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Full Length Research Paper

Acute low dose monosodium glutamate retards novelty induced behaviours in male swiss albino mice

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The study investigated the effects of acute systemic administration of monosodium glutamate (MSG) on novelty induced behaviours in male albino mice. The aim was to provide information on the neurobehavioural effects of a single i.p.(intraperitoneal) injection of MSG. Forty male swiss albino mice (age, 6 to 8 weeks; mean weight, 22.5 ± 2.5 g) were divided into four treatment groups (n = 10). Novelty induced behaviours that is horizontal locomotion (line crossing), rearing and grooming was carried out after intraperitoneal injection of varying doses of MSG (0.5, 1.0 and 1.5 m/kg) or its vehicle (normal saline). Each parameter was measured over a thirty minute period of ten minute intervals. Statistical analysis was carried out using a one way ANOVA followed by the Student-Newman-Keul's test. Compared with the control mice, a single intraperitoneal injection of MSG significantly reduced locomotor and rearing activities as horizontal locomotion and rearing was found to be significantly lower at 1.5 mg/kg when compared to their respective controls; grooming showed an initial slight increase followed by a visual but progressive reduction. The study concluded that acute administration of monosodium glutamate has a retardant effect on novelty induced behaviors in male mice.

Key words: Monosodium glutamate, novelty induced behaviours.

INTRODUCTION

Monosodium glutamate (MSG) is one of the commonest food additives in the developed world and it is a commonly used flavour enhancer. MSG can be found in various concentrations in numerous food products (Walker and Lupien, 2000). MSG is a sodium salt of glutamic acid, a naturally occurring non-essential amino acid with trade names such as Ajinomoto, Vetsin, Ac'cent and Tasting Powder. It was once made predominantly from wheat gluten, but is now made mostly from bacterial fermentation (Leung et al., 2003).

MSG added to foods produces a flavouring function similar to the glutamate that occurs naturally in foods. It adds a fifth taste, called "umami", which is best described as a savoury, broth-like or meaty taste, although traditional East Asian cuisine had often used seaweed extract, which contains high concentrations of glutamic acid, it was not until 1907 that MSG was isolated by Kikunae Ikeda. It was subsequently patented by Ajinomoto Corporation of Japan in 1909. In its pure form, it appears as a white crystalline powder that, as a salt, dissociates into sodium cations and glutamate anions while dissolving (glutamate is the anionic form of glutamic acid) (Sano, 2009).

Glutamate is a naturally occurring amino acid that is one of the most abundant amino acids in the central nervous system (CNS). It exist in atypically high concentration in brain regions that are critical in the mediation of cognitive performance such as cerebral cortex, dentate gyrus of hippocampus and striatum (Park et al., 2000) indicating the amino acid plays an important role in higher cognitive functions including memory formation (Hlinák et al., 2005). Glutamate is the primary excitatory amino acid neurotransmitter in the human brain. It is important in synaptic plasticity, learning, and development (Maragakis and Rothstein, 2001). Over the last four decades, a direct correlation between the neuroexcitatory and neurotoxic properties of glutamate

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has been linked to activation of excitatory amino acid receptors. This stimulation leads to an enzymatic cascade of events ultimately resulting in cell death (Maragakis and Rothstein, 2001).

MSG interacts with two main subtypes of membrane receptors, ionotropic and metabotropic coupled to ion channels and G proteins, respectively. The ionotropic receptors are further subdivided based on selective agonists into N-methyl-D-aspartate (NMDA), kainate and a-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) subtypes (Zomurski and Thio, 1992). Interactions of glutamate with its ionotropic, mainly NMDA, receptors have been found to lead to neurotoxic changes in some experimental situations by allowing excessive amounts of calcium to enter the neuron (Choi, 1985) Glutamate's activity at the synaptic cleft is carefully balanced by receptor inactivation and glutamate reuptake. When this balance is upset, excess glutamate can itself become neurotoxic (Maragakis and Rothstein, 2001).

The neurotoxic properties of glutamate were first demonstrated in 1957 by Lucas and Newhouse (Lucas and Newhouse, 1957) who showed that systemic administration of glutamate to infant mice caused retinal degeneration, following this discovery, a series of studies available now have demonstrated serious neurotoxico-logic effects of MSG on animals at significantly high doses. Park and his colleagues in 2000 found that single intraperitoneal injection of 4.0 mg/g bodyweight of MSG caused significant damage to hypothalamic neurons in the arcuate nucleus and impaired memory retention in adult mice (Park et al., 2000). Gonzalez-Burgos et al. (2001) also found that subcutaneous administration of 4.0 mg/g bodyweight of MSG to male neonate rats induced excitotoxicity, leading to cell death in prefrontal cerebral cortex. Another study using also high dose glutamate (4.0 mg/g body weight) reported reactivity of astrocytes and glial cells in the fronto-parietal cortex, including hyperplasia and hypertrophy (Martinez- Contreras et al., 2002). While MSG's similarity to glutamate might be the reason for its neurotoxicity, it is important to note the extremely high dosage administered in these animal studies.

