Endothelium-Dependent Responses to Platelets and Serotonin in Spontaneously Hypertensive Rats

THOMAS F. LÜSCHER AND PAUL M. VANHOUTTE

SUMMARY We studied endothelium-dependent responses to substances released from aggregating platelets in spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY). Rings of thoracic aorta with and without endothelium were taken from adult rats and suspended for isometric tension recording in organ chambers containing modified Krebs-Ringer bicarbonate solution. Aggregating platelets caused statistically similar contractions in rings without endothelium in both strains. In rings with endothelium from SHR the contractions were significantly more pronounced than in rings with endothelium from WKY. In contracted rings with endothelium, serotonin caused a slight relaxation at lower concentrations but contraction at higher concentrations; only contractions were seen in rings without endothelium. The higher concentrations of the monoamine caused contractions, which in the SHR but not in the WKY were larger in the presence than in the absence of endothelium. In both strains adenosine diphosphate induced concentration-dependent relaxation in rings with endothelium but not in those without it; at high concentrations of adenosine diphosphate, the relaxation responses were significantly smaller in the SHR than in the WKY. Endothelium-dependent relaxation in response to thrombin did not differ in the two strains. The increased contraction in response to aggregating platelets and serotonin and the decreased relaxation in response to adenosine diphosphate in the SHR suggest that functional changes occur in the endothelium in this model of hypertension, possibly because of the release of one or more endothelium-derived contracting factors. (Hypertension 8 [Suppl II]: II-55-II-60, 1986)

KEY WORDS • adenosine diphosphate • endothelium-derived relaxing factors • endothelium-derived contracting factors • hypertension • norepinephrine • rat thoracic aorta • serotonin • thrombin

THE endothelium produces vasoactive substances that may be important in the local control of the circulation.1-4 The release of one or more endothelium-derived relaxing factors may contribute to the protective role of the endothelium.5 6 In the course of hypertension the endothelium changes both morphologically and functionally.7-10 The endothelium-dependent relaxations evoked by acetylcholine are decreased in spontaneously hypertensive rats (SHR).8-10 In hypertension, the spontaneous turnover of platelets is accelerated, which suggests that continuous platelet aggregation occurs.5 11 Aggregating platelets release various substances, including adenosine diphosphate and serotonin, which in canine blood vessels can evoke endothelium-dependent relaxation.12-13 The present study was designed to determine whether or not endothelium-dependent responses to aggregating platelets and the major products they release are altered in SHR.

Methods

Preparation of Blood Vessels

The experiments were performed on the thoracic aorta taken from age-matched (30-34 weeks) and weight-matched (330-400 g) male SHR and Wistar-Kyoto (WKY) rats (Harlan Sprague Dawley, Indianapolis, IN, USA). The rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). Blood pressure was recorded in the left femoral artery or the abdominal aorta by means of a heparinized PE-50 catheter connected to a strain-gauge pressure transducer (P-23
The blood was centrifuged for 30 minutes at 100 g at ambient temperature, and the platelet-rich plasma was recovered cellulose membranes (pore size, 0.2 µm). The supernatant was discarded, and the remaining platelet pellet was resuspended in a small volume of the second centrifugation medium and kept on ice until use. The platelet count of this suspension was then determined, and the volume of the suspension adjusted so that when added to the organ chamber (in a dilution of 1:100 or higher), the final platelet concentration in the bath was 25,000/µl, 50,000/µl, or 75,000/µl. Platelet aggregation on exposure to the collagen of the blood vessel wall and the calcium-containing modified Krebs-Ringer bicarbonate solution was evidenced by clearing of the initially turbid solution and formation of visible platelet clumps. Samples of fluid (2 ml) were withdrawn from the organ baths after the addition of platelets, divided for determination of the concentrations of thromboxane B2 and serotonin, and frozen until analysis.

