Nitric Oxide Synthesis in the Adult and Developing Kidney

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Nitric oxide (NO) is synthesized within the adult and developing kidney and plays a critical role in the regulation of renal hemodynamics and tubule function. In the adult kidney, the regulation of NO synthesis is very cell type specific and subject to distinct control mechanisms of NO synthase (NOS) isoforms. Endothelial NOS (eNOS) is expressed in the endothelial cells of glomeruli, peritubular capillaries, and vascular bundles. Neuronal NOS (nNOS) is expressed in the tubular epithelial cells of the macula densa and inner medullary collecting duct. Furthermore, in the immature kidney, the expression of eNOS and nNOS shows unique patterns distinct from that is observed in the adult. This review will summarize the localization and presumable function of NOS isoforms in the adult and developing kidney.

Key Words : Kidney, Development, Renal hemodynamics, Nitric oxide

Expression of NOS isoforms in the adult kidney

Nitric oxide (NO) is a lipophilic gas with unique physiological properties and plays an important role in maintaining basal renal vascular tone and tubule function in the kidney[1-4]. NO is synthesized from the amino acid L-arginine by the action of an NO synthase (NOS). NOS exists in three isoforms of which two forms, nNOS (NOS I) and eNOS (NOS III), are consecutive and the other, iNOS (NOS II), is an inducible form kidney[5]. These three major NOS isoforms are expressed in various locations in the kidney and subject to distinct control mechanisms.

In the adult kidney, eNOS is expressed in the endothelial cells of almost all blood vessels except the venous system[6, 7]. The eNOS immunoreactivity is observed in the endothelial cells of glomeruli and peritubular capillaries in the cortex, and in the endothelial cells of vascular bundles in the medulla. Notably, the staining intensity is much stronger in the endothelial cells of the renal medulla than in those of the renal cortex (Fig. 1). Considerable eNOS immunostaining is also detected in the endothelium of arcuate and interlobular arteries. However, vascular smooth muscle cells as well as the tubular epithelial cells including proximal tubule cells and macula densa cells do not show eNOS immunoreactivity in the adult kidney[7].

There is general agreement that the macula densa is the principal site of nNOS expression in the kidney[6, 8]. Strong presence of nNOS in macula densa is found in the kidney of rat, mouse, guinea pig, and rabbit, whereas in humans and pig the signal is weaker[6]. Although high levels of nNOS mRNA are found in the

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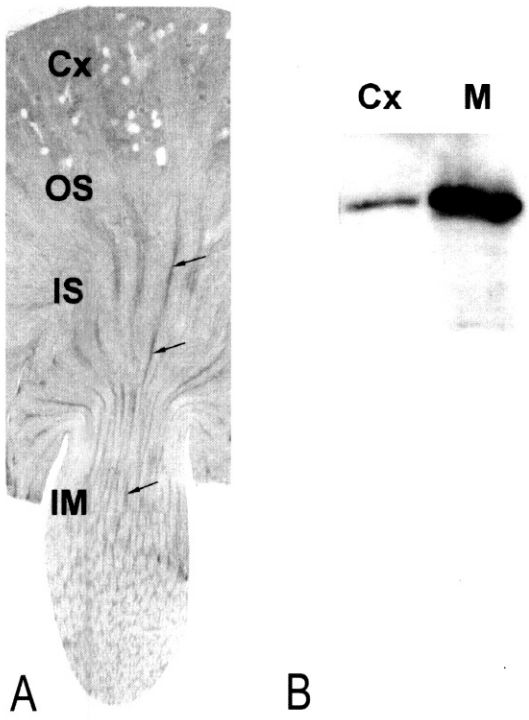

Fig. 1. Strong eNOS immunoreactivity is present in the vascular bundles (arrows) in the inner stripe of outer medulla (IS) and inner medulla (IM) (A). Immunoblot analysis demonstrated that medullary eNOS is proportionately greater than cortical eNOS in the adult kidney (B). Cx, cortex; OS, outer stripe of outer medulla. Magnifications: ×15.

inner medullary collecting duct, previous histochemical methods have been unable to detect nNOS protein in this segment. Within freshly microdissected inner medullary collecting ducts, Roczniak et al. demonstrated that cells stained strongly for nNOS with a cytoplasmic pattern[9]. The antibodies used in these studies would be expected to recognize all nNOS variants. Expression of nNOS mRNA has also been detected by RT-PCR in microdissected outer medullary collecting duct and in the thin limb of the loop of Henle[10]. Indeed, the nNOS immunoreactivity is detected in descending thin limb and inner medullary collecting duct as well as in macula densa with preembedding vibratome technique (Unpublished observations of our studies).

