Modulatory Effects of Endothelin-1 on Central Cardiovascular Control in Rats

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Abstract In urethane-anesthetized and immobilized rats, modulatory effects of endothelin-1 (ET-1) on central cardiovascular control were examined. An injection of 0.1 pmol of ET-1 into the cisterna magna caused immediate increases in arterial pressure (AP), renal sympathetic nerve activity (RSNA), and heart rate (HR) that lasted for 5–45 min. At doses of 1 and 10 pmol, intracisternal ET-1 elicited initial increases (phase I) followed by decreases in these variables below the pre-injection level (phase II). At the dose of 1 or 10 pmol, the arterial baroreceptor reflex was suppressed during the latter part of phase I and during phase II. The three variables subsequently returned to, or often exceeded, pre-injection levels in 30 to 60 min and reflex activity recovered (phase III). However, AP often remained below control throughout the 2-h observation period. Essentially identical responses to intracisternal ET-1 were observed in unanesthetized precollicular decerebrated or urethane-anesthetized rats. Application of a piece of filter paper soaked with 1 pmol of ET-1 to the ventral surface of the medulla (VSM) caused the pattern of changes similar to that following intracisternal injection. A microinjection of 4 pmol of ET-1 into the nucleus tractus solitarius (NTS) caused a moderate increase in RSNA with a minute fall in AP. Intrathecal administration of ET-1 resulted in moderate changes in AP and RSNA at the dose as high as 100 pmol. We conclude that intracisternally administered ET-1 modulates tonic and reflex control of AP and sympathetic vasomotor activity and that the VSM appears to be involved critically in this modulation.

Key words: endothelin-1, arterial pressure, renal sympathetic nerve activity, arterial baroreceptor reflex, ventral surface of the medulla.

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Endothelin (ET) refers to a family of endogenous vasoactive peptides which consists of three isoforms termed ET-1, -2, and -3 (INOUE et al., 1989). Of these, ET-1 is most richly distributed in various tissues of the rat including the vascular endothelium, lung, intestine, and brain (MATSUMOTO et al., 1989). When the vascular effect of ET-1 and ET-3 is compared, ET-1 elicits greater vasoconstriction and its threshold dose for this response is much smaller (YANAGISAWA et al., 1988). Furthermore, the presence of ET-1 mRNA has been demonstrated by in situ hybridization in rat tissues including the brain (MACCUMBER et al., 1989; YOSHIZAWA et al., 1990). These results suggest the role of ET in the central nervous system (CNS) as a regulatory peptide (YOSHIZAWA et al., 1990) and that ET-1 can be more influential than ET-3.

We previously reported that intracisternally administered ET-3 markedly modulated the tonic and reflex control of arterial pressure (AP) and sympathetic discharges by the CNS (KUWAKI et al., 1990). We further demonstrated that local application of ET-3 to the ventral surface of the medulla (VSM) resulted in a similar pattern of cardiovascular and sympathetic vasomotor responses as that following intracisternal administration.

The present study was intended to investigate modulatory effects of intracisternally administered ET-1 on the central cardiovascular control. Our specific objectives were to examine (a) the effect of intracisternal ET-1 on the tonic control of AP and sympathetic vasomotor discharges, (b) the modulatory effect of intracisternal ET-1 on the arterial baroreceptor reflex, and (c) the effect of local application of ET-1 on CNS sites in the lower brainstem which were responsive to ET-3. The general experimental scheme and methods employed were basically the same as described in our previous paper (KUWAKI et al., 1990).

MATERIALS AND METHODS

Since experimental methods in this study are almost identical to those described in our previous paper (KUWAKI et al., 1990), this section is only briefly outlined.

Preparation of animals. Experiments were performed on 54 male Sprague-Dawley rats weighing between 300 and 400 g. Except for five rats in which precocillicular decerebration was performed (see below), they were anesthetized with an intraperitoneal injection of urethane (initially 1 g/kg). When necessary, supplementary doses were intravenously given. After insertion of tracheal, arterial, and venous cannulas, the animal was paralyzed with gallamine triethiodide (initially 10 mg/rat i.v., thereafter 6–8 mg/h, i.v.) and artificially ventilated with oxygen-enriched room air. End-tidal $P_{CO_2}$ was continuously monitored and maintained between 3 and 4.5%. Body temperature was maintained between 37 ± 0.5°C.

