Central Effect of Endothelin on Neurohormonal Responses in Conscious Rabbits

Kiyoshi Matsumura, Isao Abe, Takuya Tsuchihashi, Mitsuhiro Tominaga, Kazuo Kobayashi, and Masatoshi Fujishima

It has been shown that endothelin-1 (ET-1) binding sites exist in the central nervous system and that the injection of intracerebroventricular ET-1 induces a pressor response. Therefore, we determined the neurohormonal and cardiovascular responses to intracerebroventricular ET-1 (25 pmol/kg) in conscious rabbits with chronically instrumented electrodes on the renal sympathetic nerve. Intracerebroventricular ET-1 provoked a prompt increase in arterial pressure and in renal sympathetic nerve activity within 5 minutes, and peak values were obtained at 20 and 40 minutes, respectively. Plasma epinephrine and norepinephrine reached peak values at 5–20 minutes. Plasma vasopressin and plasma glucose levels also increased significantly, but plasma osmolality, hematocrit, and serum sodium and potassium concentrations did not show any changes. Arterial blood gas analysis showed respiratory alkalosis. However, pretreatment with intravenous pentolinium (5 mg/kg), a ganglion blocking agent, abolished these neurohormonal and cardiovascular responses. Conversely, the same dose of intravenous ET-1 (25 pmol/kg) as that used in the intracerebroventricular experiment failed to cause any cardiovascular or renal sympathetic nerve responses. These results suggest that intracerebroventricular ET-1 acts in the central nervous system and causes a pressor response mainly through the enhancement of sympathoadrenal outflow. (Hypertension 1991; 17:1192–1196)

Endothelin-1 (ET-1), a novel 21–amino acid peptide isolated from the supernatant of cultured porcine endothelial cells,1 is a potent vasoconstrictor substance in vitro1,2 and in vivo.3,4 Binding sites for this peptide have been shown to exist in the central nervous system.5 Intravenous ET-1 has been reported to provoke a potent and sustained increase in blood pressure in both conscious6 and anesthetized7 rats. This pressor effect is shown to be mediated mainly by an increase in total peripheral resistance.6,7 Conversely, although a central pressor effect of ET-1 concomitant with elevation of plasma catecholamines has been reported in conscious rats,8 its mechanisms have not yet been fully elucidated. In addition, other biochemical and neurohormonal responses, including direct sympathetic nerve recording, also have not been studied. To determine what mechanisms are involved in the pressor response to intracerebroventricular ET-1 in conscious animals, we examined cardiovascular and neurohormonal responses induced by intracerebroventricular ET-1 in conscious rabbits.

Methods

Preparation of Animals

Experiments were conducted on 20 male Japanese White rabbits weighing 2.5–3.0 kg. Rabbits were anesthetized with sodium pentobarbital (30 mg/kg i.v.). Electrodes were implanted on the left renal sympathetic nerve and a stainless steel cannula was placed in the right lateral cerebral ventricle. Under aseptic conditions, the left kidney was exposed retroperitoneally, and a branch of the renal nerve was separated from the renal plexus and the surrounding connective tissues with the use of a dissecting microscope.9 Renal sympathetic nerve activity (RSNA) was recorded by a pair of electrodes made from Teflon-insulated seven-stranded steel wire (Medwire, Mt. Vernon, N.Y.). The area of the nerve and wire interface was embedded in silicone cement.

A 23-gauge stainless steel cannula was implanted into the right lateral cerebral ventricle 4 mm lateral to the bregma and 6 mm below the cerebral surface. The position of the cannula in the lateral ventricle was confirmed by the staining of all four ventricles.
after injection of 0.1 ml dye at the end of the experiment. The cannula was fixed to the skull with three jewelers' screws and dental cement. A 27-gauge obturator was used to seal the cannula. After surgery, disodium sulbenicillin (200 mg i.v.) was given to the rabbits to prevent any postoperative infection.

At least 3 days after the surgical procedures, the following experiments were carried out with a rabbit in a clear plastic box. On each experimental day, polyethylene catheters (PE-50) were inserted into the central ear artery and marginal ear vein under 1% (vol/vol) lidocaine local anesthesia. The arterial catheter was connected to a pressure transducer (P50, Gould Statham Instruments Inc., Hato Rey, Puerto Rico) to measure arterial pressure. The heart rate (HR) was monitored by a cardiotachometer (model 1332, NEC San-ei, Tokyo, Japan).

