Salt-induced hypertension in Dahl salt-resistant and salt-sensitive rats with NOS II inhibition

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Rudd, M. Audrey, Maria Trolliet, Susan Hope, Anne Ward Scribner, Geraldine Daumerie, George Toolan, Timothy Cloutier, and Joseph Loscalzo. Salt-induced hypertension in Dahl salt-resistant and salt-sensitive rats with NOS II inhibition. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H732–H739, 1999.—Although recent evidence suggests that reduced nitric oxide (NO) production may be involved in salt-induced hypertension, the specific NO synthase (NOS) responsible for the conveyance of salt sensitivity remains unknown. To determine the role of inducible NOS (NOS II) in salt-induced hypertension, we treated Dahl salt-resistant (DR) rats with the selective NOS II inhibitor 2-amino-5,6-dihydro-6-methyl-4H-1,3-thiazine (AMT) for 12 days. Tail-cuff systolic blood pressures rose 29 ± 6 and 42 ± 8 mmHg in DR rats given 150 and 300 nmol AMT/h, respectively (P < 0.01, 2-way ANOVA) after 7 days of 8% NaCl diet. We observed similar results with two other potent selective NOS II inhibitors, S-ethylisourea (EIT) and N-[3-(aminomethyl)benzyl]acetamidine hydrochloride (1400W). Additionally, AMT effects were independent of alterations in endothelial function as assessed by diameter change of mesenteric arterioles in response to methacholine using videomicroscopy. We, therefore, conclude from these data that NOS II is important in salt-induced hypertension.

salt-sensitive hypertension; Dahl rats; N\(^{\circ}\)-nitro-L-arginine methyl ester; 2-amino-5,6-dihydro-6-methyl-4H-1,3-thiazine; N-[3-(aminomethyl)benzyl]acetamidine hydrochloride

The etiology of salt-induced hypertension remains an enigma. In recent years, nitric oxide (NO) has been implicated in the development of hypertension in the Dahl rat model of salt-induced hypertension (5, 6, 46, 47). Specifically, urinary nitrite/nitrate as well as cGMP levels rise after an increase in NaCl intake in Dahl salt-resistant (DR) (6, 47) and normal Sprague-Dawley rats (46). Additionally, other investigators have shown a salt-induced increase in arterial pressure during NO synthase (NOS) inhibition with the nonselective NO synthase (NOS) inhibitor N\(^{\circ}\)-nitro-L-arginine methyl ester (L-NAME) (43). Although these studies suggested a role for NO in the development of salt-sensitive hypertension, the particular enzymatic source of NO has not been determined.

Three NOS isoforms have thus far been described (17). Neural and endothelial NOS (NOS I/Nos1 and NOS III/Nos3, respectively) are constitutively expressed and are calcium dependent, whereas the calcium-independent isoform (NOS II/Nos2) is expressed after induction with cytokines and other agonists. Interestingly, there are several tissues that appear to express NOS II constitutively, including the kidney (4, 28, 35). L-NAME inhibits all three isoforms nonselectively (34), thus making it difficult to determine which isoform may be the critical source of NO in salt-sensitive states. However, there is some suggestion that the NOS II isoform may be involved in Dahl salt-sensitive (DS) hypertension (6, 7). Chen and Sanders (6), in addition to showing that DS rats make less NO, also demonstrated that DS rats have reduced NO synthesis derived from aortic smooth muscle cell NOS II (7). These authors also reported a decrease in the prevention of salt-induced hypertension after L-arginine administration with dexamethasone treatment (6). Dexamethasone is a nonselective inhibitor of gene transcription including the Nos2 gene (55). Additionally, proximal tubule epithelial cell Na\(^{+}\)-K\(^{+}\)-ATPase is inhibited after induction of NO production with cytokine treatment, suggesting a modulatory role for NOS II-derived NO in sodium excretion (15).

We therefore proposed that salt-induced hypertension results from an impaired or deficient inducible NOS system. To test this hypothesis, we treated DR rats with the specific NOS II inhibitor 2-amino-5,6-dihydro-6-methyl-4H-1,3-thiazine (AMT) followed by a diet containing 80 g/kg (8%) NaCl (37). We reasoned that if the NOS II system were involved, the DR rats would become hypertensive during a high-salt diet and the DS rats, which presumably have an intrinsically abnormal NOS II system (7), would become more responsive to salt intake.

