Nitric Oxide in the Control of Renal Hemodynamics and Excretory Function

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Experimental evidence has now been amassed to indicate that inhibition of nitric oxide (NO) synthase reduces total or regional renal blood flow by approximately 25 to 30% and markedly increases the renal vascular resistance, demonstrating that basal release of NO helps to maintain the relatively low vascular resistance that is characteristic for the kidney. It has been demonstrated that intrarenal administration of NO synthase inhibitors causes marked reductions in sodium excretion without changes in filtered load and suppressed the arterial pressure-induced natriuretic responses in the kidney. We also demonstrated that a constant rate infusion of a NO donor in dogs pretreated with a NOS inhibitor resulted in increases in sodium excretion but failed to restore the slope of the relation between arterial pressure and sodium excretion, suggesting that an alteration in intrarenal NO production rate during changes in arterial pressure is involved in the mediation of pressure natriuresis. Further experiments in dogs performed in our laboratory have confirmed that there is a direct relationship between changes in arterial pressure and intrarenal NO production, which inhibits tubular sodium reabsorption to manifest the phenomenon of pressure natriuresis. Am J Hypertens 2001;14:74S–82S © 2001 American Journal of Hypertension, Ltd.

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The importance of various intrarenal paracrine systems and their interactive contributions to the regulation of renal hemodynamics and renal function has received increased attention in recent years. Among these paracrine agents, nitric oxide (NO), initially identified as endothelium-derived relaxing factor, has been implicated in many physiologic processes that influence both acute and long-term control of kidney function. There is now substantial evidence that the endothelial, epithelial, and other cells in the kidney can generate NO, which interacts with vascular smooth muscle, mesangial, juxtaglomerular, and tubular cells to regulate vascular and tubular function.

NO Formation in the Kidney

Nitric oxide is synthesized from the amino acid L-arginine in endothelial as well as other cell types by the action of an NO synthase (NOS). NOS exists in three isoforms of which two forms are constitutive and the other is an inducible form. These isoforms are heme-containing enzymes that catalyze the oxidation of L-arginine to NO and L-citrulline. NOS I or neuronal NOS and NOS III or endothelial NOS are constitutively present and are dependent on calcium and calmodulin for activation. An inducible NOS (NOS II) is usually expressed primarily after transcriptional induction, which seems less dependent on calcium for its activation because of its very high affinity to bind with calmodulin with which it forms a constitutive subunit. Studies have now demonstrated that all these isoforms are expressed in various locations in the kidney. The concentrations of both NOS I and NOS III are generally higher in renal medulla than in the cortex. Table 1 provides the localization of NOS isoforms in the kidney. Generally, the endothelia of the renal vasculature and also some tubular epithelial cells express NOS III. NOS I is expressed in macula densa cells and also in lesser quantities in efferent arterioles. The inducible NOS II is expressed in vascular smooth muscle, mesangial, medullary interstitial cells, as well as in tubular epithelial cells.

Because NOS reacts directly with L-arginine, the formation and release of NO can be competitively inhibited by structural analogs of L-arginine such as N-monometh-
yl-L-arginine (L-NMMA) and nitro-L-arginine (NLA). Endothelial NOS can be stimulated by agents such as acetylcholine (Ach), bradykinin, and ATP, as well as flow-induced sheer stress on the vessel wall. Agonist agents such as Ach and bradykinin have long been known to exert marked renal vasodilation. Because of the short half-life and the labile nature of NO, studies evaluating the in vivo effects of endogenous NO in the kidney have been conducted primarily by pharmacologic interventions with agents that can stimulate its release as well as those that can competitively block its formation.

