Arginase inhibition restores arteriolar endothelial function in Dahl rats with salt-induced hypertension

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1Tulane Hypertension and Renal Center of Excellence, and Department of Physiology, Tulane University Health Sciences Center, New Orleans, Louisiana; and 2Michael E. DeBakey Veterans Affairs Medical Center, and Departments of Medicine and Pharmacology, Baylor College of Medicine, Houston, Texas

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Johnson, Fruzsina K., Robert A. Johnson, Kelly J. Peyton, and William Durante. Arginase inhibition restores arteriolar endothelial function in Dahl rats with salt-induced hypertension. Am J Physiol Regul Integr Comp Physiol 288: R1057–R1062, 2005. First published December 9, 2004; doi:10.1152/ajpregu.00758.2004.—Vascular tissues express arginase that metabolizes L-arginine to L-ornithine and urea and thus reduces substrate availability for nitric oxide formation. Dahl salt-sensitive (Dahl-S) rats with salt-induced hypertension show endothelial dysfunction, including decreased vascular nitric oxide formation. This study tests the hypothesis that increased vascular arginase activity contributes to endothelial dysfunction in hypertensive Dahl-S rats. Male Dahl-S rats (5–6 wk) were placed on high (8%) or low (0.3%) NaCl diets for 4 wk. With respect to the low-salt group, mean arterial blood pressure was increased in the high-salt animals. Immunohistochemical stainings for arginase I and II were enhanced in arterioles isolated from high-salt Dahl-S rats. Experiments used isolated Krebs buffer-superseded first-order gracilis muscle arterioles with constant pressure (80 mmHg) and no luminal flow or constant midpoint but altered endpoint pressures to establish graded levels of luminal flow (0–50 μl/min). In high-salt arterioles, responses to an endothelium-dependent vasodilator acetylcholine (1 μmol/l to 3 μmol/l) and flow-induced dilations were decreased. Acute in vitro treatment with an inhibitor of arginase, 100 μmol/l (5-(2-boronoethyl)-L-cysteine, or the nitric oxide precursor, 1 mmol/l L-arginine, similarly enhanced acetylcholine and flow-induced maximal dilations and abolished the differences between high- and low-salt arterioles. These data show that arteriolar arginase expression is increased and that endothelium-dependent vasodilation is decreased in high-salt Dahl-S rats. Acute pretreatment with an arginase inhibitor or with L-arginine restores endothelium-dependent vasodilation and abolishes the differences between high- and low-salt groups. These results suggest that enhanced vascular arginase activity contributes to endothelial dysfunction in Dahl-S rats with salt-induced hypertension and identifies arginase as a potential therapeutic target to prevent endothelial dysfunction.

salt-sensitive hypertension; vascular tone; arterioles

THE RELEASE OF NITRIC OXIDE through the oxidation of L-arginine by nitric oxide synthase plays a pivotal role in the maintenance of vascular homeostasis. In response to changes in hemodynamic forces (shear stress) or receptor stimulation (e.g., acetylcholine), nitric oxide is released from the vascular endothelium to promote relaxation of vascular smooth muscle. Endothelial dysfunction resulting from impaired nitric oxide synthesis is a prominent feature in several cardiovascular diseases, including hypertension (4, 31). Given that L-arginine is the exclusive substrate of endothelial nitric oxide synthase (24), alterations in L-arginine availability may play a crucial role in regulating nitric oxide release. In this respect, endothelial cells express arginase that catalyzes the metabolism of L-arginine to L-ornithine and urea (5, 8, 32), raising the possibility that arginase and endothelial nitric oxide synthase may compete for substrate. Consistent with this proposal, inhibition of arginase activity stimulates nitric oxide production (5, 8, 32), whereas overexpression of arginase inhibits nitric oxide synthesis in cultured endothelial cells (20). Moreover, recent findings indicate that increased arginase activity blocks nitric oxide synthesis in the corpus cavernosum and promotes erectile dysfunction in diabetes (3, 19), as well as endothelial dysfunction in aging (2) and after ischemia-reperfusion (13). In addition, a recent study found that upregulation of vascular arginase expression decreases nitric oxide-mediated dilation of coronary arterioles isolated from pigs with aortic coartation hypertension (33).