**Experiment 1**

To determine the dose of ET-1 (Peptide Institute Inc., Osaka, Japan) needed to increase blood pressure, 6.25, 12.5, 25, and 50 pmol/kg of ET-1 was injected intracerebroventricularly (n=6). These doses of ET-1 were dissolved in 80 μl buffered saline. The administration of each dose of ET-1 was separated by a period of at least 24 hours.

**Experiment 2**

After a control period, a blood sample (2.4 ml) was drawn from the arterial catheter to measure plasma catecholamines (epinephrine and norepinephrine), plasma renin activity (PRA), plasma vasopressin (AVP), plasma glucose, serum sodium, serum potassium, plasma osmolality, hematocrit, and arterial blood gas; then, 25 pmol/kg ET-1 in a volume of 80 μl was injected via the intracerebroventricular cannula (n=6). Additional blood samples were drawn at 5, 20, and 60 minutes after intracerebroventricular ET-1 and were replaced by the same volume of 0.9% saline. Arterial pressure, HR, and RSNA were monitored continuously.

**Experiment 3**

After a control period, the rabbits were injected with pentolinium (Sigma Chemical Co., St. Louis) (5 mg/kg in 0.3 ml/kg i.v., n=4). Five minutes later, a blood sample (2.4 ml) was drawn from the arterial catheter, and ET-1 (25 pmol/kg) was injected intracerebroventricularly, as in experiment 2. Additional blood samples were drawn at 5 and 20 minutes after intracerebroventricular ET-1 and were replaced by the same volume of 0.9% saline.

**Experiment 4**

The same dose of ET-1 (25 pmol/kg) as that used in the intracerebroventricular experiment was injected intravenously (n=4). Arterial pressure, HR, and RSNA were monitored continuously.

**Recording Procedures of Renal Sympathetic Nerve Activity**

RSNA was amplified (model DPA-100E, Dia Medical System Co., Tokyo, Japan) and filtered (100–3,000 Hz), and the waveforms were integrated after a full wave rectification using an integrator amplifier (model 1322, NEC San-ei) with the sample-hold function reset to baseline by an internal timer set at 5 seconds.9 Absolute values for integrated RSNA were corrected before data analysis by subtracting the residual electrical output (noise level) recorded from the integrator induced by intravenous phenylephrine (16 μg/kg).

**Blood Collection and Analysis**

Blood samples for measurement of plasma catecholamines, PRA, and plasma AVP were centrifuged at 4°C. Plasma for catecholamines was stored at −80°C, and others were stored at −20°C until assay. The plasma catecholamine concentration was measured by a radioenzymatic assay,10 and PRA and plasma AVP level were measured by a radioimmunoassay.11,12 The assay sensitivities for PRA, AVP, and catecholamines were 0.1 ng/ml/hr, 0.45 pg/ml, and 20 pg/ml, respectively. The intra-assay and interassay coefficients of variation were 5.6–10.2% and 6.7–16.3%, respectively. Arterial blood gas was determined with an IL 1304 Blood Gas Analyzer (Instrumentation Laboratory Inc., Lexington, Mass.), and plasma glucose levels were measured by a Glucose Analyzer 2 (Beckman Instruments, Inc., Fullerton, Calif.). Serum sodium and potassium concentrations were measured by flame photometry (model 205D, Hitachi, Tokyo, Japan), and plasma osmolality was measured with a freezing-point osmometer (Osmotron-20, Orion Riken Co., Tokyo).

**Statistics**

All values are expressed as mean±SEM. A one-way analysis of variance for repeated measurements was performed, followed by Duncan's multiple range test to determine which means differed statistically from the control means. A value of p<0.05 was considered significant.

**Results**

**Experiment 1**

Intracerebroventricular ET-1 at the doses of 6.25 and 12.5 pmol/kg did not elicit any changes in mean arterial pressure (MAP). On the other hand, 25 pmol/kg ET-1 showed a prominent increase in MAP; however, 50 pmol/kg ET-1 did not show any further increase in MAP (44.3±9.3 and 46.0±16.8 mm Hg, respectively). Therefore, we used 25 pmol/kg ET-1 in the following studies.

