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Lipid Peroxidation and Antioxidant Status of the Cerebrum, Cerebellum and Brain Stem Following Dietary Monosodium Glutamate Administration in Mice

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ABSTRACT

The effect of the ingestion of large amounts of MSG administered with diets on different brain regions was investigated. Animals were divided into two groups of six mice each. The control group was fed with water and diet only while the experimental group was placed on water and a diet containing 30% (w/w) MSG for 14 weeks. Internationally acceptable procedures were used for all the parameters analyzed in this study. Following MSG administration, there were increases ($p < 0.01$) in the total brain weight as well as the brain weight to body weight ratio with a reduction ($p < 0.01$) in body weight. The weights of both the cerebrum and the cerebellum were increased ($p < 0.05$). Lipid Peroxidation (LP) was increased ($p < 0.05$) in the cerebrum, reduced in the brain stem and remained unchanged in the cerebellum. Catalase (CAT) activity in both the brain stem and the cerebrum was increased ($p < 0.05$) but the cerebellum was unaffected. Superoxide Dismutase (SOD) activity and Glutathione (GSH) level were reduced ($p < 0.05$) in the cerebellum but the antioxidants were not affected in the cerebrum and the brain stem. The results of the present study suggest that the ingestion of large amounts of MSG with diet causes an increase in the weight of cerebrum with simultaneous increase in LP and reduction in CAT activity.

Key words: Monosodium glutamate, brain, excitotoxicity, lipid peroxidation, catalase, flavor enhancer

INTRODUCTION

Monosodium Glutamate (MSG) ($C_5H_9NO_4NaH_2O$) is widely used as a flavour enhancer in West African and Asian diet (Farombi and Onyema, 2006; Oluwole and Iyortim, 2006). It is a sodium salt of naturally occurring (non-essential) L form of glutamic acid (Singh and Pushpa, 2005). Glutamic acid is an excitatory amino acid neurotransmitter which is abundantly present in the brain of mammals, as well as in dietary protein (Rang *et al.*, 2003) but also acts as an excitotoxin when present in excess (Egbuonu *et al.*, 2010). Monosodium glutamate utilizes glutamate receptors throughout the mammalian body. These glutamate receptors are present in the central nervous

system as the major mediators of excitatory neurotransmission and excitotoxicity. Neural injury associated with trauma, stroke, epilepsy and many neurodegenerative diseases such as Alzheimer's, Huntington's and Parkinson's diseases and amyotrophic lateral sclerosis may be mediated by excessive activation of the glutamate receptors. Neurotoxicity associated with excitatory amino acids encountered in food, such as monosodium glutamate, has also been linked to glutamate receptors. Glutamate receptors are found in the rat and monkey heart, the conducting system, nerve terminals and cardiac ganglia. They are also present in the kidney, liver, lung, spleen and testis. Therefore, food safety assessment should consider these tissues as potential target sites (Gill *et al.*, 2000).

Many years ago, a link was forged between dietary MSG and brain function through the report that repeated injection of very large doses of the amino acid into neonatal rodents could produce visible brain damage, notably in the hypothalamus (Fernstrom, 2000). However, it is worthy of note that the experimental protocols employed by these scientists were unphysiological and of little or no relevance to nutrition (Adenuga, 1994).

The present study was designed to investigate the effects of the ingestion of large amounts of MSG (30% w/w) in diet on lipid peroxidation and antioxidant status of the cerebrum, cerebellum and brain stem of mice.

MATERIALS AND METHODS

Animals, diets and chemicals: The study was carried out in 2008. Adult male mice obtained from the animal house of the University of Agriculture, Abeokuta, Nigeria were used for the experiment while the feed was obtained from Tala-Tani feed mill, Ikenne Remo, Nigeria. All reagents were obtained from Sigma Chemical Co., USA and BDH Chemicals Ltd; Poole, England.

