REVIEW OF LITERATURE
BACKGROUND
In 1980, Furchgott and Zawadzki were the first to observe that acetylcholine (Ach)-induced relaxation of vascular smooth muscle cells is dependent on a factor released by an intact endothelium (Furchgott and Zawadzki, 1980). This factor was endothelium-derived relaxing factor (EDRF), and it took seven more years to discover the identity of EDRF as nitric oxide (NO) (Palmer et al., 1987; Moncada et al., 1991). NO is produced continuously by NOS in endothelial cells in response to shear stress and factors like Ach and bradykinin (BK) (Palmer et al., 1988). The vascular wall is considered to be in a state of active vasodilatation maintained by NO. The L-arginine-NO pathway plays a role in hypertension, renal disease, inflammation and atherosclerosis (Calver et al., 1993; Moncada and Higgs, 1993; Gabbai and Blantz, 1999). This pathway also interacts with the renin-angiotensin system (RAS), the eicosanoid pathway, endothelins (ET), cytokines and regulators of inflammation (Vallance and Collier, 1994).

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 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 vasomotor 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 (Nathan, 1992; Moncada and Higgs, 1993), which laid the foundations for a plethora of scientific publications that established the importance of NO in renal physiology and pathophysiology.
SYNTHESIS OF NITRIC OXIDE

A metabolic pathway that utilizes L-arginine as an exclusive precursor is the synthesis of nitric oxide with the release of L-citrulline (Fig. 1.) by cells containing the enzyme(s) nitric oxide synthase (NOS) (Nathan, 1992; Langrehr et al., 1993; Moncada and Higgs, 1993). The formation of nitric oxide, one of the smallest (30 Da) and simplest biosynthetic products, is catalyzed by enzymes that are among the largest (300 kDa) and most complicated. NOSs are homodimers whose monomers are themselves two enzymes fused, a cytochrome reductase and a cytochrome, that require three cosubstrates [L-arginine, nicotinamide adenine dinucleotide phosphate (NADPH), and oxygen] and five cofactors of prosthetic groups [Flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), calmodulin, (6R)-tetrahydrobiopterin, and heme]. The Michaelis-Menton constant (Km) for arginine use as a substrate for NO synthase is on the order of 1-10 μM. Thus, there would appear to be a vast surplus of substrate (plasma concentration of L-arginine is 80-100μM). Nitric oxide has a half-life of only few seconds, but its biological activity may last 1-2 min because of the in vivo formation of complexes of nitric oxide with S-nitroso adducts (Stamler et al., 1992).

![Figure 1: 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 nitric oxide synthase to nitric oxide (NO) and L-citrulline (Klahr, 1999)]
Once generated, NO is not very reactive under physiologic pH, but its paramagnetic properties (odd number of electrons) account for its strong binding affinity for the heme iron and thereby its inactivation by hemoglobin and other hemoproteins (Marietta, 1993). The same mechanism applies for the activation of soluble guanylate cyclase, because NO binds to the heme group of this enzyme. Reduced iron (Fe$^{2+}$) in the form of heme is required for the activation of soluble guanylate cyclase, which represents the main molecular target of NO, although NO also interacts with a variety of other iron-sulphur enzymes (Nathan, 1992) and promotes adenosine diphosphate ribosyl transfer (Dimmeler and Brune, 1992).

**NITRIC OXIDE SYNTHASES**

The expanding family of NOS isoforms may principally be divided into inducible and constitutive isoforms. All the purified NOSs have been isolated from the cytosol. Molecular cloning and sequence analysis has revealed the existence of at least three main NOS isoforms that are probably encoded by three different genes (Alderton et al., 2001; Tatoyan and Giulivi, 1998). Neuronal and endothelial NOS, also termed NOS I and NOS III, respectively, according to the nomenclature proposed by the group of Forstermann et al. (1991) are both cNOS's that are dormant until activated briefly by the cofactor Ca$^{2+}$ which sustains the binding of calmodulin (Marsden et al., 1992; Miller et al., 1997). A third NOS, termed "inducible NOS" (iNOS) or "NOS II" is expressed only after transcriptional induction (Ruan et al., 1996). Once induced, this NOS is active for prolonged periods (Cho et al., 1992). The neuronal isoform of NOS (NOS I) was originally purified from rat, pig, and human cerebellum (Nathan, 1992; Alderton et al., 2001) and was found to be a cytosolic protein (molecular weight, to 150 to 160 kd) that is dimeric in the native state (Wang et al., 1992; Tojo et al., 1999). The endothelial isoform of NOS (NOS III) was purified by Forstermann and colleagues (Forstermann et al., 1993) and was subsequently cloned from bovine and human aortic cells (Sessa et al., 1992; Marsden et al., 1992; Nishida et al., 1992; Knowles and Moncada, 1994). From these studies, a 50% to 60% sequence identity with rat brain (NOS I) as well as murine macrophage NOS (NOS II) isoforms was calculated.
Although both NOS I and NOS III are constitutive, Ca\(^{2+}\)-calmodulin-dependent isoforms, upregulation and downregulation of their expression have been reported. The expression of NOS I in the kidney may be upregulated and, probably, downregulated depending on hemodynamic changes; likewise, the expression of NOS III can be upregulated by shear forces (Nishida et al., 1992) and downregulated by cytokines (Forstermann et al., 1993). However, it remains to be seen whether these changes involve an interaction with gene transcription (Peunova and Enikolopov, 1993; Yoshizumi et al., 1993; Kanno et al., 1993).

![Figure 2: Schematic representation of the mechanisms of activation of different NOS isoforms, and subsequent effects of NO generated](image)

Abbreviations: BH4, tetrahydrobiopterin; CaM, calmodulin; COX-2, cyclo-oxygenase type 2; g-IFN, gamma interferon; IL-1, interleukin-1; LPS, lipopolysaccharide; NF-kB, nuclear factor-kB; PARS, poly-adenosine diphosphate ribose synthase; TNF-a, tumour necrosis factor-a. nNOS, iNOS and eNOS refer to the neuronal (type I), inducible (type II) and endothelial (type III) isoforms of NOS respectively (Gabbai and Blantz, 1999).

The inducible NOS isoform (NOS II or macrophage NOS) has been purified (Yui et al., 1991) and cloned (Lowenstein et al., 1992; Xie et al., 1992; Lyons et al., 1992; Nunokawa et al., 1993) from lipopolysaccharide/γ-interferon-treated macrophages and
neutrophils as well as from vascular smooth muscle cells. Like NOS I, NOS II is a cytosolic protein (molecular weight, 130 kd) and is a dimer under native conditions (Nathan, 1992).

A fourth form of NOS, inducible hepatic NOS, has been isolated from human hepatocytes (Geller et al., 1993). Of interest, it is regulated by calmodulin mechanisms and chronically by cytokines. While most of the homology of the amino acid sequences of NOS as well as the similarities to cytochrome P450 reductase are localized in the COOH-terminal halves, the NH₂ termini of NOS differ extensively from all other reported sequences and account for much of the divergences among types of NOS (Lowenstein et al., 1992; Xie et al., 1992; Knowles and Moncada, 1994).

**LOCALIZATION OF NITRIC OXIDE SYNTHASES IN THE KIDNEY**

Earlier attempts to localize NOS activity in the kidney were based on indirect evidence for NO biosynthesis obtained by the measurement of nitrite, nitrate, and cGMP levels; specificity for NOS was assessed by L-arginine-dependent activation, cytokine and endotoxin stimulation, and/or selective pharmacologic inhibition with structural analogues to L-arginine. For these measurements, tissue slices of dissected renal zones as well as isolated glomeruli and various cell cultures were used (Forstermann et al., 1993; Mohaupt et al., 1994; Bachmann et al., 1995). It was suggested from these in vitro studies that renal NO production was not only derived from endothelial cells, but also from smooth muscle cells, mesangial cells, and tubular epithelial cells.

For general histochemical detection of NOS, the classic nitroblue tetrazolium reaction has been used (Pearse, 1957; Schmidt et al., 1992). The intensity of this reaction varies with the NADPH diaphorase activity of NOS, thereby indicating NOS enzyme activity. The NADPH diaphorase reaction has been used for many years as a histochemical marker for neurons and was recently suggested to correspond to the existence of NOS (Dawson et al., 1990; Matsumoto et al., 1993). It is believed that diaphorase staining indicates NOS activity irrespective of the particular isoforms. However, the NADPH diaphorase tetrazolium reaction is not particularly specific for NOS, but may detect other reducing enzymes as well (Pearse, 1957). Therefore, to assign the diaphorase labeling to a particular NOS isoform, immuno-histochemical methods have been applied using
isoform-specific monoclonal and polyclonal antibodies that were raised against various purified NOSs and characterized by electrophoresis and Western blot analysis (Mundel et al., 1992; Schmidt et al., 1992; Loesch et al., 1993; Liu et al., 1996). Molecular cloning of NOS isoforms has provided a number of specific sequences so that molecular probes could be derived for biochemical and histochemical analysis (Table 1).

<table>
<thead>
<tr>
<th>ISOFORMS</th>
<th>nNOS (NOS I)</th>
<th>iNOS (NOS II)</th>
<th>eNOS (NOS III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Expression</td>
<td>Constitutive</td>
<td>Inducible</td>
<td>Constitutive</td>
</tr>
<tr>
<td>2. Chromosomal Localization</td>
<td>12q 24.2</td>
<td>17q 11.2-q12</td>
<td>7q 35-q36</td>
</tr>
<tr>
<td>3. Cell Prototype</td>
<td>Neurons, Platelets, Renal Epithelial Cells</td>
<td>Hepatocytes, Macrophages, Mesangial, Endothelial Cells</td>
<td>Endothelial Cells</td>
</tr>
<tr>
<td>4. Size of Protein/Number of aa</td>
<td>160 kDa/1434</td>
<td>130 kDa/1153</td>
<td>135 kDa/1203</td>
</tr>
<tr>
<td>5. Subcellular Localization</td>
<td>Cytosolic/Membrane</td>
<td>Cytosolic</td>
<td>Membrane</td>
</tr>
<tr>
<td>6. Calcium Dependency</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>7. Calmodulin Dependency</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8. Inhibitors:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-NAME</td>
<td>Inhibits</td>
<td>Inhibits</td>
<td>Inhibits</td>
</tr>
<tr>
<td>TGF-β</td>
<td>No Effect</td>
<td></td>
<td>No Effect</td>
</tr>
</tbody>
</table>

**Nitric Oxide Synthase I:**

The constitutive, "neuronal" isoform NOS I has been localized strictly to the cells of the macula densa of the rat and mouse kidney by three different groups (Wilcox et al., 1992; Mundel et al., 1992; Schmidt et al., 1992). That a strong NADPH-diaphorase reaction in the macula densa of the juxtaglomerular apparatus is in fact due to the presence of NOS I was verified by a specific antiserum and by in situ hybridization (Mundel et al., 1992; Bachmann et al., 1992). An immunoelectron microscopic study has localised NOS I to
the cytoplasm and to cytoplasmic vesicles (Tojo et al., 1994). Of note, another well-established cytologic difference between the macula densa and the surrounding distal tubular epithelium relates to the activity of glucose-6-phosphate dehydrogenase (G6PD), an enzyme that directs the entry of glucose into the pentose shunt pathway (Hess and Gross, 1959; Norgaard, 1979, 1980). The pentose phosphate pathway is a source of NADPH that also functions as a cofactor required by NOS enzyme activity; thus, the colocalization of NOS I and G6PD may indicate a functional relationship since G6PD might supply NOS with the reduced form of the cofactor. Histochemically, only the macula densa and parts of the glomerular capsule were NOS I-labeled in rat and human kidney. In the guinea pig, however, selective immunohistochemical staining of the glomerular efferent arteriole was seen (Bachmann et al., 1995). The presence of neuronal NOS in an endothelium, where normally the endothelial NOS III-isoform would predominate (Forstermann et al., 1991), may not be a unique finding, since a study demonstrated the presence of neuronal NOS I in rabbit aortic endothelium by ultrastructural immunocytochemistry (Loesch et al., 1993). Using reverse transcription and polymerase chain reaction in individual, microdissected rat nephron segments, however, Terada et al. localized NOS I mRNA in the inner medullary collecting duct and, to a lesser extent, in the glomerulus, inner medullary thin limb, and cortical and outer medullary collecting duct, as well as in parts of the renal vasculature (Terada et al., 1992). The discrepancy between these results and the histochemical findings listed above is not clear, but may simply be a matter of sensitivity in the detection methods applied.

