Renal medullary interstitial infusion of L-arginine prevents hypertension in Dahl salt-sensitive rats

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Miyata, Noriyuki, Ai Ping Zou, David L. Mattson, and Allen W. Cowley, J r. Renal medullary interstitial infusion of L-arginine prevents hypertension in Dahl salt-sensitive rats. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1667–R1673, 1998.—Studies were designed to examine the effects of renal medullary interstitial infusion of L-arginine (L-Arg) on the development of high-salt-induced hypertension in Dahl salt-sensitive/Rapp (DS) rats. The threshold dose of L-Arg (300 µg·kg⁻¹·min⁻¹) that increased the renal medullary blood flow without altering the cortical blood flow was first determined in anesthetized DS rats. Studies were then carried out to determine the effects of this dose of L-Arg on salt-induced hypertension in DS rats. In the absence of chronic medullary L-Arg infusion, mean arterial pressure (MAP) increased in DS rats from 125 ± 2 to 167 ± 5 mmHg by day 5 of a high-salt diet (4.0%), with no change observed in Wistar-Kyoto (WKY) or Dahl salt-resistant/Rapp (DR) rats. MAP did not change significantly with medullary infusion of L-Arg alone in DR rats (control = 104 ± 1 mmHg) or in WKY rats (control = 120 ± 3 mmHg) and was not significantly changed from these levels during the 7 days of L-Arg infusion combined with high-NaCl diet. The same amount of L-Arg that prevented salt-induced hypertension in DS rats when infused into the renal medulla (300 µg·kg⁻¹·min⁻¹) failed to blunt salt-induced hypertension when administered intravenously to DS rats. DS rats receiving L-Arg (300 µg·kg⁻¹·min⁻¹ iv) exhibited an increase in plasma L-Arg from control concentrations of 138 ± 11 to 218 ± 4 µmol/l, while MAP, which averaged 124 ± 3 mmHg during the 3-day control period, rose to 165 ± 5 mmHg by day 5 of high salt (4%) intake. These results indicate that the prevention of salt sensitivity in DS rats was due specifically to the action of L-Arg on renal medullary function and that DS rats may have a deficit of medullary substrate availability and NO production.

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IT IS WELL KNOWN that inhibition of nitric oxide (NO) formation by specific inhibitors of NO synthase (NOS) leads to an increase in blood pressure (2, 9, 12, 24, 25), suggesting that NO plays an important role in the physiological regulation of blood pressure. We have shown that acute inhibition of NO in the renal medulla decreases renal medullary blood flow, with parallel changes in sodium and water excretion (17). Either chronic intravenous (19) or renal medullary interstitial (15) infusion of N⁵-nitro-L-arginine methyl ester (L-NAME) can produce a sustained decrease in renal medullary blood flow, which, in the absence of changes in cortical blood flow, results in retention of sodium and water and the development of hypertension. It is evident from these studies that NO in the renal medulla plays an important role in the regulation of blood flow to this region, which in turn influences the long-term control of arterial blood pressure.

A number of studies have also shown that either oral or intravenous administration of L-arginine (L-Arg) in large amounts can prevent salt-induced hypertension in Dahl salt-sensitive (DS) rats (3, 4, 8, 20, 21). Evidence for increased NO production with chronic L-Arg administration was supported by observations that L-Arg administration increased the urinary levels of cGMP (3). Similarly, it has been found in anesthetized DS rats that pressure natriuresis and transmission of perfusion pressure into the renal interstitium were normalized by long-term L-Arg administration (20, 21).

Other recent studies in our laboratory have found that NOS activity, protein expression (14), and NO concentrations (26) are significantly higher in the rat renal medulla than in the cortex. Taken together, these data led us to hypothesize that the major antihypertensive actions of L-Arg in DS rats may be mediated largely through actions of this NO precursor, specifically within the renal medulla. Techniques developed in our laboratory to deliver compounds chronically into the medullary interstitial space of the rat kidney were used in these studies to examine the ability of selective elevations of medullary L-Arg to blunt the hypertension normally observed in DS rats fed a high-NaCl diet. Responses were compared with Dahl salt-resistant (DR) and Wistar-Kyoto (WKY) rats.

