Nitric Oxide Synthesis in the Kidney: Isoforms, Biosynthesis, and Functions in Health

By Bruce C. Kone

Nitric oxide (NO) is a gaseous free radical that serves cell signaling, cellular energetics, host defense, and inflammatory functions in virtually all cells. In the kidney and vasculature, NO plays fundamental roles in the control of systemic and intrarenal hemodynamics, the tubuloglomerular feedback response, pressure natriuresis, release of sympathetic neurotransmitters and renin, and tubular solute and water transport. NO is synthesized from L-arginine by NO synthases (NOS). Because of its high chemical reactivity and high diffusibility, NO production by each of the 3 major NOS isoforms is regulated tightly at multiple levels from gene transcription to spatial proximity near intended targets to covalent modification and allosteric regulation of the enzyme itself. Many of these regulatory mechanisms have yet to be tested in renal cells. The NOS isoforms are distributed differentially and regulated in the kidney, and there remains some controversy over the specific expression of functional protein for the NOS isoforms in specific renal cell populations. Mice with targeted deletion of each of the NOS isoforms have been generated, and these each have unique phenotypes. Studies of the renal and vascular phenotypes of these mice have yielded important insights into certain vascular diseases, ischemic acute renal failure, the tubuloglomerular feedback response, and some mechanisms of tubular fluid and electrolyte transport, but thus far have been underexploited. This review explores the collective knowledge regarding the structure, regulation, and function of the NOS isoforms gleaned from various tissues, and highlights the progress and gaps in understanding in applying this information to renal and vascular physiology.

NITRIC OXIDE (NO) is an important molecular mediator of numerous physiologic processes in virtually every organ. In the kidney, NO plays prominent roles in the homeostatic regulation and integration of glomerular, vascular, and tubular function, as well as a variety of fundamental cellular functions, including cell proliferation, transcription, and energy metabolism.[1-4] In the vasculature, NO functions not only as a vasodilator, but also has antithrombotic, anti-inflammatory, antiproliferative, and antioxidant properties. Although NO serves beneficial roles as a messenger and host defense molecule, excessive NO production can be cytotoxic, the result of NO's reaction with reactive oxygen and nitrogen species, leading to peroxynitrite anion, nitroxyl radical, and hydroxyl radical production, and protein tyrosine nitration.[5,6] Excessive NO production contributes to the pathogenesis of a variety of renal and vascular diseases characterized by inflammation and injury, including glomerulonephritis,[7-9] tubulointerstitial renal disease,[10] postischemic renal failure,[11,12] radiocontrast nephropathy,[13] obstructive nephropathy,[14] and renal allograft rejection.[15,16] Many of these diseases are the subject of reviews in this issue of the journal.

NO exerts its actions by chemical modification of targets, preferentially interacting with thiol groups, transition metals, and free radicals. In addition to its well-characterized signaling effects mediated by cyclic guanosine monophosphate (cGMP), NO and several NO-derived species can S-nitrosylate cysteine residues in target proteins.[17] This redox-based posttranslational modification has been implicated in the cGMP-independent control of a broad spectrum of cellular functions, and may function in a manner akin to phosphorylation to regulate proteins.

NO SYNTHESIS AND NOS STRUCTURE-FUNCTION RELATIONSHIPS

NO is metabolized from L-arginine by the NOS isoforms in a complex reaction requiring molecular oxygen, the reduced form of nicotinamide-adenine dinucleotide phosphate (NADPH), flavin nucleotides, tetrahydrobiopterin (BH_4), a cytochrome P450-type heme moiety, and calmodulin (Fig 1).[18] The 3 principal NOS isoforms—neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS)—are encoded by different genes and share ~55% to 60% amino acid homology (Table 1). A mitochondrial NOS also has been reported,[19] and it consistently was identified recently as full-

From the Division of Renal Diseases and Hypertension, The University of Texas Health Sciences Center at Houston, Houston, TX.

Address reprint requests to Bruce C. Kone, MD, FACP, FCP, FAHA, Division of Renal Diseases and Hypertension, The University of Texas Health Sciences Center at Houston, 6431 Fannin, MSB 4.138, Houston, TX 77030. Email: Bruce.C.Kone@uth.tmc.edu

0270-9295/04/2404-0002$30.00/0

doi:10.1016/j.semnephrol.2004.04.002

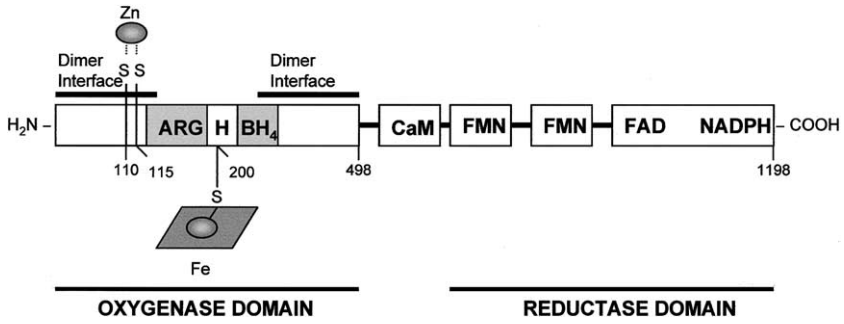

Fig 1. Structural organization of NOS. Binding sites for L-arginine (ARG), CAM, calmodulin), BH_4, flavin mononucleotide (FMN), flavin adenine nucleotide (FAD), and NADPH are indicated. The position of the heme iron (FE) group and the disulfide bond (SS) in the coordinating zinc-tetrathiolate center positioned at the interface of the 2 monomers. This zinc-tetrathiolate center plays a key role in stabilizing intersubunit contacts and in maintaining the integrity of the BH_4 binding site of iNOS are shown. Amino acids are numbered.

length nNOS with specific posttranslational modifications. nNOS and eNOS are inactive until intracellular Ca^{2+} levels increase sufficiently to promote calmodulin binding. In contrast, iNOS binds calmodulin at resting intracellular Ca^{2+} concentrations, and thus its activity has been viewed typically as Ca^{2+} calmodulin independent. The genes and complementary DNAs encoding the 3 NOS isoforms have been cloned and characterized, the reaction mechanisms and crystal structures of the NOS isoforms have been solved, and mice bearing targeted deletions of each of the 3 major NOS isoforms and an nNOS/eNOS double knockout have been generated (Table 1).

All the NOS isoforms are NADPH- and calmodulin-dependent and contain consensus binding sites for flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), BH_4, and a heme complex (Fig 1). They are only active as homodimers. Nonetheless, they differ to a large extent in their cellular localization, regulation, catalytic properties, and inhibitor sensitivity. Structurally, NOS enzymes are modular proteins in which the N-terminal oxygenase domain, which contains binding sites for heme, BH_4, and L-arginine, is linked by a calmodulin-recognition site to a C-terminal reductase domain, which contains binding sites for FAD, FMN, and NADPH.[18] Electrons are donated by NADPH to the reductase domain of the enzyme and proceed via FAD and FMN redox carriers to the oxygenase domain. There they interact with the heme iron and BH_4 at the active site to catalyze the reaction of oxygen with L-arginine, generating citrulline and NO as products. Electron flow through the reductase domain requires the presence of bound Ca^{2+}/calmodulin. At subsaturating L-arginine or BH_4 concentrations or in the presence of certain NOS inhibitors, NOS can, under at least in vitro conditions, catalyze NADPH oxidation that is uncoupled from NO formation, forming superoxide anion.[20] It remains to be established whether these uncoupled reactions are biologically meaningful in vivo.

Crystal structures have been solved for truncated oxygenase domains of murine iNOS,[21] and for full-length human iNOS,[22,23] and eNOS.[23] Studies of iNOS and eNOS show conservation of quaternary structure, tertiary topology, and substrate and cofactor binding sites, and the importance of a coordinating zinc-tetrathiolate center,[22,23] in which a zinc is positioned at the interface of the 2 monomers and coordinated by 2 cysteines from each monomer. This zinc-tetrathiolate center plays a key role in stabilizing intersubunit contacts and in maintaining the integrity of the BH_4 binding site of iNOS. In the structure of the murine iNOS oxygenase domain (amino acid residues 66-498), Glu^{371} is critical for substrate binding and interactions of Arg^{375}, Trp^{457}, Trp^{455}, and Phe^{470} influence tetrahydrobiopterin binding.[24] Site-directed mutagenesis also has shown that iNOS Cys^{200} is essential for dimer stability,[25] and the regions R^{501} to A^{532} and I^{1121} to L^{1144} function as domains for calmodulin[26] and NADPH[27] binding, respectively.

Considerable functional information regarding the contributions of the individual NOS isoforms and their compensatory responses has been generated from studies of NOS isoforms knockout mice. The principal extrarenal and renal phenotypes of these mice are summarized in Table 1 and references therein.

Table 1. Nitric Oxide Synthases

Gene	Human Locus	Primary Tissue Distribution Under Basal Conditions	Intrarenal Protein Distribution Under Basal Conditions	Phenotype of Knockout Mice
nNOS 1439 aa	12q24.21	Neurons Skeletal muscle Macula densa segment Bronchial and tracheal epithelium	Macula densa[37,185] Collecting ducts[38,186] Renal pelvis[37]	*Renal* Defective proximal tubule HCO_3^- and fluid transport[155] *Extrarenal* Hypertrophy of the pyloric sphincter[187] Increased aggressive behavior in men[188] Bladder-urethral sphincter dysfunction[189] Relative protection from ischemic neurologic events[190] Accelerated neointimal formation and constrictive vascular remodeling[191] Impaired resetting of TGF[192]
INOS 1153 aa	17q11.2-q12	Bronchial airway epithelium, alveolar macrophages Ileum Uterus Platelets	Proximal tubule (functional evidence)[35]	*Renal* Kidneys protected against ischemic injury[193] Lower fluid and bicarbonate reabsorption in proximal tubules[35] Protection or proximal tubules from hypoxic injury[194] *Extrarenal* Increased susceptibility to infection with intracellular pathogens and less susceptibility to sepsis-induced hypotension[195] Limited infarct-sparing effect of late phase of ischemic preconditioning[196] Attenuated neointima formation after perivascular arterial injury[197]
eNOS 1203 aa	7q35-q36	Endothelium Hippocampal CA1 neurons Cardiac myocytes	Endothelium, including afferent, efferent arterioles and vasa recta[37] Proximal tubules Thick ascending limbs[198] Collecting ducts	*Renal* Hypertension, hyperlipidemia, insulin resistance[199] Impaired NaCl transport in TALH[198] *Extrarenal* More vulnerable to cerebral and myocardial ischemia[200] Bicuspid aortic valves, congenital septal defects, heart failure[201,202] Pulmonary hypertension with chronic, mild hypoxia[203] Deficient growth factor–induced angiogenesis[204] Markedly decreased bleeding times[79]

All NOS isoforms potentially are subject to inhibition by endogenously produced asymmetric dimethylarginines (ADMA), which are synthesized in vivo by N-methyltransferases, a family of enzymes that methylate L-arginine residues within specific proteins. Free ADMA is released during proteolysis of methylated proteins, and it can be detected in plasma and urine. ADMA is metabolized by the enzyme dimethylarginine dimethylaminohydrolase, and inhibition of dimethylarginine dimethylaminohydrolase activity results in increased ADMA levels. Plasma levels of ADMA are increased in end-stage kidney disease, and in other conditions characterized by endothelial dysfunction such as atherosclerosis, hypercholesterolemia, hypertension, and heart failure.[28,29]

NET CONSTRAINTS ON NO IMPACTING FUNCTION

In considering the influence of NO on renal and vasculature function, it is important to consider the collective constraints on its ability to mediate a functional change. Controls on NOS synthesis and degradation, L-arginine uptake, NOS dimerization and association with BH_4, signal transduction events, and presence of ADMA are all important factors in this regard. In addition, the sensitivity of soluble guanylyl cyclase,[30] and the end-product bioavailability of NO[31] (ie, is it bioinactivated from reaction with O_2^- or other reactive species?) must be considered.

