Physiol. Res. 49: 269-277, 2000

Alkaline Phosphatase Activity of Duodenal Enterocytes After Neonatal Administration of Monosodium Glutamate to Rats

Š. MOZEŠ, Ľ. LENHARDT[1], A. MARTINKOVÁ

Institute of Animal Physiology, Slovak Academy of Sciences and [1]Department of Pathology, Veterinary University, Košice, Slovak Republic

Received April 16, 1999
Accepted July 12, 1999

Summary

In the present work neonatal male and female Wistar rats were treated intraperitoneally with monosodium glutamate (MSG 2 mg/kg b.w.) or saline (controls) daily for 4 day after birth. At the age of 30 and 80 days, the alkaline phosphatase activity (AP) in the brush border of individual enterocytes, the body fat content and Lee's index of obesity were analyzed. Microdensitometrical quantification of AP was significantly increased on day 30 in males ($P<0.01$) and on day 80 in MSG-treated male and female rats ($P<0.001$) as compared to the controls. MSG administration also increased the body fat weight and the obesity index significantly ($P<0.001$) in 80-day-old animals, but was without any significant effect on their food intake. Our results showed that a) neonatal MSG-treatment may significantly change the intestinal function and b) the investigation of the intestinal enzyme activities may be important in further studies on MSG-induced and other forms of obesity.

Key words

Alkaline phosphatase activity • Duodenal enterocytes • Monosodium glutamate • Obesity • Rat

Introduction

Monosodium glutamate (MSG) treatment of neonatal rodents results in a syndrome characterized by damage to several hypothalamic nuclei (Lemkey-Johnson and Reynolds 1974, Sun *et al.* 1991), endocrine and sexual abnormalities (Bakke *et al.* 1978, Abe *et al.* 1990, Sun *et al.* 1991), arrested skeletal growth, and obesity (Abe *et al.* 1990, Zhang *et al.* 1994). The MSG-treated rats are defined as obese on the basis of a greater percentage of body fat (Nemeroff *et al.* 1977, Zhang *et al.* 1994) and a significantly greater Lee's index of obesity in comparison with control animals (Nemeroff *et al.* 1977, Bakke *et al.* 1978, Abe *et al.* 1990). Detailed studies of neonatal MSG administration partially revealed the contribution of neuroendocrinological and behavioral disorders in situations leading ultimately to obesity in mature animals. However, the precise mechanism of increased adiposity has not yet been fully elucidated. The obesity in MSG rats is accompanied with increased plasma levels of insulin and triglycerides (Abe *et al.* 1990), a decreased volume, density and number of ventromedial hypothalamic (VMH) neurons (Sun *et al.* 1991), and with a significantly decreased functional activity of VMH (Zhang *et al.* 1994). The mechanism by which MSG may induce obesity could also involve a disruption of leptin-mediated regulation of the body fat content *via* damage of leptin receptors in the

Fax +420 2 249 20 590
E-mail: physres@biomed.cas.cz http://www.lf2.cuni.cz/physiolres/physiolres.htm

hypothalamic arcuate nucleus (Seress 1982, Schwartz *et al.* 1996). Leptin is a peptide hormone produced by adipose tissue which decreases appetite, the body fat content and increases energy expenditure (Dawson *et al.* 1997, Qian *et al.* 1998, Morris *et al.* 1998). Neonatally MSG-treated rats also exhibit altered dietary preferences, consume more of the palatable high-fat diet than the controls (Kanarek *et al.* 1979), or selectively increase carbohydrate consumption (Beck *et al.* 1997).The specific feature of obesity induced by neonatal MSG administration is that the increased adiposity occurs in the absence of hyperphagia or even with hypophagia (Abe *et al.* 1990, Betran *et al.* 1992, Zhang *et al.* 1994, Caputo and Scallet 1995, Stricker-Krongrad *et al.* 1998).

However, the nutritional deficiency induced by food restriction even in non-obese rats is connected with enhanced intestinal absorption and hypertrophy of the intestinal mucosa and also with the altered activity of brush border-bound enzymes (Leveille and Chakrabarty 1968, Pathak *et al.* 1985, Holt *et al.* 1991). It is therefore conceivable that besides neurohormonal and metabolic disturbances during the MSG syndrome, the altered intestinal funcion may be a further key factor leading to the development of obesity.

