Effect of Dietary Monosodium L-Glutamate on Some Brain and Liver Metabolites in Rats (35930)

LEON PROSKY AND ROGER G. O'DELL
(Introduced by J. C. Fritz)
Division of Nutrition, Food and Drug Administration, U.S. Department of Health, Education, and Welfare, Washington, D. C. 20204

The production of acute neuronal degeneration in the retina of mice receiving parenterally administered monosodium L-glutamate (MSG) has been described (1, 2). Brain lesions in the hypothalamus of rat, monkey, and mouse have also been associated with administration of MSG by the subcutaneous route (3–5) and in the mouse by the oral route (6). The results are in disagreement with the findings of Adamo and Ratner (7) who were unable to demonstrate any effects on brain or reproduction function in rats.

Glutamic acid is highly concentrated in nervous tissue and plays a significant role in its metabolism (8). Although no net uptake of glutamic acid in brain can be demonstrated after excessive elevation of blood glutamic acid (9, 10), the glutamic acid from blood exchanges rapidly with that from brain (11, 12). The present study is an attempt to establish a biochemical basis for the action of MSG relating to its metabolic function as an energy source in brain and as a precursor for biologically active metabolites in brain and liver.

Materials and Methods. MSG was purchased from Sigma Chemical Company. L-Glutamic acid-U-^{14}C (197 μCi/μmole) used in the *in vitro* incubation study was obtained from the New England Nuclear Corporation. Purina laboratory chow was purchased from Ralston Purina Company.

Fifty male Holtzman albino weanling rats were allotted to five groups of 10 animals each and were housed individually. The diets, Purina laboratory chow supplemented with MSG at the 0, 1, 5, 10, and 20% levels, were fed *ad libitum* for 16 weeks. Body weights were recorded weekly. At the end of the experimental period the rats were lightly anesthetized with ether and decapitated. Whole brain was rapidly excised and weighed, and a 16% homogenate was prepared in 0.1 *M* phosphate buffer (pH 6.1) for assay of glutamic acid decarboxylase (GAD), the enzyme that may be the rate-limiting step in determining the level of γ-aminobutyric acid (GABA) in particular areas of the central nervous system (13). For the assay of GAD, the final reaction mixture of 2.2 ml contained 1.4 ml of phosphate buffer (pH 6.1 at 36°), 0.2 ml of homogenate, 0.2 ml of pyridoxal phosphate (500 μg/ml), 0.1 ml of MSG (500 μmole), and 0.3 ml of MSG-^{14}C (2.2×10^6 cpm and 0.0076 μmole). The mixture was shaken at 36° in a constant-temperature water bath for 10 min; aliquots were withdrawn at 2.5 min intervals during the incubation period and added to tubes containing 1.3 vol of cold 0.6 *N* perchloric acid. After the tubes were centrifuged the supernatant was removed, neutralized, and reduced in volume under vacuum. An appropriate volume was applied to paper strips in a Durrum cell for electrophoresis in a pyridine:acetic acid:water (8:15:977) system at 300 mV for 3 hr. Areas on the paper corresponding to those of authentic GABA were cut out, placed in a vial containing 15 ml of Bray's solution (14), shaken mechanically for 30 min, and counted for radioactivity in a Nuclear Chicago Mark 1 liquid scintillation counter. Measurements were corrected for quenching as previously described (15). The remainder of the brain homogenate was saved for colorimetric assay of protein (16) and DNA (17). Glutamate (18), GABA (19), and aspartate (20) concentrations in protein-free brain extracts were determined by enzymatic methods. Glutamine was converted to glutamate with the enzyme glutaminase and assayed as described above. Succinate was

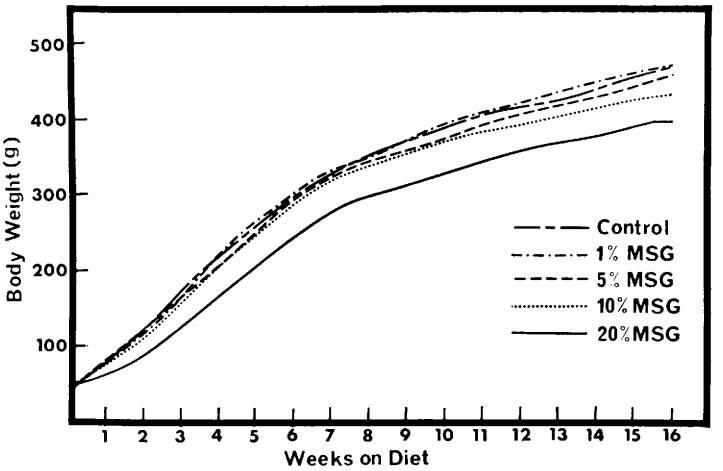

FIG. 1. The effect on body weight of feeding various levels of MSG to rats for 16 weeks.

assayed by a previously described enzymatic method (21) after its isolation by eluting from Dowex-1-formate columns (22).

