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Effect of Nitric Oxide Inhibition on Blood Pressure and Corticosterone Responses in Adult Rats Neonatally Treated with Glutamate

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Summary

The role of nitric oxide (NO) in the regulation of blood pressure and hypothalamic-pituitary-adrenal function of adult rats treated with monosodium glutamate (MSG) during the neonatal period was investigated. Blood pressure and the heart rate were registered by a computerized system of direct blood pressure measurement through an indwelling cannula in the femoral artery. The inhibition of the activity of NO synthase by acute injection of N^{ω}-nitro-L-arginine-methylester (L-NAME, 30 mg/kg, i.v.) to control rats produced a rise of blood pressure and a fall of heart rate. Both responses were reduced in MSG-treated rats. Repeated administration of L-NAME (50 mg/kg, i.p, two times daily for 4 days) increased BP in both groups of animals. Corticosterone concentrations in the plasma were significantly increased in response to repeated L-NAME administration in MSG-treated rats, while ACTH levels were similar in both groups of animals. These data suggest that some of the cardiovascular and endocrine changes in rats treated with MSG may be due to the abnormal function of the NO system.

Key words

Monosodium glutamate • Nitric oxide • L-NAME • Blood pressure • Corticosterone

Introduction

Since the report of Olney (1969) describing obesity and growth retardation in mice with destroyed hypothalamic arcuate and preoptic nuclei by injection of monosodium glutamate in the neonatal period, a body of new information concerning morphological, behavioral, and endocrine abnormalities has accumulated (Nemeroff *et al.* 1977, Hamaoka and Savada 1987, Kovacs *et al.* 1995, Otoya *et al.* 1996, Dubovický *et al.* 1997, Škultétyová *et al.* 1998 a,b, Macho *et al.* 1999). Although several deviations were described in MSG-treated rats, no definitive evidence of altered cardiovascular system regulation is available (van den Buuse *et al.* 1985, Mosqueda-Garcia *et al.* 1986, Clough *et al.* 1986, Hambley *et al.* 1987). Our previous experiments with stimulation of the cardiovascular system by phenylephrine and angiotensin II indicated a decreased responsiveness of blood pressure (BP) to vasopressor drugs in MSG-treated rats (Tokarev *et al.* 1997).

It is known that nitric oxide (NO) is a powerful vasodilator in the mammalian organisms (Palmer *et al.* 1987) and mediates several central and peripheral endocrine processes (Grossman 1994, Kadowaki *et al.*

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1994, Bugajski *et al.* 1997). Inhibition of NO formation by systemic injections of L-arginine derivatives was shown to increase blood pressure (Rees *et al.* 1990, Gardiner *et al.* 1990, Johnson and Freeman 1992, Kvetňanský *et al.* 1997) and to induce ACTH and corticosterone release (Adams *et al.* 1992, Giordano *et al.* 1996).

We have suggested that at least some of the cardiovascular and endocrine regulatory alterations observed in rats treated with MSG in the neonatal period could be related to abnormal formation or function of NO. In the present experiments, the impact of acute and repeated L-NAME treatment on blood pressure, ACTH and corticosterone release was assessed in MSG-treated and control rats.

Material and Methods

Animals

Newborn pups of the Sprague-Dawley strain (Charles River Wiga, Silzfeld, Germany) received an injection of MSG (4 mg/g of BW, i.p.) or 10 % NaCl (as isosmotic control to the MSG solution) on alternate days during the first 10 days of life. The rats were weaned at the age of 21 days and separated according to the treatment and sex. The animals were housed under controlled 12 h light-dark cycle (light at 06:00 h), constant temperature (20-22 °C) and free access to food and water. Experiments were performed on 25 male rats aged 3 months.

Experimental protocol

N^{ω}-nitro-L-arginine-methyl ester (L-NAME, Sigma Chemical Co, St. Louis, MO), was diluted in saline immediately before injection. Adult MSG-treated rats (2 groups) and control animals (2 groups) were administered L-NAME (50 mg/kg/ml, i.p.) or saline (1 ml/kg) two times daily (every 10-14 h) for 4 days. On the day before the experiment, a femoral artery and a jugular vein were cannulated under pentobarbital anesthesia. Saline-pretreated control and MSG-lesioned rats served for testing the response to acute L-NAME injection, while those pretreated with L-NAME were used for evaluation of the effects of repeated L-NAME administration.

