Interactions Between Nitric Oxide and Renal Nerves on Pressure-Diuresis and Natriuresis

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Abstract. The present study examined the effect of renal denervation on the impairment of the pressure-diuresis response produced by nitric oxide synthesis blockade. The experiments were performed in Inactin-anesthetized Munich-Wistar rats. The animals with innervated kidneys had lower baseline values of renal blood flow, GFR, sodium excretion (UNaV), and urine flow (V) than rats with denervated kidneys. Also, renal denervation shifted pressure-diuresis and natriuresis toward lower pressures. A low dose of NAME (3.7 nmol/kg per min) reduced UNaV and the fractional excretion of sodium (FENa) and blunted pressure-natriuresis only in rats with innervated kidneys, whereas it had no effects in rats with denervated kidneys. A medium dose of NAME (37 nmol/kg per min) lowered FENa only in rats with innervated kidneys. The administration of NAME (37 nmol/kg per min) blunted pressure-diuresis and natriuresis in kidneys with or without the renal nerves, but the effect was more pronounced in rats with innervated kidneys. A high dose of NAME (3.7 μmol + 185 nmol/kg per min) increased UNaV and FENa only in rats with innervated kidneys, whereas it reduced GFR, V, UNaV, and FENa in rats with denervated kidneys. However, pressure-natriuresis and diuresis were blunted by this high dose of NAME independently of the presence or absence of renal nerves. These results demonstrate that renal nerves potentiate the renal effects of low doses of NAME on renal function and pressure-diuresis and natriuresis. However, high doses of NAME abolish pressure-diuresis independently of renal nerves, and the natriuretic effect of NAME in innervated kidneys may be attributed to reflex inhibition of sympathetic tone due to the rise in arterial pressure.

Nitric oxide (NO) is a humoral factor produced within the kidney, and increasing evidence indicates that NO is one of the most important systems in controlling renal function and arterial pressure. Administration of NAME reduces NO synthesis and lowers dramatically renal blood flow (RBF) and also reduces sodium and water excretion, without affecting autoregulation of total RBF and GFR (1–3).

The changes in renal function observed after NO synthesis blockade are due, at least in part, to the fact that physiologically NO buffers the influence of endogenous vasoconstrictor systems within the kidney (4–10). Vasoconstriction may increase shear stress and NO production, which in turn acts as a regulation system by restraining the constrictor action of a variety of control systems, such as the renal sympathetic nerves. It is known that the sympathetic nervous system is important in the regulation of renal function and arterial pressure (11,12), because renal denervation shifts the pressure-natriuresis response toward lower pressures (13). Evidence indicates that renal nerve activity and the renal actions of sympathetic nerves are buffered by NO (4–9). In this regard, it has been reported that chronic intrarenal NO synthase inhibition potentiates the renal vasoconstrictor and hypertensive effects of an intrarenal infusion of norepinephrine (4). Also, it has been shown that the intrarenal infusion of an NO donor reduced the hemodynamic and excretory effects of renal nerve stimulation (5), whereas intrarenal NO synthesis inhibition potentiated the actions of renal nerve stimulation and increased renal norepinephrine secretion rate (6). In addition, NO potentiation by infusion of L-arginine increases RBF and reduces renal sympathetic nerve activity (7), whereas acute NO synthesis blockade produces renal vasoconstriction and increases renal sympathetic outflow (7–9). Taken together, those studies indicate that NO modulates both renal nerve activity and the renal actions of renal nerves.

The pressure-diuresis response in an important renal mechanism of regulation of sodium excretion, which is thought to be nonadaptive and responsible for the long-term control of arterial pressure (14). According to this hypothesis, arterial pressure is dependent on the mechanisms regulating pressure-diuresis, many of which are not completely understood. A variety of studies have shown that NO synthesis blockade resets the pressure-natriuretic response toward higher pressures (2,3,15–17). This is consistent with the observation that chronic administration of NAME produces sustained sodium-dependent arterial hypertension (18–21). Because the preglomerular autoregulatory vasoconstriction should increase endothelial shear stress and NO secretion as arterial pressure rises, it has been hypothesized that the vascular endothelium may be the sensor, and NO may be the mediator coupling elevations in
arterial pressure with reductions in tubular sodium and water reabsorption, through intrarenal hemodynamic changes (15) or direct tubular actions (22,23). This point of view is consistent with the fact that pressure-diuresis is associated with elevations in nitrate/nitrite excretion (24,25). Thus, NO appears to play a central role in the control of renal function and arterial pressure.