### Serotonin Determination

Immediately after the experiment, 1 ml of fluid collected from the organ bath was added to 240 µl of cysteine (1% by weight in distilled water) and frozen at −24°C. Before analysis proteins were precipitated by adding ZnSO4 and NaOH and centrifuging the solution at 3000 g for 30 minutes at 4°C. The supernatant was then filtered through centrifugal microfilters (Bioanalytical Systems, West Lafayette, IN, USA) with regenerated cellulose membranes (pore size, 0.2 µm). The amine in the resulting supernatant was quantitated by reverse-phase high-pressure liquid chromatography with electrochemical detection. The pH of the buffer was 4.7. Protein-free extracts (25 ml) were injected into the high-pressure liquid chromatography column. Serotonin concentrations were determined by comparing the peak height of the sample with that of a standard of serotonin. The interassay coefficient of variation was 5.6%. Corrections for intraassay variation were made by running a standard of serotonin with each series of determinations.

### Thromboxane B2 Determination

Before analysis the samples were centrifuged (3000 g, 10 minutes, 4°C) and brought to pH 3.5 with 1 N hydrochloric acid. Thromboxane B2 was extracted with octadecylsilyl silica columns (Bond Elut C18, Analyti-Chem International, Harbor City, CA, USA) by the method of Powell. Further purification was accomplished by eluting the samples with 2 ml of ethyl acetate onto silica columns (Bond Elut Si, Analyti-Chem International). After being washed with a 2-ml mixture of benzene/ethyl acetate (80:20), thromboxane B2 was eluted with a 4-ml mixture of benzene/ethyl acetate/methanol (60:40:40) and evaporated to dryness in a 37°C water bath under nitrogen. The samples were resuspended in phosphate buffer (pH 7.4). Aliquots (100 µl) of standards and diluted samples were assayed by displacement of [3H]thromboxane B2 from

### Table 1. Body Weight, Blood Pressure, Platelet Count, and Platelet Volume in Normotensive WKY and in SHR

<table>
<thead>
<tr>
<th>Rat strain</th>
<th>Body weight (g)</th>
<th>Mean BP (mm Hg)</th>
<th>Platelet count (µl)</th>
<th>Platelet volume (µm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>350±9</td>
<td>895,414±32,907</td>
<td>7.1±0.2</td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>355±8</td>
<td>591,167±21,415</td>
<td>7.0±0.2</td>
<td></td>
</tr>
</tbody>
</table>

Data are given as means ± SEM in 12 animals. BP = blood pressure.

*The difference between SHR and WKY is statistically significant (p < 0.05).
thromboxane B\textsubscript{2} antiserum in a total incubation volume of 300 \(\mu l\) at 4°C. After centrifugation (3000 g, 5 minutes, 4°C), the supernatant containing the antibody-bound \([^{3}H]\)thromboxane B\textsubscript{2} fraction was counted in a scintillation counter, and the concentration of thromboxane B\textsubscript{2} was estimated by comparison with a standard curve.\textsuperscript{17} The recovery of the cold standard averaged 94%. The intraassay coefficient of variation was 5.6%, and the interassay variation was 9.6%.

**Drugs**

The following drugs were used: acetylcholine hydrochloride (Sigma, St. Louis, MO, USA), adenosine diphosphate (Sigma), \(\text{\textit{l}}\)-norepinephrine bitartrate (Sigma), prostaglandin F\textsubscript{2\alpha} (Sigma), 5-hydroxytryptamine (serotonin, Sigma), and bovine thrombin (Sigma). All drugs were dissolved in distilled water.

**Calculations and Statistical Analysis**

Up to four rings from each rat were studied in parallel. Decreases and increases in tension were expressed as the percentage of the contraction evoked by prostaglandin F\textsubscript{2\alpha} (\(10^{-8}\) to \(3 \times 10^{-6}\) M). The concentration of prostaglandin was adjusted individually to evoke a comparable increase in tension (in grams) in rings from SHR and WKY (average increase in tension: 1.24 ± 0.05 and 1.26 ± 0.04 g, respectively, corresponding to the concentration of prostaglandin F\textsubscript{2\alpha} causing 30% of the maximal response [ED\textsubscript{30}]; \(n = 56\)). Concentrations of adenosine diphosphate evoking 30% relaxation are expressed as the negative logarithm of the molar concentration (IC\textsubscript{30}). The results are given as means ± SEM. In each series, \(n\) refers to the number of rats from which vessels were taken. Statistical evaluation was done by Student’s \(t\) test for paired and unpaired observations. When \(p\) was smaller than 0.05, means were considered to be significantly different.

