Nitric Oxide Modulation of Neurally Induced Proximal Tubular Fluid Reabsorption in the Rat

Xiao Chun Wu, Edward J. Johns

Abstract—This study investigated the role of NO in mediating the renal sympathetic nerve-mediated increases in proximal tubular fluid reabsorption (Jva). In inactin-anesthetized Wistar rats, renal sympathetic nerve stimulation (15 V, 2 ms) at 0.75 and 1.0 Hz did not change blood pressure or glomerular filtration rate but did decrease urine flow and sodium excretion in a frequency-related fashion by 40% to 50% at 1.0 Hz (both, \( P<0.01 \)). Renal nerve stimulation in control animals increased Jva by 11% at 0.75 Hz (\( P<0.05 \)) and 31% at 1.0 Hz (\( P<0.01 \)). Intraluminal \( \text{NO}_2^- \)-nitro-L-arginine methyl ester (L-NAME) resulted in a higher basal Jva (19%, \( P<0.05 \)), and renal nerve stimulation had no effect on Jva. When L-NAME plus sodium nitroprusside was present intraluminally, however, there were frequency-dependent increases in Jva that were similar in pattern and magnitude to the control rats. Introduction of the relatively selective nNOS blocker 7-nitroindazole intraluminally, at \( 10^{-6} \) and \( 10^{-4} \) M, raised basal Jva by 18% and 24%, respectively (\( P<0.01 \)), and renal nerve stimulation did not change Jva. Intraluminal aminoguanidine (\( 10^{-4} \) M), a relatively selective iNOS blocker, did not affect basal Jva, which remained unchanged during renal nerve stimulation. These data are consistent with NO exerting a tonic inhibitory action on the basal levels of Jva, which, in part, is caused by NO generated by the nNOS isoform. Moreover, the findings have revealed that the presence of NO is necessary to ensure that renal nerves can stimulate fluid reabsorption by the proximal tubules, requiring NO generated from both nNOS and iNOS.

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Key Words: nitric oxide ■ renal nerves ■ antinatriuresis ■ sodium ■ kidney

The renal sympathetic nerves play an important role in regulating renin release, tubular sodium and water reabsorption, and renal vascular resistance. Increasing levels of renal nerve activity progressively recruit these functions; that is, at low frequencies there is renin release. Thereafter, increased tubular reabsorption of fluid becomes apparent, and it is only at the higher frequencies that there are reductions in renal blood flow and glomerular filtration rate.1,2 Noradrenaline is released from the renal sympathetic nerve endings and, as at other sympathetic neuroeffector junctions, has an autoinhibitory feedback action, mediated via presynaptic \( \alpha_2 \)-adrenoceptors, to attenuate the level of neurotransmitter release.3 At the postsynaptic level, noradrenaline acts on the \( \alpha_2 \)-adrenoceptors of the epithelial cells to stimulate sodium, and hence water, transport by activation of the \( \text{Na}^+/-\text{K}^+\)-ATPase at the basolateral membrane and the \( \text{Na}^+/-\text{H}^+\)-exchanger at the apical membrane.4,5

Recently, there has been increasing interest in the role of NO in the control of renal function. NO synthase (NOS) catalyzes the generation of NO, which stimulates cyclic GMP production to modify specific aspects of renal function.6 At least 3 isoforms of NOS have been identified so far: inducible NOS (iNOS), endothelial NOS (eNOS), and neuronal NOS (nNOS), all of which are present within the kidney to varying degrees. Thus, with regard to the cortex, the endothelial cells of peritubular capillaries, macula densa, and proximal tubular cells all demonstrate NOS mRNA and NO immunoreactivity in perivascular nerve fibers, but the functional role of NO on proximal tubular function is unclear. Endothelial cell-derived NO or infusion of cGMP into perfused proximal tubules has been reported to stimulate the \( \text{Na}^+/-\text{H}^+\)-exchanger,7 which would lead to increased fluid reabsorption. Moreover, micropuncture studies have shown \( \text{N}^5\)-monomethyl-L-arginine (L-NMMA), a nonselective NOS blocker, to cause a reduction in proximal fluid reabsorption in control and sham-operated rats, but this effect was reportedly abolished by renal denervation.8 These observations would indicate that the action of NO required the participation of the renal sympathetic nerves. In contrast, it has been shown previously that intratubular administration of sodium nitroprusside (SNP) depressed basal proximal tubular fluid reabsorption, whereas it was increased by \( \text{NO}_2^- \)-nitro-L-arginine methyl ester (L-NAME) in anesthetized Sprague Dawley and Wistar rats.9,10 Together, these observations imply that the final action of NO may be variable because of its action on different components of the reabsorptive processes, ie, either on the \( \text{Na}^+/-\text{K}^+\)-ATPase or the \( \text{Na}^+/-\text{H}^+\)-exchanger at basal levels, or when these exchangers are stimulated. Our earlier report also demonstrated that the action of NO on proximal tubular fluid reabsorption (Jva) was renal sympathetic nerve-
dependent and also that the active enzyme was likely to be nNOS because administration of the relatively selective nNOS inhibitor, 7-nitroindazole (7-NI), also increased basal Jva. However, the location of this nNOS was uncertain, although the nitrenergic nerve fibers found in the kidney may be one of the important anatomical locations.11