Among enzymes of the small intestine, alkaline phosphatase (AP) is a representative brush border enzyme functionally involved in nutrient (fat) absorption and transport of long-chain fatty acids in the intestinal mucosa (Takase and Goda 1990, Bernard *et al.* 1992). In mature rats, the activity of AP increases in duodenal enterocytes after eating fat (Alpers *et al.* 1995, Zhang *et al.* 1996), displays circadian fluctuations in the duodenum closely related to the rhythm of food intake (Ishikawa *et al.* 1983) and markedly decreases after food deprivation (Ishikawa *et al.* 1983, Holt and Kotler 1987). Depending on the duration of fasting, the AP activity in rat duodenum gradually decreases starting from day 1 of food deprivation to the lowest values on day 4 (Ishikawa *et al.* 1983). Moreover, starvation altered the intestinal morphology leading to a reduction in the height and number of villi *per* serosa associated with changes in the lipid content of the brush border (Keelan *et al.*1985) and decreased the adipose tissue fatty acids (Raclot and Groscolas1995, Klain and Harmon 1977) and the size of fat cells (Yang *et al.* 1990). On the other hand, when food-deprived rats were refed, enhanced activity of duodenal AP (Holt and Kotler 1987), increased expression of the 3.0 kb of intestinal AP mRNA species (Hodin *et al.* 1994a) and a higher rate of lipogenesis in the white adipose tissue were observed (Kochan *et al.* 1997). These results indicate that there is a possible relationship between the drop in enzyme activity and the decreased mass of adipose tissue in rats with complete nutritional deficiency. However, information about the activity of intestinal AP in obese rats with free access to food is lacking.

We therefore studied the activity of duodenal alkaline phosphatase in individual duodenal enterocytes of intact male and female rats and neonatally MSG-treated animals.

Methods

Animals

A total of 50 male and 48 female Wistar rats were used in the present study. The animals were housed from birth to weaning in litters of nine pups in Plexiglass cages in a temperature-controlled environment of 22 °C with a 12L:12D regime (light on 07:00-19:00 h). The mothers had free access to a standard laboratory diet (Velaz/Altromin 1520 DOS 2b, Velaz, Prague) and tap water. After weaning on day 30, the animals were housed in Plexiglass cages (two rats per cage) under the same conditions (diet, water, temperature, light-dark regime) as before weaning.

On the first day after birth the litters were adjusted to nine pups per nest. The experimental animals received a subcutaneous injection of monosodium salt of L-glutamic acid (Sigma, St. Louis, MO) 2 mg/g b.wt. The controls were given the same volume of saline daily for 4 days after birth. MSG was dissolved in distilled water and the drug concentration adjusted so that each pup received 50 µl of solution per gram of body weight.

Separation of enterocytes

On day 30 (26 males, 24 females) and on day 80 (24 males, 24 females), the rats were killed under ether anesthesia. Small (0.5 cm) segments of the proximal duodenum were removed, everted and frozen in hexane (to –70 °C) immediately after washing in distilled water.

To collect the epithelial cells, we used the procedure of Weiser (1973) modified by us (Mozeš *et al.* 1998). It is based on the principle of sequential dissociation of epithelial cells from tissue segments depending on the duration of tissue incubation, which establishes a gradient of cells running from the villus tip to the cryptal cells. On the day of the experiment, the frozen duodenal segments were incubated for 5 min at

room temperature (20 °C) in a sodium citrate buffer (1.5 mM KCl, 96 mM NaCl, 8 mM KH_2PO_4, 5.6 mM Na_2HPO_4, 27 mM sodium citrate, pH 7.3). After this initial incubation, the tissue was transferred to plastic tubes containing 0.15 M phosphate buffer (pH 5.3) and incubated for 1.5 and 10 min. At the end of each incubation period, the tissue segment was spun briefly by a cyclomixer to dissociate the cells, then transferred into another tube containing phosphate buffer, and the incubation was continued. Our previous study (Mozeš *et al.* 1998), which compared AP activity in 8 μm cryostat sections along the villus axis (in arbitrarily divided regions of the villus, i.e. the tip, middle and cryptal part) with the activity of the individual cells obtained by successive separation, indicated similar values of the percentual quotient from the entire activities of the villi. Incubation for 1, 10 and 20 min enabled the isolation of the respective cells from the tip, middle and cryptal areas of the villus. The epithelial cells released after 10 min incubation (middle part of the villus) were centrifuged for 3 min at 800 *g*, and the pellet was mixed with distilled water. A small droplet of the cell suspension was transferred onto a glass slide, covered by a thin plastic film and squashed by pressing with another glass slide. After removing the film, the cells were air-dried.