METHODS

Animals. Male DR and DS rats (5–6 wk old) were purchased from Harlan Sprague-Dawley ( Indianapolis, IN). After an acclimation period of 3–5 days, baseline tail-cuff systolic blood pressure (BP) was obtained using an IITC model monitoring system (Woodland Hills, CA).

L-NAME study. To determine whether or not a conventional NOS inhibitor could confer salt sensitivity to control DR rats, we treated DR rats with L-NAME with and without the addition of a diet containing 80 g/kg (8%) NaCl (Harlan Teklad, Madison, WI). Animals (n = 36) received L-NAME (0.172 mmol · kg\(^{-1}\) · day\(^{-1}\) in drinking water) for a 3-wk period. During the first week of L-NAME treatment, all rats remained on a regular-salt diet [4 g/kg (0.4%) NaCl; Purina Mills, St. Louis, MO] as during the baseline measurements. However, during the last 2 wk of L-NAME treatment, the rats either remained on regular-salt (0.4% NaCl) chow (n = 9) or...
were changed to 8% NaCl rat chow (n = 12). L-NAME concentration was appropriately adjusted during the high-salt diet, when water intake was increased to maintain the assigned dosage. Tail-cuff BP measurements were made at designated times (baseline and 1, 2, and 3 wk after initiation of L-NAME treatment) throughout the 3-wk period. We were only able to examine the effect of a 10 mg/kg dose (0.172 mmol·kg$^{-1}$·day$^{-1}$) of L-NAME on salt-induced hypertension in the DS rat during low- and high-salt diets. We were unable to complete the high-dose (40 mg·kg$^{-1}$·day$^{-1}$) study because of the extensive morbidity observed within this strain during treatment. DR and DS rats maintained on regular (0.4% NaCl) rat chow or high-salt (8% NaCl) chow served as time course controls.

Selective NOS II inhibition study. All animals were maintained on a diet containing 1.2 g/kg NaCl (0.12%) as used by the Harlan facility. Once baseline pressures were established, the animals were implanted with 14-day delivery minipumps (Alzet; Alza, Palo Alto, CA) containing AMT (0, 150, or 300 nmol/h; Tocris Cookson, St. Louis, MO). These doses correspond to 0, 0.025, and 0.05 mg/h and are comparable to in vivo doses previously used (41, 51). BP was monitored on days 1, 3, and 5 of the AMT administration. On day 5 of AMT treatment, rats either remained on the low-salt (0.12%) diet (DR, n = 11) or were changed to 8% NaCl rat chow (DR, n = 18). BP was monitored on days 7, 10, and 12 of AMT administration, which corresponds to days 2, 5, and 7 of the high-salt diet.

In another series of rats, the selective NOS II inhibitors S-ethylisourea (EIT; Tocris Cookson, Baldwin, MO) and N-[3-(amonomethyl)benzyl]acetamide dihydrochloride (1400W; Calbiochem, La Jolla, CA) were given (13, 14, 37). The protocol was exactly as that used for AMT. The doses used were 0.075 mg/h (380 nmol/h) and 0.008 mg/h (35 nmol/h) for EIT (n = 3) and 1400W (n = 5), respectively. DR rats maintained on a 0.12% NaCl diet during AMT or 1400W treatment served as time course controls.

Videomicroscopy. To ascertain whether AMT, at a dose that induced an increase in BP, impaired endothelium-dependent relaxation factor or endothelial NO release, we measured mesenteric microvessel relaxation after superfusion of methacholine (Sigma, St. Louis, MO). Three treatment groups were evaluated: (1) rats given 150 nmol/h of AMT and a high-salt diet (n = 7), L-NAME and high-salt diet (n = 3), or high-salt diet alone (n = 3). The AMT-treated rats were studied between days 10 and 12 of AMT treatment, which corresponded to days 5–7 of the high-salt diet, whereas L-NAME-treated and control rats were studied between days 7 and 14 of the high-salt diet. This represented days 14–21 of L-NAME administration in the L-NAME group. Rats were anesthetized with a mixture of ketamine (87 mg/kg), xylazine (13 mg/kg), and acepromazine (2 mg/kg) given intraperitoneally. The animals were placed on a platform, and a small abdominal incision was made. A segment of the small intestine was very gently removed and spread over a pedestal on which a coverslip had been placed. Warmed saline was immediately placed over the exposed tissue. Care was taken not to traumatize or stretch the tissue. The platform was then placed on the microscope stage for viewing. The image magnified by a ×40 water-immersion objective was projected onto a monitor (Dage-MTI, Michigan City, IN) using an Attofluor high-intensity camera (Atto Instruments, Rockville, MD). The final screen image was magnified ×64,000. The mesentery was constantly superfused with 35°C saline or saline containing methacholine (0.001 or 0.01 M). The diameter of arterioles used ranged from 20 to 30 μm. Using a videocapitaler apparatus (Microcirculation Research Inst., Texas A&M College of Medicine, College Station, TX), we made baseline diameter measurements before changing to 0.001 M methacholine. Measurements were made again after 5 min of superfusion of the drug. The drug was washed out with saline before the 0.01 M dose of methacholine was given and a final diameter measurement was made.