Although most studies performed show that NO synthesis inhibition reduces renal excretion of sodium and blunts pressure-natriuresis (1-3,15-17), there are some contradictory reports indicating that NO synthesis blockade, in some conditions, may cause natriuresis and diuresis; the fact that this phenomenon is more prominent in innervated than in denervated kidneys led to the hypothesis that it may be mediated through the renal nerves (26-28). These studies were all performed using high doses of NO synthase (NOS) inhibitors, which produced rapid elevations of arterial pressure. Because the natriuresis was abolished when arterial pressure was not allowed to rise (26), this effect was interpreted as pressure-diuresis. Because the natriuretic effect of NOS inhibition is eliminated by renal denervation, a possible cause for the natriuresis produced by NOS blockade on innervated kidneys could be the reflex sympathoinhibition caused by the rise in arterial pressure. An alternative hypothesis might be that the presence of renal sympathetic tone might alter the effects of NAME on pressure-diuresis, thus explaining the greater diuresis obtained in innervated than in denervated kidneys after high doses of NOS inhibitors. However, whether NO synthesis inhibition affects pressure-natriuresis differently depending on the presence or absence of the renal nerves remains to be established. Thus, the purpose of the present study was to evaluate the effect of renal denervation on the impairment produced by different doses of an NO synthesis inhibitor on renal function and the pressure-diuresis response.

**Materials and Methods**

Experiments were performed on 56 Munich-Wistar rats (200 to 250 g body wt) purchased from Harlan Laboratories (Madison, WI) and bred in our animal care facility. All procedures followed were in accordance with the recommendations from the Declaration of Helsinki and the guiding principles in the care and use of animals approved by the Council of the American Physiological Society. The rats were anesthetized with an intramuscular injection of ketamine (30 mg/kg) and an intraperitoneal injection of Inactin (thiobutabarbital, 50 mg/kg), and placed on a heated table to maintain body temperature at 36.5°C. Cannulas were placed in the femoral vein for infusions and in the femoral artery for measurement of arterial pressure. An aortic clamp was placed above the left renal artery, and ties were loosely placed around the mesenteric and celiac arteries so that renal perfusion pressure (RPP) could be manipulated by adjusting peripheral resistance, as described previously. The left kidney was denervated in groups 5 through 8 (see below) by stripping all visible nerves from the renal artery and coating the hilar region of the kidney with a 10% solution of phenol in ethanol (13). Plasma levels of norepinephrine, aldosterone, cortisol, and vasopressin were maintained at fixed levels throughout the experiment by continuous intravenous infusion of norepinephrine (333 ng/kg per min), aldosterone (66 ng/kg per min), cortisol (33 mg/kg per min), and vasopressin (0.17 ng/kg per min) at the doses indicated (13). The rats received an intravenous infusion of a 0.9% sodium chloride solution containing all hormones indicated above and 1% bovine serum albumin, at a rate of 2 ml/100 g per h throughout the experiment.

A cannula was placed in the left ureter for collection of urine. 3H-inulin (1 μCi/ml) was included with the infusion solution to allow for measurement of GFR. An electromagnetic flow probe (Skalar, Copenhagen, Denmark) was placed around the renal artery to allow for measurement of RBF, with care taken not to damage the renal nerves in rats with innervated kidneys.

