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Soluble Aβ oligomers impair hippocampal LTP by disrupting glutamatergic/GABAergic balance

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ABSTRACT

Epileptic activity may be more prevalent in early stage Alzheimer's disease (AD) than previously believed. Several studies report spontaneous seizures and interictal discharges in mouse models of AD undergoing age-related Aβ accumulation. The mechanism by which Aβ-induced neuronal excitability can trigger epileptiform activity remains unknown. Here, we systematically examined field excitatory postsynaptic potentials (fEPSP) in stratum radiatum and population spikes (PSs) in the adjacent stratum pyramidale of CA1 in wild-type mouse hippocampal slices. Soluble Aβ oligomers (oAβ) blocked hippocampal LTP and EPSP–spike (E–S) potentiation, and these effects were occluded by prior treatment with the glutamate uptake inhibitor TBOA. In accord, oAβ elevated glutamate levels in the hippocampal slice medium. Recording the PS revealed that oAβ increased PS frequency and reduced LTP, and this LTP deficit was occluded by pretreatment with the $GABA_A$ antagonist picrotoxin. Whole-cell recordings showed that oAβ significantly increased spontaneous EPSC frequency. Decreasing neuronal activity by increasing GABA tone or partially blocking NMDAR activity prevented oAβ impairment of hippocampal LTP. Finally, treating slices with two antiepileptic drugs rescued the LTP inhibition induced by oAβ. We conclude that soluble Aβ oligomers at the low nanomolar levels present in AD brain increase neuronal excitability by disrupting glutamatergic/GABAergic balance, thereby impairing synaptic plasticity.

1. Introduction

The risk of unprovoked epileptic seizures is rather high in early-onset (often familial) Alzheimer's disease, perhaps as much as ~90-fold higher than in an age-matched reference population (Amatniek et al., 2006). Importantly, epileptic activity may also be more prevalent in the early stages of 'sporadic' late-onset AD than was previously thought (Vossel et al., 2013), but the underlying mechanisms are poorly understood. In line with the increasing awareness of the occurrence of subclinical and clinical seizures in AD patients, several laboratories have reported spontaneous seizures and interictal discharges in mouse models of AD undergoing age-related Aβ accumulation (Palop et al., 2007; Westmark et al., 2008; Minkeviciene et al., 2009; Vogt et al., 2011; Bezzina et al., 2015). Such studies suggest that the hyperexcitability induced by oligomeric Aβ assemblies (oAβ) may involve depolarization of the resting membrane potential (Minkeviciene et al., 2009), depression of the slow after-hyperpolarization (Driver et al., 2007), depletion of calbindin and ectopic expression of neuropeptide Y (Palop et al., 2007) and upregulation of α7-nAChR function (Liu et al., 2013). Although epileptiform activity and its relevant effects are common features of AD mouse models that likely contribute to Aβ-induced cognitive impairment, the neurophysiological basis by which Aβ-induced neuronal hyperexcitability can trigger epileptiform activity remains to be elucidated.

Neuronal activity in the brain depends on a balanced regulation between excitatory and inhibitory neurotransmission. A loss of excitation/inhibition balance in favor of excitation can result in epileptiform activity. The excitatory glutamatergic system plays a key role in generating and spreading epileptic discharges. Extracellular glutamate concentrations are tightly controlled by a family of membrane transporters predominantly expressed by perisynaptic astrocytes (Danbolt, 2001).

Abbreviations: AD, Alzheimer's disease; oAβ, soluble Aβ oligomers; AP5, DL-2-amino-5-phosphonopentanoic acid; fEPSP, field excitatory postsynaptic potentials; E–S, EPSP–spike; GT, glutamate transporter; IPSC, inhibitory postsynaptic currents; sEPSC, spontaneous excitatory postsynaptic current; PS, population spike; PTX, picrotoxin; TBOA, DL-threo-β-benzyloxyaspartic acid.

