Comparison of the Effect of Endothelin on Microvessels and Macrovessels in Goldblatt II and Deoxycorticosterone Acetate–Salt Hypertensive Rats

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The response to endothelin, a novel 21-amino acid peptide, is investigated in isolated aortas with and without endothelium and in mesenteric microvessels in vivo–in situ, in Goldblatt II (GII) and deoxycorticosterone acetate (DOCA)-salt hypertensive rats. Median effective concentrations and maximal responses to endothelin did not differ in aortas with endothelium isolated from GII, DOCA-salt hypertensive, and control rats. After removal of the endothelium, the potentiation of the aorta responses to endothelin was of the same magnitude in hypertensive and control rats. A closed-circuit television system was used to observe the microvascular bed of the exteriorized mesentery of anesthetized GII, DOCA-salt hypertensive, and control rats. The time necessary to induce a vasoconstrictor response was determined after the topical application of endothelin. Vessel diameters at rest and after endothelin application were also estimated. At the microcirculatory level, a greater reactivity to endothelin was observed in both hypertensive rat groups, whereas higher sensitivity to endothelin was recorded in the GII hypertensive microvessel preparations alone. It is suggested that the increased response to endothelin observed in hypertensive rats might be due to abnormal sensitivity or reactivity of the microvessels of these rats reflecting an alteration of the contractile sequence possibly at the plasma membrane level, or due to both. Endothelial dysfunction at the microcirculatory level, however, cannot be dismissed. (Hypertension 1990;15[suppl I]:I-68–I-71)

A possible factor contributing to the elevated vascular resistance seen in hypertension is altered reactivity to vasoactive substances. Increased responsiveness to vasoconstrictor agents has been demonstrated in different models of experimental hypertension such as deoxycorticosterone acetate (DOCA)–salt and Goldblatt two-kidney, one clip (GII) hypertension.2,4 Endothelin is a novel 21-amino acid peptide considered one of the most potent vasoconstrictor agents thus far described.5 It is apparently a local rather than a circulating hormone6 and, it might be involved in the control of blood flow and pressure because of a potent microvascular constrictor effect demonstrated in vivo.7 Greater reactivity to endothelin has been observed in the isolated arteries of spontaneously hypertensive rats.8 Thus, enhanced reactivity to this agent might be expected in other models of experimental hypertension.

The present study was undertaken to compare the reactivity to endothelin in GII and DOCA-salt models of hypertension using microvessel (mesenteric microvessel in vivo–in situ) and macrovessel (isolated aorta) preparations.

Methods
A total of 58 male Wistar rats, initially weighing 150–175 g, were used. All rats were derived from breeding stock maintained in our institute.

GII hypertension was induced in 15 rats by compression of a silver clip reduced to 0.2-mm aperture around the right renal artery of 8-week-old rats.

DOCA-salt hypertension was induced in 19 8-week-old male Wistar rats. After unilateral nephrectomy, the rats were given DOCA subcutaneously over a 5-week period, commencing with 5
mg in the first week, 3 mg in the second and third weeks, and 1.5 mg in the fourth and fifth weeks. These rats were provided with water containing 1% NaCl and 0.2% KCl. Age- and weight-matched controls were sham operated. Tail-cuff blood pressure was determined with an automated sphygmomanometer (Narco Bio-Systems, Houston, Texas) in rats maintained at 40° C. All rats were weighed and blood pressure determined weekly. Experimental rats were used 4–6 weeks after surgery (GII) or within 10 days of the last injection of DOCA, respectively.

Preparation of Isolated Aorta Rings

The rats were anesthetized with chloral hydrate (300 mg/kg i.p.). The thorax was opened and the descending aorta immediately excised. After removal of loose connective tissue, two transverse rings approximately 4 mm in length were cut and mounted at the optimal length for isometric tension recording in an organ chamber, according to Furchgott and Zawadzki. While one ring served as a control, the endothelium was mechanically removed from the other by gently rubbing the luminal surface with a small cylindrical piece of artificial sponge attached to a thread, thus permitting insertion into the lumen. Two L-shaped stainless steel wire hooks were used to mount each ring in the organ bath containing Krebs-Henseleit solution (37° C, pH 7.2-7.4) containing 1% gelatin. The initial diameter of the mesenteric arterioles was exteriorized and arranged for microscopic observation in situ. The rats were maintained at 37° C and saturated with a mixture of 95% O₂ and 5% CO₂. The aorta preparations were allowed to equilibrate for at least 1 hour under a resting tension of 1.5 g, which was maintained throughout the experiment. Developed tension was detected with an F-60 microdisplacement myograph and recorded on a polygraph (Narco Bio-Systems). Cumulative concentration-effect curves were constructed from the response of the tissue to endothelin dissolved in Krebs-Henseleit solution. The doses used are given as the final molar concentrations of the salt in the organ bath. Effective concentration 50% (EC₅₀) and maximal responses were determined from the cumulative concentration-effect curves.