Measurement of cardiovascular variables and renal sympathetic nerve discharges. Instantaneous and mean arterial pressures (APs), heart rate (HR), and electrocardiogram were monitored continuously in all experiments. AP was recorded from the abdominal aorta. HR was computed from the AP pulse by a tachometer.
The left renal sympathetic nerve was approached retroperitoneally, and prepared for recording from near the renal artery. To record its efferent discharges, the central cut end of the nerve was placed on bipolar Ag hook electrodes connected to an amplifier and displayed on an oscilloscope. The upper and lower cut-off frequencies of the recording system were 100 and 3,000 Hz, respectively. Renal sympathetic nerve discharges were rectified, integrated over a 10-s interval and stored in a tape recorder. The level of the instrumentation noise was determined at the end of the experiment and integrated over 10-s intervals. When the recorded nerve signal was reproduced for processing, it was subtracted by the integrated noise level. Renal sympathetic nerve discharges quantitated in this manner were called renal sympathetic nerve activity (RSNA). RSNA was used as a measure of sympathetic vasomotor activity, since it accurately reflects activity of sympathetic vasoconstrictor fibers (Dorward et al., 1986).

Intracisternal and topical applications of ET-1. Rats were placed prone in a stereotaxic frame with the bit bar set at −12 mm. The needle of a microsyringe carried in a stereotaxic micromanipulator was advanced through the exposed atlanto-occipital membrane and the tip was placed within the cisterna magna. Either vehicle (artificial cerebrospinal fluid, pH = 7.4; Lindvall et al., 1978), or ET-1 (Peptide Institute, Osaka) dissolved in 10 μl of artificial cerebrospinal fluid was injected into the cisterna magna. The injected solution contained 0.5% of Evans Blue to check, after the experiment, the extent of the brain area where the drug solution reached. Doses of ET-1 employed for the intracisternal injection were 0.1, 1, or 10 pmol.

In order to determine CNS sites responsive to ET-1, it was topically administered to the nucleus tractus solitarius (NTS), VSM, and spinal cord. The method of application of the drug was almost exactly the same as in our previous paper. In short, ET-1 was injected into the NTS by micropipettes connected by polyvinyl tubing to the pressure source of 0.7–1.5 atm. The desired volume (200 nl) was injected by adjusting manually the opening time of a solenoid valve. The VSM was exposed through a ventral approach for topical application of ET-1 and a piece of filter paper (1 × 1 mm in size) soaked with ET-1 solution was placed on it. ET-1 was given to the spinal cord by intrathecal administration through a polyethylene catheter (o.d. = 0.5 mm) passed into the subarachnoid space via an incision in the atlanto-occipital membrane. It was advanced caudally for 5–7 cm so that the tip lay at Th5–L1.

Precocollular transection of the brainstem. Under halothane anesthesia, the dorsal surface of the midbrain was exposed and the brainstem was totally transected at the precocollular level. The brain tissue rostral to transection was removed by aspiration. After the anesthesia was discontinued, a 2-h intermission was interposed to eliminate the effect of halothane.

Assessment of activity of the arterial baroreceptor reflex. The arterial baroreceptor reflex was compared before and after intracisternal administration of ET-1 by one of the following two methods. (i) The amplitude of the cardiac-related
changes of the rectified renal sympathetic discharges was determined by its post-R-wave histogram and used to assess the reflex activity. The cardiac-related changes in sympathetic discharges are known to represent baroreceptor-initiated sympathoinhibition (GEBBER and BARMAN, 1980). Since this method is valid as far as levels of AP and RSNA would not change drastically, it was applied to examine the reflex activity during phase I. (ii) During phase II, when AP often diminished below the threshold pressure of the arterial baroreceptor reflex, the reflex activity was assessed by the change in RSNA in response to a bolus i.v. injection of phenylephrine (1–10 μg) (see RESULTS for definition of phases I and II).

