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Juvenile-onset Obesity and Deficits in Caloric Regulation in MSG-treated Rats[1]

ROBIN B. KANAREK,[2] JULIE MEYERS, ROBERT G. MEADE AND JEAN MAYER

Department of Psychology, Tufts University, Medford MA 02155

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KANAREK, R. B., J. MEYERS, R. G. MEADE AND J. MAYER. *Juvenile-onset obesity and deficits in caloric regulation in MSG-treated rats.* PHARMAC. BIOCHEM. BEHAV. **10**(5)717–721, 1979.—Caloric regulation and the development of obesity were examined in rats which had received parenteral injections of monosodium glutamate (MSG) as neonates. Rats were injected with either 2 mg/g or 4 mg/g MSG on alternate days for the first 20 days of life. Lee Indices of obesity were calculated at 22, 70, and 130 days of age. Animals in the 4 mg/g group were significantly more obese than controls at all three ages. However, both food intake and body weight of this group were significantly lower than those of controls. In adulthood, the ability to regulate caloric intake was tested by allowing animals access to diets of varying caloric densities. While control animals maintained relatively constant caloric intakes across dietary conditions, MSG-treated animals demonstrated an inability to respond to caloric challenges. Treated animals decreased caloric intake on a diluted diet and consumed more calories than controls when presented with a calorically dense diet. This inability to regulate caloric intake is compared with regulatory deficits observed in animals sustaining lesions of the ventromedial hypothalamus. The value of an animal model of juvenile-onset obesity is also discussed.

Monosodium glutamate Adiposity Caloric regulation Body weight Juvenile-onset obesity

BASED on their own findings as well as previous reports indicating that monosodium glutamate (MSG) produced brain damage when injected systemically in infant mice, Arees and Mayer [2] suggested an increase in the research effort aimed at delineating the effects of MSG on the mammalian central nervous system. Initial experiments on MSG-induced neuropathology reported only damage to cells in the inner retina [8,14]. However, the observation that MSG-treated mice became obese led Olney [16] to look for neural damage in the region of the hypothalamus. Using electron microscopy, he found lesions in the arcuate nucleus and median eminence of the hypothalami of infant mice which had received a single subcutaneous injection of MSG [16]. Similar lesions were observed in adult mice, injected with MSG either as neonates or adults, and in adult rats which had received the drug during infancy [16].

To examine the obesity associated with these hypothalamic lesions more carefully, Olney [16] gave daily subcutaneous injections of MSG to neonatal mice from Day 1 to Day 10 of life. In adulthood, although animals which had received MSG weighed more, they consumed less food than controls which had received saline injections as neonates. The increase in body weight was accompanied by stunted skeletal growth and extensive accumulations of adipose tissue. Subsequent studies have confirmed that MSG administration in rodents during the first ten days of life results in a syndrome characterized by hypothalamic lesions, obesity, arrested skeletal development, hypophagia, and endocrine abnormalities (e.g. [1, 6, 15, 18, 19, 24]).

The development of obesity despite a decreased food intake in MSG-treated animals suggests that animals given MSG may be hypoactive. However, results from studies which have examined this question are contradictory. For example, while both Olney [16] and Pizzi and Barnhart [18] noted that MSG-treated mice were less active than controls, Araujo and Mayer [1] reported hyperactivity in MSG-treated mice. In rats, Nicoletseas [15] found no significant differences between animals which had received MSG and controls. Procedural differences in measuring activity levels, as well as species differences may explain some of the discrepancies among these studies.

Before MSG-induced obesity can be compared to obesity associated with other types of hypothalamic damage, it is necessary to elaborate the MSG syndrome. To this end, the present study examined both the pattern of development of obesity, and the relation between obesity and the dose of MSG. Also, as previous work has found that hypothalamically-obese animals respond differently than lean animals to dietary manipulations (e.g. [7, 17, 23]), the effects of varying the caloric density of the diet were studied in adult animals which had received neonatal MSG treatment.

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[2]Please address reprint requests to Robin B. Kanarek, Department of Psychology, Tufts University, Medford MA 02155.