### NO and Renal Vascular Resistance and Blood Flow

It is now well established that tonic release of NO plays an important role in maintaining normal vascular tone in the kidney, which is known to have substantially lower vascular resistance than most other organs or vascular beds. Intrarenal NO helps to maintain the normally low renal vascular resistance (RVR), being responsible for up to one-third of the normal renal blood flow (RBF). The contribution of endogenously released NO to the basal level of RVR has been assessed from the responses to L-arginine analogs that inhibit NO synthesis. In response to inhibition of NO synthesis, there is an increase of 30% to 50% in RVR and a decrease of 25% to 40% in RBF in anesthetized as well as in conscious animals. In experiments conducted in our laboratory, intraarterial administration of NLA at a rate of 50 μg/kg/min for 30 min in anesthetized dogs resulted in an increase of 48% in RVR and a decrease of 28% in RBF. Fig. 1 shows the average renal responses to NOS inhibition in anesthetized dogs collated from several studies from our laboratory. The responses to NOS inhibition have been shown to be reversed by the administration of NO substrate, L-arginine substantiating that the responses to NLA were due to inhibition of endogenous NO formation and release.

Renal cortical blood flow (CBF) measurements in dogs and rats, measured by laser-Doppler flowmetry (LDF) with a surface probe, as well as blood flow in single cortical capillaries in rats measured with fluorescent videomicroscopy, have shown similar decreases in renal blood flow (RBF) during NO inhibition.

### Table 1. Localization of NOS isoforms in the kidney

<table>
<thead>
<tr>
<th>Localization</th>
<th>Constitutive NOS</th>
<th>Inducible NOS</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>NOS III</td>
<td>NOS II</td>
</tr>
<tr>
<td>Endothelium</td>
<td>+ + +</td>
<td>−</td>
</tr>
<tr>
<td>Large vessels</td>
<td>+ +</td>
<td>−</td>
</tr>
<tr>
<td>Afferent arterioles</td>
<td>+ +</td>
<td>−</td>
</tr>
<tr>
<td>Efferent arterioles</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glomerular capillaries</td>
<td>+ +</td>
<td>−</td>
</tr>
<tr>
<td>Epithelium</td>
<td>−</td>
<td>+ + +</td>
</tr>
<tr>
<td>Macula densa</td>
<td>−</td>
<td>+ + +</td>
</tr>
<tr>
<td>Thick ascending limb</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Collecting duct</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Proximal tubule</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Vascular smooth muscle</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Mesangial cells</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Intrarenal neuron</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Medullary interstitial cells</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = present; − = absent; NOS = nitric oxide synthase.

FIG. 1. Summary of the relative renal responses to intraarterial infusion of nitro-L-arginine in anesthetized dogs. Data collected from several studies (Refs. 21, 22, 24, 28, 67). RVR = renal vascular resistance; RBF, CBF, MBF = renal, cortical, medullary blood flow; GFR = glomerular filtration rate; U = urine flow; $U_{Na}V$ = sodium excretion; $FE_{Na}$ = fractional sodium excretion; $U_{K}V$ = potassium excretion.
response to NOS inhibition with L-arginine analogs. In further studies, LDF with needle probes was used to assess renal CBF and medullary blood flow (MBF) responses to NOS inhibition in dogs.\textsuperscript{29,32} These studies demonstrated that blockade of NO synthesis in the kidney by NLA administration resulted in reductions in both CBF (26%) and MBF (25%), which were very similar to the reductions in total RBF (24%). However, studies performed in rats have suggested that NO may exert a greater influence on the medullary circulation than on the cortical circulation.\textsuperscript{33} Comparison of the responses to NOS inhibition of blood flow and vascular resistances in other vascular beds such as femoral, coronary, and brain have shown that the renal vasculature is more dependent on endogenous NO than other organs to maintain normal perfusion of the organ.\textsuperscript{34,35} Collectively, the data generated in various species provide clear evidence that basal release of NO helps to maintain the relatively low RVR that is characteristic for the renal circulation.

Several studies have evaluated the direct effects of elevated intrarenal NO levels using nitrovasodilators, also known as NO donor compounds. Among the commonly used nitrovasodilators, s-nitrosothiols are potent vasodilators and have been used to mimic the actions of endogenously formed NO.\textsuperscript{10,36} To evaluate the unique and specific actions of NO on RBF and RVR, experiments were performed in which s-nitroso-n-acetylpenicillamine (SNAP), an s-nitrosothiol compound, was administered intraarterially in anesthetized dogs, which were pretreated with NLA to inhibit endogenous NO release.\textsuperscript{22} Administration of SNAP at a dose of 2 µg/kg/min to NLA-treated dogs elicited renal vasodilator responses and restored RBF and RVR to values existing before NOS inhibition, confirming the specificity of the vascular actions of NO in the kidney.

