Morphological and Cell Proliferative Study on the Growth of Visceral Organs in Monosodium L-Glutamate-Treated Obese Mice

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Summary The growth pattern of visceral organs was investigated in monosodium L-glutamate (MSG)-treated obese mice with hypothalamic lesions. Male Jcl-ICR strain mice were subcutaneously injected with MSG, 2 mg/g of body weight daily, for five days after birth. The MSG-treated mice became obese after 4 weeks of age. According to patterns of weight gain compared with those in the control mice, the visceral organs in the MSG-treated mice were classified into three groups as follows: The first group of organs (heart, lungs, spleen, pancreas, kidneys, testes, brain and submandibular glands) remained absolutely lower in weight throughout their growth. The second group of organs (liver and stomach) was low in weight until 12 weeks of age, but became identical to that of the control mice after 16 weeks of age. The third group of organs (epididymal fat, small intestine and colon) showed lower weight until 4 weeks of age and were significantly heavier than those in the control mice after 8 weeks of age.

The heart in the first group of organs apparently had hypertrophic muscle cells after 8 weeks of age and became significantly hypoplastic due to decreased cell production as was revealed by the continuous suppression of mitotic activity and DNA synthesis by [3H]thymidine autoradiography. The liver in the second group of organs became significantly hypoplastic due to decreased cell production and showed the same weight with the control mice due to the development of fatty liver. The small intestine in the third group of organs became hypoplastic due to decreased cell production in the crypts until 4 weeks of age, and became hypertrophic and hyperplastic by the acceleration of cell production in the crypts from 4 to 8 weeks of age. From these findings, in the MSG-treated mice with specific growth patterns of visceral organs, it is suggested that low energy expenditure results in a relatively excessive energy supply and leads to obesity, because most of the important organs with major physiological functions became
hypoplastic. Moreover, it seems that hypertrophy and hyperplasia of the intestine suggest a possible acceleration of the absorptive function.

**Key Words** monosodium L-glutamate (MSG), neonatal hypothalamic lesion, obesity, short body length, growth, visceral organs, cell proliferation, hypoplasia

In 1969, Olney (1) reported that massive doses of monosodium L-glutamate (MSG) caused degeneration and necrosis of preoptic and arcuate nuclei of the hypothalamus in suckling animals. In the same report, he mentioned that the animals with MSG-induced hypothalamic lesions became apparently obese in and after adolescence even without hyperphagia and that these MSG-induced obese animals remained short throughout their lives (1-4). MSG-induced obese animals have been mainly investigated biochemically in order to clarify the mechanism of obesity (5-9).

In the present study, the growth pattern of the visceral organs together with the changes in body weight gain, morphological findings and cell proliferative kinetics was investigated in MSG-induced obese mice.

**MATERIALS AND METHODS**

Male Jcl-ICR strain mice were used in this study. In preparing the obese mice, a 10% MSG solution of pH 7.0-7.1 (2 mg/g of body weight) was subcutaneously injected daily for five days after birth, according to the method described by Tanaka et al. (3). Male mice without treatment were used as a control. Both groups of mice were suckled by mothers (each mother suckled a litter of 10 infants) and weaned on the 20th day. After weaning, 5 mice were housed in one cage, and maintained on food (Nihon Clea, CE-2) and tap water *ad libitum*. The room temperature was kept constant at 24°C and the lights were kept on from 9:00 to 21:00.

**Experiment 1: Physical growth.** Twenty mice each from the MSG and the control groups were examined for body weight and body length (naso-anal length) according to age. The degree of obesity was evaluated by the Lee-index. The change in body weight during the infantile period was followed every 2 days until 29 days of age and thereafter at 35 and 43 days of age.

**Experiment 2: Determination of the weight of the visceral organs with age.** Twenty mice each from the MSG and the control groups were autopsied for body weight and body length (naso-anal length) according to age. The heart, lungs, spleen, pancreas, kidneys, testes, brain, submandibular glands, liver, stomach, epididymal fat, small intestine and colon were immediately removed, and weighed.

