Antihypertensive Treatment Normalizes Decreased Endothelium-Dependent Relaxations in Rats with Salt-Induced Hypertension

THOMAS F. LÜSCHER, PAUL M. VANHOUTTE, AND LEOPOLDO RAIU

SUMMARY  Endothelium-dependent responses are impaired in various models of hypertension. The effects of antihypertensive treatment on endothelium-dependent relaxations were studied in Dahl salt-sensitive (DS) and Dahl salt-resistant rats (DR) on a high or low sodium diet. The rats were given either a diet containing 8% NaCl or 0.1% NaCl for 8 weeks or a diet containing 8% NaCl and a combination of reserpine, hydrochlorothiazide, and hydralazine for 8 or 2 weeks. DS on the 8% NaCl diet developed hypertension, while the other rats did not. Antihypertensive therapy for 8 or 2 weeks prevented or reversed hypertension in DS and lowered blood pressure in DR on the 8% NaCl diet. Aortic rings with and without endothelium were suspended in organ chambers for isometric tension recording. In all groups, acetylcholine, adenosine 5'-diphosphate, and thrombin caused endothelium-dependent relaxations. The relaxations in response to all agonists were significantly decreased in DS on 8% NaCl compared to relaxations in the other rats. Antihypertensive treatment for 8 or 2 weeks prevented or reversed the decreased endothelium-dependent relaxations in response to all agonists tested, but not those to the endothelium-independent agonist, sodium nitroprusside. These results suggest that antihypertensive treatment normalizes endothelium-dependent relaxations. This effect of antihypertensive treatment might be important for the prevention of cardiovascular complications in patients with hypertension. (Hypertension 9 [Suppl III]: III-193–III-197, 1987)

KEY WORDS  • acetylcholine • adenosine 5'-diphosphate • Dahl rats • norepinephrine • sodium nitroprusside • thrombin • thoracic aorta

Methods

Experimental Animals

Male 6-week-old Dahl salt-sensitive (DS) and salt-resistant rats (DR) weighing about 250 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 that contained either 8% NaCl or 0.1% NaCl. The rat chow was purchased from Ralston Purina (St. Louis, MO, USA). Some DS and DR on 8% NaCl also received antihypertensive drugs in the drinking water (reserpine, 1.4 mg/L; hydrochlorothiazide, 100 mg/L; hydralazine, 80 mg/L) for 8 weeks. Other DS and DR on the 8% NaCl diet received antihypertensive drugs at the end of the 7th week for 2 weeks. Diastolic blood pressure and weight were measured at the beginning of the study to obtain baseline values, at 3 or 5 weeks, and at the end of the 7th week. In animals treated for 2 weeks, blood pressure was also recorded at the end of the 9th week. Blood pressure was recorded in unanesthetized rats by the tail cuff method with a physio-
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 composed of (mM): NaCl, 118; KCl, 4.7; CaCl₂, 2.5; MgSO₄, 1.2; KH₂PO₄, 1.2; NaHCO₃, 25.0; edetate calcium disodium, 0.026; and glucose, 11.1 (control solution). The blood vessels were cleaned of adherent connective tissue and cut into rings 6 mm in length. In some rings, the endothelium was removed by gentle rubbing of the intimal surface with a small forceps. Previous studies have shown that this technique successfully removes the endothelium without damaging the arterial smooth muscle in this strain of rats. The rings were suspended between two stirrups in organ chambers filled with Krebs-Ringer bicarbonate solution composed of (mM): NaCl, 118; KCl, 4.7; CaCl₂, 2.5; MgSO₄, 1.2; KH₂PO₄, 1.2; NaHCO₃, 25.0; edetate calcium disodium, 0.026; and glucose, 11.1 (control solution). The blood vessels were cleaned of adherent connective tissue and cut into rings 6 mm in length. In some rings, the endothelium was removed by gentle rubbing of the intimal surface with a small forceps. Previous studies have shown that this technique successfully removes the endothelium without damaging the arterial smooth muscle in this strain of rats. 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 (Model UC2, Statham Universal, Oxnard, CA, USA; or Model FT 03C, Grass, Quincy, MA, USA), and changes in isometric force were recorded. The preparations were progressively stretched and exposed to norepinephrine (3 × 10⁻⁴ M) at each level of stretch until the optimal point of the length-tension relationship was reached. After this procedure, the rings were allowed to equilibrate for 45 minutes.