**Experimental Protocols**

The left kidney was denervated in groups 5 through 8. Urine flow, sodium excretion, RBF, GFR, and arterial pressure were measured during a 30-min control period. Then, either vehicle (groups 1 and 5, n = 8 and n = 13, respectively) or NAME (groups 2 and 6, 3.7 nmol/kg per min, n = 7 and n = 7; groups 3 and 7, 37 nmol/kg per min, n = 11 and n = 10; groups 4 and 8, 3.7 μmol + 185 nmol/kg per min, respectively) was administered intravenously, and after a 30-min equilibration period, urine and plasma samples were collected again in a 15-min experimental clearance period. Afterward, RPP was lowered to 100 mmHg by aortic occlusion; 10 min later, urine flow, sodium excretion, GFR, and RBF were measured during a 30-min period. RPP was then elevated by 20 mmHg by releasing the clamp on the abdominal aorta, and after a 10-min equilibration period, urine and plasma samples were collected during a 20-min experimental period. Finally, RPP was increased 20 mmHg above control by tying off the mesenteric and celiac arteries, and urine and plasma samples were again collected during a 15-min experimental period.

**Analytical Methods**

Urine volume was measured gravimetrically and factored by gram kidney weight (gk). 3H-Inulin concentrations in urine and plasma samples were determined using liquid scintillation spectrophotometry. GFR was calculated as the urine-to-plasma inulin concentration ratio times urine flow rate. The sodium concentration of urine and plasma samples was determined by flame photometry.

**Statistical Analyses**

Data are presented as mean values ± 1 SEM. The significance of differences in the measured values within groups was analyzed using a one-way ANOVA for repeated measures followed by a Fisher least significant difference test. The significance of differences in the measured values between groups was analyzed using a two-way ANOVA for repeated measures followed by a Fisher least significant difference test (29). P < 0.05 (two-tailed test) was considered statistically significant.

**Results**

The effects of renal denervation on renal function are presented in Tables 1 and 2. The rats with denervated kidney (groups 5 through 8) had higher baseline RBF (approximately 20%), GFR (approximately 20%), and sodium (approximately 15%) and water (approximately 30%) excretion than rats with innervated kidney (groups 1 through 4). The effect of renal denervation on pressure-diuresis is depicted in Figures 1 and 2. The group 5 rats (denervated kidney) excreted more sodium and water at 140 mmHg of RPP than group 1 rats (innervated kidney) due to an increased filtered load.

The interactions between renal nerves and NO on renal
Table 1. Effect of NAME (3.7 or 37 nmol/kg per min) in rats with innervated kidney\(^a\)

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP (mm Hg)</th>
<th>RBF (ml/min per gk)</th>
<th>GFR ((\mu)l/min per gk)</th>
<th>V ((\mu)l/min per gk)</th>
<th>UNaV ((\mu)Eq/min per gk)</th>
<th>FENa (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cont</td>
<td>Exp</td>
<td>Cont</td>
<td>Exp</td>
<td>Cont</td>
<td>Exp</td>
</tr>
<tr>
<td>1 Control</td>
<td>130±1</td>
<td>128±2</td>
<td>6.9±1.1</td>
<td>7.1±1.1</td>
<td>1052±38</td>
<td>1045±40</td>
</tr>
<tr>
<td>inn.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 NAME (3.7 inn.)</td>
<td>135±4</td>
<td>138±4</td>
<td>6.4±0.2</td>
<td>6.2±0.3</td>
<td>1039±101</td>
<td>1036±66</td>
</tr>
<tr>
<td>3 NAME (37 inn.)</td>
<td>132±3</td>
<td>145b±3</td>
<td>6.7±0.4</td>
<td>5.7b±0.4</td>
<td>952c±43</td>
<td>848±55</td>
</tr>
<tr>
<td>4 NAME (185 inn.)</td>
<td>137±2</td>
<td>167b±2</td>
<td>6.5±0.2</td>
<td>5.2b±0.4</td>
<td>1094±86</td>
<td>1096±58</td>
</tr>
</tbody>
</table>

\(^{a}\) Mean ± 1 SEM is presented. NAME, \(N^\circ\)-nitro-l-arginine methyl ester; MAP, mean arterial pressure; RBF, renal blood flow; V, urine flow; UNaV, absolute sodium excretion; FENa, fractional sodium excretion; gk, gram kidney weight; Cont, control; Exp, experimental; inn., innervated.

