The Effect of Monosodium Glutamate on GDH, GOT, and GPT Levels in Mice[1] (37521)

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Olney (1) reported that a single subcutaneous injection (0.5–4.0 mg/g body weight) of monosodium glutamate (MSG) in newborn mice (2–9 days old) induced acute necrosis in several regions of developing brain including the hypothalamus. The newborn mice injected daily over long periods of time showed, as adults, stunted development, marked obesity, and female sterility. Olney (1) also reported pathological changes in several organs associated with endocrine function. He postulated that the adult syndrome suggested a complicated neuroendocrine disturbance. Similar findings have been reported by Redding and Schally (2). Adamo and Ratner (3), following the experimental procedures of Olney (1), found that injections of MSG produced no changes in the brain or the reproductive function in rats. Olney and Sharpe (4) have reported acute brain damage in a newborn rhesus monkey following a single injection of MSG. Recently, Olney and Ho (5) have found that both MSG and monosodium aspartate (MSA) administered orally to infant mice (10–12 days old) caused necrosis of hypothalamic neurones. They also reported that the free amino acids, L-glutamate, L-aspartate, and L-cysteine produced the same effect. Bazzano *et al.* (6) reported that large amounts of glutamic acid incorporated in a formula diet and fed to adult humans and gerbils appeared to cause no clinical pathological changes.

The injection of large doses of monosodium glutamate by Olney (1) and others (2–4) has raised the question of the effect of MSG on the three important glutamate metabolizing enzymes, glutamic dehydrogenase (GDH, EC 1.4.1.3), aspartate aminotransferase (GOT, EC 2.6.1.1), and alanine aminotransferase (GPT, EC 2.6.1.2). The present study was designed to determine the effect of MSG on the levels of GDH, GOT, and GPT in the brains and livers of newborn and weanling mice.

Materials and Methods. Animals and treatments. Male weanling mice (25 days old) of the Charles Rivers CE-1 strain received daily subcutaneous injections of monosodium glutamate in saline (1.0 mg/g body weight) or monosodium aspartate in saline (0.92 mg/g body weight). Two control groups were used; one received saline and the other no injection. The MSG concentration used was twice that which Olney (1) has reported as a cause of brain lesions in newborn mice. The animals were sacrificed and the brains and livers excised 24 hr after the completion of daily injections given for 1, 3, 7, 13, and 21 days. Thirty days following the completion of the original experiment, the study was repeated, again using weanling mice. At a later date, the original experimental design was used to study the effects on newborn mice (1 day of age).

Both brains and livers were homogenized in 0.025 *M* phosphate buffer pH 7.4 in the proportion of 1 g tissue to 9 ml of buffer. The tissue was sonified for two minutes with a Heat Systems Model W-1855 sonifier at an

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output setting of 8. A magnesium chloride ice bath was used to prevent heating of the homogenate. The sonified tissue was centrifuged at 21,000*g* for 30 min at 0°. The enzyme assays were made on the supernatant. Glutamic dehydrogenase was assayed according to the method of Olson and Anfinsen (7, 8). The assay mixture contained 2.1 ml of 0.025 *M* phosphate buffer (pH 7.6), 0.1 ml of 3 *M* NH_4Cl, 0.5 ml of 0.47 m*M* NADH, 0.2 ml of 0.167 *M* K-*α*-ketoglutarate and 0.1 ml of the appropriate tissue supernatant. Aspartate aminotransferase activity was determined according to the method of Karmen (9). The assay mixture contained 0.5 ml of 0.2 *M* L-aspartate, 0.2 ml of 1.41 m*M* NADH, 0.1 ml of malic dehydrogenase (2000 units/ml), 0.2 ml of 0.1 *M* K-*α*-ketoglutarate, 0.8 ml of water and 0.2 ml of the appropriate tissue supernatant. The method of Wroblewski and La Due (10) was used to determine alanine aminotransferase activity. The assay mixture contained 0.5 ml of 0.2 *M* L-alanine, 0.2 ml of 1.41 m*M* NADH, 0.1 ml of lactic dehydrogenase (8000 units/ml), 0.2 ml of 0.1 *M* K-*α*-ketoglutarate, 1.8 ml of water and 0.2 ml of the appropriate tissue supernatant. The aminotransferases and glutamic dehydrogenase were assayed by measuring nucleotide oxidation at 340 nm in a recording spectrophotometer. All enzyme assays were performed at 25° in 3 ml, 1 cm cuvettes. A linear relationship was obtained between the enzyme activity and varied quantities of the tissue supernatant for each enzyme assayed.

