Nitric oxide and renal nerve-mediated proximal tubular reabsorption in normotensive and hypertensive rats

XIAO CHUN WU,1 PETER J. HARRIS,2 AND EDWARD J. JOHNS1

1Department of Physiology, Medical School, University of Birmingham, Birmingham B15 2TT, United Kingdom; and 2Department of Physiology, University of Melbourne, Parkville, Victoria 3052, Australia

Wu, Xiao Chun, Peter J. Harris, and Edward J. Johns. Nitric oxide and renal nerve-mediated proximal tubular reabsorption in normotensive and hypertensive rats. Am. J. Physiol. 277 (Renal Physiol. 46): F560–F566, 1999.—In Inactin-anesthetized Wistar rats with an intact renal innervation, intratubular nitro-L-arginine methyl ester (L-NAME, 10−4 M) increased proximal fluid uptake (Jva, at 2.47 ± 0.61 × 10−4 mm·cm−2·s−1) by 17% (P < 0.05), whereas coadministration with sodium nitroprusside (SNP, 10−4 M) decreased Jva by 18% (P < 0.01). Similar manipulations of NO generation was without effect in groups of Wistar rats subjected to acute renal denervation. Intratubular aminoguanidine (10−4 M), a selective inducible nitric oxide synthase (NOS) blocker, had no effect on Jva in intact kidneys of Wistar rats, but the neuronal NOS (nNOS) blocker, 7-nitroindazole (10−4 M and 10−6 M) increased Jva by 19–23% (both P < 0.001). In stroke-prone spontaneously hypertensive rats (SHRSP), Jva values in the innervated kidneys were lower (P < 0.05) than in the corresponding Wistar groups and were unchanged by intratubular L-NAME or L-NAME plus SNP. The tonic attenuation of proximal epithelial transport by NO was dependent on the renal sympathetic nerves and appeared to be generated by the nNOS isoform of the enzyme. This role of NO was not evident in the SHRSP.

nitric oxide synthase; renal sympathetic nerves; proximal tubular sodium reabsorption; stroke-prone spontaneously hypertensive rats

NITRIC OXIDE SYNTHASE (NOS) is a key enzyme that catalyzes the oxidation of L-arginine to L-citrulline, giving rise to NO, which acts in a paracrine and autocrine fashion on target cells to stimulate soluble guanylate cyclase and increase production of cGMP. Three isoforms of the enzyme exists, inducible (iNOS), endothelial (eNOS), and neuronal (nNOS), all of which have been shown to be present in the kidney and may be involved in the generation of NO to modulate specific aspects of kidney function (1, 4, 5, 22, 28). The eNOS has been shown to produce NO, which primarily acts to directly on the epithelial tubular cells to stimulate sodium and water transport by stimulation of the sodium/hydrogen exchanger at the apical membrane (18). Reports to date indicate that nNOS is very localized primarily to the macula densa and the perivascular nerve endings and to a small extent to the endothelial cells of the efferent arteriole (5, 22, 25). It may cause the production of NO, which is involved in the regulation of renin release and the determination of efferent arteriolar vascular tone (19, 23). In a wider context, there is increasing evidence that NO can influence the effectiveness of neurotransmission between nerve cells in the central nervous system (16) and at more peripheral sites (34) of the autonomic nervous system by facilitating norepinephrine release (35).

The kidney receives a very dense innervation of sympathetic nerves that regulate both hemodynamic and tubular function (11). It is recognized that low levels of activation of the renal nerves, which have no effect on either renal blood flow or glomerular filtration rate (GFR), cause an antinatriuresis and antidiuresis (21, 31). Micropuncture studies have shown this action of the renal nerves to be exerted primarily at the proximal tubule (17) and the thick limb of the ascending loop of Henle (12). At these sites, norepinephrine acts directly on the epithelial tubular cells to stimulate sodium and water transport by stimulation of the basolateral sodium-potassium-ATPase as well as the sodium/hydrogen exchanger at the apical membrane (30). In the spontaneously hypertensive rat, renal sympathetic nerve activity is elevated compared with normotensive Wistar rats (26), but the impact of the renal nerves on proximal tubular fluid reabsorption in the genetic hypertensive rat has received little attention.