**Experiment 3: Histological and cell proliferative examination of the heart, liver and jejunum.** Five mice from each group were autopsied at 2, 4, 8, 12 and 20 weeks of age, and the heart and liver were removed. The jejunum was removed from mice.
autopsied at 4, 8 and 12 weeks of age, and was cut into regional segments. These organs and tissue were fixed in a 10% solution of formalin, dehydrated in graded ethanol series and embedded in paraffin. Serial paraffin sections, 5 microns in thickness, were stained with hematoxylin-eosin (H-E) and examined by light microscopy.

In the jejunum, serial sections including the vertical cut surface of the villi and crypts 2 cm distal from the Treitz's muscle were prepared. The height of the villi, the depth of the crypts and the number of proliferating cells in one crypt were examined by light microscopy. The number of proliferating cells in the crypt were counted excluding Paneth's cells located in the bottom of the crypt. These morphological characteristics in the jejunal mucosa were expressed in terms of the arithmetical means of the measurements made on 50 villi and crypts for each mouse. Statistical significance between these means was determined by the Student's t test.

In order to examine the cell proliferative activity, three MSG and three control mice were autopsied on specified days of age. The heart and liver were fixed in a 10% solution of formalin and embedded in paraffin. Serial sections, 5 microns in thickness, were stained with H-E and the mitotic activity of myocardial cells in the left ventricle and hepatic cells was measured by light microscopy. The mitotic index was expressed by the ratio of the number of the mitotic myocardial cells or hepatic cells to a total of 8,000 cells examined in each organ.

Moreover, in order to examine the DNA synthetic activity of myocardial cells in the left ventricle, hepatic cells and proliferating cells in the crypts, flash labeling autoradiography was carried out. Three MSG and three control mice on specified days of age received a single intraperitoneal injection of 2μCi [3H]thymidine ([3H]TdR; [6-3H]thymidine, Radiochemical Centre, Amersham, England, specific activity, 10 Ci/mM) per gram of body weight, and were autopsied 40 min after the injection. The heart and liver were fixed in a 10% solution of formalin and embedded in paraffin. The serial sections, 5 microns in thickness, were dipped in Sakura NR-M2 nuclear emulsion and developed in FD-111 after a 4 week exposure, and then the sections were stained with H-E. The labeling index was expressed by the ratio of the number of labeled cells to a total of 8,000 cells. Mice younger than 5 days of age were used for autoradiography, 4 h after the injection of MSG. [3H]TdR labeling index of the proliferating cells in the crypts was obtained by the same method. In this investigation three mice each from the MSG and the control groups were used at 4, 8 and 12 weeks of age. The labeling index was calculated as the ratio of the labeled cells to all cells in a crypt.

RESULTS

Experiment 1

The changes in body weight during the infantile period in the MSG group showed a slow increase from 11–13 to 29 days of age. After 29 days, however, weight gain accelerated rapidly (Fig. 2A). As shown in Fig. 2B, there was no
GROWTH OF ORGANS IN MSG OBESE MICE

Fig. 1. Weight gain patterns of the visceral organs in the MSG-treated and control mice. A: The first group of organs (heart, lungs, spleen, pancreas, kidneys, testes, brain and submandibular glands). B: The second group of organs (liver and stomach). C: The third group of organs (epididyman fat, small intestine and colon).

The difference in the Lee-index in both groups until 3 weeks of age, showing that the MSG-treated mice were not yet obese. The Lee-index in the MSG group was significantly higher than that in the control group at 4 weeks of age, being $332 \pm 4.5$ (mean $\pm$ SD) in the MSG group and $325 \pm 6.4$ in the control group. Thereafter, the Lee-index in the MSG group became higher than that in the control group.

Experiment 2

In comparison with the control group, the visceral organs of the MSG group were classified into three groups, according to the patterns of weight gain (Fig. 1). The weights of the organs of the first group (heart, lungs, spleen, pancreas, kidneys,
Fig. 2.  A: Changes in body weight during suckling and young periods in the MSG-treated and control mice. B: Lee-index of the MSG-treated and control mice.

