Physiol. Res. 46: 165 – 171, 1997

Treatment of Neonatal Rats with Monosodium Glutamate Attenuates the Cardiovascular Reactivity to Phenylephrine and Angiotensin II

D. TOKAREV, V. KRISTOVÁ[1], M. KRIŠKA[1], D. JEŽOVÁ

Institute of Experimental Endocrinology, Slovak Academy of Sciences and [1]Department of Pharmacology, Medical School, Comenius University, Bratislava, Slovak Republic

Received March 20, 1996
Accepted December 18, 1996

Summary

In rats, neonatal administration of monosodium glutamate (MSG) causes serious damage in some hypothalamic and circumventricular areas. The resulting loss of appropriate neurons important for the regulation of blood pressure (BP) may modulate cardiovascular system receptivity in these animals. In the present study, the reactivity of the cardiovascular system to intravenous injection of α_1-adrenergic receptor agonist phenylephrine (200 μg/kg/ml) and angiotensin II (500 ng/kg in 0.6 ml for 2 min) was investigated in adult rats which had been neonatally treated with MSG or vehicle. BP parameters measured directly in conscious cannulated rats were continuously registered using a computerized system. Under basal conditions, MSG-treated rats had slightly lower systolic, diastolic and mean BP with significant differences in pulse pressure (systolic – diastolic BP). In MSG-treated animals, the maximal increase of mean arterial BP after phenylephrine and the duration of BP elevation after both agents were significantly reduced. Slopes of the linear portion of baroreceptor function curves in control and MSG-treated rats did not differ significantly, indicating that baroreflex efficacy was unchanged. The results obtained by perfusion of the hindlimb vascular bed *in situ* showed that the pressure responses to increasing doses of noradrenaline in MSG-treated rats were reduced. These findings demonstrate that neonatal treatment of rats with MSG lowers the responsiveness of the cardiovascular system, particularly in response to α-adrenergic stimulation. It is suggested that the attenuation of cardiovascular reactivity in MSG-treated rats is, at least partly, caused by diminished vascular responsiveness.

Key words
Blood pressure – MSG treatment – Phenylephrine – Angiotensin II – Vascular responsiveness

Introduction

Repeated administration of monosodium glutamate (MSG) to neonatal rodents induces a large spectrum of endocrine, neurochemical and behavioural changes (Olney 1969, Nemeroff *et al.* 1977, Spinedi *et al.* 1984, Lorden and Caudle 1986, Magarinos *et al.* 1988, Boudouresque *et al.* 1991, Fisher *et al.* 1991, Caputo and Scallet 1995). Growth retardation and obesity belong to the typical characteristics of adult animals neonatally treated with MSG. The main damage is located in the preoptic and arcuate nuclei of the hypothalamus (Olney 1969) and some circumventricular regions, such as the area postrema (Phelix and Hartle 1990) and median eminence (Olney 1969, Nemeroff *et al.* 1977).

The above mentioned regions contain receptors for several vasoactive substances and they are known to be involved in the regulation of cardiovascular function (Saavedra 1990, Williams *et al.* 1992, Bohus and Koolhaas 1993). Moreover, Hamaoka and Savada (1987) observed morphological alterations in the heart of MSG-treated mice, e.g. a smaller number and hypertrophy of myocardial cells. On the other hand, either unchanged or only slightly diminished blood pressure (BP) was demonstrated in adult rats treated with MSG in the neonatal period (Clough *et al.* 1986, Mousqueda-Garcia *et al.* 1986, Hambley *et al.* 1987).

We hypothesized that possible alterations of the cardiovascular system in MSG-treated rats might be revealed by evaluating its function after appropriate stimulation. All previously published values of blood pressure in MSG-treated rats were registered by the indirect tail-cuff method. In our paper, we present the data obtained by direct measurement of blood pressure parameters in response to α_1-adrenergic receptor agonist phenylephrine and angiotensin II administration in rats neonatally treated with MSG. A computerized system for the measurement and analysis of blood pressure parameters was used.