Animals were randomly assigned to two groups, A and B with six (6) mice per group. Animals in group A served as the control and were fed with water and diet only while animals in group B were placed on water and a diet containing 30% (w/w) monosodium glutamate. Food and water for both groups were supplied *ad libitum* and were housed in wire cages. At the end of fourteen weeks, the animals were sacrificed. The heads were dissected and brains were removed and immediately separated into cerebrum, cerebellum and brain stem. These different parts of the brains were kept frozen in the freezer at -4°C for analyses. All experimental procedures were performed in compliance with international guidelines on Animal Ethics.

Preparation of brain homogenate: Two sets of brain homogenates were prepared. 0.1 g of each part of the brain of individual sample was homogenized in 1 mL 0.01 M phosphate buffer, pH 7.4, kept frozen in the freezer and used for Catalase, Glutathione and Lipid Peroxidation analyses while another 0.1 g of each of the brain sample was homogenized in 1ml of isolation buffer (250 mM Sucrose, 5 mM Tris, 1 mM Mercaptoethanol and 0.5 mM Phenylsulphonyl-flouride (PMSF) pH 7.4) also kept frozen in the freezer and used for Protein determination and Superoxide dismutase analyses.

Lipid peroxidation assay: Brain lipid peroxidation was carried out by measuring the Thiobarbituric Acid-Reactive (TBAR) products using the procedure of Vashney and Kale (1990). The method is based on formation of pink colored product when Malondialdehyde treated with 2-thiobarbituric acid which has a maximum absorbance at 531.87 nm.

Glutathione assay: Assay for Glutathione (GSH) was done by the method of Beutler *et al.* (1963). This method is based on the development of a stable yellow color when a 2-nitrobenzoic acid is added to sulfhydryl compounds.

Catalase assay: The catalase activity of each brain samples was determined by the method of Sinha (1972) but with a slight modification. 0.1 mL of each brain part was mixed with 4.9 mL of distilled water. One milliliter of the mixture was added to H₂O₂-phosphate buffer mixture. The principle is based on the formation of chromic acetate when hydrogen peroxide (H₂O₂) reacts with dichromate-glacial acetic mixture at 100°C. The decomposition of H₂O₂ when acted upon by catalase and reduction in the green coloration is measured spectrophotometrically at 570 nm.

Superoxide Dismutase (SOD) assay: SOD activity was determined by the method of Del-Maestro *et al.* (1983). This assay is based on the ability of SOD to scavenge superoxide anion radical (O₂⁻) which by shortening reaction chains, decreases the overall rate of pyrogallol autoxidation.

Statistical analysis: The statistical analysis was carried out using independent t test at 99% confidence level for Table 1. For Table 2, One-way Analysis of Variance (ANOVA) was used and the differences between means were determined by the use of Duncan multiple range tests at 95% confidence level (SPSS software).

RESULTS

As shown in Table 1, there was a significant reduction ($p < 0.01$) in the body weight (18.92 ± 3.17) of MSG fed mice and a significant increase ($p < 0.01$) in the total brain weight (1.75 ± 0.23) as well as the brain weight to body weight ratio (0.095 ± 0.023) when compared with the control (26.11 ± 2.40 , 1.09 ± 0.32 and 0.043 ± 0.015), respectively. In Table 2, however, results show that there is a significant increase ($p < 0.05$) in lipid peroxidation in the cerebrum (3.11 ± 0.49) as against

Table 1: Effect of MSG oral administration on body weight, brain weight and brain weight/body weight of adult mice

Treatment	Body weight (g)	Brain weight (g)	Brain weight/Body weight (g)
Control (n = 5)	26.11±2.40	1.09±0.32	0.043±0.015
SG fed mice (n = 7)	18.92±3.17**	1.75±0.23**	0.095±0.023**

Oral administration of MSG 30% (w/w) was given for a period of 12 weeks. ** $p < 0.001$. Values are presented as Mean±SD