### NO and RBF Autoregulation

The autoregulatory adjustments in the renal vasculature that occurs in response to changes in arterial pressure are associated with changes in blood flow velocity through the small arteries and arterioles. Because the entire preglomerular arteriolar vasculature changes dimensions in response to changes in arterial pressure,\textsuperscript{1,37} there would be sustained shear stress-induced alterations in NO release, which could modulate renal vascular tone.\textsuperscript{10} As the diameters of preglomerular vessels decrease in response to increases in arterial pressure, the blood flows at an increased velocity thus causing increased shear stress on the vessel wall leading to increases in NO synthesis.\textsuperscript{29,32} Several studies in dogs and rats have been performed to determine how NO might contribute to renal autoregulatory behavior.\textsuperscript{19–22,24} There is now general agreement that NOS inhibition does not interfere with the ability of the kidney to autoregulate RBF in response to alterations in renal arterial pressure (RAP); however, there is suppression of the autoregulation plateau due to the decrease in basal RBF during NOS inhibition.\textsuperscript{19–21} In experiments conducted in anesthetized dogs in our laboratory,\textsuperscript{22} RBF and RVR responses to stepwise reductions in RAP were evaluated before, after NOS inhibition, and during administration of the NO donor SNAP. As illustrated in Fig. 2, autoregulatory efficiency of RBF remained intact during both NOS inhibition and after NO replacement with SNAP. Most reports in both anesthetized and unanesthetized animals have reported similar results.

Studies using LDF in anesthetized dogs have shown that autoregulatory efficiency of CBF remains highly efficient during intrarenal infusion of NOS inhibitors.\textsuperscript{25,29,38} There are mixed reports with regard to MBF. It has been reported that an impaired autoregulatory efficiency of papillary blood flow in volume-expanded rats, measured by LDF, could be restored by administering the NOS inhibitors.\textsuperscript{39} This finding indicates that an enhancement of NO production during volume expansion in rats may be responsible for impairment of autoregulatory efficiency in MBF, which was not observed in euvolumic rats.\textsuperscript{40,41} Studies in dogs have indicated, however, that MBF maintains efficient autoregulatory responses under conditions of euvolumic and volume expansion.\textsuperscript{42,43} Collectively, the data indicate that intrarenal NO activity does not influence normal autoregulatory efficiency of RBF but does affect the plateau of autoregulation. Although increases in RAP

![Figure 2](https://academic.oup.com/ajh/article-abstract/14/S3/74S/205791/205791)

**FIG. 2.** Summary of renal autoregulatory behavior of RBF, CBF and MBF in response to nitro-L-arginine (NLA) and to the NO donor s-nitroso-n-acetylpenicillamine (SNAP) (○ control; □ NLA; △ NLA+SNAP). RAP = renal arterial pressure; other abbreviations as in Fig. 1. (Data derived from Refs. 22 and 38.)
cause increased NO release from the vascular endothelium due to increased shear stress, the amount released is apparently not sufficiently powerful to counteract the autoregulatory mechanism.

**Renal Microvascular Actions of NO**

Studies involving direct assessment of renal microvascular responses to NOS inhibition in rats and rabbits have demonstrated that tonically released NO regulates both pre- and postglomerular vascular resistance. Results from both in vivo micropuncture experiments and in vitro studies have consistently demonstrated decreases in the vessel diameter and increases in vascular resistance in the afferent arterioles; the efferent arteriolar responses have been more variable. Using a perfused isolated rabbit afferent arteriole–glomerulus preparation, Ito and Ren reported no change in efferent arteriolar diameter during arterioles. Similar to this finding, Deng and Baylis reported that intraarterial infusion of NO inhibition did not cause detectable increases in efferent arteriolar resistance, glomerular pressure, or glomerular flow, although systemic infusion of NO inhibitors did cause efferent arteriolar constriction. However, studies using the in vitro blood-perfused juxtaglomerular nephron preparation demonstrated that both afferent and efferent arteriolar diameters in rat kidneys decreased equally by about 15% to 18% after superfusion of renal tissue with 0.1 to 1 mmol/L NLA. Similar observations were also reported in several other studies. Decreases in glomerular flow and associated increases in resistance of afferent and efferent arterioles were also noted during systemic administration of L-arginine analogs in vivo micropuncture studies. In addition, inhibition of NOS I activity by the specific inhibitor S-methyl-L-thiocitrulline (L-SMTC) in an in vitro juxtaglomerular nephron preparation in rats elicited decreases in efferent and afferent arteriolar diameter by about 15%. Thus, on balance, the results support the conclusion that intrarenal NO contributes to the regulation of vascular tone of both preglomerular and efferent arterioles.

