Endothelium-Dependent Responses in Carotid and Renal Arteries of Normotensive and Hypertensive Rats

THOMAS F. LÜSCHER, DENNIS DIEDERICH, ERIKA WEBER, PAUL M. VANHOUTTE, AND FRITZ R. BÜHLER

SUMMARY Endothelium-dependent relaxations are impaired in the aorta of various models of hypertension, but no data are available regarding the cerebral or renal circulation. Endothelium-dependent relaxations were studied in the carotid and renal artery of Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). Rings with and without endothelium were suspended in organ chambers for isometric tension recording. Acetylcholine and adenosine 5'-diphosphate (ADP) caused endothelium-dependent relaxations in both arteries that were impaired in the carotid, but not in the renal artery, of the SHR, similar to those to the endothelium-independent vasodilator sodium nitroprusside. Indomethacin did not affect relaxations to acetylcholine in the carotid artery, but it significantly augmented them in the renal artery. This finding suggests that an impaired vascular responsiveness to endothelium-derived relaxing factor is responsible for the decreased relaxations in the carotid artery of the SHR. In the renal artery, acetylcholine appears to release both endothelium-derived relaxing factor and a vasoconstrictor prostanoid. Carotid arteries of SHR were more sensitive to the constrictor effects of serotonin than were those of WKY. Endothelium removal caused a twofold to eightfold increase in sensitivity to serotonin in both strains. Thus, endothelium-dependent relaxations to acetylcholine and ADP are reduced and constrictions to serotonin are enhanced in the carotid, but not in the renal, artery of the SHR. (Hypertension 11: 573-578, 1988)

KEY WORDS • acetylcholine • adenosine 5'-diphosphate • indomethacin • prostaglandins • spontaneously hypertensive rats • serotonin • sodium nitroprusside • Wistar-Kyoto rats

THE endothelium releases vasoactive substances that modulate vascular tone.1,2 This regulatory mechanism is impaired in the aorta of hypertensive rats.3-8 In the aorta of the spontaneously hypertensive rat (SHR), acetylcholine may induce endothelium-dependent contractions mediated by a vasoconstrictor prostanoid.9

Altered endothelium-dependent responses in hypertensive blood vessels would be of primary pathophysiological importance if they were present in those vascular beds where hypertensive sequelae occur or where peripheral vascular resistance is regulated (or both). In SHR, stroke is the most common complication of the hypertensive process.10 For long-term blood pressure regulation, vascular tone in the renal circulation is important. Thus, the present study was designed to investigate whether endothelium-dependent vascular responses are altered in the common carotid or the main renal artery (or both) of the adult SHR.

Materials and Methods

Experimental Animals

Commercially available male 24-week-old Wistar-Kyoto rats (WKY) and SHR weighing 220 to 300 g were used (Madoerin, Füllinsdorf, Switzerland). The rats were fed standard rat chow (Nafag, Gossau, Switzerland). Systolic blood pressure was recorded in unanesthetized rats by the tail-cuff method. An average of three readings was analyzed; values above 200 mmHg were considered hypertensive.
Hg were read as 200 mm Hg. Blood pressure was 144 ± 7 mm Hg in WKY and 191 ± 14 mm Hg in SHR (p < 0.05).

Experimental Setup

The common carotid and main renal arteries were removed from rats anesthetized with pentobarbital sodium (50 mg/kg i.p.), cleaned of adherent connective tissue under a dissection microscope (Wild and Leitz AG, Zürich, Switzerland), and cut into rings. Rings were placed into 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). In some rings, the endothelium was removed by gentle rubbing of the intimal surface with a small wire. Removal of the endothelium was confirmed by the absence of a relaxation to acetylcholine.

The rings were suspended in organ chambers filled with 25 ml of control solution (37°C), aerated with 95% O₂, 5% CO₂, and connected to force transducers (Statham Universal UC2, Oxnard, CA, USA); changes in isometric force were recorded. The preparations were progressively stretched and exposed to norepinephrine (3 x 10⁻⁷ 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. Some rings were incubated with indomethacin (10⁻⁵ M; 30 minutes) to block the production of prostaglandins.

Drugs

Acetylcholine hydrochloride, adenosine 5'-diphosphate (ADP), indomethacin, l-norepinephrine bitartrate, serotonin (5-hydroxytryptamine creatinine sulfate), and sodium nitroprusside were obtained from Sigma Chemical (St. Louis, MO, USA). Concentrations (M) in the bath solution are given. Drugs were dissolved in distilled water, except for indomethacin, which was dissolved in distilled water containing 5 x 10⁻⁴ M NaHCO₃ and sonicated before use.