METHOD

Animals

Seventy-two male and female Sprague-Dawley rats were used. These animals were born in the laboratory to nine dams purchased from Charles River Breeding Labs (Wilmington MA). At birth, litters were reduced in size so that there were eight pups in each litter. All animals were housed in a temperature-controlled room (22°C) maintained on a 12–12 hr light–dark cycle (lights on: 0800 hr).

Procedure

Preweaning period. Three days after birth, neonates were divided into three groups of three litters each. Pups in Group 1 received subcutaneous injections of 2 mg/g body weight of the monosodium salt of L-glutamic acid (MSG) (Sigma Chemical Co., St. Louis MO). Pups in Group 2 were injected with 4 mg/g body weight MSG. Pups in Group 3 received injections of distilled water. MSG was dissolved in distilled water. Drug concentrations were adjusted such that each pup received 0.01 ml of solution per gram of body weight. Injections were given on alternate days from Day 3 to Day 19 of life. Rats remained nursing with their mothers throughout the injection period.

Postweaning period. At weaning, 22 days of age, all rats were separated from their mothers and housed individually in standard laboratory cages equipped with Wahmann (Timonium, MD) LC-306A food cups. Female animals were used in an experiment which investigated the effects of MSG on reproductive behavior, to be reported elsewhere. The forty-two male rats were used for the following investigations of the effects of neonatal MSG on growth and consummatory behaviors. There were 14 animals in the group which had received 4 mg/g MSG, 17 animals in the group which had received 2 mg/g MSG, and 10 animals in the group which had received water. Body weights, and food (ground Purina Rodent Chow 5001) (3.6 kcal/g), and water intakes were measured every other day until the animals reached 92 days of age. Measurements were then taken daily for the remainder of the experiment. On Day 100, the animals in each of the preweaning treatment groups were divided into an experimental and a control group, matched on the basis of body weight. The animals in the experimental groups were given a calorically diluted diet (2.7 kcal/g) as their sole source of food for ten days. This diet contained 75% ground Purina Rodent Chow and 25% celluflour (General Biochemicals, Chagrin Falls OH). At the end of this period, experimental animals were returned to the standard ground Purina diet for 24 days. On Day 135, experimental groups were given a high fat diet (5.4 kcal/g) containing 67% ground Purina Chow and 33% vegetable fat (Crisco). Animals remained on this diet for two weeks. Animals in the control groups were maintained on ground Purina Rodent Chow throughout the experiment.

Lee Indices of obesity ($\sqrt[3]{\text{body weight (g)}}$/naso-anal length (cm), which have been shown to correlate well with carcass fat content [3,13], were calculated for all animals at 22, 70 and 130 days of age.

Statistical comparisons among groups employed one-way analyses of variance for unequal groups. A posteriori comparisons between groups were made in accordance with Scheffe's procedure [21].

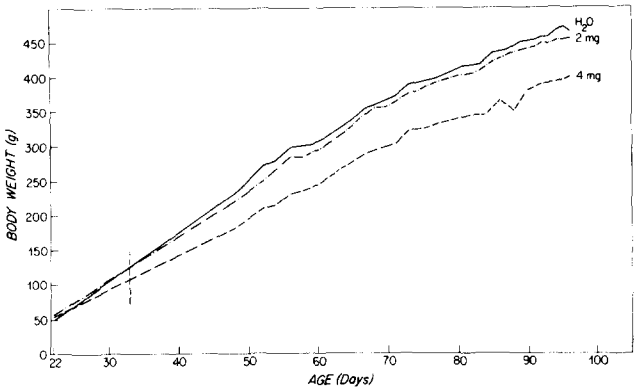

FIG. 1. Mean body weights as a function of age for groups of animals receiving injections of either 4 mg/g MSG, 2 mg/g MSG or water as neonates. Dashed vertical line indicates the earliest age at which body weight of 4 mg/g group was first significantly lower than that of the other two groups.

