Role of Endothelium in the Response to Endothelin in Hypertension

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The relation between endothelin and acetylcholine (ACh) was examined and compared in aortas from Wistar-Kyoto (WKY) rats and from stroke-prone spontaneously hypertensive rats (SHRSP). The relaxation produced by ACh in an endothelin-induced contraction was less in aortas from WKY rats than in those from SHRSP. In aortas from WKY rats but not in those from SHRSP, the contraction produced by endothelin was augmented when the intact aortic rings were treated with methylene blue (10^{-5} M). This augmentation was also found in preparations of the WKY rat aortic rings in which the endothelium had been removed. The augmentation was not present in SHRSP aortic rings that had been similarly denuded. Treatment with indomethacin (5 \times 10^{-6} M) had no effect on endothelin-induced contraction in either WKY rat or SHRSP aortic rings. Our findings indicate that endothelin and ACh have in common the ability to release endothelium-derived relaxing factor (EDRF) in WKY rat aortic rings. The reduced endothelium-dependent relaxation in response to ACh in the WKY rat probably reflects the fact that endothelin had already released the EDRF in rings from this strain of rats. The release of EDRF by endothelin is less in SHRSP than it is in WKY rats. Because of this failure of endothelin to release EDRF in SHRSP, endothelin may contribute to the increase in total peripheral resistance in this form of hypertension. (Hypertension 1990;16:677–681)

Endothelial cells in situ or in vitro release vasoactive substances that mediate either vasoconstriction or vasodilation. The relaxing effect of acetylcholine (ACh) on rabbit aorta is mediated by the endothelium-derived relaxing factor (EDRF). Endothelin, a peptide first obtained from the culture medium of vascular endothelial cells, induces a potent vasoconstriction of a variety of blood vessels from numerous species. This peptide-evoked vasoconstriction is not mediated either by \alpha\-adrenergic, histaminergic, or serotoninergic receptors or by cyclooxygenase or lipoxygenase metabolites, suggesting that it acts directly on vascular smooth muscle cells. Vasoconstriction induced by endothelin is dependent on extracellular Ca^{2+}, suggesting that influx of extracellular Ca^{2+} is essential for its vascular action. It is possible that this peptide may be associated with the pathogenesis of hypertension and pathological vascular spasm.

Recently, it has been reported that in addition to this direct vasoconstrictor action, endothelin can also release potent vasodilator substances such as prostacyclin (PGI_{2}) in isolated lungs of rats and EDRF from the rat isolated perfused mesentery. The present study examined the ability of ACh to cause a further release of EDRF in vascular smooth muscle that has already been stimulated with endothelin. It also compared this ACh-induced relaxation (EDRF release) of vascular smooth muscle from Wistar-Kyoto (WKY) rats and with that from stroke-prone spontaneously hypertensive rats (SHRSP).

Rings from SHRSP and WKY rats were studied in the same muscle bath. Results of the concentration–response curve to endothelin (10^{-11} to 3 \times 10^{-8} M) are expressed as percent of maximal response to KCl (60 mM). In relaxation studies, the rings were contracted with a concentration of endothelin (3 \times 10^{-9} M) or serotonin (3 \times 10^{-6} M) that caused an active tension of about 1.0–1.5 g. Results of these studies are expressed as percent relaxation of the contraction caused by endothelin or serotonin. Experiments with methylene blue, indomethacin, or endothelial denudation were performed in parallel with control rings from the same animals.

Data are expressed as mean±SEM. Statistical evaluation was done with Student's t test. Significance was set at the p<0.05 level.
Drugs

Acetylcholine chloride, indomethacin, sodium nitroprusside, 5-hydroxytryptamine (serotonin) creatinine sulfate, and methylene blue were purchased from Sigma Chemical Co., St. Louis, Mo. Endothelin (human) was purchased from Peptides International Inc., Louisville, Ky. Drug concentrations are expressed as final molar concentrations in the bath solution. Stock solutions were made in distilled water except indomethacin, which was dissolved in Na₂CO₃ (1 mg/ml, pH 9.0).