Measurement of blood pressure and heart rate

All experimental procedures were performed in the morning hours between 08:00-12:00 h. On the day of the experiment, 14 h after finishing the repeated L-NAME administration, blood samples (0.8 ml) were withdrawn for ACTH and corticosterone determination one hour before the BP measurement. Blood pressure was measured simultaneously on two rats by means of a computerized direct BP system (DBP001, Kent Scientific Corporation) which was coupled with a MacIntosh IIvx computer. Arterial cannulas were connected to the TRN050 BP transducers the signal of which was processed by TRN005 amplifiers and a T51-R terminal panel. Basal BP parameters were measured for 10 min with registration every 20 s. Without interrupting the BP recordings, L-NAME (30 mg/kg/ml, i.v.) was injected through the cannula in the jugular vein and BP parameters were measured during the next 30 min.

Hormone measurements

Plasma samples were analyzed by radioimmunoassay for ACTH (Ježová *et al.* 1987) without extraction using a double antibody technique to separate free and bound fractions. Plasma corticosterone was measured by radioimmunoassay after previous extraction in dichlormethane (Ježová *et al.* 1994).

Statistical analysis

The data were statistically evaluated using one-way analysis of variance (ANOVA), Student's t-test and post-hoc Scheffe F-test for evaluating differences between the groups.

Results

Effect of acute administration of L-NAME on blood pressure in control and MSG-treated rats

An acute injection of L-NAME to control rats induced a pronounced increase of mean blood pressure (MBP) and a decrease in heart rate (HR) (Fig.1). The degree of hypertension accompanied by bradycardia can be described by the logarithmic function A + B*LOG(X) (R^2 values were 0.76 and 0.69, respectively). The plateaus were reached after 10-11 min, with maximal changes +45.7±2.9 mm Hg for MBP and –43.5±8.4 beats/min for HR.

In MSG-treated rats, markedly diminished BP responses to an acute L-NAME injection were demonstrated as compared to those in the control group (Fig. 1). MBP ($R^2 = 0.118$) and HR ($R^2 = 0.305$) reached their maximal values after 3 min (+19.6±4.1 mm Hg and –38±20 beats/min, respectively).