Dahl/Rapp salt-sensitive (Dahl-S) rats are models of salt-induced hypertension (25). Attenuated endothelium-dependent vasodilation has been repeatedly demonstrated in blood vessels isolated from hypertensive Dahl-S rats (4, 16, 21, 34); however, the pathological basis remains uncertain. Although plasma substrate levels for nitric oxide synthesis are normal in these animals (7), salt-induced hypertension (6, 14), as well as vascular endothelial dysfunction (34), can be corrected by the administration of L-arginine, suggesting that impaired intracellular L-arginine availability may be a contributing factor. Because enhanced arginase activity has been shown to decrease nitric oxide production and promote endothelial dysfunction in various pathological conditions, we hypothesized that arginase may contribute to endothelial dysfunction in Dahl-S rats with salt-induced hypertension. To test this hypothesis, we evaluated arteriolar arginase I and II content using immunohistochemistry in Dahl-S rats on high- and low-salt diets. To examine endothelial function, we conducted experiments using skeletal muscle arterioles taken from these Dahl-S rats and examined the responses to an endothelium-dependent vasodilator and increases in luminal flow while in the presence or absence of an inhibitor of arginase or L-arginine.

METHODS

Materials. S-(2-boronoethyl)-L-cysteine (BEC) was purchased from Calbiochem (EMD Biosciences, San Diego, CA). Hematoxylin solution, thiothabarbital sodium (Inactin), L-arginine, and acetylcholine were obtained from Sigma Aldrich (St. Louis, MO).
enhanced 3,3′-diaminobenzidine kit was purchased from Pierce Biotechnology (Rockville, IL), and arginase I and II antibodies were obtained from Santa Cruz Biotechnologies (Santa Cruz, CA). All other chemicals were obtained from Fisher Scientific (Houston, TX). Acetylcholine (10 mmol/l) stock solution was prepared in saline and diluted in modified Krebs buffer immediately before use. Inactin was prepared fresh daily in saline (50 mg/ml), and maintained 80 mmHg constant luminal pressure with no flow. After pretreatment and the distal micropipette to a closed stopcock to achieve was connected to a pressure servo-controller (Living Systems Instrumentation). The arginase inhibitor (100 μmol/l BEC), the arginase and nitric oxide synthase substrate (1 mmol/L L-arginine), or vehicle was included in the luminal perfusion buffer. During a 60-min stabilization period, both proximal and distal pressures were adjusted to 80 mmHg with no luminal flow. During the experiments, proximal and distal pressures were adjusted equally in opposite directions to maintain the midline pressure at 80 mmHg and establish graded levels of luminal flow (0–50 μl/min in 5 μl/min increments). Each flow was tested for 5 min, internal diameter was recorded every minute, and the average of the last two measurements was used to determine the response.

To study flow-induced dilation, both the proximal and distal micropipettes were connected to pressure servo-controllers and an inline microflowmeter (Living Systems Instrumentation). The arginase inhibitor (100 μmol/l BEC), the arginase and nitric oxide synthase substrate (1 mmol/L L-arginine), or vehicle was included in the luminal perfusion buffer. During a 60-min stabilization period, both proximal and distal pressures were adjusted to 80 mmHg with no luminal flow. During the experiments, proximal and distal pressures were adjusted equally in opposite directions to maintain the midline pressure at 80 mmHg and establish graded levels of luminal flow (0–50 μl/min in 5 μl/min increments). Each flow was tested for 5 min, internal diameter was recorded every minute, and the average of the last two measurements was used to determine the response.

