Nitric oxide (NO) is an important signaling and effector molecule that plays critical roles in a remarkable array of essential biological processes, ranging from neurotransmission and the control of vascular tone to apoptosis and inflammation (26, 66). Through covalent modifications of target proteins and redox reactions with oxygen, superoxide radical, and transition metals, NO influences a number of biosynthetic, metabolic, and membrane transport processes important to normal renal function. Given the diverse actions of this molecule and the multiplicity of cell phenotypes in the kidney, it is not surprising that NO has become the darling of renal physiologists seeking to explain complex, integrated functions of the kidney. For example, work to date has implicated NO in the paracrine control of basal renal and glomerular hemodynamics (140, 161), the tubuloglomerular feedback (TGF) response (186, 208), and pressure natriuresis (55, 76). Although promising advances in our understanding of the roles of NO in renal health and disease have been made, it has been difficult for investigators to keep pace with the explosion of new information about the molecular diversity and regulatory complexity of NO biosynthesis. At the current rate of over 4,000 publications per year on NO research, attempted encyclopedic reviews of the NO field will be incomplete and risk cursory analysis of the relevant data. Therefore this review explores selected, newly recognized developments in the biology and homeostatic functions of NO in the kidney and places particular emphasis on involvement of NO in the control of normal renal hemodynamics, the glomerular microcirculation, and renal salt excretion.

**NITRIC OXIDE BIOSYNTHESIS**

*Nitric Oxide Synthases*

General structure and biochemistry. NO is metabolized from L-arginine by NO synthases (NOS) in a complex reaction requiring molecular oxygen, reducing equivalents from NADPH as cosubstrates, tetrahydrobiopterin, a cytochrome P-450-type heme moiety, calmodulin, and several tightly bound redox cofactors.
The reaction catalyzed by nitric oxide synthases (NOS). CaM, calmodulin; BH₄, tetrahydrobiopterin; L-NMA, N⁶-methyl-L-arginine; FMN, flavin mononucleotide; FAD, flavin adenine dinucleotide.

Fig. 1. The reaction catalyzed by nitric oxide synthases (NOS). CaM, calmodulin; BH₄, tetrahydrobiopterin; L-NMA, N⁶-methyl-L-arginine; FMN, flavin mononucleotide; FAD, flavin adenine dinucleotide. (26) (Fig. 1). The reaction can be inhibited to a variable degree by N⁶-substituted L-arginine analogs, such as N⁶-nitro-L-arginine methyl ester, as well as relatively NOS-isozyme-selective inhibitors, such as 7-nitroindazole (for neuronal NOS) and aminoguanidine (for inducible NOS). To date, three broad categories of NOS isozymes, referred to in this review as neuronal (nNOS), endothelial (eNOS), and inducible NOS (iNOS), have been purified and characterized from rodents and humans (Table 1). Although these isozymes have different molecular weights and variable cofactor requirements, all are NADPH- and calmodulin-dependent and contain consensus binding sites for flavin adenine dinucleotide, flavin mononucleotide (FMN), tetrahydrobiopterin, and a heme complex (Fig. 2). The two major "constitutive" NOS isozymes, nNOS and eNOS, exhibit strict dependency on intracellular Ca²⁺/calmodulin levels, produce "puffs" of NO for signaling purposes, and are expressed in a limited tissue distribution. Although they are classified as constitutive enzymes, expression of nNOS and eNOS is regulated by specific physiological and pathophysiological stimuli (see below). A novel 105-kDa (native M₄) protein with biochemical properties similar to nNOS was recently purified from rat cerebellum (36). This protein, termed nNOS-II, utilizes both L-arginine and the oligopeptide bradykinin as substrates in biochemical assays. The reaction it catalyzes is calmodulin-dependent with L-arginine, but calmodulin-independent with bradykinin as substrate.

In contrast to these isozymes, iNOS, originally cloned from murine macrophage cell lines (211), is expressed in virtually all nucleated cells subjected to immunologic

<table>
<thead>
<tr>
<th>Isozyme</th>
<th>Monomeric Size</th>
<th>Human Chromosomal Location</th>
<th>Number of Exons</th>
<th>Principal Tissue Distribution Under Basal Conditions</th>
<th>Principal Phenotype of Knockout Mice</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>nNOS</td>
<td>160 kDa 1,439 aa</td>
<td>12q24.21</td>
<td>29</td>
<td>Neurons, Skeletal muscle, Macula densa segment, Bronchial and tracheal epithelium</td>
<td>Pyloric stenosis, Protection from cerebral ischemic injury</td>
<td>7, 21, 25, 32, 71, 79, 86, 138, 141, 143, 173, 178, 188, 208</td>
</tr>
<tr>
<td>nNOS-μ</td>
<td>164 kDa 1,468 aa</td>
<td>12q24.21</td>
<td>29</td>
<td>Skeletal muscle, Heart, Penis, lower urinary tract, Endothelium, Hippocampal CA1 neurons, Cardiac myocytes</td>
<td>Improved aggresive behavior in males</td>
<td>116, 177</td>
</tr>
<tr>
<td>eNOS</td>
<td>133 kDa 1,203 aa</td>
<td>7q35</td>
<td>26</td>
<td>Heart, Endothelium, Hippocampal CA1 neurons</td>
<td>Hypertensive, More susceptible to cerebral ischemic injury</td>
<td>7, 56, 80, 82, 94, 120, 144, 172</td>
</tr>
<tr>
<td>iNOS</td>
<td>135 kDa 1,153 aa</td>
<td>17q11.2</td>
<td>26 or 27</td>
<td>Kidney vascular and epithelial cells, Bronchial airway epithelium, alveolar macrophages, Ileum, Uterus, Platelets</td>
<td>Increased susceptibility to intracellular pathogens, Blunted hypotensive response to LPS</td>
<td>1, 17, 30, 32, 54, 62, 68, 78, 115, 117, 119, 128, 131, 133, 145, 151, 164, 188, 206, 211</td>
</tr>
</tbody>
</table>

NOS, nitric oxide synthase (n, neuronal; e, endothelial; i, inducible); aa, amino acids; LPS, lipopolysaccharide.
or certain nonimmunologic stimuli. Once expressed, iNOS remains tonically activated, although subject to feedback inhibition (2), producing large amounts of NO for cytoactive purposes. Although its activity was originally believed to be Ca2+/calmodulin independent, owing to its tight binding of calmodulin, recent studies with purified, recombinant iNOS protein indicate that iNOS is twice as active in the presence of Ca2+ as in its absence (198). Therefore, the common practice of measuring 1-[^3H]arginine to 1-[^3H]citrulline conversion in the presence and absence of Ca2+ to distinguish iNOS from nNOS and eNOS may yield erroneous estimates of the contributions of these isozymes to overall NOS activity. Similarly, although initially thought to be a soluble enzyme, iNOS has been shown to be associated with 50- to 80-nm vesicles in primary mouse macrophages (202). The ability to associate with membranes of vesicular iNOS is apparently the result of a yet to be characterized posttranslational modification that increases the mass of the protein by 4.5 kDa (202).