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Glutamate transporter (GT) expression has been found to be decreased in postmortem AD brain and in APP transgenic (tg) mouse models of AD (Masliah et al., 1996, 2000; Jacob et al., 2007). As a potentially primary or early pathogenetic factor, oAβ was shown by us to inhibit glutamate uptake in mouse hippocampal synaptosomes, similar to the effect of a known glutamate uptake blocker, TBOA (Li et al., 2009). Several mouse models of seizure states have been generated by GT impairments (Danbolt, 2001; Maragakis and Rothstein, 2004; Milh et al., 2007), and increases in extracellular glutamate concentrations have been observed in the tissues of epileptic mice and humans (Danbolt, 2001; Eid et al., 2008). On the other hand, reducing GABAergic tone favors increased excitability, as some reports have shown that neuronal hyperactivity is associated with decreased GABAergic inhibition, and GABA administration significantly improved cognitive function in APP Tg mice (Busche et al., 2008; Sun et al., 2012). Recent findings also suggest that Aβ-mediated synaptic suppression occurs in part through inhibition of the $GABA_A$ receptor (Orr et al., 2014). These results suggest that oAβ can interrupt glutamate uptake, increase extracellular glutamate levels and thereby activate extrasynaptic GluN2B receptors, and can also reduce $GABA_A$ receptor-mediated inhibition, thus promoting neuronal hyperexcitability. Here, we demonstrate directly that soluble oAβ increase neuronal excitability and impair hippocampal LTP and E–S coupling by inducing an imbalance between glutamatergic and GABAergic transmission. Further, we show that the application of antiepileptic drugs to the hippocampus can prevent oAβ-mediated impairment of LTP.

2. Materials and methods

2.1. Aβ preparations

Secreted human Aβ peptides were collected and prepared from the conditioned medium (CM) of a CHO cell line (7PA2) that stably expresses human APP751 containing the V717F AD mutation (Podlisny et al., 1995; Welzel et al., 2014). Cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum, 1% penicillin/streptomycin, 2 mM L-glutamine, and 200 mg/mL G418 for selection. Upon reaching ~95% confluency, the cells were washed and cultured overnight (~15 h) in serum-free medium. CM was collected, spun at 1500 ×*g* to remove dead cells and debris, and stored at 4 °C. The CM was concentrated 10-fold with a YM-3 Centricon filter (Walsh et al., 2005). Aliquots of concentrated 7PA2 CM were stored at −80 °C. Once the 7PA2 CM is added to the ACSF perfusate, the total Aβ concentration is around 5.5 ± 0.9 ng/mL as measured by Aβ ELISA (Walsh et al., 2002).

Synthetic Aβ(1–40)S26C peptide was synthesized and purified using reverse phase HPLC by Dr. James I. Elliott at Yale University, (New Haven, CT). Peptide mass and purity (>99%) were confirmed by electrospray/ion trap mass spectrometry and reverse phase HPLC. Oxidatively cross-linked Aβ(1–40)S26C dimer ($[Aβ_{40}S26C]_2$) was prepared as described previously (O'Nuallain et al., 2010; O'Malley et al., 2014). Following oxidation the $[Aβ_{40}S26C]_2$ was treated with 7 M guanidinium HCl to remove aggregates and dimer isolated using a Superdex 75 10/30 HR column (GE Healthcare Biosciences, Pittsburgh, PA) eluted in 20 mM sodium phosphate, pH 7.4. The peak fraction of $[Aβ_{40}S26C]_2$ was collected, the concentration determined ($\varepsilon_{275} = 2272\ M^{-1}\ cm^{-1}$) and the sample diluted to 20 μM in elution buffer. $[Aβ_{40}S26C]_2$ readily assembles to form kinetically trapped protofibrillar assemblies under quiescent conditions (O'Nuallain et al., 2010; O'Malley et al., 2014); thus aliquots (120 μL) of the diluted sample were incubated in the wells of a black polystyrene, 96-well plate (Fisher Scientific) at 37 °C for 5 days until a maximal ThT fluorescence was attained. Thereafter, material from 40 wells was pooled, gently mixed and aliquoted into 50 μL lots and stored at −80 °C until required.