Nitric Oxide Synthase III:
The second constitutive-type NOS, NOS III, also termed “particulate NOS” (Forstermann et al., 1991) or “endothelial NOS,” is present in the endothelium of renal arterioles as an NADPH-diaphorase reaction product was detected to a varying extent in renal vascular endothelia. However, to date, an NOS III-specific immunohistochemical labeling (Bachmann et al., 1995) has not yet been performed for the renal vasculature, nor has an in situ hybridization been accomplished.
Nitric Oxide Synthase II:
In situ labeling of renal structures for the inducible NOS II has been shown in preliminary reports using specific antisera to NOS II. Nitric oxide synthase II immunoreactivity was localized to a preglomerular portion of the afferent arteriole, including the granular cells (Hess and Gross, 1959). Under LPS stimulation there was a significantly enhanced NOS II immunoreactivity while the neuronal isoform was unaffected (Tojo et al., 1994). In primary cultures prepared from microdissected tubule segments, direct evidence for the transcription of NOS II mRNA was demonstrated in rat proximal and inner medullary collecting duct cells; these segments generated NO on exposure to TNF-α and IFN-γ in a time- and dose-dependent manner (Markewitz et al., 1993). Increases in NO synthesis were associated with significant increases in NOS II mRNA as revealed by reverse transcription and polymerase chain reaction, suggesting that the proximal tubule and inner medullary collecting duct contain the inducible isoform of NOS.

REGULATION OF NITRIC OXIDE SYNTHASES: Physiology
The physiologic agents that regulate NO synthesis may be divided into upregulating or stimulating and downregulating or inhibiting agents. Substances considered to be agonists may activate NO synthesis within seconds or a few minutes, acting on pre-existing cNOSs without interfering with transcription or translation. Their effect requires calmodulin binding, an event controlled by the level of intracellular Ca2+. Among these agonists, acetylcholine, bradykinin, leukotrienes, platelet-activating factor, excitatory amino acids, and calcium ionophores as well as electrical stimulation play important roles in endothelium dependent vasodilation (Nathan, 1992; Forstermann et al., 1993). Other cell types known to accomplish a rapid and transitory release of NO in response to such agonists include mast cells, peripheral blood neutrophils and some neurons (Snyder, 1992; Nathan, 1992; Forstermann et al., 1993).

There is little evidence for a downregulation of cNOSs once these enzymes have been activated; a feedback inhibition by NO or citrulline in cNOSs has not been reported. It appears, however, that at the mRNA level, tumor necrosis factor-α (TNF-α) may down regulate cNOS activity in vascular endothelial cells by shortening its mRNA half-life.
Moreover, NO itself has been shown to downregulate NOS from cerebellum. On the other hand, agents considered to be inductants may activate NO synthesis from iNOS within hours. Their action may be prevented by inhibition of transcription and translation using actinomycin D and cycloheximide, respectively. Once induced, NO release from NOS II catalytic activity may continue for days; in this condition, excessive amounts of NO are released (Moritoki et al., 1992; Lamas et al., 1992; Nicolson et al., 1993). As revealed in experiments with rat and mouse macrophages, inflammatory neutrophils, hepatocytes, vascular smooth muscle, and glomerular mesangial cells, NOS II activity may be induced by endotoxin (bacterial lipopolysaccharide [LPS]) and by cytokines such as γ-interferon (IFN-γ), as sole agents, as well as by the synergistic combinations of IFN-γ plus either LPS, TNF-α, TNF-β, or interleukin (IL)-1, and by IFN-α and IFN-β combined with LPS (Nathan, 1992; Pfeilschifter et al., 1993; Cattell and Cook, 1993; Xie et al., 1994). Very complex regulation conditions must be considered in endothelia, in which reports have shown effects of factors influencing cNOS that otherwise act only on iNOS (Marsden et al., 1992; Tsukahara et al., 1994).

Suppression of cytokine-stimulated iNOS can be effected by glucocorticoids and by a variety of cytokines, such as transforming growth factor (TGF)-β, macrophage deactivation factor, IL-4, IL-10 and platelet-derived growth factor (Nathan, 1992; Pfeilschifter et al., 1993; Cattell and Cook, 1993).

**REGULATION OF NITRIC OXIDE SYNTHASES: Pharmacology**

The effect of NO synthases may be mimicked by organic nitrates, such as nitroglycerin, nitrosothiols, and sodium nitroprusside, that exert an endothelium-independent vasodilating effect by their local activation to NO in the vascular wall (Furchgott and Vanhoutte, 1989; Kowaluk et al., 1992). In addition, there also are several classes of pharmacologic inhibitors of NOS (Nathan, 1992). Among these, the most commonly used are substrate analogues such as N⁢⁢⁢⁢⁢G-nitro-L-arginine methyl ester (L-NAME) and N⁢⁢⁢⁢⁢G-monomethyl-L-arginine (L-NMMA). The most likely mechanism of action of these substances is competitive inhibition of the conversion of L-arginine to NO. Both the inducible and the constitutive isoforms of NOS reveal characteristic differences in
susceptibility to the inhibition by various L-arginine analogues (Leiper and Vallance, 1999). A second class of inhibitors is represented by diphenylene iodonium and its congeners (Nathan, 1992). In addition, carbon monoxide inhibits NOS enzyme activity by (incompletely) binding the heme (White and Marletta, 1992), and a fourth class consists of substituted pyrimidine that limits the formation of tetrahydrobiopterin (Werner-Felmeyer et al., 1990). However, only the first class of inhibitors is widely used for experimental purpose.

**NOS IN THE RENAL VASCULATURE**

The iNOS is calcium-and calmodulin-independent and is not constitutive but can be induced by certain cytokines and bacterial products (Millatt et al., 1999). The amount of NO generated by eNOS is small (nmol), and its effects are transient since NO is rapidly inactivated by superoxide anions and binding to haemoglobin (Gow et al., 1999). There is continual basal production of NO by vascular endothelium (Moncada et al., 1991). iNOS, by contrast, synthesises NO in large (mmol) quantities. Since iNOS is regulated at the transcriptional level, the initiation of NO synthesis/release is delayed after the stimulus by several hours, but once initiated, the synthesis of NO lasts for hours (Millat et al., 1999).

Originally purified from neurons, vascular endothelium and cytokine-induced macrophages, these three isoforms are now understood to be distributed across a wide spectrum of cell types and tissues. All three enzymes have been localized in the kidney and have been implicated in the control of renal function. Constitutive nNOS has been localized to the cells of macula densa of the rat and mouse kidney (Mundel et al., 1992). Using the reverse transcriptase-polymerase chain reaction (RT-PCR) in individual, microdissected rat nephron segment, Terada et al. (1992) localized nNOS mRNA in the inner medullary collecting duct and, to a lesser extent, in the glomerulus, inner medullary thin limb, and the cortical and outer medullary collecting duct, as well as in parts of the renal vasculature. Messenger RNA for eNOS has been demonstrated by RT-PCR in the glomerulus and afferent and efferent arterioles (Ujiie et al., 1994). These two forms of NOS are crucially involved in the renal pathophysiology, and local release of NO serves to control renal blood flow (Ito et al., 1995) and modulate the activity of
tubuloglomerular feedback (TGF). Two structurally distinct forms of iNOS are expressed during physiological conditions at the juxtaglomerular apparatus and in tubules (Mohaupt et al., 1994). After immense stimulation, iNOS has been located in glomerular mesangial cells and several locations in the tubules (Pfeilschifter et al., 1993; Morrissey et al., 1994; Saura et al., 1995). The steady-state amounts of iNOS mRNA and proteins in normal rat kidney is highest in tubules of the outer medulla, and in vitro studies of rat renal homogenates showed that the specific activity of NOS in the medulla was three times that of the cortex (McKee et al., 1994). The significance of the high expression of iNOS in induces natriuresis by antagonizing the effect of angiotensin II on sodium reabsorption in proximal tubules (Ito et al., 1995) and by directly inhibiting renal tubular Na+/K+ adenosinetriphosphatase (ATPase) (Guzman et al., 1995).

**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 (Giulivi et al., 1998). Moreover intrarenal NO regulates macrovascular and microvascular hemodynamics in the kidney (Majid and Navar, 2001). NOS inhibition decreases basal renal blood flow although preserving auto-regulatory responses (Ichihara and Navar, 1999) (Table 2). 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 renal medullary blood flow and promote salt retention and hypertension in animal models (Mattson et al., 1994). 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 HCO$_3^-$ reabsorption by stimulating Na$^+$/H$^+$ exchanger (Wang et al., 2000). 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 of 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 HCO$_3^-$ reabsorption in the
medullary thick ascending loop of Henle (mTALH) by inhibiting the Na⁺K⁺2Cl⁻ co-transporter and Na⁺/H⁺ exchanger activity (Plato et al., 1999; Ortiz and Garvin, 2000). On the other hand, NO stimulated K⁺ channel activity in the apical membrane of the mTALH segment (Lu et al., 1998). 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 (Lu et al., 1997). NO also inhibited Na⁺ and ADH-sensitive water permeability in the principal cells (Garcia et al., 1996) and H⁺-ATP-ase in the interstitial cells of CCD (Tojo et al., 1994).

**Table 2: 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

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 (Majid et al., 1993). Furthermore many studies have confirmed the role of intra renal NO in mediating tubulo-glomerular feedback. Wilcox et al. (1992) 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 studies support the inhibitory role of NO from eNOS and iNOS in mTALH segment on TGF effects (Wang et al., 2002).

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\(_3^-\) reabsorption by stimulating Na\(^+\)/H\(^+\) exchanger and through augmenting basolateral electrogenic Na\(^+\)-3HCO\(_3^-\) co-transporter (Wang, 1997). 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\(_3^-\) reabsorption through electrogenic H\(^+\) secretion. In the medullary thick ascending limb of loop of Henle (mTALH), NO inhibited net Cl\(^-\) and HCO\(_3^-\) absorption by modulating the activity of the Na\(^+\)/H\(^+\) exchanger at both apical and basolateral sides of the mTALH and Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter (Ortiz et al., 2001). Till date, 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 (Tojo et al., 1994).

In summary, intra renal NO 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\(^+\) ATP-ase activity. In the mTALH segment NO inhibits Cl\(^-\) and HCO\(_3^-\) 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.

Renal Cortical Hemodynamics:

It is now widely accepted that EDRF is an important mediator of renal blood now and that regional microcirculation may be mediated by endogenously produced NO (Baylis et al., 1990; King and Brenner, 1991; Luscher 1991; Lahera et al., 1991; Romero et al., 1992; Beierwaltes et al., 1992). Like endothelium-independent nitrovasodilators, such as sodium nitroprusside or molsidomine and its active metabolite SIN-I, endothelium-derived NO may relax vascular smooth muscle by activation of soluble guanylate cyclase, which leads to an intracellular accumulation of cGMP (Holzmann, 1982; Kukovetz and Holzmann, 1985). In the isolated perfused rat kidney and in isolated human renal arteries, renal vasodilation has been elicited after treatment with acetylcholine, bradykinin, histamine, and thrombin as well as flow-induced shear stress on the vessel wall (Biondi et al., 1990; Radermacher et al., 1990; Luscher et al., 1991). These results have been obtained by microangiography or by measuring cGMP formation, since the kidney, like large blood vessels, produces large amounts of cGMP under stimulated
conditions. In the isolated perfused kidney, the NO dependent increase in urinary cGMP levels may be suppressed by the NO synthesis inhibitor methylene blue or by gossipol inhibiting guanylyl cyclase (Radermacher et al., 1990; Pfeilschifter et al., 1993; Mayer et al., 1993). Pharmacologically, the enzymatic synthesis of NO can be inhibited by the structural analogues of L-arginine, such as L-NAME or L-NMMA leading to an increase in renal vascular resistance and a decrease in renal blood flow, urine flow, and sodium excretion (Tolins et al., 1990; Lahera et al., 1990). In catheterized rats, a moderate dose or L-NAME (10 mg/kg body weight) led to a large, sustained increase in blood pressure associated with a large increase in renal vascular resistance, a decrease in renal plasma flow, and a marked natriuresis that was due to reduced fractional reabsorption of sodium; the effects were attenuated by the infusion of excess L-arginine (Baylis et al., 1992).

Renal cortical blood flow in rats is much more sensitive to inhibition by L-NMMA than is mean arterial blood pressure (Walder et al., 1992). Acetylcholine-induced effects on renal hemodynamics and urinary cGMP excretion in anesthetized rats were all inhibited by L-NMMA, suggesting that these acetylcholine-mediated effects were NO dependent (Tolins et al., 1990). Similar results were obtained in the dog (Lahera et al., 1990). Taken together, these studies indicate that, probably due to a high-based rate of vascular NO formation, the renal cortical vasculature substantially contributes to the NO dependent regulation and maintenance of renal hemodynamics and electrolyte balance.