Several studies have investigated the possible role of NO in modulating proximal tubular function, but conflicting views have arisen. Intravenous administration of N⁵-monomethyl-L-arginine (L-NMMA) into Sprague-Dawley rats increased fractional excretions of sodium and lithium, suggesting that NO decreases fluid and sodium reabsorption by the proximal tubules (29). However, this interpretation was put into doubt by the fact that L-NMMA caused a concomitant rise in blood pressure, which would act directly, via a pressure natriuresis effect, and indirectly, via withdrawal of renal sympathetic nerve activity, to decrease fluid reabsorption. In a more focused micropuncture examination of proximal tubular function, Eitle et al. (13) found that NO was able to depress proximal tubular fluid reabsorption when the NO donor sodium nitroprusside (SNP) was given either intraluminally or via the peritubular capillaries. Thus NO would appear to exert...
a tonic inhibitory action on fluid reabsorption on this nephron segment.

The aims of this investigation were, first, to investigate whether NO contributed to the renal nerve-dependent component of sodium reabsorption by the proximal tubule and, if so, to elucidate the isoform of NOS that was involved and, second, to determine whether NO might also be involved in modulating proximal tubular fluid reabsorption in a genetic rat model of hypertension. This was done by measuring the changes in proximal tubular fluid reabsorption in response to intraluminal blockade of NO production using selective NOS isoform inhibitors and NO donor administration in groups of intact or denervated kidneys of normotensive Wistar rats and stroke-prone spontaneously hypertensive rats (SHRSP).

METHODS

General Preparation

All experimentation was carried out under the auspices of the United Kingdom Government project license PIL 40/1367 and personal investigator license PIL 40/00371 and 40/03881 to E. J. J ohns and X. C. Wu, respectively. Male Wistar rats (280 ± 30 g) and SHRSP (275 ± 25 g) had their food removed overnight before use but were given free access to water. Anesthesia was induced using an intraperitoneal injection of Inactin (100 mg/kg), and the animal was placed on a heating table to maintain the body temperature around 36–36.5°C by means of a rectal thermistor feedback system (Harvard Instruments). The trachea was cannulated, and the animal breathed spontaneously. Cannulas were placed in the right femoral vein for infusion of saline (150 mM NaCl) and inulin, in the femoral artery for monitoring mean blood pressure, and in the bladder to allow the urine to drain. The left kidney was exposed using a flank incision, and the capsule was removed. The kidney was stabilized with cotton wool and in a kidney cup, which was attached to the operating table. The left ureter was cannulated for urine collection. A small area of the agar was removed to allow access to the underlying tubules. The artificial proximal tubule was punctured with a double-barreled glass micropipette, one barrel of which contained Sudan black-stained castor oil, whereas the other contained an artificial proximal tubular fluid. A column of castor oil, at least 20 tubule diameters in length, was injected into the distal end, and then a small volume of test solution was injected to split the oil column upstream from the point of puncture. Images of the shrinking split-drop were captured digitally, at 1- or 2-s intervals, by a video camera (Leica). This was connected to a microcomputer (Gateway G5/233) implementing a digital image capture program, which stored and analyzed the images and thereby enabled calculation of the rate of proximal tubular fluid reabsorption expressed per unit area of epithelium (20).

Whole Kidney Function

Glomerular filtration rate. Clearance periods of 15 min were collected. The plasma and urine samples were assayed for inulin using previously established methods (9). The GFR was determined as microliters per minute per gram body weight.

Urine sodium excretion. The urine sodium concentration was measured by flame photometry (Corning model 410C flame photometer), and absolute sodium excretion was calculated using urine volume values.

Systemic hemodynamics. Blood pressure was measured using a computer-based LabVIEW system and simulated polygraph. Average blood pressure values were recorded over each clearance period.

