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Cognitive and biochemical effects of monosodium glutamate and aspartame, administered individually and in combination in male albino mice

Gasem M. Abu-Taweel [a], Zyadah M. A. [a], Jamaan S. Ajarem [b], Mohammad Ahmad [c,*]

[a] Department of Biology, Dammam University, Saudi Arabia
[b] Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia
[c] Department of Medical Surgical Nursing, College of Nursing, King Saud University, Riyadh, Saudi Arabia

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ABSTRACT

The present study was designed to investigate the in vivo effects of monosodium glutamate (MSG) and aspartame (ASM) individually and in combination on the cognitive behavior and biochemical parameters like neurotransmitters and oxidative stress indices in the brain tissue of mice. Forty male Swiss albino mice were randomly divided into four groups of ten each and were exposed to MSG and ASM through drinking water for one month. Group I was the control and was given normal tap water. Groups II and III received MSG (8 mg/kg) and ASM (32 mg/kg) respectively dissolved in tap water. Group IV received MSG and ASM together in the same doses. After the exposure period, the animals were subjected to cognitive behavioral tests in a shuttle box and a water maze. Thereafter, the animals were sacrificed and the neurotransmitters and oxidative stress indices were estimated in their forebrain tissue. Both MSG and ASM individually as well as in combination had significant disruptive effects on the cognitive responses, memory retention and learning capabilities of the mice in the order (MSG + ASM) > ASM > MSG. Furthermore, while MSG and ASM individually were unable to alter the brain neurotransmitters and the oxidative stress indices, their combination dose (MSG + ASM) decreased significantly the levels of neurotransmitters (dopamine and serotonin) and it also caused oxidative stress by increasing the lipid peroxides measured in the form of thiobarbituric acid-reactive substances (TBARS) and decreasing the level of total glutathione (GSH). Further studies are required to evaluate the synergistic effects of MSG and ASM on the neurotransmitters and oxidative stress indices and their involvement in cognitive dysfunctions.

1. Introduction

Food additives that are intended for human use are generally approved after testing for their toxicity through animal toxicity tests (Kokoski et al., 1990). The overall goal of such tests is twofold: to assess the additive's potential for causing toxic effects in humans and to determine if safe conditions of use can be established (Kokoski et al., 1990). However, evaluation for the safe consumption of such food additives is usually based on their toxicity data obtained from animal studies since human data are scantily available (Lin et al., 1992).

Monosodium glutamate (MSG) is one of the most popular flavoring agents of modern time and is widely used in many commercially packed food and restaurant and household cooking. It is reported that neonatal exposure to MSG (4 mg/g body weight) in rats and mice causes many effects like learning difficulty (Olvera-Cortes et al., 2005), obesity (Nagasawa et al., 1974), and gonadal dysfunction (Pizzi et al., 1978). Brain damage induced by the neurotoxicity of MSG has also been established in experimental chicken (Robinzon et al., 1974). MSG injected i.p. at 2 and 4 mg neonatally in mice produced lesions in the arcuate nucleus region of the brain affecting the regulation of water drinking (Morley and Flood, 1989). Some of the neurotransmitters like norepinephrine, serotonin, dopamine and their metabolites in the hypothalamus region were found to be depleted in MSG treated rats (Nakagawa et al., 2000). MSG administration (4 mg/g) has also been associated with oxidative stress in the hepatic tissue of young rats (Diniz et al., 2004). Elevation of serum alanine aminotransferase (ALAT) and aspartic aminotransferase (ASAT) with degenerative changes in hepatocytes after a single high dose intraperitoneal injection of MSG was noted in rats (Ortiz et al., 2006).Hepatocellular damage due to long term exposure to MSG (2 mg/g body weight) was also reported in albino mice after neo-natal exposure (Bhattacharya et al., 2011). On the contrary, some researchers reported that MSG taken with food showed no adverse effect (Stegink et al., 1985).

Aspartame (ASM) is a dipeptide (L-aspartyl-L-phenylalanine methyl ester) and is used as an artificial sweetener. ASM is used in a variety of

* Corresponding author at: Department of Medical Surgical Nursing, College of Nursing, King Saud University, P.O. Box 642, Riyadh 11421, Saudi Arabia. Tel.: +966 505195887; fax: +966 11 4693770.
E-mail address: mbadshah@ksu.edu.sa (M. Ahmad).

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food products; however, ASM-related neurological disturbances such as dizziness, headaches, gastrointestinal symptoms, mood alterations, allergic type reactions and alterations in menstrual patterns have also been reported (Coulombe and Sharma ,1986). Studies in mice have reported that alterations in brain neurotransmitters are responsible for the behavioral effects associated with ASM consumption at varying doses of 13, 130 or 650 mg/kg (Coulombe and Sharma, 1986). ASM consumption has also been reported to affect motor behavior in rats (Dourish et al., 1983). Furthermore, consumption of ASM by rats during pregnancy and lactation also affects their offspring's morphological and reflex development (Brunner et al., 1979). Oral intake of ASM in mice has been reported to be the cause of neuronal necrosis in several regions of the brain including the hypothalamus (Reynolds et al., 1976; Olney et al., 1980). In another study, Holder and Yirmiya (1989) reported that ASM had adverse effects in rats when injected intraperitoneally and not when administered orally. Possible epileptogenic or neurotoxic effects of ASM (34 mg/kg) have also been reviewed in experimental models (Stegink, 1987; Janssen and van der Heijdan, 1988).

