EFFECT OF ADMINISTRATION ROUTES OF MONOSODIUM GLUTamate ON PLASMA GLUTAMATE LEVELS IN INFANT, WEANLING AND ADULT MICE

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Summary—The time course of concentrations of glutamate in plasma was
examined following a single dose of 1 g/kg body weight of monosodium glutamate
(MSG) by intraperitoneal or subcutaneous injection or peroral intubation in 10-
day, 23-day and 4-month-old mice of ICR strain. Ad libitum dietary adminis-
tration in shorter period of time and effects of food accompaniment were also
investigated. Levels of plasma glutamate rapidly elevated and reached maximum
from 10 to 30 minutes after administration of an aqueous solution of MSG and
then returned to normal levels by 90 minutes. Peak values in peroral intubation
were significantly lower than those in intraperitoneal or subcutaneous injection,
especially in infants and weanlings. Food accompaniment markedly suppressed
elevations of plasma glutamate. In dietary administration, maximum levels of
plasma glutamate, which were remarkably low as compared with those in food
accompaniment, never exceeded 5-fold of base-line values.

INTRODUCTION

In 1969 Olney reported that a single subcutaneous injection of a massive dose of
monosodium L-glutamate (MSG) in newborn mice induced neuronal necrosis in the
hypothalamic region. Since then, many researchers have carried out the experiments on
brain lesions caused by MSG using mice (Olney, 1969; Burde et al., 1971; Takasaki,
1977a), rats (Burde et al., 1971), guinea pigs (Olney et al., 1973b), fowls (Snapir et al.,
1973), dogs (Oser et al., 1975) and monkeys (Olney et al., 1969; 1972; Reynolds et al.,
1971). In these experiments subcutaneous injection was commonly applied as an adminis-
tration route of MSG, though gastric intubation was used in some works. Some inves-
tigators demonstrated acute neuronal necrosis in the developing brain but other people
denied that a single dose of MSG could produce any brain damage in infant mice, rats,
dogs and monkeys (Newman et al., 1973; Oser et al., 1975).

There are several works in which dietary administration of a large amount of MSG
was carried out to mice, rats and monkeys (Wen et al., 1973; Semprini et al., 1974;
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Huang et al., 1976; Yonetani et al., 1977; Takasaki, 1977b). Histopathological observation of the hypothalamus revealed that no lesions were detected following a feeding period of time in any experiments. This strongly suggests that induction of the brain lesions may highly depend upon the administration route of MSG.

Perez et al. (1972) demonstrated that the transient accumulation of glutamate in the arcuate nucleus of the hypothalamus was attained following the forerunning elevations of plasma glutamate after a single subcutaneous injection of MSG to mice, suggesting that this might relate to the selective destruction of arcuate neurons. This indicates that a plasma glutamate concentration over a certain level, which may depend upon animal species or strains, is necessary to induce the brain lesions. However, there is little information about the relationship between plasma glutamate levels and administration conditions of MSG.

The purpose of the present work was to examine systematically plasma glutamate levels after intraperitoneal, subcutaneous, peroral (by stomach tube) and dietary administration of MSG in infant, weanling and adult mice.

MATERIALS AND METHODS

MSG used in this study was a food additive grade “Ajinomoto” which was commercially available. As clear soup, “Condensed beef consommé soup” produced by Campbell Soup Co. was used. Total glutamate content in this soup was 0.59% (w/v) as served basis. Infant formula “SMA-S-26™”, a product of Wyeth Japan Co., was employed. The infant formula prepared according to the directions had 0.306% (w/v) of total glutamate content.

Animals used in this experiment were mice of ICR strain which were originally supplied by Charles River Japan Co. and Nippon CLEA Co. and were bred in our laboratory. Mice were housed in polycarbonate cages in the air-conditioned room where temperature and relative humidity were regulated at 23 ± 2°C and 60 ± 10%. Lighting was also regulated at 12 hr on and 12 hr off throughout the experiment. Mice were fed a commercial laboratory chow (CE-2 of Nippon CLEA Co.; Content of total glutamate 4.2% (w/v)) and given water ad libitum in all experiments unless otherwise noted elsewhere.

Routes of administration of the test material applied in this study were subcutaneous (sc) and intraperitoneal (ip) injections, and peroral (po) and dietary administrations. A po administration was restricted to intragastric intubation by a stomach tube, while dietary administration was defined as ingestion of the test material mixed with the diet ad libitum.

