Role of Neuronal Nitric Oxide Synthase in Dahl Salt-Sensitive Hypertension

Dunyong Y. Tan, Shumei Meng, R. Davis Manning, Jr

Abstract—The goal of this study was to determine the role of neuronal nitric oxide synthase (nNOS) in the arterial pressure, renal hemodynamic, and renal excretory changes that occur in Dahl salt-resistant (DR) and salt-sensitive (DS) rats during changes in Na intake. Fifty-three DR and DS rats/Rapp strain of 7 to 8 weeks of age with indwelling arterial and venous catheters were subjected to low (0.87 mmol/d) or high (20.6 mmol/d) Na intake beginning 2 days before the start of the control period. Measurements were made during a 5-day control period followed by a 5-day period of nNOS inhibition with intravenous 7-nitroindazole (7NI, 1.67 mg · kg⁻¹ · h⁻¹) or vehicle infusion. After 5 days of 7NI, mean arterial pressure increased to 120±6% control in the DR-high Na, 7NI rats compared with 98±1% control (P<0.05) in the DR-high Na alone rats. After 5 days of 7NI, DS-high Na rats, which had a control arterial pressure 31 mm Hg higher than the comparable DR rats, increased their arterial pressure to 114±3% control, which was not significantly different from the DS-high Na alone pressure of 110±2% control. No significant changes occurred in glomerular filtration rate, effective renal plasma flow, urinary Na excretion, or urine volume because of 7NI. However, plasma renin activity decreased significantly in DR and DS rats on low Na intake with 7NI infusion. The data demonstrate that the highly salt-resistant DR rat became salt-sensitive during nNOS inhibition with 7NI. However, the arterial pressure of the DS rat was not affected by 7NI. This suggests that nitric oxide produced by nNOS in the DR rat normally helps to prevent salt-sensitive hypertension and that low functional levels of nNOS in the DS rat may contribute to its salt-sensitivity. (Hypertension. 1999;33[part II]:456-461.)

Key Words: arterial pressure ■ glomerular filtration rate ■ renal hemodynamics ■ sodium ■ renin ■ urine

The arterial pressure of some people with hypertension is very sensitive to changes in Na intake and they have been classified as "salt-sensitive," but the cause of the salt-sensitivity is not known. A recent preliminary report showed that salt-sensitive humans release less nitric oxide (NO) during NO agonist administration compared with patients with salt-resistant essential hypertension. 1 In Dahl salt-sensitive (DS) rats, our laboratory and others2,3 showed that NO production is decreased during high Na intake compared with Dahl salt-resistant (DR) rats. L-Arginine administration to DS rats increased NO production and prevented salt-sensitive hypertension.3,4 Therefore, a decrease in NO production may be partly responsible for salt-sensitive hypertension in humans and DS rats. However, the relative importance of the various isoforms of NO synthase in causing salt-sensitive hypertension is not known.

Recent studies have shown that NO produced by neuronal NO synthase (nNOS) may play a significant role in preventing salt-sensitive hypertension in normal rats. Increases in Na intake caused an increase in renal medullary nNOS protein in Sprague-Dawley (SD) rats.5 Even though the SD rat is normally salt-resistant, inhibition of nNOS in the renal medulla of these rats on a high Na diet caused salt-sensitive hypertension.6 However, whether nNOS plays an important role in Dahl salt-sensitive hypertension is not known.

Preliminary results in our laboratory (R.D. Manning, unpublished data, 1998) showed that a high Na diet resulted in a much greater increase in renal medullary nNOS protein in DR rats than in DS rats. We hypothesize that NO produced by nNOS in the DR rat helps to prevent salt-sensitive hypertension, and nNOS inhibition in the DR rat will make it salt-sensitive like the DS rat. Studies were conducted in DR and DS rats, Rapp strain, during a 5-day control period and a 5-day period of nNOS inhibition with continuous intravenous infusion of 7-nitroindazole Na salt (7NI) at 1.67 mg · kg⁻¹ · h⁻¹. Rats were subjected to either low or high Na intake, and cardiovascular and renal functional measurements were made throughout the experiment.

