Neonatal influence of monosodium glutamate on the somatometric parameters of rats

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Abstract. The glutamate receptors are expressed in various cell types including bone and adipose cells. The effects of neonatal administration of monosodium glutamate (MSG) on the "programming" of somatometric parameters in Wistar rats during the period up to 14th week of life were estimated. The rats were treated subcutaneously with five doses of 4 mg/g MSG (10 μl/g body mass) during the first 10 postnatal days (group M). The control (group C) was treated in the same manner with normal saline solution. During three months, body mass, naso-anal length and tail length were measured in 14 days intervals, while femoral and tibial masses and lengths, and testicular mass were measured following sacrificing. The body mass at the end of this period in the M group of males was higher than the body mass in the group C. Reduction in relative bone length, body and tail lengths and the relative as well as absolute testicular mass were registered in MSG-treated rats. A significant reduction in somatometric parameters was registered only in female MSG-treated rats during period of sexual maturation compared to controls.

Key words: Monosodium glutamate — Obesity — Bones — Rat — Somatometry

Introduction

Monosodium glutamate (MSG) (EU food additive code-E 621) is a substance that has toxic effects on numerous animal species. It is widely used as a food and pharmaceutical additive. Experimental models have revealed various harmful effects of MSG. The main toxicity affected loci are the hypothalamic nuclei and the median eminence. The consecutive changes in central nervous system (CNS) and hypothalamo-pituitary axis are the cause of generalized effects. The most prominent manifestations of the neuroendocrine-metabolic disorders caused by these lesions are: growth retardation, obesity and infertility (Olney 1969; Nemeroff et al. 1977; Klingberg et al. 1987). MSG-treatment in rats during the neonatal period causes morphologic and functional changes in the endocrine glands (Terry et al. 1981; Shapiro et al. 1989; Miskowiak et al. 1993). Today, increasing evidence demonstrates that adipose tissue is an endocrine organ secreting a large number of signaling proteins (adipokines) such as leptin and adiponectin, also nerve growth factor and brain-derived neurotrophic factor (this issue), which affect bone and cardiovascular system (Hamerman 2002; Chaldakov et al. 2004). *Via* the intracerebroventricular pathway, leptin enhances the bone loss and causes sympathetic hyperactivity and hypertension in rats (Ducy et al. 2000; Ren 2004). Large adipose content is associated with high leptin levels. The high level of leptin is found in MSG-treated rats, also (Camihort et al. 2005). Adipose tissue is also linked to adiponectin release (Chandran et al. 2003), which has important implications to atherosclerosis and bone mineral density.

Glutamate signal system was discovered in the CNS first. It is now clear that this system is also functional in the non-neural tissues such as the bones, liver, pancreas and skin (Skerry and Genever 2001). Both the metabotropic and ionotropic glutamate receptors and transporters are expressed in osteoblasts (Laketic-Ljubojevic et al. 1999; Gu et al. 2002) and osteoclasts (Epsinosa et al. 1999; Peet et al. 1999).

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The aim of our work was to study the effects of neonatal administration of MSG on somatometric parameters in Wistar rats during their growth up to 14th week of life and to estimate the effects on morphometric parameters of long bones (femur and tibia).

Materials and Methods

The research was carried out on 40 neonatal Wistar rats of both sexes. The animals were housed in the Institute for Biomedical research vivarium and all procedures on animals followed Guideline for work on experimental animals approved by Ethic Committee of School of Medicine in Niš. The animals were housed in standard plastic cages under controlled conditions (room temperature 23 ± 2°C; air humidity 50 ± 5%; photoperiod of 12 h of light from 6:00 a.m. to 6:00 p.m.). The animals were divided into two groups. In each group of rats: the experimental (group M) and control (group C) was 10 males and 10 females. The pups were injected with either 4 mg/g body mass of MSG or an equal volume of normal saline solution subcutaneously to the interscapular region on alternate days for the first 10 days of life. The pups were weaned at age of 28 days. They had free access to tap water and to standard laboratory rat food ("Veterinarski zavod" Zemun). Morphometric parameters (body mass, naso-anal and tail length) of the animals were measured in 14 days intervals. The animals were sacrificed following an intraperitoneal application of ketamine at age of 14 weeks. The last measurements were performed before anesthetized animals were sacrificed. After the sacrificing of the animals, the rear extremities were separated in the hip joint level. The bone surrounding tissues of the femurs and tibiae were carefully removed and bone mass and bone lengths were measured. The testes were carefully removed, cleaned of the surrounding tissue and mass was measured.

