RESEARCH ARTICLE

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Gestational Monosodium Glutamate Exposure Effects on Anogenital Distance of Male Rat Pups

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Abstract

High-dose Monosodium Glutamate (MSG) expo sure increases the estrogen level in pregnant rats. However, there are limited data available on whether the MSG-related maternal hormonal effects can affect male litters' genitalia phenotype. This study aimed to analyze the impact of MSG on estrogen level in pregnant rats and anogenital distance in male pups. Experiment for this study was performed at the animal facility of Biomedical Laboratory at the Faculty of Medicine, Universitas Andalas, from April 2019 to February 2020. Pregnant Wistar rats were given MSG orally at 2 and 4 mg/g body weight (BW) for 20 days. On day 21, pregnant rats were sacrificed and blood was drawn intracardially. Estradiol serum level was measured by ELISA. Male pups were counted, and the anogenital distance (AGD) was measured. Maternal serum estradiol levels were statistically analyzed by One-Way ANOVA and the AGD of male litters were analyzed by the Kruskal-Wallis test. Results showed that perinatal MSG exposure increased the estradiol level (26.3±4.5 pg/mL; 37.5±6.7 pg/mL; 62.1±8.2 pg/mL in control, 2 mg/gBW, and 4 mg/gBW group, respectively [mean±SD; p=<0.001]) and decreased the AGD (4 mm; 3 mm; 1.5 mm in control, 2 mg/gBW, and 4 mg/gBW group, respectively [median; p=<0.01]) in a dose-dependent manner. Thus, MSG exposure during pregnancy is a risk factor for male rat feminization.

Keywords: Anogenital distance, estrogen, gestation, male, monosodium glutamate

Efek Paparan Monosodium Glutamat Pada Masa Gestasi Terhadap Jarak Anogenital Bayi Tikus Jantan

Abstrak

Paparan monosodium glutamat (MSG) pada dosis tinggi diketahui meningkatkan kadar estrogen pada tikus bunting. Namun masih sedikit data mengenai pengaruh efek hormonal MSG pada induk terhadap fenotip genitalia bayi jantan. Penelitian ini bertujuan menganalisis pengaruh MSG terhadap kadar estrogen tikus bunting dan jarak anogenital bayi jantan. Penelitian ini dilakukan di fasilitas hewan Laboratorium Biomedik Fakultas Kedokteran Universitas Andalas dari April 2019 sampai Februari 2020. Tikus Wistar bunting diberi MSG secara oral pada dosis 2 and 4 mg/gBB selama 20 hari. Pada hari ke-21, tikus bunting dikorbankan dan darahnya diambil intrakardial. Kadar estradiol serum diukur menggunakan ELISA. Bayi jantan dihitung dan jarak anogenital (JAG) diukur. Kadar estradiol serum induk dan JAG bayi dianalisis secara statistik. Hasil penelitian menunjukkan bahwa paparan MSG pada masa gestasi meningkatkan kadar estradiol level (26.3±4.5 pg/mL; 37.5±6.7 pg/mL; 62.1±8.2 pg/mL pada kelompok kontrol, 2 mg/gBB, dan 4 mg/gBB, berturut-turut [rerata±SD; p=<0.001; *One-Way* ANOVA]) dan menurunkan JAG (4 mm; 3 mm; 1.5 mm pada kelompok kontrol, 2 mg/gBB, dan 4 mg/gBB, berturut-turut [median; p=<0.01; Kruskal-Wallis]) sesuai peningkatan dosis. Simpulan, paparan MSG selama kehamilan merupakan faktor risiko feminisasi pada anak tikus jantan.

Kata kunci: Estrogen, gestasi, jantan, jarak anogenital, monosodium glutamat

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Introduction

Monosodium glutamate (MSG) is a form of glutamic acid salt. Glutamic acid can be found in food products containing high protein, such as meat, fish, cheese, and vegetables. The average estimated daily MSG consumption per person is 0.65 g in Indonesia, 1.90 g in Japan, 1.00 g in the US, and 0.57 g in Canada.[1]

MSG at a dose of 0.2 g/kg body weight (BW) given for 14 days has been shown to induce severe ovarian damage in rats.[2] MSG exposure at the amount of 3 and 6 g/kg BW for 30 days has been shown to disrupt testicular morphology, affecting testosterone level and sperm count, potentially causing partial infertility in men.[3]

A research on male rabbits found that MSG given at a dose of 1 g/kgBW for 56 days suppressed the level of luteinizing hormone (LH), decreased testosterone level without pathological testicular lesions.[4] MSG impairs ovarian function by inducing the secretion of LH and follicle-stimulating hormone (FSH) from the anterior pituitary and estradiol from ovarian follicles by acting as a neurotransmitter.[5] Monosodium glutamate exposure at 0.08 mg/kg BW for 14 days on female rats can cause degenerative and atrophic changes in the fallopian tubes.[6]

Increased estrogen or estrogen-like compound exposure affects male reproductive phenotype, such as shorter anogenital distance (AGD), hypospadias,[7] and feminization of seminal vesicle.[8] MSG exposure has been shown to induce estrogen levels in adult female rats.[9] The MSG effect on female reproductive organs through interaction with the endocrine system by increasing estradiol level may impact the hormone-sensitive development of male offsprings' genitalia. However, there is limited data on whether gestational MSG exposure can decrease male AGD, a feminization marker.

