Monosodium Glutamate Induces Oxidative Stress and Affects Glucose Metabolism in the Kidney of Rats

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Research Article

ABSTRACT

Monosodium glutamate (MSG) is a widely used flavour enhancer with a number of adverse effects. Earlier studies have shown the induction of oxidative stress in some organs of experimental animals after chronic administration of MSG. Some reports have also shown some alterations in hepatic glucose metabolism as a result of MSG administration. In this study, we have tested the hypothesis that alteration in glucose metabolism following MSG administration might be a contributor to the changes in the markers of oxidative stress observed in the animals. Twenty four male Wistar rats were divided into two groups. MSG was orally administered to one group of rats at a dose of 4g/kg body weight for ten days while the other group received normal saline. MSG-treated rats showed a significant alteration (P<0.05) in a number of oxidative stress parameters and a significant (P<0.05) increase in the activity of glucose-6- Phosphatase (G6Pase), corroborating earlier observations. In addition, there was a decrease in the activity of glucose-6-phosphate dehydrogenase (G6PD) and a significantly (P<0.05) higher blood glucose and renal glucose concentration in MSG-treated rats. There was no change in renal glycogen concentration following MSG administration. The pattern of induction of oxidative stress and alteration of glucose metabolic enzymes in the animals is an indication that oxidative stress induced by MSG in the renal tissues of rats might be contributed by increased tissue glucose concentration resulting from enhanced renal gluconeogenesis.

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1. INTRODUCTION

Monosodium glutamate (MSG) is the sodium salt of glutamic acid, an amino acid considered to be a potential renal gluconeogenic precursor. Although about 25% of the human population is reported to be sensitive to MSG (Kerr et al., 1979), MSG still remains a widely used flavour enhancer. The group of people that reacts to MSG does so, even at doses recommended in food (Schaumburg et al., 1969). Earlier studies, which have also been confirmed on more recent reports on the adverse effects of MSG laid emphasis on its effects on the hypothalamus-pituitary axis of the brain, leading to its neuro-excitatatory/neuro-endocrine effects and induction of obesity (Feldman and Weidenfeld, 2005; Hee et al., 2010). More recent studies have examined other metabolic and toxic effects of MSG, with a number of the reports showing that showing the induction of oxidative stress in different tissues of experimental animals after administration of chronic doses of MSG (Onyema et al., 2006; Farombi and Onyema, 2006; Diniz et al., 2004; Singh et al., 2003). MSG administration has also been associated with hyperglycemic conditions based on parameters tested in the brain, serum and liver of experimental animals (de Andrade et al., 2006; Diniz et al., 2005; Diniz et al., 2004); increased glucose-6-phosphatase activity and decreased hexokinase activity in rat liver (Diniz et al., 2004). Administration of monosodium glutamate has also been associated with increased body weight (Egbuonu et al., 2010), adversely affected locomotor activities (Eweka and Om'Iniabohs, 2008) and altered lipid metabolism (Egbonu and Osakwe, 2011). However, limited information exists on the role of the kidney in MSG metabolism *vis-à-vis* the effects of MSG on the renal physiology. Stumvoll (1998) and Stumvoll et al. (1999) had suggested the need for studies on the regulation of renal glucose metabolism by substrate availability, and changes that occur in the kidney during increased gluconeogenesis. This made it imperative for a study on the relationship between the effects of MSG on biochemical parameters of glucose metabolism and oxidative stress in the kidney.

Hyperglycemia and oxidative stress are two important factors that contribute to renal damage as shown in different pathological disorders (Koya et al., 2003). The kidney could produce as well as utilize a significant amount of glucose (Schoolwerth and Drewnowska, 1993), and is therefore involved in the regulation of the amount of glucose in circulation. Glutamic acid has been suggested as one of the amino acids utilized by the kidney during gluconeogenesis since the net uptake of important gluconeogenic precursors such as lactate, glycerol, glutamate, glutamine and other amino acids by the kidney accounts for the turnover of glucose by the kidney (Bjorkman and Felig, 1982; Brundin and Wahren, 1994). Increased influx of substances into (and increased uptake of substances by) the kidney has been associated with various changes and oxidative stress (Braunlich et al., 1994). This has been corroborated in more recent reports in which hyperglycemia caused oxidative stress in the kidney via the formation of free radicals (koya et al., 2003) and altered the antioxidant reactions catalyzed by ROS scavenging enzymes (Uchimura et al., 1999). Hyperglycemia is also known to increase glucose auto-oxidation (Wolff and Dean 1987) and labile glycation (Mullarkey et al., 1990), or intracellular activation of the polyol pathway (Williamson et al., 1993), with the subsequent oxidative degradation of the glycated protein enhancing the production of reactive oxygen species (Klein, 1995; Standl et al., 1996).

