

Prolonged NOS inhibition in the brain elevates blood pressure in normotensive rats

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Sakima, Atsushi, Hiroshi Teruya, Masanobu Yamazato, Rijiko Matayoshi, Hiromi Muratani, and Koshiro Fukiyama. Prolonged NOS inhibition in the brain elevates blood pressure in normotensive rats. *Am. J. Physiol.* 275 (*Regulatory Integrative Comp. Physiol.* 44): R410–R417, 1998.—Systemic inhibition of nitric oxide synthase (NOS) evokes hypertension, which is enhanced by salt loading, partly via augmented sympathetic activity. We investigated whether inhibition of brain NOS elevates blood pressure (BP) in normotensive rats and, if so, whether the BP elevation is enhanced by salt loading. After a 2-wk low-salt (0.3%) diet, male Sprague-Dawley (SD) rats were divided into four groups. *Groups 1* and *2* received a chronic intracerebroventricular infusion of 0.5 mg·kg⁻¹·day⁻¹ of N^G-monomethyl-L-arginine (L-NMMA), and *groups 3* and *4* were given artificial cerebrospinal fluid (aCSF). *Groups 1* and *3* were placed on a high-salt (8%) diet, whereas *groups 2* and *4* were on a low-salt diet. On *day 9* or *10*, *group 1* showed significantly higher mean arterial pressure (MAP) in a conscious unrestrained state (129 ± 3 mmHg vs. 114 ± 3, 113 ± 1, and 108 ± 3 mmHg in *groups 2, 3, and 4*, respectively, *P* < 0.05). On a high-salt diet, response of renal sympathetic nerve activity but not of BP to air-jet stress was significantly larger in rats given L-NMMA than in rats given aCSF (29 ± 4% vs. 19 ± 3%, *P* < 0.05). When the intracerebroventricular infusions were continued for 3 wk, MAP was significantly higher in rats given L-NMMA than in rats given aCSF irrespective of salt intake, although the difference was ~7 mmHg. Thus chronic inhibition of NOS in the brain only slightly elevates BP in SD rats. Salt loading causes a more rapid rise in BP. The mechanisms of the BP elevation and its acceleration by salt loading remain to be elucidated.

N^G-monomethyl-L-arginine; central nervous system; intracerebroventricular infusion; renal sympathetic nerve activity; salt

NITRIC OXIDE (NO), which was originally considered to be an endothelium-derived relaxing factor (18), is now known to be synthesized in the central nervous system as well as in the vascular endothelial cells (2). In the brain, NO acts as a neurotransmitter or neuromodulator to exert a regulatory function in central cardiovascular control (3, 7, 8, 21, 28, 30, 33). Chronic systemic administration of NO synthase (NOS) inhibitor increases blood pressure in rats (5) and dogs (15). According to previous reports (4, 16, 24, 32), the sympathetic nervous system seems to play a critical role in the development of hypertension induced by systemic NOS inhibition. Ganglionic blockade caused a significantly greater decrease in mean arterial pressure (MAP) in rats receiving chronic systemic administration of N^G-

nitro-L-arginine methyl ester (L-NAME) than in the control group (4). The fall in blood pressure induced by an acute administration of phentolamine was significantly larger in L-NAME-treated rats than in the control rats (32). Development of the L-NAME-induced hypertension was delayed by renal denervation (16) and blunted by pharmacological blockade of the sympathetic nervous system (24). In addition, the blood pressure response to systemic NOS inhibition was enhanced by salt loading (26, 29).

Because acute central administration of NOS inhibitor elevates blood pressure and enhances renal sympathetic nerve activity (RSNA) in anesthetized rats (28, 30), cats (33), and rabbits (8), suppression of NOS in the brain seems to contribute to the sympathetically mediated elevation of blood pressure caused by systemic administration of NOS inhibitor. In fact, NOS activity in the cerebral tissue was significantly lower in the L-NAME-treated rats than in the control animals (32). Moreover, recent studies suggest a close relationship between salt sensitivity of blood pressure and NO production in the brain. Deoxycorticosterone acetate (DOCA)-salt hypertensive rats exhibited significantly lower gene expression of constitutive NOS in the hypothalamus than normotensive rats (17). In normotensive rats, chronic salt loading enhanced NOS gene expression in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus (10). Chronic intracerebroventricular infusion of N^G-monomethyl-L-arginine (L-NMMA), a NOS inhibitor, augmented hypertension in DOCA-salt hypertensive rats (25).

We hypothesized that chronic inhibition of NOS in the brain elevates blood pressure and that the pressor effect is accelerated or enhanced by salt loading. In the present study, we examined the effects of prolonged intracerebroventricular infusion of L-NMMA on blood pressure in normotensive rats placed on either a high-salt or a low-salt diet.

METHODS

Animals. Male 4-wk-old Sprague-Dawley (SD) rats were purchased from Charles River. All procedures were in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. The protocol was approved by the Animal Care and Use Committee, University of the Ryukyus.

