Renal endothelial and macula densa NOS: integrated response to changes in extracellular fluid volume

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Braam, Branko. Renal endothelial and macula densa NOS: integrated response to changes in extracellular fluid volume. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1551–R1561, 1999.—If, only 20 years ago, anyone had postulated that the absence of nitric oxide gas (NO) would lead to severe hypertension and destruction of the vascular bed of the kidney within weeks, it is not unlikely that smiles of pity would have appeared on the faces of fellow researchers. By now, this has become common knowledge, and hundreds of reports have appeared on the regulation of vascular and renal function by nitric oxide. The amount of information complicates the design of a concept on how NO participates in control of extracellular fluid volume (ECFV) by the kidney. This review analyzes the function of endothelial and macula densa NO synthase (NOS) in the regulation of renal function. From this analysis, endothelial NOS (eNOS)-derived NO is considered a modulator of vascular responses and of renal autoregulation in particular. Increases in renal perfusion pressure and sodium loading will increase eNOS activity, resulting in vasodilatation and depression of tubuloglomerular feedback system responsiveness. Endothelium-derived NO seems important to buffer minute-to-minute variations in perfusion pressure and rapid changes in ANG II activity. In contrast, macula densa NOS is proposed to drive adaptations to long-term changes in distal delivery and is considered a mediator of renin formation. Increases in perfusion pressure and distal delivery will depress the activity and expression of the enzyme that coincides with, and possibly mediates, diminished renin activity. Together, the opposite responses of eNOS and macula densa NOS-derived NO to changes in ECFV lead to an appropriate response to restore sodium balance. The concept that the two enzymes with different localizations in the kidney and in the cell are producing the same product, displaying contrasting responses to the same stimulus but nevertheless exhibiting an integrated response to perturbation of the most important regulated variable by the kidney, i.e., the ECFV, may be applicable to other tissues.

renal function; autoregulation; tubuloglomerular feedback; angiotensin; nitric oxide; nitric oxide synthase; volume regulation

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Is there any danger that our need to progress will go unsatisfied, . . . and that the advance of science will come to an end because science has completed its task? I hardly think so, thanks to the infinity of our ignorance.

K. R. Popper

The discovery of the minuscule molecule nitric oxide (NO) has dramatically changed our view on the regulation of blood flow during the last 10 years. The endothelium is no longer the passive inside of a blood vessel, but is now seen as a highly active, diffuse organ system that importantly determines vascular tone and flow. Application of L-arginine analogs that inhibit formation of NO have been demonstrated to decrease cerebral (35), mesenterial (38), coronary (82), and renal blood flow (7, 23, 39, 40, 42, 43, 59, 80) in animals, as well as forearm (72), coronary (49), and renal blood flow in humans (17). Meanwhile, detailed information on the interaction between vasoactive hormones and NO has become available (53). The focus of this review is on the regulation of renal function by NO and its interaction with the renin-angiotensin system (RAS). The amount of information on the interaction between these sys-
tems is overwhelming, which complicates the design of a uniform framework in terms of regulation of renal function. This review analyzes responses of the endothelium- and macula densa-derived NO related to changes in renal perfusion pressure and distal delivery on the one hand and changes in ANG II activity on the other. From this analysis, the concept is developed that the kidney possesses at least two different functional NO systems, with opposite responses to the same stimulus primarily formed by extracellular fluid volume (ECFV) expansion and increases in renal perfusion pressure and distal delivery. Despite the contrasting responses of endothelial and neuronal NO synthase (NOS) (eNOS and nNOS, respectively), the analysis indicates an integrated response to changes in ECFV, leading to restoration of sodium balance.

**ENDOTHELIAL NOS**

The enzyme eNOS is closely associated with the cell membrane and located in caveolae. Recent insights indicate that enzyme activity is closely and inversely related to the binding of caveolin (18). Among the cofactors for the enzyme are calcium/calmodulin (61) and tetrahydrobiopterin (BH$_4$). The latter supposedly is involved in the ratio of superoxide to NO generated by the enzyme (83). There are at present no reports indicating that renal vascular or glomerular eNOS has other properties than the eNOS in the rest of the vasculature. eNOS has been detected throughout the vasculature, including the glomerulus (3). The main stimuli for eNOS activation are probably shear stress and pressure stretch (14). Scarce information is available on the factors that regulate expression of the enzyme or one of its critical cofactors within the kidney. Shear stress (90), ANG II (26), and transforming growth factor-β (89) have been demonstrated to increase renal eNOS mRNA acutely and eNOS protein levels chronically. However, the exact pathway by which promoter activity is affected by these stimuli is unclear (19).

