Evidence that renal arginine transport is impaired in spontaneously hypertensive rats

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1Department of Physiology, Monash University, 2Department of Biochemistry and Molecular Biology, Monash University, and 3Baker IDI Heart and Diabetes Institute, Melbourne, Australia, and 4Department of Physiology, Medical College of Wisconsin, Milwaukee, Wisconsin

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Rajapakse NW, Kuruppu S, Hanchapola I, Venardos K, Mattson DL, Smith AI, Kaye DM, Evans RG. Evidence that renal arginine transport is impaired in spontaneously hypertensive rats. Am J Physiol Renal Physiol 302: F1554–F1562, 2012.—Low renal nitric oxide (NO) bioavailability contributes to the development and maintenance of chronic hypertension. We investigated whether impaired L-arginine transport contributes to low renal NO bioavailability in hypertension. Responses of renal medullary perfusion and NO concentration to renal arterial infusions of L-arginine concentration to renal arterial infusions of the L-arginine transport inhibitor L-lysine (10 μmol·kg−1·min−1; 30 min) and subsequent superimposition of L-arginine (100 μmol·kg−1·min−1; 30 min), the NO synthase inhibitor Nω-nitro-L-arginine (2.4 mg/kg; iv bolus), and the NO donor sodium nitroprusside (0.24 μg·kg−1·min−1) were examined in Sprague-Dawley rats (SD) and spontaneously hypertensive rats (SHR). Renal medullary perfusion and NO concentration were measured by laser-Doppler flowmetry and polarographically, respectively, 5.5 mm below the kidney surface. Renal medullary NO concentration was less in SHR (53 ± 3 nM) compared with SD rats (108 ± 12 nM; 𝑃 = 0.004). L-Lysine tended to reduce medullary perfusion (−15 ± 7%; 𝑃 = 0.07) and reduced medullary NO concentration (−9 ± 3%; 𝑃 = 0.03) while subsequent superimposition of L-arginine reversed these effects of L-lysine in SD rats. In SHR, L-lysine and subsequent superimposition of L-arginine did not significantly alter medullary perfusion or NO concentration. Collectively, these data suggest that renal L-arginine transport is impaired in SHR. Renal L-[3H]arginine transport was less in SHR compared with SD rats (𝑃 = 0.01). Accordingly, we conclude that impaired arginine transport contributes to low renal NO bioavailability observed in the SHR kidney.

kidney; nitric oxide, L-arginine transport, hypertension

THE ROLE OF NITRIC OXIDE (NO) in kidney function and long-term regulation of arterial pressure is now well established (3, 13). Low NO bioavailability contributes to the development and maintenance of hypertension and related renal damage (17). L-Arginine is the substrate for NO formation in the vascular (2). Cationic amino acid transporters (CAT) are responsible for transporting extracellular L-arginine into the cell. CAT-1 is the predominate L-arginine transporter expressed in the kidney (8). The expression of CAT-1 in the kidney is greatest in the renal medulla, with little or no CAT-1 detectable in the renal cortex (8).

L-Arginine can cause renal vasodilatation (4) and increase renal NO levels (8), while amino acids that compete with L-arginine for cellular uptake can reduce renal NO levels in normotensive Sprague-Dawley (SD) rats (8). Chronic renal medullary infusions of L-ornithine or L-lysine, which compete with arginine for cellular uptake, have been shown to increase arterial pressure and reduce renal medullary NO concentration in SD rats (9). Chronic renal medullary infusion of L-arginine has been shown to reverse the effects of L-ornithine and L-lysine in these rats (9). Collectively, these data indicate that under normal physiological conditions, L-arginine transport plays a critical role in regulating basal renal medullary NO bioavailability and medullary perfusion. Given the critical role of medullary perfusion in regulation of renal excretory function (3), L-arginine transport may have an important role in long-term control of blood pressure.

A growing body of evidence suggests that L-arginine transport is impaired in chronic hypertension (21, 27). For example, L-arginine transport in red blood cells is reduced in spontaneously hypertensive rats (SHR) (27) and hypertensive patients (20, 21) compared with appropriate controls. Impairments in arginine uptake mechanisms have been shown to be linked with increased plasma L-arginine concentration in chronic hypertension (20). L-Arginine transport in peripheral blood mononuclear cells is blunted not only in hypertensive patients but also in normotensive individuals at high risk of the development of hypertension compared with normotensive subjects (21). This latter observation indicates that impairments in L-arginine uptake are not merely a consequence of hypertension but are likely to be causative in the development of hypertension.