Renal Medullary Hemodynamics:

Renal medullary blood flow has been shown to respond to endothelium-dependent vasodilatation (Lahera et al., 1991; Walder et al., 1992; Alberola et al., 1992; Mattson et al., 1992). It also has been suggested that endothelial cells of the vasa recta were able to produce NO (Biondi et al., 1990). Mattson et al. (1992) reported that in the dog kidney, a nonpressor dose of the NO inhibitor N$^o$-nitro-L-arginine selectively applied to act in the renal medulla reduced papillary blood flow quite effectively. This was accompanied by decreased sodium and water excretion, and it was concluded that NO formed in the renal medulla exerts a tonic influence on the local circulation. To establish regional NO activity in the dog kidney, Biondi et al. (1992) dissected the dog kidney into six different zones and measured highest cGMP production in the middle and inner portion of the inner medulla and lowest concentration in the cortex.
**Review of Literature**

**Tubuloglomerular Feedback:**
New insights into the mediation of the tubuloglomerular feedback mechanism have been obtained in a micropuncture study of single nephron microperfusion using an inhibiting NO substrate analogue (Johnson et al., 1992). This study revealed that local generation of NO can vasodilate the afferent arteriole and increase glomerular capillary pressure; these effects were inhibited by furosemide. It was therefore concluded that NOS in macula densa cells is activated by tubular solute reabsorption to release NO as a vasodilating component of the tubuloglomerular feedback response. In microperfused glomeruli of rabbit kidney it was shown that afferent arteriolar constriction induced by high concentrations of NaCl at the macula densa may he modulated by macula densa-derived NO. It was speculated that an increase in intracellular calcium released through the action of the Na⁺/ K⁺/2Cl⁻ cotransport process may link tubular fluid reabsorption to an activation of the Ca²⁺-dependent constitutive NOS I (Wilcox et al., 1992). Moreover, these findings present another, albeit indirect support for a selective NO effect on the afferent arteriole.

**Autoregulation of Renal Blood Flow:**
It is generally thought that the mechanism for autoregulation is triggered by the stretch of arterial walls during increased renal perfusion pressure leading to autonomic contraction of the smooth muscle. It has been debated as to how arterial contraction is adjusted to maintain constant renal blood flow (Romero et al., 1992). It has been proposed that NO is involved in mediating the myogenic response of autoregulation. Studies addressing the role of NO/EDRF in autoregulation of renal blood flow and glomerular filtration rate have been performed in the rat (Beierwaltes et al., 1992; Sigmon and Beierwales, 1993) and dog (Majid and Navar, 1992; Baumann et al., 1992). Inhibition of EDRF by L-NAME markedly lowered renal blood flow in these studies, but left autoregulation of renal blood flow intact. Thus there is agreement that although endothelial NO formation apparently helped to maintain renal blood flow and glomerular filtration rate, there was no indication of involvement of EDRF in the mediation of intrinsic autoregulatory responses.
**Angiotensin II Interaction:**

Blockade of EDRF at both the systemic and single nephron levels resembled the administration of angiotensin II in various respects (Baylis and Brenner, 1978; Baylis et al., 1993). Inhibition of EDRF synthesis using L-NAME or L-NMMA decreased renal blood flow (Baylis et al., 1990; Beierwaltes et al., 1992). This increase in renal vascular resistance may be due to removal of intrinsic EDRF-mediated vasodilation, permitting endogenous vasoconstrictors such as angiotensin II to predominate (Sigmon and Beierwaltes, 1993). Therefore, studies were performed to elucidate the interaction of EDRF and angiotensin II in the control of glomerular microvascular diameter (Ohishi et al., 1992; Ito et al., 1993) and in conditions of NO blockade-related diuresis and natriuresis (Baylis et al., 1993). In isolated microperfused rabbit glomeruli, L-NAME pretreatment of the glomerular vasculature augmented the vasoconstrictor action of angiotensin II in the afferent but not the efferent arteriole, thus indicating that endogenous NO may selectively modulate afferent arteriolar angiotensin II actions (Ito et al., 1993). Ohishi et al. (1992) showed that angiotensin II blockade attenuated the arteriolar vasoconstriction elicited by EDRF blockade, suggesting that EDRF interacts with the renin-angiotensin system to control glomerular arteriolar resistance. There is still some controversy, however, as to the interpretation of these findings, as Baylis et al. (1993) found that simultaneous application of EDRF blockade and angiotensin II receptor antagonists did not enhance the effect of EDRF blockade alone; these investigators concluded that the hemodynamic effects of acute EDRF blockade were not mediated by angiotensin II. A differential regulation of renal blood flow has been described in renal vascular hypertension induced by unilateral renal artery stenosis (two kidney-one clip Goldblatt model) (Sigmon and Beierwaltes, 1993). This study indicated an important interaction between angiotensin II and EDRF in the regulation of hemodynamics, since NO counteracted elevated angiotensin II levels to regulate perfusion in the non-clipped kidney, whereas angiotensin-dependent vasoconstriction predominated in the clipped kidney.

**Renin Secretion:**

The vascular mechanisms that render renin release dependent on the myogenic response to changes in renal perfusion pressure have been studied (Romero et al., 1992); several in
vitro studies have suggested that renal vascular endothelial may directly modulate renin release. On the other hand, the presence of NOS I in the macula densa was thought to be related to the macula densa-dependent release of renin (Wilcox et al., 1992; Mundel et al., 1992; Schmidt et al., 1992; Bachmann et al., 1995). At present it is controversial as to whether EDRF has an inhibitory or a stimulatory effect on renin secretion (Kurtz et al., 1991; Romero et al., 1992; Scholz and Kurtz, 1993). It has been shown by using renal cortical slices that EDRF released from rat and dog renal vasculature inhibits renin release and that EDRF inhibition stimulates its basal release (Beierwaltes and Carretero, 1991). Likewise, when controlling changes in renal perfusion pressure and β-adrenergic activity, inhibition of EDRF synthesis with L-NAME increased plasma renin levels (Sigmon et al., 1992). It was concluded that via the formation of the inhibitory messenger cGMP, EDRF could serve as an inhibitory modulator of renin release (Sigmon et al., 1992); however, it must be added that the role of cGMP in the control of renin release is not yet unequivocally understood (Scholz et al., 1993). Other studies found stimulatory effects or EDRF on basal renin release (Majid and Navar, 1992; Gardes et al., 1992; Scholz and Kurtz, 1993). In these studies, a stimulatory effect of EDRF on renin secretion was evidenced by the observation that the increase in renin secretion in response to a reduction of the renal artery pressure was markedly attenuated under EDRF blockade. A stimulatory effect of NO was also reported from a study using isolated rat glomeruli (Munter and Hackenthal, 1991).

Similar to the role of NO proposed for the tubuloglomerular feedback mechanism, macula densa-derived NO may mediate, as a paracrine factor, the macula densa-regulated renin secretion, although the two possible sources of NO at the juxtaglomerular apparatus (vascular endothelium and macula densa) are difficult to distinguish. Histochemically, it has been reported that macula densa NOS I activity and afferent arteriolar renin status (i.e., the amount or renin stored in these cells) vary in parallel under renal and systemic hemodynamic changes, thus pointing to a possible NO-related stimulation of renin synthesis (Bachmann et al., 1995). In the isolated juxtaglomerular apparatus, elevated renin secretion induced by a low NaCl concentration at the macula densa was largely abolished by application of an inhibitory NO substrate analogue, thus pointing to a stimulatory role of NO. With regard to the controversy of inhibitory versus stimulatory
effects or NO on renin secretion, it is currently thought that this may depend on the experimental setting and that different results may be due to time dependent changes: an initial NO effect is probably inhibitory, whereas a long-term NO effect might stimulate renin secretion (Schricker and Kurtz, 1993). Another interpretation involves an intermediate messenger of yet unknown nature that may be triggered by NO from either macula densa or vascular endothelium and may influence renin secretion.

**Glomerular Arterioles:**

Much work has been devoted to clarify the role of NO in the local regulation of glomerular arteriolar resistance. Micropuncture studies in rat kidney (Zatz and Nucci, 1991) revealed that EDRF blockade led to complex changes in glomerular hemodynamics with increases in both preglomerular and efferent arteriolar resistances such that glomerular blood pressure increased significantly. Moreover, in a chronic NO inhibition study, a significant decrease in the glomerular capillary ultrafiltration coefficient was observed (Baylis et al., 1992). While the latter effect was ascribed to changes in mesangial cell tone, there is some controversy as to a selective effect of the NO blockade on either afferent or efferent arteriolar tone (Ito et al., 1993; Deng and Baylis, 1993). In a juxtamedullary rat nephron preparation, Ohishi et al. (1992) found that a sufficient quantity of EDRF is continuously released in both the afferent and efferent arterioles. Using a similar microvascular preparation, Imig and Roman (1992) suggested that NO primarily alters afferent vascular tone, thereby modifying the ability of the preglomerular vasculature to autoregulate glomerular capillary pressure. Similarly, Deng and Baylis (1993) stated that local NO controls afferent arteriolar resistance, whereas efferent resistance is not under tonic control by NO. In isolated microperfused rabbit glomeruli, inhibition of EDRF caused a reduction of the basal luminal diameter of the afferent arteriole (Ito et al., 1993). However, studies in the avascular hydronephrotic kidney have indicated a preferential effect of EDRF blockade on the efferent arteriole (Hoffend et al., 1993).

**Mesangial Cells:**

The glomerular mesangial is regarded as a major regulator of glomerular filtration rate. Mesangial cells resemble vascular smooth muscle cells. They may contract on hormonal stimuli (i.e., arginine-vasopressin, angiotensin II). Changes in mesangial tone may
modulate the capillary ultrafiltration coefficient (Marsden et al., 1992; Baylis et al., 1992). Using cell cultures, a number of studies have elucidated the physiologic and pathophysiologic significance of the local glomerular effects of NO. Angiotensin II caused mesangial contraction via a phospholipase C-inositol 1,4,5-trisphosphate pathway (Pfeilschifter et al., 1993), an effect antagonized by vasodilating atrial natriuretic peptide or the NO agonist sodium nitroprusside (Appel et al., 1986). Using aortic and glomerular endothelial cells, it was demonstrated that endothelium-derived NO in co-culture experiments increased mesangial cGMP levels (Shultz et al., 1990; Marsden et al., 1992); angiotensin II-mediated mesangial contraction could be inhibited by endothelium-derived NO (Shultz et al., 1990).

In addition to smooth muscle like properties, mesangial cells also possess a number of similar functions as macrophages: they may produce eicosanoids, cytokines, various growth factors, and oxygen radicals (Sigmon et al., 1992). Like macrophages, mesangial cells probably express the inducible form of NOS (NOS II), since cytokines (IL-β, TNF-α) and lipopolysaccharide have been shown to stimulate mesangial cGMP production (Pfeilschifter and Schwarzenbach, 1990; Marsden and Ballermann, 1990; Nicolson et al., 1993). Moreover, the effect of cytokines was blunted by L-NMMA, indicating that NO was involved in the increase of cGMP (Shultz et al., 1991; Nicolson et al., 1993).

Glomerular Epithelial Cells:

Similar to mesangial cells, glomerular podocytes possess contractile filaments (Drenchkahn and Franke, 1988). It has been suggested that an angiotensin II-mediated adjustment of the glomerular capillary ultrallitration coefficient could be effected by the glomerular epithelium; it was also suggested that its contractile apparatus might alter the size and length or the glomerular slit pores of the podocyte foot processes, thereby altering the filtration surface (Shake et al., 1992). In this respect, a presumptive NO receptor (i.e., the α1-suhunit of soluble guanylate cyclase) was recently shown by immunocytochemistry to be present in glomerular podocytes in abundant amounts (Mundel et al., 1992). Thus, it hereby appears plausible that NO produced in the glomerulus may, via activation or its receptor and subsequent generation of cGMP, modulate the glomerular filtration coefficient, possibly in a manner antagonistic to the effect of angiotensin II.
Tubular Function:

Several studies have emphasized an effect of NO/EDRF not only on glomerular, but also on tubular function. Studies in dogs (Alberola et al., 1992; Majid et al., 1993), rats and in the isolated perfused rat kidney (Radermacher et al., 1992) have elucidated the effects of either arginine depletion or NOS inhibition on natriuresis and diuresis under various physiologic conditions. In dogs, NO synthesis inhibition by intrarenal infusion of nitro-L-arginine caused a significant increase in renal vascular resistance, a decrease in renal blood flow and urine flow and a marked reduction in sodium excretion, suggesting that NO may participate in tubular effects that regulate the pressure natriuretic response (Majid et al., 1993). Alberola et al. (1992) also suggested that a reduced natriuresis and diuresis in response to volume expansion observed under NOS inhibition in the dog might be in part mediated by altered proximal tubular reabsorption. In contrast, there was a reduction in the fractional reabsorption or sodium after NO inhibition in the isolated perfused rat kidney, presumably due to a specific tubular effect of EDRF (Baylis et al., 1990; Radermacher et al., 1992; Lahera et al., 1990). Similarly, De Nicola et al. (1992) observed a significant decrease of absolute proximal tubular reabsorption under L-NMMA treatment, both under control conditions and during glycine-induced changes in glomerular filtration rate. The differences in the effects of NO blockade on natriuresis may partly be the consequence of differences in the experimental settings: the natriuresis seen after acute inhibition of EDRF in rats was possibly the result of a pressure natriuretic response to the abrupt increase in blood pressure (Baylis et al., 1990), whereas constant infusions of NOS inhibitors tend to reduce sodium excretion and eventually lead to hypertension (Baylis et al., 1992; Nakamura et al., 1993).

