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Effect of Neonatal MSG Treatment on Day-Night Alkaline Phosphatase Activity in the Rat Duodenum

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Summary

The day-night variation of food intake and alkaline phosphatase (AP) activity was studied in the duodenum of rats neonatally treated with monosodium glutamate (MSG) and saline-treated (control) rats. The animals were kept under light-dark conditions (light phase from 09:00 h to 21:00 h) with free access to food. AP activity was cytophotometrically analyzed in the brush-border of enterocytes separated from the tip, middle and cryptal part of the villi every 6 h over a 24-hour period. In comparison with the controls, MSG-treated rats consumed about 40 % less food during the dark period and their 24-hour food intake was thus significantly lowered ($P<0.001$). On the other hand, the nocturnal feeding habit showed a similar pattern: food consumption was high during the night (65 % vs. 75 %) and the lowest consumption was found during the light phase (35 % vs. 25 %) in MSG-treated and control rats, respectively. In agreement with the rhythm of food intake, the highest AP activity was observed during the dark phase and was lowest during the light phase in both groups of animals. These significant day-night variations showed nearly the same pattern in the enterocytes of all observed parts along the villus axis. In comparison with the controls, a permanent increase of AP activity was observed in neonatal MSG-treated rats. This increase was more expressive during the dark phase of the day in the cryptal ($P<0.001$) and middle part of the villus ($P<0.01$). From the viewpoint of feeding, this enzyme in MSG-treated rats was enhanced in an inverse relation to the amount of food eaten i.e. despite sustained hypophagia the mean AP activity in the enterocytes along the villus axis was higher than in the control animals during all investigated periods. The present results suggest that the increased AP activity in MSG-treated rats is probably not a consequence of actual day-night eating perturbations but could be a component of a more general effect of MSG. This information contributes to better understanding of the function of intestinal AP and its relation to day-night feeding changes especially in connection with the MSG syndrome.

Key words

Light-dark rhythm • Alkaline phosphatase • Duodenal enterocytes • Neonatal MSG treatment

Introduction

The circadian rhythm of AP activity, as well as of other digestive enzymes has been observed in the small intestine. In rats kept under a normal light-dark cycle and fed *ad libitum*, AP is highest at night and low during daytime, whereby this fluctuation is closely related to the rhythm of food intake (Saito *et al.* 1975, Ishikawa *et al.*

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1983). In agreement with the nocturnal feeding habits of rats, a high level of AP activity is observed during the dark period when food intake is highest and total dry matter of the intestinal content reaches its maximum in the duodenum (Vachon and Savoil 1987). Such a relationship between AP activity and food intake supports the suggestion that when the feeding time is shifted to the light-phase, maximum enzyme activity is always observed throughout the time of feeding (Saito *et al.* 1975, Ishikawa *et al.* 1983). Moreover, it was also observed that the AP rhythm requires the presence of food in the alimentary tract of the animals (Stevenson *et al.* 1980).

Neonatal monosodium glutamate treatment (MSG) results in a syndrome characterized by damage of several hypothalamic structures, endocrine abnormalities and obesity (Zhang *et al.* 1994, Sun *et al.* 1991). Neuronal damage and loss of neuromodulators in the circumventricular organs (Dawson *et al.*1989, Meister *et al.* 1989) observed after this treatment are also associated with disturbances in feeding behavior. The MSG-treated rats exhibit altered dietary preferences (Kanarek *et al.* 1979, Beck *et al.* 1997) and decreased food intake (Stricker-Krongrad *et al.* 1998). It has also been demonstrated that early postnatal administration of MSG to rat pups induced profound changes in the feeding pattern and feeding rhythms of mature rats. In consequence of pronounced nocturnal hypophagia, these rats kept on a 12:12 light-dark cycle consumed a significantly smaller amount of food during 24 h than the controls (Stricker-Krongrad *et al.*1998).