Ten-day-old mice were used as infants in this experiment. Infant mice were not deprived of their mother’s milk before administration of MSG, which was carried out between 10:00 and 13:00 in every route of administration. (Lighting commenced at 6:00.) Animals were killed at serial post-administration intervals from 10 to 180 minutes. Infant formula was prepared by mixing infant formula powder with water according to
the directions for human baby and then MSG was dissolved in it to make infant formula containing 10% (w/v) of MSG.

In the experiment of weanling mice, 23-day-old animals, which had been separated from their mother and given the diet and water ad libitum for 3 days were used. They were deprived of the diet since 2 hrs before the commencement of dark period and were given MSG by sc or ip injection or po administration at 3 hrs after the commencement of dark period. In the dietary feeding of MSG, the diet containing 10% of MSG was fed to mice so that 1 g/kg body weight of this material could be consumed within 15 or 30 minutes. This method of administration of MSG was roughly regarded as a single dose. Animals were killed at serial post-administration intervals from 10 to 180 minutes. Zero time was set at the beginning of feeding in dietary administration.

Four-month-old mice were used in this experiment as adult animals. MSG was given at 2 hrs before dark period after 10 hrs of fasting by sc or ip injection or po administration. In other respects methods of administration were essentially the same as stated above in weanlings.

Blood samples were collected from the right ventricle under light anesthesia by the special heparinized micro-hematocrit capillary tube for infants or heparinized syringe for weanlings and adults. Plasma was obtained by centrifugation of the sealed capillary tube or sealed syringe. After adding a solution of DL-norleucine to mice plasma as internal standard, plasma samples were deproteinized with sulfosalicylic acid and then analyzed by an amino acid analyzer (Hitachi KLA-5). For amino acid analysis, plasma samples of 50 μl for infants, 0.3 ml for weanlings and 0.5 ml for adults were applied.

RESULTS

Daily Variations in Plasma Glutamate Levels

Adult mice were fed the basal diet and given tap water ad libitum. Circadian variations in concentrations of glutamate in plasma with feeding pattern and lighting schedule are shown in Fig. 1. Elevations of plasma glutamate were attained in the dark period. Ratio of the highest level to the lowest was around 2. As shown in Fig. 1, the results of feeding pattern indicated that adult mice began to eat the diet from about 2 hrs before the dark period and that food intake was practically limited in the dark period.

Time Course of Plasma Glutamate after Administration of MSG

Infant, weanling and adult mice were given a single dose of 1 g/kg body weight of MSG by various routes of administration. Animals were killed at serial post-administration intervals from 10 to 180 minutes. The time course of concentrations of glutamate in plasma is presented in Figs. 2a, 2b and 2c. Levels of plasma glutamate rapidly elevated and reached maximum from 10 to 30 minutes after administration of MSG and then returned to normal levels by 90 minutes. In sc and ip injections, older mice showed less elevations of glutamate levels in plasma as a whole. Table 1 shows maximum concentrations of plasma glutamate in respective treatments. Infant mice showed a significant difference in maximum concentrations of plasma glutamate between ip and sc injections.
Fig. 1. Circadian changes of plasma glutamate levels in adult mice. Blood samples were obtained from the right ventricle at each given time.
Top: Lighting schedule
Middle: Changes of plasma glutamate levels; Each point represents mean±S.E.
Bottom: Histogram of food consumption

However, no significant difference was observed between ip and sc injections in both weanlings and adults. In po administration, no significant difference in peak values of plasma glutamate was revealed between infants and weanlings and between infants and adults, but a significant difference was shown between weanlings and adults. In adult animals as well as infant and weanling mice, maximum concentrations of glutamate in plasma by po administration were far lower than those by ip or sc injections, especially in infants and weanlings.

Effect of MSG Ingestion by Food Accompaniment and Dietary Administration on Plasma Glutamate Levels

A single dose of 1 g/kg body weight of MSG was administered by mixing with infant formula to infant mice and with clear soup to adult animals by gastric intubation. The time course of glutamate concentrations in plasma were compared with that in po administration of an aqueous solution (10% w/v) of MSG. Results are presented in Figs. 3a and 3c. Weanling and adult animals were fed 1 g/kg body weight of MSG by dietary administration. Results are shown in Figs. 3b and 3c.

Infant formula accompaniment markedly suppressed elevations of glutamate levels
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Fig. 2. Time course of plasma glutamate levels after administration of monosodium glutamate (MSG). Infant (A), weanling (B), and adult (C) mice were given a single dose of 1 g/kg body weight of MSG by sc (○), ip or po (●) administration. A 4% (w/v) MSG aqueous solution was used for sc and ip routes and a 10% (w/v) MSG aqueous solution was used for po administration. Each point represents mean ± S.E.