Animal Groups

Our previous reports examining the neural control of whole kidney function (36) had compared Wistar and SHRSP, and these strains were utilized in the present investigation. The animals were divided into 14 groups.

Wistar rats. These rats were divided into the following nine groups (groups 1–9): group 1, Wistar rats with innervated kidneys; group 2, Wistar rats subjected to left renal denervation; group 3, Wistar rats with innervated kidneys given intratubular nitro-L-arginine methyl ester (L-NNAME, 10^-4 M); group 4, Wistar rats subjected to renal denervation given intratubular L-NNAME; group 5, Wistar rats with innervated kidneys given intratubular L-NNAME (10^-4 M) plus SNP (10^-4 M); group 6, Wistar rats with innervated kidneys given intratubular 7-nitroindazole (7-NI, 10^-4 M); group 7, Wistar rats with innervated kidneys given intratubular 7-nitroindazole (7-NI, 10^-4 M) plus SNP (10^-4 M); group 8, Wistar rats with innervated kidneys given intratubular 7-NI (10^-6 M); and group 9, Wistar rats with innervated kidneys given intratubular aminoguanidine (10^-4 M).

SHRSP rats. SHRSP rats were divided into the following five groups (groups 10–14): group 10, SHRSP with innervated kidneys; group 11, SHRSP subjected to renal denervation; group 12, SHRSP with intact renal innervation given intratubular L-NNAME (10^-4 M); group 13, SHRSP subjected to renal denervation given intratubular L-NNAME (10^-4 M); and group 14, SHRSP with intact renal innervation given intratubular L-NNAME (10^-4 M) plus SNP (10^-4 M).

Protocol

Two to five tubules were punctured, and the rate of reabsorption of the intratubular solution (Jwa) was recorded. Immediately, a 15-min urine collection was commenced for whole kidney function and blood pressure measurements. At the end of this period, an arterial blood sample (0.3 ml) was withdrawn via the femoral artery for inulin and electrolyte estimations. The sequence of Jwa measurements was then repeated but using different sets of two to five tubules. A time control study was performed in which the same intratubular solution was given to ensure that fluid uptake rate was stable over the duration of the study. The drugs were presented to the different sets of tubules in random fashion, being either in the first or second measurement period. The time between the first clearance and second clearance periods was no more than 2 h.

Compounds

Inactin, 7-NI, and aminoguanidine were obtained from RBI (Research Biochemicals International, Natick, MA) and at
the doses used were soluble in the artificial proximal tubular fluid after 5 min of sonication. There was a possibility that 7-NI at $10^{-4}$ M might not be particularly selective for nNOS. In an attempt to overcome this limitation, a lower dose, of $10^{-6}$ M, was used, thereby decreasing the chance of its inhibiting other NOS isoforms. L-NAME, SNP, and castor oil were obtained from Sigma (Poole, Dorset, UK); all other compounds were obtained from BDH (Poole, Dorset, UK).

**Calculation of Data**

The proximal tubular fluid uptake per unit surface area of epithelium ($J_{\text{va}} \times 10^{-4} \text{mm}^{-3} \cdot \text{mm}^{-2} \cdot \text{s}^{-1}$) was determined in each of the tubules, which were subjected to the split-droplet procedure two to three times to ensure consistent measurements and the mean values were then taken.

**Statistical Analysis**

All data were calculated as means ± SE. The differences within groups were analyzed using a paired Student's t-test, and differences between groups using a one-way ANOVA. The percentage values quoted were based on the mean values obtained, but significance values were based on calculations from the absolute values recorded. Significance was taken at the 5% level.