**Results**

**Aggregating Platelets**

Rings with and without endothelium from SHR and WKY were contracted with prostaglandin F\textsubscript{2\alpha} and exposed to aggregating platelets collected from rats of the same strain. In rings from both strains, the platelets (25,000/\(\mu l\), 50,000/\(\mu l\), and 75,000/\(\mu l\)) evoked further increases in tension, which were significantly greater in rings without endothelium than in rings with endothelium. In rings without endothelium from both strains and in rings with endothelium from SHR, the increases in tension evoked by aggregating platelets were significantly more pronounced with higher concentrations (50,000/\(\mu l\) and 75,000/\(\mu l\)) than with lower concentrations (25,000/\(\mu l\)). In rings with but not without endothelium from SHR, aggregating platelets induced significantly greater contractions than in rings from WKY (Figure 1). The difference in responsiveness to aggregating platelets between rings with and without endothelium was significantly smaller in SHR than in WKY, with all concentrations of platelets used. Similar results were obtained in rings from SHR exposed to aggregating platelets from WKY and vice versa (data not shown). The amounts of serotonin and thromboxane B\textsubscript{2} released by platelets were comparable in the two rat strains (Table 2).

**Serotonin**

During contractions to prostaglandin F\textsubscript{2\alpha}, serotonin (\(10^{-7}\) to \(10^{-4}\) M) was added. Lower concentrations (\(10^{-8}\) to \(10^{-4}\) M) of serotonin induced a slight relaxation of rings with endothelium, but higher concentrations (\(10^{-7}\) to \(10^{-4}\) M) induced contraction (Figure 2). In rings without endothelium, serotonin caused only contractions. In the SHR but not in the WKY, the increase in tension evoked by serotonin (3 \(\times\) \(10^{-8}\) to 3 \(\times\) \(10^{-3}\) M) was statistically significant.

**Table 2. Concentration of Serotonin and Thromboxane B\textsubscript{2} in the Bath Solution After the Addition of Platelets (75,000/\(\mu l\))**

<table>
<thead>
<tr>
<th>Aorta preparation</th>
<th>Serotonin (ng/ml)</th>
<th>Thromboxane B\textsubscript{2} (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SHR</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rings with endothelium</td>
<td>124 ± 27</td>
<td>4500 ± 685</td>
</tr>
<tr>
<td>Rings without endothelium</td>
<td>117 ± 39</td>
<td>3106 ± 297</td>
</tr>
<tr>
<td><strong>WKY</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rings with endothelium</td>
<td>124 ± 27</td>
<td>6970 ± 1409</td>
</tr>
<tr>
<td>Rings without endothelium</td>
<td>124 ± 27</td>
<td>3008 ± 762</td>
</tr>
</tbody>
</table>

The amounts of serotonin and thromboxane B\textsubscript{2} released from aggregating platelets taken from SHR and WKY did not differ statistically. Data are given as means ± SEM (\(n = 5\)).
was significantly more pronounced in rings with endothelium than in those without it (see Figure 2).

In rings contracted with prostaglandin F$_{2\alpha}$, the S$_2$-serotonergic antagonist ketanserin ($10^{-7}$ M) significantly reduced the increases in tension induced by serotonin ($10^{-6}$ to $3 \times 10^{-5}$ M, $n = 6$). WKY rings with endothelium had significantly less pronounced contractions to serotonin than rings without endothelium. In contrast, rings with endothelium from SHR exhibited significantly enhanced contractions to the monoamine, as compared to rings without endothelium. Hence, the endothelial response (increase in tension in rings with endothelium minus increase in tension in rings without endothelium) was qualitatively different in the two strains (Figure 3).