This study is therefore an attempt to gain further insight into the role of the kidney in gluconeogenesis, and the impact of gluconeogenesis on oxidative stress in the kidney of MSG-treated rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals

A total of twenty four male Wistar rats (180-200g) were used in this study. The animals were three months old. The experimental animals were kept in neat metallic cages in a properly ventilated animal house as the study lasted. They received humane care in accordance with the National Institute of health USA guideline, had access to a daily cycle of 12 hours of day light and 12 hours of darkness and were provided with standard rat chow (Ladokun Feeds, Ibadan, Nigeria) and tap water *ad labitum*.

2.2 Animal Treatment

The animals were randomly divided into two groups comprising twelve animals each. One group was administered monosodium glutamate (MSG) at a dose of 4g/kg body weight. This is the sub chronic MSG dose, and has been widely used in studying MSG induced toxic reactions in experimental animals (Diniz et al., 2004; de Andrade et al., 2006; Farombi and Onyema, 2006). Normal saline was used as the vehicle for MSG. Thus, the other group of animals was given normal saline. This served as the control group. All the substances were administered to the animals by oral gavages, once daily between the hours of 10.00 and 11.00 G.M.T. Fresh samples of MSG were always prepared prior to administration.

After acclimatization, the animals were subjected to a 24 hour fasting period. Blood was collected from the tail vein of the rats for the determination of fasting blood glucose concentration. The substances were administered to the rats for ten consecutive days. We have previously used the ten days administration period (Onyema et al., 2006; Farombi and Onyema, 2006). At the end of administration, the animals were subjected to another 24 hour fasting period. Blood samples were also collected for the determination of fasting blood glucose concentration. The animals were sacrificed by cervical dislocation and their kidneys carefully excised. The kidneys from each rat were separately washed in ice cold 1.15% KCl, dried in filter paper, weighed and homogenized in ice cold buffer (tris buffer, pH 7.4). After centrifuging the homogenate at 10,000rpm for 15 minutes at 0-4°C, we decanted the supernatants and kept them under frozen conditions for all the analysis on renal tissue. The concentration of protein in the samples was determined using the Lowry's method as described by Lowry et al. (1951). Bovine serum albumin (BSA) was used as the standard.

2.3 Determination of Oxidative Stress Markers

To determine the level of lipid peroxidation in the renal fractions, an aliquot of each tissue fraction was deproteinized in trichloroacetic acid and the concentration of thiobarbituric acid reactive substances (TBARS) in the deproteinized tissue fractions was measured spectrophotometrically at 532nm as previously described by Varsney and Kale (1990). The result was expressed as malondialdehyde (MDA) formed using an extinction coefficient of $1.56 \times 10^5 M^{-1} cm^{-1}$ for MDA.

The concentration of non-protein thiol compounds (NPSHs) was determined using the method of Jollow et al. (1974). This is an indirect way of measuring glutathione since NPSHs

in tissues are mostly glutathione. An aliquot of the tissue fraction was deproteinized in 4% sulphosalicylic acid and the absorbance of 2-nitro-5-thiobenzoate produced when Ellman's reagent reacted with NPSHs in the deproteinized tissues was read at 412nm.

GST activity was determined according to the method of Habig et al. (1974) as modified by Rajurkar et al. (2003). GST catalyzed the conjugation of Glutathione with 1-chloro-2, 4-dinitrobenzene, forming a complex that absorbed at 340nm. The activity was calculated using an extinction coefficient of $9.6 mMcm^{-1}$ for 1-chloro-2, 4-dinitrobenzene.

The activity of superoxide dismutase (SOD) was determined according to the method of Misra and Fridovich (1972). This was based on the rate of inhibition of the autooxidation of adrenaline to adenochrome by SOD. The optical density of adenochrome was measured at 480nm.

The activity of catalase in the renal tissues was measured using the method of Sinha (1972). The absorbance of the residual hydrogen peroxide after tissue catalase has acted on a given sample of hydrogen peroxide was read at 570nm. Hydrogen peroxide was used as the standard.

2.4 Determination of Parameters of Glucose Metabolism

Glucose-6-phophatase (G6Pase) activity was determined as previously described by Swanson (1950). The absorbance of inorganic phosphate liberated during the dephosphorylation of glucose-6-phosphate by G6Pase was measured in a solution of 1% ammonium molybdate at 700nm using appropriate blanks. The enzyme activity was calculated from the absorbance value.