The proximal tubule itself may synthesize NO, since a cultured proximal tubular epithelial cell line (LLC-PK I) is capable of NO production (Ishii et al., 1991). Moreover, as mentioned above, both NOS I (Ignarro et al., 1990) and NOS II (Markewitz et al., 1993) mRNA was detected in the proximal tubule. Interestingly, as the kidney is one of the most important sources of body arginine (Perez et al., 1978), a study by Levillain et al. (1993) pointed to the proximal convoluted tubule as the predominant site of renal tubular synthesis of arginine possibly serving as substrate for local NOS actions. Since angiotensin II influences proximal tubular reabsorption in a concentration-dependent
manner, it was suggested that NO might mediate the angiotensin II effects between its extremes that range between inhibition and stimulation of tubular reabsorption (De Nicola et al., 1992; Seikaly et al., 1990). Alternatively, NO might exert a direct effect on collecting duct reabsorption, as was shown in collecting duct cells co-cultured with vascular endothelial cells that released NO on stimulation with bradykinin (Stoos et al., 1992); the short-circuit current in the collecting duct cells was inhibited, thus pointing to an NO-dependent inhibition of solute transport (Stoos et al., 1992) that may also account in vivo for altered solute reabsorption under NO blockade (Majid et al., 1993). Immunohistochemical findings indicated a significant presence of the β2-subunit of guanylate cyclase in principal cells of the rat collecting duct (Mundel et al., 1992). Thus, similar to the results reported by Stoos (Stoos et al., 1992), increases in cGMP may account for an NO-dependent transport reduction in the cortical and medullary collecting duct. Similarly, atrial natriuretic peptide acts via cGMP to inhibit transport in the collecting duct (Antonino and Kudo, 1990). Taken together, it appears that NO contributes to renal volume control via a tubular effect, possibly by directly influencing tubular reabsorption.

**RENAL PATHOPHYSIOLOGY AND NITRIC OXIDE**

*Hypertension and Hypotension*

It is now widely accepted that L-arginine-derived NO/EDRF plays a significant role in the regulation of renal and systemic hemodynamics by means of a relaxing effect on the microvasculature. It has been suggested that a deficiency in the synthesis of NO may constitute an important factor in the development of systemic hypertension, as NO interferes with the ability of the kidney to excrete sodium and water and because NO is thought to mediate the renal responses to volume expansion (Baylis et al., 1990; Zatz and Nucci, 1991; Mattson et al., 1992; Majid et al., 1993; Cattell and Cook, 1993; Ikenaga et al., 1993). At present, however, little direct evidence is available concerning the particular NO-related renal mechanisms(s) that may be involved in the pathophysiology of hypertension (De Nicola et al., 1992; Alberola et al., 1992; Majid et al., 1993). Rats with genetic, salt-sensitive hypertension fail to adjust renal hemodynamics appropriately to changes in salt intake and have a blunted renal and systemic vascular response to
Review of Literature

inhibition of NOS (Chen and Sanders, 1991). Similarly, a study in the spontaneously hypertensive rat has shown that an NO-mediated dysfunction was partly responsible for an impaired pressure natriuresis, and it was concluded that a disturbance in the endothelial control of renal function could play a role in the pathogenesis of hypertension (Ikenaga et al., 1993). However, as the NO system is intimately interacting with the renin-angiotensin system and with the prostaglandins, endothelin, and atrial natriuretic peptide (Vidal et al., 1988; Romero et al., 1992; Chevalier et al., 1992; Ito et al., 1993; Ikenaga et al., 1993; Jover et al., 1993; Manning et al., 1993; Pollock et al., 1993), more detailed analyses are still required.

Experimental NO blockade has shed some light on NO-mediated mechanisms that may be involved in the development of hypertension. Studies in anesthetized rabbits, guinea pigs, rats, and dogs have demonstrated that acute blockade of NO synthesis leads to marked increases in blood pressure and consistent increases in renal vascular resistance (Baylis et al., 1990; Tolins et al., 1990; Nathan, 1992; Baylis et al., 1992; Salom et al., 1992). As mentioned above, some studies have reported that inhibition of NO synthesis causes no effect or increases sodium excretion; most studies, however, agree that NO blockade causes reduction in sodium excretion (Baylis et al., 1990; Romero et al., 1992; Beierwaltes et al., 1992; Johnson et al., 1992). Reduction in the filtered load of sodium, increase in tubular reabsorption of sodium, or altered medullary blood flow (Mattson et al., 1992) may be involved in the reduction of sodium excretion; moreover, decreases in renal interstitial hydrostatic pressure may also mediate sodium retention during nitric oxide blockade (Nakamura et al., 1993). However, the exact mechanisms causing the reduced sodium excretory function during NOS inhibition are not fully understood.

A new model of hypertension has been obtained by the chronic inhibition of NO synthesis using the orally active inhibitor L-NAME (Baylis et al., 1992; Ribeiro et al., 1992; Jover et al., 1993; Pollock et al., 1993; Ward and Angus, 1993). Chronic NO blockade causes dose-dependent increases in systolic blood pressure reaching values. In this condition, marked renal vasoconstriction was also observed with reductions in glomerular capillary ultrafiltration coefficient (Baylis et al., 1992). The prolonged hypertension was also associated with a mild degree or renal failure indicated by the occurrence of proteinuria and glomerular sclerosis (Baylis et al., 1992; Ribeiro et al., 1992; Ribeiro et al.,
1992). It appears, however, that increasing the dose of L-NAME, and thereby increasing systolic blood pressure, does not lead to an aggravation of renal damage, possibly because a parallel increase in preglomerular afferent constriction may protect the glomerulus from a deleterious transmission of the systemic blood pressure.

Apart from the mentioned renal effects, the increases in systolic blood pressure under blockade of the tonic vasorelaxing effect of NO are thought to derive from a predominance or vasoconstricting agents. To assess whether activation of the renin-angiotensin system accounts, at least in part, for the pathophysiologic effects of NO blockade, the effects of combined NO blockade and inhibition of the renin-angiotensin system by angiotensin II-antagonists has been studied; inhibition of the renin-angiotensin system was shown to prevent L-NAME-induced hypertension and to reverse established hypertension in the rat, thus indicating that angiotensin II plays a critical role in the development of L-NAME-induced hypertension (Pollock et al., 1993).

Finally, in contrast to hypertension-related effects of NO, a macrophage-dependent NO overproduction due to induction of NOS by cytokines was proposed as a possible cause for hypotension in chronic hemodialysis patients (Beasley and Brenner, 1992). Among the various factors involved in endotoxin shock caused by sepsis syndrome, cytokines have been suggested to increase cGMP in vascular smooth muscles via NO formation (Schini et al., 1991). A study in cultured smooth muscle cells demonstrated that IL-1β induced de novo synthesis of iNOS; subsequent release of NO was suggested to contribute to endotoxin-related hypotension (Moritoki et al., 1992; Kanno et al., 1993).

**Nitric oxide and acute renal failure**

Acute renal failure, either posts ischemic or nephrotoxic is characterized by circulatory changes and tubular injury that result in filtration failure. There are no unique mechanisms involved in various types of experimental ARF and many factors contribute to the initiation and maintenance of the disease.

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 (Brodsy et al., 2002). 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 (Conger et al., 1995). The imbalance results from enhanced iNOS activity and attenuated eNOS activity in the kidney. Employing antisense-oligodeoxynucleotides to iNOS, Noiri et al (1996) demonstrated that high output NO production by iNOS might suppress the activity of eNOS, a scheme 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 neutrophilic adhesion while enhanced iNOS activity could explain loss of vasomotion, enhanced neutrophilic motility, tubular cell injury and suppression of eNOS activity. Generation of superoxide and NO in ischemic reperfusion injury results underscore the need for more mechanistic studies to gain better insight into pathogenic role of NO in tubulointerstitial disorders.

Figure 3. Potential effects of increase in NO levels in progression of ARF (Valdivielso and Blantz, 2002)
NO in ischemic ARF
The involvement of NO in ischemic ARF has been established since 1988 (Conger et al., 1988; Lieberthal et al., 1989). However, the controversial issue is whether the production of NO is increased or decreased in this model of ARF and whether this increase is beneficial or deleterious. Several models of ischemic ARF indicate that NOS activity increases. This increase has been reported as glomerular nitrite production (Rivas-Cabanero et al., 1995; Valdivielso et al., 2000), renal nitrite content (Weight et al., 1998; 1999), urinary nitrite excretion (Peer et al., 1996), electron paramagnetic resonance in renal tissue (Lui et al., 1999), arginine-citrulline conversion rates (Shoskes et al., 1997), cGMP production, NADPH diaphorase activity (Valdivielso et al., 2001), and direct electrode measurements of NO in renal cortex (Saito and Miyagawa, 2000). The source of the increased NO is still a controversial issue. Several authors have described an increase in eNOS protein content in glomerular tuft (Shoskes et al., 1997; Saito and Miyagawa, 2000; Valdivielso et al., 2001), and also an increase in iNOS activity has been described in tubular cells (Peer et al., 1996; Valdivielso et al., 2001). Furthermore, Yu et al. (1994) demonstrated that freshly isolated proximal tubules responded to hypoxic periods with increases in Ca\(^{2+}\)-independent NO release. The nonselective inhibition of NO synthesis in rats with ischemic ARF leads to a worsening in renal function. Several groups have reported that the inhibition of NO synthesis worsens GFR after ischemic ARF (Chintala et al., 1993; Mashiach et al., 1998; Weight et al., 1999; Valdivielso et al., 2001). Cristol et al. (1993) found that L-NAME decreased RBF in rats with ischemic ARF. This issue has been addressed also by several others (Conger et al., 1995; Mashiach et al., 1998). Jerkic et al. (1999) also showed a further decrease in RBF of ischemic rats treated with L-NAME, which was reversed with L-arginine coadministration. Atanasova and his group (1995) reported that, in anesthetized rats, L-NAME combined with renal ischemia not only induces a deleterious renal effect, but also leads to a pronounced cardiac depression and a major increase in systemic vascular resistance and pulmonary vascular resistance, associated with high mortality. Also, inhibition of NO synthesis with L-NAME during the reperfusion period increases morphological damage (Noiri et al., 1996; Jerkic et al., 1999; Kakoki et al., 2000; Valdivielso et al., 2001). The application of iNOS specific inhibitors has been reported to be beneficial in vivo reducing plasma
creatinine concentration and tubular damage (Walker et al., 2000). Also, various strategies utilized to reduce iNOS-derived NO have been reported to be beneficial. Ling et al. (1999) induced renal ischemia in the iNOS knockout mouse and observed both less reduction in renal function and morphological damage than in wild-type animals. Noiri et al. (1996), using a different approach, also observed an improvement in renal function and morphological structure in rats treated with antisense oligodeoxynucleotides against iNOS. Weight et al. (1999) found that administration of aminoguanidine, a purported iNOS inhibitor, exerts a protective effect on renal function at day 2 after ischemia, but had no beneficial effect by day 7. In contrast, attempts to stimulate NO synthesis with L-arginine infusion have provided controversial results. Whereas some authors have found that L-arginine administration exerts beneficial effects (Chintala et al., 1993; Schramm et al., 1996; Shoskes et al., 1997; Mashiah et al., 1998; Jerkic et al., 1999; Tome et al., 1999), others report no response (Cristol et al., 1996; Kakoki et al., 2000) or detrimental to variable effects, depending on the dose, time, and the presence of superoxide dismutase (Caramelo et al., 1996; Weight et al., 1999). Also, Dagher and co-workers (1995) observed a beneficial effect of L-arginine infusion when given before the ischemic insult. Studies in isolated tubules have led to more consistent results. Ischemia does stimulate iNOS (Yu et al., 1994), and this NO generated seems to exert a deleterious effect. Addition of L-NAME to tubules decreases the degree of damage induced by ischemia (Yu et al., 1994; Paller et al., 1998; Tome et al., 1999) and L-arginine (Tome et al., 1999; Yu et al., 1994) or NO donors (Yu et al., 1994) increase injury.

**NO and Nephrotoxic ARF**

**Radiocontrast:**
Radiocontrast-induced nephropathy remains one of the most serious and frequent complications of contrast imaging. ARF following the administration of radiocontrast agents may range in severity from nonoliguric, asymptomatic, and transient renal dysfunction to oliguric, severe, and protracted renal failure requiring dialysis. The role of NO in radiocontrast-induced ARF has not been fully elucidated. In a study by Agmon et al. (1994), rats pretreated with L-NAME, indomethacin, or both showed greater morphological and functional damage in response to contrast media (iothalamate). Furthermore, hemodynamic studies using laser-Doppler probes have shown that, when
injected alone, contrast doses of iothalamate increase outer medullary blood flow. Pretreatment with L-NAME, indomethacin, or both reduces basal medullary blood flow and transforms the medullary vasodilator response to radiocontrast into vasoconstriction. These results suggest that prostanoids and NO play important protective roles in the renal responses to contrast substances. A study by Heyman et al. (1998), differing effects in cortical and medullary NOS activity were reported. Whereas cortical NOS activity was decreased 30 min after contrast media administration, outer medullary NOS activity remained unchanged. The decrease in cortical NOS activity was exacerbated by pretreatment with an endothelin-I antagonist. NO levels, measured with an electrode, were decreased in cortical tissue, but were increased in the outer medullary area, despite the absence of changes in NOS activity. The authors explained this paradox as an effect of increased NO bioavailability in a region of reduced O$_2$ tension. It has been described that in a solution containing a NO donor, NO concentration increased with the reduction of O$_2$ content (Heyman et al., 1997). As the infusion of contrast media reduced outer medullary oxygenation, they proposed that contrast agent-induced hypoxia enhances NO bioavailability that leads to regional vasodilatation. Schwartz et al. (1994) induced ARF with radio contrast in normal and salt-depleted rats. L-Arginine administration before ARF abolished the decrease in renal plasma flow (RBF) and improved GFR. Administration of a NOS inhibitor did not modify the effect of contrast media on GFR. Andrade et al. (1998) induced ARF with contrast media in hypercholesterolemic salt-depleted rats. In this model, endothelium dependent relaxation is reduced and vasoconstriction is increased, and NO has been implicated in its pathophysiology. Under these circumstances, L-arginine, but not D-arginine, restored renal function completely. The effect of hypo- and hyperosmotic contrast media in cultured mesangial cells has also been examined (Wollman et al., 1995). Both types of radiocontrast media reduced basal and lipopolysaccharide- stimulated (LPS) NO production by these cells.