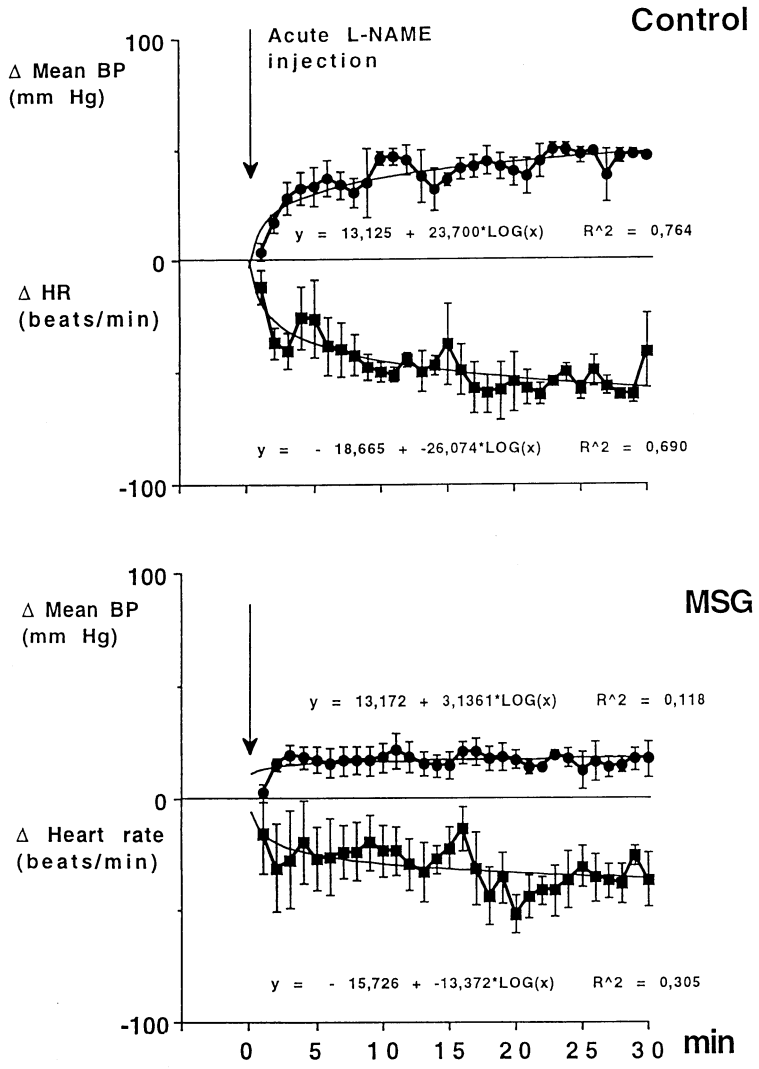

Fig. 1. *Effect of an acute L-NAME injection (30 mg/kg, i.v.) on mean BP (full dots) and heart rate (full squares) in control and MSG-treated rats. BP parameters were registered during 30 min after the injection. Responses were described by logarithmic curves. In each rat, means of 3 registrations during one minute were calculated. Data represent means ± S.E.M. of 4-5 rats.*

Table 1. Hemodynamic parameters measured after repeated administration of saline and L-NAME in control and MSG-treated rats

	Saline		L-NAME	
	Controls (n=4)	MSG (n=5)	Controls (n=6)	MSG (n=6)
Systolic BP (mm Hg)	126.0±4.0	127.6±3.9	140.1±4.1	145.5±3.2*
Diastolic BP (mm Hg)	95.4±2.5	96.6±3.1	114.8±5.3*	124.9±3.1*
Mean BP (mm Hg)	109.8±2.6	111.8±3.1	127.8±4.7*	135.7±3.0*
Heart rate (beats/min)	285.1±8.5	312.7±5.7	300.8±8.3	323.1±15.1

L-NAME – N^{ω}-nitro-L-arginine-methylester; MSG – monosodium glutamate; BP – blood pressure. Data are means ± SEM. Asterisks indicate significant difference ($P<0.05$) between corresponding groups of saline and L-NAME treated rats.

Effect of repeated administration of L-NAME on blood pressure in control and MSG-treated rats

As shown in Table 1, the administration of L-NAME for four days induced BP increase in both control and MSG-treated rats in comparison with the corresponding saline-treated groups. Significant changes were observed in systolic, diastolic and MBP values of MSG-treated rats as well as in diastolic and MBP values of control rats. The heart rate was not affected by L-NAME in any of the groups. There was a significant reduction of pulse pressure in MSG-treated rats with repeated L-NAME injections (20.5±2.0 mm Hg) in comparison with saline-administered MSG-treated rats (30.8±2.8 mm Hg).