Finally, the paradigm that iNOS requires a provocative immune stimulus for induction has been challenged by the observation that iNOS is expressed in several normal adult and fetal tissues (30, 54, 68, 128, 151, 164, 206, 216), including the adult and fetal kidney (1, 17, 131, 133). Indeed, the first 3.8 kb upstream of the transcription initiation site of the human iNOS gene exhibited basal promoter activity when expressed by transfection in a human liver epithelial cell line (47).

**Molecular diversity.** The encoding DNAs and genes for the major NOS isozymes have been cloned and characterized (Table 1), recombinant proteins have been expressed in heterologous systems (137), and targeted disruption of each gene, as well as an nNOS/eNOS “double knockout” (179), has been accomplished in mice. In addition, alternative splice variants of nNOS and iNOS as well as iNOS-like genes have recently been identified, adding further to the structural diversity of this multigene family and to the complexity of analyzing their distribution and function.

Complementary DNA encoding nNOS was originally cloned from rat brain (25), but cDNAs have since been cloned from several tissues in rodents and humans (138). The ~10-kb nNOS transcripts in rats and humans encode proteins of 1,429 and 1,434 amino acids, respectively, that share 93% sequence identity. The NOS gene spans ~200 kb on human chromosome 12, and it contains 28 coding exons (71). Expression of the human nNOS gene is regulated by closely linked promoter/enhancer region contains potential cis-regulatory elements, including Sp1, AP-1, NF-1, adenosine 3',5'-cyclic monophosphate (cAMP)-responsive element (CRE), shear stress, and sterol regulatory motifs. eNOS-deficient mutant mice are hypertensive and more susceptible to global cerebral ischemia than wild-type littermates (80, 82), but systematic analysis of their renal function has not yet been performed.

iNOS cDNAs have been cloned from several species, including humans. Structurally, the deduced proteins of the mouse (103, 211) and rat (145) iNOS cDNAs share roughly 92% amino acid sequence identity with one another, but only 80% amino acid identity with the predicted human iNOS protein (62, 117). Using reverse transcription-polymerase chain reaction (RT-PCR), DNA sequence analysis, and diagnostic restriction endonuclease digestion of amplicons, we found that rat kidney expresses two distinct, highly homologous iNOS isoforms that are differentially expressed along the nephron and that respond differently to identical immunologic stimuli (131). One isoform, which we named VSM-NOS, is identical in sequence to the cDNA cloned from rat vascular smooth muscle cells (145), whereas the other isoform, which we named MAC-NOS, is more closely related in nucleotide sequence to the iNOS cloned from cultured murine macrophages (211). These findings were substantiated by Lau et al. (107), who demonstrated that cytokines differentially regulate the expression of these two iNOS isoforms in cultured rat medullary interstitial cells. MAC-NOS and VSM-NOS appear to represent distinct proteins encoded by separate genes (B. C. Kone, unpublished observations). However, this specific genetic variation may be re-
stricted to the rat, since only a single iNOS peptide has been identified in mouse and humans. Such species-specific genetic heterogeneity has been observed for many other genes, including the angiotensin II (ANG II) receptor family and the renin genes. Furthermore, the functional and regulatory properties of the two isoforms and their distinctive contributions to renal physiology and disease are as yet unknown. The most direct evidence for functional roles of both iNOS isoforms in rat comes from recent studies of postischemic acute renal failure (142). Treatment of rats with antisense oligodeoxynucleotides specific for VSM-NOS and MAC-NOS prevented renal dysfunction after the ischemic insult. In contrast, when MAC-NOS was allowed to function unopposed in rats treated with antisense oligodeoxynucleotides specific for VSM-NOS, much more severe renal failure ensued. These data suggest that VSM-NOS serves a protective role, and MAC-NOS serves a destructive role, in the pathogenesis of experimental postischemic renal failure in the rat. Clearly, further investigation of these isoforms is needed.

Genetic heterogeneity may exist for human iNOS as well, although the prevailing consensus of opinion favors a single iNOS-encoding gene. Molecular genetic analysis identified at least three distinct iNOS-like sequences mapping to different human chromosomal locations (19, 214). The major human iNOS gene, residing on chromosome 17, comprises 26 (32, 119) or 27 (214) exons that span ~37 kb, and a closely related iNOS gene has been mapped to human chromosome 14 (214). Multiple transcription initiation sites, including those far upstream of the TATA box, and alternative splicing give rise to multiple forms of exon 1 (38). In addition, four distinct alternative splice variants of human iNOS mRNA (Fig. 2), bearing deletions of exon 5, exons 8 and 9 (encoding amino acids 241-235), exons 9, 10, and 11 (encoding amino acids 289-427), and exons 15 and 16 (encoding amino acids 604-678, including the FMN binding site), respectively, have been identified (52). Transcripts for the exon 5-, exons 8- and 9-, and exons 9, 10, , and 11 variants were detected in whole kidney (52). Although the functions of these variants and their translation in vivo remain to be established, the fact that they are differentially distributed among tissues and inducible by lipopolysaccharide (LPS) and cytokines suggests that they may have important biological roles.

The complete rodent iNOS genes have not yet been cloned, but ~4 kb of the 5' control region for the mouse iNOS gene (207) and ~1.6 kb of the 5'-flanking region of the rat VSM-NOS gene have been characterized (50). The rodent and human genes contain a TATA box and numerous consensus binding elements for transcription factors involved in bacterial LPS, hypoxia, cAMP, or cytokine inducibility of other genes (Fig. 3). However, there are major differences in sequence and potential cis-acting elements in the 5'-promoter regulatory regions cloned from rodents and humans (as detailed below); whereas only ~1 kb of the proximal 5'-flanking region of the murine iNOS gene was necessary to confer inducibility to LPS and interferon (IFN)-γ (113, 213), cytokine-responsive elements of the human iNOS 5'-flanking region were distributed over at least 16 kb (47). In addition, the human iNOS gene, but not the rodent counterparts, contains a shear-stress responsive element (GAGACC) that merits further investigation (146). iNOS knockout mice (Table 1) exhibit increased susceptibility to intracellular pathogens and tumors and a blunted hypotensive response to bacterial LPS (115). Again, results from studies of the renal function of these animals have not been reported.

Intrarenal Distribution of NOS Isoforms

Biochemical studies have shown that the renal medulla has a greater capacity to generate NO than the renal cortex. For example, McKee et al. (126) observed that the renal medulla basally expresses considerable NOS activity (~20% of the highly NOS-enriched cerebellum) and that the basal medullary NOS activity is threefold greater than that of renal cortex. NADPH-dependent diaphorase activity, a histochemical index of catalytic NOS activity, has also been used to determine renal cell types likely to express NOS isoymes. Two groups reported strong NADPH-dependent diaphorase activity in the rat macula densa segment (MDS) (208), and McKee et al. (126) reported that the medullary thick ascending limb (MTAL) exhibited the greatest NADPH-dependent diaphorase staining of any nephron segment in the rat.

