The effects of intraluminal and extraluminal endothelin-1 and its interactions with endothelium-derived relaxing factor were studied in perfused mesenteric resistance arteries of Wistar-Kyoto rats and spontaneously hypertensive rats. Changes in intraluminal diameter were recorded. In adult Wistar-Kyoto rats, but not spontaneously hypertensive rats, low concentrations of intraluminal endothelin-1 (10^{-10} to 10^{-9} M) caused relaxations of quiescent arteries blocked by indomethacin. After endothelial removal, intraluminal endothelin-1 evoked concentration-dependent contractions in both strains. Extraluminal endothelin-1 caused greater contractions of arteries with endothelium than intraluminal endothelin-1, and the sensitivity was lower in adult hypertensive rats; endothelial removal enhanced the contractions to extraluminal endothelin-1 to a greater extent in hypertensive than in normotensive rats. In arteries without endothelium, intraluminal and extraluminal endothelin-1 caused comparable contractions, but the sensitivity was reduced in adult but not young hypertensive as compared with normotensive rats. Both young spontaneously hypertensive and normotensive rats exhibited a high sensitivity to the peptide. In arteries precontracted with endothelin-1, endothelium-dependent relaxation to intraluminal acetylcholine was reduced in hypertensive as compared with normotensive rats, whereas relaxations to extraluminal acetylcholine were increased in hypertensive rats. Thus, endothelin-1 interacts with both vascular smooth muscle and the endothelium. The sensitivity of vascular smooth muscle to endothelin-1 is reduced in adult hypertensive rats. Intraluminal activation of the endothelium by endothelin-1 or acetylcholine is reduced in spontaneously hypertensive rats, whereas extraluminal activation causes more pronounced responses in hypertensive than in normotensive rats, suggesting a prominent dysfunction of the intraluminal surface of the endothelium in hypertension. (Hypertension 1991;18:543-549)
the anatomic origin of the blood vessels, the experimental conditions most likely are a major factor. Indeed, although endothelin infused in vivo first reaches the endothelium of the blood vessel wall, vascular smooth muscle and the endothelium are simultaneously activated in organ chamber systems using ring preparations. The arteriograph system where arteries are perfused allows application of substances intraluminally and extraluminally and closely mimics physiological conditions.

Thus, we studied 1) the inhibitory role of the endothelium against the effects of endothelin-1 and 2) the interaction of the peptide with EDRF in perfused mesenteric resistance arteries of normotensive and hypertensive rats.

**Methods**

**Experimental Animals**

Male Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR), 8 (young) and 16–20 weeks of age (adult), were obtained from Charles River Wiga GmbH, Sulzfeld, FRG. The mesentery was removed from rats anesthetized with pentobarbital (50 mg/kg i.p.) and was placed into cold buffer solution (mM): NaCl 118.6, KCl 4.8, CaCl2 2.5, MgSO4 1.2, KH2PO4 1.2, NaHCO3 25.1, edetate calcium disodium 0.026, glucose 10.1.

**Experimental Set-up**

A 3-mm-long segment of the third branch of the mesenteric artery was isolated under a dissection microscope. The artery was transferred to an arteriograph chamber filled with buffer solution (95% O2–5% CO2, 37°C). The proximal end of the artery was cannulated with the afferent cannula and secured with a surgical nylon suture. The distal end was attached to the inside of the efferent cannula. The artery was perfused with buffer solution containing 1% bovine serum albumin (supplied from a 500 ml reservoir) and equilibrated under an optimal transmural pressure of 30 mm Hg for 1 hour before the experiment. A polyethylene catheter (i.d. 50 μm) was placed in the afferent cannula and connected to a pressure transducer for measurement of transmural pressure or used for intraluminal application of drugs. Transmural pressure was adjusted by changing the height of the reservoir supplying the perfusate. Flow through the vascular segment was repeatedly determined by counting the drops at the efferent cannula. In this experimental set-up, 20 drops corresponded to 1 ml; accordingly the volume of 1 drop equaled 0.05 ml. Depending on the contractile state of the blood vessel, the intraluminal flow rates ranged from 0.03–0.6 ml/min. The concentrations of intraluminally applied drugs required to construct a concentration–response curve were calculated for the actual level of flow under each experimental condition, and the infusion rate (through the polyethylene catheter) of the solution containing the drugs was adjusted accordingly (syringe infusion pump 22, Harvard Apparatus, Boston, Mass.; infusion rates 1–50 μl/min).