Endothelium-derived NO and renal perfusion pressure. Like most vascular beds, the preglomerular vasculature of the kidney has the intrinsic property to react to a decrease in perfusion pressure with vasodilation and to an increase in perfusion pressure with vasoconstriction (52, 67). At present, it is still unclear whether this myogenic mechanism is mediated by pressure stretch-activated ion channels or by shear stress-sensitive transmembrane phospholipids and ion channels (16, 44). However, blockade of calcium entry into the cell completely abolishes autoregulation of renal blood flow and glomerular pressure (15, 27, 37, 47). Shear stress- and pressure stretch-mediated NO release from endothelial cells has also been coupled to calcium entry, followed by activation of eNOS (16, 44). Increasing renal perfusion pressure will induce increases in shear stress, because the autoregulatory response of the vasculature reduces vessel wall radius and because imperfectness of autoregulation will lead to blood flow increases. Numerous studies have indicated that the renal vasculature is under continuous and strong influence of NO. It has thus been proposed that, during increases in renal perfusion pressure, NO will be released and oppose the autoregulatory process (Fig. 1) (52).

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**Fig. 1.** Diagram representing dampening effect of endothelial nitric oxide (NO) formation on renal vasoconstriction during increases in renal perfusion pressure. VSM, vascular smooth muscle; NOS, NO synthase.
Several studies have addressed this issue at the microvascular level. Using the isolated perfused hypertrophic kidney preparation, Hayashi et al. (24) studied afferent arteriolar diameter during changes in perfusion pressure. NO inhibition resulted in autoregulatory adjustments in afferent arteriolar diameter that were extended to lower perfusion pressures in rats treated with N-nitro-L-arginine (L-NNA) than in untreated rats. Similar results were obtained by Imig and Roman (28). Juncos et al. (31) reported that, in isolated afferent arterioles, the pressure-induced vasoconstriction was weaker in perfused than in nonperfused afferent arterioles. Both disruption of the endothelium and administration of nitro-L-arginine methyl ester (L-NAME) augmented the pressure-induced vasoconstriction in the perfused but not in the nonperfused afferent arterioles. Both the intrinsic property of the renal vasculature (myogenic response) and the tubuloglomerular feedback (TGF) system participate in autoregulation of renal blood flow (RBF) and glomerular filtration rate (GFR) (9, 13, 67). Systemic (32, 77) or local (11, 12, 30, 75, 76, 88) application of NO synthesis inhibitors consistently results in a strong enhancement of TGF responsiveness, indicating tonic depression of TGF responses by NO under physiological conditions. Attenuation of TGF responses by NO will contribute to the effects of NO to oppose autoregulation of RBF and GFR.

At the whole kidney level, a positive relation between renal perfusion pressure and urinary NO$_3$/NO$_2$ excretion as well as output current from a NO-sensitive electrode inserted in the renal cortex were reported (41). Madrid et al. (39) have investigated autoregulation in the cortex and medulla of volume-expanded Munich-Wistar rats under conditions of fixed levels of norepinephrine, aldosterone, cortisol, and vasopressin. Acute, intravenous administration of L-NAME resulted in significant increases in autoregulatory index in papillary but not cortical blood flow in both innervated and denervated kidneys. Two studies in normotensive rats indicated a decrease in the lower limit of autoregulation; however, the data were not analyzed to that purpose (7, 51). In the study by Ortiz et al. (58), a clear increase in autoregulatory efficacy can be observed during NO synthesis inhibition. Again, no separate analysis on the data was employed to study changes in the autoregulatory index. At whole kidney level, we were unable to prove significant changes in autoregulatory behavior, although the lower limit of autoregulation after NO inhibition in normotensive rats tended to decrease (13a). In the contralateral kidney of 2K1C rats, we were able to show a significant decrease in the lower limit of autoregulation and a significant increase in autoregulatory efficacy (13a). Finally, Majid and Navar (40) observed almost perfect autoregulation in the absence of NO inhibition, and neither the slope nor the lower limit of the autoregulation curve seemed to be modified by NO inhibition. However, fixing NO levels in the kidney with a NO-clamp technique by Majid et al. (42) resulted in super-autoregulation of RBF in dogs.