Implantation of intracerebroventricular cannula. Each rat was anesthetized by pentobarbital sodium (50 mg/kg intraperitoneally, supplemented as required). Rats were placed on a stereotaxic frame (Narishige Scientific Instruments, Tokyo, Japan). The skin overlying the midline of the skull was incised, and a small hole was drilled through the appropriate portion of the skull. A 27-gauge (0.2/0.4 mm inner/outer diameter) stainless-steel cannula to which a silicone tube was

† Deceased 16 January 1998.

attached was lowered to the right lateral ventricle according to the coordinates of Paxinos and Watson (1.3 mm posterior to the bregma, 2.0 mm lateral to the midline, and 4.0 mm ventral to the skull surface) (19) and fixed to the skull with cyanoacrylate adhesive (Aron Alpha; Toa Gosei Chemical Industries, Tokyo, Japan). The silicone end of the cannula was connected to a miniosmotic pump (type 2002 or type 2004; Alza, Palo Alto, CA) filled with L-NMMA or artificial cerebrospinal fluid (aCSF). L-NMMA was dissolved in aCSF. The miniosmotic pump of type 2002 was placed in sterile 0.9% saline at 37°C for 5 h before subcutaneous implantation into the interscapular region. The miniosmotic pump of type 2004 was incubated for 48 h before the implantation. After surgery, each rat received an intramuscular injection of 40,000 U/kg body wt of penicillin G for prophylaxis.

Verification of the position of the intracerebroventricular cannula. At the end of the experiments, the position of the intracerebroventricular cannula was verified by injection of 5% solution of methylene blue and observing the staining on the ventricular surface. If the ventricular surface was not stained, data from that animal were excluded.

Implantation of arterial and venous catheters. In rats receiving intravenous infusion, a silicone catheter was inserted into the inferior caval vein through the left femoral vein, and the end of the catheter was attached to a miniosmotic pump filled with L-NMMA solution or 0.9% saline. For intravenous infusion, L-NMMA was dissolved in 0.9% saline at the same concentration as the intracerebroventricular infusion. After surgery, each rat received an intramuscular injection of 40,000 U/kg body wt of penicillin G for prophylaxis. One or two days before the direct measurement of MAP, each rat was again anesthetized by administration of pentobarbital sodium (50 mg/kg ip), and one arterial catheter (PE-10 fused with PE-50, Clay Adams) was inserted through the right femoral artery to record arterial blood pressure and heart rate. The end of catheter was exteriorized through the interscapular region through a subcutaneous tunnel.

Implantation of nerve electrode. Separate groups of rats given intracerebroventricular infusion of L-NMMA ($n = 12$) or aCSF ($n = 12$) were fed a high-salt (8% NaCl; Oriental Yeast, Tokyo, Japan) diet. On day 8 or 9 of the intracerebroventricular infusion, they were again anesthetized with pentobarbital sodium (50 mg/kg body wt ip and 10–15 mg/kg body wt intravenously every hour as a supplemental dose). One arterial catheter was inserted through the right femoral artery to record arterial blood pressure and heart rate, and one venous catheter (PE-10 fused with PE-50) was inserted into the inferior vena cava via the right femoral vein for administration of drugs. The left renal nerves were exposed through a retroperitoneal approach. A branch of the nerves was separated from surrounding fat and connective tissue and placed on a bipolar silver wire electrode (no. 7855; A-M Systems, Everett, WA). When an optimal neurogram was obtained, the nerve and electrode were embedded in silicone gel (Sil-Gel 604; Wacker, Munich, Germany) and allowed to harden. Catheters and the lead wires from the recording electrode were exteriorized through the dorsal skin of the neck and fixed to the skin. After closure of the left flank incision, each rat received an intramuscular injection of 40,000 U/kg body wt of penicillin G for prophylaxis.

Experimental protocol 1: Effects of intracerebroventricular infusion of L-NMMA for 9–10 days on blood pressure, heart rate, body weight, and sodium and water balance. After purchase, rats were fed a low-salt diet (0.3% NaCl, Oriental Yeast) for 2 wk. They were allowed free access to food and tap water. They were then divided into four groups. Groups 1 ($n = 11$) and 2 ($n = 11$) received an intracerebroventricular

infusion of $0.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ of L-NMMA (Research Biochemicals International, Natick, MA), whereas groups 3 ($n = 9$) and 4 ($n = 8$) received an intracerebroventricular infusion of aCSF (in mM: 133.3 NaCl, 3.4 KCl, 1.3 CaCl₂, 1.2 MgCl₂, 0.6 mM NaH₂PO₄, 32.0 NaHCO₃, and 3.4 glucose; 10 $\mu\text{l/day}$). L-NMMA was dissolved in aCSF ($0.05 \text{ mg} \cdot \text{kg}^{-1} \cdot \mu\text{l}^{-1}$, 10 $\mu\text{l/day}$). The dose of L-NMMA was determined based on preliminary experiments in which 0.08, 0.2, or 0.5 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ of L-NMMA or aCSF was infused for 10 days into the lateral ventricle of SD rats (3 or 4 rats for each group) on a high-salt diet. Only $0.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ of L-NMMA elevated blood pressure. During the infusion period, groups 1 and 3 were placed on a high-salt diet and groups 2 and 4 were on a low-salt diet.