LOCALIZATION OF NOS ISOFORMS IN KIDNEY

A variety of methods ranging from protein and messenger RNA (mRNA) localization methods to functional studies in isolated or in situ microperfused nephron segments have been used to identify NOS expression in the kidney. These results have been somewhat variable and not always internally consistent in terms of a correlation between the gene product identified and its function (usually judged by inhibitor sensitivity or function in knockout compared with wild-type mice). Ideally, multiple independent observers unequivocally would identify NOS isoform protein expression in specific regions of the intact kidney, and this would be correlated with functional studies of that region using NOS isoform-selective inhibitors, which are available for iNOS and nNOS, and/or knockout mice. This full set of criteria has been met infrequently (Table 1). The following examples highlight the problem. First, data generated about mRNA expression and distribution have not always been corroborated by protein expression data. For example, our laboratory[32,33] and others[34] reported basal expression of iNOS mRNA in the rat kidney, yet there has been no consistent demonstration of iNOS protein in the normal rat kidney. Although this simply may be an issue of sensitivity of the assays used for mRNA versus protein expression, it does highlight the need for caution for interpreting gene expression data. Second, there have been numerous examples of reports of NOS isoform protein expression in the kidney that have varied depending on the antibody used for the study. Finally, there have been a few functional studies that strongly have implicated the activity of a specific NOS isoform in a specific nephron segment, which has not been shown convincingly to express protein for that isoform. For example, studies in the in situ perfused proximal tubule in iNOS knockout mice clearly have shown a role for iNOS in fluid and HCO_3^- transport in this segment,[35] yet iNOS protein has not been observed consistently there.

With those caveats in mind, immunocytochemical studies have shown that all 3 isoforms of NOS are expressed in the kidney (Table 1). eNOS protein is expressed in renal vascular endothelial cells,[36] and nNOS protein has been found predominantly in epithelial cells of the macula densa,[36,37] principal cells of the collecting duct,[38] and renal pelvic sensory nerves.[39] iNOS protein is widely expressed after induction with endotoxin or proinflammatory stimuli in tubule epithelia, including the proximal tubule, thick ascending limb, and distal convoluted tubule.[34] Renal proximal tubules and inner medullary collecting duct cells can produce NO by means of expression of iNOS, but the true intrarenal locale of the expressed iNOS in vivo is unclear.[32]

NOS GENE PRODUCTS AND THEIR REGULATION

nNOS

Structure and Variants

The human nNOS gene (designated *NOS1* by the Human Genome Nomenclature Committee) resides at chromosome 12q24.2 and spans over 240 kb.[40] nNOS is expressed basally in diverse cell types and tissues, but predominantly neurons, skeletal muscle, and the macula densa segment. Expression derived from alternative promoters of the human nNOS gene direct tissue- and cell-specific expression. A transcription cluster of exon 1 variants enriched in neuronal tissues reside in one genomic region, whereas those enriched in skeletal muscle are grouped together in another genomic region 75 kb upstream.[41,42] A third transcriptional cluster remote from the other 2 clusters directs Leydig cell–specific transcription.[43] In addition, a calcium-responsive exon 2 promoter recently has been characterized in rodent nNOS.[44]

The major neuronal transcript comprises 29 exons and encodes a 160-kd protein. Several splice variants also yield functional protein. An insertion between exons 16 and 17 encodes 34 additional

amino acids.[45-47] This variant, termed *nNOS-μ*, is expressed in muscle complexed with α1-syntrophin and is co-expressed with nNOS in the pelvic plexus and bladder.[45,46] The testis-specific transcription cluster gives rise to an mRNA transcript comprised of 2 new 5′ exons (Tex1 and Tex2) spliced to exon 4 of the full-length nNOS, and encodes a 125-kd protein that lacks the protein inhibitor of NOS binding, and the PDZ protein interaction domain implicated in membrane localization. When stably expressed in Chinese hamster ovary–K1 cells, the 125-kd protein encoded by testis-specific nNOS (TnNOS) possesses NOS enzymatic activity comparable with that of the full-length nNOS (160 kd).

Regulation

Originally believed to be a constitutively expressed enzyme, nNOS is now known to be regulated by a variety of physiologic and pathologic stimuli. The neuronal transcriptional cluster of the human nNOS gene contains binding sites for several transcription factors, including activating protein 2 (AP-2), nuclear factor κ B (NF-κB), cAMP response element binding protein (CREB), and Ets.[40] Studies of nNOS promoter activity in neuronal and fibroblast cell lines revealed that Oct-2 transactivates the downstream promoter (designated 5.1),[48] whereas nerve growth factor induces transcription through the upstream promoter (designated 5′2).[49] CREB binds to 2 sites within the exon 2 5′-untranslated region (UTR) of mouse nNOS to activate transcription in cortical neurons.[44] Steroidogenic factor-1 also transactivates the nNOS gene through the exon 2 promoter.[50] The human testis transcriptional cluster also contains potential *cis*-regulatory elements,[51] but no functional characterization of this region has been published yet to our knowledge. nNOS also is subject to unique controls on translational efficiency. The highly structured nNOS 5′-UTRs contain *cis* RNA elements that modulate translational efficiency in vitro and in vivo.[52] Although nNOS expression appears to be regulated in the kidney during certain physiologic and pathophysiologic states, virtually nothing is known about the specific mechanisms involved.

Phosphorylation also may regulate nNOS. Protein kinases A, G, or C, or Ca^{2+}/calmodulin-dependent protein kinase all have been shown to phosphorylated purified nNOS and reduce its catalytic activity, whereas calcineurin-mediated dephosphorylation enhances nNOS catalytic activity.[53] Adenosine monophosphate (AMP)-activated protein kinase increases nNOS phosphorylation in human skeletal muscle, but its effects on nNOS activity are unknown.[54] Protein phosphatase-2 has been shown to dephosphorylate nNOS in vitro.[55] Further studies are needed to clarify the role and regulation of nNOS phosphorylation in intact renal cells.

Higher-order interactions of protein complexes with nNOS regulate the spatial distribution and activity of nNOS in various cell types. As for all the NOS isoforms, calmodulin serves as an allosteric activator. The N-terminus of nNOS contains a PDZ domain that associates with multiple proteins including α1-syntrophin,[56] postsynaptic density proteins PSD-93 and PSD-95,[57,58] and the muscle isoform of phosphofructokinase, the inhibitor protein CAPON (for *ca*rboxy-terminal *P*DZ ligand of nNOS),[59] carboxy-terminal-binding protein,[60] islet cell autoantigen 512,[61] and Ca^{2+}-adenosine triphosphatase plasma membrane Ca^{2+} ATPases (PMCA).[62] nNOS also interacts with caveolin-3 in skeletal muscle, where it appears to comprise a component of the dystrophin complex.[63-65] An 89 amino acid light-chain dynein protein termed *PIN* (for protein inhibitor of nNOS) binds to the N-terminus of nNOS and inhibits its activity.[66,67] The molecular chaperone heat shock protein (hsp) 90 has been shown to complex with nNOS and to activate NO production.[68] Finally, bradykinin B2 receptors[69] and α1A-adrenergic receptors[70] have been shown to interact with nNOS in vitro, although the full physiologic relevance of these associations are unknown. Presumably these interactions provide a mechanism by which the activity, spatial distribution, and proximity of nNOS to regulatory proteins and intended targets is governed, which provides specificity and controls potential cytotoxicity.

iNOS

The human iNOS gene on chromosome 17 spans 37 kb and contains 27 exons, with translation initiation encoded by exon 2.[71] Multiple transcription initiation sites and alternative splicing give rise to several forms of exon 1. In addition, cassette deletions give rise to multiple alternative splice variants of human iNOS.[72] The functions of these variants in vivo remain to be established, but one

of them, which lacks exons 8 and 9, exhibits a functional reductase domain but fails to dimerize and to produce NO when heterologously expressed in cultured cells.[73]

Regulation

Multiple layers of control govern iNOS expression and activity, including changes in iNOS gene transcription; mRNA stability, translation, and degradation; substrate and cofactor binding and availability; and dimerization. Human iNOS is regulated tightly at the level of transcription, and the mechanisms of iNOS induction are cell, species, and stimulus specific. Cytokine combinations leading to iNOS induction vary between species and between cell types in the same species,[74,75] suggesting that tissue-specific regulation of human iNOS is important in determining its local physiologic and pathophysiologic roles. For example, the JAK/STAT pathway mediates the lipopolysaccharide plus interferon-γ–induced iNOS expression in RAW 264.7 macrophage cells,[74,75] yet inhibition of the Janus-activated kinase (JAK)/signal transducers and activators of transcription (STAT) pathway enhances iNOS induction by these same stimuli in rat aortic smooth muscle cells.[76] Moreover, the human iNOS promoter exhibits differential responsiveness to mixtures of the same 2 cytokines in different cell lines.[77] In one report, different patterns of altered chromatin structure were identified in different nonrenal epithelial cell lines treated with the same combination of cytokines.[78] Finally, depending on the cell type studied, different regions of the 5′-flanking sequence mediate cytokine induction of the human iNOS gene.[77-80] All of these findings suggest that cell- and stimulus-specific control of the human iNOS promoter may be important in determining its functional roles in different tissues in humans. Very little is known, however, about the mechanisms underlying this specificity.