Tissue protein was determined by the method of Lowry *et al.* (11) utilizing crystalline bovine serum albumin as the standard.

Results. The injections of monosodium glutamate, monosodium aspartate, and saline for 21 days had no significant effect on the levels of GDH, GOT or GTP in the brains and livers of weanling mice. The combined data for both experiments using weanling mice are presented in Table I. The enzyme activities reported are the mean values of the five injection periods. As can be seen, there were no significant differences in enzyme activities between treatments at the $p < 0.05$ level (Duncan's New Multiple Range Test). It was also found that there were no differences in enzyme activities between treatments after 1, 3, 7, 13 or 21 injections. Body, liver or brain weights were not significantly different between treatments.

The data for the experiments using newborn mice are presented in Tables II and III. These data represent the mean enzyme activity values for livers and brains after one injection (Table II) and after 21 injections (Table III). Enzyme activities were compared at the 0.05 level using Duncan's New Multiple Range Test.

Since newborn mice (one day of age) were used in this experiment, it was considered essential that one group of animals receive no treatment. This determined any enzymatic changes due to postnatal development and any changes caused by the stress of handling and administering the subcutaneous injections.

There were no significant differences ($p < 0.05$) in the enzymes studied between the nontreated controls and the saline-treated controls after either one or 21 injections of

TABLE I. Effect of Monosodium Glutamate on Enzyme Levels in Weanling Mice.

Enzyme system	Tissue	Treatment			
		None	Saline	MSA	MSG
GDH	Brain	3.25 ± 0.27[a,b]	3.19 ± 0.29	2.80 ± 0.25	2.84 ± 0.26
GDH	Liver	11.41 ± 0.56	11.09 ± 0.49	11.09 ± 0.43	11.23 ± 0.60
GPT	Brain	5.09 ± 0.28	4.95 ± 0.29	4.54 ± 0.22	4.57 ± 0.22
GPT	Liver	16.09 ± 0.76	16.31 ± 0.69	15.42 ± 0.59	16.15 ± 0.68
GOT	Brain	45.92 ± 2.18	45.56 ± 1.92	43.04 ± 2.52	43.16 ± 2.02
GOT	Liver	37.85 ± 1.65	38.11 ± 1.47	37.64 ± 1.78	38.00 ± 1.30

[a] Values are means ± SE, $n = 6$.

[b] Specific activity, micromoles NADH oxidized per hour per milligram protein.

TABLE II. Effect of Monosodium Glutamate on Enzyme Levels in Newborn Mice After One Injection.

Enzyme system	Tissue	Treatment			
		None	Saline	MSA	MSG
GDH	Brain	0.68 ± 0.04[a,b]	1.18 ± 0.05	0.57 ± 0.07	1.18 ± 0.06
GDH	Liver	7.71 ± 0.33	7.92 ± 0.09	9.35 ± 0.14	9.54 ± 0.30
GPT	Brain	1.24 ± 0.10	1.27 ± 0.16	2.97 ± 0.29	2.47 ± 0.06
GPT	Liver	12.12 ± 0.07	11.19 ± 0.22	14.77 ± 0.46	16.48 ± 0.79
GOT	Brain	8.47 ± 0.63	7.45 ± 0.46	14.21 ± 0.22	13.08 ± 0.37
GOT	Liver	33.10 ± 0.15	34.05 ± 0.26	41.32 ± 0.83	46.84 ± 3.03

[a] Values are means ± SE, $n = 6$.
[b] Specific activity, micromoles NADH oxidized per hour per milligram protein.

the saline except for GDH in the brain after one injection. The same difference is observed between the MSA and MSG treated mice after one injection. After three injections, the GDH level was 1.15 ± 0.10, 1.23 ± 0.06, 1.50 ± 0.03, and 1.17 ± 0.01 μmoles NADH oxidized per hour per mg protein, respectively, for the non-treated controls, saline-treated controls, MSA-treated and MSG-treated mice. The authors feel that since after three injections there were no differences found in GDH levels between treatments, that the differences found after one injection were due to the variability in GDH levels in the developing brain of one-day-old mice and/or stress on the animals. It can be seen that these differences only occurred in the brain. The data shows that the largest increase in enzyme activity in the control groups occurred in the brain indicating a very rapid change in this tissue. Brain GDH, GOT and GPT levels in both the saline-treated controls and the non-treated controls increased significantly with time. The increase in enzyme activity closely parallels the increase in glutamic acid, aspartic acid, and α-aminobutyric acid concentrations reported by Agrawal *et al.* (12) in developing mouse brain. Also, the increase in enzyme activity is similar to that found by Wang (13) in newly hatched chicks.