Statistics. All data are expressed as means ± SE. Vascular response data were analyzed by ANOVA using a computer statistical package (SigmaStat 3.0). When significant differences were observed, orthogonal contrasts were performed as a post hoc analysis (27). All other data were analyzed by t-tests. A value of P < 0.05 was considered statistically significant.

RESULTS

Blood pressure measurements. Table 1 summarizes mean arterial pressure, heart rate, and body and organ weights for Dahl-S rats on high- and low-salt diets. After 4 wk of high-salt diet, mean arterial pressure was increased in Dahl-S rats compared with the low-salt group, but there was no difference in heart rate. Dahl-S rats after 4 wk of high-salt diets had lower body but higher kidney and heart weights compared with low-salt diet controls.

Arginase I and II immunohistochemistry. First-order gracilis muscle arterioles isolated from Dahl-S rats on low-salt diets exhibited immunostaining for both arginase I and II. Arterioles isolated from Dahl-S rats on high-salt diets showed enhanced immunostaining for arginase I as well as arginase II in the vascular endothelium and smooth muscle compared with the low-salt group (Fig. 1). Control sections, where the arginase antibody was omitted, did not show signs of immunostaining.

Isolated microvessel experiments. An endothelium-dependent vasodilator, acetylcholine (1 mmol/l to 3 μmol/l) promoted concentration-dependent increases in diameter of arterioles isolated from Dahl-S rats after 4 wk of high- or low-salt diets (Fig. 2, top). However, acetylcholine-induced vasodilation was greatly attenuated in high-salt arterioles compared with the low-salt group (low-salt Δmax: 36 ± 8 μm, n = 5, vs. high-salt Δmax: 16 ± 4 μm, n = 7; P < 0.05, where Δmax is maximum

<table>
<thead>
<tr>
<th>Variable</th>
<th>Low-Salt (n = 18)</th>
<th>High-Salt (n = 20)</th>
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<tbody>
<tr>
<td>MAP, mmHg</td>
<td>136±3</td>
<td>171±6*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>429±7</td>
<td>400±12</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>327±6</td>
<td>307±7*</td>
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<tr>
<td>Kidney weight, g</td>
<td>1.20±0.02</td>
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<tr>
<td>Kidney/body weight, %</td>
<td>0.37±0.01</td>
<td>0.62±0.02*</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>1.11±0.02</td>
<td>1.45±0.02*</td>
</tr>
<tr>
<td>Heart/body weight, %</td>
<td>0.34±0.01</td>
<td>0.48±0.01*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of rats. Dahl-S rats, Dahl salt-sensitive rats.
MAP, mean arterial pressure; HR, heart rate. *P < 0.05 compared with low-salt group.
change). Acute in vitro pretreatment with an inhibitor of arginase, 100 μmol/l BEC, enhanced maximal responses and abolished the difference between high- and low-salt arterioles (low-salt Δ_max: 57 ± 2 μm, n = 6, vs. high-salt Δ_max: 49 ± 3 μm, n = 6) (Fig. 2, middle). Similarly, acute in vitro pretreatment with the arginase and nitric oxide synthase substrate, 1 mmol/l L-arginine, enhanced maximal responses in both groups and abolished the difference between high- and low-salt arterioles (low-salt Δ_max: 54 ± 3 μm, n = 6, vs. high-salt Δ_max: 48 ± 8 μm, n = 6) (Fig. 2, bottom).

Increases in luminal flow (0–50 μl/min) promoted vasodilation in arterioles isolated from Dahl-S rats on low-salt diets but not in arterioles from high-salt animals (low-salt Δ_max: 20 ± 1 μm, n = 7, vs. high-salt Δ_max: −1 ± 1 μm, n = 6; P < 0.05) (Fig. 3, top). Acute in vitro pretreatment with an inhibitor of arginase, 100 μmol/l BEC, restored flow-induced responses in high-salt arterioles and abolished the difference between high- and low-salt groups (low-salt Δ_max: 22 ± 3 μm, n = 5, vs. high-salt Δ_max: 19 ± 1 μm, n = 4) (Fig. 3, middle). Similarly, acute in vitro pretreatment with the arginase and nitric oxide synthase substrate, 1 mmol/l L-arginine, restored flow-induced dilation in high-salt arterioles and abolished the difference between high- and low-salt groups (low-salt Δ_max: 22 ± 1 μm, n = 4, vs. high-salt Δ_max: 20 ± 1 μm, n = 4) (Fig. 3, bottom).

