Chronic Effects of Intracerebroventricular Endothelin-1 on Blood Pressure and Water and Sodium Handling in the Rat

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We examined the chronic effects of intracerebroventricular (ICV) endothelin on blood pressure (BP) and water and sodium metabolism in rats. Endothelin-1 (ET-1) at doses of 10, 30, 100 ng/h or artificial cerebrospinal fluid (CSF) at a dose of 1 μl/h was administered continuously into the lateral ventricle for 7 days (n=6 each). Intravenous (IV) administration of either ET-1 (100 ng/h) or saline (control) was additionally done in two other groups of rats. The highest dose of ICV ET-1 produced a mild but significant increase in systolic BP on days 6 and 7 (from 125 ± 3 to 138 ± 5 mmHg, m ± SE). It transiently increased urine volume on day 1 and day 2, and sodium excretion on day 1 without stimulating water intake. These changes were not produced by the lower doses of ICV ET-1 or ICV artificial CSF. The same dose of IV ET-1 (100 ng/h) also failed to produce these effects. On day 7, the pressor effect of the highest dose of ICV ET-1 was confirmed by direct BP measurement in conscious rats. Plasma norepinephrine level was significantly higher in the ICV ET-1 (100 ng/h) group than the control group (384 ± 61 vs. 222 ± 40 pg/ml, p < 0.05), while plasma vasopressin was similar in the two groups. Depressor response to intravenous hexamethonium (20 mg/kg) was significantly greater in the ICY ET-1 group than the control group. These results suggest that chronic ICV ET-1 elevates BP mainly due to activation of the sympathetic nervous system. Endothelin may also act on the brain to increase water and sodium excretion without stimulating water intake. (Hypertens Res 1994; 17: 23-28)

Key Words: endothelin, brain, blood pressure, water and sodium metabolism, sympathetic nervous system, vasopressin

Endothelin (ET) was originally isolated from vascular endothelial cells, and has a potent vasoconstrictive action (1). Although ET appears to play an important role in regional hemodynamics (2, 3), there is evidence from several lines of investigations suggesting a role for ET in central cardiovascular regulation. Immunoreactive ET and ET messenger RNA are present in the brain with relatively higher levels in the pituitary, brainstem, and hypothalamus (4-6). It has been shown that ET receptors are concentrated in discrete brain regions which are associated with cardiovascular and body fluid regulation, such as the subfornical organ, supraoptic nucleus, and nucleus tractus solitarii (7, 8). Investigations from several sources, including our own, have shown that acute intracerebroventricular (ICV) administration of ET elevates blood pressure (BP) through activation of the sympathetic nervous system and release of vasopressin (9-12).

However, there have been only a few studies relating to the chronic effects of ICV ET on BP (13), and no reports about the effects of chronic ICV ET on water and sodium metabolism. The chronic effects of centrally acting substances on BP and neurohumoral factors may be substantially different from their acute effects. For example, vasopressin may play an important role in acute but not in chronic hypertension induced by ICV infusions of angiotensin II and hypertonic NaCl (14, 15). To assess the chronic influences of ET within the central nervous system on cardiovascular and body fluid regulation, we examined the effects of continuous ICV ET-1 on BP and water and sodium handling in the rat. We also determined the plasma levels of catecholamines, vasopressin, and renin activity at the end of the treatment.

Methods

Experimental Procedures

Male Wistar rats aged 12-14 weeks were used for the experiments. The rats were housed individually in metabolic cages and were fed regular rat chow and tap water. The environmental conditions were...
maintained at a constant temperature and humidity with a 12-h light/dark cycle. Following a short period of acclimation to laboratory conditions, measurements of the baseline levels were established for body weight, systolic BP, heart rate, water intake, and urine volume for a period of two days.

