Sperm Concentration and its Acrosome Status of Monosodium Glutamate-Treated Rats

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Background and Objective: This present study was aimed to investigate the sensitivity of the testis, epididymis, seminal vesicle, and the sperm acrosome reaction to monosodium L-glutamate (MSG) in rats.

Method: Rats were divided into four groups (three treated and on control groups) and each group was fed with non-acidic MSG at 0.25, 3, or 6 g/KgBW for 30 days or without MSG. Possible morphological changes in male reproductive organs were studies. In addition, the sperm concentration and acrosome reaction status were assayed.

Result: When compared to the control without administration of MSG, no significant changes were discerned in gross morphology and weights of testes. In contrast, significant decreases were detected in the weight of epididymis plus vas deferens and sperm concentration of rats treated with 6 g/KgBW of MSG. In addition, the weight loss was evident in the seminal vesicle in all groups of MSG-administered rats. In the 6 g/KgBW of MSG group, the sperm concentration was significantly decreased, compared with the control or two lower dose MSG groups. In the acrosome reaction assays, there was no statistically significant difference among MSG-rats and normal rats.

Conclusion: High dose MSG decreased sperm concentration and the weight of epididymis plus vas deferens and seminal vesicle while it did not affect acrosome reaction in rats.

Key word: Monosodium L-glutamate (MSG, epididymis plus vas deferens, seminal vesicle, sperm acrosome reaction

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Introduction

Monosodium L-glutamate (MSG) is a major flavor enhancer used as the food additive. The concentrations of MSG used as the food additive were varying in different foods[1]. Currently, the safe concentration of MSG in foods and its toxicity in human is still a controversial issue[2]. In animals, MSG at higher doses was demonstrated to be a neurotoxic salt that could alter the hypothalamic-pituitary-adrenal axis (HPA) and damage neurons in the hypothalamic nuclei[3, 4]. In addition, the damages of liver and kidney could be induced by excessive MSG administration[5]. Those finding imply that free glutamate dissociated from MSG may act on their specific receptors in the central neurons or some peripheral cells, resulting in their pathological alterations.

There have recently been several reports indicating that administration of MSG can affect the sperm count in both neonatal and adult animals[6] and the glutamate receptors and transporters are expressed in the testis and sperm of mouse, rat, and human[7]. However, the roles of glutamate receptors present in male reproduc-

tive organs including the sperm have not been elucidated. Moreover, no systemic examination of male reproductive organs and of the sperm physiology, like endogenous sperm capacitation and acrosome reaction in MSG-treated rats, has been performed. Therefore, this study was attempted to examine the sensitivity of entire male reproductive systems and the sperm acrosome reaction to varied-doses MSG administrations.

Objective

This present study was aimed to investigate the sensitivity of the testis, epididymis, seminal vesicle, and the sperm acrosome reaction to MSG in rats.

Methods

1. Animals and treatment

Sprague-dawley male rats (8-weeek-old) were purchased from Laboratory Animal Unit, faculty of Medicine, Khon Kaen university, Khon Kaen, Thailand. Thirty-two animals were randomly divided into four groups (n = 8 for each) and kept in groups of four in a cage (60 x 30 x 20) with food and water available ad libitum. The control group was treated with distilled water (vehicle) and the treated groups were administered with non-acidic MSG solutions at concentrations of 0.25 [pH 7.3], 3 [pH 7.17], or 6 [pH 7.24] g/KgBW by gavage for consecutive 30 days. The selected doses were based on the toxicity levels reported by previous authors[8, 9]: 0.25 g/KgBW for non-toxic dose MSG, 3 and 6 g/KgBW for slightly toxic and high toxic doses, respectively. The animals were administered twice a day (between 0 and 8 h) with a half of given concentrations of MSG for each feeding period to avoid the damages of rat's stomach from single excess administration of MSG, resulting in unhealthy animals (our preliminary results). This study was approved by the Animal Ethics Committee of Khon Kaen University, based on the Ethics of Animal Experimentation of National Research Council of Thailand (ref. no. 0514.1.12.2/70).

