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*Research Article*

# Effects of Monopotassium Glutamate on Mitochondrial Membrane Permeability Transition and Lipid Peroxidation- an *In-vitro* Study

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**Abstract**

Stimulation of Mitochondrial Membrane Permeability Transition (MMPT) pore opening as well as membrane lipid peroxidation by apoptotic and necrotic stimuli has been identified as the possible effectors of cell death in numerous pathological disorders. Studies have reported that ingestion of monosodium glutamate (MSG) stimulate MMPT pore opening *in vivo*. Consumption of potassium supplements and salt substitutes may cause a shift in potassium homeostasis resulting in a number of complications like paralysis and cardiac arrhythmia. Monopotassium glutamate (MPG) is used generally as flavor enhancer in food and salt substitute by people who are sodium restricted but there is dearth of information on the effect of MPG on MMPT pore opening. This study was therefore designed to assess the *in vitro* effect of MPG on MMPT pore opening and lipid peroxidation. Liver mitochondria were prepared from liver excised from male Wistar rats (100-120g) under ether anesthesia. Varying concentration of potassium and sodium glutamate (100, 200, 300 and 400 µg/ml) were tested on MMPT and lipid peroxidation in rat liver. The MMPT pore was estimated by measuring mitochondrial swelling while lipid peroxidation was estimated spectrophotometrically. Data were analyzed by one-way ANOVA and level of significance taken at $P<0.05$. Monopotassium and monosodium glutamate did not induce MMPT pore opening but induced generation of lipid peroxides. Monopotassium glutamate induced higher percentage of lipid peroxidation ($p<0.05$) compared to MSG. Monopotassium glutamate significantly induced lipid peroxidation but did not stimulate mitochondrial membrane permeability transition pore opening.

**Keywords:** Mitochondrial Membrane Permeability Transition (MMPT) pore, monopotassium glutamate, monosodium glutamate, lipid peroxidation

## INTRODUCTION

Glutamate is a neurotransmitter in the central nervous system (CNS), where it acts through inotrophic (iGluR) and metabotrophic (mGluR) glutamate receptors. It is required for neurotransmission, neuronal growth, axon guidance, brain development and maturation, and synaptic plasticity in health and disease (Monaghan *et al*., 1989; Madden, 2002). In addition to it being required in the CNS, studies have also shown the expression of glutamate receptors on different non-neuronal cells such as lymphocytes, hepatocytes and testicular cells in humans, mice and rodents (Storto *et al*., 2001; Lomardi *et al*.,2004). Studies have reported that glutamate mediates signaling pathway in both physiological and pathological conditions (Storto *et al*., 2001; Lomardi *et al*., 2004).

Over the years, neurotoxicity mediated by excessive activation of glutamate receptors has been associated with diverse neurodegenerative diseases. Recent studies showed that ingestion of glutamate in the form of monosodium glutamate (MSG) as food additive interferes with brain chemistry; thymocyte and lymphocyte function and induce obesity and fatty liver (Lomardi *et al*., 2004; Pavlovic *et al*., 2007; de Oliveira *et al*., 2011). The exact mechanism of glutamate toxicity still remains unknown. However, evidence have suggested necrosis and apoptosis as possible mechanisms implicating activation of mitochondrial membrane permeability transition (MMPT) pore and mitochondrial reactive oxygen species as the possible downstream pathway (Kanki *et al*., 2004; Zorov *et al*., 2010). Activation of MMPT pore involves glutamate-induced $Ca^{2+}$ influx or retention in the mitochondria with subsequent loss of permeability barrier, release of apoptotic protein such as cytochrome c, activation of degenerative enzymes such as proteases and generation of free radicals which promote apoptotic or necrotic pathways resulting in cell death (Kanki *et al*., 2004; Pavlvovic *et al*., 2007; Baines, 2010; de Oliveira *et al*., 2011).

Monopotassium glutamate is a general flavor enhancer and salt substitute for people who are sodium restricted. According to Hoskote *et al*. (2008), normal plasma potassium levels are important in maintaining cell growth, enzymatic function, DNA and protein synthesis. However, the risk of increased plasma potassium levels in sodium-restricted persons consuming potassium supplements or salt substitutes is high (Hollander-Rodriguez and Calvert, 2006). There are

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more scientific reports on the toxicity of sodium glutamate but there is dearth of information on the safety of monopotassium glutamate. This study was therefore designed to compare the effects of monosodium glutamate and monopotassium glutamate on MMPT pore opening and mitochondrial membrane lipid peroxidation with a view to ascertaining its safety.