Given the central role of the kidney in regulating blood pressure, it is also likely that altered L-arginine and NO metabolism in the kidney could contribute to the pathogenesis of hypertension. However, the lack of reliable techniques to measure renal NO levels in real time, in vivo, has precluded accurate investigations of the roles of NO in regulating renal perfusion and function (6). This is well reflected by the numerous previous in vivo studies, including some of our previous work (18, 19), which have examined the effects of NO on renal perfusion and/or function (5, 8, 25, 28, 29), without directly measuring NO levels in real time. To overcome this issue, we employed a newly developed NO sensor in the current study to measure renal medullary NO levels in real time. In conjunction, we characterized arginine uptake in renal tissue.

The aim of the current study was to compare the roles of renal arginine transport in regulating renal medullary and cortical perfusion and renal medullary NO content in SHR relative to normotensive SD rats. We hypothesized that L-argi-
nine transport is critical in regulating renal medullary NO content and perfusion in normotensive rats. We also hypothesized that impaired l-arginine transport limits NO bioavailability, and thus the contribution of NO to regulation of renal medullary perfusion, in SHR.

METHODS

In vivo experiments were carried out to assess the functional significance of l-arginine transport in maintaining renal cortical and medullary perfusion in SD rats and SHR. We validated a novel NO probe (amiNO-IV, Innovative Instruments, Tampa, FL) for measurement of renal NO content in vivo in real time in the current study. With the use of this new technique, we determined the physiological significance of l-arginine transport in maintaining renal NO bioavailability in SHR and SD rats. Western blotting and l-[3H]arginine uptake experiments were then performed to determine the expression of CAT-I and renal l-arginine transport activity, respectively, in the SD and SHR kidney.

Calibration of AmiNO-IV Probe

The amiNO-IV needle probe (Innovative Instruments) was placed in a calibration solution containing 100 mM sulfuric acid and 6 mM potassium nitrite. Addition of potassium nitrite to this solution results in a calibration solution containing 100 mM sulfuric acid and 6 mM

Calibration of AmiNO-IV Probe

Thus the amount of NO generated is equal to the amount of nitrite added.

The current produced by the NO probe (pA) in response to four different concentrations of potassium nitrite (50, 100, 150, and 200 nM) was determined. The sensitivity (pA/nM) of the probe was then calculated by dividing the current produced (pA) by the respective concentration of potassium nitrite (nM) which produced that current. As per the manufacturer’s instructions, this calibration procedure was performed before each in vivo experiment.

Sensitivity of Amino-IV Probe to Changes in Oxygen, Carbon Dioxide, Osmolarity, and Temperature

To test for the effects of different concentrations of oxygen and carbon dioxide on the sensitivity of the NO probe, the calibration of the probe was performed while the calibration solution was placed in room air, or being bubbled with oxygen or carbon dioxide. To test for the effects of osmolarity on the sensitivity of the NO probe, the calibration of the NO probe was performed in calibration solutions prepared in distilled water, 154 mM and 308 mM NaCl. According to the manufacturer’s instructions, the inNO software program (Innovative Instruments) can automatically compensate for any shifts in current in response to changes in temperature. To test their claim, the NO probe was calibrated at 35 and 37°C.

In Vivo Experiments in SD Rats and SHR

Total renal blood flow (RBF), cortical laser Doppler flux (CLDF), medullary laser Doppler flux (MLDF), and renal medullary NO concentration were measured in SD rats and SHR under control conditions, during renal arterial infusions of l-lysine (which inhibits arginine transport by competing with l-arginine for cellular arginine uptake), and during subsequent superimposition of an infusion of l-arginine. These infusions were then terminated, and after all variables returned to their control level, we tested the effects of a bolus dose of the nitric oxide synthase inhibitor Nω-nitro-l-arginine-methyl ester (l-NAME) and subsequent infusion of the nitric oxide donor sodium nitroprusside (SNP).

Animals

Male SD rats (12 wk; 301–380 g) and SHR (12 wk old, 300–350 g) were used in this study. The animals were provided with food and water ad libitum. All experiments were conducted according to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved in advance by the Animal Ethics Committee of the Monash University School of Biomedical Sciences, or the Alfred Medical Research and Education Precinct Animal Experimentation Ethics Committee.