Changes in renal medullary blood flow and sodium handling have been implicated in the natriuretic response to increases in renal perfusion pressure (22, 63). Majid et al. (43) have reported that pressure natriuresis is diminished significantly by systemic administration of L-NNA in dogs, which did not affect GFR. The authors suggested that this effect on the pressure-natriuresis curve was mediated by alterations in tubular transport and not a result of cascading effects of changes in renal arterial pressure and RBF. Salom et al. (64) have reached similar conclusions in slightly different settings. The natriuretic response of the kidney after acute volume expansion with isotonic saline was strongly inhibited by L-NAME in another two studies (2, 36). In the former study the increase in fractional lithium clearance during L-NAME was lower than in the control kidneys, which suggests interference of NO synthesis inhibition with sodium reabsorption in the proximal part of the nephron (2). In the latter study from these investigators, the increase in renal interstitial hydrostatic pressure (RIHP) was slightly but not significantly less in L-NAME-treated kidneys than in control kidneys, and the authors suggested that modulation of RIHP was not involved in the mediation of natriuresis during sodium loading (64). Altogether, these data indicate that the natriuresis in response to an acute increase in renal perfusion pressure is importantly dependent on the presence of NO. These data illustrate that there may well be a complex synergism between the endothelial NO system in regulating RBF and the medullary NOS systems in mediating pressure natriuresis. The extent to which endothelial NO and the medullary NOS systems participate in pressure natriuresis has not yet been resolved.

Taken together, these data indicate that NO inhibition can affect afferent arteriolar autoregulatory adjustments. At whole kidney level, NO inhibition seems to decrease the lower limit of autoregulation and increase the autoregulatory index. These observations support that NO activity and renal perfusion pressure are positively correlated and that NO opposes the vasoconstriction elicited by autoregulatory adjustments (Fig. 2A).

NO and acute changes in angiotensin activity. Numerous studies have been directed to elucidate the interaction between ANG II and NO. Ito et al. (29) demonstrated in rabbit isolated perfused afferent arterioles that the sensitivity to ANG II was enhanced in the afferent arteriole during blockade of NOS. Conversely, in the juxtamedullary nephron preparation, the vasoconstrictive action of increasing doses of L-NNA was attenuated during ANG II blockade with angiotensin-converting enzyme (ACE) inhibition or angiotensin type 1 (AT$_1$)-receptor blockade (55). In both studies perfusion pressure was controlled at a fixed level. In in vivo micropuncture experiments, combined infusion of ANG II and L-NNA into peritubular capillaries resulted in a dramatic decrease in glomerular pressure, as estimated from stop-flow pressure (12). These studies indicate that, during pharmacological blockade of NO,
the effects of ANG II are enhanced, so that under physiological conditions NO blunts the vasoconstrictor response to ANG II. Conversely, the tonic influence of NO is decreased during acute inhibition of ANG II activity.

Studies in our laboratory focused on the question of whether this balance can also be found for the TGF system. It has been well established that both increasing ANG II levels and decreasing NO levels enhance the responsiveness of the TGF system (11, 12, 29, 75, 88). We compared maximum TGF-mediated decreases in stop-flow pressure under control conditions during peritubular capillary infusion of ANG II and L-NNA alone and during combined infusion of ANG II and L-NNA. Both ANG II and L-NNA increased TGF responsiveness; however, combined infusion of the compounds increased TGF responses further (29). Conversely, local application of L-NNA hardly affected TGF responses under conditions of blockade of the RAS by AT1-receptor blockade (29). Similarly, Kawata et al. (32) reported that the enhancement of TGF responses during systemic L-NAME was attenuated by concomitant systemic infusion of an ANG II receptor antagonist. Thus, not only for the isolated vasculature, but also for the dynamic regulation of glomerular pressure by the TGF system, ANG II and NO seem to be in balance, and increasing ANG II levels will evoke an increase in NO dependency of the TGF system.

