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, is a potent vasoconstrictor substance in vitro and in vivo. Binding sites for this peptide have been shown to exist in the central nervous system. Intravenous ET-1 has been reported to provoke a potent and sustained increase in blood pressure in both conscious and anesthetized rats. This pressor effect is shown to be mediated mainly by an increase in total peripheral resistance. Conversely, although a central pressor effect of ET-1 concomitant with elevation of plasma catecholamines has been reported in conscious rats, 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. 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.

**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 osmolality 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 illicit 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
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>
</thead>
<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>424.7±49.0</td>
<td>1,129.8±269.4*</td>
<td>1,132.2±199.8*</td>
<td>684.8±90.7*</td>
</tr>
<tr>
<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>
</tr>
<tr>
<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>
</tr>
<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>
</tr>
<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>
</tr>
<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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<tr>
<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>22.3±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>
</tr>
<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 epineph-rine, 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-
tricular experiment was injected intravenously. Arterial pressure, HR, and RSNA did not show any response (data not shown).

**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 induced the enhancement of the sympathetic outflow to the kidney, renin release was expected to increase. However, in the present study, increases in both arterial pressure and plasma AVP levels may suppress PRA response. Conversely, the release of AVP can be influenced by changes in plasma osmolality and blood volume. In the present study, plasma osmolality, serum sodium concentra-

tion, and hematocrit did not show any responses. In addition, because infusions of epinephrine and nor-epinephrine were reported to increase venous return, the changes in the central venous pressure were not considered to be involved in the release of AVP. Therefore, it seems that ET-1 acted on the central nervous system and evoked the release of AVP to systemic circulation.

Intracerebroventricular ET-1 also induced hyperglycemia. Because hyperglycemia has been shown to be evoked by an increase in plasma epinephrine levels, the hyperglycemia was likely attributable to the increased plasma epinephrine concentration in this study. This was confirmed by the evidence that hyperglycemia was prevented by pretreatment with pentolinium. In the present study, we also serially measured arterial blood gas. PaO_2 increased and PaCO_2 decreased, both significantly, whereas pH became alkaline. These serial changes in PaO_2 and PaCO_2 were not affected by pentolinium pretreatment, suggesting that intracerebroventricular ET-1 might stimulate the respiratory center of the central nervous system.

As described above, intracerebroventricular ET-1 induced pressor responses, increases in RSNA, and hyperglycemia, which all were attributed to the enhanced sympathoadrenal outflow, and intravenous pentolinium suppressed these responses. Although this study did not clarify what exact mechanisms are at work in pressor responses to intracerebroventricular ET-1, a receptor-mediated action of ET-1 in the central nervous system might be a candidate for the mechanisms. Binding sites for ET-1 exist in 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 tissues or organs. On the other hand, a recent report demonstrated that canine basilar arteries are contracted by intracisternal injection of ET-1. We did not examine 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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