**DISCUSSION**

In the present study, we found that arginase I and II are expressed in Dahl-S rat skeletal muscle arterioles and that the expression of both arginase isoforms is increased with salt-induced hypertension. In addition, we discovered that acute in vitro pretreatment with an arginase inhibitor restores endothelium-dependent vasodilator responses and abolishes the differences between high- and low-salt arterioles. Similarly, we...
found that acute in vitro pretreatment with the arginase and nitric oxide synthase substrate, l-arginine, restores endothelium-dependent responses and eliminates the differences between the two salt groups. These data suggest that enhanced vascular arginase activity contributes to endothelial dysfunction in Dahl-S rats with salt-induced hypertension.

Arginase is the central enzyme in the urea cycle, metabolizing l-arginine to l-ornithine and urea. Two distinct isoforms of arginase, arginase I and II, have been identified (9, 29). These isozymes possess similar enzymatic properties, but differ with regard to their tissue distribution and subcellular localization (15). Type I arginase is a cytosolic enzyme that is highly expressed in the liver, whereas arginase II is a mitochondrial isoform that is found predominantly in extrahepatic tissue. Previous studies have documented the presence of arginase I and II in cultured vascular smooth muscle and endothelial cells as well as the expression of arginase I in the aorta and coronary microvasculature (2, 10, 13, 20, 30). Our present findings extend this work by demonstrating the expression of arginase I and II in first-order gracilis muscle arterioles of Dahl-S rats. Moreover, we show that salt-induced hypertension in these animals enhances the arteriolar expression of both arginase isoforms. Interestingly, increases in vascular arginase activity have also been detected in DOCA-salt hypertensive rats (26) and in pigs with aortic coarctation (33), suggesting that up-regulation of arginase is a general feature of salt-sensitive forms of hypertension.

The Dahl-S rat is a genetic model of salt-induced hypertension (25) that exhibits cardiac hypertrophy (12) and renal injury accompanied by an increase in renal weight (11). After 4 wk of a high-salt diet, mean arterial pressure and kidney and heart weights were significantly increased in Dahl-S rats compared with the low-salt group. We and others have previously shown that Dahl-S rats with salt-induced hypertension develop vascular endothelial dysfunction (4, 16, 21, 34) but do not display impaired responsiveness to an endothelium-independent activator of soluble guanylate cyclase (16). We again found that gracilis muscle arterioles isolated from high-salt Dahl-S rats exhibit attenuated endothelium-dependent vasodilator responses to acetylcholine compared with low-salt Dahl-S animals. Muscarinic agonists, such as acetylcholine, are widely used to assess nitric oxide-dependent endothelial function (16, 21, 28, 34). An alternative means for assessing endothelial function is to generate nitric oxide in response to increased shear forces along the vascular endothelium (24). Our group previously reported (18) that in skeletal muscle arterioles isolated from normotensive male Sprague-Dawley rats flow-induced dilation could be completely abolished by nitric oxide synthase inhibition. We found that the arterioles from high-salt Dahl-S rats fail to dilate in response to flow. To test whether elevated arginase expression contributes to the impairment in endothelial function in arterioles from high-salt animals, we employed the arginase inhibitor BEC. This boronic acid-based L-cysteine analog is a highly potent and specific competitive inhibitor of both arginase I and II and has no direct effect on nitric oxide synthase (19). We found that acute in vitro pretreatment with the arginase inhibitor BEC augments acetylcholine and flow-induced dilation in arterioles from both low- and high-salt groups. Furthermore, BEC restores acetylcholine and flow-mediated dilation in high-salt arterioles to levels observed in low-salt vessels. These findings suggest that elevated arginase expression contributes to arteriolar nitric oxide dysfunction in Dahl-S rats with salt-induced hypertension. It was further shown that constitutive arginase expression may counteract nitric oxide-mediated vasodilatory function in microves-

