Endothelium-Dependent Vascular Responses in Normotensive and Hypertensive Dahl Rats

THOMAS F. LÜSHER, LEOPOLDO RAJ, AND PAUL M. VANHOUTTE

SUMMARY Experiments were designed to study endothelium-dependent responses in salt-sensitive (DS) and salt-resistant Dahl rats (DR). The rats were fed a low sodium (0.1% NaCl) or high sodium (8% NaCl) diet for 8 weeks. Blood pressure in DS fed a high sodium diet was higher than that in the remaining animals. Aortic rings with and without endothelium were suspended for isometric tension recording. Acetylcholine, adenosine 5'-diphosphate, and thrombin induced endothelium-dependent relaxations that were significantly depressed in the aorta of DS fed a high sodium diet. The relaxations in response to sodium nitroprusside were only slightly, but significantly, depressed in DS fed a high sodium diet. Removal of the endothelium greatly enhanced the response to serotonin and norepinephrine. In rings with, but not without, endothelium taken from rats fed a high sodium diet, the tension developed in response to serotonin and norepinephrine was significantly greater than that in animals fed a low sodium diet. These experiments indicate that (1) endothelium-dependent relaxations to acetylcholine, adenosine 5'-diphosphate, and thrombin are depressed in hypertensive Dahl rats; (2) this effect probably reflects a decreased release of endothelium-derived relaxing factor(s), although structural changes might contribute; and (3) the responsiveness to vasoconstrictor agents is increased in DS and DR fed a high sodium diet. These findings may indicate differential effects of blood pressure and dietary salt on endothelial function. (Hypertension 9: 157-163, 1987)

KEY WORDS • acetylcholine • adenosine 5'-diphosphate • aorta (thoracic) • endothelium-dependent relaxation • norepinephrine • thrombin • serotonin

In hypertension, arterial endothelial cells exhibit morphological and functional changes. The renin-angiotensin system, mineralocorticoid hormones, and catecholamines, as well as high salt intake, have been implicated in hypertensive vascular injury. Endothelium-dependent relaxations to acetylcholine are depressed in spontaneous hypertension, in aortic coarctation, and in mineralocorticoid hypertension of the rat. No information is available on endothelium-dependent responses of blood vessels in salt-induced hypertension.

Dahl developed two strains of rats with different propensities toward hypertension if fed a high sodium diet: a salt-sensitive strain, which becomes hypertensive when fed a high sodium diet, and a salt-resistant strain, which does not. This model may have similarities with a subgroup of human hypertension in which sodium contributes to the development of high blood pressure. The present study was designed to investigate whether endothelium-dependent vascular responses are altered in salt-induced hypertension of the rat.

Materials and Methods

Male 6-week-old Dahl salt-sensitive (DS) and salt-resistant rats (DR) weighing 257 ± 7 g were purchased from Brookhaven National Laboratories (Brookhaven, NY, USA). All rats were housed two to a cage and had free access to water. For 8 weeks both DS and DR were fed standard rat chow (Ralston-Purina, St. Louis, MO, USA) that contained either 8% NaCl or 0.1% NaCl. Four groups of rats were used in the study: DS fed 8% NaCl, DS fed 0.1% NaCl, DR fed 8% NaCl, and DR fed 0.1% NaCl. Systolic blood
TABLE 1. Blood Pressure, Age, and IC₅₀ Values of Acetylcholine and Sodium Nitroprusside in Contracted Dahl Rat Thoracic Aortas

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet</th>
<th>Weight (g)</th>
<th>Systolic blood pressure (mm Hg)</th>
<th>IC₅₀ (−log M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Acetylcholine</td>
</tr>
<tr>
<td>DS</td>
<td>8% NaCl</td>
<td>416 ± 15</td>
<td>183 ± 7*</td>
<td>6.3 ± 0.3*</td>
</tr>
<tr>
<td>DS</td>
<td>0.1% NaCl</td>
<td>463 ± 17</td>
<td>147 ± 5</td>
<td>7.4 ± 0.1</td>
</tr>
<tr>
<td>DR</td>
<td>8% NaCl</td>
<td>435 ± 13</td>
<td>127 ± 1</td>
<td>7.4 ± 0.04</td>
</tr>
<tr>
<td>DR</td>
<td>0.1% NaCl</td>
<td>479 ± 16</td>
<td>123 ± 5</td>
<td>7.2 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SEM of six experiments except for DR fed 8% NaCl (n = 5). Blood pressure values were taken 7 weeks after the introduction of a high or low sodium diet.

