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# NMDA Receptor and NO Mediate ET-1-Induced Behavioral and Cardiovascular Effects in Periaqueductal Gray Matter of Rats

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Endothelin-1 (ET-1), a novel and potent vasoconstrictor in blood vessel, is known to have some functions in the rat central nervous system (CNS). In order to investigate the central functions of ET-1, ET-1 was administered to the periaqueductal gray area (PAG) of anesthetized rats to induce barrel rolling and increase the arterial blood pressure (ABP). ET-1 had a modulatory effect on central cardiovascular and behavioral control. The selective N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 (3 μmol/kg, i.p.) blocked the ET-1 induced responses, and both the nitric oxide synthase (NOS) inhibitor L-NAME (N-nitro-L-arginine methyl-ester 1 mmol/rat) and the nitric oxide (NO) scavenger hemoglobin (15 nmol/rat) had similar effects in reducing the ET-1 (10 pmol/rat)-induced behavioral changes and ABP elevation. However, NO donor sodium nitroprusside (SNP 10 μg, 1 μg/rat) decreased the ET-1 induced ABP elevation, and recovered the ET-1-induced barrel rolling effect that was reduced by MK-801. These results suggest that ET-1 might have neuromodulatory functions such as ABP elevation and barrel rolling induction in the PAG of the rats via the NMDA receptor and NO.

**Key words:** Endothelin-1, Nitric oxide, NMDA receptor, Barrel rolling, Arterial blood pressure

## INTRODUCTION

Endothelin-1 (ET-1), a 21-amino acid peptide, was originally isolated and sequenced from the supernatant of cultured porcine aortic endothelial cells. It is considered to be the most potent vasoconstrictor (Yamagisawa *et al.*, 1988). Though ET-1 cannot traverse the blood-brain barrier, experimental evidence suggests that ETs are localized in the central nervous system (CNS) and modulate several CNS functions (Gulati and Rebello, 1992; Koseki *et al.*, 1989). In addition, studies using immunohistochemistry, northern blot, and *in situ* hybridization techniques provided evidence of ET gene expression in a variety of the regions of the human brain and spinal cord (Mosqueda-Garcia *et al.*, 1995). However there is a paucity of reports regarding the mechanisms and functions of ET in the CNS that compare those in the peripheral tissue or blood vessel.

ET-1 is known to induce several behavioral changes barrel rolling, head twitch, ataxia, teething, rearing, grooming, scratching, running (Kuwaki *et al.*, 1990; D'Amico *et al.*, 1995) and an arterial blood pressure (ABP) increase (Minamisawa *et al.*, 1989; Matsumura *et al.*, 1991) in the periaqueductal gray matter (PAG). In this region NMDA (N-methyl-D-aspartate) binding sites exist and nitric oxide synthase (NOS) was found to be localized (Onstott *et al.*, 1993; Snyder, 1992). In the view that ET-1 activates the NMDA receptor, Rossi et al suggested that the NMDA receptor is involved in ET-1-induced physiological change (D'Amico *et al.*, 1995). In addition, several studies reported that nitric oxide (NO) production was increased when the NMDA receptor was stimulated. It may be possible that NO behaves as a second messenger of the NMDA receptor (Garthwaite and Boulton, 1995; Dawson and Dawson, 1996).

This study aims to show that in the CNS, particularly in the PAG, ET-1 exerts behavioral and cardiovascular effects via the NMDA receptor and NO is related to these responses.

## MATERIALS AND METHODS

### Animals

Male Sprague-Dawley rats weighing 150~250 g were used in the study. The animals were maintained in a tempera-

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ture (20°C)-humidity (55%) controlled room under a regular 24 h night/day schedule and were provided food and water *ad libitum*. They were then fasted for 24 h prior to the experiments. Six animals were used in each group.

### Stereotaxic surgical process

One day before drug injection, the animals were stereotaxically (Narishige, Tokyo, Japan) implanted with a guide cannula (23 G) in the dorsal PAG area (AP: -7.8; L: 0.8; V: 4.5) under pentobarbital anesthesia (60 mg/kg, i.p.) (D'Amico *et al.*, 1995). After implantation, the cannula was fixed to skull with dental cement and three metal screws and was protected by a plastic cap. Following surgery, the animals were placed in 40°C to recover, and housed in colony cages. They were fasted for 24 h before administration of the drugs used in each experiment.

### Drug treatment

Endothelin-1, hemoglobin (Hb), sodium nitroprusside, N-nitro-L-arginine methylester (L-NAME) were administrated into the PAG by an intracerebral microinjection. The intracerebral microinjection was conducted by a polyethylene tube (PE-10) connected to a 10 μl Hamilton syringe at 5 μl/ml. The total volume of drugs injected was not greater than 10 μl. Both Hb and L-NAME were injected 10 min before the ET-1 treatment. Sodium nitroprusside (SNP) was injected 20 min before the ET-1 treatment. Only MK-801 was intraperitoneally injected 30 min before the ET-1 injection (Sripada *et al.*, 1998; Wong *et al.*, 1986).

