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# Nitric oxide mediates central activation of sympathetic outflow induced by interleukin-1β in rats

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**Abstract**

The excitatory mechanism of central sympathetic outflow induced by interleukin-1β was investigated in urethane-anesthetized rats. Intracerebroventricular administration of interleukin-1β induced a gradually developing elevation of plasma noradrenaline levels in a dose-dependent manner (50, 100 and 200 ng/animal), while the levels of adrenaline were not affected. The elevation of noradrenaline levels induced by interleukin-1β (100 ng/animal i.c.v.) was abolished by the following treatments with: (1) a chemical sympathectomizer, 6-hydroxydopamine (15 mg/kg i.v., 3 days before); (2) a prostaglandin synthesis inhibitor, indomethacin (500 μg/animal i.c.v.); (3) a nitric oxide synthase inhibitor, L-$N^G$-nitroarginine methyl ester (100 μg plus 10 μg/min i.c.v.); and (4) a nitric oxide scavenger, oxyhemoglobin (32.3 μg plus 3.23 μg/min i.c.v.). In contrast to these results, D-$N^G$-nitroarginine methyl ester, an inactive isomer of L-$N^G$-nitroarginine methyl ester, and methemoglobin, a metabolite of oxyhemoglobin, were without effect. Furthermore, prostaglandin $E_2$ (100 ng/animal i.c.v.) rapidly and markedly elevated the plasma level of noradrenaline but not adrenaline. This prostaglandin $E_2$-induced elevation of plasma noradrenaline levels was not attenuated by L-$N^G$-nitroarginine methyl ester (100 μg plus 10 μg/min i.c.v.). The present results suggest that nitric oxide is involved in the interleukin-1β-induced central activation of sympathetic outflow. Furthermore, there probably exists nitric oxide-linked prostaglandin-generating system in the brain.

*Keywords:* Nitric oxide (NO); Interleukin-1β; Prostaglandin; Central nervous system; Plasma noradrenaline

## 1. Introduction

Several independent lines of evidence indicates a close functional link between the immune system and the brain. Interleukin-1, a mediator of immune signals, are produced not only in the immune system (e.g. lymphocytes and macrophages) but also in the brain (neuronal and glial cells) (Fontana et al., 1982). Furthermore, centrally applied interleukin-1β releases corticotropin releasing hormone and activates the splenosympathetic nerves (Shimizu et al., 1994).

We reported that central administration of interleukin-1β inhibited acid secretion in anesthetized rats, and this inhibition was blocked by both sympathectomy and indomethacin, an inhibitor of prostaglandin synthesis (Yokotani et al., 1995b). Prostaglandin $E_2$ in the brain induces sympathetic outflow, and elevates plasma levels of noradrenaline but not adrenaline (Yokotani et al., 1995a). We therefore suggested that interleukin-1β induces a prostaglandin-mediated central excitation of the sympathetic nervous system (Yokotani et al., 1995b). Precise mechanisms of this cytokine's central action in the brain are, however, still in obscure.

Interleukin-1β generates nitric oxide by activating nitric oxide synthase, an enzyme which converts L-arginine to nitric oxide (Palmer et al., 1988). Nitric oxide produces cGMP (Ariano, 1983; Ignarro et al., 1987), and affects various functions such as smooth muscle relaxation (Buga et al., 1989) and the release of neurotransmitters (Wiklund et al., 1993; Guevara-Guzman et al., 1994). It has been demonstrated that nitric oxide generates prostaglandins by elevating the activity of cyclooxygenase (Salvemini et al., 1993).

In the present study, therefore, we investigated possible roles of nitric oxide in the interleukin-1β-induced central activation of sympathetic outflow in rats anesthetized with urethane.

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## 2. Materials and methods

### 2.1. Subjects

Male Wistar rats weighing about 350 g were maintained in a room at 22–24°C under a constant day-night rhythm for > 2 weeks, and given food (laboratory chow, CE-2, Clea, Japan) and water ad libitum. Under urethane anesthesia (1.2 g/kg i.p.), the femoral vein was cannulated for infusion of saline (1.4 ml/h) and the femoral artery was cannulated for collecting blood samples. After these procedures, the animal was placed in a stereotaxic apparatus.

