

Original Article

The effects of monosodium glutamate and aspartame on rat hippocampal N-methyl-D-aspartate receptor subunits and oxidative stress biomarkers

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Abstract: Object: Food additives have been used widely for promotion of food safety, flavoring and coloring. We aimed to investigate the effects of Monosodium Glutamate and Aspartame on hippocampal N-methyl D-aspartate receptor subunits and serum antioxidant enzymes and total oxidative stress indices. Materials and methods: Rats were randomly divided into four groups: Control, MSG (Monosodium Glutamate: 120 mg/kg), ASP (Aspartame: 40 mg/kg) and MSG + ASP for 8 weeks. NMDA receptor subunits, the oxidative stress indices and serum parameters were measured in hippocampal tissue and serum. Results: MSG and ASP consumption significantly increased MDA and NMDA receptor subunit levels (NR1, NR2A and NR2B) in hippocampus and decreased serum SOD and GSH-Px activities. There was no significant difference in serum MDA levels and total oxidative stress indices. Conclusion: MSG and ASP consumption alters NMDA receptor levels and may contribute to the pathogenesis of some neurodegenerative disorders via excitotoxicity.

Keywords: Monosodium glutamate, aspartame, NMDA receptors, oxidative stress, neurotoxicity

Introduction

Food additives (FA) have been used widely for promotion of food safety, flavoring and coloring. Two main groups of food additives are aroma enhancers and sweeteners (e.g. Monosodium glutamate, Aspartame). Food additives that are intended for human usage are generally approved after testing for their toxicity through animal toxicity tests. Safety evaluation protocols of these FA are well established [1].

Monosodium glutamate (MSG), the sodium salt of glutamate, is one of the most commonly used aroma enhancing agent for increasing flavor in meals and its consumption has increased in daily food intake recently. It produces a unique taste referred as a fifth taste, umami, which cannot be provided by other basic tastes. After absorption, glutamate is taken up from the extra-cellular region and transported into synaptic vesicles of astrocytes and subsequently released by exocytosis in central nervous system (CNS). It is used for some func-

tions such as serving as a substrate for glutathione synthesis and a neurotransmitter in CNS.

Aspartame, which is an artificial sweetener -200 times sweeter than sucrose- is comprised of aspartic acid, phenylalanine and methanol. It is used in different types of food products including desserts, multi-vitamins, chewing gums, pharmaceuticals and particularly in diet beverages.

Aspartame is hydrolyzed to three types of products mainly: two amino acids, phenylalanine (50%) and aspartic acid (40%), and methanol (10%). After ingestion, concentration of aspartic acid increases both in blood and brain. In astrocytic and neuronal cells, aspartic acid is then converted to aspartate and act as an excitatory neurotransmitter [2].

Both glutamate and aspartate are known as the principal excitatory neurotransmitters which are responsible for glutamatergic transmission in the CNS and show their effects via

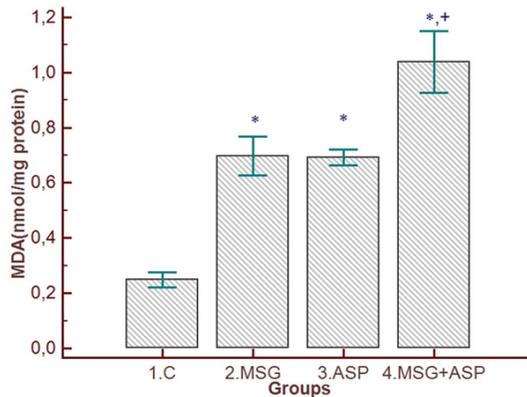


Figure 1. MDA levels of hippocampus tissue. ‘*’ Denotes significant difference at a P < 0.05 in comparison with control group. ‘+’ Denotes significant difference at a P < 0.05 in comparison with MSG or ASP group.

N-methyl-D-aspartate receptors (NMDARs). NMDARs are in the ionotropic group of the glutamate receptors. They are comprised of three main subunit classes: NR1, NR2 and NR3 which are involved in many CNS functions, including synaptic plasticity, learning, and memory [3].