**RESULTS**

Table 1 shows the mean blood pressure, GFR, urine flow, sodium excretion, and $J_{\text{va}}$ in groups of Wistar and SHRSP with both innervated (groups 1 and 10) or denervated (groups 2 and 11) kidneys, and it is evident that these variables were stable over time in all groups of rats. Renal denervation had no effect on the level of blood pressure or GFR in either the Wistar rats or the SHRSP (Table 1). Moreover, in the time control studies, the two consecutive $J_{\text{va}}$ estimations were virtually the same in the innervated Wistar and SHRSP (Table 1). The intratubular administration of the different compounds alone or in combination (groups 3–7 and 11–13) had no effect on mean blood pressure or whole kidney GFR, urine flow, or sodium excretion in any of the groups of animals, with all variables remaining at a stable level (Tables 2 and 3).

**Wistar Rats**

In the group of rats subjected to renal denervation, urine flow and absolute sodium excretion were higher (both $P < 0.001$) by 179 and 119%, respectively (Table 1), compared with the values in the group of animals with an intact innervation, whereas $J_{\text{va}}$ was elevated by ~25% ($P < 0.05$, Table 4). Measurement of $J_{\text{va}}$ with $10^{-4}$ M L-NAME present in the tubular fluid, to block NO production locally (group 3), caused a significant ($P < 0.05$) increase of ~17% in the group of animals with innervated kidneys (Table 4, Fig. 1). By contrast, in the group of animals subjected to renal denervation (group 4), inclusion of L-NAME in the tubular lumen had no consistent effect on $J_{\text{va}}$ (Table 4, Fig. 1), which was significantly ($P < 0.05$) different from the responses in the innervated kidneys. Further studies were performed in which the NO donor, SNP, was perfused together with the L-NAME, and it was found that $J_{\text{va}}$ was significantly ($P < 0.01$) reduced by 18% (Table 4, Fig. 1) in the group of rats with innervated kidneys (group 5), whereas in the animals with denervated kidneys (group 6), $J_{\text{va}}$ was unchanged (Table 4, Fig. 1). An attempt was made to identify the type of NOS isoform mediating the effect on $J_{\text{va}}$ by infusing relatively selective NOS inhibitors. In a further group of rats (group 7) with an intact renal innervation, tubules were perfused with 7-NI at $10^{-4}$ M, a relatively selective nNOS blocker, which caused a significant ($P < 0.001$) increase in $J_{\text{va}}$ of 19% (Table 4, Fig. 2). A second group of animals with innervated kidneys were used and received 7-NL at $10^{-6}$ M (group 8) intraluminally, which resulted in a significantly ($P < 0.001$) higher $J_{\text{va}}$ of ~23% (Table 4, Fig. 2). However, in the group of innervated rats (group 9) in which the blocker of iNOS, aminoguanidine, was included in the tubular perfusate, $J_{\text{va}}$ was unchanged (Table 4, Fig. 2).

**SHRSP Rats**

Both groups of hypertensive rats (groups 10 and 11) with innervated and denervated kidneys had significantly higher blood pressures, similar GFRs, and significantly lower urine flows and sodium excretions (all $P < 0.001$, Table 1) compared with the corresponding Wistar groups (Table 1). It was evident that in the SHRSP subjected to denervation, urine flow and absolute sodium excretions were significantly (both $P < 0.01$) higher compared with the innervated groups, by ~40–50%. These differences in excretory variables between innervated and denervated kidneys were greater in the