Statistical analysis. Time course of BP responses and microvessel diameter changes were analyzed by one-way ANOVA, and treatment time course and microvessel response comparison analysis were performed using two-way ANOVA. Multiple comparison was made using either the Dunnett’s or Newman-Keuls test where appropriate. Values represent means ± SE. Multiple-time course treatment comparison was done by two-way ANOVA analysis.

RESULTS

L-NAME study. Because L-NAME treatment was previously shown to produce hypertension in Sprague-Dawley rats given a high-salt diet, we initially administered L-NAME [40 mg·kg$^{-1}$·day$^{-1}$ (0.172 mmol·kg$^{-1}$·day$^{-1}$)] to DR rats for a 3-wk period. One week of L-NAME administration increased systolic BP from a baseline of 119 ± 2 mmHg to 149 ± 4 mmHg in DR rats (Fig. 1). The addition of 8% NaCl further increased BP to 187 ± 8 mmHg after 7 days of the diet and 194 ± 7 mmHg by day 14 of the diet (P < 0.01, 1-way ANOVA). This represents a 72-mmHg increase from baseline and a 33-mmHg increase from the 7-day L-NAME treatment alone.

Because the 40 mg/kg dose caused a significant rise in baseline BP in DR rats, we also gave a dose of L-NAME that had minimal effects on baseline pressure. The dose chosen was 10 mg·kg$^{-1}$·day$^{-1}$. As seen in Fig. 1, this lower dose did not alter basal pressure. However, BP was significantly elevated after 14 days of high-salt diet (171 ± 7 vs. 108 ± 3 mmHg at baseline; P < 0.01, 1-way ANOVA). Because there appeared to be a delay in the development of hypertension in this group of animals, we continued the treatment until day 28. BP
increased to 193 ± 6 mmHg on day 28 (P < 0.01, 1-way ANOVA and Dunnett's test).

There was also a significant increase in BP in DR rats remaining on a regular-salt diet (0.4% NaCl) with the 40 mg/kg dose of L-NAME (Table 1; P < 0.01 vs. baseline, 1-way ANOVA and Dunnett's test); BP increased in these animals to 180 ± 3 mmHg by the third week of L-NAME administration during the regular-salt diet (P < 0.01, 1-way ANOVA). The lower-dose L-NAME (10 mg/kg) treatment did not produce a significant change in baseline BP in DR rats given 0.4% NaCl rat chow.

We also examined the effect of L-NAME on BP in DS rats on low- and high-salt diets. As shown in Table 2, BP increased in DS rats given 10 mg/kg L-NAME and 0.4% NaCl and remained elevated throughout the study. BP was also increased after 7 and 14 days of high-salt diet, which corresponds to days 14 and 21 of L-NAME (10 mg·kg⁻¹·day⁻¹) administration. Although BP rose with high-salt diet in DS rats, the rise was less than that seen with 0.4% NaCl. It is not clear why this is the case. Heart rate did not change significantly over time during L-NAME administration. It should be noted that the animals appeared less active and began losing weight when the high-salt diet was initiated.

There was no significant change in BP of DR rats on 0.4% NaCl rat chow; however, the 8% NaCl diet in DS rats caused a significant rise in BP by 15.5 mmHg on day 7 (P < 0.01, 1-way ANOVA). We were unable to continue the treatment beyond 7 days because of animal morbidity requiring euthanasia.