Methods

Neonatal treatment with MSG

Pregnant Sprague-Dawley rats (Charles River Wiga, Silzfeld, Germany) were housed individually under standard laboratory conditions with 12 h light:darkness cycles (light on at 06:00 h), controlled temperature (23±2 °C) and free access to food and water. After parturition, the newborn pups received an intraperitoneal injection of MSG (4 mg/g of body weight) (Sigma, St Louis, MO) diluted in saline (0.9 % solution of NaCl, 100 μl) or 10 % NaCl as an iso-osmotic control on alternate days for the first 10 days of life. The animals were weaned at 21 days of age, divided into groups of 6 males and used for experimentation at the age of 10 weeks.

Cannulation

One day before the measurements, a femoral artery and a jugular vein of both MSG and control rats were catheterized with polyethylene cannulas (Intramedic PE 50, Clay Adams, USA) under pentobarbital anaesthesia (50 mg/kg i.p.). These cannulas were filled with heparinized saline (300 IU/ml), tunneled under the skin of the back and led out through the top of the cage using a steel spring. After the cannulation procedure was completed, the animals were housed individually and the cages were transferred into the experimental room.

Measurement of blood pressure and drug treatments

Measurements of blood pressure parameters were performed simultaneously on two rats by a computerized direct blood pressure system (DBP001, Kent Scientific Corporation, USA) that was configurated with a MacIntosh IIvx personal computer. Arterial cannulas were connected to transducers and basal BP parameters were registered for 10 min. Thereafter, the α_1-adrenergic receptor agonist phenylephrine (200 μg/kg/ml) was injected intravenously without interruption of the recordings which were taken at 1-second intervals. Fifteen minutes later, when arterial pressure came back to normal values, angiotensin II (500 ng/kg dissolved in 0.6 ml of saline) was infused (2 min) *via* the cannula in the jugular vein. Phenylephrine (Sigma Chemical Co., St. Louis, MO) and angiotensin II (Peninsula Laboratories, Belmont, Canada) were diluted in saline.

Hindlimb vascular bed preparation

Rats were anaesthetized with pentobarbital sodium and laparotomy was performed. All visceral organs were ligated and completely resected. The abdominal aorta and inferior vena cava were carefully isolated. After administration of heparin the aorta was ligated distal to the renal artery, cannulated and connected to the perfusion system. The outflow from vena cava was directed into a perfusion reservoir. The vascular bed was perfused at a constant flow rate of 3 ml/min with registration of perfusion pressure changes. Noradrenaline (doses 0.01, 0.1, 1.0 and 10 μg) was injected directly into the cannula in a volume of 0.1 ml.

The experimental data were evaluated by analysis of variance (ANOVA) and unpaired Student's t-test. Data are expressed as means ± S.E.M.

Results

Basal levels of blood pressure and heart rate

Under resting conditions, MSG-treated rats showed diminished BP parameters compared to those in control rats (Table 1). Significant differences were observed in pulse pressure (systolic − diastolic BP) as shown in Figure 1. Heart rate values were similar in both groups (Table 1).

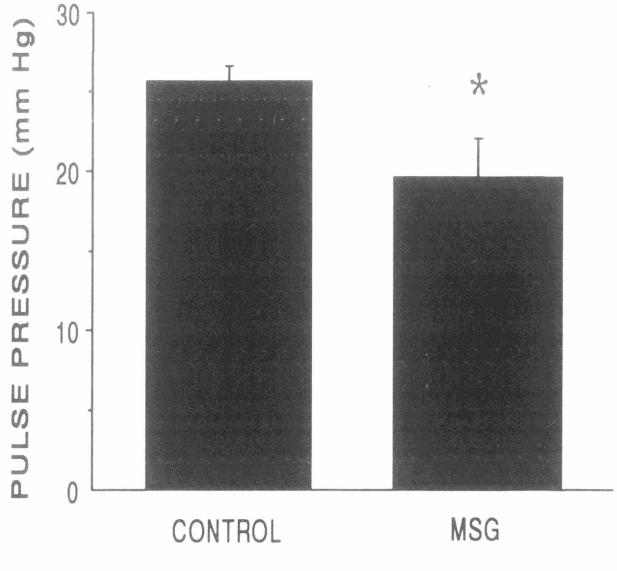

Fig. 1. *Pulse pressure (systolic − diastolic BP) in control (n=6) and MSG-treated (n=7) rats measured under basal conditions. Statistical significance: * $p<0.05$.*