The characteristic pattern of AP distribution along the villus axis of the rat duodenum has been reported by several authors who observed maximum enzyme activity in the middle part of the villus and minimum activity in the cryptal zone (Weiser 1973, Gutschmidt *et al.* 1980, Vallet *et al.* 1994, Mozeš *et al.* 1998). This distribution to some extent reflects the developmental changes of enzyme activity during epithelial cell migration from the crypt to the tip of the villi (Leblond and Messie 1958, Altmann and Leblond 1982). However, the distribution of AP activity along the villus has not yet been described in a quantitative manner nor has it been related to the day-night feeding disturbances in neonatally MSG-treated rats.

The exact role of AP under normal physiological conditions has not been fully elucidated, although it has been suggested that it might be functionally involved in lipid digestion and absorption (Takase and Goda 1990, Bernard and Carlier 1991, Yamagishi *et al.* 1994). In contrast to rats fed *ad libitum*, nutritional deficiency induced by food restriction even in non-obese animals is connected with a marked decrease of intestinal AP activity (Holt and Kotler 1987, Thompson *et al.* 1989). However, there exists lack of information about the intestinal AP activity in hypophagic MSG-treated obese rats. It is therefore conceivable that investigation of AP activity during light-dark period may help us to understand the feeding perturbations induced by neonatal MSG administration and their relation to intestinal function. Accordingly, AP activity was compared in individual enterocytes from the tip, middle and cryptal part of the villi every 6 h over a 24-hour period in neonatally MSG-treated and control rats.

Methods

Experimental paradigma

A total of 71 male Wistar rats were used in the present study. The animals from birth to weaning were housed in Plexiglass cages with a temperature-controlled environment of 22±2 °C with a relative humidity of 60±15 % and a 12L:12D regime (light on 09:00-21:00 h). The mothers had free access to a standard laboratory diet (Velaz/Altromin 1520 DOS 2b, Velaz, Prague) and tap water. On the first day after birth, the litters were adjusted to nine pups (4-5 males + 4-5 females) per nest. In each nest, the males and females were divided into two groups. The first group (MSG-treated) and the second group of animals (saline-treated) received a s.c. injection of monosodium salt of L-glutamic acid (Sigma, St. Louis, MO) 2 mg/g b.wt or an equivalent volume of saline daily at 09.00 h for 4 days after birth. MSG was dissolved in distilled water and the drug concentration adjusted so that each pup received 50 μl of the solution/gram of body weight. After weaning (on day 30) the female rats were excluded from the experiment and the male rats were housed by two in Plexiglas cages under the same conditions (diet, water, temperature, light-dark regime) as before weaning.

From day 75 to day 80 the MSG-treated (n=10) and control rats (n=10) were housed individually with free access to food and water. Food intake was measured at 6-h intervals as the difference between the amount offered and that remaining in the cups.

By day 80, the body weight of MSG-treated rats (n=40) was significantly decreased (307.5±16.7 g vs. 371.5±12.5 g, $P<0.001$), and their body fat content

(epididymal + retroperitoneal fat pads) was significantly higher in comparison with the controls (n=31, 3.9±0.2 g vs. 2.7±0.1 g, P<0.01).

Separation of enterocytes

On day 80 the rats were killed under ether anesthesia at 09:00, 15:00, 21:00 and 03:00 h. Small (0.5 cm) segments of the proximal duodenum were removed, everted and frozen in hexane (−70 °C) immediately after washing in distilled water.

To collect epithelial cells we used Weiser's procedure (Weiser 1973) modified in our laboratory (Mozeš *et al.* 1998). This was 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 and 27 mM sodium citrate, pH 7.3). After this initial incubation, the tissue was transferred into plastic tubes containing 0.15 M phosphate buffer (pH 5.3) and incubated for 1, 10 or 20 min. At the end of each incubation period, the tissue segment was spun briefly by a cyclomixer to dissociate the cells, then transferred to another tube containing the phosphate buffer and the incubation was continued. The epithelial cells released after 1, 10, and 20 min of incubation (tip, middle, and cryptal part of the villus) were centrifuged for 3 min at 800 x*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. The cells were air-dried after removing the film.