### 2.2. Details of specific experiments

3 h were allowed to elapse before the start of each experiment, for stabilization of the basal plasma levels of catecholamine. For i.c.v. administration of test substances, a stainless-steel cannula (0.35 mm o.d.) or a double lumens cannula (0.50 mm o.d.) was inserted into the right lateral ventricle at co-ordinates AP +8.0 mm from the interaural line, L 1.5 mm from the midline, H 3.5 mm below the surface of the brain according to the rat brain atlas by Paxinos and Watson (1986). Interleukin-1$\beta$ was dissolved in sterile saline, prostaglandin $E_2$ was dissolved in saline containing 0.5% of ethanol. These test substances were slowly injected into the right lateral cerebral ventricle in a volume of 10 μl using 50-μl Hamilton syringe. L-$N^G$-nitroarginine methyl ester or D-$N^G$-nitroarginine methyl ester dissolved in saline (10 μg/μl) was administered i.c.v. throughout the experiment using double lumens cannula; a bolus injection (100 μg) 30 min before administration of interleukin-1$\beta$ was followed by a continuous infusion (10 μg/min). Oxyhemoglobin or methemoblobin dissolved in saline (3.23 μg/μl) was administered i.c.v. throughout the experiment; a bolus injection (32.3 μg) 30 min before administration of interleukin-1$\beta$ was followed by a continuous infusion (3.23 μg/min). 6-Hydroxydopamine (15 mg/kg) dissolved in sterile saline containing 0.5% ascorbic acid was administered i.v. 3 days before experiments with interleukin-1$\beta$. Indomethacin (500 μg/animal) dissolved in sterile saline was administered i.c.v. in a volume of 10 μl, 15 min before administration of interleukin-1$\beta$.

### 2.3. Measurement of plasma catecholamines

Blood samples (400 μl) were collected through an arterial catheter. Catecholamines in the plasma were extracted by the method of Anton and Sayre (1962) with slight modifications, and assayed electrochemically using high-performance liquid chromatography, as shown in our previous paper (Okuma et al., 1991). Briefly, plasma (150 μl) was transferred to a centrifuge tube containing 30 mg of activated alumina, 2 ml of twice deionized water and 1 ml of 1.5 M Tris buffer (pH 8.6) containing 0.1 M disodium EDTA, after which the preparation was shaken for 10 min. After several washes with 4-ml aliquots of ice-cold twice deionized water, catecholamines adsorbed onto alumina were eluted with 500 μl of 4% of acetic acid containing 0.1 mM disodium EDTA. A pump (880 PU; Japan Spectroscopic, Japan) and an electrochemical detector (ECD-100, Eicom, Japan) equipped with a graphite electrode were used with high-performance liquid chromatography. Analytical conditions were as follows: detector, +700 mV potential; column, Cosmosil-packed column (ODS) 4.6 × 150 mm (Nacalai Tesque, Japan); mobile phase, 0.1 M phosphate buffer pH 3.5, 20 mM EDTA, 4 mM 1-octane sulfate sodium (Nacalai Tesque) containing 16% methanol. The amount of catecholamines in each sample was calculated by using the peak height ratio relative to 3,4-dihydroxy benzylamine, an internal standard. By this assay method, 5 pg of noradrenaline and adrenaline could be determined accurately.

### 2.4. Treatment of data and statistics

All data were analyzed by repeated-measure ANOVA using Statview 4.0 (Abacus Concepts, CA, USA), followed by post-hoc analysis with Bonferroni method for comparing a control to all other means except when only two means were compared. In the latter case, Student's $t$-test was employed for significantly different variations between two groups. $P$ values of $< 0.05$ were taken to indicate significance.