Whole liver, removed at the time of sacrifice, was frozen in Freon and weighed, and sections were taken for protein, RNA (15), and DNA determinations. The remainder of the liver was acid extracted (22), and the supernatant fraction was assayed for glutamate, lactate (23), malate (24), aspartate, and α-glycerophosphate (α-GPO_4) (25).

Data were analyzed by the Student t test.

Results. Figure 1 shows that rats fed the 20% MSG-supplemented diet did not grow as well as control rats or rats fed the chow diet supplemented with MSG at the 1, 5, or 10% levels. By week 16, body weights of rats fed the 20% MSG diet were depressed by 15% compared with those of control rats ($p<$ .001).

The data in Table I show some of the effects of feeding MSG on its metabolites in rat brain. GABA concentrations in brains of rats fed the 1% MSG diet were decreased by 17% from control values ($p<.025$) with a maximum decrease of 20% in rats receiving the highest dietary level of MSG ($p<.005$). Hyperirritability, an excessive response to handling, was observed in all groups of rats consuming diets supplemented with MSG. Succinate concentrations in brain were elevated as dietary levels of MSG were increased; in rats fed the highest level of MSG, the succinate was increased 20% compared to control values ($p<.05$). No changes were noted in brain weight, protein, or DNA. Despite the high MSG intakes, brain glutamate remained relatively constant as did GAD, the enzyme responsible for the formation of GABA. Glutamine and aspartate concentrations in brain were also unresponsive to dietary levels of MSG.

Table II shows some of the effects of feeding MSG on metabolites in rat liver. The liver weights of rats fed the 20% MSG diet were depressed by 9% ($p<.025$); however, liver:body weight ratio was not significantly different from that of controls. Aspartate concentrations in all rats receiving MSG were elevated compared with those of controls; the maximum elevation was 25% in rats given the highest level of dietary MSG ($p<.025$). No significant changes were observed in liver protein, RNA-P, or DNA-P. Liver glutamate and α-GPO_4 remained unchanged, although an upward trend in their concentrations was observed. Lactate and malate levels were not significantly changed in liver.

Discussion. Glutamic acid in the form of the sodium salt is widely used as a seasoner or flavor enhancer for many foods, and as a drug in the treatment of ammonia accumulation in hepatic failure and various behavioral aberrations (26, 27). Glutamate enters the brain rapidly (11, 12) and is quickly metabolized to other substances; there was no net increase in brain glutamate levels of rats ingesting the 20% MSG diet. Figure 2 shows that increased concentrations of brain succinate can be derived from MSG by two

TABLE I. The Effects of Various Dietary Levels of MSG on Some Biochemical Parameters of Rat Brain.[a]

Dietary MSG (%)	Brain wt (g)	Protein (mg/g)	GAD[b]	DNA (mg/g)	(μmole/g) Glutamate	GABA	Glutamine	Aspartate	Succinate[c]
Control	2.01 ±0.05	114 ±3	69 ±8	1.18 ±0.05	9.20 ±0.56	2.59 ±0.10	1.12 ±0.20	2.43 ±0.09	0.401 ±0.033
1	1.97 ±0.05	118 ±3	64 ±5	1.25 ±0.04	9.38 ±0.34	2.16[d] ±0.11	1.37 ±0.22	2.30 ±0.18	0.467 ±0.055
5	1.89[e] ±0.01	117 ±1	70 ±7	1.28 ±0.05	9.38 ±0.08	2.11 ±0.31	1.21 ±0.18	2.38 ±0.03	0.460[e] ±0.008
10	1.90 ±0.07	116 ±3	61 ±5	1.19 ±0.07	9.78 ±0.27	2.20[f] ±0.08	1.72 ±0.15	2.41 ±0.10	0.508 ±0.063
20	1.92 ±0.03	116 ±1	64 ±5	1.30 ±0.07	9.25 ±0.18	2.07[g] ±0.08	1.07 ±0.28	2.31 ±0.05	0.508[e] ±0.030

[a] Each value is the mean of 8 to 10 animals and is given with the SE.