**NO and Glomerular Filtration Rate**

Although administration of NOS inhibitors consistently results in significant reductions in RBF, the glomerular filtration rate (GFR) responses have been less consistent. Several experiments conducted in dogs as well as in humans showed that GFR is well maintained during treatment with NOS inhibitors (Fig. 1). However, other studies report reductions in GFR in response to NOS inhibition. The decreases in GFR are comparatively less than the decreases in RBF leading to increases in filtration fraction during NOS inhibition. It seems that the reductions in GFR in response to NOS inhibition depends on the dose as well as the routes of administration of NOS inhibitors used. Systemic infusion of NOS inhibitors have consistently caused substantial increases in arterial pressure, which may have an indirect effect on kidney function as it was shown that a lower systemic dose (1 μg/kg/min), which had minimal effects on arterial pressure, did not cause significant changes in GFR. It was also observed that if the renal perfusion pressure was not allowed to increase during treatment of NOS inhibition by the use of an aortic snare, there were greater decreases in GFR. However, studies in conscious rats and dogs demonstrated that NOS inhibition resulted in no change or only slight reductions in GFR depending on the dose and duration.

**NO and Tubuloglomerular Feedback Responses**

Several studies have indicated that NO may also be an important modulator of tubuloglomerular feedback (TGF) responsiveness. Studies in which NOS inhibitors were infused intravenously or microperfused into the interstitium or into the tubular segments encompassing the macula densa cells have shown that local NO production can attenuate TGF-mediated vasoconstriction of the afferent arterioles. Using the doubly perfused isolated glomerular segment with macula densa attached, Ito and Ren demonstrated that addition of an NOS inhibitor to the macula densa perfusate led to afferent arteriolar constriction only when the tubules were perfused with isotonic Krebs-Ringer’s solution but not when perfused with a hypertonic solution. Furthermore, Wilcox and Welch reported that the TGF response is enhanced by NOS blockade during high salt but not in low salt intake rats. Blockade of macula densa reabsorption by furosemide was also shown to abolish the enhancement of TGF responsiveness by NOS inhibition. Thus, it is generally thought that increased macula densa NaCl is associated with increased NO synthesis and solute reabsorption by the macula densa cells and the increased NO release partially counteracts the vasoconstrictor stimuli mediating TGF responses. NO is not the mediator of the TGF mechanism but serves to modulate the responsiveness. Recently, Ichihara et al demonstrated in the blood-perfused rat juxtaglomerular nephron preparations that superfusion with the specific neuronal NOS inhibitor L-SMTC decreased afferent and efferent arteriolar diameters, but only under conditions of maintained tubular flow to the macula densa, as these decreases in arterial diameters were prevented during interruption of distal volume delivery by papillectomy. Afferent, but not efferent, arteriolar vasoconstrictor responses to L-SMTC were also enhanced during increases in volume delivery to the macula densa segment by the use of acetazolamide and this effect was completely prevented after papillectomy. In contrast, the arteriolar diameter responses to the nonselective NOS inhibitor NLA were not significantly attenuated by papillectomy. These findings indicate that neuronal NOS in the macula densa exerts a modulating influence on TGF-mediated
adjustments in afferent arteriolar tone. Recent studies suggest that NO, produced in the macula densa by the action of neuronal NOS (NOS I) and not endothelial NO, serves as an important modulator of TGF responsiveness by stimulating soluble guanylate cyclase generating cGMP and activating cGMP-dependent protein kinase within the macula densa cells.60