Methods

Animal Preparation, Experimental Measurements, and Instrumentation

Experiments were conducted in 53 conscious 7- to 8-week-old male DR or DS rats, Rapp strain (Harlan Sprague Dawley, Indianapolis, IN). The project had the approval of the local Institutional Animal Committee. Rats were received when they were 5 to 6 weeks old,
surgery was performed when the rats reached a weight of 200 g, and experiments were begun 1 week later when the rats had a weight of \(\approx 220\) g. Aortic and vena cava catheters were implanted as we have done before, and 15 mL/d of either hypotonic or hypertonic saline containing antibiotics (Mezlin, 30 mg/d; Miles; penicillin G, 5000 U/d) was infused intravenously. Rats were placed in a temperature-controlled room with a 12-hour light/dark cycle.

Both catheters exited the body in the scapular region and were connected to a dual-channel infusion swivel (Instech Laboratories, Inc). Saline solutions were infused with a Harvard apparatus syringe pump through a 0.22-µm filter (Cathivex, Millipore Corp). The arterial catheter was filled with 1000 U/mL heparin and connected to a Cobe pressure transducer and in turn to a pressure amplifier. Pulsatile arterial pressure signals from the amplifier were sent to a digital computer through an analog-to-digital converter and were sampled at 500 Hz for 4 seconds of each minute throughout the entire 24-hour period. Heart rate and arterial pressure were determined from these data samples.

The arterial and urinary output volume was measured daily. Urinary Na concentration was determined by flame photometry and plasma renin activity by radioimmunoassay. Urinary nitrate plus nitrite excretion (UNOx) was determined using the Greiss reaction and nitrate reductase from *Escherichia coli* as before. Technical problems prevented determination of UNOx in DS-high Na, 7NI rats. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were determined by measuring the radioactivity and aminohippurate concentration of a 4-hour fasting plasma sample after a 24-hour period of intravenous infusion of [125I]iothalamin (Isotec Diagnostics) and aminohippurate Na (Merck). Steady state is easily achieved in less than 12 hours of intravenous infusion. A sample of the infused was analyzed for [125I] and aminohippurate concentration, and infusion rates of iothalamin and hippurate were calculated and substituted for the urinary excretion rates of these substances. This constant-infusion method for determining GFR and ERPF gives the same values as urinary clearance techniques.

### Experimental Protocols

The following 8 groups of rats were studied: DR-low Na, 7NI (n = 7); DS-low Na, 7NI (n = 7); DR-high Na, 7NI (n = 8); DS-high Na, 7NI (n = 8); DR-low Na alone (n = 7); DS-low Na alone (n = 5); DR-high Na alone (n = 6); and DS-high Na alone (n = 7). During a 7-day surgical recovery period, all rats were fed a low Na food (Teklab Test Diets), and a low Na intake of 0.87 mmol/d was maintained by infusing intravenously 15 mL/d of 0.3% NaCl plus ingestion of 0.10 mmol/d of the low Na food. Some of the rats received a high Na intake of 20.6 mmol/d of Na (15 mL/d IV of 8% NaCl plus the low Na food beginning 2 days before the control period). Data were collected during a 5-day control period followed by a 5-day period of vehicle infusion or nNOS inhibition with intravenous infusion of 7NI.

### Selectivity of 7NI

Several studies have indicated that 7NI selectively inhibits nNOS without affecting endothelial NO synthase (eNOS) or inducible NO synthase (iNOS). Inhibition of nNOS with 7NI had no effect on the carbachol-induced vasorelaxation in aortic rings or in acetylcholine-induced vasodilation in anesthetized rats infused intravenously with a large dose of 7NI (25 mg/kg). Administration of 40 mg·kg⁻¹·d⁻¹ of 7NI caused no significant changes in either arterial pressure or renal blood flow in SD rats. The dose of 7NI in the present study was the same as that used previously, and no change in renal plasma flow occurred. The above studies suggest that the 7NI doses used did not affect eNOS.