Surgical Preparations

Adult male SD and SHR were anesthetized (Inactin; thiobutabarbital, 100 mg/kg ip; Sigma, St Louis, MO). A catheter was placed in the trachea to facilitate breathing. Body temperature was maintained at 37°C during surgery and throughout the experiment. Catheters were placed in the femoral artery and vein to facilitate measurement of mean arterial pressure (MAP) and infusion of BSA, respectively. BSA (2% in 154 mM NaCl) was infused (iv at a rate of 5 ml·kg⁻¹·h⁻¹ during surgery and at a rate of 3 ml·kg⁻¹·h⁻¹ postsurgery until the end of the experiment to maintain fluid levels). We have previously found that rats are euvoicemic (hematocrit 40–43% and urine flow rate 5.8 μl/min) under these experimental conditions (7, 16). A catheter was placed in the left renal artery through the abdominal aorta (10) to facilitate infusion of drugs. This catheter was placed ~0.5 mm into the renal artery, and saline was infused continuously (10 μl/min) to maintain catheter patency. An adjustable clamp was placed around the suprarenal abdominal aorta to maintain renal perfusion pressure at its pretreatment level. The left kidney was exposed via a left flank incision and was freed from the surrounding fat tissue and placed in a stable cup. A transit-time ultrasound flow probe (type 0.7VB, Transonic Systems, Ithaca, NY) was placed around the renal artery to measure total RBF. A needle-type laser Doppler flow probe (26 gauge, DP4s, Moor Instruments, Millwey, Devon, UK) was inserted 5.5 mm into the kidney, with the use of a micromanipulator (Narishige, Tokyo, Japan) to measure MLDF. Another laser Doppler flow probe (DP2b, Moor Instruments) was placed on the dorsal surface of the kidney to measure CLDF. The amiNO-IV needle probe and the associated temperature sensor were then inserted 0.5 mm into the kidney with the use of a micromanipulator to measure cortical NO concentration. Once a stable baseline current was obtained in the cortex (30–40 min), the amiNO-IV probe and the associated temperature sensor (Innovative Instruments) were advanced 5.5 mm into the kidney so that the tip of the probe was placed in the renal medulla. When all surgical preparations were completed, the exposed left kidney was covered with cotton wool soaked in saline to keep it moist. After a 60- to 90-min equilibration period, the experimental protocols commenced.

Experimental Protocol

Rats received an intrarenal infusion of l-lysine (10 μmol·kg⁻¹·min⁻¹; 30 min; Sigma-Aldrich, Castle Hill, NSW) followed by superimposition of an infusion of l-arginine (100 μmol·kg⁻¹·min⁻¹; 30 min; Sigma-Aldrich). After a 30-min recovery period during which rats received an intra-arterial infusion of saline (10 μl/min), and all measured variables returned to close to their original baseline levels (see Table 1), the animals were given a bolus dose of l-NAME (2.4 mg/kg iv Sigma-Aldrich). When all the variables were stable, a renal arterial infusion of SNP (0.24 μg·kg⁻¹·min⁻¹; 30 min; Sigma-Aldrich) commenced. Twenty minutes after the completion of the SNP infusion, rats were euthanized with an overdose of pentobarbital sodium.

Recording of Hemodynamic Variables

Signals were acquired and processed as described previously (19) to obtain 2-s averages of MAP (mmHg), CLDF (perfusion units), and MLDF (perfusion units). Signals from the amiNO IV probe were acquired and processed separately. Two-second averages of current (pA; detected by the amiNO-IV probe) and temperature (°C; detected by the temperature sensor) were acquired and processed with
Table 1. Effects of l-lysine and subsequent superimposition of l-arginine, and l-NAME and subsequent sodium nitroprusside on renal perfusion and medullary nitric oxide concentration in Sprague-Dawley and spontaneously hypertensive rats

<table>
<thead>
<tr>
<th>Effects of l-Lysine and Subsequent l-arginine in SD Rats</th>
<th>Pretreatment</th>
<th>l-Lysine</th>
<th>l-arginine + l-arginine</th>
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<tr>
<td>MAP, mmHg</td>
<td>97 ± 5</td>
<td>98 ± 3</td>
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<td>RBF, ml/min</td>
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<td>CLDF, units</td>
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<td>319 ± 35</td>
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<td>MLDF, units</td>
<td>75 ± 10</td>
<td>64 ± 11</td>
<td>78 ± 13†</td>
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<td>Medullary [NO], units</td>
<td>108 ± 12</td>
<td>97 ± 11*</td>
<td>109 ± 11*</td>
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<th>Pre-l-NAME Levels</th>
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<th>Sodium Nitroprusside</th>
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<tr>
<td>MAP, mmHg</td>
<td>96 ± 4</td>
<td>98 ± 4</td>
<td>95 ± 5</td>
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<td>RBF, ml/min</td>
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<tr>
<td>CLDF, units</td>
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<td>MLDF, units</td>
<td>75 ± 10</td>
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<td>69 ± 13†</td>
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<td>Medullary [NO], units</td>
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<td>97 ± 10*</td>
<td>112 ± 11*</td>
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<td>RBF, ml/min</td>
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<td>CLDF, units</td>
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<td>252 ± 38</td>
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<td>MLDF, units</td>
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<td>Medullary [NO], units</td>
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<th>Effects of l-NAME and Subsequent Sodium Nitroprusside in SHR</th>
<th>Pre-l-NAME Levels</th>
<th>l-NAME</th>
<th>Sodium Nitroprusside</th>
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<tr>
<td>MAP, mmHg</td>
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<td>138 ± 4</td>
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<tr>
<td>RBF, ml/min</td>
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<td>CLDF, units</td>
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<td>261 ± 10*</td>
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<tr>
<td>MLDF, units</td>
<td>66 ± 9</td>
<td>54 ± 9‡</td>
<td>61 ± 11†</td>
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<tr>
<td>Medullary [NO], units</td>
<td>53 ± 3</td>
<td>50 ± 2*</td>
<td>65 ± 5€</td>
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</table>