**NO Influences on Water and Electrolyte Excretion**

The results from many studies have implicated intrarenal NO as an important regulator of water and sodium excretion by the kidney.2,3,5–7 NOS inhibitors, when administered intrarenally, elicit substantial reductions in urine flow and in sodium and potassium excretion, suggesting that basal release of NO helps to maintain normal excretory function of the kidney.1,17,21,22,24,25,28 Fig. 1 illustrates the average responses to NOS inhibitors on renal excretory function in anesthetized dogs observed in experiments performed in our laboratory. Administration of a NO donor compound directly into the kidney reversed the effects of NLA and caused increases in urine flow and in sodium excretion confirming that NO serves as a diuretic and natriuretic agent.22,38 These effects are not consistently associated with significant reductions in GFR, indicating that NO exerts a tubular effect to inhibit epithelial transport. It is also possible that the effects of NOS inhibitors or NO donors on tubular reabsorptive function are mediated indirectly by the associated changes in peritubular hemodynamics or interstitial pressure.8,38,39 A direct effect is supported further by in vitro studies performed in cultured cortical collecting duct cells and in isolated perfused collecting duct segments,5 demonstrating that NO exerts a direct inhibitory effect on epithelial transport mechanisms. Interestingly, it has also been reported that systemic doses of NO inhibitors in rats induce diuretic and natriuretic responses.23,61,62 Although the reasons for these contrasting findings remain unclear, one explanation is that natriuretic and diuretic responses during systemic NOS inhibition may be mediated by other factors associated with the concomitant increases in systemic arterial pressure, reflex inhibition of sympathetic nerve activity to the kidney, and release of humoral agents from extrarenal tissues.61,62

**NO and Pressure Natriuresis**

Nitric oxide has been strongly implicated in mediating the progressive increases in sodium excretion that occur in response to increases in RAP, which is known to occur even under conditions where RBF and GFR autoregulatory efficiency are essentially perfect.53,64 It was previously suggested that some humoral factor capable of detecting altered preglomerular arterial pressure and transmitting signals to the tubules could play a role in this RAP-induced natriuretic response.63 During recent years, the hypothesis that NO serves as this humoral agent responsible for the arterial pressure-induced changes in sodium excretion has received growing support. Initial studies as well as others demonstrated that NOS inhibition caused marked attenuation of the relationship between arterial pressure and sodium excretion.22,24,28,32 We also observed that a constant rate intrarenal infusion of an NO donor agent in dogs pretreated with NOS inhibitors failed to restore the slope of the pressure–natriuresis relationship, although it caused increased sodium excretion. These findings consequently led to the suggestion that an alteration in intrarenal NO activity during changes in RAP, rather than just the presence of NO, is directly involved in mediating pressure–natriuresis.

To examine possible alterations in renal NO activity during changes in RAP, experiments were conducted in anesthetized dogs evaluating the changes in urinary excretion rate of NO metabolites, nitrate and nitrite (NOX), at different levels of RAP.28 Changes in urinary excretion rates of nitrate and nitrite provide an index of the changes in endogenous formation of NO provided other exogenous factors such as dietary intake of nitrates and nitrites are maintained constant.65 As shown in Fig. 3 in response to acute changes in RAP, we observed a positive correlation between RAP and the changes in urinary NOX excretion rate.28 These changes in urinary NOX excretion also showed a positive correlation with urinary sodium excretion.28 To provide a more direct measure of intrarenal NO, we examined the changes in renal tissue NO activity during changes in RAP using an NO selective electrode inserted into the tissues of renal cortical and medullary regions.29,32 These data are summarized in Fig. 4 and show that acute reductions in RAP induce concomitant decreases in intrarenal NO activity. This direct relationship was observed in the presence of efficient autoregulation of cortical and MBF, as determined by single fiber laser-Doppler needle probes.29,32