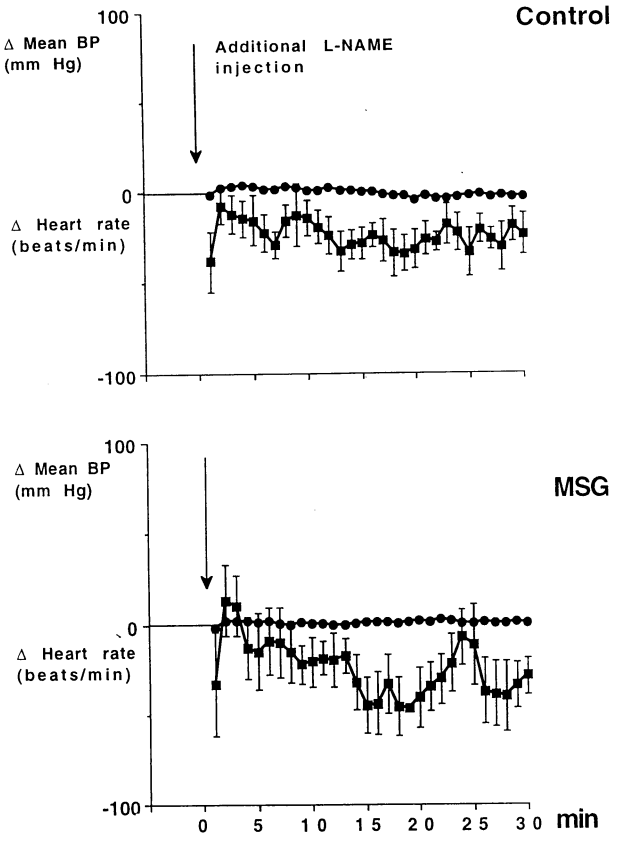

Fig. 2. *Effect of intravenous L-NAME injection (30 mg/kg) on mean BP (full dots) and heart rate (full squares) in control and MSG-treated rats pretreated with L-NAME (50 mg/kg, i.p, two times daily during 4 days). Responses were registered during 30 min. In each rat, means of 3 registration during one minute were calculated. Data represent means ± S.E.M. of 6 rats.*

Additional intravenous injection of L-NAME to rats with repeated L-NAME administration decreased the heart rate in both control and MSG-treated rats, while the MBP, elevated by the previous treatment, remained unchanged (Fig. 2).

Effect of repeated administration of saline or L-NAME on plasma ACTH and corticosterone in MSG-treated and control rats

Plasma concentrations of ACTH and corticosterone were within the normal range after saline administration to MSG-treated and control rats. In rats with repeated administration of L-NAME, plasma ACTH levels in MSG-treated and control animals were 157±77.9 and 175±39.7 pg/ml, respectively. In contrast, plasma corticosterone concentrations were significantly increased in MSG-treated rats compared to the controls (Fig. 3). In MSG-lesioned rats treated with L-NAME, morning corticosterone concentrations in the plasma were significantly higher compared to those in all other groups of rats, while no significant differences in ACTH release were observed.

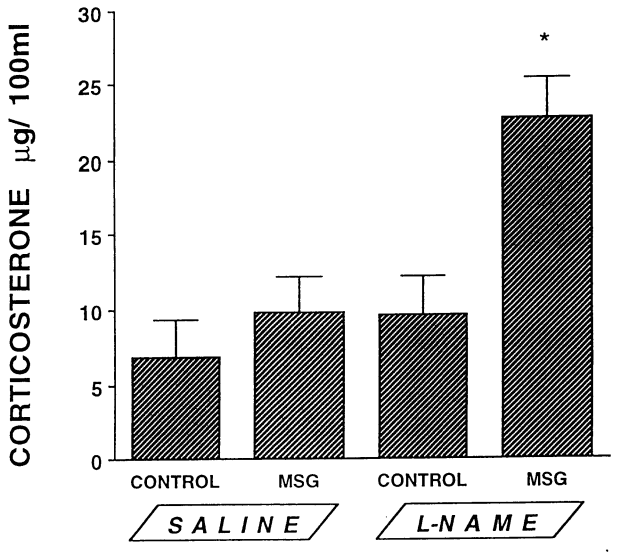

Fig. 3. *Morning plasma corticosterone levels in control and MSG-treated rats 14 h after the end of repeated administration of saline and L-NAME (50 mg/kg i.p, two times daily during 4 days). Each bar represents mean ± S.E.M. of the data obtained in 4-6 animals. Asterisk indicates significant difference ($P<0.05$) compared to all other groups (one-way ANOVA followed by Scheffe F-test).*