Preparation of Mesenteric Microvessels In Vivo

In another series of experiments, the mesentery was exteriorized and arranged for microscopic observation in situ. The rats were maintained under chloral hydrate anesthesia (400–450 mg/kg s.c.) at 37° C on a special board containing a transparent plate on which the tissue to be transilluminated was placed. The mesentery was kept moist and warm by irrigating the tissue with Ringer-Locke solution (37° C, pH 7.2–7.4) containing 1% gelatin. A 500-line television camera was coupled to a tricorocular microscope to facilitate observation of the image enlarged x3,400 on a video screen. An image-splitting micrometer was adapted to the phototube of the microscope, shearing the optical image into two separate, displaced images. By rotation of the image splitter in the phototube, the shearing was maintained at right angles to the long axis of the vessel. The displacement of coincidence of one image from the other permitted measurement of the vessel diameter.

First, the rats were anesthetized with chloral hydrate (400–450 mg/kg i.p.) at 37° C on a special board containing a transilluminating screen in an organ chamber according to Furchgott and Zawadzki. While one ring served as a control, the endothelium was mechanically removed from the other by gently rubbing the luminal surface with a small cylindrical piece of artificial sponge attached to a thread, thus permitting insertion into the lumen. Two L-shaped stainless steel wire hooks were used to mount each ring in the organ bath containing Krebs-Henseleit solution (37° C, pH 7.2-7.4) containing 1% gelatin. The initial diameter of the mesenteric arterioles was exteriorized and arranged for microscopic observation at a magnification of x3,400 on a video screen. An image-splitting micrometer was adapted to the phototube of the microscope, shearing the optical image into two separate, displaced images. By rotation of the image splitter in the phototube, the shearing was maintained at right angles to the long axis of the vessel. The displacement of coincidence of one image from the other permitted measurement of the vessel diameter.

First-order arterioles were selected for study and changes in vessel diameter estimated after the topical application of endothelin. The preparation was standardized on the basis of the constrictor response to a fixed dose of endothelin added topically. The response was characterized by the complete cessation of blood flow within 20–50 seconds in at least two vessels (capillaries excluded) of the microscopic field observed at a magnification of x100; the time necessary to impede blood flow (latency) was determined. Each given section of the vascular bed was tested only once, and no more than three endothelin concentrations were tested on a single rat. Endothelin, dissolved in Ringer-Locke solution, was added to the preparation in a standard volume of 0.01 ml and was removed by washing out with warm Ringer-Locke solution.

Synthetic porcine endothelin was obtained from Scientific Marketing Associates, London and DOCA from Berlimed, Brazil.

Statistical Analysis

Data are given as the mean±SEM. A one-way analysis of variance for repeated measurements and the Student's t test for unpaired data were used where appropriate. The minimum acceptable level of significance was p at a value less than or equal to 0.05.

Results

Four weeks after surgery, blood pressure was significantly elevated in clipped rats as also noted 10 days after the suspension of treatment in the DOCA-salt group (Tables 1 and 2).

Effect of Endothelin in Isolated Aortas

Cumulative concentration-effect curves for endothelin were obtained simultaneously from the responses of two portions of a single artery, one intact and the other lacking endothelium. The effect of endothelin on endothelium-intact preparations from hypertensive rats was similar to that seen in normal rats. Although removal of the endothelium shifted the concentration-effect curves to the left as compared with endothelium-intact preparations, no difference was observed between the response to endothelin in aortas from hypertensive (GII and DOCA-salt) and control rats lacking endothelium (Table 1).