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Fig. 3. Structural organization of the murine iNOS 5'-flanking region. Consensus sites for transcription factors, the transcription start site (tsp), and the translation initiation codon (ATG) are indicated. Regulatory domains identified by analyses of iNOS promoter-reporter gene constructs (3, 119, 121, 129, 212, 213) are shown. For the purposes of illustrating consensus binding regions, the drawing is not to scale. HRE, hypoxia response element; TNF-RE, TNF-α response element; IFN-γ, interferon-γ; γ-IRE, interferon-γ response element; LPS, lipopolysaccharide; GAS, γ-activated site; ISRE, interferon-stimulated response element.
Several laboratories have used immunohistochemistry or in situ hybridization with NOS isoform-specific antibodies or nucleic acid probes to localize the major NOS isoforms within the kidney (Table 2). As alluded to above, however, renal functional and localization studies of the recently discovered nNOS and iNOS alternative forms have not been reported, and the specificity of the various reagents used to localize the NOS isoforms in earlier reports must now be reexamined. Colocalization of nNOS transcripts and immunoreactivity in the MDS have been demonstrated in several mammalian species (7), and nNOS immunoreactivity has been shown in the MDS in humans (7); thus there is general agreement that the MDS is the principal site of nNOS gene expression in the rat kidney (7, 178, 208). In addition, nNOS immunoreactivity was observed in the endothelium of the rat efferent arterioles, in single cells of the glomerular visceral epithelium, perivascular nerves surrounding the arcuate and interlobular arteries, in nerve fibers underneath the pelvic epithelium, and in a subset of cells in the MTAL that did not exhibit immunoreactivity for Tamm-Horsfall protein (7). Using RT-PCR of microdissected renal segments, Terada et al. (184) observed nNOS mRNA principally in the inner medullary collecting duct (IMCD) and, to a lesser degree, the glomerulus, inner medullary thin limb, cortical and outer medullary collecting duct, and renal vasculature. Since these structures exhibited neither nNOS immunoreactivity nor significant NADPH-dependent diaphorase staining, the amount of nNOS mRNA detected by RT-PCR in these segments may not be physiologically meaningful or may reflect expression of an alternative nNOS form not detected by available antibodies.

eNOS mRNA and protein have been reported in renal cortex and medulla of the rat (Table 2). Using RT-PCR of microdissected renal structure, Ujic et al. (195) detected eNOS mRNA in glomeruli, preglomerular vasculature, proximal tubules, thick ascending limbs (TAL), and collecting ducts. Immunohistochemical studies localized eNOS protein expression principally to the endothelium of the glomerular capillaries, the afferent and efferent arteriole, the intrarenal arteries, and the medullary vasa recta (7).

iNOS mRNA expression has been identified in the kidneys of normal rats (Table 2). Using competitive RT-PCR combined with diagnostic restriction endonuclease digestion, we demonstrated that the MAC-NOS isoform was expressed basally in several renal tubule segments, with highest expression in the MTAL, whereas the VSM-NOS isoform was principally expressed in the glomeruli and interlobular and arcuate arteries of the normal rat kidney (131). Morrissey et al. (133) independently reported abundant iNOS mRNA in the MTAL of the normal rat. Since previous in situ hybridization and immunohistochemical studies of nNOS and eNOS expression (7) failed to detect mRNA or protein for these isozymes in the MTAL, and since the MTAL is the tubule segment expressing the most abundant iNOS mRNA, it is logical to conclude that the abundant NADPH-diaphorase activity present in the MTAL (126) represents constitutively functional MAC-NOS. In situ hybridization studies of normal rat kidney with a riboprobe common to MAC- and VSM-NOS revealed signal in the S3 segment of the proximal tubule, the cortical and medullary TAL, the distal convoluted tubule, and the cortical collecting duct and IMCD of the normal rat (1). In contrast to earlier immunohistochemical studies (167, 168), no significant iNOS labeling was observed in the afferent arteriole. These results indicated that iNOS mRNA is tonically and differentially expressed along the normal rat nephron, with highest abundance in the MTAL.

The localization of iNOS immunoreactivity in the kidney has been more problematic. Conflicting results have been reported with different anti-iNOS antibodies. The afferent arteriole of the normal rat was labeled with one anti-iNOS antibody (188, 189), intercalated cells of the normal collecting duct with another antibody (187), and glomeruli in a rat model of immune complex glomerulonephritis with a third anti-iNOS antibody (93). Although the discrepancies among these data and between these results and the mRNA localization studies may reflect the inability of the available antibodies to detect both rat iNOS isoforms, further studies will be needed to reach a consensus on the intrarenal distribution of iNOS protein.

### Table 2. Basal expression of nitric oxide synthase isoforms in the kidney

<table>
<thead>
<tr>
<th>Isozyme</th>
<th>Renal Intrarenal Distribution</th>
<th>Evidence</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>nNOS</td>
<td>Macula densa, Glomerular parietal epithelium, Endothelium of glomerular efferent arterioles, Renal pelvic nerves, Perivascular nerves, CCD, OMCD, IMCD</td>
<td>RT-PCR, IH</td>
<td>7, 178, 188, 208, 7, 195</td>
</tr>
<tr>
<td>eNOS</td>
<td>Endothelium of: Glomerular capillaries, Afferent and efferent arterioles, Intrarenal arteries, Descending vasa recta, Hilar veins, Proximal tubule, MTAL, collecting duct</td>
<td>RT-PCR, IH</td>
<td>7, 195</td>
</tr>
<tr>
<td>iNOS</td>
<td>MTAL, S3 proximal tubule, CCD, OMCD, IMCD, Arcuate arteries, Vasa recta bundles</td>
<td>RT-PCR, IH</td>
<td>1, 131, 133, 1, 131, 133, 187, 131, 133</td>
</tr>
</tbody>
</table>

**References:**
- RT-PCR: Reverse transcriptase polymerase chain reaction.
- IH: Immunohistochemistry (protein localization).
- MTAL: Medullary thick ascending limb of Henle's loop.
- CCD: Cortical collecting duct.
- OMCD: Outer medullary collecting duct.
- IMCD: Inner medullary collecting duct.
**Regulation of NO Biosynthesis**

With the exception of nNOS-II, which employs bradykinin as substrate (33), all reported nNOS and eNOS isoforms are regulated by Ca²⁺ via calmodulin. Thus Ca²⁺ influx into neural or endothelial cells prompted by acetylcholine, bradykinin, or shear stress, for example, will stimulate NO biosynthesis. In addition, a host of physiological and pathophysiological stimuli have been shown to modulate the expression, intracellular distribution, or activity of the various NOS isoforms through transcriptional, posttranscriptional, translational, and/or posttranslational mechanisms. These data have been detailed in several recent reviews (23, 72, 101, 139). Below, we highlight recently described regulatory mechanisms that remain to be explored in the kidney.