To cause neuronal damage in animals, MSG plasma level needed to be100 to 130 mumol/dl in neonates and >630 mumol/dl in adults (Ng, 2002), hence the choice of low doses in our study. Neurobehavioural effects of glutamate also at high doses have been studied with the respect to variables such as age, time course of administration and neurobehavioural models. Carter and Levesque (1979) studying the effects of MSG on neurobehaviour in rats described a significant decrease in open-field activity at the 2 mg/g MSG group, with the decreases seen in the 4 mg/g MSG group being only visual. In 1999, Dubovicky et al. investigated the effect of monosodium-L-glutamate (MSG) administration in the neonatal period on habituation of exploratory behavior related to gender differences, rats of both sexes received intraperitoneal injection of MSG (4 mg/g) or vehicle on postnatal days 2, 4, 6, 8 and 10 and then exposed to the open field apparatus on the 65th postnatal day for four consecutive days to test their habituation with respect to exploratory behaviour, compared to intact controls, there were no significant differences found in interrupted habituation, neither in males nor in females.

Hlinák et al. (2005) evaluated the long-term behavioral consequences of neonatal MSG treatment in rats. The pups received subcutaneous injections of MSG at 3 mg/g daily from postnatal day 5 to 12. Data from an automatic activity monitor showed that locomotion of MSG-treated females and males aged 56 and 84 days was significantly reduced. Beginning from postnatal day (PD 120), three different behavioral tests were performed, in the open field test; a significant decrease in the habituation rate was found in MSG-treated animals. Sex-dependent differences in behavioral performance were suggested in the open field and elevated plus maze tests. These findings although inconsistent with dubovisky's findings in 1999 buttresses the fact that rats showed behavioural changes to MSG administration at high doses irrespective of the presence of gross morphological changes. Kiss et al. (2007) studied the changes in open-field activity and novelty-seeking behavior in periadolescent rats neonatally treated with monosodium glutamate; newborn rats were treated with 4 mg/g MSG subcutaneously on postnatal days 1, 3, 5, 7 and 9. Open-field behavior was tested at 2, 3, 4, 6 and 8 weeks of age, they found that MSG administration led to only temporary increases in locomotor behavior, which was more pronounced during the first few postnatal weeks, followed by a subtle hypoactivity at 2 months of age. Novelty-seeking was tested in four 5 min trials at 3 weeks of age and the conclusion was that the behavioral pattern of MSG-treated rats was the opposite in all tested signs in the novelty exploration test compared to control pups.

Investigation of novelty-seeking behavior is of growing importance for its relationship with sensitivity to psychomotor stimulants (Kiss et al., 2007) and though a lot has been done studying the effects of MSG on novelty induced behaviours at high doses, in this present study we decided to evaluate the possible acute neurobehavioural effects of MSG (if any) at doses otherwise recognized as being within normal dietary allowances.

MATERIALS AND METHODS

Equipments and apparatus

Electronic precision balance, plastic animal cages, sterile disposable syringes (1, 5, and 10 ml) and needles, cotton wool, stop watch and open field box.

Reagents and drugs

99% monosodium glutamate (Ajinomoto brand) was purchased

from the open market, weighed and dissolved in measured volume of isotonic saline solution to get desired concentrations MSG at the varying doses (0.5, 1.0 and 1.5 mg/kg) was administered intraperitoneally using an insulin syringe. The selection of this doses was arbitrary although cognizance was given to the LD 50 (i.p) of monosodium glutamate in mice which is 6900 mg/g (Yanagisawa et al., 1961) and also to previous reports that had used much higher doses successfully (Park et al., 2000; Hlinák et al., 2005; Kiss et al., 2007).

Animals

Healthy adult male swiss albino mice purchased from the Empire Animal farms in Osogbo, Osun State, Nigeria were used (age in the range of six to eight weeks) with weight in the range of 20 to 25 g. After being weighed on an electronic scale, the animals were randomly divided into four treatment groups. The animals were housed in plastic cages measuring 16"x12"x10" (10 mice in each cage). All animals had free access to food and water *ad libitum*. They were maintained under standard laboratory conditions that is a well aerated room with alternating light and dark cycles of 12 h each and at room temperature of 25°C. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC). All rules applying to animal safety and care were observed.