Values are means ± SE. l-NAME, N\(^\text{3}\)-nitro-l-arginine methyl ester; MAP, mean arterial pressure; RBF, renal blood flow; CLDF, cortical laser Doppler flux; MLDF, medullary laser Doppler flux; medullary [NO], medullary nitric oxide concentration; SD, Sprague-Dawley rats; SHR, spontaneously hypertensive rats. *P ≤ 0.05, †P ≤ 0.01, ‡P ≤ 0.001 compared with the levels observed during the previous treatment period.

To determine renal medullary l-arginine uptake, 100 µg of protein from the renal medullary cell suspension was incubated with l\(^{-1}\)Hl-arginine and unlabeled arginine over a range of 2–1,000 µM in a final incubation volume of 50 µl HEPES buffer (pH 7.4). All reaction tubes were run in triplicate. After incubation of the reaction tubes at 37°C for 1 min, l\(^{-1}\)Hl-arginine uptake was terminated by addition of 200 µl of ice-cold HEPES buffer containing 25 mM unlabeled l-arginine. Parallel incubations were performed at 0°C to determine nonspecific l-arginine uptake. All samples were centrifuged at 10,000 g at 4°C for 7 min, and the pellet was resuspended in 200 µl of ice-cold HEPES buffer containing 25 mM unlabeled l-arginine. Samples were then centrifuged at 10,000 g at 4°C for 2 min, the pellet was resuspended in 500 µl of lysis buffer (0.2% wt/vol SDS and 0.2 M NaOH), and 45 min were allowed for cells to solubilize. Samples (400 µl) were then transferred into scintillation vials, and radioactivity in each sample was determined using a liquid scintillation counter. To determine specific uptake in cells, nonspecific uptake at 0°C was subtracted from the total uptake at 37°C.

Data Analysis

Data are expressed as means ± SE. Changes in MAP, RBF, CLDF, MLDF, and medullary NO levels during the final 3 min of various treatment periods were compared with the average levels of these variables during the 3-min period immediately preceding the commencement of each treatment. For example, the average levels of different variables during the final 3 min of l-lysine infusion were compared with the levels of these variables during the final 3 min of the control period, immediately preceding the commencement of l-lysine infusion. The average levels of variables during the final 3 min of l-arginine + l-lysine infusion were compared with the levels of these variables during the final 3 min of the l-lysine alone infusion. Kinetic analysis of renal l-l-arginine transport in Sprague-Dawley rats, which was then blocked with 5% wt/vol nonfat dry milk in TBS to block nonspecific binding. The PVDF membrane was incubated with a 1:1,000 dilution of a custom-made polyclonal rabbit anti-CAT-1 primary antibody (24) (Rockland Immunochemicals) in 5% wt/vol BSA in TBS overnight at 4°C. The membrane was then incubated with horseradish peroxidase-labeled sheep anti-rabbit IgG (Chemicon) in 5% wt/vol skim milk in TBS. The CAT-1 protein was detected with the use of enhanced chemiluminescence (Amersham Biosciences) on X-ray film. Membranes were stripped with Tris-buffered solution containing 2% SDS and 100 mM β-mecaptoethanol and reprobed for β-actin.

Preparation of Inner Medullary Cells for Measurements of l\(^{-1}\)Hl-arginine Uptake

Inner medullary cells were prepared as described previously (26). This preparation was a mix of cells from the inner medulla which predominately consisted of inner medullary collecting duct cells (26). Rats were euthanized with an overdose of pentobarbital sodium (100 mg/kg ip), and the kidneys were rapidly removed and cut in half along the longitudinal axis. The renal papilla was excised and immediately placed in ice-cold HEPES buffer (135 mM NaCl, 3 mM KCl, 1.5 mM CaCl\(_2\), 1 mM MgSO\(_4\), 1 mM KH\(_2\)PO\(_4\), 5.5 mM glucose and 20 mM HEPES, pH 7.4). The papilla was cut into small pieces in a petri dish filled with ice-cold HEPES buffer and then transferred into a conical plastic tube containing 10 ml of HEPES buffer solution containing 2 mg/ml collagenase. The tissue was incubated at 37°C for 60 min while being continuously bubbled with carbogen and was aspirated through a Pasteur pipette several times to break up any visible cell clumps. The cell suspension was then centrifuged at 180 g for 2 min, and the pellet was resuspended in HEPES buffer and this procedure was repeated. The protein concentration of the cell suspension was determined using a Bio-Rad protein assay kit (Bio-Rad, Hercules, CA) with BSA as a standard.