<p>| Table 1. Time controls for blood pressure, GFR, fluid excretion, and proximal tubular fluid reabsorption in innervated and denervated kidneys of Wistar and SHRSP |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th><strong>Blood Pressure</strong>, mmHg</th>
<th><strong>GFR</strong>, µl·min$^{-1}$·g$^{-1}$</th>
<th><strong>Urine Flow</strong>, µl·min$^{-1}$·g$^{-1}$</th>
<th><strong>Sodium Excretion</strong>, µmol·min$^{-1}$·g$^{-1}$</th>
<th><strong>J$_{va}$</strong>, ×$10^{-4}$ mm$^{-3}$·mm$^{-2}$·s$^{-1}$</th>
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<tbody>
<tr>
<td><strong>Wistar</strong></td>
<td></td>
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<tr>
<td>Innervated</td>
<td>Control 1 Control 2</td>
<td>Control 1 Control 2</td>
<td>Control 1 Control 2</td>
<td>Control 1 Control 2</td>
</tr>
<tr>
<td>8</td>
<td>107 ± 3 98 ± 2</td>
<td>3.5 ± 0.4 3.6 ± 0.5</td>
<td>46.5 ± 7.2 35.4 ± 4.2</td>
<td>12.8 ± 1.2 11.5 ± 1.7</td>
</tr>
<tr>
<td>Denervated</td>
<td>10 104 ± 1 97 ± 2</td>
<td>3.6 ± 0.3 4.1 ± 0.5</td>
<td>129 ± 8.6 125 ± 9.7</td>
<td>28.0 ± 1.8 29.8 ± 1.9</td>
</tr>
<tr>
<td><strong>SHRSP</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Innervated</td>
<td>8 148 ± 2 139 ± 2</td>
<td>4.2 ± 0.4 4.6 ± 0.4</td>
<td>30.3 ± 3.3 32.2 ± 1.4</td>
<td>3.7 ± 0.7 5.3 ± 0.69</td>
</tr>
<tr>
<td>Denervated</td>
<td>6 145 ± 7 136 ± 8</td>
<td>4.0 ± 0.5 3.7 ± 0.3</td>
<td>42.2 ± 5.3 55.7 ± 4.9</td>
<td>7.1 ± 1.4 10.7 ± 1.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$ = number animals. SHRSP, stroke-prone spontaneously hypertensive rats; GFR, glomerular filtration rate; $J_{va}$, proximal tubular fluid uptake. *$P < 0.05$, †$P < 0.01$, and ‡$P < 0.001$, innervated compared with denervated Wistar or SHRSP. **$P < 0.05$ and §$P < 0.001$, innervated or denervated Wistar compared with innervated or denervated SHRSP.
Wistar than SHRSP (both P < 0.01, Table 1). At the tubular level, J\textsubscript{\text{Vw}} was ~30% higher (P < 0.05) in innervated vs. denervated kidneys (Table 1) but was significantly (P < 0.05) less than that obtained in the corresponding Wistar groups (groups 1 and 2; Table 1, P < 0.05). Intratubular perfusion with L-NAME had no effect on J\textsubscript{\text{Vw}} in either the intact (group 12; Table 4, Fig. 3) or denervated kidneys (group 13; Table 4 and Fig. 3). Moreover, the coadministration of L-NAME with SNP (group 14) in the intratubular fluid was without effect on J\textsubscript{\text{Vw}} in the group of SHRSP with innervated kidneys (Table 4, Fig. 3). The lack of influence of L-NAME and L-NAME plus SNP in the SHRSP was very different from the increases and decreases observed when these agents were administered into the proximal tubules of Wistar rats.

**DISCUSSION**

There is a body of evidence indicating that NO can act as a modulator of neurotransmission within the central nervous system, at sympathetic ganglia, and at peripheral neuroeffector junctions (16). Because the renal sympathetic nerves have an important influence on proximal tubular fluid reabsorption (11), the question arose as to whether NO could contribute to the impact of the nerves at this site. In a recent report (8), it has been shown that the effect of NO on the vasculature of the spontaneously hypertensive rat (SHR) was enhanced, and it is possible that a similar situation might pertain in the renal tubules of the SHR.