Effect of Endothelin on Mesenteric Microvessels In Vivo

The initial diameter of the mesenteric arterioles was not different in either GII rats (14.9±0.8 μm, n=9) and their respective controls (13.4±0.9 μm, n=8), or in DOCA-salt rats (13.8±0.6 μm, n=13) and their respective controls (13.3±0.5 μm, n=10).
TABLE 1. Effective Concentrations 50% and Maximal Responses to Endothelin in Aortas Isolated From Goldblatt II and Deoxycorticosterone Acetate–Salt Hypertensive Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>BP (mm Hg)</th>
<th>EC50×10⁻¹⁰ M</th>
<th>Maximal responses (g)±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With</td>
<td>Without</td>
<td></td>
</tr>
<tr>
<td></td>
<td>endothelium</td>
<td>endothelium</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>120.8±4.2</td>
<td>5.41* (2.90–10.09)</td>
<td>3.0±0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.13* (1.59–2.87)</td>
<td>5.6±0.2*</td>
</tr>
<tr>
<td>Gil</td>
<td>172.5±12.0†</td>
<td>6.00 (3.79–9.47)</td>
<td>3.3±0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.71* (2.16–3.71)</td>
<td>4.8±0.4*</td>
</tr>
<tr>
<td>DOCA-salt</td>
<td>195.8±7.7†</td>
<td>7.35 (2.66–20.29)</td>
<td>2.7±0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.57* (2.01–6.35)</td>
<td>3.9±0.4*</td>
</tr>
</tbody>
</table>

Six animals were used in each group. Effective concentration 50% (EC50) are expressed as geometric means of the individual determinations and their respective 95% confidence interval. BP, Blood pressure; Gil, Goldblatt two-kidney, one clip hypertensive rats; DOCA, deoxycorticosterone acetate.

*p<0.05 in comparison with corresponding preparations with endothelium.
†p<0.05 in comparison with control values.

In the control rat preparations, cessation of blood flow through the mesenteric microvessels was only achieved when endothelin was applied topically at 10⁻⁸ M; in Gil hypertensive rats, 10⁻⁹ M endothelin induced cessation of blood flow (Table 2). Thus, microvessel preparations from Gil rats are more sensitive to endothelin than are control preparations. This increased sensitivity to endothelin was also observed at 10⁻¹⁰ M, where a greater reduction in the initial diameter of arterioles from Gil rats (39.8±13.4%) was seen as compared with controls (15.7±2.3%).

Microvessel preparations from DOCA-salt rats exhibited sensitivity to endothelin similar to control preparations because complete interruption of blood flow was induced by 10⁻⁸ M endothelin in both cases. The latency to induce this response, however, was significantly decreased in preparations from DOCA-salt hypertensive rats as compared with control rats. Thus, greater reactivity to endothelin was detected in DOCA-salt as compared with control rats. In contrast, at lower endothelin concentrations (10⁻⁹ and 10⁻¹⁰ M), similar decreases in initial diameter (approximately 15%) were obtained in DOCA-salt and control preparations, indicating that sensitivity did not differ between these groups.

TABLE 2. Latency to Induce Cessation of Blood Flow in Mesenteric Microvessels In Vivo–In Situ of Goldblatt II and Deoxycorticosterone Acetate–Salt Hypertensive Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>BP (mm Hg)</th>
<th>10⁻⁸ M</th>
<th>10⁻⁹ M</th>
<th>10⁻¹⁰ M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=8)</td>
<td>116.7±4.6</td>
<td>44.5±5.5</td>
<td>&gt;90*</td>
<td>&gt;90</td>
</tr>
<tr>
<td>Gil (n=9)</td>
<td>180.0±8.2†</td>
<td>28.1±3.5†</td>
<td>47.2±2.3†</td>
<td>&gt;90</td>
</tr>
<tr>
<td>Control (n=10)</td>
<td>116.7±5.4</td>
<td>40.2±6.0</td>
<td>&gt;90</td>
<td>&gt;90</td>
</tr>
<tr>
<td>DOCA-salt (n=13)</td>
<td>178.5±8.0†</td>
<td>23.8±3.3†</td>
<td>&gt;90</td>
<td>&gt;90</td>
</tr>
</tbody>
</table>

All values are mean±SEM; values are in seconds. BP, Blood pressure; Gil, Goldblatt two-kidney, one clip hypertensive rats; DOCA, deoxycorticosterone acetate.

*p<0.05 in comparison with control values (Student’s t test).