**nNOS.** The expression of nNOS is developmentally regulated in brain (144) and lung (143), and pulmonary expression of nNOS mRNA and protein is enhanced after prolonged in vivo hypoxia (173). These findings suggest that this gene can be transcriptionally controlled. The nNOS 5'-flanking region contains a TATA box, CAAT box, and consensus binding sites for AP-2, CREB/activating transcription factor, NF-κB, nuclear respiratory factor 1, and other potential factors, but involvement of these or other transcription factors in the control of nNOS gene expression has not been established. The recent reports that changes in dietary salt intake produce inverse variations in nNOS gene expression selectively in macula densa cells (21, 178) invite detailed studies of nNOS transcriptional control in this setting. Moreover, transgenic animal methodology should be useful in determining the cis-elements governing the renal cell-type-specific expression of this gene.

In vitro studies have demonstrated that nNOS catalytic activity is regulated by the phosphorylation state of the enzyme. Whether this mode of nNOS regulation occurs in vivo remains to be convincingly established. In vitro phosphorylation of nNOS by protein kinase A, protein kinase G, protein kinase C, or Ca²⁺/calmodulin-independent protein kinase reduces catalytic activity (24, 42, 48, 136), whereas calcineurin-mediated dephosphorylation of the enzyme enhances catalytic activity (42). In addition, in vitro phosphorylation studies of wild-type and NH₂-terminal deletion mutants of nNOS indicate that the wild-type enzyme autophosphorylates, presumably at S¹⁷² (204). Although functional activity was not assessed in these studies, the nNOS-specific autophosphorylation pathway might regulate enzyme activity (204).

Although originally believed to be a cytosolic enzyme, nNOS has been found associated with integral membrane and cytoskeletal proteins of the skeletal muscle sarclemma (28) and with the rough endoplasmic reticulum in neurons (74). The NH₂-terminus of nNOS contains a PDZ domain (also known as GLGF-repeat) that binds α₁-syntrophin (28), a dystrophin-associated protein, and two postsynaptic density proteins, PSD-93 and PSD-95 (27). PDZ domains mediate protein-protein interactions that have been implicated in clustering of signaling and receptor molecules and the coupling of receptors to effector enzymes (73). Since proteins bearing PDZ domains typically localize to specialized cell-cell contacts (23) and the juxtaglomerular apparatus comprises different cellular interfaces, it will be interesting to determine whether nNOS is spatially distributed within the MDS and whether such geometry is important to MDS function. Similarly, the recent characterization of a highly conserved 10-kDa peptide, named PIN, which is widely distributed among tissues and binds to and inhibits the activity of nNOS, introduces another level of complexity to the regulation of this isoform for consideration (91). Detailed studies of PIN expression and regulation in the kidney, particularly in the MDS, are clearly needed.

**eNOS.** Virtually nothing is known about the molecular mechanisms controlling eNOS expression in renal vascular or glomerular endothelium, so inferences drawn from other tissues and systems should be helpful in guiding future research in the kidney. In vivo data indicate that eNOS gene expression can be modulated by chronic hypoxia (173), chronic exercise (171), and during fetal development (143, 144). Endothelial cell culture studies suggest that shear stress (92, 162), oxygen tension (5), transforming growth factor-β₁ (85), protein kinase C (147), tumor necrosis factor-α (218), lysophosphatidylcholine (220), and the degree of cellular proliferation (4) all influence the level of eNOS gene expression via changes in gene transcription rates and/or mRNA stability. Structure-function analysis of the eNOS promoter/enhancer region has provided evidence for cis-elements involved in the control of eNOS gene transcription. The Sp₁ element appears to be necessary for basal eNOS gene transcription in endothelial cells, and GATA-2 binding, synergistically with Sp₁ factors, appears to modulate the level of eNOS gene expression (321). NF-κB binding to a putative transforming growth factor-β₁ promoter response element is important for transforming growth factor-β₁ transactivation of the bovine eNOS gene (85). Additional structure-function studies of the eNOS gene are being pursued in several laboratories.

eNOS undergoes several posttranslational modifications, including phosphorylation, N-myristoylation, and cysteine palmitoylation, that regulate both activity and subcellular localization of the enzyme. Studies in culture bovine aortic endothelial cells demonstrated that eNOS can be tyrosine phosphorylated, a response that is enhanced by exposure of the cells to hydrogen peroxide or protein tyrosine phosphatase inhibitors. Moreover, the increased tyrosine phosphorylation of eNOS promoted by these maneuvers was associated with a substantial decrease in eNOS-specific activity (60). Other studies in bovine aortic endothelial cells revealed that bradykinin tyrosine phosphorylates a 90-kDa protein, eNOS-associated protein 1 (ENAP-1), that specifically interacts with eNOS and promotes association of eNOS with the membrane cytoskeleton (199). Conceivably, tyrosine phosphorylation-dependent interaction of eNOS with the cytoskeleton might direct NO production to specific subcellular locations.
Similarly, N-myristoylation is required for the enzyme's membrane association and trafficking into the Golgi complex, whereas both myristoylation and palmitoylation are required for efficient targeting of the enzyme to plasmalemmal caveolae (112, 174). Caveolins, a family of transmembrane proteins that constitute the principal structural components of caveolae, have been shown to interact functionally with several classes of signaling molecules, including trimeric G protein subunits and Src family tyrosine kinases (109, 110). Thus one proposed function of caveolae is to concentrate signaling proteins within a discrete microdomain of the plasma membrane. Indeed, immunoprecipitation studies of bovine lung microvascular endothelial cells demonstrated that tyrosine-phosphorylated eNOS coprecipitates with caveolin-1 (60). Thus tyrosine phosphorylation may influence not only eNOS functional activity as detailed above, but also potentially its intracellular routing and interaction with other caveolin-associated proteins. The functional importance of palmitoylation and subcellular targeting of eNOS to caveolae is evidenced by the fact that human embryonal kidney 293 cells transfected with palmitoylation-deficient mutants of eNOS released less NO compared with wild-type eNOS recipients, despite the fact that the purified mutant and wild-type enzymes were kinetically identical (112). These provocative results suggest that optimal NO release from intact cells is governed by palmitoylation of eNOS and the membrane microdomain in which the enzyme resides. Since eNOS palmitoylation can be dynamically regulated by bradykinin and presumably other agonists (165), and since the interaction of eNOS with distinct caveolin isoforms occurs in a tissue-specific manner (56), these pathways represent important biosynthetic control points in eNOS-expressing cells.

The mechanisms by which shear stress stimulates NO production by vascular endothelial cells are also complex. Differential display studies of cultured human endothelial cells demonstrated that laminar shear stress induces eNOS mRNA expression (192). In cultured aortic endothelial cells, shear stress induced de novo synthesis of eNOS mRNA and NO, effects that were prevented by actinomycin D and the K+ channel antagonist tetraethylammonium (TEA) chloride (194). These results suggested that shear stress activates K+ channels, which initiate a signaling cascade resulting in enhanced eNOS gene transcription and NO production. Protein kinase C inhibition did not prevent these effects, suggesting that c-Fos/c-Jun activation and binding to the AP 1 elements in the eNOS promoter were not involved. In another in vitro model of shear stress, however, depolarization with KCl or TEA did not alter cGMP production, an index of NOS activity, in either flow-treated or stationary cultures of human umbilical vein endothelial cells (64). The reasons for these discordant results are unclear. Tyrosine phosphorylation (6, 39), and perhaps changes in intracellular pH (6), also appear to be involved in a mechanotransduction cascade that activates eNOS in the absence of increased intracellular Ca2+ levels. Whether ENAP-1 is also involved in the shear-stress signaling pathway remains to be explored.