Table 1. Effect of L-NAME on systolic blood pressure in DR rats given a regular (0.4% NaCl)-salt diet

<table>
<thead>
<tr>
<th>L-NAME</th>
<th>n</th>
<th>Baseline</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mg/kg</td>
<td>3</td>
<td>115 ± 6</td>
<td>101 ± 11</td>
<td>108 ± 7</td>
<td>117 ± 15</td>
</tr>
<tr>
<td>40 mg/kg</td>
<td>6</td>
<td>118 ± 3</td>
<td>148 ± 6*</td>
<td>161 ± 8*</td>
<td>180 ± 3*</td>
</tr>
</tbody>
</table>

Values represent means ± SE; n, no. of rats. *P < 0.05 vs. baseline, 1-way ANOVA and Dunnett's test.

Table 2. Systolic blood pressure in Dahl salt-sensitive rats given L-NAME and regular (0.4% NaCl)- or high (8% NaCl)-salt diets

<table>
<thead>
<tr>
<th>L-NAME</th>
<th>NaCl</th>
<th>n</th>
<th>Baseline</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mg/kg</td>
<td>0.4%</td>
<td>3</td>
<td>133 ± 10</td>
<td>142 ± 8*</td>
<td>173 ± 8*</td>
<td>200 ± 3*</td>
</tr>
<tr>
<td></td>
<td>8%</td>
<td>6</td>
<td>124 ± 4</td>
<td>135 ± 8</td>
<td>159 ± 15*</td>
<td>185 ± 12*</td>
</tr>
<tr>
<td>40 mg/kg</td>
<td>0.4%</td>
<td>6</td>
<td>132 ± 4</td>
<td>196 ± 6</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values equal means ± SE; n, no. of rats. ND, not determined. 8% NaCl was begun on day 7 of L-NAME and continued until day 21. *P < 0.05 vs. baseline, 1-way ANOVA and Dunnett's test; † P < 0.05, t-test.

NOS II inhibition series. Because L-NAME effectively inhibits all three isoforms of NOS, we also treated animals with AMT, a selective inhibitor for the inducible form of NOS (15). AMT has been shown to be at least 100-fold more selective than conventional NOS inhibitors and 10- and 40-fold more selective for NOS II than NOS I and NOS III, respectively (15). The inhibitor was administered for 14 days by Alzet minipump in varying doses (0, 150, and 300 nmol/h). As shown in Fig. 2, these doses did not significantly alter BP in DR rats while they were maintained on a low-salt diet (0.12% NaCl) during the 12 days of BP monitoring. However, when DR rats were given an 8% NaCl diet, there was a dose-dependent increase in BP during AMT administration (Fig. 3). BP rose from a baseline of 126 ± 5 mmHg to 155 ± 6 and 166 ± 4 mmHg (150 and 300 nmol/h, respectively) by day 7 of an 8% NaCl diet (P < 0.05; 1-way ANOVA and Dunnett's test) compared with time course controls (P < 0.01, 2-way ANOVA and Newman-Keuls test).

To determine whether the effects of AMT were specific to NOS II inhibition, we also examined the effect of EIT and 1400W on BP response in DR rats on high-salt diets. Previous reports show that 1400W has a 285- and ~7,000-fold greater selectivity for NOS II than NOS I or NOS III, respectively, whereas EIT has a 20- and 30-fold greater affinity for NOS II than NOS I and NOS III, respectively (13, 37). The apparent inhibitory con-
stants for all three inhibitors are comparable (AMT, 3.6 nM; EIT, 13 nM; 1400W, 7 nM).

After the initiation of a high-salt diet, 1400W increased BP from a baseline of 118 mmHg to 139 ± 6 and 136 ± 2 mmHg on days 10 and 12, respectively (P < 0.05 vs. baseline) of drug treatment, which corresponded to days 5 and 7 of the 8% NaCl diet. Additionally, BP was significantly increased on day 12 of EIT from a baseline of 102 ± 9 mmHg to 128 ± 4 mmHg (P < 0.05, 1-way ANOVA, Dunnett's test). BP was unchanged in DR rats remaining on the low-salt diet (116 ± 2, 124 ± 4, 122 ± 2, and 129 ± 3 mmHg at days 0, 5, 10, and 12, respectively, of 1400W; P = not significant, 1-way ANOVA).

DS rats were also treated with AMT. Figure 4 shows the BP time course response in DS rats on a low-salt diet. The BP response in DS receiving 150 nmol/h of AMT was not statistically significant from that of DS rats on a low-salt (0.12 % NaCl) diet. Interestingly, although the lower dose of AMT did not produce an effect on systolic BP, the 300-nmol dose caused a significant increase in BP on days 10 and 12 while the animals were maintained on a low-salt diet (161 ± 4 and 164 ± 6 mmHg, respectively, vs. baseline; P < 0.05, 1-way ANOVA).