**Cisplatin:**

Cisplatin is an effective chemotherapeutic agent for a number of cancers. However, it exerts significant nephrotoxic effects (Cornelison and Reed, 1993), and the exact mechanism of cisplatin-induced renal injury is unclear. Although few publications have addressed the role of NO in cisplatin nephrotoxicity, it has been suggested that cisplatin-
induced tubular and glomerular injury might be modulated by NO production. Srivastava et al. (1996) showed that cisplatin treatment in rats increased the activity of calcium independent NOS in kidneys. The administration of L-NAME decreased renal toxicity and levels of blood urea nitrogen and creatinine. In a previous report, Son and Kim (1995) showed that macrophages exposed in vivo to cisplatin express iNOS. Alternatively, in cisplatin-treated rats, the infusion of glycine increases GFR and RBF (Heyman et al., 1991; Li et al., 1994) in a NO-dependent manner. The administration of L-NAME abolishes the improvement in RBF induced by glycine in these animals (Li et al., 1994).

**Cyclosporine:**

Although cyclosporine A (CsA) and tacrolimus (FK506) do not share similar chemical structures and react with different biochemical targets, they produce similar signal transduction effects in T lymphocytes, resulting in the desired immunosuppressive effect. Their toxicity spectra are similar, and indeed the main problem with both drugs is nephrotoxicity (Bennett et al., 1994). It is currently believed that in CsA nephrotoxicity there is an alteration of the L-arginine-NO pathway in blood vessels. It is well known that CsA administration causes hypertension both in humans and in laboratory animals (Hamilton et al., 1982). A number of explanations for this phenomenon have been proposed, but none of them definite. Data have been presented suggesting that CsA produces endothelial damage, which is both time-and dose-dependent (Zoja et al., 1986). CsA impairs endothelium dependent relaxation in response to acetylcholine (ACh) in rat arteries and isolated resistance vessels from humans (Richards et al., 1989). Also, aortic rings isolated from rats treated with CsA demonstrated impaired endothelial-dependent relaxation, achieving levels similar to that observed in endothelium-denuded rings (Noiri et al., 1996). Nitrite/nitrate levels were also similar. The ability of the CsA-treated rat aortic rings to relax after addition of a NO donor was preserved and was not suppressed by addition of CsA (Oriji and Keiser, 1998). When CsA was added acutely to intact aortic rings from normal rats, tension increased and nitrite/nitrate and cGMP production decreased, suggesting a decrease in NO production. However, in a different publication by Navarro- Antolin et al. (1998), 24-h incubation of bovine aortic endothelial cells with CsA produced an increase in eNOS mRNA, probably due to an increase in the
transcription rate. Stroes et al. (1997) also found an increase in eNOS in human endothelial cells incubated with CsA. This effect appears mediated by an increase in reactive oxygen species. Additional evidence for the involvement of the NO pathway in CsA-induced nephropathy was generated by Potier et al. (Potier et al., 1996). In this study, incubation of normal glomeruli or mesangial cells with CsA induced an increase in contractility, which was blunted by coincubation with a NO donor. Wu et al., in 1998 (Wu et al., 1998) demonstrated that incubation of medullary thick ascending limb cells with CsA decreased LPS-stimulated NO production. Protein kinase C is involved in this process. In two different studies, Amore et al. (2000) and Esposito et al. (2000) demonstrated that CsA induces apoptosis in various cultured renal cells (endothelial, epithelial and tubular). This effect is mediated by increases in iNOS-derived NO, because CsA induces an increase in iNOS mRNA, and apoptosis can be inhibited by L-NAME and enhanced by NO donors. Analysis of NOS expression in kidneys of animals treated with CsA produces controversial results. Whereas Gonzalez-Santiago et al. (2000) found a decrease in eNOS mRNA in aorta and renal cortex of animals treated for 30 days with CsA, and Vaziri et al. (1998) found decreases in iNOS mRNA and protein and no changes in eNOS, Sanchez-Lozada et al. (2000) observed an increase of eNOS mRNA in cortex and reductions in iNOS and neuronal NOS (nNOS) in the medulla of 7 day treated animals. The increase in eNOS, in this case, was related to an increase in shear stress due to vasoconstriction. In a different study, Bobadilla et al. (1998) also showed an increase in eNOS in cortex and also in the medulla, with decreases in nNOS and iNOS in medulla after 7 days of treatment. Rao et al. (1996) found an increase in NOS activity in animals treated with CsA. Tack et al. (1997) did not find any changes in glomerular eNOS mRNA 6-9 h after a single dose of CsA, despite the fact that those glomeruli produced higher amounts of cGMP. In vivo L-NAME treatment and in vitro calcium depletion blunted this increase in cGMP. In isolated perfused kidneys, CsA has been shown to reduce the vasodilator response to ACh and nitroprusside (Cairns et al., 1989) and L-arginine reverses cyclosporine-induced vasoconstriction, whereas L-NAME has no additional effect (Bloom et al., 1995). However, in another study, Stephan et al. (1995) showed that in isolated kidneys of rats treated with subacute doses of CsA, ACh-induced relaxation was blunted (with a defect in NO mediated relaxation), whereas endothelium-
related relaxation (after addition of nitroprusside or fenoldopam) was totally preserved in micropuncture studies performed by De Nicola et al. (1993), CsA decreased NO-dependent glycine vasodilatation, suggesting a decrease in NO production. Administration of CsA to animals in vivo has been reported to increase urinary nitrite excretion in one case (Assis et al., 1997), to decrease nitrite and cGMP excretion in some others (Oriji and Keiser, 1998; Vaziri et al., 1998), and to produce no changes in another (Bobadilla et al., 1994). The renal dysfunction induced by CsA has been improved by dietary supplementation with L-arginine (Amore et al., 1995; Andoh et al., 1997; Assis et al., 1997; Yang et al., 1998; Shihab et al., 2000). Administration of L-arginine causes an increase in urinary nitrite excretion (Bobadilla et al., 1994; Andoh et al., 1997; Assis et al., 1997); therefore, an increase in NO production appears involved in the improvement in renal function, probably by decreasing renal vascular resistances and increasing glomerular plasma flow (Bobadilla et al., 1994). These results agree with those from Andres et al. (1997) in renal transplant patients, in which administration of L-arginine increases RBF, GFR, and natriuresis. In other studies, administration of L-NAME to CsA-treated animals has produced a worsening of renal function (Bobadilla et al., 1994; Amore et al., 1995; Andoh et al., 1997; Bobadilla et al., 1998; Shihab et al., 2000). This effect of L-NAME in CsA-treated animals is a consequence of an increase in afferent arteriolar resistance and a decrease in glomerular capillary pressure (Bobadilla et al., 1998). Furthermore, administration of L-NAME to CsA-treated rats worsened tubulointerstitial fibrosis (Andoh et al., 1997; Shihab et al., 2000). Little is known about the relationship between FK506 and NO. Two studies have been published concluding that FK506 induces a decrease in both eNOS mRNA and activity in endothelial cells (Agmon et al., 1994; Atanasova et al., 1995), possibly due to an inhibition in NOS phosphorylation (Dawson et al., 1993).

Myohemoglobinuric ARF:
The classical experimental model is glycerol-induced myohemoglobinuria, usually in water-deprived rats. It seems that the hemodynamic changes and not the tubular obstructions are the major factors responsible for the filtration failure in this model. Maree et al., (1994) administered L-arginine before and immediately after glycerol and achieved a near normalization of GFR and RBF 24 hr after the induction of ARF in rats.
with or without, previous dehydration. NOS-inhibited animals developed a more severe ARF. L-arginine increased significantly the urinary cGMP before the glycerol administration but in the first hour after the induction of ARF the urinary cGMP dropped to very low levels.

**Urinyl nitrate ARF:**

It is a model of non oligouric ARF induced by a bolus injection of uranyl nitrate. Schramm et al., (1994) administered L-arginine, NOS inhibitor or both 180 mins after the induction of ARF. The L-arginine produced a significant improvement in GFR. No change in urinary flow or sodium excretion was observed. Because the uranyl nitrate is considered to act via a reduction in the glomerular ultrafiltration coefficient ($K_f$), it seems that L-arginine may act by the augmentation of NO production by the messenger cells, improving $K_f$ in this model.

**Gentamicin ARF:**

Rivas-Cababero et al. (1994) studied glomeruli isolated from normal and gentamicin-induced ARF rats. They found evidence of significant increase in the NO production in gentamicin treated rats. In these rats, the glomeruli had 10 times more basal production of cGMP, thought to be due to the high NO. The cause of high NO production is not clear. It may be the result of infiltrating glomerular macrophages in the gentamicin-treated rats.

**Renal NO pathway in chronic renal failure (CRF)**

There is increasing evidence that inadequate NO production within the kidney plays a key role in causing and/or mediating the complex hemodynamic disorders associated with the progression of chronic renal disease. Most of these studies (Benigni et al., 1999; Takamitsu et al., 2003) have been focused on the rat experimental model of chronic renal failure obtained by extensive renal mass reduction (RMR). These animals develop systemic hypertension and exhibit a decrease in both glomerular filtration rate (GFR) and renal blood flow (RBF), despite an initial adaptive increase in single-nephron GFR and plasma flow per nephron. Animals with RMR also develop severe proteinuria and structural changes in the kidney, including glomerulosclerosis, which eventually lead to renal insufficiency. In rats with RMR, renal generation of NO, measured as the ex vivo conversion of 3H L-arginine to 3H L-citrulline, per mg protein was markedly lower than
normal (Aiello et al., 1997). The abnormality was evident 7 days after RMR surgery and progressively worsened with time, in close correlation with signs of renal injury, such as glomerulosclerosis and tubular damage (Aiello et al., 1997; Santmyire et al., 1998). Similar results were obtained by the in situ detection of NOS catalytic activity by NADPH-diaphorase staining, which demonstrated a progressive loss of renal NOS activity in RMR rats in all the structures examined including glomeruli (except the macula densa), proximal tubules, and collecting ducts (Aiello et al., 1997). Consistent with the in vitro and ex vivo results, urinary excretion of the stable NO oxidation metabolites, NO2/NO3− was significantly reduced in rats with RMR as compared with control animals (Ashab et al., 1995; Aiello et al., 1997; Vaziri et al., 1998). It has been suggested that in renal failure NO deficiency could result from reduced arginine availability, since the kidney is a major site of endogenous arginine synthesis (Chan et al., 1974; Mitch and Chesney, 1983). However, in one study Boudy et al. (1993) showed that total renal arginine synthesis and arginine plasma levels are not diminished in the remnant kidney model despite a significant reduction in GFR. This is possibly due to a combination of three factors: hypertrophy of proximal tubules in remnant nephrons; hyperfiltration that increases the amount of citrulline filtered per nephron; and an increase in plasma citrulline concentration that enhances arginine synthesis because of the concentration dependency of this synthesis in the kidney. Based on these studies in experimental animals, it seems unlikely that the substrate supply of arginine would be rate limiting for NOS even with the severe reduction of functional renal mass, unless there were extraordinary arginine demands (i.e., sepsis or vigorous cytokine-induced NOS activation). Alternatively, decreased NOS activity could be due to a decrease of NOS isoenzyme content. Using the immunoperoxidase technique, reduced iNOS expression was found in the proximal tubules and collecting ducts of rats with RMR (Aiello et al., 1997). Endothelial NOS (eNOS) staining was comparable in RMR and control kidneys. However, in RMR rats the eNOS signal was reduced in glomeruli affected by sclerosis, possibly because of loss of functional endothelium (Aiello et al., 1997). Another study showed reduced iNOS and eNOS proteins in the kidneys of RMR rats, confirming the decreased availability of both NOS enzyme isoforms (Vaziri et al., 1998). In another model of experimental progressive renal disease induced by chronic
administration of cyclosporine (CsA), fall in GFR and hypertension were associated with a marked reduction of urinary excretion of NO metabolites and of renal iNOS mRNA and iNOS protein content, suggesting that CsA-induced hypertension and renal dysfunction may be, in part, related to impaired NO production (Vaziri et al., 1998). There is also evidence indicating that renal NO deficiency occurs in patients with chronic renal failure (CRF). Thus, daily urinary excretion of $NO_2^-/NO_3^-$ was significantly lower in patients with moderate and severe renal failure, as compared with those with mild renal failure and normal controls; the lowest values were found in the severe renal failure group (Blum et al., 1998). Altogether these findings indicate that CRF is a condition of renal NO deficiency, both in experimental animals and in patients.