Results of statistical analysis are expressed as means ± SD (standard deviation) and statistical significance was determined with Independent samples Student's *t*-test. Differences were considered significant at $p < 0.05$ level. All statistical analyses were performed using the SPSS statistical software (version 15).

Results

Somatometric parameters were measured periodically in Wistar rats of both sexes.

Body mass

The body masses in MSG-treated and control Wistar rats are shown in Table 1. There were no statistically significant differences in body mass of the male rats between C and M groups. Significantly lower body mass was registered in MSG-treated females then in C group, both at the age of six weeks (132.0 ± 15.3 g vs. 158.2 ± 15.0 g; $p < 0.001$) and at the age of eight weeks (153.0 ± 20.2 g vs. 188.6 ± 15.1 g; $p < 0.001$). Both MSG-treated females at age of six weeks and at age of eight weeks had significantly lower body mass then MSG-treated males (132.0 ± 15.3 g *vs.* 156.0 ± 23.7 g; $p < 0.01$; 153.0 ± 20.2 g vs. 180.3 ± 27.4 g; $p < 0.01$). MSG-treated females had significantly lower body mass than MSG-treated males at age of twelve and fourteen weeks (272.3 ± 26.1 g vs. 318.3 ± 31.2 g; $p < 0.05$). The females from C group had significantly lower body mass then males from C group both at age of ten weeks (199.2 ± 23.5 g vs. 225.0 ± 13.4 g; $p < 0.05$) and twelve weeks (219.2 ± 26.3 g vs. 250.8 ± 13.2 g; $p < 0.05$).

Naso-anal body length

The naso-anal body length values are shown in the Table 2. Lower values of naso-anal lengths were recorded both in males and females of the M groups compared to the C groups. Statistically significant differences were found in the females at the ages of: four weeks (15.1 ± 0.80 cm *vs.* 16.0 ± 0.72 cm; $p < 0.05$), six weeks (16.8 ± 0.67 cm vs. 17.7 ± 0.75 cm; $p < 0.01$), and eight weeks (18.1 ± 0.84 cm vs. 19.2 ± 0.46 cm; $p < 0.01$). Related to sex, significantly shorter naso-anal

Table 1. Body mass in MSG-treated and control Wistar rats of both sexes measured at different age

Groups		Age (weeks)						
		2	4	6	8	10	12	14
♂	C	52.4 ± 10.7	126.9 ± 9.5	167.9 ± 13.5	196.9 ± 16.4	225.0 ± 13.4	250.8 ± 13.2	311.6 ± 20.7
	M	48.6 ± 8.3	117.4 ±2 1.9	156.0 ± 23.7	180.3 ± 27.4	217.0 ± 28.5	252.5 ± 29.9	318.3 ± 31.2
♀	C	48.8 ± 10.8	123.9 ± 25.9	158.2 ± 15.0	188.6 ± 15.1	199.2 ± 23.5[a]	219.2 ± 26.3[a]	288.4 ± 22.3
	M	45.6 ± 8.6	105.2 ± 13.5	132.0 ± 15.3[b,c]	153.0 ± 20.2[b,c]	185.0 ± 31.6	211.0 ± 28.6[d]	272.3 ± 26.1[d]

The values are expressed as mean ± SD in grams; ♂, males; ♀, females; M, rats treated with monosodium glutamate; C, rats treated with normal saline. Statistically significant difference between: [a] C♀ and C♂ for the level of $p < 0.05$; [b] M♀ and C♀ for the level of $p < 0.001$; [c] M♀ and M♂ for the level of $p < 0.01$; [d] M♀ and M♂ for the level of $p < 0.05$.