To exclude the possibility that L-NMMA chronically infused into the lateral ventricle exerts systemic effects through leakage into the peripheral circulation, we examined effects of chronic intracerebroventricular infusion of L-NMMA at the same dose as the intracerebroventricular infusion: this was group 5 ($n = 8$). Another group of rats receiving chronic intravenous infusion of 0.9% saline served as a control of group 5; this was group 6 ($n = 7$). Groups 5 and 6 were placed on a high-salt diet. Throughout the experiments, all rats were maintained individually at constant temperature ($24 \pm 1^\circ\text{C}$), humidity ($50 \pm 5\%$), and light cycle (0900 to 2100).

Systolic blood pressure (SBP) was measured before the beginning and on days 5 and 8 of either intracerebroventricular or intravenous infusion by using the tail-cuff method (UR-5000; Ueda Electronic Works, Tokyo, Japan). Body weight was measured before the beginning and on day 8 of infusion. On day 5, each rat from groups 1–4 was placed in a separate metabolic cage. Food and water intake and urine volume were measured on days 6 and 7, as were urinary excretions of water and sodium. Urinary sodium concentration was measured by the ion-selective electrode method (Sera 210; Horiba, Kyoto, Japan). Urine samples were centrifuged immediately at 4°C and stored at -80°C until assay. On day 9 or 10, at least 24 h after insertion of the arterial catheter, rats were placed in a plastic bowl with a diameter and depth of 18 cm, and the arterial catheter was connected to a strain-gauge transducer (P23 ID; Gould, Oxnard, CA) to measure the phasic arterial blood pressure, MAP, and heart rate. These parameters were recorded on a chart recorder (RJG-4128; Nihon Kohden, Tokyo, Japan). Resting values of MAP and heart rate were recorded after a stabilization period of at least 30 min.

Experimental protocol 2: Effects of intracerebroventricular infusion of L-NMMA for 3 wk on blood pressure and heart rate. The effects of prolonged intracerebroventricular infusion of L-NMMA for 3 wk on blood pressure and heart rate were examined in 26 rats. Groups 7 ($n = 7$) and 8 ($n = 6$) received intracerebroventricular infusion of $0.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ of L-NMMA for 3 wk, while groups 9 ($n = 6$) and 10 ($n = 7$) received an intracerebroventricular infusion of aCSF. During the infusion period, groups 7 and 9 were placed on a high-salt diet and groups 8 and 10 were on a low-salt diet. On day 21 or 22 of the intracerebroventricular infusion, at least 24 h after insertion of the arterial catheter, resting MAP and heart rate were recorded.

Experimental protocol 3: Effects of chronic intracerebroventricular infusion of L-NMMA on the responses of MAP and RSNA to air-jet stress and acute ganglionic blockade. The responses to air-jet stress were examined in 12 rats receiving intracerebroventricular infusion of L-NMMA and 12 rats receiving intracerebroventricular infusion of aCSF. These rats were placed on a high-salt diet during the infusion period. On day 9 or 10 of the intracerebroventricular infusion,

at least 24 h after implantation of nerve electrode and arterial and venous catheters, when rats appeared to be in good condition and had resumed their regular eating, drinking, and grooming habits, experiments were carried out in a conscious and unrestrained state. The animals were placed in a plastic bowl with a diameter of 18 cm. After a stabilization period of at least 30 min, resting MAP, heart rate, and RSNA were recorded for at least 30 min. Then, each rat was exposed to 30 s of noisy air-jet stress applied to the face from a distance of ~50 cm. The strength of the stimulus was adjusted so that the rats would not increase gross locomotor activity. For measurement of RSNA, original renal nerve signals were amplified by a biophysical amplifier (DPA-100E; Dia Medical System, Tokyo, Japan) and filtered (100–1,000 Hz). The output from the amplifier was fed into a spike counter (DSE-325, Dia Medical System), which identified spikes exceeding a preselected level. The renal neurogram along with the arterial pressure pulse was stored on a magnetic tape recorder (RD-130 TE; TEAC, Tokyo, Japan) for later analysis. The cutoff level of the spike counter was set to filter out the background noise that persisted after intravenous injection of trimethaphan (15 mg/kg body wt). The number of nerve spikes per 1 or 2 s was continuously displayed on a chart recorder (RJG-4128, Nihon Kohden) together with pulsatile pressure, MAP, and pulse rate, which was triggered by arterial pressure pulse. Changes in RSNA were expressed as percent changes from basal spike counts.