RESULTS

Developmental Effects of MSG Administration

No differences in body weight were observed among the three groups at the time of weaning (Fig. 1). Body weights of animals given injections of either 2 mg/g MSG or water as neonates continued to remain similar throughout the experiment. Animals which had received 4 mg/g MSG, however, gained substantially less weight than animals in the other two groups. Body weights of the animals in the 4 mg/g MSG group were significantly lower ($p<0.01$) than those of animals in the other two groups by 33 days of age. At 100 days of age, mean body weight of the 4 mg/g MSG group was only 86% that of controls.

On the three occasions when Lee Indices of obesity were computed, animals which had been injected with 4 mg/g MSG had significantly greater Indices than animals injected with water (Table 1). This difference indicated that animals in the 4 mg/g MSG group had more body fat than controls. At 70 and 130 days of age, Lee Indices also were significantly greater for animals given 4 mg/g MSG than for animals given 2 mg/g MSG. Lee Indices were significantly greater at weaning and 130 days of age in the 2 mg/g group than in the water group.

While there were no differences in body lengths at weaning, at both 70 and 130 days of age, the 4 mg/g MSG group was significantly shorter than the 2 mg/g MSG group, which in turn, was significantly shorter than the water group (Table 1).

The difference in Lee Indices observed among groups cannot be solely accounted for by differences in body length, as the 4 mg/g MSG group was not only shorter, but also, weighed less than controls.

Mean daily food intake from weaning to 100 days of age, averaged 26.0 g, 29.8 g, and 29.8 g respectively for the 4 mg/g MSG, 2 mg/g MSG, and water groups. While food intake was similar in the 2 mg/g MSG and water groups, animals in the 4 mg/g MSG group consumed significantly less food than other animals ($p<0.01$).

It is interesting to note, that although animals given 4 mg/g MSG ate less, their fluid intake was greater than that of controls or of the 2 mg/g MSG group (Table 2). This differ-

TABLE 1

MEAN BODY WEIGHTS, BODY LENGTHS, AND LEE INDICES AT WEANING AND 70 AND 130 DAYS OF AGE FOR ANIMALS GIVEN WATER OR MSG AS NEONATES

Age	Neonatal Treatment	Body Weight (g)	Body Length (cm)	Lee Index
Weaning	Water	53.7	12.0	0.314
	2 mg/g MSG	57.6 (NS)	11.8 (NS)	0.326 (.05)
	4 mg/g MSG	55.9	11.7	0.326 (.05)
70 days	Water	390.3 (.01)	24.2 (.05)	0.302 (.01)
	2 mg/g MSG	372.9 (.01)	23.5 (.01)	0.307 (.01)
	4 mg/g MSG	316.1	21.4	0.316
130 days	Water	535.1 (.01)	26.7 (.05)	0.304 (.01)
	2 mg/g MSG	533.8 (.01)	25.7 (.01)	0.316 (.01)
	4 mg/g MSG	479.3	23.8	0.328

TABLE 2

DAILY WATER INTAKE OF ANIMALS GIVEN INJECTIONS OF MSG OR WATER AS NEONATES

Neonatal Treatment	Mean Daily Water Intake (ml)	Water Intake (ml)/ g Food Intake	Water Intake (ml)/ 100 g Body Weight
Water	46.2	1.55	9.71
2 mg/g MSG	43.6	1.52	9.44
4 mg/g MSG	47.4	1.81	11.81

ence was even more pronounced when water intake was expressed as a function of either body weight or food intake.

Effects of Neonatal MSG Administration on Responses to Dietary Manipulations

Animals injected with water as neonates increased food intake when given the calorically diluted diet and decreased intake when given the calorically dense diet. As a function of these compensatory changes in food intake, total daily caloric intake remained relatively constant across dietary conditions (Fig. 2—top). In contrast, animals injected with MSG did not increase food intake when given the celluflour-diluted diet, resulting in a decrease in caloric intake. While MSG animals did decrease food intake slightly on the high-fat diet, the reduction in intake was not sufficient to compensate for the increase in the caloric density of the diet (Fig. 2—middle and bottom panels). In comparison to their caloric intake from Purina Chow during the 14 days prior to dietary manipulations, animals which had received 2 mg/g MSG decreased caloric intake by 16% on the celluflour-diluted diet and increased caloric intake by 16% on the high-fat diet. The corresponding manipulations in the 4 mg/g MSG group resulted in a 9% decrease in caloric intake on the diluted diet and a 44% increase on the high-fat diet.