\(^{b}\) Significant difference from the control value of the same group.

\(^{c}\) Significant difference from the control value of rats with innervated kidney.

Table 2. Effect of renal denervation on the actions of NAME (3.7 or 37 nmol/kg per min)\(^a\)

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP (mm Hg)</th>
<th>RBF (ml/min per gk)</th>
<th>GFR ((\mu)l/min per gk)</th>
<th>V ((\mu)l/min per gk)</th>
<th>UNaV ((\mu)Eq/min per gk)</th>
<th>FENa (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cont</td>
<td>Exp</td>
<td>Cont</td>
<td>Exp</td>
<td>Cont</td>
<td>Exp</td>
</tr>
<tr>
<td>5 Control</td>
<td>133±3</td>
<td>130±4</td>
<td>8.1±0.6(^b)</td>
<td>8.3±0.5(^b)</td>
<td>1205±63(^b)</td>
<td>1220±55(^b)</td>
</tr>
<tr>
<td>den.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 NAME (3.7 den.)</td>
<td>138±3</td>
<td>142±4</td>
<td>8.2±0.5(^c)</td>
<td>7.4±0.5</td>
<td>1204±67(^c)</td>
<td>1047±38</td>
</tr>
<tr>
<td>7 NAME (37 den.)</td>
<td>135±4</td>
<td>143±4(^d)</td>
<td>8.6±0.6(^e)</td>
<td>6.3±0.5(^d)</td>
<td>1103±81(^e)</td>
<td>1025±52</td>
</tr>
<tr>
<td>8 NAME (185 den.)</td>
<td>133±3</td>
<td>169±3(^d)</td>
<td>8.4±0.3(^f)</td>
<td>5.0±0.3(^d)</td>
<td>1010±40</td>
<td>751±14(^d)</td>
</tr>
</tbody>
</table>

\(^{a}\) Mean ± 1 SEM is presented. den., denervated. Other abbreviations as in Table 1.

\(^{b}\) Significant difference from the control value of rats with innervated kidney.

\(^{c}\) Significant difference from the same value of rats with innervated kidney and treated with NAME (3.7 nmol/kg per min).

\(^{d}\) Significant difference from the control value of the same group.

\(^{e}\) Significant difference from the same value of rats with innervated kidney and treated with NAME (37 nmol/kg per min).

\(^{f}\) Significant difference from the same value of rats with innervated kidney and treated with NAME (185 nmol/kg per min).
Nitric Oxide and Pressure-Diuresis

Figure 2. Comparison of the relationship of renal perfusion pressure with renal blood flow (top) and GFR (bottom) of control rats with innervated (Control inn.) or denervated (Control den.) kidneys, and also of rats treated with NAME with innervated (3.7 nmol/kg per min inn.) or denervated (3.7 nmol/kg per min den.). Mean ± 1 SEM is presented. *, significant difference from the same value of control rats with innervated kidney. †, significant difference from the same value of rats with innervated kidney and treated with NAME (3.7 nmol/kg per min). #, significant difference from the same value of control rats with denervated kidney.

Figure 1. Comparison of the relationship of renal perfusion pressure with urine flow (top), sodium excretion (middle), and fractional sodium excretion (bottom) of control rats with innervated (Control inn.) or denervated (Control den.) kidneys, and also of rats treated with N-nitro-L-arginine methyl ester with innervated (NAME, 3.7 nmol/kg per min inn.) or denervated (NAME, 3.7 nmol/kg per min den.) kidney. Mean ± 1 SEM is presented. #, significant difference from the same value of control rats with innervated kidney. †, significant difference from the same value of rats with innervated kidney and treated with NAME (3.7 nmol/kg per min).