METHODS

Experimental Animals

Experiments were performed using inbred DS rats (270–350 g), DR rats (255–270 g), WKY rats (260–350 g), and Sprague-Dawley (SD) rats (365–460 g) purchased from Harlan Laboratories (Madison, WI). All animals were housed individually in the Animal Resource Center at the Medical College of Wisconsin. DS and DR rats were maintained on an ad libitum low-NaCl (0.4%) or high-NaCl (4.0%) diet, and water was provided. WKY and SD rats were maintained on a normal-NaCl (1.0%) or high-NaCl (4.0%) diet and water. All animal procedures were approved by the Medical College of Wisconsin Institutional Animal Care and Use Committee.

Surgical Preparation for Acute Study

Rats were anesthetized with ketamine (30 mg/kg im) and Inactin (50 mg/kg ip). Rats had been maintained on a low-salt diet (0.4% NaCl; Dyets, Bethlehem, PA) before the study. The rats were placed on a heated surgical table, and their body temperature was maintained at 36.5°C. Catheters were placed in the femoral vein for infusions, in the femoral artery for
blood pressure measurements, in the renal medulla for the L-Arg infusion, and in the ureter for the collection of urine. The rats received an intravenous infusion of 2% BSA in a 0.9% NaCl solution at a rate of 2 ml·100 g⁻¹·h⁻¹ throughout the experiment. The renal medullary interstitial catheter, which we have described previously (11), was fabricated from a piece of PE-10 polyethylene tubing (Clay Adams, Parsippany, NJ), which was extruded over hot air to give a tip diameter of 100 µm. The other end of the PE-10 tubing was heat fused to a 25-cm piece of PE-50 tubing and connected to the infusion pump. The catheter was inserted into the kidney through a small hole in the renal capsule made with a 27-gauge needle. The catheter tip was placed at or near the border of the inner and outer medulla and anchored in place on the kidney surface with cyanoacrylate adhesive. Sterile saline as vehicle or L-Arg in saline was infused throughout the study at a rate of 8.3 µl/min.

Optical fibers were inserted (500-µm diameter) into the cortical and medullary regions of the left kidney, and the blood flow of these renal regions was measured with a laser-Doppler flowmeter (model P1f, Perimed) as previously described (10). The cortical fiber was implanted to a depth of 2 mm from the surface of the cortex, and the medullary fiber was implanted to a depth of 5 mm, which detected changes in blood flow within the inner (white) medulla. Cortical and medullary blood flows were expressed as voltage units. Placement of the interstitial catheter tip and optical fibers was confirmed at the end of the experiment by careful visual inspection, and rats with incorrectly placed catheters or fibers were discarded from the study.

Surgical Preparation for Studies in Conscious Animals

DS and DR rats were anesthetized with ketamine (30 mg/kg im) and xylazine (2 mg/kg im). WKY rats were anesthetized with a mixture of ketamine (40 mg/kg im) and acepromazine (1 mg/kg im). All surgical procedures were performed under aseptic conditions. The right kidney was removed, and rats were allowed 7–10 days for recovery. Unilateral nephrectomy enabled delivery of L-Arg or vehicle (isotonic saline) to the remaining left kidney and prevented compensatory responses from the contralateral kidney.

Rats were again anesthetized in the same manner for implantation of indwelling catheters. The femoral artery was exposed for insertion of the aortic catheter as described previously (15). The left kidney was then exposed via a flank incision, and the medullary interstitial catheter was implanted as described in previous studies from our laboratory (15). Placement of the interstitial catheter tip was confirmed at the end of the chronic experiment by careful visual inspection. All catheters were tunneled subcutaneously to the back of the neck, where they were exteriorized through a midscapular incision and passed through a spring for protection. Buprenex, a narcotic analgesic, was administered after the recovery from anesthesia. After closure of the incisions, the rats received 200,000 U/kg im of penicillin G. Rats were housed individually in metabolic cages, with the spring protecting the catheters attached to a swivel that allowed the animal to move about the cage. Infusion swivels of our own design were used for the continuous infusion of 0.9% NaCl saline or L-Arg (300 µg·kg⁻¹·min⁻¹) at 8.3 µl/min into the renal medullary interstitial space. The arterial catheters were filled with 1,000 U/ml of heparin to prevent clotting. Animals were allowed 7 days to recover from this procedure before study.