Methods

Experiments were performed on the thoracic aorta of adult (28–36 weeks old) male normotensive WKY rats and age- and sex-matched hypertensive SHRSP. The rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.). Segments of thoracic aorta were dissected free, excised, and placed in a cold physiological salt solution (PSS). The composition of the PSS was (mM): NaCl 130, KCl 4.7, KH₂PO₄ 1.18, MgSO₄ · 7H₂O 1.17, CaCl₂ · H₂O 1.60, NaHCO₃ 14.9, dextrose 5.5, CaNa₂ · EDTA 0.026. The blood vessels were cleaned of adherent connective tissue and cut into rings (4–5 mm long). In some rings the endothelium was removed by gently rubbing the intimal surface. In the remaining rings care was taken not to touch the inner surface of the blood vessel. The presence or absence of endothelium was confirmed by the presence or absence of relaxation in response to ACh (5×10⁻⁷ M). The rings were suspended in a 40 ml organ bath containing oxygenated (95% O₂-5% CO₂) PSS maintained at pH 7.4 and 37° C and connected to force transducers (FT03, Grass Instrument Co., Quincy, Mass.); the responses were recorded on a Grass Model 7 polygraph. The rings from both SHRSP and WKY rats were stretched with an initial passive force of 4 g. At this initial passive force, the rings developed maximal active force in response to 60 mM KCl. After being equilibrated for 60–90 minutes, the active force generated in response to this stimulation was recorded. The tissues were rinsed with fresh PSS, and the muscle was allowed to relax to baseline levels.

Results

Effects of Acetylcholine on Endothelin- and Serotonin-Induced Contraction of Rat Intact Aorta

The aortic rings were stimulated with endothelin (3×10⁻⁹ M) and serotonin (3×10⁻⁶ M), causing an active force of approximately 1.0–1.5 g. Superimposed on this contraction, ACh (10⁻⁶ M to 10⁻⁵ M) caused a concentration-dependent relaxation of the rings from both SHRSP and WKY rats. When the preparations were exposed to endothelin, this relaxation was significantly greater in rings from SHRSP than in those from WKY rats at concentrations of ACh from 10⁻⁹ to 10⁻⁵ M (Figure 1A). Conversely, this relaxation was greater in rings from WKY rats than in those from SHRSP at the same range of concentrations of ACh when the rings were exposed to serotonin (Figure 1B).

Effects of Sodium Nitroprusside on Endothelin- and Serotonin-Induced Contraction

The effect of sodium nitroprusside on endothelin- or serotonin-induced contraction was examined in intact rings from both SHRSP and WKY rats. An increase in sodium nitroprusside caused a concentration-dependent relaxation in rings from both SHRSP and WKY rats. The relaxation caused by sodium nitroprusside was not significantly different between intact rings of SHRSP and WKY rats with either the endothelin- or serotonin-induced contractile response (Figure 2, panels A and B).

Concentration–Response Contraction to Endothelin in Intact and in Endothelium-Denuded Aortic Rings

The contractile response to endothelin was concentration-dependent in the intact and endothelium-denuded rings from both SHRSP and WKY rats. Removal of the endothelium caused a shift to the left of the concentration–response curve to endothelin in WKY rats but not in SHRSP. The concentration–response curve and the maximal contractile response to endothelin were similar in the intact rings from SHRSP and WKY rats (Figure 3).
Effects of Indomethacin on Relaxation Response to Acetylcholine of an Endothelin-Induced Contraction

After aortic rings were exposed to indomethacin (5×10⁻⁶ M) for 30 minutes, the relaxation response of the endothelin-induced contraction to ACh was examined. Figure 4 showed that the relaxation caused by ACh was significantly greater in WKY rats than that in SHRSP. Comparing the response to ACh in the presence (Figure 4) and in the absence (Figure 1A) of indomethacin, we found indomethacin did not affect the relaxation caused by ACh in aortic rings from either SHRSP or WKY rats.

Effects of Pretreatment With Indomethacin or Methylene Blue on Endothelin Response

Pretreatment of the aortic rings with indomethacin (5×10⁻⁶ M) caused no significant change in endothelin-induced contraction of rings from either SHRSP or WKY rats (Table 1). Furthermore, pretreatment with indomethacin did not alter the relative effectiveness of ACh in releasing EDRF in SHRSP or WKY rat aortas that were contracted with endothelin (compare Figure 1A with Figure 4). Methylene blue (10⁻⁵ M) significantly enhanced the contractions evoked by endothelin (3×10⁻⁹ M) in rings from WKY rats but had little effect on the contraction of the rings from the SHRSP (Table 1).

Responses to Endothelin by Intact and Denuded Aortic Rings

The contraction caused by endothelin (3×10⁻⁹ M) was significantly enhanced in WKY rat aortic rings without endothelium, but the response was little changed in the SHRSP rings without endothelium (Table 1).

Discussion

Endothelial cells release EDRF and endothelium-derived constricting factors (EDCF), causing relaxation or constriction of the underlying smooth muscle.²⁻¹¹⁻¹³ The present study examined the effects of EDRF released by ACh and by endothelin on aortic rings from SHRSP and WKY rats. Our results demonstrated that ACh was less effective in causing relaxation of an endothelin-induced contraction in rings from WKY rats than in those from SHRSP.