Analysis of data and histological examination. While reproducing mean AP (MAP), HR, and RSNA, they were sampled at the rate of 1 Hz by an analog-to-digital converter (CANOPUS Electronics ADX-98H). The mean values of these variables over the period of successive 1- or 5-min intervals were then calculated by a computer (NEC PC-9801RX). The baseline level was defined as the average of each variable over the 10-min interval immediately before administration of ETs.

Statistical analysis of the results was carried out using Student’s t-test for paired data before and after administration of ET-1 or that for unpaired data between two groups of experiments. To compare the slope of regression lines, analysis of covariance was used. Differences in data were considered to be significant when \( p < 0.05 \). Results were expressed as mean ± S.E.

At the end of each experiment, the animal’s brain was fixed and excised. Histological examinations were done as previously described to identify the sites of injected dye.

RESULTS

Effects of intracisternal injection of ET-1 on AP, RSNA, and HR

An intracisternal injection of 0.1 pmol of ET-1 resulted in an immediate and modest increases in AP, RSNA, and HR (Fig. 1B and Table 1). The response lasted for 5–45 min and each variable returned thereafter to the pre-injection level. The duration of the ET-induced pressor response was \( 21 \pm 7 \) min (\( n = 6 \)). At the dose of 1 or 10 pmol, however, this initial response was curtailed by falls in the three variables below the control level (Fig. 1C and Table 1). As in our previous study (KUWAKI et al., 1990), the period of initial and subsequent responses was termed phases I and II, respectively.

Phase II was usually terminated in 30–60 min and AP, RSNA, and HR exceeded the baseline levels. However, these variables, especially AP, sometimes remained below the control level until the end of the 2-h observation period. In those experiments in which AP returned to the baseline level within 2 h, the duration of the depressor response was \( 50 \pm 5 \) min (\( n = 4 \)). On the other hand, intracisternal administration of artificial cerebrospinal fluid as vehicle did not result in changes in AP, RSNA, and HR corresponding to phase I or II (Fig. 1A).

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Table 1. Peak changes in cardiovascular variables and renal sympathetic nerve activity induced by intracisternal administration or topical application of ET-1.

<table>
<thead>
<tr>
<th>DOSE (pmol)</th>
<th>n</th>
<th>MAP (mmHg)</th>
<th>RSNA (% of control)</th>
<th>HR (beats/min)</th>
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<tr>
<td></td>
<td></td>
<td>Control</td>
<td>$\Delta$MAP</td>
<td>Control</td>
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<td></td>
<td></td>
<td>Max in</td>
<td>Min in</td>
<td>Max in</td>
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<td></td>
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<td>phase I</td>
<td>phase II</td>
<td>phase I</td>
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<td>Intracisternal injection in urethane-anesthetized rats</td>
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<tr>
<td>0.1</td>
<td>6</td>
<td>98 ± 4</td>
<td>+8 ± 2*</td>
<td>443 ± 17</td>
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<td></td>
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<td></td>
<td></td>
<td>+12 ± 5</td>
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<td>1</td>
<td>6</td>
<td>98 ± 5</td>
<td>+9 ± 6</td>
<td>471 ± 16</td>
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<td></td>
<td></td>
<td></td>
<td>-49 ± 6</td>
<td>+9 ± 4*</td>
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<tr>
<td>10</td>
<td>6</td>
<td>97 ± 10</td>
<td>+7 ± 4</td>
<td>497 ± 8</td>
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<tr>
<td>(ACSF)</td>
<td>6</td>
<td>93 ± 5</td>
<td></td>
<td>442 ± 25</td>
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<td>Intracisternal injection in unanesthetized and precollricular decerebrated rats</td>
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<tr>
<td>1</td>
<td>5</td>
<td>96 ± 7</td>
<td>+14 ± 6</td>
<td>505 ± 10</td>
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<td></td>
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<td></td>
<td>-53 ± 6</td>
<td>+8 ± 3</td>
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<tr>
<td>Topical application to the VSM in urethane-anesthetized rats</td>
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<tr>
<td>1</td>
<td>5</td>
<td>85 ± 4</td>
<td>+19 ± 9</td>
<td>512 ± 5</td>
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<td></td>
<td></td>
<td></td>
<td>-31 ± 8</td>
<td>+16 ± 5*</td>
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Values are means ± S.E. n = number of experiments. Abbreviations: ACSF, artificial cerebrospinal fluid; ET-1, endothelin-1; HR, heart rate; MAP, mean arterial pressure; RSNA, renal sympathetic nerve activity; VSM, ventral surface of the medulla. $\Delta$ denotes change in each variable from control. For definition of phases I and II, see text. Significance of difference from the pre-injection control value is denoted: * $p<0.05$; ** $p<0.01$ (paired $t$-test).
Fig. 1. Polygraph records illustrating responses of arterial pressure (AP), renal sympathetic nerve activity (RSNA), and heart rate (HR) to intracisternal (i.c.) administration of artificial cerebrospinal fluid (ACSF; A) or 0.1 pmol (B) or 10 pmol (C) of ET-1.