Within the water-injected group, there were no differences in body weights between animals given the experimental diets and animals maintained on Purina Chow. Fluctuations in caloric intake in the MSG-injected groups resulted in experimental animals gaining less weight than controls on the calorically diluted diet, and more weight than controls on the calorically dense diet. The changes in body weight in the MSG-treatment groups accurately reflected the observed variations in caloric intake.

DISCUSSION

The presence of obesity with a concomitant decrease in body weight and food intake in rats given MSG as neonates is consistent with results of previous studies [15,19]. In addition, the present results indicate that the syndrome associated with neonatal administration of MSG is not an all-or-none phenomenon, but is related to the dose of MSG. This dose-related effect was evidenced by the differences observed in Lee Indices of obesity and body length. Animals

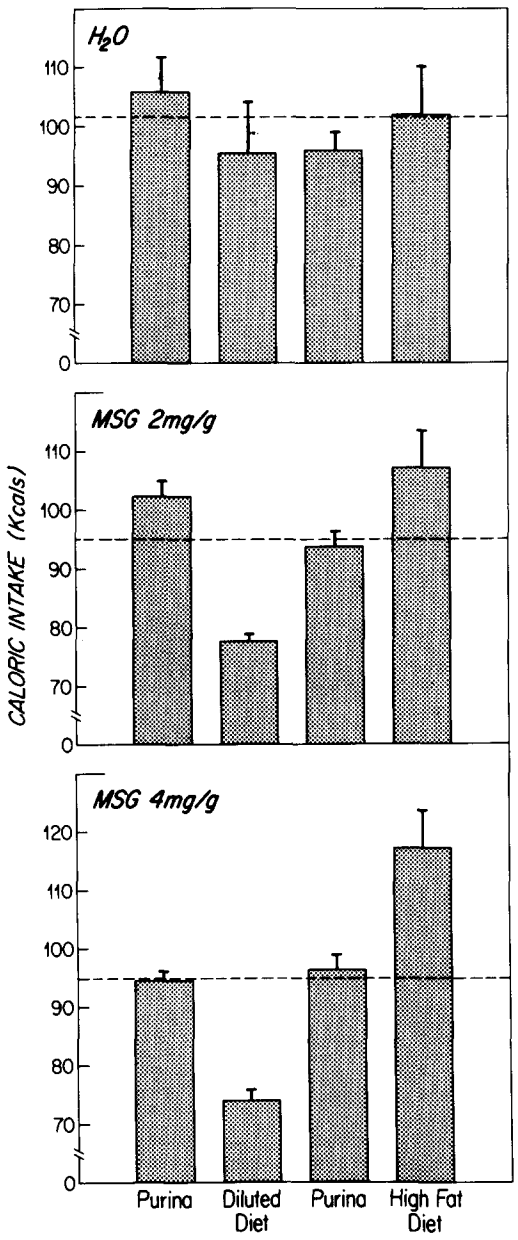

FIG. 2. Mean(±SE) daily caloric intakes of ground Purina Chow (3.6 kcal/g), the calorically diluted diet (2.7 kcal/g), and the high fat diet (5.4 kcal/g), for groups of animals receiving injections of either water, 2 mg/g MSG or 4 mg/g MSG as neonates. Dashed horizontal line in each panel indicates the caloric intake of animals in that group maintained throughout the experiment on ground Purina Chow.

in the 4 mg/g group consistently had higher Lee Indices and were shorter than animals in the 2 mg/g group, which had higher Lee Indices and were shorter than animals in the water group.