### 2.5. Compounds

The following drugs were used: bovine hemoglobin and 6-hydroxydopamine (Sigma, USA); human recombinant interleukin-1$\beta$ was kindly provided by Dr. T. Hiyama (Otsuka Pharmaceutical, Japan); water-soluble indomethacin sodium trihydrate was kindly provided by Merck Sharp & Dohme (USA); All other reagents were the highest grade available (Nacalai Tesque).

Oxyhemoglobin and methemoglobin were prepared by the method of Martin et al. (1985) and Kelm and Schrader (1988). Briefly, oxyhemoglobin was prepared by treating commercial hemoglobin with a 10-fold molar excess of sodium dithionite, methemoglobin was prepared with a 2-fold molar excess of potassium ferricyanide, followed by dialysis with 100 vols. of distilled water for 2 h at 4°C. The purity of hemoglobin in the solution was determined spectrophotometrically, and the solution was stored at −80°C.

## 3. Results

### 3.1. Effect of interleukin-1β on plasma levels of catecholamines

Basal plasma levels of noradrenaline and adrenaline were 358 ± 16 and 335 ± 50 pg/ml ($n = 16$), respec-

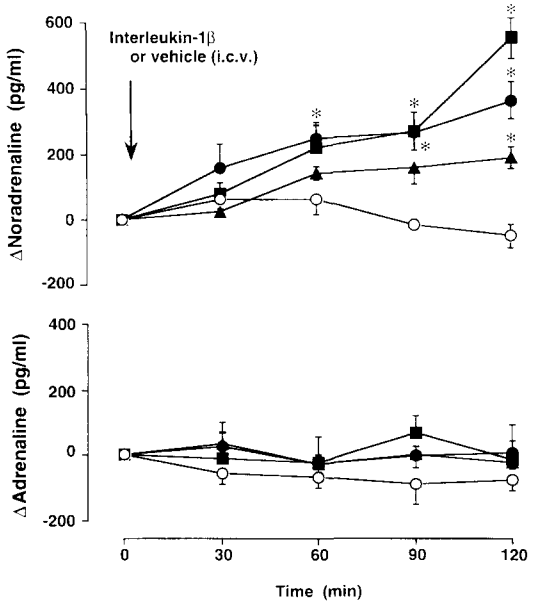


Fig. 1. Effect of i.c.v. administration of interleukin-1β on plasma levels of catecholamines. (○) vehicle (saline) 10 μl/animal ($n = 5$); (▲) interleukin-1β 50 ng/animal ($n = 4$); (●) interleukin-1β 100 ng/animal ($n = 4$); (■) interleukin-1β 200 ng/animal ($n = 4$). Δ Noradrenaline: net changes in plasma levels of noradrenaline from their respective basal levels. Δ Adrenaline: net changes in plasma levels of adrenaline from their respective basal levels. * Significantly different ($P < 0.05$) from the respective vehicle-treated controls.

tively. Blood sampling 5 times at 0, 30, 60, 90 and 120 min after i.c.v. administration of vehicle (10 μl saline) did not affect the plasma levels of noradrenaline and adrenaline (Fig. 1). Because of the relatively large individual variation in the basal levels of catecholamines as compared with their changes induced by interleukin-1β, results were expressed as the mean ± S.E.M. of the net changes from the respective basal values.

Administration of interleukin-1β (50, 100 and 200 ng/animal i.c.v.) induced a gradually developing and dose-dependent elevation of plasma levels of noradrenaline, while the levels of adrenaline were not affected (Fig. 1). On the other hand, i.v. administration of interleukin-1β (200 ng/animal) did not affect the plasma levels of both noradrenaline and adrenaline (data not shown).