$$\text{Lee-index} = \frac{3}{\text{naso-anal length (cm)}} \times 10^3$$

The weights of the organs of the second group (liver and stomach) were lower until 12 weeks of age, then became comparable to those in the control group after 16 weeks of age. The weights of the organs of the third group testes, brain and submandibular glands) were lower than those in the control group throughout the experiment.
Fig. 3. Photomicrographs of the left ventricular muscle cells at 20 weeks of age (H.
E., × 350). a, control mice; b, MSG-treated mice. The left ventricular muscle cells in
the MSG-treated mice are apparently hypertrophic compared with those in the
control mice.
(epididymal fat, small intestine and colon) were lower than those in the control group until 4 weeks of age. These organs, however, increased rapidly in weight from 4 to 8 weeks of age and became significantly heavier compared to those in the control group after 8 weeks of age. It was concluded that all organs of the first group in the MSG group were absolutely lower in weight than those in the control group throughout the course of the experiment, because the ratios of organ weight/body length in the MSG group were significantly lower than those in the control group throughout this study.

**Experiment 3**

The heart in the MSG group was apparently smaller than that of the control group macroscopically. In the MSG group, myocardial degeneration was not found by light microscopy throughout the experiment. After 12 weeks of age, however, the myocardial cells of the left ventricle in the MSG group were apparently hypertrophic compared with those of the control group (Fig. 3). As shown in Figs. 4A and 4B, the mitotic indices and labeling indices in the MSG group were significantly lower than those in the control group throughout the experiment. The myocardial cells with mitotic figures disappeared on the 14th and 16th days of age in the MSG and control groups, respectively. The \[^3\]HTdR labeled cells disappeared at 6 and 7 weeks of age in the MSG and control groups, respectively.

In the MSG group, the liver became yellowish and enlarged as obesity developed and the hepatocytes showed vacuolar changes centrilobularly after 8 weeks of age. The hepatocytes in the MSG group were significantly larger than those in the control group, suggesting the development of fatty liver (Fig. 5).
mitotic and $[^3]H$TdT labeling indices of the hepatocytes in the MSG group were lower than those in the control group until the 8th day and 3rd week of age, respectively. The mitotic indices in the MSG group decreased gradually without a catch-up phenomenon after the 8th day of age and the hepatocytes with mitotic figures disappeared at 3 and 4 weeks of age in the MSG and control groups, respectively (Fig. 6A). After the MSG treatment, the $[^3]H$TdT labeling indices of the liver remained lower, until 3 weeks of age. Then, from 3 to 4 weeks of age, the labeling index in the MSG group increased from $0.84 \pm 0.2$ (mean $\pm$ SD) to $2.17 \pm 0.5\%$, being identical to the $2.44 \pm 0.2\%$ in the control group. The labeling indices decreased gradually after 4 weeks of age and labeled cells were not found in either group at 6 weeks of age (Fig. 6B).
Fig. 5. Photomicrographs of the liver at 20 weeks of age (H.E., x 35). a, control mice; b, MSG-treated mice. The hepatocytes in the MSG-treated mice show vacuolar changes centrilobularly and are significantly larger than those in the control mice.

The histological findings in the jejunal mucosa in both groups are summarized in Table 1. Although it seemed that the heights of the villi and the depth of the crypts in the MSG group were slightly shorter than those in the control group at 4 weeks of age, the differences between both groups were not statistically significant. The villi and crypts in the MSG group grew rapidly from 4 to 8 weeks of age. Thereafter, at 8 and 12 weeks of age, the height of the villi and depth of the crypts in the MSG group became larger than those in the control group. The differences between both groups, however, were not statistically significant. The proliferative cells in
Fig. 7. Photomicrographs of the jejunal mucosa in the MSG-treated and control mice at 20 weeks of age. a, control mice; b, MSG-treated mice. The jejunal villi in the MSG-treated mice become thicker than those in the control mice in the mature stage.

Table 1. Morphological growth records of the jejunal mucosa in the MSG-treated and control mice.

