO production. Dobrian et al[86] recently reported that the peroxisome proliferator-activated receptor-γ agonist and insulin sensitizer pioglitazone prevented the development of hypertension in obese hypertensive rats. This therapeutic effect was at least partially attributed to increased renal NO production and bioavailability caused by decreased superoxide generation. However, the exact site of NO production is unknown, because PPARγ receptors are present in many renal cells that express NOS and produce NO.[87–90] Finally, reactive oxygen species production by the kidney and their role in scavenging NO have recently received a great deal of attention.[91] Superoxide plays a quintessential role in determining NO bioavailability and thus its effect. This topic is beyond the scope of this review and has recently been reviewed.[91,92]

Perspectives

The complexity of the kidney, with its nearly 20 different tissue types arranged in a geometry that also impacts on function, has slowed progress of both cellular and whole-animal approaches to understanding the role of NO in regulating renal function. To study the contribution of the various NOS isoforms, 2 lines of research have been followed: (1) assessment of renal function using isolated cells and individual segments; and (2) studies of renal function in vivo. Part of our limitation in understanding NOS regulation comes from our inability to measure biologically active NO in intact systems. This is important because: (1) protein expression does not necessarily correlate with enzyme activity and thus NO production; (2) the assay normally used to assess enzyme activity (conversion of L-arginine to L-citrulline) requires the addition of substrate and cofactors, and thus regulation of enzyme activity by decreased substrate or cofactor availability may be missed; and (3) enzyme activity may not represent bioavailable NO, because NO can rapidly be scavenged by other substances such as superoxide. Although much progress has been made regarding our understanding of NOS regulation within the kidney, in some instances the information was obtained from nonrenal cells and in vitro systems, and thus more research is required to fully understand the mechanisms whereby NOS is regulated within the different structures in the kidney as well as their physiological significance. New technology that allows deletion or expression of a particular gene in specific tissues in the kidney is an important scientific advance. Over the past few years, various animal models have been developed using such technology[13,93–95] that provide new means of studying the physiological actions of a single NOS isoform in a single cell type at a specific point in time in vivo.

Acknowledgments

This work was supported by grants from the National Institutes of Health (HL 28982 and HL 070985) to J.G.

References

1. Persson PB. Nitric oxide in the kidney. *Am J Physiol Regul Integr Comp Physiol*. 2002;283:R1005–R1007.
2. Jin XH, McGrath HE, Gildea JJ, Siragy HM, Felder RA, Carey RM. Renal interstitial guanosine cyclic 3′,5′-monophosphate mediates pressure-natriuresis via protein kinase G. *Hypertension*. 2004;43:1133–1139.
3. Wang H, Carretero OA, Garvin JL. Nitric oxide produced by THAL nitric oxide synthase inhibits transforming growth factor (TGF). *Hypertension*. 2002;39:662–666.
4. Majid DS, Nishiyama A. Nitric oxide blockade enhances renal responses to superoxide dismutase inhibition in dogs. *Hypertension*. 2002;39:293–297.
5. Mattson DL, Lu S, Nakanishi K, Papanek PE, Cowley AW Jr. Effect of chronic renal medullary nitric oxide inhibition on blood pressure. *Am J Physiol*. 1994;266:H1918–H1926.
6. Racasan S, Braam B, van der Giezen DM, Goldschmeding R, Boer P, Koomans HA, Joles JA. Perinatal L-arginine and antioxidant supplements reduce adult blood pressure in spontaneously hypertensive rats. *Hypertension*. 2004;44:83–88.
7. Nishimoto Y, Tomida T, Matsui H, Ito T, Okumura K. Decrease in renal medullary endothelial nitric oxide synthase of fructose-fed, salt-sensitive hypertensive rats. *Hypertension*. 2002;40:190–194.
8. Szentivanyi M Jr, Zou AP, Mattson DL, Soares P, Moreno C, Roman RJ, Cowley AW Jr. Renal medullary nitric oxide deficit of Dahl S rats enhances hypertensive actions of angiotensin II. *Am J Physiol Regul Integr Comp Physiol*. 2002;283:R266–R272.