Discussion

The present study shows that the treatment of rats in the neonatal period with MSG resulted in an attenuated BP response to acute injection of L-NAME which inhibits the formation of NO, identified as the endothelium-derived relaxing factor (Palmer *et al.* 1988, Rees *et al.* 1990). In contrast to control animals, which responded by a logarithmic rise of mean BP with reflex bradycardia during the whole time period of BP recording (30 min), MSG-treated rats showed a reduced and constant BP response to acute L-NAME injection. Since endothelial NO appears to be one of the important modulators of vascular smooth muscle tone (Palmer *et al.* 1988, Vanhoutte 1989), the reduced BP response to L-NAME observed in MSG-treated rats may be related to both altered endothelial NO formation and smooth muscle tone abnormalities. In support of the latter assumption, previous experiments demonstrated reduced BP responses to the α_1-adrenergic receptor agonist phenylephrine and to angiotensin II, as well as lower reactivity of the hind limb vascular bed to noradrenaline and serotonin in MSG-treated rats (Tokarev *et al.* 1997, Kristová *et al.* 1998). An alternative and equally plausible explanation is that the extensive neurological damage caused by MSG might have altered the sympathetic output.

In contrast to the different effects of acute L-NAME injection on BP in MSG-treated and control rats, repeated L-NAME treatment induced BP increase, but did not change the heart rate in both groups of animals. Sustained hypertension induced by chronic L-NAME appears to involve different mechanisms (Johnson and Freeman 1992). Activation of the renin-angiotensin and sympathoadrenal systems by chronic NO blockade may represent additional factors in the mechanisms responsible for BP elevation (Navarro *et al.* 1994, Iwai *et al.* 1995, Zanchi *et al.* 1995, Jablonskis and Howe 1995). Kvetňanský *et al.* (1997) reported that prolonged (4 days) administration of L-NAME increased the levels of plasma epinephrine, norepinephrine and dopamine metabolites, indicating activation of the sympathoadrenal system in rats.

In recent years, information suggesting that endogenous NO may inhibit ACTH and corticosterone secretion has accumulated (Adams *et al.* 1992, Volpi *et al.* 1996, Bugajski *et al.* 1997). Acute L-NAME injection was shown to induce ACTH and corticosterone release in mice (Giordano *et al.* 1996). In the present study, changes in plasma ACTH and corticosterone levels were evaluated after repeated administration of L-NAME or saline. In control rats, repeated L-NAME treatment failed to modify plasma ACTH and corticosterone concentrations. In spite of normal ACTH levels, high corticosterone concentrations in plasma were observed in MSG-treated rats. We have previously observed (Škultétyová *et al.* 1998a) that corticosterone responses to several stress stimuli in MSG-treated rats are prolonged. Moreover, the mentioned study demonstrated a decreased clearance rate of corticosterone in rats treated with MSG. It is possible that the reduced clearance rate of corticosterone contributed to the high levels of this steroid in the plasma of MSG-treated rats after repeated L-NAME treatment. Potential changes in NO synthase (NOS) activity in the adrenal cortex (Tsuchiya *et al.* 1997) may also be involved.

Neurotoxic lesions induced by MSG in the neonatal period may result in long-term changes of central regulatory mechanisms. The administration of glutamate to neonatal rats is associated with the activation of the stress system including alterations at the level of gene expression (Ježová *et al.* 1998). The inhibition of NO synthesis has recently been found to have a neuroprotective action against neonatal excitotoxic lesions (Marret *et al.* 1999). Moreover, neonatal mice lacking neuronal NOS were shown to be more vulnerable to hypoxic-ischemic injury (Ferriero *et al.* 1996). Although NOS-immunoreactive neurons in selected parts of the brain survive neonatal glutamate treatment (Xue *et al.* 1997), long-lasting functional changes cannot be excluded. In the light of both cardiovascular and adrenal responses observed in the present study, it is likely that central NO may play a role through regulation of sympathetic activity and/or hypothalamo-pituitary-adrenocortical axis.

Our findings revealed that adult rats treated with MSG in the neonatal period had reduced BP response to acute L-NAME injection and high plasma corticosterone concentrations after prolonged inhibition of NO synthesis. These data provide indirect evidence that MSG-treated rats have altered NO production and suggest that some of the cardiovascular and endocrine changes in rats treated with MSG may be due to abnormal function of the NO system.

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