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Aspartame and the hippocampus: Revealing a bi-directional, dose/time-dependent behavioural and morphological shift in mice

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ABSTRACT

Changes, in behaviour, oxidative markers of stress and hippocampal morphology were evaluated following aspartame administration. Mice, (20–22 g each) were given vehicle (10 ml/kg) or aspartame (20, 40, 80 and 160 mg/kg) daily for 28 days. They were tested in the Y-maze, radial-arm maze and elevated plus-maze (EPM) after the first and last dose of vehicle or aspartame; and then sacrificed. Hippocampal slices were analysed for aspartic acid, nitric oxide (NO) and superoxide dismutase (SOD); and processed for general histology and neuritic plaques. Glial fibrillary-acid protein (GFAP) expression and neuron-specific enolase (NSE) activities were determined. Radial-arm maze scores increased significantly after acute administration at 80 and 160 mg/kg. Repeated administration at 20 and 40 mg/kg (Y-maze) and at 40 mg/kg (radial-arm maze) was also associated with increased scores, however, performance decreased at higher doses. EPM tests revealed anxiogenic responses following both acute and repeated administration. Significant increase in SOD and NO activities were observed at 40, 80 and 160 mg/kg. Neuron counts reduced at higher doses of aspartame. At 40, 80 and 160 mg/kg, fewer GFAP-reactive astrocytes were observed in the cornus ammonis, but increased GFAP-reactivity was observed in the dentate gyrus subgranular zone. NSE-positive neurons were readily identifiable within the dentate gyrus at the lower doses of aspartame; but at 160 mg/kg, there was marked neuron loss and reduction in NSE-positive neurons. Oral aspartame significantly altered behaviour, anti-oxidant status and morphology of the hippocampus in mice; also, it may probably trigger hippocampal adult neurogenesis.

1. Introduction

Aspartame, an intense food and drink sweetener, has continued to enjoy worldwide popularity since its discovery. It is approved for use in thousands of food products and can also be found in a wide variety of pharmaceuticals (AFSSA (Agence Française De Sécurité Sanitaire Des Aliment), 2002). Despite ongoing debates concerning its safety in humans; aspartame continues to appeal to our taste, and its avid consumers include weight-watchers and diabetics.

Aspartame's effects on the brain had been the subject of several studies that had generated an array of contradicting results (Abd El-Samad, 2010; Abdel-Salam et al., 2012; Ashok, Sheeladevi, & Wankhar, 2013; Bergstrom, Cummings, & Skaggs, 2007; Christian et al., 2004). Some studies have reported evidence of neuronal injury in several brain regions (Omar, 2009; Abd El-Samad, 2010; Abu-Taweel, Zyadah, Ajarem, & Ahmad, 2014), while others have reported no evidences of such (Burdock, 2005; Finkelstein, Daabees, Stegink, & Applebaum, 1988). There are also studies supporting (Beck, Burlet, Max, & Stricker-Krongrad, 2002; Goerss, Wagner, & Hill, 2000), or refuting (Reilly and Lajtha, 1995; Reilly et al., 1990) the occurrence of aspartame-induced alterations in brain neurochemistry and neurotransmitter activities; while Fernstrom, Fernstrom, and Gillis (1983) was of the opinion that only transient elevations occur.

Effects of aspartame or its metabolites (Goerss et al., 2000) on brain neurochemistry, memory and anxiety-related behaviours are of concern, due to the increasing consumption of aspartame and aspartame-containing products worldwide (AIC (Aspartame Information Centre), 2015). Learning and memory studies conducted in both animals (Christian et al., 2004; Collison et al., 2012) and humans (Konen JA, Czuchry M, Bahr GS, & Dansereau DF, 2000; Gendle, Smucker, Stafstrom, Helterbran, & Glazer,

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2009; Lindseth, Coolahan, Petros, & Lindseth, 2014) have generated mixed results. In some of these studies, aspartame consumption did not have any adverse implications on memory (Shaywitz et al., 1994; Spiers et al., 1998); while in a few others, learning and memory deficits following consumption of aspartame (Christian et al., 2004; Collison et al., 2012) were reported. Effects of aspartame on anxiety-related behaviours have also been studied, although a dearth of information still exists. LaBuda and Hale (2000) reported that aspartame did not affect anxiety-related behaviours, while Ashok et al. (2013) reported increased anxiety following long-term administration. From the point of view of human health, any report on effects of aspartame on the brain, is of great interest; considering the fact that worldwide consumption of aspartame-containing products is on the rise, especially in children and young adults (Padridge, 1986; AIC (Aspartame Information Centre), 2015). Hence, there is a need for continued research into the potential health implications of aspartame consumption.