The issue of interaction between NO and ANG II has also been extensively studied in whole kidney experiments. In one study in dogs and one in conscious rats, the renal vasoconstrictive actions of ANG II were more pronounced during NOS inhibition (5, 65), which is in line with the above-mentioned studies. In our laboratory, low-dose L-NNA decreased RBF, and we could restore RBF to baseline levels with the AT1 antagonist losartan. However, no effect of losartan was observed after systemic infusion of high doses of L-NNA (80). Similarly, the renal effects of systemic L-NAME infusion were not different from the renal effects of combined L-NAME and losartan or L-NAME and enalaprilat infusion in conscious rats (4). Takenaka et al. (73) showed that blood pressure responses to systemic L-NAME infusion were similar in losartan-treated and untreated rats; however, decreases in RBF were attenuated, and decreases in GFR were absent in losartan-treated rats.

Summarizing, acute variations of ANG II activity are paralleled by changes in NO activity and vice versa (Fig. 2A). From the studies it remains unclear whether ANG II primarily regulates NO activity in the endothelium; however, increases in shear stress and direct stimulation of eNOS by ANG II can form the basis for this relationship. Because this balance is observed during acute changes in local ANG II and NO activity, it is likely that this interaction takes place in the vasculature. It should be emphasized that the relationships between the variables are simplified; in this respect it is not clear that all of the relationships are indeed linear.

Acute responses of the RAS to changes in renal perfusion pressure. It is common knowledge that with increasing renal perfusion pressure, renal renin release decreases. More complicated is whether NO modulates the negative relation between pressure and renin release. Persson et al. (60) have shown, in the conscious dog, that the negative relation between pressure and renin release is attenuated during systemic blockade of NO synthesis (Fig. 2A). This may imply that NO stimulates renin release. Whether eNOS or nNOS mediates these changes is discussed in further detail below.

Summarizing, NO and renal perfusion pressure and ANG II activity are in balance. As is well known, activity of the RAS and renal perfusion pressure are negatively related. Thus one could define the relationships between acute changes in NO, renal perfusion pressure, and ANG II as they are depicted in Fig. 2A. This representation underscores that a certain perfusion pressure dictates ANG II and NO activity and that both the renal autoregulatory vasoconstrictive
response and the vasoconstriction by increased ANG II activity are dampened by NO, probably released by the endothelium. It should be mentioned that the assumed linearity of the relationships, as depicted in Fig. 2, is probably a simplification of the real situation.

MACULA DENS A NOS

The enzyme. In contrast to eNOS, nNOS is present within the cytosol and not closely located to the cell membrane. nNOS is also calcium/calmodulin dependent and BH₄ dependent (20). Many different splice variants have been detected, and the kidney NOS variants have a specific first exon and are generally lacking exon 2 (54). At present it is unknown whether the different splice variants have different function and localization (54). nNOS is expressed in the juxtaglomerular apparatus (JGA) (3, 10, 33, 50, 87), in the renal medulla (45, 62), and in nerve terminals (3). nNOS is abundantly present in the cytosol of macula densa and efferent arteriolar cells but not in afferent arteriolar cells (3). nNOS has also been detected in medullary interstitial cells (1) and in principal cells of the collecting duct (85). By use of RT-PCR, nNOS was demonstrated in microdissected nephron segments, in particular in the glomerulus and the initial and terminal parts of the inner medullary collecting duct (74). Finally, nNOS has been detected in nitrinergic nerve fibers along the interlobular and arcuate arteries, which in some instances have been observed to end in the afferent arteriole (3). Macula densa nNOS expression is regulated in parallel with renin expression under a variety of conditions (10, 68, 71). The medullary and macula densa nNOS do not seem to be regulated by the same stimuli, because increases in sodium intake seem to increase the activity of medullary nNOS (46). Neither the transcription factor for macula densa nNOS nor the transcription factor for medullary nNOS are clear at present. Even less information is available on the influence of nitrinergic nerve fibers on renal function and of stimuli regulating the neuronal activity. It has been suggested in two papers that nitrinergic nerve fibers exert a presynaptic inhibition on perivascular sympathetic vasoconstrictor nerves innervating the renal artery (56, 84). The extent of nitrinergic control of renal function is far from clear.