Enzyme assay

Demonstration of alkaline phosphatase activity was performed using a modified simultaneous azo-coupling method (Lojda *et al.* 1979). The incubation medium contained 0.4 mM naphthol AS-BI phosphate (Sigma, Deisenhofen, Germany), 0.8 mM Hexazotized New Fuchsin (Serva, Heidelberg, Germany), 20 mM N,N-dimethylformamide and 0.05 M veronal acetate buffer (pH 9.2). The incubation was performed at 37 °C in the presence of 13 % w/v polyvinyl alcohol (PVA, Sigma). Enzyme activity was cytophotometrically analyzed with a Vickers M85a microdensitometer. The measurements were performed using a 40x objective, an effective scanning area of 28.3 μm^2 and a scanning spot 0.5 μm. Integrated absorbance was measured at a wavelength of 520 nm (Frederiks *et al.* 1987). The mask was set over the brush border of individual cells obtained from the middle part of the villi (third cell fraction incubated for 10 min). The AP activity was calculated as the absorbance values recorded by the instrument in $min/\mu m^3$ brush border ± S.E.M. In each animals 30 cells were always measured and their mean value referred to one animal.

Epididymal and retroperitoneal fat in males and parametrial and retroperitoneal fat in females was removed after the animals were killed and the wet weight of the whole pad was obtained. The Lee's index of obesity was calculated from data $\sqrt[3]{}$body weight (g) / naso-anal length (cm).

Statistical analysis

The two-way ANOVA was used to test if sex affected body weight, fat pad weight, or AP activity after neonatal MSG-treatment. Tukey's test was used to compare the differences between the groups.

Table 1. Alkaline phosphatase (AP) activity, body fat parameters, and body weight in neonatally MSG-treated and control (saline-treated) rats at the age of 30 days.

	MALES		FEMALES	
	MSG (n=13)	Controls (n=13)	MSG (n=12)	Controls (n=12)
AP activity	13.3± 0.3**	11.9 ± 0.3	12.9± 0.3	12.2±0.3
Fat (% b.w.)	1.1±0.07	0.9 ± 0.05	0.62±0.05	0.55±0.02
Lee's index	0.311±0.003	0.313±0.002	0.321±0.003	0.314±0.002
Naso-anal length (cm)	13.5±0.2	13.9±0.3	13.3±0.1*	14.1±0.3
Tail length (cm)	9.9±0.2	10.4±0.3	9.8±0.2*	10.8±0.3
Body weight (g)	73.7±1.8	82.3±4.0	77.3±2.9	87.1±4.2

*Data are means ± S.E.M. AP activity is given as the integrated absorbance in $min/\mu m^3$ brush border of duodenal enterocytes at a wavelength of 520 nm. Fat % represents epididimal + retroperitoneal pads in males and parametrial + retroperitoneal pads in females. Lee's index = $\sqrt[3]{}$body weight (g)/naso-anal length (cm). Significant differences from controls: * $P<0.01$, ** $P< 0.001$ (Tukey's test).*

Results

At weaning on day 30, no significant differences in body weight and Lee's index of obesity were found between MSG and control rats (Table 1). MSG-treated females were significantly shorter than the saline-treated controls, while the growth parameters in males were unchanged (Table 1).

The animals given MSG as neonates, in comparison to their controls, showed a 20 % and 13 % increase in fat pad weight in male and female rats, respectively, however, these differences were not statistically significant (Table 1). Two-way ANOVA showed no sex-specific changes in the fat content (interaction F = 0.905, NS). The AP activity in the brush border of individual duodenal enterocytes was significantly increased in MSG-treated males but no differences was observed in females in comparison with their controls (Table 1). The differences of AP activity between experimental and control rats were about 12 % and 6 % in males and females, respectively. Two-way ANOVA showed no sex-specific changes (interaction F=0.760, NS).

On day 80, the neonatally MSG-treated male rats weighed significantly less than the controls, while the body weight in MSG females was unchanged (Table 2). Two-way ANOVA showed no sex-specific changes in body weight (interaction F = 3.806, P = 0.057, NS). The mean daily food intake (measured in individual rats from day 75 to 80) was slightly decreased in male and female MSG-treated animals when expressed in grams of food per rat in comparison with the controls (Table 2), and slightly increased in males when expressed in grams of food/kg b.wt. (80.4 vs 75.9 in males; 85.1 vs 87.3 in females). The body fat and Lee's index of obesity were significantly higher (P<0.0001) in both male and female MSG-treated rats. The increase in body fat content ranged from 40 % to 110 % in males and females, respectively, when compared to control rats. Two-way ANOVA revealed that there were no sex-specific changes in fat content (interaction F = 3.750, P = 0.059, NS). The significant differences in duodenal alkaline phosphatase activity corresponded with the observed differences in body fat. In male and female rats neonatally treated with MSG, a significant (P<0.0001) increase of AP activity was found in comparison with saline-treated controls on day 80 (Table 2). The AP activity values were higher in MSG-treated males and females (about 13 and 17 %) relative to their controls. Two-way ANOVA showed no sex-specific changes (interaction F = 1.419, NS).