As we can see from the above literature survey, plenty of studies on MSG and ASM individual exposures have been reported in experimental animals at neonatal stages and have looked for various deleterious effects at the adolescent and/or adult stages. However, the combined effects of MSG and ASM have not been studied in experimental models as widely as their individual exposures; and the combined effects of MSG and ASM still remain unclear. Olney and Ho (1970) and Olney et al. (1980) reported in neonatal mice that MSG and ASM in combination doses of 34 mg/kg each produced neuronal necrosis in brain tissue. Very recently, Collison et al. (2012) reported that MSG and ASM (120 and 50 mg/kg body weight/day respectively) administered in neonatal mice impaired their glucose and insulin homeostasis. On the contrary, little hazard has been reported from injection of combined doses of MSG and ASM in rodents and primates (Reynolds et al., 1976). Studies related to exposures to food additives in combined doses at adulthood stages are wanting. Furthermore, studies on the effects of MSG and ASM exposures (singly or in combination) on behavior and neurotransmitters and oxidative stress in brain tissue are also much needed in order to understand their biochemical correlation with the memory retention system.

Thus, it was hypothesized that consumption of MSG and ASM in combination could be comparatively more deleterious than exposure to them individually. Although no effort has been made to compare the doses of MSG and ASM used herein with doses that a human would be exposed to, the present study used doses that fall within the range of the doses used for a previous study conducted in adult humans (Stegink et al., 1982). Furthermore, the present study was hypothetically designed to investigate the in vivo toxic effects of MSG and ASM individually and in combination on cognitive behavior and to find out their correlation with biochemical parameters like some neurotransmitters and some oxidative stress indices in forebrain tissue regions that are reportedly responsible for cognitive activities.

2. Materials and methods

2.1. Experimental animals

Forty male Swiss–Webster strain mice (8–10 weeks old, bred and reared under controlled conditions) were housed in opaque plastic cages measuring 30 × 12 × 11 cm (5 animals per cage) under hygienic conditions in the animal facility of the Zoology Department, King Saud University, Riyadh, Saudi Arabia. All animals were maintained under reversed lighting conditions with white lights on from 22.00 to 10.00 h local time. The ambient temperature was regulated between 20 and 22 °C. Food (Pilsbury's Diet) and water were available ad libitum, unless otherwise indicated. All procedures were carried out in accordance with the ethical guidelines for care and use of laboratory animals, and all protocols were approved by the local Ethics and Care of Experimental Animals Committee.

2.2. MSG and ASM administration

All animals were randomly divided into four different groups with ten animals each. Group I consisted of untreated mice and served as naïve controls since they were given only plain tap water. Group II was treated with monosodium glutamate (MSG) at a dose of 8 mg/kg body weight/day, dissolved in drinking water. Group III was treated with aspartame (ASM) at a dose of 32 mg/kg body weight/day, dissolved in drinking water. Group IV was treated with MSG and ASM together in the same doses as in groups II and III dissolved together in drinking water. The doses were selected on the basis of our pilot studies and from available literature. All exposures were through oral administration in their drinking water that formed the only source of drinking fluid for a period of one month. Our pilot studies have shown that a normal adult mouse on average consumes 30 ml of water per day. Thus, all doses of MSG and ASM were prepared in such a manner that the required doses of MSG and ASM (individually and in combination) were consumed by the animals per day through their daily consumption of water. MSG and ASM of analytical grade, from Sigma Chemical Company, USA, were used in this study. After the exposure period of one month, the animals were subjected to cognitive behavioral tests in a shuttle box and a water maze. Subsequently, the animals were sacrificed and the neurotransmitters and oxidative stress parameters were estimated in their forebrain tissue.

2.3. Body weight observation

Throughout the exposure period, all animals were subjected to body weight observations and their body weight was recorded on day 1, day 6, day 18, day 24 and day 30 of the treatment period.

2.4. Cognitive behavioral studies

The learning capabilities of all animals were measured in the same order in the shuttle-box followed by the water maze test.

2.4.1. Shuttle-box test (active avoidance responses)

The active avoidance responses were measured in the animals using a shuttle-box (Ugo Basile, Comerio-Varese, Italy). The rectangular shaped shuttle-box was divided into two chambers of equal size by a stainless steel partition with a gate providing access to the adjacent chambers. Before starting the trial sessions, each animal was allowed to adapt and acquaint itself with the shuttle-box for 2 min without any stimulus. A light bulb (21 W) for 6 s duration and a buzzer (670 Hz and 70 dB) were switched on consecutively and used as a conditioned stimulus (CS). The CS preceded the onset of the unconditioned stimulus (US) by 5 s. The US was an electric scrambler shock (1 mA for 4 s) applied to the metallic grid floor that was hinged in the middle with a fulcrum (8 cm height) located in the middle half of the floor below the metallic gate. Because of the fulcrum the entire metallic grid floor worked like a see-saw. The floor was lowered on the side where the animal entered through the gate. Thus, the floor was a two way procedure and the shock (US) was delivered on either side of the metallic grid floor after the light and sound stimuli (CS). If the animal avoided the US by running into the other compartment within 5 s after the onset of the CS, the microprocessor recorder unit of the shuttle-box recorded an avoidance response and this was considered as a conditioned avoidance response to avoid the electric shock. Each animal was given 50 trials with a fixed intertrial interval of 15 s. During the 50 trial sessions of the individual animals, the total number of avoidance was measured. The total time taken until the animal entered the other chamber to avoid the shock treatment (latency of avoidance response or escape latency in seconds) was also measured for each animal. The recorder unit