l-Arginine Transport Assay

To determine renal medullary l-arginine uptake, 100 µg of protein from the renal medullary cell suspension was incubated with l\(^{-1}\)Hl-arginine and unlabeled arginine over a range of 2–1,000 µM in a final incubation volume of 50 µl HEPES buffer (pH 7.4). All reaction tubes were run in triplicate. After incubation of the reaction tubes at 37°C for 1 min, l\(^{-1}\)Hl-arginine uptake was terminated by addition of 200 µl of ice-cold HEPES buffer containing 25 mM unlabeled l-arginine. Parallel incubations were performed at 0°C to determine nonspecific l-arginine uptake. All samples were centrifuged at 10,000 g at 4°C for 7 min, and the pellet was resuspended in 200 µl of ice-cold HEPES buffer containing 25 mM unlabeled l-arginine. Samples were then centrifuged at 10,000 g at 4°C for 2 min, the pellet was resuspended in 500 µl of lysis buffer (0.2% wt/vol SDS and 0.2 M NaOH), and 45 min were allowed for cells to solubilize. Samples (400 µl) were then transferred into scintillation vials, and radioactivity in each sample was determined using a liquid scintillation counter. To determine specific uptake in cells, nonspecific uptake at 0°C was subtracted from the total uptake at 37°C.
nine uptake was performed using the nonlinear regression function of GraphPad Prism version 5.04.

Densitometric Analysis

The density of immunoreactive bands was quantified by performing densitometric scans with the use of NIH Image J (V. 1.41o) public domain software. Membrane background was subtracted, and band intensity was calculated relative to the expression of β-actin protein (loading control). Results were then expressed relative to β-actin.

Statistics

Repeated-measures ANOVA was used to assess the effects of L-lysine and L-arginine infusions. Specific contrasts were then made between levels of hemodynamic variables during the final 3 min of J) the pretreatment period and the period during L-lysine infusion, 2) the L-lysine infusion period and the period during subsequent superimposition of L-arginine, and 3) the pretreatment period and the period during subsequent superimposition of an infusion of L-arginine. To reduce the risk of a comparison-wise type 1 error, P values were conservatively adjusted using the Greenhouse-Geisser correction.

Two-way ANOVA followed by Bonferroni post hoc tests were used to determine whether renal responses to J) L-lysine and subsequent superimposition of L-arginine infusion and 2) L-NAME and subsequent SNP were different in SD rats compared with SHR.

Student’s t-tests were used for dichotomous comparisons and specifically to test 1) before and after effects of a single treatment on baseline levels of variables within the same animal, 2) whether the effects of L-NAME on medullary perfusion or NO content differed between SD rats and SHR, 3) whether expression of CAT-1 protein in the kidney differed between the two strains, and 4) whether renal L-[3H]arginine uptake in the kidney differed between SD rats and SHR. Two-tailed \( P \leq 0.05 \) was considered statistically significant.

RESULTS

Effects of Temperature, Osmolarity, and Oxygen and Carbon Dioxide Content on NO Probe Sensitivity

A calibration curve was constructed by plotting the current levels (pA) obtained in response to known concentrations of a potassium nitrate solution (50–200 nM). Addition of increasing volumes of a nitrate standard produced concentration-dependent increases in current (Fig. 1).

When temperature compensation mode was turned on, changes in temperature in the calibration solution (35–37°C) had little effect on the concentrations of NO produced (\( P = 0.76 \); data not shown). Similarly, changes in oxygen and carbon dioxide concentrations had little effect on the sensitivity of the NO probe (\( P = 0.49 \); data not shown). In contrast, the sensitivity of the probe was significantly less in the presence of isotonic (154 mM; \( P = 0.0003 \)) and hypertonic (308 mM; \( P = 0.002 \)) NaCl than in the presence of distilled water (Fig. 1). Nevertheless, there was little difference in sensitivity between the two concentrations of saline (\( P = 0.17 \); Fig. 1).