*p < 0.05, compared with remaining groups.
†p < 0.05, compared with DS fed 0.1% NaCl.

pressure and weight were measured weekly. Blood pressure was recorded in unanesthetized rats by the tail cuff method with a physiograph MK IV (Narco Biostystems, Houston, TX, USA) as described previously. The blood pressure was measured in the morning in a quiet environment, and an average of three successive readings was recorded. In DS fed an 8% NaCl diet, blood pressure increased gradually and leveled off between 5 and 8 weeks (Table 1).

The experiments were performed in rings of thoracic aorta. The rats were anesthetized with pentobarbital sodium (50 mg/kg i.p.). The thoracic aorta was dissected free, excised, and placed into cold modified Krebs-Ringer bicarbonate solution of the following composition (mM): NaCl, 118; KCl, 4.7; CaCl₂, 2.5; MgSO₄, 1.2; KH₂PO₄, 1.2; NaHCO₃, 25.0; edetate calcium disodium, 0.026; glucose, 11.1 (control solution). The blood vessels were cleaned of adherent connective tissue and cut into rings (6 mm long). In some rings, the endothelium was removed by gently rubbing the intimal surface with a small forceps. In certain experiments, the presence or absence of endothelial cells was confirmed histologically (Figure 1).

The rings were suspended between two stirrups in organ chambers filled with 25 ml of control solution (37°C) aerated with 95% O₂, 5% CO₂. They were connected to force transducers (Statham Universal UC2, Oxnard, CA, USA; or Grass FT 03C, Quincy, MA, USA), and changes in isometric force were recorded. The preparations were progressively stretched and exposed to norepinephrine (3 × 10⁻⁷ M) at each level of stretch until the optimal point of the length-tension relationship was reached; the optimal basal tension did not differ significantly in DS (4.0 ± 0.1 g on 8% NaCl and 4.3 ± 0.1 g on 0.1% NaCl) and DR (4.2 ± 0.1 g on 8% NaCl and 4.2 ± 0.1 g on 0.1% NaCl). After this procedure the rings were allowed to equilibrate for 45 minutes. In certain experiments, some rings were incubated with indomethacin (10⁻⁵ M) for 45 minutes.

**Drugs**

The following drugs were used: acetylcholine hydrochloride (Sigma, St. Louis, MO, USA); adenosine 5'-diphosphate (ADP; Sigma); sodium heparin (Elkins Sinn, Cherry Hill, NJ, USA); 5-hydroxytryptamine (serotonin; Sigma); indomethacin (Sigma); L-norepinephrine bitartrate (Sigma); prostaglandin F₂α (Sigma); sodium nitroprusside (Sigma); and thrombin (bovine;
Sigma). The concentrations of the drugs are expressed as final molar concentrations [M] in the bath solution. All drugs were dissolved in distilled water, except indomethacin, which was dissolved in 10 ml of distilled water containing $5 \times 10^{-3}$ M NaCO$_3$ and was sonicated before use. The drugs were added to the organ chambers in volumes of 500 µl or less.

Drugs were always added in the same order. Some rings were exposed to ADP, thrombin, and serotonin, and other rings to acetylcholine, serotonin, norepinephrine, and sodium nitroprusside.

Calculations and Statistics

Rings from DS and DR fed an 8% NaCl or 0.1% NaCl diet were studied in parallel. Contractions are expressed in absolute tension (grams). To avoid multiple statistical comparisons at each concentration of the agonists, equipotent dosages (ED in grams; i.e., the concentration of an agonist needed to evoke a certain increase in tension) were calculated for a level of absolute tension (grams) reached by all rings of a given experiment. In experiments in which relaxations were studied, the rings were contracted with the individual concentration ($10^{-9}$ to $5 \times 10^{-8}$ M) of norepinephrine or prostaglandin F$_2\alpha$ ($10^{-8}$ to $5 \times 10^{-8}$ M) causing an increase in tension of approximately 1.2 g; sodium nitroprusside was added after the concentration-response curve to norepinephrine had been established. The results are expressed as a percentage relaxation of that contraction. The concentration exhibiting 50% relaxation in contracted rings (IC$_{50}$) or a given increase in absolute tension (grams) in quiescent rings is expressed as negative log M. The results are given as means ± SEM. In all experiments, n equals the number of rats used. Since the parameters blood pressure and salt were systematically interrelated in the study design, t-tests for unpaired observations for comparison of the four groups of animals rather than two-way analysis of variance were used for statistical analysis. To account for multiple comparisons, the p value was multiplied by 4 (Bonferroni rule) and a value smaller than 0.05 was considered to indicate a statistical difference.