9. Cervenka L, Kramer HJ, Maly J, Heller J. Role of nNOS in regulation of renal function in angiotensin II-induced hypertension. *Hypertension*. 2001;38:280–285.
10. Navar LG, Inscho EW, Majid SA, Imig JD, Harrison-Bernard LM, Mitchell KD. Paracrine regulation of the renal microcirculation. *Physiol Rev*. 1996;76:425–536.
11. Gabbai FB, Blantz RC. Role of nitric oxide in renal hemodynamics. *Semin Nephrol*. 1999;19:242–250.
12. Ortiz PA, Garvin JL. Role of nitric oxide in the regulation of nephron transport. *Am J Physiol Renal Physiol*. 2002;282:F777–F784.
13. Ortiz PA, Hong NJ, Wang D, Garvin JL. Gene transfer of eNOS to the thick ascending limb of eNOS-KO mice restores the effects of L-arginine on NaCl absorption. *Hypertension*. 2003;42:674–679.
14. Sasaki S, Siragy HM, Gildea JJ, Felder RA, Carey RM. Production and role of extracellular guanosine cyclic 3′,5′ monophosphate in sodium uptake in human proximal tubule cells. *Hypertension*. 2004;43:286–291.
15. Schnackenberg CG, Tabor BL, Strong MH, Granger JP. Inhibition of intrarenal NO stimulates renin secretion through a macula densa-mediated mechanism. *Am J Physiol*. 1997;272:R879–R886.
16. Juncos LA, Garvin J, Carretero OA, Ito S. Flow modulates myogenic responses in isolated microperfused rabbit afferent arterioles via endothelium-derived nitric oxide. *J Clin Invest*. 1995;95:2741–2748.
17. Brands MW, Bell TD, Gibson B. Nitric oxide may prevent hypertension early in diabetes by counteracting renal actions of superoxide. *Hypertension*. 2004;43:57–63.
18. Thomson SC, Deng A, Komine N, Hammes JS, Blantz RC, Gabbai FB. Early diabetes as a model for testing the regulation of juxtaglomerular NOS I. *Am J Physiol Renal Physiol*. 2004;287:F732–F738.

19. Li C, Liu J, Saavedra JE, Keefer LK, Waalkes MP. The nitric oxide donor, V-PYRRO/NO, protects against acetaminophen-induced nephrotoxicity in mice. *Toxicology*. 2003;189:173–180.
20. Prabhakar SS. Role of nitric oxide in diabetic nephropathy. *Semin Nephrol*. 2004;24:333–344.
21. Noiri E, Satoh H, Taguchi J, Brodsky SV, Nakao A, Ogawa Y, Nishijima S, Yokomizo T, Tokunaga K, Fujita T. Association of eNOS Glu298Asp polymorphism with end-stage renal disease. *Hypertension*. 2002;40: 535–540.
22. Alexander BT, Llinas MT, Kruckeberg WC, Granger JP. L-arginine attenuates hypertension in pregnant rats with reduced uterine perfusion pressure. *Hypertension*. 2004;43:832–836.
23. Conrad KP. Possible mechanisms for changes in renal hemodynamics during pregnancy: studies from animal models. *Am J Kidney Dis*. 1987; 9:253–259.
24. Alexander BT, Miller MT, Kassab S, Novak J, Reckelhoff JF, Kruckeberg WC, Granger JP. Differential expression of renal nitric oxide synthase isoforms during pregnancy in rats. *Hypertension*. 1999;33: 435–439.
25. Sladek SM, Magness RR, Conrad KP. Nitric oxide and pregnancy. *Am J Physiol*. 1997;272:R441–R463.
26. Kassab S, Miller MT, Hester R, Novak J, Granger JP. Systemic hemodynamics and regional blood flow during chronic nitric oxide synthesis inhibition in pregnant rats. *Hypertension*. 1998;31:315–320.
27. Nakane H, Miller FJ, Jr., Faraci FM, Toyoda K, Heistad DD. Gene transfer of endothelial nitric oxide synthase reduces angiotensin II-induced endothelial dysfunction. *Hypertension*. 2000;35:595–601.