Table 2. Alkaline phosphatase (AP) activity, body fat parameters, body weight and food intake in neonatally MSG-treated and control (saline-treated) rats at the age of 80 days.

	MALES		FEMALES	
	MSG (n=12)	Controls (n=12)	MSG (n=12)	Controls (n=12)
AP activity	14.8±0.2***	13.1±0.2	14.7±0.3***	12.6±0.2
Fat (% b.w.)	3.9±0.4***	2.8±0.2	5.7±0.7***	2.7±0.2
Lee's index	0.318±0.002***	0.308±0.001	0.313±0.002***	0.296±0.001
Body weight (g)	307.1±5.5***	358.9±6.2	227.8±8.7	246.2±4.7
Food intake (g/day)	24.7±1.4	26.8±1.1	19.4±0.9	21.6±1.4

*Data are means ± S.E.M. AP activity is given as the integrated absorbance in min/μm^3 brush border of duodenal enterocytes at a wavelength of 520 nm. Fat % represents epididimal + retroperitoneal pads in males and parametrial + retroperitoneal pads in females. Lee's index = $\sqrt[3]{}$body weight (g)/naso-anal length (cm). Significant differences from controls: ***P<0.0001 (Tukey's test).*

Discussion

Significantly increased body fat, Lee's index of obesity, decreased body weight, and normophagia in MSG-treated rats observed in our experiment are in agreement with earlier studies (Nemeroff *et al.* 1977, Bakke *et al.* 1978, Abe *et al.* 1990, Zhang *et al.* 1994). Moreover, in younger rats aged 30 days a similar MSG

influence was confirmed by a moderately enhanced adiposity, a mild fall of body growth and weight gain. The fact that no sex-specific differences of the MSG effect were found indicates that the susceptibility of both males and females to glutamate is similar. This is at variance with the work of Sun *et al.* (1991), who reported more severe neuronal damage in several hypothalamic nuclei and more prominent changes in the feeding and drinking behavior of MSG-treated male rats than in MSG-treated females. This different susceptibility might be due to the fact that these authors used a different strain of rats (Long-Evans vs Wistar) or higher doses of MSG (4 mg/g vs 2 mg/g b.w.).

In several respects, the neurohormonal effects of MSG treatment on body fat increase are similar to those found in severe forms of obesity. Among CNS structures, the VMH is considered to be a site of the neural control of fat metabolism but, furthermore, the role of insulin as the key hormone in fat storage is also well-known. Evidence has been provided that destruction of the VMH produces hyperinsulinemia, hyperphagia and obesity (Hustvedt *et al.* 1984, Bray 1986). On the other hand, similarly as in normophagic neonatally MSG-treated rats (Abe *et al.* 1990), hyperinsulinemia and increased adiposity was also observed in VMH-lesioned weanling rats prior to developing hyperphagia (Frohman *et al.* 1969). However, hyperphagia was prevented in adult rats by pair-feeding (Hustveldt and Lovo 1972) as well as in food-restricted genetically obese rats (Cleary *et al.* 1980).

The present experiment showed that the mean daily food intake of obese male and female rats did not differ from that of saline-treated controls. This is consistent with the concept that MSG-induced obesity could be affected by any one or all of these lipogenic factors rather than by an elevated food intake. On the other hand despite the absence of hyperphagia in MSG rats the observed increase of duodenal AP activity clearly demonstrated that obesity in these animals may also be associated with an altered efficiency of intestinal function. According to this hypothesis, increased consumption of a high-fat diet as well as selectively enhanced carbohydrate consumption was observed in MSG-treated rats (Kanarek *et al.* 1979, Beck *et al.* 1997).

