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Vitamin E can improve behavioral tests impairment, cell loss, and dendrite changes in rats' medial prefrontal cortex induced by acceptable daily dose of aspartame

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ABSTRACT

Aspartame is an artificial sweetener used in about 6000 sugar-free products. Aspartame consumption could be associated with various neurological disorders. This study aimed to evaluate the effect of aspartame onmedial Prefrontal Cortex (mPFC) as well as neuroprotective effects of vitamin E. The rats were divided into seven groups, including distilled water, corn oil, vitamin E (100 mg/kg/day), and low (acceptable daily dose) and high doses of aspartame (40 and 200 mg/kg/day) respectively, with or without vitamin E consumption, for 8 weeks. Behavioral tests were recorded and the brain was prepared for stereological assessments. Novel objects test and eight-arm radial maze showed impairmentoflong- and short-termmemoriesin aspartame groups. Besides, mPFC volume, infralimbic volume, neurons number, glial cells number, dendrites length per neuron,and number of spines per dendrite length were decreased by 7–61% in the rats treated with aspartame. However, neurons' number, glial cells number, and rats' performance in eight-arm radial mazes were improved by concomitant consumption of vitamin E and aspartame. Yet, the mPFC volume and infralimbic cortex were protected only in the rats receiving the low dose of aspartame + vitamin E. On the other hand, dendrites length, spines number,and novel object recognition were not protected by treatment with vitamin E + aspartame. The acceptable daily dose or higher doses of aspartame could induce memory impairments and cortical cells loss in mPFC. However, vitamin E could ameliorate some of these changes.

1. Introduction

Aspartame is an artificial sweetener,which is around 200 times sweeter than sugar. Therefore, it is used in about 6000 sugar-free products, including carbonated soft drinks, hot chocolate, chewing gums, candies, deserts, tabletop sweeteners, vitamins, and cough drops as a substitution for sugar (Adaramoye and Akanni, 2016; Sahar and Omar, 2009). Aspartame is absorbed from the intestine and is quickly metabolized to its fundamental constituents by the liver in rodents, primates, and humans, which may generatepoisonous or adverse effects in animal model systems (Finamor et al., 2014). Aspartame consumption was reported to be associated with various symptoms in the centralnervous system, such as seizures, memory deficits, cholinergic symptoms, headaches, personality disorders, visual problems, dizziness, and ontogenesis (Sahar and Omar, 2009; Christian et al., 2004). Sahar and Omar (2009) also indicated that aspartame could affect the frontal cortex. The hippocampal region and prefrontal cortex are the fundamental parts of the brain,which are involved in learning and memory activities. The effects of aspartame on these structures are mainly focused on the hippocampal region; however, the prefrontal cortex hasreceived less attention. In addition, the majority of academic theses have evaluated biochemical and electrophysiological activities of these regions, and structural changes of prefrontal cortex have not received adequate attention (Simintzi et al., 2007a,b).Therefore, the first aim of the present study is toevaluate the possible toxic effects of long-term aspartame consumption (8 weeks) on the medial Prefrontal Cortex (mPFC),which takes part in memory and learning. Rats' mPFC is mainly subdivided into three cytoarchitectonic subareas, namely Anterior Cingulate Cortex (ACC), Infralimbic Cortex (ILC), and Prelimbic Cortex (PLC) (Dalton et al., 2016; Rafati et al., 2017).This study also aims to find a protective agent to be used in case of exposure to aspartame. Vitamin E is a recognized neuroprotectant and has been used as a

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therapeutic agent in neurodegenerative diseases.Vitamin E can be found naturally in some foods and is available as a nutritional supplement. Therefore, it may fulfill aputative protective function in the present study (Yin et al., 2015; Sakr et al., 2015).The acceptable daily intake (ADI) for aspartame is 40 mg/kg body weight (Ashok and Sheeladevi, 2014). Hence, the low and high doses of aspartame in this study were considered to be 40 mg/kg/day and 200 mg/kg/day, respectively. Considering the fact that individuals' exact in take throughout the day and in diverse dietary habits are not known, the high dose was selected as 5 times higher than the ADI. Dosage of vitamin E was also selected according to previous studies, which reported the neuroprotective effects of this agent (Yin et al., 2015; Sakr et al., 2015). Overall, the present survey was conducted on a rat model of aspartame consumption to find answers to the following questions:

Do the rats' visual learning and memory change after exposure tolow and high doses of aspartame? Is the rats' spatial learning and memory affected differently following exposure to low and high doses of aspartame? Do the rats' motor skills change after aspartame consumption? Does the volume of the mPFC (anterior cingulated, prelimbic, and infralimbic cortices) change after treatment with aspartame? Can the number of neurons and glial cells in the mPFC change after exposure to aspartame? Are the dendrites length and spines (mushroom, thin, and stubby subtypes) modified after exposure to aspartame? Can vitamin E prevent alteration in behavioral tests? Can vitamin E prevent changes in the mPFC structure in the animals treated with aspartame? To assess the structural changes of mPFC, the tissue was assessed using stereological methods.