Another area of controversy concerns the response of the eNOS gene to hypoxia. Given the relatively hypoxic milieu of the renal medulla (29, 100), this issue is of particular relevance to the homeostatic control of medullary vascular tone (discussed below). In vivo studies showed that eNOS expression is upregulated in the pulmonary vasculature of the chronically hypoxic rat (108, 173). However, in vitro studies of the effects of hypoxia on eNOS expression arrived at conflicting results. Three studies (111, 127, 154) reported that hypoxia suppresses eNOS gene expression in cultured human and bovine endothelial cells. This suppressive effect appeared to result from decreased eNOS gene transcription and accelerated mRNA decay (111, 127), and it was not mimicked by normoxic exposure of the cells to reducing agents (154). In contrast to these data, studies of porcine coronary arteriolar endothelial cells (215) and bovine aortic endothelial cells in culture (5) indicated that hypoxia promotes eNOS expression. Indeed, the hypoxic induction of an eNOS promoter-reporter gene transfected into bovine aortic endothelial cells suggested that hypoxia, via as yet undefined transcription factors, trans-activates the eNOS gene in these cells (5). The reasons for these discrepant results are unclear, but likely reflect differences in the experimental protocols and the tissue origin of the endothelial cells studied. A resolution of this controversy, as well as in vitro and in vivo studies in the kidney, is surely needed.

\textbf{iNOS.} The regulation of iNOS expression and activity in response to inflammatory cytokines and LPS has received considerable attention in a variety of cell types and tissues. The influence of these mediators on iNOS gene transcription, mRNA stability, translation rates, protein stability, substrate availability, and enzymatic activity has been detailed (103, 132, 135, 139, 150, 183). This regulatory complexity presumably reflects the necessity for cell-type-specific control of this ubiquitous enzyme. For example, our studies in murine MTAL cells indicate that NF-κB p50/p65 proteins, but not c-Rel, are involved in LPS inducibility of the iNOS gene (103), whereas NF-κB p50-e Rel mediates this response in murine macrophages (212). Similarly, the mechanism by which NF-κB activates the iNOS promoter in vascular smooth muscle cells in response to cytokines appears to be markedly different from that which confers LPS inducibility in macrophages (180).

Detailed analyses of the highly complex 5' gene control region of the iNOS gene has been undertaken in several laboratories (Fig. 3). Mutational analysis of the murine iNOS 5' flanking region fused to reporter genes and transfected into RAW 264.7 cells indicated that the downstream one-third (region I, -48 to -209) and a more distal region [region II, variously reported as -1029 to -913 (Ref. 113) or -955 to -722 (Ref. 212)] of the iNOS promoter contain putative transcriptional
elements that mediate the inductive effects of LPS, whereas the synergistic effects of IFN-γ are restricted only to region II. The action of LPS in these cells is dependent on NF-κB heterodimers (p50-c-Rel) binding to the downstream iNOS NF-κB sequence (nucleotides −85 to −75; Ref. 212), whereas the synergetic effect of IFN-γ to activate iNOS transcription requires inclusion of the distal half of the promoter and, at a minimum, binding of interferon regulatory factor-1 (IRF-1) to its cognate sequence (−923 to −913) in the promoter/enhancer (113, 121, 212, 213). Region I appears to serve as a core promoter module (3), whereas the region from −1588 to −722 functioned in an orientation- and position-independent manner, suggesting that it serves as a classic enhancer element (3). Substitution of heterologous DNA between the two regions did not alter maximal promoter activity, suggesting that no other regulatory elements reside in the intervening region (−722 to −209). In vivo footprinting verified protein occupation of both NF-κB sites, an IRF-1 site, NF-IL6 [homologous to CCAAT/enhancer binding protein (C/EBP)] sites, and several other sites that do not correspond to known consensus sequences (63). Indeed, our preliminary work suggests that C/EBP-β trans-activates the iNOS gene in LPS-treated MTAL cells (69). A cytokine-responsive element (−890 to −1002) and a hypoxia-responsive enhancer element (HRE; −227 to −229), bearing close homology to the erythro-poetin-responsive enhancer element (ERE), have also been identified in the murine iNOS promoter; the latter appears to account for hypoxia inducibility of iNOS gene transcription in ANA-1 macrophages (129). Silencing mechanisms have been implicated in iNOS transcriptional control as well. A full-length iNOS promoter construct bearing mutation of the −913 to −923 IRF-1 element yielded greater LPS-induced expression of reporter gene activity than did the wild-type promoter, indicating that this element may also function as a silencer (121). Since this mutation also rendered IFN-γ suppressive, rather than stimulatory, an IFN-γ-acti-vated silencing mechanism must also be operative outside the IRF-1 element. However, the hazards of extending conclusions drawn from these in vitro studies to the in vivo setting are highlighted by the fact that a single NF-κB site was sufficient for LPS induction of iNOS promoter-reporter gene constructs transfected into RAW 264.7 cells (as detailed above), yet knockout mice lacking genes encoding IFN-γ (41), IRF-1 (98), or an IFN-γ receptor subunit (81) exhibited little or no LPS induction of iNOS. Thus the IFN-γ signaling pathway appears to be critical for LPS induction of murine iNOS in vivo.

Analysis of the human iNOS promoter revealed marked differences from murine iNOS in its transcriptional regulation. In contrast to murine iNOS, the first 3.8 kb upstream of the human iNOS gene demonstrated basal promoter activity but was unresponsive to LPS and cytokines. However, three cytokine-responsive regions in the human iNOS gene were identified at sites (−3.8 to −5.8, −5.8 to −7.0, and −7.0 to −16.0 kb) remote from the core promoter module (47). Structure-function studies revealed that the proximal region of the human iNOS promoter contains several inactivating nucleotide substitutions compared with the corresponding (LPS-responsive) region of the mouse iNOS gene (222). These structural differences, coupled with the apparent lack of LPS-inducible NF-κB/Rel complexes in human macrophages, appear to contribute to the inability of LPS, with or without IFN-γ, to activate strongly iNOS gene transcription in human monocytes and macrophages (222).

In contrast to the wealth of data concerning control of iNOS induction in pathological states, little is known about homeostatic regulation of iNOS expression in tissues, like the kidney, that constitutively express this enzyme. Presumably, iNOS expression in these settings results from basal autocrine or paracrine production of cytokines, growth factors, or hormones. The fact that iNOS expression is developmentally modulated in several tissues (17, 30, 216), hormonally regulated in the female reproductive tract (78), and varies in the renal medulla with changes in salt balance (124) indicates that nonimmune regulation of iNOS contributes to the normal function of several organs. Transforming growth factor-β (TGF-β) appears to be one factor that negatively modulates renal iNOS expression in vivo; transforming growth factor-β knockout mice exhibit enhanced iNOS expression in kidney and heart (201). Thus local control exerted by this growth factor may be one mechanism for cell-type-specific expression of iNOS in the kidney. Tyrosine kinases and phosphatases appear to be involved in posttranslational modification of iNOS, and may potentially play a role in modulating the functional activity of the enzyme (150). Since shear stress activates tyrosine kinases in endothelial cells (6, 39) and the 5′ control region of the human iNOS gene contains a shear-stress response element (146), it will be interesting to determine whether shear stress activates iNOS activity or expression in the macro- and microvascular endothelial cells of the kidney.