The bulk of analysis of iNOS gene expression, including our own, has focused on the murine gene. Structure-function studies of the murine iNOS promoter have shown that the proximal 1 kb is sufficient to confer inducibility to lipopolysaccharide and interferon-$\tilde{\gamma}$[27] In contrast, a large span of the 5′-flanking region (from −3.8 to −16 kb) is required for cytokine-mediated iNOS induction in humans. A limited deletion analysis of the 5′-flanking sequence of the human iNOS gene has been performed by transfection of promoter-reporter gene constructs in cell lines from liver, lung, and colonic epithelium, but not kidney. From these limited data, the regulation of the human iNOS gene regulation appears to differ markedly from its rodent counterparts and the specific enhancers appear to be cell specific. More than 3.8 kb of the human iNOS 5′-flanking sequence is required for cytokine induction of reporter genes in both liver and colonic cell lines.[75,80] In DLD-1 colonic cells, sequences between −8.7 and −10.7 kb upstream from the transcription initiation site were necessary for cytokine responsiveness. In contrast, Spitsin et al[79] reported that sequences between −0.4 and −1.6 kb supported modest cytokine responsiveness in the A549 lung epithelial adenocarcinoma cell line, whereas Nunokawa et al[81] showed that 3.2 kb of the iNOS 5′-flanking sequence supported robust reporter gene expression in these same cells. Functionally important NF-κB–like sequences have been identified in the region of −5.2 to −6.1 kb[82] and −8.2 kb[83] in the human iNOS promoter. In addition, inducible AP-1 binding sites have been reported at −5.1 and −5.3 kb. Stat1, in response to interferon-γ, binds to a *cis*-acting DNA element at −5.2 kb in the human iNOS promoter, and a DNA element at −5.8 kb serves as a bifunctional motif that binds either STAT1 and/or NF-κB in response to cytokines.[84] Chromatin structure analysis in A549 and AKN-1 cells showed cytokine-inducible DNase I hypersensitive sites and in vivo footprints in the region of −5 to −5.5 kb.[78] Finally, in addition to coordinating interaction of transcription factors to impact iNOS gene transcription, we recently showed that hyperacetylation, governed by limiting or facilitating interactions with histone deacetylases, diminishes cytokine induction of murine iNOS transcriptional activity, at least in part, by restricting the functional efficacy of NF-κB.[85]

Changes in mRNA stability of the iNOS gene also have been implicated in the control of iNOS gene expression. The 3′-UTR of the human iNOS mRNA contains 4 AUUUA motifs and 1 AUUUUA motif. The embryonic lethal abnormal vision–like protein HuR was found to bind with high affinity to the AU-rich elements of the iNOS 3′-UTR and to stabilize this mRNA.[86] In other examples, interleukin-1β stabilizes iNOS mRNA in pancreatic β cells,[87] β-adrenergic stimulation enhances interleukin-1β induction of iNOS in cardiac fibroblasts by stabilizing the iNOS message,[88]

and BH_4 stabilizes the transcript in rat vascular smooth muscle cells.[89] In contrast, increased Ca^{2+} concentrations limited iNOS mRNA half-life in human articular chondrocytes,[90] and atrial natriuretic peptide (ANP) accelerated iNOS mRNA decay in macrophages.[91] In addition to these mechanisms, NO itself, in an autoinhibitory feedback loop mediated by cGMP, appears to destabilize its own mRNA.[92] Tyrosine kinases and phosphatases appear to be involved in posttranslational modification of iNOS as well, and potentially may play a role in modulating the functional activity of the enzyme.[93] Finally, it has been established that the proteasome is the primary degradation pathway for iNOS,[94] although it also has been observed that caveolin-1 complexes with iNOS and promotes iNOS proteolysis in human carcinoma cell lines.[95]

Several proteins that interact and regulate iNOS have been identified. Murine macrophages express a 110-kd protein that interacts with the N-terminus of iNOS, termed *NOS-associated protein-110 kd*, which inhibits iNOS catalytic activity by preventing dimerization.[96] A neural-specific cytosolic protein, kalirin, interacts with the first 70 amino acids of iNOS in yeast 2-hybrid assays and inhibits iNOS activity by preventing the formation of iNOS homodimers.[97] Our laboratory recently defined a regulatory interaction of iNOS with the Rac family of Rho-like guanosine triphosphatases (GTPases) that augments iNOS activity and controls its spatial distribution in activated macrophages.[98] Finally, iNOS, via its C-terminal final 3 amino acids, physically interacts with the apical membrane PDZ-domain protein, ezrin-radixin-moesin-binding phosphoprotein 50, in human proximal tubule epithelial cells.[99] This ezrin-radixin-moesin-binding phosphoprotein 50–iNOS interaction apparently serves to direct iNOS to the apical membrane and drive vectorial NO production at this surface, facilitating NO delivery to targets residing in this membrane microdomain, such as ion transporters.[99]

eNOS

The eNOS gene localizes to 7q35-36, spans approximately 21 kb of genomic DNA, contains 26 exons, and encodes an mRNA of 4,052 nucleotides.[100] No alternative splice variants for this isoform have been characterized yet. The gene is expressed in the endothelium of a variety of tissues, as well as in cardiac and myometrial myocytes, platelets, and in airway epithelium. Several genetic polymorphisms of the eNOS gene have been identified and their association with human disease states as susceptibility genes, including end-stage kidney disease and hypertension, have been studied. A GT substitution in exon 7 (at position 894) in codon 298 of the human eNOS gene alters the amino acid at this residue from glutamate to aspartate.[101] In vitro studies have shown that this mutation results in diminished NO production compared with the wild-type gene.[102] The Glu298Asp variant has been reported to correlate with increased coronary spasm, myocardial infarction, and essential hypertension in various populations.[103,104] Noiri et al[102] reported that the Glu298Asp variant of eNOS is a predisposing factor in end-stage renal disease, especially end-stage renal disease secondary to diabetic nephropathy. However, the population of subjects studied was relatively small, studies of much larger groups of patients are needed.

At the transcriptional level, the eNOS promoter lacks a TATA sequence and contains a single major transcription initiation site. The promoter/enhancer region includes a CCAAT box, several half-palindrome sequences for estrogen response elements, a shear stress response element, and potential binding sites for Sp1, AP-1, cyclic adenosine monophosphate response element, GATA, nuclear factor 1, γ-interferon response element, and NF-κB. Structure-function studies indicate that basal promoter activity in transfected endothelial cells requires an upstream Sp1 binding site, a GATA site at position -230, and a PEA3 site at -26.[105,106] Two tightly clustered positive regulatory domains at $-104/-95$ and $-144/-115$, to which Ets family members, Sp1, variants of Sp3, MAZ, and YY1 bind, regulate human eNOS promoter activity in endothelial cells (Fig 2).[107] A third positive regulatory domain resides 4.9 kb upstream from the transcription start site, and binds MZF-like, AP-2, Sp-1–related, and Ets-related factors.[108] Finally, studies in mice transgenic for a promoter-reporter construct containing 5.2 kb of the native murine eNOS promoter showed transgene expression that was restricted to large- and medium-sized blood vessels of the heart, lung, kidney, liver, spleen, and brain. Interestingly, the renal microvasculature, with the exception of the vasa recta of the renal medulla, showed no eNOS transgene expression.[109]

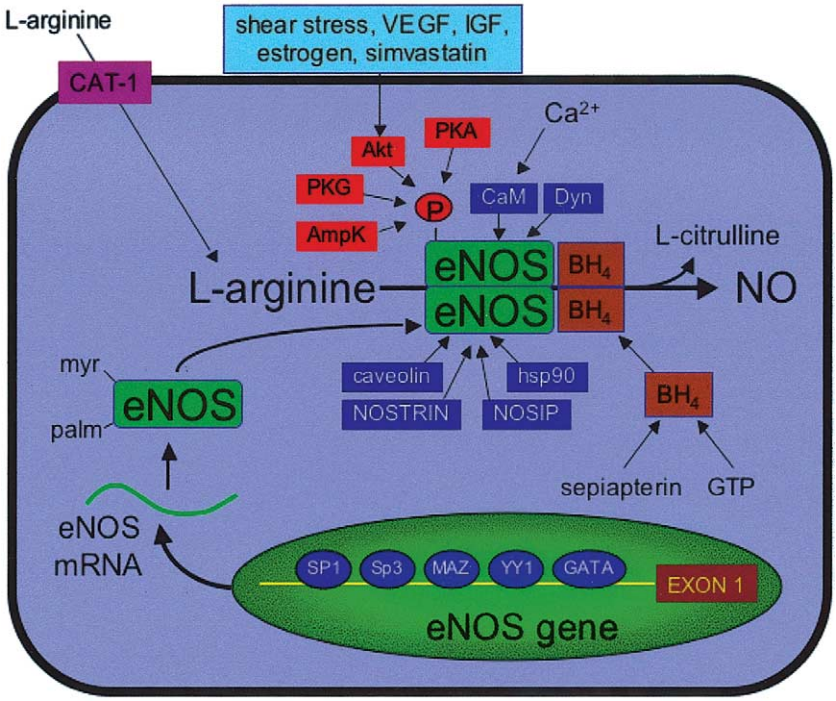

Fig 2. Regulation of endothelial NOS synthesis and activity. The eNOS gene is under transcriptional control by a variety of stimuli, including estrogen and glucocorticoids. Binding of several transcription factors to positive regulatory elements in the promoter/enhancer region of the eNOS gene regulate its transcription. Once synthesized, eNOS protein undergoes myristoylation (MYR) and palmitoylation (PALM), which efficiently targets the protein to the plasma membrane caveolae. There, eNOS interacts with a variety of regulatory proteins shown in blue boxes. Under basal conditions, eNOS is tethered to caveolin and inactive. With agonist-induced increases in intracellular Ca^{2+}, calmodulin binds eNOS and displaces caveolin in a mechanism facilitated by cooperative binding of hsp90, leading to activation of eNOS activity. Binding of the GTPase dynamin-2 (DYN) to eNOS also appears to be triggered by intracellular Ca^{2+} transients and to enhance eNOS activity. eNOS traffic inducer (NOSTRIN) and eNOS interacting protein (NOSIP) serve to promote translocation and inactivation of eNOS from the caveolae. ENOS also may be activated through phosphorylation by the protein kinases AKT, protein kinase A (PKA), protein kinase G (PKG), and AMP-dependent protein kinase (AMPK). In addition, the delivery of the substrate L-arginine via CAT-1–mediated uptake and the synthesis and binding of the important cofactor BH_4 are critical and regulated events for eNOS activity. See text for additional details and citations.