Experimental Design

Protocol 1: Renal medullary interstitial infusion of L-Arg in anesthetized DS rats. DS rats were prepared as described in Surgical Preparation for Acute Study. One hour after implantation of the optical fibers and interstitial infusion catheter, two 20-min control measurements of MAP, cortical blood flow, and medullary blood flow were obtained. After the control periods, L-Arg (160, 320, 640, and 1,000 µg·kg⁻¹·min⁻¹) was infused into the renal medulla for 60 min at each dose. L-Arg hydrochloride was obtained from Sigma (St. Louis, MO). The pH of the L-Arg solution (5.77) was found to be nearly identical to the saline vehicle solution (5.68). Cortical blood flow, medullary blood flow, and MAP were quantified during the final 30 min of each infusion period. Timed urine samples were collected during two 20-min control periods (while 0.9% NaCl solution was infused into the medullary interstitium at a rate of 8.3 µl/min) and during the final 30 min of each of the L-Arg infusion periods. Urine sodium concentrations were determined with a flame photometer (model 143, Instrumentation Laboratory, Lexington, MA).

Protocol 2: Effects of high salt intake on blood pressure in DS, DR, and WKY rats receiving a chronic medullary interstitial infusion of L-Arg or vehicle. After the surgical recovery period of 10 days, while rats were maintained on a low-NaCl (0.4%) diet, blood pressure was measured daily for 2 h in three groups of rats: DS (n = 6), DR (n = 5), and WKY (n = 6). Pressure was recorded at the same time each day (8:00–11:00.
AM), with rats maintained in their home cages. During the recovery and throughout the control period, all rats received a continuous infusion of the vehicle (saline) at a rate of 8.3 µl/min into the renal medullary interstitial space. After 3 days of stable control blood pressure measurements, the interstitial infusion solution was changed from vehicle to L-Arg (300 µg·kg⁻¹·min⁻¹) and MAP was recorded daily. After 4 days of interstitial infusion of L-Arg, the NaCl of the diet was raised to 4% for 7 days. At the end of this period, sodium intake was returned to 0.4% for another 2 days to determine short-term recovery responses. At the end of the experimental protocol, the animals were euthanized and the position of the interstitial catheter was determined after fixation of the kidney in a 10% Formalin solution for 24 h.

Protocol 3: Effects of acute and chronic renal medullary interstitial infusion of L-Arg on systemic plasma concentrations of L-Arg. Experiments were performed on 7 SD rats prepared as described in Surgical Preparation for Acute Study. The rats received an intravenous infusion of 1% BSA in a 0.9% NaCl solution at a rate of 1 ml·100 g⁻¹·h⁻¹ throughout the experiment. One hour after completion of the surgical procedures, an arterial blood sample (300 µl) was collected and MAP was measured for 30 min. After the control period, L-Arg (300 µg·kg⁻¹·min⁻¹) was infused into the renal medulla at a rate of 8.3 µl/min for 2 h with continuous measurement of MAP. At the end of this period, an arterial blood sample was collected (300 µl) for measurement of plasma L-Arg.

Experiments were also performed on 5 DS rats surgically prepared for chronic study as described in Surgical Preparation for Studies in Conscious Animals above. During the recovery and throughout the control, saline was infused (8.3 µl/min) into the renal medullary interstitial space. After three stable control days of MAP measurements, a 300-µl blood sample was taken from the arterial catheter for determination of plasma L-Arg. The interstitial infusion solution was then changed from vehicle to L-Arg (300 µg·kg⁻¹·min⁻¹), and after 3 days of interstitial L-Arg infusion, another 300-µl blood sample was taken for L-Arg analysis.