Therefore, this study aimed to investigate the effect of MSG exposure during the gestational period on maternal serum estrogen level and AGD of male offspring.

Methods

The institutional research ethics committee of the Faculty of Medicine Universitas Andalas approved this study (No. 581/KEP/FK/2019). The animal experimentation was conducted at the animal house facility of Faculty Medicine Universitas Andalas on December 2019 to January 2020. Adult Wistar rats (8-10 week-old) weighing 170-190 grams were kept in cages at a temperature of 20º-25ºC and a 12/12 hour light/dark cycle. Rats were fed standard chow (All feed-3®, PT. Central Proteina Prima Tbk, Medan) *ad libitum.*

Male and female rats were mated; pregnancy was confirmed by checking vaginal plugs (gestational day 1). Pregnant rats were randomLy divided into three groups (n=6 each). The control group was fed standard chow and water. The treatment group was given standard chow and given MSG orally at 2 and 4 mg/g BW every 10 AM using oral gavage for 20 days.[10] On day 21, animals were anesthetized using Ketamine 0.15 mL/200 gBW and Xylazil 0.25 mL/200 g BW intra-peritoneally. Then, 2 mL of blood was drawn through the heart and into a vacutainer, centrifuged at 3,000 rpm for 15 minutes to separate the serum. Sera were stored in a freezer at -20°C until analyzed for estradiol by the ELISA method. All experiment was performed in duplicate.

Following blood drawing from the dams, all litters were surgically removed from the womb, and were placed in 10% neutral buffered formalin. Their genitalia were identified and male litters were separated, placed in supine position, and AGD was measured (the distance between the anal rim and the genital papilla) using a caliper and millimeter paper. Data on serum estradiol had a normal distribution, while the AGD had a non-normal distribution. Therefore, comparison of serum estradiol level and AGD between groups were analyzed by One-Way ANOVA (followed by Bonferroni *post hoc* test) and Kruskal-Wallis (followed by Mann-Whitney U test), respectively. Statistical significance was considered at a p-value <0.05. All analyses were performed by using IBM SPSS Statistics for Windows ver.25 (IBM Corporation, Armonk, NY).

Results

Bodyweight changes were measured periodically to monitor animal well-being during the experiment until the end of the study, and the result is presented in Figure 1. As shown in Figure 1, body weight increased in all groups during the experiment, with a statistically significant increase in the group receiving MSG at 4 mg/g BW/day compared to receiving 2 mg/gBW/day.

This study orally exposed female rats to two doses of MSG during the gestational period to

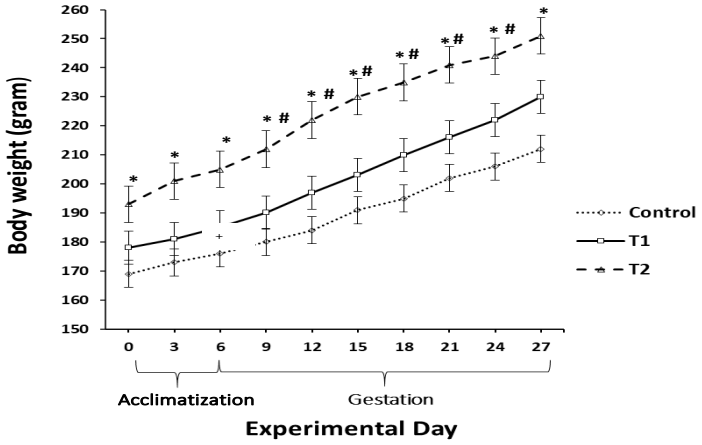

Figure 1 Bodyweight Changes (gram) of Female Rats During the Acclimatization and Gestational Period

Intergroup Comparison for Each Point in time was Analyzed by One-Way ANOVA followed by Bonferroni test. Results are Presented in mean±SD*difference from control at p<0.05; #difference from T_1 at p<0.05

validate the previous finding that MSG induced estradiol serum levels.[9] This study corroborated the previous study, and the result is presented in Figure 2. This study found a statistically significant elevation in serum estradiol level in pregnant rats orally exposed to MSG compared to the control group.