Shear stress, hypoxia, tumor necrosis factor-α, lysophosphatidylcholine, oxidized low-density lipoprotein, platelet-derived growth factor, basic fibroblast growth factor (bFGF), vascular endothelial growth factor, transforming growth factor-β1, oxidative stress, cyclosporine A, and angiotensin II[110] all have been shown to influence the level of eNOS gene expression in cultured endothelial cells. Estradiol has been shown to activate human eNOS promoter activity, in part via activation of the transcription factor Sp1, in cultured endothelial cells.[111,112] Similarly, a platelet-derived growth factor response element between −744 and −1,600 of the human eNOS gene appears to be important for eNOS transactivation in endothelial cells.[113] In contrast to these activating stimuli, glucocorticoids have been shown to decrease GATA binding activity and thereby limit the activity of a 3.5-kb human eNOS promoter-reporter gene construct. Finally, changes in mRNA stability also affect eNOS mRNA expression in several settings. Tumor necrosis factor α has been shown to destabilize eNOS mRNA,[152] whereas HMG-CoA reductase inhibitors, by blocking the geranylgeranylation of the GTPase Rho,[114] and oxidant stress,[115] prolong eNOS mRNA half-life.

At the posttranslational level, N-myristoylation and palmitoylation of eNOS are required for efficient eNOS targeting to caveolae of the endothelial cell membrane[116,117] and appear to facilitate optimal NO release (Fig 2). Similarly complex interactions with caveolins, calmodulin, and hsp90 gov-

ern eNOS activity (Fig 2). At resting intracellular calcium concentrations, eNOS is inhibited tonically through a stable interaction with caveolins.[65,118-121] Agonists, such as bradykinin or fluid shear stress, increase intracellular Ca^{2+} concentrations and cause calmodulin to bind and caveolin to dissociate from eNOS, resulting in an activated eNOS-calmodulin complex. Hsp90 appears to facilitate the calmodulin interaction with eNOS.[122] Inhibition of eNOS also occurs through interactions with membrane-proximal regions of intracellular domain 4 of several G protein–coupled receptors (the bradykinin B2, the angiotensin II AT, and the endothelin-1 endothelin B [ETB] receptors). Phosphorylation of the eNOS-interacting region of the bradykinin B2 receptor limits the binding interaction and reverses the inhibitory effect.[123] Other proteins, including eNOS interacting protein, a 34-kd protein that avidly binds to the carboxyl-terminal region (amino acids 366-486) of the eNOS oxygenase domain,[124] eNOS traffic inducer,[125] and the GTPase dynamin-2[126] apparently participate in the complex regulation of eNOS trafficking, targeting, and activity (Fig 2).

Phosphorylation plays an important role in eNOS activity (Fig 2). AMP-activated protein kinase has been shown to phosphorylate eNOS on Ser1177 in vitro and in rat heart during ischemia.[127] The enzyme also is phosphorylated in vitro on Ser633, Ser1177, and activated by cyclic adenosine monophosphate–dependent kinase and cGMP-dependent protein kinase II.[128] Bradykinin,[129] vascular endothelial growth factor, and shear stress trigger Akt-mediated phosphorylation of serine 1177/1179 on eNOS, leading to eNOS activation in vitro and in vivo.[130-132] Estrogen, through activation of phosphatidylinositol-3-OH kinase, stimulates Akt and, thereby, eNOS.[133] Ceramide and sphingosine-1-phosphate activate eNOS via Ca^{2+}-independent pathways.[134,135]

FUNCTIONS OF NO IN THE KIDNEY AND VASCULATURE

Effects of NO on Cellular Energetics

Studies in isolated mitochondria and intact cells showed that NO modulates mitochondrial QO_2, membrane potential, adenosine triphosphate production, and free radical generation (Fig 3).[136-142] NO potently, rapidly, and reversibly inhibits cytochrome oxidase and reduces the affinity of the enzyme for O_2.[136,143,144] In addition, full-length nNOS with unique posttranslational modifications is expressed in mitochondria of various tissues, including kidney, and produces NO under physiologic conditions.[145,146] Collectively, these data suggest that NO might serve as a physiologic regulator of cellular respiration. Garvin and Hong[147] reported that nanomolar concentrations of NO could potently and reversibly inhibit respiration renal tubules and isolated mitochondria. The sensitivity to NO-mediated inhibition of respiration was comparable between outer medullary and cortical tubules. These investigators concluded that competition between NO and O_2 to control respiration in the low P_{O_2} environment of the renal outer medulla might contribute to the vulnerability of this region to hypoxia.

NO's ability to inhibit cytochrome oxidase also shifts the electron transport chain to a more reduced state, which favors superoxide anion (O_2^-) formation at the level of complexes I[148] and III (Fig 3).[149] Depending on intracellular redox conditions, the O_2^- then can be converted by superoxide dismutase into hydrogen peroxide or react with NO to form peroxynitrite ($ONOO^-$).[150] These highly reactive species can alter solute transport pathways,[151,152] nitrate proteins, damage cell membranes, cause DNA fragmentation, and promote apoptosis (Fig 3).[153]

Effects of NO on Renal Solute Transport

By regulating the local renal circulation, the renal efferent and afferent nerve activity, and by direct tubular effects on fluid and electrolyte reabsorption, NO plays an important role in fluid and solute transport.[154] In the proximal tubule, NO has been reported to stimulate[155] net fluid and HCO_3^- flux, whereas only inhibitory effects of NO have been found on the Na^+/H^+ exchanger[156] and Na^+, K^+-adenosine triphosphatase activity[157,158] in this segment. Both nNOS and iNOS appear to participate in these responses. In nNOS knockout mice, both HCO_3^- absorption ($JHCO_3^-$) and fluid flux (Jv) in the proximal tubule are reduced significantly, and these animals develop metabolic acidosis (Table 1).[155] In iNOS knockout mice, $JHCO_3^-$ and Jv were each about 35% less than their wild-type controls. Moreover, addition of the iNOS-selective inhibitor L-*N*6-(1-iminoethyl) lysine, reduced both Jv and $JHCO_3^-$ significantly in wild-type, but not in iNOS knockout, mice.[35] In

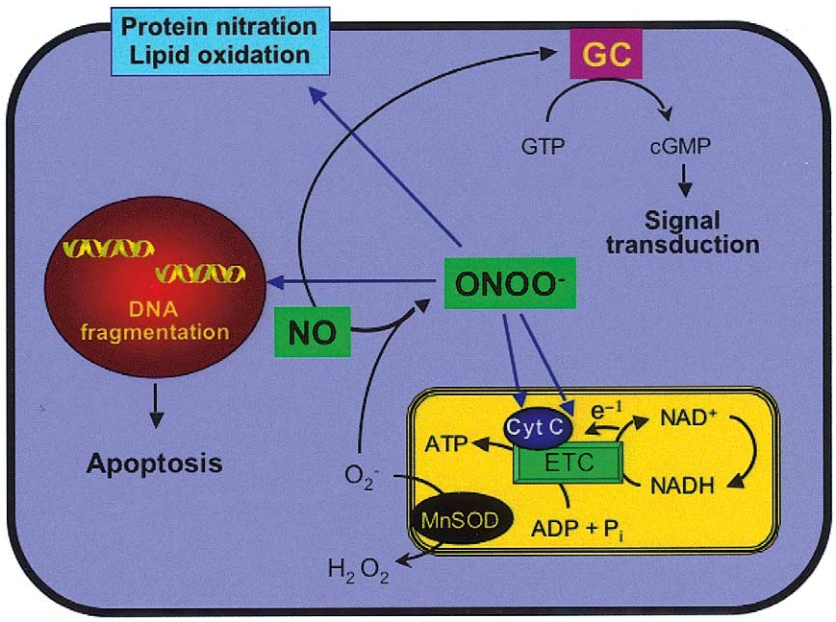

Fig 3. Effects of NO and peroxynitrite ($ONOO^-$) on cellular function. Many of NO's effects are mediated through its actions on guanylate cyclase (GC), which produces the second messenger molecule cGMP that elicits vasodilation and other responses. NO also inhibits cytochrome oxidase (CYT C) in the mitochondria, shifting the electron transport chain (ETC) to a more reduced state, which favors superoxide anion (O_2^-) formation. Depending on intracellular redox conditions, the O_2^- can then be converted by superoxide dismutase (MNSOD) into hydrogen peroxide or react with NO to form peroxynitrite ($ONOO^-$) These highly reactive species can cause DNA fragmentation and apoptosis, as well as protein nitration and lipid peroxidation, which damage cells.

contrast, neither $JHCO_3^-$ nor *J*v was significantly different from wild type in eNOS knockout mice.[35] In the aggregate, these results indicated that endogenous NO derived from both nNOS and iNOS serves to enhance fluid absorption and HCO_3^- transport, whereas eNOS does not directly modulate these parameters in the proximal tubule.

In the medullary thick ascending limb of Henle's loop (MTAL), NO inhibits net Cl^- and HCO_3^- absorption,[159-161] effects in part mediated by a direct inhibitory action of NO on the Na^+-K^+-$2Cl^-$ cotransporter[147] and the Na^+/H^+ exchanger.[159] In contrast, NO stimulates the activity of apical K^+ channels in this segment.[162] In the collecting duct, NO inhibits Na^+ absorption[163] and vasopressin-stimulated osmotic water permeability.[164] In addition, Lu et al[165,166] showed that NO inhibits apical Na^+ channels in the cortical collecting duct (CCD), and linked this mechanism to the inhibition of the basolateral small-conductance K^+ channel. Moreover, NO also has been reported to inhibit the H^+-adenosine triphosphatase of intercalated cells of the collecting duct,[167] and to mediate the stimulatory effect of angiotensin II on basolateral K-channel activity in the CCD.[168]

Effects of NO on the Renal Microcirculation

NO is an important modulator of glomerular and renal hemodynamics.[169] Intrarenal NO is responsible for up to one third of the normal renal blood flow and helps to maintain the low renal vascular resistance under normal conditions. Although NOS inhibition does not interfere with the basic autoregulatory mechanism of the glomerulus, it does decrease the absolute renal blood flow.[170] Studies of the renal microvascular responses to NOS inhibition in animal models have shown that tonically released NO regulates both the resistances of both the afferent and efferent arterioles. As a regulator of tubular reabsorptive function, NO also serves as a major mediator of arterial pressure–induced natriuretic responses in the kidney (see later).[171]

NO plays a key role in regulating perfusion of the renal medulla.[172-175] Local infusion of NOS inhibitors into animals reduces medullary blood flow, promotes salt retention, and leads to hyper-

tension. Conversely, L-arginine infusion increases NO, enhances medullary blood flow, and abrogates hypertension in these models. High salt intake has been shown to result in increased NO concentrations and NOS expression and activity selectively in the renal medulla.[172-175] Conversely, Dahl salt-sensitive rats exhibit a deficiency of NOS activity that is confined to the renal medulla.[176] Thus, NO acts in the renal medulla to control sodium excretion during variations in salt intake and therefore helps to modulate arterial blood pressure.