Results

Endothelium-Dependent Relaxations

Rings with and without endothelium of DS and DR fed either diet (8% and 0.1% NaCl, respectively) were contracted with norepinephrine and then exposed to increasing concentrations of acetylcholine ($10^{-9}$ to $10^{-4}$ M). In all strains, acetylcholine caused endothelium-dependent relaxations. The relaxations in response to acetylcholine were significantly depressed in DS fed 8% NaCl as compared with the remaining groups (Figure 2). The IC$_{50}$ value of acetylcholine in DS fed 8% NaCl was higher (6.3 ± 0.3 vs 7.4 ± 0.1; log shift, 13-fold) and the maximal relaxations less pronounced than those in the other groups of animals (see Table 1). Indomethacin ($10^{-4}$ M) did not significantly affect the relaxations to acetylcholine (data not shown). Bovine thrombin (1 U/ml) caused transient relaxations in rings with endothelium in all groups of rats. These relaxations were significantly less pronounced in DS fed 8% NaCl as compared with the remaining groups (Figure 3). Endothelium-dependent relaxations in response to ADP were significantly depressed at $10^{-9}$ to $10^{-4}$ M in DS fed 8% NaCl as compared with DS fed 0.1% NaCl (Figure 4). In DR, endothelium-dependent relaxations did not differ in animals fed 8% NaCl or 0.1% NaCl.

Serotonin

In unstimulated rings and in those contracted with prostaglandin F$_2\alpha$, serotonin ($10^{-9}$ to $10^{-4}$ M) caused increases in tension at higher concentrations that were significantly more pronounced in rings without than in rings with endothelium in all groups of animals (Fig-
Endothelium-dependent relaxations in response to ADP in Dahl rat thoracic aortas. Experiments were done in DS or DR fed a high (8% NaCl) or low sodium diet (0.1% NaCl). Data are given as means ± SEM of five or six experiments. An asterisk denotes that the difference between rings with endothelium of DS fed 8% NaCl or 0.1% NaCl is statistically significant (p < 0.05).

In DS and DR fed 8% NaCl, the increase in tension in response to serotonin was more pronounced in rings with, but not without, endothelium as compared with that in DS and DR fed 0.1% NaCl (see Figures 5 and 6). In contracted rings the equipotent concentration of serotonin was significantly lower in DS and DR fed 8% NaCl (ED 1.4 g: 5.9 ± 0.04 and 5.3 ± 0.1, i.e., the concentration of serotonin needed to evoke a contraction of 1.4 g) than in DS and DR fed 0.1% NaCl (ED 1.4 g: 4.9 ± 0.9 and 4.9 ± 0.3). Similarly, in unstimulated rings, the equipotent concentration of serotonin was significantly lower and the maximal response to the monoamine was significantly enhanced in Dahl rats fed 8% NaCl as compared with Dahl rats fed 0.1% NaCl. Indomethacin (10⁻⁴M) did not significantly affect the contractions in response to serotonin in rings with and without endothelium in DS fed 8% NaCl.

Norepinephrine and Sodium Nitroprusside
Norepinephrine (10⁻⁹ to 10⁻⁴ M) caused concentration-dependent contractions in all groups of rats. The absolute tension developed in response to norepinephrine (3 × 10⁻⁸ to 3 × 10⁻⁵ M) was significantly increased in rings without as compared with those with endothelium. The equipotent concentrations of norepinephrine evoking an increase in tension of 0.25 g was significantly lower in rings with, but not without, endothelium in DS and DR fed 8% NaCl as compared with DS and DR fed 0.1% NaCl. The maximal response to norepinephrine in rings with endothelium was significantly lower as compared with rings without endothelium in DS and DR fed 0.1% NaCl but did not differ statistically in DS and DR fed 8% NaCl (see Table 2).