Table 2. Naso-anal length in MSG-treated and control Wistar rats of both sexes measured at different age

Groups		Age (weeks)			
		4	6	8	14
♂	C	16.0 ± 0.37	18.2 ± 0.33	20.0 ± 0.53	22.8 ± 0.82
	M	16.0 ± 0.93	17.7 ± 0.85	19.7 ± 0.71	22.2 ± 0.75
♀	C	16.0 ± 0.72	17.7 ± 0.74	19.2 ± 0.46[c]	21.5 ± 0.89[a]
	M	15.1 ± 0.79[e,b]	16.8 ± 0.67[f, b]	18.1 ± 0.83[f,d]	21.2 ± 1.30

The values are expressed as mean ± SD in centimeters; ♂, males; ♀, females; M, rats treated with monosodium glutamate; C, rats treated with normal saline. Statistically significant difference between: [a] C♀ and C♂ for the level of $p < 0.05$; [b] M♀ and M♂ for the level of $p < 0.01$; [c] C♀ and C♂ for the level of $p < 0.01$; [d] M♀ and M♂ for the level of $p < 0.001$; [e] M♀ and C♀ for the level of $p < 0.05$; [f] M♀ and C♀ for the level of $p < 0.01$.

Table 3. Tail length in MSG-treated and control Wistar rats of both sexes measured at different age

Groups		Age (weeks)			
		4	6	8	14
♂	C	13.0 ± 0.71	15.8 ± 1.02	17.1 ± 0.82	18.4 ± 0.37
	M	12.7 ± 1.32	15.5 ± 0.98	17.1 ± 0.73	18.3 ± 1.32
♀	C	13.4 ± 0.84	16.0 ± 0.81	17.0 ± 0.47	18.9 ± 0.73
	M	12.8 ± 0.90	14.9 ± 1.08[a]	15.7 ± 0.93[b,c]	17.9 ± 2.26

The values are expressed as mean ± SD in centimeters; ♂, males; ♀, females; M, rats treated with monosodium glutamate; C, rats treated with normal saline. Statistically significant difference between: [a] M♀ and C♀ for the level of $p < 0.05$; [b] M♀ and M♂ for the level of $p < 0.05$; [c] M♀ and C♀ for the level of $p < 0.001$.

body lengths were measured in MSG-treated females then in MSG-treated males at the ages of: four weeks (15.1 ± 0.79 cm vs. 16.0 ± 0.93 cm; $p < 0.01$), six weeks (16.8 ± 0.67 cm vs. 17.7 ± 0.85 cm; $p < 0.01$) and eight weeks (18.1 ± 0.83 cm vs. 19.7 ± 0.71 cm; $p < 0.001$). Also, significantly shorter naso-anal body lengths were recorded in females then in males of C groups: in the eighth (19.2 ± 0.46 cm vs. 20.0 ± 0.53 cm; $p < 0.01$) and fourteenth week of age (21.5 ± 0.89 cm vs. 22.8 ± 0.82 cm; $p < 0.05$).

Tail length

Tail length values are shown in Table 3. There were no significant differences in tail length in the males between C and M group. The mean tail length of the females treated with MSG was significantly shorter compared to the females of C groups both at the age of six weeks (14.9 ± 1.08 cm vs. 16.0 ± 0.82 cm; $p < 0.05$) and at the age of eight weeks (15.7 ± 0.93 cm vs. 17.0 ± 0.47 cm; $p < 0.05$). MSG-treated females had significantly shorter tails than MSG-treated males at the age of eight weeks (15.7 ± 0.93 cm vs. 17.1 ± 0.73 cm; $p < 0.05$).