Protocols, Calculations, and Statistics

In most experiments, rings of SHR and WKY with and without endothelium or rings treated with or without indomethacin were studied in parallel. Contractions are expressed in absolute tension (grams) or as a percentage of the maximal response. The concentration exhibiting 30 or 50% of the maximal response to the agonist (EC₃₀ and EC₅₀, respectively) or that required to evoke an increase in absolute tension of 0.25 or 0.4 g (ED₁₀ or ED₅₀, respectively) was calculated for each ring and expressed as negative log M. To study relaxations, rings were contracted with norepinephrine (3 x 10⁻⁷ M), and relaxations are expressed as a percentage of that contraction. The level of contraction (grams) did not differ statistically in WKY and SHR. In rings treated with indomethacin (10⁻⁵ M), contractions evoked by norepinephrine were less than those elicited under control conditions. Thus, rings treated with indomethacin were contracted with 3 x 10⁻⁷ to 3 x 10⁻⁶ M norepinephrine to match the level of tension of control rings. Sodium nitroprusside was added in rings contracted with 3 x 10⁻⁷ or 10⁻⁶ M norepinephrine. The concentration exhibiting 30 or 50% relaxation (IC₃₀ and IC₅₀) is expressed as negative log M. With ADP in both arteries and acetylcholine in the carotid artery, the area under the concentration-response curve was used for statistical comparison, since IC₃₀ and IC₅₀ values could not be obtained in all rings.

The data are given as means ± SEM. In all experiments, n equals the number of rats used. Student's t test for paired or unpaired observations was used for statistical analysis. A p value smaller than 0.05 was considered to indicate a statistical difference.

Results

Common Carotid Artery

Endothelium-Dependent Relaxations

In rings contracted with norepinephrine, acetylcholine (10⁻⁹ to 10⁻⁴ M) and ADP (10⁻⁹ to 10⁻⁴ M) caused relaxations in rings with, but not without, endothelium (Figure 1). The relaxations were reduced in SHR as
compared with WKY (p<0.05; Figures 2 and 3). In both strains, indomethacin (10^{-5} M) did not enhance the relaxations induced by acetylcholine (n = 8).

**Endothelium-Independent Relaxations**

In SHR, the concentration-relaxation curve to sodium nitroprusside was shifted to the right as compared with WKY both in rings maximally contracted with norepinephrine (see Figure 2) or with 3 \times 10^{-7} M of the catecholamine (n = 4; data not shown). The IC_{50} value was 7.3 ± 0.1 in WKY and 6.5 ± 0.2 in SHR (p<0.05). Indomethacin (10^{-5} M) did not affect the relaxations in either strain (n = 4 and 5, respectively).

**Contractions to Serotonin and Norepinephrine**

The maximal contraction to serotonin was similar in WKY and SHR and was not affected by removal of the endothelium (Table 1). Removal of the endothelium caused a shift to the left of the concentration-response curve to the monoamine in both strains (2.5-fold to 3.2-fold log shift at ED_{50} and ED_{90}, p<0.05; Figure 4; see Table 1). Rings with and without endothelium of SHR were more sensitive to serotonin than were those of WKY (twofold to 7.9-fold log shift at ED_{50} and ED_{90}, p<0.05; see Figure 4). The concentration required to induce an increase in tension of 0.25 and 0.4 g was lower in SHR than in WKY (ED_{0.25} g: 6.3 ± 0.1 and 5.8 ± 0.1, respectively; ED_{0.4} g: 6.0 ± 0.1 and 5.4 ± 0.2, respectively; p<0.05).

The maximal contraction induced by norepinephrine did not differ in WKY and SHR, and the ED_{50} and ED_{90}

**Table 1. Contractile Responses to Serotonin and Norepinephrine in Common Carotid Artery Rings With and Without Endothelium Taken from WKY and SHR**

