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Monosodium Glutamate (MSG) Promotes the Contraction of Duodenal Visceral Smooth Muscle *Ex Vivo* in Rat

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ABSTRACT

Monosodium glutamate (MSG), popularly trading in the name of AJI-NO-MOTO, is one of the world's most extensively used taste enhancing food additive. It is used in cooking as a flavor enhancer with an umami taste that intensifies the meaty and savory flavor of food. Thus, humans are often exposed to MSG through consumption of MSG tainted food stuffs and the small intestine gets primarily exposed to it. In order to examine any effect of MSG on the contractile activity of the small intestinal visceral smooth muscle (SiVSM), the movement of the duodenum *ex vivo* in response to MSG in single dose experiments have been recorded using an isotonic transducer (IT-2245) coupled with an RMS-Polyrite D machine (RMS, Chandigarh, India). Significant increase in the amplitude and frequency of contraction of the duodenum in comparison with control tracings in a dose-response manner were observed. From the results, it can be suggested that MSG potentiates the contractile activity of duodenal visceral smooth muscle probably by increasing the amplitude and frequency of the contractions of the visceral smooth muscle located in the wall structure of the duodenum, probably by facilitating the activity of excitatory intrinsic cholinergic efferents and/or inhibiting the activity of inhibitory adrenergic or nitrergic myenteric efferents. The MSG induced impairment in the contraction of the dVSM results in impaired digestive and absorptive functions of the duodenum (small intestine).

Keywords: MSG, contractile activity, SiVSM, food additive, myenteric efferents

INTRODUCTION

Monosodium glutamate (MSG) is a hydrated sodium salt of naturally occurring L-glutamic acid. It is generally used as a flavor-enhancing food additive to processed fast foods. It has been reported that MSG altered the renal cortical structure and degenerative nephron structure in the kidney of MSG exposed groups of rats (Eweka, 2007). It has also been reported that MSG significantly induced the weight gain of the animal feed with MSG added diet as results of the induction in the appetite process (Moore, 1999). Endocrine disruptions in MSG exposed laboratory animals have also been reported (Samuels, 1999). It has been also reported that locomotion disorders were observed in MSG-exposed animals (Ali et al., 2000).

Humans are often exposed to MSG through consumption of MSG tainted food stuffs and the small intestine gets primarily exposed to it. The small intestine helps in digestion and absorption with the help of the contractions of the visceral smooth muscles located in the muscularis externa layer of the small intestine that provides motility to it. Any impairment in the contractions of the SiVSM due to exposure to external agents might impair the digestive and absorptive functions of the small intestine. So, the present study was carried out to examine the effect of MSG on the contractile activity of the duodenal VSM *ex vivo* in male albino rats.

MATERIALS AND METHODS

Chemical and Reagents

Chemical used for this acute study was Monosodium glutamate (MSG) with 99% purity. It was purchased

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Sigma-Aldrich, USA. All the experimental reagents were of analytical grade. They were such as 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) were obtained E- Merck, India, and Sisco Research Laboratory (SRL), India respectively.

Animal Handling

Charles Foster male albino rats weighing 110-130 gm were used for this experimental study. Ethical clearance regarding animal acclimatization was gained by the Kalyani University Animal Ethics Committee according to national guidelines.

Experimental Set Up for the Post-treatment of the Animal

For this post-treatment experiment, animals were sacrificed by cervical dislocation after acclimatization duration. The abdomen was opened, duodenal segments were taken off by transverse incision and were prepared to record the contraction ofthe duodenum. For this acute study, a graded dosage of MSG was applied directly in the 50 ml organ bath and the contraction of the duodenum was recorded.

Table 1.1: Experimental set up for Acute Study

Animal grouping	Application of dosages (µM)
I	Application of 2.1µM dose of MSG on isolated duodenal (n=10)preparation.
II	Application of 4.2µM dose of MSG on isolated duodenal (n=10) preparation.
III	Application of 8.5µM dose of MSG on isolated duodenal (n=10) preparation.
IV	Application of 17µM dose of MSG on isolated duodenal (n=10) preparation.
V	Application of 34µM dose of MSG on isolated duodenal (n=10) preparation.