Table 2: Effects of MSG oral administration on antioxidant levels and other biochemical parameters in the brain of adult mice

Sections of brain	Treatments	Weight	Lipid peroxidation (MDA)×10 ⁵	Catalase activity (units g ⁻¹ tissue)×10 ⁶	Superoxide dismutase (ng SOD mg ⁻¹ protein)	GSH (mg g ⁻¹ tissue)
Cerebrum	Control	0.65±0.29 ^c	1.99±0.35 ^a	5.33±0.44 ^d	2.30±0.39 ^b	47.94±2.21 ^b
	Treated	0.89±0.20 ^a	3.11±0.49 ^f	2.04±0.47 ^a	2.28±0.52 ^b	46.95±2.15 ^b
Cerebellum	Control	0.19±0.06 ^a	2.15±0.46 ^{ab}	3.12±0.79 ^b	6.71±1.85 ^c	57.67±0.72 ^c
	Treated	0.46±0.16 ^{bc}	2.03±0.57 ^a	3.86±0.90 ^{bc}	1.22±1.00 ^{ab}	43.31±3.29 ^b
Brain stem	Control	0.25±0.20 ^{ab}	2.65±0.42 ^c	4.45±0.09 ^f	0.33±0.41 ^a	23.97±10.33 ^a
	Treated	0.40±0.10 ^{ab}	1.83±0.25 ^a	3.64±0.46 ^b	0.57±0.40 ^a	22.77±4.37 ^a

Experimental animals were given water and diet containing 30% (w/w) MSG for a period of 12 weeks while the control animals fed on the diet and water only. The superscripts a, b, c, d ($p < 0.05$) indicate the differences from the control. Values are presented as Mean±SD

(1.99±0.35). No significant change was observed in the cerebellum but a reduction in lipid peroxidation in brain stem was observed (1.83±0.25) when compared with the control (2.65±0.42). Also, there were significant decreases ($p<0.05$) in the activity of catalase in the cerebrum (2.04±0.47) and the in brain stem (3.64±0.46) at ($p<0.05$) but no significant effect was observed in the cerebellum when compared with the control cerebrum and brain stem (5.33±0.44 and 4.45±0.09), respectively. In addition, MSG induced significant reductions ($p<0.05$) in SOD activity (1.22±1.00) and in GSH level (43.31±3.29) at ($p<0.05$) of the cerebellum as against their control (6.71±1.85 and 57.67±0.72), respectively while there were no significant differences observed in both SOD activity and GSH level of both the cerebrum and the brain stem when compared with control. Also, there were significant increases ($p<0.05$) in the weight of both the cerebrum (0.89±0.20) and cerebellum (0.46±0.16) when compared to the control (0.65±0.29 and 0.19±0.06), respectively whereas the increase in brain stem weight was not significant.

DISCUSSION

Glutamic acid is in a class of chemicals known as excitotoxins. Abnormally high levels of excitotoxins have been shown in hundreds of animal studies to cause damage to areas of the brain unprotected by the blood brain barrier and that a variety of chronic diseases can arise out of this neurotoxicity (Meldrum, 1993). For example, MSG treatment of neonatal rats causes neuronal degeneration in various brain areas and leads to several neurochemical, endocrinological and behavioral alterations (Kiss *et al.*, 2005).