2. Materials and methods

2.1. Animals

This study was performed on 70 male Sprague-Dawley rats weighing 250–280 g and attwo months of age. They were obtained from the University's Center of Comparative and Experimental Medicine. All animal were handled according to the Animal Care and Ethics Committee of the University (Agreement License No. 95–7663).

2.2. Experimental design

The animals were randomly categorized into seven groups each containing 10 rats. The rats in groups I to VII received daily oral gavages of distilled water, corn oil, vitamin E (100 mg/kg/day = 149 IU,CAS No. 10191-41-0· T3251 · synthetic, ≥96% Sigma-Aldrich, Germany), low dose of aspartame(40 mg/kg/day CAS No 22839-47-0,purity 98%, Sigma-Aldrich, Germany), high dose of aspartame (200 mg/kg/day), low dose of aspartame plus vitamin E, and high dose of aspartame plus vitamin E for 8weeks. Distilled water and corn oil were the solvents of aspartame and vitamin E, respectively. The low dose of aspartame was chosen based on the acceptable daily intake,-which is 40 mg/kg/day (Sahar and Omar 2009; Sakr et al., 2015; Simintzi et al, 2007c) and the high dose was considered to be 5 timesgreater than the ADI. Behavioral tests were initiated at the end of the 4th week and the animals were sacrificed with deep anesthesia (ketamine/xylazine) at the end of the 8th week. All the 10 rats in each group underwent novel object and eight-arm radial maze evaluations. 6 rats per group were assessed using stereological methods. At the end of each week all the animals were weighed.

2.3. Novel object recognition test

To assess visual memory recognition,the rats were subjected to the novel object recognition test according to the details presented in previous studies (Stranahan, 2011). During the habituation phase (phase I), the animals were allowed to explore an empty box. During the acquisition phase(phase II), the animals were exposed to two familiar objects on the next day. During the short-term memory test (phase III) and long-term memory test (phase IV), the rats faced a familiar and a novel object. After each experiment, the objects and the box were wiped with ethanol to weaken olfactory cues. Every task was recorded using a camera for offline judgment (Stranahan, 2011). In addition, Effective recognition was examined by preferential exploration of the novel object. Difference of visual novelty was assessed by a preference index, determined as: (time near the new–time near the old object)/(time near the new + time near the old object) (Noorafshan et al., 2017).

2.4. Eight-arm radial maze test

The rats' spatial learning and memory were assessed in an eight-arm maze consisting of a stage and eight arms according to the details suggested by previous studies (Karkada et al., 2012). After 15% loss in the rats' body weight, phase I or adaptation phase was started.In phase II (acquisition phase or learning career), two trials were done per day until the rats achieved the learning criterion (accomplishment of 80% of correct choices). This session took eight to fifteen days in different groups. Entrance into unbaited arms were considered as reference memory errors. On the other hand, reentrance into baited arms was considered as working memory errors. Finally, phase III (retention phase) was initiated ten days after acquisition. The mean number of correct choices and errors of phases II and III in the two trials were considered for analysis (Karkada et al., 2012).

2.5. Tissue preparation

At the end of the 8th week, the animals were sacrificed with deep anesthesia (Ketamine/xylazine) and the brains werefixed by cardiac perfusion (paraformaldehyde solution). The tissue was processed for paraffin embedding, sectioned serially in the coronal plane (25 μm thick), and stained with Giemsa (Lifshitz and Lisembee, 2010).The sections were used to estimate the number of neurons and glial cells as well as the volume of the mPFC. The left hemispheres were also prepared for Golgi impregnation procedure (Zhang et al., 2011) and were used to estimate the dendritic length and morphology of the dendritic spines

2.6. Estimation of the mPFC volume

The most anterior section of mPFC was defined by the appearance of the white matter (Bregma 4.20 mm). The sections were followed until the appearance of the corpus callosum decussating fibers (Bregma 2.28 mm) according to the rat's brain atlas (Paxinos and Watson 2006). In this study, 10–13 sections per all regions of the mPFC were sampled and the boundaries were assessed at the final magnification of 25× (Fig. 1A).Generally, the mPFC can be subdivided into ACC, PLC, and ILC categories(Fig. 1 B) (Dalton et al., 2016). The area of eachcortexwas measured and the total volume was estimated using Cavalieri's method (Kristiansen and Nyengaard, 2012; Von Bartheld, 2012; Geuna and Herrera-Rincon, 2015; Koss et al., 2012; Rafati et al., 2017). Using the software designed at the Histomorphometry and Stereology Research centerof the University,stereological probes were overlaid on the images.The area was estimated using point-counting method. The area per point (a/p) was 0.04 mm^2 and on average 450–500 points were counted per animal. Then, areas of each cortex and the mPFC, "$\Sigma A(mPFC)$", were estimated on the sampled sections using the software and were multiplied by the distance between the sampled sections (T):

$$V(mPFC) = \Sigma A(mPFC) \times T$$

2.7. Estimation of the number of neurons and glial cells

Neural cells were identified by their large size, and by having a