**Experiment 2**

Intracerebroventricular injection of 25 pmol/kg ET-1 provoked a prompt increase in MAP and in RSNA, and peak values were obtained after 20 and...
1194 Hypertension
Vol 17, No 6, Part 2 June 1991

FIGURE 1. Panel A: Line graphs showing time course of mean arterial pressure (MAP), heart rate (HR), and integrated renal sympathetic nerve activity (RSNA) in six rabbits given 25 pmol/kg endothelin-1 into the right lateral cerebral ventricle. Values are expressed as mean±SEM. *p<0.05 compared with control period by Duncan's multiple range test. Panel B: Representative original recordings show increases in RSNA induced by intracerebroventricular endothelin-1.

TABLE 1. Effects of Intracerebroventricular Endothelin-1

<table>
<thead>
<tr>
<th>Variable</th>
<th>0</th>
<th>5</th>
<th>20</th>
<th>60</th>
</tr>
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<tbody>
<tr>
<td>Epinephrine (pg/ml)</td>
<td>101.1±35.9</td>
<td>2,048.0±493.2*</td>
<td>1,252.3±246.7*</td>
<td>446.7±145.1*</td>
</tr>
<tr>
<td>Norepinephrine (pg/ml)</td>
<td>112.9±269.4*</td>
<td>1,132.2±199.8*</td>
<td>684.8±90.7*</td>
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<td>PRA (ng/ml/hr)</td>
<td>1.8±0.7</td>
<td>2.6±1.1</td>
<td>1.5±0.6</td>
<td>1.9±1.1</td>
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<td>AVP (pg/ml)</td>
<td>1.8±0.8</td>
<td>8.1±5.6*</td>
<td>13.4±5.1*</td>
<td>8.2±4.7*</td>
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<tr>
<td>Sodium (meq/l)</td>
<td>141.0±1.4</td>
<td>142.3±1.5</td>
<td>142.3±1.3</td>
<td>139.7±1.2</td>
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<tr>
<td>Potassium (meq/l)</td>
<td>4.1±0.4</td>
<td>4.2±0.4</td>
<td>3.4±0.2</td>
<td>3.2±0.1</td>
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<tr>
<td>Glucose (mg/dl)</td>
<td>130.0±5.1</td>
<td>145.2±10.5</td>
<td>208.0±26.1*</td>
<td>201.3±16.2*</td>
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<tr>
<td>Hematocrit (%)</td>
<td>33.2±0.9</td>
<td>32.5±1.1</td>
<td>31.6±1.1</td>
<td>32.4±1.3</td>
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<tr>
<td>Osmolality (mosm/l)</td>
<td>293.0±9.3</td>
<td>292.7±16.1</td>
<td>284.8±11.4</td>
<td>278.7±8.1</td>
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<td>pH</td>
<td>7.43±0.02</td>
<td>7.50±0.03</td>
<td>7.51±0.04</td>
<td>7.63±0.05*</td>
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<tr>
<td>HCO₃⁻ (meq/l)</td>
<td>21.2±1.5</td>
<td>17.9±2.2*</td>
<td>13.4±1.8*</td>
<td>17.4±1.8*</td>
</tr>
<tr>
<td>PaO₂ (mm Hg)</td>
<td>91.5±2.8</td>
<td>115.8±2.0*</td>
<td>116.2±3.7*</td>
<td>99.2±9.0</td>
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<tr>
<td>PaCO₂ (mm Hg)</td>
<td>32.8±1.8</td>
<td>22.4±2.5*</td>
<td>16.5±1.9*</td>
<td>16.4±2.1*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. PRA, plasma renin activity; AVP, vasopressin. *p<0.05 compared with control period by Duncan's multiple range test.

40 minutes, respectively (Figure 1). After peak values were obtained, MAP gradually decreased and returned to baseline levels at 60-120 minutes. On the other hand, HR showed tachycardia at 90-120 minutes. Table 1 showed the effects of intracerebroventricular ET-1 on hormones and other variables. Plasma epinephrine and norepinephrine levels showed significant increases at 5 minutes, and peak values were obtained at 5-20 minutes, later returning to levels that still were significantly higher than control levels. PRA showed a small increase at 5 minutes, but did not reach a significant level. On the other hand, plasma AVP levels showed a significant increase, and a peak value was obtained at 20 minutes. Plasma osmolality, hematocrit, and serum sodium and serum potassium concentrations did not show any changes. Plasma glucose levels increased significantly at 20 and 60 minutes. Arterial blood gas analysis showed an increase in PaO₂ and a decrease in PaCO₂, whereas pH became more alkaline.

