Role of Nitric Oxide Synthesis in Salt-Sensitive Hypertension in Dahl/Rapp Rats

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Nitric oxide is a potent endogenous vasodilator that regulates arterial tone. A family of nitric oxide synthases uses l-arginine and l-homoarginine stereospecifically as substrates for nitric oxide production in vivo. By preventing expression of inducible but not constitutive nitric oxide synthases, glucocorticoids differentiate which enzyme in this family is the predominant source of nitric oxide generation in a given situation. We proposed that defective production of nitric oxide produces salt-sensitive hypertension in the Dahl/Rapp rat. Plasma concentrations of l-arginine, citrulline, and ornithine of salt-sensitive (SS/Jr) and salt-resistant (SR/Jr) rats on 8% sodium chloride chow for 1 week did not differ. However, intravenous infusion of l-arginine and l-homoarginine, but not d-arginine, increased urinary excretion of nitrate, the degradation product of nitric oxide, and simultaneously lowered blood pressure in hypertensive SS/Jr rats. Oral l-arginine also prevented development of hypertension and increased urinary excretion of cyclic GMP and nitrate in these rats. Dexamethasone, in a dose that prevented hypotension from parenteral injection of lipopolysaccharide, completely prevented the increase in excretion of cyclic GMP and nitrate, and hypertension resulted despite concomitant treatment with l-arginine. These studies supported an important role of dexamethasone-suppressible nitric oxide synthesis in the prevention of salt-sensitive hypertension in the Dahl/Rapp rat. (Hypertension. 1993;22:812-818.)

KEYWORDS • nitric oxide • arginine • homoarginine • dexamethasone • nitrates • guanosine cyclic monophosphate • hypertension, sodium-dependent

The description of endothelium-derived relaxing factor in 1980 by Furchgott and Zawadzki has initiated a revolution in understanding blood pressure regulation. Endothelium-derived relaxing factor, identified as nitric oxide (NO), produces vasorelaxation by activating soluble guanylate cyclase and increasing cyclic GMP (cGMP) in vascular smooth muscle. The family of NO synthases that produces NO in vivo consists of inducible and constitutive classes of enzymes. Several isoforms of these enzymes have been identified: a cytosolic, constitutive NO synthase; two inducible NO synthases; and a constitutive, noninducible, plasma membrane-bound NO synthase obtained from endothelial cells. NO synthases use l-arginine and a few other similar substrates, such as l-homoarginine but not d-arginine, to synthesize NO. Both inducible and constitutive NO synthases are present in the arterial wall and dramatically lower blood pressure in certain circumstances. Glucocorticoids differentiate between NO production by these two classes of enzymes, because they do not alter the function of the constitutive forms but prevent expression of inducible NO synthases both in vivo and in vitro. Because blood pressure represents the net effect of vasodilating and vasoconstricting influences, hypertension can reflect either defective vasodilation or enhanced vasoconstriction. We have shown that salt-sensitive hypertension in the Dahl/Rapp rat is completely prevented by parenteral and oral administration of l-arginine but not d-arginine. In contrast, l-arginine does not affect development of hypertension in the spontaneously hypertensive rat. L-Arginine also prevents associated renal failure and death from hypertensive nephrosclerosis in salt-sensitive rats. An increase in dietary sodium chloride increases NO production in the salt-resistant Dahl/Rapp (SR/Jr) rat and the Sprague-Dawley rat, but not in the salt-sensitive (SS/Jr) rat, unless provided l-arginine. These studies suggest that SS/Jr rats possess a unique defect in nitrovasodilation that manifests as hypertension while on a high-salt diet and corrects with administration of the biologic substrate for NO synthesis. The purpose of the current series of experiments was to examine further the important antihypertensive role of the l-arginine–NO pathway in salt-sensitive hypertension in the Dahl/Rapp rat.