Animals were anesthetized with ether for the surgical procedure. The skull of the rat was exposed and leveled between the bregma and lambda using a stereotaxic apparatus (Narishige, Tokyo, Japan). After the 24-h ICV infusions, body weight, systolic BP, heart rate, water intake, and urine volume were determined for a period of two days. Intra-arterial BP was monitored using a Statham P23ID transducer (Gould, Oxnard, USA) and a multichannel recorder (model 7758, Hewlett-Packard, Waltham, USA). The level of mean BP was determined when it was stabilized at least 10 min after starting the BP monitoring. Blood samples were collected into tubes containing heparin and EDTA, and were centrifuged immediately. Aliquots of plasma and urine samples were stored at -20°C until assay. Sodium concentration was measured by flame photometry (model 7775, Hitachi, Tokyo, Japan). Plasma catecholamines were assayed by high performance liquid chromatography and trihydroxyindole fluorometry. Plasma vasopressin and plasma renin activity were determined by radioimmunoassay. The intra-assay coefficient of variation for these assays was less than 10%, and the inter-assay coefficient of variation was less than 12%.

All data were expressed as mean±SEM. Statistical analysis was performed by two-way analysis of variance and subsequent Tukey's multiple comparison test for serial data and data of three or more groups. Comparisons between the two groups were made using Student's t test when appropriate. A value of p < 0.05 was considered statistically significant.

**Results**

Table 1 shows the effects of the 7-day ICV infusions of artificial CSF (control group) or ET-1 at doses of 10, 30, and 100 ng/h on systolic BP and heart rate. The highest dose of ICV ET-1 did not change BP during the first several days, but elevated BP significantly on days 6 and 7. The chronic ICV infusions of artificial CSF alone or lower doses of ET-1 did not change BP significantly during the infusion period. On days 6 and 7, systolic BP was higher in the 100 ng/h ET-1 group than the control group. There were no significant changes in heart rate in either the ET-1 groups or the control group.

Body weight decreased in all the groups after starting the ICV infusions as a result of the surgical stress (Table 2). During later periods of the ICV infusions, body weight returned to the baseline level, although the recovery was delayed in the group which was given the highest dose of ICV ET-1. Daily water intake decreased on days 1 and 2 in all the groups, and then it returned to baseline level. As shown in Table 3, urine volume increased sig-
significantly on days 1 and 2 during the ICV infusions of 100 ng/h of ET-1, and then it returned to baseline level. In contrast, urine volume did not change during the infusion period in the control group or in the groups given lower doses of ET-1. In the ICV 100 ng/h ET-1 group, urinary sodium excretion increased significantly on day 1 but decreased on days 2 and 3. Only a decrease in urinary sodium excretion was observed in the other 3 groups.

The effects of IV infusions of ET-1 at a dose of 100 ng/h are shown in Table 4. Chronic IV ET-1 treatment failed to produce the hypertensive, diure-

tic, or natriuretic responses which were seen during the ICV infusions of the same dose of ET-1. Chronic IV infusions of isotonic saline also did not change systolic BP during the infusion period.

On day 7, intra-arterial mean BP was significantly higher in the ICV 100 ng/h ET-1 group than the control group (128 ± 3 vs. 116 ± 3 mmHg, Fig. 1). Although the low dose ET-1 groups showed slightly higher BP than the control group, the differences were not statistically significant.

Figure 2 shows plasma levels of norepinephrine, epinephrine, vasopressin, and renin activity at the
end of ICV infusions of ET-1 at a dose of 100 ng/h or artificial CSF. Plasma norepinephrine level was significantly higher in the ET-1 group than the control group (384 ± 61 vs. 222 ± 40 pg/ml, p < 0.05). Although plasma epinephrine was higher, and plasma renin activity was lower in the ICV ET-1 group, these changes were not statistically significant. Plasma vasopressin levels were similar between the ET-1 group (0.80 ± 0.06 pg/ml) and the control group (0.78 ± 0.08 pg/ml). Plasma norepinephrine levels in the lower ET-1 infusion groups (10 ng/h: 236 ± 46 pg/ml, 30 ng/h: 281 ± 49 pg/ml) were not significantly different from the control group.

Intravenous administration of hexamethonium caused greater depressor response in the ICV ET-1 (100 ng/h) group than in the control group (-68 ± 4 vs. -58 ± 3 mmHg, p < 0.05). The nadir of mean BP was similar in the ET-1 group (62 ± 2 mmHg) and the control group (60 ± 2 mmHg).

Blood pressure and plasma levels of catecholamines, vasopressin, and renin activity after one-day ICV infusion of ET-1 (100 ng/h) or artificial CSF are shown in Table 4. There were no significant differences in these variables between the two groups.