Drugs

The following drugs were used: acetylcholine hydrochloride (Sigma, St. Louis, MO, USA), adenosine 5'-diphosphate (Sigma), sodium heparin (Elkin Sinn, Cherry Hill, NJ, USA), sodium nitroprusside (Sigma), and bovine thrombin (Sigma). The concentrations of the drugs are expressed as final molar concentrations (M) or as U/ml in the bath solution. All drugs were dissolved in distilled water. 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 adenosine 5'-diphosphate and thrombin in other rings to acetylcholine, norepinephrine, and sodium nitroprusside.

Calculations and Statistics

In most experiments, rings from DS and DR on the 8% NaCl or 0.1% NaCl diet were studied in parallel. Contractions are expressed in absolute tension (grams). In experiments where relaxations were studied, the rings were contracted with the individual concentration (10⁻⁹ to 5 × 10⁻⁴ M) of norepinephrine, causing an increase in tension of approximately 1.1 to 1.4 g; the absolute level of tension did not differ statistically between the different groups of animals. Sodium nitroprusside was added after the concentration-response curve to norepinephrine (10⁻⁹–10⁻⁴ M). The response induced by sodium nitroprusside is expressed as the percentage of relaxation of the maximal contraction to norepinephrine (10⁻⁴ M). The concentration causing 50% relaxation in contracted rings (IC₅₀) is expressed as negative log M. In the case of acetylcholine and sodium nitroprusside, IC₅₀ values were used for statistical comparison. With adenosine 5'-diphosphate, IC₅₀ values could not be obtained in some rings. Therefore, the area under the concentration-response curve was used for statistical comparison (i.e., the more pronounced the relaxations, the smaller the area). The data are given as means ± SEM. In all experiments, n equals the number of rats used. Since blood pressure, salt, and hypertensive therapy were systematically interrelated in the study design, Student's t test for unpaired observations was used for comparison of the four groups of animals rather than two-way analysis of variance. To account for multiple comparisons, the p value was multiplied by 8 (Bonferroni rule) and a value smaller than 0.05 was considered to indicate a significant difference.

Results

Blood pressure was measured at the beginning of the study, at 3 or 5 weeks, and at the end of the 7th week. In the animals treated with antihypertensive drugs for 2 weeks after 7 weeks of the high sodium diet, blood pressure was also determined at 9 weeks (Figure 1). The high sodium diet caused a marked rise in blood pressure in DS but not in DR. Antihypertensive therapy normalized blood pressure or prevented hypertension in DS on the 8% NaCl diet, but also lowered blood pressure slightly but significantly in DR on 8% NaCl.

In rings contracted with norepinephrine to a similar level of tension, acetylcholine (10⁻⁴–10⁻⁴ M), adenosine 5'-diphosphate (10⁻⁴–10⁻⁴ M) and thrombin (1 U/ml) caused endothelium-dependent relaxations that were significantly decreased in DS on the high sodium diet. In DR given antihypertensive treatment for 2 or 8 weeks, endothelium-dependent relaxations to all agonists were comparable to those of DR and DS on the low-sodium diet. The IC₅₀ of acetylcholine was
5.8 ± 0.3 in hypertensive DS, and 7.2 ± 0.1 and 7.8 ± 0.1 in DS treated with antihypertensive drugs for 2 or 8 weeks, respectively (log shift at IC50: 26-fold and 100-fold, respectively; Figure 2 and Table 1). In treated DS, the relaxation response to acetylcholine did not differ statistically from that of DS on the low sodium diet. In DR, antihypertensive therapy slightly lowered the IC50 value for acetylcholine (log shift at IC50: 1.9-fold; see Figure 2 and Table 1). The endothelium-dependent relaxations in response to adenosine 5'-diphosphate in DS on the high sodium diet were significantly enhanced by antihypertensive treatment for 2 and 8 weeks (Figure 3). DS treated for either 2 or 8 weeks did not differ from DS on the low sodium diet.