### Arterial blood pressure (APB) measurement

The right carotid artery was catheterized to measure blood pressure using pressure transducer connected to a DC amplifier and the heart rate (HR) was measured by a tachograph. The changes in ABP and HR were displayed and recorded on a polygraph (Model 7E, Grass Co., Boston, MA). The animals were artificially ventilated (A rodent ventilator, Ugo Basile, Italy) via a tracheal cannula at a rate of 60 breaths/min with a stroke volume of 1 ml per 100 g body weight to maintain the arterial $pCO_2$ at 30~35 mmHg.

After surgery they were adapted to the ABP measuring condition for about 20 min. The instantaneous and mean arterial blood pressure (MAP), and the heart rate were continuously monitored for 30 min.

### Barrel rolling inspection

Maione *et al.*, (1993) have defined barrel rolling as a longitudinal rolling of the body. To inspect the barrel rolling response of the rats, the animals were placed in 60 × 40 cm box and watched for 30 min after drug injection into the brain.

### Drugs

Endothelin-1, hemoglobin, sodium nitroprusside, N-nitro-L-arginine methylester were purchased from the Sigma Chemical Co. (St. Louis, MO, USA), and MK-801 was purchased from Tocris Cookson Ltd. (Langford, UK). All drugs were adjusted to pH 7.

### Statistical analysis

The inhibitory or inducing effects of the individual drugs on the ET-1-induced barrel rolling response were evaluated with $x^2$-test. An ANOVA test was applied to test the significance of difference between the values of the groups in an ABP measurement test. p values less than 0.05 were considered 'significant'.

## RESULTS

### ET-1- induced barrel rolling

The barrel rolling responses of rats treated with each drug are shown in Table I. By administering ET-1 into the PAG of rats, some other stereotyped behaviors besides barrel rolling (head twitch, ataxia, teething, grooming, scratching, and running) were observed. While these responses did not appear in all animals tested, all ET-1 (10 pmol)-treated rats (n=6) showed a barrel rolling response.

In this test, MK-801, a selective NMDA receptor antagonist (3 μmol/kg, i.p.), prevented the ET-1-induced barrel rolling response. To elucidate the participation of NO in this response, a NO scavenger, Hb (15 nmol/rat) and a NOS inhibitor, L-NAME (1 μmol/rat) were injected into the rats' PAG. Both drugs inhibited the barrel rolling response significantly. An additional study was performed with a NO donor, SNP. When SNP (10 μg/rat) was injected with both ET-1 and MK-801, an increased number of rats showed the barrel rolling response, which was reduced by MK-801.

### Arterial blood pressure measurement

Fig. 1 shows the ET-1-induced changes in both the APB and heart rate. The ET-1-induced ABP changes were recorded for 30 min. There were three phases in the pressure

**Table I.** Duration and induction number of barrel rolling response in rats

| Group | Duration (min) | Induction No./ Animal No. |
|---|---|---|
| Vehicle | 0 | 0/6 |
| ET-1 | 13 ± 1.33* | 6/6 |
| ET-1+MK-801 | 0.6 ± 0.19§ | 4/6 |
| ET-1+Hb | 0§ | 0/6 |
| ET-1+L-NAME | 0.33 ± 0.15 § | 2/6 |
| ET-1+MK-801+SNP | 0.67 ± 0.15 | 5/6 |

*$p<0.05$ versus vehicle and §$p<0.05$ versus control (ET-1) (n=6). Vehicle was PBS. MK-801 was injected 30 min before ET-1 treatment. Hb and L-NAME were injected 10 min before ET-1 treatment, and SNP was injected 20 min before ET-1 treatment.

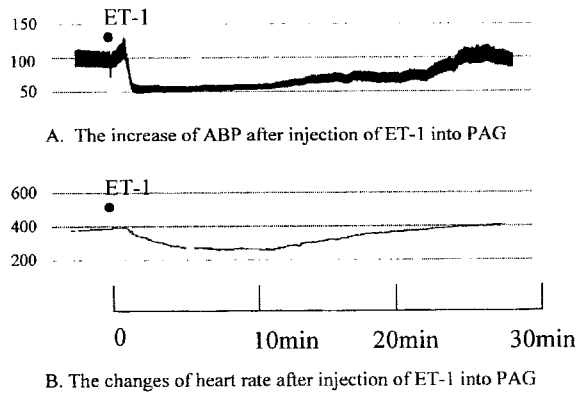


**Fig. 1.** Changes in arterial blood pressure and heart rate induced by an ET-1 injection into the PAG. The record was continued for 30 min. The change in ABP was about 23 mmHg, and the heart rate changes seemed to be contrary to that in the ABP.