<table>
<thead>
<tr>
<th></th>
<th>4 week</th>
<th>8 week</th>
<th>12 week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height of villus (µm)</td>
<td>Control</td>
<td>256 ± 33 (n.s.)</td>
<td>262 ± 24 (n.s.)</td>
</tr>
<tr>
<td></td>
<td>MSG</td>
<td>239 ± 30</td>
<td>264 ± 24</td>
</tr>
<tr>
<td>Depth of G-zone (µm)</td>
<td>Control</td>
<td>49.9 ± 7.1 (n.s.)</td>
<td>61.8 ± 9.7 (n.s.)</td>
</tr>
<tr>
<td></td>
<td>MSG</td>
<td>45.7 ± 7.1</td>
<td>69.7 ± 10.9</td>
</tr>
<tr>
<td>G-cell number in G-zone</td>
<td>Control</td>
<td>29.9 ± 3.0 *</td>
<td>32.1 ± 3.4 ***</td>
</tr>
<tr>
<td></td>
<td>MSG</td>
<td>21.0 ± 2.3</td>
<td>38.2 ± 4.2</td>
</tr>
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* p < 0.001, ** p < 0.005, *** p < 0.05 (mean ± SD).

Fig. 8. Incorporation of [³H]thymidine into nuclear DNA of the proliferating cells in the crypts in the MSG-treated and control mice.

One crypt in the MSG group were significantly smaller in number at 4 weeks of age (p < 0.001), but became greater in number than those in the control group after 8 weeks of age (p < 0.05 at 8 weeks and p < 0.005 at 12 weeks). The jejunal villi in the MSG group became thicker than those in the control group in the mature stage (Fig. 7).

The [³H]TdR labeling indices of the proliferating cells in the crypts are shown in Fig. 8. The labeling indices in the MSG group were significantly lower than those in the control group until 4 weeks of age. After 8 weeks of age, however, the indices
became significantly higher than those in the control group.

DISCUSSION

In this study, we examined the characteristic growth pattern including the growth of the visceral organs in terms of the changes in body weight and organ weight gain, histological findings and cell proliferative kinetics in the MSG-treated mice.

The MSG-treated mice had a pattern of body weight gain, which was slow from 11–13 to 29 days of age, but which accelerated after 4 weeks of age. As shown in the Lee-index, obesity began at 4 weeks of age in the MSG-treated mice. The visceral organs of the MSG-treated mice were divided into three groups according to their weight gain patterns, and compared with those in the control mice. The weight gain was repressed in all organs examined in the MSG-treated mice until 4 weeks of age, but thereafter each organ increased in weight independently, depending on the group described above. These patterns of body and organ weight gains around 4 weeks of age in the MSG-treated mice suggest dramatic changes in the interaction of the nervous, endocrine and metabolic systems involved in the regulation of the physiological and biochemical processes.

The low weight of the organs is usually caused by hypoplastic or atrophic changes. On the other hand, overweight may be caused by multiple factors such as congestion, hyperplasia, hypertrophy, tumorous change, accumulation of foreign substances, edema etc. Therefore, in order to clarify the mechanism of the specific patterns of organ weight gain in the MSG-treated mice, we examined the changes in histological findings and cell proliferative kinetics on the heart, liver and small intestine selected from each group of organs.

In the MSG-treated mice, the heart was apparently smaller and lighter than that of the control mice throughout their growth. No degenerative changes were demonstrated by light microscopy, and the myocardial cells apparently became hypertrophic in the mature stage, showing that the low weight of the heart is not ascribed to atrophic changes. The proliferative activity of the myocardial cells estimated by the mitotic index and the [H]TdR labeling index was depressed throughout the experiment and disappeared at an early stage of growth without a late catch-up phenomenon. These results confirm that the heart in the MSG-treated mice consists of much smaller numbers of myocardial cells than those in the control mice, and that this hypoplastic change in the cause of the low heart's weight.

The liver in the MSG-treated mice gradually became yellowish and enlarged as obesity developed. During the stages from adolescence to maturation, the hepatocytes in the MSG-treated mice showed vacuolar changes and apparently became larger than those in the control mice, indicating the development of a fatty liver. The weight gain pattern and cell proliferative kinetics of the liver in the MSG-treated mice, however, have not yet been reported, but it has been demonstrated in the present investigation that the liver with fatty infiltration in the MSG-treated mice.
did not become overweight. The results revealed an apparent suppression of cell proliferative activity of the liver in the MSG-treated mice. As observed in the heart, the liver also became hypoplastic, leading to a poor weight gain in the early stage. The histological findings suggest that the weight of liver in the MSG-treated mature mice became identical to that of the control mice due in the development of fatty liver.