This study measured male litter's AGD at birth to elucidate whether maternal gestational exposure to MSG affects male offspring's reproductive phenotype. The result is presented in Figure 3. The current study found a statistically significant shortening of male AGD in group from dams exposed to 2 mg/gBW/day (median 3.0 mm, interquartile range [IQR] 1.0 mm) and 4 mg/gBW/day (median 1.5 mm, IQR 0.25 mm), compared to the control group (median 4.0 mm, IQR 0.5 mm). Representative pictures

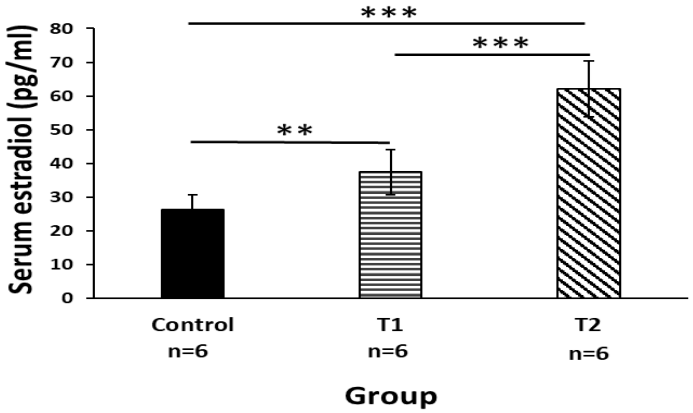

Figure 2 Serum Estradiol Level of Female Rats Exposed to MSG During Pregnancy (20 days)

Control Group was Given Standard Chow. Treatment Groups were Given Standard Chow and MSG orally at 2 mg/gBW/day (T_1) and 4 mg/gBW/day (T_2). Intergroup Comparison was Analyzed by One-Way ANOVA followed by Bonferroni test, Presented as mean±SD; **p<0.01; ***p<0.001

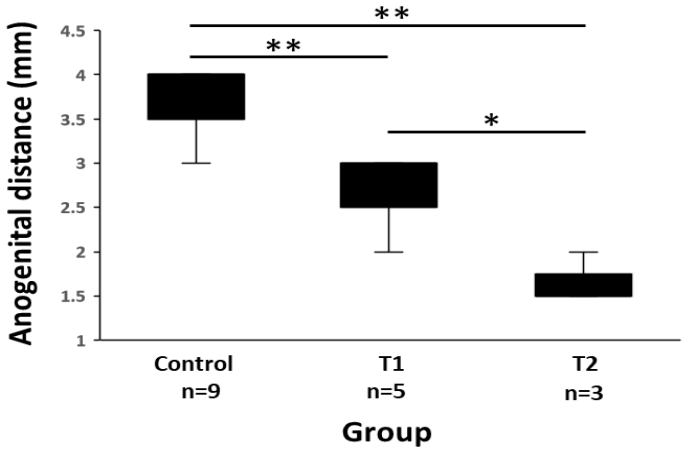

Figure 3 Anogenital Distance (AGD) of Male Pups Born from Dams Exposed to MSG During Pregnancy.
AGD (mm) was measured at birth. Control group was given standard chow. Treatment groups were given standard chow and MSG orally at 2 mg/gBW/day (T_1) and 4 mg/gBW/day (T_2). Intergroup comparison was analyzed by Kruskal-Wallis followed by Mann-Whitney U test and presented as boxplot (the box is formed by interquartile range and the whiskers showed minimum and maximum value);*p<0.05; **p<0.01

of measurement for each group are shown in Figure 4.

To understand the effect of maternal gestational MSG exposure on maternal fertility, we counted the number of total pups in each dam and the result is presented on Figure 5. Compared to the control group, gestational exposure to MSG resulted in lesser number of pups in both exposed groups, but only statistically significant in group exposed to MSG at 4 mg/gBW/day (p<0.01; Mann-Whitney U test).

Discussion

This study indicates that treatment groups exposed to MSG during the gestational period had a higher body weight than the control group, particularly exposure to higher dose of MSG (Figure 1). MSG can cause lesions in the hypothalamus, therefore interfering with hypothalamic signals and leptin resistance.[11] The exact mechanism for the effect of MSG on obesity is not known. One plausible explanation is that MSG can trigger leptin resistance. Leptin is a hormone produced by fat cells in the body. In normal people, leptin will increase during meals, and the leptin in the blood serves as a signal for the brain to stop eating. However, in people with leptin resistance, the brain does not respond to leptin signals, and satiety is not reached, leading to more food consumption.[11]

