Endothelium-Dependent Contractions to Acetylcholine in the Aorta of the Spontaneously Hypertensive Rat

THOMAS F. Lüscher and PAUL M. Vanhoutte

SUMMARY To study the mechanism of decreased endothelium-dependent relaxations in spontaneously hypertensive rats (SHR), rings of thoracic aorta with and without endothelium were taken from age-matched male SHR and normotensive Wistar-Kyoto rats (WKY) and suspended for isometric tension recording. Acetylcholine caused endothelium-dependent contractions in quiescent rings from SHR but not in those from WKY. These contractions were inhibited by atropine but not by hexamethonium and were prevented by inhibitors of phospholipase A$_2$ or cyclooxygenase but not by inhibitors of prostacyclin synthetase, thromboxane synthetase, or leukotriene synthetase. Prostaglandin D$_2$, E$_1$, E$_2$, and F$_2$ caused concentration-dependent contractions in rings without endothelium from both SHR and WKY; the responses to the highest concentration (10$^{-5}$ M) of the individual prostaglandins were comparable in both strains. Endothelium-dependent relaxations evoked by high but not by low concentrations of acetylcholine were significantly depressed in SHR as compared with those in WKY (p < 0.05). Indomethacin normalized endothelium-dependent relaxations in SHR. Thus, acetylcholine can activate muscarinic receptors that evoke endothelium-dependent contractions in the aorta of SHR but not in that of WKY. The contraction probably is mediated by a cyclooxygenase product(s) other than prostacyclin or thromboxane A$_2$. The reduced endothelium-dependent relaxations to acetylcholine in the SHR probably are not due to a decreased release of endothelium-derived relaxing factor(s) but to the simultaneous release of endothelium-derived contracting substance(s).

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KEY WORDS • acetylcholine • cyclooxygenase • endothelium • endothelium-dependent contractions • endothelium-dependent relaxations • hypertension • prostaglandins • aorta

THE endothelium may contribute in several ways to the local regulation of vascular function, since endothelial cells produce prostaglandin I$_2$ (prostacyclin) and endothelium-derived relaxing factor(s) and also contain enzymes that can activate or degrade vasoactive hormones. Under certain conditions endothelial cells can also release contracting substance(s).

In hypertension, morphological changes occur in endothelial cells. Decreased endothelium-dependent relaxations to acetylcholine and the calcium ionophore A23187 have been reported in blood vessels of adult spontaneously hypertensive rats (SHR). The present experiments were designed to determine the mechanism underlying these decreased endothelium-dependent relaxations.

Materials and Methods

The experiments were performed on the thoracic aorta from age-matched (30–34 weeks) and weight-matched male SHR and Wistar-Kyoto rats (WKY; Harlan Sprague Dawley, Indianapolis, IN, USA; Table 1). The rats were anesthetized with pentobarbital sodium (50 mg/kg i.p.). Blood pressure was recorded in the abdominal aorta or the left femoral artery by means of a heparinized PE-50 catheter connected to a strain gauge pressure transducer (Statham P-23 Oxnard, CA, USA; see Table 1). Immediately after the arterial blood pressure was recorded, 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; CaCl$_2$, 344
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TABLE I Blood Pressure, Body Weight, Basal Tension, and Maximal Response to Norepinephrine in SHR and Normoten-

tive WKY

<table>
<thead>
<tr>
<th>Variable</th>
<th>WKY (n = 36)</th>
<th>SHR (n = 70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>81 ± 3</td>
<td>139 ± 3*</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>362 ± 6</td>
<td>372 ± 4</td>
</tr>
<tr>
<td>Aortic rings</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal tension (g)</td>
<td>3.5 ± 0.1</td>
<td>3.4 ± 0.1</td>
</tr>
</tbody>
</table>
| Response to 10^-4 M norepineph-

rine (g) | 4.0 ± 1.3 | 2.8 ± 0.8* |

Values are means ± SEM.
*p < 0.05, compared with values in WKY

2.5; MgSO4, 1.2; KH2PO4, 1.2; NaHCO3, 25.0; ede-
tate 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). The
endothelium was removed by gently rubbing the in-
ternal 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 endo-
thelial cells was confirmed histologically in certain exper-
iments and in others by the presence or absence of
responses to acetylcholine (10^-7 M).

