Endothelin-1 Augments Pressor Response to Angiotensin II Infusion in Rats

Kazunori Yoshida, Minoru Yasujima, Masahiro Kohzuki, Masayuki Kanazawa, Kaoru Yoshinaga, and Keishi Abe

To assess possible roles of endothelin in the regulation of blood pressure, we studied effects of a subpressor dose of endothelin-1 (3 μg/kg/day) on chronic blood pressure responses to infusion of angiotensin II and norepinephrine in rats. Rats were infused with angiotensin II at a subpressor dose (400 μg/kg/day i.p.) or with norepinephrine at a subpressor dose (360 μg/kg/day i.p.) for 6 days. Systolic blood pressure was significantly elevated during combined infusion of endothelin-1 and angiotensin II, whereas endothelin-1 alone or angiotensin II alone failed to induce any significant changes in systolic blood pressure compared with vehicle alone. This effect was sustained for the whole experimental period and was not associated with any significant changes in body weight, fluid intake, urine volume, or urinary electrolyte excretion. In contrast, combined infusion of endothelin-1 and norepinephrine failed to elevate systolic blood pressure, and no significant difference in systolic blood pressure was observed for the whole experimental period among the four groups of rats with endothelin-1 in combination with norepinephrine, endothelin-1 alone, norepinephrine alone, and vehicle alone. The present results indicate that angiotensin II and endothelin-1, but not norepinephrine and endothelin-1, work synergistically to raise the blood pressure and also suggest the possibility that endothelin-1 may modulate blood pressure control. (Hypertension 1992;20:292-297)

KEY WORDS • blood pressure • angiotensin II • Sprague-Dawley rats • norepinephrine • endothelins

Endothelin (ET), an endothelium-derived 21-residue peptide vasoconstrictor, has been isolated from the culture supernatant of porcine aortic endothelial cells and has also been shown to be one of the most potent vasoconstrictors in a variety of blood vessels from various species. Continuous infusion of endothelin-1 (ET-1) at a dose of 60 μg/kg per day for up to 6 days induced a significant increase in systolic blood pressure in conscious rats, whereas ET-1 at doses of smaller than 6 μg/kg per day did not induce any significant increase in systolic blood pressure. It is also postulated that ET-1–induced smooth muscle contraction is mediated by an increase in intracellular calcium concentration. The relation of the ET system to the sympathetic nervous system and the renin-angiotensin system in the regulation of blood pressure and sodium-water metabolism still remains to be determined. Therefore, we assessed the chronic effects of ET-1, angiotensin II (Ang II), and norepinephrine (NE) on systolic blood pressure and sodium-water metabolism in conscious rats and also evaluated the interaction between ET-1 and Ang II and between ET-1 and NE.

Methods

Male Sprague-Dawley rats (Funabashi, Chiba, Japan) weighing approximately 200 g were used. All rats were maintained in a humidity- and temperature-controlled room. Each rat was housed in a metabolic cage designed to prevent feces-urine contact (model ST, Sugiyama gen, Tokyo) during the study. The rats were fed on a regular diet (Oriental CMF, 0.20 meq sodium per gram, 0.27 meq potassium per gram; Oriental Yeast Co., Tokyo) and had free access to tap water. Two study protocols were performed to assess the effect of ET-1 on chronic blood pressure responses to Ang II and NE in rats.

Study 1: Effects of Endothelin-1 in Ang II-Infused Rats

Rats were divided into four groups. Group 1 rats (n=7) received a subpressor dose of Ang II (400 μg/kg per day i.p.) only. Group 2 rats (n=7) received a subpressor dose of ET-1 (3 μg/kg per day i.v.) only. Group 3 rats (n=7) received simultaneously both the subpressor dose of Ang II (400 μg/kg per day i.p.) and the subpressor dose of ET-1 (3 μg/kg per day i.v.). Group 4 rats (n=7) received vehicle (physiological saline) only.

