Short Term and Long Term Effect of Monosodium Glutamate (Ajinomoto) on Ingestive Behavior and Estrous Cycle in Female Wistar Rats

AK Nayanatara, B Sandhya, N Harini, C Ramaswamy, NA Vinodini, R Sheila Pai, M Ramesh Bhat

Abstract

Pathological processes frequently involve the body's normal responses to abnormal environmental influences. Various environmental chemicals, industrial pollutants and food additives have been implicated as causing harmful effects. Monosodium glutamate (MSG) a sodium salt of naturally occurring (non-essential) L-form of glutamic acid is one of the main flavor enhancer used as an ingredient in various food products. The purpose of the present study was to evaluate the short term and long term effect of monosodium glutamate on estrous cycle and ingestive behavior in female rats. Adult (two months old) female wistar rats (n = 6/group) received MSG 4 g/kg BW for 15 days (short term) and 30 days (long term) respectively. The food intake, water intake and vaginal smears of these rats were monitored everyday in both the groups. The short term exposure showed a significant (P<0.0001) increase in the water intake with no significant change in the food intake. Whereas, long term exposure did not show any significant changes in the ingestive behavior. During short term exposure in the first week, the estrous cycle changes showed a significant increase in the proestrous phase (P<0.0001) and complete absence of diestrous, estrous and metaestrous phase. Whereas, in the second week there was a significant increase (P<0.0001) in the pro estrous phase and a decrease in the diestrous (P<0.0001), estrous (P<0.05) and metaestrous phase (P<0.05). During the third week, a significant increase (P<0.0001) in the proestrous phase and a significant decrease in the diestrous phase (P<0.001) followed by no significant change in estrous and metaestrous phase was observed. However, after third week (long term) the estrous cycle became regular. Therefore the results of the present study suggest that short term exposure to MSG might have some immediate effect on reproductive system and ingestive behavior.

Key Words: *MSG, estrous cycle, ingestive behavior*

Pathological processes frequently involve the body's normal responses to abnormal environmental influences. Various environmental chemicals, industrial pollutants and food additives have been implicated as causing harmful effects. Most food additives act either as preservatives or enhancer of palatability. One of such food additive is monosodium glutamate (MSG). Monosodium glutamate a sodium salt of naturally occurring (non-essential) L-form of glutamic acid is one of the main flavor enhancer used as an ingredient in various food products.[1,2] Its palatable and favorite flavor is a must in almost all Chinese and South-Asian dishes, where it is known by the names of *Ajinomoto, Sasa, Vetsin,Miwon and Weichaun* etc.[2] It has been reported that MSG is used as a flavor enhancer in all Chinese, Japanese and ready served foods like 2-minute noodles, soups, sauces, chips etc.[3] Though MSG improves taste stimulation enhances apetite, reports indicate that it is toxic to humans and experimental animals. The interest in the toxicity of MSG, a flavor enhancer increased due to its association with Chinese restaurant syndrome. The safety of MSG's usage has generated much controversy locally and globally.[4] Reports also show that MSG also enhances hunger and as such could be one of the key factors behind the problem of obesity, especially among children. It has been reported that MSG has neurotoxic effects resulting in various endocrine disorders.[5-7] Neonatal administration of MSG reduced the reproductive ability in female and male mice.[7] Reports also shows that neonatal administration of MSG leads to a syndrome of endocrine dysfunction characterized by reduced growth and hypogonadism.[8,9] The short length of the estrous cycle of rats makes them ideal for investigation of changes occurring during the reproductive cycle. The present study was aimed to investigate the short term and long term effects of MSG on ingestive behavior and oestrous cycle in female rats.

From the Departments of Physiology, Center for Basic Sciences, Kasturba Medical College, Bejai, Mangalore 575004, Karnataka, India.