**Adenosine Diphosphate**

In rings from both rat strains adenosine diphosphate ($10^{-8}$ to $10^{-4}$ M) caused concentration-dependent relaxation (only in the presence of endothelium) during contractions evoked by prostaglandin F$_{2\alpha}$. At $3 \times 10^{-5}$ M and $10^{-4}$ M, the relaxations were significantly more pronounced in rings from WKY than in those from SHR (Figure 4). The IC$_{50}$ values for adenosine diphosphate were comparable in the two strains ($5.6 \pm 0.3$ and $5.9 \pm 0.2$ in SHR and WKY, respectively).

**Thrombin**

In rings with endothelium contracted with prostaglandin F$_{2\alpha}$, thrombin (1 U/ml) caused transient relaxation. The nadir and the time course of the endothelium-dependent response to thrombin did not differ statistically between rings from SHR and those from WKY (Figure 5).

**Discussion**

In contrast to observations in the coronary artery of the dog$^5$, the aggregating platelets at the concentrations used in this study did not cause relaxation of the contracted aorta of the rat when endothelial cells were present. However, our experiments demonstrate that in this blood vessel, as in the canine coronary artery,$^5$ the presence of endothelial cells markedly reduces the contractions evoked by platelets, illustrating for this species the protective role of the endothelium when
platelets aggregate in a blood vessel with intact intima. The absence of systematic relaxation of rings with endothelium when exposed to aggregating platelets may reflect the fact that thromboxane $A_2$ is a strong activator of vascular smooth muscle in arteries of the rat and that rat platelets release larger amounts of the vasoconstrictor prostaglandin $F_2$-$\alpha$ (PGF$_{2\alpha}$). The present experiments demonstrate that in the aorta of SHR, endothelium-dependent relaxation in response to adenosine nucleotides and serotonin is identical in normotensive and hypertensive animals. These studies strongly suggest that the blunting of the endothelium-dependent relaxation evoked by acetylcholine in the aorta of SHR is due to the concomitant release of one or more endothelium-derived contracting factors. The present results, then, would best be explained if aggregating platelets were to cause the release of such a factor (or factors) in the aorta of hypertensive but not normotensive rats. The release of one or more endothelium-derived contracting factors has been demonstrated in canine veins exposed to arachidonic acid and in various blood vessels of the same species during hypoxia.

Alternatively, serotonin, adenosine diphosphate, and thrombin might release different endothelium-dependent relaxing factors. If so, differences in endothelium-dependent responses in WKY and SHR may at least in part reflect a selective loss from a heterogeneous population of relaxing factors.

The major finding of this study is that the protective role of the endothelium (i.e., the diminished contraction to platelets in rings with endothelium, as compared to rings without endothelium) is less pronounced in the aorta of SHR than in that of normotensive animals. Indeed, in this strain, the presence of the endothelium did not have a major effect on contractions evoked by aggregating platelets. This observation is in line with reports that endothelium-dependent relaxation in response to acetylcholine and the calcium ionophore A23187 is reduced in the aorta of SHR. The reduced inhibition of the response to aggregating platelets in the presence of endothelium could be due to a decreased ability to release one or more endothelium-derived relaxing factors or a reduced sensitivity of vascular smooth muscle to such a factor (or factors). These explanations are made unlikely by the observations that endothelium-dependent relaxation responses to thrombin, to low and moderate concentrations of adenosine diphosphate, and to low concentrations of serotonin are comparable in the aorta of normotensive and hypertensive animals. Earlier work has shown that, provided inhibitors of cyclo-oxygenase are present, endothelium-dependent relaxation in response to acetylcholine is identical in normotensive and hypertensive animals. These studies strongly suggest that the blunting of the endothelium-dependent relaxation evoked by acetylcholine in the aorta of SHR is due to the concomitant release of one or more endothelium-derived contracting factors. The present results, then, would best be explained if aggregating platelets were to cause the release of such a factor (or factors) in the aorta of hypertensive but not normotensive rats. The release of one or more endothelium-derived contracting factors has been demonstrated in canine veins exposed to arachidonic acid and in various blood vessels of the same species during hypoxia.

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References

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