Methods

Substrate Analysis

Animal care and use were approved by the Animal Resources Advisory Committee at the University of Alabama at Birmingham. Male, 21-day-old SS/Jr and SR/Jr rats (Harlan Sprague Dawley Inc, Indianapolis,
Ind) were maintained on an 8.0% sodium chloride diet (Dyets Inc, Bethlehem, Pa) and tap water ad libitum for 10 days. These rats were then prepared surgically in a standard fashion. Briefly, after anesthesia with ethyl 1-methylpropylthiobarbiturate (Inactin, BYK-Gulden, Hamburg, FRG) (100 mg/kg body wt IP), the animals were placed on a servo-controlled heated table that maintained rectal temperature at 37°C. Tracheostomy was performed with polyethylene PE-240 tubing, followed by catheterization of both external jugular veins with PE-50 tubing. One venous catheter was used for infusion of Ringer-bicarbonate containing 8% polyfructosan (Inutest, Laevosan-Gesellschaft, Linz, Austria) at 1.2 mL/100 g body wt per hour after a 1 mL bolus; this infusion was maintained throughout the experiment. The second venous catheter was used for administration of pharmacologic agents. The right femoral artery was catheterized through a suprapubic route for urine collection. A unigraph (model ICT-1H, Gilson Medical Electronics, Middleton, Wis) and for blood sampling. The bladder was catheterized through a suprapubic route for urine collection. After a 60-minute equilibration period, arterial blood samples were collected for determination of hematocrit and plasma inulin concentration. A 30-minute collection of urine was obtained to determine inulin clearance and urinary nitrate excretion rate. Mean arterial pressure (MAP) recorded at the end of the clearance served as baseline MAP. One group of SS/Jr rats then received an intravenous injection of L-arginine (Sigma Chemical Co, St Louis, Mo) (1.5 mmol/kg body wt). SR/Jr rats were handled similarly to this group and were given an equivalent amount of L-arginine. The other two groups of SS/Jr rats received either D-arginine or L-homoaarginine (both from Sigma) (1.5 mmol/kg body wt). A second 30-minute collection of urine was obtained for determination of inulin clearance and urinary nitrate excretion. Final MAP was recorded at the end of this clearance period.

**Amino Acid Analysis**

Twenty-one-day-old SS/Jr and SR/Jr rats were maintained on 0.3% sodium chloride chow and tap water ad libitum for 2 weeks. Rats then received 8.0% sodium chloride chow for an additional week. After anesthesia with ethyl 1-methylpropylthiobarbiturate (100 mg/kg body wt IP), the animals were placed on a servo-controlled heated table that maintained rectal temperature at 37°C. The right femoral artery was cannulated for recording of arterial blood pressure for 10 minutes. Immediately after blood pressure determination, heparinized aortic blood was obtained. After centrifugation and removal of cells, the plasma was stored at −20°C until analyzed.

**Dexamethasone Protocols**

Male, 21-day-old SS/Jr rats were maintained on a 0.3% sodium chloride diet (Dyets Inc) and tap water ad libitum for 2 weeks. They were then anesthetized with ether, and an osmotic minipump (Alzet Corp, Palo Alto, Calif) was implanted subcutaneously at the nape of the neck for continuous infusion of either dexamethasone (American Regent Laboratory, Inc, Shirley, NY) (5 μg/d), or vehicle (deionized water) alone. This amount of dexamethasone was chosen because of a previous study that demonstrated progressive weight loss in rats when higher doses were used. In one set of experiments, the left femoral artery was catheterized with polyethylene tubing, which was tunneled subcutaneously to the nape of the neck. After a 24-hour recovery period, the rats were divided into three groups. Group D+A (0.3%) received dexamethasone and remained on the low-salt chow. Group D+A (8.0%) was given dexamethasone, and the dietary salt was increased to 8.0% sodium chloride (Dyets). The third group, A (8.0%), received vehicle alone by the osmotic minipump and was placed on the high-salt chow. In the first set of experiments, all three groups received daily intraperitoneal injections of 250 mg/kg L-arginine. In the second series of experiments, the rats received 1.25 g/L L-arginine in the drinking water, and arterial catheters were not placed. After 5 days on either protocol, rats were prepared surgically as described above. A 30-minute collection of urine served to determine inulin clearance and the excretion rates of nitrate and cGMP.