Animals given MSG were more obese than controls at weaning. This finding has since been replicated several times in this laboratory. To our knowledge, this is the earliest age at which obesity has been reported following physiological manipulations. In other studies employing preweaning physiological intervention, the onset of obesity has not been noted until much later in development [9, 15, 16, 18]. For example, Fisher *et al.* [9] found that obesity did not develop until at least 80 days of age in animals hypothalamically (VMN) lesioned as preweanlings. In other experiments in which MSG was administered neonatally, the existence of obesity was not determined until animals were adults (e.g. [15, 16, 18]). Age of onset is an important variable in the study of obesity. Differences in physiological characteristics are noted between juvenile and maturity obesity in both humans and experimental animals (e.g. [5,20]). For example, in general, juvenile-onset obesity is associated with hyperplasia of adipocytes, whereas, maturity-onset obesity is accompanied by hypertrophy of these cells [5,20]. At the present time, it is not known whether the obesity associated with MSG administration is the result of an increase in fat cell number and/or an increase in fat cell size, however, the early age of onset suggests a hyperplastic obesity.

Although animals given 4 mg/g MSG were more obese than controls, the MSG animals, in fact, weighed less than controls. This difference in body weight was significant by 33 days of age. Other studies on MSG-induced obesity in rats also have found that the body weights of treated animals are lower than those of controls [15,19]. In contrast, experiments employing MSG treatment in mice, consistently report not only increased adiposity, but also increased body weights in treated animals [1, 16, 18].

The present data, as well as results of previous studies, question the utility of body weight as a measure of obesity. For example, whereas in the adult ventromedial hypothalamically (VMH) lesioned animal obesity is correlated with increased body weight, in the weanling animals VMH lesions result in obesity without a concomitant increase in body weight [4, 10, 11, 12]. Additionally, as previously mentioned, depending upon species, obesity may or may not be accompanied by increased body weight in animals given neonatal MSG treatment. Thus, increased body weight is not indicative of obesity. In fact, as the present results demonstrate, increased adiposity may occur with lowered body weights.

Although on an absolute basis, the 4 mg/g MSG group was hypophagic compared to controls and the 2 mg/g MSG group, when caloric intake was calculated on a per gram body weight basis this difference was eliminated. The difference in percent body fat between MSG animals and controls, however, suggests that more of these calories are stored in the carcasses of MSG animals. Direct measurements of body composition are necessary to determine more precisely utilization of calories consumed. These determinations are in progress in this laboratory.

The failure of MSG-treated animals to control energy intake when given diets of varying caloric densities is similar to the behavior observed in VMH-lesioned animals (e.g. [7,17]). It is unclear whether the fluctuations in caloric intake result from a true regulatory deficit or are a function of diet palatibility. In the VMH-lesioned animal, the inability to regulate intake seems to be primarily due to the orosensory properties of the diets. This may be deduced from the observations that while VMH animals given powdered diets do not maintain constant caloric intakes, they accurately regulate energy consumption when provided with either liquid [25] or pelleted diets [22]. Unpublished data from this laboratory indicate that MSG animals, like VMH-lesioned animals, may be more responsive to changes in diet palatability than normal animals. MSG-treated animals were more sensitive to

quinine adulteration of the diet than controls. The ability of MSG-treated animals to control intake on both pelleted and liquid diets is currently being investigated to determine if the lack of caloric regulation is specific to powdered diets.

The discovery that MSG treatment resulted in increased adiposity as early as weaning was unexpected. This treatment, thus, provides a paradigm for elucidating the influence of juvenile-onset obesity on behavior. Prior to this time, juvenile-onset obesity in animals has been detailed only in genetically obese rodents. Comparisons now can be made between physiological and genetic forms of obesity in juvenile animals. Further, behavior differences between juvenile and later onset obesity may be examined in animals of similar genetic constitutions.