Glutamate and aspartate are also recognized as excitotoxins. The excess of these amino acid, mainly glutamate, causes hyper excitability and induces death of neurons and astrocytes which are related to various pathological processes, such as memory impairment and Alzheimer’s disease [4]. Excitotoxicity occurs due to increased influx of calcium ions via glutamate-gated channels, which triggers catabolic processes and releases reactive oxygen species (ROS) which causes oxidative stress that lead to neuronal injury [5, 6]. Glutamatergic neuronal cell death occurs mainly in hippocampal region of patients with AD. However the data is limited about the effects of MSG and ASP consumption on neurotoxicity, oxidative stress and metabolic biochemical parameters. Although their widely consumption, the effects of MSG and ASP, singly or in combination, on NMDAR subunits have not been studied in experimental models and especially the combined effects of MSG and ASP usage still remain unclear.

Thus, the main aim of the present study is to investigate the effects of MSG and ASP consumption on glutamate receptor subunits NR1, NR2A and NR2B and oxidative stress in the hip-

pocampal tissues. Additionally, we aimed to examine the effects of these food additives on total oxidative stress and biochemical parameters in serum.

Materials and methods

Experimental animals

Wistar albino rats (n = 40) were randomly divided into four groups (n = 10): Control, (Serum physiologic), MSG (Monosodium Glutamate: 120 mg/kg-body weight), ASP (Aspartame: 40 mg/kg- body weight) and MSG + ASP. Experimental groups were received food additives with doses equivalent to the acceptable daily intake (ADI) established by Joint Expert Committee on Food Additives (JECFA) daily by oral gavage dissolved in tap water for 8 weeks. The ambient temperature was regulated between 20 and 22°C and water was available ad libitum. The experimental procedures were approved by The Animal Ethics Committee of Mustafa Kemal University (Code: 2014/2-6) and all procedures were carried out in accordance with the ethical guidelines for care and use of laboratory animals.

Chemicals

Anti-NR1, Anti-NR2A anti-NR2B and T-PER® (Tissue Protein Extraction Reagent) were purchased from Thermo (Thermo Fisher Scientific Inc., Waltham, MA USA), anti-Rabbit IgG and Protease Inhibitor Cocktail were purchased from Sigma® (Sigma-Aldrich Co., Missouri, MO USA). All other reagents were of analytical grade or the highest grade available.

Blood and tissue collection

Blood samples were collected from all animals through cardiac puncture and sera portioned after centrifugation at 5000 rpm for 10 minutes. After sacrificing the animals by decapitation, the brain was dissected and hippocampal regions were then isolated in cold conditions, washed in ice-cold phosphate-buffered saline (PBS) and rapidly frozen in liquid nitrogen. All blood and tissue samples were stored at -80°C until analyses.

Western blotting procedures

Hippocampus tissues were homogenized (1/20, w/v) with a glass-teflon homogenizer in