In an earlier report, it was shown that MSG ingested in large amounts with diet had no significant effect on brain weight; body weight as well as brain weight to body weight ratio and concluded that the ingestion of MSG even in massive doses has no harmful effect on the brain (Adenuga, 1994). However, the present results show that the ingestion of large amounts of MSG with diet caused a significant reduction in body weight. The difference in results might be due to the 100% increase in the amount of MSG added to the diet in the present study (30% (w/w)) as against 15% (w/w) in the former study. This toxicity also affected the brain as its weight was significantly increased (Table 1). Recent advances indicate that glutamate, the predominant excitatory neurotransmitter in mature neurons, can influence immature neural cell proliferation and differentiation (Garcia-Bueno *et al.*, 2007). The increase in brain weight observed in this result supports the finding and shows that glutamate can influence proliferation and neuronal commitment as well and acts as a positive regulator of neurogenesis. It has been reported that brain injuries like ischemia, epilepsy or stress lead to severe neuronal death and additionally, influence neurogenesis. Glutamate homeostasis is altered under these pathological circumstances, implying that therapeutic treatments mediating glutamate signaling might be useful to increase neuronal replacement after loss in the brain (Schlett, 2006).

Also, the result of this study about increase in the weight of the brain in some regions and non-effect in another is consistent with Erb hypothesis that monosodium glutamate as food and vaccine additive triggers unchecked brain cell growth which results in an overgrowth of certain areas of the brain rendering them damaged or destroyed, while accelerating the development of other areas (Courchesne *et al.*, 2003). The organ to body weight ratio of the MSG-treated mice is also higher (121% increase) than the value obtained for untreated control. This further buttresses the fact that MSG abnormally accelerates the growth of neurons and stimulates brain cell proliferation. The increase in brain weight is however, not restricted to a particular brain region as it could be seen on Table 2 though it was not significant in the brain stem.

MSG administered with diet at a dose of 30% (w/w) significantly increase ($p < 0.05$) lipid peroxidation in the cerebrum with no significant difference in lipid peroxidation of cerebellum when compared with control. However, the lipid peroxidation in the brain stem was found to be lower than the control mice. The increase lipid peroxidation observed in the cerebrum might be as a result of increase glutamate level leading to excitotoxic neuronal death through the activation of N-methyl-D aspartate (NMDA) and non NMDA glutamatergic receptors in the CNS (Gonzalez-Burgos *et al.*, 2004; Segura-Torres *et al.*, 2006). The increase in lipid peroxidation could also be initiated by changing the redox potential of the cell and thus favoring lipogenesis (Sushma *et al.*, 2006). While the results show that MSG has no significant effect on lipid peroxidation in cerebellum, the reason for reduction in lipid peroxidation of MSG treated mice when compared with the control, in brain stem, remains yet unclear.

The antioxidant status of the various brain regions varies widely. The results of the effect of MSG on the brain enzymes in this study were at variance with previous study. This may be due to the fact that, like earlier said, 100% increase in the amount of MSG was used in this study. In addition, it was the effect of MSG on total brain that was earlier reported whereas different sections of the brain were analyzed in this study. The result of this study shows a decrease in the activity of Catalase (CAT) in the cerebrum and brain stem which could be due to less availability of NADPH, as MSG favors lipogenesis by increasing the level of glutamine (Choudhary *et al.*, 1996; Abdel Beky *et al.*, 2009). However, the activity of catalase in the cerebellum was not affected. These results are consistent with the present results on the levels of lipid peroxidation in the cerebrum and cerebellum. Reports have shown an association between reduced catalase activity and neurodegenerative diseases (Adenuga *et al.*, 2009; Batcioglu *et al.*, 2005). The result of this study established the fact that MSG affects the various regions of the brain differently. The decrease in catalase activities in cerebrum and brain stem suggest a repression of catalase synthesis by MSG administration. Meanwhile, the activity of SOD and GSH levels were not significantly affected in both the cerebrum and the brain stem while a significant reduction in cerebellum was recorded when compared with the control. These observations might account for the report of Olney (1969) that glutamate treatment did indeed cause brain lesions, particularly acute neuronal necrosis in several regions of the developing brain of neonatal mice and acute lesions in the brains of adult mice.

CONCLUSION

The results of the present study suggest that the ingestion of large amounts of MSG with diet causes an increase in the weight of cerebrum with simultaneous increase in lipid peroxidation and reduction in catalase activity in that region of the brain of mice.

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