Mechanisms leading to impaired renal NO synthesis in CRF:

Various studies have addressed the progressive decrease in iNOS and reduced NO formation in CRF. In glomeruli, soon after surgical ablation of renal mass, inflammatory mediators, such as platelet-derived growth factor (PDGF) and transforming growth factor-ß (TGF-ß), are formed in excessive amounts (Floege et al., 1992; Lee et al., 1995). PDGF and TGF-ß are both potent inhibitors of NO synthesis and block dose dependently interleukin-1 ß-induced iNOS mRNA in rat mesangial cells (Ketteler et al., 1994). It is conceivable that increased concentrations of PDGF and TGF-ß in the glomeruli of rats with RMR downregulate glomerular iNOS. Other reports (Beck et al., 1995; Markewitz et al., 1997) have raised the possibility that excessive renal synthesis of the potent vasoconstrictor and promitogenic peptide endothelin-1 (ET-1) is a major determinant for the progressive decrease in iNOS in the kidney of RMR rats. In rats with RMR, despite no increase in plasma ET-1 levels, renal prepro-ET-1 gene expression and urinary excretion of the peptide increased time dependently, the latter correlating with signs of renal injury (Orisio et al., 1993). Strong ET-1 mRNA and peptide staining was found in proximal tubular cells in rats with RMR in contrast to the weak focal reactivity of proximal tubular cells detectable in control kidneys; some specific ET-1 expression was also found in RMR glomeruli (Bruzzi et al., 1997). Either PDGF or TGF-ß could be responsible for the higher glomerular ET-1 production in the remnant kidney (Zoja et al., 1991; Sakamoto et al., 1992), while a large body of in vitro and in vivo evidence supports the concept that tubular ET-1 formation is a direct consequence of excessive tubular
reabsorption of filtered proteins (Abbate et al., 1999). In situ hybridization analysis of serial sections of renal tissue from RMR rats showed a colocalization of intense ET-1 mRNA signal and loss of iNOS in the same cortical tubules (Benigni et al., 1999). This observation is of particular interest since recent data demonstrated that ET–1 strongly inhibits cytokine induced NO formation in cultured mesangial (Beck et al., 1996; Hirahashi et al., 1996), epithelial (Markewitz et al., 1997), and vascular smooth muscle cells (Ikeda et al., 1997), by blocking iNOS transcription. This action of ET-1 is probably mediated by interaction with the endothelin subtype A (ET\(_A\)) receptor; indeed, the ET\(_A\) receptor antagonist BQ123 can overcome the inhibitory effect of ET-1 on iNOS expression and NO release (Beck et al., 1996; Markewitz et al., 1997). Thus, it is conceivable that the increase of ET-1 in cortical tubules in RMR rats may block iNOS transcription leading to a progressive decrease in iNOS protein and NO production. The administration of a selective ET\(_A\) receptor antagonist to RMR rats ameliorated renal function and histology (Benigni et al., 1999) and significantly increased ex vivo renal NO generation, as compared with untreated RMR animals (Aiello et al., 1997). These data provide in vivo evidence that increased renal ET-1 release in RMR rats, besides being noxious per se, could also be responsible, at least in part, for the defective NO availability. On the other hand, it has also been shown that the endothelin subtype B (ET\(_B\)) receptors are functionally coupled to eNOS and stimulate the release of NO in cultured endothelial cells (Tsukahara et al., 1994). In perfused rabbit kidney, a selective ET\(_B\) receptor antagonist potentiates the increase in perfusion pressure induced by ET-1 (D’Orleans et al., 1994). In addition, infusion of a specific ET\(_A\), receptor agonist in isolated rat renal vessels induces dose-dependent NO release (Hirata et al., 1995). This suggests that the renal effects of ET-1 resulting from the predominance of vascular constriction mediated by ETA receptors are partially counteracted by an ET\(_A\) receptor dependent activation of eNOS in the endothelium. Thus, ET-1 could have a dual role in modulating the renal NO pathway in the setting of the RMR model. On one hand, through an ETA, receptor-mediated mechanism, it maintains glomerular eNOS activity, at least in the early phases of the disease, when the glomerular capillary structure and eNOS expression are still intact. On the other hand, ET–1 blocks tubular and mesangial
iNOS activity through ETA receptors. In this regard, selective ETA receptor antagonists would represent a better therapeutic approach than nonselective ETA/ETB antagonists.

**RENAL TRANSPLANTATION AND NITRIC OXIDE SYSTEM**

The protective role of constitutive NO in transplanted kidneys was well documented in several studies (Ishimura et al., 2002). 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 potential for graft dysfunction in the early post-transplant phase (Vos et al., 2000) 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 (Shears et al., 1997). Endothelial injury is a key component in the pathogenesis of acute renal allograft rejection. Nuclear factor kB (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

![Figure 4: Interaction of NO and NF kappa B with other cytokines and pathophysiological processes in allograft dysfunction.](image-url)
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.

THERAPEUTIC POTENTIAL OF NITRIC OXIDE DONORS AND INHIBITORS

**NO Donors:**

Because of the limited utility of authentic NO gas in many experimental systems and the short half-life of NO in vivo, chemicals that have the capacity to release NO (with or without a requirement for enzymatic metabolism) have been widely employed as pharmacological tools to investigate the role of NO in physiology and pathophysiology. Of course, several NO donors have been used in clinical settings for decades (e.g., nitroglycerin, nitroprusside). The growth of interest in the physiology of NO since the mid-1980s led to the development of a variety of new NO donors that offered several advantages over what had previously been available, such as compounds that spontaneously release NO in solution (e.g., S-nitrosopenacillamine), compounds that can release NO over a prolonged period of time at predictable rates (e.g., NCX-4016), and compounds that selectively release NO in certain tissues (Saavedra et al., 1997). It is these newer compounds that represent useful therapeutic agents for the treatment of various disorders.

**L-arginine:**

L-arginine is a semi essential amino acid and is also substrate for the synthesis of nitric oxide (NO), 4 polyamines, and agmatine and influences hormonal release and the synthesis of pyrimidine bases (Reyes et al., 1994; Moncada, 1997). This places L-arginine, its precursors, and its metabolites at the center of the interaction of different metabolic pathways and interorgan communication. L-arginine is the main source for the generation of NO via NO synthase (NOS) (Moncada, 1997). Approximately 1% of the daily L-arginine intake is metabolized through this pathway (Castillo et al., 1995). The 3 NOS isoforms have been found to be expressed in the kidney (Moncada, 1997). L-arginine is also the substrate for arginases, a group of enzymes that are involved in tissue repair processes and that metabolize L-arginine to L-ornithine. Arginase I is expressed in the liver while arginase II is expressed in the kidney and macrophages (Jenkinson et al., 1996). L-ornithine is the first step in the synthesis of the polyamines putrescine,
spermine, and spermidine via the enzyme ornithine decarboxylase (Shih, 1981). Increased activity of this enzyme has been associated with tissue repair and cell growth (Reyes et al., 1994) and may play an important role in renal disease (Cook et al., 1994). More recently another L-arginine metabolizing pathway has been identified. This pathway involves the generation of agmatine via the enzyme L-arginine decarboxylase (ADC). Agmatine can activate $\alpha$-1 adrenoreceptors and imidazol guanidine receptors (Li et al., 1994) and when infused into the renal interstitium increases glomerular filtration and tubular reabsorption (Schwartz et al., 1997). ADC activity is high in the normal kidney (Morrissey et al., 1996) and therefore it is possible that agmatine may be mediating some of the biological effects of L-arginine supplementation in renal disease. All the above-mentioned L-arginine metabolites may participate in pathogenesis of renal disease and constitute the rationale for manipulating L-arginine metabolism as a strategy to ameliorate kidney disease. A large number of studies have been performed in experimental models of kidney disease with sometimes conflicting results, which underlie the complexity of L-arginine metabolism and our incomplete knowledge of all the mechanisms involved.

**L-arginine in chronic kidney disease:**

Beneficial effects of L-arginine supplementation have been reported in several models of chronic kidney disease (CKD) including renal ablation, ureteral obstruction, puromycin induced nephrosis and nephropathy secondary to diabetes, salt sensitive hypertension, radiocontrast agents, and aging (Reyes et al., 1994; Katoh et al., 1994; Ashab et al., 1995, Amore et al., 1995; Morrissey et al., 1996). In the remnant rat model of CKD, a decrease of 85 to 90% of the total kidney mass results in a series of functional changes including increases in single nephron glomerular filtration rate (SNGFR), progressive reductions in glomerular filtration rate (GFR) and renal plasma flow (RBF), hypertension, proteinuria and structural changes including epithelial cell protein reabsorption droplets, foot process fusion, mesangial expansion, and progressive glomerulosclerosis. In this model, the renal synthesis of NO decreases in parallel with the decline of renal function (Aiello et al., 1997). Based on this observation, L-arginine supplementation has been used as a strategy to ameliorate the progression of kidney disease in this model. Administration of L-arginine in the drinking water for 6 wk to rats
with subtotal nephrectomy resulted in higher GFR and RBF compared to their untreated counterparts (Reyes et al., 1994). L-arginine–treated rats also had a larger number of normal or minimally abnormal glomeruli and also had less severe tubulointerstitial changes. Importantly, these beneficial effects of L-arginine were independent of blood pressure or protein intake. Other studies have also shown similar effects in a similar model of remnant kidneys but utilizing L-arginine supplementation at a dose approximately one tenth of that used in the above-mentioned studies (Ashab et al., 1995). In these studies, L-arginine supplementation had beneficial effects equivalent to those of angiotensin converting enzyme (ACE) inhibition with captopril including reductions in proteinuria, increase in GFR and increase in the urinary excretion of the stable NO metabolites NO$_2$/NO$_3$. Bilateral ureteral obstruction (BUO) is another model of chronic kidney disease characterized by low GFR and RBF, interstitial fibrosis, hypertension, leukocyte infiltration, and increased tubular apoptosis (Klahr, 1998; Miyajima et al., 2001). Increased eNOS and iNOS expression (Salvemini et al., 1994) and low plasma levels of L-arginine have been reported to occur in this animal model. Administration of L-arginine in the drinking water to rats with BUO ameliorates the decreases in GFR and elevations of blood pressure in these rats (Reyes et al., 1994) and is associated with increased urinary NO$_2$/NO$_3$ excretion (Ito et al., 2004) and significant reductions in interstitial volume, monocyte infiltration, and collagen IV deposition. Based on these findings it has been postulated that reduced NO synthesis, because of decreased availability of substrate and in spite of increased eNOS and iNOS expression, may be playing a pathogenic role in this model of kidney disease. The effects of L-arginine administration on the renal function of untreated diabetic rats have also been examined. L-arginine administration in the drinking water of diabetic rats reduced the urinary excretion of cyclic GMP (cGMP), prevented the development of hyperfiltration and significantly reduced proteinuria (Morrissey et al., 1996). The mechanisms involved were not investigated.

The Dahl salt sensitive (DS) rat is a paradigm of low plasma renin, salt sensitive hypertension (Leclercq et al., 2002). DS rats downregulate eNOS and develop impaired NO-mediated endothelial function, accompanied by cardiovascular and renal injury. Recent studies support the notion that salt sensitive hypertension might represent a
vascular diathesis linked to functional upregulation of angiotensin II (Ang II) actions, accompanied by insufficient NO bioavailability and increased oxidative stress (Zhou et al., 2003). Ang II activates NADH/NADPH oxidase in endothelial cells, vascular smooth muscle cells, adventitial fibroblasts, and glomerular mesangial cells (Jaimes et al., 1998). NADH/NADPH oxidase in turn produces superoxide anion (O$_2^-$), which avidly interacts with NO, reduces its bioactivity, and generates peroxynitrite (Griendling et al., 2000).

Renal expression of NADH/NADPH oxidase has been found to be increased in hypertensive salt loaded DS rats and associated with increased production of superoxide anion and decreased NO bioactivity (Zhou et al., 2003). In hypertensive Dahl/Rapp rats the administration of L-arginine but not of D-arginine results in significant reductions in blood pressure and increases the urinary excretion of cGMP (Chen and Sanders, 1991). More recent studies in hypertensive DS rats have shown that supplementation with L-arginine increases plasma NO$_2$/NO$_3$ and produces significant reductions in blood pressure, renal NADH/NADPH oxidase expression, proteinuria, and superoxide anion production (Fujii et al., 2003). These effects of L-arginine suggest that in the hypertensive DS rat there is a reduction in the availability of L-arginine that may be responsible at least in part for the increases in oxidative stress and reductions in NO bioavailability that play an important role in the pathogenesis of renal injury in this model. L-arginine supplementation has also been investigated in the DOCA-salt hypertensive rat, a model of severe hypertension accompanied by severe renal injury. In this model L-arginine supplementation for 8 wk (0.5 g/L in drinking water) was shown to improve endothelial function and increase the renal production of NO. These changes were not associated with improvements in glomerular injury or the severity of hypertension (Hayakawa et al., 1994). However, other studies utilizing higher doses of L-arginine (3 g/kg/day) for 6 wk have reported significant reductions in blood pressure in the same animal model (Abreu et al., 1999).