Table 1
Basal characteristics of MSG-treated (n=7) rats and age-matched controls (n=6)

	Control	MSG
Body weight (g)	396.7±4.0	302.1±8.6**
Systolic BP (mm Hg)	138.7±4.2	129.6±1.9
Diastolic BP (mm Hg)	113.5±4.4	111.9±1.4
Mean BP (mm Hg)	125.9±4.3	121.3±1.2
Heart rate (beats/min)	319.9±12.4	326.0±8.7

** *p<0.01, significant difference from the control group*

Vasopressor response to phenylephrine

A typical example of BP registration before and after phenylephrine injection is presented in Figure 2. Phenylephrine increased BP in both control and MSG-treated rats. However, the vasopressor response to phenylephrine was significantly decreased (mean and diastolic BP) in MSG-treated rats compared to that in the control group (Table 2). Duration of the response of BP parameters to phenylephrine was significantly reduced in MSG-treated rats in comparison with control rats (Table 3).

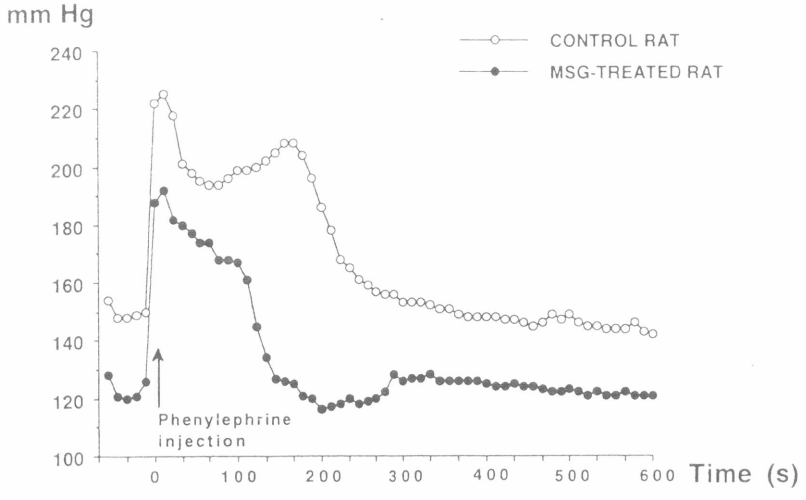

Fig. 2. *Typical systolic BP recording before and after intravenous injection of phenylephrine. BP measured directly in conscious cannulated rats was continuously registered using a computerized system.*