Sodium nitroprusside (10⁻⁶ to 10⁻³ M) inhibited the contractions induced by norepinephrine in a concentration-dependent manner. The IC₅₀ values of sodium nitroprusside in DS fed 8% NaCl were slightly but significantly higher than those in DS fed 0.1% NaCl (log shift, threelfold; see Table 1). In DR fed 8% NaCl or 0.1% NaCl, the IC₅₀ values of sodium nitroprusside did not differ. The aortas of all four groups of animals relaxed to baseline with sodium nitroprusside (Figure 7).
TABLE 2. Maximal Response to Serotonin and Norepinephrine in Thoracic Aortas With and Without Endothelium Taken from DS and DR Fed Either 8% or 0.1% NaCl

<table>
<thead>
<tr>
<th>Agonist and preparation</th>
<th>Response in DS (g)</th>
<th>Response in DR (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8% NaCl diet</td>
<td>0.1% NaCl diet</td>
</tr>
<tr>
<td>Serotonin</td>
<td>1.9±0.4*</td>
<td>0.6±0.1</td>
</tr>
<tr>
<td>With endothelium</td>
<td>2.0±0.2*</td>
<td>1.1±0.3</td>
</tr>
<tr>
<td>Without endothelium</td>
<td>4.8±0.4f</td>
<td>4.3±0.2f</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>4.4±0.4†</td>
<td>4.5±0.7†</td>
</tr>
<tr>
<td>With endothelium</td>
<td>2.7±0.4</td>
<td>1.4±0.3</td>
</tr>
<tr>
<td>Without endothelium</td>
<td>4.3±0.6†</td>
<td>3.9±0.5†</td>
</tr>
</tbody>
</table>

Values are means ± SEM of six experiments except for DR fed 8% NaCl (n = 5).

* p < 0.05, compared with values in rats of the same strain fed 0.1% NaCl.
† p < 0.05, compared with respective values in rings with endothelium.

Discussion

The major findings of the present study are the depressed endothelium-dependent relaxations in response to acetylcholine, ADP, and thrombin in hypertensive Dahl rats and the increased responsiveness to serotonin and norepinephrine in rings with, but not without, endothelium in normotensive and hypertensive Dahl rats fed a high sodium diet.

Endothelium-dependent relaxations in response to acetylcholine are depressed in other experimental models of hypertension of the rat.5, 8-11, 12 This study demonstrates a similar phenomenon in the DS. In the aorta of the spontaneously hypertensive rat, the endothelium-dependent relaxations in response to acetylcholine are decreased at least in part because of the simultaneous release of an endothelium-derived constricting factor(s) rather than due solely to an altered release of endothelium-derived relaxing factor(s).11 In the Dahl rat, no endothelium-dependent contractions in response to acetylcholine were noted. Moreover, blockade of cyclooxygenase by indomethacin did not enhance endothelium-dependent relaxations to acetylcholine as it does in the spontaneously hypertensive rat.11 This finding suggests that different mechanisms may be responsible for the decreased endothelium-dependent relaxations to acetylcholine in spontaneous and salt-induced hypertension. Theoretically, the relaxations to acetylcholine in the hypertensive Dahl rat could be depressed because of decreased release of endothelium-derived relaxing factor(s), impaired diffusion of the factor(s) from the endothelium to the smooth muscle, or decreased responsiveness of the vascular smooth muscle to the factor(s). Subendothelial thickening occurring during the hypertensive process could indeed impair the diffusion of substances released from the endothelium.13 As judged from experiments done in the spontaneously hypertensive rat, in which endothelium-dependent relaxations were normal after blockade of cyclooxygenase, this is unlikely to be a major factor.11 In addition, a marked shift of the concentration-response curve to acetylcholine would be expected at low concentrations of the muscarinic agonist under these circumstances. The relaxations, however, were more impaired at higher than at lower concentrations of acetylcholine. The relaxations induced by endothelium-derived relaxing factor(s) are mediated through an increase of cyclic guanosine 3', 5'-monophosphate (cyclic GMP) levels in vascular smooth muscle cells.14 Hence, to test vascular smooth muscle responsiveness to endothelium-derived relaxing factor(s), sodium nitroprusside, a substance that also acts through cyclic GMP, was used. The relaxations in response to sodium nitroprusside were slightly impaired in hypertensive Dahl rats. However, the shift at IC50 observed with sodium nitroprusside was much smaller than that seen with acetylcholine, and maximal relaxations were not affected. If the mode of action of sodium nitroprusside and endothelium-derived relaxing factor(s) is indeed similar, this would suggest that the release of the latter is decreased in hypertensive Dahl rats, although structural changes and, in turn, a decreased responsiveness of vascular smooth muscle cells to the endothelium-derived relaxing factor(s) may contribute.15