Summarized in Table 1 are peak increases in mean AP (MAP), RSNA, and HR during phase I, and peak decreases in them during phase II in response to intracisternal administration of ET-1. At the dose of 0.1 pmol, peak increases in MAP and RSNA from control were both statistically significant. At the dose of 1 or 10 pmol of ET-1, peak decreases in the three variables from control were all statistically significant.

At the doses of 1 and 10 pmol of ET-1, initial increases in RSNA and HR were observed in all the 12 rats, whereas that of MAP occurred in all but two cases. However, these increases in MAP and RSNA were not statistically significant (Table 1). At these doses, phase I was terminated by sudden and drastic decreases in these variables (Fig. 1C). Since the time of initiation of phase II following the drug

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administration varied considerably among the rats (range 2–19 min; n=12), individual variations of peak increases in these variables were greater than those at the dose of 0.1 pmol. Consequently, peak increases in MAP and RSNA at these doses of ET-1 were statistically insignificant, although their mean values were comparable to or even greater than those at 0.1 pmol. The brain surface stained with Evans Blue simultaneously injected with ET-1 usually extended to the dorsal, lateral, and ventral aspects of the brainstem, the ventral aspect of the cerebrum, and the upper segments of the spinal cord. The surface of the cerebroventricular space including the floor of the fourth ventricle was not visibly stained.

Effect of precollicular decerebration on the response to ET-1

In five precollicular decerebrated rats, 1 pmol of ET-1 was injected intracisternally. The pattern of changes in AP, RSNA, and HR (Fig. 2) and peak changes in the three variables (Table 1) were similar to those in urethane-anesthetized rats with intact neuraxis. Actually, between unanesthetized precollicular decerebrated and urethane-anesthetized rats, there was no statistically significant difference in peak changes of each variable induced by 1 pmol of intracisternal ET-1. The difference in pre-injection values between these two groups of rats was statistically insignificant either (p > 0.05, unpaired t-test). In the present experiments, therefore, it appeared that urethane did not seriously distort cardiovascular and sympathetic vasomotor responses induced by ET-1. For this reason, and for its relatively stable and long-lasting anesthetic effect, urethane was used as general anesthesia in the present experiments.

Effects of intracisternal ET-1 on the arterial baroreceptor reflex

In 25 rats, the effect of intracisternal ET-1 on the arterial baroreceptor reflex was examined by the two methods explained in MATERIALS AND METHODS.

![Graph](image-url)

**Fig. 2.** Effects of intracisternal administration of 1 pmol of ET-1 on mean AP (MAP), RSNA, and HR in an unanesthetized and precollicular decerebrated rat. The three variables were expressed as percent of pre-injection values.
An injection of 0.1 pmol of ET-1 did not conspicuously affect the amplitude of the cardiac-related changes in rectified renal sympathetic discharges. Actually, the amplitude of the cardiac-related changes relative to the pre-injection control during the former and latter halves of phase I were $101 \pm 5$ and $102 \pm 7\%$ ($n=6$), respectively. These values were not significantly different from the pre-injection value.

