



## Monosodium Glutamate Potentiates the Force of Contraction of Uterine Smooth Muscle in Rat by Augmenting Acetylcholine Mediated Neuromuscular Transmission

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### ABSTRACT

The objective of the study was to examine the effects of Monosodium glutamate (MSG) on the contraction of uterine smooth muscles in non-pregnant rat *ex-vivo*. The experiments were carried out in virgin female adult rats (Charles Foster strain). The experimental groups of rat were received three graded doses (0.8, 1.6, 2.4gm/kg body weight/day) for two different exposure durations (30 days and 40 days). The amplitude of the contraction of uterus was potentiated and the frequency of contractions was decreased in a dose response manner in both exposure durations in comparison with the amplitude and frequency of the contractions of uterus in control rats. Besides, the enzymatic activity of acetylcholinesterase of uterine smooth muscle was decreased dose dependently compared to the activity of acetylcholinesterase in smooth muscles of the uterus in control rats. Considering the results, we may conclude that MSG potentiates the amplitude of the contraction of uterine smooth muscle probably by prolonging the action of acetylcholine, the neurotransmitter substance that transmits motor impulse from cholinergic autonomic efferents to the smooth muscle cells.

**Keywords:** Monosodium glutamate, uterine movement, acetylcholine, acetylcholinesterase.

### INTRODUCTION

Monosodium glutamate (MSG) is one of the controversial food additives used to enhance the flavor of the food and augment the appetite of the human beings. Though, the use of MSG was initially introduced in the Chinese restaurant, subsequently the concept of using MSG to expand the market of the food products spreaded to most all Countries in the World<sup>1, 2</sup>. Recently, the experimental results in animal models has revealed the link between MSG accumulation in tissues and symptoms exhibited by the Chinese eaters due to consumption of MSG adulterated food items<sup>3, 4</sup>. Besides, the broad spectrum harmful effects of MSG, including the effects on liver functions<sup>5</sup>; function on the endocrine glands<sup>6, 7</sup>; functions on the male reproductive organ system<sup>8, 9</sup>; and central nervous system functions<sup>11-14</sup>; have been reported by many research groups based on their experiments in animals models.

The uterus is one of the major female reproductive organs for delivering female reproductive functions. The uterine tube shows rhythmic motility in basal state in non-pregnant animal including human beings. The basal movement of the uterus is provided by the visceral smooth muscle found in the wall structure of uterus. The probable toxic effect of MSG on the contraction of uterine smooth muscles has not been reported till recently. Therefore, the objective of the present study was to examine the effects of MSG on the contraction of uterine smooth muscle *ex vivo* in female rat model.

### MATERIALS AND METHODS

#### Reagents and Chemicals

Reagents and chemicals used for this study were of analytical grade. MSG ( $\leq 99\%$ ) was purchased from Sigma-Aldrich, USA. Sodium chloride (NaCl), potassium chloride (KCl), magnesium chloride ( $MgCl_2$ ), calcium chloride ( $CaCl_2$ ), sodium bicarbonate ( $NaHCO_3$ ), glucose, sodium dihydrogen phosphate ( $NaH_2PO_4$ ); and 5, 5'-dithiobis-2-nitrobenzene (DTNB), acetyl thiocholine iodide were procured from EMerck, India and SRL, India respectively.

#### Animals

Studies were performed on three to four months old female virgin albino rats of Charles Foster strain weighing about 110-120 gm. Animals were maintained in Animal House as per guidelines of the Animal Ethics Committee of Kalyani University according to recommendations of the national guidelines. The Animals were kept in equal day-night cycle (12L: 12D) and fed standard laboratory chow and water *ad libitum*.