L-arginine in glomerular diseases:

One of the central characteristics of immune-mediated renal injury is the local induction of iNOS resulting in the production of large amounts of NO. iNOS induction has been demonstrated in a variety of models including anti-thymocyte serum (ATS) induced glomerulonephritis, immune-complex glomerulonephritis, and transplant rejection (Cook...
et al., 1994; Peters et al., 1999). In human renal disorders, increased expression of iNOS mRNA and protein has been documented in mesangiproliferative glomerulonephritis (Kashem et al., 1996), Wegener’s granulomatosis (Heeringa et al., 2001), lupus nephritis (Furusu et al., 1998), and kidney transplant rejection (Albrecht et al., 2000) and correlated with histologic signs of renal damage and loss of renal function. The ATS model is a rat model of mesangial proliferative glomerulonephritis induced by the injection of an antibody to a Thy 1-like epitope that causes dose-dependent complement mediated glomerular injury. This model is characterized by initial mesangiolysis followed by mesangial cell proliferation and accumulation of mesangial matrix with subsequent resolution and the return to almost normal histology (Jafferson et al., 1999).

In ATS glomerulonephritis, NOS inhibition before ATS administration produces a dramatic reduction in mesangial cell lysis, which implies that increased NO production, may play an important pathogenic role at least in the injury phase of this model (Walpen et al., 2001). Moreover, supplementation with 1% L-arginine in the drinking water during the induction phase of ATS glomerulonephritis results in significant increases in glomerular NO production, increased proteinuria and fibrosis (Peters et al., 1999), suggesting that in this model, L-arginine supplementation can enhance NO-dependent tissue injury by providing increased substrate for iNOS. In support of this notion, restriction of dietary L-arginine intake in the same model, even when total protein intake is normal, results in decreased proteinuria, decreased expression of TGF-β, and decreased extracellular matrix deposition, indicating that dietary L-arginine restriction may be limiting the substrate for NO generation and via this mechanism limiting glomerular injury (Narita et al., 1998). In contrast, when L-arginine is administered after the injury phase is completed, it results in significant reductions in extracellular matrix accumulation, TGF-β expression, and fibrosis, suggesting that NO may have a role regulating TGF-β overexpression during the fibrotic phase of this model of glomerulonephritis (Peters et al., 2000). In contrast with ATS glomerulonephritis, in experimental immune-complex glomerulonephritis increased NO generation via iNOS induction does not seem to promote glomerular injury (Waddington et al., 1996; Cattell, 1995). In a model of nephritis induced by administration of nephrotoxic globulin to preimmunized rats, L-arginine depletion via systemic administration of arginase
worsened the severity of the glomerular injury especially if hypertension was present, suggesting that rather than favoring tissue injury, NO may have a role limiting the magnitude of glomerular injury in this model (Waddington et al., 1996). Moreover, in a mouse model of accelerated anti-glomerular basement membrane glomerulonephritis (anti-GBM), genetic disruption of iNOS did not result in significant changes in albuminuria or glomerular inflammation compared with their heterozygous littermates, indicating that iNOS does not play an essential role in this form of glomerulonephritis in the mouse (Cattell et al., 1998). L-arginine supplementation has also been used in models such as the lupus nephritis in MRL/lpr mice, a model of chronic and progressive glomerulonephritis in which glomerular iNOS expression and activity are increased (Weinberg et al., 1994). In this model, administration of 1% L-arginine in the drinking water was associated with a high death rate, increased albuminuria, extracellular matrix accumulation, TGF-β expression, and blood and urine NO$_2$/NO$_3$ levels, suggesting that L-arginine worsens renal fibrosis and increases death rate probably by enhancing cytotoxic NO generation via iNOS (Peters et al., 2003). A few clinical studies have also investigated the effects of L-arginine supplementation on the progression of non-diabetic glomerular disease with disappointing results. Oral administration of L-arginine (0.2 g/kg/day) for 6 months to subjects with different types of glomerulonephritis resulted in significant increases in the plasma levels of L-arginine and a delayed increase in the plasma levels of the stable NO metabolites NO$_2$/NO$_3$, but no change in proteinuria, glomerular filtration rate, and renal plasma flow compared to a control group receiving placebo (De Nicola et al., 1999). In another study, L-arginine supplementation for 8 wk in patients with chronic rejection led to significant increases in plasma levels of L-arginine that were not associated with changes in renal function or peripheral vascular disease (Eisenberger et al., 1999).

**L-arginine in acute renal failure:**

Acute renal failure (ARF) is a common clinical condition associated with a high morbidity and mortality (Kelly and Molitoris, 2000). Multiple strategies including L-arginine supplementation have been attempted to ameliorate the course of ARF. In several studies administration of exogenous L-arginine has been shown to protect the kidney against toxic or ischemic injury (Lopau et al., 2000; Valdivielso et al., 2000;
Schramm et al., 2002). However, the molecular mechanisms for these beneficial effects are unclear and occur even though the intrarenal levels of L-arginine are well above the Km value for NOS saturation of 3 to 5 μmol/L (Harrison, 1997). This phenomenon has been described as the “L-arginine paradox” (Boger and Bodge-Boger, 2001).

Defects in the L-arginine: NO pathway have been proposed to play an important role in the pathogenesis of ARF (Schramm et al., 1996; Goligorsky and Noiri, 1999). Several studies have suggested that NO bioactivity is reduced in models of postischemic ARF as assessed by a blunted response to endothelium-dependent vasodilators such as acetylcholine and bradykinin (Sternbergh et al., 1993; Conger et al., 1995) and increased constrictor responses to renal nerve stimulation and Ang II. Interestingly, these reductions in NO bioactivity are accompanied by increases in iNOS expression and activity (Schneider et al., 2003) and in the case of eNOS there was either no change in its expression (Conger et al., 1995) or a transitory increase followed by a reduced expression (Schneider et al., 2003). In models of ischemic ARF these changes in iNOS expression are accompanied by increased production of superoxide anion and peroxynitrite (ONOO⁻) leading to nitrosative stress. Nitric oxide rapidly reacts with O₂⁻ resulting in the generation of the highly reactive ONOO⁻ and reduced NO bioactivity. ONOO⁻ itself can produce lipid peroxidation and DNA damage and in addition can produce NOS uncoupling by affecting the NOS dimeric structure. Increased O₂⁻ production has been found in several models of ischemic ARF. Among the potential sources of O₂⁻ in the kidney are NADPH oxidase, xanthine oxidase, or uncoupled NOS. In addition, reduced expression of the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase, and catalase may also contribute to increase oxidative stress in ischemic ARF (Conti et al., 2000). Inhibition of iNOS has been shown to be beneficial in models of ischemia reperfusion, which suggests that increased iNOS expression and activity may have roles in the pathogenesis of ischemic ARF (Chatterjee et al., 2002). In contrast, NOS inhibition during endotoxemia results in glomerular thrombosis suggesting that increased iNOS activity may also have a protective role under specific conditions (Jaimes et al., 2001). Exogenous L-arginine supplementation for 14 d in a model of ischemic acute renal failure has been shown to have a beneficial effect on GFR, RBF, reduced O₂⁻ production and prevention of iNOS upregulation as well as reduced nitrotyrosine
formation. These beneficial effects may suggest a functional NO deficiency in ischemic ARF, probably as a result of inactivation by $O_2^-$ and subsequent ONOO$^-$ generation. Decreased renal L-arginine production has been found to occur in models of renal ischemia/reperfusion that may be contributing to the reductions in the tissue levels of L-arginine observed in ARF (Schramm et al., 2002). Although reduced, these levels are still well above the $K_m$ for NOS, which suggests that other cytoprotective mechanisms may be involved (Jerkic et al., 1999). As an alternative explanation for these beneficial effects, it has been suggested that L-arginine compartmentalization in the cytoplasm may result in lower levels of L-arginine in the vicinity of eNOS and a relative L-arginine deficiency in spite of appropriate tissue or plasma levels of L-arginine (McDonald et al., 1997). As an alternative explanation, it has also been suggested that L-arginine excess may be beneficial by overcoming the activity of the endogenous NOS inhibitor asymmetric dimethylarginine (ADMA) which has been reported to be increased in renal failure (Vallance et al., 1992). In addition, a relative L-arginine deficiency may lead to NOS uncoupling (Xia et al., 1998), as has been described for tetrahydrobiopterin (BH4), leading to the production of $O_2^-$ instead of NO by NOS. This mechanism could explain the lowered renal production of $O_2^-$ in models of ARF treated with L-arginine.

**Molsidomine and its metabolites:**

![Molsidomine and its metabolites](image)

N-(ethoxycarbonyl)-3-(4-morpholinyl)-sydnone imine

1) Mechanism of action

Molsidomine is the endogenous precursor of the NO donor, 3-morpholinosydnonomine (SIN-1) which has been shown to stimulate sGC activity by an NO-mediated pathway in blood vessels (Rinaldi and Cingolani, 1983; Plane et al., 1998; Bassenge et al., 1989). Conversion of molsidomine to SIN-1 occurs primarily in the liver in vivo, and subsequent transformation of SIN-1 to SIN-1A occurs spontaneously in the blood via a hydroxylation
reaction (Noack and Feelisch, 1989). Molecular oxygen initiates conversion of SIN-1A to SIN-1C, resulting in the release of NO (Bohn and Schonafinger, 1989), a reaction that is accelerated by O₂ (Feelisch et al., 1989). Formation of S-nitrosoglutathione has also been implicated as an intermediary step prior to activation of sGC (Schrammel et al., 1998).

![Activation mechanism of molsidomine.](image)

The beneficial effects of molsidomine were recognized before endogenous NO was identified and were first applied in the treatment of angina in 1978. However, the mechanism by which molsidomine and SIN-1 cause vascular relaxation and inhibit platelet activation is a controversial issue because, although the effects of these compounds are associated with increased cGMP, there is a poor correlation between both the time course and extent of cGMP elevation and the observed hemodynamic effect (Rinaldi and Cingolani, 1983). A possible explanation may revolve around activation of K⁺ channels (Peng et al., 1996; Plane et al., 1996; Mistry and Garland, 1998). Molsidomine has a slower onset and longer duration of action than conventional nitrates due to the relatively slow rate of conversion to SIN-1. SIN-1 itself has a rapid onset and short duration of action. Interestingly, molsidomine and SIN-1 do not appear to induce tolerance and are not cross-tolerant with conventional nitrates (Govantes et al., 1996; Sutsch et al., 1997).

2) Therapeutic applications

Molsidomine and SIN-1 are therapeutic alternatives to traditional organic nitrates in stable angina (Messin et al., 1995), coronary vasospasm (Danchin et al., 1991) and heart failure (Unger et al., 1994; Lehmann et al., 1995), with the obvious benefit of avoiding tolerance. However, large-scale trials have failed to reach firm conclusions as to the effect of molsidomine and nitrates on survival following myocardial infarction. Continuous chronic administration might offer other benefits, especially through inhibition of platelet activation (Gerzer et al., 1989; Bult et al., 1989): SIN-1 has been
shown to protect against thrombotic occlusion of blood vessels following balloon angioplasty (Groves et al., 1993; Provost et al., 1997) and in stenosed coronary arteries (Just and Martorana, 1989) in experimental models. Unlike organic nitrates, molsidomine and SIN-1 appear to have significant antiplatelet activity at therapeutic doses (Drummer et al., 1991). SIN-1 might also help to prevent atherosclerosis by inhibiting oxidation of low density lipoprotein (Rikitake et al., 1998) and smooth muscle cell proliferation (Groves et al., 1995), but its antiatherogenic benefits are questionable (De Meyers et al., 1995; 1997). SIN-1 has also been shown to have beneficial effects in reperfusion injury, reducing neutrophil sequestration and vascular resistance (Clark et al., 1999), as well as preserving endothelial function (Johnson et al., 1998).

In view of the dilatory effect of NO in cavernous smooth muscle relaxation, NO donor drugs have an obvious application in the treatment of erectile dysfunction. SIN-1 is probably the most extensively studied to date but the results are disappointingly mixed (Wegner et al., 1995). In this setting, it is apparent that SIN-1 is less effective than prostaglandin E1 (Segarra et al., 1999) and, probably, phosphodiesterase inhibitors.

In addition to the cardiovascular system, molsidomine and SIN-1 have both been shown to dilate human isolated bronchus and guinea pig isolated trachea through stimulation of sGC (Zhang et al., 1993). The potential of NO donor drugs in treatment of respiratory diseases has not been fully explored to date.

