INTRACEREBROVENTRICULAR INJECTIONS OF ENDOTHELIN INCREASE ARTERIAL PRESSURE IN CONSCIOUS RATS

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We investigated the possible effects of endothelin (ET) on the central regulation of arterial pressure by injecting ET intracerebroventricularly (ICV) into conscious rats. ICV injections of ET caused dose-dependent elevation of arterial pressure and increase of heart rate. The first reaction was abolished by bunazosin, an α_1 -adrenergic blocker, injected intravenously. However, the increase in arterial pressure and heart rate caused the rats, injected with ET ICV, to roll to the left on their long axis for 20–30 min, followed by prolonged sedation. Furthermore, the pressor responses and tachycardia were significantly attenuated by the ICV pretreatment with nicardipine, a calcium channel blocker, and nicorandil, a nitrate derivative, respectively.

These results suggest that ET may elevate the intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) of sympathetic nerve activity regulatory neurons of the brain, leading to an accelerated outflow of sympathetic nerve activity and cause the elevation of arterial pressure.

ENDOTHLIN (ET) is one of the most potent vasoconstrictors ever discovered!

An endothelium-derived 21-residue peptide, ET was isolated by Yanagisawa et al! The precise mechanisms of vasoconstriction have not yet been clearly demonstrated, and the vasoconstrictive response is resistant to the known receptor-antagonists for pressor agents such as α -adrenergic, H_1 -histaminergic, and serotonergic blockers! It is found, however, that ET increases the intracellular Ca^{2+} concentration, $[\text{Ca}^{2+}]_i$, presumably by stimulating the endothelin-specific receptors?^{2–4}

Key words:

Endothelin
Intracerebroventricular injection
Arterial pressure
Sympathetic nerve activity
Intracellular calcium concentration

When administered intravenously in rats, ET elicits complex cardiovascular responses with several phases of arterial pressure and heart rate responses^{5–7} These findings indicate that ET affects not only the contraction of vascular smooth muscle cells but also several mechanisms for controlling the cardiovascular system.

Recently, it is suggested that the central nervous system contains a significant amount of ET-like immunoreactivity, particularly in the lower brain stem when measured with a radioimmunoassay for ET⁸

Furthermore, specific binding sites for ET have been demonstrated in the brain⁹ The plasma concentration of ET is very low, when measured with a ET-radioimmunoassay,¹⁰ and ET is supposed not to cross the blood-brain barrier because of its large

(Received May 30, 1989; accepted November 29, 1989)

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Fig.1. Arterial pressure and heart rate responses to intracerebroventricular injections (arrow) of endothelin (ET, 5 pmol) in conscious rats. From top to bottom, tracings of phasic arterial pressure (AP, mmHg), mean arterial pressure (MAP, mmHg) and heart rate (HR, beats/min) are shown.

Fig.2. Effects of intracerebroventricular injections of ET, 0.1–10 pmol, on mean arterial pressure (mmHg) and heart rate (beats/min) in conscious rats. (* $p < 0.05$, ** $p < 0.01$, vs. vehicle)

molecular weight. We, therefore, supposed that ET might be acting as a neurotransmitter or a neuromodulator in the central nervous system to affect the central regulation of arterial pressure.

Present study aimed to investigate the possible role of ET on the central regulation of arterial pressure by injecting ET intracerebroventricularly (ICV) in unres-trained, conscious rats.

MATERIALS AND METHODS

Twelve-week old, male Wistar rats (254 ± 2 g, $n=106$) were purchased from Nippon Rat Laboratories (Osaka, Japan). For experiments using conscious rats, a stainless steel cannula and an arterial, polyethylene catheter were implanted into the right lateral ventricle and the femoral artery, respectively, as described below. After the rats had been allowed to recover from the operation for 2 days, arterial pressure and heart rate were recorded continuously for 60 min following ICV injections of drugs.