The weight of the small intestine in the MSG-treated mice increased markedly from 4 to 8 weeks of age. Mino (10, 11) reported that the jejunal mucosa in the MSG-treated mature mice was hyperplastic and that the life span of the absorptive epithelial cells was prolonged. His investigation, however, was performed on mature mice where the obesity had already been established. Therefore, we examined in detail the histological changes in the mucosal structure and cell proliferative kinetics in the crypts during the course of growth. Although the jejunal mucosa in the MSG-treated mice was hypoplastic at 4 weeks of age, it became hyperplastic and thick with the acceleration of cell production in the crypts after 8 weeks of age. These results explain the pattern of weight gain of the small intestine in the MSG-treated mice.

As mentioned above, all organs of the first group and the liver in the MSG-treated mice remained hypoplastic throughout their growth. On the other hand, the intestine, classified in the third group, showed the dramatic change from hypoplasia to hyperplasia.

MSG treatment in infant animals was reported to produce a few acute toxic effects such as an irreversible neuronal degeneration in the inner retina (12–15) and hypothalamus (1, 3, 16), transiently depressed extramedullary hematopoiesis (17), and a slight vacuolar change in the hepatocytes (17). No degenerative change as a result of direct toxicity has yet been described in other organs by light microscopy. On the other hand, MSG may suppress cell proliferation in the visceral organs. According to the biochemical study in our laboratory, however, glutamate concentration in blood reached a peak level 60 min after the injection of MSG and it returned to the pre-injection level within 6–8 h. From this study, it seems that the suppression of cell proliferative activity in all organs was not due to the direct toxicity of MSG.

In the MSG-treated mature animals, reduction of the growth hormone (6–8) and thyroid hormone (5–9) in the blood as well as an imbalance in the autonomic nervous system (5–9) were reported. Moreover, irreversible hypothalamic lesions by MSG histologically were manifested immediately after the treatment (18, 19), suggesting possible functional disturbance in the hypothalamus-hypophysis system and a subsequent functional imbalance in the nervous, endocrine and metabolic systems. Therefore, the changes in neuroendocrine factors due to neonatal hypothalamic lesions may play a major role in causing the short body length and the characteristic growth patterns of visceral organs throughout the experiment. Cell proliferative kinetics in some organs is characterized by their excessive gain in weight after 4 weeks of age. It should be taken into account that some effects are due
to hypothalamic lesions.

Although the MSG-treated mice became obese after 4 weeks of age, most of the visceral organs remained low in weight throughout their growth and only adipose tissue and intestines became overweight. In connection with the development of obesity, the balance between energy supply and expenditure should be taken into consideration. In regard to the energy supply, no hyperphagia was demonstrated in the MSG-treated mice (2, 3, 20). Hyperplasia of the intestine, noted after 8 weeks of age, suggests a possible acceleration of the absorptive function. The intestine, however, remained hypoplastic at 4 weeks of age when the acceleration of body weight gain and the development of obesity started. Accordingly, the acceleration of the absorptive function of the intestine should be ruled out. Energy expenditure includes basal metabolism, diet-induced thermogenesis and physical activity. It was reported that the MSG-treated mice had lower spontaneous activity after 30 days of age (5, 20) and that the growth hormone (6-8) and thyroid hormone (5-9), which affect energy expenditure including basal metabolism, were also reduced in the blood. In addition to these findings, we have confirmed that most of the important organs with major physiological functions became hypoplastic in the MSG-treated mice. Therefore, it seems that low energy expenditure, although hyperphagia is lacking, results in a relatively excessive energy supply and leads to obesity in the MSG-treated mice. The hypertrophic and hyperplastic intestine may induce a possible acceleration of absorptive function, resulting in the progress of obesity.

The present study showed that the mice treated with massive doses of MSG in the neonatal period became obese, coupled with growth stunting and characteristic growth pattern of the organs, and that lasting suppression in cell proliferation caused hypoplasia in most of the organs. From the viewpoint of interactions between the hypothalamus-hypophysis system and the visceral organs, further investigation including the periodical estimation of energy balance and the changes in the nervous, endocrine and metabolic systems is required. A detailed investigation of these interactions after MSG treatment may make it possible to understand the roles of the central nervous system in visceral and/or cellular growth.

REFERENCES