Expression of iNOS may be distributed quite widely in tubular epithelium. Ahn et al. observed iNOS mRNA signal in the S3 segment of the proximal tubule, the cortical and medullary thick ascending limb, the distal convoluted tubule, and the cortical collecting duct and inner medullary collecting duct in the normal rat kidney[11]. Expression of iNOS protein is identified in the proximal tubule, the thick ascending limb, and intercalated cells of the collecting duct in histochemical studies of normal rats[12]. However, there is still controversy whether iNOS protein is expressed in the specific renal cell types and plays a homeostatic role under normal conditions.

NO and renal functions in the adult kidney

It is well established that endogenous release of NO plays an important role in the regulation of renal blood flow ankidney[2, 13, 16]. d glomerular filtration rate (GFR) in the adult Experimental evidence indicated that specific inhibition of NO synthesis with competitive L-arginine analogues including *N*-nitro-L-arginine methylester (L-NAME), *N*-nitro-L-arginine (L-NNA), and *N*-monomethyl-L-arginine (L-NMMA) reduces regional renal blood flow and markedly increases the renal vascular resistance (RVR), suggesting that NO maintains the low vascular tone that is characteristic for the kidney. In response to systemic infusion of competitive L-arginine analogues, there is an increase of 30% to 50% in RVR and a decrease of 25% to 40% in renal blood flow in both conscious and anesthetized animals[15, 17, 18]. Studies in rats have suggested that NO may exert a greater influence on the medullary blood flow than on the cortical blood flow in the adult kidney[19]. However, the effect of NOS inhibitors on the GFR seems to be less consistent. Several experiments showed that GFR is well maintained with treatment of NOS inhibitors[17, 20], while other studies reported significant reductions in GFR[13, 15]. The reductions in GFR in response to NOS inhibition may depend on the dose as well as the routes of administration of NOS inhibitors used.

The renal hemodynamic responses to inhibition of NO synthesis are modulated by intrarenal interactions between the renin angiotensin system and NO[20].

Long-term inhibition of endogenous NO production produces an angiotensin II-dependent form of hypertension and angiotensin II directly interacts with NO at the arteriolar level[21, 22]. In the rabbit kidney, L-NAME decreases basal diameter of the glomerular arteriole and augments angiotensin II action on the afferent arteriole[21]. Although it is not clear whether NO stimulates or inhibits renin release in the kidney, inhibition of NO synthesis with L-NAME may increase plasma renin activity, at least, under certain conditions[23]. All these results suggest that renin angiotensin system modulate the renal hemodynamics responses to NO synthesis inhibition by either increasing RVR through the elevated action of intrarenal angiotensin II and/or modulating renin release.

In addition to regulating vascular tone, NO is suggested to affect tubule function in the kidney[8, 14, 20, 24, 25]. There is now substantial evidence that not only the endothelial cells but also the epithelial cells in the kidney can generate NO. Macula densa cells show the most prominent levels of the nNOS expression among the epithelial locations in the kidney[6]. A number of in vivo studies have reported that NO may function as an important mediator or modulator of the tubuloglomerular feedback (TGF) response and renin secretion in the macula densa cells. Addition of NOS inhibitor to the macula densa perfusate led to afferent arteriolar constriction only when the tubules were perfused with isotonic Ringer's solution but not when perfused with a hypotonic solution[8]. Inhibition of NOS enhanced the TGF response in high salt intake rats but not in low salt animals[25]. NO also regulates tubular water and electrolytes excretion in the kidney. Inhibition of NOS reduces sodium and potassium excretion as well as urine flow, whereas administration of a NO donor compound increases the response of urine flow and sodium excretion to NOS inhibitors. These reverse effects are not consistently associated with significant reductions in GFR, indication that NO may directly affect tubular epithelia transport[20].