Function are presented in Tables 1 and 2. The low dose of NAME used (3.7 nmol/kg per min) had no effect on arterial pressure, RBF, or GFR in rats with innervated or denervated kidney (groups 2 and 6). However, this low dose of NAME reduced absolute (approximately 31%) and fractional (approximately 39%) sodium excretion only in rats with the renal nerves intact (group 2), but it had no effect in rats with denervated kidney (group 6). The medium dose of NAME used (37 nmol/kg per min) increased arterial pressure slightly and reduced RBF in rats with innervated or denervated kidney (groups 3 and 7, respectively). Although this medium dose of NAME reduced sodium (approximately 22%) and water (approximately 27%) excretion in rats with denervated kidney, this effect was more intense in rats with innervated kidneys (−50% and −45% for sodium and water excretion, respectively). NAME (3.7 and 37 nmol/kg per min) reduced fractional sodium (FENa) excretion only in rats with innervated kidney (groups 2 and 3, −49% and −44%, respectively), but it had no effect on FENa excretion in rats with denervated kidney. The high dose of NAME used (3.7 μmol + 185 nmollkg per min) increased arterial pressure by approximately 30 mmHg and lowered RBF in groups 4 and 8. GFR fell by 26%, and urine flow decreased by 44% only in rats with denervated kidneys. In addition, although NAME (3.7 μmol + 185 nmollkg per min) increased absolute (+30%) and fractional (+28%) sodium excretion in rats with innervated kidneys, it lowered absolute (−45%) and fractional (−29%) sodium excretion in rats with denervated kidneys.
The interactions between renal nerves and NO on pressure-diuresis are presented in Figures 1 through 6. NAME (3.7 nmol/kg per min) reduced absolute and fractional sodium excretion at high RPP (−21% and −25%, respectively) only in rats with innervated kidney (group 2, Figure 1). This low dose of NAME also reduced RBF in innervated and denervated kidneys at all RPP studied by approximately 2 ml/min per gk, but it did not affect GFR (Figure 2, groups 2 and 6). The medium dose of NAME used (37 nmol/kg per min) lowered absolute sodium and water excretion and also fractional sodium excretion in rats with innervated (−50%, −38%, and −40% at 140 mmHg of RPP, respectively) and denervated (−35%, −37%, and −22%, respectively) kidney (groups 3 and 7, Figure 3). This effect of NAME on sodium excretion was more pronounced in rats with innervated kidney, in which the pressure-diuresis and natriuresis response was severely blunted. NAME (37 nmol/kg per min) also decreased RBF (approximately −2 ml/min per gk) and GFR (approximately −15%) at all RPP studied in groups 3 and 7 (Figure 4). The high dose of NAME used in the present study (3.7 μmol + 185 nmol/kg per min) reduced urine flow and absolute and fractional sodium excretion at all RPP studied, essentially abolishing the pressure-diuresis and natriuresis response (Figure 5) in rats with innervated or denervated kidney.

Discussion

In the present study, the control group of rats with innervated kidney (group 1) had lower baseline values of RBF, GFR, and sodium and water excretion compared to rats with denervated kidney (group 5). In addition, renal denervation shifted the pressure-natriuresis response toward lower pressures, demonstrating that in this experimental model renal sympathetic tone is not abolished, and is modulating renal function, despite the fact that these animals were infused with norepinephrine. Similar results were reported previously by Roman and Cowley (13), who originally described the experimental model used in the present study. In this model, RPP is varied by manipulation of peripheral resistances while the hormonal influences on the kidney are maintained constant at physiologically high levels by intravenous infusion of norepinephrine, vasopressin, aldosterone, and cortisol (13). The infusion of norepinephrine is necessary to raise and maintain elevated arterial pressure above baseline by occluding the mesenteric and celiac arteries; without the hormonal intravenous infusion, arterial baroreflexes buffer all changes in vascular resistances and it is impossible to increase and hold constant RPP long enough to perform a clearance period. In a previous study, we have shown that this hormonal clamp does no appear to affect the response of the renal cortical and medullary circulations to NAME or to changes in RPP (10). Although it cannot be excluded that the infusion of norepinephrine may reduce the endogenous sympathetic tone, there is no other method widely accepted to study pressure-diuresis in rats.