The rings were suspended in organ chambers, which
contained 25 ml of control solution (37 °C) aerated
with 95% O2, 5% CO2, and connected to force trans-
ducers (Statham Universal UC2 or Grass FT 03C,
Quincy, MA, USA); changes in isometric force were
then recorded. Before the actual experiments began,
the preparations were progressively stretched and ex-
posed to norepinephrine (3 × 10^-7 M) at each level of
tension until the optimal point of the length-tension
relationship was reached. The optimal basal tension
did not differ significantly between groups (see Table
1). After this procedure the rings were allowed to
equilibrate for 45 minutes. All rings were then exposed
to norepinephrine (10^-4 M) to determine maximal re-
sponsiveness. The response to the catecholamine was
significantly larger in rings from WKY than in those
from SHR (see Table 1).

The following drugs were used: acetylcholine HCl,
atropine sulfate, diethylcarbamazine, indomethacin,
L-norepinephrine bitartrate, prostacyclin, prostaglan-
din D2, prostaglandin E1, prostaglandin E2, prostaglan-
din F2α, quinacrine (Sigma Chemical, St. Louis, MO,
USA); heparin (Elkin Sinn, Cherry Hill, NJ, USA);
hexamethonium bromide (K + K Laboratories, Plain-
view, NY, USA); imidazole (Aldrich Chemical, Mil-
waukee, WI, USA); sodium meclofenamate (Parke,
Davis, Detroit, MI, USA); and tranylcypromine
(Smith, Kline & French Laboratories, Philadelphi-
PA, USA). Drug concentrations are expressed as final
molar concentrations in the bath solution. All drugs
were dissolved in distilled water except indomethacin,
which was dissolved in 10 ml of distilled water con-
taining 5 × 10^-3 M NaCO3 and was sonicated before
use. Prostacyclin was dissolved in 0.15 M NaHCO3
solution.

Rings from SHR and WKY were studied in parallel. Experiments with inhibitors were performed on rings from the same animals studied in parallel. In the ex-
periments in which concentration-response curves
were determined in quiescent preparations, the results
are expressed as percent of the maximal response to
norepinephrine (10^-4 M). In the experiments in which
relaxations were studied, the rings were contracted
with the individual concentration (10^-8-3 × 10^-7 M)
of norepinephrine causing an increase in tension of
about 1.4 g; the results are expressed as percent relax-
ation of that contraction. Results are given as means ±
SEM. Statistical evaluation was done by Student's t


test for paired or unpaired observations. Means were
considered significantly different when p was less than
0.05.

Results

Under basal conditions acetylcholine had no signifi-
cant effect on rings from SHR without endothelium
(n = 7) or on rings from WKY with or without endo-
thelium (n = 4). In rings from SHR with endothelium,
10^-8 to 3 × 10^-8 M acetylcholine caused no signifi-
cant changes in tension, while 3 × 10^-7 to 10^-5 M
acetylcholine evoked concentration-dependent con-
tractions accompanied by rhythmic oscillations in ten-
sion (Figure 1). The absolute tension developed at
10^-5 M acetylcholine averaged 0.9 ± 0.6 g (n = 28).

FIGURE 1. Quiescent rings with and without endothelium were studied in parallel and exposed to increasing concentrations of
acetylcholine (10^-8-10^-4 M). Results are expressed as percent of the maximal response to norepinephrine (10^-4 M). Asterisk indicates significant difference between rings from SHR with and without endothelium (p < 0.05, n = 7). Inset: Original recordings of isometric tension in rings (with endothelium) of
aortas from SHR and WKY.
Atropine (10^{-7} M) caused a significant shift to the right of the concentration-response curve to acetylcholine (log shift at the 10% effective dose [ED_{10}] level, relative to the maximal response to norepinephrine: 2.04 ± 0.24; ≈ 200-fold), while hexamethonium (10^{-7} M) had no significant effect (Figure 2).

Quinacrine (10^{-5} M) significantly depressed the contractions evoked by acetylcholine in rings from SHR with endothelium; 10^{-4} M quinacrine abolished the response (Figure 3). The endothelium-dependent contractions to acetylcholine were prevented by a 30-minute incubation with the cyclooxygenase inhibitors indomethacin (10^{-5} M; n = 4) and meclofenamate (10^{-4} M, n = 4; see Figure 2). Inhibitors of leukotriene synthetase (diethylcarbamazine, 10^{-4} M; n = 6), prostacyclin synthetase (tranylcypromine, 10^{-4} M; n = 3), or thromboxane synthetase (imidazole, 10^{-4} M; n = 6) did not significantly affect the contractions (Table 2). Basal tension was not significantly affected by inhibitors of arachidonic acid metabolism.