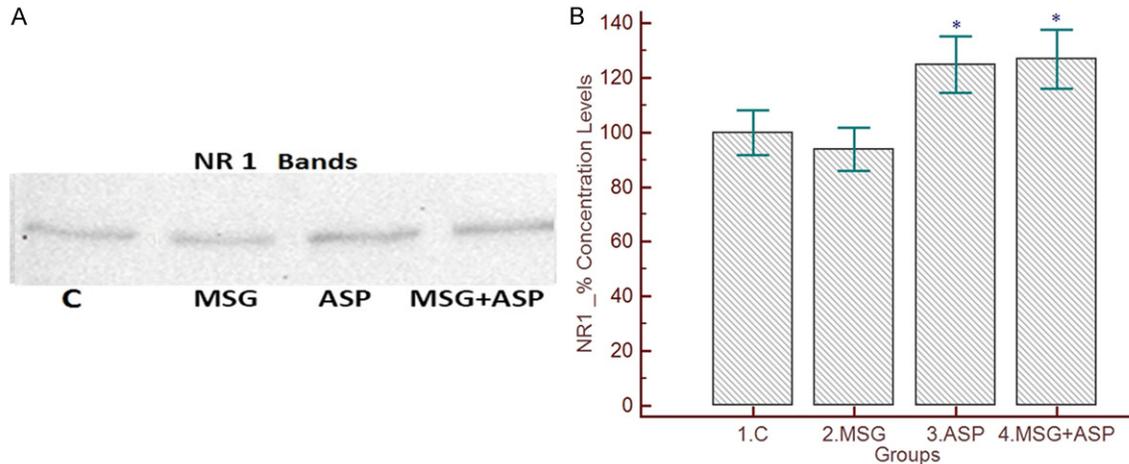


Figure 2. A. Western Blotting bands of NR1 subunit from hippocampi. First lane contains control group (C), second lane: Monosodium Glutamate group (MSG), third lane: Aspartame group (ASP), fourth lane: Monosodium Glutamate plus Aspartame group (MSG+ASP). B. Optic densities of NR1 subunit protein levels. Mean of control group data was assumed as 100 and % concentration values of experiment groups were given (Data are presented as mean \pm standard deviation). ** Denotes significant difference at a $P < 0.05$ in comparison with control group.

1% Protease inhibitor cocktail added T-PER[®] solution in an ice bath and frozen in liquid nitrogen and maintained at -80°C until analyses. Homogenates were centrifuged (14,000 g, 15 min, 4°C) and the supernatants were collected. Protein concentration of the homogenate was determined by Bradford assay [7]. Equal amounts of protein for each sample (50 μg per lane) were separated by SDS-PAGE on 7.5% mini gels. The proteins then blotted to PVDF membrane and incubated in Tris-buffered saline (TBST) with Tween 20 containing 3% Bovine Serum Albumin (BSA) for 60 min. Membranes were incubated overnight with anti-NR1 (1/2000), anti-NR2A (1/1000), anti-NR2B (1/1000) and anti-Beta Actin (1/250) in 1% BSA. Membranes were then washed three times (10-minutes periods) with TBST. Membranes were incubated with monoclonal anti-rabbit IgG (1/80,000) in 1% BSA for 1 h at room temperature and membranes were then washed three times (5-minutes periods) with TBST. The membranes incubated with ECL solution and visualized by ChemiDoc Imaging Systems (Bio-Rad Inc., USA). The results are expressed as arbitrary units corresponding to the subunit/Beta-Actin ratio of the individual samples.

Oxidative stress parameters

Hippocampal tissue protein levels were measured by the method of Bradford [7] and

Malondialdehyde (MDA) levels in tissue and serum by the method of Esterbauer and Cheeseman [8]. Serum Glutathione peroxidase (GSH-Px) enzyme activities were estimated by the method of Paglia and Valentine [9]; Total superoxide dismutase (Cu-Zn-SOD and MnS-OD) enzyme activities by the method of Sun et al. [10] and Catalase (CAT) enzyme activities by the method of Aebi [11] using a UV-Spectrophotometer (UV-1800 Shimadzu, Kyoto, Japan).

Biochemical analyses

Serum Total Antioxidant Status (TAS) and Total Oxidant Status (TOS) were measured by using commercially available kits (Rel Assay Diagnostic, Gaziantep, Turkey). Oxidative Stress Index (OSI) was calculated according to the following formula: OSI (arbitrary unit) = TOS ($\mu\text{mol H}_2\text{O}_2 \text{ Eq/L}$)/TAS (mmol Trolox Eq/L) [12]. Serum ALT (alanine aminotransferase), AST (aspartate aminotransferase) activities were measured spectrophotometrically by an auto-analyzer (Abbot Architect c8000, Abbott Park IL, USA).

Statistical analyses

All data were analyzed statistically using MEDCALC statistics program. The differences between groups were analyzed with Kruskal-Wallis test, and then multiple comparisons were performed with the Mann-Whitney U-test.