The BP time course response in the DS rats receiving the lower dose of AMT plus 8% NaCl diet was not different from that of DS animals on the 8% NaCl diet alone (Fig. 5). However, the higher dose caused an earlier and more pronounced rise in BP over time. Interestingly, the 300-nmol AMT dose, unlike all other groups, caused a significant rise in BP from baseline (from 135 ± 1 to 156 ± 6 mmHg; P < 0.05, 1-way ANOVA using Dunnett's multiple-comparison analysis). By days 5 and 7, BP had risen to 208 ± 6 and 209 ± 6 mmHg, respectively (P < 0.01 vs. baseline, 1-way ANOVA using Dunnett's test).

Videomicroscopy. To determine whether AMT was affecting endothelial function, we measured in vivo vessel relaxation after methacholine administration in DR rats receiving 150 nmol/h AMT and 8% NaCl diet. This dose did not cause a rise in systolic BP in DR rats on the low-salt diet (0.12% NaCl) but resulted in a rise in BP after an increase in dietary salt. As shown in Fig. 6, methacholine (0.001 M) produced vessel relaxation (as indicated by a positive percent change in diameter) in the AMT-treated group on the high-salt diet. Interestingly, AMT plus high-salt diet tended to have a greater vessel relaxation response with increasing doses of methacholine compared with high-salt diet alone. However, the dose response to methacholine was not statistically different between AMT-treated and untreated DR rats on 8% NaCl and, importantly, clearly showed no attenuation of endothelium-dependent relaxation in the presence of AMT. In contrast, L-NAME at a dose (10 mg/kg) that had little effect on basal BP in rats failed to show a vasodilatory response to methacholine. Thus there was profound inhibition of endothelium-depen-
We observed similar results with vasculature (1, 2, 22, 33). Mohaupt and colleagues (33) have demonstrated salt-induced hypertension with NO inhibition in DS rats on a high-salt diet, suggesting a role of TGF in DS rats. In addition, Singh and associates (48) observed an inverse relationship of NOS I to salt intake. Consequently, all three NOS isoforms contribute significantly to renal function and salt and water homeostasis.

However, NO concentration is highest within the medullary region of the kidney (15, 31). This is the principal location of NOS II and, thus, may explain the high NO levels because NOS II is a high-capacity enzyme for NO generation (24, 35). Other investigators found that cytokine-induced NO inhibits Na⁺/K⁺-ATPase (15, 31) and that the MTAL regulates sodium reabsorption by tonically expressed NOS II. Mattson and colleagues (30) reported that intramedullary infusion of L-NAME increases BP and achieves positive sodium balance independent of cortical blood flow, suggesting that the NOS II isoform may be very important in both volume and BP regulation.

Recently, Deng and Rapp reported that the Nos2 gene, which encodes for the NOS II protein, cosegregates with BP in the DS rat, whereas the Nos1 and Nos3 genes, which encode for NOS I and NOS III, respectively, do not (12, 34). However, Deng (9) subsequently reported that NOS II also was not associated with hypertension in DS rats but suggested that NO has important secondary physiological actions. Interestingly, Chen and Sanders (6) observed that dexamethasone, a nonspecific inhibitor of NOS II, prevented the L-arginine-induced increase in nitrate and cGMP excretion in DS rats on a high-salt diet, suggesting a role of NOS II in the salt-induced hypertension. These investigators also found that NO synthesis after cytokine treatment of smooth muscle cells is impaired in the DS rat (7). Additionally, Mattson and colleagues (30) reported that renal medullary NOS could significantly contribute to BP regulation, possibly through renal tubular actions (29). Several investigators showed that NO production after cytokine stimulation in renal tissue inhibits Na⁺/K⁺-ATPase activity (15, 31). Therefore, the NOS II isoform may be critically involved in both salt and water homeostasis, as well as BP regulation.