### *3.2. Effect of chemical sympathectomy with 6-hydroxydopamine on interleukin-1β-induced elevation of plasma noradrenaline levels*

In a preliminary experiment, i.v. administration of 6-hydroxydopamine (15 mg/kg, 3 days before experiments) decreased the contents of noradrenaline in the sympathetically innervated organs such as spleen and stomach to < 20% of those in controls treated with vehicle (saline containing 0.5% ascorbic acid). The basal plasma noradrenaline levels were 196 ± 24 pg/ml for 6-hydroxydopamine-treated animals and 284 ± 29 pg/ml for vehicle-treated animals, respectively. In 6-hydroxydopamine-treated animals, the interleukin-1β (100 ng/animal i.c.v.)-induced elevation of plasma noradrenaline was not observed (Fig. 2).

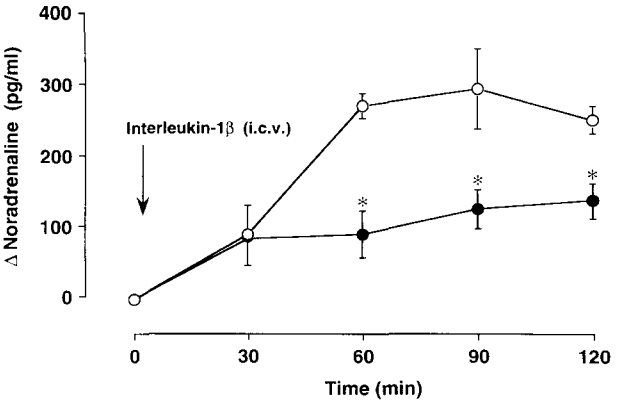


Fig. 2. Effect of chemical sympathectomy with 6-hydroxydopamine on the interleukin-1β-induced elevation of plasma noradrenaline levels. Pre-treatment with 6-hydroxydopamine (15 mg/kg i.v.) or vehicle (saline containing 0.5% ascorbic acid i.v.) was performed 3 days before administration of interleukin-1β (100 ng/animal i.c.v.). (○) vehicle-treated control group ($n = 5$), (●) 6-hydroxydopamine-treated group ($n = 5$). * Significantly different ($P < 0.05$) from vehicle-treated control. Other conditions were the same as for Fig. 1.

### *3.3. Effect of indomethacin on interleukin-1β-induced elevation of plasma noradrenaline levels*

Intracerebroventricular pre-treatment with indomethacin (500 μg/animal) or vehicle (10 μl saline) did not significantly affect the basal plasma noradrenaline levels. Basal plasma noradrenaline levels at 0 min were 530 ± 77 pg/ml for indomethacin-treated animals and 384 ± 12 pg/ml for vehicle-treated animals, respectively. Pre-treatment with indomethacin abolished the interleukin-1β (100 ng/animal i.c.v.)-induced elevation of plasma noradrenaline levels (Fig. 3).

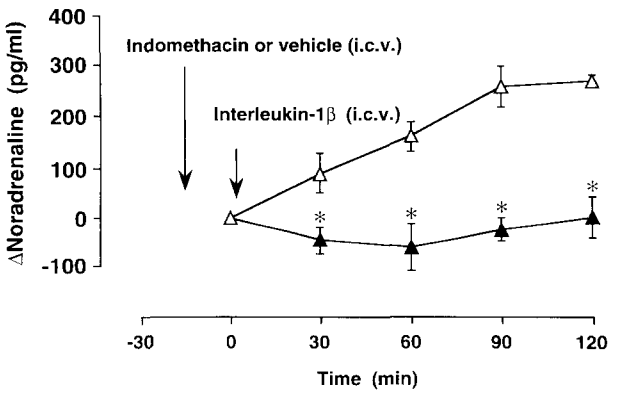


Fig. 3. Effect of indomethacin on interleukin-1β-induced elevation of plasma noradrenaline levels. Indomethacin (500 μg/animal) or vehicle (10 μl saline) was i.c.v. administered 15 min before administration of interleukin-1β (100 ng/animal i.c.v.). (△) vehicle-treated control group ($n = 5$); (▲) indomethacin-treated group ($n = 4$). * Significantly different ($P < 0.05$) from vehicle-treated control. Other conditions were the same as for Figs. 1 and 2.