28. Plato CF, Shesely EG, Garvin JL. eNOS mediates L-arginine-induced inhibition of thick ascending limb chloride flux. *Hypertension*. 2000;35: 319–323.
29. Liu R, Persson AE. Angiotensin II stimulates calcium and nitric oxide release from macula densa cells through AT1 receptors. *Hypertension*. 2004;43:649–653.
30. Zhang Z, Pallone TL. Response of descending vasa recta to luminal pressure. *Am J Physiol Renal Physiol*. 2004;287:F535–F542.
31. Nathan C, Xie QW. Regulation of biosynthesis of nitric oxide. *J Biol Chem*. 1994;269:13725–13728.
32. Wang H, Carretero OA, Garvin JL. Inhibition of apical Na+/H+ exchangers on the macula densa cells augments tubuloglomerular feedback. *Hypertension*. 2003;41:688–691.
33. Liu R, Carretero O, Ren YL, and Garvin J. Increased intracellular pH at the macula densa activates nNOS during tubuloglomerular feedback. *Kidney Int*. 2005;41(3 Pt 2):688–91.
34. Prabhakar SS. Inhibition of mesangial iNOS by reduced extracellular pH is associated with uncoupling of NADPH oxidation. *Kidney Int*. 2002; 61:2015–2024.
35. Gorren AC, Schrammel A, Schmidt K, Mayer B. Effects of pH on the structure and function of neuronal nitric oxide synthase. *Biochem J*. 1998;331:801–807.
36. Mori T, Cowley AW Jr. Renal oxidative stress in medullary thick ascending limbs produced by elevated NaCl and glucose. *Hypertension*. 2004;43:341–346.
37. Taylor N, Cowley AW Jr. Mechanism of NOS uncoupling in the renal medulla of Dahl S rats. *58th annual fall conference and scientific sessions of the council for high blood* p*ressure research.* 2004;Abstract.
38. Fujii S, Zhang L, Igarashi J, Kosaka H. L-arginine reverses p47phox and gp91phox expression induced by high salt in Dahl rats. *Hypertension*. 2003;42:1014–1020.
39. Kakoki M, Wang W, Mattson DL. Cationic amino acid transport in the renal medulla and blood pressure regulation. *Hypertension*. 2002;39: 287–292.
40. Low BC, Grigor MR. Angiotensin II stimulates system y+ and cationic amino acid transporter gene expression in cultured vascular smooth muscle cells. *J Biol Chem*. 1995;270:27577–27583.
41. Fisslthaler B, Dimmeler S, Hermann C, Busse R, Fleming I. Phosphorylation and activation of the endothelial nitric oxide synthase by fluid shear stress. *Acta Physiol Scand*. 2000;168:81–88.
42. Jaffrey SR, Snyder SH. PIN: an associated protein inhibitor of neuronal nitric oxide synthase. *Science*. 1996;274:774–777.
43. Hemmens B, Woschitz S, Pitters E, Klosch B, Volker C, Schmidt K, Mayer B. The protein inhibitor of neuronal nitric oxide synthase (PIN): characterization of its action on pure nitric oxide synthases. *FEBS Lett*. 1998;430:397–400.
44. Sato Y, Sagami I, Shimizu T. Identification of caveolin-1-interacting sites in neuronal nitric-oxide synthase. Molecular mechanism for inhibition of NO formation. *J Biol Chem*. 2004;279:8827–8836.
45. Venema VJ, Ju H, Zou R, Venema RC. Interaction of neuronal nitric-oxide synthase with caveolin-3 in skeletal muscle. Identification of a novel caveolin scaffolding/inhibitory domain. *J Biol Chem*. 1997;272: 28187–28190.
46. Kone BC, Kuncewicz T, Zhang W, Yu ZY. Protein interactions with nitric oxide synthases: controlling the right time, the right place, and the right amount of nitric oxide. *Am J Physiol Renal Physiol*. 2003;285: F178–F190.
47. Roczniak A, Levine DZ, Burns KD. Localization of protein inhibitor of neuronal nitric oxide synthase in rat kidney. *Am J Physiol Renal Fluid Electrol Physiol*. 2000;278:F702–F707.