The exact mechanism which induces an increase of duodenal AP activity in neonatally MSG-treated rats has not been fully elucidated. Among the hormones that may have influenced the intestinal enzymes and thus explained the increased AP activity in the present experiment, insulin, corticosteroids and thyroid hormone should be considered as eligible candidates. There are many data supporting the possible role of insulin as a regulatory factor in enterocyte maturation. Insulin given to 8-day-old rat pups increased the specific activity of several brush border enzymes and elicited the premature appearance of sucrase activity in villus and cryptal cells (Buts *et al.* 1988, 1990). The small intestine possesses insulin receptors and their concentration during ontogenesis was found to be higher during the suckling period than after weaning. This is in contrast to plasma insulin, the levels of which display a reversed developmental pattern (Forgue-Lafitte *et al.* 1970, Fernandez-Moreno *et al.* 1988). There are also further arguments supporting the role of insulin as the most important modulator of intestinal AP. First, it was found that a reduction of insulin binding sites (Young *et al.* 1987) or decreased insulin levels in the circulation elicited by alloxan administration diminish intestinal AP activity (Dutt and Sarkar 1993). Second, MSG-treated rats are hyperinsulinemic while the serum levels of other hormones such as thyrotropin (TSH), thyroxin (T4) and growth hormone (GH) are decreased (Abe *et al.* 1990, Bakke *et al.* 1978). Finally, in suckling rats neither thyreoidectomy at 14 days nor thyroid hormone (T3) administration at 10 days of age changed the AP activity or 3.0 kb AP mRNA levels in the duodenum of rats (Subramoniam and Ramakrishnan 1980, Hodin *et al.* 1994b).

The maturation of rat intestinal AP is also primarily regulated by glucocorticoids. The change of AP from the suckling to the adult type was accelerated by injection of cortisone on day 9-10 or hydrocortisone on day 12-13 after birth (Uezato and Fujita 1983, Tojoy *et al.* 1985). Neonatal MSG administration disrupts the regulation of the hypothalamic-pituitary-adrenal axis. In MSG-treated rats, a morphometric study of the adrenals revealed a hyperfunctional state associated with an increase in the basal plasma corticosterone concentration (Dolnikoff *et al.* 1988).

Leptin receptor expression has recently also been found in the small intestine (Lostao *et al.* 1998, Morton *et al.* 1998), suggesting that the intestinal epithelium is a direct target of leptin action. From this point of view leptin might affect the lipid metabolism or lipid absorption in the intestine. However, the inability of chronic leptin treatment to decrease fat pad weight and insulin levels in MSG-treated rats suggests that the lack of leptin or resistance to leptin action in the small intestine may lead to the development of obesity in these animals (Dawson *et al.* 1997).

Our results have revealed permanent alterations of the intestinal function in MSG-treated rats which have been demonstrated by the significantly increased AP activity at 30 days of age; these increased levels were also maintained over the next 50 days. In MSG-treated rats kept under similar light-dark conditions and with free access to food, AP activity was maintained in enterocytes separated every 6 h over a 24 h period from the tip, middle and cryptal parts of villi in comparison with the controls (unpublished observation). It is well known that enterocytes continually differentiate in the crypt and migrate up the villus tip. As to the crypt-villus position, AP activity in intact rats was lowest in the cryptal zone, peaked in the middle part and declined in the villus tip (Mozeš *et al.* 1998). However, despite the persistence of increased AP activity values, neonatal MSG administration did not change the distribution pattern of AP along the crypt-villus axis, i.e. the highest enzyme activity was observed in enterocytes separated from the middle parts and the lowest activity was found in epithelial cells obtained from the cryptal parts of villi. Such stability of the AP gradient suggests that this parameter is not influenced by MSG treatment. The permanent increase of enzyme activity might rather be a part of a more general effect of MSG characterized by neurohormonal disturbances.

In comparison with younger rats, a substantial increase of body fat and the Lee's index of obesity was observed in 80-day-old animals. These late changes in the body fat content are in agreement with the earlier observation that in neonatally MSG-treated rats obesity becomes fully manifest in mature rats (Bakke *et al.* 1978). One potentially important aspect of the present results may be the determination whether the changes in duodenal AP activity of MSG-treated rats could also be related to the body fat changes. However, according to our knowledge no reports have studied the effect of MSG on intestinal AP in rats. The increase AP activity and body fat content are of interest with respect to the reports which demonstrated a close relationship between body fat and the ventromedial hypothalamic structure. We have previously shown the existence of a negative correlation between the body fat content and the functional activity of VMH in intact non-obese rats (Kuchár *et al.* 1993). Such an inverse relationship was also confirmed by experiments in which decreased body fat and increased VMH activity were observed after neonatal undernutrition (Mozeš *et al.* 1991, Kuchár *et al.* 1992). Moreover, by using the same experimental procedure as in the present study, increased body fat and decreased activity of VMH neurons were demonstrated after neonatal MSG administration in male and female rats (Zhang *et al.* 1994). It is therefore tempting to speculate that the significantly increased AP activity in MSG-treated obese rats as a result of decreased VMH function and the prominent activation of hormonal signals to the intestine may be of importance for the development of obesity.