For determination of whether the amount of dexamethasone administered was sufficient to prevent induction of NO synthase by endotoxin, 2 days before study osmotic minipumps containing either 5 μg/d dexamethasone or vehicle were inserted subcutaneously into SS/Jr rats that had been maintained on the low-salt chow. Rats were continued on 0.3% sodium chloride chow. The day before study, the right femoral artery was cannulated as described. After blood pressure had been determined in conscious, unrestrained rats, lipopolysaccharide from *Escherichia coli*, serotype 0111:B4 (Sigma) (3 mg/kg body wt), was injected intraperitoneally. This dose of lipopolysaccharide has been shown to produce hypotension within 3 hours in rats; this effect was inhibited in a dose-dependent fashion by N6-methyl-L-arginine. Blood pressure was examined 3 hours after injection. After completion of the study, the animals were anesthetized with 50 mg/kg body wt pentobarbital and killed.

**Analytic Techniques**

Concentration of inulin in plasma and urine was determined by the anthrone colorimetric method standardized in our laboratory. Clearance of inulin (Cl) was calculated from these values and factored by body weight. Measurement of cGMP was accomplished by a cGMP enzyme-linked immunosorbent assay kit (Cayman Chemical Co, Ann Arbor, Mich). A modification of the method described by Granger et al was used for determination of urinary nitrate and nitrite. All reagents were from Sigma. For determination of nitrite concentration, urine samples and sodium nitrate standards (0 to 200 μmol/L) were simultaneously reduced for 1 hour at 37°C by *Escherichia coli* (ATCC 25922, American Type Culture Collection, Rockville, Md) grown under anaerobic conditions. After centrifugation for 10 minutes at 1000g, 50 μL of supernatant was added to 50 μL of 1.0% sulfanilamide in 30% acetic acid and 50 μL of 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride in 60% acetic acid (Griess reagent). After mixing, the optical density was read at 540 nm using a microplate reader (ThermoMax, Molecular Devices Corp, Menlo Park, Calif). For determination of nitrate concentration, the protocol was repeated using the same samples, except *Escherichia coli* was not added.
and sodium nitrite (0 to 200 \( \mu \text{mol/L} \)) served as the standard.

For amino acid analysis, plasma was deproteinized with sulfosalicylic acid, vortexed, and centrifuged at 3500 rpm for 20 minutes. After addition of 100 \( \mu \text{L} \) of internal standard (amino ethyl-L-cysteine) to 100 \( \mu \text{L} \) of the supernatant, the solution was filtered. Separation and quantitation of plasma amino acids were accomplished with an automated amino analyzer (System 6300, Beckman Instruments, Inc, Palo Alto, Calif), which used cation exchange with postcolumn ninhydrin derivatization, and a computer software package (Beckman System Gold).

**Statistical Analysis**

All values are expressed as mean±SEM. Comparisons within a group were analyzed for statistical significance by the Student's paired \( t \) test. Comparisons among groups were analyzed by analysis of variance and the Scheffé F test using statistical software (STATVIEW 512+, Abacus Concepts, Inc, Berkeley, Calif). Significance was set at the 5% level.

**Results**

**Substrate Analysis**

Mean body weights and \( C_{\text{in}} \) at the time of study did not differ among the four groups of rats in this study (Table 1). As expected, after 5 days of high-salt chow, blood pressure increased in SS/Jr and remained normal in SR/Jr rats. Equimolar amounts of L-arginine and L-homoarginine lowered (\( P<.05 \)) blood pressure and simultaneously increased (\( P<.05 \)) urinary nitrate excretion rate, whereas D-arginine did not change blood pressure and urinary nitrate excretion rate (Fig 1). In SR/Jr rats, L-arginine decreased (\( P<.05 \)) blood pressure slightly and increased (\( P<.05 \)) urinary nitrate excretion. In all four groups, urinary nitrite concentration was undetectable.