48. Zhang W, Kuncewicz T, Yu ZY, Zou L, Xu X, Kone BC. Protein-protein interactions involving inducible nitric oxide synthase. *Acta Physiol Scand*. 2003;179:137–142.
49. Kuncewicz T, Balakrishnan P, Snuggs MB, Kone BC. Specific association of nitric oxide synthase-2 with Rac isoforms in activated murine macrophages. *Am J Physiol Renal Physiol*. 2001;281:F326–F336.
50. Ju H, Zou R, Venema VJ, Venema RC. Direct interaction of endothelial nitric-oxide synthase and caveolin-1 inhibits synthase activity. *J Biol Chem*. 1997;272:18522–18525.
51. Garcia-Cardena G, Fan R, Shah V, Sorrentino R, Cirino G, Papapetropoulos A, Sessa WC. Dynamic activation of endothelial nitric oxide synthase by Hsp90. *Nature*. 1998;392:821–824.
52. Dedio J, Konig P, Wohlfart P, Schroeder C, Kummer W, Muller-Esterl W. NOSIP, a novel modulator of endothelial nitric oxide synthase activity. *FASEB J*. 2001;15:79–89.
53. Zimmermann K, Opitz N, Dedio J, Renne C, Muller-Esterl W, Oess S. NOSTRIN: a protein modulating nitric oxide release and subcellular distribution of endothelial nitric oxide synthase. *Proc Natl Acad Sci U S A*. 2002;99:17167–17172.
54. Ortiz PA, Hong NJ, Garvin JL. Luminal flow induces eNOS activation and translocation in the rat thick ascending limb: role of PI3-kinase and Hsp90. *Am J Physiol Renal Physiol*. 2004;287:F281–F288.
55. Michell BJ, Chen Z, Tiganis T, Stapleton D, Katsis F, Power DA, Sim AT, Kemp BE. Coordinated control of endothelial nitric-oxide synthase phosphorylation by protein kinase C and the cAMP-dependent protein kinase. *J Biol Chem*. 2001;276:17625–17628.
56. Fleming I, Busse R. Molecular mechanisms involved in the regulation of the endothelial nitric oxide synthase. *Am J Physiol Regul Integr Comp Physiol*. 2003;284:R1–R12.
57. Cai Z, Xin J, Pollock DM, Pollock JS. Shear stress-mediated NO production in inner medullary collecting duct cells. *Am J Physiol Renal Physiol*. 2000;279:F270–F274.
58. Ortiz PA, Hong NJ, Garvin JL. Luminal flow induces eNOS activation and translocation in the rat thick ascending limb. *Am J Physiol Renal Physiol*. 2004;287:F274–F288.
59. Bauer PM, Fulton D, Boo YC, Sorescu GP, Kemp BE, Jo H, Sessa WC. Compensatory phosphorylation and protein-protein interactions revealed by loss of function and gain of function mutants of multiple serine phosphorylation sites in endothelial nitric-oxide synthase. *J Biol Chem*. 2003;278:14841–14849.
60. Lee DL, Sasser JM, Hobbs JL, Boriskie A, Pollock DM, Carmines PK, Pollock JS. Posttranslational regulation of NO synthase activity in the renal medulla of diabetic rats. *Am J Physiol Renal Physiol*. 2005;288: F82–F90.
61. Lin MI, Fulton D, Babbitt R, Fleming I, Busse R, Pritchard KA, Jr., Sessa WC. Phosphorylation of threonine 497 in endothelial nitric-oxide synthase coordinates the coupling of L-arginine metabolism to efficient nitric oxide production. *J Biol Chem*. 2003;278:44719–44726.
62. Song T, Hatano N, Horii M, Tokumitsu H, Yamaguchi F, Tokuda M, Watanabe Y. Calcium/calmodulin-dependent protein kinase I inhibits neuronal nitric-oxide synthase activity through serine 741 phosphorylation. *FEBS Lett*. 2004;570:133–137.
