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Changes in the physiology of CA1 hippocampal pyramidal neurons in preplaque CRND8 mice

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Abstract

Amyloid-β protein (Aβ) is thought to play a central pathogenic role in Alzheimer's disease. Aβ can impair synaptic transmission, but little is known about the effects of Aβ on intrinsic cellular properties. Here we compared the cellular properties of CA1 hippocampal pyramidal neurons in acute slices from preplaque transgenic (Tg+) CRND8 mice and wild-type (Tg−) littermates. CA1 pyramidal neurons from Tg+ mice had narrower action potentials with faster decays than neurons from Tg− littermates. Action potential-evoked intracellular Ca^{2+} transients in the apical dendrite were smaller in Tg+ than in Tg− neurons. Resting calcium concentration was higher in Tg+ than in Tg− neurons. The difference in action potential waveform was eliminated by low concentrations of tetraethylammonium ions and of 4-aminopyridine, implicating a fast delayed-rectifier potassium current. Consistent with this suggestion, there was a small increase in immunoreactivity for Kv3.1b in stratum radiatum in Tg+ mice. These changes in intrinsic properties may affect information flow through the hippocampus and contribute to the behavioral deficits observed in mouse models and patients with early-stage Alzheimer's disease.

Keywords: Alzheimer's disease; Hippocampus; Pyramidal neuron; Intracellular calcium; Abeta; Beta-amyloid; Dendrite; Potassium channel

1. Introduction

The brains of patients with Alzheimer's disease (AD) contain elevated levels of β-amyloid protein (Aβ; Masters; et al., 1985). Aβ is a peptide of 38–43 amino acids, which is liberated by sequential cleavage of amyloid precursor protein (APP). Aβ peptides can be neuropathogenic and aggregate to form senile plaques, a hallmark of postmortem AD brains (Hardy, 2006). The discovery that Aβ can be neurotoxic led to the amyloid hypothesis, which proposes that Aβ-induced cellular dysfunction and, eventually, cell death is central to AD (Hardy and Selkoe, 2002).

An improved understanding of the effects of Aβ has followed the development of transgenic mouse lines overexpressing human APP genes with mutations identified from patients with familial AD. In many of these mouse lines, Aβ accumulation causes synaptic dysfunction, learning deficits, and plaque deposition. This progression mirrors AD in humans, where the early stages are often termed "mild cognitive impairment" and are characterized by subtle cognitive dysfunction in the absence of neuronal loss. Hence APP overexpressing mice are a good model in which to investigate the early effects of chronic Aβ exposure in which a functional deficit, rather than a loss of neurons, underlies cognitive dysfunction.

As in AD, one of the first regions of the brain to show elevated levels of Aβ in many of these mouse lines is the hippocampus, which is central to memory formation and essential for many forms of learning. Within hippocampus the principal excitatory cell type is the pyramidal neuron, and synaptic transmission between pyramidal neurons in CA3 and CA1 regions of the hippocampus has been exten-

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sively studied in APP overexpressing mice (Palop and Mucke, 2010; Small et al., 2001). Many authors have reported deficits in long-term potentiation (LTP) and long-term depression (LTD) in the CA3–CA1 pathway in APP overexpressing mice (e.g., Brown et al., 2005; Chapman et al., 1999; Dewachter et al., 2007; Jacobsen et al., 2006; Jolas et al., 2002; Knobloch et al., 2007). LTP and long-term depression are changes in the strength of synaptic connections that are often considered to be electrophysiological correlates of learning and memory. Many of these effects occur before plaque formation (e.g., Hsia et al., 1999; Jolas et al., 2002; Larson et al., 1999). Aβ can also exert rapid effects on synaptic plasticity when applied to tissue from wild-type mice (Kim et al., 2001; Li et al., 2009; Nomura et al., 2005; Shankar et al., 2008; Townsend et al., 2006; Walsh et al., 2002; Wang et al., 2002, 2004; although see Raymond et al., 2003). Hence recent research has emphasized the adverse effects of Aβ on synapses, rather than direct effects on the intrinsic properties of neurons: their ion channels and membrane physiology.