Pressure-Natriuresis and Tubuloglomerular Feedback

Long-term control of arterial pressure is in part governed by the pressure-natriuresis mechanism, which couples increases in renal perfusion pressure to increases in renal sodium excretion. Experiments in dogs confirmed that intrarenal NO activity directly correlates with changes in arterial pressure and changes in urinary excretion rates of sodium, suggesting that acute changes in arterial pressure alter intrarenal NO production, which inhibits tubular sodium reabsorption and effects pressure natriuresis.[168,171,177]

NOS activity contributes to the activity of tubuloglomerular feedback (TGF), which couples tubular reabsorption to the regulation of glomerular ultrafiltration. A growing body of evidence suggests that NO produced by nNOS in the macula densa stimulates soluble guanylate cyclase, generating cGMP and activating cGMP-dependent protein kinase within the macula densa cells, which then modulate TGF responsiveness. Micropuncture studies in vivo have shown that NO blunts the TGF response that causes vasoconstriction of the renal afferent arteriole in response to sodium chloride reabsorption at this site, and possibly regulates renin release from the juxtaglomerular apparatus.[178-181] Oxidative stress produced in hypertensive rat models greatly limits NO bioactivity in the juxtaglomerular apparatus[182,183] (see article by Modlinger et al, in this issue). Recent studies indicate that NO produced by either eNOS or iNOS in the medullary thick ascending limb of Henle also may inhibit TGF. In the presence of the nNOS-selective inhibitor 7-nitroindazole, a nonselective NOS inhibitor significantly enhanced TGF when the macula densa was perfused orthograde via the medullary thick ascending limb of Henle, whereas the blocker had no effect on TGF when the macula densa was perfused retrograde via the distal tubule.[184]

CONCLUSIONS AND PERSPECTIVES

The field of NO biology has advanced at a dramatic pace and has offered new tools and mechanistic insights into the regulation of NO biosynthesis, NO targets, pharmacologic and endogenous inhibitors, and biological functions mediated. Methods for detecting NO and reactive nitrogen species in biological systems are being refined, gene expression profiling is identifying target genes susceptible to regulation by NO in various cells, proteomic methods are being exploited to identify protein targets of nitration and S-nitrosylation, the phenotypes of NOS isoform knockout and compound knockout mice are being dissected in greater detail, and more pharmacologic inhibitors with greater isoform selectivity are being generated. The challenge for renal investigators is to exploit these data, reagents, methods, and animal models to address with a high degree of sophistication the impact of NO on renal health and disease, and to generate new information in renal cells that may be more broadly applicable to other cell types and tissues.

REFERENCES

1. Liang M, Knox FG: Production and functional roles of nitric oxide in the proximal tubule. Am J Physiol 278:R1117-R1124, 2000

2. Wilcox CS, Deng X, Welch WJ: NO generation and action during changes in salt intake: Roles of nNOS and macula densa. Am J Physiol 274:R1588-R1593, 1998

3. Kone BC: Nitric oxide in renal health and disease. Am J Kidney Dis 30:311-333, 1997

4. Kone BC, Baylis C: Biosynthesis and homeostatic roles of nitric oxide in the normal kidney. Am J Physiol 272:F561-F578, 1997

5. Babior BM: Phagocytes and oxidative stress. Am J Med 109:33-44, 2000

6. Levonen AL, Patel RP, Brookes P, et al: Mechanisms of cell signaling by nitric oxide and peroxynitrite: From mitochondria to MAP kinases. Antioxid Redox Signal 3:215-229, 2001

7. Goto S, Yamamoto T, Feng L, et al: Expression and localization of inducible nitric oxide synthase in anti-Thy-1 glomerulonephritis. Am J Pathol 147:1133-1141, 1995

8. Narita I, Border WA, Ketteler M, et al: Nitric oxide mediates immunologic injury to kidney mesangium in experimental glomerulonephritis. Lab Invest 72:17-24, 1995

9. Jansen A, Cook T, Taylor GM, et al: Induction of nitric oxide synthase in rat immune complex glomerulonephritis. Kidney Int 45:1215-1219, 1994

10. Gabbai FB, Hammond TC, Thomson SC, et al: Effect of acute iNOS inhibition on glomerular function in tubulointerstitial nephritis. Kidney Int 61:851-854, 2002

11. Noiri E, Peresleni T, Miller F, et al: In vivo targeting of inducible NO synthase with oligodeoxynucleotides protects rat kidney against ischemia. J Clin Invest 97:2377-2383, 1996

12. Noiri E, Nakao A, Uchida K, et al: Oxidative and nitrosative stress in acute renal ischemia. Am J Physiol 281:F948-F957, 2001

13. Heyman SN, Goldfarb M, Carmeli F, et al: Effect of radiocontrast agents on intrarenal nitric oxide (NO) and NO synthase activity. Exp Nephrol 6:557-562, 1998

14. Fitzgerald J, Chou SY, Wahid A, et al: Regional expression of inducible nitric oxide synthase in the kidney in dogs with unilateral ureteral obstruction. J Urol 166:1524-1529, 2001

15. Joles JA, Vos IH, Grone HJ, et al: Inducible nitric oxide synthase in renal transplantation. Kidney Int 61:872-875, 2002

16. Romagnani P, Pupilli C, Lasagni L, et al: Inducible nitric oxide synthase expression in vascular and glomerular structures of human chronic allograft nephropathy. J Pathol 187:345-350, 1999

17. Stamler JS, Lamas S, Fang FC: Nitrosylation. The prototypic redox-based signaling mechanism. Cell 106:675-683, 2001

18. Alderton WK, Cooper CE, Knowles RG: Nitric oxide synthases: Structure, function and inhibition. Biochem J 357: 593-615, 2001

19. Tatoyan A, Giulivi C: Purification and characterization of a nitric-oxide synthase from rat liver mitochondria. J Biol Chem 273:11044-11048, 1998

20. Miller RT, Martasek P, Roman LJ, et al: Involvement of the reductase domain of neuronal nitric oxide synthase in superoxide anion production. Biochemistry 36:15277-15284, 1997

21. Crane BR, Arvai AS, Ghosh S, et al: Structures of the N(omega)-hydroxy-L-arginine complex of inducible nitric oxide synthase oxygenase dimer with active and inactive pterins. Biochemistry 39:4608-4621, 2000

22. Li H, Raman CS, Glaser CB, et al: Crystal structures of zinc-free and -bound heme domain of human inducible nitric-oxide synthase. Implications for dimer stability and comparison with endothelial nitric-oxide synthase. J Biol Chem 274:21276-21284, 1999

23. Fischmann TO, Hruza A, Niu XD, et al: Structural characterization of nitric oxide synthase isoforms reveals striking active-site conservation. Nat Struct Biol 6:233-242, 1999

24. Ghosh S, Wolan D, Adak S, et al: Mutational analysis of the tetrahydrobiopterin-binding site in inducible nitric-oxide synthase. J Biol Chem 274:24100-24112, 1999

25. Cubberley RR, Alderton WK, Boyhan A, et al: Cysteine-200 of human inducible nitric oxide synthase is essential for dimerization of haem domains and for binding of haem, nitroarginine and tetrahydrobiopterin. Biochem J 323:141-146, 1997

26. Ruan J, Xie Q, Hutchinson N, et al: Inducible nitric oxide synthase requires both the canonical calmodulin-binding domain and additional sequences in order to bind calmodulin and produce nitric oxide in the absence of free Ca^{2+}. J Biol Chem 271:22679-22686, 1996

27. Xie Q-W, Kashiwabara Y, Nathan C: Role of transcription factor NF-κB/Rel in induction of nitric oxide synthase. J Biol Chem 269:4705-4708, 1994

28. Boger RH, Bode-Boger SM: Asymmetric dimethylarginine, derangements of the endothelial nitric oxide synthase pathway, and cardiovascular diseases. Semin Thromb Hemost 26:539-545, 2000

29. Leiper J, Vallance P: Biological significance of endogenous methylarginines that inhibit nitric oxide synthases. Cardiovasc Res 43:542-548, 1999

30. Napoli C, Ignarro LJ: Nitric oxide and atherosclerosis. Nitric Oxide 5:88-97, 2001

31. Crow JP, Beckman JS: Reactions between nitric oxide, superoxide, and peroxynitrite: footprints of peroxynitrite in vivo. Adv Pharmacol 34:17-43, 1995

32. Ahn KY, Mohaupt MG, Madsen KM, et al: In situ hybridization localization of mRNA encoding inducible nitric oxide synthase in rat kidney. Am J Physiol 267:F748-F757, 1994

33. Mohaupt MG, Elzie JL, Ahn KY, et al: Differential expression and induction of mRNAs encoding two inducible nitric oxide synthases in rat kidney. Kidney Int 46:653-665, 1994

34. Morrissey JJ, McCracken R, Kaneto H, et al: Location of an inducible nitric oxide synthase mRNA in the normal kidney. Kidney Int 45:998-1005, 1994

35. Wang T: Role of iNOS and eNOS in modulating proximal tubule transport and acid-base balance. Am J Physiol 283:F658-F662, 2002

36. Terada Y, Tomita K, Nonoguchi H, et al: Polymerase chain reaction localization of constitutive nitric oxide synthase and soluble guanylate cyclase messenger RNAs in microdissected rat nephron segments. J Clin Invest 90:659-666, 1992

37. Bachmann S, Bosse HM, Mundel P: Topography of nitric oxide synthesis by localizing constitutive NO synthases in mammalian kidney. Am J Physiol 268:F885-F898, 1995

38. Wang X, Lu M, Gao Y, et al: Neuronal nitric oxide synthase is expressed in principal cell of collecting duct. Am J Physiol 275:F395-F399, 1998

39. Liu L, Liu GL, Barajas L: Distribution of nitric oxide synthase-containing ganglionic neuronal somata and postganglionic fibers in the rat kidney. J Comp Neurol 369:16-30, 1996

40. Hall AV, Antoniou H, Wang Y, et al: Structural organization of the human neuronal nitric oxide synthase gene (NOS1). J Biol Chem 269:33082-33090, 1994

41. Brenman JE, Xia H, Chao DS, et al: Regulation of neuronal nitric oxide synthase through alternative transcripts. Dev Neurosci 19:224-231, 1997

42. Xie J, Roddy P, Rife TK, et al: Two closely linked but separable promoters for human neuronal nitric oxide synthase gene transcription. Proc Natl Acad Sci U S A 92:1242-1246, 1995

43. Wang Y, Newton DC, Miller TL, et al: An alternative promoter of the human neuronal nitric oxide synthase gene is expressed specifically in Leydig cells. Am J Pathol 160:369-380, 2002

44. Sasaki M, Gonzalez-Zulueta M, Huang H, et al: Dynamic regulation of neuronal NO synthase transcription by calcium influx through a CREB family transcription factor-dependent mechanism. Proc Natl Acad Sci U S A 97:8617-8622, 2000

45. Magee T, Fuentes AM, Garban H, et al: Cloning of a novel neuronal nitric oxide synthase expressed in penis and lower urinary tract. Biochem Biophys Res Commun 226:145-151, 1996