## MATERIAL AND METHODS

### Animals

Experiment was performed on male Wistar rats (100-120g) obtained from Pre-Clinical Animal house, University of Ibadan, Nigeria and housed in well-ventilated cages under standard laboratory conditions. They were maintained on standard rat chow (Vital Feeds, Nigeria) and water *ad libitun*. All experiments were performed in accordance with the public health policy on Humane Care and Use of Laboratory Animals of National Institute of Health, USA.

### Preparation of rat liver mitochondria

Low ionic strength mitochondria were isolated using the method of Johnson and Lardy (1957). Liver tissue was excised from ether-anaesthetized rats and homogenized in 9X buffer (containing 0.12g Herpes, 3.83g mannitol, 2.4g sucrose and 0.038 EGTA in 100mls of distilled water, pH 7.4). Homogenate was serially washed and centrifuged before finally rinsed in another buffer (containing 0.12g Herpes, 3.83g mannitol, 2.4g sucrose and 0.5g bovine serum albumin (BSA) in 100mls of distilled water, pH 7.4 .All reagent were of analytical grade and obtained from Sigma Aldrich, USA

### Determination of mitochondrial protein content

Mitochondrial protein content was estimated by the method of Lowry *et al*. (1951). A volume of 50µl of stock mitochondrial preparation and 1ml of 1mg/ml bovine serum albumin (BSA) were diluted 20X with distilled water, after which 3mls of alkaline $CuSO_4$ was added. After 10mins, 0.3ml of Folin-Ciocalteau reagent was added and shaken vigorously. Absorbance at 750nm was measured after 30mins of incubation at room temperature against distilled water as blank. A standard protein curve was obtained using BSA (Sigma Aldrich, USA).

### Assessment of Mitochondrial Membrane Permeability Transition (MMPT) pore in rat liver mitochondria

Mitochondrial membrane permeability transition was assessed by determining mitochondrial swelling according to the method of Lapiudus and Sokolove (1993). Mitochondrial fraction was pre-incubated in 2200µl swelling buffer (containing 210mM mannitol, 70mM sucrose, 5mM Hepes in 100mls of distilled water, pH 7.4), 0.8µm rotenine and MSG/MPG (Sigma Aldrich, France) at varying concentration (100, 200, 300, 400 µl/ml) for 3mins after which $Ca^{2+}$ (triggering agent) was added. After 30seconds, 5mM of succinate was added to energize the reaction and absorbance measured at 520nm every 30s for 12mins.Spermine (4mM)was used as inhibitor and $CaCl_2$ to generate $Ca^{2+}$(triggering agent).

### Assessment of mitochondrial lipid peroxidation

Lipid peroxidation was assessed by measuring the thiobarbituric acid-reactive (TBARS) products (Ruberto *et al*., 2000). Mitochondrial fraction (0.4mg/ml) was incubated in varying concentrations of MSG/MPG (0.25, 0.5, 1, 2 and 4mg/ml) and 0.5mls of 0.07M $FeSO_4$ at room temperature for 30mins. Thereafter, 1.5mls of 20% acetic acid (pH 3.5) and 1.5mls of 0.8% (w/V) TBA in 1.1% sodium dodecyl sulphate were added, vortexed and heated at 95°C for 60mins. After cooling, 5.0mls of buta-1-ol were added and centrifuged at 3000rpm for 10mins. Absorbance of the organic upper layer was measured at 532nm.

Percentage inhibition by the salt (MSG and MPG) was calculated as

$$\frac{1-(E-C)}{C} \times 100$$

C – Absorbance value of the fully oxidized control
E – Absorbance value in the presence of MSG or MPG

### Statistical Analysis

Data were analyzed using one-way ANOVA and expressed as mean ± standard deviation. Significance was considered at $P < 0.05$ using Newman-Keuls' post-hoc test (Graphpad Prism 5.01, USA).

## RESULTS

Mitochondrial membrane permeability transition (MMPT) pore opening was triggered by $Ca^{2+}$. There was a progressive increase in pore opening as calcium concentration increases until the increase becomes steady. This was however reversed by spermine, an inhibiting agent (Figure 1).