The arteriograph was placed on a stage of a microscope that had a TV camera attached to the viewing tube. The signal derived from the video image of the vessel was processed by an electronic system (Living Systems Instrumentation, Burlington, Vt.) for continuous measurement and recording of intraluminal diameter.

**Endothelium Removal**

To remove the endothelium, 0.5% 3-[3-cholamidopropyldimethylammonio]-1-propanesulfonate (CHAPS) was infused for 30 seconds. The resting intraluminal diameter and the contraction to KCl (100 mM) was not affected by endothelial removal. The presence or absence of the endothelium was confirmed by acetylcholine (10–6 M).

**Protocols**

Concentration–response curves to intraluminal or extraluminal endothelin-1 were obtained in arteries with and without endothelium by cumulative application of the drug. In some experiments, 30 minutes before the experiment indomethacin (10–5 M) or Nω-monomethyl-L-arginine (LNMMA) (10–4 M) was infused intraluminally to inhibit the production of prostaglandins or nitric oxide, respectively. To study endothelium-dependent relaxations, arteries were precontracted with a concentration of extraluminal endothelin-1 corresponding to the mean pD2 (i.e., the negative log molar concentration causing half-maximal contraction) value of the peptide as determined in parallel experiments. As repetitive exposures to endothelin-1 were not possible under these experimental conditions, the pD2 value could not be determined in the same blood vessel before the actual experiment. Contractions or relaxations were expressed as percent decrease or increase in intraluminal vascular diameter. The diameter at the resting level was taken as 100%.

**Drugs**

The following drugs were used (obtained from Sigma Chemical Co., St. Louis, Mo., unless otherwise stated): acetylcholine hydrochloride, 3-[3-cholamidopropyldimethylammonio]-1-propanesulfonate (CHAPS), endothelin-1 (Peptide Institute, Osaka, Japan), indomethacin, Nω-monomethyl L-arginine (LNMMA) (Calbiochem, Lucerne, Switzerland), L-norepinephrine, papaverine, 3-morpholino-sydnonimine (SIN-1, Hoechst Pharmaceuticals, Paris). Endothelin-1 was dissolved in distilled water containing 0.05% albumin, all other drugs in distilled water. The concentrations are expressed as final molar concentration.

**Calculations and Statistics**

Data are given as mean±SEM. The concentration of an agonist causing half-maximal contraction (ED50 value) or half-maximal inhibition of a contraction of the solution containing the drugs was adapted accordingly (syringe infusion pump 22, Harvard Apparatus, Boston, Mass.; infusion rates 1–50 μl/min).

The arteriograph was placed on a stage of a microscope that had a TV camera attached to the viewing tube. The signal derived from the video image of the vessel was processed by an electronic system (Living Systems Instrumentation, Burlington, Vt.) for continuous measurement and recording of intraluminal diameter.
(IC_{50} value) was calculated for each experiment and was expressed as negative log molar (pD_{2} value). The shift of concentration–response curves was expressed as concentration shift at pD_{2} values. In each set of experiments, n equals the number of animals studied. Statistical evaluation was done by paired and unpaired Student's t test. Means were considered significantly different with a value of p<0.05.

Results

Extraluminal Endothelin-1

In adult rats, extraluminal endothelin-1 (10^{-11} to 3\times 10^{-8} M) evoked concentration-dependent contractions of arteries with endothelium (Figure 1, left panel). The maximal contraction did not differ in WKY rats and SHR, but the sensitivity to endothelin-1 was lower in SHR than in WKY rats (Table 1; concentration shift, 32-fold; p<0.005). Removal of the endothelium augmented the contraction to endothelin-1 (Figure 1, right panel). In WKY rats, the sensitivity was enhanced sixfold (p<0.05) and the maximal response was greater in vessels without (p<0.005) as compared with those with endothelium (Table 1). In SHR, the sensitivity was increased 25-fold after removal of the endothelium (p<0.005), and the maximal response was similarly augmented (Table 1; p<0.05). In arteries without endothelium, the sensitivity to endothelin-1 remained lower in SHR than in WKY rats (10-fold; p<0.05), while the maximal response was not different.