Implantation of Arterial and Venous Catheters

During catheter implantation, rats were anaesthetized with sodium pentobarbital (40 mg/kg, intraperitoneally). Arterial pressure was measured directly from a femoral, arterial catheter. The catheter was modified

Fig.3. Effects of intravenous pretreatment with bunazosin ($50 \mu\text{g}$, first arrow) on arterial pressure and heart rate responses to intracerebroventricular injections of endothelin (ET, 5 pmol , second arrow). From top to bottom, tracings of phasic arterial pressure (AP, mmHg), mean arterial pressure (MAP, mmHg) and heart rate (HR, beats/min) are shown.

Fig.4. Effects of intravenous pretreatments with bunazosin (BZ, $50 \mu\text{g}$) and vehicle (V) on mean arterial pressure and heart rate responses to intracerebroventricular injections of endothelin (ET, 5 pmol).

by making its body entirely of PE-20 polyethylene tubing (16 cm) and the terminal PE-10 was formed into a loop about 6

mm in diameter between the point of entry into the femoral artery and PE-20. The other end was exteriorised by pulling it

Fig.5. Effects of intracerebroventricular pretreatments with nicardipine (NC, 100 nmol), nicorandil (ND, 100 nmol) or pinacidil (PC, 100 nmol) on arterial pressure and heart rate responses to intracerebroventricular injections of endothelin (ET, 5 pmol) in conscious rats. From top to bottom, tracings of phasic arterial pressure (AP, mmHg) and heart rate (HR, beats/min) are shown. The first arrow indicates the pretreatment, and the second the injection of ET.

subcutaneously through a cut in the skin on the back of the neck. The catheter was held in place with wound clips, and was filled with heparinized saline. The tip was plugged with a stylet (stainless steel, 21 gauge). A venous catheter made of PE-20 tubing was inserted into the right femoral vein.

Implantation of Cerebroventricular Cannulas

The stainless steel cannula (23 gauge, 1.4 cm long) was inserted into the right lateral cerebral ventricle stereotactically (stereotaxic coordinates to bregma: -0.8 mm antero-

posterior; $+1.6$ mm lateral; $+4.5$ mm dorsoventral; with the upper incisor bar set 3.3 mm below the interaural line). The cannula was fixed to the skull using three small screws and dental cement. Drugs were injected by inserting an injection cannula (30 gauge, stainless steel tubing) connected to a $25 \mu\text{l}$ microsyringe through the guide cannula. Each injection had a volume of $10 \mu\text{l}$, and was delivered manually within 10 seconds. After each experiment, methylene blue was injected through the injection cannula to verify the correct placement of the cannula tip within the lateral ventricle.

Arterial pressure was recorded continuously during the experiments by connecting the catheter to a small volume displacement pressure transducer (TP-200T, Nihon Kohden, Tokyo). Heart rate (pulse rate) was calculated automatically from aortic pulse waves with a tachometer (AT-601 G, Nihon Kohden, Tokyo).

Drug Injections

For ICV and intravenous injections, synthetic, porcine endothelin (ET-1, PEP-TIDE INSTITUTE, INC., Osaka), nicardipine (Yamanouchi pharmaceutical Co., Tokyo), nicorandil (Chugai Pharmaceutical Co., Tokyo) and bunazosin (Eizai Co., Tokyo) were dissolved in saline, respectively. Pinacidil (Shionogi Pharmaceutical Co., Tokyo) was dissolved in 0.1N HCl and neutralized with 0.1N NaOH. Respective solvents were used as the vehicle. The vehicle and every drug solution were adjusted to pH 7.4 by addition of 0.2N Na_2HPO_4 .

Statistical Analysis

Data expressed as mean $\pm$ SEM were compared using analysis of variance, and for F ratios significant at less than 5% ($p < 0.05$) differences between pairs of means were examined with a Duncan's new multiple range test. The differences between pairs of mean values were compared using a Student's t-test to compare independent samples; differences at less than 5% ($p < 0.05$) were considered significant.

RESULTS

Intracerebroventricular Injections of ET (n=40)

Fig. 6. Effects of intracerebroventricular pretreatments with nicardipine (NC, 100 nmol), nicorandil (ND, 100 nmol), pinacidil (PC, 100 nmol), or vehicle (V) on the pressor responses to intracerebroventricular injections of endothelin (5 pmol or 10 pmol) in conscious rats.