Enzyme assay

AP activity was assayed by 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). 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 x 40 objective, an effective scanning area of 28.3 μm^2 and a scanning spot of 0.5 μm. The 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 first, second, and third cell fraction. AP activity was calculated as the absorbance values recorded by the instrument in min/μm^{-3} brush border ± S.E.M. Thirty cells were always measured in each animal and their mean values referred to one animal and one villus position.

Statistical analysis

Statistical evaluation of the results was carried out by one-way analysis of variance (ANOVA). The significance of differences between the means was calculated by Tukey's test.

Results

Table 1 shows a similar day-night variation of food intake in control and MSG-treated rats, i.e. maximum consumption during the dark phase (21:00-09:00 h) and minimum food consumption during the light phase (09:00-21:00 h). The expression of day-night consumption as the percentage of the 24-hour food intake also showed a similar tendency. MSG-treated and control rats consumed the highest proportion of food during the night (65 % vs. 75 %) and the lowest percentage of food consumption was seen during the light-phase (25 % vs. 35 %). However, the mean daily food intake of these animals was different. As compared to the controls, the MSG-treated rats consumed about 40 % less food during the dark period. Due to this decrease, their mean 24-hour food intake was significantly lower (19.2±1.3 g vs. 27±0.7 g, P<0.001) than in the control rats.

Table 1. Food intake pattern (g/rat) in MSG-treated and control rats.

Time	MSG-treated rats (n 10)	Controls (n 10)
09:00 – 15:00	2.2±0.4	2.0±0.3
15:00 – 21:00	5.1±0.8	6.1±0.3
21:00 – 03:00	6.3±0.8**	9.7±0.6
03:00 – 09:00	5.5±0.5***	9.4±0.5

*Data are means ± S.E.M.; ** P<0.01; ***P<0.001; statistical differences between MSG-treated and control rats were assessed by one-way ANOVA*