Finally, the increase of brush border bound duodenal AP activity in the present experiments extends our knowledge on the intestinal function in MSG obese rats. From this point of view, the investigation of intestinal enzyme activities may be important for the study of MSG-induced and another forms of obesity.

Acknowledgements

This work was supported by grant 2/6108 of the Slovak Academy of Sciences.

References

ABE M, SAITO M, SHIMAZU T: Neuropeptide Y in the specific hypothalamic nuclei of rats treated neonatally with monosodium glutamate. *Brain Res Bull* **4:** 289-291, 1990.

ALPERS DH, ZHANG Y, AHNEN DJ: Synthesis and parallel secretion on rat intestinal alkaline phosphatase and surfactant-like particle protein. *Am J Physiol* **268:** E1205-E1214, 1995.

BAKKE JL, LAWRENCE N, BENNETT J, ROBINSON S, BOWERS CY: Late endocrine effects of administering monosodium glutamate to neonatal rats. *Neuroendocrinology* **26:** 220-228, 1978.

BECK B, STRICKER-KRONGRAD A, BURLET A, MUSSE N, NICOLAS JP, BURLET C: Dietary preferences in monosodium glutamate-lesioned rats: age-variable influence of hypothalamic neuropeptide Y. *Neurosci Lett* **225:** 153-156, 1997.

BERNARD A, CASELLI C, BLOND JP, CARLIER H: Diet fatty acid composition, age and rat jejunal microvillus enzyme activities. *Comp Biochem Physiol A* **101:** 607-612, 1992.

BETRAN MA, ESTORNELL E, BARBER T, CABO J: Nitrogen metabolism in obesity induced by monosodium-L-glutamate in rats. *Int J Obes* **16:** 555-564, 1992.

BRAY GA: Autonomic endocrine factors in the regulation of energy balance. *Fed Proc* **45:** 1404-1410, 1986.

BUTS JP, DE KEYSER N, DIVE C: Intestinal development in the suckling rat: effect of insulin on the maturation of villus and crypt cell functions. *Eur J Clin Invest* **18:** 391-398, 1988.

BUTS JP, DE KEYSER N, KOLANOWSKY J, VANTTOOF F: Hormonal regulation of the rat small intestine responsiveness of villus and crypt cells to insulin during the suckling period and unresponsiveness after weanling. *Pediatr Res* **27:** 161-164, 1990.

CAPUTO FA, SCALLET AC: Postnatal MSG treatment attenuates angiotensin II (AII) induced drinking in rats. *Physiol Behav* **58:** 25-29, 1995.

CLEARY MP, VASSELLI JR, GREENWOOD MRC: Development of obesity in Zucker obese (fa/fa) rat in absence of hyperphagia. *Am J Physiol* **238:** E284-E292, 1980.

DAWSON R, PELLEYMOUNTER MA, MILLARD W., LIU S, EPPLER B: Attenuation of leptin-mediated effects by monosodium glutamate-induced arcuate nucleus damage. *Am J Physiol* **273:** E202-E206, 1997.

DOLNIKOFF MS, KATER CE, EGAMI M, DE ANDRADE IS, MARMO MR: Neonatal treatment with monosodium glutamate increases plasma corticosterone in the rat. *Neuroendocrinology* **48:** 645-649, 1988.

DUTT P, SARKAR AK: Alterations in rat intestine sucrase and alkaline phosphatase activities in aloxan induced experimental diabetes. *Indian J Biochem Biophys* **30:** 177-180, 1993.

FERNANDEZ-MORENO MD, FERNANDEZ-GONZALEZ MA, DIAZ-JUAREZ JL, LOPEZ-LUNA MP, PRIETO JC: Interaction of insulin with small intestine epithelial cells from developing rats. *Biol Neonate* **54:** 289-293, 1988.

FREDERIKS WM, MARX P, JONGES GN, VAN NORORDEN CFJ: Quantitative histochemical study of acid phosphatase activity in rat liver using a semipermeable membrane technique. *J Histochem Cytochem* **35:** 175-180, 1987.

