Nitric oxide and the kidney

SP Sharma
Associate Professor, Division of Nephrology, Department of Internal Medicine,
Texas Tech Health Science Center, Lubbock, USA

Abstract

Recent years have witnessed a phenomenal scientific interest in the vascular biology, particularly the relevance of nitric oxide (NO) in cardiovascular and renal physiology and pathophysiology. Although hemodynamic actions of NO received initial attention, a variety of non-hemodynamic actions are now known to be mediated by NO in the normal kidney, which include tubular transport of electrolyte and water, maintenance of acid-base homeostasis, modulation of glomerular and interstitial functions, renin-angiotensin activation and regulation of immune defense mechanism in the kidney. In most pathological states, the role of NO is dependent by the stage of the disease, the nitric oxide synthase (NOS) isoform involved and the presence or absence of other modifying intrarenal factors. Additionally NO may have a dual role in several disease states of the kidney such as acute renal failure, inflammatory nephritides, diabetic nephropathy and transplant rejection. These controversies along with some preliminary experiences with therapeutic potential for manipulation of L-arginine-nitric oxide axis in renal disease states are discussed in the following treatise.

Key Words: Nitric oxide, nitric oxide synthase, L-arginine, renal physiology.

Introduction

One of the major scientific advances in the past decade in understanding of the renal function and disease is the prolific growth of literature incriminating nitric oxide (NO) in renal physiology and pathophysiology. NO was first shown to be identical with endothelial derived relaxing factor (EDRF) in 1987 and this was followed by a rapid flurry of information defining the significance of NO in not only vascular physiology and hemodynamics but also in neurotransmission, inflammation and immune defense systems. Although most actions of NO are mediated by cyclic guanosine monophosphate (cGMP) signaling, S-nitrosylation of cysteine residues in target proteins constitutes another well defined non-cGMP dependent mechanism of NO effects. While NO is considered beneficial in general in regulation of vasmotor tone, immune defense modulation and neurotransmission, excessive NO generation is cytotoxic due to the effects on generation of reactive oxygen and nitrogen species and nitrosylation of proteins. The physiologic role of NO in kidney function was suggested by preliminary observations in early nineties, which laid the foundations for a plethora of scientific publications that established the importance of NO in renal physiology and pathophysiology. While the vast amount of NO literature has enhanced our understanding of its relevance in kidney disease and health, it has also contributed to significant confusion in view of the conflicting data. In this review, I will attempt to summarize the available information in this area and present a fair and balanced view concerning the physiological and pathological role of NO in nephrology.

Role of nitric oxide in renal physiology

Although NO was described initially as a vasodilatory chemokine, it plays a major role in vascular biology in terms of anti-thrombosis, anti-inflammatory, antiproliferative and antioxidative effects. Nitric oxide is synthesized exclusively from its precursor L-arginine under the catalytic effect of nitric oxide synthase (NOS), of which there are three isoforms—neuronal NOS (or NOS I), inducible NOS (or NOS II) and endothelial NOS (or NOS III). The three isoforms are encoded by different genes and share less than 60% structural homology. The L-arginine→NO conversion is a complex reaction that requires, in addition to NOS enzyme, molecular oxygen, nicotinamide dinucleotide phosphate (NADPH), tetrahydrobiopterin, flavin adenine dinucleotide (FAD)

Address for Correspondence:
Dr S Prabhakar Sharma
Associate Professor
Division of Nephrology
Department of Internal Medicine
Texas Tech Health Science Center
3601, 4th street Suite 4C-178
Lubbock, TX 79430 USA
E-mail:sharma.prabhakar@ttuhsc.edu

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flavin mononucleotide (FMN) and calmodulin. In addition NOS I and NOS II require increase in cytosolic calcium (Ca++) to promote calmodulin binding while NOS III doesn’t require additional Ca++ for calmodulin binding and hence relatively calcium-independent.