The aim of this study was to examine the potential influence of NO on the increased proximal tubular fluid reabsorption resulting from low level renal nerve stimulation and to elucidate which isoforms of NOS might be involved. This was performed by measuring the changes in proximal tubular fluid reabsorption in response to renal nerve stimulation following blockade of NOS using a selective (L-NAME) and selective (7-NI and aminoguanidine) NOS inhibitors, as well as an NO donor (SNP) alone and together with L-NAME.

Methods

Experiments were performed under the UK project license PPL 40/1367 and the personal investigator licenses PL 40/00371 and 40/03881 to E.J. Johns and X.C. Wu. Male Wistar rats (240±30 g) were fasted for 12 h before use and given free access to water. The inanesthetized (100 mg/kg IP) animals were maintained at 36°C to 36.5°C. Cannulae were placed in the right femoral vein for infusion of saline (150 mmol/L NaCl) and inulin, in the femoral artery for monitoring blood pressure, and in the bladder for urine drainage. The left kidney was exposed and placed in a kidney cup, and its ureter was cannulated.10 Thereafter, a 2-mL bolus of inulin (15% 4 M), (4) 7-NI (10 6 M), (5) APTF plus aminoguanidine (10 6 M), (6) APTF plus L-NAME plus SNP caused only a small reduction in basal levels of proximal tubular fluid reabsorption, which were the same as those obtained when APTF was present in the tubules.

Stimulation of the renal sympathetic nerves when APTF was present in the tubules increased proximal tubular fluid reabsorption by 11% at 0.75 Hz (P<0.05) and 31% at 1 Hz (P<0.01), compared with basal values. Sodium excretion was also reduced during renal nerve stimulation, by 29% at 0.75 Hz (P<0.05) and 49% at 1 Hz (P<0.01), compared with basal levels.

Administration of L-NAME intraluminally (Figure 1) increased basal Jva by 19% compared with that measured when APTF was given alone (3.27±0.20×10 4 versus 2.67±0.10×10 4 mmol·min·mm 2·s 1, P<0.05), whereas coadministration of L-NAME plus SNP caused only a small reduction in basal levels of proximal tubular fluid reabsorption (Figure 1), 7-NI given into the tubules at 10 4 and 10 6 (Figure 2) increased basal Jva by 18% and 27%, respectively (P<0.01 and P<0.001), from 2.52±0.10×10 4 to 2.99±0.12×10 4 mmol·min·mm 2·s 1 and from 2.4±0.07×10 4 to 3.05±0.08×10 4 mmol·min·mm 2·s 1, respectively. By contrast, intraluminal aminoguanidine (Figure 2) had no effect on basal levels of proximal tubular fluid reabsorption, which were the same as those obtained when APTF was present in the tubules.

Results

Electrical stimulation of the renal sympathetic nerves at both 0.75 and 1.0 Hz had no effect on blood pressure (98±1 mm Hg) or glomerular filtration rate, which remained stable over the experimental periods (Table). Urine flow was reduced progressively when the nerves were stimulated, by 28% at 0.75 Hz (P<0.05) and 39% at 1 Hz (P<0.01), compared with basal values. Sodium excretion was also reduced during renal nerve stimulation, by 29% at 0.75 Hz (P<0.05) and 49% at 1 Hz (P<0.01), compared with basal levels.

Blood Pressure and Whole Kidney Glomerular Filtration Rate, Urine Flow, and Sodium Excretion During Renal Nerve Stimulation in Wistar Rats

<table>
<thead>
<tr>
<th>Stimulation Frequency</th>
<th>Blood Pressure (mm Hg)</th>
<th>Glomerular Filtration Rate (µL·min⁻¹·g⁻¹)</th>
<th>Urine Flow (µL·min⁻¹·g⁻¹)</th>
<th>Sodium Excretion (µmol·min⁻¹·g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Hz</td>
<td>99±2</td>
<td>6.6±0.6</td>
<td>90.5±9.0</td>
<td>14.4±1.6</td>
</tr>
<tr>
<td>0.75 Hz</td>
<td>97±2</td>
<td>6.6±0.7</td>
<td>64.9±9.0*</td>
<td>10.3±1.5*</td>
</tr>
<tr>
<td>1 Hz</td>
<td>99±2</td>
<td>6.4±0.9</td>
<td>55.5±7.0†</td>
<td>7.9±0.8*</td>
</tr>
</tbody>
</table>

*P<0.05, compared with 0 Hz; †P<0.01, compared with 0 Hz; n=10.