In our study, all groups of rats having innervated or denervated kidneys had blood pressure and renal hemodynamic and excretory functions that were stable during the course of the measurements. Acute denervation of the kidneys in the Wistar rats resulted in some two- to threefold higher levels of urine flow and sodium excretion with no differences in the level of glomerular filtration, representing the well-known phenomena of denervation diuresis and natriuresis (11). These differences were reflected at the proximal tubular level where fluid uptake rate, which was stable over the time course of the experiment, was lower in the denervated compared with the innervated kidneys. These data are consistent with earlier micropuncture studies (6, 7), which provided the initial evidence for a direct action of the renal nerves on proximal tubular fluid reabsorption. The situation in the SHRSP was somewhat different, because despite the higher blood pressure, sodium excretion was only about one-half that observed in the Wistar rats. A lower sodium output in the SHR has been previously reported (32), and relative sodium retention has been considered to be an important contributor to the development of the hypertensive state. One possible reason for the depressed sodium excretion in the SHRSP could have been the raised renal sympathetic nerve activity present in this strain (26, 36), but this appears not to be the case, since although denervation resulted in natriuresis, the level of sodium excretion was still well below that observed in the renally denervated Wistar rats.

An even more intriguing observation was that even though GFRs were similar in both strains of rat, proximal tubular fluid reabsorption rate was lower in the SHRSP compared with the Wistar. One possibility might be that more fluid would pass from the proximal tubule into the more distal parts of the nephron and would suggest that a relatively greater fluid reabsorption occurred at these nephron regions in the SHRSP compared with the Wistar rats. Again, the reasons for this are unclear, but in these mature hypertensive rats,

### Table 2. Blood pressure and whole kidney GFR, urine flow, and sodium excretion during intraluminal L-NAME and SNP administration in Wistar rats

<table>
<thead>
<tr>
<th></th>
<th>Blood Pressure, mmHg</th>
<th>GFR, µl·min\textsuperscript{-1}·g\textsuperscript{-1}</th>
<th>Urine Flow, µl·min\textsuperscript{-1}·g\textsuperscript{-1}</th>
<th>Sodium Excretion, µmol·min\textsuperscript{-1}·g\textsuperscript{-1}</th>
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<tr>
<td></td>
<td>n</td>
<td>Control</td>
<td>Exptl</td>
<td>n</td>
</tr>
<tr>
<td>Innervated + L-NAME</td>
<td>7</td>
<td>103 ± 2</td>
<td>102 ± 2</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>Denervated + L-NAME</td>
<td>8</td>
<td>97 ± 2</td>
<td>98 ± 2</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>Innervated + L-NAME + SNP</td>
<td>8</td>
<td>98 ± 3</td>
<td>100 ± 2</td>
<td>3.9 ± 0.8</td>
</tr>
<tr>
<td>Denervated + L-NAME + SNP</td>
<td>10</td>
<td>102 ± 1</td>
<td>101 ± 1</td>
<td>4.2 ± 0.5</td>
</tr>
<tr>
<td>Innervated + 7-nitroindazole</td>
<td>10</td>
<td>101 ± 3</td>
<td>100 ± 2</td>
<td>3.8 ± 0.5</td>
</tr>
<tr>
<td>Innervated + amino guanidine</td>
<td>10</td>
<td>100 ± 1</td>
<td>102 ± 2</td>
<td>3.7 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = number of animals. SNP, sodium nitroprusside; L-NAME, nitro-L-arginine methyl ester; Exptl, experimental group. *P < 0.05 and ‡P < 0.001, innervated L-NAME compared with either denervated L-NAME or innervated + L-NAME + SNP.

### Table 3. Blood pressure and whole kidney GFR, urine flow, and sodium excretion during intraluminal L-NAME and SNP administration in SHRSP

<table>
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<tr>
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<th>Blood Pressure, mmHg</th>
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<tr>
<td></td>
<td>n</td>
<td>Control</td>
<td>Exptl</td>
<td>n</td>
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<tr>
<td>Innervated + L-NAME</td>
<td>9</td>
<td>160 ± 4</td>
<td>154 ± 4</td>
<td>3.4 ± 0.2</td>
</tr>
<tr>
<td>Denervated + L-NAME</td>
<td>6</td>
<td>153 ± 6</td>
<td>152 ± 6</td>
<td>4.7 ± 1.3</td>
</tr>
<tr>
<td>Innervated + L-NAME + SNP</td>
<td>8</td>
<td>161 ± 4</td>
<td>148 ± 4</td>
<td>3.3 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = number of animals.
in which the kidneys have been exposed to a higher blood pressure for some time, it is recognized that hypertrophic vascular changes take place, and the possibility arises that compensatory and protective mechanisms could also have come into play in relation to sodium and water absorption in the distal nephron segments.