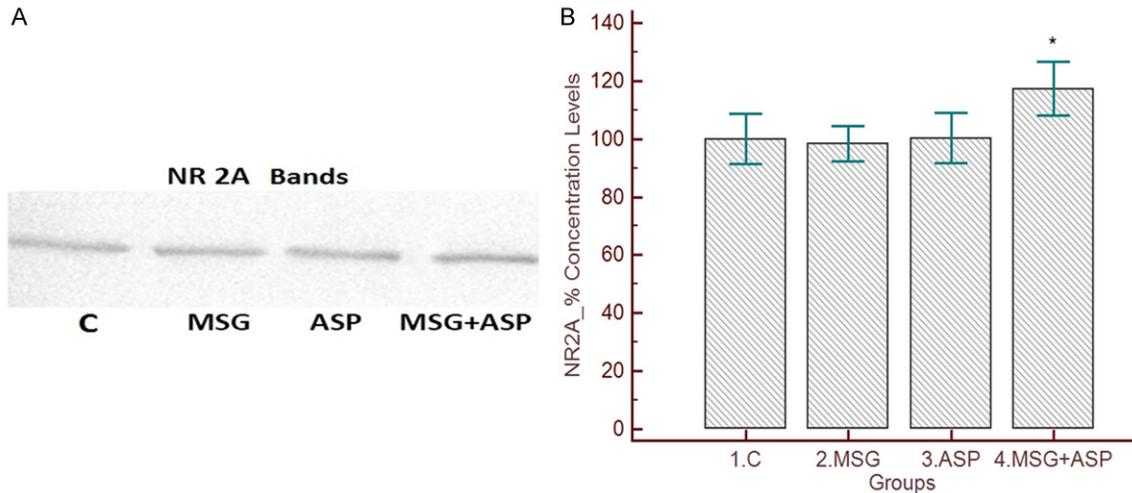


Figure 3. A. Western Blotting bands of NR2A subunit from hippocampi. First lane contains control group (C), second lane: Monosodium Glutamate group (MSG), third lane: Aspartame group (ASP), fourth lane: Monosodium Glutamate plus Aspartame group (MSG+ASP). B. Optic densities of NR2A subunit protein levels. Mean of control group data was assumed as 100 and % concentration values of experiment groups were given (Data are presented as mean \pm standard deviation). Asterisk '*' denotes significant difference at a $P < 0.05$ in comparison with control group.