2.2. Hippocampal slice preparation

Mice (C57BL/6 × 129, wild-type, both genders), were sacrificed by isoflurane anesthesia at age 3–4 weeks for patch-clamping or at 6–8 weeks for field recordings. Brains were quickly removed and submerged in ice-cold oxygenated sucrose-replaced artificial cerebrospinal fluid (ACSF) cutting solution containing (in mM) 206 sucrose, 2 KCl, 2 $MgSO_4$, 1.25 NaH_2PO_4, 1 $CaCl_2$, 1 $MgCl_2$, 26 $NaHCO_3$, 10 D-glucose, pH 7.4, 315 mOsm. Transverse slices (350 μm thick) were cut with a vibroslicer from the middle portion of each hippocampus. After dissection, slices were incubated in ACSF containing (in mM): 124 NaCl, 2 KCl, 2 $MgSO_4$, 1.25 NaH_2PO_4, 2.5 $CaCl_2$, 26 $NaHCO_3$, 10 D-glucose, pH 7.4, 310 mOsm, in which they were allowed to recover for at least 90 min before recording. A single slice was then transferred to the recording chamber and submerged beneath continuously perfused ACSF saturated with 95% O_2 and 5% CO_2. Slices were incubated in this chamber for 20 min before stimulation at RT (~24 °C).

2.3. Electrophysiology

Standard field excitatory postsynaptic potentials (fEPSPs) and population spikes (PSs) were recorded in the CA1 region of hippocampus. A bipolar stimulating electrode (FHC Inc., Bowdoin, ME) was placed in the Schaffer collaterals to deliver test and conditioning stimuli. Two borosilicate glass recording electrodes filled with ACSF were positioned in stratum radiatum and stratum pyramidale of CA1, 200–300 μm from the stimulating electrode. fEPSP (from stratum radiatum) and population spikes (from stratum pyramidale) in CA1 were induced by test stimuli at 0.05 Hz with an intensity that elicited a fEPSP amplitude of 40–50% of maximum. Test responses were recorded for 30–60 min prior to beginning the experiment, to ensure stability of the response. To induce LTP, two consecutive trains (1 s) of stimuli at 100 Hz separated by 20 s, a protocol that induces LTP lasting ~1.5 h in wild-type mice of this genetic background were applied to the slices. The field potentials were amplified 100× using Axon Instruments 200B amplifier and digitized with Digidata 1322A. The data were sampled at 10 kHz and filtered at 2 kHz. Traces were obtained by pClamp 9.2 and analyzed using the Clampfit 9.2.

Whole-cell recordings were made from the somata of visually identified CA1 pyramidal neurons. Patch pipettes (5–7 MΩ) were filled with an internal solution containing (in mM): 120 CsGluconate, 5 $MgCl_2$, 0.6 EGTA, 30 HEPES, 4 MgATP, 0.4 Na_2GTP, 10 phosphocreatine-Tris, 5 QX-314; 290 mOsm; pH was adjusted to 7.2 with C_5OH. Spontaneous excitatory postsynaptic currents (sEPSCs) were collected at a membrane holding potential of −70 mV, which is close to the calculated reverse potential of GABA. In order to measure simultaneously the excitatory and inhibitory inputs on the same neuron, spontaneous inhibitory postsynaptic currents (sIPSCs) were also measured on the same neuron by raising the holding potential to 0–10 mV, a potential close to the reverse potential of excitatory input, without visualizing a negative rebound deflection. Recorded neuronal activities were detected by custom software (DClamp: available at www.ieeg.org/?q=node/34). Evoked excitatory postsynaptic currents (eEPSCs) was induced by tungsten wire electrodes placed in stratum radiatum ~300 μm away from the recording pyramidal neuron in CA1. Electrical stimulation was delivered every 0.05 Hz. NMDA-mediated EPSCs were recorded at +45 mV in ACSF containing 10 μM bicuculline and 10 μM NBQX, or else at −70 mV in 0.1 mM Mg^{2+} ACSF containing bicuculline and NBQX. In some experiments, we blocked active synaptic NMDAR by applying MK-801 (20 μM) in the above buffer and changed the stimulation protocol to 0.125 Hz using paired-pulse stimulation (100 ms interpulse interval), because this effects presynaptic release of glutamate, which in turn facilitates the activation of synaptic NMDAR currents. After washout of the MK801, the remaining EPSC can be considered as extrasynaptic NMDA-currents. Series resistance was kept at 15–30 MΩ and monitored throughout each recording. Neurons with negative resting membrane