46. Silvagno F, Xia H, Bredt DS: Neuronal nitric oxide

synthase-μ, an alternatively spliced isoform expressed in differentiated skeletal muscle. J Biol Chem 271:11204-11208, 1996

47. Larsson B, Phillips SC: Isolation and characterization of a novel, human neuronal nitric oxide synthase cDNA. Biochem Biophys Res Commun 251:898-902, 1998

48. Deans Z, Dawson SJ, Xie J, et al: Differential regulation of the two neuronal nitric-oxide synthase gene promoters by the Oct-2 transcription factor. J Biol Chem 271:32153-32158, 1996

49. Rife TK, Xie J, Redman C, et al: The 5′2 promoter of the neuronal nitric oxide synthase dual promoter complex mediates inducibility by nerve growth factor. Brain Res Mol Brain Res 75:225-236, 2000

50. Wei X, Sasaki M, Huang H, et al: The orphan nuclear receptor, steroidogenic factor 1, regulates neuronal nitric oxide synthase gene expression in pituitary gonadotropes. Mol Endocrinol 16:2828-2839, 2002

51. Wang Y, Goligorsky MS, Lin M, et al: A novel, testis-specific mRNA transcript encoding an NH2-terminal truncated nitric-oxide synthase. J Biol Chem 272:11392-11401, 1997

52. Newton DC, Bevan SC, Choi S, et al: Translational regulation of human neuronal nitric-oxide synthase by an alternatively spliced 5′-untranslated region leader exon. J Biol Chem 278:636-644, 2003

53. Dawson TM, Steiner JP, Dawson VL, et al: Immunosuppressant FK506 enhances phosphorylation of nitric oxide synthase and protects against glutamate neurotoxicity. Proc Natl Acad Sci U S A 90:9808-9812, 1993

54. Choi YB, Tenneti L, Le DA, et al: Molecular basis of NMDA receptor-coupled ion channel modulation by S-nitrosylation. Nat Neurosci 3:15-21, 2000

55. Komeima K, Watanabe Y: Dephosphorylation of nNOS at Ser(847) by protein phosphatase 2A. FEBS Lett 497:65-66, 2001

56. Brenman JE, Chao DS, Xia H, et al: Nitric oxide synthase complexed with dystrophin and absent from skeletal muscle sarcolemma in Duchenne muscular dystrophy. Cell 82:743-752, 1995

57. Brenman JE, Chao DS, Gee SH, et al: Interaction of nitric oxide synthase with the postsynaptic density protein PSD-95 and α1-syntrophin mediated by PDZ domains. Cell 84:757-767, 1996

58. Brenman JE, Christopherson KS, Craven SE, et al: Cloning and characterization of postsynaptic density 93, a nitric oxide synthase interacting protein. J Neurosci 16:7407-7415, 1996

59. Jaffrey SR, Snowman AM, Eliasson MJ, et al: CAPON: A protein associated with neuronal nitric oxide synthase that regulates its interactions with PSD95. Neuron 20:115-124, 1998

60. Riefler GM, Firestein BL: Binding of neuronal nitric-oxide synthase (nNOS) to carboxyl-terminal-binding protein (CtBP) changes the localization of CtBP from the nucleus to the cytosol: A novel function for targeting by the PDZ domain of nNOS. J Biol Chem 276:48262-48268, 2001

61. Ort T, Maksimova E, Dirkx R, et al: The receptor tyrosine phosphatase-like protein ICA512 binds the PDZ domains of beta2-syntrophin and nNOS in pancreatic beta-cells. Eur J Cell Biol 79:621-630, 2000

62. Schuh K, Uldrijan S, Telkamp M, et al: The plasma membrane calmodulin-dependent calcium pump: A major regulator of nitric oxide synthase I. J Cell Biol 155:201-205, 2001

63. Venema RC, Ju H, Zou R, et al: Subunit interactions of endothelial nitric-oxide synthase. Comparisons to the neuronal and inducible nitric-oxide synthase isoforms. J Biol Chem 272: 1276-1282, 1997

64. Venema VJ, Ju H, Zou R, et al: Interaction of neuronal nitric-oxide synthase with caveolin-3 in skeletal muscle. Identification of a novel caveolin scaffolding/inhibitory domain. J Biol Chem 272:28187-28190, 1997

65. Garcia-Cardena G, Martasek P, Masters BS, et al: Dissecting the interaction between nitric oxide synthase (NOS) and caveolin. Functional significance of the NOS caveolin binding domain in vivo. J Biol Chem 272:25437-25440, 1997

66. Jaffrey SR, Snyder SH: PIN: An associated protein inhibitor of neuronal nitric oxide synthase. Science 274:774-777, 1996

67. Jeong Y, Won J, Yim J: Cloning and structure of a rabbit protein inhibitor of neuronal nitric oxide synthase (PIN) gene and its pseudogene. Gene 214:67-75, 1998

68. Bender AT, Silverstein AM, Demady DR, et al: Neuronal nitric-oxide synthase is regulated by the Hsp90-based chaperone system in vivo. J Biol Chem 274:1472-1478, 1999

69. Golser R, Gorren AC, Leber A, et al: Interaction of endothelial and neuronal nitric-oxide synthases with the bradykinin B2 receptor. Binding of an inhibitory peptide to the oxygenase domain blocks uncoupled NADPH oxidation. J Biol Chem 275:5291-5296, 2000

70. Pupo AS, Minneman KP: Interaction of neuronal nitric oxide synthase with alpha1-adrenergic receptor subtypes in transfected HEK-293 cells. BMC Pharmacol 2:17, 2002

71. Chartrain NA, Geller DA, Koty PP, et al: Molecular cloning, structure, and chromosomal localization of the human inducible nitric oxide synthase gene. J Biol Chem 269:6765-6772, 1994

72. Eissa NT, Strauss AJ, Haggerty CM, et al: Alternative splicing of human inducible nitric oxide synthase mRNA tissue specific regulation and induction by cytokines. J Biol Chem 271:27184-27187, 1996

73. Eissa NT, Yuan JW, Haggerty CM, et al: Cloning and characterization of human inducible nitric oxide synthase splice variants: A domain, encoded by exons 8 and 9, is critical for dimerization. Proc Natl Acad Sci U S A 95:7625-7630, 1998

74. Guo FH, Comhair SA, Zheng S, et al: Molecular mechanisms of increased nitric oxide (NO) in asthma: Evidence for transcriptional and post-translational regulation of NO synthesis. J Immunol 164:5970-5980, 2000

75. De Vera ME, Taylor BS, Wang Q, et al: Dexamethasone suppresses iNOS gene expression by upregulating I-kappa B alpha and inhibiting NF-kappa B. Am J Physiol 273:G1290-G1296, 1997

76. Marrero MB, Venema VJ, He H, et al: Inhibition by the JAK/STAT pathway of IFNgamma- and LPS-stimulated nitric oxide synthase induction in vascular smooth muscle cells. Biochem Biophys Res Commun 252:508-512, 1998

77. de Vera ME, Shapiro RA, Nussler AK, et al: Transcriptional regulation of human inducible nitric oxide synthase (NOS2) gene by cytokines: Initial analysis of the human NOS2 promoter. Proc Natl Acad Sci U S A 93:1054-1059, 1996

78. Mellott JK, Nick HS, Waters MF, et al: Cytokine-induced changes in chromatin structure and in vivo footprints in the inducible NOS promoter. Am J Physiol 280:L390-L399, 2001

79. Spitsin SV, Koprowski H, Michaels FH: Characterization and functional analysis of the human inducible nitric oxide synthase gene promoter. Mol Med 2:226-235, 1996

80. Linn SC, Morelli PJ, Edry I, et al: Transcriptional regulation of human inducible nitric oxide synthase gene in an intestinal epithelial cell line. Am J Physiol 272:G1499-G1508, 1997

81. Nunokawa Y, Oikawa S, Tanaka S: Expression of human inducible nitric oxide synthase is regulated by both promoter and 3′-regions. Biochem Biophys Res Commun 233:523-526, 1997

82. Taylor BS, de Vera ME, Ganster RW, et al: Multiple NF-kappaB enhancer elements regulate cytokine induction of the human inducible nitric oxide synthase gene [in process citation]: J Biol Chem 273:15148-15156, 1998

83. Chu SC, Marks-Konczalik J, Wu HP, et al: Analysis of the cytokine-stimulated human inducible nitric oxide synthase (iNOS) gene: Characterization of differences between human and mouse iNOS promoters. Biochem Biophys Res Commun 248:871-878, 1998

84. Ganster RW, Taylor BS, Shao L, et al: Complex regulation of human inducible nitric oxide synthase gene transcription by Stat 1 and NF-kappa B. Proc Natl Acad Sci U S A 98:8638-8643, 2001

85. Yu Z, Zhang W, Kone BC: Histone deacetylases augment cytokine induction of the iNOS gene. J Am Soc Nephrol 13:2009-2017, 2002

86. Rodriguez-Pascual F, Hausding M, Ihrig-Biedert I, et al: Complex contribution of the 3′-untranslated region to the expressional regulation of the human inducible nitric-oxide synthase gene. Involvement of the RNA-binding protein HuR. J Biol Chem 275:26040-26049, 2000

87. Carpenter L, Cordery D, Biden TJ: Protein kinase Cdelta activation by IL-1beta stabilizes inducible NO synthase mRNA in pancreatic beta-cells. J Biol Chem 276:5368-5374, 2000

88. Gustafsson AB, Brunton L: β-adrenergic stimulation of rat cardiac fibroblasts enhances induction of nitric-oxide synthase by interleukin-1β via message stabilization. Mol Pharmacol 58:1470-1478, 2000

89. Saura M, Perez Sala D, Canada FJ, et al: Role of tetrahydrobiopterin availability in the regulation of nitric oxide synthase expression in human mesangial cells. J Biol Chem 271:14290-14295, 1996

90. Geng Y, Lotz M: Increased intracellular Ca^{2+} selectively suppresses IL-1-induced NO production by reducing iNOS mRNA stability. J Cell Biol 129:1651-1657, 1995

91. Kiemer AK, Vollmar AM: Autocrine regulation of inducible nitric-oxide synthase in macrophages by atrial natriuretic peptide. J Biol Chem 273:13444-13451, 1998

92. Perez-Sala D, Cernuda-Morollon E, Diaz-Cazorla M, et al: Posttranscriptional regulation of human iNOS by the NO/cGMP pathway. Am J Physiol 280:F466-F473, 2001

93. Pan J, Burgher KL, Szczepanik AM, et al: Tyrosine phosphorylation of inducible nitric oxide synthase: Implications for potential post-translational regulation. Biochem J 314:889-894, 1996