63. Komeima K, Hayashi Y, Naito Y, Watanabe Y. Inhibition of neuronal nitric-oxide synthase by calcium/ calmodulin-dependent protein kinase II alpha through Ser847 phosphorylation in NG108–15 neuronal cells. *J Biol Chem*. 2000;275:28139–28143.
64. Arima S, Kohagura K, Xu HL, Sugawara A, Uruno A, Satoh F, Takeuchi K, Ito S. Endothelium-derived nitric oxide modulates vascular action of aldosterone in renal arteriole. *Hypertension*. 2004;43:352–357.

65. Taylor TA, Gariepy CE, Pollock DM, Pollock JS. Gender differences in ET and NOS systems in ETB receptor-deficient rats: effect of a high salt diet. *Hypertension*. 2003;41:657–662.
66. Plato CF, Pollock DM, Garvin JL. Endothelin inhibits thick ascending limb chloride flux via ET(B) receptor-mediated NO release. *Am J Physiol Renal Fluid Electrol Physiol*. 2000;279:F326–F333.
67. Mori T, Dickhout JG, Cowley AW Jr. Vasopressin increases intracellular NO concentration via Ca(2+) signaling in inner medullary collecting duct. *Hypertension*. 2002;39:465–469.
68. Mattson DL, Higgins DJ. Influence of dietary sodium intake on renal medullary nitric oxide synthase. *Hypertension*. 1996;27:688–692.
69. Herrera M, Garvin JL. A high-salt diet stimulates thick ascending limb eNOS expression by raising medullary osmolality and increasing release of endothelin-1. *Am J Physiol Renal Physiol*. 2005;288:F58–F64.
70. Ramirez V, Uribe N, Garcia-Torres R, Castro C, Rubio J, Gamba G, Bobadilla NA. Upregulation and intrarenal redistribution of heat shock proteins 90alpha and 90beta by low-sodium diet in the rat. *Cell Stress Chaperones*. 2004;9:198–206.
71. Fontana J, Fulton D, Chen Y, Fairchild TA, McCabe TJ, Fujita N, Tsuruo T, Sessa WC. Domain mapping studies reveal that the M domain of hsp90 serves as a molecular scaffold to regulate Akt-dependent phosphorylation of endothelial nitric oxide synthase and NO release. *Circ Res*. 2002;90:866–873.
72. Herrera M, Garvin JL. Endothelin stimulates endothelial nitric oxide synthase expression in the thick ascending limb. *Am J Physiol Renal Physiol*. 2004;287:F231–F235.
73. Ye Q, Chen S, Gardner DG. Endothelin inhibits NPR-A and stimulates eNOS gene expression in rat IMCD cells. *Hypertension*. 2003;41:675–681.
74. Chen S, Cao L, Intengan HD, Humphreys M, Gardner DG. Osmoregulation of endothelial nitric-oxide synthase gene expression in inner medullary collecting duct cells. Role in activation of the type A natriuretic peptide receptor. *J Biol Chem*. 2002;277:32498–32504.
75. Noh H, Ha H, Yu MR, Kang SW, Choi KH, Han DS, Lee HY. High glucose increases inducible NO production in cultured rat mesangial cells. Possible role in fibronectin production. *Nephron*. 2002;90:78–85.
76. Poljakovic M, Nygren JM, Persson K. Signalling pathways regulating inducible nitric oxide synthase expression in human kidney epithelial cells. *Eur J Pharmacol*. 2003;469:21–28.
77. Yu Z, Kone BC. Hypermethylation of the inducible nitric-oxide synthase gene promoter inhibits its transcription. *J Biol Chem*. 2004;279:46954–46961.
78. Vallance P, Leiper J. Cardiovascular biology of the asymmetric dimethylarginine:dimethylarginine dimethylaminohydrolase pathway. *Arterioscler Thromb Vasc Biol*. 2004;24:1023–1030.
79. Osanai T, Saitoh M, Sasaki S, Tomita H, Matsunaga T, Okumura K. Effect of shear stress on asymmetric dimethylarginine release from vascular endothelial cells. *Hypertension*. 2003;42:985–990.