Protocol 4: Effect of continuous intravenous infusion of L-Arg on high-NaCl-diet-induced hypertension in DS rats. Studies were carried out in a smaller group of DS rats to determine if renal medullary infusion of L-Arg could influence blood pressure by escape and recirculation. Ten DS rats were surgically prepared for chronic study as described in Surgical Preparation for Studies in Conscious Animals. During this experiment, all rats received a continuous infusion of saline as vehicle (8.3 µl/min) into the renal medullary interstitial space throughout the experiment. After the recovery period of 7 days, MAP was measured for 2 h daily for 3 control days during the low-NaCl control. At this time, the rats received either an intravenous infusion of saline as vehicle (n = 3), L-Arg (30 µg·kg⁻¹·min⁻¹, n = 3), or L-Arg (300 µg·kg⁻¹·min⁻¹, n = 4) in a 0.9% NaCl solution at a rate of 8.3 µl/min. After 4 additional days of blood pressure recordings, the rats were fed a high-NaCl (4%) diet for 7 days and then returned to a low-NaCl diet. At the end of the experimental protocol, the animals were killed and the position of the interstitial catheter was determined after fixation of the kidney in a 10% Formalin solution for 24 h. A blood sample (300 µl) was taken from the arterial catheter on the third control day and the third day of L-Arg infusion for measurement of plasma L-Arg.

Statistical Analysis

Data are expressed as means ± SE. Within-group changes in MAP were evaluated with a one-way ANOVA for repeated measures and Duncan’s post hoc test. The unpaired t-test was used to examine the plasma concentration of L-Arg. The level of significance was P < 0.05.

RESULTS


Figure 1 summarizes the effects of medullary interstitial infusion of L-Arg on MAP and cortical and medullary blood flow of the kidney. MAP, which averaged 118 ± 4 mmHg (n = 8) during the control period, decreased significantly at the two highest doses of L-Arg, falling to 108 ± 4 mmHg at a dose of 1,000 µg·kg⁻¹·min⁻¹. The cortical flow signal during control periods averaged 1.4 ± 0.1 V (n = 8), and the medullary flow signal averaged 0.6 ± 0.1 V (n = 8). The cortical blood flow was not affected significantly by the interstitial infusion of L-Arg at any dose administered. In contrast, a threshold dose of 320 µg·kg⁻¹·min⁻¹, L-Arg increased medullary blood flow significantly by 16.7%, which increased progressively, reaching 32.7% (n = 8) at the dose of 1,000 µg·kg⁻¹·min⁻¹. Associated effects of L-Arg on urine flow and sodium excretion are seen in Fig. 2. Control urine flow averaged 18.0 ± 1.8 µl·min⁻¹·g⁻¹ kidney weight (n = 8), and sodium excretion averaged 2.85 ± 0.28 µeq·min⁻¹·g⁻¹ kidney weight.

![Image][3]

**Fig. 1.** Changes in mean arterial pressure (MAP, top, n = 8), cortical blood flow (middle, n = 8), and medullary blood flow (bottom, n = 8) during a control period and renal medullary interstitial infusion of increasing dose of L-arginine (L-Arg) in anesthetized Dahl salt-sensitive (DS) rats. Values are means ± SE. ri, Renal medullary interstitial infusion. *P < 0.05, significantly different from second control (C) period.
At the same threshold dose of L-Arg (320 µg·kg\(^{-1}\)·min\(^{-1}\)) that increased medullary blood flow, a nearly 40% increase of urine flow and sodium excretion was observed, which rose even further at the highest concentration of infused L-Arg (1,000 µg·kg\(^{-1}\)·min\(^{-1}\)).