Macula densa NO and distal delivery. The responses of the RAS with respect to changes in macula densa NO activity are far from clear. At the translational level, Bosse et al. (10) demonstrated that, in rats treated with furosemide for 5 days and in rats placed on a low-sodium diet, both macula densa nNOS and renin expression increased and nNOS protein increased. Conversely, in rats treated with L-NAME, nNOS and renin mRNA levels and nNOS protein levels were reduced (10). Similarly, Schricker et al. (68) demonstrated upregulation of macula densa nNOS in rats treated with furosemide. No significant changes in eNOS or inducible NOS (iNOS) activity were observed in this study. The authors of the latter study also emphasized that the ratio of nNOS to eNOS mRNA approximated 1:50 and considered it likely that, given a similar translational efficacy, the amount of eNOS protein largely exceeds the amount of nNOS (68). In renal cortical slices of salt-restricted, normal, and salt-loaded rats, nNOS, renin, and angiotensinogen mRNA expression were found to be negatively related to the level of sodium intake (71). In AT₁a receptor-deficient mice, both nNOS and angiotensinogen mRNA have been shown to be upregulated in renal cortical slices, and high sodium intake resulted in a decrease in nNOS and angiotensinogen expression (34). Thus macula densa nNOS expression parallels renin gene expression during variations in distal delivery, and even in the absence of AT₁ receptors, nNOS is sensitive to changes in sodium intake. This is in line with the hypothesis that NO derived from the macula densa stimulates renin formation.

At the functional level, the increase in renin release during an acute decrease in perfusion pressure is inhibited by nonselective NOS inhibition, as mentioned above (60). Administration of 7-nitroindazole (7-N1), which is a relatively selective inhibitor of nNOS, fully inhibited the increase in renin secretion rate induced by furosemide treatment in vivo experiments; however, it failed to affect renin secretion during an acute decrease in perfusion pressure (6). In an attempt to distinguish the effect of NO delivered from the vasculature to the macula densa from the effect of NO formed within the macula densa by changes in macula densa sodium reabsorption, He et al. (25) performed experiments in isolated perfused rabbit JGA. Here, application of l-NNA to the tubular lumen significantly reduced the increase in renin secretion rate as a result of a reduction in luminal sodium concentration. Remarkably, administration of the NO donor sodium nitroprusside (SNP) to the bath also reduced the increase in renin secretion in response to a decrease in luminal sodium level (25).

From these observations, it can be derived that ANG II, macula densa NO, and distal delivery are in such a balance that increases in distal delivery will result in a simultaneous decrease in macula densa NO and ANG II activity by inhibition of renin release and formation. Increases in distal delivery will diminish macula densa nNOS activity, whereas sustained increases in distal delivery will decrease macula densa nNOS expression. This is depicted in Fig. 2B. In a recent review, the exact pathways that are involved in the regulation of renin by NO are extensively discussed (66). Inhibition of renin release is likely to be mediated by increases in guanosine 3',5'-cyclic monophosphate after activation of guanylate cyclase by NO. Stimulation of renin release by NO could occur 1) through the formation of an intermediate factor, such as prostaglandins; 2) through reduction of intracellular calcium; or 3) through the protein kinase A pathway (66). One option not mentioned in that review is that NO generated by the endothelium inhibits macula densa NOS directly. This mechanism, called NO-autoinhibition, has been implicated in the decrease in GFR after lipopolysaccharide. In this situation, endothelium-dependent relaxation was decreased, which may result from inhibition of eNOS by NO.
produced by iNOS (69). Although an attractive explanation, it is as yet hard to prove that NO produced by eNOS actually is capable of reaching macula densa NOS. As depicted in Fig. 3, top, an increase in perfusion pressure will evoke a decrease in renin release as a result of activation of eNOS and also as a result of a concomitant increase in distal delivery and reduction of macula densa NOS. The complex pathways that have been mentioned to regulate renin release are depicted in Fig. 3, bottom.

Complicated issue of TGF and nNOS. Several studies have demonstrated that nonselective NOS inhibition increases TGF responsiveness (11, 12, 75, 77, 81, 86, 88). In fact, the enhancement of TGF-mediated decreases in glomerular pressure on increases in distal delivery is of such a magnitude that NO can be considered an important modulator of TGF function. Relevant in the current context is whether macula densa nNOS is responsible for the NO production that alters TGF responsiveness. Studies in our laboratory have indicated that NO is released upon acute increases in macula densa delivery. NOS was inhibited by systemic infusion of L-NNA, and an intrarenal infusion of SNP was used to fix NO at that level, where renal vascular resistance and other renal function parameters returned to baseline. Under these conditions of fixed NO levels, TGF responses were significantly enhanced. This study indicated that NO forms a dynamic and integral part of the feedback loop (81). However, uncertainty remained after this study as to whether NO was formed at the afferent arteriolar endothelium by increased shear stress caused by TGF-mediated vasoconstriction of the afferent arteriole or in the macula densa as a direct result of increased macula densa transport.

