Reduced Influence of Nitric Oxide on Arteriolar Tone in Hypertensive Dahl Rats

Matthew A. Boegehold

The aim of this study was to evaluate the influence of endogenous nitric oxide on resting microvascular tone in the Dahl salt-sensitive (DS) rat and to determine how this influence is altered in salt-induced hypertension. Intravital microscopy was used to examine the arteriolar network in the spinotrapezius muscle of DS rats maintained on low (0.45% NaCl) or high (4% NaCl) salt diets for 6–7 weeks. Mean arterial pressure for DS rats on high salt (163±3 mm Hg) was significantly greater than that for DS rats on low salt (128±4 mm Hg). Inhibition of microvascular nitric oxide synthesis with N\textsuperscript{G}-nitro-L-arginine-methyl ester caused arteriolar constriction in normotensive DS but not in hypertensive DS rats. Application of L-arginine consistently caused arteriolar dilation in normotensive DS but not hypertensive DS rats. In contrast, arteriolar responses to iontophoretically applied acetylcholine and sodium nitroprusside were similar in both groups. These results indicate that basal release of nitric oxide, presumably from the endothelium, normally influences arteriolar tone in skeletal muscle of DS rats and that this influence is suppressed in established salt-induced hypertension. However, the normal arteriolar response to acetylcholine in hypertensive DS rats suggests that a generalized impairment of endothelial function may not occur in the microcirculation of these animals. Unaltered arteriolar responsiveness to sodium nitroprusside in hypertensive DS rats also suggests that salt-induced hypertension is not accompanied by a change in the responsiveness of arteriolar smooth muscle to nitric oxide. (Hypertension 1992;19:290–295)

KEYWORDS • hypertension • microcirculation • endothelium • nitric oxide • endothelium-derived relaxing factor

When fed a high salt diet, the Dahl salt-sensitive (DS) rat develops hypertension that is chiefly due to increased total peripheral resistance.\textsuperscript{1,2} Accordingly, the vascular tone of large arterioles is increased in skeletal muscle of hypertensive DS rats, reducing the average internal diameter of these vessels after 4 weeks of high salt intake.\textsuperscript{3}

Since acetylcholine (ACh) was first shown to cause vasodilation through the release of a relaxing factor from the endothelium,\textsuperscript{4} other endothelium-derived factors have been identified and their importance in regulating vascular tone has been recognized.\textsuperscript{5–7} The factor released by ACh is thought to be nitric oxide (NO) or a compound that spontaneously generates NO.\textsuperscript{5–7} Chronic hypertension alters endothelial cell morphology and function,\textsuperscript{8–10} and a reduced relaxation response to ACh and other endothelium-dependent agonists has been observed in large arteries from the hypertensive DS rat and other models of hypertension.\textsuperscript{11–15} However, because large arteries contribute little to vascular resistance, the relevance of these findings to elevated total peripheral resistance in hypertension remains unclear. Endothelium-derived factors also influence arteriolar tone,\textsuperscript{16–27} but there is limited information on how this influence is altered in hypertensive animals.\textsuperscript{17,23,27}

The present study was undertaken to determine if a reduced influence of endogenous NO contributes to increased arteriolar tone in salt-induced hypertension. Arteriolar responses to ACh, L-arginine (L-Arg, the endogenous substrate for NO\textsuperscript{5–6}), and sodium nitroprusside (NP, an agonist that spontaneously generates NO\textsuperscript{6}) were evaluated in DS rats fed low and high salt diets. In addition, the contribution of NO to resting arteriolar tone was assessed by inhibition of its synthesis with N\textsuperscript{G}-nitro-L-arginine-methyl ester (L-NAME).

Methods

Male Brookhaven DS rats averaging 25 days of age were obtained from Harlan Sprague Dawley, Indianapolis, Ind., and were placed on a whole-grain diet containing 0.45% NaCl by weight (TD8831, Teklad, Madison, Wis.). After 1 week, one half of the animals were placed on a 4% NaCl diet with the other half remaining on the 0.45% NaCl diet. The 4% NaCl diet was made by adding NaCl to the Teklad diet. From this time until a week before study, systolic blood pressure was monitored weekly via tail-cuff plethysmography. Weekly values were obtained by averaging five successive measurements in resting, fully conscious animals that had been warmed in a 35°C incubator.
Rats on both diets were studied 6–7 weeks after initiation of the 4% NaCl diet. Anesthesia was induced with sodium thiopental (100 mg/kg i.p.), and supplemental doses (20% of initial) were given as needed during the experimental period. The rat was placed on a heating mat to maintain a 37°C rectal temperature, and the trachea was intubated to ensure a patent airway. The right carotid artery was cannulated for arterial pressure measurement, and the right spinal accessory muscle was exteriorized as previously described. After the muscle was continuously superfused with an electrolyte solution (mM: NaCl 119, NaHCO_3 25, KCl 6, and CaCl_2 3.6) warmed to 35°C and equilibrated with a mixture of 95% N_2–5% CO_2 (pH, 7.35–7.40), superfusate flow was 4–6 ml/min to minimize equilibration with atmospheric oxygen.