Since basal iNOS gene expression is highest in the MTAL and IMCD (126, 131, 133), segments that normally function in a relatively hypoxic environment (29, 100), the recent discovery that iNOS is an hypoxia-inducible gene (108, 129) provides at least one mecha-nism that may account for constitutive expression of iNOS in these segments. It is tempting to speculate that "constitutive" iNOS expression in the MTAL and IMCD relates to tonic activation of factors that bind the iNOS-I/IRE and trans-activate the iNOS gene. Such a construct would predict that iNOS expression would vary directly with oxygen utilization and the demand for active transport in these nephron segments. Indeed, iNOS protein levels were 50% greater in the inner medulla of rats chronically adapted to a high-salt diet compared with rats on a low-salt diet (124). More detailed mechanistic studies, currently underway in our laboratory, should clarify this position.

**HOMEOSTATIC ROLES OF NO IN THE NORMAL KIDNEY**

**NO and Renal Hemodynamics**

There is now abundant evidence that tonically generated NO plays a major role in maintenance of renal
perfusion and glomerular filtration in the normal kidney. Increased NO production has also been implicated in the renal vasodilatory responses to normal pregnancy and protein feeding/ amino acid infusion, discussed elsewhere (162).

In several species, including humans, acute systemic NO synthesis inhibition with L-arginine analogs produces dose-dependent increases in arterial blood pressure (BP) and renal vascular resistance (RVR) that are reversible with excess L-arginine (161, 209). When BP increases, some of the increase in RVR is autoregulatory; however, intrarenal local and low-dose systemic NOS inhibition produces renal vasoconstriction in the absence of increased BP (65, 161, 209). Thus NO tonically generated within the kidney lowers RVR. Increases in RVR due to NO synthesis inhibition cause reductions in renal plasma flow (RPF) and glomerular filtration rate (GFR), although GFR decreases proportionately less than RPF because filtration fraction rises (161).

These vasoconstrictor effects persist for the duration of NO synthesis inhibition, even in the conscious animal where all buffer mechanisms are operating. This result suggests that NO is not part of the feedback control of BP and vascular tone but rather sets the level of tone at which the other control systems operate. There is, however, no consensus as to how much of the vasoconstriction due to NOS inhibition results from withdrawal of an active NO vasodilatory stimulus and how much is secondary to amplification of underlying vasoconstrictor systems. The variability in the literature probably reflects differences in experimental preparations. It is likely that the role of vasoconstrictor systems in mediating the responses to NO synthesis inhibition is less important in the unstressed, conscious preparation than in surgically stressed, anesthetized animals.

The response to systemic NOS inhibition closely resembles the response to ANG II infusion, suggesting that ANG II participates in NOS inhibition-induced vasoconstriction (161). When the ANG II system is acutely activated (by volume depletion, surgical stress, etc.), or when exogenous ANG II levels are raised by infusion, the renal vasoconstrictor response to acute NOS inhibition is partly due to ANG II (12, 161, 176, 190). In the conscious, unanesthetized rat, however, endogenous levels of ANG II are low, and are not tonically controlling renal hemodynamics. In this preparation, blockade of the endogenous ANG II system has little or no effect on NOS inhibition-induced renal vasoconstriction (10, 161, 176). Because of these observations, we previously concluded that ANG II is not inevitably involved in the vasoconstrctor responses to NOS inhibition. However, this position has now been modified (see below).

There is also controversy about the role of the sympathetic nervous system (SNS) in the vasoconstrictor responses to acute NOS inhibition. Some workers report that SNS inhibition (ganglion blockade, pithing, or adrenergic receptor inhibition) has little effect on the increase in BP and RVR seen with acute NOS inhibition (161). In contrast, others claim that the hypertension and renal vasoconstriction are partly due to central and peripheral sympathetic activation (31, 105, 161, 193). Again, the experimental preparation, and thus level of activation of the SNS, may be a key factor in determining the contribution of the SNS. In the conscious, chronically catheterized rat, where efferent renal sympathetic nerve activity is low, renal denervation has no impact on the renal hemodynamic responses to either NOS inhibition or NOS stimulation with L-arginine (22). This suggests that neither direct nitrergic innervation (149) nor attenuation of renal efferent sympathetic nerve activity is involved in maintenance of renal perfusion by NO. We recently observed that in the conscious rat, acute systemic α-adrenoceptor blockade does not attenuate either pressor or renal vasoconstrictor responses to acute NOS inhibition. The obvious interpretation, that the SNS is not involved in expression of the vasoconstrictor effects of NOS inhibition, proved to be incorrect. Although inhibition of ANG II or the SNS alone has little impact on the pressor response to acute NOS inhibition in the conscious rat, combined ANG II and SNS inhibition attenuates the rise in BP while leaving the renal vasoconstriction intact (161, 169). The mechanism of the interaction between ANG II and the SNS is not clear but may involve actions of NO in the brain to inhibit central sympathetic outflow (31, 193). Thus even in the unstressed state, both ANG II and the SNS are important mediators of the pressor response to acute NOS inhibition, via a complex interaction with the SNS. The findings in normal rats with acute NOS inhibition are remarkably similar to those we reported earlier in the rat with chronic NOS inhibition (169), despite the significant vascular and renal pathology that had also developed with long-term NOS blockade.

Of importance, there is a dissociation between the pressor and renal vascular responses to NOS inhibition, in terms of interactions with ANG II and the SNS. Differences in NO dependent regulation of tone in kidney versus peripheral blood vessels were also observed by us in other contexts (12, 13, 159), suggesting that there are fundamental differences in the way NO regulates vascular tone in the kidney compared with other organs. Perhaps NO lowers systemic vascular resistance and thus BP largely by antagonizing vasoconstrictor tone, whereas locally generated endothelial NO tonically relaxes the renal vasculature.

Endothelin (ET) is a potent vasoconstrictor peptide with widely distributed receptors throughout the peripheral and renal vasculature (61, 205). Acute systemic NOS inhibition potentiates the vasoconstrictor actions of ET and also enhances the synthesis and release of ET (104). There are both ET_A and ET_B receptors in the kidney, although there is controversy about the physiological role of these ET receptors in regulation of peripheral and renal vascular tone (61, 205). In the normal, conscious rat we found that the pressor response to acute NOS inhibition was attenuated by concomitant inhibition of ET (158). A slight reduction in
the renal vasoconstriction was also seen but only as a secondary response to the blunted pressor effect (158).