There is a wide consensus that the hippocampus is strongly involved in spatial navigation, learning and memory processes (Buzsáki & Moser, 2013; Eichenbaum & Cohen, 2014; Gruart, Leal-Campanario, López-Ramos, & Delgado-García, 2015; Rendeiro et al., 2009), as well as emotional and motivational behaviours such as anxiety (Bannerman et al., 2004). However, the extent of its involvement, as well as how hippocampal circuitry and neurotransmitter response mediate these behaviours are contentious (Rendeiro et al., 2009). The hippocampus is highly susceptible to neurotoxic damage; but it is also capable of continuous generation of neurons, even in adulthood. In this study, an opportunity arises to observe not only the effects of, but also the response to a probable excitotoxin within the same brain structure. Therefore, it is desirable to know, the effects (if any) that continuous administration of aspartame (at the doses given in the study) may have on this part of the brain.

The rationale for this study was the need to determine the effects (in mice) of repeated aspartame consumption, at an human equivalent dose of between 1.63 and 13.1 mg/kg, {doses within the recommended maximum daily intake of 40 mg/kg (Europe) and 50 mg/kg (America)} on hippocampal morphology, histomorphometry and behaviours that are known to be hippocampal-dependent. We tested the hypothesis, that acute or repeated oral administration of increasing doses of aspartame could significantly alter spatial working-memory, level of anxiety, hippocampal biochemical parameters of oxidative stress and hippocampal morphology in mice.

2. Methods

2.1. Drugs

Aspartame tabletop sweetener (99.9% purity, NutraSweet®, Nutra Sweet Company, Illinois, USA).

2.2. Animals

Male, six-month old Swiss mice (Empire Breeders, Osogbo, Osun State, Nigeria), weighing 20–22 g each were used for this study. Mice were housed in groups of 6 in plastic cages (with exercise ladders, shredded-paper beddings, artificial burrows made of plastic tubes, and platforms) located in a temperature-controlled quarters (22–25 °C) with 12 h of light daily (lights on at 7 a.m.). The animals were fed with standard mice chow and water *ad libitum*, except during the behavioural tests. Mice received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (2013). All procedures were conducted in accordance with the approved institutional protocols and within the provisions for animal care and use prescribed in the scientific procedures on living animals, European Council Directive (EU2010/63).

2.3. Experimental method

144 male mice, randomly assigned into two main groups {memory and anxiety test groups respectively (72 each)} were used for this study. They were further divided into 6 groups of 12 animals each. Mice were administered vehicle (distilled water), a standard drug (diazepam at 0.5 mg/kg for the anxiety test and scopolamine at 1 mg/kg for cognition test) or one of four oral doses of aspartame (20, 40, 80 and 160 mg/kg body weight) daily for 28 days (Onaolapo, Onaolapo, Akanmu, & Olayiwola, 2016). Doses of aspartame were calculated by dissolving measured quantities of the sweetener in distilled water.

2.3.1. Behavioural tests

Behavioural tests were conducted on days 1 (after first dose) and 28 (after last dose); thirty minutes after administration of vehicle or aspartame. Tests were conducted in a quiet room between the hours of 8 a.m. and 2 p.m. On each of the test days, mice were transported in their home-cages to the behavioural testing laboratory, allowed 30 min to acclimatize, and then administered aspartame or vehicle. Animals were allowed to explore the Y-maze, radial arm maze or elevated plus-maze for 5 min each. At the beginning of the behavioural tests, each animal was placed in the apparatus and its behaviour videotaped for subsequent analysis. After testing, each mouse was removed from the maze and returned to its home cage, and all interior surfaces were cleaned thoroughly with 70% ethanol, and then wiped dry to remove any trace of conspecific odour. The behavioural parameters were later scored by two independent observers who were blind to the groupings.

2.3.1.1. Memory (Y-maze, Radial-arm maze). Y-maze and the radial-arm maze were used to measure general activity and spatial memory. Spontaneous alternation is a measure of spatial working-memory, and the Y–maze spontaneous alternation has been used extensively as a measure of working-memory (Farley, McKay, Disterhoft, & Weiss, 2011). Spontaneous alternation in the Y-maze was assessed using a maze composed of three equally spaced arms (120°, 41 cm long and 15 cm high, 5 cm wide). Each mouse was placed in one of the arm compartments and allowed to move freely until its tail completely entered another arm. The sequence of arm entries was recorded. An alternation was defined as entry into all three arms consecutively. The number of actual alternations is number of sequential arm entries into three arms, designated A, B and C. The percentage alternation was calculated as {(Actual alternations/Total arm entry minus two) × 100} in a 5 min period (Onaolapo, Onaolapo, & Nwoha, 2016).

Working-memory in the radial arm maze was measured as sequential arm entries before error. The apparatus consists of eight equidistantly spaced arms, each about 33 cm long, all radiating from a small circular central platform. Working-memory was assessed when the mouse enters each arm a single time. re-entry into the arms would result in a working-memory error (Onaolapo, Onaolapo, Akanni, & Eniafe, 2014).

2.3.1.2. Anxiety Model: Elevated plus-maze. The elevated plus-maze is a plus-shaped apparatus with four arms at right angles to each other; which has been validated for use in mice. The elevated plus-maze relies upon rodents' proclivity toward dark, enclosed spaces (approach) and an unconditioned fear of heights/open