Here we describe the changes in the intrinsic cellular function of CA1 pyramidal neurons in the CRND8 mouse model of APP overexpression. CRND8 mice carry a double human APP mutation (KM670/671NL "Swedish" and V717F "Indiana") and overexpress APP, resulting in Aβ accumulation (Chishti et al., 2001). Plaque deposition first occurs at 2–3 months of age in CRND8 mice, becomes pronounced only after ~6 months of age, and plaque load increases rapidly thereafter (Chishti et al., 2001; Hyde et al., 2005; Jolas et al., 2002). CRND8 mice demonstrate cognitive impairments that initially precede plaque deposition (e.g., Ambrée et al., 2006) and progress with age. Changes in synaptic transmission also begin in CRND8 mice before plaque formation, with basal synaptic transmission being depressed at 6–9 weeks of age (Ye et al., 2010) and LTP being enhanced in both pre- and postplaque mice (Jolas et al., 2002). To identify the earliest, preplaque effects of Aβ, we therefore studied CA1 pyramidal neurons in acute slices from 1-week to 4-month-old CRND8 mice.

We measured a large number of intrinsic cellular parameters in CA1 hippocampal pyramidal neurons, using electrophysiological and imaging techniques and comparing the properties of young adult transgenic (Tg)+ mice and age-matched wild-type littermates. We found changes in intrinsic cellular properties that precede synaptic dysfunction. Our results suggest that Aβ affects both intrinsic cellular properties and, subsequently, synaptic dysfunction and that these effects combine to shape the network and behavioral deficits observed in CRND8 mice and perhaps in AD.

2. Methods

All experiments and procedures were approved by the Northwestern University Institutional Animal Care and Use Committee (IACUC).

2.1. CRND8 mice

A colony of CRND8 mice was established from founders provided by the University of Toronto. Mice were bred and genotyped as in previous publications (Ambrée et al., 2006; Chauhan et al., 2004; Chishti et al., 2001; Hyde et al., 2005; Jolas et al., 2002), crossing heterozygous male CRND8 mice with wild-type B6C3H females, which yields F1 mice with the transgene and wild-type littermates. The presence or absence of the transgene was determined by polymerase chain reaction (PCR) analysis of genomic DNA from tail clips, using primers for the APP Swedish (K670M/N671L) and Indiana (V717F) transgenes. All recordings were from male mice. Where possible, recordings were obtained from Tg+ and Tg− littermates and recording and analysis were performed blind to the genotype of the animal.

2.2. Acute slice preparation

Mice were deeply anesthetized with ketamine/xylazine (intraperitoneally) and transcardially perfused with cold (4 °C) modified artificial cerebrospinal fluid (ACSF) containing high sucrose and low sodium (in mM): 85 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 20 $NaHCO_3$, 10 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 25 glucose, 75 sucrose, 0.5 $CaCl_2$, 4 $MgCl_2$, pH 7.3, oxygenated with 95% O_2/5% CO_2. The mouse was then decapitated and the brain rapidly removed into cold sucrose-ACSF. Horizontal hippocampal slices 300 μm thick were prepared using a vibrating slicer (Vibratome, St. Louis, MO, USA) and transferred to a holding chamber containing modified ACSF at 35 °C. After 20 minutes slices were transferred to a holding chamber containing ACSF at room temperature (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 20 $NaHCO_3$, 5 HEPES, 25 glucose, 2 $CaCl_2$, 1 $MgCl_2$, pH 7.3, oxygenated with 95% O_2/5% CO_2. Slices were used for recordings 0.5–4 hours after preparation. For recording, slices were transferred to the stage of an upright microscope (BX51W, Olympus, Center Valley, PA, USA) and constantly perfused with ACSF at 36 ± 1 °C. Where appropriate, synaptic transmission was blocked by adding 10 μM 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX), 50 μM DL-2-amino-5-phosphonopentanoic acid (AP5), and 10 μM gabazine (6-imino-3-[4-methoxyphenyl]-1[6H]-pyridazinebutanoic acid hydrobromide) to the ACSF. After recording and imaging, slices were fixed for histological processing. Slices were fixed overnight in 4% (wt/vol) paraformaldehyde in phosphate buffer (PB; 75 mM Na_2HPO_4, 25 mM NaH_2PO_4, pH 7.4).

2.3. Electrophysiology

Somatic whole-cell recordings were obtained under visual control using infrared difference interference contrast optics (IR-DIC). Pipettes were 3–6 MΩ when filled with intracellular solution. Current-clamp recordings were obtained with an Axoclamp-2A amplifier (Molecular Devices,