In Vivo Studies: Baseline Hemodynamics

SD rats and SHR. Baseline levels of MAP, RBF, CLDF, MLDF, and medullary NO content in SD rats and SHR are shown in Table 1. Basal MAP was significantly greater in SHR than in SD rats (\( P \leq 0.002 \)), but the basal levels of MLDF were similar in the two strains of rats. Basal levels of RBF and CLDF were significantly less in SHR compared with the respective levels of these variables in SD rats (\( P \leq 0.004 \); Table 1).

Basal medullary and cortical NO concentrations were significantly less in SHR compared with the respective levels of these variables in SD rats (\( P \leq 0.004 \); Table 1).

Renal Responses to L-Arginine Transport Inhibitor L-Lysine and Subsequent Superimposition of L-Arginine

Responses of RBF and CLDF. L-Lysine and subsequent superimposition of L-arginine infusion did not significantly alter RBF or CLDF, in both SD rats and SHR (\( P \geq 0.07 \); Fig. 3).

Responses of MLDF and medullary NO content. Figure 4 indicates a representative trace of medullary NO in response to L-lysine and subsequent superimposition of an infusion of L-arginine in a SD rat. In these rats, L-lysine tended to reduce MLDF (23%; \( P = 0.07 \)) and significantly reduced medullary NO content (12%; \( P = 0.03 \)). In these L-lysine-pretreated rats, subsequent superimposition of L-arginine increased MLDF (23%); \( P = 0.01 \) and medullary NO content (12%; \( P = 0.02 \)), so that levels of these variables were not significantly different from the respective control levels (\( P \geq 0.16 \); Fig. 5). In SHR, neither L-lysine nor subsequent superimposition of L-arginine significantly altered MLDF or medullary NO content (\( P \geq 0.25 \); Fig. 5). Responses of MLDF and medullary NO concentration to L-lysine and subsequent superimposition of L-arginine were significantly different in SD rats compared with SHR (\( P \leq 0.05 \)).
Renal Responses to NOS inhibitor L-NAME and Subsequent NO Donor SNP

Responses of RBF and CLDF. In SD rats, L-NAME administration was followed by reductions in RBF (−25 ± 6%; \( P = 0.01 \)) and CLDF (−32 ± 8%; \( P = 0.01 \)). In these L-NAME-pretreated rats, subsequent SNP infusion then increased RBF (34 ± 5%; \( P = 0.04 \)) and CLDF (39 ± 8%; \( P = 0.03 \)) so that the levels of these variables during SNP infusion were comparable to the respective control levels observed before L-NAME administration (\( P \geq 0.33 \); Fig. 6).

In SHR, the L-NAME bolus reduced RBF (−11 ± 3%; \( P = 0.003 \)) and CLDF (−15 ± 4%; \( P = 0.02 \)). In these L-NAME-pretreated rats, subsequent SNP infusion increased RBF (\( P = 0.01 \)) and CLDF (\( P = 0.04 \)), so that levels of these variables were not significantly different from the respective control levels observed before L-NAME administration (\( P \geq 0.11 \)).

Responses of MLDF and medullary NO content. In SD rats, L-NAME administration was followed by reductions in MLDF (−37 ± 9%; \( P = 0.005 \)) and medullary NO concentration (−9 ± 2%; \( P = 0.03 \)). In these L-NAME-pretreated rats, subsequent SNP infusion then increased MLDF and medullary NO content by 42 ± 8 (\( P = 0.005 \)) and 16 ± 6 (\( P = 0.05 \)), respectively, so that the levels of MLDF and medullary NO content during the final 3 min of SNP infusion were similar to the respective control levels of these variables before administration of L-NAME (Fig. 7; \( P \geq 0.11 \)).

In SHR, L-NAME administration was followed by reductions in MLDF (−19 ± 2%; \( P = 0.001 \)) and medullary NO concentration (−5 ± 1%; \( P = 0.02 \)). However, the reduction in MLDF in response to L-NAME was significantly less in SHR (−19 ± 2%), compared with SD rats (−37 ± 9%; \( P = 0.03 \)). In L-NAME-pretreated SHR, subsequent SNP infusion increased MLDF by 13 ± 2% (\( P = 0.004 \); Fig. 7), so that the level of MLDF was not significantly different from the control
level observed before L-NAME administration. In L-NAME-pretreated SHR, subsequent SNP infusion increased medullary NO concentration by 29% ($P < 0.01$; Fig. 7), so that the level of this variable was 23% greater than its control level before L-NAME administration ($P < 0.01$).

**CAT-1-Immunoreactive Protein in Kidneys of SD Rats and SHR**

Previous studies have shown that CAT-1 is the predominant L-arginine transporter expressed in the SD rat kidney (9, 26). Consistent with previous data (24), we observed two bands at 80 kDa corresponding to glycosylated forms of CAT-1 protein in the kidney (Fig. 8A). Densitometric analysis indicated that CAT-1-immunoreactive protein expression is significantly greater in the kidney of the 12-wk-old SHR than in age-matched SD rats (Fig. 8B).