Protocol 2: Effect of High NaCl Intake on Blood Pressure in DS, DR, and WKY Rats Receiving Continuous Medullary Infusion of Vehicle or L-Arg

Figure 3 summarizes the blood pressure responses in DS rats to a high NaCl intake during medullary infusion of vehicle (n = 6) or L-Arg (300 µg·kg\(^{-1}\)·min\(^{-1}\)) (n = 6). In the vehicle-infused DS group, MAP averaged 125 ± 2 mmHg during the low-NaCl (0.4%) control period. DS rats receiving the medullary infusion of vehicle throughout the study exhibited significant elevations of pressure with high NaCl (4%) intake. MAP rose to 148 ± 2 mmHg after 1 day of the 4% NaCl diet and continued to rise to a level of 167 ± 5 mmHg by day 7. MAP remained at this level during the 2 days of measurement after rats were returned to a low-NaCl (0.4%) diet.

The dose of L-Arg chosen for these chronic experiments was the threshold dose found to increase medullary blood flow and sodium excretion in the acute rat studies (300 µg·kg\(^{-1}\)·min\(^{-1}\); see Figs. 1 and 2). As seen in Fig. 3, during the low-NaCl (0.4%) control days with vehicle (saline) continuously infused (8.3 µl/min) into the renal medulla, the MAP averaged 123 ± 2 mmHg (n = 6). Four days of medullary L-Arg infusion with rats on low salt intake resulted in no changes of MAP. Most importantly, the DS rats receiving a renal medullary interstitial infusion of L-Arg did not exhibit significant elevations of pressure throughout the 7-day period of high salt intake.

Figure 4 summarizes the effects of the same high salt intake in DR and WKY rats receiving the same medullary interstitial infusion of L-Arg (300 µg·kg\(^{-1}\)·min\(^{-1}\)). During the control period, with saline continuously infused (8.3 µl/min) into the renal medulla, the average MAP of DR rats (receiving a 0.4% salt diet; n = 5) was 104 ± 2 mmHg, and WKY rats (receiving a 1% salt diet; n = 6) averaged 120 ± 3 mmHg. During the period of high salt intake (4%), neither group of rats exhibited sustained elevations in blood pressure. Thus medullary administration of L-Arg appears to have little effect on these two salt-insensitive control strains of rats during a low or high level of daily salt intake.

Protocol 3: Effects of Acute and Chronic Renal Medullary Interstitial Infusion of L-Arg on Plasma Levels of L-Arg

To determine if renal medullary infusion of L-Arg could be influencing blood pressure by escape and recirculation, we first determined the effects of a 2-h medullary infusion of 300 µg·kg\(^{-1}\)·min\(^{-1}\) L-Arg on...
plasma L-Arg concentrations in anesthetized SD rats (Table 1). Renal medullary interstitial infusion of L-Arg increased plasma levels of L-Arg from 99 ± 8 to 121 ± 5 µmol/l \((n = 7)\), which was a 23% elevation. MAP before and after the 2-h medullary infusion of L-Arg averaged 118 ± 4 mmHg and 110 ± 5 mmHg, respectively \((n = 7)\). Chronic medullary interstitial infusion of L-Arg \((300 \mu g \cdot kg^{-1} \cdot min^{-1})\) resulted in similar changes, as determined on the third day of continuous infusions. Plasma levels of L-Arg increased from 115 ± 11 \((n = 5)\) to 149 ± 29 µmol/l \((n = 5)\), a 30% elevation.

**Protocol 4: Effects of Intravenous Infusion of L-Arg on High-Salt-Induced Hypertension in DS rats**

To determine whether a 23% increase of plasma L-Arg concentration could prevent the high-salt-induced hypertension in DS rats, we determined the rate of intravenously infused L-Arg required to increase plasma concentration by 23% (Table 1). Chronic intravenous infusion of L-Arg \((30 \mu g \cdot kg^{-1} \cdot min^{-1})\) increased the plasma levels of L-Arg by 23% from 141 ± 5 µmol/l \((\text{third control day, } n = 3)\) to 172 ± 19 µmol/l \((\text{third day of L-Arg infusion, } n = 3)\). Vehicle infusion \((8.3 \mu l/min)\) did not significantly influence the plasma levels of L-Arg, which averaged 159 ± 20 µmol/l \((\text{third control day, } n = 3)\) and 145 ± 14 µmol/l \((\text{third day of vehicle infusion})\).