Other studies indicate that 7NI has little effect on iNOS. Selective inhibition of nNOS compared with iNOS was achieved with 7NI in rat lung cells. Infusion of either 7NI or antisense nucleotide for nNOS mRNA into the renal medulla caused salt-sensitive hypertension in SD rats. Both groups experienced very similar increases in arterial pressure and decreases in total medullary NO synthase activity, and no change occurred in iNOS activity in the antisense group. This suggests that the increase in salt-sensitivity was dependent on decreases in nNOS activity and not iNOS activity.

### Data Analysis

Data from DR-7NI groups were statistically compared with the DR-Na alone groups at the same experimental time in each experimental period. This was also done for each DS rat group. Both DR- and DS-Na alone groups served as timed controls for their respective 7NI groups. In addition, statistical comparisons were also made between DR- or DS-high Na, 7NI groups and comparable low Na, 7NI groups at the same experimental time. Statistics were performed by first using a 2-way analysis of variance for repeated measures followed by a 1-way analysis of repeated measures for each group and a Newman-Keuls test for post hoc analysis at each experimental time point. Data were considered to be statistically different from control when \(P<0.05\). All data are expressed as mean±SE.

### Results

#### Arterial Pressure Responses to nNOS Inhibition

The top panel of Figure 1 shows that 7NI caused mean arterial pressure (MAP) of the DR rats to increase significantly during high Na intake, and by day 10 MAP reached a value of 120±6% control compared with a pressure of 98±1% control (\(P<0.05\)) in DR-high Na alone rats. Note also that DR-low Na, 7NI rats did not significantly increase their MAP when compared with DR rats on low Na alone. Therefore, the DR rat became salt-sensitive during nNOS inhibition.

The bottom panel of Figure 1 shows that by day 10 MAP increased to 114±3% control in the DS-high Na, 7NI rats and 110±2% control in the DS-high Na alone group, but their responses were not significantly different from each other. Table 1, which shows control values for MAP, GFR, and ERPF, indicates that MAP was elevated in all DS rats on high Na, because they had been on high Na intake starting 2 days
before the control period. In fact, the arterial pressure on day 10 in the DS-high Na alone rats was 40 mm Hg higher than the control pressure of the DS-low Na alone group, demonstrating the salt-sensitivity of the DS rats.

GFR Responses to nNOS Inhibition
The top and bottom panels of Figure 2 show that GFR in all 8 groups of rats stayed close to their respective control values during the nNOS inhibition. Neither the DR- nor DS-high Na, 7NI groups were significantly different from either the DR- or DS-high Na alone groups, respectively, or their corresponding low Na, 7NI groups.

ERPF Responses to nNOS Inhibition
The top and bottom panels of Figure 3 show that ERPF for all 8 groups of rats remained close to their respective control values during the nNOS inhibition period. The ERPF of the DR-high Na, 7NI group was not significantly different from the DR-high Na alone group or the DR-low Na, 7NI group. Likewise the DS groups did not experience significant changes in ERPF during the nNOS inhibition period.

Urinary Na Output Responses to nNOS Inhibition
Figure 4 shows that urinary Na excretion in the DR-high Na, 7NI rats was not significantly different from the Na excretion of the DR-high Na alone rats. Likewise the urinary Na excretion of the DR-low Na, 7NI rats was not significantly different from the DR-low Na group. In a similar way, 7NI had no significant effect on Na excretion of DS rats on low or high Na intake.

Urinary Volume Output Responses to nNOS Inhibition
Figure 5 shows that there were no significant effects of 7NI on urinary volume output in either the DR or DS groups on either high or low Na intake when compared with their respective timed control Na alone groups. Urine volume was significantly higher in the DR- and DS-high Na groups than in the low Na groups as expected.