Studies by others have addressed whether the source of the NO-dampening TGF responses is macula densa NOS by use of 7-NI. This compound has been used as a selective nNOS inhibitor. It should be mentioned at this point that 7-NI has been shown to be able to bind eNOS also, although with a lower affinity than nNOS (8). Because it has been assessed that the amount of eNOS mRNA in the kidney largely exceeds the amount of nNOS mRNA, it is possible that, despite the selectivity of 7-NI, eNOS is also inhibited (68). In our laboratory, we observed significant decreases in RBF during 7-NI administration (unpublished data). Systemic application and intratubular 7-NI infusion have been shown to increase TGF responses (57, 76), which may indicate that macula densa nNOS is involved in TGF modulation. Wilcox and Welch (86) demonstrated that, in rats fed a low-sodium diet, TGF responses are slightly increased compared with rats on a high-sodium diet; however, the latter are more sensitive to NO inhibition. Obviously this is in contrast with the findings that macula densa nNOS mRNA is increased in rats maintained on a low-sodium diet, as mentioned above, which would attenuate TGF responses if macula densa NOS were the source of the NO modulating the TGF response (71). Thus although the data are not conclusive because of the properties of 7-NI, they suggest that blockade of macula densa NOS will enhance TGF responses.

Probably the NO dependency will depend on total NO production in the proximity of the afferent arteriole by both nNOS and eNOS rather than on the contribution of the individual sources. From the studies mentioned...
above, it seems likely that NO produced by eNOS will affect TGF responses during acute increases in shear stress and during acute increases in distal delivery by TGF-induced vasoconstriction of the afferent arteriole. Endothelial NO will exert a dualistic effect on the TGF system during changes in renal perfusion pressure, because low perfusion pressures increase ANG II production, which enhances endothelial NO production. Macula densa NO production will increase during sustained decreases in distal delivery and modulate TGF under these conditions. Taken together, production of NO by both nNOS and eNOS will counteract the effects of high ANG II under conditions of sustained low perfusion pressure and distal delivery, whereas acute production of NO by eNOS will account for NO dependency under conditions of high perfusion pressure (Fig. 4). We were unable to demonstrate alterations in NO dependency of the TGF system during changes in sodium intake (unpublished data), whereas others showed enhanced NO dependency of the TGF system during sodium loading (86). This may well be explained by this complex balance between distal delivery, perfusion pressure, and NO production by eNOS and nNOS and ANG II activity. Experiments to further clarify this issue and test the present hypothesis will require extremely specific nNOS inhibitors.

HYPOTHESIS

Opposite responses of eNOS and macula densa nNOS to increases in renal perfusion pressure. From this analysis, it becomes clear that the two different NOS enzymes and their different localizations drive different responses to changes in ECFV and perfusion pressure. The eNOS is a fast-reacting system, which is stimulated by shear stress and/or pressure stretch and the increased levels of ANG II. By its nature, the system is involved in adaptations to acute changes in perfusion pressure. The endothelial NO system reflects an adaptive mechanism, dampening perturbations in perfusion pressure and ANG II. The macula densa NO system is suppressed in response to increases in distal delivery and is regulated in parallel with renin release. It probably mediates renin release and participates in long-term adaptations to changes in sodium intake. The macula densa NO system thus functions as a mediator system for changes in renin formation and release. The main characteristics of the systems are summarized in Table 1.

Table 1. NO systems in the kidney

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<th>NO Systems</th>
<th>Characteristics</th>
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<tr>
<td>Endothelial NO</td>
<td>Participates in short-term regulation of renal blood flow</td>
</tr>
<tr>
<td>Macula densa NO</td>
<td>Mediated by eNOS, reacts to acute changes in ANG II actions, participates in short-term regulation of renal blood flow</td>
</tr>
<tr>
<td></td>
<td>Reacts to long-term changes in distal delivery</td>
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<td>May well regulate renin formation and secretion</td>
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In an attempt to integrate the functions of these systems with the ECFV control by the kidney, a scheme was constructed (Fig. 5). In this scheme an increase in sodium intake will be accompanied by a modest increase in ECFV and systemic arterial pressure. The vascular NO system will be activated by modest increases in shear stress and pressure stretch, leading to an increase in RBF. Concomitantly, NO formed by the vasculature may depress TGF responsiveness, which will contribute to maintain a high distal delivery without concomitant depression of single nephron glomerular filtration rate. The increase in perfusion pressure and the vasodilation will lead to a sustained increase in distal delivery that will deactivate the macula densa NO system. As a consequence renin release will decrease, leading to diminished ANG II formation. Diminished ANG II will contribute to increased sodium excretion by causing vasodilation, decreasing proximal tubular reabsorption and attenuation of TGF responsiveness. Both NO systems will be restored to normal activity at the moment that perfusion pressure is normalized.