Expression of NOS isoforms in the developing kidney

During development, the consecutive isoforms of NOS are expressed in immature renal blood vessels and tubules, and show unique patterns distinct from that are observed in the adult[7, 26, 30]. Expression of eNOS is first observed in the endothelial cells of the undifferentiated intrarenal capillary network at embryonic day 14. In the fetuses and newborn, the intensity of eNOS immunostaining is much stronger in the developing renal vascular system in the renal cortex than in the medulla (Fig. 2). At embryonic day 16, expression of eNOS is strong in the endothelium of capillaries surrounding vesicles (stage I nephron) and

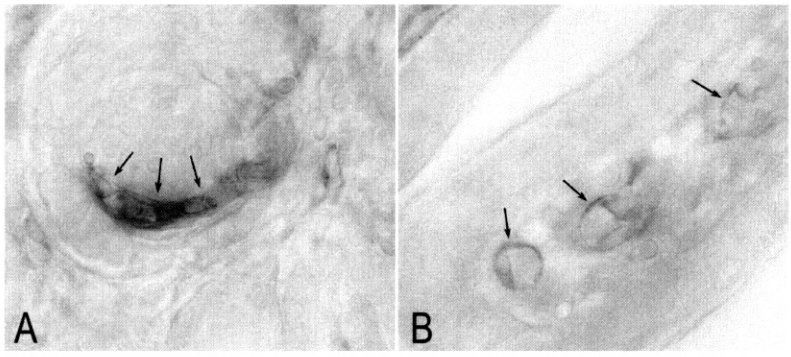

Fig. 2. *Light micrographs of 50 μm thick vibratome sections from a 3-day-old rat kidney illustrating immunostaining for eNOS. Strong eNOS immunoreactivity is observed in the endothelial cells (arrows) of the immature glomerulus (A). The endothelial cells (arrows) in the medulla show less intense labeling than in the cortex (B). Magnifications : ×480.*

in the cleft of S-shaped bodies (stage II nephron), but is less intense in more mature glomeruli of stage III nephrons at embryonic day 18 and 20. As the kidney matures after birth, the strong eNOS immunostaining gradually decreases in the cortex. In contrast, in the renal medulla, the expression of eNOS gradually increases after birth in the endothelial cells of the vascular bundles and capillaries, and becomes highest in the adult kidney[7, 29]. Strikingly, there is also strong labeling of eNOS in the proximal tubules at postnatal day 1. Electron microscopy studies revealed that the eNOS immunostaining is located primarily in vacuoles and lysosome-like structures in the proximal tubules indicating that it is reabsorbed from the glomerular filtrate. The immunoreactivity in the proximal tubules is not observed at other ages during development[7].

The immunohistochemical distribution and intensity of nNOS in the immature kidney also differ from the adult. In the developing pig, nNOS localizes in portions of the thick ascending limb leading to the macula densa in a pattern not seen in the adult[27]. Fisher et al. demonstrated that nNOS positive cells are present in the developing distal tubule from the earliest stages of nephrongenesis, the S shaped body, and appear to be involved with the developmental organization of the macula densa in rats 2, 6, and 15 days of age[16]. These observations are consistent with the results of our studies in rat embryos and newborns (Unpublished data). Expression of nNOS first appears in the distal tubule anlage at embryonic day 16, and in all epithelial cells of immature thick ascending limbs as well as macula densa at embryonic day 17 and 18. After birth, nNOS expression is detected in the newly formed cortical thick ascending limb located in the medullary ray, whereas it gradually decreases in the medullary thick ascending limb of mature juxtamedullary nephrons and becomes restricted to the macula densa. In the inner medullary collecting duct, nNOS immunoreactivity appears first, especially in the papilla region, at postnatal day 7 and gradually ascends to the border between the outer and inner medulla. In the descending thin limb, nNOS is expressed first at postnatal day 14 and gradually increases to adult levels (Unpublished observations of our studies).

The intrarenal localization of iNOS has not been established in the developing kidney.