We also examined the effects of acute ganglionic blockade. Separate groups of rats given intracerebroventricular infusion of L-NMMA ($n = 9$) or aCSF ($n = 8$) for 10 days were fed a high-salt diet. At least 24 h after insertion of arterial and venous catheters, resting MAP and heart rate were recorded for at least 30 min. Then, 15 mg/kg body wt trimethaphan were intravenously administered and the changes in MAP were recorded.

Statistical analysis. Values are expressed as means \pm SE. In the rats receiving intracerebroventricular infusion of L-NMMA or aCSF, differences among the groups were tested by two-way analysis of variance with or without repeated measures. Subsequent analysis for significant difference was performed using Duncan's multiple-range test. In rats receiving intravenous infusion of L-NMMA or saline, in the rats with recorded RSNA, and in the rats receiving a bolus intravenous injection of trimethaphan, differences between two groups were tested by one-way analysis of variance or

unpaired Student's *t*-test. A value of $P < 0.05$ was considered significant.

RESULTS

Effect of chronic intracerebroventricular infusion of L-NMMA on SBP and body weight. Baseline SBPs measured by the tail-cuff method were similar among groups 1–4 (135 ± 1 , 135 ± 2 , 135 ± 2 , and 136 ± 1 mmHg in groups 1, 2, 3, and 4, respectively). On days 5 and 8 of the intracerebroventricular infusion, group 1 exhibited significantly higher SBP than the other three groups (Fig. 1, left). Although body weight measured on day 8 of the infusion was lower in groups 1 and 2 than in groups 3 and 4, the difference among the four groups was not significant ($P = 0.07$, Table 1).

Effects of chronic intracerebroventricular infusion of L-NMMA and high-salt diet on MAP and heart rate. On day 9 or 10 of the intracerebroventricular infusion, MAP measured directly in a conscious unrestrained state was also significantly higher in group 1 (129 ± 3 mmHg) than in the other three groups (114 ± 3 , 113 ± 1 , and 108 ± 3 mmHg in groups 2, 3, and 4, respectively, Fig. 2, left). However, on day 9 or 10 of the intravenous infusion, MAP showed no difference between the rats receiving L-NMMA and rats receiving normal saline (113 ± 2 mmHg in group 5 and 115 ± 4 mmHg in group 6). No significant difference was found in heart rate among the six groups (Fig. 2, right).

When the intracerebroventricular infusion was prolonged for 3 wk, MAP values in the 4 groups were 114 ± 2 (group 7), 112 ± 2 (group 8), 108 ± 2 (group 9), and 105 ± 4 (group 10) mmHg (Fig. 3, left). The two-way analysis of variance showed that MAP was significantly higher in rats receiving L-NMMA than those receiving aCSF ($P < 0.05$). The high-salt diet did not affect the MAP level. Heart rate was not significantly different among the four groups, and the influence of L-NMMA and the high-salt diet was not statistically significant (Fig. 3, right).

Fig. 1. Systolic blood pressure (left) measured by the tail-cuff method and body weight (right) in rats given chronic intracerebroventricular (i.c.v.) infusion of *N*^G-monomethyl-L-arginine (L-NMMA) or artificial cerebrospinal fluid (aCSF) on a high-salt or low-salt diet. Group 1 (L-NMMA and high-salt diet) had a significantly higher systolic blood pressure than the other 3 groups. However, no significant difference was found in the growth curve among the groups. Values are means \pm SE. * $P < 0.05$.

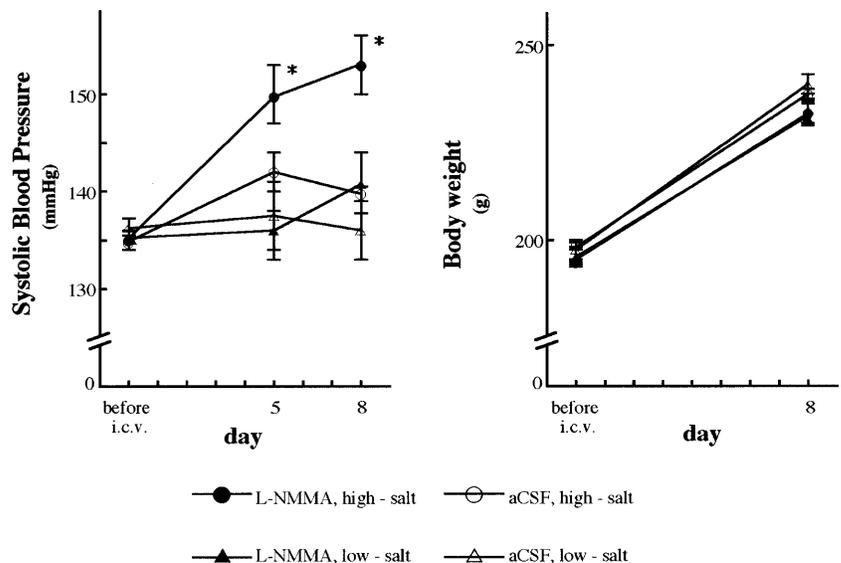


Table 1. *Body weight change and effects of chronic intracerebroventricular infusion of L-NMMA or aCSF on metabolic study*