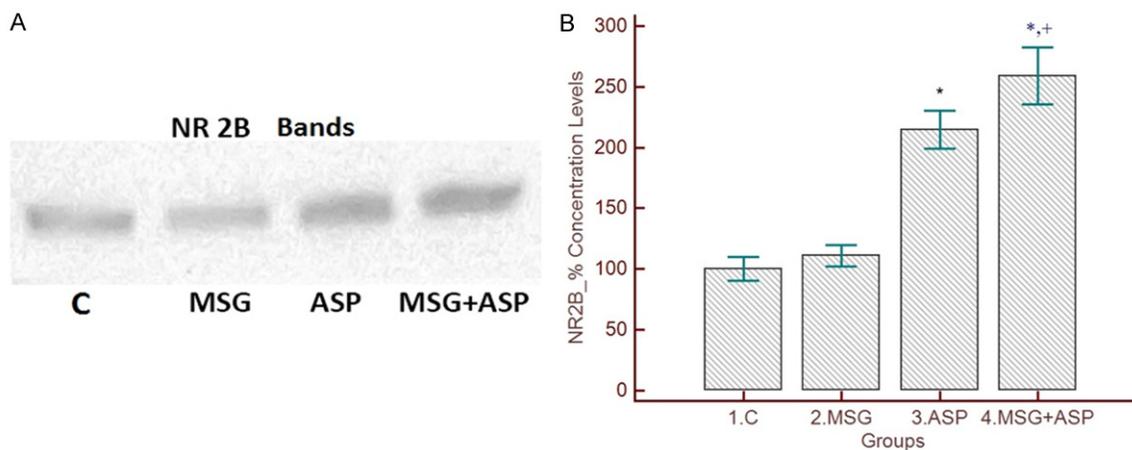


Figure 4. A. Western Blotting bands of NR2B subunit from hippocampi. First lane contains control group (C), second lane: Monosodium Glutamate group (MSG), third lane: Aspartame group (ASP), fourth lane: Monosodium Glutamate plus Aspartame group (MSG+ASP). B. Optic densities of NR2A subunit protein levels. Mean of control group data was assumed as 100 and % concentration values of experiment groups were given (Data are presented as mean \pm standard deviation). '*' Denotes significant difference at a $P < 0.05$ in comparison with control group, '+' denotes significant difference at a $P < 0.05$ in comparison with MSG or ASP group.

The results were given as mean \pm standard deviation (SD). A p value of less than 0.05 was considered statistically significant.

Results

MDA levels of the hippocampal tissue were increased significantly in the MSG and ASP groups whereas; MSG+ASP group's MDA level

were significantly higher from both MSG and ASP groups (**Figure 1**).

NR1, NR2A and NR2B receptor protein levels were significantly higher in MSG+ASP group compared to control group. NR1 and NR2B receptor protein levels were significantly higher in ASP group compared to control group whereas there was no significant difference between

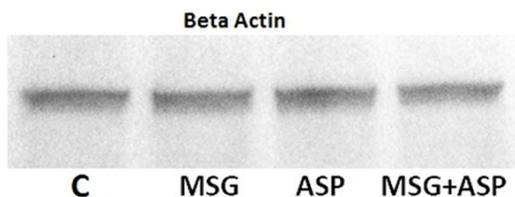


Figure 5. Western Blotting Bands of Beta Actin.

MSG and control group (**Figures 2A-4A, 2B-4B, 5**).

No significant difference was observed among all groups for serum total oxidative stress biomarkers (TAS, TOS and OSI). Significantly higher serum SOD and GSH-Px enzyme activities were observed in MSG, ASP and MSG+ASP groups compared to control but no significant difference was seen in serum MDA levels among all groups. Serum alanine and aspartate transaminase (ALT, AST) activities did not show any significant difference among groups (**Table 1**).

Discussion

Our results demonstrated that both MSG and ASP consumption individually as well as in combination, caused oxidative stress and this has been documented with increased levels of MDA in hippocampus. The receptor levels of all NMDAR subunits (NR1, NR2A and NR2B) increased significantly with MSG+ASP consumption.

Although, the safety assessment procedures are clearly documented and ADI values of MSG and ASP are well established, the doubt about the safety and toxicity of these food additives especially on brain tissue have gradually increased recently and the data in this field are controversial. It has been previously reported that dietary glutamate did not cause serious medical problems related to neurodegenerative diseases [13]. On the other hand, Olvera-Cortes et al. reported that exposure to MSG neonatally causes different effects such as difficulty in learning processes in rats [14]. In another study, Olney et al. reported that oral glutamate and aspartate usage caused brain damage in infant mice [15]. It has been shown in a recent study that 10 days MSG treatment, orally or subcutaneously, increased beta-amyloid accumulation in rat hippocampus [16].