This observation contradicts earlier observations that EDRF was more effective on an α-adrenergic-induced contraction in rings from WKY rats than in those from SHR.¹⁴⁻¹⁸ Our current finding indicates that the lesser relaxation produced by ACh is specific...
for endothelin but not for other vasoconstrictors in the normotensive rat. Conversely, this finding is also specific for ACh, as the endothelium-independent vasodilator sodium nitroprusside did not differ when acting on an endothelin-induced contraction. The response to sodium nitroprusside acting on an endothelin-induced contraction was found to be similar to a serotonin-induced contraction. In an attempt to explain this paradox, we carried out studies to determine whether endothelin itself was altering the effect of ACh by acting on the endothelium.

A recent report by de Nucci et al. showed that endothelin caused the release of PGI\textsubscript{2} in isolated lung from the rat and EDRF from the isolated perfused mesentery of this animal. Later, other investigators showed that endothelin caused the release of EDRF from the perfused aorta of the rabbit and that it inhibited platelet function in vivo due to the release of PGI\textsubscript{2}. We hypothesized that endothelin might be altering the response to ACh by itself causing the release of EDRF. To test this hypothesis, we studied the effect of procedures that eliminate the action of EDRF on the endothelin response.

Treatment of the aortas with methylene blue, which blocks the vasodilator action of EDRF, enhanced the basal tension by 3±1% and 8±2% (of maximum contraction of 60 mM KCl) in SHRSP and WKY rats, respectively (data not shown). This implies that the basal release of EDRF is less in SHRSP than in WKY rats. Our results showed that both methylene blue and removal of the endothelium virtually doubled the response to endothelin of rings from the WKY rats. These same procedures had little effect on the response to endothelin of rings from the SHRSP. We interpret this observation as indicating that in the intact aortic rings from the WKY rats, the endothelin-induced contraction was attenuated by the EDRF that it released. The spontaneous release of EDRF may also contribute to the difference between the aortas from SHRSP and WKY rats. However, the relaxation of the rings in response to ACh and the contraction of the rings in response to endothelin were not altered by indomethacin. This finding suggests that the EDRF induced by both ACh and endothelin is not PGI\textsubscript{2}.

This study further showed that removal of the endothelium caused a shift to the left of the concentration-response curve to endothelin in WKY rats but not in SHRSP. Another study demonstrated that low concentrations of endothelin caused relaxation of prostaglandin F\textsubscript{2\alpha}-precontracted intact aortic rings from normotensive guinea pigs (I.G. Joshua and D.F. Bohr, unpublished data). We conclude that endothelin causes the release of EDRF in aortic rings in the WKY rat but not in those of the SHRSP. This interpretation would explain the impaired relaxation caused by ACh, but not that caused by sodium nitroprusside, of the endothelin contraction in the WKY rat aortic rings. In these rings, the endothelin itself had already caused the EDRF to be released. The additional EDRF released by ACh was therefore reduced in rings from the WKY rats.

The failure of endothelin to cause EDRF release in the SHRSP aortic rings may be responsible for the greater sensitivity of vascular smooth muscle from SHR to the constrictor action of this peptide. Because of its failure to cause EDRF release in SHRSP, endothelin could contribute to the increase in total peripheral resistance of this form of hypertension.

In summary, our observations provide evidence that in the WKY rat aorta, endothelin and ACh have in common the ability to release EDRF that is not PGI\textsubscript{2}, and that the ability of endothelial cells to release EDRF is attenuated in SHRSP.

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**TABLE 1. Effects of Indomethacin Pretreatment, Methylene Blue Pretreatment, or Endothelial Removal on the Endothelin (3 × 10^{-9} M) Response of Intact Aortic Rings**

<table>
<thead>
<tr>
<th>Intervention</th>
<th>SHRSP</th>
<th>WKY</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indomethacin (5 × 10^{-6} M)</td>
<td>94±20</td>
<td>89±18</td>
<td>5</td>
</tr>
<tr>
<td>Endothelin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylene blue (10^{-3} M)</td>
<td>113±15</td>
<td>206±17*</td>
<td>5</td>
</tr>
<tr>
<td>Endothelium removed</td>
<td>108±12</td>
<td>188±22*</td>
<td>4</td>
</tr>
</tbody>
</table>

Values denote percent of contractile response to endothelin alone in intact aortic rings. SHRSP, stroke-prone spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.

*p<0.05 significant difference between SHRSP and WKY rats.
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