The muscle was transilluminated with halogen light and was observed with an intravital microscope (model BHMJ, Olympus, Hyde Park, N.Y.) fitted with a Newvicon video camera (Panasonic, Secaucus, N.J.). Video images were displayed on a Panasonic high resolution television monitor and stored on videotape for offline analysis. Observations were made with a ×10 eyepiece and Nikon ×10 or ×20 water immersion objectives (final video magnification, ×730 or ×1,460). Arteriolar inner diameters were measured offline with a video image shearing monitor (model 908, I.P.M., San Diego, Calif.).

ACH, L-Arg, and NP (Sigma Chemical Co., St. Louis, Mo.) were applied to individual arterioles by iontophoresis. Glass micropipettes were beveled at a 23–25° angle to an outer tip diameter of 2–4 μm and filled with a 0.05 M (for ACh and NP) or 0.10 M (for L-Arg) solution of the vasoactive agent in distilled water. A current programmer (model 260, WPI, New Haven, Conn.) was used to deliver currents of up to 100 nAmp for the ejection or retention of each ionized agent.

**Experimental Protocol**

The large feed arterioles entering the rat spinotrapezius muscle from its anterior and posterior borders meet to form an "arcade bridge" that gives rise to a network of arcading arterioles. After a postsurgical equilibration period of 45 minutes, a segment of the arcade arterioles was selected to evaluate arteriolar responses to ACh or L-Arg. A micromanipulator was used to place the pipette tip on the outer wall of the vessel, and a retaining current of 80–100 nAmp was applied to prevent passive diffusion of the agent from the tip. After a 2-minute control period, ACh or L-Arg was continuously applied to the vessel for 2 minutes by administering a net ejection current of 5, 10, 20, 40, or 80 nAmp. A subsequent recovery period of at least 2 minutes was allowed for the vessel to regain preapplication diameter before beginning the control period for the next application. This sequence was repeated five times, so that all five of the above current doses were applied to the vessel in random order.

A second arcade bridge segment was selected to compare arteriolar responses to ACh and to NP. The ACh pipette was moved to this segment, and after a 2-minute control period, ACh was applied for 2 minutes at 40 nAmp. After the recovery period, the pipette was removed, and a second pipette containing NP was positioned at the same site on the vessel wall. The control, application (40 nAmp), and recovery periods were then repeated. An additional application of NP at 10 nAmp was made to evaluate arteriolar responsiveness to a more modest challenge of this agent.

The contribution of endogenous NO to resting arteriolar tone was assessed by exposing the vascular bed to L-NAME (Bachem Biosciences, Philadelphia, Pa.), an L-Arg analogue that inhibits endothelial NO production. During normal superfusion, 4–7 arcade arterioles (including the two previously studied) were selected and their diameters were measured over a 2-minute control period. L-NAME was then added to the superfusate (final concentration, 10⁻⁴ or 10⁻³ M), and diameters were remeasured after 5, 10, and 15 minutes of continuous exposure to this solution. To determine the extent of NO inhibition under these conditions, arteriolar responses to 20-nAmp ACh were studied before and during L-NAME superfusion. At the end of each experiment, the diameter of each arteriole was remeasured after microvascular tone had been abolished with a superfusate containing 10⁻⁴ M adenosine (Sigma).

**Data Analysis and Statistics**

Arteriolar responses to ACh, L-Arg, and NP were quantified by comparing the mean internal diameter over the final 30 seconds of application with that for the control period immediately preceding application. Because of the normal variation in resting diameter among arcade arterioles in this muscle (25–63 μm in this study), the magnitude of each response was normalized by expressing it as percent change from control diameter. The magnitude of response was also compared with the vessel's total capacity for dilation by expressing the response as percent of the maximal dilation induced by adenosine. Percent of maximal dilation (%Δmax) was calculated as follows:

\[
\%\Delta_{\text{max}} = \left(\frac{D_{\text{max}} - D_{\text{out}}}{D_{\text{mix}} - D_{\text{out}}}\right) \times 100
\]

where \(D_{\text{in}}\) is steady-state diameter over the last 30 seconds of application, \(D_{\text{out}}\) is preapplication control diameter, and \(D_{\text{mix}}\) is passive diameter under adenosine.