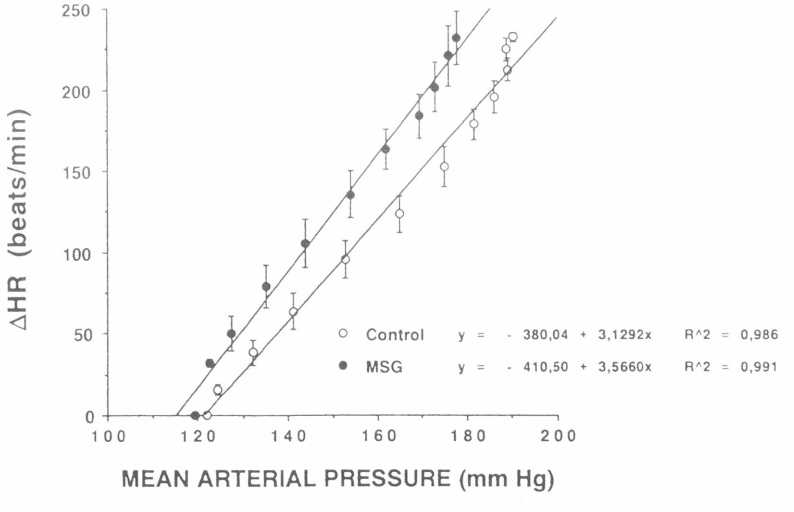

Fig. 3. *The efficiency of the baroreceptor reflex to phenylephrine (200 μg/kg/ml, i.v.) in control (open circles, n=5) and MSG-treated (closed circles, n=5) rats. Each point represents the reflex decrease in heart rate produced by an increase in blood pressure. The slopes of the linear portion of the baroreceptor function curve were determined by linear regression analysis.*

The reflex fall of heart rate in response to the phenylephrine-induced rise in BP had similar characteristics in both groups of animals. When the decrease in HR was plotted against mean arterial BP, there were no significant differences in the slope of the linear portion of the obtained baroreceptor function curves between the two groups of animals (Fig. 3).

Table 2
Maximal increment of BP parameters to intravenous injection of phenylephrine (200 μg/kg/ml) and angiotensin II (500 ng/kg/ml/2 min) in control (n=6) and MSG-treated (n=7) rats

	Phenylephrine		Angiotensin II	
Parameters (mm Hg)	Control	MSG	Control	MSG
Systolic BP	108.3±6.6	93.6±7.7	74.5±4.9	67.2±3.8
Diastolic BP	65.3±5.8	49.4±4.4*	56.2±3.8	50.6±7.3
Mean BP	80.2±5.9	61.9±4.5*	64.8±3.8	59.6±4.2

* *p<0.05, significant differences between corresponding control and MSG-treated groups*

Table 3
Duration of response (seconds) of BP parameters to intravenous injection of phenylephrine (200 μg/kg/ml) and angiotensin II (500 ng/kg/ml/2 min) in control (n=6) and MSG-treated (n=6) rats

	Phenylephrine		Angiotensin II	
Parameters (mm Hg)	Control	MSG	Control	MSG
Systolic BP	467.0±70.6	160.8±15.3**	282.4±33.4	247.6±52.6
Diastolic BP	299.3±41.2	153.8±27.3*	302.2±34.7	222.7±53.5
Mean BP	332.5±45.9	151.2±12.8**	592.6±102.2	209.0±50.2*

* *p<0.05,* ** *p<0.01, significant differences between corresponding control and MSG-treated groups*

Vasopressor response to angiotensin II

The maximal increase of BP in response to the infusion of angiotensin II was similar in both groups of animals (Table 2). However, MSG-treated rats demonstrated a significant reduction in the duration of the mean BP response (Table 3). This effect was similar in magnitude as that observed after adrenergic stimulation by phenylephrine.

Hindlimb vascular bed preparation

In the control group, the hindlimb vascular bed preparation responded to the administered doses of noradrenaline by increased constriction. Vasoconstrictor responses to noradrenaline in MSG-treated rats were diminished for all doses employed with significant differences at doses of 0.01, 0.1 and 1.0 μg (Table 4).

Table 4
Constrictor responses of hindlimb vascular bed preparation to noradrenaline under conditions of perfusion method in control (n=8) and MSG-treated (n=8) rats

Noradrenaline (μg)	Perfussion pressure (mm Hg) Control	MSG
0.01	28.2±3.7	6.7±0.5**
0.1	50.1±5.3	18.0±1.7**
1.0	84.7±8.6	41.6±5.1**
10.0	114.7±12.3	99.4±11.7

** *p<0.01, significant difference from the control group*

Discussion

The results obtained in the present study indicate that adult rats treated with MSG in the neonatal period show a diminished BP response to two different pressor agents – to α_1-adrenoceptor agonist phenylephrine and to angiotensin II. Moreover, a decreased pressor responsiveness to adrenergic stimulation at the vascular level was observed in experiments on perfused hindlimb vascular bed.