After one injection, the brain GDH level was significantly lower in the nontreated controls and MSA-treated animals than in the MSG-treated animals, and the saline-treated controls. GDH activity in the liver after one injection was significantly higher in the MSG-treated animals than in the nontreated controls, but was not different from either of the other treatments. The GOT and GPT levels in both the brains and livers were significantly lower in the saline-treated controls and the nontreated controls than in the MSA and MSG-treated animals. MSA and MSG-treated animals were not significantly different

TABLE III. Effect of Monosodium Glutamate on Enzyme Levels in Newborn Mice After Twenty-one Injections.

Enzyme system	Tissue	Treatment			
		None	Saline	MSA	MSG
GDH	Brain	2.52 ± 0.18[a,b]	3.08 ± 0.29	4.89 ± 0.70	5.32 ± 0.28
GDH	Liver	11.90 ± 0.14	10.24 ± 1.17	17.86 ± 0.34	17.43 ± 0.44
GPT	Brain	3.37 ± 0.56	2.86 ± 0.24	7.14 ± 0.71	7.25 ± 0.08
GPT	Liver	12.59 ± 0.52	8.96 ± 0.36	34.87 ± 1.59	31.25 ± 1.01
GOT	Brain	26.62 ± 0.85	27.97 ± 0.48	45.04 ± 0.22	48.28 ± 0.64
GOT	Liver	34.42 ± 0.54	31.08 ± 1.05	71.79 ± 0.96	65.61 ± 0.79

[a] Values are means ± SE, $n = 6$.
[b] Specific activity, micromoles NADH oxidized per hour per milligram protein.

from each other in GOT and GPT activities in either brains or livers. After 21 injections, enzyme activities (GDH, GPT or GOT) in the brains and livers of MSA and MSG-treated animals were not significantly different from each other. However, MSA and MSG-treated animals had significantly higher enzyme activities (GDH, GPT, and GOT) in both brains and livers than the saline-treated controls and the nontreated controls.

The GDH, GPT, and GOT activities in the brains and livers were affected by both MSA and MSG. In both cases, there was a 2–3-fold increase in activity of the three enzymes studied. The GDH, GPT, and GOT activities following injections for 7 and 13 days also showed an increase with MSA and MSG treatment, but of a lesser degree. As in the weanling mice, no differences were observed in body, brain, and liver weights.

Discussion. These data indicate that monosodium glutamate (1 mg/g body weight) does not affect the enzyme activity of GDH, GOT, or GPT in weanling mice. These results appear to indicate that the weanling mouse has the biochemical capacity to metabolize large doses of monosodium glutamate. Although no differences in enzyme activities were found 24 hr after 1, 3, 7, 12, or 21 injections, there may be an effect at shorter time periods. Olney (1) found brain lesions three hours after monosodium glutamate injections. Larger doses of monosodium glutamate may also have an effect on GDH, GOT, and GPT activities.

As previously stated, both monosodium glutamate and monosodium aspartate caused a 2–3-fold increase in GDH, GOT, and GPT activities in newborn mice. These results indicate that newborn mice may not have the capacity to metabolize large doses of monosodium glutamate or monosodium aspartate and, therefore, must adapt to the higher exogenous levels that were administered. Kraus (14) has shown that L-glutamate (1 mg/g body weight) caused induction of L-glutamate decarboxylase (EC 4.1.1.15) in mice brains. Therefore, it appears that MSG and MSA may also cause an induction of GDH, GOT and GPT in mouse brain and liver in a similar manner.

Summary. Monosodium glutamate and monosodium aspartate were injected subcutaneously in newborn and weanling mice. The brains and livers were excised and GDH, GOT, and GPT levels were measured. Monosodium glutamate or monosodium aspartate administration resulted in 2–3-fold increases in activities in both the brains and livers of newborn mice. However, the injection of either monosodium glutamate or monosodium aspartate, resulted in no significant effect on the enzymes measured in the brains and livers of weanling mice.

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