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SHORT COMMUNICATION

Low brain ascorbic acid increases susceptibility to seizures in mouse models of decreased brain ascorbic acid transport and Alzheimer's disease

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Summary Seizures are a known co-occurring symptom of Alzheimer's disease, and they can accelerate cognitive and neuropathological dysfunction. Sub-optimal vitamin C (ascorbic acid) deficiency, that is low levels that do not lead the sufferer to present with clinical signs of scurvy (*e.g.* lethargy, hemorrhage, hyperkeratosis), are easily obtainable with insufficient dietary intake, and may contribute to the oxidative stress environment of both Alzheimer's disease and epilepsy. The purpose of this study was to test whether mice that have diminished brain ascorbic acid in addition to carrying human Alzheimer's disease mutations in the amyloid precursor protein (APP) and presenilin 1 (PSEN1) genes, had altered electrical activity in the brain (electroencephalography; EEG), and were more susceptible to pharmacologically induced seizures. Brain ascorbic acid was decreased in APP/PSEN1 mice by crossing them with sodium vitamin C transporter 2 (SVCT2) heterozygous knockout mice. These mice have an approximately 30% decrease in brain ascorbic acid due to lower levels of SVCT2 that supplies the brain with ASC.

Abbreviations: ASC, ascorbic acid; AD, Alzheimer's disease; APP, amyloid precursor protein; EEG, electroencephalography; EMG, electromyography; KA, kainic acid; MDA, malondialdehyde; PSEN1, presenilin 1; PTZ, pentylenetetrazol; SVCT2, sodium dependent vitamin C transporter.

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SVCT2+/−APP/PSEN1 mice had decreased ascorbic acid and increased oxidative stress in brain, increased mortality, faster seizure onset latency following treatment with kainic acid (10 mg/kg i.p.), and more ictal events following pentylenetetrazol (50 mg/kg i.p.) treatment. Furthermore, we report the entirely novel phenomenon that ascorbic acid deficiency alone increased the severity of kainic acid- and pentylenetetrazol-induced seizures. These data suggest that avoiding ascorbic acid deficiency may be particularly important in populations at increased risk for epilepsy and seizures, such as Alzheimer's disease.

Introduction

Seizures are a co-occurring adverse event in Alzheimer's disease (AD), related to amyloid precursor protein (APP) and presenilin 1 (PSEN1) mutations in familial AD, but also affecting many sporadic AD cases, with estimates of prevalence of up to 64% (Friedman et al., 2012). Non-convulsive seizures (*e.g.* absence or partial seizures) are harder to distinguish from other abnormal behaviors (Pandis and Scarmeas, 2012) and may be under-reported in AD, particularly by non-medical caregivers.

Ascorbic acid (ASC, vitamin C) is a critical antioxidant in the brain. ASC levels are depleted or deficient in up to 30% of Western populations, particularly in the elderly and hospitalized (Harrison, 2012). ASC is carefully controlled in the brain parenchyma *via* the sodium dependent vitamin C transporter, SVCT2, which transfers ASC at the choroid plexus from blood into cerebral spinal fluid, and also from extracellular fluid into neurons. This two-step transport process allows accumulation in the brain to far exceed that in blood, except under conditions of prolonged insufficient intake.

Pre- and post-seizure treatments with exogenous ASC moderate the severity of seizures and resultant neurological damage in rodent models (Dong et al., 2013; Gonzalez-Ramirez et al., 2010; Naseer et al., 2011; Santos et al., 2009; Xavier et al., 2007), but ASC deficiency, the more common state in humans, has not been investigated in epilepsy. The objective of this study was to demonstrate whether chronic ASC deficiency increased seizure susceptibility and severity in a mouse model of AD by measuring mortality, behavioral response, and electroencephalography (EEG) responses to pharmacologically induced seizures, targeting two neurotransmitter systems, GABAergic and glutamatergic.

Methods

Animals

Heterozygous SVCT2 knockout mice (SVCT2+/−; Sotiriou et al., 2002) were crossed with a bigenic mouse carrying two mutations known to cause familial (early-onset) AD (APP_{SWE}/$PSEN1_{dE9}$; Jackson Laboratories, stock #005864; Fig. 1A). Mice aged 12–18 weeks, were maintained in a temperature and humidity controlled environment with *ad libitum* access to food and water. These mice can synthesize ASC and received no additional supplementation. All procedures were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the NIH *Guide for the Care and Use of Laboratory Animals*. All experiments were run, and data analyzed, with the experimenter blinded to genotype.

Kainic acid (KA) seizure induction

Seizure induction was performed in female mice ($n = 28$) by administration of KA 10 mg/kg, i.p. (Sigma–Aldrich, St. Louis, MO). Seizure-related activity was scored according to a modified Racine scale (Fig. 1B).

EEG headmount affixation surgery, recording and analysis

Male mice ($n = 24$), were fitted with a prefabricated headmount (Pinnacle Technology Inc.) comprised of three channels; 2 EEG to assess the electrical impulses of the brain, and 1 EMG (electromyography) to measure the muscular activity evoked in the nuchal muscles. Two mice died following surgeries (wild-type and SVCT2+/−APP/PSEN1). Following a 1-week recovery period, synchronized video-EEG/EMG recordings were conducted to assess baseline activity over a 24-h period, quantified in uniform 5-min segments each hour (Arain et al., 2012). Each mouse was then injected with a single dose of the $GABA_A$ receptor antagonist, pentylenetetrazol (PTZ; Sigma–Aldrich, St. Louis, MO) 50 mg/kg, i.p. to induce seizure-related activity and monitored during the first 15 min after administration (Binder et al., 2004; Rauca et al., 1999).

A trained observer assessed the spike-and-wave discharges (SWDs) including specific seizure-related events (absence seizures, myoclonic jerks) following previously determined guidelines (Akman et al., 2010; Chung et al., 2009; Snead et al., 1999). SWDs associated with absence seizures and myoclonic jerks were correlated with the appropriate behavioral manifestations in the accompanying video of the EEG/EMG recordings. Abnormal discharges (absence seizure-like activity) and spike discharges (myoclonic jerk-like activity) were quantified regardless of a detectable associated behavior with specific seizure-related events.

Ascorbic acid and malondialdehyde (MDA)

ASC was measured by an ion pair HPLC and electrochemical detection as previously described (Harrison et al., 2008). MDA was measured as thiobarbituric reactive substances as previously described (Harrison et al., 2010).