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 • 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.3,12,13 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...
De, Statham Instruments, Oxnard, CA, USA; Table 1). In 12 animals 1 ml of blood was drawn for the determination of platelet count and platelet volume (Coulter S+ IV, Miami, FL, USA; see Table 1). The thoracic aorta was dissected free with a dissecting microscope (Carl Zeiss Instruments, Overkochen, Federal Republic of Germany), excised, and placed in 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 in length). In certain rings, the endothelium was removed by gentle rubbing of the intimal surface with a small forceps; in the remaining rings care was taken not to touch the inner surface of the blood vessel. The presence or absence of endothelial cells was confirmed either histologically or by the presence or absence, respectively, of relaxation responses induced by acetylcholine (10⁻⁷ M), thrombin (1 U/ml), or adenosine diphosphate (10⁻⁵ M).³

Organ Chamber Experiments

The rings were suspended in organ chambers filled with control solution kept at 37 °C and were aerated with a 95% O₂, 5% CO₂ gas mixture. They were connected to force transducers (Statham Universal UC2 or Grass FT 03C, Quincy, MA, USA), and changes in isometric force were recorded. Before the actual experiments, the preparations were progressively stretched and repeatedly exposed to norepinephrine (10⁻⁷ M or 3 × 10⁻⁷ M) at different levels of tension until the optimal point of the length-tension relationship was reached; the mean optimal basal tension (±SEM) was 3.6 ± 0.1 g in rings from both WKY and SHR (n = 72). After this procedure, the rings were allowed to equilibrate for 45 minutes.

Platelet Preparation

In SHR and WKY blood was drawn from the abdominal aorta into citrate anticoagulant to yield final concentrations of 9.3 mM sodium citrate, 0.7 mM citric acid, and 14 mM dextrose.¹⁴ In each experiment blood was collected from three rats of the same strain. The blood was centrifuged for 30 minutes at 100 g at room temperature, and the platelet-rich plasma was removed with a pipette. An equal volume of cold citrate anticoagulant mixture was then added to the platelet-rich plasma, and the mixture was centrifuged for 20 minutes at 500 g. The supernatant was discarded, and the remaining platelet pellet was resuspended in a small volume of the second citrate anticoagulant mixture and kept on ice until use. The platelet count of this suspension was then determined, and the volume of the suspension adjusted to 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 distilled water (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 amines in the resulting supernatant were quantitated by reverse-phase high-pressure liquid chromatography with electrochemical detection.¹⁵ 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 1N hydrochloric acid. Thromboxane B₂ was extracted with octadeucysilsilica columns (Bond Elut C18, Analyti-Chem International, Harbor City, CA, USA) by the method of Powell.¹⁶ 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-mI 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 redissolved in phosphate buffer (pH 7.4). Aliquots (100 μl) of standards and diluted samples were assayed by displacement of [³H]thromboxane B₂ from

<table>
<thead>
<tr>
<th>Body Weight, Blood Pressure, Platelet Count, and Platelet Volume in Normotensive WKY and in SHR</th>
<th>Rat strain</th>
<th>Body weight</th>
<th>Mean BP</th>
<th>Platelet count</th>
<th>Platelet volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>12</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>12</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 B₂ antiserum in a total incubation volume of 300 μl at 4°C. After centrifugation (3000 g, 5 minutes, 4°C), the supernatant containing the antibody-bound [³H]thromboxane B₂ fraction was counted in a scintillation counter, and the concentration of thromboxane B₂ was estimated by comparison with a standard curve. 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), l-norepinephrine bitartrate (Sigma), prostaglandin F₂o (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 F₂o (10⁻⁸ to 3 × 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 F₂o causing 30% of the maximal response [IC₃₀]; n = 56). Concentrations of adenosine diphosphate evoking 30% relaxation are expressed as the negative logarithm of the molar concentration (IC₃₀). 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 F₂o and exposed to aggregating platelets collected from rats of the same strain. In rings from both strains, the platelets (25,000/μl, 50,000/μl, and 75,000/μl) 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/μl and 75,000/μl) than with lower concentrations (25,000/μl). 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 B₂ released by platelets were comparable in the two rat strains (Table 2).