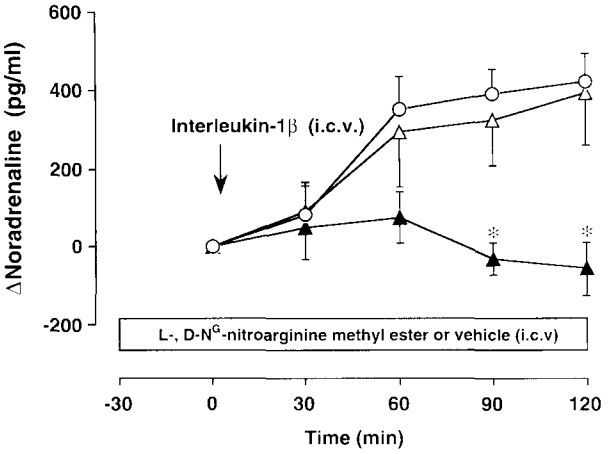


Fig. 4. Effects of L-$N^G$-nitroarginine methyl ester and its D-isomer on the interleukin-1$\beta$-induced elevation of plasma noradrenaline levels. Administration of L-$N^G$-nitroarginine methyl ester, D-$N^G$-nitroarginine methyl ester (100 $\mu$g plus 10 $\mu$g/min i.c.v.) or vehicle (saline, 10 $\mu$l plus 1 $\mu$l/min i.c.v.) was started 30 min before i.c.v. administration of interleukin-1$\beta$ (100 ng/animal). (▲) L-$N^G$-nitroarginine methyl ester-treated group ($n = 4$); (△) D-$N^G$-nitroarginine methyl ester-treated group ($n = 4$); (○) vehicle-treated group ($n = 4$). * Significantly different ($P < 0.05$) from vehicle-treated group. Other conditions were the same as for Figs. 1–3.

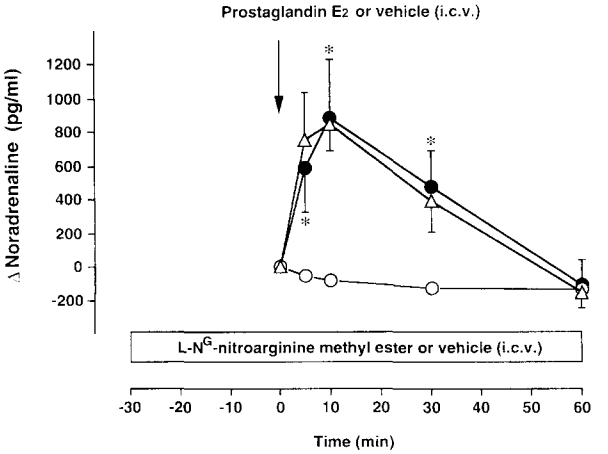


Fig. 6. Effect of L-$N^G$-nitroarginine methyl ester on prostaglandin $E_2$-induced elevation of plasma noradrenaline levels. Administration of L-$N^G$-nitroarginine methyl ester (100 $\mu$g plus 10 $\mu$g/min i.c.v.) or vehicle (saline, 10 $\mu$l plus 1 $\mu$l/min i.c.v.) was started 30 min before i.c.v. administration of prostaglandin $E_2$ (100 ng/animal i.c.v.). (●) prostaglandin $E_2$ and L-$N^G$-nitroarginine methyl ester-treated group ($n = 4$); (△) prostaglandin $E_2$ and vehicle-treated group ($n = 4$); (○) vehicle and L-$N^G$-nitroarginine methyl ester-treated group ($n = 4$). * Significantly different ($P < 0.05$) from vehicle and L-$N^G$-nitroarginine methyl ester-treated group. * Significantly different ($P < 0.05$) from vehicle and L-$N^G$-nitroarginine methyl ester-treated group. Other conditions were the same as for Figs. 1–5.