In a previous study, we assessed the effect of continuous Ang II infusion at doses of 10, 75, 100, and 150 ng/min for up to 6 days in conscious rats. Systolic blood pressure increased dose-dependently at Ang II levels of 100 and 150 ng/min, whereas the changes induced by 10 and 75 ng/min did not differ from those induced by vehicle infusion. In a previous study, we have shown...
that chronic infusion of Ang II at a rate of 100 ng/min induced a sustained increase in systolic blood pressure in rats, and in preliminary experiments, we confirmed that continuous infusion of Ang II at a rate of 75 ng/min did not significantly affect the blood pressure in rats (Figure 1A). The infusion dose at a rate of 400 µg/kg per day (56 ng/min) was then chosen as a subpressor dose. In preliminary experiments, the increased value of Ang II up to approximately 44% was confirmed in Ang II-infused rats (400 µg/kg per day for 6 days). Ang II (Peptide Institute, Inc., Osaka, Japan) was dissolved in 0.01N acetic acid and delivered intraperitoneally by osmotic minipumps (Alzet, Palo Alto, Calif.) placed in the abdominal cavity while the rats were under ether anesthesia. ET-1 (Peptide Institute, Inc.), dissolved in physiological saline or vehicle, was administrated via osmotic minipumps into the jugular vein (intravenously) for up to 6 days. The vascular catheter (PE-60) was tunneled subcutaneously to the osmotic minipump and implanted in the interscapular region of the rat's back under ether anesthesia.

Study 2: Effects of Endothelin-1 in Norepinephrine-Infused Rats

Rats were divided into four groups. Group 1 rats (n=7) received a subpressor dose of NE (360 µg/kg per day i.p.) only. In previous studies, we have shown that chronic infusion of NE at a rate of 1.8 mg/kg per day induced a sustained increase in systolic blood pressure in rats,9 and in preliminary experiments we confirmed that continuous infusion of NE at a rate of 720 µg/kg per day did not significantly affect the blood pressure in rats (Figure 1B). NE (noradrenaline bitartrate, Wako Pure Chemical Industries LTD., Osaka, Japan) was dissolved in 5 mM glutathione containing ascorbic acid (50 µg/ml) and delivered intraperitoneally by osmotic minipumps placed in the abdominal cavity. Group 2 rats (n=7) received a subpressor dose of ET-1 (3 µg/kg per day i.v.) only. Group 3 rats (n=7) and Group 4 rats (n=7) received both Ang II (400 µg/kg/day i.p.) and ET-1 (3 µg/kg/day i.v.) simultaneously.

Figure 1. Line graph shows effect of angiotensin II (Ang II) (panel A) and norepinephrine (panel B) on systolic blood pressure in conscious rats. Daily systolic blood pressure in rats infused with Ang II (75, 100, and 150 ng/min i.p.), norepinephrine (NE) (720, 1,440, 2,160, and 2,880 µg/kg/day i.p.), or vehicle alone. Data are mean±SEM. A II, angiotensin II.

Figure 2. Line graph shows effect of angiotensin II (Ang II) and endothelin-1 (ET-1) on systolic blood pressure in conscious rats. Daily systolic blood pressure in rats infused with Ang II (400 µg/kg/day i.p.) (○), with ET-1 (3 µg/kg/day i.v.) (●), with both Ang II (400 µg/kg/day i.p.) and ET-1 (3 µg/kg/day i.v.) (▲), and with vehicle alone (▲). Data are mean±SEM. *p<0.05 compared with Ang II alone; †p<0.05 compared with ET-1 alone; §p<0.05 compared with vehicle alone. A II, angiotensin II.
TABLE 1. Effect of Continuous Infusion of Endothelin-1, Angiotensin II, and Endothelin-1 Combined With Angiotensin II in Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>BW (g)</th>
<th>WI (ml/day)</th>
<th>Day -1</th>
<th>BW (g)</th>
<th>WI (ml/day)</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET-1</td>
<td>7</td>
<td>219.3±6.2</td>
<td>21.3±2.6</td>
<td></td>
<td>240.8±11.3</td>
<td>22.3±3.1</td>
<td></td>
</tr>
<tr>
<td>Ang II</td>
<td>7</td>
<td>214.8±3.7</td>
<td>21.8±2.6</td>
<td></td>
<td>232.0±11.4</td>
<td>25.8±0.8</td>
<td></td>
</tr>
<tr>
<td>ET-1+Ang II</td>
<td>7</td>
<td>213.0±6.1</td>
<td>18.2±1.7</td>
<td></td>
<td>227.7±5.1</td>
<td>24.7±2.2</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>7</td>
<td>217.8±6.9</td>
<td>20.5±1.4</td>
<td></td>
<td>238.0±6.0</td>
<td>23.8±1.5</td>
<td></td>
</tr>
</tbody>
</table>

Results are mean±SEM. BW, body weight; WI, water intake; ET-1, endothelin 1; Ang II, angiotensin II.

received simultaneously both the subpressor dose of NE (360 μg/kg per day i.p.) and the subpressor dose of ET-1 (3 μg/kg per day i.v.). Group 4 rats (n=7) received vehicle only.