Recording of Contraction of Duodenal Movement

Segments of the duodenum were used for this study. After overnight fasting, each rat was sacrificed by cervical dislocation. The abdomen of the sacrificed rat was then opened immediately, and the duodenal segments were collected by transverse incision. After dissecting out the segment of the duodenal muscle, it was kept in Tyrode's solution made up of NaCl, KCl, $MgCl_2$, NaH_2PO_4, Na_2HPO_4, glucose. The lower hook was fixed at the bottom of the organ bath and the upper hook was connected to a lever connecting to an isotonic transducer (IT-2245). They were blotted dry and after that, placed in the 50 ml organ bath containing Tyrode's solution. The temperature of the bath was maintained (37˚C±0.5) and 95% CO_2, 5% O_2 were supplied continuously (2-3 bubbles/second). The initial preparations were allowed to equilibrate for at least 40 mins by applying an initial load of 0.1 gm. During this period, the experimental preparations underwent repeated and continuous washes with Tyrode's solution to avoid an accumulation of metabolites in the organ bath. Contraction of the duodenum was carried out by isotonic transducer (IT-2245) coupled to RMS Polyrite-D. Recording was taken at sweep speed= 0.937 mm/sec, deflection= 1000 mm, low filter= 0.2 Hz, high filter= DC and sensitivity= 10 µV (Mondal et al., 2018).

Statistical Analysis

The data were presented as Mean ± SEM of the value of exposed groups and control group of rats. The values of the data of the experimental groups were represented as percent changes of the values of a control group of rats. Statistical analysis of the results was evaluated using students''t' test or analysis of variance (ANOVA) in the Graph Pad Prism 5.03 (Graph Pad Software, Inc). The level of significance was applied at $p \leq 0.05$. In the results, the number of treated preparations of the duodenum was considered by 'n'.

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RESULTS AND DISCUSSION

To examine the response produced by MSG on the contractions of duodenal VSM, the movement of duodenum has been recorded *ex vivo* in response to application of graded doses of MSG. In this experiment, a significant increase in the amplitude and frequency of contraction of the duodenum in comparison with control tracings in a dose-response manner have been observed (Fig. 1.1: Tracings A-F; 1.2 and Table 1.2).

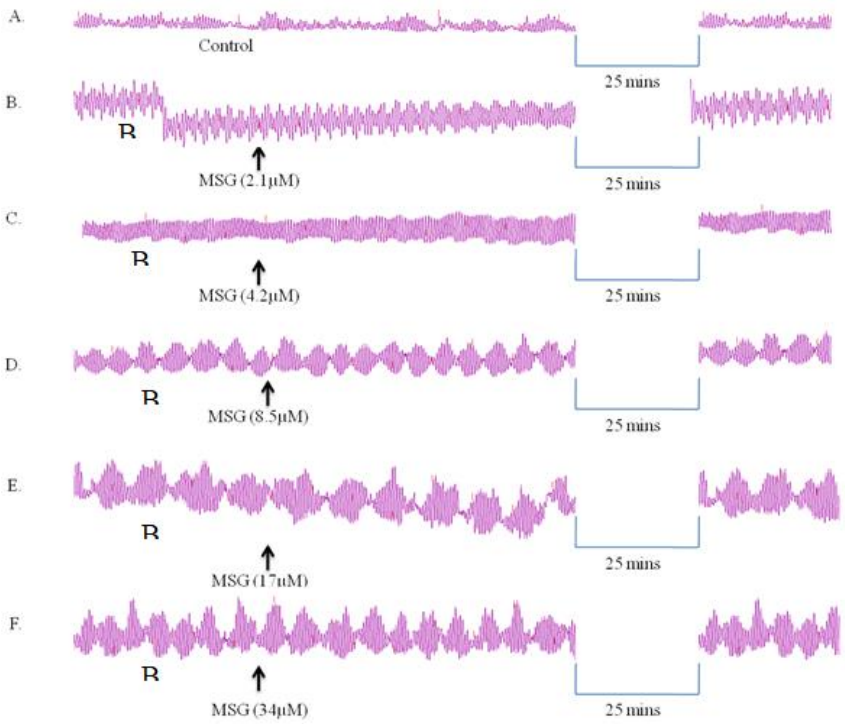
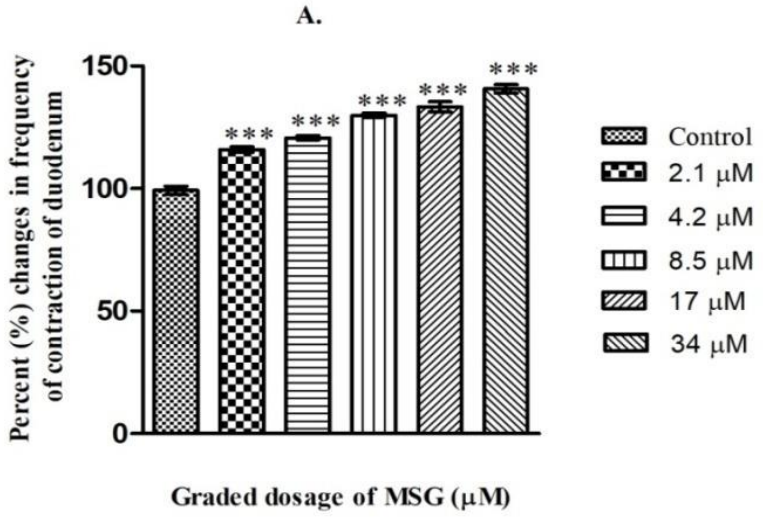