In DR, antihypertensive therapy for 8 but not for 2 weeks also tended to enhance endothelium-dependent relaxations in response to adenosine 5'-diphosphate (see Figure 3). However, the difference did not reach the level of statistical significance. The reduced endothelium-dependent relaxations in response to thrombin in DS on the high sodium diet were significantly enhanced by antihypertensive treatment for 2 or 8 weeks (Figure 4). Treated DS did not differ statistically from DS on the low sodium diet. In DR, the endothelium-dependent relaxations in response to thrombin were not significantly affected by antihypertensive therapy for either 2 or 8 weeks (see Figure 4).

In DS on the high sodium diet, the concentration-

![Graph showing endothelium-dependent relaxations in response to acetylcholine in thoracic aortas of Dahl rats.](http://hyper.ahajournals.org/)

**FIGURE 2.** Endothelium-dependent relaxations in response to acetylcholine in thoracic aortas of Dahl rats. The experiments were performed on DS and DR on a high sodium (8% NaCl) or low sodium (0.1% NaCl) diet with or without antihypertensive drug (AHD) treatment. The data are shown as means ± SEM. The IC50 values for acetylcholine in DS on the 8% NaCl diet were significantly higher than IC50 values in the other groups of rats.

![Graph showing endothelium-dependent relaxations in response to adenosine diphosphate in thoracic aortas of Dahl rats.](http://hyper.ahajournals.org/)

**FIGURE 3.** Endothelium-dependent relaxations in response to adenosine 5'-diphosphate in thoracic aortas of Dahl rats. The experiments were performed on DS and DR on a high sodium (8% NaCl) or low sodium (0.1% NaCl) diet with or without antihypertensive drug (AHD) treatment. The data are given as means ± SEM. The area under the concentration-response curve was significantly higher in the hypertensive rats than in the other rats.

**TABLE 1. Effect of Antihypertensive Therapy on IC50 Values for Acetylcholine and Sodium Nitroprusside and Maximal Contractions in response to Norepinephrine**

<table>
<thead>
<tr>
<th>Agonist (M)</th>
<th>Control</th>
<th>Antihypertensive therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DS 8% NaCl</td>
<td>DR 8% NaCl</td>
</tr>
<tr>
<td>Acetylcholine (10^-5 - 10^-4 M)</td>
<td>5.8±0.3</td>
<td>7.4±0.03</td>
</tr>
<tr>
<td>Sodium nitroprusside (10^-5 - 10^-3 M)</td>
<td>7.7±0.1</td>
<td>7.7±0.2</td>
</tr>
<tr>
<td>Maximal contractions (g) to norepinephrine (10^-4 M)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With endothelium</td>
<td>2.7±0.4</td>
<td>4.4±0.4</td>
</tr>
<tr>
<td>Without endothelium</td>
<td>4.3±0.5</td>
<td>5.2±0.3</td>
</tr>
</tbody>
</table>

Values are means ± SEM. AHD = antihypertensive drug.

*Significant difference between rings taken from rats on the high or low sodium diet (p<0.05 using the Bonferroni rule).
†Significant difference between treated and untreated rings (p<0.05 using the Bonferroni rule).
response curve to sodium nitroprusside was slightly but significantly shifted to the right compared to the curve for DS on the low sodium diet (threelfold log shift at IC_{50}; see Table 1). The IC_{50} values for sodium nitroprusside in treated DS and DR on the high sodium diet did not differ significantly from those of untreated rats. In DS and DR, the antihypertensive therapy did not significantly affect the maximal contraction response to norepinephrine in rings with and without endothelium (see Table 1).

Discussion

The present study demonstrated that decreased endothelium-dependent relaxations in the aortas of hypertensive Dahl rats can be prevented and normalized by antihypertensive therapy. We previously reported that endothelium-dependent relaxations in response to acetylcholine, adenosine 5'-diphosphate, and thrombin were depressed in salt-induced hypertension of Dahl rats. Since a high sodium diet alone did not significantly affect endothelium-dependent relaxations, this seemed to be related to high blood pressure rather than the intake of salt. In the present study, the animals were treated with antihypertensive drugs either for 8 weeks (to prevent the development of hypertension in rats on the high sodium diet) or for 2 weeks after hypertension had developed. Both regimens were effective in normalizing endothelium-dependent relaxations in response to all agonists tested. Although the relaxations tended to be more pronounced in rats treated for 8 weeks than in those treated for 2 weeks, this difference was not statistically significant. This indicates that changes in endothelium-dependent relaxations occurring during the hypertensive process can be both prevented and reversed by antihypertensive therapy. The results further demonstrate that endothelium-dependent responses recover rather quickly (i.e., within 2 weeks) after normalization of blood pressure. Similar data have been obtained by Lockette and colleagues after reversal of deoxycorticosterone acetate-salt hypertension in the rat.