Furthermore, we also examined the chronic effects of continuous 3-day intravenous infusions of L-Arg \((300 \mu g \cdot kg^{-1} \cdot min^{-1})\) on the plasma levels of L-Arg to determine what the expected responses would have been if all the L-Arg chronically infused into the medullary interstitium had escaped into the peripheral circulation. Chronic intravenous infusion of L-Arg \((300 \mu g \cdot kg^{-1} \cdot min^{-1})\) increased the plasma levels of L-Arg by 58% from 138 ± 11 µmol/l \((\text{third control day})\) to 218 ± 4 µmol/l \((\text{third day of L-Arg infusion; } n = 4)\), as seen in Table 1.

The effects of a high-salt diet were then determined in a small group of rats using a protocol identical to that described in the aforementioned studies, except that L-Arg was infused intravenously at a rate of either 30 \(\mu g \cdot kg^{-1} \cdot min^{-1}\) or 300 \(\mu g \cdot kg^{-1} \cdot min^{-1}\). Figure 5 summarizes the arterial pressure responses to high sodium intake in rats receiving either intravenous L-Arg or vehicle \((8.3 \mu l/min)\). The average control MAP was nearly identical in all groups of DS rats \((130 ± 1, 125 ± 2, \text{ and } 124 ± 3 \text{ mmHg in vehicle-infused group and } 30 \mu g \cdot kg^{-1} \cdot min^{-1} \text{ iv and } 300 \mu g \cdot kg^{-1} \cdot min^{-1} \text{ iv L-Arg-infused groups, respectively})\). All groups also exhibited nearly identical elevations of MAP when placed on a high-NaCl diet \((4%)\), reaching 165 ± 5 mmHg \((\text{vehicle, } n = 3)\), 170 ± 4 mmHg \((30 \mu g \cdot kg^{-1} \cdot min^{-1} \text{ L-Arg iv, } n = 3)\), and 164 ± 3 mmHg \((300 \mu g \cdot kg^{-1} \cdot min^{-1} \text{ L-Arg iv, } n = 4)\) by day 5. These levels of pressure fell only slightly during the 2 days after return to a low salt intake. These results indicate that even if all of the medullary infused L-Arg had escaped and recirculated, the systemic actions of L-Arg alone could not prevent the salt-induced hypertension in the DS rats.

**DISCUSSION**

A number of investigators have observed that intraperitoneal injections and intravenous or even oral administration of L-Arg can prevent salt-induced hypertension in DS rats, whereas similar effects were not observed with D-Arg \((3, 4, 8, 20, 21)\). Patel and associates \((21)\) found that L-Arg normalized pressure natriuresis and improved transmission of perfusion pressure into the renal interstitium \((20)\) in the anesthetized DS rats. Hu and Manning \((8)\) reported that continuous intravenous infusion of large doses of L-Arg \((4,000 \mu g \cdot kg^{-1} \cdot min^{-1})\) prevented the shift of the long-term pressure-natriuresis relationship and prevented salt-induced hypertension in DS rats. They also showed that urinary NO\(_2/\)NO\(_3\) excretion was lower in DS rats than in DR rats and that intravenous infusion of L-Arg resulted in a greater increase in the urine excretion of NO\(_2/\)NO\(_3\) in DS rats compared with that of the control DR rats. Although these studies indicate a reduced capability of DS rats to produce NO, they do not provide data for the site of action of the antihypertensive effects of L-Arg. The present studies were designed to determine whether an elevation of L-Arg within the region of

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**Table 1. Arterial plasma L-Arg concentrations during both renal medullary interstitial and intravenous infusion in DS rats**