**NOS isoforms in the kidney:**

The information on the presence of different isoforms in the kidney is derived from mRNA localization and NOS expression studies. Unfortunately such studies did not yield consistent results. For instance, iNOS mRNA has been identified in rat kidney while iNOS expression has not been demonstrated in normal rat kidney. Furthermore the NOS expression studies have not always corroborated with functional studies in the kidney. This is illustrated by the fact that while studies from in situ perfused proximal tubule derived from iNOS knockout mice had shown a role for iNOS in fluid and HCO₃⁻ transport, iNOS protein had not been shown here. Notwithstanding these discrepancies, all 3 NOS isoforms have been demonstrated in the kidney by immunohistochemical studies. While eNOS is abundantly expressed in renal microvasculature, glomerular endothelial cells, proximal tubular cells, thick ascending limb of Henle’s loop and collecting tubule, nNOS is expressed in macula densa, principal cells of the collecting duct and in pelvic renal nerves. Functional studies support the presence of iNOS in proximal tubule and in vitro studies in cultured cells indicate the presence of iNOS in glomerular mesangial and inner medullary collecting duct cells, although the exact localization of iNOS in vivo is still unknown.

**Functional role of NO in the kidney:**

Studies in intact cells as well as in cellular organelles indicate that nitric oxide modulates mitochondrial respiration, membrane transport and cellular ATP generation. Garvin and Hong showed in renal tubules and isolated renal mitochondria that NO inhibited mitochondrial respiration. Many studies established that intrarenal NO regulates macromolecular and microvascular including glomerular hemodynamics in the kidney. NOS inhibition decreases basal renal blood flow although preserving autoregulatory responses (Table 1). Animal studies involving NOS inhibitors suggest that intrarenal NO regulates both afferent and efferent arteriolar tone. One of the major roles of NO is the regulation of medullary perfusion. L-arginine infusion enhances and NOS inhibitors reduce medullary blood flow and promote salt retention and hypertension in animal models. These data underscore the significance of role of NO in renal medullary perfusion and the renal hemodynamics in development of hypertension.

The effects of NO on renal fluid and electrolyte transport result from the net effects on renal hemodynamics, renal nerves and direct tubular transport properties. In the proximal tubule, NO stimulates fluid, sodium and HCO₃⁻ reabsorption by stimulating Na⁺/H⁺ exchange. Endogenous NO derived from nNOS (NOS I) and iNOS (NOS III) mediate these effects in the proximal tubule as demonstrated by studies from knockout models and specific inhibitors nNOS and iNOS. To this date, there is no evidence that supports the role of eNOS in directly modulating proximal tubular transport. Several studies indicated that NO decreased Cl⁻ and HCO₃⁻ reabsorption in the medullary thick ascending loop of Henle (mTALH) by inhibiting the Na⁺-K⁺-2Cl⁻ co-transporter and Na⁺/H⁺ exchanger. On the other hand NO stimulated K⁺ channel activity in the apical membrane of the mTALH segment. NO exhibits several effects in the collecting tubule. In the cortical collecting duct (CCD), NO inhibited apical Na⁺ conductance which is related to inhibition of basolateral K⁺ conductance. NO also inhibited Na⁺ and ADH-sensitive water permeability in the principal cells and H⁺-ATP-ase in the interstitial cells of CCD.

One of the renal regulatory mechanisms related to maintenance of arterial blood pressure involves the phenomenon of pressure-natriuresis in response to elevation of arterial pressure. This effect implies inhibition of tubular sodium reabsorption resulting in natriuresis, in an effort to lower arterial pressure. Experimental evidence from dog studies indicates that intra-renal NO modulates pressure natriuresis. Furthermore many studies have confirmed the role of intra renal NO in mediating tubulo-glomerular feedback (TGF). Wilcox et al. have demonstrated through in vivo micropuncture studies that NO derived from nNOS in macula densa specifically inhibits the TGF responses leading to renal afferent arteriolar vasoconstriction in response to sodium reabsorption in the distal tubule. Other recent studies support the inhibitory role of NO from eNOS and iNOS in mTALH segment on TGF effects.