Assuming that ET-1, Ang II, and NE did not degrade during the study and that the pumps dispensed fluid at a preset rate of 1 μl/hr, the infusion rates of ET-1, Ang II, and NE were calculated. The stability of ET-1, Ang II, and NE in the osmotic minipumps was examined by comparing the blood pressure-elevating activities remaining in the solutions recovered from the minipumps in the rats after 6 days with those of freshly dissolved ET-1, Ang II, and NE. Systolic blood pressure in rats was recorded daily by the indirect tail-cuff method (UEDA UR 1000, Ueda Industries Co., Tokyo) without anesthesia.9 The systolic blood pressure measured by this method correlated well with direct systolic blood pressure.9 The daily body weight, fluid intake, urine volume, urinary sodium excretion, and urinary potassium excretion were also determined. Urinary sodium and potassium were measured with ion-specific electrodes (CIM-104 Na/K ION METER, Shimadzu Co., Kyoto, Japan). Data are expressed as mean±SEM. Statistical differences were determined by analysis of variance and Student’s unpaired t test between groups. A probability less than 0.05 was considered statistically significant.

Results

Study 1: Effects of Endothelin-1 in Ang II-Infused Rats

Body weight, systolic blood pressure, fluid intake, urine volume, urinary sodium excretion, and urinary potassium excretion were not significantly different among the groups before the infusion of Ang II alone, ET-1 alone, Ang II and ET-1, or vehicle alone. As shown in Figure 2, continuous infusion of Ang II (400 μg/kg per day) did not induce any significant changes in systolic blood pressure compared with that of vehicle-infused rats. Similarly, continuous infusion of ET-1 (3 μg/kg per day) did not induce any significant changes in systolic blood pressure compared with that of vehicle-infused rats. On the contrary, the combined infusion of ET-1 (3 μg/kg per day) induced a significant increase in systolic blood pressure compared with that of ET-1-infused rats and vehicle-infused rats on day 1 and compared with that of Ang II-infused rats on day 2, and the effect continued thereafter. On day 6, systolic blood pressure in Ang II-infused rats, ET-1-infused rats, and vehicle-infused rats was 148.0±3.0, 142.7±2.9, and 143.5±3.0 mm Hg, respectively (NS). On the other hand, systolic blood pressure in Ang II- and ET-1-infused rats was 189.0±12.5 mm Hg (p<0.05) on day 6. The infusion of

FIGURE 3. Bar graphs show daily urine volume, urinary sodium excretion, and urinary potassium excretion in rats infused with endothelin-1 (ET-1), angiotensin II (Ang II), ET-1 combined with Ang II, and vehicle alone. A II, angiotensin II.
Ang II alone, ET-1 alone, and the two combined did not induce any significant changes in body weight, fluid intake, urine volume, urinary sodium excretion, and urinary potassium excretion (Table 1 and Figure 3).

**Study 2: Effects of Endothelin-1 in Norepinephrine-Infused Rats**

As shown in Figure 4, continuous infusion of NE (360 μg/kg per day) did not induce any significant changes in systolic blood pressure compared with the vehicle-infused rats. Continuous infusion of ET-1 (3 μg/kg per day) did not induce any significant changes in systolic blood pressure as observed in study 1. In contrast to study 1, the combined infusion of the subpressor dose of NE (360 μg/kg per day) and the subpressor dose of ET-1 (3 μg/kg per day) failed to induce any significant changes in systolic blood pressure compared with the other three groups throughout the study. There were no significant differences in body weight, fluid intake, urine volume, urinary sodium excretion, and urinary potassium excretion among the four groups (Table 2 and Figure 5). We examined higher doses of NE infusion (two or four times the dose of 360 μg/kg per day) in the previous experiment and assessed the synergistic effect of ET-1 and NE on blood pressure (Figure 6). Continuous infusion of ET-1 (3 μg/kg per day) did not affect any significant changes in systolic blood pressure in rats infused with various doses of NE.

**Discussion**

In the present study, we clearly demonstrated that the subpressor dose of Ang II in combination with the subpressor dose of ET-1 administered for 6 days induced a significant increase in systolic blood pressure in conscious rats, whereas the subpressor dose of NE in combination with the same dose of ET-1 failed to elevate systolic blood pressure. This synergistic effect of ET-1 and Ang II on blood pressure was not accompanied by any significant changes in body weight, fluid intake, urine volume, urinary sodium excretion, and urinary potassium excretion, suggesting that the significant increase in blood pressure is not secondary to the renal effects of these peptides.