Fig. 1.1: Representative records show the effects of control and single graded dosages of MSG (i.e. 2.1, 4.2, 8.5,17, 34 µM) on the contraction of duodenum *ex vivo*, n=10.A) Tracings of the record of the duodenum in absence of MSG, B) Tracings of the effect of a 2.1µM dose of MSG, C) Tracings of the effect of a 4.2 µM dose of MSG, D) Tracings of the effect of an 8.5 µM dose of MSG, E) Tracings of the effect of a 17 µM dose of MSG. F) Tracings of the effect of a 34 µM dose of MSG. BC indicates a basal contraction of the duodenum. Arrowheads indicate the point of MSG application.



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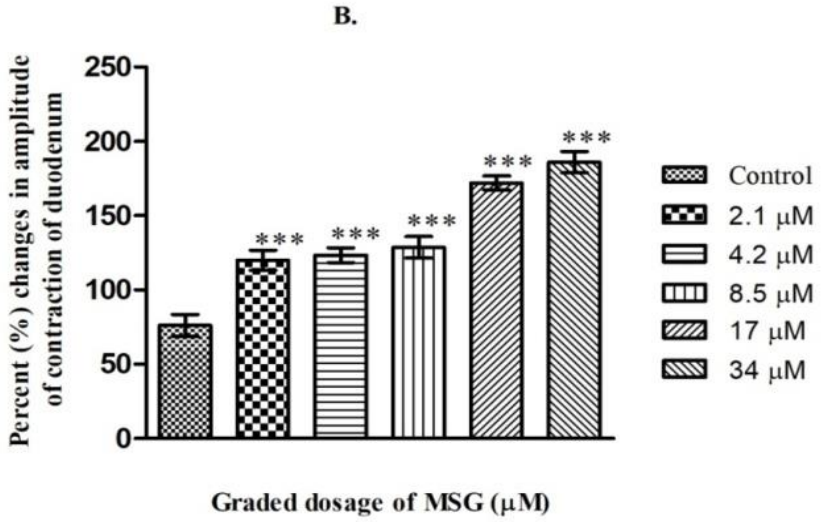

Fig. 1.2: Diagrammatic representations of the changes in frequency (A) and amplitude (B) of the contraction in absence of MSG and presence of MSG at single different dosages (i.e. 2.1, 4.2, 8.5,17, 34 µM) *ex vivo.* Data are expressed as Mean ± SEM, ***p<0.001 vs. control, n=10.

