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$_2\alpha$ 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 prostacyclin (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
sodium meclofenamate (Parke, Davis, Detroit, MI, USA); imidazole (Aldrich Chemical, Milwaukee, WI, USA); hexamethonium bromide (K + K Laboratories, Plainview, NY, USA); imidazole (Aldrich Chemical, Milwaukee, WI, USA); heparin (Elkin Sinn, Cherry Hill, NJ, USA); hexamethonium bromide (K + K Laboratories, Plainview, NY, USA); imidazole (Aldrich Chemical, Milwaukee, WI, USA); sodium meclofenamate (Parke, Davis, Detroit, MI, USA); and tranylcypromine (Smith, Kline & French Laboratories, Philadelphia, 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 containing 5 × 10⁻³ M NaHCO₃ 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 experiments 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 (10⁻⁶-3 × 10⁻⁷ M) of norepinephrine causing an increase in tension of about 1.4 g; the results are expressed as percent relaxation 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 significant effect on rings from SHR without endothelium (n = 7) or on rings from WKY with or without endothelium (n = 4). In rings from SHR with endothelium, 10⁻⁸ to 3 × 10⁻³ M acetylcholine caused no significant changes in tension, while 3 × 10⁻⁷ to 10⁻⁵ M acetylcholine evoked concentration-dependent contractions accompanied by rhythmic oscillations in tension (Figure 1). The absolute tension developed at 10⁻⁵ M acetylcholine averaged 0.9 ± 0.6 g (n = 28).
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 [ED₁₀] 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 (10⁻⁵ M; n = 4; see Figure 2). Inhibitors of leukotriene synthetase (diethylcarbamazine, 10⁻⁴ M; n = 6), prostacyclin synthetase (tranylcypromine, 10⁻⁴ M; n = 5), 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 D₂, E₁, E₂, F₂α, and I₁. Prostaglandins D₂, E₁, E₂, F₂α caused concentration-dependent tension increases in rings from both SHR and WKY according to the following order of potency: F₂α > E₂ > D₂ ≥ E₁ (Table 3). The only significant difference between the two strains was that rings from SHR were significantly more responsive to low concentrations of prostaglandin F₂α (10⁻⁸-10⁻⁷ 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)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Acetylcholine (log M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-6.5</td>
</tr>
<tr>
<td>Control (n = 17)*</td>
<td>2.6±0.7</td>
</tr>
<tr>
<td>10⁻⁴ M diethylcarbamazine (n = 6)</td>
<td>0.8±0.6</td>
</tr>
<tr>
<td>10⁻⁴ M tranylcypromine (n = 5)</td>
<td>4.6±2.1</td>
</tr>
<tr>
<td>10⁻⁴ M imidazole (n = 6)</td>
<td>4.2±2.1</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><strong>D₂</strong></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><strong>E₁</strong></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><strong>E₂</strong></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><strong>F₂0</strong></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><strong>l₂</strong></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).² 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,⁴⁻¹¹⁻¹³ 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 (tranylcypromine¹⁴) and thromboxane synthetase (imidazole⁵⁻¹⁵) 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

![Figure 4](https://hyper.ahajournals.org/content/25/6/1062/F4.large.jpg)
normotensive and hypertensive rats) or thromboxane A₂. 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 F₂α 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 F₂α 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 Su⁷ and Winquist et al.⁸ 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 mediators⁹, ¹⁰ and to prostaglandin F₂α. 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 F₂α would not fully explain the differences observed between the SHR and the WKY.

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