Antihypertensive therapy may enhance endothelium-dependent relaxations by four mechanisms. First, antihypertensive therapy may reverse functional changes of the endothelium related to high blood pressure. Second, it may reverse subendothelial thickening and improve transit of endothelium-derived vasoactive substances. Third, it may reverse structural vascular changes and normalize the responsiveness of vascular smooth muscle cells to endothelium-derived vasoactive substances. Fourth, antihypertensive drugs may have direct effects that are not related to the change in blood pressure that they induce.

The vascular endothelium undergoes functional changes during the development of hypertension. For example, the clearance of serotonin by the pulmonary vascular endothelium is reduced in spontaneously hypertensive rats. The production and/or release of endothelium-derived relaxing factor or factors might also be reduced in salt-induced hypertension of the rat. Antihypertensive therapy could normalize the function of endothelium that is exposed to high blood pressure. Indeed, it is reported that even after mechanical denudation, the endothelial layer is reconstituted within days. In the coronary artery of the pig, endothelium-dependent relaxations were found to recover 1 week after denudation with a balloon catheter. The rapidity of these changes might in part explain why the altered endothelium-dependent responses in hypertension can be normalized after only 2 weeks of antihypertensive therapy.

In hypertensive Dahl rats, focal expansion of the subendothelial space represents the earliest morphological changes of the intima. Increased blood pressure stimulates the biosynthesis of collagen and fibro-genesis in the arterial wall. Reduction of blood pressure with antihypertensive drugs reduces collagen formation. Expanded or increased collagen content in the subendothelial space might impair the transit of endothelium-derived relaxing factors. Therefore, reversal of these changes might improve diffusion of the factors. However, evidence that relaxations in response to endothelium-derived relaxing factors are normal in the spontaneously hypertensive rat in spite of subendothelial thickening indicates that this mechanism might not be important.

The relaxations in response to various substances acting directly on vascular smooth muscle cells are impaired in hypertension. In the aorta of the hypertensive Dahl rat, the relaxations in response to sodium nitroprusside are slightly impaired. In this study, relaxations in response to sodium nitroprusside were not significantly affected by antihypertensive therapy. Since endothelium-derived relaxing factors, like sodi-
um nitroprusside, cause relaxations by increasing cyclic guanosine 3',5'-monophosphate levels in vascular smooth muscle cells, an improved responsiveness to endothelium-derived relaxing factors is an unlikely explanation for the observed effects of the antihypertensive therapy.

In the rabbit aorta, the relaxations induced by hydralazine are in part endothelium-dependent. Hence, it is conceivable that antihypertensive drugs could exert a direct effect on endothelial cells. In the rat aorta, however, the relaxing effects of hydralazine are endothelium-independent.

Also, we found that the enhancement of endothelium-dependent relaxations was more pronounced in animals with a marked decrease in blood pressure during antihypertensive therapy (i.e., DS on 8% NaCl) than in rats with only a slight decrease in blood pressure (i.e., DR on 8% NaCl). This might indicate that the normalization of endothelium-dependent relaxations during antihypertensive therapy in hypertensive Dahl rats is primarily related to changes in transmural pressure. This conclusion is in line with results obtained in rats with coarctation of the aorta.

In summary, we found that antihypertensive therapy normalizes endothelium-dependent relaxations in response to acetylcholine, adenosine 5'-diphosphate, and thrombin in the aortas of hypertensive Dahl rats. This effect of antihypertensive drugs is probably related to their ability to lower blood pressure. Control of the blood pressure may improve endothelial function or, by reversing structural subendothelial changes, favor the transit of endothelium-derived vasoactive substances from the endothelium to the underlying vascular smooth muscle.

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