94. Kolodziejski PJ, Musial A, Koo JS, et al: Ubiquitination of inducible nitric oxide synthase is required for its degradation. Proc Natl Acad Sci U S A 99:12315-12320, 2002

95. Felley-Bosco E, Bender FC, Courjault-Gautier F, et al: Caveolin-1 down-regulates inducible nitric oxide synthase via the proteasome pathway in human colon carcinoma cells. Proc Natl Acad Sci U S A 97:14334-14339, 2000

96. Ratovitski EA, Bao C, Quick RA, et al: An inducible nitric-oxide synthase (NOS)-associated protein inhibits NOS dimerization and activity. J Biol Chem 274:30250-30257, 1999

97. Ratovitski EA, Alam MR, Quick RA, et al: Kalirin inhibition of inducible nitric-oxide synthase. J Biol Chem 274: 993-999, 1999

98. Kuncewicz T, Balakrishnan P, Snuggs MB, et al: Specific association of nitric oxide synthase-2 with Rac isoforms in activated murine macrophages. Am J Physiol 281:F326-F336, 2001

99. Glynne PA, Darling KE, Picot J, et al: Epithelial inducible nitric-oxide synthase is an apical EBP50-binding protein that directs vectorial nitric oxide output. J Biol Chem 277: 33132-33138, 2002

100. Marsden PA, Heng HH, Scherer SW, et al: Structure and chromosomal localization of the human constitutive endothelial nitric oxide synthase gene. J Biol Chem 268:17478-17488, 1993

101. Yoshimura M, Yasue H, Nakayama M, et al: A missense Glu298Asp variant in the endothelial nitric oxide synthase gene is associated with coronary spasm in the Japanese. Hum Genet 103:65-69, 1998

102. Noiri E, Satoh H, Taguchi J, et al: Association of eNOS Glu298Asp polymorphism with end-stage renal disease. Hypertension 40:535-540, 2002

103. Hingorani AD: Endothelial nitric oxide synthase polymorphisms and hypertension. Curr Hypertens Rep 5:19-25, 2003

104. Nassar BA, Bevin LD, Johnstone DE, et al: Relationship of the Glu298Asp polymorphism of the endothelial nitric oxide synthase gene and early-onset coronary artery disease. Am Heart J 142:586-589, 2001

105. Zhang R, Min W, Sessa WC: Functional analysis of the human endothelial nitric oxide synthase promoter. Sp1 and GATA factors are necessary for basal transcription in endothelial cells. J Biol Chem 270:15320-15326, 1995

106. Cieslik K, Zembowicz A, Tang JL, et al: Transcriptional regulation of endothelial nitric-oxide synthase by lysophosphatidylcholine. J Biol Chem 273:14885-14890, 1998

107. Karantzoulis-Fegaras F, Antoniou H, Lai SL, et al: Characterization of the human endothelial nitric-oxide synthase promoter. J Biol Chem 274:3076-3093, 1999

108. Laumonnier Y, Nadaud S, Agrapart M, et al: Characterization of an upstream enhancer region in the promoter of the human endothelial nitric-oxide synthase gene. J Biol Chem 275:40732-40741, 2000

109. Teichert AM, Miller TL, Tai SC, et al: In vivo expression profile of an endothelial nitric oxide synthase promoter-reporter transgene. Am J Physiol 278:H1352-H1361, 2000

110. Forstermann U, Boissel JP, Kleinert H: Expressional control of the "constitutive" isoforms of nitric oxide synthase (NOS I and NOS III). FASEB J 12:773-790, 1998

111. Kleinert H, Euchenhofer C, Fritz G, et al: Involvement of protein kinases in the induction of NO synthase II in human DLD-1 cells. Br J Pharmacol 123:1716-1722, 1998

112. Kleinert H, Wallerath T, Euchenhofer C, et al: Estrogens increase transcription of the human endothelial NO synthase gene: Analysis of the transcription factors involved. Hypertension 31:582-588, 1998

113. Guillot PV, Guan J, Liu L, et al: A vascular bed-specific pathway. J Clin Invest 103:799-805, 1999

114. Laufs U, Liao JK: Post-transcriptional regulation of endothelial nitric oxide synthase mRNA stability by Rho GTPase. J Biol Chem 273:24266-24271, 1998

115. Drummond GR, Cai H, Davis ME, et al: Transcriptional and posttranscriptional regulation of endothelial nitric oxide synthase expression by hydrogen peroxide. Circ Res 86:347-354, 2000

116. Shaul PW, Smart EJ, Robinson LJ, et al: Acylation targets endothelial nitric-oxide synthase to plasmalemmal caveolae. J Biol Chem 271:6518-6522, 1996

117. Liu JW, Garciacardena G, Sessa WC: Palmitoylation of endothelial nitric oxide synthase is necessary for optimal stimulated release of nitric oxide: Implications for caveolae localization. Biochemistry 35:13277-13281, 1996

118. Michel JB, Feron O, Sacks D, et al: Reciprocal regulation of endothelial nitric-oxide synthase by Ca^{2+}-calmodulin and caveolin. J Biol Chem 272:15583-15586, 1997

119. Michel JB, Feron O, Sase K, et al: Caveolin versus calmodulin. Counterbalancing allosteric modulators of endothelial nitric oxide synthase. J Biol Chem 272:25907-25912, 1997

120. Feron O, Dessy C, Opel DJ, et al: Modulation of the endothelial nitric-oxide synthase-caveolin interaction in cardiac myocytes. Implications for the autonomic regulation of heart rate. J Biol Chem 273:30249-30254, 1998

121. Feron O, Saldana F, Michel JB, et al: The endothelial nitric-oxide synthase-caveolin regulatory cycle. J Biol Chem 273:3125-3128, 1998

122. Gratton JP, Fontana J, O'Connor DS, et al: Reconstitution of an endothelial nitric-oxide synthase (eNOS), hsp90, and caveolin-1 complex in vitro. Evidence that hsp90 facilitates calmodulin-stimulated displacement of eNOS from caveolin-1. J Biol Chem 275:22268-22272, 2000

123. Marrero MB, Venema VJ, Ju H, et al: Endothelial nitric oxide synthase interactions with G-protein-coupled receptors. Biochem J 343:335-340, 1999

124. Dedio J, Konig P, Wohlfart P, et al: NOSIP, a novel modulator of endothelial nitric oxide synthase activity. FASEB J 15:79-89, 2001

125. Zimmermann K, Opitz N, Dedio J, et al: NOSTRIN: A protein modulating nitric oxide release and subcellular distribution of endothelial nitric oxide synthase. Proc Natl Acad Sci U S A 99:17167-17172, 2002

126. Cao S, Yao J, McCabe TJ, et al: Direct interaction between endothelial nitric-oxide synthase and dynamin-2. Implications for nitric-oxide synthase function. J Biol Chem 276: 14249-14256, 2001

127. Chen ZP, Mitchelhill KI, Michell BJ, et al: AMP-activated protein kinase phosphorylation of endothelial NO synthase. FEBS Lett 443:285-289, 1999

128. Butt E, Bernhardt M, Smolenski A, et al: Endothelial nitric-oxide synthase (type III) is activated and becomes calcium independent upon phosphorylation by cyclic nucleotide-dependent protein kinases. J Biol Chem 275:5179-5187, 2000

129. Harris MB, Ju H, Venema VJ, et al: Reciprocal phosphorylation and regulation of endothelial nitric-oxide synthase in response to bradykinin stimulation. J Biol Chem 276:16587-16591, 2001

130. Michell BJ, Griffiths MJ, Mitchelhill KI, et al: The Akt kinase signals directly to endothelial nitric oxide synthase. Curr Biol 9:845-848, 1999

131. Luo Z, Fujio Y, Kureishi Y, et al: Acute modulation of endothelial Akt/PKB activity alters nitric oxide-dependent vasomotor activity in vivo. J Clin Invest 106:493-499, 2000

132. Fulton D, Gratton JP, McCabe TJ, et al: Regulation of endothelium-derived nitric oxide production by the protein kinase Akt. Nature 399:597-601, 1999

133. Simoncini T, Hafezi-Moghadam A, Brazil DP, et al: Interaction of oestrogen receptor with the regulatory subunit of phosphatidylinositol-3-OH kinase. Nature 407:538-541, 2000

134. Igarashi J, Thatte HS, Prabhakar P, et al: Calcium-independent activation of endothelial nitric oxide synthase by ceramide. Proc Natl Acad Sci U S A 96:12583-12588, 1999

135. Igarashi J, Michel T: Agonist-modulated targeting of the EDG-1 receptor to plasmalemmal caveolae. eNOS activation by sphingosine 1-phosphate and the role of caveolin-1 in sphingolipid signal transduction. J Biol Chem 275:32363-32370, 2000

136. Cleeter MW, Cooper JM, Darley-Usmar VM, et al: Reversible inhibition of cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide. Implications for neurodegenerative diseases. FEBS Lett 345: 50-54, 1994

137. Lizasoain I, Moro MA, Knowles RG, et al: Nitric oxide and peroxynitrite exert distinct effects on mitochondrial respiration which are differentially blocked by glutathione or glucose. Biochem J 314:877-880, 1996

138. Clementi E, Brown GC, Feelisch M, et al: Persistent inhibition of cell respiration by nitric oxide: Crucial role of S-nitrosylation of mitochondrial complex I and protective action of glutathione. Proc Natl Acad Sci U S A 95:7631-7636, 1998

139. Riobo NA, Clementi E, Melani M, et al: Nitric oxide inhibits mitochondrial NADH: ubiquinone reductase activity through peroxynitrite formation. Biochem J 359:139-145, 2001

140. Moncada S, Erusalimsky JD: Does nitric oxide modulate mitochondrial energy generation and apoptosis? Nat Rev Mol Cell Biol 3:214-220, 2002

141. Beltran B, Quintero M, Garcia-Zaragoza E, et al: Inhibition of mitochondrial respiration by endogenous nitric oxide: A critical step in Fas signaling. Proc Natl Acad Sci U S A 99:8892-8897, 2002

142. Beltran B, Mathur A, Duchen MR, et al: The effect of nitric oxide on cell respiration: A key to understanding its role in cell survival or death. Proc Natl Acad Sci U S A 97:14602-14607, 2000

143. Brown GC, Cooper CE: Nanomolar concentrations of nitric oxide reversibly inhibit synaptosomal respiration by competing with oxygen at cytochrome oxidase. FEBS Lett 356:295-298, 1994

144. Loke KE, McConnell PI, Tuzman JM, et al: Endogenous endothelial nitric oxide synthase-derived nitric oxide is a physiological regulator of myocardial oxygen consumption. Circ Res 84:840-845, 1999

145. Giulivi C, Poderoso JJ, Boveris A: Production of nitric oxide by mitochondria. J Biol Chem 273:11038-11043, 1998

146. Elfering SL, Sarkela TM, Giulivi C: Biochemistry of mitochondrial nitric-oxide synthase. J Biol Chem 277:38079-86, 2002