**Serotonin**

During contractions to prostaglandin F₂o, serotonin (10⁻⁷–10⁻⁴ M) was added. Lower concentrations (10⁻⁷–10⁻⁴ M) of serotonin induced a slight relaxation of rings with endothelium, but higher concentrations (10⁻⁷–10⁻⁴ 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 × 10⁻⁴ to 3 × 10⁻³ M)

**Table 2. Concentration of Serotonin and Thromboxane B₂ in the Bath Solution After the Addition of Platelets (75,000/μl)**

<table>
<thead>
<tr>
<th>Aorta preparation</th>
<th>Serotonin (ng/ml)</th>
<th>Thromboxane B₂ (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 ± 42</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 B₂ 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$_{2a}$, 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$_{2a}$. 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$_{2a}$, 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$^{14}$ 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, the presence of endothelial cells markedly reduces the contractions evoked by platelets, illustrating for this species the protective role of the endothelium when present.
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 A₂ is a strong activator of vascular smooth muscle in arteries of the rat and that rat platelets release larger amounts of the vasoconstrictor prostanoi than canine platelets (D.S. Houston, personal communication, 1986). Alternatively, the experimental conditions used may not have been sensitive enough to detect endothelium-dependent relaxation, since serotonin and other platelet-derived substances reach both the endothelium and vascular smooth muscle.

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. These studies strongly suggest that the blunting of the endothelium-dependent relaxation evoked by adenosine diphosphate 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.

Of the various substances released by aggregating platelets, those most likely to play a major part in endothelium-dependent responses are adenosine nucleotides and serotonin. The present experiments demonstrate that in the aorta of SHR, endothelium-dependent relaxation in response to adenosine nucleotides is reduced, and the presence of endothelial cells potentiates the contractions evoked by serotonin. If S₂-serotonergic receptors on vascular smooth muscle are blocked to minimize the direct contractile effects of serotonin, these qualitative differences of the endothelial response to the monoamine in WKY and SHR are even more apparent and provide further support for the concept of endothelium-dependent contractions in response to serotonin in SHR. Thus, both mediators could contribute to the release of one or more endothelium-derived contracting factors by aggregating platelets. Since the endothelium-dependent responses to serotonin are affected at lower concentrations than those to adenosine diphosphate, the former may play a more important part than the latter in the augmented contractions evoked by aggregating platelets in aortic rings with endothelium from hypertensive rats. The present experiments do not provide information on other mediators (e.g., platelet-activating factor or thromboxane A₂) released from aggregating platelets that may also have a role in the altered endothelial responses of SHR. Our observations do, however, exclude a major role for thrombin formed during the aggregation process.

Acknowledgments

The authors wish to thank Dr. J. Carlos Romero for determining the levels of thromboxane B₂ and Dr. Gertrude M. Tyce for measuring serotonin. We also thank Dr. Donald S. Houston for advice and for reading the manuscript, Mr. Robert R. Lorenz and Mrs. Helen Hendrickson for preparing the figures, and Mrs. Janet Beckman for typing the manuscript.

References

1. Moncada S, Vane JR. Prostacyclin (PGI₂), the vascular wall and vasodilation. In: Vanhoutte PM, Leusen I, eds. Meca-


10. Lüscher TF, Vanhoutte PM. Endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat. Hypertension 1986;8:344-348


Endothelium-dependent responses to platelets and serotonin in spontaneously hypertensive rats.

T F Lüscher and P M Vanhoutte

Hypertension. 1986;8:II55
doi: 10.1161/01.HYP.8.6_Pt_2.II55

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1986 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/8/6_Pt_2/II55

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at: http://hyper.ahajournals.org/subscriptions/