**Renal L-Arginine Transport in SD rats and SHR**

L-[3H]arginine uptake in renal medullary cells of SD rats and SHR is shown in Fig. 9. The kinetic analysis of renal L-[3H]arginine uptake demonstrated a maximal velocity ($V_{\text{max}}$) of 651 pmol·mg protein$^{-1}$·min$^{-1}$ and a Michaelis constant ($K_{\text{m}}$) of 148 μM for SD rats. In SHR, $V_{\text{max}}$ and $K_{\text{m}}$ of renal L-[3H]arginine uptake were 456 pmol·mg protein$^{-1}$·min$^{-1}$ and 275 μM, respectively. In these rats, $V_{\text{max}}$ of renal L-[3H]arginine uptake was less while $K_{\text{m}}$ of renal L-[3H]arginine transport was greater compared with SD rats ($P \leq 0.01$).

**DISCUSSION**

There were four major novel findings from our in vivo study. First, we found that basal renal cortical and medullary NO content was significantly less in SHR compared with SD rats. Second, we found that the L-arginine transport inhibitor L-lysine tended to reduce MLDF and significantly reduced medullary NO content in SD rats. In these L-lysine-pretreated SD rats, subsequent superimposition of an infusion of L-arginine increased MLDF and medullary NO content, so that levels of these variables were not significantly different from their respective control levels. In SHR, neither L-lysine nor subsequent L-arginine infusion significantly altered MLDF or medullary NO content. These data suggest that L-arginine transport, which plays a critical role in modulating basal renal medullary perfusion and NO content in SD rats (8), has little impact on renal medullary perfusion and NO concentration in SHR. Consistent with this proposition, in our in vitro studies we found evidence for lesser L-arginine uptake in medullary collecting duct cells from SHR than SD rats. Third, we found that L-lysine and subsequent L-arginine did not alter CLDF in both SD rats and SHR, suggesting that L-arginine transport has little role in regulating renal cortical perfusion in both these rat strains. Finally, our data indicate that the amiNO-IV NO sensor is capable of measuring changes in renal medullary NO concentration in vivo in real time.

Our present findings are consistent with previous findings which indicate a role for arginine transport in modulating NO
levels in the renal medulla of SD rats (8). CAT-1 is the principal l-arginine transporter expressed in the SD rat kidney (8). Thus it is likely that CAT-1 was responsible for modulating renal medullary NO content and perfusion in response to l-lysine and subsequent l-arginine. In SD rats, CAT-1 is chiefly localized to vasa recta and collecting ducts in the renal medulla and little or no CAT-1 protein is detected in the renal cortex (8). This provides a possible explanation for our observation that l-lysine and subsequent l-arginine had little effect on CLDF. Collectively, these data indicate that arginine transport plays a critical role in regulating basal renal medullary, but not cortical, perfusion and NO content.

l-Arginine transport inhibition with l-lysine and subsequent reactivation with l-arginine modulated MLDF and medullary NO concentration in normotensive SD rats as has been previously reported (8). In contrast, these amino acids did not significantly alter NO concentration and perfusion in the renal medulla in SHR, suggesting that there are functional impairments in renal arginine transport in these rats. Consistent with this, previous data indicate that chronic l-arginine administration fails to reduce arterial pressure in SHR (11), suggesting that l-arginine fails to increase NO bioavailability in this strain of rats. This contrasts with our previous findings, which indicate that even acute infusions of l-arginine can increase renal medullary NO levels in normal SD rats (8). eNOS and CAT-1 are colocalized in the plasma membrane caveolae of endothelial cells (14), and eNOS is suggested to preferentially utilize extracellular l-arginine transported by CAT-1 to produce NO (14). Recent evidence suggests that renal eNOS expression and activity are either not different or even upregulated in SHR compared with age-matched normotensive rats (12). Thus it is unlikely that the lack of effect of l-lysine and subsequent l-arginine on renal medullary perfusion and NO content in SHR reflects lesser expression/activity of eNOS in the SHR kidney compared with the SD rat kidney.