Yang et al. reported that in human arteries, threshold concentrations of ET-1 amplify the contractions induced by NE via a calcium-dependent mechanism. Recently Tabuchi et al. reported that the subpressor dose of ET-1 enhanced the pressor response to NE in isolated perfused rat mesenteric arteries in vitro. However, the subpressor dose of ET-1 inhibited the pressor response of rat mesenteric arteries to electrical sympathetic nerve stimulation. They also reported that ET-1 inhibited NE release from sympathetic nerve endings of rat mesenteric arteries. Thus, the synergistic effect between NE and ET on vascular resistance has so far been controversial. Moreover, we cannot find in the literature any in vivo long-term study on interaction between NE and ET-1. As described previously, chronic combined infusion of the subpressor dose of ET-1 and the subpressor dose of NE failed to induce any significant changes in systolic blood pressure in conscious rats. Assuming that the dose of Ang II and the dose of NE used in the present experiment were equipotent in the preliminary study, our present study shows that ET-1 preferentially potentiates pressor responsiveness to Ang II when compared with that of NE.

There are few reports on the relation between the renin-angiotensin system and the ET system in the regulation of blood pressure and sodium-water metabolism. Kawaguchi et al. reported that endothelin stimulates angiotensin I to Ang II conversion in cultured pulmonary artery endothelial cells. In addition to its direct effect on vascular smooth muscle, it is suggested that ET-1 may play an important role in regulating vascular tone by modulating angiotensin converting enzyme activity. Rakugi et al. also reported that ET activates the release of Ang II from the rat mesenteric arteries. On the other hand, it has also been reported that ET inhibits renin release from isolated rat glomer-

### Table 2. Effect of Continuous Infusion of Endothelin-1, Norepinephrine, and Endothelin-1 Combined With Norepinephrine in Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Day -1</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BW (g)</td>
<td>WI (ml/day)</td>
</tr>
<tr>
<td>ET-1</td>
<td>7</td>
<td>232.8±6.0</td>
<td>22.3±0.2</td>
</tr>
<tr>
<td>NE</td>
<td>7</td>
<td>235.5±5.0</td>
<td>22.8±1.2</td>
</tr>
<tr>
<td>ET-1+NE</td>
<td>7</td>
<td>231.5±7.4</td>
<td>22.7±0.8</td>
</tr>
<tr>
<td>Vehicle</td>
<td>7</td>
<td>235.7±6.6</td>
<td>21.9±0.6</td>
</tr>
</tbody>
</table>

Results are mean±SEM. BW, body weight; WI, water intake; ET-1, endothelin 1; NE, norepinephrine.
Therefore, the synergistic effect between ET and the renin-angiotensin system has not been established. To our knowledge, this is the first report to demonstrate the long-term synergistic effect of ET-1 and Ang II on blood pressure in vivo. The mechanisms by which Ang II and ET-1 exert their pressor effects synergistically were not clear in the present experiments. Although actual intracellular mechanisms remain to be determined, the present results suggest the possibility that there are some common pathways in the hypertension-producing mechanism of these two peptides. More recently, Nakamoto et al. reported that pressor responses to NE and Ang II were significantly attenuated in dogs infused with 40 fmol/kg min⁻¹ ET, but pressor responses to NE and Ang II were not changed significantly in dogs infused with 400 fmol/kg min⁻¹ ET. They suggested that an increase in antihypertensive substances, probably induced by low-dose ET administration, might have produced the decreased pressor responsiveness to the vasoactive substances.

ET is one of the most potent vasoconstrictors and may be involved in the regulation of blood pressure. We have already reported that continuous infusion of ET-1 into the jugular vein for up to 6 days induced a significant increase in systolic blood pressure in conscious rats. However, the circulating levels of ET in rats are very low. Low levels of ET-1 could play an important role if ET-1 sensitizes the blood vessel wall to the effects of some other specific vasoconstrictor substances. Even a slight elevation of circulating and, more importantly, local ET-1 concentration may profoundly affect vascular tone to regulate the local blood flow. Therefore, the present observation may again cast a new light on the significance of circulating ET in the regulation of blood pressure.

In conclusion, the present results suggest the possibility that Ang II and ET-1 potentiate synergistically each other's pressor effect when administered chronically, and their combined actions might play a role in controlling blood pressure. However, it still remains to be clarified why ET-1 and Ang II exert their pressor effects synergistically while ET-1 and NE do not.

References


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