NO and renal functions in the developing kidney

Although the role of NO in the regulation of renal function has been well established in the adult, recent studies point to perhaps an even more critical role in the developing kidney[27, 31-35]. During development, the immature kidney shows low renal blood flow and high RVR[36, 37]. This hemodynamic condition contributes to the newborn's low GFR, the altered tubular sodium handling of the newborn, and significant clinical complications including acute renal failure. Highly activated vasoconstrictor mechanisms such as the renin angiotensin system are believed to maintain the high RVR during renal maturation, and recent studies have demonstrated critical hemodynamic interactions between NO and the renin angiotensin system in the developing kidney[38-40]. Studies by Sener and Smith measured renal blood flow and GFR in the developing sheep kidney and showed age-dependent NO effects on renal hemodynamics, which provided evidence that the physiological effects of NO produced by the constitutive isoforms of NOS are developmentally regulated[41, 42]. Inhibition of NOS produced greater changes in renal hemodynamics in studies of piglets. Intrarenal infusion of L-NAME, at a dose that does not alter systemic pressure, produced significant decreases of 28% to 45% in renal blood flow and increases of 47% to 128% in RVR in the developing pig than in the adult[27, 30]. Preinfusion with a specific angiotensin II AT1 receptor antagonist, A-89291 (ATX), significantly attenuates the hemodynamic responses to intrarenal L-NAME in the developing kidney. However, the developing animal's responses of renal blood flow and RVR to L-NAME are still greater than the adult changes following ATX treatment, indicating other vasoconstric-

tors must be participating in the immature renal response to inhibition of NOS synthesis[43]. Functioning as a critical vasodilator, NO might counterbalance the activated vasoconstrictors in the fetal and postnatal maturing kidney.

This greater functional role for NO in the developing kidney may be due to the developmental differences of NOS isoforms expression. Whereas nNOS may participate in renal function throughout the period of renal development, the most important impact of eNOS may occur in a narrower postnatal window, in the first days of the immature kidney[7, 28, 29]. The highest expression of nNOS occurs immediately at birth in the pig, especially in the renal medulla, and progressively declines with age to the lowest levels in the adult[28, 29]. At birth, eNOS shows critical expression in the newborn pig kidney but rapidly decreases after birth reaching its lowest point at 7 days of age and returning by 14 days to adult levels[7, 29]. Moreover, cortical eNOS is proportionately greater than medullary eNOS in the immature kidney, whereas medullary eNOS is greater than cortical in the adult[7, 29]. Fisher et al. investigated the age-dependent intrarenal distributions of both nNOS and renin signals in the rat[16]. Although the intrarenal distribution of nNOS and renin signals showed a parallelism, the intensity of nNOS and renin expression along the developmental time axis was not parallel. The abundance of nNOS reached its maximum on postnatal day 6, whereas renin expression was highest on day 2[16, 28, 40]. Interestingly, eNOS is localized in proximal tubules at postnatal day 1 during renal development of the rat[7]. Although the physiological role is not clear, the transient pattern of eNOS expression in renal tubular segments may be associated with a critical expression of renin in the newborn kidney. Taken together, these findings suggest that eNOS may be more critical participant, than nNOS, in regulating renal cortical hemodynamics and GFR during early renal maturation.

There is evidence that the developing animal is associated with altered sodium homeostasis when compared with that in adults. In response to an acute saline load, the piglets excreted significantly less sodium than the adults without significant changes in GFR[44]. However, little is known about the regulation of NO in tubule transport processes in the immature kidney.

Conclusion

Recent studies have provided substantial evidence demonstrating the important role of NO in the regulation of renal hemodynamics and tubule function. As a vasodilator, NO helps to maintain the renal blood flow and GFR. NO also regulates tubular reabsorptive function and serves as a major mediator of natriuretic responses in the adult kidney. On the other hand, the developing kidney demonstrates a greater role of NO in the regulation of renal blood flow, RVR, and GFR than the adult during renal maturation. More detailed microanatomic studies will expend our understanding of the interplay among these renal NOS isoforms and the unique control modes in the developing kidney as well as in the adult kidney.

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