2. Morphological study

On the day next to the termination of the MSG-administration, all animals were euthanized by cervical dislocation and gently sacrificed to collect the male reproductive organs (testes [Te], epididymis [Ep]), vas deferens [Vd], and seminal vesicle [Sv].

3. Sperm count and acrosome reaction assay

Rat sperms were collected from the left caudal epididymis plus vas deferens and put into 1 ml phosphate buffered saline (PBS, 37ฐC, pH 7.4) and further centrifuged (500 xg, 37ฐC, 5 min) to separate the sperm pellet from the epididymal fluid. For the sperm concentration analysis, the sperm pellets were resuspended with 1 ml potassium-enriched simplex optimized medium (KSOM) and supplemented (EmbryoMax KSOM Powdered Mouse Embryo Culture Medium; Millipore catalogue number: R-MR-020P-5D) with 0.3% bovine serum albumin (BSA). The sperm solutions (1:20 dilution) were counted according to the standard procedure in a Neubauer counting chamber . To evaluate the percentage of the endogenous acrosome reaction (AR), a small aliquot of the sperm suspension was subjected to Coomassie blue staining as previously described[10].

4. Statistical analysis

One-way ANOVA and Student's t-test were used to examine the significant differences between two or more sets of data, and between two data points, respectively, using the program of SigmaStat version 3.1.1. All quantitative results are presented as the mean $\pm$SD.

Results

1. The weight of male reproductive organs

The weight of the different organs from the different groups was measured (Table 1). No significant differences were found among testis of any MSG administered groups as compared with the control group ($p > 0.05$) (Fig. 1 and Table 1). The weight of the epididymis plus vas deferens was significantly decreased only in 6 g/

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KgBW of MSG group as compared with the control ($p< 0.05$), while the weight of seminal vesicle decreased in all MSG-treated groups with a markedly decrease in 6 g/KgBW of MSG group ($p< 0.01$).

2. Sensitivity of sperm count and acrosome reaction to MSG

The epididymal sperm concentration and the percentages of sperms representing the endogenous acrosome reaction were shown in Table 2. The administration of 0.25 and 3 g/KgBW of MSG did not affect the sperm concentration when compared with the control group. In contrast, the sperm concentration was significantly decreased in the 6 g/KgBW of MSG group, compared with the control or two lower dose MSG groups. In the acrosome reaction assays, there was no statistically significant difference between the control and three different MSG treated groups ($p> 0.05$).

Conclusion

MSG at a high dose level could decrease sperm concentration and the weight of epididymis plus vas deferens and seminal vesicle. Although glutamate receptors and transporters have been localized in the mature sperm, their functions responsible for acrosome reaction are not elucidated. In this study, we first showed that no endogenous sperm acrosome reaction status was observed in MSG-treated rats. The analysis of the amount of free glutamate present in the epididymal fluid of MSG rats and the in vitro acrosome reaction assay by incubation with washed epididymal sperms and pure glutamate need to be further clarified.

Table 1 Effect of monosodium glutamate on testicular, epididymis plus vas deferens, or seminal vesicle weight (g) in the rats (Mean ±SD, n=8 in each group)

Groups	Weights (g)		
	Testes	Epididymis plus Vas deferens	Seminal vesicle
Control	1.6829±0.05	0.5068±0.01	0.8769±0.04
0.25 g MSG/KgBW	1.6812±0.03	0.4803±0.02	0.6836±0.07*
3 g MSG/KgBW	1.5988±0.06	0.4727±0.01	0.6579±0.06*
6 g MSG/KgBW	1.5874±0.04	0.4283±0.02*	0.3904±0.08**

* $p < 0.05$; ** $p < 0.01$

Table 2 Effect of monosodium glutamate on sperm concentration and acrosome reaction in rats (Mean ± SD, n = 8 in each group)

Groups	Sperm concentration(x 10^6 sperm/ml)	Acrosome reacted sperm(%)
Control	36.88 ± 2.4	4.1
0.25 g MSG/KgBW	34.25 ± 4.01	5.7
3 g MSG/KgBW	36.93 ± 3.09	6.4
6 g MSG/KgBW	24.70 ± 6.02*	4.4

* $p < 0.05$

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