Method

The animals were randomly assigned into four groups. Group I (n=10) was the control group, they were administered equivalent volume of intraperitoneal injection of normal saline (0.01 ml/g). Group II, III and IV (n=10) received intraperitoneal injections of MSG at 0.5, 1.0 and 1.5 mg/kg respectively. Behavioral testing was commenced immediately after administering monosodium glutamate or its vehicle (normal saline).Thirty minute period of the following behavioral states; horizontal locomotion, rearing and grooming were observed and scored at 10 min interval. This was used to characterize behavioral changes in the mice when placed in the open field.

Behavioural tests: The behavioural tests were conducted in a large quite room between the hours of 8 a.m. and 5 p.m. Novelty induced behaviours were evaluated using the open field box. Behaviours were scored by the authors using a stop watch; all animals in a group were tested on the same day (10 animals per day). All events were observed manually.

The open field box: The open field box is a rectangular area composed of a hard floor measuring 36 x 36 x 26 cm and made of white painted wood. The floor was divided by permanent red markings into 16 equal squares at the bottom. Generally, spontaneous motor activity was monitored for 30 min in the open field as described by Ajayi and Ukponmwan (1994). After treatment as explained earlier, each mouse was introduced into the field and the total locomotion (number of floor units entered with all paws), rearing frequency (number of times the animal stood on its hind limbs or with its fore limbs against the walls of the observation box or free in the air) and frequency of grooming (number of body cleaning with paws, picking of the body and pubis with mouth and face washing actions) within each 10 min interval were recorded. The arena was cleaned with 70% alcohol to eliminate olfactory bias and the arena allowed to dry before introducing a fresh animal.

Statistical analysis

All behavioral data were analyzed using the one way analysis of variance (ANOVA) followed by post hoc tests (Student Newman Keul's) carried out to determine the source of a significant effect.

Results are expressed as Mean ±S.E.M. $p<0.05$ was taken as accepted level of significant difference from control.

RESULTS

Effects of monosodium glutamate (0.5, 1.0 and 1.5 mg/Kg) on locomotor activity

Following thirty minutes of exposure in the open field, there was significant ($F (3, 39) = 3.94$, $p<0.05$) retardant effect on locomotor activity between the drug treated animals and vehicle treated animals at dose level of 1.5 mg/kg although this effect is seen also at dose levels of 0.5 and 1.0 mg/kg, respectively. It is however only visual as shown in Figure 1.

Effects of monosodium glutamate (0.5, 1.0 and 1.5 mg/Kg) on rearing activity

Following thirty minutes of exposure in the open field, there was significant ($F (3, 39) = 3.08$, $p<0.05$) retardant effect on rearing activity between the drug treated animals and vehicle treated animals at dose level of 1.5 mg/kg although this effect is seen also at dose levels of 0.5 and 1.0, respectively it is however, only visual (Figure 2).

Effects of monosodium glutamate (0.5, 1.0 and 1.5 mg/Kg) on grooming behaviour

Following thirty minutes of exposure in the open field, there was no significant ($F (3, 39) = 1.31$, $p = 0.285$) difference in grooming behaviour between the drug treated animals and vehicle treated animals at any of the dose levels. Visual increases in grooming behaviour were seen at all doses compared to control, although compared with the effect seen at the 0.5 mg/kg dose the responses seen at the 1.0 and 1.5 mg/kg doses were reduced as shown in Figure 3.

DISCUSSION

A number of studies in the past have been conducted to investigate the effects of glutamate administration on various body organs especially, the brain. This study however, tried to investigate the acute neurobehavioural effects of MSG using the open field. The study differs in at least one important aspect; lower doses of glutamate were used. It is already known that glutamate administered parentrally increases the brain free glutamate levels (Bogdanov et al., 1996), is neurotoxic at high concentrations and contributes to the developments of a number of neurodegenerative diseases (Narayanan et al., 2010). Our study investigated possible