#### Animal exposure and grouping

After one week of acclimatization to the laboratory environment, the animals was randomly distributed into following 8 (eight) groups for sub-chronic MSG treatment. The different doses of MSG were selected in this study according to the graded percentage of  $LD_{50}$  value of MSG in rat model mentioned by JECFA 1988<sup>15, 16</sup>. The rats of the first group were received distilled water and marked as control. The rats of the second, third and fourth groups were received 0.8gm, 1.6gm and 2.4gm of



MSG/kgBW/Day (i.e., approximately 5%, 10% and 15% of LD<sub>50</sub> of MSG) respectively by oral gavages for 30 and 40 days treatment durations; and were marked as Treated I, Treated II and Treated III respectively. The animals were sacrificed by cervical dislocation on the 24<sup>th</sup> hour after the completion of last dose.

### Recording of uterine smooth muscle contraction

After overnight fasting, each rat was sacrificed by cervical dislocation. The abdomen of the sacrificed rat was then opened immediately and the uterus was removed by transverse incision. A segment of uterine tube (2 to 3cm) was placed longitudinally in 50 ml organ bath of Dale's apparatus containing Tyrode's solution consisting of 8.0 g/l NaCl, 0.2 g/l KCl, 0.2 g/l CaCl<sub>2</sub>, 0.1 g/l MgCl<sub>2</sub>, 1.0 g/l NaHCO<sub>3</sub>, 0.05 g/l NaH<sub>2</sub>PO<sub>4</sub> and 1.0 g/l glucose (pH-7.4). The temperature of the bath was maintained within a range of 37±0.5°C and continuously bubbled with 95% O<sub>2</sub> and 0.5% CO<sub>2</sub>. The contractions of uterine segment were obtained with isotonic transducer (IT-2245) coupled to RMS-Polyrite –D instrument (RMS, Chandigarh, India) by our standard laboratory protocol<sup>17</sup>. The uterine preparations were allowed to equilibrate for at least 40mins by applying an initial load 0.1gm and during this period, the preparations underwent repeated and continuous washes with Tyrode's solution. The data were stored and subsequently retrieved through RMS-Polyrite-D software.

### Sample collection for enzymatic assay

The control and test rats were sacrificed by cervical dislocation on the 24<sup>th</sup> hour after completion of the last dosage of both exposure durations (i.e., 30 and 40 days). The uterus of the rats in marked groups were removed and washed in ice cold phosphate buffer solution, then dried and stored in -20°C for AChE activity study.

### Homogenate preparation of uterine smooth muscle

For the homogenate preparation, the uterine segments collected from the experimental and control groups of rats were excised meticulously and the myometrium was minced in ice-cold saline. The minced muscle tissue was then homogenized in 10 ml buffer solution containing 0.1M phosphate buffer (pH 8.0), 0.5% Triton-X-100 and 2 mM EDTA with the help of a tissue homogenizer. After that, the homogenized muscle tissue sample was centrifugated at first at 8000 rpm for 10 mins and then the supernatant obtained was re-centrifugated at 12000 rpm for 10mins to obtain the intended supernatant. The final supernatant was then stored in laboratory at -20°C for AChE activity study.

### Acetylcholinesterase (AChE) assay

AChE activity of uterine smooth muscle was estimated from the homogenate by the method of Ellman et al., 1961<sup>18</sup> followed by Srikumar et al., 2004<sup>19</sup>. For this study, 0.4ml aliquot of uterine muscle tissue homogenate was added to a cuvette containing 2.6ml phosphate buffer (0.1 M, pH-8.0) and 0.1ml of DTNB. The content in the

cuvette were mixed properly and optical density of the solution in cuvette was measured by spectrophotometer (Genesys 10S UV-VIS Spectrophotometer, Thermo Fisher-Scientific, India) at 412nm. Then, 20µl of acetyl thiocholine iodide (substrate) was added to the solution in cuvette and six observations of the changes in absorbance of the solution were serially obtained each with a period of 10 mins having 2 mins interval from initial to final observations. The enzyme activity was calculated and expressed as micromoles of substrate hydrolyzed/minute/mg protein.