Although, the differences in some somatometric parameters were not significant between MSG-treated and control groups the most of animals, especially females from M group had increased content of abdominal fat tissue (Fig. 1).

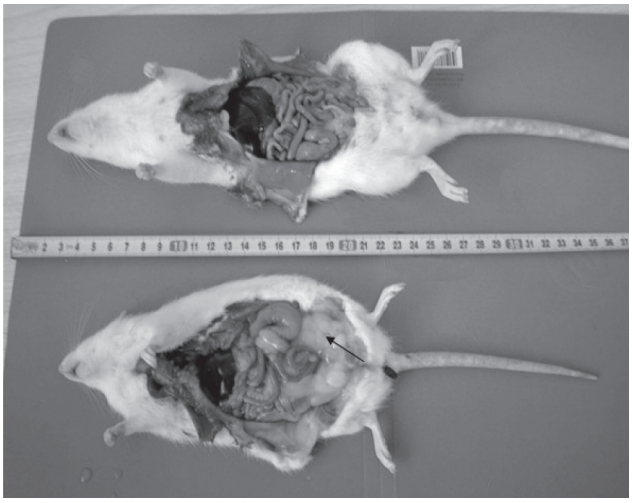

Figure 1. Differences in both naso-anal and tail lengths and fat tissue (arrow) content between normal saline-treated females (up) and MSG- treated females (down).

Bone lengths

Mean femoral and tibial lengths in both the females and males are shown in Table 4. The differences in mean absolute and relative femoral and tibial lengths were not significant between

Table 4. Absolute and relative femoral and tibial lengths in MSG-treated and control Wistar rats of both sexes

Groups		Femoral length		Tibial length	
		Absolute	Relative	Absolute	Relative
♂	C	34.0 ± 0.89	10.92	37.8 ± 0.95	12.13
	M	33.9 ± 1.47	10.64	37.2 ± 1.47	11.67
♀	C	33.0 ± 1.57	11.44	36.8 ± 1.72	12.70
	M	32.0 ± 0.45[a]	11.75	36.1 ± 1.11	13.27

Absolute lengths are expressed as mean ± SD in millimeters; ratios are expressed in percents related to body mass; ♂, males; ♀, females; M, rats treated with monosodium glutamate; C, rats treated with normal saline; [a] statistically significant difference between M♀ and M♂ for the level of $p < 0.05$.

Table 5. Absolute and relative femoral and tibial masses in MSG-treated and control Wistar rats of both sexes

Groups		Femoral mass		Tibial mass	
		Absolute	Relative	Absolute	Relative
♂	C	0.65 ± 0.06	0.21 ± 0.03	0.54 ± 0.06	0.18 ± 0.03
	M	0.59 ± 0.11	0.18 ± 0.02	0.46 ± 0.06	0.15 ± 0.09
♀	C	0.63 ± 0.05	0.22 ± 0.02	0.51 ± 0.04	0.18 ± 0.01
	M	0.51 ± 0.02[b]	0.18 ± 0.01[b]	0.41 ± 0.02[a]	0.14 ± 0.01[a]

Absolute masses are expressed as mean ± SD in grams; relative masses are expressed as mean ± SD in %; ♂, Males; ♀, Females; M, rats treated with monosodium glutamate; C, rats treated with normal saline; statistically significant difference between: [a] M♀ and C♀ group for the level of $p < 0.01$; [b] M♀ and C♀ group for the level of $p < 0.05$

Table 6. Absolute and relative testicular mass in MSG-treated and normal saline-treated Wistar rats

Groups	Absolute mass	Relative mass
C	3.32 ± 0.18	1.06 ± 0.09
M	2.93 ± 0.35[a]	0.92 ± 0.09[a]

Absolute masses are expressed as mean ± SD in grams; relative masses are expressed as mean ± SD in %; M, rats treated with monosodium glutamate; C, rats treated with normal saline; [a] statistically significant difference between M and C group for the level of $p < 0.05$.