Not included in the hypothesis are the medullary NOS enzymes. From experiments using the microdialysis technique, it has become clear that the renal medulla has a significant capacity to produce NO (91). All NOS enzymes have been detected in medullary structures (3, 48, 74). In particular, eNOS has been detected in the endothelium of medullary vasculature (3), nNOS and iNOS in several tubule segments, and iNOS in the medullary vasculature (48, 74). NO is supposedly involved in the regulation of medullary blood flow and in sodium handling. Reports in the literature indicate tubular effects of medullary iNOS and nNOS, vascular effects of medullary nNOS and eNOS, and enhanced medullary NO activity of nNOS, iNOS, and eNOS during sodium loading. Nevertheless, increases in perfusion pressure and ECFV activate the medullary NOS enzymes, and their concerted action...
leads to vasodilation and sodium excretion. The extent to which each of the enzymes is involved in regulation of medullary reabsorption and hemodynamics, however, is not clear.

It should be emphasized that the two systems can interact at several places. On activation of the endothelial NO system, renin release can be suppressed by both the relative vasodilation and by the increase in distal delivery. Also, as has been mentioned, it is conceivable that endothelial NO directly interferes with macula densa NOS activity. On the other hand, the macula densa NO system may act as a negative feedback system on eNOS. As ANG II levels decrease, the TGF system will be attenuated, and the vasoconstriction and accompanying shear stress by afferent arteriolar constriction by the TGF mediator will decrease. Obviously, the fact that there are no specific eNOS inhibitors and only partially selective nNOS blockers hinders experiments to resolve interactions between the two systems.

Application of the model. The hypothesis of different renal NO systems can be used to explain the events in the contralateral kidney of two-kidney, one-chip (2K1C) hypertensive rats. The model predicts that in this kidney, endothelial NO activity will be enhanced as a result of an increase in renal perfusion pressure and elevated circulating ANG II levels (21). Sigmon and Beierwaltes (70) have demonstrated that the decrease in RBF after NO synthesis inhibition is positively related with the degree of stenosis in the ipsilateral kidney. In our laboratory, we examined the lower limit of autoregulation and the autoregulatory index in kidneys of normotensive rats and in the contralateral kidney of 2K1C hypertensive rats. 2K1C rats exhibited decreased autoregulatory efficacy compared with controls, which was significantly improved by NO inhibition with L-NNA, and the lower limit significantly decreased after L-NNA (unpublished observations). Furthermore, in the contralateral kidney of the 2K1C Goldblatt hypertensive rat, intraluminal L-NNA infusion resulted in a larger increase in TGF responses compared with sham-operated rats, indicating that, in this state with increased circulating ANG II levels, the NO dependency of TGF responses was also enhanced (78).

The model would predict that in response to a sustained increase in distal delivery macula densa nNOS is decreased. Indeed, two studies evaluated the expression of macula densa nNOS in contralateral kidneys of 2K1C rats. In both studies nNOS mRNA was decreased in parallel with renin mRNA (10, 68). The observation that the influence of NO on TGF responses is more pronounced in the 2K1C contralateral kidneys may indicate that, under these conditions, NO generated by the endothelium is responsible for the modulation of TGF responses.
CONCLUSION

This paper presents a hypothesis on the function of the endothelial and macula densa NO systems in the kidney. The systems are driven by different enzymes, with different localization in the cell and responding in an opposite fashion to changes in ECFV. The endothelial NO system modulates the renal vasoconstrictive response to increases in renal perfusion pressure, mitigates the actions of ANG II, and attenuates TGF responsiveness. The macula densa NO system likely mediates the decrease in renal secretion during long-term increases in renal perfusion pressure and distal delivery. Despite the differences between the systems, both participate in the restoration of volume balance in response to a change in ECFV.

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