**Amino Acid Analysis**

After 7 days of the high-salt diet, blood pressure under sedation was higher (\( P<.005 \)) in SS/Jr (139±4 mm Hg) than SR/Jr (100±3 mm Hg) rats. However, plasma concentrations of L-arginine (142±9 versus 154±5 \( \mu \text{mol/L} \)), citrulline (107±17 versus 108±15 \( \mu \text{mol/L} \)), and ornithine (44±5 versus 58±3 \( \mu \text{mol/L} \)) did not differ (Fig 2).

**Dexamethasone Protocol**

In the first set of experiments, indwelling arterial catheters were placed to monitor MAP in conscious, unrestrained SS/Jr rats. L-arginine was administered parenterally. Initial MAP did not differ among the three groups (Table 2). Blood pressure increased progressively in the D+A (8.0%) group but did not change in the D+A (0.3%) and A (8.0%) groups (Fig 3). After 5 days, mean \( C_{\text{in}} \) and hematocrit did not differ among these groups (Table 2). Under anesthesia, MAP remained elevated (\( P<.05 \)) in the D+A (8.0%) group (148±3 mm Hg) compared with the D+A (0.3%) group (112±2 mm Hg) and A (0.3%) group (116±2 mm Hg). Urinary cGMP excretion was greatest (\( P<.05 \)) in the A (8.0%) group (Table 2). Urinary nitrate and nitrite were not determined in these groups.

In the second set of experiments, an indwelling arterial catheter was not placed, and L-arginine was given orally. Each group contained six rats. After 5 days on the protocol, SS/Jr rats in the A (8.0%) group were normotensive. The D+A (8.0%) group had elevated (\( P<.05 \)) MAP and, compared with the A (8.0%) group, lower (\( P<.05 \)) urinary excretion rates of both cGMP and nitrate (Fig 4). In these experiments, when data from individual rats were examined, a direct correlation (\( r^2=0.439 \)) was found between urinary excretion of cGMP and nitrate (Fig 5). Urinary nitrite concentration was negligible in all three groups. In separate studies, pretreatment of rats with 5 \( \mu \text{g/d} \) dexamethasone prevented hypotension that occurred 3 hours after injection of endotoxin (Fig 6).