**Table 1: Functions of NO in the kidney**

| 1. Renal macrovascular and microvascular dilatation (afferent > efferent) |
| 2. Regulation of mitochondrial respiration |
| 3. Modulation renal medullary blood flow |
| 4. Stimulation of fluid, sodium and HCO₃⁻ reabsorption in the proximal tubule |
| 5. Stimulation of renal acidification in proximal tubule by stimulation of NHE activity |
| 6. Inhibition of Na⁺, Cl⁻ and HCO₃⁻ reabsorption in the mTALH |
| 7. Inhibition of Na⁺ conductance in the CCD |
| 8. Inhibition of H⁺-ATPase in CCD |
There is abundant literature supporting a role for intrarenal NO in regulating renal acidification mechanisms. Despite conflicting data, majority of evidence showed that NO stimulated Na’ and HCO\textsubscript{3} reabsorption by stimulating Na’/H’ exchanger and through augmenting basolateral electrogenic Na’-3HCO\textsubscript{3} co-transporter\textsuperscript{28}. Thus NO participates in the proximal tubular acidification mechanisms by at least two distinct mechanisms. Currently there is no data implicating NO modulation of HCO\textsubscript{3} reabsorption through electrogenic H’ secretion. In the medullary thick ascending limb of Henle (mTALH), NO inhibited net Cl’ and HCO\textsubscript{3} absorption by modulating the activity of the Na’/H’ exchanger at both apical and basolateral sides of the mTALH and Na’-K’-2Cl’ cotransporter\textsuperscript{29}. At the present time, there are no reports of NO modulating H’ secretion in the distal tubule. In collecting duct, NO inhibits H’ ATPase activity in the intercalated cells of the collecting duct\textsuperscript{24}.

In summary, intra renal NO activity is a major regulator of the glomerular hemodynamics, tubular transport and TGF responses. NO relaxes both afferent and efferent arterioles and regulates renal medullary blood flow. In the proximal tubule, NO promotes fluid and bicarbonate reabsorption and inhibits Na’/H’ exchanger and Na’-K’ ATPase activity. In the mTALH segment NO inhibits Cl’ and HCO\textsubscript{3} reabsorption while in collecting duct NO decreases Na’ and fluid reabsorption. The net result of these changes is increased renal and glomerular perfusion, natriuresis and diuresis.

**Nitric oxide and acute renal failure:**

Recent observations in vascular biology have yielded new information that endothelial dysfunction early in the course might contribute to the pathophysiology of acute renal failure\textsuperscript{30}. Structural and functional changes in the vascular endothelium are demonstrable in early ischemic renal failure. Altered NO production and/or decreased bioavailability of NO comprise the endothelial dysfunction in acute renal failure. Several studies have indicated imbalance of NOS activity with enhanced expression and activity of iNOS and decreased eNOS in ischemic kidneys\textsuperscript{31}. The imbalance results from enhanced iNOS activity and attenuated eNOS activity in the kidney. Employing antisense-oligodeoxynucleotides to iNOS, Noiri et al\textsuperscript{32} demonstrated that high output NO production by iNOS might suppress the activity of eNOS, a schema that could potentially explain the abnormal vascular phenomena of acute ischemic renal failure. For example suppressed eNOS activity could explain loss of anti-thrombogenic properties of endothelium, vasoconstriction and enhanced neutrophil adhesion while enhanced iNOS activity could explain loss of vasomotion, enhanced neutrophil motility, tubular cell injury and suppression of eNOS activity. Generation of superoxide and NO in ischemic reperfusion injury results in the formation of peroxynitrite, which is cytotoxic by causing lipid peroxidation, injuring DNA, and nitrotyrosination of proteins. Noiri et al\textsuperscript{33} showed in a rat model that while renal ischemia increased oxidative and nitrosative stress by enhanced peroxynitrite formation, rats treated L-NiI, an iNOS-specific inhibitor improved renal function by inhibiting oxidative stress. Recently Brodsky et al\textsuperscript{34} have demonstrated that transplantation of functionally competent mature endothelial cells into circulation of post-ischemic rats provided dramatic renal protection against ischemic injury.

**Nitric oxide in inflammatory nephritides**

Many experimental studies support a contributory role for NO in glomerulonephritis (GN). Evidence from recent studies pointed out that NO may be involved in peroxynitrite formation, pro-inflammatory chemokines and signaling pathways in addition to direct glomerular effects that promote albumin permeability in GN\textsuperscript{35}. Although originally macrophages and other leukocytes were first considered as the source renal NO production in GN, it is now clear iNOS derived NO from glomerular mesangial cells are the primary source of NO in GN\textsuperscript{36}. Several experimental models of GN such as anti-Thy antibody nephritis, nephrotoxic serum nephritis are associated with increased expression of iNOS and possible nNOS but normal and reduced expression of eNOS activity\textsuperscript{37,38}. These changes are particularly relevant in the acute phase of the illness. Human studies in IgA and lupus nephritis support these observations\textsuperscript{39}. However a few animal and human studies showed decreased NOS expression and NO levels\textsuperscript{40}. Thus considering the currently available literature, it is likely that iNOS derived NO may promote inflammatory injury in GN, while eNOS derived NO maintains endothelial function and protects against further glomerular damage.