Neurotoxic effects of MSG in the central nervous system appear to have little impact on basal BP regulatory mechanisms. Other authors working with different normotensive and hypertensive rat strains reported a reduced (Van den Buuse *et al.* 1985, Clough *et al.* 1986) or unchanged (Mosqueda-Garcia *et al.* 1986, Habley *et al.* 1987) systolic BP in rats treated with MSG. The results of these studies also differed according to the gender and age of the animals. In male 10-week-old Sprague-Dawley rats used in the present experiments, the values of systolic, diastolic and mean BP were lower in MSG-treated rats as compared to the controls, but these differences were not statistically significant. However, the computerized method of direct BP measurement made it possible to reveal a significant reduction in pulse pressure. The reduced weight and possible morphological changes in the heart (Hamaoka and Sawada 1987) may be responsible, at least partly, for reduced pulse pressure observed in our MSG-treated rats.

In contrast to small changes in basal BP values, the vasopressor response of MSG-treated rats to angiotensin II and particularly to phenylephrine was clearly reduced. This was evident not only from the reduced maximal BP rise after phenylephrine but also from the shorter duration of BP elevation after both agents. To our knowledge, the effects of these substances on BP control in MSG-treated rats have not yet been evaluated. Similarly, only limited data are available on the cardiovascular changes induced by other vasoactive drugs in rats neonatally exposed to MSG.

Several factors might contribute to the reduced BP responsiveness to intravenously injected vasoactive agents in MSG-treated rats. As the experiments were carried out in conscious rats in which the baroreflex was counteracting the pressor reaction, the possibility that the baroreflex was operating more effectively in MSG-treated animals than in the controls should be considered. This hypothesis is strengthened by the findings of Mosqueda-Garcia *et al.* (1986) who measured the BP response to clonidine and by indirect evidence reported by Clough *et al.* (1986) of a possible resetting of the baroreflex to a lower BP level in rats treated with MSG. The evaluation of baroreflex efficacy in this study failed to present supportive evidence to the mentioned suggestions. The slopes and shift of the baroreceptor function curves in control and MSG-treated rats were not significantly different. Thus, major changes in baroreflex function do not seem to be the cause of reduced BP responsiveness in MSG-treated rats.

It has been indicated that adult rats neonatally exposed to MSG had a decreased catecholamine content and slower noradrenaline turnover in the heart (Rehorek *et al.* 1987, Dulloo and Young 1991) as well as diminished excretion of adrenaline (Leigh *et al.* 1992). Our recent studies showed reduced activity of adrenal tyrosine hydroxylase and a slight decrease in adrenal tyrosine hydroxylase mRNA levels (Tokarev *et al.* 1996). The reduced pressor response to adrenergic stimulation, observed in the present experiments, might partly be related to impaired sympathoadrenal regulatory mechanisms.

After attempting to evaluate the factors responsible for the observed changes in vasopressor reactivity, we suggest that the impairment occurs at the vascular level. Such an impairment may occur anywhere between receptor level and contractile protein function. Indeed, our data indicate that the observed abnormalities in MSG-treated rats may be caused by diminished vascular responsiveness. Supporting evidence was obtained in the hindlimb vascular bed preparation which exhibited reduced pressure responses to increasing doses of noradrenaline in MSG-treated rats. Moreover, preliminary experiments demonstrated a similar reduction in responsiveness in isolated renal artery segments (Kristová *et al.* 1995). It cannot be excluded that, besides neurotoxic effects in the brain, the intraperitoneal administration of MSG to neonatal animals induces some damage to the development of peripheral vessels.

The reduced cardiovascular reactivity of MSG-treated animals may also be accounted for by the consequences of their changed metabolic state. Rats treated with neurotoxic doses of MSG in the early postnatal period developed obesity and hyperinsulinaemia with signs of insulin resistance (Nenoff *et al.* 1993, Zórad *et al.* 1996). Interestingly, lower values of systolic BP (Yu and McNeill, 1992) and depressed pressor responses to noradrenaline and angiotensin II were reported in rats with experimentally induced diabetes (Jackson and Carrier 1983). The involvement of similar mechanisms in the reduced BP responsiveness operative both in MSG-treated and in diabetic rats cannot be excluded.