All data are expressed as mean±SEM. Comparisons of mean values between dependent and independent sample pairs were made with the paired and unpaired Student's t test, and significance was assessed at \(p<0.05\). Reported \(N\) values represent the number of animals studied, and \(n\) values represent the number of vessels studied for each treatment.

**Results**

Fifty DS rats (29 on 0.45% NaCl, 21 on 4% NaCl diet) were used in this study. On the first day of high salt intake (average age of all rats was 34±0.5 days), the mean systolic pressure of those rats placed on 4% NaCl was not different from that of those remaining on 0.45% NaCl (Figure 1). In both groups, systolic pressure increased with age, but the increase was greater in the rats on 4% NaCl. After 1 week on this diet, systolic pressure of high salt rats was significantly higher than that of the low salt rats, and this difference persisted for the remainder of the dietary period. At the time of study (1–2 weeks after the last tail-cuff measurements), mean arterial pressure under anesthesia averaged
163±3 mm Hg for DS rats on 4% NaCl and 128±4 mm Hg for DS rats on 0.45% NaCl (p < 0.001). Mean body weight of DS rats on 4% NaCl was significantly lower than that of DS rats on 0.45% NaCl (328±7 versus 360±6 g, p < 0.001).

The mean resting diameter of all arterioles studied was 33.5±1.3 µm in DS rats on 0.45% NaCl (n=84) and 32.5±1.5 µm in DS rats on 4% NaCl (n=68). These values were not significantly different. During adenosine superfusion, the mean passive diameter of these vessels was also not different between DS rats on 0.45% and 4% NaCl (67.0±2.2 versus 65.9±2.6 µm, respectively). Figure 2 illustrates the mean arteriolar response to each current dose of ACh in the two experimental groups. In both groups, the magnitude of dilation was dose-dependent, with the mean increase in diameter ranging from approximately 20% at 5 nAmp to 100% at 80 nAmp (Figure 2, top panel). There was no significant difference between groups in the mean response to any level of ACh. These responses were also not different when expressed as percent maximal dilation (Figure 4). Consistent with the data in Figure 2, there was also no difference between groups in the response of these vessels to ACh.

After 5 minutes of continuous superfusion with 10⁻⁴ M L-NAME, resting arteriolar diameters in DS rats on 0.45% NaCl diet were significantly reduced to an average of 93.8±1.9% of preexposure diameter (Figure 5). No further diameter changes were observed over the remainder of the exposure period. In contrast, arterioles in DS rats on 4% NaCl diet were unaffected by L-NAME, with diameters after 5 minutes of exposure averaging 104.2±4.3% of preexposure diameter, and no subsequent changes were observed. There was no difference between dietary groups in the effect of L-NAME on arteriolar responses to ACh. The data for both groups were therefore pooled and are shown in Figure 6. The average arteriolar dilation to 20 nAmp ACh was reduced by 72% with 10⁻⁴ M L-NAME and by 80% with 10⁻³ M L-NAME. In DS rats on 0.45% NaCl diet, superfusion with 10⁻³ M L-NAME had no significant effect on arteriolar diameter. In DS rats on 4% NaCl diet, superfusion with 10⁻³ M L-NAME had no significant effect on arteriolar diameter.

**Discussion**

In this study, inhibition of endogenous NO synthesis reduced arteriolar diameters in DS rats fed a low salt.
diet but not in DS rats with salt-induced hypertension. L-Arg, the precursor of endothelium-derived NO, consistently caused arteriolar dilation in DS rats on low salt but not in hypertensive DS rats. Despite these differences, arteriolar responses to ACh and NP were similar in both groups.

Synthesis of NO was suppressed with L-NAME, one of a series of L-Arg analogues that competitively inhibit NO generation from L-Arg in the vascular endothelium. The analogue N\(^{\text{G}}\)-monomethyl-L-arginine (L-NMMA) increases renal, mesenteric, and hind limb vascular resistance in the rat, and both L-NAME and L-NMMA reduce arteriolar diameters in rat and rabbit skeletal muscles. The current finding that L-NAME reduces arteriolar diameters in the spinotrapezius muscle of DS rats on 0.45% NaCl diet (Figure 5), together with these previous observations, strongly suggests that endogenous NO influences arteriolar tone in a number of vascular beds. The ineffectiveness of L-NAME on arterioles in hypertensive DS rats argues against a tonic influence of NO in established salt-induced hypertension. Similarly, L-NMMA reduces arteriolar diameters in the spinotrapezius muscle of normotensive rats but not in rats with one-kidney, one clip hypertension. This abnormality could reflect a suppression of NO synthesis/release from the endothelium, impaired coupling between endothelium and arteriolar smooth muscle, or a reduced smooth muscle responsiveness to NO. Smooth muscle responsiveness to NO is most likely not reduced in the hypertensive DS rats studied here because the responsiveness of these vessels to NP, an endothelium-independent agonist that spontaneously generates NO, was identical to that of arterioles in normotensive DS rats (Figure 4). Impaired endothelial–smooth muscle coupling in hypertensive DS rats would result in an attenuated response to all endothelium-dependent agonists. However, the ACh dose–response relations for the normotensive and hypertensive animals are virtually superimposable (Figure 2), suggesting that diffusion of NO from endothelium to smooth muscle is not impaired in the hypertensive animals. Therefore, the most likely explanation for the lack of effect of L-NAME on resting arteriolar diameter in hypertensive DS is that the basal synthesis or release of NO is impaired in these animals.