80. Kielstein JT, Simmel S, Bode-Boger SM, Roth HJ, Schmidt-Gayk H, Haller H, Fliser D. Subpressor dose asymmetric dimethylarginine modulates renal function in humans through nitric oxide synthase inhibition. *Kidney Blood Press Res*. 2004;27:143–147.
81. Takiuchi S, Fujii H, Kamide K, Horio T, Nakatani S, Hiuge A, Rakugi H, Ogihara T, Kawano Y. Plasma asymmetric dimethylarginine and coronary and peripheral endothelial dysfunction in hypertensive patients. *Am J Hypertens*. 2004;17:802–808.
82. Fujiwara N, Osanai T, Kamada T, Katoh T, Takahashi K, Okumura K. Study on the relationship between plasma nitrite and nitrate level and salt sensitivity in human hypertension: modulation of nitric oxide synthesis by salt intake. *Circulation*. 2000;101:856–861.
83. Osanai T, Fujiwara N, Saitoh M, Sasaki S, Tomita H, Nakamura M, Osawa H, Yamabe H, Okumura K. Relationship between salt intake, nitric oxide and asymmetric dimethylarginine and its relevance to patients with end-stage renal disease. *Blood Purif*. 2002;20:466–468.
84. Rodriguez F, Lamon BD, Gong W, Kemp R, Nasjletti A. Nitric oxide synthesis inhibition promotes renal production of carbon monoxide. *Hypertension*. 2004;43:347–351.
85. Wang T, Sterling H, Shao WA, Yan Q, Bailey MA, Giebisch G, Wang WH. Inhibition of heme oxygenase decreases sodium and fluid absorption in the loop of Henle. *Am J Physiol Renal Physiol*. 2003;285:F484–F490.
86. Dobrian AD, Schriver SD, Khraibi AA, Prewitt RL. Pioglitazone prevents hypertension and reduces oxidative stress in diet-induced obesity. *Hypertension*. 2004;43:48–56.
87. Nicholas SB, Kawano Y, Wakino S, Collins AR, Hsueh WA. Expression and function of peroxisome proliferator-activated receptor-gamma in mesangial cells. *Hypertension*. 2001;37:722–727.
88. Yang T, Michele DE, Park J, Smart AM, Lin Z, Brosius FC, III, Schnermann JB, Briggs JP. Expression of peroxisomal proliferator-activated receptors and retinoid X receptors in the kidney. *Am J Physiol*. 1999;277:F966–F973.
89. Guan Y, Zhang Y, Schneider A, Davis L, Breyer RM, Breyer MD. Peroxisome proliferator-activated receptor-gamma activity is associated with renal microvasculature. *Am J Physiol Renal Physiol*. 2001;281:F1036–F1046.
90. Guan Y, Zhang Y, Davis L, Breyer MD. Expression of peroxisome proliferator-activated receptors in urinary tract of rabbits and humans. *Am J Physiol*. 1997;273:F1013–F1022.
91. Ortiz PA, Garvin JL. Interaction of O2− and NO in the thick ascending limb. *Hypertension*. 2002;39:591–596.
92. Wilcox CS, Welch WJ. Interaction between nitric oxide and oxygen radicals in regulation of tubuloglomerular feedback. *Acta Physiol Scand*. 2000;168:119–124.
93. Lavoie JL, Lake-Bruse KD, Sigmund CD. Increased blood pressure in transgenic mice expressing both human renin and angiotensinogen in the renal proximal tubule. *Am J Physiol Renal Physiol*. 2004;286:F965–F971.
94. Ahn D, Ge Y, Stricklett PK, Gill P, Taylor D, Hughes AK, Yanagisawa M, Miller L, Nelson RD, Kohan DE. Collecting duct-specific knockout of endothelin-1 causes hypertension and sodium retention. *J Clin Invest*. 2004;114:504–511.
95. Ge Y, Ahn D, Stricklett PK, Hughes AK, Yanagisawa M, Verbalis JG, Kohan DE. Collecting duct-specific knockout of endothelin-1 alters vasopressin regulation of urine osmolality. *Am J Physiol Renal Physiol.* 2005. In press.