<table>
<thead>
<tr>
<th>Infusion method</th>
<th>Control L-Arg, µmol/l</th>
<th>L-Arg Infusion, µmol/l</th>
<th>n</th>
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<tbody>
<tr>
<td>Medullary interstitial infusion ((300 \mu g \cdot kg^{-1} \cdot min^{-1}))</td>
<td></td>
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<tr>
<td>Infusion duration</td>
<td></td>
<td></td>
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<tr>
<td>2 h</td>
<td>99 ± 8</td>
<td>121 ± 5(^a)</td>
<td>7</td>
</tr>
<tr>
<td>3 days</td>
<td>115 ± 11</td>
<td>149 ± 29(^a)</td>
<td>5</td>
</tr>
<tr>
<td>Intravenous infusion (3 days)</td>
<td></td>
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<tr>
<td>Infusion dose</td>
<td></td>
<td></td>
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<tr>
<td>Vehicle</td>
<td>159 ± 20</td>
<td>145 ± 14</td>
<td>3</td>
</tr>
<tr>
<td>30 (\mu g \cdot kg^{-1} \cdot min^{-1})</td>
<td>141 ± 5</td>
<td>172 ± 19</td>
<td>3</td>
</tr>
<tr>
<td>300 (\mu g \cdot kg^{-1} \cdot min^{-1})</td>
<td>138 ± 11</td>
<td>218 ± 4(^a)</td>
<td>4</td>
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Values are means ± SE. L-Arg, L-arginine; DS, Dahl salt sensitive.

\(^a\)Significance at P < 0.05 using a paired t-test.
the renal medulla alone could prevent the salt-induced hypertension in DS rats.

Possible Mechanism of Antihypertensive Effects of L-Arg in DS Rats

The results of chronic medullary infusion of L-Arg indicate that the antihypertensive effects of excess L-Arg are indeed mediated through the effects of this amino acid selectively within the renal medulla. The data indicate that the amount of L-Arg chronically infused into the renal medulla did not exert antihypertensive effects systemically by escape and recirculation of the compound into the general circulation. Even when the total amount of L-Arg that was infused into the renal medulla (300 µg·kg⁻¹·min⁻¹) was administered systemically (intravenously), there was no blunting of the salt-induced hypertension of the DS rats. The antihypertensive actions of L-Arg therefore appear to be localized to the renal medulla.

The results of the present study and of others (9) show that there is a threshold plasma level that must be achieved before the antihypertensive effects of intravenously infused L-Arg are observed in the DS rats. The amount of intravenously infused L-Arg (30 µg·kg⁻¹·min⁻¹), which was selected to produce an increase of plasma L-Arg concentrations from 141 to 172 µmol/l (the rise which occurred during the chronic delivery of 300 µg·kg⁻¹·min⁻¹ L-Arg into the renal medulla) was a dose well below the threshold required to prevent hypertension. Hu and Manning (8) found that intravenous administration of 4,000 but not 2,000 µg·kg⁻¹·min⁻¹ prevented salt-induced hypertension in DS rats. It was also found by these investigators that only the 4,000-µg·kg⁻¹·min⁻¹ dose lowered the control blood pressure of the DS rats. These data indicate that L-Arg in ~1/3 the effective dose given intravenously had antihypertensive effects in the DS rat when given into the renal medulla.

The precise mechanism(s) whereby excess levels of medullary L-Arg prevent salt-induced hypertension in DS remain to be determined. It is evident from the present study that medullary administration of L-Arg in amounts that prevented the hypertension resulted in significant increases of medullary blood flow. Changes in medullary blood flow can play an important role in the long-term regulation of arterial blood pressure (5). Furthermore, the renal medulla normally exhibits greater basal activity of the NO synthetic pathways, and medullary NOS activity plays an important role in the regulation of sodium and water excretion and the long-term control of arterial pressure (19, 26). Specifically, we have found that expression of endothelial NOS, inducible NOS, and neuronal NOS is much greater in the renal medulla than in the renal cortex of normal SD rats (14). We have also found that tissue NO concentration in the renal medulla is substantially higher than in the cortex, as determined by microdialysis oxyhemoglobin NO-trapping techniques (26). Last, chronic low-dose intravenous infusion of L-NAME was found to result in a selective reduction of renal medullary blood flow, retention of sodium and water, and development of hypertension in the absence of changes of renal cortical blood flow (19).