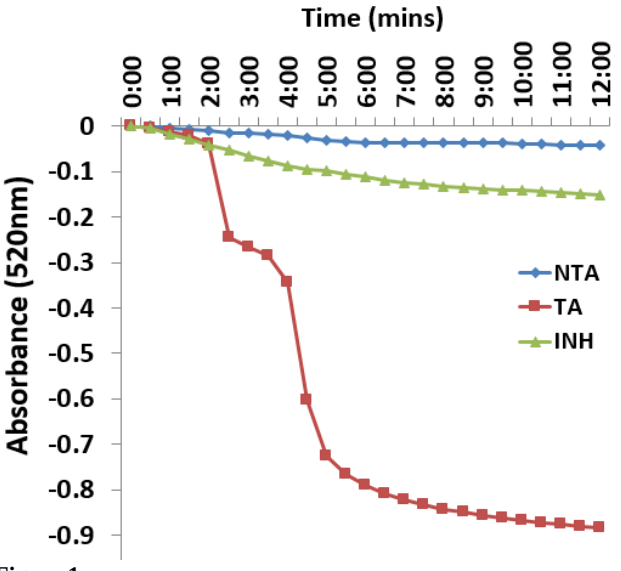


**Figure 1:**
Calcium-induced mitochondrial membrane permeability transition pore opening and its reversal by spermine (inhibiting agent)
NTA – No Triggering Agent; TA – Triggering Agent (Calcium)

Monopotassium glutamate and monosodium glutamate did not induce mitochondrial swelling and/or pore opening at all the concentrations studied when compared to $Ca^{2+}$-stimulated pore opening (Figures 2 and 3).

Monopotassium glutamate significantly ($p<0.05$) induced production of lipid peroxides at 0.25, 0.5, 1 and 2mg respectively compared to MSG. At 4mg, MPG and MSG induced lipid peroxides which were not significantly different from each other (Figure 4).

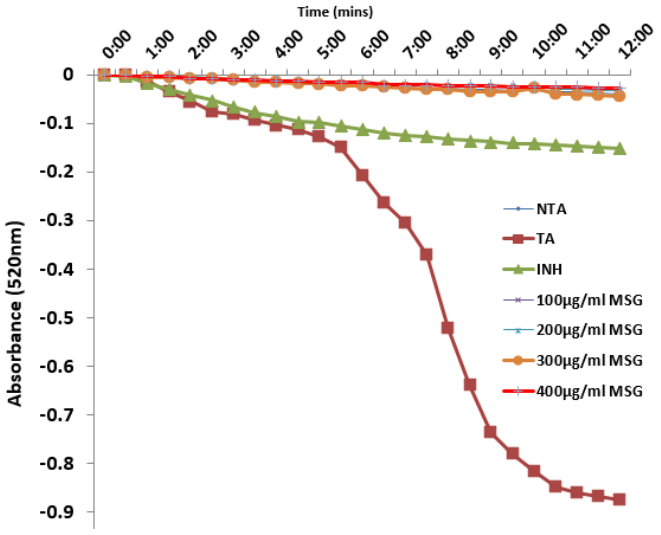


**Figure 2:**
Effect of Monosodium glutamate (MSG) on mitochondrial membrane permeability transition pore opening
NTA – No Triggering Agent
TA – Triggering Agent (Calcium)
INH – Inhibiting Agent (Spermine)

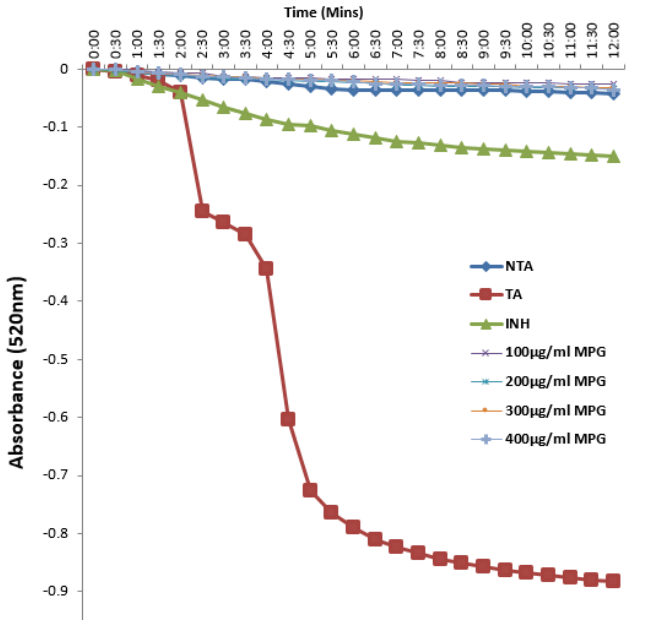


**Figure 3:**
Effect of Monopotassium glutamate (MPG) on mitochondrial membrane permeability transition pore opening
NTA – No Triggering Agent
TA – Triggering Agent (Calcium)
INH – Inhibiting Agent (Spermine)