In conclusion, the measurement of BP responses to the vasoactive agents phenylephrine and angiotensin II revealed the impaired BP regulation as a consequence of the neurotoxic action of MSG administered to rats in the early postnatal period. It is being suggested that the attenuation of cardiovascular reactivity in MSG-treated rats is, at least partly, caused by diminished vascular responsiveness.

Acknowledgements
The skillful assistance of I. Szalayová in animal surgery and that of E. Hejdová in hindlimb perfusion technique is gratefully acknowledged. The study was supported by a grant of the European Community (CIPACT930227, ERB 3510 PL 92 4137) and of VEGA (2/1288; 5305/272).

References

BOHUS B., KOOLHAAS J.M.: Stress and the cardiovascular system: central and peripheral physiological mechanisms. In: *Stress. From Synapse to Syndrome.* S.C. STANFORD, P. SALMON, (eds), London, Academic Press, 1993, pp. 76–117.

BOUDOURESQUE F., CHAUTARD T., JEŽOVÁ D., BECQUET D., GUILLAUME V., HÉRY F., GIRAUD P., GRINO M., OLIVER C.: Excitatory amino acids and adenopituitary hormone secretion in mammals, with special reference to development. *Endocr. Regul.* **25:** 44–52, 1991.

CAPUTO F.A., SCALLET A.C.: Postnatal MSG treatment attenuates angiotensin II (AII) induced drinking in rats. *Physiol. Behav.* **58:** 25–29, 1995.

CLOUGH R.W., ARAVICH P.F., SLADEK C.D.: Monosodium glutamate neurotoxicity: a sex specific impairment of blood pressure but not vasopressin in developing rats. *Brain Res. Bull.* **17:** 51–58, 1986.

DULLOO A.G., YOUNG J.B.: Effect of monosodium glutamate and gold thioglucose on dietary regulation of sympathetic nervous system activity in rodents. *Metab. Clin. Exp.* **40:** 113–121, 1991.

FISHER K.N., TURNER R.A., PINEAULT G., KLEIM J., SAARI M.J.: The postweaning housing environment determines expression of learning deficit associated with neonatal monosodium glutamate. *Neurotoxicol. Teratol.* **13:** 507–513, 1991.

HAMAOKA K., SAWADA T.: Hypoplastic heart induced by neonatal hypothalamic lesions in mice. *J. Mol. Cell. Cardiol.* **19:** 741–749, 1987.

HAMBLEY J.W., JOHNSTON G.A.R., ROGERS L.J.: Blood pressure development in SHR and WKY rats: Effects of neonatal monosodium glutamate treatment and evidence for transient hypertension in WKY rats. *Neurosci. Lett.* **83:** 190–194, 1987.

JACKSON C.V., CARRIER G.O.: Influence of short-term experimental diabetes on blood pressure and heart rate in response to norepinephrine and angiotensin II in the conscious rat. *J. Cardiovasc. Pharmacol.* **5:** 260–265, 1983.

KRISTOVÁ V., KRIŠKA M., HEJDOVÁ E., KASZÁSOVÁ E., BABÁL P., JEŽOVÁ D.: Changes in the reactivity of rat hind limb vascular bed after neonatal treatment with monosodium glutamate (Abstract in Slovak). *Čs. fyziol.* **44**: 39, 1995.

LEIGH F.S., KAUFMAN L.N., YOUNG J.B.: Diminished epinephrine excretion in genetically obese (ob/ob) mice and monosodium glutamate-treated rats. *Int. J. Obes. Metab. Disord.* **16:** 597–604, 1992.

LORDEN J.F., CAUDLE A.: Behavioral and endocrinological effects of single injections of monosodium glutamate in the mouse. *Neurobehav. Toxicol. Teratol.* **8:** 509–519, 1986.