The baseline of arterial pressure and heart rate were as follows: vehicle; 120 ± 1.5 mmHg, 398 ± 11.6 beats/min (bpm), 0.1 pmol; 127 ± 2.5 mmHg, 420 ± 9 bpm, 1 pmol; 116 ± 3.2 mmHg, 404 ± 19 bpm, 5 pmol; 114 ± 1 mmHg, 401 ± 18 bpm, 10 pmol; 124 ± 4.8 mmHg, 420 ± 15 bpm. Within 1 min of the injection doses of 1–10 pmol/rat, arterial pressure began to rise, attaining the peak value 2–5 min later (Fig. 1, 2). The pressor phase lasted for only 5–10 min. Within the dose range tested, the magnitude of the peak pressor effect of ET was dose-dependent (vehicle; $+4 \pm 2$ mmHg, ET, 0.1 pmol; $+5 \pm 2$ mmHg, 1 pmol; $+12 \pm 2$ mmHg, 5 pmol; $+21 \pm 4$ mmHg, 10 pmol; $+62 \pm 11$ mmHg) (Fig. 2).

The heart rate tended to increase for 1–5 min after ICV injection of ET at doses of 1–10 pmol/rat corresponding to the pressor phase, but the difference was not significant compared to the vehicle injection. Then, heart rate began to decrease, and significant bradycardia was recorded in only 60 min following the ICV injection of 10 pmol of ET, compared with the vehicle injection (Fig. 2).

Intravenous injections of ET, 10 pmol, affected neither arterial pressure ($+1 \pm 2$ mmHg) nor heart rate (-5 ± 4 bpm) as seen in the vehicle injection ($+2 \pm 2$ mmHg, -3 ± 5 bpm).

Together with the elevation of arterial pressure, dose-related behavioral excitation was observed; immediately after the arterial pressure rose, the rats began to gnash their teeth and then to roll to the left on their long axis for 20–30 min, which was followed by prolonged sedation of more than 20 min. Similar injections of the vehicle without ET were ineffective.

Effects of Intravenous Pretreatment with α_1 Adrenergic Blocker, Bunazosin (n=12)

The baseline arterial pressure and heart rate before ICV injections of ET were 116 ± 4 mmHg, 420 ± 6 bpm for the vehicle, and 118 ± 3 mmHg, 418 ± 4 bpm for the bunazosin pretreatment. Pressor responses to ICV injections of ET, 5 pmol/rat, were significantly decreased by bunazosin, 50 μ g, injected intravenously (vehicle; $+26 \pm 4$ mmHg, bunazosin; $+6 \pm 2$ mmHg, $p < 0.01$).

On the other hand, the heart rate increased in the bunazosin group, and the magnitude of tachycardia was comparable to the vehicle group (vehicle; $+90 \pm 21$ bpm, bunazosin; $+107 \pm 12$ bpm). The striking behaviors, described above, however, were not influenced by the intravenous pretreatment with bunazosin (Fig. 3, 4).

Effects of ICV Pretreatments with Nicardipine, Nicorandil or Pinacidil

Fig. 7. Effects of intracerebroventricular pretreatments with nicardipine (NC, 100 nmol), nicorandil (ND, 100 nmol), pinacidil (PC, 100 nmol), or vehicle (V) on heart rate responses to intracerebroventricular injection of endothelin (5 pmol or 10 pmol) in conscious rats.

(Fig. 5, 6 and 7)

ICV pretreatments either with nicardipine, 100 nmol, a voltage-operated Ca^{2+} channel blocker ($n=6$), or with nicorandil, 100 nmol, a nitrate derivative ($n=6$), significantly attenuated the vasopressor responses and tachycardia to ICV injections of ET, 5 pmol, compared to the pretreatment with the vehicle ($n=6$, each). The baseline arterial pressure and heart rate were as follows: 118 ± 4 mmHg and 392 ± 12 bpm for the nicardipine group, 116 ± 5 mmHg and 420 ± 6 bpm for the vehicle of nicardipine, 116 ± 3 mmHg and 406 ± 17 bpm for the nicorandil, 112 ± 3 mmHg and 410 ± 13 bpm for the vehicle of nicorandil. The magnitude of peak changes of arterial pressure was $+10 \pm 3$ mmHg in the nicardipine group ($p < 0.01$), $+5 \pm 3$ mmHg in the nicorandil group ($p < 0.01$) compared to $+49 \pm 9$ mmHg in the vehicle group, and that for heart rate was $+38 \pm 12$ bpm in the nicardipine group ($p < 0.1$), $+8 \pm 10$ bpm in the nicorandil group ($p < 0.01$) and $+82 \pm 24$ bpm in the vehicle group, respectively. And the behavioral changes were also attenuated significantly in both groups treated with nicardipine and nicorandil.