To determine the mechanisms underlying the relative insensitivity of the renal medullary circulation of SHR to l-arginine transport inhibition with l-lysine and subsequent reactivation with l-arginine, we examined CAT-1 expression and l-[3H]arginine uptake by renal medullary cells.
uptake in kidney tissue from SD rats and SHR. We hypothesized that lesser expression of the predominant arginine transporter CAT-1 in the SHR kidney may be responsible for impaired renal arginine transport observed in these rats. Surprisingly, we found that the CAT-1 protein expression in the kidney was significantly greater in SHR compared with age-matched SD rats. The greater CAT-1 expression in the SHR kidney may reflect a compensatory increase in CAT-1 expression in response to low renal NO bioavailability in SHR. Our current findings indicate that $V_{\text{max}}$ and $K_m$ of renal L-arginine transport in SD rats were 651 pmol·mg protein$^{-1}$·min$^{-1}$ and 148 μM, respectively. The $K_m$ value we obtained for renal L-arginine transport is consistent with previous studies that characterized a system y$^+$ transport mechanism in cultured porcine aortic endothelial cells ($K_m$ 140 μM; $V_{\text{max}}$ ~2.08 nmol/5 × 10^6 cells/min) (1) and rat cardiac myocytes ($K_m$ 125 μM; $V_{\text{max}}$ ~44 pmol/2 × 10^5 cells/min) (22). Plasma L-arginine concentration in adult Wistar-Kyoto (WKY) rats and SHR are ~125 and 100 μM, respectively (23). Our current data indicate that the $K_m$ of renal L-arginine transport in SD rats and SHR are 148 and 275 μM, respectively. These data indicate that reduced cellular L-arginine uptake in SHR may alter the intracellular availability of L-arginine and lead to an impairment in NO production in the SHR. Collectively, these data indicate that CAT-1-mediated L-arginine uptake is impaired in the SHR kidney relative to the SD rat kidney despite upregulation of CAT-1 expression in the SHR kidney. Our current data are consistent with previous findings which indicate that arginine transport is impaired in hypertension. For example, it has been shown that arginine transport via the y$^+$L system is lesser in red blood cells from hypertensive patients and SHR compared with normotensive subjects and WKY rats, respectively (15). Impaired L-arginine transport observed in hypertensive patients is associated with increased plasma concentrations of L-arginine (15, 20). Arginine transport was shown to be impaired not only in hypertensive patients but also in normotensive subjects genetically predisposed to hypertension (21). This latter observation suggests that impaired L-arginine transport is not merely a consequence of hypertension but may rather be a cause for the development of this disease. Our current findings are significant in this context as they provide evidence of impaired L-arginine transport-dependent NO formation in renal vascular and/or tubular elements critical in regulation of medullary perfusion. Our data indicate that the amiNO-IV probe is capable of measuring changes in NO levels in the kidney under in vivo experimental conditions. A major strength of this new method is its ability to simultaneously measure temperature within the kidney along with NO measurements and compensate for any shifts in the current resulting from changes in temperature. This is critical since changes in temperature within the kidney can greatly interfere with NO measurements and are difficult to avoid during renal arterial drug infusions that alter renal perfusion. Our data indicate that the amiNO-IV NO probe is also insensitive to changes in oxygen and carbon dioxide levels. We found that the sensitivity of the amino-IV NO probe was reduced in the presence of ionic solute compared with its sensitivity in distilled water. However, we observed little change in the sensitivity of this probe when measuring NO levels under isotonic and hypertonic conditions. These latter findings suggest that NO measurements obtained in the current study are unlikely to be confounded by regional differences in osmolarity in the kidney. We observed ~40 and 20% reductions in medullary perfusion in response to L-NAME in SD rats and SHR, respectively. However, the corresponding decreases in medullary NO content were ~5% in both strains of rats. Thus the NO probe may have failed to detect the true magnitude of the reduction in medullary NO content in response to the NO synthase inhibitor L-NAME. Alternatively, it may simply be that NO makes a lesser contribution to the regulation of medullary perfusion in the SHR than the SD rat. This notion is supported by the observation of lesser NO content in the medulla and cortex of SHR than SD rats. We conclude that renal NO bioavailability is less in the SHR kidney compared with the normotensive SD rat kidney. Renal arginine transport plays a critical role in maintaining basal renal medullary NO bioavailability and perfusion in normotensive SD rats but not in SHR. This appears to be due to a defect in arginine transport in the SHR kidney.

**Perspectives**

Our data indicate that the capacity of renal arginine transport to regulate NO content is compromised in SHR compared with SD rats, consistent with a major impairment of arginine transport in the former strain compared with the latter. This is consistent with previous data, which indicate that arginine transport is impaired in human hypertension. Previous data demonstrate that antioxidants do not improve NO-dependent renal vasodilatation in the SHR kidney and this is likely because renal NO formation is already compromised in these rats. In this context, our data provide strong evidence that resurrecting renal arginine transport may help restore renal NO content and improve renal endothelial function in hypertension. Given the central role of the kidney in blood pressure regulation, such treatments should also ameliorate hypertension. Accordingly, we suggest that manipulation of renal arginine transport or metabolism may be a useful target for the development of further therapies in which renal blood flow and NO homeostasis are disturbed.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

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