In the control rat preparations, cessation of blood flow through the mesenteric microvessels was only achieved when endothelin was applied topically at 10⁻⁸ M; in Gil hypertensive rats, 10⁻⁹ M endothelin induced cessation of blood flow (Table 2). Thus, microvessel preparations from Gil rats are more sensitive to endothelin than are control preparations. This increased sensitivity to endothelin was also observed at 10⁻¹⁰ M, where a greater reduction in the initial diameter of arterioles from Gil rats (39.8±13.4%) was seen as compared with controls (15.7±2.3%).

Microvessel preparations from DOCA-salt rats exhibited sensitivity to endothelin similar to control preparations because complete interruption of blood flow was induced by 10⁻⁸ M endothelin in both cases. The latency to induce this response, however, was significantly decreased in preparations from DOCA-salt hypertensive rats as compared with control rats. Thus, greater reactivity to endothelin was detected in DOCA-salt as compared with control rats. In contrast, at lower endothelin concentrations (10⁻⁹ and 10⁻¹⁰ M), similar decreases in initial diameter (approximately 15%) were obtained in DOCA-salt and control preparations, indicating that sensitivity did not differ between these groups.

**Discussion**

A modulatory role for the vascular endothelium on the underlying smooth muscle layer has been suggested by many authors (see Reference 12 for review). Removal of endothelial cells renders preparations more sensitive to vasoconstrictor agents. In the present study, both greater sensitivity to (lower EC50) and enhanced maximal response to endothelin were observed in aorta rings lacking endothelium prepared from control and hypertensive (Gil and DOCA-salt) rats as compared with responses in preparations in which the endothelium was left intact. The difference observed between preparations with and without endothelium was similar in both control and hypertensive groups. Thus, the modulation of the contractile response to endothelin by the endothelium is unaltered in these models of hypertension.

An enhanced response to vasoconstrictor agents is a frequent finding in different models of experimental hypertension. A greater sensitivity to noradrenaline has been demonstrated in vessels from DOCA-salt hypertensive rats, and in renal hypertension, and in spontaneously hypertensive rats. Greater reactivity, sensitivity, or reactivity and sensitivity to endothelin in aortas from DOCA-salt or Gil hypertensive rats, however, was not encoun-
tered. The difference in response to the different agonists might indicate a specific alteration at the plasma membrane level of the vessels in these hypertensive rats that affects the response to some but not all vasoconstrictor agents.

Hypertension did interfere, however, with the action of endothelin at the microcirculatory level. Different mechanisms, therefore, might be involved regarding the effect of endothelin on small vessels in situ and large arteries in vitro. Despite the fact that macrovessels and microvessels are subjected to high blood pressure levels, different adjustment mechanisms might be involved.

In the present study, reactivity to endothelin (referred to here as the magnitude of the response as proposed by Mulvany) was increased in the hypertensive state (GI and DOCA-salt microvessel preparations), whereas a higher sensitivity (used here to describe the facility with which the contractile process in vascular smooth muscle is activated) was observed in preparations from GI but not DOCA-salt hypertensive rats.

The observation of an increased vascular response to vasoactive stimuli leads to the hypotheses that the increased total vascular resistance might result from abnormalities in smooth muscle sensitivity or that the reactivity or contractility of the vessel wall is enhanced.

The present study suggests the increased response of aorta preparations to endothelin observed in GI and DOCA-salt hypertensive rats might result from an abnormal sensitivity, reactivity, or sensitivity and reactivity of the microvessels that might reflect an alteration of the contractile sequence possibly at the plasma membrane level; however, an endothelial dysfunction at the microcirculatory level cannot be dismissed. In contrast to the notion that the enhanced response to norepinephrine observed in DOCA-salt hypertensive rats is due to endothelial cell dysfunction, the present data suggest that such a mechanism is not involved in the altered response to endothelin, at least in large vessels.

Acknowledgment

The secretarial assistance of Yara Corradini is gratefully acknowledged.

References


Key Words: • vasoactive compounds • vasoconstriction • endothelin • aorta • microcirculation
Comparison of the effect of endothelin on microvessels and macrovessels in Goldblatt II and deoxycorticosterone acetate-salt hypertensive rats.
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*Hypertension*. 1990;15:I68
doi: 10.1161/01.HYP.15.2_Suppl.I68

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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