Data were calculated as mean±SEM. Differences within groups were analyzed using the paired Student’s t test; between groups, using a 1-way ANOVA. Significance was taken at the 5% level.

An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.
dine was present intraluminally, stimulation of the renal nerves was unable to change proximal tubular fluid reabsorption (Figure 2).

Discussion
It was important to establish that the low level of renal nerve stimulation had minimal effects on renal hemodynamics. Indeed, neither stimulation frequency altered blood pressure or whole kidney glomerular filtration rate at a time when there were frequency-related decreases in urine flow and sodium excretion. The same stimulation parameters caused frequency-dependent increases in Jva, which was indicative of the renal nerves acting directly on the fluid reabsorptive processes of the proximal epithelial cells, compatible with earlier micropuncture studies.13,14

NO is involved in determining sympathetic outflow15 and modulating neuroeffector junction efficiency.16 At the kidney, Barajas et al13 demonstrated diaphorase-staining nerve fibers and somata to be present, which is consistent with nitrergic neurones, and they frequently colocalized with the sympathetic innervation of the kidney. The exact mechanism by which the nitrergic and adrenergic nerves might interact at the neuroeffector junction is still not resolved. Yamamoto et al17 found that field stimulation of rat mesenteric arteries caused noradrenaline release, which was decreased by some 50% in the presence of Nω-nitro-L-arginine (30 μmol/L), suggesting NO was necessary for effective neurotransmission. Conversely, Egi et al18 and Maekawa et al,19 using the dog, found that intrarenal blockade of NO generation was associated with an increase, whereas NO donors led to a suppression of noradrenaline spillover from the kidney. Despite these diverse reports, there is a view that NO released from the nerve terminals can act presynaptically to exert a tonic inhibitory action on transmitter release.

We reported previously10 that intratubular administration of the NOS blocker L-NAME increased proximal tubular fluid reabsorption, compatible with NO exerting a tonic inhibitory action on basal epithelial cell transport processes. Moreover, this appeared to be dependent on the nNOS isoform, as proximal reabsorption was also increased after the relatively selective nNOS blocker 7-NI but not after aminoguanidine, the relatively selective iNOS blocker. This concept was reinforced by the observations that application of a NO donor to the epithelial cells caused Jva to decrease9,10 and the present study showing fluid reabsorption to be increased by L-NAME and 7-NI. Interestingly, in our previous report,10 the tonic inhibitory action of NO

Figure 1. This illustrates the effect of renal nerve stimulation on Jva under basal conditions (open bars) and when the renal sympathetic nerves were stimulated at 0.75 Hz (stippled bars) and at 1.0 Hz (slashed bars). APTF present in the tubules (control), L-NAME 10−4 M present intraluminally (L-NAME x 10−4), and L-NAME and SNP present in the tubules (L-NAME & SNP x 10−4). *P<0.05 vs basal, and †P<0.05 vs basal control, with the basal in the presence of L-NAME or L-NAME & SNP.

Figure 2. This demonstrates the influence of intraluminal 7-NI at 10−6 M (7-NI x 10−6 M), 7-NI at 10−4 M (7-NI x 10−4 M), and aminoguanidine (aminoguanidine x 10−4) on basal Jva (open bars) and demonstrates the responses to renal nerve stimulation at 0.75 Hz (stippled bars) and 1.0 Hz (slashed bars). †P<0.05 vs basal values, with APTF against 7-NI or aminoguanidine.
was prevented by renal denervation, suggesting an interaction between NO and noradrenergic stimulation of proximal fluid reabsorption. Two possible mechanisms can be considered. First, there might be a tonic inhibitory action of NO only on that component of epithelial cell transport processes determined by the renal nerves. Second, the NOS blockers might be diffusing further through the epithelial cells to the varicosities of the sympathetic fibers to modulate transmission at the neuroeffector junction. To further investigate this interaction, the converse approach was taken of directly stimulating the renal sympathetic nerves.

Low-level renal nerve stimulation increased proximal fluid reabsorption in a frequency related manner, which was effectively blocked by intraluminal administration of L-NAME. This suggested that the presence of NO was essential for the renal nerves to increase fluid transport by the epithelial cells. This was supported by the observation that the concomitant administration of L-NAME plus the NO donor SNAP restored the ability of the renal nerves to increase tubular fluid reabsorption. This effect of NO appeared to be mediated, in part, by NO generated by the nNOS isoform as not only were basal levels of Jva increased by both low and high doses of 7-NL, but also the neurally induced increases in Jva were prevented by the compound.