The initial observations in the normotensive Wistar rats were that local tubular blockade of NO achieved by intraluminal administration of L-NAME resulted in a rise in $J_v$, and conversely the NO donor, SNP, depressed proximal tubular fluid reabsorption. This was consistent with the view that NO exerts a tonic inhibitory action on proximal tubular fluid reabsorption and supported the earlier report of Eitle et al. (13) using intact Sprague-Dawley rats. These findings contrast with the micropuncture studies of Gabbai et al. (14), who also measured proximal fluid reabsorption and found L-NMMA to depress fluid transport, an effect blocked by renal denervation. The difficulty with these observations is that the L-NMMA was given systemically and blood pressure was allowed to rise, which in itself would have depressed fluid reabsorption, and the L-NMMA-induced pressure rise was less in the animals subjected to renal denervation than the sham controls. The experimental design and findings of the present study overcome the confounding problem of changes in blood pressure. However, if the kidneys were subjected to acute denervation, then the manipulation of NO generation, with L-NAME alone or together with intraluminal SNP, was without effect on proximal tubular fluid reabsorption. These findings imply that NO was modulating at least one component of fluid uptake, that is one which was dependent on background levels of renal nerve activity. The mechanisms by which this could occur remain to be defined, but two possibilities arise. The NO could act within the epithelial cells to modulate selectively that component of the sodium-transporting pathway which is regulated by catecholamines released from the renal nerves. Alternatively, NO might be generated at the nerve fiber varicosities and act to regulate neurotransmitter release via an auto-inhibitory mechanism. However, this latter option is questionable, because the restricted application of the NOS blocker and donor within the tubules might not allow them to penetrate sufficiently far to influence the nerve fiber varicosities.

Subsequent studies were designed to investigate the specific isof orm of NOS that was involved, as L-NAME is known to inhibit activity of all three isoforms of the enzyme. The animal preparation, laparotomy, and exposure of the kidney together could have been sufficient to activate iNOS, but it was apparent that iNOS was not implicated, as the iNOS-selective blocker, aminoguanidine, was without effect on $J_v$. A number of reports have indicated that the substituted nitroindazole, 7-NI, demonstrates some selectivity for the nNOS with a $K_i$ in vitro of $\sim 10^{-5}$ M (3). However, even at a concentration of $10^{-4}$ M, 7-NI was found to have no effect on the acetylcholine-induced vasorelaxation of preconstricted aortas (27), yet this is a concentration at which L-NAME can completely block the acetylcholine-mediated relaxation of renal arcuate arteries (33). Because of the concern over selectivity, two concentrations of 7-NI were utilized, $10^{-4}$ M and $10^{-6}$ M, and it was apparent that both concentrations caused significant elevations in $J_v$. Together these experimental findings strongly suggest that the nNOS isof orm was responsible, as $J_v$ was increased by intraluminal infusion of 7-NI to the same extent as had been achieved with...
expected that the tonic inhibitory action of NO on 
inhibition of fluid reabsorption in Wistar and SHRSP 
demonstrated that in the Wistar rat, NO exerted a tonic 
In summary, we have investigated the interactions 
with innervated kidneys in absence (open bars) and presence (solid bars) of L-NAME (10^{-4} M) to block all isoforms of nitric oxide synthase (NOS), aminoguanidine (AGD, 10^{-4} M) to selectively block the inducible isofom of NOS, and 7-nitroindazole (7-NI, 10^{-4} M and 10^{-6} M) to selectively block the neuronal isoform of NOS: n is the number of animals in each group, and comparisons were made between pretreatment and with-treatment measurements in each group.*P < 0.05, ‡P < 0.001.