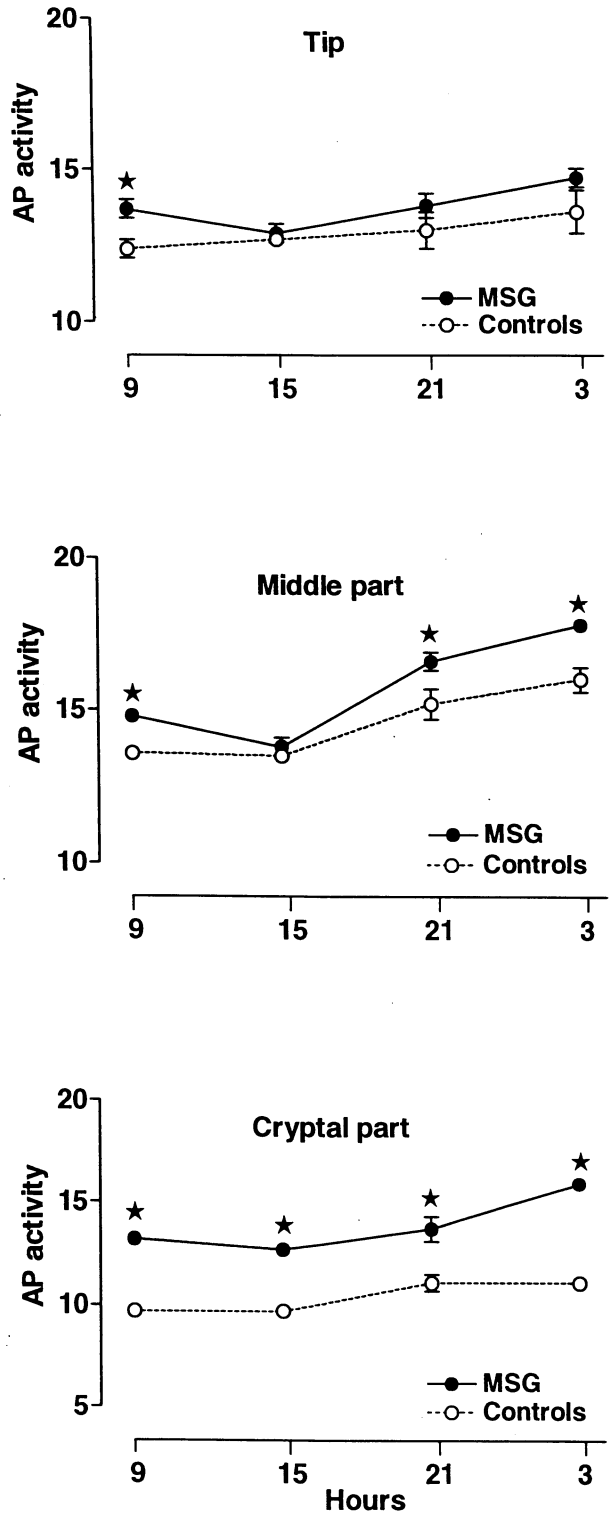

Fig. 1. *Alkaline phosphatase activity in isolated duodenal enterocytes from the tip, middle and cryptal part of the villus in control and MSG-treated rats at different times of the day. Each point is given as the integrated absorbance in min/μm^3 brush-border of duodenal cells at a wavelength of 520 nm. Significantly different from the controls * P<0.001.*

Figure 1 summarizes the day-night AP values and their distribution along the villus axis in the controls and MSG-treated rats. The highest enzyme activity within 24 h was observed at 03:00 h and the lowest values were seen at 15:00 h in both groups of animals. These significant night-day variations showed nearly the same pattern in the enterocytes along the villus axis in MSG-treated rats and in the cryptal and middle part of the villi in the control animals. When the AP activities of enterocytes from the tip, middle, and cryptal part of the villus were expressed as the percentage of the entire villus activities, the enzyme distribution displayed a similar pattern without any significant differences between the control and experimental animals throughout all investigated periods. Peak activity was observed in the middle part (38 % and 36 %) whereas the lowest activity was seen in the cryptal part of the villus (27 % and 32 %) in control and MSG-treated rats, respectively.

The AP activity was closely related to the night-day pattern of food intake in both groups of rats but not to the amount of food consumed (Table 1, Fig. 1). This was apparent in MSG-treated rats, where despite sustained hypophagia the mean AP activity in enterocytes along the villus axis was higher at all times examined than in the control animals. As compared to the controls, mean AP activity in MSG-treated rats was significantly higher in the middle ($P<0.001$) and cryptal part ($P<0.001$) of the villi during the dark phase, whereas mean food intake was significantly decreased ($P<0.001$, 11.8±0.7 g vs. 19.1±0.6 g in the control animals). Such a significant increase of enzyme activity continued in the light phase but only in the cryptal and at 09:00 h in the middle and tip part ($P<0.001$) despite the insignificant differences in food intake observed between these groups (7.3±0.6 g in MSG vs. 8.1±0.3 g in the controls).

Discussion

The present experiment showed a similar day-night variation of duodenal AP activity in MSG-treated and control rats. This is in agreement with previous experiments recording the rhythmic changes of intestinal AP activity in the rats fed *ad libitum* kept under normal light-dark conditions (Saito *et al.* 1975, Ishikawa *et al.* 1983). Our results also demonstrated that the nocturnal feeding pattern in both groups of rats remained relatively intact, i.e. their food intake was significantly higher at night than during the light phase. This indicates that the observed rhythmic rise and fall in AP activity

might be a consequence of increased or decreased food consumption. On the other hand, it was suggested that the day-night fluctuation of AP is controlled by an anticipatory mechanism in which anticipated feeding acts as a trigger of enzyme changes (Saito *et al.* 1976). The presence of such a component was confirmed by the observation that AP activity had already increased one hour prior to the start of feeding in rats kept under a 6-hour feeding schedule (Ishikawa *et al.* 1983). The results of the present study agree to some extent with these experiments. AP activity in MSG-treated and control rats showed relatively high values at the start of the dark phase (21:00 h). However, a prominent increase of food consumption was observed later.