FORGUE-LAFITTE ME, MARESATT MR, CHAMBERLAIN MC, ROSSELIN G: Evidence for the presence of insulin binding sites in isolated rat intestinal epithelial cells. *Diabetologia* **19:** 373-378, 1970.

FROHMAN LA, BERNARDIS LL, SCHNATZ JD, BUREK L: Plasma insulin and triglyceride levels after hypothalamic lesions in weanling rats. *Am J Physiol* **216:** 1496-1501, 1969.

HODIN RA, GRAHAM JR, MENG S, UPTON MP: Temporal pattern of rat small intestinal gene expression with refeeding. *Am J Physiol* **226:** G83-G89, 1994a.

HODIN RA, MENG S, CHAMBERLAIN SM: Thyroid hormone responsiveness is developmentally regulated in the rat small intestine: a possible role for the alpha-2 receptor variant. *Endocrinology* **135:** 564-568, 1994b.

HOLT PR, KOTLER DP: Adaptive changes of intestinal enzymes to nutritional intake in the aging rat. *Gastroenterology* **93:** 295-300, 1987.

HOLT PR, HELLER TD, RICHARDSON AG: Food restriction retards age-related biochemical changes in rat small intestine. *J Gerontol* **46:** 89-94, 1991.

HUSTVEDT BE, LOVO A: Correlation between hyperinsulinemia and hypephagia in rats with ventromedial hypothalamic lesions. *Acta Physiol Scand* **84:** 29-33, 1972.

HUSTVEDT BE, JESKA J, CHRISTOPHERSEN A, LOVO A: Energy metabolism in rats with ventromedial hypothalamic lesions. *Am J Physiol* **246:** 319-326, 1984.

ISHIKAWA Y, SUZUKI M, ONO A, NAKABOU Y, HAGIHIRAH: Memory of the rhythmic change in activity of duodenal alkaline phosphatase in rats. *J Nutr Sci Vitaminol* **29:** 85-90, 1983.

KANAREK RB, MEYERS J, MEADE RG, MAYER J: Juvenile-onset obesity and deficits in caloric regulations in MSG-treated rats. *Pharmacol Biochem Behav* **10:** 717-721, 1979.

KEELAN M, WALKER K, THOMSON BR: Intestinal brush border membrane marker enzymes, lipid composition and villus morphology: effect of fasting and diabetes mellitus in rats. *Comp Biochem Physiol A* **82:** 83-89, 1985.

KLAIN GJ, HARMON JP: Differential response of rat brown and white adipose tissue to environmental or nutritional stress. *Comp Biochem Physiol B* **58:** 227-230, 1977.

KOCHAN Z, KARBOWSKA J, SWIERCYNSKI J: Unusual increase of lipogenesis in rat white adipose tissue after multiple cycles of starvation-refeeding. *Metabolism* **46:**10-17, 1997.

KUCHÁR S, MOZEŠ Š, RYBOŠOVÁ Z, NOVÁKOVÁ V, MICHALOV M: Influence of early undernutrition in rats on their body fat and RNA content in VMH neurons. *Physiol Behav* **52:** 695-698, 1992.

KUCHÁR S, MOZEŠ Š, ZHANG WM, RYBOŠOVÁ Z: Body fat and RNA content of VMH neurons in rats. *Brain Res Bull* **32:** 211-213, 1993.

LEMKEY-JOHNSON N, REYNOLDS WA: Nature and extent of brain lesions in mice related to ingestion of monosodium glutamate. *J Neuropathol Exp Neurol* **33:** 74-79, 1974.

LEVEIILE GA, CHAKRABARTY K: Absorption and utilization of glucose by meal-fed and nibbling rats. *J Nutr* **96:** 69-75, 1968.

LOJDA Z, GROSSRAU R, SCHIBLER TH: *Enzyme Histochemistry*. Springer-Verlag, Berlin, 1979, pp 59-70.

LOSTOA MP, URDANETA E, MARTINEZ-ANSO E, BARBER A, MARTINEZ JA: Presence of leptin receptors in rat small intestine and leptin effect on sugar absorption. *FEBS Lett* **423:** 302-326, 1998.

MORRIS MJ, TORTELLI CF, FILIPS A, PROIETTO J: Reduced BAT function as a mechanism for obesity in the hypophagic, neuropeptide Y deficient monosodium glutamate-treated rat. *Regul Pept* **75-76:** 441-447, 1998.

MORTON NM, EMILSON V, LIU YL, CAWTHORNE MA: Leptin action in intestinal cells. *J Biol Chem* **273:** 26194-26201, 1998.