Plasma Renin Activity Responses to nNOS Inhibition
In the DR-low Na, 7NI rats, plasma renin activity decreased significantly on day 10 compared with the DR-low Na group (Figure 6). Also, on day 10 the renin activity of the DS-low Na, 7NI group was significantly less than that of the DS-low Na alone group. The average control renin activity in the DR-low Na group was 3.2±0.1 ng angiotensin I/mL·h, which was significantly different from the average control value of 2.0±0.1 ng AI/mL·h in the DS-low Na group.

Table 2 shows that there were no significant effects of 7NI on heart rate in the DR or DS rats during high or low Na intake.
intake when compared with their respective timed control Na alone groups. On day 10 high Na intake increased UNOx significantly in rats that received Na alone and in 7NI rats. UNOx in the DR-high Na, 7NI group was not significantly different from the DR-high Na alone group.

**Discussion**

The major new finding in this study is that nNOS inhibition makes the normally salt-resistant DR rat salt-sensitive. This fact was confirmed by the increase in MAP in the DR-high Na, 7NI group and the lack of increase in MAP in both the DR rats on high Na alone and DR rats on low Na plus 7NI. However, MAP of the DS rat was not significantly affected by 7NI during high Na intake, which suggests that the functional effects of nNOS may be blunted in the DS rat compared with the DR rat. Inhibition of nNOS did not significantly change GFR, ERPF, urinary Na excretion, or urinary volume in the DR or DS rats, but plasma renin activity decreased during 7NI infusion in both DR and DS rats on low Na intake.

Our recent studies have shown that NO production is decreased in DS rats on high Na intake compared with DR rats, and the UNOx data in Table 2 confirm these previous findings. Infusion of L-arginine intravenously increased NO release in DS rats and prevented the blunted renal pressure natriuresis.

Therefore, NO produced by one or more of the NOS isoforms can decrease salt-sensitivity, and previous studies have shown that iNOS may achieve this. Our data suggest that NO produced by nNOS also reduces salt-sensitivity in DR rats, because nNOS inhibition in DR rats on high Na intake caused increased Na-sensitivity.

The increase in salt-sensitivity in DR rats during nNOS inhibition could have been related to several factors. First, changes in nNOS activity in the brain likely occurred, which could have increased sympathetic nervous system output. However, a previous study in our laboratory showed that the effects of $N^\omega$-nitro-L-arginine methyl ester on the arterial pressure of conscious dogs was not affected by pretreatment with either α- or β-adrenergic blockers or a combination of these blockers.

Second, renal nNOS activity may have decreased during 7NI infusion. Changes in renal NO synthase, including changes in renal nNOS, can have profound effects on renal excretory ability. Both nNOS protein and mRNA have been found in several locations in the kidney, including inner and outer medullary collecting ducts, macula densa, glomerulus, vasa recta, and renal nerves. Functionally, nNOS blunts the tubuloglomerular feedback control of afferent arteriolar resistance and mediates the macula densa control of renin secretion. However, the effect of Na intake on renal nNOS synthesis is controversial. Renal cortical mRNA for nNOS increased in one study, but the functional effects of macula...
TABLE 2. Responses of Heart Rate (HR) and Urinary Nitrate+Nitrite Excretion (UNOx)