REFERENCES

1. Araujo, P. E. and J. Mayer. Activity increase associated with obesity induced by monosodium glutamate in mice. *Am. J. Physiol.* **225:** 764–765, 1973.
2. Arees, E. and J. Mayer. Monosodium glutamate-induced brain lesions: Electron microscopic examination. *Science* **170:** 549–550, 1970.
3. Bernardis, L. L. and B. D. Patterson. Correlation between 'Lee Index' and carcass fat content in weanling and adult female rats with hypothalamic lesions. *J. Endocr.* **40:** 527–528, 1968.
4. Bernardis, L. L. and F. R. Skelton. Growth and obesity in male rats after placement of ventromedial hypothalamic lesions at four different ages. *J. Endocr.* **38:** 351–362, 1967.
5. Bray, G. A. *The Obese Patient.* Philadelphia: W. B. Saunders Co., 1976.
6. Burde, R. M., B. Schanker and J. Kayes. Acute effects of oral and subcutaneous administration of monosodium glutamate on the arcuate nucleus of the hypothalamus in mice and rats. *Nature, Lond.* **233:** 58–60, 1971.
7. Carlisle, H. J. and E. Stellar. Caloric regulation and food preference in normal, hyperphagic, and aphagic rats. *J. comp. physiol. Psychol.* **69:** 107–114, 1969.
8. Cohen, A. I. An electron microscopic study of the modification by monosodium glutamate of the retinas of normal and "rodless" mice. *Am. J. Anat.* **120:** 319–356, 1967.
9. Fisher, R. S., C. R. Almli and S. Parsons. Infant rats: VMH damage and the ontogeny of obesity and neuroendocrine dysfunction. *Physiol. Behav.* **21:** 369–382, 1978.
10. Han, P. W. Hypothalamic obesity in rats without hyperphagia. *Trans. N.Y. Acad. Sci.* **30:** 229–243, 1967.
11. Han, P. W., C. Lin, K. Chu, J. Mu and A. Liu. Hypothalamic obesity in weanling rats. *Am. J. Physiol.* **209:** 627–631, 1965.
12. Kennedy, G. C. The hypothalamic control of food intake in rats. *Proc. Roy. Soc. B.* **137:** 535–549, 1950.
13. Lee, M. O. Determination of the surface area of the white rat with its application to the expression of metabolic results. *Am. J. Physiol.* **89:** 24–31, 1929.
14. Lucas, D. R. and J. P. Newhouse. The toxic effects of sodium L-glutamate on the inner layers of the retinas. *AMA Arch. Ophthamol.* **58:** 193–201, 1957.
15. Nicoletseas, M. M. Obesity in exercising, hypophagic rats treated with monosodium glutamate. *Physiol. Behav.* **19:** 767–773, 1977.
16. Olney, J. W. Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. *Science* **164:** 719–721, 1969.
17. Parsons, W., J. L. Camp, III and K. R. Crispell. Dietary dilution studies in mice with gold thioglucose-induced obesity and in mice with the hereditary obesity-diabetes syndrome. *Metabolism* **3:** 351–356, 1954.
18. Pizzi, W. J. and J. E. Barnhart. Effects of monosodium glutamate on somatic development, obesity, and activity in the mouse. *Pharmac. Biochem. Behav.* **5:** 551–557, 1976.
19. Redding, T. W., A. V. Schally, A. Arimura and I. Wakabayashi. Effect of monosodium glutamate on some endocrine functions. *Neuroendocrinology* **8:** 245–255, 1971.
20. Salans, L. B., S. W. Cushman and R. E. Weismann. Studies on human adipose tissue. Adipose cell size and number in nonobese and obese patients. *J. Clin. Invest.* **52:** 929–941, 1973.
21. Scheffe, H. *The Analysis of Variance.* New York, New York: John Wiley and Sons, 1959.
22. Smutz, E. R., E. Hirsch and H. L. Jacobs. Caloric compensation in hypothalamically obese rats. *Physiol. Behav.* **14:** 305–310, 1975.
23. Strominger, J. L., J. R. Brobeck and R. L. Cort. Regulation of food intake in normal rats and in rats with hypothalamic hyperphagia, *Yale J. Biol. Med.* **26:** 55–74, 1953.
24. Tafelski, T. J. and A. A. Lamperti. The effects of a single injection of monosodium glutamate on the reproductive neuroendocrine axis of the female hamster. *Biol. Reprod.* **17:** 404–411, 1977.
25. Thomas, D. W. and J. Mayer. Meal taking and regulation of food intake by normal and hypothalamic hyperphagic rats. *J. comp. physiol. Psychol.* **66:** 642–653, 1968.