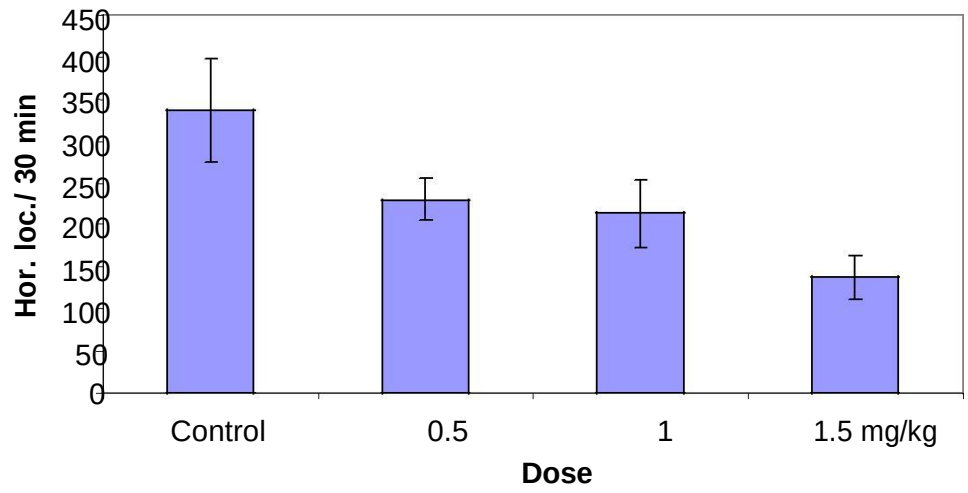

Figure 1. Effects of monosodium glutamate (0.5, 1.0 and 1.5 mg/kg i.p.) on horizontal locomotor activity in male mice over 30 min. Each bar represents, Mean ± S.E.M,* $p<0.05$ compared to control, n = 10.

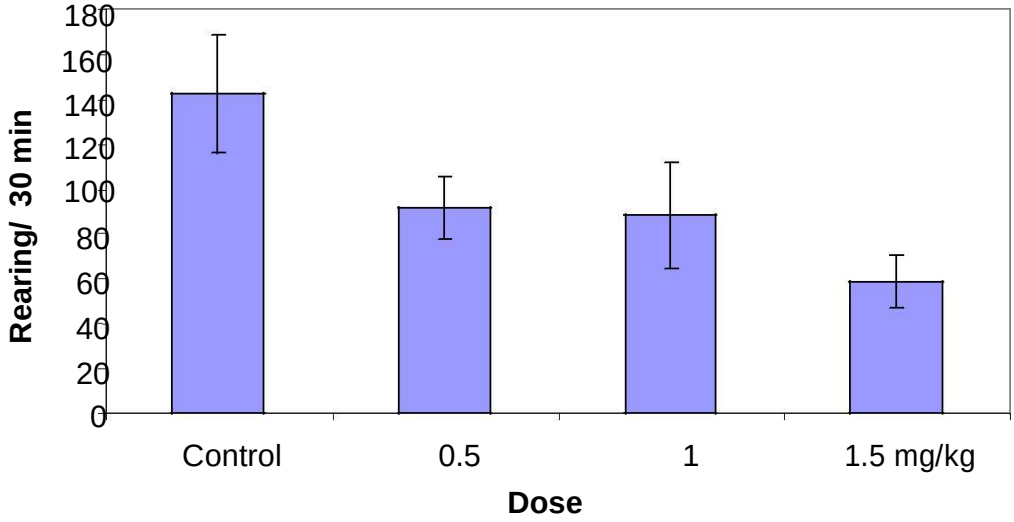

Figure 2. Effects of monosodium glutamate (0.5, 1.0 and 1.5 mg/kg i.p.) on rearing activity in male mice over 30 min. Each bar represents, Mean ± S.E.M, ¢ $p<0.05$ compared to control, n = 10.

neurobehavioural effects of MSG at dose levels otherwise accepted as being within normal limits with respect to a possible increase in brain free glutamate level, although we did not quantify level of free glutamate in the brain.

The data showed a reduction in horizontal and vertical locomotor (rearing) activity in the MSG treated at all dose groups during the open field tests. This effect of MSG on locomotion could be attributed to the modulatory effects of MSG on the hypothalamic brain regions. Reports suggest that activation of the rostro-caudal axis of the arcuate nucleus leads to their neurobehavioural reactions as seen in anxiety like behaviours in the elevated plus maze (Cortese and Phan, 2005) which we propose may be similar to the reactions seen here although studies that focus on the novelty induced behavioural effects of glutamate in adult animals do not appear to be common and results regarding changes of exploratory behaviour after neonatal MSG treatment are controversial (Dubovický et al., 1999). Some authors have described increased exploratory behaviour in mice (Dawson and Annau, 1983). However, Grimm and Frieder (1985) reported reduced exploratory behaviour, which is consistent with our findings.