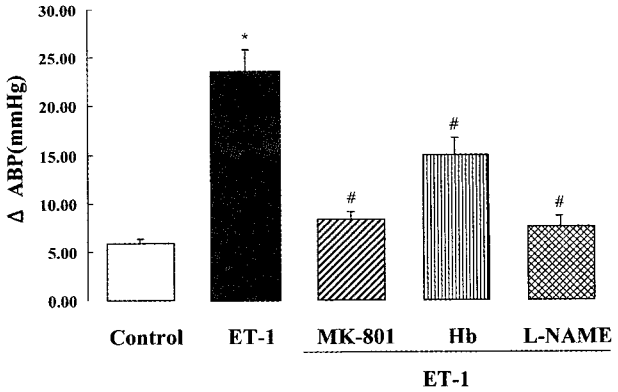


**Fig. 2.** Effects of MK-801, Hb and L-NAME on the ET-1-induced arterial blood pressure change. MK-801 was injected 30 min before ET-1 treatment. Hb and L-NAME were injected 10 min before ET-1 treatment. ET-1 increased arterial blood pressure. However, MK-801, L-NAME, and Hb significantly inhibited the increase in arterial blood pressure. *$p<0.05$ vs. control and # $<0.05$ vs. ET-1 alone (n=6). ANOVA test was applied to test significance.

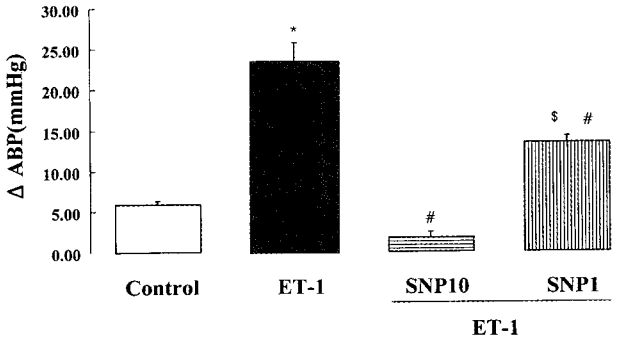


**Fig. 3.** Effect of SNP on the ET-1 induced arterial blood pressure change. SNP was injected 20 min before ET-1 treatment. ET-1 increased an arterial blood pressure. However, SNP (1, 10 μg) significantly inhibited the increase in arterial blood pressure. *$p<0.05$ vs. control, #$<0.05$ vs. ET-1 alone and \$$<0.05$ vs. SNP (10 μg)(n=6). ANOVA test was applied to test significance.

change; initially falling, rising and steeply falling. The duration of the rising phase was about 2 min. After the pressure fell, it slowly returned to normal levels.

The APB changes induced by each drug in the rats are shown in Fig. 2 and 3. The results were comparable to the barrel rolling test. When ET-1 was administered, the ΔABP was increased by $23 \pm 2.41$ mmHg. It was a significant increase in ABP compared with the control group. When the animals were pretreated with MK-801, the increase in DABP was reduced to $8.33 \pm 0.74$ mmHg. In order to investigate the roles of NO in this region, Hb (15 nmol/rat) and L-NAME (1 μmol/rat) were injected into the rats PAG. Both Hb and L-NAME reduced the DABP significantly ($p<0.05$ by ANOVA test): $15 \pm 1.67$ mmHg and $7.5 \pm 1.11$ mmHg, respectively (Fig. 2). We showed that pretreatment with SNP (1, 10 μg/rat) also inhibited ET-1-induced elevation in ABP significantly (Fig. 3). Their values were $1.67 \pm 0.74$ and $13.33 \pm 0.78$ mmHg, respectively. This suggests that there is another pathway related to NO in this region.

The heart rate was also recorded, and the (data not shown) decreases in the HR observed occurred concomitantly with the increases in ABP following the central ET injection.

## DISCUSSION

The PAG is the midline structure of the brain stem that encircles the mesencephalic aqueduct from the posterior commissure to the rostral locus coeruleus (George, 1995). This area is involved in analgesia, the autonomic system, the rage reaction, fear, and eye movements (George, 1995). It is probable that the analgesic, autonomic, and behavioral reactions evoked from the PAG are best conceptualized as components of coordinated responses that are necessary for the animal's survival (Bandler and Depaulis, 1991). The finding that NMDA binding sites are highest in the dorsolateral PAG is of interest in light of the fact that NMDA receptors are known to activate nitric oxide production. Nitric oxide synthase is localized predominantly to the dorsolateral PAG (Onstott *et al.*, 1993; Snyder, 1992).