Corresponing Author:
Nayanatara Arun Kumar, PhD
Department of Physiology, Center for Basic Sciences, Kasturba Medical college, Bejai, Mangalore 575004 , Karnataka, India.
Email: nayanaarun@hotmail.com

Methods

Laboratory bred adult two month old female Wistar strain albino rats weighing 120-200 g were selected for the experiment. However, the animals were matched with age and by body weight across treatment groups at the start of the experiment. The rats were maintained in the central animal house, KMC Mangalore with 12:12

light: dark cycle at room temperature 26±1°C. They were housed in separate polypropylene cages containing sterile paddy husk as bedding material. The animals were provided with standard pellet diet "Gold Mohar" rat feed (Hindustan Lever Company, Mumbai) and water ad libitum. All experimental procedures and animal maintenance conformed to the strict guidelines of Institutional Animal Ethics Committee and that of Federal laws for the use of animals in experiments. The animals were divided into two groups the experimental group and control group. Each group was divided into short term group and long term group. The experimental group was injected with MSG for 15 days in short term (4 g/kg BW, i.p.) and 30 days in long term (4 g/kg BW, i.p.). Each experimental group had separate control group which received the same volume of distilled water intraperitoneally.

Estrous cycle evaluation: Daily vaginal smears were performed every morning in between 8:00 and 9:00 a.m. Vaginal secretion was collected with a plastic pipette filled with 10 ml of normal saline (NaCl 0.9%) by inserting the tip into the rat vagina, but not deeply. Vaginal fluid was placed on glass slides. A different glass slide was used for each animal. One drop was collected with a clean tip from each rat. Unstained material was observed under a light microscope, without the use of the condenser lens, with 10 and 40x objective lenses. A normal estrous cycle in rats was defined as 4–5 days. Three types of cells could be recognized: round and nucleated ones are epithelial cells; irregular ones without nucleus are the cornified cells; and the little round ones are the leukocytes. The proportion among them was used for the determination of the estrous cycle stages. The estrous cycle stages are (1) proestrus (mainly epithelial cells), (2) estrus (mainly cornified cells), (3) metestrus (cornified and leukocyctes), and (4) diestrus (mainly leukocytes) present. Animals showing normal and regular estrous cycle (4 to 5 days) for at least three consecutive times were selected for the experiment Cycles were considered prolonged if the rat remained in one phase for 4 days and acyclic if rat remained in one phase for less than 15 days. At the end of the experimental period, animals were anesthetized (Pentobarbitone sodium, 40 mg/kg, i.p.) and then sacrificed by the terminal dose of the same.

Statistical analysis

The data were expressed as mean ± SD from 6 animals per group. The differences between the groups were compared for statistical significance by the Student's *t* test with the level of significance set at $P<0.05$.

Results

The short term exposure showed a significant ($P<0.0001$) increase in the water intake with no significant change in the food intake, whereas long term exposure did not show any significant changes in the ingestive behavior. During short term exposure in the first week, the estrous cycle changes showed a significant increase in the proestrous phase ($P<0.0001$) and complete absence of diestrous, estrous and metaestrous phase, whereas in the second week there was a significant increase ($P<0.0001$) in the pro estrous phase and a decrease in the diestrous ($P<0.0001$), estrous ($P<0.05$) and metaestrous phase ($P<0.05$). During the third week, a significant increase ($P<0.0001$) in the proestrous phase and a significant decrease in the diestrous phase ($P<0.001$) followed by no significant change in estrous and metaestrous phase was observed. However, after third week (long term) the estrous cycle became regular.

Discussion

In the present study the short term and long term injection of MSG did not show any changes in the food intake. Whereas, short term injection of monosodium glutamate showed a significant increase in the water intake. Even though monosodium glutamate influences the appetite positively, and induces weight gain.[10-12] Studies show that there are differences between genders of rats on the effect of glutamate on food intake.[10,13] Our study is in accordance with the reports done by Racotta, R and Hernandez Garcia et al. who observed similar kind of observation.[13] The significant increase in the water

Table 1. Effect of short term (15 days) MSG injection on ingestive behavior

Parameters	Normal	Treated
Food intake	10.8226±0.04320	10.8650±0.0197
Water intake	17.4646±0.00301	38.8083±0.0392*

* $P<0.0001$ short term control versus short term experimental

Table 2. Effect of long term (30 days) MSG injection on ingestive behavior

Parameters	Normal	Treated
Food intake	10.8133±0.03266	10.8183±0.02857
Water intake	17.4363±0.00328	17.4623±0.02053

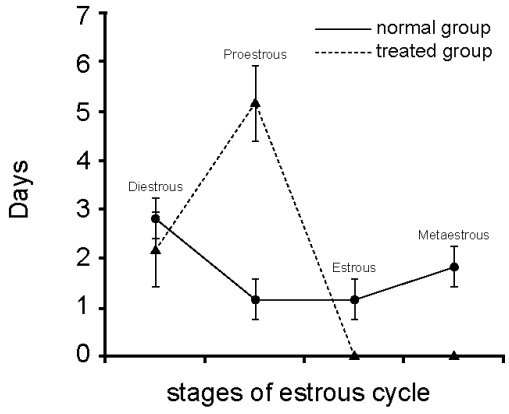

Figure 1. The effect of MSG injection on the first week of the estrous cycle in Wistar rats