A rapidly growing body of evidence supports a critical role for NO in tubulointerstitial nephritis (TIN). In the rat model of autoimmune TIN, Gabbai et al\textsuperscript{41} demonstrated increased iNOS expression in the kidney and NO metabolites in urine and plasma. However the effects of iNOS on renal damage in TIN seem to have a biphasic effect- since iNOS specific inhibitors (eg. L-Nil) are renoprotective in the acute phase while they actually accelerated the renal damage in the chronic phase. Thus chronic NOS inhibition is used to induce chronic tubulointerstitial injury and fibrosis along with mild glomerulosclerosis and hypertension. Tubulointerstitial damage as a result of urinary obstruction represents another clinical syndrome where NO may play a role. In a rat model of obstruction, L-arginine ameliorated while L-NAME accelerated tubulointerstitial damage\textsuperscript{42}. On the other hand in models of drug induced TIN such as cyclosporine nephrotoxicity, NO donors promote and NOS inhibitors ameliorate tubulointerstitial damage\textsuperscript{43}. These data while lucidly incriminating NO in TIN,
underscore the need for more mechanistic studies to gain better insight into pathogenic role of NO in tubulointerstitial disorders.

**Nitric oxide in diabetic nephropathy:**

Endothelial dysfunction is the predominant pathophysiological marker for micro- and macrovascular complications of diabetes including diabetic nephropathy. Conventionally, hyperglycemia, hypertension, and activation of intra-renal renin-angiotensin system have been considered contributory to the development and progression of diabetic renal disease. All these three factors potentially can alter NO production in the kidney. A vast array of literature has built up in the past decade that incriminated abnormalities of intra-renal nitric oxide generation in the pathogenesis of diabetic nephropathy. Unfortunately most these studies have added to the confusion that exists in this field in view of the conflicting results that these studies yielded. The differences can be partly explained on the basis of the in vitro versus in vivo studies, methodologies used for NO measurements, the NOS isoforms studied and many more. Despite the conflicting reports, some reasonable generalizations can be made about the role of NO in diabetic nephropathy.

Diabetic nephropathy represents a complex metabolic arena characterized with patho-physiological events that both stimulate and depress intrarenal NO production. The net effect on renal NO production depends on the mechanisms that prevail in a given stage of the disease (Figure 1). Most in-vitro studies which examined NO synthesis in diabetic state, evaluated NO generation in cell cultures exposed to high ambient glucose concentrations and found reversible NO inhibition. But many in-vivo animal studies documented the role of NO and NOS isoforms. The available evidence suggests that early diabetic nephropathy is associated with increased constitutive NO production derived primarily from eNOS and possibly nNOS and this effect may causally be related to intraglomerular hemodynamic changes seen in early diabetic renal disease leading to microalbuminuria and hyperfiltration. On the contrary, later stages of diabetic nephropathy characterized by hypertension, progressive renal insufficiency and heavy proteinuria is associated with decreased NO production (especially iNOS derived) resulting in a NO deficient state. Several intra-renal pathophysiological phenomena may contribute such NO deficiency, including advanced glycation end-products, increased formation of reactive oxygen and nitrogen species, activation of TGF- beta and protein kinase C. Hyperglycemia and angiotensin activation may lead to stimulation of transforming growth factor- beta (TGF-b) which in turn may mediate more distal events in the cascade of pathophysiology of diabetic nephropathy. We have recently shown that angiotensin II inhibited inducible NO production from cytokine stimulated mesangial cells and this effect was associated with increased TGF-b expression shown by immunocytochemistry (Figure 2). The role of hyperinsulinemia, which is regarded an independent cardiovascular risk factor in diabetes, on intrarenal NO production...
production are largely unknown. We have recently demonstrated that insulin in physiological concentrations enhanced iNOS activity in normal human mesangial cells and this effect was mediated by augmented cellular uptake of NO-substrate, L-arginine. However in the clinical diabetic state, cellular resistance to the action of insulin may not permit increased NO activity despite hyperinsulinemia. Finally several studies linked genetic pleomorphisms in the eNOS (NOSIII) isoform especially Glu298asp mutation at exon 7, to susceptibility to diabetic nephropathy (Figure 3A & Fig 3B). A recent review summarizes all the above mentioned factors that contribute to NO abnormalities in diabetic nephropathy.