Quiescent aortic rings without endothelium taken from SHR and WKY were exposed to increasing concentrations (10^{-9}-10^{-5} M) of prostaglandin E_{2}, E_{6}, F_{2a}, and I_{2}. Prostaglandins E_{2}, E_{6}, F_{2a} caused concentration-dependent tension increases in rings from both SHR and WKY according to the following order of potency: F_{2a} > E_{6} > E_{2} > D_{1} (Table 3). The only significant difference between the two strains was that rings from SHR were significantly more responsive to low concentrations of prostaglandin F_{2a} (10^{-5}-10^{-7} M) than were those from WKY. The contractions obtained with 10^{-5} M of each of the prostaglandins did not differ significantly in the two strains (see Table 3). Prostacyclin elicited a biphasic response that was comparable in SHR and WKY: slight relaxations at lower concentrations (10^{-5}-10^{-6} M) and weak contractions at higher concentrations (3 × 10^{-6}-10^{-5} M).

During contractions evoked by norepinephrine, acetylcholine caused relaxation only in rings with endothelium. From 3 × 10^{-9} to 10^{-7} M acetylcholine, the relaxations were comparable in rings from SHR and WKY. At higher concentrations (3 × 10^{-7}-10^{-5} M)

![Figure 2](https://example.com/figure2.png)  
**Figure 2.** Effect of atropine (10^{-7} M) and hexamethonium (10^{-7} M) on endothelium-dependent contractions induced by acetylcholine in aortas of SHR. Results are expressed as percent of the maximal response to norepinephrine. Asterisk indicates significant difference between the two groups (p < 0.05, n = 8).

![Figure 3](https://example.com/figure3.png)  
**Figure 3.** Effects of quinacrine, meclofenamate, and indomethacin on endothelium-dependent contractions that are evoked by acetylcholine in aortas of SHR. Results are expressed as percent of the maximal response to norepinephrine. Asterisk indicates significant difference from control (p < 0.05, n = 4).

**Table 2.** Effect of Inhibitors of Prostacyclin Synthetase (Tranylcypromine), Thromboxane Synthetase (Imidazole), and Leukotriene Synthetase (Diethylcarbamazine) on Endothelium-Dependent Contractions to Acetylcholine in Aortas of SHR

<table>
<thead>
<tr>
<th>Variable</th>
<th>Acetylcholine (log M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 17)*</td>
<td>-6.5</td>
</tr>
<tr>
<td>10^{-4} M diethylcarbamazine (n = 6)</td>
<td>2.6 ± 0.7</td>
</tr>
<tr>
<td>10^{-4} M tranylcypromine (n = 5)</td>
<td>0.8 ± 0.6</td>
</tr>
<tr>
<td>10^{-4} M imidazole (n = 6)</td>
<td>4.6 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>9.8 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>14.7 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>14.9 ± 2.0</td>
</tr>
</tbody>
</table>

Values are means ± SEM, expressed as percent of the maximal response to 10^{-4} M norepinephrine

*Statistical analysis performed between control and treated rings of the same rats revealed no statistical differences
Table 3. Vascular Responsiveness to Prostaglandins in Quiescent Aortic Rings Without Endothelium from SHR and WKY

<table>
<thead>
<tr>
<th>Prostaglandin</th>
<th>Concentration (log M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-9</td>
</tr>
<tr>
<td>D₂</td>
<td></td>
</tr>
<tr>
<td>WKY (n = 6)</td>
<td>0</td>
</tr>
<tr>
<td>SHR (n = 6)</td>
<td>0</td>
</tr>
<tr>
<td>E₁</td>
<td></td>
</tr>
<tr>
<td>WKY (n = 5)</td>
<td>0</td>
</tr>
<tr>
<td>SHR (n = 5)</td>
<td>0</td>
</tr>
<tr>
<td>E₂</td>
<td></td>
</tr>
<tr>
<td>WKY (n = 4)</td>
<td>0.01±0.01</td>
</tr>
<tr>
<td>SHR (n = 4)</td>
<td>0.07±0.03</td>
</tr>
<tr>
<td>F₂₁₀</td>
<td></td>
</tr>
<tr>
<td>WKY (n = 7)</td>
<td>0.1±0.1</td>
</tr>
<tr>
<td>SHR (n = 7)</td>
<td>0.6±0.2</td>
</tr>
<tr>
<td>I₂</td>
<td></td>
</tr>
<tr>
<td>WKY (n = 5)</td>
<td>0</td>
</tr>
<tr>
<td>SHR (n = 5)</td>
<td>-0.02±0.02</td>
</tr>
</tbody>
</table>