It is well known that enterocytes continuously differentiate in the crypt and migrate towards the villus tip where they are extruded into the lumen. Concerned to the crypt-villus position, AP activity in intact rats was minimal in the cryptal zone, peaked in the middle part, and declined in the villus tip (Weiser 1973, Gutschmidt *et al.* 1980, Vallet *et al.* 1994, Mozeš *et al.* 1998). Our results show that the microstructure of this parameter was affected neither by day-night enzyme variations nor by neonatal MSG administration. In all the periods examined and in both the MSG-treated and control groups AP activity reached its maximum in the middle part of the villus, whereas its activity always remained lower in the cryptal and tip part (Fig. 1). This stability of the AP activity gradient suggests that this parameter is not under circadian control. According to this hypothesis the same phenomenon was seen in rats deprived of food for 72 h where the enzyme gradient along the villus axis remained preserved after disappearance of the day-night rhythm of AP activity and despite decreased enzyme values (unpublished observations).

Our results revealed that the feeding pattern observed under a normal light-dark cycle changed in neonatally MSG-treated rats. MSG-treated rats consumed less food throughout the 24-hour period than control animals mainly as the direct consequence of significant hypophagia during the dark phase, which is in agreement with previous studies (Stricker-Krongrad *et al.* 1998). On the other hand, despite the decrease in total food consumption their nocturnal feeding habit did not disappear. In MSG-treated rats, similarly as in the controls, the major part of the total daily food intake was eaten during the dark period (about 70 %) and a lower percentage of food (about 30 %) was consumed during the light phase. In contrast to the data of Stricker-Krongrad *et al.* (1998), who reported mild but significant hyperphagia during the light phase in MSG rats, no marked changes were found in our experiment in food consumption during the same period. These differences might be due to the different strains of rats employed (Long-Evans vs. Wistar) or higher doses of MSG (4 mg/g vs. 2 mg/g b.wt.).

Although it can be assumed that the day-night AP variations are food-dependent, some differences between experimental and control rats do exist. As compared to the controls, MSG-treated rats exhibited a significantly higher AP activity during the dark phase, despite the hypophagia. The same phenomenon of increased AP activity occurred in MSG-treated rats during all the investigated periods in comparison with the lean controls. The exact mechanism inducing these changes has not been fully elucidated. It is known that food restriction in non-obese rats is related to enhanced intestinal absorption and altered activity in brush-border enzymes (Pathak *et al.* 1981, Holt *et al.* 1991, Casirola *et al.* 1996). Furthermore, it was also demonstrated that obesity is associated with an elevated rate of uptake of nutrients and with their more effective absorption in the upper part of the small intestine (Wisen and Johansson 1992). It could be speculated whether the effectiveness of nutrient absorption, increased AP activity or both may be of importance for the development of obesity in MSG-treated rats. It is possible that enhanced body fat accumulation in these animals may be associated with a permanent alteration of the intestinal function due to significantly increased AP activity on day 30 in neonatally MSG-treated rats as compared to the controls (unpublished data). The MSG syndrome also involves a disruption of leptin-mediated control of body fat content *via* damage of leptin receptors in hypothalamic arcuate nucleus (Serres 1982, Schwartz *et al.* 1996). Moreover, leptin receptor expression has also been recently 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 ineffectiveness of chronic leptin treatment to decrease fat pad weight in MSG-treated rats suggests that lack of leptin or the resistance to leptin action in the small intestine may result in the development of obesity in these animals (Dawson *et al.* 1997).

The detailed analysis of brush border-bound duodenal alkaline phosphatase activity studied in the present experiment has provided information about the relation between the day-night feeding pattern and

intestinal function especially concerning the study of the MSG syndrome. These results have revealed that the permanent increase of enzyme activity in MSG-treated rats is probably not a consequence of actual day-night food intake variations but could be a part of a more general effect of MSG characterized by neurohormonal and metabolic disturbances. The increased AP activity in our experimental rats suggests that, despite sustained hypophagia, their increased body fat content could also be caused by changes of intestinal function. However, further research is required to confirm the causal relationship between increased AP activity and the development of obesity in neonatally MSG-treated rats.

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