In young rats, the sensitivity (pD_{2}, 9.4±0.1 in WKY rats and 9.3±0.2 in SHR) and maximal response to endothelin-1 (WKY, 76±2% and SHR, 80±1%) did not differ in arteries with endothelium (Figure 2). Endothelial removal similarly enhanced the sensitivity and maximal response in WKY rats and SHR. After endothelial removal, the pD_{2} value was 14.0±1.3 ( TABLE 1. Sensitivity and Maximal Response to Extraluminal and Intraluminal Endothelin-1 and Acetylcholine in Perfused Mesenteric Resistance Arteries of 16–20-Week-Old Wistar-Kyoto Rats and Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Application and type of drugs</th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraluminal endothelin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With endothelium</td>
<td>10.1±0.2</td>
<td>8.6±0.1*</td>
</tr>
<tr>
<td>Without endothelium</td>
<td>11.0±0.3†</td>
<td>10.0±0.1‡</td>
</tr>
<tr>
<td>Intraluminal endothelin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With endothelium</td>
<td>8.4±0.01</td>
<td>8.5±0.03*</td>
</tr>
<tr>
<td>Without endothelium</td>
<td>ND</td>
<td>7.8±0.1§</td>
</tr>
<tr>
<td>Extraluminal acetylcholine</td>
<td>7.0±0.1</td>
<td>7.8±0.1§</td>
</tr>
<tr>
<td>Intraluminal acetylcholine</td>
<td>8.2±0.1</td>
<td>7.8±0.1§</td>
</tr>
</tbody>
</table>

pD_{2} value was calculated as the negative log Molar concentration of the agonist evoking a half-maximal contraction (endothelin-1) or inhibition of a previous contraction (acetylcholine). Maximal response (Max) is expressed as percent of baseline internal diameter (endothelin-1) or percent of inhibition of a previous contraction (acetylcholine). WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats.

tp<0.05, *p<0.005 as compared with preparation with endothelium.

§p<0.05, ¶p<0.005 as compared with Wistar-Kyoto rats.

The pD_{2} value was not determined (ND) because in some experiments the contractions induced by 10^{-10} M of endothelin-1 exceeded 50% of maximal contraction.
the maximal response of arteries with \((p<0.005)\) and without \((pD_2, p<0.005; \text{max, } p<0.05)\) endothelium were smaller in adult than young rats.

**Intraluminal Endothelin-1**

In adult rats, low concentrations of intraluminal endothelin-1 caused a relaxation in arteries with endothelium of WKY rats but not SHR (Figure 3). Papaverine \((10^{-4} \text{ M})\) caused a comparable relaxation of quiescent arteries of WKY rats \((14\pm 4\%); n=4; \text{NS})\. In WKY rats, endothelium-dependent relaxation to intraluminal endothelin-1 \((10^{-8} \text{ M})\) was not affected by LNMMA \((10^{-4} \text{ M})\) but was prevented by indomethacin \((10^{-5} \text{ M})\) (Figure 4). Higher concentrations of intraluminal endothelin-1 \((10^{-8} \text{ M})\) caused similar contractions in WKY rats and SHR, but the contractions were smaller than those evoked by extraluminal endothelin-1 \((p<0.05)\). In SHR, the response to intraluminal endothelin-1 was not altered in the presence of LNMMA or indomethacin. After endothelial removal, the contractions to intraluminal and extraluminal endothelin-1 were comparable both in WKY rats and SHR (Figure 3). The inhibitory effect of the endothelium against contractions to intraluminal endothelin-1 was greater in WKY rats than in SHR (Figure 3).

**Figure 2.** Line graphs show concentration-response curves to extraluminal endothelin-1 in mesenteric resistance arteries with (left panel) and without (right panel) endothelium of 8-week-old Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR). Sensitivity and maximal response to endothelin-1 were not different in WKY rats and SHR.

**Figure 3.** Bar graphs show effects of intraluminal and extraluminal endothelin-1 in mesenteric resistance arteries with and without endothelium obtained from 16–20-week-old Wistar-Kyoto (WKY) rats (left panel) and spontaneously hypertensive rats (SHR) (right panel). Intraluminal application of peptide in arteries with endothelium caused a small relaxation in WKY rats but not SHR. Removal of endothelium augmented contractions to intraluminal endothelin-1 to a greater extent in WKY rats than in SHR. With extraluminal endothelin-1, removal of endothelium caused a more pronounced increase in contractility in SHR than in WKY rats. In preparations without endothelium obtained from both animals, intraluminal and extraluminal endothelin-1 caused comparable contractions. \(*p<0.05, \quad \cdot p<0.005 \quad \text{intraluminal vs. extraluminal}; \quad +p<0.05, \quad ++p<0.01, \quad +++p<0.005 \quad \text{preparations with vs. without endothelium.} \)