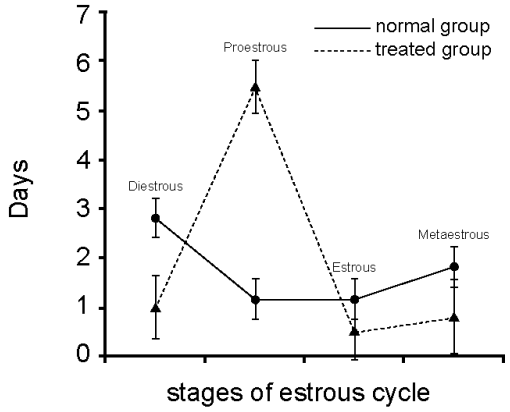

Figure 2. The effect of MSG injection on the second week of the estrous cycle in Wistar rats

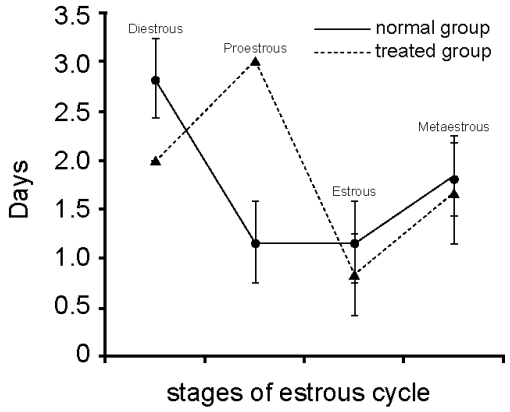

Figure 3. The effect of MSG injection on the third week of the estrous cycle in Wistar rats

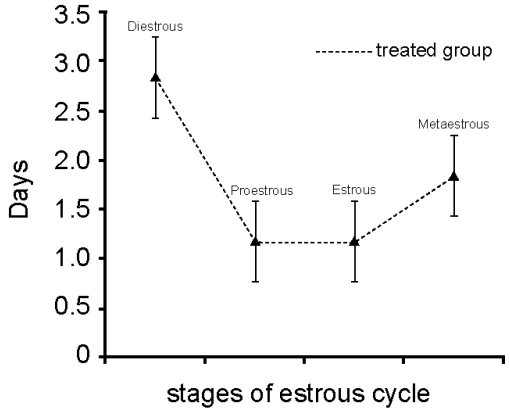

Figure 4. The effect of MSG injection on the fourth week of the estrous cycle in Wistar rats

intake direct effect on subfornical organ which is outside the blood brain barrier. It is known that the brain, even in the adult, has several areas that normally do not have a barrier system, called the circumventricular organs. These include the hypothalamus, the subfornical organ, organium vasculosum, area postrema, pineal gland, and the subcommisural organ. Of these, the most important is the hypothalamus, since it is the controlling centre for all neuroendocrine regulation, sleep wake cycles, emotional control, caloric intake regulation, immune system regulation and regulation of the autonomic nervous system. Interestingly, the glutamate is the most important neurotransmitter in the hypothalamus. Therefore, careful regulation of blood levels of glutamate is very important, since high blood concentrations of glutamate can easily increase hypothalamic levels as well .Based on these reports we suggest that MSG might have directly stimulated the subfornical region and caused increase in the water intake. The normal food intake and water intake during the long term exposure might be due to process of adaptability to taste sensation.