Table 1.2: Tabular presentation of percent changes in the amplitude and frequency of the contraction of duodenum *ex vivo* of rat in response to the graded dosages of MSG application. Data are expressed as Mean±SEM, ***p<0.001 vs. control, n=10.

Graded dosages of MSG (µM)	Amplitude of contraction	Frequency of contraction
Control	76.125±7.273	99.257 ±1.728
2.1	119.980±6.826***	115.921± 1.111***
4.2	123.400±5.017***	120.735± 0.820***
8.5	128.718±7.194***	129.993± 0.865***
17	172.075±4.679***	133.329± 2.066***
34	185.975±7.216***	140.736± 1.746***

In the present investigation, MSG potentiates the contraction of duodenal visceral smooth muscle (VSM) in exposed rats. The MSG-induced potentiation of contraction of VSM, might be due to facilitation of the contraction of duodenal visceral smooth muscle probably by augmenting the activity of cholinergic myenteric efferents innervating the smooth muscle and/or inhibiting the activity of adrenergic or nitrergic myenteric efferents as result of intoxication by MSG (Fig 1.3). Further, the increase in the frequency by MSG might be due to facilitation in the propagation of slow waves (SWs) and basal electrical rhythm (BER) in the Interstitial Cells of Cajal (ICCs) that determines the rhythmicity of the contraction of the dVSM. The MSG induced hypermotility was characterized by increased, excessive movement of the small intestine, which can cause diarrhea, nutrient malabsorption, bloating, and pain due to improper digestion or absorption. Improper digestion would lead to several gastrointestinal problems; and malabsorption of nutrients will cause severe deficiencies that could degrade the physical health.

CONCLUSION

In conclusion, it can be suggested that Monosodium glutamate is a potent toxicant and it impairs the basal contractions of dVSM in rat by increasing the frequency and contractions of the dVSM. The MSG induced

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facilitation of the contractions of the dVSM might be due to promotion of the activity of cholinergic myenteric efferents and/or facilitation of the activity of nitrergic and/or adrenergic myenteric efferents innervating the dVSM. The results obtained from the study could be extrapolated in humans. Thus, alteration of the contractile function of the dVSM on MSG exposure due to consumption of MSG tainted foods will result in the impairment of the digestive and absorptive functions of the small intestine.

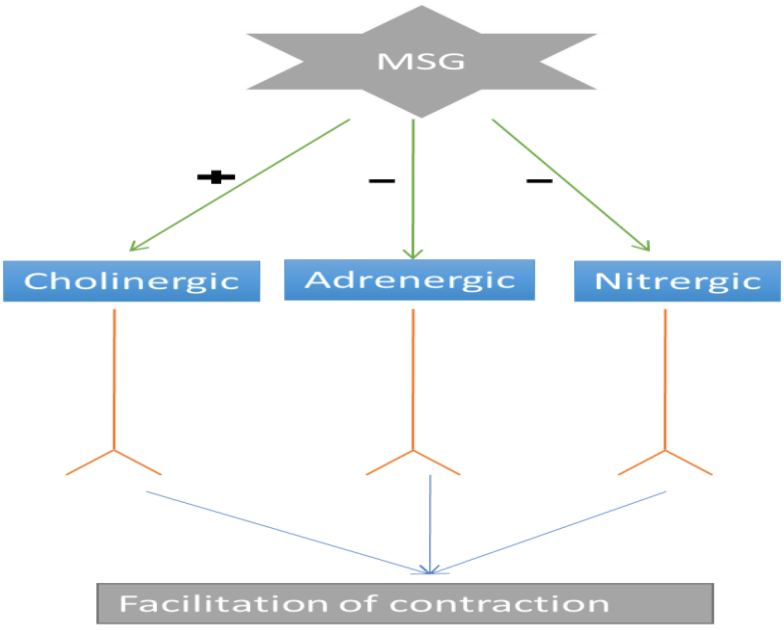

Fig1.3:Schematic representation showing probable neurocrine mechanism involved in MSG induced potentiation of contraction of duodenal visceral smooth muscle.+ indicates stimulation, - indicates inhibition, × indicates no effect.

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