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Short Communication

Altered Neurotransmission Prior to Cognitive Decline in AβPP/PS1 Mice, a Model of Alzheimer's Disease

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Abstract. Indirect evidence supports altered glutamate signaling with Alzheimer's disease, however, it is not known if glutamate neurotransmission is impacted prior to cognitive decline. We examined cognition and glutamate neurotransmission in 2–4 month AβPP/PS1, an Alzheimer's disease model, and age-matched control mice. There were no differences in learning and memory as assessed by Morris water maze. However, *in vivo* electrochemical measures of potassium-evoked glutamate release in the CA1, but not the CA3 or dentate, was significantly elevated in AβPP/PS1 mice. These data support changes in the glutamatergic system that precedes cognitive decline in a mouse model of Alzheimer's disease.

Keywords: Alzheimer's disease, biological markers, cognition, glutamic acid

INTRODUCTION

Alzheimer's disease (AD) is an age-related neurodegenerative disorder resulting in severe impairment in new learning and memory, and eventually progressive dementia. In the United States, AD is the 6th leading cause of death, and over 5.2 million Americans have the disease [1]. There has not been a new FDA approved drug to treat AD since 2003, therefore, it is imperative that we find a novel therapeutic target or opportune treatment window that exists prior to cognitive decline with the potential to slow or stop AD progression. Increasing evidence supports the glutamatergic system as a possible early biomarker that meets these criteria [2–11].

Glutamate (Glu) is the major excitatory neurotransmitter in the mammalian central nervous system (CNS), and several studies have implicated its involvement in AD pathologies including soluble amyloid-β $(Aβ)_{42}$, deposition of Aβ in plaques, and hyperphosphorylated tau protein in tangles [6, 12–28]. Recent evidence from AβPP/PS1 mice, a model of AD, and postmortem human CNS tissue suggest that altered extracellular Glu levels and function change as AD progresses. For example, vesicular Glu transporter 1 boutons are elevated in pre-clinical AD cases (possibly through interaction with soluble Aβ) but are significantly reduced and associated with Aβ plaque accumulation in end-stage AD cases and AβPP/PS1 mice [12]. Given these findings, we believe that altered Glu neurotransmission may be instrumental not only in presenting symptoms, such as memory loss, but that extracellular Glu levels may also be a useful early biomarker and early therapeutic target of AD.

AβPP/PS1 mice, an established model of AD, are cognitively normal and lack AD neuropathology at 2–4 months of age, but begin showing cognitive decline and amyloid plaque accumulation at approximately 6–8 months of age [18, 29–36]. In the present studies,

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we examined cognition (Morris water maze; MWM) and Glu neurotransmission (*in vivo* electrochemistry) in the CA1, CA3, and dentate gyrus (DG) regions of the hippocampus in 2–4 month anesthetized AβPP/PS1 mice.

MATERIALS AND METHODS

Animals

AβPP/PS1 mice are double transgenic mice expressing a chimeric mouse/human amyloid-β protein precursor (Mo/HuAPP695swe) and a mutant human presenilin 1 (PS1-dE9). Both mutations were directed to CNS neurons and have been associated with early-onset AD. Two to four month old group housed, male AβPP/PS1 (B6.Cg-Tg(APPswe, PSEN1dE9)85Dbo/J; 034832) and C57BL/6J (000664) mice were obtained from Jackson Laboratory (Bar Harbor, ME) and used for all experiments. Protocols for animal use were approved by the Laboratory Animal Care and Use Committee at Southern Illinois University School of Medicine. Animals were housed according to approved guidelines, on a 12 : 12 h light: dark cycle, and food and water were available *ad libitum*.

Morris water maze

MWM consisted of five consecutive learning days where mice were placed in a large tank of opaque water at various starting positions. The mice were trained to navigate to a platform at a fixed point based on visual cues, and remain there for 10 s. Mice underwent three swimming trials on each of the five training days, with a minimum of 20 min between trials. Mice had two rest days prior to undergoing a single probe trial. During the probe, mice were placed in the tank of water for 60 s, without the platform. Data parameters were recorded using video tracking software with the ANY-maze system (Stoelting Co., Wood Dale, IL, USA).