The lower dose of NAME used (3.7 nmol/kg per min) reduced absolute and fractional sodium excretion only in rats with innervated kidney, but it had no effects in rats that underwent renal denervation. In addition, this low dose of NAME shifted the pressure-natriuresis relationship to the right, reducing absolute and fractional sodium excretion at high RPP (140 mmHg) only in rats with the renal nerves intact. These results indicate that the antinatriuretic effects of NAME are potentiated by the presence of renal nerves, and they are in

![Figure 3. Comparison of the relationship of renal perfusion pressure with urine flow (top), sodium excretion (middle), and fractional sodium excretion (bottom) of control rats with innervated (Control inn.) or denervated (Control den.) kidneys, and also of rats treated with NAME with innervated (37 nmol/kg per min inn.) or denervated (37 nmol/kg per min den.) kidney. Mean ± 1 SEM is presented. *, significant difference from the same value of control rats with innervated kidney. †, significant difference from the same value of control rats with denervated kidney. ‡, significant difference from the same value of rats with innervated kidney and treated with NAME (37 nmol/kg per min).]
agreement with previous reports showing that acute NO synthesis blockade produced renal vasoconstriction and increased renal sympathetic outflow (7,8).

The medium dose of NAME used (37 nmol/kg per min) raised arterial pressure by approximately 10 mmHg, and it reduced RBF and sodium and water excretion in rats with innervated or denervated kidneys. However, fractional sodium reabsorption was reduced only in innervated kidneys by NAME (37 nmol/kg per min). In addition, NO synthesis blockade with NAME (37 nmol/kg per min) shifted pressure-diuresis and natriuresis toward higher pressures in rats with or without the renal nerves, but these effects were more pronounced in innervated than in denervated kidneys. Although RBF fell similarly after NAME in rats with or without the renal nerves, as reported previously (30), at high RPP (140 mmHg) GFR, water and sodium excretion, and also fractional sodium excretion were lower in rats with the renal nerves intact. In fact, the pressure-diuresis and natriuresis responses were severely impaired in rats with innervated kidneys after NAME (37 nmol/kg per min). These observations are compatible with the hypothesis that the effects of NO synthesis blockade on pressure-natriuresis are partly mediated through potentiation of the renal sympathetic nerves.

The results of the present study obtained using low (3.7 nmol/kg per min) and medium (37 nmol/kg per min) doses of NAME are in apparent contradiction with previous reports showing that NO synthesis inhibition caused diuresis and natriuresis due to decreased proximal tubular reabsorption when RPP is allowed to rise (26–28). The fact that this phenomenon is more prominent in innervated kidneys led to the hypothesis
renal nerves (10). This effect may contribute to the more intense effect of NAME (3.7 and 37 nmol/kg per min) on pressure-diuresis on innervated than in denervated kidneys, because the impairment of pressure-diuresis produced by NAME has been attributed to its medullary vasoconstrictor effect (15). However, the data in the present study are compatible with the point of view that high doses of NAME produce such an intense peripheral vasoconstriction and arterial hypertension that sympathetic outflow is reflexly suppressed, and the intense impairment of pressure-diuresis caused by NOS blockade is in this case unaffected by renal denervation.

In the past few years, considerable advances have been made in our understanding of the role of the pressure-diuresis and natriuresis response controlling sodium excretion in normal conditions and in hypertension. It has been recently postulated that NO may be the mediator linking increases in arterial pressure with reduced tubular sodium reabsorption (2,3,15). According to this hypothesis, arterial pressure is dependent on the mechanisms regulating the renal actions of NO. The results of the present study indicate that low doses of NO synthesis antagonists shift pressure-diuresis toward higher pressures and may cause arterial hypertension partly by potentiation of the renal sympathetic nervous system. However, high doses of NOS blockers severely impair pressure-diuresis with no participation of renal nerves.

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**References**