MSG-treated and normal saline-treated males and females. The femurs in MSG-treated females were significantly shorter than in MSG-treated males (32.0 ± 0.45 mm vs. 33.9 ± 1.47 mm; $p < 0.05$). Tibial length differences between groups were not statistically significant. Females had shorter tibias compared to males, but those differences were not significant too.

Bone mass

Mean absolute and relative femoral and tibial masses for both males and females are shown in Table 5. The differences in absolute femoral mass between M and C group of females were not significant. MSG-treated females had significantly lowered absolute and relative femoral mass compared to normal saline-treated females (0.18 ± 0.01 vs. 0.22 ± 0.02; $p < 0.05$). Absolute and relative tibial masses were significantly lower in MSG-treated females than in normal saline-treated females (0.41 ± 0.02 vs. 0.51 ± 0.04; $p < 0.01$ and 0.14 ± 0.01 vs. 0.18 ± 0.01; of $p < 0.01$) respectively. The differences between both MSG-treated males and females and normal saline-treated males and females were not significant.

Testicular mass

Mean values of absolute and relative testicular masses in MSG-treated and normal saline-treated Wistar rats at age of 100 days are shown in Table 6. Absolute and relative masses of MSG treated rats were significantly lower than in normal saline-treated rats.

Discussion

Neonatal MSG-treatment causes morphometric changes in rats. The majority of previous studies recorded a body mass reduction in the rats (Terry et al. 1981; Millard et al. 1982; Shapiro et al. 1989; Waxman et al.1990). The reduction in body mass in MSG-treated males was not registered by Miskowiak et al. (1993) and Bojanić (1998). Significantly lower body mass was registered in MSG-treated than in normal

saline-treated female Wistar rats (Bojanić 1998). The results of our study showed that MSG-treated males had lower body mass during the first ten weeks of life. MSG-treated rats overgrew normal saline-treated rats in the twelfth week of life.

At the other hand, retardation in mass gain of MSG-treated females compared to controls was especially expressed and statistically highly significant at the age of 6 and 8 weeks ($p < 0.001$). The differences in body mass between M and C groups decreased, or disappeared, or even the rats of M group overgrew the rats from C group in later period.

In peripubertal period the differences between sexes within identically treated groups of males and females were also recorded. Differences between sexes are especially emphasized in group M. Body mass of MSG-treated females was significantly lower than in MSG-treated males.

Numerous studies showed stunted growth in rats. Both female and male MSG-treated rats had significantly shorter both naso-anal and tail length than normal saline-treated rats (Terry et al. 1981; Shapiro et al. 1989; Waxman et al.1990; Miskowiak et al. 1993; Bojanic 1998). In our study, the most prominent changes in body mass and naso-anal and tail lengths of MSG-treated rats compared to normal saline-treated rats were at the age of six and eight weeks. As well, MSG-treated females compared to males showed the most prominent differences in mentioned parameters in the same period.

We can conclude that neonatal exposition to MSG affected rat growth and development, especially during period of sex maturation and that the females are more sensitive to MSG treatment than males. This means that pubertal period is characterized with pronounced vulnerability and sensitivity for expression of external influences, particularly in females.

Animal linear growth decrease can be attributed to depressed activity of growth and sexual hormones. Because, MSG application in the neonatal period reduces growth in males and females, the therapy with growth hormone (GH) was used in attempt to make correction of this disorder (Rol de Lama et al. 1998a). GH substitution therapy is effective for growth acceleration after the age of 30 days, with a partial recovery in males and a complete recovery in females (Rol de Lama et al. 1998a). MSG treatment reduced GH and insulin-like growth factor-1 (IGF-1) levels in rats of both sexes, causing a less pronounced reduction in tibial growth and body mass gain in females compared to males (Rol de Lama et al. 1998b). In MSG-treated males reduction of pituitary LH content was found what was different than in females. This dimorphic action of MSG on the gonadal axis could be explanation for the observed differences in growth rate (Rol de Lama et al. 1998b).