Furthermore, Gasem et al. reported that consumption of MSG and ASP in combination causes oxidative stress by increasing lipid peroxidation products in brain [17]. Similar with previous reported In the present study, we found that MSG and ASP consumption, both individually and in combination, caused increase in MDA levels-which is an indicator of oxidative stress. They also reported that both MSG and ASP had significant deleterious effects on cognitive functions and memory. The underlying mechanism of the effects of these food additives in brain tissue especially in hippocampal region can be explained by glutamate excitotoxicity. Glutamate, the most common excitatory neurotransmitter in the brain, considered as an excitotoxic agent and recognized as the most important mediator in neurodegenerative diseases especially in AD [18]. Aspartic acid, a metabolite of aspartame digestion, can stimulate neuronal NMDA receptors. Additionally, aspartic acid serves a source of endogenous glutamate, activates presynaptic and postsynaptic NMDA receptors especially NR2B subunit leading to hyper excitation of hippocampal neurons [2]. Therefore, consumption of MSG and ASP causes elevations of these excitotoxic agents in blood and CNS leading increased stimulation of postsynaptic NMDA receptors. Overstimulation of these receptors causes increased Ca^{+2} ion concentration inside the neuronal cells. Intracellular Ca^{+2} overload stimulates several proteases and triggers complicated metabolic pathways which lead to depletion of ATP stores and production of reactive oxygen species. As a result, excessive activation of NMDA receptors causes destruction of the neurons in hippocampal region [19]. This well-known phenomenon is called as excitotoxicity. Our findings support the fact that the use of ASP and MSG when administered especially in combination can be more effective than their individual use because of their cumulative effects on NMDA receptor subunits. In a previous study Mourad et al. used same dose of aspartame with our study and observed significant increases in the levels of oxidative stress indices in the brain [20]. Similarly, we observed significant increases in MDA levels of the hippocampal tissues.

We also measured the total oxidative stress indices in serum. According to our knowledge, this is the first study evaluating the relationship

Table 1. Total oxidative stress parameters, antioxidant enzyme activities and liver transaminases

Serum Parameters	Groups			
	C	MSG	ASP	MSG+ASP
Antioxidant Enzymes				
MDA (Nmol/mL)	3.95±0.39	4.11±0.41	3.8±0.39	4.18±0.40
SOD (U/mL)	12.9±1.9	7.9±1.5*	12.1±1.1*	7.8±0.8*
GPx (U/L)	366±50	261.8±29.4*	274.9±11.9*	252.8±36.9*
Systemic Oxidative Parameters				
TAS (Trolox equivalent/L)	1.5±0.3	1.4±0.1	1.2±0.1	1.5±0.1
TOS (µmol H ₂ O ₂ Eqv/L)	19.3±10	24.6±16.8	11.8±3.6	15.7±11.1
OSi (AU)	12.4±4.3	16.2±10.5	9.2±3.1	10±5.4
Liver Transaminases				
ALT (U/L)	57.6±15	61±11.6	69.7±10.9	68.3±13.8
AST (U/L)	142.9±38.9	153.5±63.1	119±16.2	141.5±28.8

*Denotes significant difference at a P < 0.05 in comparison with control group.

between food additives and systemic oxidative stress biomarkers. We observed that serum TAS, TOS levels and calculated OSI index did not show any significant difference among the study groups but serum SOD and GSH-Px activities were found to be decreased, similar with a previous study by Diniz et al. [21]. Our results indicate that increased oxidative stress due to consumption of MSG and ASP, both individually and in combination, was limited to hippocampal tissue and didn't cause a systemic oxidative damage. The low serum antioxidant enzyme activities might be originated from various tissues or organs. Therefore, we can say that MSG and ASP consumption can lead to different effects in different tissues. We suggest that further studies, examining the antioxidant enzyme activities of various organs and tissues, especially whole brain and liver, should be conducted to evaluate the systemic reflection of local tissue damages by MSG and ASP.

No significant difference was seen in serum ALT and AST activities so we can conclude that chronic consumption of MSG and ASP did not cause any damage in liver. However, Diniz et al. reported that MSG administration was associated with oxidative stress in the hepatic tissue of young rats [21]. Elevation of serum ALT and AST levels with degenerative changes in hepatocytes after a single high dose intraperitoneal injection of MSG was noted in rats [22]. Hepatocellular damage due to long term exposure to MSG was also reported in albino mice after neonatal exposure [23]. We can argue that the effects of these food additives on hepatic tissue are needed to elucidate by dif-

ferent study designs including more biochemical and histopathological parameters.

We conclude that chronic and combined MSG and ASP consumption may alter neuronal NMDA receptors and lead to excitotoxicity, free radical formation and oxidative stress in hippocampal tissues. Additionally they can contribute to the pathogenesis of some neurodegenerative disorders.

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Disclosure of conflict of interest

None.

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