The relatively selective iNOS blocker, aminoguanidine, also prevented the neurally induced rise in Jva but had no effect on basal levels. These observations might suggest that NO derived from iNOS was involved in mediating part of the neurally stimulated Jva, although there may be some question as to the selectivity of the compound at this concentration.20 There is evidence that iNOS is expressed constitutively at low levels in the kidney and may generate NO, which contributes to the neural stimulation of Jva. Surprisingly, these observations indicate a further mechanism by which NO might modulate the ability of noradrenaline to increase epithelial cell transport processes; ie, NO was in some way facilitating the neural stimulation of Jva. Neurally stimulated Jva, although there may be some question as to the selectivity of the compound at this concentration.20

References
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EXTENDED METHODS

The experimental procedures were carried out under the terms of Project Licence No. 40/1367 and Personal Investigator Licences 40/37 and 40/3881 issued to E.J. Johns and X.C. Wu. Male Wistar rats (240±30 g) and SHRSP (254±23 g) were placed on restricted food intake but water and libitum over the night prior to use. The animals were anaesthetised (Inactin 100 mg/kg ip) and placed on a heated table and body temperature maintained at 36-36.5°C using a rectal thermister feedback system (Harvard Apparatus, Kent, UK). Cannulae were placed in the trachea, femoral artery, to monitor blood pressure, femoral vein for infusion of saline (150 mM NaCl) and the bladder to provide urine drainage. A flank incision was used to expose the left kidney, the capsule was removed and it was then placed in a kidney cup, stabilised with cotton wool and agar and its ureter cannulated. A small area of agar was removed to ensure access to the superficial proximal tubules and it was then covered with paraffin oil. The renal sympathetic nerves were dissected out, placed onto bipolar stimulating electrodes, sealed into place with Wacker silgel and attached to a Grass S8 stimulator. Once surgery was completed, a 2ml bolus of inulin in saline (15%) was infused iv and followed by 1.6 ml/h/100 g body wt. One hour of stabilisation was taken before the experimental study began. The measurement of proximal tubular fluid reabsorption was performed as previously described [9]. Briefly, tubules were punctured and a column of Sudan black caster oil, of some 20 tubule diameters in length, was injected and then a small volume of artificial proximal tubular fluid (APTF) injected to split the column. Images were captured of the shrinking split droplet at 2s intervals using a video camera (Leica, UK). A digital image capture programme stored and analysed the images which then calculated the rate of proximal tubular fluid reabsorption expressed per unit area of epithelium (Jva x 10⁴ min⁻¹ mm⁻² s⁻¹). Each tubule was subjected to the shrinking split droplet procedure two to three times to ensure that measurements were consistent and average values were taken.
Whole kidney glomerular filtration rate was evaluated using 15 min urine collections and the calculation of inulin clearance [8,9]. Urine flow rate was estimated gravimetrically and sodium content assessed using flame photometry (Corning model 410C, Halstead, Essex, UK). Blood pressure was measured via a simulated polygraph using LabVIEW software (National Instruments, Austin, Texas, USA).

The following groups of rats were studied:

A) Wistar rats:
(1) Wistar Control rats were given intratubular infusions of artificial proximal tubular fluid (APTF); (2) Wistar rats given intratubular APTF plus superoxide dismutase (SOD $10^{-4}$M).

B) SHRSP rats:
(3) SHRSP control rats were given artificial proximal tubular fluid; (4) SHRSP rats given intratubular APTF plus L-NAME ($10^{-4}$M); (5) SHRSP rats given intratubular APTF containing SOD ($10^{-4}$M); (6) SHRSP rats given intratubular APTF plus SNP ($10^{-4}$M); (7) SHRSP rats given intratubular APTF plus SOD ($10^{-4}$M) plus SNP($10^{-4}$M).

A minimum of one pair of surface nephrons were used per rat. Basal measurements of Jva were performed and then the second and third measurements were taken from the same nephron during which the renal sympathetic nerves were stimulated at either 0.75 or 1 Hz (2 ms, 15V) in random order. After a recovery period of 15 min, a second set of estimations were undertaken using a different nephron. The drugs under investigation were presented to the tubules in random order on either the first or second set of measurements. Once the Jva estimations were completed, 15 min clearance periods were undertaken for whole kidney function measurements. Arterial blood samples were taken for inulin and electrolyte evaluation.
L-NAME, sodium nitroprusside (SNP), superoxide dismutase (SOD) and caster oil were purchased from Sigma (Poole, Dorset, UK) and other compounds were obtained from BDH (Poole, Dorset, UK).

Statistics. Data are presented as means ± SEM. The paired Students ‘t’ test was used to compare differences within groups and a one-way ANOVA for differences between groups. The percentage changes reported were calculated from the absolute values obtained. Significance was taken when P<0.05.