**Figure 4.** Bar graphs show effects of inhibitor of cyclooxygenase indomethacin \((10^{-5} \text{ M})\) and that of endothelial nitric oxide formation \(N^\text{G}-\text{monomethyl L-arginine (LNMMA; 10}^{-4} \text{ M})\) on response to intraluminal endothelin-1 in mesenteric resistance arteries with endothelium obtained from 16–20-week-old Wistar-Kyoto (WKY) rats (left panel) and spontaneously hypertensive rats (SHR) (right panel). Relaxation to endothelin-1 \((10^{-8} \text{ M})\) was prevented by indomethacin but not LNMMA in Wistar-Kyoto (WKY) rats. \(*p<0.01 \text{ vs. control.} \)
In young rats, low concentrations of endothelin-1 (10^{-11} to 10^{-10} M) did not change vascular diameter in both strains (Figure 5). Contractions induced by 10^{-9} M intraluminal endothelin-1 (WKY, 29±5%; SHR, 24±7%) were smaller than in those evoked by extraluminal endothelin-1 (WKY, 58±3%; SHR, 53±4%; p<0.005) but did not differ in WKY rats and SHR.

**Contraction to Norepinephrine**

Extraluminal norepinephrine evoked contraction in arteries without endothelium with a similar pD_2 value and maximal response in WKY rats (6.1±0.1% and 86±1%) and SHR (5.8±0.1% and 83±2%; NS).

**Acetylcholine and Endothelin-1**

Intraluminal acetylcholine (10^{-9} to 10^{-6} M) evoked endothelium-dependent relaxation of arteries contracted with extraluminal endothelin-1 at its mean pD_2 concentration (Figure 6, left panel). The sensitivity and maximal response to acetylcholine were smaller in SHR than WKY rats (p<0.01; Figure 6, left panel and Table 1). In contrast, with extraluminal acetylcholine the relaxation was greater in SHR than WKY rats (Figure 6, right panel and Table 1).

**3-Morpholino-sydnonimine and Endothelin-1**

Arteries without endothelium precontracted with extraluminal endothelin-1 (10^{-8} M) relaxed to SIN-1 (10^{-8} to 10^{-4} M). The pD_2 value and maximal response did not differ in WKY rats (5.9±0.2% and 87±5%) and SHR (5.7±0.1% and 98±5%; NS).

**Discussion**

This study demonstrates asymmetrical responses of the blood vessel wall of perfused rat mesenteric resistance arteries to endothelin-1 that are related to differential effects on the endothelium and vascular smooth muscle. In established hypertension, endothelium-dependent inhibition of contractions to endothelin-1 is reduced with intraluminal but augmented with extraluminal activation, whereas the contractile effects at the level of smooth muscle are reduced under both conditions.

As with norepinephrine, removal of the endothelium enhanced the contraction to extraluminal endothelin-1 (Figure 1). This suggests that formation of EDRFs inhibits the effects of the peptide at the level of vascular smooth muscle. The inhibitory role of the endothelium against intraluminal endothelin-1 was much greater than that against extraluminal endothelin-1. Thus, this difference cannot be explained only by basal formation of EDRFs. Intraluminal application of low concentrations of the peptide caused endothelium-dependent relaxations of quiescent arteries indicating that endothelin-1 can release EDRFs. Since this response was blocked by the cyclooxygenase inhibitor indomethacin (Figure 4) but not that of nitric oxide formation LNMMA, endothelin-1 must activate endothelial receptors linked to the production of prostacyclin or prostaglandin E_2. Indeed, endothelin-1 activates phospholipase A_2 in cultured cells and releases prostacyclin in intact organs. Similarly, indomethacin augments the pressor effects of the peptide in the rat. The endothelium-dependent relaxation to intraluminal endothelin-1 was not observed in young rats possibly because of the high sensitivity of vascular smooth muscle to the peptide. In mesenteric resistance arteries without endothelium, the contractile responses to extraluminal and intraluminal endothelin-1 were similar, confirming a previous report in large arteries.
and veins. Thus, the asymmetrical responses of mesenteric resistance arteries to endothelin-1 are related to endothelial effects of the peptide. The pronounced activation of the endothelium with intraluminal rather than with extraluminal application of the peptide may be due to a different expression of endothelin receptors on the intraluminal and extraluminal surface of the cells or to a different accessibility of the two surfaces under both conditions.

