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Monosodium glutamate neurotoxicity increases beta amyloid in the rat hippocampus: A potential role for cyclic AMP protein kinase

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ABSTRACT

Background: Glutamate excitotoxicity and cyclic AMP-activated protein kinase (AMPK) are both recognized as important mediators in neurodegenerative disorders including Alzheimer's disease (AD).
Objectives: To investigate whether oral or subcutaneous monosodium glutamate (MSG) neurotoxicity mimics some features of AD and whether these can be reversed by the AMPK activator Pioglitazone.
Methods: Male Wistar rats aged 5 weeks were administered oral or subcutaneous MSG for 10 days with or without daily oral Pioglitazone. Two additional groups given only saline orally or subcutaneously acted as controls. At age 10 weeks the rats were subjected to neurobehavioral testing, then sacrificed for measurement of AMPK, β-amyloid and Fas ligand in the hippocampus.
Results: Oral and subcutaneous MSG both induced a lowering of hippocampal AMPK by 43% and 31% respectively ($P < 0.05$ for both) and >2-fold increase in hippocampal Fas ligand, a mediator of apoptosis ($P < 0.001$ for both). MSG treatment also induced a significant increase in β-amyloid in the hippocampus by >4-fold and >5-fold in the oral and subcutaneous groups. This was associated with increased latency before crossing to the white half in the black–white alley and before the first rear in the holeboard test, suggesting increased anxiety. Pioglitazone decreased hippocampal β-amyloid accumulation and Fas ligand, but did not ameliorate the neurobehavioural deficits induced by MSG.
Conclusions: MSG treatment enhances β-amyloid accumulation in the rat hippocampus. Our results suggest a role for AMPK reduction in mediating the neurotoxic effects of glutamate, including β-amyloid accumulation.

1. Introduction

Accumulating evidence supports a role for excitotoxins in pathophysiology of neuro-degenerative disorders (Mehta et al., 2013). Alzheimers disease (AD) is a progressive and debilitating neuro-degenerative disorder, and the leading cause of dementia. The hallmark of AD is accumulation, in the neocortex and hippocampus, of β-amyloid peptides, that contribute to several aspects of AD neurodegeneration including direct induction of neuronal apoptosis (Yao et al., 2005).

Glutamate is the principal excitatory neurotransmitter, with a key role in neuronal survival and differentiation but is a recognized excitotoxin (Meldrum, 2000). Glutamate excitotoxicity is thought to play a key role in AD pathogenesis via excessive stimulation of cortical glutamate receptors (Hynd et al., 2004). A central mechanism of excitotoxicity is increased calcium influx via glutamate-gated channels, which triggers catabolic processes that result in neuronal injury and apoptosis (Ndountse and Chan, 2009). The neuronal cell loss observed in AD generally occurs in cell bodies and dendrites of glutamatergic neurons in the cortex and hippocampus (Braak and Braak, 1998).

The sodium salt of glutamate, monosodium glutamate (MSG), is used as a flavor enhancer in human diets, and is generally considered safe (Beyreuther et al., 2007). However, neurotoxicity of MSG has been intensively studied following early reports that MSG administration in neonatal mice induced acute neuronal necrosis (Olney, 1969). Two approaches to induce MSG neurotoxicity in rats were then described, using oral and subcutaneous (SC) regimes (Burde et al., 1971). Both resulted in acute necrotic lesions in hypothalamic neurons (Burde et al., 1971). Subsequent studies were often conducted using either route alone, and with some

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differences in dosage and duration, resulting in diverse neurological phenotypes being reported for each regimen (Ali et al., 2000; Lopez-Perez et al., 2010; Beas-Zarate et al., 2002; Kiss et al., 2005).

Acute increases in hippocampal and intracerebroventricular glutamate concentrations are observed following MSG administration (Lopez-Perez et al., 2010). MSG excitotoxicity is believed to result from increased calcium influx through excessive stimulation of glutamate receptors, with induction of Fas ligand and mitogen-activated protein kinase pathways, and consequent nuclear hyperchromatism, neuronal shrinkage, and apoptosis (Rivera-Cervantes et al., 2004; Segura Torres et al., 2006). Glutamergic receptor activation using N-methyl D-aspartate (NMDA) in rats enhanced neuronal and glial amyloid precursor protein, an effect that was attenuated by calcium channel blockade (Harkany et al., 2000). In AD patients, the NMDA receptor antagonist drug, Memantine, ameliorates several neurocognitive deficits (Wilkinson et al., 2014), which underscores the causal role of glutamate toxicity in AD. Yet despite the apparent converging mechanisms in neurotoxicity due to AD and MSG, the effect of MSG administration on characteristic AD-type neuropathology has not been explored.

AMP-activated protein kinase (AMPK) regulates signaling pathways involved in cell survival and apoptosis, and has been implicated in enhancing hippocampal neurogenesis (Moon et al., 2013). Pharmacologic AMPK activation directly inhibits β-amyloid accumulation *in vitro* (Vingtdeux et al., 2011; Cai et al., 2012). In a transgenic mouse model of AD, AMPK activation by oral Reservatol also reduced the number and size of β-amyloid plaques in the cerebral cortex (Vingtdeux et al., 2010). In the present study, we compared the effects of oral low-dose (Ali et al., 2000) and subcutaneous high-dose (Kiss et al., 2005) MSG on hippocampal AMPK levels, β-amyloid accumulation and neuronal apoptosis. We also evaluated the neurobehavioural consequences of MSG toxicity, and tested whether these mediators or phenotypes could be reversed by the AMPK activator Pioglitazone (Zhang et al., 2012).