Group	n	Body Weight, g		Water Intake, ml/day	Urine Volume, ml/day	Food Intake, g/day	U _{Na} V, mmol/day
		Before infusion	Infusion day 8				
1) L-NMMA, high-salt	11	190 ± 6	215 ± 5	87 ± 5*	68 ± 3*	18 ± 0.8	15.7 ± 1*
2) L-NMMA, low-salt	11	191 ± 5	214 ± 6	25 ± 0.9	6.0 ± 0.5	18 ± 0.5	0.4 ± 0.03
3) aCSF, high-salt	9	196 ± 4	225 ± 3	91 ± 3†	66 ± 3†	19 ± 0.9	14.7 ± 0.8†
4) aCSF, low-salt	8	195 ± 5	230 ± 5	26 ± 0.4	6.4 ± 0.7	19 ± 0.6	0.2 ± 0.03

Values shown are means ± SE. L-NMMA, N^G-monomethyl-L-arginine; aCSF, artificial cerebrospinal fluid; U_{Na}V, urinary sodium excretion. * $P < 0.001$ group 1 vs. group 2, † $P < 0.001$ group 3 vs. group 4.

Balance study. In groups 1–4, we performed a balance study on days 6 and 7 of the intracerebroventricular infusion of L-NMMA or aCSF. Food intake was similar among the four groups. Water intake and urinary excretions of sodium and water were higher in rats placed on the high-salt diet (groups 1 and 3) than in rats on the low-salt diet (groups 2 and 4) ($P < 0.001$, Table 1). The intracerebroventricular infusion of L-NMMA did not alter food and water intake and urinary excretions of sodium and water of any rat on either diet (Table 1).

MAP and RSNA in response to air-jet stress and acute ganglionic blockade. To evaluate the contribution of the sympathetic nervous system to the blood pressure elevation in rats receiving intracerebroventricular infusion of L-NMMA, we examined the responses of MAP and RSNA to noisy air-jet stress in separate groups of rats. In this series of experiments, the animals were placed on a high-salt diet and received intracerebroventricular infusion of L-NMMA or aCSF for 9 or 10 days. At least 24 h after the implantation of the nerve electrode and the catheters, resting MAP was significantly higher in rats receiving L-NMMA than in those receiving aCSF (113 ± 3 vs. 104 ± 2 mmHg, $P < 0.05$), but there was no significant difference in the resting heart rate between the two groups of rats (407 ± 8 and 425 ± 9 beats/min, respectively). Representative traces of pulsatile arterial pressure, MAP, and RSNA recorded before, during, and after the noisy air-jet stress are shown in Fig. 4. During the stress period, the increase in RSNA in rats receiving L-NMMA was significantly

larger than in those receiving aCSF (29 ± 4 vs. $19 \pm 3\%$, $P < 0.05$, Fig. 5, left), whereas there was no difference in the maximal change in MAP between the two groups of rats (13 ± 2 vs. 14 ± 3 mmHg, respectively, Fig. 5, right).

We further examined the effects of acute ganglionic blockade. Before the intravenous administration of 15 mg/kg body wt trimethaphan, MAP was 114 ± 1 mmHg in the rats receiving L-NMMA ($n = 9$) and 105 ± 2 mmHg in the rats receiving aCSF ($n = 8$). After the trimethaphan administration, it decreased to 65 ± 2 mmHg in the rats receiving L-NMMA and to 63 ± 2 mmHg in the rats receiving aCSF. The change in MAP was significantly larger ($P < 0.05$) in the rats receiving L-NMMA, and the attained MAP level was similar between the two groups. There was no significant difference in heart rate response between the two groups (from 409 ± 8 to 354 ± 9 beats/min in the rats receiving L-NMMA and from 414 ± 11 to 371 ± 12 beats/min in the rats receiving aCSF).

DISCUSSION

In the present study, chronic intracerebroventricular infusion of L-NMMA for 9 or 10 days significantly increased blood pressure in rats on the high-salt diet but not on the low-salt diet. When aCSF was chronically infused intracerebroventricularly, blood pressure was similar between rats on the high-salt diet and the low-salt diet. Chronic intravenous infusion of L-NMMA at the same dose as the intracerebroventricular infu-