MOZEŠ Š, KUCHÁR S, MICHALOV M, NOVÁKOVÁ V: Influence of litter size on the RNA content of VMH and LHA in preweaning rats. *Physiol Behav* **50:** 1133-1136, 1991.

MOZEŠ Š, LENHARDT L, MARTINKOVÁ A: A quantitative histochemical study of alkaline phosphatase activity in isolated rat duodenal epithelial cells. *Histochem J* **30:** 583-589, 1998.

NEMEROFF CB, GRANT LD, BISSETTE G, ERVIN GN, HARRELL LE, PRANGE AJ: Growth, endocrinological and behavioral deficits after monosodium-L-glutamate in the neonatal rat: possible involvement of arcuate dopamine neuron damage. *Psychoneuroendocrinology* **2:** 179-196, 1977.

PATHAK RM, MAHMOOD A, DUDEJA PK, SUBRAHMANYAM D: Intestinal brush border membrane structure and function: effect of early postnatal undernutrition. *Pediatr Res* **15:** 112-114, 1981.

QIAN H, AZAIN MJ, HARTZELL DL, BAILE CA: Increased leptin resistance as rats grow to maturity. *Proc Soc Exp Biol Med* **219:** 160-165, 1998.

RACLOT T, GROSCOLAS R: Selective mobilization of adipose tissue fatty acids during energy depletion in the rat. *J Lipid Res* **36 :** 2164-2173, 1995.

SCHWARTZ MW, SEELEY RJ, CAMPFIELD LA, BURN P, BASKIN DG: Identification of targets of leptin action in rat hypothalamus. *J Clin Invest* **98:** 1101-1106, 1996.

SERESS L: Divergent effects of acute and chronic monosodium L-glutamate treatment on the anterior and posterior parts of the arcuate nucleus. *Neuroscience* **7:** 2207-2216, 1982.

STRICKER-KRONGRAD A, BURLET C, BECK B: Behavioral deficits in monosodium glutamate rats: specific changes in the structure of feeding behavior. *Life Sci* **62:** 2127-2132, 1998.

SUBRAMONIAM A, RAMAKRISHNAN C.V: Influence of thyroxine on suckling rat intestinal alkaline phosphatase and effects of undernutrition on the same. *J Exp Zool* **214:** 317-322, 1980.

SUN Y.M, HSU H.K, LUE S.I, PENG M.T: Sex-specific impairment in sexual and ingestive behaviors of monosodium glutamate-treated rats. *Physiol Behav* **50:** 873- 880, 1991.

TAKASE S, GODA T: Effects of medium-chain triglycerides on brush border membrane-bound enzyme activity in rat small intestine. *J Nutr* **120:** 969-976, 1990.

TOJOY Y, SAIDA K, SUZUKI A: Hydrocortisone-evoked molecular conversion of alkaline phosphatase in suckling rat small intestine. *Jpn J Pharmacol* **39:** 105-108, 1985.

UEZATO T, FUJITA M: Development transition of alkaline phosphatase from suckling to adult type in rat small intestine: molecular species and effect of injected cortisone and thyroxine. *J Biochem* **94:** 1483-1488, 1983.

WEISER MM: Intestinal epithelial cell surface membrane glycoprotein synthesis. I. An indication of cell differentiation. *J Biol Chem* **248:** 2536-2541, 1973.

YANG MU, PRASTA E, BJORNTROP P: Refeeding after fasting in rats: effects of duration of starvation and refeeding on food efficiency in diet-induced obesity. *Am J Clin Nutr* **51:** 970-978, 1990.

YOUNG GP, MORTAN CL, ROSE IS, TARANTO TM, BHATHAL PS: Effects of intestinal adaptation on insulin binding to villus cell membranes. *Gut* **28:** 57-62, 1987.

ZHANG WM, KUCHÁR S, MOZEŠ Š: Body fat and RNA content of the VMH cells in rats neonatally treated with monosodium glutamate. *Brain Res Bull* **35:** 383-385, 1994.

ZHANG Y, SHAO J-S, XIE Q-M, ALPERS DH: Immunolocalization of alkaline phosphatase and surfactant-like particle proteins in rat duodenum during fat absorption. *Gastroenterology* **110:** 478-488, 1996.

Reprint requests

Dr. Š. Mozeš, Institute of Animal Physiology, Slovak Academy of Sciences, Šoltesovej 4, 040 01 Košice, Slovak Republic. E mail: mozes@mail.saske.sk , martink@mail.saske.sk