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

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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 α1-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 α₁-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 configured with a Macintosh IVx personal computer. Arterial cannulas were connected to transducers and basal BP parameters were registered for 10 min. Thereafter, the α₁-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.](image)
Table 1
Basal characteristics of MSG-treated (n=7) rats and age-matched controls (n=6)

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

** 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

<table>
<thead>
<tr>
<th>Parameters (mm Hg)</th>
<th>Control</th>
<th>MSG</th>
<th>Control</th>
<th>MSG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP</td>
<td>108.3±6.6</td>
<td>93.6±7.7</td>
<td>74.5±4.9</td>
<td>67.2±3.8</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>65.3±5.8</td>
<td>49.4±4.4*</td>
<td>56.2±3.8</td>
<td>50.6±7.3</td>
</tr>
<tr>
<td>Mean BP</td>
<td>80.2±5.9</td>
<td>61.9±4.5*</td>
<td>64.8±3.8</td>
<td>59.6±4.2</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>Parameters (mm Hg)</th>
<th>Control</th>
<th>MSG</th>
<th>Control</th>
<th>MSG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP</td>
<td>467.0±70.6</td>
<td>160.8±15.3**</td>
<td>282.4±33.4</td>
<td>247.6±52.6</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>299.3±41.2</td>
<td>153.8±27.3*</td>
<td>302.2±34.7</td>
<td>222.7±53.5</td>
</tr>
<tr>
<td>Mean BP</td>
<td>332.5±45.9</td>
<td>151.2±12.8**</td>
<td>592.6±102.2</td>
<td>209.0±50.2*</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>Noradrenaline (µg)</th>
<th>Perfusion pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>0.01</td>
<td>28.2±3.7</td>
</tr>
<tr>
<td>0.1</td>
<td>50.1±5.3</td>
</tr>
<tr>
<td>1.0</td>
<td>84.7±8.6</td>
</tr>
<tr>
<td>10.0</td>
<td>114.7±12.3</td>
</tr>
</tbody>
</table>

** 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 α1-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 (Tokarcv 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 (Kristóvá 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 hyperinsulinemia with signs of insulin resistance (Nenoff et al. 1993, Zórád 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.
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References


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