At the dose of 1 or 10 pmol, their amplitude was conspicuously reduced during the latter half of phase I (Fig. 3A, b). At the dose of 1 pmol of ET-1, the relative amplitude of the cardiac-related changes during the former and latter halves of

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Fig. 3. Inhibition of the arterial baroreceptor reflex by intracisternal administration of 10 pmol of ET-1. In A, cardiac-related fluctuations of rectified renal sympathetic nerve discharges (RSND), obtained as the post R-wave time histogram over 32 successive sweeps, were determined during the control period (a; MAP = 100 mmHg), latter part of phase I (b; MAP = 130 mmHg), phase II (c; MAP = 50 mmHg), and phase III (d; MAP = 100 mmHg) and shown along with electrocardiogram (ECG). In B, the relationship between the reflex changes in RSNA and alterations of MAP induced by graded bolus injection of phenylephrine, both expressed as the change from pre-injection level, was obtained during the control period (solid line) and phase II (broken line). In B, data points were collected from four experiments.
phase I was 106±5 and 41±9% (n=6), respectively. The corresponding values at the dose of 10 pmol of ET-1 were 110±6 and 32±4% (n=6), respectively. At both doses, the values during the former half of phase I were not significantly different from pre-injection values, whereas those during the latter half were significantly smaller (p<0.01).

During phase II following intracisternal administration of 1 or 10 pmol of ET-1, the cardiac-related changes were not detected because MAP decreased below the threshold pressure of the arterial baroreceptor reflex (Fig. 3A, c). Therefore, reflex changes in RSNA induced by bolus intravenous injections of phenylephrine at different doses were plotted against alterations in MAP. When such data were

![Graph A: ET-1 to VSM](image)

**Fig. 4.** A: Effects of topical application of ET-1 to the ventral surface of medulla (VSM). A piece of filter paper (1 x 1 mm) soaked with artificial cerebrospinal fluid containing 1 pmol of ET-1 was placed on the exposed VSM as shown by the dotted area in B. Abbreviations in this and following figures are as follows. Basilar a., basilar artery; DMN, dorsal motor nucleus of vagus nerve; ION, inferior olivary nucleus; LRN, lateral reticular nucleus; NA, nucleus ambiguus; NTS, nucleus of solitary tract; NV, spinal nucleus of trigeminal nerve; NXII, nucleus of hypoglossal nerve; Pyr, pyramidal tract; TS, solitary tract; V, trigeminal nerve; X, vagus nerve; XII, hypoglossal nerve.
pooled from three rats to which 1 pmol of ET-1 was administered, the slope of the regression line was $-0.22 \text{ mmHg}^{-1}$ during phase II as against $-0.95 \text{ mmHg}^{-1}$ during the pre-injection period. The difference was statistically significant ($p < 0.01$). Likewise, with respect to the pooled data from four rats that were given 10 pmol of ET-1, the corresponding values were $-0.26$ and $-0.90 \text{ mmHg}^{-1}$, respectively (Fig. 3B). The difference was statistically significant ($p < 0.01$). During phase III, the reflex was restored as evidenced by reappearance of the cardiac-related rhythm in RSNA (Fig. 3A, d). In summary, at the dose of 1 or 10 pmol of intracisternal ET-1, the arterial baroreceptor reflex was inhibited during the latter half of phase I and during phase II.

**Effects of local CNS application of ET-1**

We sought to determine CNS sites possibly involved in the cardiovascular and

**ETs into NTS**

**A. ET-1 + ET-3**

![Graph A](image1)

**B. ET-3 + ET-1**

![Graph B](image2)

**Fig. 5. Comparison of effects of local microinjection of ET-1 and ET-3 into the NTS.**

In A, ET-1 was injected into the left NTS at the moment indicated by the vertical broken line. Forty-five min after that, ET-3 was injected into the right NTS. In B, ET-3 was injected into the left NTS first. Forty min after that, ET-1 was given to the contralateral NTS. The amount of injected ET was always 4 pmol in 200 nl of artificial cerebrospinal fluid. Changes from pre-injection values in MAP, RSNA, and HR were expressed in percent and illustrated on the left panels. Injection sites in each experiment were shown by the shaded area on the frontal section of the medulla at the obex level.
sympathetic responses to an intracisternal administration of ET-1. The VSM, NTS, and spinal cord were examined, since these sites were all responsive to ET-3 (Kuwaki et al., 1990).