### 3.4. Effects of L-$N^G$-nitroarginine methyl ester (nitric oxide synthase inhibitor) and oxyhemoglobin (nitric oxide scavenger) on interleukin-1$\beta$-induced elevation of plasma noradrenaline levels

Intracerebroventricular treatment with L-$N^G$-nitroarginine methyl ester, D-$N^G$-nitroarginine methyl ester or vehicle alone did not significantly affect the basal plasma noradrenaline levels. The basal noradrenaline levels at 0 min were 535 ± 66 pg/ml for L-$N^G$-nitroarginine methyl ester-treated animals, 634 ± 58 pg/ml for D-$N^G$-nitroarginine methyl ester-treated animals and 654 ± 162 pg/ml for vehicle-treated animals, respectively. Treatment with L-$N^G$-nitroarginine methyl ester abolished the interleukin-1$\beta$ (100 ng/animal i.c.v.)-induced elevation of plasma noradrenaline levels, while treatment with the D-$N^G$-nitroarginine methyl ester was without effect (Fig. 4).

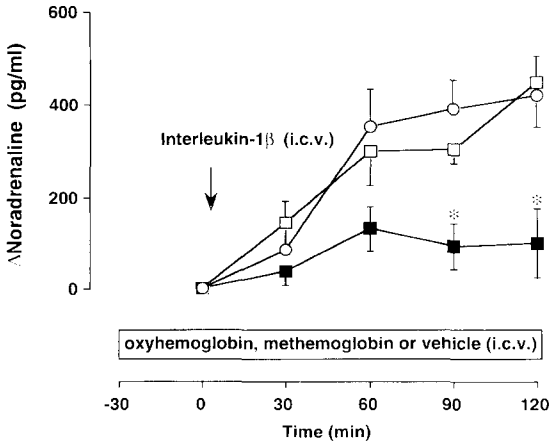


Fig. 5. Effects of oxyhemoglobin and methemoglobin on the interleukin-1$\beta$-induced elevation of plasma noradrenaline levels. Administration of oxyhemoglobin, methemoglobin (32.3 $\mu$g plus 3.23 $\mu$g/min i.c.v.) or saline was started 30 min before i.c.v. administration of interleukin-1$\beta$ (100 ng/animal). (■) oxyhemoglobin-treated group ($n = 4$); (□) methemoglobin-treated group ($n = 4$); (○) vehicle-treated group (cited from Fig. 4). * Significantly different ($P < 0.05$) from vehicle-treated group. Other conditions were the same as for Figs. 1–4.

Intracerebroventricular treatment of oxyhemoglobin or methemoglobin alone did not significantly affect the basal plasma noradrenaline levels. The basal noradrenaline levels at 0 min were 469 ± 69 pg/ml for oxyhemoglobin-treated animals, 329 ± 50 pg/ml for methemoglobin-treated animals and 654 ± 162 pg/ml for vehicle-treated animals, respectively. Treatment with oxyhemoglobin abolished the interleukin-1$\beta$ (100 ng/animal i.c.v.)-induced increases in the plasma noradrenaline levels, while treatment with methemoglobin was without effect (Fig. 5).

### 3.5. Effect of L-$N^G$-nitroarginine methyl ester (nitric oxide synthase inhibitor) on prostaglandin $E_2$-induced elevation of plasma noradrenaline levels

The basal noradrenaline levels at 0 min were 433 ± 82 pg/ml for L-$N^G$-nitroarginine methyl ester-pre-treated animals and 433 ± 107 pg/ml for vehicle (saline)-pre-treated animals, respectively.

In vehicle (saline)-pre-treated control animals, prostaglandin $E_2$ (100 ng/animal i.c.v.) caused a rapid and

marked elevation of plasma noradrenaline levels (Fig. 6), as observed in our previous study (Yokotani et al., 1995a). Intracerebroventricular pre-treatment with L-$N^G$-nitroarginine methyl ester did not modify this prostaglandin $E_2$-induced elevation of plasma noradrenaline levels. Furthermore, i.c.v. administration of saline containing 0.5% ethanol as the vehicle for prostaglandin $E_2$ did not affect the basal plasma levels of noradrenaline in the L-$N^G$-nitroarginine methyl ester-pre-treated animals. Plasma adrenaline levels were not affected by prostaglandin $E_2$ (data not shown).