Renal Transplantation and Nitric oxide system

The protective role of constitutive NO in transplanted kidneys was well documented in several studies. Deficient NO in transplanted kidneys as a result of genetic deficiency of eNOS or NOS inhibitor therapy is associated with hypertension and graft dysfunction. On the other hand, iNOS mediated NO has dual roles with

**Figure 3A**

**Figure 3B**

Figure 3A. Effects of tetrahydrobiopterin (BH₄) on NOx accumulation in MMC. **P < 0.001. L+T vs. LG and HG+BH₄+L+T vs. HG+L+T, n = 12 in each group.

Figure 3B. Western blotting showing effects of HG on iNOS protein expression. The immunoblot shown above represents similar results from 3 other experiments. Densitometric image analysis revealed no significant differences between the bands seen in lane LG+(L+T) and lane HG+(L+T).
potential for graft dysfunction in the early post-transplant phase mediated by peroxynitrite formation secondary to NOS co-factor deficiency, while iNOS offers renal graft protection in the later phase by suppressing inflammatory cell recruitment and smooth cell proliferation. Endothelial injury is a key component in the pathogenesis of acute renal allograft rejection. Nuclear factor kappa B (NFkB) is the most critical upstream event that leads to recruitment of allo-activated T cells since it is activated by reactive oxygen species and cytokines such as tumor necrosis factor – alpha and interleukin-1. Further sequence in the cascade of events involve transcriptional activation of iNOS and other cytokines and cell adhesion molecules by NFkB which ultimately lead to inflammation and graft dysfunction. In addition, NO may also exercise a negative feedback effect on NFkB, suggesting that NO is an important modulator of graft function. All these pathophysiological signaling processes are schematically represented in Figure 4.

Figure 4: Interaction of NO and NF kappa B with other cytokines and pathophysiological processes in allograft dysfunction. iNOS, inducible NO synthase; ROS, reactive oxygen species; APC, antigen presenting cells; MHC, major histocompatibility complex; NF-kappaB, nuclear factor kappa B.

Figure 5: Major pathways of L-arginine metabolism. L-arginine may be metabolized by the urea cycle enzyme arginase to L-ornithine and urea by arginine decarboxylase to agmatine and CO₂ or by NOS to nitric oxide (NO) and L-citrulline. Adapted from Klahr S: Can L-arginine manipulation reduce renal disease? Semin Nephrol 61:304-309, 1999.
L-Arginine therapy in renal diseases:

The recent unfolding of diverse functions of NO in the kidney has kindled major enthusiasm in exploring L-arginine as a therapeutic tool especially in those chronic renal conditions associated with NO deficiency. L-arginine is the sole precursor of NO but is also the source for creatinine, polyamines, urea and agmatine (Figure 5). Our current knowledge of therapeutic potential of L-arginine administration is largely based on animal studies. Reno-protective effects, amelioration of pathological changes and functional improvement in the kidney have been reported with L-arginine therapy in rat models of renal failure and diabetic renal disease and several other models. Preliminary clinical studies indicate that intra-coronary or systemic L-arginine infusion improves endothelial dysfunction in human subjects with hyperlipidemia and angina pectoris. Although L-arginine therapy is safe and inexpensive, a more comprehensive understanding of the metabolites of arginine especially agmatine, is warranted before L-arginine therapy could be considered.

Summary

The foregoing discussion attests the explosive growth in literature pertaining to nitric oxide in normal kidney physiology as well pathophysiology of various kidney diseases. It is obvious that kidney is not only a major source of arginine and nitric oxide but NO plays an important role in the water and electrolyte balance and acid-base physiology and many other homeostatic functions in the kidney. Unfortunately we are far from a precise understanding of the significance of NO alterations in various disease states primarily due to conflicting data from the existing literature. More studies are required to elucidate the abnormalities in NO metabolism in renal diseases and to confirm the therapeutic potential of L-arginine.

References