Glutamate recordings

Enzyme-based microelectrode arrays (MEA) with platinum recording sites were obtained from Cen-MeT (Lexington, KY). Twenty MEAs were used for this study with an average Glu sensitivity of 11.7 pA/μM ($R^2 = 0.996$), selectivity ratio over ascorbic acid of 386 : 1, and a signal-to-noise ratio of 0.5 μM. A single barrel glass micropipette was attached to the MEA assembly to locally apply isotonic KCl (pH 7.4) [37, 38]. Mice were anesthetized with isoflurane and the MEA assemblies were lowered into the CA1 (AP: −2.0, ML: ± 1.0, DV: −1.7 mm), DG (AP: −2.0, ML: ± 1.0, DV: −2.2 mm, or CA3 (AP: −2.0, ML: ± 2.0, DV: −2.2 mm) based on coordinates from Paxinos and Franklin [39]. Solutions were pressure ejected from the micropipette using a Picospritzer (Parker Hannafin, Morton, IL) and volumes were monitored using a stereomicroscope fitted with a reticule. A constant volume of 70 mM KCl (∼100–200 nL) was used to elicit Glu release to allow for direct comparisons between the groups. Amperometric data, time, and pressure ejection marks were recorded and analyzed using a FASTmkIII system and FAST Analysis Version 5.2 software (Quanteon, LLC, Nicholasville, KY, USA).

Data analysis

Data from MWM training days was analyzed using a two-way ANOVA followed by a Fisher's LSD *post-hoc* test and probe data was analyzed using an unpaired Student's *t*-test. Glu data was analyzed using a one-way ANOVA followed by a Fisher's LSD *post-hoc* test. Correlations between MWM and Glu data were established using Pearson's correlation. Data was reported as mean ± SEM and significance was defined as $p < 0.05$. Seven to ten mice were used per group.

RESULTS

Morris water maze

All mice underwent cognitive testing using the MWM prior to *in vivo* Glu measures. Parameters analyzed for the initial five training days included duration, distance, average speed, path efficiency, and time spent in target quadrant. Parameters analyzed for the single probe trial included those previously mentioned and the number of platform entries, time spent in annulus 40, latency, distance, and path efficiency to first platform entry. During the single day probe session, AβPP/PS1 mice (0.261 ± 0.005 m/s) swam significantly ($p < 0.05$) faster compared to C57BL/6J mice (0.240 ± 0.009 m/s). To account for differences in swimming speed, we normalized probe data to total distance swam (in meters) for each mouse. We did not observe differences between genotypes during training or probe in any of the parameters measured (data not shown) as reported elsewhere [35].

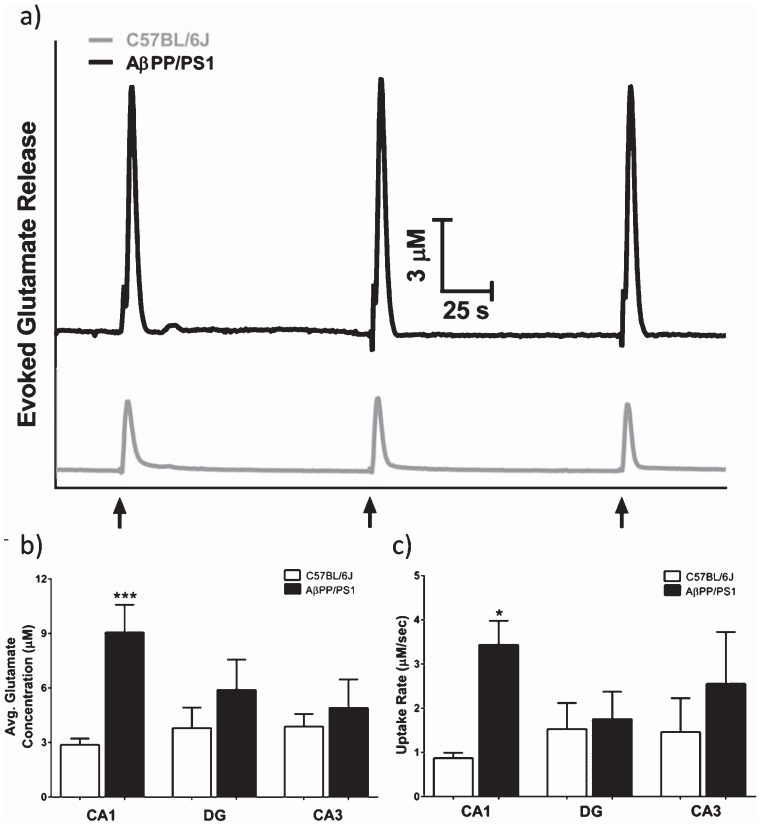

Fig. 1. KCl-evoked Glu release and uptake in the hippocampus. a) Representative traces taken from the CA1 of AβPP/PS1 mice (black line) and C57BL/6J mice (gray line). Arrows indicate local application of 70 mM KCl (150-200 nl). b, c) KCl-evoked Glu release and uptake, respectively, was significantly elevated in the CA1 region of the hippocampus of AβPP/PS1 mice compared to C57BL/6J mice, but not in the DG or CA3. Seven to ten mice were used per group. $^{*}p<0.05$; $^{***}p<0.001$ based on a one-way ANOVA with a Fisher's LSD *post-hoc*.