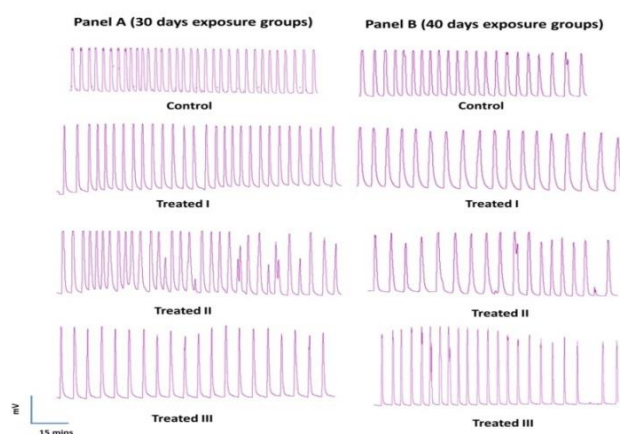
### Statistical analysis

All the data obtained from this study were expressed as mean ± SEM. The force of contraction of uterus was expressed by measuring the amplitude of contraction and presented in the graph as percent changes in amplitude. The frequency of the contraction of uterus was presented in the graph as percent changes in frequency. The frequency of the contraction was counted by a standard scale drawn from RMS Polyrite D software based on recorded movements of uterus. Statistical analysis of the data obtained from control and in treated groups were done by Student's 't' test or analysis of variance (ANOVA) whichever is applicable and the analysis was done by GraphPad Prism 5.03 (GraphPad software, Inc). p≤0.05 was considered as the level of significance.

## RESULTS

### Effects of MSG on the contraction of uterus *ex vivo* in rats

We observed a significant potentiation of the amplitude of the contractions of uterus in MSG exposed groups of rats recorded *ex vivo* in a dose response manner in both exposure durations (30 and 40 days) compared to the amplitude of contraction of uterus in control rats (Figure 1 and Figure 2 A) in both exposure durations. We also observed a significant decrease in frequency of contraction of uterus compared to the frequency of contraction of uterus *ex vivo* in control rats (Figure 1 and Figure 2 B) in both exposure durations. The effect was more pronounced in 40 days exposure groups of rats.

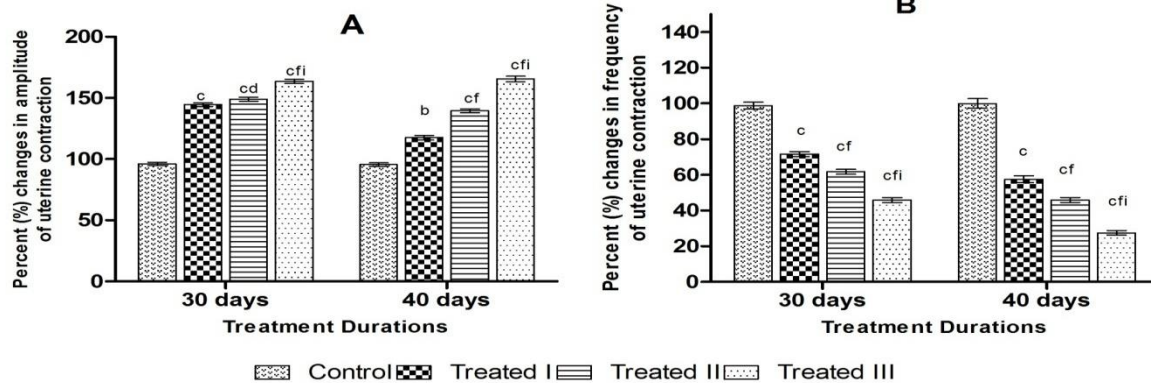


**Figure 1:** Representative records of the isolated uterine contraction of control and MSG exposed rats *ex vivo* for

both 30 days (Panel A) and 40 days (Panel B) exposure durations.

Panel A (30 days exposure groups): Treated I received 0.8gm of MSG/KgBW/Day, Treated II received 1.6gm of MSG/KgBW/Day, Treated III received 2.4gm of

MSG/KgBW/Day. Panel B (40 days exposure groups): Treated I received 0.8gm of MSG/KgBW/Day, Treated II received 1.6gm of MSG/KgBW/Day, Treated III received 2.4gm of MSG/KgBW/Day.