147. Garvin JL, Hong NJ: Nitric oxide inhibits sodium/

hydrogen exchange activity in the thick ascending limb. Am J Physiol 277:F377-F382, 1999

148. Turrens JF, Boveris A: Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. Biochem J 191:421-427, 1980

149. Boveris A: Mitochondrial production of superoxide radical and hydrogen peroxide. Adv Exp Med Biol 78:67-82, 1977

150. Packer MA, Porteous CM, Murphy MP: Superoxide production by mitochondria in the presence of nitric oxide forms peroxynitrite. Biochem Mol Biol Int 40:527-534, 1996

151. Ortiz PA, Garvin JL: Interaction of O(2)(-) and NO in the thick ascending limb. Hypertension 39:591-596, 2002

152. Ortiz PA, Garvin JL: Superoxide stimulates NaCl absorption by the thick ascending limb. Am J Physiol 283:F957-F962, 2002

153. Halestrap AP, Doran E, Gillespie JP, et al: Mitochondria and cell death. Biochem Soc Trans 28:170-177, 2000

154. Ortiz PA, Garvin JL: Role of nitric oxide in the regulation of nephron transport. Am J Physiol 282:F777-F784, 2002

155. Wang T, Inglis FM, Kalb RG: Defective fluid and HCO_3^- absorption in proximal tubule of neuronal nitric oxide synthase-knockout mice. Am J Physiol 279:F518-F524, 2000

156. Roczniak A, Burns KD: Nitric oxide stimulates guanylate cyclase and regulates sodium transport in rabbit proximal tubule. Am J Physiol 270:F106-F115, 1996

157. Guzman NJ, Fang MZ, Tang SS, et al: Autocrine inhibition of Na^+/K^+-ATPase by nitric oxide in mouse proximal tubule epithelial cells. J Clin Invest 95:2083-2088, 1995

158. Linas SL, Repine JE: Endothelial cells regulate proximal tubule epithelial cell sodium transport. Kidney Int 55:1251-1258, 1999

159. Ortiz PA, Hong NJ, Garvin JL: NO decreases thick ascending limb chloride absorption by reducing Na^+-K^+-$2Cl^-$ cotransporter activity. Am J Physiol 281:F819-F825, 2001

160. Ortiz PA, Garvin JL: Autocrine effects of nitric oxide on HCO_3^- transport by rat thick ascending limb. Kidney Int 58:2069-2074, 2000

161. Plato CF, Stoos BA, Wang D, et al: Endogenous nitric oxide inhibits chloride transport in the thick ascending limb. Am J Physiol 276:F159-F163, 1999

162. Lu M, Wang X, Wang W: Nitric oxide increases the activity of the apical 70-pS K^+ channel in TAL of rat kidney. Am J Physiol 274:F946-F950, 1998

163. Stoos BA, Garcia NH, Garvin JL: Nitric oxide inhibits sodium reabsorption in the isolated perfused cortical collecting duct. J Am Soc Nephrol 6:89-94, 1995

164. Garcia NH, Stoos BA, Carretero OA, et al: Mechanism of the nitric oxide induced blockade of collecting duct water permeability. Hypertension 27:679-683, 1996

165. Lu M, Giebisch G, Wang W: Nitric oxide links the apical Na^+ transport to the basolateral K^+ conductance in the rat cortical collecting duct. J Gen Physiol 110:717-726, 1997

166. Lu M, Giebisch G, Wang W: Nitric oxide-induced hyperpolarization stimulates low-conductance Na+ channel of rat CCD. Am J Physiol 272:F498-F504, 1997

167. Tojo A, Garg LC, Guzman NC, et al: Nitric oxide inhibits bafilomycin-sensitive H^+-ATPase activity in rat cortical collecting duct. Am J Physiol 267:F509-F515, 1994

168. Wei Y, Wang W: Angiotensin II stimulates basolateral K channels in rat cortical collecting ducts. Am J Physiol 284: F175-F181, 2003

169. Majid DS, Navar LG: Nitric oxide in the control of renal hemodynamics and excretory function. Am J Hypertens 14:74S-82S, 2001

170. Ichihara A, Navar LG: Neuronal NOS contributes to biphasic autoregulatory response during enhanced TGF activity. Am J Physiol 277:F113-F120, 1999

171. Majid DSA, Williams A, Navar LG: Inhibition of nitric oxide synthesis attenuates pressure-induced natriuretic responses in anesthetized dogs. Am J Physiol 264:F79-F87, 1993

172. Mattson DL, Wu F: Control of arterial blood pressure and renal sodium excretion by nitric oxide synthase in the renal medulla. Acta Physiol Scand 168:149-154, 2000

173. Mattson DL, Higgins DJ: Influence of dietary sodium intake on renal medullary nitric oxide synthase. Hypertension 27:688-692, 1996

174. Mattson DL, Lu S, Nakanishi K, et al: Effect of chronic renal medullary nitric oxide inhibition on blood pressure. Am J Physiol 266:H1918-H1926, 1994

175. Zou AP, Cowley AW Jr: Nitric oxide in renal cortex and medulla. An in vivo microdialysis study. Hypertension 29:194-198, 1997

176. Szentivanyi M Jr, Zou AP, Mattson DL, et al: Renal medullary nitric oxide deficit of Dahl S rats enhances hypertensive actions of angiotensin II. Am J Physiol 283:R266-R272, 2002

177. Majid DS, Godfrey M, Grisham MB, et al: Relation between pressure natriuresis and urinary excretion of nitrate/nitrite in anesthetized dogs. Hypertension 25:860-865, 1995

178. Wilcox CS, Welch WJ: Macula densa nitric oxide synthase: Expression, regulation, and function. Kidney Int 67:S53-S57, 1998 (Suppl)

179. Thorup C, Persson AEG: Inhibition of locally produced nitric oxide resets tubuloglomerular feedback mechanism. Acta Physiol Scand 153:43-49, 1995

180. Wilcox CS, Welch WJ, Murad F, et al: Nitric oxide synthase in macula densa regulates glomerular capillary pressure. Proc Natl Acad Sci U S A 89:11993-11997, 1992

181. Schnermann J, Traynor T, Yang T, et al: Tubuloglomerular feedback: New concepts and developments. Kidney Int 67:S40-S45, 1998 (Suppl)

182. Wilcox CS, Welch WJ: Interaction between nitric oxide and oxygen radicals in regulation of tubuloglomerular feedback. Acta Physiol Scand 168:119-124, 2000

183. Welch WJ, Tojo A, Lee JU, et al: Nitric oxide synthase in the JGA of the SHR: Expression and role in tubuloglomerular feedback. Am J Physiol 277:F130-F138, 1999

184. Wang H, Carretero OA, Garvin JL: Nitric oxide produced by THAL nitric oxide synthase inhibits TGF. Hypertension 39:662-666, 2002

185. Tojo A, Bredt DS, Wilcox CS: Distribution of postsynaptic density proteins in rat kidney: Relationship to neuronal nitric oxide synthase. Kidney Int 55:1384-1394, 1999

186. Roczniak A, Zimpelmann J, Burns KD: Effect of dietary salt on neuronal nitric oxide synthase in the inner medullary collecting duct. Am J Physiol 275:F46-F54, 1998

187. Huang PL, Dawson TM, Bredt DS, et al: Targeted disruption of the neuronal nitric oxide synthase gene. Cell 75:1273-1286, 1993

188. Demas GE, Eliasson MJ, Dawson TM, et al: Inhibition

of neuronal nitric oxide synthase increases aggressive behavior in mice. Mol Med 3:610-616, 1997

189. Sutherland RS, Kogan BA, Piechota HJ, et al: Vesicourethral function in mice with genetic disruption of neuronal nitric oxide synthase. J Urol 157:1109-1116, 1997

190. Huang Z, Huang PL, Panahian N, et al: Effects of cerebral ischemia in mice deficient in neuronal nitric oxide synthase. Science 265:1883-1885, 1994

191. Morishita T, Tsutsui M, Shimokawa H, et al: Vasculoprotective roles of neuronal nitric oxide synthase. FASEB J 16:1994-1996, 2002

192. Vallon V, Traynor T, Barajas L, et al: Feedback control of glomerular vascular tone in neuronal nitric oxide synthase knockout mice. J Am Soc Nephrol 12:1599-1606, 2001

193. Ling H, Edelstein C, Gengaro P, et al: Attenuation of renal ischemia-reperfusion injury in inducible nitric oxide synthase knockout mice. Am J Physiol 277:F383-F390, 1999

194. Ling H, Gengaro PE, Edelstein CL, et al: Effect of hypoxia on proximal tubules isolated from nitric oxide synthase knockout mice. Kidney Int 53:1642-1646, 1998

195. Gross SS, Kilbourn RG, Griffith OW: NO in septic shock: Good, bad or ugly? Learning from iNOS knockouts. Trends Microbiol 4:47-49, 1996

196. Guo Y, Jones WK, Xuan YT, et al: The late phase of ischemic preconditioning is abrogated by targeted disruption of the inducible NO synthase gene. Proc Natl Acad Sci U S A 96:11507-11512, 1999

197. Chyu KY, Dimayuga P, Zhu J, et al: Decreased neointimal thickening after arterial wall injury in inducible nitric oxide synthase knockout mice. Circ Res 85:1192-1198, 1999

198. Plato CF, Shesely EG, Garvin JL: eNOS mediates L-arginine-induced inhibition of thick ascending limb chloride flux. Hypertension 35:319-323, 2000

199. Duplain H, Burcelin R, Sartori C, et al: Insulin resistance, hyperlipidemia, and hypertension in mice lacking endothelial nitric oxide synthase. Circulation 104:342-345, 2001

200. Huang Z, Huang PL, Ma J, et al: Enlarged infarcts in endothelial nitric oxide synthase knockout mice are attenuated by nitro-L-arginine. J Cereb Blood Flow Metab 16:981-987, 1996

201. Feng Q, Song W, Lu X, et al: Development of heart failure and congenital septal defects in mice lacking endothelial nitric oxide synthase. Circulation 106:873-879, 2002

202. Lee TC, Zhao YD, Courtman DW, et al: Abnormal aortic valve development in mice lacking endothelial nitric oxide synthase. Circulation 101:2345-2348, 2000

203. Fagan KA, McMurtry I, Rodman DM: Nitric oxide synthase in pulmonary hypertension: Lessons from knockout mice. Physiol Res 49:539-548, 2000

204. Fukumura D, Gohongi T, Kadambi A, et al: Predominant role of endothelial nitric oxide synthase in vascular endothelial growth factor-induced angiogenesis and vascular permeability. Proc Natl Acad Sci U S A 98:2604-2609, 2001