Application of a piece of filter paper soaked with 1 pmol of ET-1 in five rats resulted in a pattern of changes in AP, RSNA, and HR consisting typically of phases I–III (Fig. 4), just as following an intracisternal administration. Increases in AP, RSNA, and HR during phase I were observed in all rats. However, increases in MAP and RSNA were statistically not significant (Table 1). On the other hand, decreases in AP and RSNA during phase II were statistically significant. Although the actual amount of ET-1 released from the filter paper and acting on the VSM cannot be determined, we can still say that topical application of ET-1 to the VSM elicits the pattern of cardiovascular and sympathetic vasomotor changes similar to that following an intracisternal injection. Topical application of the filter paper soaked in artificial cerebrospinal fluid to the VSM in three rats did not produce appreciable changes in the three variables.

An injection of ET-1 into the left NTS at a dose of 4 pmol resulted in a long-lasting but moderate increase in RSNA with a little change in AP (Fig. 5A). This pattern of changes was not identical to that following intracisternal administration or topical application to the VSM. Forty-five min after the injection, changes in RSNA and MAP from control were 16 ± 5% and −10 ± 4 mmHg (n = 5), respectively. Increase in RSNA was statistically significant (p < 0.05). Absence of

![Diagram of ET-1 100 pmol i.th.](image_url)

Fig. 6. Effects of intrathecal administration of ET-1 (100 pmol) observed in three rats. Curves represent mean values and vertical bars are S.E.
drastic changes does not seem to be ascribable to an inappropriate site of microinjection, since a subsequent injection of 4 pmol of ET-3 into the corresponding site in the contralateral NTS resulted in substantial increases in RSNA and MAP. Furthermore, in separate experiments in 2 rats, 4 pmol of ET-3 was first microinjected into the left NTS and increases in RSNA and AP were elicited. A subsequent injection of ET-1 into the contralateral NTS did not cause conspicuous changes (Fig. 5B). In these experiments, simultaneously injected Evans Blue stained the area around the tractus solitarius usually covering dorsal, medial, and ventrolateral subnuclei of the NTS (Kalia et al., 1984). Considering a relatively large amount of locally administered ET-1 and modest responses to it, it appears that, as far as the regulation of AP and RSNA is concerned, ET-1 is not very influential on the NTS.

Finally, intrathecal injection of 100 pmol of ET-1 into the spinal cord in three rats elicited modest changes in AP, RSNA, and HR. The pattern of these changes was different from that following intracisternal administration (Fig. 6).

**DISCUSSION**

Major findings of the present study are summarized as follows. In urethane-anesthetized and paralyzed rats, an intracisternal injection of 0.1 pmol of ET-1 caused a pressor response with sympathoexcitation and tachycardia. At a dose of 1 or 10 pmol, a subsequent depressor response with sympathoinhibition and bradycardia appeared. The same pattern of responses was produced in unanesthetized and decerebrated rats. In addition to modulation of tonic control of cardiovascular and sympathetic vasmotor activity by the CNS, 1 or 10 pmol of intracisternally administered ET-1 modulated the reflex control as evidenced by suppression of the arterial baroreceptor reflex. Topical application of ET-1 to the VSM elicited the same pattern of changes in AP, RSNA, and HR as that following intracisternal administration, whereas microinjection into the NTS or intrathecal administration did not result in this particular pattern of changes.