### Table 4. Effects of Chronic Intravenous ET-1 (100 ng/h) on Blood Pressure, Heart Rate, Urine Volume, and Urinary Sodium Excretion

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>130 ± 5</td>
<td>128 ± 4</td>
<td>130 ± 5</td>
<td>122 ± 5</td>
<td>124 ± 6</td>
<td>128 ± 6</td>
<td>128 ± 4</td>
<td>130 ± 5</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>389 ± 18</td>
<td>365 ± 14</td>
<td>378 ± 20</td>
<td>375 ± 18</td>
<td>383 ± 13</td>
<td>399 ± 10</td>
<td>393 ± 22</td>
<td>390 ± 15</td>
</tr>
<tr>
<td>Urine volume (ml/day)</td>
<td>11 ± 1</td>
<td>10 ± 2</td>
<td>11 ± 1</td>
<td>9 ± 2</td>
<td>10 ± 1</td>
<td>12 ± 1</td>
<td>11 ± 1</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>Urinary sodium excretion (meq/day)</td>
<td>1.2 ± 0.1</td>
<td>1.1 ± 0.2</td>
<td>0.8 ± 0.2*</td>
<td>0.8 ± 0.2*</td>
<td>1.0 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>1.0 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
</tbody>
</table>

**Discussion**

In the present study, 7-day administration of ET-1 at a dose of 100 ng/h into the brain ventricle of the rat elevated BP in the late stages. The high BP in the ET-1 treated rats was associated with an increase in plasma norepinephrine and a greater depressor response to intravenous hexamethonium. The same dose of intravenously administered ET-1 failed to increase BP. These results are consistent with a report by Nishimura et al. (13), and suggest that ET within the central nervous system may have a chronic influence on BP regulation.

Several investigations, including our own previous studies, have shown that ET-1 administered into the brain ventricle elevates BP acutely in a dose-dependent manner (9-12). The acute pressor response to ICV ET-1 appears to be mediated by the sympathetic nervous system and vasopressin, since it was accompanied by increases in circulating catecholamines (10, 12) and vasopressin (12, 17), and was inhibited by sympatholytic agents (9, 10) and a vasopressin V₁ antagonist (9, 17). The central cardiovascular effect of ET can be differentiated clearly from the peripheral effect because the latter elevates BP through direct vasoconstriction (1) and requires much larger doses to produce a significant pressor response (9, 10).

It has been shown that immunoreactive ET (4-6),
ET messenger RNA (4), and ET binding sites (7, 8) are present in the brain. We observed that the contents of immunoreactive ET-1 in several brain regions were different between the spontaneously hypertensive rat and the normotensive Wistar-Kyoto rat (5). Koseki et al. (7) and Niwa et al. (8) reported that ET receptors are concentrated in those regions of the brain which are involved in cardiovascular and body fluid regulation, such as the subfornical organ, supraoptic nucleus in the hypothalamus, and nucleus tractus solitarii in the medulla oblongata in the rat. These findings suggest that endogenous ET in the central nervous system may play a role in cardiovascular regulation.

The increase in BP caused by the chronic ICV infusion of ET-1 was relatively small in our study. Nishimura et al. (13) reported that BP rose during the late stages of 7-day ICV ET-1 treatment at a dose of 10 pmol/h (about 25 ng/h) in the rat. In our present study, the pressor effect of ET-1 was significant only at a dose of 100 ng/h, but not at the lower doses (10, 30 ng/h). Although we did not examine the effects of ICV ET-1 for more than 7 days, we studied the effects of a larger dose (300 ng/h) of chronic ICV ET-1 in six rats (unpublished data). At this dose, one half of the group of rats died within the first 24 h, and the remaining rats failed to produce sustained hypertension during the 7 days of the infusion period. Therefore, the increase in brain ET-1 may be able to produce a chronic elevation of BP but may not be enough to produce prominent hypertension in intact normotensive rats.