<table>
<thead>
<tr>
<th>Variable</th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serotonin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ED_{50} with endothelium</td>
<td>5.6 ± 0.1</td>
<td>5.9 ± 0.1*</td>
</tr>
<tr>
<td>ED_{50} without endothelium</td>
<td>6.0 ± 0.1†</td>
<td>6.8 ± 0.2†</td>
</tr>
<tr>
<td>ED_{90} with endothelium</td>
<td>6.0 ± 0.1</td>
<td>6.3 ± 0.2*</td>
</tr>
<tr>
<td>ED_{90} without endothelium</td>
<td>6.4 ± 0.1†</td>
<td>7.3 ± 0.1†</td>
</tr>
<tr>
<td>Maximal response</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With endothelium (g)</td>
<td>0.8 ± 0.1</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Without endothelium (g)</td>
<td>0.9 ± 0.2</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td><strong>Norepinephrine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ED_{50} with endothelium</td>
<td>7.4 ± 0.1</td>
<td>7.2 ± 0.2</td>
</tr>
<tr>
<td>ED_{50} without endothelium</td>
<td>7.6 ± 0.1†</td>
<td>7.9 ± 0.2†</td>
</tr>
<tr>
<td>ED_{90} with endothelium</td>
<td>7.7 ± 0.1</td>
<td>7.6 ± 0.1</td>
</tr>
<tr>
<td>ED_{90} without endothelium</td>
<td>7.8 ± 0.1</td>
<td>8.1 ± 0.2</td>
</tr>
<tr>
<td>Maximal response</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With endothelium (g)</td>
<td>0.5 ± 0.04</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Without endothelium (g)</td>
<td>0.5 ± 0.1</td>
<td>0.7 ± 0.1</td>
</tr>
</tbody>
</table>

Data (-log M) are means ± SEM of 4 to 24 experiments.

* p<0.05, compared with WKY values.
† p<0.05, compared with rings with endothelium.
values of norepinephrine in rings with endothelium were similar (see Table 1).

Main Renal Artery

Endothelium-Dependent Relaxations

In rings contracted with norepinephrine, acetylcholine (10^-9 - 10^-4 M) induced relaxations in rings with, but not without, endothelium. Renal arteries were more sensitive to acetylcholine, and the maximal relaxation was larger than that seen in carotid arteries. The IC50 and IC10 values of acetylcholine did not differ in WKY (7.5 ± 0.2 and 7.0 ± 0.2) and SHR (7.6 ± 0.1 and 7.1 ± 0.1). At higher concentrations (10^-6 - 10^-4 M), acetylcholine induced endothelium-dependent contractions that tended to be more pronounced and associated with rhythmic oscillations in tension in SHR (p > 0.05; Figures 5 and 6). In both strains, indomethacin (10^-5 M) enhanced the relaxations at higher concentrations of acetylcholine (10^-6 - 10^-4 M; p < 0.05) without affecting the IC50 and IC10 values of the muscarinic agonist.

In rings contracted with norepinephrine, ADP (10^-6 - 10^-4 M) evoked endothelium-dependent relaxations. The relaxations were less pronounced than those seen in the carotid artery, but they did not differ in WKY and SHR (n = 11 and 16, respectively).

Endothelium-Independent Relaxations

In rings contracted with norepinephrine (3 × 10^-7 M), the IC50 value of sodium nitroprusside did not differ in WKY (7.2 ± 0.2) and SHR (7.1 ± 0.3) both under control conditions (n = 7 and 8, respectively) and with indomethacin (10^-5 M; n = 5 and 6, respectively).

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**Figure 4.** Contractile responses to serotonin (5-hydroxytryptamine) in the common carotid artery of WKY and SHR. The sensitivity in rings with endothelium was enhanced in SHR as compared with WKY (twofold log shift at ED50; p < 0.05). Removal of the endothelium caused a leftward shift of the concentration-response curve in both strains (2.5-fold to 10-fold log shift; p < 0.05).

**Figure 5.** Endothelium-dependent contractions to acetylcholine in the renal artery of one SHR. Note the marked contractions with superimposed oscillatory activity in the ring with, but not in that without, endothelium.

**Figure 6.** Endothelium-dependent responses to acetylcholine in the renal artery of WKY and SHR. Note the endothelium-dependent relaxations at lower concentrations and the increase in tension at higher concentrations of acetylcholine. Indomethacin inhibited the increase in tension occurring at higher concentrations of acetylcholine (10^-6 - 10^-4 M; p < 0.05).
Contractions to Serotonin and Norepinephrine

Contractions to serotonin did not differ in WKY and SHR (n = 11 and n = 13, respectively; Table 2). Removal of the endothelium caused a slight, but not significant shift to the left of the concentration-response curve in both strains (twofold log shift at \( EC_{50} \); \( p > 0.05 \)) in WKY and SHR, the maximal response to serotonin was greater and the \( EC_{50} \) value of the monoamine was lower in the renal as compared with the carotid artery (\( p < 0.05 \)). The \( EC_{50} \) value was lower in the renal as compared with the carotid artery both in WKY ( \( 7.0 \pm 0.1 \) vs \( 5.4 \pm 0.2 \); \( p < 0.05 \)) and in SHR ( \( 6.9 \pm 0.1 \) vs \( 6.0 \pm 0.1 \); \( p < 0.05 \)).