L-glutamate receptors may also influence the effect of MSG on body weight in the

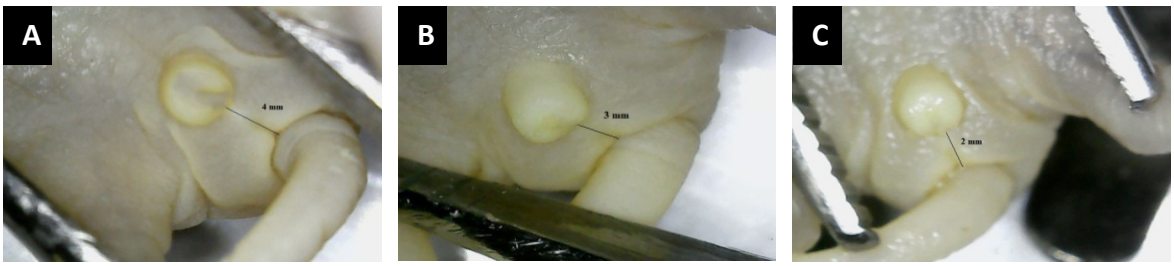

Figure 4 The Anogenital Distance at Birth (mm) in Male Pups Born from Dams Exposed to MSG During Pregnancy
A. Control. B. Born from dam exposed to MSG 2 mg/gBW/day for 20 days. C. Born from dam exposed to MSG 4 mg/gBW/day for 20 days. Picture is representative of each group

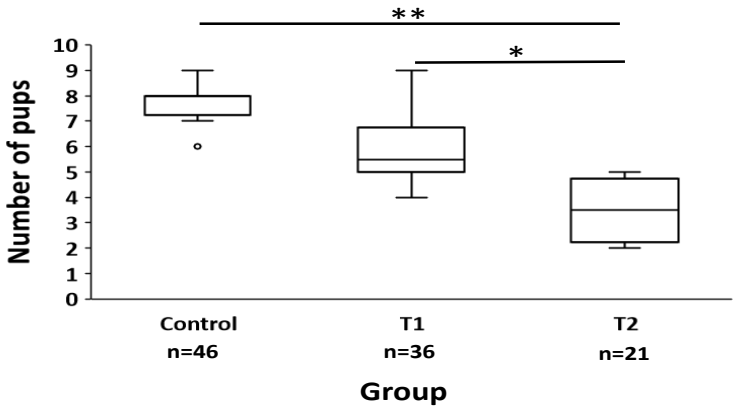

Figure 5 Number of Total Pups in Dams Exposed to MSG During Pregnancy. Control Group was Given Standard Chow

Treatment groups were given standard chow and MSG orally at 2 mg/gBW/day (T_1) and 4 mg/gBW day (T_2). Intergroup comparison was analyzed by Kruskal-Wallis followed by Mann-Whitney U test and presented as boxplot (the box is formed by interquartile range and the whiskers showed minimum and maximum value). *p<0.05; **p<0.01

gastrointestinal tract connected to afferent fibers from the vagal nerve.[12] Our finding is in line with previous research in rats given MSG, where an increase in body weight was observed,[13] possibly due to the impairment of the hypothalamus and arcuate nucleus, resulting in a lack of control in energy absorption and expenditure. The increase in body weight may also occur because MSG stimulates the pancreas, causing hyperinsulinemia, leading to increased sugar conversion to glycogen and subsequent deposition in adipose tissue.[14] Monosodium glutamate given at 4 mg/gBW/day during gestation in this study influenced the number of pups conceived by the dams. It is possible that gestational MSG exposure induced maternal obesity and metabolic syndrome, affecting ovarian functions. MSG-exposed animals may suffer from irregular cycle and less ovulated oocyte,[15] hence lesser number of pups.

Monosodium glutamate impairs ovarian function by increasing LH and FSH's secretion from the anterior pituitary and estradiol from ovarian follicles through action as a neurotransmitter. Monosodium glutamate may stimulate glutamatergic neurons in the hypothalamus. In return, these neurons induce the synthesis and release of gonadotropin-releasing hormone (GnRH), followed by increased LH and FSH secretion from the anterior pituitary through a positive feedback mechanism.[5] The result of this study is similar to a previous study in rats given oral MSG at a 1, 2, and 4 mg/gBW for 21 days, where an increase in serum estrogen was observed in a dose-dependent manner.[16]

A previous study on male rats given diethylstilbestrol showed decreased testosterone levels and the seminal vesicles' weight, leading to a significant reduction in AGD.[17] The anogenital distance is a useful biological marker for detecting androgen deficiency in the fetus and detecting disruptive endocrine effects.[18] In humans, this will increasingly serve as a prospective biomarker, where if the male AGD is shorter than average, it is a warning of complications or reproductive problems in adulthood.[19]

In conclusion, there is an effect of gestational exposure to MSG on maternal serum estradiol and male pups' anogenital distance.

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