Bones of MSG-treated rats in our research were shorter and had lower mass in animals of both sexes. Kovács et al. (1996) summarized that neonatal MSG -treatment had a negative effect on bone growth and was associated with reduced levels of pituitary and serum GH, serum IGF-1 and pituitary GH receptors concentration. GH administration increased leg length in the animals of both sexes treated with MSG, but more effectively in females, in spite of a similar IGF-1 levels in both sexes (Rol de Lama et al. 1998b). Decreased femoral strength following MSG- treatment is addressed to reduced activity of the hypothalamo-pituitary-GH-IGF-1 system (Stevenson et al. 2009). The crucial effect of GH decreased level on the body growth is due to MSG- dependent anatomic and functional hypothalamic and adenohypophyseal reorganization. In this way neonatal treatment with MSG made programming of further animal development.

Increased fat content in MSG-treated rats (Fig 1.) should be reconsidered in attempt to explain possible effects on bones. It's well known that obese animals have high leptin level which has effect on bones. Leptin expression in bone basic cellular system, which is primarily involved in bone formation and/or bone resorption, indicates the possibility that leptin is involved in modulating of the initial phases of bone modeling and remodeling processes (Morroni et al. 2004). Mainly, leptin has reputation of antiosteogenic mediator (Cock and Auwerx 2003). Guidobono et al. (2006) found that leptin reduced the accretion of total femoral bone mineral content and bone mineral density of both femur and tibia in growing rats after long-term continuous intracerebroventricular infusion. One of the ways of leptin's action is over alpha-Melanocyte-stimulating hormone. This hormone regulates appetite and body mass, and it can decrease the bone volume directly affecting the skeleton (Cornish et al. 2003).

The studies in leptin receptor-deficient Zucker (fa/fa) rats indicate that leptin could have positive effect on bones (Tamasi 2003). Anyway, the obesity could influence bone tissue by leptin independent mechanisms. Cornish et al. (2002) offered the explanation for diversity of findings about action of leptin on bone tissue indicating on the differences between its central and peripheral effects. Obviously, the effect of leptin on bones is determined by kind of experimental model, doze and route of application. It should be mentioned, that in our experimental model, there was a relatively short period for development of obesity, because the full effect of MSG-treatment should be expected later.

There is much controversy about the effect of MSG on testis. Olney (1969) found no difference in testis hystology between mice neonatally treated with MSG and mice of control group. His findings were supported by reports of some other authors (Adamo and Ratner 1970; Nikoletseas 1977). Waxman et al. (1990) emphasized that rats neonatally treated with MSG were fertile with normal secretion of gonadotropines and sex steroids. Redding et al. (1971) reported significantly reduced gonadal weights in MSG-treated rats at 40 and 110 days of age and a marked decrease in GH and

luteinizing hormone content in the anterior pituitaries of male MSG-treated rats at 40 days. Reproductive dysfunction was seen by Pizzi et al. (1977) in male mice. Males treated with MSG showed reduced fertility and decreased testis weights. Miskowiak et al. (1993) registered desquamation of spermatosoal cells in seminal tubules of four months old rats neonatally treated with MSG. Testicular atrophy in Wistar rats neonatally treated with MSG was reported by Bojanić (1998). França et al. (2006) revealed a significant ($p < 0.05$) reduction in testis weight and the number of Sertoli (SC) and Leydig cells (LC) per testis in prepubertal MSG rats. They concluded that testis development as well as SC and LC proliferation was disturbed. In adult MSG-treated rats no apparent alterations were observed in testis structure. But, the number of SCs per testis was significantly ($p < 0.05$) reduced in the adult males. Decreased gland mass is indirect indicator of decreased function of the gland. So, neonatal "castration" with MSG, *via* the hypothalamus and the hypophysis, significantly modifies the somatic status. Any way MSG-induced somatometric alterations could be addressed to neonatal reprogramming of developmental and maturation processes due to changed hypothalamo-pituitary-gonadal secretory functions.

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