The changes in tissue NO activity in response to acute changes in RAP were strongly correlated with the concomitant changes in urinary excretion rate of NOX.29,32 These findings demonstrate that intrarenal NO activity is altered by changes in RAP and support the hypothesis that NO is involved in the mediation of pressure natriuretic responses. On the basis of well-known evidence that flow-induced shear stress stimulates the release of NO from the vascular endothelium, it seems likely that alterations in shear stress occurring as a consequence of the changes in resistance of the preglomerular vessels during changes in RAP induce alterations in the release on NO.2,66 As shown in Fig. 5, increases in NO production during increases in RAP would boost prevailing tissue NO levels, which in turn would mediate natriuretic responses by either influencing tubular transport directly or possibly altering the intrarenal hemodynamic environment. Increases in intrarenal NO activity during raises in RAP may partly influence tubular transport by eliciting concomitant increases in renal interstitial hydrostatic pressure (RIHP).8 We have recently demonstrated that intrarenal NO is linked with
changes in RIHP that occur in response to alterations in RAP.\textsuperscript{38}

As mentioned earlier, it has also been shown that NO exerts a direct inhibitory effect on epithelial transport mechanisms.\textsuperscript{5} To examine the tubular sites responsive to changes in RAP, we also examined the possible role of distal nephron sodium entry pathways in mediating pressure natriuresis. The two major sodium transport pathways in the distal nephron were blocked using pharmacologic agents.\textsuperscript{67} We observed that blockade of apical sodium entry pathways in the distal nephron by intraarterial infusion of amiloride and bendroflumethiazide caused a marked attenuation of the pressure natriuretic responses particularly at higher pressure levels.\textsuperscript{67} These findings suggest that the distal nephron sodium entry pathways, which have been shown to be responsive to NO, are principally involved in mediating arterial pressure-induced changes in urinary sodium excretion.

The source of enhanced NO production in the kidney during increases in RAP has not yet been determined. However, because increases in RAP caused changes in blood flow velocity and sheer stress in the preglomerular vessels, it is reasonable to assume that renal endothelial NOS in preglomerular arterioles is responsible for enhanced NO production rate. There are abundant close contacts between afferent arterioles and distal tubules\textsuperscript{68} and it is possible that the NO released during increases in RAP would have a relatively short linear diffusion pathway from the preglomerular arterioles to the tubular segments. Thus, as shown in Fig. 5, it is reasonable to propose that enhancement of renal tissue NO levels in response to acute increases in RAP may directly inhibit distal tubular sodium transport leading to an increase in sodium excretion. In addition, collecting duct cells also contain substantial quantities of NOS\textsuperscript{9,15} and it is possible that NO formed in collecting duct cells affect tubular sodium transport directly. The link between the changes in RAP and direct effects on NO production by tubular NOS is unclear, but tubular NO may alter the magnitude of the pressure natriuresis phenomenon. As mentioned earlier, macula densa cells contain neuronal NOS, which has been shown to play a role in modulating tubuloglomerular feedback responses.\textsuperscript{9,15,57,59} Thus, it is also possible that signals from preglomerular vessels due to changes in RAP cause an enhancement of NO release from the macula densa that may travel downstream to the distal segment causing alterations in the sodium reabsorption rate.

**Conclusion**

Recent developments have provided substantial evidence demonstrating the important role of NO in the regulation

\*FIG. 3. Summary of changes in fractional sodium excretion (FE\textsubscript{Na}) and urinary nitrate/nitrite excretion (UNOX\textsubscript{V}) in response to change in renal arterial pressure (RAP) during control conditions (○), during infusion of NLA (●), and during concomitant infusion of NLA and SNAP (○). (Data derived from Refs. 29 and 38.)

\*FIG. 4. Changes in cortical and medullary tissue nitric oxide (NO) activity (assessed using nitric oxide electrode) in response to changes in renal arterial pressure (RAP) (○ cortex; ● medulla). (Data derived from Refs. 29 and 32.)
of renal function. Nitric oxide helps to maintain the normally low RVR necessary to ensure adequate perfusion of the kidney. Nearly one-third to one-fourth of total RBF is dependent on an intact NO influence. Nitric oxide regulates both pre- and postglomerular vascular resistances. However, the basic autoregulatory mechanism is not dependent on an intact NO system, although NOS blockade does decrease the absolute RBF. There is also substantial evidence that NO is an important local regulator of tubular reabsorptive function and serves as a major mediator of arterial pressure-induced natriuretic responses in the kidney.

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References