MAGARINOS A.M., ESTIVARIZ F., MORADO M.I., DE NICOLA A.F.: Regulation of the central nervous system-pituitary-adrenal axis in rats after neonatal treatment with monosodium glutamate. *Neuroendocrinology* **48:** 105–111, 1988.

MOSQUEDA-GARCIA R., ESKAY R., ZAMIR N., PALKOVITS M., KUNOS G.: Opioid-mediated cardiovascular effects of clonidine in spontaneously hypertensive rats: elimination by neonatal treatment with monosodium glutamate. *Endocrinology* **118:** 1814–1822, 1986.

NEMEROFF C.B., KONKOL R.J., BISSETTE G., YOUNGBLOOD W., MARTIN J.B., BRAZEAU P., RONE M.S., PRANGE A.J., BREESE G.R., KIZER J.S.: Analysis of the disruption in hypothalamic-pituitary regulation in rats treated neonatally with monosodium L-glutamate (MSG): evidence for the involvement of tuberoinfundibular cholinergic and dopaminergic systems in neuroendocrine regulation. *Endocrinology* **101:** 613–622, 1977.

NENOFF P., REMKE H., MULLER F., ARNDT T., MOTHES T.: In vivo assessment of insulin binding in different organs of growing and adult glutamate-induced obese rats. *Exp. Clin. Endocrinol.* **101:** 215–221, 1993.

OLNEY J.W.: Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. *Science* **164:** 719–721, 1969.

PHELIX C.F., HARTLE D.K.: Systemic glutamate induces degeneration of a subpopulation of tyrosine hydroxylase-immunoreactive neurones in the rat area postrema. *Brain Res.* **516:** 335–340, 1990.

REHOREK A., KERESCEN L., MULLER F.: Measurement of tissue catecholamines of obese rats by liquid chromatography and electrochemical detection. *Biomed. Biochim. Acta* **46:** 823–827, 1987.

SAAVEDRA J.M.: Interactions between the circulating hormones angiotensin and atrial natriuretic peptide and their receptors in brain. In: *Circulating Regulatory Factors and Neuroendocrine Function.* J.C. PORTER, D. JEŽOVÁ, (eds), New York, Plenum Press, 1990, pp. 191–210.

SPINEDI E., JOHNSTON C., NEGRO-VILAR A.: Increased responsiveness of the hypothalamic-pituitary axis after neurotoxin-induced hypothalamic denervation. *Endocrinology* **115:** 267–272, 1984.

TOKAREV D., ŠKULTÉTYOVÁ I., KRISTOVÁ V., JAROŠČÁKOVÁ I., KRIŠKA M., KVETŇANSKÝ R., JEŽOVÁ D.: Reactivity of cardiovascular system to phenylephrine, angiotensin II and cold stress in rats neonatally treated with monosodium glutamate. In: *Stress: Molecular Genetics and Neurobiological Advances.* R. MCCARTY, G. AGUILERA, E. SABBAN, R. KVETŇANSKÝ (eds), New York, Gordon and Breach Science Publishers, 1996, pp. 317–326.

VAN DEN BUUSE M., VERSTEEG D.H.G., DE JONG W.: Effect of neonatal treatment with monosodium glutamate in spontaneously hypertensive rats. *Dev. Brain Res.* **19:** 135–138, 1985.

WILLIAMS J.L., BARNES K.L., BROSNIHAN K.B., FERRARIO C.M.: Area postrema: a unique regulator of cardiovascular function. *NIPS* **7:** 30–34, 1992.

YU Z., MCNEILL J.H.: Blood pressure and heart rate response to vasoactive agents in conscious diabetic rats. *Can. J. Physiol. Pharmacol.* **70:** 1542–1548, 1992.

ZÓRAD Š., FICKOVÁ M., JEŽOVÁ D., MACHO L.: Presence of insulin resistance in fat tissue of rats with obesity induced by glutamate (in Slovak). In *Abstracts of Slovak and Czech Physiological Society Meeting,* Bratislava, February 5–7, 1996, p. 150.

Reprint Requests

Dr. D. Ježová, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Vlárska 3, 833 06 Bratislava, Slovak Republic.