To determine the activity of glucose-6-phosphate dehydrogenase (G6PD) in the renal tissue, the method of Bergmeyer (1984) was used. The increase in the absorbance of NADPH generated when the enzyme catalyzed the oxidation of glucose-6-phosphate in the presence of NADP at 340nm was the criterion used for the determination.

The glycogen content of the renal tissue was determined by boiling an aliquot of the homogenate at 100°C. Glycogen was precipitated with ethanol, followed by pelleting, washing and resolubilization in distilled water. The resolubilized solution was treated with anthrone reagent before the absorbance was read at 625nm (Ong and Khoo, 2000).

Renal glucose concentration was determined based on the glucose oxidase method. The absorbance of the coloured complex formed was read at 625nm. This was compared with a glucose standard treated in the same manner (Marks, 1959). The blood glucose concentration prior to MSG administration and before the animals were sacrificed was determined using a glucometer (One Touch Ultra Blood Glucose Monitoring System from Lifescan, Johnson and Johnson Company, Milpitas CA, USA). Blood samples were collected from the tail vein by making a cut near the tip of the tail each time the test was done.

2.5 Statistical Analysis

Experimental results were analyzed using the unpaired one-tailed student's t-test ($P<0.05$). All parameters of oxidative stress and glucose metabolism were expressed in units relative to the total protein concentration.

3. RESULTS

There was no change in the body weight, organ weight and protein concentration between MSG treated animals and the control (Table 1). However, we found significant differences between the two groups in the various parameters of oxidative stress we determined. MSG significantly ($P<0.05$) induced lipid peroxidation in the kidney of the experimental animals. The administration of monosodium glutamate also significantly ($P<0.05$) reduced the level of GSH (as a function of NPSHs) in the kidney of rats; while the activity of GST was significantly ($P<0.05$) increased in the renal tissues of MSG-treated animals when compared with the control. The activities of SOD and catalase in the renal tissues of rats that were treated with MSG were significantly ($P<0.05$) reduced when compared with their activities in the control group (Table 2).

Table 1. Effects of monosodium glutamate on animal weight, kidney weight and protein concentration

Groups	Initial animal weight (g)	Final animal weight (g)	Kidney weight (mg/g animal weight)	Protein concentration (mmol/L)
MSG	190 ± 10.00	189 ± 12.43	11.57 ± 2.25	19.00 ± 6.48
Normal saline	190 ± 10.00	191 ± 9.58	12.36 ± 3.66	18.33 ± 3.86

*Values given as mean $\pm$ standard deviation of twelve animals.*

Table 2. Effects of monosodium glutamate on some oxidative stress markers (lipid peroxidation, glutathione, glutathione S-transferase (GST) activity, superoxide dismutase (SOD) activity and catalase activity) in the renal tissue of rats*

Groups	Lipid peroxidation (nmolMDA/mg protein)	Glutathione (µmole/mg protein)	GST Activity (units/mg protein)	SOD activity (units/mg protein)	Catalase activity (units/mg protein)
MSG	$190.80^{\#} \pm 1.65$	$40.00^{\#} \pm 4.90$	$10.52^{\#} \pm 3.46$	$4.86^{\#} \pm 2.59$	$1.45^{\#} \pm 0.03$
Normal saline	108.20 ± 19.57	63.33 ± 15.69	4.06 ± 0.40	10.04 ± 2.89	6.03 ± 1.19

Values given as mean $\pm$ standard deviation of twelve animals.
[#]Significant difference compared at $P < 0.05$