However, when the higher dose of ET, 10 pmol/rat, was introduced, ICV pretreatments with both nicardipine, 100 nmol ($n=6$) and nicorandil, 100 nmol ($n=6$)

were ineffective (vehicle: $+74 \pm 9$ mmHg, $+110 \pm 26$ bpm, nicardipine: $+76 \pm 9$ mmHg, $+110 \pm 21$ bpm, nicorandil: $+75 \pm 10$ mmHg, $+108 \pm 14$ bpm). The baseline arterial pressure and heart rate before injections of ET 10 pmol were as follows; 116 ± 3 mmHg and 402 ± 11 bpm for the vehicle group, 120 ± 3 mmHg and 399 ± 12 bpm for the nicardipine, 122 ± 9 mmHg and 411 ± 3.4 bpm for nicorandil, respectively.

ICV pretreatment with pinacidil, 100 nmol ($n=6$), which is considered to open K^+ channels of the membrane and to cause hyperpolarization of resting membrane potentials, could not modify the vasopressor responses and tachycardia to ICV injections of ET, 5 pmol (vehicle; $+46 \pm 7$ mmHg, $+97 \pm 20$ bpm, pinacidil; $+48 \pm 4$ mmHg, $+113 \pm 15$ bpm). The baselines of arterial pressure and heart rate were 115 ± 4 mmHg and 369 ± 17 bpm for the pinacidil injection, 118 ± 4 mmHg and 382 ± 17 bpm for the vehicle injection. Even when the dose of pinacidil was increased to $1 \mu\text{mol}$, vasopressor responses to ICV ET, 5 pmol were not affected.

DISCUSSION

We have previously reported that intravenous injections of ET caused the long-lasting vasopressor effect following initial,

transient vasodepression in rats.⁵ Initial vasodepression is probably due either to direct action of ET on the peripheral vascular bed or to the release of vasodilating substances, because ICV injections of ET did not cause initial vasodepression. We have considered that central nervous system might participate at the second phase of the vasopressor response, because sympathetic nerve activity increased at that stage.⁵

The present study, using conscious, non-restrained rats, demonstrated that ICV injections of very minute amounts of ET caused dose-dependent elevation of arterial pressure and heart rate. These effects of ET must be of central origin, because the same amount of ET could not elicit these changes by the intravenous injection.

These responses of ICV injection of ET would be caused by the acceleration of sympathetic nerve activity since intravenous injections of bunazosin, α_1 -adrenergic blocker, significantly decreased the vasopressor responses without affecting the heart rate responses. Because the pressor response was not completely abolished by the α_1 -blockade, an alternative mechanism such as vasopressin release might be involved in the pressor response. We did not measure plasma vasopressin level in this particular experiment. Because α_1 -receptors in the heart mediate negative chronotropic action,^{12,13} pretreatment with bunazosin would increase the heart rate.

Specific binding sites for ¹²⁵I-ET have been demonstrated widely in the brain; particularly in areas such as the subfornical organ (SFO), the supraoptic nucleus (SON), widely in the hypothalamus, the solitary tract nucleus (NTS) and purkinje cells of the cerebellum.⁹ Because SFO, SON, NTS and the hypothalamus are believed to be central sites for the regulation of cardiovascular and metabolic phenomena, it is assumed that ET plays an important role on central cardiovascular regulation.

ET may increase the vagal nerve activity by stimulating NTS, since bradycardia occurred in the late phase with ICV injections of ET. It is also possible that the pressor effect of ET did not last for a long period due to the increase of vagal nerve activity.