Glutamate measures

Basal Glu levels were similar in all hippocampal subregions (~1.0 μM; data not shown). We observed significantly more KCl-evoked Glu release in the CA1 of AβPP/PS1 mice compared to age-matched controls (9.1 ± 1.5 μM versus 2.9 ± 0.3 μM, respectively; $p<0.001$), however, no differences were observed in the DG or CA3 (Fig. 1a,b). Glu clearance (time from peak amplitude back to basal levels) from the extracellular space was similar in the CA1 of the AβPP/PS1 mice compared to controls (representative traces, Fig. 1a). However, there was an approximate three-fold increase in Glu uptake rate in the CA1 of AβPP/PS1 mice compared to controls (3.4 ± 0.5 μM/s versus. 0.9 ± 0.1 μM/s, respectively; $p<0.05$), which accounts for similar clearance times (Fig. 1c). Additionally, we observed a strong negative correlation ($r=-0.621$) between KCl-evoked Glu release in the CA1 and the number of platform entries per meter swam during the probe trial in the AβPP/PS1 mice (Fig. 2, solid line; $p=0.10$), while no correlation ($r=-0.036$) was observed in the C57BL/6J control mice (Fig. 2; dashed line; $p=0.93$).

DISCUSSION

Similar to previous reports, we did not observe learning and/or memory differences in 2–4 month old AβPP/PS1 mice compared to age-matched controls, supporting that AβPP/PS1 mice in this age range remain cognitively intact [32]. While basal Glu levels were similar in all hippocampal subregions, we did observe increased potassium-evoked Glu release in 2–4 month AβPP/PS1 mice. Consistent with our basal Glu findings, researchers using magnetic resonance spectroscopy (MRS) in 2–4 month AβPP/PS1 mice have shown no differences in hippocampal Glu metabolite levels compared to age-matched controls [40, 41]. However, stimulus evoked release was not examined in these MRS studies nor is MRS specific

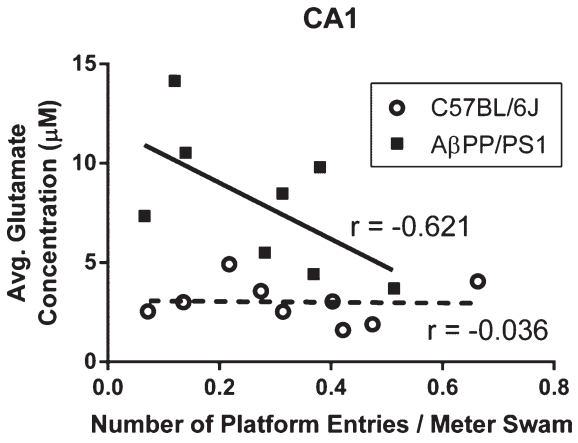

Fig. 2. KCl-evoked Glu release in the CA1 versus number of platform entries per meter swam. Correlation of KCl-evoked Glu release in the CA1 (Fig. 1b) with the number of platform entries per meter swam during the probe session of MWM. Pearson's correlation when evaluating AβPP/PS1 mice was $r = -0.621$ ($p = 0.10$; solid line). Pearson's correlation when evaluating C57BL/6J mice was $r = -0.036$ ($p = 0.93$; dashed line).

enough to elucidate the subregional hippocampal differences observed in the present study. Only changes in CA1 evoked-Glu release were observed, which corresponds to the neuropathological progression of AD. Magnetic resonance imaging studies have shown that hippocampal atrophy begins in the CA1 subfield of mild cognitively impaired patients and then affects other subregions as the diseases progresses to AD [42], a finding consistent with the Braak stages of AD [43].

Interestingly, there was no difference in the time it took to clear Glu from the extracellular space in the CA1 despite evoked Glu release being approximately three times greater in the AβPP/PS1. This was likely due to an approximate three-fold increase in uptake rate in the CA1 of AβPP/PS1 mice, which may indicate that the numbers of glial Glu transporters are increased as a compensatory mechanism to prevent excitotoxicity.

The increased KCl-evoked Glu release in the CA1 negatively correlated with performance on the memory portion of the MWM task. This correlation supports a specific threshold of Glu release in AβPP/PS1 mice that negatively impacts memory formation or information recall, possibly due to overactivation of N-methyl-D-aspartate (NMDA) receptors that causes impairments in new learning and memory before the onset of excitotoxicity [44].

Taken together, these data support that altered Glu neurotransmission precedes neuropathology and cognitive decline in AβPP/PS1 mice and may be a potential early biomarker for AD. Future studies will evaluate Glu levels and kinetics during and after cognitive decline in AβPP/PS1 mice to understand how Glu neurotransmission changes during AD progression as well as exploring potential therapeutic treatments.

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