Table 1. Maximum concentrations of plasma glutamic acid in mice given a single dose of monosodium glutamate.

Infant, weanling and adult mice were given a single dose of 1 g/kg body weight of MSG with or without food components by sc, ip, po, or dietary administration.

<table>
<thead>
<tr>
<th>Route</th>
<th>Test Preparation</th>
<th>Infant</th>
<th>Weanling</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Treatment</td>
<td>—</td>
<td>16.9 ± 1.0*</td>
<td>10.0 ± 0.9</td>
<td>9.7 ± 1.2</td>
</tr>
<tr>
<td>SC</td>
<td>4% (w/v) MSG aqueous solution</td>
<td>1058.2 ± 81.5a</td>
<td>760.0 ± 52.2b</td>
<td>538.5 ± 25.2d</td>
</tr>
<tr>
<td>IP</td>
<td>4% (w/v) MSG aqueous solution</td>
<td>715.4 ± 44.8b</td>
<td>729.1 ± 52.2b</td>
<td>521.6 ± 29.5p</td>
</tr>
<tr>
<td>PO</td>
<td>10% (w/v) MSG aqueous solution</td>
<td>314.0 ± 66.2e</td>
<td>219.2 ± 31.0p</td>
<td>343.7 ± 37.22d</td>
</tr>
<tr>
<td>PO</td>
<td>Infant formula containing 10% (w/v) of MSG</td>
<td>126.1 ± 25.1e</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PO</td>
<td>Soup containing 10% (w/v) of MSG</td>
<td>—</td>
<td>—</td>
<td>191.6 ± 11.9a</td>
</tr>
<tr>
<td>Dietary</td>
<td>Diet containing 10% (w/v) of MSG</td>
<td>—</td>
<td>44.7 ± 7.6d</td>
<td>43.4 ± 5.6e</td>
</tr>
</tbody>
</table>

a Plasma glutamate levels at 20 minutes after administration of MSG.
b Plasma glutamate levels at 10 minutes after administration of MSG.
c Plasma glutamate levels at 30 minutes after administration of MSG.
d Plasma glutamate levels at 15 minutes after administration of MSG.
e Plasma glutamate levels at 45 minutes after administration of MSG.

* mean ± S.E.
Fig. 3. Effects of monosodium glutamate (MSG) load by food accompaniment or dietary administration on time course of plasma glutamate levels in mice. Mice were given a single dose of 1 g/kg body weight of MSG with or without food accompaniment by po or dietary administration. Infant mice (A) were given a 10\% (w/v) MSG aqueous solution (○) or an infant formula containing 10\% (w/v) of MSG (●) or an infant formula only (●) by po administration. Weanling mice (B) were given a 10\% (w/v) MSG aqueous solution (○) by po route, or were fed a basal diet containing 10\% (w/w) of MSG (●) or a basal diet only (●) by dietary administration. Adult mice (C) were given a 10\% (w/v) aqueous solution (○) or a clear soup containing 10\% (w/v) of MSG (●) or a clear soup only (●) by po route, or were fed a basal diet containing 10\% (w/v) of MSG (●) or a basal diet only (●) by dietary administration. Each point represents mean ± S.E.

in plasma in infant animals. Peak values in the food accompaniment were less than a half of those in an aqueous solution and time of maximum glutamate levels was delayed as compared with that in an aqueous solution. Maximum concentrations of plasma glutamate in adult mice administered MSG with clear soup showed essentially the same tendency as in the experiment of food accompaniment in infant mice. In the dietary administration of 1 g/kg body weight of MSG, elevations of glutamate levels in plasma were slight and attained peak values of less than 45 \( \mu \)moles/dl in both weanlings and adults.

**DISCUSSION**

Daily variations of concentrations in plasma glutamate were observed under *ad*
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*ad libitum* food and water conditions. Fernstrom et al. (1971) reported the same findings in rats, although their ranges of variations were narrower than those in the present experiment. The time of administration was designated so that mice were given MSG when they ate the diet under *ad libitum* food and water conditions. Deprivation of food before the administration might affect the plasma glutamate levels in weanling mice alone, if any, whose time of administration was set at 3 hrs after the dark portion because of successful performance of dietary administration.