Both young WKY rats and SHR showed much higher sensitivity to endothelin-1 than adult rats of both strains. Indeed, particularly after removal of the endothelium, the threshold concentration of the peptide was markedly lower than in adult rats (Figure 2). In adult SHR, the sensitivity but not maximal response of arteries without endothelium to extraluminal endothelin-1 was paradoxically reduced as compared with WKY rats (Figure 1). Since the response to norepinephrine did not differ, the reduced sensitivity, as in old rats, is specific for the peptide. Since the response to endothelin-1 was unaltered in young SHR, this may represent premature aging of hypertensive arteries and may be related to a downregulation of endothelin receptors on vascular smooth muscle. In line with that interpretation, plasma concentrations of endothelin increase with age and in certain hypertensive patients, and the density of endothelin binding sites is decreased in aortic smooth muscle cells in culture and ventricular membranes of SHR. Downregulation of endothelin receptors occurs in cultured smooth muscle cells after exposure to the peptide. However, other investigators reported normal endothelin levels in hypertensive patients. Alternatively, different subpopulations of endothelin receptors may be expressed in SHR and WKY rats.

In large arteries of SHR, a decreased, normal, and increased sensitivity to endothelin-1 has been reported. This may be related to different anatomic origins of the arteries or different experimental conditions. In ring preparations, endothelin-1 acts simultaneously from the adventitial and luminal side. Thus, the responses must be different from those obtained in perfused blood vessels. Indeed, the sensitivity of arteries with endothelium to intraluminal endothelin-1 was increased in adult SHR as compared with WKY rats while that to extraluminal endothelin-1 was reduced.

In adult SHR, the enhancement of the sensitivity to extraluminal endothelin-1 by endothelial removal was much greater than in WKY rats (Figure 1). This may be related to a different basal or stimulated (by extraluminal endothelin-1) release of EDRFs in SHR and WKY rats. Since the basal release of EDRFs during contractions to extraluminal norepinephrine is smaller in SHR, extraluminal endothelin-1 most likely stimulates the release of EDRFs to a greater extent in SHR than in WKY rats. Indeed, extraluminal acetylcholine caused a more pronounced relaxation in SHR than in WKY rats (Figure 6). In contrast, with intraluminal application of endothelin-1, the relaxations induced by low concentrations of the peptide were absent in the SHR (indicating a defective receptor-coupled stimulation of the arachidonic acid pathway), and the relaxations evoked by intraluminal acetylcholine were reduced as compared with WKY rats. Thus, the normotensive endothelium exhibits a greater sensitivity to acetylcholine when activated from the intraluminal as compared with the extraluminal side, whereas in SHR acetylcholine was similarly effective whether applied intraluminally or extraluminally. In spite of a greater sensitivity to extraluminal acetylcholine in SHR, the intraluminal activation of the endothelium with acetylcholine was blunted in SHR; this indicates that the intraluminal surface of the hypertensive endothelium does exhibit an abnormal formation of EDRF and vasodilator prostanoids in response to receptor-operated agonists. As the response to the nitric oxide donor SIN-1 was identical in SHR and WKY rat arteries contracted with endothelin-1, these differences cannot be attributed to effects of the peptide on vascular smooth muscle. Furthermore, in perfused mesenteric resistance arteries of the SHR, cyclooxygenase inhibitors, in contrast to other vascular beds or experimental conditions, do not affect endothelium-dependent relaxations to acetylcholine; thus, a contribution of endothelium-derived contractile prostanoids can also be excluded. Thus, in line with previous observations the intraluminal surface, which is most exposed to high blood pressure, but not the extraluminal side of the endothelium exhibits functional changes in adult SHR; a contribution of differences in the activity of acetylcholinesterase in the blood vessel wall of both strains cannot be excluded, however. The fact that even in the presence of LNMMA and indomethacin (to inhibit EDRF and prostacyclin released from the endothelium), the responses to intraluminal endothelin are much more pronounced in the absence than in the presence of endothelial cells, indicates that this structure of the blood vessel wall may also act as a physical barrier reducing the capability of intraluminal endothelin to reach vascular smooth muscle cells. The increased depressor response to intravenously injected endothelin in SHR must be related to other factors such as an increased release of atrial natriuretic peptide.

In conclusion, endothelin-1 can interact with vascular smooth muscle (where it causes contraction) and the endothelium (where it stimulates the release of EDRFs) of rat mesenteric resistance arteries. This explains the marked differences of intraluminal and extraluminal endothelin-1. In adult SHR, the endothelium-dependent relaxation occurring with intraluminal endothelin-1 and acetylcholine are blunted, and the sensitivity of vascular smooth muscle to endothelin-1 is reduced. In contrast, extraluminal endothelin-1 and acetylcholine cause a more pronounced activation of the endothelium in adult SHR.
than in WKY rats. Thus, hypertension is associated with a prominent dysfunction of the intraluminal surface of the endothelium.

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Key Words: • acetylcholine • endothelin • prostaglandins • age • spontaneously hypertensive rats • Wistar-Kyoto rats
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