![Graph](image-url)
sels during normotensive states. Interestingly, increased arginase activity has recently been demonstrated to impair nitric oxide-mediated dilation in coronary arterioles following ischemia-reperfusion (13), in aortic vessels from old animals (2), and in coronary arterioles of pigs with aortic coarctation hypertension (33). Thus arginase may trigger endothelial dysfunction in various pathological conditions.

Our finding that arginase inhibition increases endothelium-dependent vascular relaxation suggests that arginase may regulate L-arginine availability for endothelial nitric oxide synthase. Although the affinity of L-arginine is much higher for purified nitric oxide synthase ($K_m$ of $\approx 2–20 \mu$mol/l) than for arginase ($K_m$ of $\approx 2–20$ mmol/l), the maximum activity of arginase is more than 1,000 times that of nitric oxide synthase, suggesting similar rates of substrate utilization at physiological L-arginine concentrations (31). Consistent with a role for arginase in depleting substrate for endothelial nitric oxide synthase, we found that the acute exogenous administration of L-arginine mimics the actions of BEC. L-Arginase enhances acetylcholine and flow-induced dilation in arterioles from low- and high-salt groups and normalizes the impaired endothelium-dependent relaxation in arterioles from high-salt animals. Competition between arginase and nitric oxide synthase has also been described in cultured endothelial cells and in other blood vessels (2, 5, 8, 13, 20, 32), further highlighting the potential importance of such an interaction in the vasculature. Interestingly, increased microvascular arginase expression and the subsequent depletion of intracellular L-arginine may also explain the ability of chronic oral L-arginine supplementation to improve endothelial dysfunction in Dahl-S rats (34). Furthermore, arginase-mediated reduction in intracellular L-arginine stores may also serve to sensitize endothelial cells to the endogenous nitric oxide synthase inhibitor, $N^\omega$-dimethyl-L-arginine, which is elevated in hypertensive Dahl-S rats (22). Previous reports suggested that limited L-arginine availability may promote uncoupling of endothelial nitric oxide synthase, which results in oxygen free radical formation rather than nitric oxide (1). Because oxygen free radicals can inactivate nitric oxide, the possibility exists that increased vascular arginase activity and the resulting intracellular L-arginine depletion may also decrease nitric oxide bioavailability. Thus it appears that increased vascular arginase expression may promote endothelial dysfunction via various mechanisms.

In summary, we found that arginase I and II are expressed in Dahl-S rat skeletal muscle arterioles and that the expression of both arginase isoforms is increased with salt-induced hypertension. In addition, acute in vitro pretreatment with an arginase inhibitor restores endothelium-dependent vasodilatation responses and abolishes the differences between high- and low-salt arterioles. Similarly, we found that acute in vitro pretreatment with the arginase and nitric oxide synthase substrate L-arginine restores endothelium-dependent responses and eliminates the differences between the two salt groups. These data suggest that enhanced vascular arginase activity contributes to endothelial dysfunction in Dahl-S rats with salt-induced hypertension. Because skeletal muscle arterioles represent a substantial portion of total peripheral resistance, they are major determinants of blood pressure. Hence, the possibility exists that arteriolar endothelial dysfunction contributes to hypertension in Dahl-S rats on high-salt diets. Therefore, our present study might provide additional insights into the well-demonstrated blood pressure-lowering effects of L-arginine in hypertensive Dahl-S rats (6, 7, 14).

GRANTS

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