Base-line values of plasma glutamate in infant mice showed higher tendency as compared with those in weanling and adult animals (Fig. 1 and Table 1). The fact that the peak values of glutamate in plasma after sc or ip injections were significantly higher than those after po administration in mice of each age strongly suggests that the gastrointestinal tract may play an important part as a barrier. Moriyuki et al. (1978) demonstrated that oral LD$_{50}$ values of MSG in mice as well as rats were more than twice as large as those of sc and ip routes. This is consistent with the result of the present investigation. In sc injections there was a significant difference in maximum glutamate levels among animals of respective ages: those in infants were highest, and adults lowest. Moreover, long retention of glutamate levels in plasma over base-line values was observed in infant animals. These may be mainly due to the development of hepatic function as a barrier with age. A statistically significant difference in peak values of plasma glutamate between sc and ip injections in infant animals cannot be explained. There were individual fluctuations in concentrations of plasma glutamate following administration of MSG. Plasma glutamate levels in infant mice 30 minutes after po administration, for example, were in the range of 190.3 to 449.8 μmoles/dl. Elevations of aspartate were also observed following administration of MSG, especially in po, but concentrations of aspartate in plasma were at most less than one fifth of those of glutamate.

A transient peaking of plasma glutamate as well as a sustained elevation seem to have influence on production of neuronal necrosis in the arcuate nucleus of the hypothalamus. The relationship between concentrations of plasma glutamate and brain damage is discussed with priority given to a transient peak value of plasma glutamate. Little information on the relationship between plasma glutamate levels and neuronal necrosis of the arcuate nucleus of the hypothalamus is available. From the experimental results of administration of casein and fibrin hydrolysates to mice by Olney et al. (1973a) and Stegink et al. (1974), Stegink et al., concluded that blood glutamate levels of 40 μmoles/dl were likely to be safe for even the acutely sensitive infant mouse, while small amount of neuronal damage to a few animal may occur at glutamate levels of approximately 50 μmoles/dl. Casein and fibrin hydrolysates contained free aspartate of approximately one fourth and fourfifths of free glutamate content, respectively. Moreover, as the plasma glutamate levels measured by them were those of pooled plasma of mice, it is presumed from the results of the present experiment that there might be remarkable variations in concentrations of glutamate in plasma among individuals of mice. Olney (1976) described that a transient peaking of plasma glutamate to 20-fold resting levels could induce neuronal
necrosis in infant mice. Consequently, a transient peak value of plasma glutamate which is required to induce neuronal necrosis seems to be higher than the value estimated by Stegink et al. (1974).

Olney (1976) reported the lowest effective dose to induce necrotic neuronal profiles in mice of 10, 21, 45 and 60 days of age. The lowest effective dose in 60-day-old mice was reported to be a single po administration of 2 g/kg body weight of MSG. In the present experiments with adult mice approximately 340 μmoles/dl (approximately 35 fold resting level) of plasma glutamate was attained after po administration of 1 g/kg body weight of MSG. This clearly shows that a transient maximum concentration of plasma glutamate which is required to induce the brain damage depends upon ages of animals, indicating that much higher levels of plasma glutamate is needed to produce the brain damage in older mice. This strongly suggests that certain mechanism for protection of the brain damage develops in the brain with age. Stegink et al. (1975) reported that MSG administered by gastric tube to infant monkeys at a dose of 4 g/kg body weight produced approximately 400 μmoles/dl (33-fold resting level) of plasma glutamate with highest values of 1,600 μmoles/dl in some animals and that these animals never demonstrated neurotoxicity. Consequently, a transient peaking of plasma glutamate for production of the brain damage may also depend upon species of animals.

Infant mice given MSG with infant formula and adults administered MSG with clear beef soup by gastric tube showed markedly low concentrations of plasma glutamate in comparison with those given an aqueous solution of the same dose of MSG. Inconsistent with these observations, McLaughlan et al. (1970) indicated that food accompaniment did not significantly alter the magnitude of the plasma glutamate elevations in weanling rats following ingestion of free glutamate alone or mixed with food. Stegink et al. (1973) reported the same findings in neonatal pigs given MSG with or without infant formula. The causes of these discrepancies are not clear.

No neuronal necrosis was observed in mice (Wen et al., 1973; Semprini et al., 1974; Takasaki, 1977 b; Yonetani et al., 1977), rats (Wen et al., 1973; Semprini et al., 1974; Huang et al., 1976), and monkeys (Wen et al., 1973) following dietary administration of MSG for a certain period of time. In the present experiment, elevations of plasma glutamate levels were remarkably suppressed in dietary administration of MSG, which was roughly regarded as a single dose. The experimental results that no brain lesions were demonstrated in animals daily given MSG by dietary administration can be thoroughly explained from the present findings that peak values of plasma glutamates following dietary administration of 1 g/kg body weight of MSG were less than 3.5 fold of those under ad libitum basal diet and water condition.

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