<table>
<thead>
<tr>
<th>UNOx, nmol/d</th>
<th>Low Na, 7NI</th>
<th>High Na, 7NI</th>
<th>Low Na Alone</th>
<th>High Na Alone</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, bpm</td>
<td></td>
<td></td>
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<tr>
<td>DR rats-control</td>
<td>437±9</td>
<td>420±9</td>
<td>420±6</td>
<td>414±3</td>
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<td>DR rats-day 10</td>
<td>427±11</td>
<td>418±7</td>
<td>422±3</td>
<td>404±7</td>
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<tr>
<td>DS rats-control</td>
<td>434±14</td>
<td>445±8</td>
<td>420±9</td>
<td>429±7</td>
</tr>
<tr>
<td>DS rats-day 10</td>
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<td>440±8</td>
<td>413±7</td>
<td>430±7</td>
</tr>
<tr>
<td>DR rats-control</td>
<td>5630±624</td>
<td>10 994±772</td>
<td>4790±801</td>
<td>10 059±1416</td>
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<tr>
<td>DR rats-day 10</td>
<td>6785±674</td>
<td>9122±1304†</td>
<td>5104±725</td>
<td>10 464±1685‡</td>
</tr>
<tr>
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<td>...</td>
<td>4934±660</td>
<td>6008±452</td>
</tr>
<tr>
<td>DS rats-day 10</td>
<td>7602±871</td>
<td>...</td>
<td>5411±195</td>
<td>6608±469‡</td>
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</tbody>
</table>

All data are mean±SE. There were no significant effects between 7NI and Na alone groups. †P<0.05, comparison of 7NI effects in high and low Na groups in the same type of rat; ‡P<0.05, high Na alone responses compared with low Na alone responses in the same type of rat on day 10.

densa nNOS increased in SD rats on high Na intake.20 Renal nNOS activity decreased after 4 weeks of high Na intake in DS rats of the Iwai strain,21 but these results are complicated by another study that showed that 3 weeks of high Na intake in DS rats caused renal damage,22 which could affect NO production.

Most studies on the renal effects of nNOS performed to date have focused on the role of macula densa nNOS. However, recent studies in SD rats have shown that the highest concentration of nNOS protein is in the renal medulla and that high Na intake markedly upregulates medullary nNOS protein.5 Also, renal medullary infusion of 7NI in SD rats on high Na intake caused a decrease in medullary nNOS activity and a salt-sensitive hypertension.6 Our preliminary experiments (R.D. Manning, unpublished observation, 1998) suggest that nNOS protein during high Na intake is lower in the medulla of the DS rat than in the DR rat. The above result suggests that upregulation of medullary nNOS protein during high Na intake may help to prevent salt-sensitive hypertension in the DR rat. This would also be consistent with results of the present study, which showed an increase in salt-sensitivity of DR rats during 7NI infusion without significant changes in renal hemodynamics, since changes in cortical nNOS would be expected to cause renal hemodynamic changes.

Changes in macula densa nNOS activity could also play a role in the present experiment; however, this activity, in contrast to medullary nNOS activity, may be more important during low Na intake. Indeed, both DR and DS rats on low Na intake demonstrated a significant decrease in plasma renin activity during 7NI, confirming the results of another study which showed that increases in renin release in Na-deprived rats were blocked by 7NI.13

Several other mechanisms could have been involved in increasing the salt-sensitivity of the DR rat during nNOS inhibition. Medullary nNOS inhibition may have increased Na retention in the distal part of the nephron, thus increasing blood volume. Arterial pressure would thus increase, which would elevate Na excretion back to normal. Therefore, nNOS inhibition would result in normal Na excretion but at the expense of an elevated arterial pressure, and indeed, this occurred in the present experiment, because an increased arterial pressure was necessary in the DR rat to excrete the high Na load during 7NI infusion. In addition, increased tubuloglomerular feedback could have occurred in the DR rats on 7NI and high Na, thus increasing renal vascular resistance,20 but whether this would increase arterial pressure in a salt-sensitive manner is not clear.

In summary, nNOS inhibition in the DR rat caused a salt-sensitive hypertension with no significant changes in GFR, ERPF, urinary Na excretion, or urinary volume, but a significant decrease in plasma renin activity in low Na groups. This suggests that nNOS normally plays an important role in the DR rat in preventing salt-sensitive hypertension and that a decrease in nNOS in the DS rat may be partly responsible for its salt-sensitivity.

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References


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