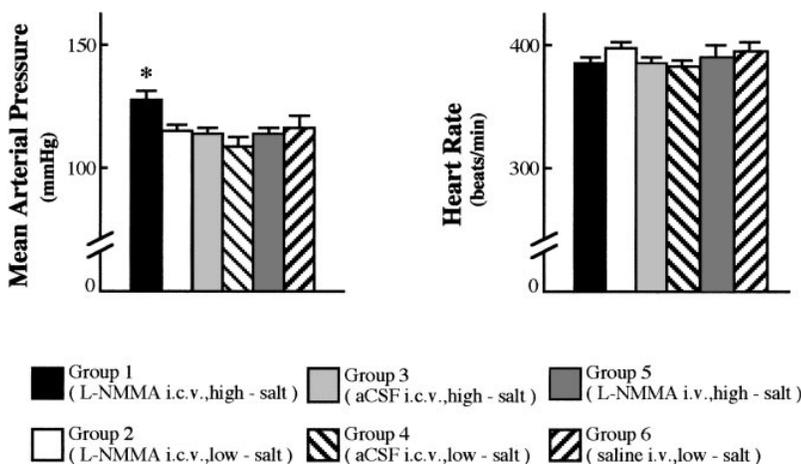
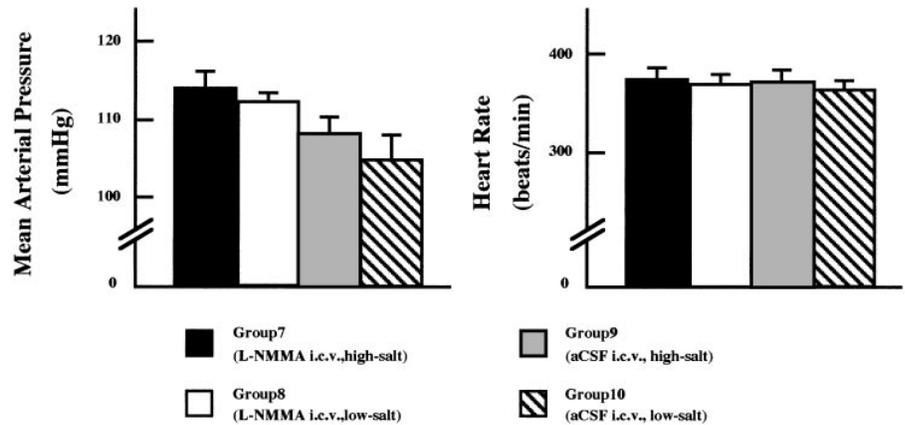


Fig. 2. Mean arterial pressure (MAP, left) and heart rate (right) measured in conscious, unrestrained states on day 9 or 10 of the infusion. Group 1 showed a significantly higher MAP than the other 3 groups. Heart rate was similar among the 4 groups. Values are means ± SE. * $P < 0.05$.

Fig. 3. MAP (left) and heart rate (right) measured in conscious, unrestrained states on day 21 or 22 of the intracerebroventricular infusion. Two-way analysis of variance showed that MAP was significantly higher in rats receiving L-NMMA on either diet than those receiving aCSF ($P < 0.05$). However, the effect of the high-salt diet on MAP was not statistically significant. Values are means \pm SE.



sion had no influence on blood pressure. These findings indicate that the blood pressure elevation observed was mediated by a central mechanism(s). Furthermore, after the prolonged intracerebroventricular infusion for 3 wk, blood pressure was significantly higher in the rats receiving L-NMMA than in rats receiving aCSF irrespective of salt intake. Thus our data indicate that long-term inhibition of NOS in the brain elevates blood pressure in SD rats. High-salt diet seems to cause a more rapid increase in blood pressure in the NOS inhibitor-treated rats.

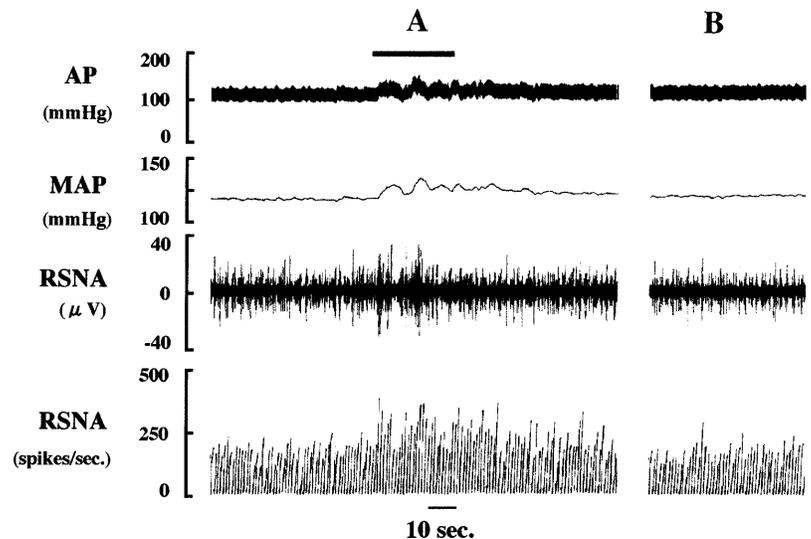
Pressor effects of acute inhibition of NOS in the central nervous system have often been reported (3, 7, 8, 28, 30, 33). Intracisternal injection (28) or microinjection into the nucleus of the solitary tract (8, 30) or the rostral ventrolateral medulla (30, 33) of NOS inhibitor evoked an increase in arterial blood pressure and RSNA in anesthetized rats (28, 30), cats (33), and rabbits (8). Short-term intracerebroventricular infusion of 5 and 15 $\mu\text{g}/\text{min}$ of N^G -nitro-L-arginine (L-NNA), a NOS inhibitor, elevated arterial pressure in a dose-dependent manner in normotensive rats, and the elevation of blood pressure was significantly attenuated by L-arginine, whereas an intravenous infusion of the same dose of L-NNA did not elevate blood pressure (7).