Interesting observations were made in the various parameters of glucose metabolism we studied as shown in Figure 1 (a-e). The activity of glucose-6-phosphatase in the kidney of MSG-treated rats increased significantly ($P<0.05$) when compared with the control while the activity of Glucose-6-phosphate dehydrogenase was significantly ($P<0.05$) reduced by MSG treatment. There was no change in the renal glycogen concentration between the group administered MSG and the control group. We observed that MSG significantly ($P<0.05$) increased the renal glucose level. Our results also show that before the administration of substances to the animals, there was no change in the blood glucose concentration between the two groups. At the end of administration, the blood glucose concentration was significantly ($P<0.05$) higher in the group that received MSG.
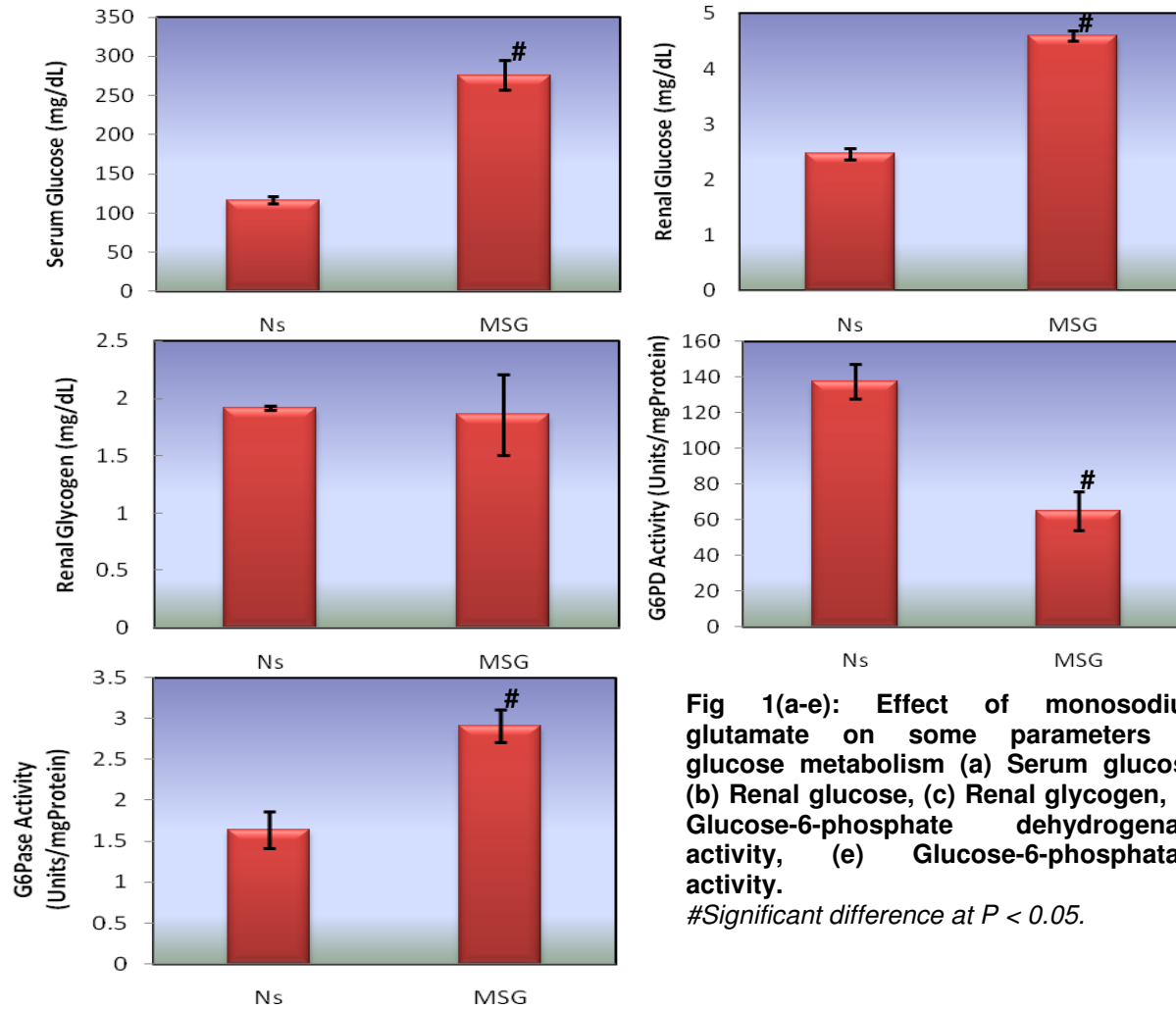
Fig 1(a-e): Effect of monosodium glutamate on some parameters of glucose metabolism (a) Serum glucose, (b) Renal glucose, (c) Renal glycogen, (d) Glucose-6-phosphate dehydrogenase activity, (e) Glucose-6-phosphatase activity.
#Significant difference at P < 0.05.

4. DISCUSSION

Monosodium glutamate is a popular food enhancer, which many manufacturers believe, can be used, as the consumer likes (Diniz et al., 2005). In this study, we investigated the effects of monosodium glutamate on markers of oxidative stress as well as some parameters of glucose metabolism in the kidney of rats.

Lipid peroxidation is a major indicator of oxidative damage initiated by ROS and causes impairment of membrane function (Selvakumar et al., 2006). The increase in lipid peroxidation observed in this study may be attributed to a direct effect of increased generation of ROS resulting from MSG treatment. Similar observations have earlier been reported in studies involving other organs (Onyema et al., 2006; Onyema and Farombi, 2006; Diniz et al., 2005; Diniz et al., 2004). A decrease in antioxidant status in an attempt by the tissues to restore their normal oxidative state may also subject the tissues to lipid peroxidation. Decreased concentration of GSH (measured as a function of NPSHs) in the kidney of our experimental animals, are similar to earlier observations that MSG induced oxidative stress in other tissues (Onyema et al., 2006; Onyema and Farombi, 2006; Singh et al., 2003). GSH is an important cellular antioxidant. Its depletion in this study correlates with the increase in lipid peroxidation observed in the kidney tissues.