The biochemical and cellular mechanisms whereby selective administration of L-Arg into the renal medulla of DS rats prevents salt-induced hypertension must now be determined. Studies carried out in cultured and native bovine endothelial cells suggest that intracellular concentrations of L-Arg are sufficiently high to saturate the constitutive NOS. Intracellular levels of L-Arg have been found to be substantially higher (~100 µM) in cultured endothelial cells (17) than the substrate concentration at which the reaction velocity is one-half maximal (K_m, 2.9 µM) (22). Similar data are not available for cells of the renal medulla (e.g., vascular, tubular, interstitial cells), and it is unknown whether the “functional” K_m of intact cells is the same as the purified enzyme that was used to determine K_m in previous studies.

Recent studies in our laboratory suggest that NO production in the renal medulla of rats may be substrate limited. Specifically, it was shown that intravenous L-Arg administration resulted in a significant increase of medullary NO concentration (from 50 to 125 nmol/l) and medullary nitrite concentrations (from 2.0 to 3.7 µmol/l) in normal SD rats (26). Moridani and Kline (18) have also found that L-Arg concentrations in the renal medulla were only about one-fourth of those found in the renal cortex. Because substrate levels are lowest in the medulla where NOS activity levels are the highest and because L-Arg administration increases tissue NO concentration even in normal rats (26), we believe that it is likely in the present study that medullary NO production of DS rats was enhanced by medullary L-Arg administration.

It is possible that an inhibitor of the NOS enzyme is in greater abundance in DS rats, causing an increased requirement for L-Arg. There is indeed evidence for such an inhibitory factor obtained from cultured bovine aortic endothelial cells and in rabbit aortic rings (1). By varying extracellular L-Arg concentrations in the absence of L-glutamine, no increase of NO was observed in response to bradykinin. However, when L-glutamine was added in amounts seen in vivo, L-Arg produced a concentration-dependent increase in NO production and vascular relaxation. The inhibitory effect of L-glutamine was completely reversed by L-Arg. It is clear that in vivo production of NO occurs through diverse and complex pathways. Posttranslational processing of NOS, phosphorylation pathways, etc., must all be explored to fully elucidate how L-Arg may influence the production of NO in the renal medulla of normal and DS rats.

Responses of Non-Salt-Sensitive Control Rats

DR and WKY rats were both used as non-salt-sensitive control rats in the present study. DR rats were originally derived from the SD rats (as were DS rats) and are homozygous at nearly all genetic loci (23). WKY rats were chosen because alleles of the inducible NOS locus were reported to cosegregate with blood pressure in the F₂ population derived from a cross of DS rats...
with WKY rats (6). It was found in the present study that MAP did not change in DR or WKY rats with renal medullary interstitial infusion of L-Arg during the control period when rats were on a low-NaCl diet and increased <10 mmHg during the combined 7 days L-Arg with high-NaCl (4%) diet. This suggests that DR and WKY already have abundant L-Arg and NO production, whereas DS rats may lack either sufficient L-Arg concentration in the renal medulla or appropriate L-Arg cell uptake for adequate NO production. We have previously shown that selective reduction of medullary NOS activity results in lowering of the medullary blood flow, antinatriuresis, and hypertension (15, 19) and that intravenous administration of L-Arg increases medullary NO concentrations (26). We hypothesize that elevation of medullary NO in the DS kidney by L-Arg infusion prevents a salt-induced reduction of medullary blood flow and abolishes hypertension.

The overall issue related to the role of NO production in salt-sensitive forms of hypertension remains unclear at this time. Studies in rats have shown that chronic NOS inhibition results in a salt-sensitive form of hypertension (14, 15, 19) and that SD rats exhibit increased renal NOS activity and protein expression when subjected to a high salt intake (14). In contrast, Manning et al. (13) have found no association between NO production and salt sensitivity in dogs. Direct tissue measurements of renal NO tissue concentrations under varying conditions of sodium intake have not yet been reported.

In summary, our results show that increased L-Arg substrate availability in only the renal medulla of DS rats can prevent high-salt-induced hypertension. This suggests that there may be a deficit of medullary NO production or substrate availability in DS rats.

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