These reports suggest that NO acts centrally to modulate sympathetic outflow and blood pressure.

Whether chronic inhibition of NOS in the brain causes hypertension remains controversial. Chronic intracerebroventricular infusion of L-NMMA further elevated blood pressure in DOCA-salt hypertensive rats (25), but not in spontaneously hypertensive rats (SHR) (27). In the present study, rats receiving intracerebroventricular infusion of L-NMMA showed blood pressure elevation that was accelerated by salt loading. Expression of mRNA of constitutive NOS was suppressed in the hypothalamus of DOCA-salt hypertensive rats (17). In normotensive rats, chronic salt loading induces increase in NOS gene expression in the PVN and SON of the hypothalamus (10). Taken together, decreased brain NO production may, at least in part, be involved in the pathogenesis of salt-sensitive hypertension.

On the other hand, chronic intracisternal infusion of L-NMMA for 7 days did not alter blood pressure in normotensive rats (31). In the present study, the intracerebroventricular infusion of L-NMMA for 3 wk elevated blood pressure irrespective of salt intake, whereas short-term infusion of L-NMMA for up to 10 days elevated blood pressure only in rats placed on a high-

Fig. 4. Representative chart of pulsatile arterial pressure (AP), MAP, and renal sympathetic nerve activity (RSNA). A: noisy air-jet stress increased these parameters. B: 20 min after air-jet stress, AP, MAP, and RSNA returned to baseline levels.



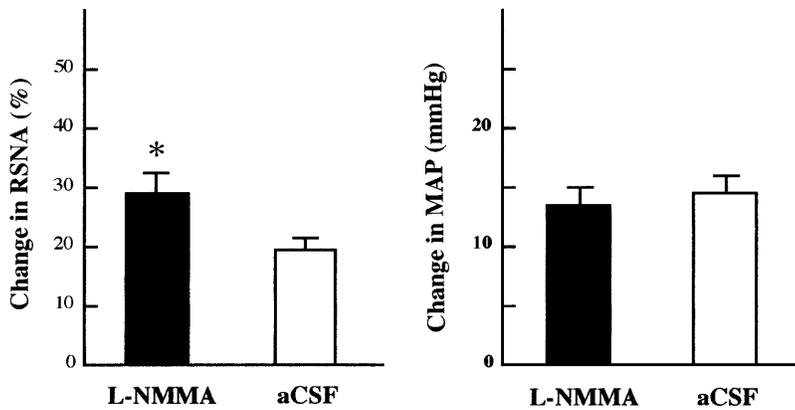


Fig. 5. Maximal changes in RSNA (*left*) and MAP (*right*) evoked by the noisy air-jet stress in rats receiving chronic intracerebroventricular infusion of L-NMMA or aCSF on a high-salt diet. Values are means \pm SE. * $P < 0.05$ vs. rats receiving aCSF.

salt diet. Therefore, prolonged inhibition of NOS in the brain for more than 10 days may be necessary to affect blood pressure in normotensive rats on a low- or normal-salt diet. In addition, the change in blood pressure caused by the intracerebroventricular infusion of L-NMMA was relatively small. Chronic inhibition of NOS in the brain is probably insufficient to cause definite hypertension. However, we cannot exclude the possibility that suppression of NOS in the brain was incomplete in the present study. As stated in METHODS, in a series of preliminary experiments the intracerebroventricular infusion of 0.08 or 0.2 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ of L-NMMA did not elevate blood pressure. Therefore, 0.5 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ of L-NMMA may be near to the threshold dose of the NOS inhibitor to cause blood pressure elevation. Blood pressure response to higher dose of L-NMMA should be examined in future studies.

Immunohistochemical studies have demonstrated the existence of NOS in the cortex, cerebellum, hypothalamus, brain stem (2), and cerebral ventricular system, including circumventricular organs (20). A NOS inhibitor infused into the lateral ventricle may spread to reach these sites (23). Decrease in NOS activity in different brain structures are reported after intracerebroventricular administration of L-NAME (1, 22). Because the NOS gene expression was suppressed in the hypothalamus of DOCA-salt hypertensive rats (17) and enhanced by salt loading in the PVN and the SON of normotensive rats (10), it is plausible that L-NMMA infused into the lateral ventricle acts on the hypothalamic nuclei to modulate the salt sensitivity of blood pressure. However, one could not exclude a possibility that L-NMMA infused into the lateral ventricle acted on vasculature, including brain vessels and choroidal vessels, to reduce arterial blood flow to specific brain areas or the cerebrospinal fluid-brain barrier. Further studies are necessary to elucidate the precise site(s) of action of the NOS inhibitor infused into the lateral ventricle.