An estrous cycle is a rhythmic reproductive cycle occurring in sexually mature female mammals which depend upon the periodic release of gonadotropic releasing hormones, gonadotropins and sex hormones[14] The estrous cycle of female rat is characterized as proestrus, estrus, metestrus and diestrus.[15] The ovulation occurs from the beginning of proestrus to the end of estrus.[16] From the onset of sexual maturity up to the age of 12 months, the mean cycle length in the female rat is 4 days[15,16] and this short cycle length makes the rat an ideal animal for investigation of changes occurring during the reproductive cycle.[17,18] In the present study there was a significant decrease in the number of estrous cycle and changes in the proestrous, estrous and metaestrous phase in the first three weeks. During the fourth week the estrous cycle was normal. This clearly indicates that short term exposure to MSG drastically affects the estrous cycle. The reason for interruption of estrous cycle during the intitial exposure might be due to the hormonal imbalance. In the present study there was a drastic increase in the proestrous phase and decrease in the estrous, metaestrous phases during the initial exposure to MSG. Based on these result we suggest that the MSG might cause an increase in the estrogen level which might have inhibited the LH surge and prevent ovulation. The normal estrous cycle in the long term exposure shows the process of adaptability. Therefore the results of the present study suggest that short term exposure to MSG might have some immediate effect on reproductive system and ingestive behavior.

References

1. Belluardo M, Mudo G and Bindoni M: Effect of early Destruction of the mouse arcuate nucleus by MSG on age Dependent natural killer activity: Brain Res. 1990;534: 225-333.
2. Moore KL: congenital malformations due to environmental; Developing Humans. W.B. Saunders co. Ltd. Philadelphia. 2003; 2nd ed.pp.173-183.
3. Geha, R.S., Beiser, A.et al. Review of alleged reaction to MSG and outcome of a multicentre double blind placebo-controlled study. J Nutr. 2000; 130,1058s-1062s.
4. Biodun D, Biodun A: A spice or poison? Is monosodium glutamate safe for human consumption? National concord 1993; 4th Jan. p5.
5. Miśkowiak B, Partyka M : Effects of neonatal treatment with MSG (monosodium glutamate) on hypothalamo-

pituitary-thyroid axis in adult male rats. Histol Histopathol. 1993;8(4):731-4.
6. Adrienne Samuels. The Toxicity/Safety of MSG; A study in suppression of information. Accountability in Research: 1999;6(4):259-310.
7. Mozes S, Sefcikova Z: Obesity and changes of alkaline phosphatase activity the small intestine of 40 and 80-day old rats subjected to early postnatal overfeeding of monosodium glutamate. Physiol Res. 2000;(2),177-86.
8. Redding TW, Schally AV, Arimura A, Wakabayashi I .Effect of monosodium glutamate on some endocrine functions. Neuroendocrinology. 1971;8:245-255.
9. Spinedi E, Johnston C, Negro-Vilar A. Increased responsiveness of the hypothalamic-pituitary axis after neurotoxin-induced hypothalamic denervation. Endocrinology. 1984;115:267-272.
10. Nascimento Curi CM, Marmo MR et al. Effect of monosodium glutamate treatment during neonatal development on lipogenesis rate and lipoprotein lipase activity in adult rats.Biochem Int. 1991;24(5):927-935.
11. Ahluwalia, P. and Malik, V.B.T. Effects of monosodium glutamate (MSG) on serum lipids,blood glucose and cholesterol in adult male mice. Toxicol Lett. 1989;45:195-198.
12. Malik, V.B.T. and Ahluwalia, P. Studies on the effect of monosodium glutamate (MSG) on various fractions of lipids and certain carbohydrate metabolic enzymes in liver and blood of adult male mice. Toxicol Lett. 1994;69-77.
13. Racotta, R, Hernandez Garcia, A Gender differences between the effect of monosodium glutamate on food intake in rats. Physiol-Behav. 1989;46(2):331-2.
14. Lerner, L.J. The biology of non-steroidal antifertility IN: Contraception chemical control of fertility Ed Daniel Lednicel "Marcel Derker" Inc, New York. Lowry, H.Rosebrough, N. I. Far, A. L. and Ranall, R. J.. Protein measurement with folinphenol reagent. J Biol Chem. 1969;193:265-275.
15. Freeman ME.The ovarian cycle of the rat. In: E. Knobil & J. Neil (eds.), Physiology of reproduction. 1988, Raven Press Ltd., New York. 1893-1928.
16. Schwartz NB. Acute effects of ovariectomy on pituitary LH, uterine weight, and vaginal cornification. Am J Physiol. 1964;107:1251-1259.
17. Marcondes FK, Miguel K et al., Estrous cycle influences the response of female rats in the elevated plus-maze. Physiol Behav. 2001;74(4-5):435-440.
18. Spornitz UM, Socin CD et al., Estrous stage determination in rats by means of scanning electron microscopic images of uterine surface epithelium. The Anat Rec. 1999;254: 116-126.