Contractions to norepinephrine did not differ in WKY and in SHR (n = 20 and 22, respectively; see Table 2). Removal of the endothelium did not alter the sensitivity to the catecholamine in either strain ( \( n = 7 \) and 8, respectively). In both groups, the maximal response to and the \( EC_{50} \) value of norepinephrine were greater in the renal than in the carotid artery (\( p < 0.05 \)).

Discussion

The present study demonstrates that endothelium-dependent relaxations to acetylcholine and ADP are decreased in the common carotid, but not in the renal, artery of SHR. In contrast to its effect in the aorta,6 indomethacin did not affect endothelium-dependent relaxations to acetylcholine in the carotid artery of SHR. This finding suggests that the relaxations to acetylcholine are mediated by endothelium-derived relaxing factor (EDRF) rather than by prostacyclin and that the muscarinic agonist does not release major amounts of prostanoidlike endothelium-derived constrictor factor in this blood vessel.2,6,9 The relaxations to the endothelium-independent vasodilator sodium nitroprusside and to acetylcholine were similarly impaired. Sodium nitroprusside and EDRF cause relaxation by increasing cyclic guanosine 3',5'-monophosphate in vascular smooth muscle cells.11 The diminished relaxations to both sodium nitroprusside and acetylcholine in the carotid artery of SHR, therefore, would be consistent with an impaired vascular responsiveness rather than with a reduced release of EDRF. Indeed, a normal release of EDF has been demonstrated in the aorta of hypertensive rats.9,12 A decreased vascular responsiveness to EDRF would also be consistent with the finding that endothelium-dependent relaxations to ADP and to acetylcholine were similarly impaired. Like the aorta of the hypertensive rats,7,6,13 the carotid artery was more sensitive and exhibited more tension in response to serotonin as compared with WKY. Removal of the endothelium caused a twofold to eightfold increase in sensitivity to the monoamine in both groups of rats, demonstrating the protective role of the endothelium against vasoconstrictor stimuli. Similar effects were found in the canine coronary circulation after mechanical removal of the endothelium and after endothelial damage caused by brief hypertension.14,15 This inhibition of the contractile response to serotonin in rings with endothelium would best be explained by basally released EDRF.9,16,17

Decreased endothelium-dependent relaxations to ADP and enhanced contractions to serotonin (both substances released from aggregating platelets5,18) in SHR would—at sites where platelets are activated—shift the balance between vasodilator and vasoconstrictor responses toward vasoconstriction. The increased serotonin release and serotonin-induced aggregation of hypertensive platelets would further contribute to this phenomenon.19

Endothelium-dependent relaxations to acetylcholine (but not those to ADP) were stronger and occurred at lower concentrations in the renal than in the carotid artery. The potency of endothelium-dependent relaxations to these agonists in the rat renal artery is comparable to that observed in human renal arteries.20 As in the aorta of SHR, acetylcholine evoked endothelium-dependent contractions at higher concentrations. In contrast to the aorta,6 however, only some SHR exhibited marked endothelium-dependent contractions. This response could, at least in part, be related to the younger age of the rats in the present study. Indomethacin, to block the production of prostaglandins,4 inhibited endothelium-dependent contractions and augmented the relaxations to acetylcholine in both strains. Thus, as in the aorta of SHR, acetylcholine evokes the release of both EDRF and a vasoconstrictor prostanoid in the renal artery.5 In the presence of indomethacin, the relaxations induced by acetylcholine were identical in both strains, indicating a normal release of and vascular responsiveness to EDRF in the renal artery of SHR. Accordingly, the response to another agonist of EDRF like ADP did not differ in normotensive and hypertensive rats.

Contractile responses to serotonin and norepinephr-
rine were much greater in the renal as compared with the carotid artery in both strains, which is remarkable considering the small size of the renal as compared with the carotid artery. The inhibitory effect of the endothelium on contractile responses, on the other hand, was almost absent in the renal artery. Possibly, the inhibition of basally released EDRF is less effective in arteries with marked contractile responses such as the rat renal artery.

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References
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