Values are means ± SEM, expressed as absolute changes in tension (g). A minus sign indicates relaxation. *p < 0.05, compared with values in WKY.

the relaxations were significantly greater in rings from WKY than in those from SHR (Figure 4). In rings from SHR with endothelium incubated for 30 minutes with 10⁻⁵ M indomethacin the relaxations evoked by acetylcholine did not differ statistically from those observed in rings from WKY (see Figure 4).

Discussion

The present experiments demonstrate that acetylcholine can cause endothelium-dependent contractions in the aorta of adult SHR but not in that of normotensive control animals. The contractions occurred with concentrations of acetylcholine higher than those required to trigger the release of endothelium-derived relaxing factor(s). As the endothelium-dependent contractions were inhibited by atropine but were not affected by hexamethonium, the endothelial receptor involved must be muscarinic in nature.

The endothelium-dependent contractions evoked by acetylcholine were abolished by the phospholipase A₂ inhibitor quinacrine, which may suggest the involvement of arachidonic acid or one of its metabolites, although inhibition of muscarinic receptors or Ca²⁺ entry cannot be excluded. Since indomethacin and meclofenamate, which share the property of inhibiting cyclooxygenase, abolished the endothelium-dependent contractions to acetylcholine, it is probable that a product(s) of the metabolism of arachidonic acid through the cyclooxygenase pathway is responsible for the contractions. Since inhibitors of prostacyclin synthetase (tranylcypromine) and thromboxane synthetase (imidazole) did not reduce the contractions evoked by acetylcholine, the metabolite of arachidonic acid is not likely to be prostacyclin (which actually only caused minimal contractions of the aorta of both SHR and WKY that were contracted with norepinephrine (10⁻⁴ M). Results are expressed as percent of the maximal response to the catecholamine norepinephrine (10⁻⁴ M). Asterisk indicates significant difference from control (p < 0.05, n = 6).
normotensive and hypertensive rats) or thromboxane $A_2$. A similar conclusion has been reached as regards endothelium-dependent contractions to exogenous arachidonic acid in canine veins.\(^7\)

The other prostaglandins tested evoked contractions in rings without endothelium, but slight differences in responsiveness to the various metabolites of arachidonic acid were noted between aortas of SHR and WKY. At a level of tension comparable to that evoked by acetylcholine in quiescent rings from SHR with endothelium, only prostaglandin $F_{2\alpha}$ caused significantly greater contractions of the aortas from SHR than of those from WKY; however, the rings from the normotensive animals reacted with significant contractions as well. Prostaglandin $F_{2\alpha}$ also was the most potent agonist of the prostaglandins tested. Thus, the present experiments do not allow speculation on the exact nature of the product of cyclooxygenase involved in the endothelium-dependent aortic contractions in the hypertensive animals.

Konishi and Su\(^9\) and Winquist et al.\(^10\) have reported a decreased amplitude of endothelium-dependent relaxations in response to acetylcholine in contracted aortas from SHR. The present study confirms these observations but strongly suggests that this difference is due to the simultaneous occurrence of endothelium-dependent relaxations and contractions in the blood vessel wall of the hypertensive animal. This conclusion is based on the similarity of the response to lower concentrations of acetylcholine in rings with endothelium taken from normotensive and hypertensive rats and on the disappearance of the difference observed between the two strains with higher concentrations after incubation with indomethacin. The present findings do not exclude the possibility that acetylcholine causes the release of endothelium-derived constricting factor(s) in the aorta of normotensive rats as well. Indeed, even if the release were similar in both strains, the smooth muscle of the hypertensive rats could be hypersensitive to the endothelium-derived constricting substance(s) as it is to many other neurohumoral mediators\(^6,11\) and to prostaglandin $F_{2\alpha}$. If a prostaglandin was involved, however, an increase in sensitivity of the smooth muscle to the substance(s) of the same magnitude as that observed for prostaglandin $F_{2\alpha}$ would not fully explain the differences observed between the SHR and the WKY.

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### References

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