Glutathione functions as an antioxidant in many ways. It can function as a direct radical scavenger and can also stabilize membrane structure through the removal of acyl peroxides formed during lipid peroxidation reaction (Price et al., 1990). This may have contributed to the decreased concentration of NPSHs observed in MSG treated animals. Glutathione depletion is a positive indicator of tissue degeneration and the magnitude of depletion parallels the severity of the damage (Andersen, 2004). The increase in the activity of glutathione S-transferase (GST) following MSG administration might have contributed to the depletion of tissue glutathione. GST catalyzes both glutathione-dependent conjugation and reduction (Ketterer et al., 1993). It detoxifies endobiotic and xenobiotic compounds by covalently linking glutathione to a hydrophobic substrate, forming less reactive and more polar glutathione S-conjugate (Neuefeind et al., 1997).

The activities of superoxide dismutase (SOD) and catalase decreased significantly ($P<0.05$) in MSG treated rats. This corroborated with earlier studies (Singh et al., 2003). The decrease in the activity of these enzymes could result from their inactivation by ROS or by their glycation. Products of glucose autoxidation cause protein glycation. Reduction in the activity of these enzymes might have contributed to the increased level of lipid peroxidation and decreased concentration of GSH. As these enzymes become inactivated, more GSH would be utilized in neutralizing acyl radicals and other ROS.

Increase in the renal tissue and blood glucose concentrations of rats administered with MSG can be associated with MSG administration (Malik et al., 1994, Chevassus et al., 2002). Thus, the increased blood glucose level following MSG administration was attributed to increased gluconeogenesis from glutamate and glutamine (Malik et al., 1994). In addition, significant ($P<0.05$) increase in the activity of glucose-6-phosphatase and a significant ($P<0.05$) decrease in the activity of glucose-6-phosphate dehydrogenase in MSG-treated rats are suggestive of decrease glucose utilization through the glycolytic pathway (Diniz et al., 2004). Diniz et al. (2004) had suggested a possible deterioration of glucose tolerance in rats following MSG administration. The abnormal glucose tolerance could be attributed to decreased cellular insulin sensitivity even under conditions of hyperinsulinemia observed in animals treated with MSG (Macho et al., 2000). Under conditions of hyperinsulinemia, cells

could switch to pathways that favour gluconeogenesis to compensate for the increased insulin release. Thus, our observations that MSG administration could not alter the tissue glycogen concentration but increased the activity of glucoce-6-phosphatase while decreasing the activity of glucose-6-phosphate dehydrogenase could be a response to hyperinsulinemia associated with MSG intake. This observation also shows that glycogenolysis does not play a role in renal glucose regulation. However, in situations where hyperinsulinemia and decreased insulin sensitivity occur simultaneously, the entrance of glucose into the cells diminishes and could lead to increased cellular glucose concentration. Decreased insulin sensitivity in MSG treated animals under conditions of hyperinsulinemia has been attributed to decreased expression of glucose transporter (GLUT4) proteins and insulin receptors (Macho et al., 2000). Thus, by promoting gluconeogenesis, MSG intake will increase the tissue glucose concentration and eventually lead to hyperglycemia. Hyperglycemia increases glucose autoxidation and the glycation of proteins (Kakkar et al., 1997). In addition to increasing the glucose level and the possible autoxidation of glucose that will eventually contribute to oxidative stress, the increased activity of glucose-6-phosphatase and decreased activity of glucose-6-phosphate dehydrogenase would also cause a decreased influx of glucose-6-phosphate into the pentose phosphate pathway. Consequently, there would be a decreased generation of NADPH needed for the regeneration of glutathione and other reducing agents needed for the maintenance of tissue integrity and oxidative balance. This shows a possible relationship between oxidative stress and altered glucose metabolism, and may have contributed to the increased renal oxidative stress in MSG-treated rats. MSG administration could therefore deplete the generation of reducing potentials required for the maintenance of tissue integrity, thus increasing the oxidative burden of the tissues.

5. CONCLUSION

Our present data indicated that chronic administration of MSG induced oxidative stress, and altered glucose metabolic processes in the renal tissues of rats. Oxidative stress induced by MSG in the renal tissues of rats resulted from increased tissue glucose concentration resulting from renal gluconeogenesis following MSG administration. Subjects with known problem of altered glucose metabolism and/or renal stress should be careful with consumption of monosodium glutamate.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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