Figure 7. Relaxations in response to sodium nitroprusside in thoracic aortic rings without endothelium taken from DS fed a high sodium diet (8% NaCl) and DS fed a low sodium diet (0.1% NaCl). Data are given as means ± SEM of six experiments. The IC50 values of sodium nitroprusside were significantly higher in DS fed 8% NaCl as compared with DS fed 0.1% NaCl.
Substances released from aggregating platelets, such as ADP and serotonin, or formed during coagulation, such as thrombin, can react with the endothelium. \(^{16-21}\) Endothelium-dependent relaxations to high concentrations of ADP, but not of thrombin, are depressed in the spontaneously hypertensive rat. \(^{22}\) In hypertensive Dahl rats, endothelium-dependent relaxations to both thrombin and ADP were considerably decreased, suggesting a heterogeneity in the alteration of endothelial function in different models of hypertension.

In contrast to canine coronary arteries, \(^{19,21,22}\) serotonin did not cause endothelium-dependent relaxations in contracted aortas of the Dahl rat. Similarly, in the aorta of spontaneously hypertensive rats, endothelium-dependent relaxations induced by low concentrations of serotonin are minimal. \(^{22}\) However, serotonin evoked considerably more tension in rings in which the endothelium had been removed, which indicates either that the monoamine causes release of endothelium-derived relaxing factor(s) or that its action on smooth muscle is reduced by basally released factor(s). The tension developed in response to serotonin was significantly increased in rings with endothelium in both DS and DR fed on high sodium diet. In contrast, the contractile responses of rings without endothelium to either serotonin or norepinephrine were not significantly affected by the amount of salt in the diet. Hence, these findings suggest a differential effect of salt and blood pressure on endothelium-dependent responses in these animals. These findings could reflect either a decreased release of endothelium-derived relaxing factor(s) in Dahl rats fed a high sodium diet or an increased release of endothelium-derived constrictor factor(s), as occurs in the aorta of the spontaneously hypertensive rat. \(^{11,22,24}\) The finding that indomethacin did not significantly affect the response to serotonin in rings with endothelium would support the former interpretation, although a release of an indomethacin-insensitive endothelium-derived constricting factor(s) cannot be excluded. The decreased endothelium-dependent relaxations to ADP and thrombin and the increased responsiveness in rings with endothelium to serotonin may reflect a diminished protective function of the endothelium when platelet aggregation occurs in hypertensive Dahl rats. This endothelial dysfunction appears to be related to both hypertension and high dietary sodium.

As with serotonin, rings without endothelium were more sensitive and developed more tension in response to norepinephrine than rings with endothelium. The enhanced responsiveness of rings without endothelium to norepinephrine and serotonin suggests a basal release of endothelium-derived relaxing factor in the aorta of the Dahl rat, as has been demonstrated in the canine femoral artery, and in the aorta of Wistar-Kyoto rats. \(^{25-27}\) A decreased basal release of endothelium-derived relaxing factor in Dahl rats fed a high sodium diet could enhance the responsiveness of rings with endothelium to vasoconstrictor stimuli. However, a decreased activity of endothelial monoamine oxidase and, in turn, a decreased endothelial degradation of serotonin and norepinephrine cannot be excluded. \(^{24}\) In the case of norepinephrine, endothelial \(\alpha_{1}\)-adrenergic receptors might mediate the release of endothelium-derived relaxing factor(s). \(^{20,28}\) This interpretation also would be in line with the concept of a decreased release of endothelium-derived relaxing factor(s) in rats fed a high sodium diet.

In conclusion, results of the present study suggest that blood pressure and salt intake may independently and differentially affect endothelial function.

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