The reason why chronic ICV infusion of ET-1 could produce only a mild increase in BP is not clear from the present study, but there are several possibilities. It is known that there are alterations in renal function in most models of experimental hypertension (18). Since chronic ICV ET-1 was given to normotensive Wistar rats in our study, the normal kidneys may have acted against a larger increase in BP. In the present study, urinary sodium excretion increased at the early phase of ICV ET-1. The loss of sodium also may have led to inhibition of the development of hypertension during chronic ICV ET-1. Another possibility is suggested by evidence of both pressor and depressor actions of ET in the brain. ET may produce a sustained reduction in BP after a brief pressor episode when administered into the cisterna magna or to the ventral surface of the rostral medulla (19). The mild elevation in BP caused by chronic ICV ET-1 might represent the net effect of the pressor and depressor actions.

Our study suggests that the sympathetic nervous system plays an important role in the chronic pressor effect of ICV ET-1 since plasma norepinephrine level at the end of the infusion period was significantly higher in the high dose ET-1 group than the control group, and the depressor effect of hexamethonium was greater in the ET-1 group. Although vasopressin contributes to the acute pressor response to ICV ET-1 (9, 12), its role may diminish in the chronic stage because of the normal plasma vasopressin levels attained at the end of the 7-day ICV infusion of ET-1. The peripheral renin-angiotensin system does not appear to contribute to the BP elevation since plasma renin activity tended to be suppressed by chronic ICV ET-1 treatment. In this respect, our results are not consistent with the earlier report of Nishimura et al. who observed increases in both urinary catecholamines and urinary vasopressin during the late stages of chronic ICV ET-1 treatment (13). Although the reason for the differences in vasopressin response between our study and theirs is not clear, an increased sympathetic outflow may have been primarily responsible for the chronic elevation in BP during ICV infusion of ET-1. It has been shown that vasopressin plays a role in the acute but not in the chronic phase of hypertension induced by ICV infusion of angiotensin II (14) or hypertonic NaCl (15).

Although ET-1 is a potent vasoconstrictor, the chronic IV infusion of ET-1 at a dose of 100 ng/h did not affect BP in this study. It has been shown that chronic IV ET-1 elevates BP in rats (20, 21); however, higher doses (250–1,000 ng/h) were used in those studies. Our results indicate that the pressor effect of chronic ICV ET-1 is not due to the leakage of ET-1 into the peripheral circulation but is caused by its action within the brain.

In the present study, the high dose (100 ng/h) of ICV ET-1 increased urine volume and urinary sodium excretion without an increase in water intake in the early phase of the 7-day infusion period. Since BP did not rise at this phase, these responses could not be attributed to the pressor diuresis and natriuresis. The exact mechanisms of the diuretic and natriuretic responses are uncertain. However, suppression of the renin-angiotensin system might contribute to the ET-induced natriuresis since plas-

**Table 5. Blood Pressure, Heart Rate, and Neurohormonal Variables after 1-Day ICV Infusions of Artificial CSF (Control) or ET-1 (100 ng/h)**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ET-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean blood pressure (mmHg)</td>
<td>121 ± 4</td>
<td>120 ± 6</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>357 ± 7</td>
<td>340 ± 10</td>
</tr>
<tr>
<td>Plasma norepinephrine (pg/ml)</td>
<td>316 ± 69</td>
<td>317 ± 66</td>
</tr>
<tr>
<td>Plasma epinephrine (pg/ml)</td>
<td>1,048 ± 172</td>
<td>1,076 ± 134</td>
</tr>
<tr>
<td>Plasma vasopressin (pg/ml)</td>
<td>1.5 ± 0.4</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>Plasma renin activity (ng/ml/h)</td>
<td>9.8 ± 2.4</td>
<td>7.0 ± 1.4</td>
</tr>
</tbody>
</table>

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The role of brain ET in body fluid regulation is not well understood. Samson et al., have shown that acute ICV ET-3 inhibits water drinking stimulated by hyperosmolality and angiotensin II (23). However, brain ET does not appear to play an important role in the drinking behavior because the daily water intake was similar in the ICV ET-1 groups and the control group. The central effect of ET on drinking behavior is also different from other centrally acting pressor agents, such as angiotensin II, carbachol, and hypertonic NaCl, since these agents have dipsogenic action.

In conclusion, our study indicates that chronic ICV ET-1 elevates BP with activation of the sympathetic nervous system. ET may also act on the brain to increase water and sodium excretion without stimulating water intake during the early phase of chronic ICV infusion.

References