## 4. Discussion

In the present study, i.c.v. administration of interleukin-1$\beta$ elevated plasma levels of noradrenaline but not adrenaline. This interleukin-1$\beta$-induced elevation of plasma noradrenaline levels was abolished in the chemically sympathectomized animals with 6-hydroxydopamine. I.v. administration of 6-hydroxydopamine does not affect the contents of catecholamines in the brain and the adrenal medullae (Okuma et al., 1987). In addition, interleukin-1$\beta$ did not elevate the plasma levels of adrenaline even in these chemically sympathectomized animals (data not shown). The elevation of plasma noradrenaline levels induced by interleukin-1$\beta$ is, therefore, due to the release of noradrenaline from the sympathetic nerve terminals but not from the adrenal medullae. The selective increase in the plasma noradrenaline by interleukin-1$\beta$ was blocked by indomethacin. These results confirm our previous reports that this cytokine inhibits acid secretion by central prostaglandin-mediated sympathetic outflow and prostaglandin $E_2$ in the brain elevates plasma levels of noradrenaline but not adrenaline (Yokotani et al., 1995a,b). Furthermore, the present results correspond well with the evidence that indomethacin suppresses the interleukin-1-induced secretion of adrenocorticotropic hormone and febrile response (Rivier and Vale, 1991; Rotondo et al., 1988). Interleukin-1$\beta$ releases prostaglandin $E_2$ from the rat hypothalamus both in vivo and in vitro experiments (Navarra et al., 1992; Komaki et al., 1992). It is, therefore, likely that the interleukin-1$\beta$-induced elevation of plasma noradrenaline is mediated by prostaglandins (probably prostaglandin $E_2$) produced in the brain.

Recently, it has been demonstrated that nitric oxide is involved in the interleukin-2-induced releases of hypothalamic hormones, such as corticotropin-releasing hormone (Karanth et al., 1993; Brunetti et al., 1993; Sandi and Guaza, 1995; Raber et al., 1995) and vasopressin (Raber and Bloom, 1994). Nitric oxide synthase is abundantly contained in the hypothalamus, particularly in the paraventricular nucleus, supraoptic nucleus, nucleus circularis and lateral hypothalamus (Bredt et al., 1990). Furthermore, the paraventricular nucleus of hypothalamus sends axons to the median eminence and to the sympathetic pre-ganglionic neurons in the spinal cord (Vandesande et al., 1977; Saper et al., 1976; Swanson and Sawchenko, 1983). In the present study, the elevation of plasma noradrenaline levels induced by interleukin-1$\beta$ was abolished by treatment not only with L-$N^G$-nitroarginine methyl ester (a nitric oxide synthase inhibitor), but also with oxyhemoglobin (a nitric oxide scavenger), while the elevation induced by prostaglandin $E_2$ was not attenuated by L-$N^G$-nitroarginine methyl ester. Furthermore, the elevation of plasma noradrenaline levels induced by prostaglandin $E_2$ was much more rapid than that induced by interleukin-1$\beta$. These results suggest that prostaglandin produced by nitric oxide in the brain may be involved in interleukin-1$\beta$-induced central activation of sympathetic outflow.

It has been reported that centrally applied interleukin-1$\beta$ increases plasma levels of noradrenaline and adrenaline (Hashimoto et al., 1993). In the present study, however, interleukin-1$\beta$ selectively elevated plasma levels of noradrenaline without affecting those of adrenaline, as described above. This difference in the results may be due to different experimental conditions. They used conscious rats, while we used anesthetized rats. However, whether this experimental difference rationalize the difference between the present results and the results by them remains unresolved.

In conclusion, we demonstrate here that i.c.v. administration of interleukin-1$\beta$ in the anesthetized rats increases sympathetic outflow. Futhermore, nitric oxide and prostaglandin in the brain are probably involved in this central activation of sympathetic outflow.

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