Rolling behaviors of rats on ICV injections of ET may be interpreted to mean

that ET has affected the the purkinje cells of the cerebellum, because ET receptors are rich in these cells.⁹ And this specific behavior of excitement would not be a cause of elevating arterial pressure, since pressor responses preceded the changes in behavior.

ET is thought to increase intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$).²⁻⁴ In the central nervous system, $[\text{Ca}^{2+}]_i$ is supposed to play important roles in brain function via the acceleration of neurotransmission,¹¹ and that the increase of $[\text{Ca}^{2+}]_i$ in the neurons related to cardiovascular functions in the brain will induce systemic cardiovascular responses. ICV pretreatments with nicardipine or nicorandil could inhibit the vasopressor responses and tachycardia only when the dose of ET was 5 pmol. Since nicardipine is a voltage-operated Ca^{2+} channel blocker and almost all of these Ca^{2+} channels in the brain are supposed to be blocked in this dose of nicardipine, 100 nmol, ET would have increased the influx of Ca^{2+} by activating these Ca^{2+} channel.

Nicorandil, which decreases $[\text{Ca}^{2+}]_i$ by activating Ca^{2+} pumps of endoplasmic reticulum and plasma membrane^{14,15} and increases K^+ conductance of the plasma membrane¹⁶ could also inhibit the responses to ICV injections of ET, 5 pmol. However, neither nicardipine nor nicorandil could inhibit the responses to ICV injections of ET, 10 pmol. This phenomenon may be due to the fact that nicardipine blocks only the late phase of the calcium increase in the cultured, vascular smooth muscle cell but not the initial phase³; a large amount of ET could have increased significantly the amount of intracellular calcium through the initial phase of the calcium increase.

On the other hand, Pinacidil, which would increase K^+ conductance of the plasma membrane¹⁷ could not influence the vasopressor responses to ET, 5 pmol.

These data suggest that ET injected ICV increases $[\text{Ca}^{2+}]_i$ in neurons by promoting both the Ca^{2+} influx through voltage-operated Ca^{2+} channels and release of Ca^{2+} from intracellular storage sites for Ca^{2+} , such as endoplasmic reticulum. Although the precise mechanisms of ET to increase $[\text{Ca}^{2+}]_i$ still remain to be undetermined, one of these must be very potent because the small quantity of ET, 10 pmol, caused

distinct responses even following the ICV injections of nicardipine or nicorandil.

To our knowledge, the most potent mechanism which causes the increase of $[Ca^{2+}]_i$ is a phosphoinositide metabolism.

It is recently reported that ET stimulates phospholipase C in cultured vascular smooth muscle cells,¹⁸ which is a key enzyme of phosphoinositide hydrolysis.

Sarafotoxin, a potent vasoconstrictive peptide, in which the amino acid sequence is almost the same as ET, also has an ability to hydrolyze phosphoinositide in the rat brain.¹⁹ Therefore, it could be assumed that ET plays an important role in the central regulation of cardiovascular function and metabolism by stimulating phosphoinositide metabolism. However, there may be other alternative mechanisms of ET to elevate arterial pressure without an increase in intracellular Ca^{2+} .

Because circulating ET is thought to be substantially removed by pulmonary circulation²⁰ and the plasma concentration of ET is very low,¹⁰ ET is thought to act as an autocrine-paracrine hormone in organs and tissues. Considering the existence of specific binding sites for ET in the brain⁹ in spite of the blood-brain-barrier, ET may exist originally in the brain and act as a neurotransmitter or neuromodulator to affect the central regulation of cardiovascular responses mainly via the autonomic nervous system. Furthermore, since circumventricular organs, which are rich in ET receptors⁹, are found outside the barrier, even a very small amount of circulating ET may affect the central nervous system to increase blood pressure.

It is conceivable that these pressor effects may be induced by cerebral ischemia via vasoconstriction with ET. Because a potent vasodilator, pinacidil, could not attenuate the pressor effect of ICV injections of ET, the possibility of cerebral ischemia on the pressor response seems to be remote.

However, this issue will not be resolved without further investigations.

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