**Discussion**

The past decade has witnessed the emergence of NO as an important hormone in the regulation of blood pressure.\(^2\) Recently, NO was implicated as an important natriuretic hormone. Shultz and Tolins\(^3\)\(^5\) demonstrated that an increase in dietary sodium chloride increased plasma nitrate concentration and urinary nitrate excretion. These parameters correlated with renal sodium excretion. We suggested previously that an increase in dietary sodium chloride increased NO production in Sprague-Dawley and SR/Jr rats but not in SS/Jr rats. SS/Jr rats developed salt-sensitive hypertension because of this inability to increase NO production.\(^2\)\(^9\)\(^20\) Plasma concentrations of L-arginine of SS/Jr and SR/Jr rats on the high-salt diet did not differ. However, short-term
Fig 1. Plots show effect of short-term administration of D-arginine, L-homoarginine, and L-arginine on mean arterial pressure (MAP) and urinary nitrate excretion in Dahl salt-sensitive (SS/Jr) and salt-resistant (SR/Jr) rats after 10 days on a high-salt diet. D-Arginine had no effect on blood pressure and nitrate excretion, but both L-homoarginine and L-arginine decreased blood pressure and simultaneously increased urinary nitrate excretion in SS/Jr rats. Blood pressure fell gradually after injection of these compounds and was maximal by 30 minutes. Infusion of L-arginine in SR/Jr rats decreased blood pressure slightly and increased urinary nitrate excretion. *P<.05 from baseline value. Comparison of baseline excretions of nitrate was not performed because variables such as blood pressure and diet, which also contained nitrite, were not strictly controlled among groups.
intravenous administration of L-arginine and L-homoarginine, substrates for NO synthesis, lowered blood pressure to normotensive levels (mean percent decrease, 21.9±2.0% with L-arginine and 15.7±3.0% with L-homoarginine) and simultaneously increased urinary nitrate excretion. Both urinary nitrate and cGMP excretion have been shown to be biologic markers of endogenous NO production. When these values were determined in the same SS/Jr rat, we found a direct correlation between urinary cGMP and nitrate. Thus, provision of precursors for NO synthesis increased NO production and concomitantly decreased arterial pressure in SS/Jr rats. L-Arginine supplementation also prevented development of hypertension in SS/Jr rats given a high-salt diet (Fig 3). In previous studies, we demonstrated that prolonged administration of L-arginine completely prevented hypertension and attendant hypertensive nephrosclerosis selectively in SS/Jr rats.29-30 The combined data suggested that administration of L-arginine to SS/Jr rats corrected a defect in NO production that manifests while rats are on a high-salt diet and results in hypertension. In SR/Jr rats, L-arginine increased urinary nitrate production but lowered blood pressure only slightly (mean percent decrease, 1.4±0.4%). This finding, along with the demonstration that L-arginine did not cause hypotension in these rats, supported the concept that multiple factors involved in the prevention of salt-sensitive hypertension in SS/Jr rats. Endotoxin has been shown to induce NO synthase,10,12,25,32 which produces hypotension in rats 3 hours after injection of endotoxin.32 Induction of NO synthase by lipopolysaccharide was prevented with dexamethasone.26,27 In the current study, pretreatment with 5 µg/d dexamethasone prevented hypotension in rats injected with endotoxin (Fig 6). When dexamethasone was added just before dietary sodium chloride was increased to 8.0%, despite supplementation with L-arginine, hypertension developed rapidly over 5 days of study (Fig 3) in a fashion typical of salt-sensitive hypertension in these rats.29-30 In addition, urinary excretion of cGMP and nitrate in the dexamethasone-treated rats was significantly lower than corresponding values obtained from vehicle-treated rats on the same diet (Fig 4). Administration of dexamethasone to SS/Jr rats on the low-salt chow did not increase blood pressure over the same observation period. These studies support an important role for dexamethasone-suppressible NO production in the prevention of salt-sensitive hypertension in these rats.

From these studies and our previous work,29-30 we suggest that in the pathogenesis of hypertension, particularly of the genetically predisposed, salt-sensitive hypertension, provision of precursors for NO synthesis in vivo contributed to blood pressure regulation in vivo.

### Table 2. Mean Physiological Parameters of Dahl Salt-Sensitive Rats Used In Dexamethasone Study

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial MAP, mm Hg</th>
<th>Final MAP, mm Hg</th>
<th>CIN (µL/min)/100 g BW</th>
<th>Hematocrit, º</th>
<th>Urinary cGMP, pmol/mL CIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>D+A (0.3%) (n=6)</td>
<td>111±3</td>
<td>108±2</td>
<td>930±92</td>
<td>44±1</td>
<td>15.4±2.5</td>
</tr>
<tr>
<td>D+A (0.8%) (n=10)</td>
<td>111±1</td>
<td>136±2*</td>
<td>996±26</td>
<td>44±1</td>
<td>14.1±1.8</td>
</tr>
<tr>
<td>A (0.8%) (n=5)</td>
<td>114±3</td>
<td>110±3</td>
<td>899±48</td>
<td>43±0</td>
<td>24.2±3.3*</td>
</tr>
</tbody>
</table>

MAP indicates mean arterial pressure; CIN, inulin clearance; and BW, body weight.

*Value different from other data in that category.
variety, a defect of NO production appears to be present. This defect is overcome by addition of substrates for NO synthesis. Although dexamethasone may also affect other features of the L-arginine-NO pathway, we propose that an inducible NO synthase found in the arterial wall is a candidate gene in hereditary salt-sensitive hypertension in the Dahl/Rapp rat.

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