Experiment 3

After pentolinium administration, MAP fell from 80.8±5.4 to 53.3±2.8 mm Hg, and HR increased from 195.0±18.3 to 262.0±21.4 beats/min within 5 minutes. However, intracerebroventricular ET-1 failed to cause any further responses in MAP and HR, and RSNA was almost completely suppressed throughout the entire experimental period. PRA fell significantly at 5 and 20 minutes. On the other hand, plasma epinephrine, norepinephrine, and plasma AVP levels did not show any significant changes. Plasma glucose, serum sodium, serum potassium, plasma osmolality, and hematocrit also showed no changes. Arterial blood gas analysis showed a significant increase in PaO₂ and a decrease in PaCO₂ (Table 2).

Experiment 4

The same dose of intracerebroventricular ET-1 (25 pmol/kg) as that used in the intracerebroven-
Intracerebroventricular Endothelin in Rabbits

Effects of Intracerebroventricular Endothelin-1 After Pentolinium Treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
<tr>
<td>Epinephrine (pg/ml)</td>
<td>44.3±5.6</td>
</tr>
<tr>
<td>Norepinephrine (pg/ml)</td>
<td>141.8±40.9</td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>6.9±1.3</td>
</tr>
<tr>
<td>AVP (pg/ml)</td>
<td>11.6±7.3</td>
</tr>
<tr>
<td>Sodium (meq/l)</td>
<td>147.0±2.2</td>
</tr>
<tr>
<td>Potassium (meq/l)</td>
<td>3.5±0.1</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>129.0±10.7</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>31.3±0.5</td>
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<tr>
<td>Osmolality (mosm/l)</td>
<td>270.0±7.2</td>
</tr>
<tr>
<td>pH</td>
<td>7.44±0.03</td>
</tr>
<tr>
<td>HCO₃⁻ (meq/l)</td>
<td>25.2±1.9</td>
</tr>
<tr>
<td>PaO₂ (mm Hg)</td>
<td>108.3±4.6</td>
</tr>
<tr>
<td>Paco₂ (mm Hg)</td>
<td>36.2±0.8</td>
</tr>
</tbody>
</table>

Values are mean±SEM. PRA, plasma renin activity; AVP, vasopressin.

*p<0.05 compared with control period by Duncan's multiple range test.

Discussion

This is the first study to show the central effect of ET-1 on hormonal and other biochemical responses including a direct sympathetic nerve recording in conscious animals. Intracerebroventricular ET-1 caused increases in arterial pressure and RSNA as well as an elevation of plasma catecholamines. Pretreatment with pentolinium abolished these neurohormonal and cardiovascular responses. Thus, the central pressor effect of ET-1 can be attributed mainly to the enhanced sympathetic outflow. Because the intravenous injection of the same dose of ET-1 as used in the intracerebroventricular injection did not cause any cardiovascular or neuronal responses, it is unlikely that these effects were caused by a leakage of intracerebroventricular ET-1 into the systemic circulation.

We also showed an increase in plasma AVP levels but not in PRA. It has been reported that intravenous ET-1 increased plasma AVP and PRA in conscious and anesthetized dogs and that vasoconstriction of the renal vessels proximal to the juxtaglomerular cells or a reduction in the amount of sodium reaching the macula densa might be involved to activate renin release. Because intracerebroventricular ET-1 also induced increases in both arterial pressure and plasma AVP levels, it is likely that ET-1 acted on the central nervous system, such as in the hypothalamic and thalamic areas, lateral ventricular region, and subfornical organ; therefore, intracerebroventricular ET-1 might act on circumventricular organs or organs. On the other hand, a recent report demonstrated that canine basilar arteries are contracted by intracisternal injection of ET-1. We also examined the intracranial vessels in rabbits, but a vasoconstriction of the cerebral arteries might be involved in neurohormonal and cardiovascular responses to intracerebroventricular ET-1.

In conclusion, ET-1 exerts a potent central pressor action mainly mediated by the enhanced sympathoadrenal outflow, and these effects are accompanied by respiratory alkalosis, hyperglycemia, and increased plasma AVP levels. ET-1 might play an important role in central blood pressure regulation, although the physiological implications have not yet been determined.

References


KEY WORDS • endothelin • sympathetic nervous system • catecholamines • central nervous system
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