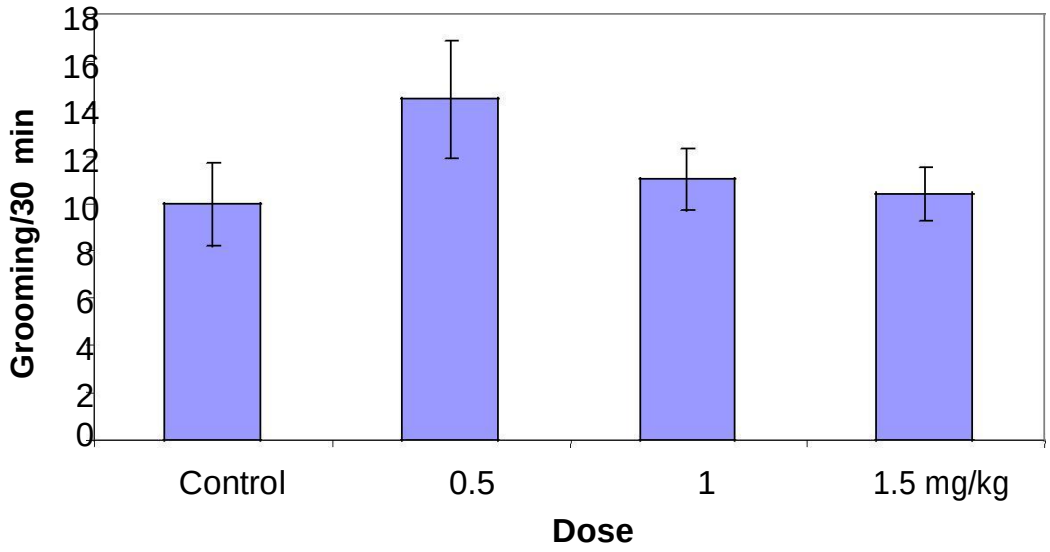

Figure 3. Effects of monosodium glutamate (0.5, 1.0 and 1.5 mg/kg i.p.) on grooming behaviour in male mice over 30 min. Each bar represents, Mean ± S.E.M, $p<0.05$ compared to control, n = 10.

It has been suggested that glutamate may play a more important role in general locomotion than previously thought. Glutamate is now known to exerts its actions in the brain by affecting the release of other neurotrans-mitters including monoamines and GABA (Cortese and Phan, 2005). Injection of glutamate antagonists had been shown to lead to induction of locomotion (Dalia et al., 1996) although this induction of locomotion was abolished by dopamine antagonists thereby suggesting that glutamate retards locomotion by interaction with dopamine. *In vivo* microdialysis experiments in awake, freely moving rats (Jedema and Moghddam, 1996, Takahata and Moghaddam, 1998) have demonstrated that both mild stress (that is handling) and intra-striatal infusion of AMPA/kainate agonists facilitate the presynaptic synthesis and release of dopamine in prefrontal cortex, while infusion of an NMDA agonist resulted in a trend toward a decrease in prefrontal cortical dopamine release (Jedema and Moghddam, 1996).

There was a visual increase in grooming activity at all doses studied although this increase was more marked at the lowest dose compared to the other doses, the increased grooming behaviour observed in a novel environment has been attributed to release of peptides derived from proopiomelanocortin (POMC), such as adrenocorticotropic hormone (ACTH), -melanocyte-stimulating hormone (-MSH), or -endorphin, which themselves can elicit grooming (Dunn et al., 1985). This is because novelty-induced grooming is attenuated both by hypophysectomy and by antiserum to ACTH injected into the cerebral ventricles. Expression of grooming in rodents is also known to be dependent on dopamine (Cools et al., 1988). Dunn et al. (1985) reported that neonatal treatment with otherwise high doses of MSG did not alter grooming behavior in either home or novel environment in adult rats, there were also no differences between MSG and saline-treated rats in the grooming scores observed following graded doses of ACTH administered intracerebroventricularly, therefore the visual enhancement of grooming activity seen at 0.5 mg/kg and then suppression at other doses relative to the 0.5 mg/kg dose may suggests that acute glutamate administration initially enhanced and then suppressed dopaminergic transmission as well as the release of other peptides. Could the mild increase seen be a response to the novel environment? Or could this be due to stimulation of dopamine receptors by free glutamate or release of other peptides derived from proopiomelanocortin family? These are the questions we intend to answer in further experiments.

Conclusion

These results provide evidence for the involvement of glutamatergic input in the control of behaviours such as locomotion and grooming. We therefore suggest that more attention should be paid to studies that investigate effects of glutamate at doses that may not cause obvious structural brain damage but nevertheless may have important behavioral effects.

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