Superfusion with 10\(^{-4}\) M L-NAME blocked most but not all of the arteriolar response to ACh in both groups (Figure 6). A 10-fold increase in L-NAME concentration had only a slightly greater effect, suggesting that this incomplete blockade is probably not due to an insufficient amount of L-NAME reaching the arterioles. As previously suggested, a portion of the endogenous L-Arg pool may be inaccessible to L-NAME. Alternatively, a portion of the ACh response may be mediated by a second factor that is not formed from L-Arg, such as endothelium-derived hyperpolarizing factor. Indomethacin does not alter the arteriolar ACh response in rat spinotrapezius muscle, ruling out the possibility that release of a vasodilator prostanoid may be involved. Arterioles in DS rats on 0.45% NaCl diet dilated in response to L-Arg (Figure 3), suggesting that basal NO
production in these vessels may be limited by the availability of endogenous substrate. L-Arg also dilates arterioles in the rat cremaster muscle, and this response has been shown to be endothelium dependent. In addition, L-Arg decreases renal, mesenteric, and hindquarter vascular resistance in the rat, suggesting that substrate availability may limit basal NO production in a number of organs. Because arterioles in hypertensive DS rats were unaffected by L-Arg at concentrations that caused dilation in normotensive DS rats (Figure 3), the absence of basal NO synthesis/release in these vessels is probably not due to a lack of available substrate.

Despite the apparent suppression of basal NO production in arterioles of hypertensive DS rats, these vessels responded normally to ACh, which acts through the release of endothelial-derived NO or prostanoids in the spinotrapezius muscle. The attenuation of responses mediated through such different pathways suggests a generalized impairment of endothelial function in these forms of hypertension. In contrast, the unaltered arteriolar response to ACh in DS rats with salt-induced hypertension may reflect a more selective alteration of endothelial function in these animals. Therefore, although a reduced responsiveness to endothelium-dependent dilators is a characteristic common to large arteries in various hypertension models, the occurrence of this deficit at the microvascular level may be more variable, depending on the form of hypertension studied.

In contrast to previous findings in hypertensive DS rats fed a high salt diet for 4 weeks,23 arcade arteriole diameters were not reduced in the current group of DS rats after 6–7 weeks of high salt intake. Longitudinal studies in DS rats and other hypertension models indicate that as hypertension progresses, the vascular alterations in a particular organ can change, often with a shift in the segments of the arterial tree that contribute to increased vascular resistance. After 6–7 weeks on a high salt diet, increased vascular resistance in hypertensive DS rats is apparently due to changes in vessels proximal or distal to the arcade arterioles. However, arcade arteriolar tone may still be elevated in these animals. In DS rats after 4–5 weeks on a high salt diet, arcade arteriolar pressure is increased by 30–50%, and this pressure may still be elevated after an additional 1–2 weeks on a high salt diet. If so, increased smooth muscle force development would be required to maintain normal luminal diameters in the face of this increased distending pressure, and a suppressed influence of NO could contribute to such an increase in smooth muscle tone. However, because microvascular...
pressures were not measured in the current study, the possibility that arcade arteriolar tone is normal in the hypertensive DS rats studied here cannot be excluded. In summary, in the spinotrapezius muscle of DS rats fed a low salt diet, inhibition of microvascular NO synthesis causes arteriolar constriction and application of the NO precursor \( L-\text{Arg} \) causes arteriolar dilation. In contrast, these treatments have no effect on arterioles in DS rats made hypertensive by high salt intake, suggesting that the establishment of salt-induced hypertension in this model is accompanied by a suppression of basal NO synthesis/release in these vessels that is not due to a lack of available substrate. The magnitude of arteriolar dilation induced by \( \text{ACh} \) was similar in both groups, suggesting that not all aspects of endothelial function may be impaired in the microcirculation of the hypertensive DS rat. Arteriolar responses to \( \text{NO} \) were also not different between groups, indicating that the responsiveness of arteriolar smooth muscle to \( \text{NO} \) is not altered in DS rats with salt-induced hypertension.

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M A Boegehold

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