We performed balance study on *days 6* and *7* of the intracerebroventricular infusion period. At that time, the food and water intake and urinary excretions of sodium and water were similar between the rats receiving intracerebroventricular infusion of L-NMMA and the rats receiving aCSF either on the high-salt diet or the low-salt diet (Table 1), suggesting that rats already

achieved sodium balance. The results seem to be in accordance with earlier report that chronic intracerebroventricular infusion of low-dose L-NMMA ($0.08 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) further elevates blood pressure in DOCA-salt hypertensive rats without altering sodium and water balance (25). However, we did not measure daily intake of food and water and urinary sodium excretions through *days 1–5*. Therefore, one could not exclude the possibility that the blood pressure elevation in rats receiving intracerebroventricular infusion of L-NMMA on the high-salt diet was associated with altered sodium and water balance during the early phase.

In the experiments of trimethaphan administration, the baseline MAP was significantly higher and the fall in MAP in response to the ganglionic blockade was significantly larger in the L-NMMA-treated rats. However, the fall in MAP was profound in either rats chronically given L-NMMA or aCSF, and the attained blood pressure level was a so-called spinal level. Therefore, the trimethaphan administration does not simply eliminate the component of blood pressure related to the L-NMMA treatment. Although we could not exclude a contribution of sympathetic nervous system to the blood pressure elevation in rats given chronic intracerebroventricular infusion of the NOS inhibitor, the precise mechanism should be elucidated in future studies.

The augmented response of RSNA to air-jet stress in the rats given L-NMMA and the high-salt diet are consistent with a previous study (14) in which Dahl salt-sensitive and salt-resistant rats placed on a high-salt diet showed an increase in RSNA in response to air stress for 10 min. In this report, the air stress-induced increases in RSNA and renal sodium retention were greater in the salt-sensitive rats than in the salt-resistant rats and were abolished by bilateral renal denervation in both strains (14). On the other hand, renal plasma flow and glomerular filtration rate were not altered by the environmental stress (14). Because the magnitude of the air stress-induced increase in RSNA significantly correlated with the decrease in urinary sodium excretion (14), environmental stress may increase renal tubular sodium reabsorption, which is mediated by the central mechanism(s) (6), and the RSNA response and the resultant renal sodium retention were enhanced in hypertensive Dahl salt-sensitive rats fed on a high-salt diet (6, 14). In our present study,

we did not examine the effects of the stress on renal sodium and water handling, because the duration of the air-jet stress was 30 s.

The blood pressure response to air-jet stress was similar between these two groups of rats. We do not have a clear explanation for the absence of increase in blood pressure response. One possibility is that the stress-induced increase in sympathetic outflow to the vascular bed other than kidney was not exaggerated in rats receiving the combined treatment. The contribution of the sympathetic nervous system to the regulation of mesenteric and hindquarter vascular resistance may be different from that to the renal vascular resistance (11). In addition, experimental conditions might have influenced the MAP and RSNA responses to air-jet stress. In a previous study (12), air stress increased RSNA in SHR rats but not in normotensive Wistar-Kyoto (WKY) rats, whereas the increases in MAP in response to stress were similar between SHR and WKY rats. MAP did not respond to air-jet stress in Dahl salt-sensitive and salt-resistant rats placed on a high-salt diet (14) and DOCA-salt hypertensive and sham DOCA-salt normotensive rats (13). However, other investigators reported that the responses of MAP and RSNA to air stress were significantly larger in SHR than in WKY rats (9). The experimental conditions were different among these previous reports (9, 12–14) and the present study. A higher MAP response to air stress in SHR (9) was observed 4 h after recovery from the anesthesia, and in the other previous studies (12–14), rats placed in Lucite cylinders, which permitted only forward and backward movement, were exposed to air-jet stress for 10 min. On the other hand, in the present study, the responses to a 30-s period of noisy air-jet stress were examined in unrestrained rats at least 24 h after surgery.

In summary, in rats placed on a high-salt diet but not on a low-salt diet, blood pressure was significantly elevated in rats receiving chronic intracerebroventricular infusion of L-NMMA for up to 10 days compared with rats receiving aCSF. Chronic intravenous infusion of L-NMMA at the same dose as the intracerebroventricular infusion had no influence on blood pressure. After 3 wk of intracerebroventricular infusion, blood pressure was only slightly but still significantly higher in the rats receiving L-NMMA than in rats receiving aCSF, irrespective of salt intake. The results suggest that in normotensive SD rats, prolonged inhibition of NOS in the brain increases blood pressure. However, even when combined with salt loading, brain NOS inhibition may be insufficient to cause definite hypertension in normotensive SD rats. Although the blood pressure elevation is mediated by central mechanism, the detail of the mechanism should be elucidated in future studies.

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