3) Limitations

Cogeneration of superoxide is a major concern which is likely to limit the therapeutic potential of molsidomine and its metabolites; SIN-1 can even be used as a peroxynitrite generator (Holm et al., 1998; Blaylock et al., 1998; Lomonosova et al., 1998; Hermann et al., 1999). Indeed, SIN-1 increases fatty streak development (De Meyers et al., 1997) and fails to inhibit neointimal proliferation (De Meyers et al., 1995) in rabbit models of atherosclerosis, possibly as a result of the O$_2^-$ generation associated with the release of NO, despite the protective effects of endogenous electron acceptors like ferricytochrome C and heme proteins present in the blood (Singh et al., 1999). Furthermore, although SIN-1 is considered resistant to tolerance, its short duration of action necessitates an increased dose frequency which might prove inconvenient in a clinical setting (Lehmann et al., 1998). Although the full potential of molsidomine and SIN-1 in cardiovascular
disease has not yet been explored, the possibility of oxidative damage with prolonged administration would require cotreatment with antioxidants (Dikshit et al., 1993). At a practical level, the light sensitivity of molsidomine necessitates careful protection of infusion bags and tubing during administration (Vandenbossche et al., 1993).

**Nebivolol:**
Among several new drugs already available that can fulfill the requirement of increasing NO synthesis, a new class is especially interesting. Beta 1-adrenoceptor antagonists with NO-releasing properties, such as nebivolol, could be important tools to improve the arterial hypertension and associated abnormalities secondary to the chronic deficiency of NO, because of two main effects. One is the above-mentioned increase in NO production; the other is the plasma renin activity (PRA)–suppressing effect. Nebivolol is a new selective β₁-adrenergic blocking agent that possesses a peculiar pharmacodynamic profile and an original chemical structure, by which it differs from traditional β₁-blockers. Nebivolol is endowed with peripheral vasodilating properties mediated by the modulation of the endogenous production of nitric oxide (NO), as well as additional antioxidative effects (Mangrellaa et al., 1998). In particular, it has been demonstrated that nebivolol vasodilates human forearm vasculature via the L-arginine/NO pathway (Mangrella et al., 1998). Nebivolol, and a chemical racemate that contains equal proportions of D- and L-enantiomers, was found to induce endothelium-dependent arterial relaxation in dogs in a dose-dependent fashion (Gao et al., 1991). The endothelium-dependent relaxation induced by nebivolol is abolished by N⁵-nitro-L-arginine methyl ester, an inhibitor of NO synthase. These experimental studies were further supported by studies involving infusion of nebivolol into the phenylephrine-preconstricted superficial human hand veins of healthy volunteers, yielding dose-dependent venodilation (Bowman et al., 1994). Similarly, nebivolol increased forearm blood flow, measured by venous occlusion plethysmography, by ≥90%. (Cockcroft et al., 1995). Whether this compound or related compounds can enhance NO production in essential hypertensives or in other individuals with cardiovascular diseases remains undetermined at this time. Nebivolol does not significantly decrease airway conductance compared with atenolol and propranolol (Mangrella et al., 1998). In 6 healthy volunteers,
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unlike propranolol and atenolol, it did not antagonize salbutamol effects (Mohammed et al., 1991). Nebivolol has been investigated in the respiratory tolerance in 12 asthmatic patients (Cazzola and Matera, 2000). It slightly affected airway function. However, although its effect on FEV1 was statistically significant, the mean percent decrease (−8.4%) was small.

Resveratrol:

Resveratrol (trans 3,5,4'-trihydroxy stilbene), a naturally occurring phenolic compound, abundantly available in grape skins and in wines, has been found to protect the heart from ischemic-reperfusion injury (Das et al., 1999; Ray et al., 1999). It possesses diverse biochemical and physiological actions, which includes estrogenic, antiplatelet, and anti-inflammatory properties (Bertelli et al., 1996; Ferrero et al., 1998). Recently, resveratrol was found to protect kidney, heart and brain from ischemic-reperfusion injury (Das et al., 1999; Ray et al., 1999; Bastianetto et al., 2000; Giovannini et al., 2001). In kidney cells, resveratrol was found to exert its protective action through upregulation of NO (Giovannini et al., 2001). The ability of resveratrol to stimulate NO production during ischemia-reperfusion is believed to play a crucial role for its ability to protect kidney cells from ischemic reperfusion injury (Bertelli et al., 1996; Bhat et al., 2001).

Ray et al. (1999) have demonstrated the ability of resveratrol to protect the ischemic reperfused myocardium by improving postischemic ventricular function and by attenuating myocardial infarction due to both necrosis and apoptosis. Although the in vivo antioxidant ability is believed to be at least partially responsible for the cardioprotective properties of resveratrol, the mechanism(s) of action was not completely understood. Recently, a study by Imamura et al (2002) demonstrated that resveratrol was unable to precondition iNOS knockout mouse hearts, whereas it could successfully
precondition the wild-type mouse hearts, indicating an essential role of iNOS in resveratrol preconditioning of the heart.

**Statins:**

The efficacy of the widely prescribed 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) in decreasing the incidence of cardiac events and mortality is likely enhanced by their possible antioxidant properties (Wilson *et al.*, 2001) and their ability to upregulate eNOS expression and activity. Statins also reverse the downregulation of eNOS expression induced by hypoxia and oxidized LDL (Laufs *et al.*, 1997), which may underlie their capacity to improve the vascular bioactivity of NO (Laufs *et al.*, 1998) and plaque stability (Jarvisalo *et al.*, 1999; Napoli and Ignarro, 2001) independent of their lipid-lowering effects. By inhibiting 3-mevalonate synthesis, statins also reduce the synthesis of farnesylpyrophosphate and geranylgeranyl pyrophosphate (GGPP) (Laufs *et al.*, 2000). GGPP is important in the posttranslational modification of a variety of proteins, including eNOS and Ras-like proteins, such as Rho. Inhibition of Rho results in a 3-fold increase in eNOS expression and nitrite generation. The effect of statins on eNOS expression is reversed by GGPP but not by farnesylpyrophosphate or LDL cholesterol (Weiss *et al.*, 1999). Thus, an important non–cholesterol-lowering effect of statins is the upregulation of eNOS expression via the inhibition of Rho. Moreover, statins prevent the downregulation of eNOS induced by tumor necrosis factor-α (Endres *et al.*, 1998) and antioxidant effects in the vascular wall by increasing eNOS activity (Laufs *et al.*, 1998; Kureishi *et al.*, 2000) and decreasing ROS production. In particular, statins are able to prevent lipid oxidation (Giroux *et al.*, 1993; Aviram *et al.*, 1998; Suzumura *et al.*, 1999) and they decrease AT-1 receptor dependent ROS generation.
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(Wassmann et al., 2001). Statins inhibit NAD(P)H oxidase expression (Wassmann et al., 2001), activity (Christ et al., 2002), and assembly by preventing isoprenylation of the small p21 rac protein (Wagner et al., 2000). These effects may play an important role in the setting of chronic statin therapy for the primary and secondary prevention of coronary heart disease.

Simvastatin was recently found to attenuate brain injury and cerebral infarct size in mice (Endres et al., 1998) and to limit cardiac dysfunction after ischemia/reperfusion injury (Simons, 2000). In these latter studies, the protective effects of simvastatin were related to the increased efficiency of the NO pathway. Thus, the statins can now be considered as agents that both modulate the synthesis and enhance the bioactivity of endogenous NO. Simvastatin can also activate the protein kinase Akt to promote new blood vessel growth (Kureishi et al., 2000), which may serve as an additional beneficial mechanism in individuals with atherothrombotic disease.

NO Inhibitors:
Most of the animal models of human diseases in which the occurrence of large amounts of iNOS-derived NO has been observed make use of "NO inhibitors" to study the involvement of NO overproduction as either the cause or the consequence of the particular disease. Even before the chemical identification of endothelium-derived relaxing factor as being NO, hemoglobin and superoxide anion (O2−) were known to block the vasodilator effects of this labile factor. Because L-arginine was identified as the naturally occurring substrate for NO synthesis, several L-arginine-derived analogs (e.g., N-monomethyl-L-arginine and nitro-L-arginine methyl ester) were synthesized and characterized as competitive nitric oxide synthase (NOS) inhibitors. These early inhibitors shared a common feature of nonselectivity with respect to the source of NO (i.e., isoform of NOS).

Selective NOS inhibitors: Many NOS inhibitors considered as "isoform selective" are now available, mainly exhibiting their selectivity for the neuronal isoform of NOS (nNOS; e.g., 7-nitroindazole and analogs) or iNOS [e.g., aminoguanidine, L-Nω-(1-iminoethyl)lysine, S-alkylated isothiourea derivatives]. A comprehensive review of iNOS inhibitors has recently been published (Southan and Szabo, 1996). It is toward iNOS that
most efforts to find a selective inhibitor are being aimed, largely because of the importance of NO derived from iNOS in mediating the cardiovascular disturbances that characterize septic shock. These agents are also being tested in the context of intestinal inflammation, such as in experimental models of colitis. Earlier studies using nonselective NOS inhibitors produced discrepant results. Some studies demonstrated that NOS inhibitors reduced the severity of experimental colitis, whereas others demonstrated exacerbation of colitis (Kubes and Wallace, 1995; Miller and Grisham, 1995). These discrepant results are almost certainly due to the dual ability of NO to mediate tissue injury and tissue defense. Even more recent studies that utilized more selective iNOS inhibitors and iNOS-deficient mice have failed to produce convincing and consistent evidence for a role of iNOS-derived NO in colitis (McCafferty et al., 1997; Ribbons et al., 1997; Southey et al., 1997).

Tetrahydrobiopterin (THB) is a common cofactor for all of the NOS isoforms. However, its role in the NO synthesis mechanism depends on the type of NOS isoform. For example, it contributes to the stabilization of nNOS and iNOS isoform dimerization, helps to prevent NOS inhibition by NO through a redox mechanism involving the prosthetic heme Fe atom, modulates the relative production of NO and $O_2^{-}$ by endothelial NOS, and helps to protect endothelial cells by scavenging $H_2O_2$. At present, attempts are being made to identify THB antagonists that are able to effectively block iNOS activity without significant effects on the other isoforms.

Peroxynitrite "scavenging." NO reacts with $O_2^{-}$ in alkaline solutions to render the peroxynitrite (PN) anion (ONOO$^-$. This reaction can also occur in biological systems (Beckman et al., 1990). The formation of PN has been reported as a secondary and parallel event in several pathological situations in which large amounts of NO derived from iNOS have been identified. With the discovery of PN, previous work on the involvement of iNOS-derived NO in pathological processes had to be revised, and the reaction of PN formation from NO and $O_2^{-}$

$$\text{NO} + O_2^{-} \rightarrow \text{ONOO}^-$$

The identification of proteins containing 3-nitrotyrosine residues is the most widely employed strategy to detect the formation of PN in biological systems (Beckman et al., 1994) given the highly reactive and unstable nature of this anion under physiological
conditions (half-life of 1.9 s). As outlined above, NO derived from iNOS has been suggested to play a role in the tissue injury associated with experimental colitis and human inflammatory bowel disease. PN has also been implicated as a mediator of tissue injury in these disorders, through demonstration of increased formation (i.e., nitrotyrosine staining) in inflamed ileum and colon from guinea pigs (Miller et al., 1995) and humans (Kimura et al., 1998), respectively. It is important to bear in mind that nitrotyrosine staining may occur independently of PN formation (Eiserich et al., 1998). On the other hand, mercaptoethylguanidine has recently been shown to exert beneficial effects in hapten-induced colitis in the rat, and the effects of this agent were attributed to its dual ability to inhibit iNOS and quench PN (Zingarelli et al., 1998). Sandoval et al. (1997) have demonstrated that 5-aminosalicylic acid, one of the most widely used drugs for treating inflammatory bowel disease, is a very potent scavenger of PN. It is possible that this activity contributes significantly to the beneficial effects of this drug in the treatment of inflammatory bowel disease.

Endogenous, unrelated compounds such as uric acid, porphyrins, and vitamins C and E have been shown to offer effective protection against the PN toxicity in different biological systems. Kamisaki et al. (1998) have found that rat spleen homogenates are able to reverse the PN-induced nitration of protein tyrosine residues and that this "nitrotyrosine denitrase" activity is enhanced in endotoxin-treated animals. The search for the expression of this (or a similar) activity in other tissues and the knowledge of their mechanisms of induction will be of fundamental importance for a rational pharmacological manipulation of PN-induced toxicity in an NOS-independent way.

NO scavengers. The high reactivity of NO with diverse chemical groups (such as reduced thiols, primary amines, and transition metals) is the main basis for the design of drugs to be used as NO scavengers. Ruthenium compounds (mainly as coordination complexes) have been proposed as efficient NO scavengers in animal models of sepsis. Fricker et al. (1997) have shown encouraging results regarding the recovery of the hemodynamic functions in the rat after the administration of one such ruthenium compound (JM-1226) 20 h after challenge with endotoxin. 2-Phenyl-4,4,5,5-tetramethylimidazolin-1-oxyl-3-xide (PTIO) is another well-known NO scavenger widely used in several animal models that are characterized by the participation of iNOS-derived NO (Maeda et al., 1994).