[b] Glutamic decarboxylase activities (μmoles of GABA formed/g of brain/hr).

[c] These values represent three brain extracts; each extract was derived from two brains.

[d] Significantly different from control, $p < .025$; [e] $p < .05$; [f] $p < .01$; [g] $p < .005$.

TABLE II. The Effects of Various Dietary Levels of MSG on Some Biochemical Parameters of Rat Liver.[a]

Dietary MSG (%)	Liver wt (g)	(mg/g)			(μmole/g)				
		Protein	RNA-P	DNA-P	Glutamate	Aspartate	Lactate	Malate	α-GPO$_4$
Control	16.56 ±0.56	202 ±3	0.699 ±0.017	0.106 ±0.006	1.58 ±0.10	0.44 ±0.02	11.25 ±0.49	0.53 ±0.03	0.94 ±0.05
1	16.46 ±0.40	194 ±2	0.699 ±0.024	0.118 ±0.005	1.51 ±0.07	0.53[b] ±0.03	10.80 ±0.51	0.58 ±0.03	0.96 ±0.03
5	16.85 ±1.01	194 ±5	0.701 ±0.028	0.110 ±0.006	1.64 ±0.06	0.54[b] ±0.02	11.33 ±0.56	0.59 ±0.03	1.04 ±0.11
10	15.93 ±0.55	198 ±4	0.719 ±0.028	0.118 ±0.007	1.70 ±0.11	0.56[c] ±0.05	10.75 ±0.45	0.57 ±0.03	1.22 ±0.13
20	15.02[b] ±0.28	200 ±2	0.729 ±0.017	0.107 ±0.006	1.76 ±0.16	0.58[b] ±0.03	11.28 ±0.59	0.59 ±0.03	1.18 ±0.11

[a] Each value is the mean of eight to ten animals and is given with the SE.
[b] Significantly different from control: $p < .025$; [c] $p < .05$.

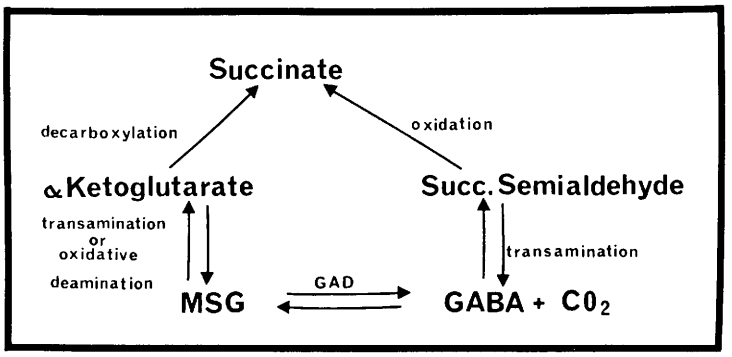

FIG. 2. The metabolic pathway of MSG in the rat brain.

widely accepted reactions: (a) oxidative deamination or transamination of glutamate to α-ketoglutarate, which is further decarboxylated; and (b) transamination and oxidation of GABA, a decarboxylation product of glutamate. However, the most important finding is the decreased concentrations of GABA in the brains of rats in all groups fed MSG diets. These rats displayed increased irritability to a type similar to that seen in vitamin B_6 deficiency. In the type of irritability induced by vitamin B_6 deficiency or isoniazid treatment, GAD activity is decreased because of insufficient coenzyme, resulting in decreased production of GABA. Supplementation with the vitamin reversed this effect (28, 29). In our experiments, decreased concentrations of brain GABA were not associated with a vitamin B_6 deficiency or with GAD activity (added vitamin B_6 did not increase enzyme activity in the assay), which remained constant in all groups of rats. It is possible that an initial and transient increase in GABA production stimulated those enzymes responsible for its degradation.

Decreased protein and DNA concentrations in brain have been associated with neuron deficiency (30); however, in our studies, these parameters remained constant in rats ingesting MSG, indicating that there was no impairment in brain development.