Overall, withdrawal of NO amplifies any vasoconstrictor systems that are currently active. In the basal, relaxed state, when tonic vasoconstrictor tone is low, marked renal vasoconstrictor responses to acute NOS inhibition persist, even after attenuation of the pressor response, with combined ANG II/SNS blockade. These data suggest that, tonically, NO exerts a direct vasodilatory effect on the renal microcirculation, possibly via eNOS, whereas the action of NO on the general circulation is more complex, involving attenuation of the activity of ANG II/SNS with a small ET contribution.

**NO and Glomerular Microcirculation**

In vivo glomerular micropuncture studies have shown that during systemic NOS inhibition, when BP rises, marked increases occur in both preglomerular \( R_A \) and efferent arteriolar \( R_E \) resistances (Fig. 4) (43, 140, 161, 219). As a result, glomerular plasma flow falls, but single-nephron GFR is maintained, because of the large rise in glomerular blood pressure \( (P_G) \) resulting from the increased BP and \( R_E \). In addition, the glomerular capillary ultrafiltration coefficient \( (K_f) \) is reduced (43, 219), probably mediated by mesangial cell contraction, since in vitro NO relaxes the glomerular mesangial cell (161). As discussed above, systemic NOS inhibition produces widespread vasoconstriction leading to a pressor response that produces an autoregulatory rise in RVR separate from the direct, local effects of intrarenal NOS inhibition (140, 161). Accordingly, local intrarenal inhibition of NO generation causes smaller increases in RVR than are seen during systemic NOS inhibition (43, 65). As shown in Fig. 4, local intrarenal NOS inhibition in the cortical glomerular microcirculation increases \( R_A \) but has no effect on \( R_E \). A similar \( K_f \)-reducing effect is seen with both local and systemic NOS inhibition, suggesting equivalent levels of intrarenal NOS inhibition (43). In vitro studies on isolated microperfused cortical arterioles from rabbits also suggest that local generation of NO plays a greater role in control of \( R_A \) than \( R_E \) in cortical vessels (51, 87, 88). In contrast, local NOS inhibition increases both \( R_A \) and \( R_E \) of the in vitro juxtaglomerular nephron preparation (140, 161), suggesting that tonically produced NO controls both \( R_A \) and \( R_E \) in deep glomeruli. The cortical efferent arteriole is certainly capable of making and responding to NO when exposed to agonists, high shear rates, and in the hydronephrotic kidney (9, 58, 75), and NOS is present in abundance in the efferent arteriole (7). Why NO is not tonically released in the cortical efferent arterioles under normal conditions, even when NOS is present, is not clear, but it may be part of the complex system allowing independent control over tone in \( R_A \) and \( R_E \) (87, 140, 161) and thus exquisite control over glomerular hemodynamics. What causes the increased \( R_E \) with systemic NO inhibition when blood pressure rises is also unknown, since inhibition of locally generated NO is not responsible. Presumably, some secondary vasoconstrictor systems are activated, and we have preliminary data suggesting that both ANG II and ET may be involved (160) (see below).

In vivo studies on the glomerular microcirculation must be conducted under general anesthesia, which inevitably activates pressor systems. A number of micropuncture studies have implicated ANG II and the SNS in the glomerular microcirculatory changes seen with systemic NOS inhibition (59, 140, 161, 185, 196). There is, however, little agreement on the exact locations at which NO interacts with these pressor systems. In vitro and in vivo studies in cortical vessels in the rat suggest that ANG II exerts predominantly efferent arteriolar vasoconstrictor actions during NOS inhibition (46, 53), whereas in the rabbit isolated arterioles and in vivo in dogs, NO and ANG II interact primarily at the afferent site (87, 88, 170). In the rat juxtaglomerular glomeruli and as recently observed by us in the cortical microcirculation, NO and ANG II are shown to interact at both afferent and efferent arterioles (148, 160). In the case of the SNS, renal nerve activity appears to play a small role in NOS inhibitor-induced increases in vivo in both \( R_A \) and \( R_E \) in the rat cortical microcirculation (59), although in rabbit isolated afferent arterioles, NOS inhibition does not amplify the vasoconstriction produced by applied norepinephrine (88). ET-induced constriction of the isolated rabbit afferent arteriole is
blunted by tonically produced NO (89). In the hydronephrotic rat kidney, ET and NO also interact in control of afferent but not efferent arteriolar tone (67). In vivo studies in normal rat, ET mediates some of the increased tone in both afferent and efferent vessels following NOS inhibition (160). In fact, the combined actions of ANG II and ET appear to be responsible for the increased $R_E$ seen with acute, pressor doses of NOS inhibitors (160).

Thus the relationship between tonically produced NO and the vasoconstrictor systems of ANG II, SNS, and ET is complex and highly variable. In anesthetized, surgically stressed animals all three vasoconstrictor systems are apparently active tonically in the renal vasculature. NO attenuates their various vasoconstrictor actions within the renal microcirculation, although the precise segmental arteriolar location of interactions between NO and ANG II, SNS, or ET remains a mystery. What is clear from our studies in the conscious, chronically catheterized rat, however, is that the increase in RVR due to acute NOS inhibition does not require participation of these vasoconstrictor systems. This separates the renal vasculature from the general systemic circulation. Micropuncture studies suggest that tone in the glomerular mesangial cell is controlled by interactions between NO and both ANG II and ET (46, 160, 161), although it is impossible to assess these relationships in the conscious animal.

The regulation of the glomerular microcirculation by NO is quite complex. In addition to direct interactions between NO and vasoconstrictors in control of tone, NO also controls glomerular hemodynamics via the TGF system (140, 161). NOS is abundant at the juxtaglomerular apparatus, and NO generated within the macula densa (from nNOS) may control glomerular hemodynamics by providing vasodilatory attenuation of TGF-induced increases in $R_A$ (186, 208). Vallon and Thomson (197) agree that NO attenuates the TGF-induced increase in $R_A$ but argue that this is not mediated via macula densa signaling. In vitro studies suggest that NO blunts the TGF-induced increase in $R_A$ only when the macula densa is perfused with high NaCl (90). NO may also influence the myogenic component of autoregulation, but while there is a suggestion that NO contributes to low-pressure dilatation of $R_A$, renal autoregulatory ability is relatively intact during NO inhibition, although RVR is reset to a higher value (140, 161).

**Effects of Long-Term NOS Inhibition**

It is possible to produce a sustained hypertension by chronic administration of NOS inhibitors. In the absence of severe pathology, the glomerular hemodynamic responses to chronic, partial NOS inhibition for 8 wk are remarkably similar to those seen with acute systemic NOS inhibition (43, 161): that is, systemic hypertension, renal vasoconstriction involving both afferent and efferent resistance vessels, and reductions in $K_E$ (14). Because of the systemic hypertension and increase in $R_E$, $P_{OC}$ is chronically elevated, and these rats display moderate proteinuria and histological evidence of structural damage with a mild increase in focal and segmental glomerular sclerosis (14). More complete NOS inhibition leads to severe hypertension and further elevations in $P_{OC}$, which probably contributes to the increased glomerular injury in the more severe models (14, 16, 161). In addition to glomerular hypertension, NO deficiency can lead to mesangial cell expansion and overproduction of extracellular matrix, which also predisposes to glomerular injury (49).