![Graph showing interaction of NO and renal sympathetic nerves on proximal tubular fluid reabsorption rate (Jva), presented for groups of Wistar rats with innervated kidneys in absence (open bars) and presence (solid bars) of L-NAME (10^{-4} M) to block all isoforms of nitric oxide synthase (NOS), aminoguanidine (AGD, 10^{-4} M) to selectively block the inducible isoform of NOS, and 7-nitroindazole (7-NI, 10^{-4} M and 10^{-6} M) to selectively block the neuronal isoform of NOS: n is the number of animals in each group, and comparisons were made between pretreatment and with-treatment measurements in each group.*P < 0.05, ‡P < 0.001.

Fig. 3. Interaction of NO and renal sympathetic nerves on proximal tubular fluid reabsorption rate (Jva), presented for groups of Wistar rats with innervated kidneys in absence (open bars) and presence (solid bars) of L-NAME (10^{-4} M) to block all isoforms of nitric oxide synthase (NOS), aminoguanidine (AGD, 10^{-4} M) to selectively block the inducible isoform of NOS, and 7-nitroindazole (7-NI, 10^{-4} M and 10^{-6} M) to selectively block the neuronal isoform of NOS: n is the number of animals in each group, and comparisons were made between pretreatment and with-treatment measurements in each group.*P < 0.05, ‡P < 0.001.

NO AND RENAL NERVES

Perspectives

These studies have highlighted the fact that nNOS plays a functional role in generating NO, which has a tonic influence to attenuate the basal action of the renal nerves on proximal tubular fluid reabsorption. Thus it would help to modulate the impact of the nerves at the level of epithelial cells. The mechanism by which NO exerts this effect is not clear. It might act by regulating the release of neurotransmitter from the nerve cells or by acting intracellularly within the epithelial cells to prevent the response to activation of the adrenoceptors. This needs to be resolved by further investigation. A second significant point is that this interaction between NO and the neural control of fluid reabsorption did not occur in the genetically hypertensive rat, indicating that the tubules would be subjected to a less dynamic neural control of fluid handling, perhaps exacerbating the deficit in fluid volume homeostasis in these animals. Interestingly, these observations support earlier immunohistochemical studies that have not provided evidence for the presence of nNOS in proximal epithelial cells, and although there is strong support for the localization of nNOS at the macula densa region (5, 22, 25), this is unlikely to be relevant to the present findings. Clearly, the functional evidence of the present study is not entirely consistent with the data concerning localization of the NOS enzyme, perhaps reflecting the limitations of current immunohistochemical techniques.

Neither L-NAME nor SNP given into the lumen altered the level of Jva in innervated kidneys of the SHRSP. This would indicate that the tonic inhibitory effect of NO on the renal nerve component of Jva was not functional in the hypertensive rat. It is recognized that renal sympathetic nerve activity is raised in the genetically hypertensive rat (26), which has been considered to be responsible, in part, for sodium retention in this model of hypertension (11). It might have been expected that the tonic inhibitory action of NO on Jva found to exist in the normotensive rats would have been greater in the hypertensive rats such that the excessive neural control could have been buffered. The fact that this was not the case would suggest that this deficit in the neural regulation of fluid reabsorption by NO would tend to exacerbate the hypertensive state. This apparent deficiency in NO action at the tubular level contrasts with findings of Bergstrom et al. (8), who demonstrated in the rat that the contribution of NO to the neural control of renal hemodynamics was greater in the SHR compared with the normotensive animals. Moreover, this defect in the neural impact on proximal tubular fluid reabsorption could go part way to explain the blunted renal nerve-dependent antinatriuretic responses to reflex activation of the renal nerves reported earlier by this group in the SHRSP (10, 36).
whole kidney findings of a defect in the renal nerve-dependent control of fluid excretion in the SHRSP.

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Address for reprint requests and other correspondence: E. J. Johns, Dept. of Physiology, Medical School, University of Birmingham, Birmingham B15 2TT, UK. (E-mail: E.J.Johns@bham.ac.uk).

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