The liver also metabolizes glutamate at a rapid rate, as shown by the consistent values for glutamate in the livers of rats fed MSG in the diet, even at the highest level. Aspartic acid, which was elevated in the livers of MSG-fed rats, was probably derived from the main degradative pathway of glutamate, transamination with oxaloacetate. Oxidative pathways for the production of energy, CO_2, and water by way of the citric acid cycle were also unaffected by dietary MSG; levels of lactate, malate, and α-GPO_4 in the liver remain constant.

Summary. Rats in each group receiving MSG exhibited hyperirritability. While levels of glutamate, glutamine, aspartate, DNA, protein, and GAD in brain remained relatively constant, a significant decrease in GABA levels was noted along with a significant increase in succinate levels. Analysis of liver indicated no effect of dietary MSG on protein, RNA-P, DNA-P, glutamate, lactate, malate, or α-GPO_4. Aspartate levels were significantly increased. The decrease in brain GABA levels may be related to the observed increased irritability.

1. Lucas, D. R., and Newhouse, J. P., AMA Arch. Ophthalmol. **58**, 193 (1957).

2. Olney, J. W., J. Neuropathol. Exp. Neurol. **28**, 455 (1969).

3. Olney, J. W., Science **164**, 719 (1969).

4. Olney, J. W., and Sharpe, L. G., Science **166**, 386 (1969).

5. Arees, E. A., and Mayer, J., Science **170**, 549 (1970).

6. Olney, J. W., and Sharpe, L. G., Science **167**, 1017 (1970).

7. Adamo, N. J., and Ratner, A., Science **169**, 674 (1970).

8. Strecker, H. J., *in* "Metabolism of the Nervous System" (D. Richter, ed.), p. 459. Pergamon, New York (1957).

9. Schwerin, P., Bessman, S. P., and Waelsch, H., J. Biol. Chem. **184**, 37 (1950).

10. Himwich, W. A., Petersen, J. C., and Allen, M. L., Neurology **7**, 705 (1957).

11. Lajtha, A., Berl, S., and Waelsch, H., J.

Neurochem. 3, 322 (1959).

12. Roberts, R. B., Flexner, J. B., and Flexner, L. B., J. Neurochem. 4, 78 (1959).

13. Roberts, E., and Simonsen, D. G., Biochem. Pharmacol. 12, 113 (1963).

14. Bray, G. A., Anal. Biochem. 1, 279 (1960).

15. Prosky, L., Roberts, B., Jr., O'Dell, R. G., and Imblum, R. L., Arch. Biochem. Biophys. 126, 393 (1968).

16. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. 193, 265 (1951).

17. Burton, K., Biochem. J. 62, 315 (1956).

18. Holzer, H., Soling, H.-D., and Witt, I., *in* "Methods of Enzymatic Analysis" (H.-U. Bergmeyer, ed.), p. 392. Academic Press, New York (1963).

19. Jakoby, W. B., Methods Enzymol. 5, 777 (1962).

20. Pfleiderer, G., *in* "Methods of Enzymatic Analysis" (H.-U. Bergmeyer, ed.), p. 381. Academic Press, New York (1963).

21. Clark, B., and Porteous, J. W., Biochem. J. 93, 21c (1964).

22. Englard, S., and Hanson, K. R., Methods Enzymol. 13, 567 (1962).

23. Holzer, H., and Soling, H.-D., *in* "Methods of Enzymatic Analysis" (H.-U. Bergmeyer, ed.), p. 275. Academic Press, New York (1963).

24. Holzer, H., and Soling, H.-D., *in* Methods of Enzymatic Analysis" (H.-U. Bergmeyer, ed.), p. 332. Academic Press, New York (1963).

25. Hohorst, H.-J., *in* "Methods of Enzymatic Analysis" (H.-U. Bergmeyer, ed.), p. 215. Academic Press, New York (1963).

26. Himwich, H. E., Wolff, K., Hunsicker, A. L., and Himwich, W. A., J. Nerv. Ment. Dis. 121, 40 (1955).

27. Albert, K., Hock, P., and Waelsch, H., J. Nerv. Ment. Dis. 114, 471 (1951).

28. Roberts, E., Younger, F., and Frankel, S., J. Biol. Chem. 191, 277 (1951).

29. Killam, K. F., and Bain, J. A., J. Pharmacol. Exp. Ther. 119, 255 (1957).

30. Zamenhof, S., Marthens, E. V., and Margolis, F. L, Science 160, 322 (1968).

Received June 2, 1971. P.S.E.B.M., 1971, Vol. 138.