As with acute systemic NOS inhibition, other systems contribute to some of the vasoconstriction that occurs with chronic NOS inhibition. Acute receptor blockade of α- or β-adrenoceptors has little impact on either the hypertension or renal vasoconstriction (8, 159), although acute ganglionic blockade produces an exaggerated fall in BP in rats with chronic NOS inhibition (40). Acute ANG II blockade has little effect on BP or RVR (8, 159), although the vasoconstriction of chronic NOS inhibition is clearly ANG II dependent, since chronic ANG II blockade ameliorates or reverses the hypertension and renal dysfunction (96, 134, 155, 163). Furthermore, when acute ANG II and α-adrenoceptor blockade are combined, an exaggerated fall in BP (but not RVR) occurs in conscious, NOS-inhibited rats (150), very similar to our observations with acute NOS inhibition (169) (see above). Overall, these observations suggest a key role for interactions between ANG II and the SNS in the hypertension induced by chronic NOS inhibition, perhaps via increased central sympathetic drive (40).

**NO in Regulation of Salt Balance**

As discussed above, eNOS, nNOS, and iNOS isoforms are all constitutively expressed in various locations throughout the tubule epithelium. There is strong in vitro evidence that NO has direct tubular actions to inhibit sodium reabsorption in proximal tubules and collecting duct by inhibition of Na⁺/H⁺ exchange, Na⁺-K⁺-adenosinetriphosphatase (Na⁺-K⁺-ATPase), and amiloride-sensitive Na⁺ channels (37, 102, 114, 126, 167, 181, 182). Our recent studies in an iNOS-transfected MTAL cell line (102) indicate that constitutive NO expression limits Na⁺-K⁺-ATPase subunit gene expression and Na⁺-K⁺-ATPase activity, the principal driving forces for Na⁺ reabsorption, consistent with a role for NO in facilitating Na⁺ excretion (Fig. 5). NO is also important in mediating pressure natriuresis (118, 168), probably via control of medullary blood flow and renal interstitial hydrostatic pressure (55, 70, 125). In apparent conflict with these studies, where NOS inhibition prevents or attenuates the pressure natriuresis (118, 168), earlier work by us and others demonstrated a marked natriuretic response in the rat during systemic NOS inhibition (11, 95). Since this was accompanied by an abrupt rise in BP, we interpreted this as a pressure natriuresis. Recent observations, however, suggest that the natriuresis seen with acute, systemic NOS inhibition can be dissociated from the rise in BP and is somehow related to the activity of the renal nerves (22, 169).

There is also some evidence that increased dietary salt intake in normal animals promotes NO synthesis,
playing a vasodilatory and possibly natriuretic role (175, 191). The stimulus to enhanced vasodilatory NO production is apparently confined to the kidney (45). NO deficiency has been causally implicated in development of hypertension in salt-sensitive Dahl rats fed a high-salt diet (33–35), and the protective effect of L-arginine is associated with normalization of the pressure natriuresis and altered epithelial transport (99, 153). In normal animals, several studies suggest that chronic NOS inhibition leads to volume-dependent hypertension. A low dose of NOS inhibition produces a vasoconstrictor response that is confined to the kidney (15, 203). These low doses produce no pressor effect in rats or dogs on normal salt diet, but elevations in BP do occur with high salt intake by a volume-dependent effect (106, 217). Local intrarenal NOS inhibition for 5 days in rats lowers medullary blood flow leading to sodium retention and increased BP (125). Volume dependency is only evident during selective, intrarenal NOS inhibition, since widespread NOS inhibition causes an immediate vasoconstriction that leads to hypotension in the absence of volume expansion and even during volume contraction (77, 125, 157).

NOS inhibition/deficiency-induced hypertension may result from reductions in NO synthesis from a variety of cells and NOS isoforms. Widespread loss of NO generated from the eNOS produces hypertension in eNOS knockout mice (80), whereas nNOS knockouts are normotensive (86), suggesting that NO from eNOS is of primary importance in BP regulation. However, human studies indicate no genetic linkage between eNOS and essential hypertension (20, 83). In contrast, functional studies with pharmacological or antisense nNOS inhibition, discussed above, suggest that nNOS plays an important role in BP control, possibly by modulation of the SNS (31, 105, 159, 161, 169, 193). There is some functional evidence that a defect in NO originating from an iNOS is associated with salt-dependent hypertension in the Dahl-Rapp rat (34).

Alleles of the iNOS, but not nNOS locus cosegregated with blood pressure in an F2 population derived from a cross of salt-sensitive Dahl-Rapp rats and normotensive strains (44). However, a role for nNOS deficiency in salt-dependent hypertension has also been suggested, since a reduction in nNOS, but not eNOS or iNOS, activity has been reported in the kidneys of Dahl salt-sensitive versus salt-resistant rats fed a high-salt diet (84). Furthermore, dietary salt loading promotes increased nNOS activity in the hypothalamus of normal rats (97, 200) and enhanced expression of nNOS (but not eNOS of iNOS) mRNA in whole cortex and MDS, and selective renal medullary nNOS inhibition produces a salt-dependent hypertension (123, 124). Chronic iNOS inhibition (with aminoguanidine) in the rat on a normal dietary salt intake has no effects on BP or renal hemodynamics (156), whereas inhibition of iNOS in rats on a high-salt diet leads to a paradoxical hypotension (18). The possible role of NO originating from the two rat iNOS isoforms (1, 131) in the response to changes in salt intake remains to be determined.

**CONCLUSIONS AND INVITATIONS**

Despite the tremendous amount of extant data on NO biology, we stand at the threshold of gaining great insight into the roles and regulation of NO biosynthesis in the kidney and the functional consequences of NO deficiency or excess. NOS isoform-specific inhibitors are under development and nearing release; these reagents will allow precise pharmacological distinction of the properties, regulation, and functions of each NOS isoform in the kidney. Renal physiological studies of the available NOS gene knockout and double-knockout mice should yield important new or confirmatory information not only about the role of NO in the control of renal glomerular and tubular function but also of its integration with other system components (e.g., ANG II, SNS, ET) regulating these processes. The generation and functional analysis of a complete battery of double-knockout mice and chimeric mice bearing regional targeted deletions of the NOS isoform genes (122) should provide additional insights into the homeostatic roles of NO in the kidney.

With the continued discovery of new members and variants of the NOS gene family and of NOS regulatory proteins, such as PIN (91), large gaps in our understanding of NOS expression and regulation in the kidney remain. Detailing the renal distribution and modulation of these NOS gene products will require development and application of additional specific molecular probes, antibodies, and inhibitors. The recent advances demonstrating the functional importance of the intracellular distribution of nNOS and eNOS should prompt similar investigations in renal epithelial and vascular cells. Studies of the molecular requisites for development and cell-type-specific expression of the NOS isoforms in the kidney are feasible with available technology. Finally, and most importantly, extension of these findings to human renal physiology is clearly needed. Success in meeting these challenges will require a true
marriage of physiological, cell biological, and molecular biological approaches.

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