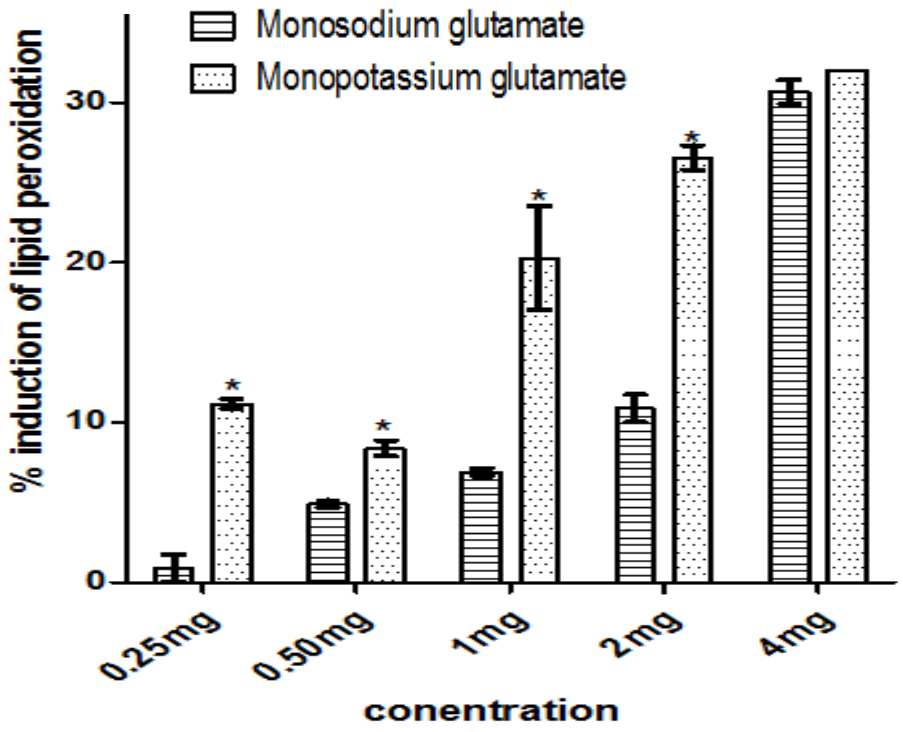


**Figure 4:**
Comparative percentage induction of lipid peroxidation by monosodium glutamate and monopotassium glutamate at varying concentrations
*Values are Mean ± SD, P<0.05 *significantly higher than monosodium glutamate*

## DISCUSSION

The reports of Kanki *et al*. (2004) and de Oliveira *et al*. (2011) stated that MSG mediates $Ca^{2+}$-induced mitochondrial pore opening *in vivo* whereas results obtained from this study did not show MMPT pore activation with MSG and MPG. This observation could possibly be due to absence of other factors that could be present *in vivo* in the mitochondrial microenvironment such as mitochondrial-associated endoplasmic reticulum membrane which is important in $Ca^{2+}$

signaling (Chipuk *et al*., 2006). Bonfocco *et al*. (1998) reported mild and intense effects of N-methyl-D-aspartate in cortical cell culture. They stated that apoptosis and necrosis of the cortical cells depended on the severity of stimulation by N-methyl-D-aspartate. It could be that a similar situation occurred in this study that is, concentrations of MSG and MPG used in this study may not be enough to stimulate MMPT pore opening but were adequate to stimulate generation of lipid peroxides. This is in support of the reports of Bindokas *et al*. (1996) and Zorov *et al*. (2010). However generation of free radicals was significantly increased with MPG than with MSG which could be due to increased uptake of electrons from membrane lipids and decreased antioxidant enzyme activities. Giebisch, (1998) reported that normal potassium levels is required for optimal enzymatic activities and any increase by 1% could result in severe hyperkalamia. Consumption of potassium supplements and salt substitutes such as MPG has been reported as possible factors that can cause increase in body potassium levels (Hoskote *et al*., 2008). This implies that generation of lipid peroxides stimulated by MPG could be connected with alterations of mitochondrial enzyme activities induced by increased extracellular potassium level. In addition, Bindokas *et al*. (1996) and Schelman *et* al., (2004) reported that increased generation of free radicals and imbalance between Bax and Bcl-2 apoptotic proteins also promote MMPT pore opening and cell damage which means, MMPT pore opening may not be caused by $Ca^{2+}$ influx alone. This study shows that MSG and MPG stimulate production of lipid peroxides *in vitro* but did not activate MMPT pore opening. However, the effect of monopotassium glutamate on MMPT pore opening needs to be investigated *in vivo*

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