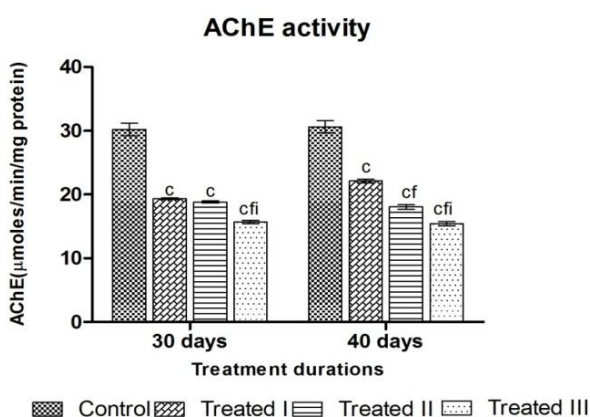


**Figure 2:** Showing the percent changes in the amplitude (A) and frequency (B) of isolated uterine contraction *ex vivo* of rats exposed to three graded dosages of MSG for 30 days and 40 days exposure durations compared to amplitude and frequency of contraction of control rats (in metestrus phase).

Values are represented as mean±SEM of seven observations, <sup>a,b,c</sup>*p*<0.05, 0.01, 0.001vs. Control, <sup>d,e,f</sup>*p*<0.05, 0.01, 0.001vs. Treated I, <sup>g,h,i</sup>*p*<0.05, 0.01, 0.001vs. Treated II.

**Effects of MSG on the enzyme activity of acetylcholinesterase (AChE) of uterine smooth muscle**

We observed a significant inhibition of the activity of AChE in MSG exposed uterine muscle tissue homogenate in both dosage and duration response compared to the AChE activity in uterine tissue homogenate of control rats (Figure 3).



**Figure 3:** Showing the acetylcholinesterase (AChE) activity in control and MSG exposed groups of rats for 30 days and 40 days exposure durations.

Values are represented as mean±SEM of seven observation, <sup>a,b,c</sup>*p*<0.05, 0.01, 0.001vs. Control, <sup>d, e, f</sup>*p*<0.05, 0.01, 0.001vs. Treated I, <sup>g,h,i</sup>*p*<0.05, 0.01, 0.001vs. Treated II.

**DISCUSSION**

The objective of the study was to examine the probable toxic effects of MSG on the contractile activity of visceral smooth muscles found in the wall structure of the uterus. In our study, MSG produced a significant potentiation of amplitude and inhibition of frequency of the contraction of uterus recorded *ex vivo* dose dependently in both exposure durations. This result suggests that MSG potentiate the amplitude of the contraction probably by promoting the force of contraction of the uterine smooth muscle. MSG might potentiate the contraction by augmenting the cholinergic neurotransmission from facilitatory cholinergic autonomic efferents to the smooth muscle at the synaptic junctions in synapse en-passant system and/or promoting the intracellular mechanism responsible for cross-bridge interactions.

In order to examine the effect of MSG on the neuromuscular transmission, the activity of AChE in MSG exposed smooth muscle homogenate of rat’s uterus has been determined. It was observed that the activity of the AChE decreases dose dependently in all exposure durations in MSG exposed rats compared to the control group of rats. This result suggests that, MSG might augment the neuromuscular transmission by inhibiting the AChE. AChE is an enzyme which is found predominantly in the membrane of the smooth muscle at the end-plate region. It terminates the acetylcholine mediated neurotransmission from cholinergic efferents to smooth muscle cells by hydrolyzing the acetylcholine into acetate and choline. In our study the activity of AChE in MSG exposed smooth muscle was decreased. Thus, we may hypothesize that MSG might augment the acetylcholine mediated neurotransmission by inhibiting the activity of AChE at the myo-neural junction.



Moreover, the frequency of uterine movement was decreased in MSG exposed rats. The decrease in frequency of uterine movement in MSG exposed rats might be due to inhibition of the intrinsic basal rhythmicity of the contraction of uterine smooth muscles. Considering the entire results, we may conclude that MSG potentiates the force of contraction of uterine smooth muscles probably by augmenting the cholinergic transmission in synapse en-passant junctions.

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