In the present studies, we used AMT, a specific NOS II inhibitor shown to be as much as 1,000-fold more selective than prototypical inhibitors, to study the role...
of inducible NOS in the development of salt-induced hypertension (37). At the doses used, there was no significant change in systemic BP in DR. However, when DR rats were placed on a high-salt (8% NaCl) diet, their systolic BP rose significantly by day 5. The response in DS rats was more pronounced, with systolic BP rising significantly during high-dose AMT administration even the rats were maintained on a low-salt (0.12% NaCl) diet. This response in the DS rats may be caused by a lower NO reserve or capacity as a result of an impaired NOS II system (7) or to a loss of selectivity of AMT at higher doses, with some inhibition of NOS III in DS rats potentiating the hypertensive response.

Although AMT is selective for NOS II, it is conceivable that other NOS isoforms may have been inhibited in vivo. For this reason we assessed in situ endothelial function directly using a videomicroscopy system. Methacholine superfusion revealed no diminished endothelium-dependent vasodilatation in the AMT-treated DR rats. Additionally, we used other NOS II inhibitors such as 1400W and EIT. 1400W has a 285- and 7,000-fold greater selectivity for NOS II than NOS I or NOS III, respectively. EIT increased BP only on day 12 of drug administration, which corresponds to day 7 of high-salt treatment. Thus vascular reactivity data suggest that the effects of AMT are not likely to be caused by NOS III inhibition. It should be noted, however, that the lowest dose of L-NAME that had little effect on basal pressure significantly impaired the vascular endothelial response to methacholine. Presently, there is no available technique to assess NOS I function in situ or in vivo during AMT treatment. Therefore, we are unable to rule out the possibility that NOS I may be involved in the results we observe in our present studies.

Neither the critical site(s) or location nor the degree of NOS II inhibition required to explain our observations is defined by these studies. It is likely that the target tissue would have to express NOS II constitutively. There are only a few tissues known to date that constitutively express NOS II. These are the spleen, fallopian tubes, and kidney (4, 28, 35). Alternatively, the elevated-salt diet may initiate or induce the expression of NOS II in tissues that do not constitutively express NOS II. However, there are no data at present that have shown such an effect of salt on NOS II expression. We believe the kidney is the most likely candidate for the site of action for these effects of AMT for the following reasons. First, the kidney constitutively expresses NOS II. Second, the kidney appears to be more responsive to NO inhibition than the systemic circulatory system (24). Specifically, investigators have noted that the kidney responds with an increase in renal vascular resistance (RVR) and a decrease in GFR, at NOS inhibitor concentrations that have little or no systemic effect (24, 45, 57). Simchon et al. (47) demonstrated an increase in RVR in DS rats on 8% NaCl for 8 wk but no appreciable change in nonrenal resistance. Last, the kidney is intimately involved in salt and water homeostasis as well as BP regulation (22, 30, 31, 47). Further investigation, however, is required before we can definitively prove that the renal NOS II system is most critical for the response to salt feeding.

The mechanism by which NOS II inhibition predisposes to salt-sensitive hypertension was not addressed in these studies. Results from numerous studies underline the importance of several neuroendocrine systems in the regulation of sodium excretion and BP, and NO has been shown to interact with several of these systems. There is evidence to suggest that NO modulates the renin-angiotensin system; however, this area of research remains quite controversial (16, 19, 20, 36, 39, 40, 45, 48). Additionally, others have demonstrated an intimate interaction of NO and the adrenergic system, particularly within the kidney (11, 12, 23, 38, 49). Other hormonal systems potentially involved in salt sensitivity, e.g., atrial natriuretic factor, prostaglandins, and endothelin, also have been shown to be modulated by NO (3, 18, 21, 26, 27, 32, 44, 50). Clearly, further studies are needed to determine whether the actions of NOS II involve one or more of these systems during salt-induced hypertension.

In summary, AMT (a novel NOS II inhibitor) conveys salt sensitivity to normally salt-resistant DR rats. This response is independent of alterations in endothelial NO activity. The response to AMT and increased salt was heightened or enhanced in the DS rats. We conclude from these data that NOS II is important in the development of salt-induced hypertension. However, more studies are needed to determine the precise molecular mechanism(s) by which NOS II inhibition leads to a predisposition to salt sensitivity.

The authors express gratitude to Jalna Ross and Stephanie Tribuna for expert and valued assistance in the preparation of this manuscript.

This work was supported in part by National Heart, Lung, and Blood Institute Grants P50-HL-55993-01, HL-58976, HL-53919, and HL-48743 and by a Merit Review Award from the US Department of Veterans Affairs.

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Received 23 December 1998; accepted in final form 22 March 1999.

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