ET-1, which was administered into the PAG, induced the barrel rolling response and increased arterial blood pressure. These results indicate that the PAG may regulate some cardiovascular functions and trigger some stereotyped behaviors by the effect of ET-1 on this area. Following ET-1 administration into the PAG, several effects were observed. These effects were marked ataxia and tendency to direct movements to one side. Some animals showed head twitch, teething, grooming, scratching and running. Since these responses did not appear in all the animals tested, we regarded barrel rolling as the standard of ET-1 induced behavioral change. 'Barrel rolling' is a unique motor phenomenon observed in rats where the animal

twists about its long axis and makes repeated lateral rolls (Burke *et al.*, 1982). Barrel rolling has also been reported following intraventricular injection of chlorpromazine and vasopressin (Wurpel *et al.*, 1986). Its mechanism of action may differ in each substance, but the exact mechanisms are not clear. The barrel rolling response may be a disturbance of motor coordination due to interference with the cerebral function or to an injury of neuronal pathways that regulate motor coordination (D'Amico *et al.*, 1995). However, D'Amico *et al* reported that the L-arginine-NO pathway exerted a functional antagonism on ET-1 induced barrel rolling in the PAG area. The reason for the discrepancy may be differences in the rats ages and anesthetic drugs used.

Many studies have been done regarding ET-induced cardiovascular changes. Matsumura *et al.* (1991) reported that intracerebroventricular ET-1 caused increases in both APB and renal sympathetic nerve activity in addition to an elevation in plasma catecholamines. Minamisawa *et al.* (1989) found that i.c.v. ET administration causes hypertension in conscious rats. Its mechanism is not clear, but is probably related to sympathetoexitation. The observation that decreases in the HR tended to occur concomitantly with increases in the BP following an ET central injection indicates that the vagal component the baroreceptor reflex is not so attenuated. Thus the transient fall in the APB can be interpreted as an accentuated vagal-sympathetic antagonism, which is a phenomenon that involves the negative chronotropic and ionotropic effects of tonic sympathetic activity (Minamisawa *et al.*, 1989). ET-1 might have caused vasoconstriction of cerebral arteries, but the pattern of cerebral ischemia in both the ABP and HR changes is different from that of the ET-induced changes that are typically characterized by three phases (Kuwaki *et al.*, 1990).

D'Amico *et al.* (1996) suggested that in barrel rolling, ET-1 acts via the $ET_B$ receptors associated with the glutamatergic system. In addition, others have suggested that the $ET_A$ receptor subtype mediates the cardiovascular actions of ET (Mosqueda-Garcia *et al.*, 1995).

Our results showed that these cardiovascular and behavior changes might be mediated by glutamatergic neurons, and NO might act as a second messenger and a negative feedback modulator. MK-801pretreatment prevented the ET-1-induced barrel rolling response and the increase in ABP. It was reported that ET-1 bound to the ET-1 receptor that is located in glutamatergic neurons, which was followed by a glutamate release. NMDA receptor activation occurred on the postsynaptic neurons (Horie *et al.*, 1995; Kataoka *et al.*, 1995). From this we deduced that the ET-1-indued responses were mediated by the NMDA receptor. However, D'Amico *et al.*, observed that non-NMDA antagonist CNQX (6-cyano-7-nitroquinoxaline-2, 3-dione) treatment had no effect on glutamate-induced hypertension in the PAG (Maione *et al.*, 1992). Hb and L-NAME, interfering with NO release, decreased those responses. This indicates that NO participated in the NMDA receptor-mediated responses in some way.

Many studies focusing on the relationship between the NMDA receptor and NO have been reported. NMDA receptor stimulation increases intracellular $[Ca^{++}]$, and an increase in $[Ca^{++}]_i$ activates NOS to release NO. In several regions where is NO released, it functions as a second messenger of the NMDA receptor by increasing c-GMP, for example the long term potentiation in the hippocampus (Schuman and Madison, 1994; Garthwaite and Boulton, 1995), and NO-mediated neurotoxicity in primary brain cultures (Dawson *et al.*, 1993).

In our study, SNP treatment alone resulted in a decrease in barrel rolling and ABP. This indicates that excess extracellular NO might inhibit the ET-1-induced changes in both ABP and HR. It implies that NO acts on the NMDA receptor in a different way. NO can inhibit the NMDA receptor by redox oxidation. NO generation oxidizes the thiol groups in the redox modulatory site of the NMDA receptor to form a disulfide bond producing a relatively persistent inhibition of the NMDA-evoked response (Lei *et al.*, 1992).

From these results, we suggest that the effect of ET-1 is related to both the behavior and the cardiovascular system. The effects of ET-1 were mediated through the NMDA receptor, which uses NO as second messenger. NO has the role as not only a second messenger but also a feedback inhibitor of the NMDA receptor in the PAG. However, it is not known whether the effects of ET-1 appear in the normal physiological condition or pathological environment.

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