Although ET-1 and ET-3 are both members of the same ET family, distinct receptors have been demonstrated for the two isopeptides (Kloog et al., 1989; Masuda et al., 1989). Distribution of their binding sites and tissue contents within various portions of the rat's brain differ considerably between ET-1 and ET-3 (Fuxe et al., 1989; Matsumoto et al., 1989). Nevertheless, as demonstrated in our present and previous studies (Kuwaki et al., 1990), intracisternal administration of ET-1 and ET-3 elicited a very similar pattern of changes in AP, RSNA, and HR. ET-1, however, was much more potent in eliciting these changes than ET-3. Namely, the dose of ETs that caused a pressor response with sympathoexcitation and tachycardia was 0.1 pmol with ET-1 and 10 pmol with ET-3. On the other hand, 1 or 10 pmol of ET-1 and 100 pmol of ET-3 both elicited changes in AP, RSNA, and HR characterized by phases I and II. As discussed below, similarity of the response pattern and difference in the potency appears to be primarily attributable to the responsiveness of the VSM to the two peptides.
Precollicular decerebration did not seriously alter cardiovascular and sympathetic changes induced by ETs. Furthermore, intracisternally administered ET-1 and ET-3 both affected tonic sympathetic discharges as well as the arterial baroreceptor reflex. However, the effect of intrathecal injection of these peptides on AP, RSNA, and HR was minute, if any. Consequently, it was expected that the major effective CNS site of intracisternally administered ETs met the following criteria. (i) It was within the midbrain or ponto-medullary region. (ii) It participated in generation or regulation of tonic sympathetic discharges. (iii) It was located along the central pathway subserving or modulating the arterial baroreceptor reflex. The VSM, a restricted area in the ventrolateral surface of the medulla oblongata (Bruce and Cherniack, 1987) which is functionally and neuroanatomically associated very closely to the rostral ventrolateral medulla (Benarroch et al., 1986), meets all these criteria. Namely, mechanically or pharmacologically induced impairment of the VSM results in a marked decrease in AP with sympathoinhibition ( Guerrizentstein and Silver, 1974; Wennergren and Öberg, 1980) and impairs the arterial baroreceptor-sympathetic vasomotor reflex (Saeki et al., 1988). As expected, topical application of 1 pmol of ET-1 or 40 pmol of ET-3 to the VSM resulted in the pattern of changes almost identical to those following intracisternal injection, although the actual amount of ETs that was released from the filter paper topically applied to the VSM and that acted on the VSM was unknown.

On the other hand, a local microinjection of ET-1 into the NTS resulted in no more than a moderate increase in RSNA with a small decrease in AP. The result is rather unexpected in view of abundant presence of binding sites for ET-1 in the rat’s NTS (Koseki et al., 1989). During the course of this study, however, we recognized that simultaneously recorded phrenic nerve discharges increased drastically (unpublished observation). Conceivably, ET-1 acts primarily on the NTS neurons involved in the respiratory control, whereas ET-3 is effective to those related to sympathetic vasomotor control.

In the excised spinal cord of the newborn rat, ET-1 added to the perfusion medium caused depolarization of the ventral root potential in a dose-dependent manner (Yoshizawa et al., 1989). Presence of ET-1 mRNA in certain regions of the spinal cord including the intermediolateral nucleus was reported by a study employing in situ hybridization technique (Giard et al., 1989). Therefore, it is puzzling that intrathecially administered ET-1, as with ET-3, was effective only at doses as high as 100 pmol in the present study. Two possible explanations, not mutually exclusive though, are (i) neonatal rats are more susceptible to ETs than adult counterparts, and (ii) intrathecially administered ETs in our experiments did not permeate deep enough to reach the intermediolateral nucleus.

It may be argued that intracisternally injected ET-1 might have caused vasoconstriction of cerebral arteries (Ide et al., 1989) and ensuing cerebral ischemia elicited the observed cardiovascular and sympathetic changes. However, the acute effect of cerebral ischemia on circulation has been shown to be a powerful pressor response with sympathoexcitation (Dampney et al., 1979) rather than the pattern
of ET-induced changes in AP, RSNA, and HR characterized typically by three phases.

Interestingly, if ET-1 is given to the lateral or third ventricle of the rat, the dose required to elicit a pressor response comparable to those of phase I in the present study was in the order of 10 to 100 pmol (MINAMIZAWA et al., 1989; OUCHI et al., 1989) as against 0.1 to 10 pmol for intracisternal administration in our study. This result suggests that the effective site of cardiovascular and sympathetic vasomotor actions of ET-1 in the cerebrospinal fluid, either endogenously or exogenously supplied, is in the lower brainstem rather than the forebrain.

In conclusion, intracisternally administered ET-1 affected both tonic and reflex control of AP and sympathetic discharges as evidenced by a peculiar pattern of changes in AP, RSNA, and HR and modulation of the arterial baroreceptor-sympathetic vasomotor reflex. Although the same is true with ET-3, the potency of ET-1 for these effects was much greater.

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