2. Materials and methods

The study was conducted on male Wistar rats aged 5 weeks and weighing 40–60 g. The rats were obtained from litters of eight female rats in the Animal House Breeding Facility at the Faculty of Medicine-Alexandria University. After weaning, pups were kept in the same conditions as their mothers with a controlled 12-h light–dark cycle, and fed a standard chow diet with free access to water till randomization. The experimental protocol was approved by the Faculty of Medicine-Alexandria University Ethics Committee.

2.1. Monosodium glutamate treatment

At 5 weeks of age the rats were randomly assigned to one of 6 groups ($n = 6$ each). The oral MSG group was administrated a single daily dose of 2 g/kg body weight MSG (Sigma–Aldrich) in 10% aqueous solution by oral gavage for 10 consecutive days (Ali et al., 2000). A control group was treated with daily saline by oral gavage for 10 days. The subcutaneous MSG group was injected subcutaneously with 4 g/kg body weight MSG (10% aqueous solution) on alternate days for 10 days (Kiss et al., 2005), with a group of rats injected subcutaneously with saline on alternate days for 10 days acting as controls. Two additional groups were administered oral or subcutaneous MSG as described above, and co-treated with Pioglitazone (Actos-Lilly) at 10 mg/kg body weight daily by oral gavage for 10 days starting from the first day of MSG administration (Ikeda et al., 1990).

At the age of 10 weeks all groups were subjected to a battery of neurobehavioral tests as described below. The rats were then sacrificed using terminal anesthesia. After craniotomy, the brain was rapidly removed and the hippocampus and cortex dissected on an ice-cold plate and stored at −80 °C for neurochemical assays.

2.2. Tissue neurochemical assays

Hippocampal samples (used for β-amyloid, AMPK and Fas-ligand determinations) were homogenized in a buffer containing 30 mM tris–HCl, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA,1% Triton X-100 and 0.5 mL/mL of protease inhibitor cocktail F (Bio Basic Inc., Canada). The homogenate was then centrifuged at 100,000 × (Beckman ultracentrifugation) for 45 min at 4 °C and supernatants were collected (Tehranian et al., 2008). Protein content was assayed using the Lowry method (Lowry et al., 1951).

2.2.1. Measurement of β-amyloid

Concentration of β-amyloid peptide 1–42 (β-amyloid 1–42) was measured using a rat ELISA Kit (WKEA MED SUPPLIES CORP, USA) according to the manufacturer's protocol. Color change was measured spectrophotometrically at a wavelength of 450 nm. The concentration of β-amyloid 1–42 was calculated based on standards and expressed in pg/mg of total protein.

2.2.2. Detection of Fas ligand

Concentration of the Fas ligand was measured by ELISA using the rat FASL immunoassay kit (WKEA MED SUPPLIES CORP, USA) according to the manufacturer's protocol. The optical density of each sample was determined using a Spectra Max microplate reader at 450 nm and the concentration of Fas ligand was calculated based on standards and expressed in pg/mg of total protein.

2.2.3. Measurement of AMPK

Total AMPK level was determined by a dot immunoblot assay. Briefly, equivalent concentrations of protein (50 μg/sample) were spotted into a nitrocellulose filter and incubated with blocking solution for 1 h at room temperature. The filters were incubated with the AMPK antibodies (R&D system, UK) at 1 μg/mL for 1 h. The blot was washed three times with 1× PBST (0.05% Tween in phosphate buffered saline PBS), then washed for 10 min with distilled water, with shaking. Filters were then incubated with secondary antibodies (Koma Biotech Inc., Korea) at a 1:2000 dilution for 1 h, and the membrane subsequently washed as described above. 3,3′-Diaminobenzidine (DAB) substrate solution was prepared, and 30 μL hydrogen peroxide added. After developing the color of the blot, the reaction was stopped after appearance of the expected bands by pouring out the substrate and rinsing repeatedly with distilled water. The membrane was dried and placed in the dark. Pictures were taken and processed by Corel Paint Shop Pro X2 software (Corel, Ottawa, Canada). The color intensity of each band was converted to a number in red green blue (RGB) units and divided by the sample protein concentration to yield a reading in units/mg protein.

2.2.4. Glutamate assay

Glutamate was measured in the left cerebral cortex by HPLC with UV detection using an Agilent HPLC system, based on the method of Clarke et al. (2007). The cortex was chosen over the hippocampus since it has been shown that no persistent elevation of hippocampal glutamate content occurs beyond the acute phase of MSG treatment (Lopez-Perez et al., 2010). Chemicals were purchased from Sigma–Aldrich (Cairo, Egypt). Briefly, brains were weighed and homogenized in 2 volumes of 0.4 M perchloric acid, followed by centrifugation at 14,000 rpm for 15 min. Aliquots (100 μL) of sample supernatant and glutamic acid internal standard working solution were added to 900 μL borate buffer (0.1 M, pH 9.5), 200 μL potassium cyanide (10 mM in HPLC grade