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Medial septal dysfunction by Aβ-induced KCNQ channel-block in glutamatergic neurons

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Abstract

Amyloid β (Aβ) peptides play a central role in the pathophysiology of Alzheimer's disease (AD). The cellular mechanisms underlying Aβ toxicity, however, are poorly understood. Here we show that $A\beta_{25\text{-}35}$ and $A\beta_{1\text{-}40}$ acutely and differentially affect the characteristics of 3 classes of medial septum (MS) neurons in mice. In glutamatergic neurons Aβ increases firing frequency and blocks the A- and the M-current (I_A and I_M, respectively). While the I_A block is similar in other MS neuron classes, the block of I_M is specific to glutamatergic neurons. I_M block and a simulated Aβ block mimic the Aβ-induced increase in spontaneous firing in glutamatergic neurons. Calcium imaging shows that under control conditions glutamatergic neurons rarely fire while nonglutamatergic neurons fire coherently at theta frequencies. Aβ increases the firing rate of glutamatergic neurons while nonglutamatergic neurons lose theta firing coherence. Our results demonstrate that Aβ-induced dysfunction of glutamatergic neurons via I_M decrease diminishes MS rhythmicity, which may negatively affect hippocampal rhythmogenesis and underlie the memory loss observed in Alzheimer's disease.

Keywords: Amyloid β peptide; Medial septum; Theta oscillations; Glutamatergic neurons; Cholinergic neurons; GABAergic neurons; M-current; A-current

1. Introduction

Nonfibrillar, water-soluble low-molecular weight assemblies of the amyloid β (Aβ) protein are believed to play an important role in Alzheimer's disease (AD) (Klein et al., 2001). Biochemical analysis of brain indicates that the levels of nonfibrillar forms of Aβ correlate well with synaptic loss and presence of dementia (Lue et al., 1999) and that ex vivo such assemblies can impair synaptic form and function (Shankar et al., 2008). While the predominant forms of Aβ found in the human brain are $A\beta_{1-40}$ and $A\beta_{1-42}$ the existence of shorter forms such as $A\beta_{25-35}$ with their own potent effects on neuronal physiology cannot be ruled out (Millucci et al., 2010).

Several studies have demonstrated the effect of Aβ oligomers on synaptic transmission with glutamatergic synapses appearing to be particularly vulnerable (Santos-Torres et al., 2007). In the medial septum/diagonal band of Broca (MS/DB) the synthetic Aβ oligomer 25–35 ($A\beta_{25-35}$) causes a decrease in glutamatergic excitatory postsynaptic potential (EPSC) amplitude. In the hippocampus, $A\beta_{25-35}$ disrupts network activity by decreasing both subthreshold oscillations in CA1 pyramidal neurons and synaptic input to these neurons (Peña et al., 2010). In contrast, it has also been reported that $A\beta_{25-35}$ in fibril form causes increased excitation (Nimmrich et al., 2008).

The advantage of being able to use both long ($A\beta_{1-40}$ and $A\beta_{1-42}$) and short forms ($A\beta_{25-35}$) of Aβ lies in the fact that it allows for investigating whether Aβ directly affects the intrinsic neuronal machinery such as various ion currents and contrast it with the recently reported ability of Aβ to form de novo ion-permeable pores in neuronal mem-

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branes (Jang et al., 2010). To exclude the formation of ion-permeable pores by Aβ we initially used the short form $A\beta_{25-35}$, which likely is too short to form membrane-spanning complexes (but see Chang et al., 2011) and lacks the region of the long Aβ peptide responsible for the change of secondary peptide structure from alpha-helix to beta-sheet necessary to form beta-sheet barrel-type pores (Jang et al., 2010; Nerelius et al., 2009). We then confirmed the results obtained with $A\beta_{25-35}$ using the long form $A\beta_{1-40}$ showing that it, too, affects the intrinsic neuronal machinery in the same way as $A\beta_{25-35}$.

The MS/DB plays a critical role in hippocampal theta rhythm generation (Bland and Colom, 1993; Bland et al., 1999; Colom and Bland, 1991; Vinogradova, 1995). Classically, acetylcholine and gamma amino butyric acid (GABA) were considered the main neurotransmitters involved in theta generation, but recent studies suggest that glutamate (Huh et al., 2010), the most abundant excitatory neurotransmitter in the central nervous system, is also essential for theta oscillations. In the MS and the hippocampus application of NMDA antagonists significantly decreased theta wave amplitudes, and intrahippocampal AMPA antagonist application alters animal behavior dramatically (Leung and Shen, 2004). Microinfusions of NMDA on apical hippocampal neurons induce long-lasting trains of theta field activity (Bland et al., 2007) and glutamate injected in the MS induces hippocampal theta (Carre and Harley, 2000). There is a large population of glutamatergic neurons in the MS and the majority of these neurons seem to be part of intra-MS circuits (Colom et al., 2005). However, there is a population of glutamatergic neurons (approximately 1/5) that send axons to the hippocampus and these neurons constitute around a quarter of the projections terminating in the CA1, CA3, and dentate region of the hippocampus (Colom et al., 2005). Septal glutamatergic neurons and their projections are particularly well placed to control hippocampal excitability (Colom, 2006) and are highly vulnerable to Aβ (Colom et al., 2010).

Here we show that $A\beta_{25-35}$ and $A\beta_{1-40}$ have a near-immediate and differential affect on the 3 classes of medial septum/diagonal band of Broca (MS/DB) neurons. In glutamatergic neurons $A\beta_{25-35}$ increases firing frequency from a near-quiescent control state and blocks the A- and the M-current (I_A and I_M, respectively). While the I_A block is similar in glutamatergic, cholinergic, and GABAergic neurons, the block of I_M is greater in glutamatergic neurons. An I_M antagonist and a simulated Aβ block mimic the Aβ-induced increase in spontaneous firing in glutamatergic neurons. Unlike glutamatergic neurons, cholinergic and GABAergic neurons fire coherently at theta frequencies in control conditions but lose theta firing coherence in the presence of Aβ. This diminished synchrony may underlie the memory deficits typically observed in AD. Our data show that both $A\beta_{25-35}$ and $A\beta_{1-40}$ have the ability to directly modulate components of the intrinsic neuronal machinery such as I_M. Explanations of Aβ cytotoxicity therefore may not have to exclusively rely on the recently reported ability of Aβ to form ion-permeable pores in neuronal membranes (Jang et al., 2010). Furthermore our results suggest that a decrease in I_M may be an integral part of AD pathophysiology, explaining why I_M blockers fail to improve cognition in AD clinical trials (Rockwood et al., 1997).

2. Methods

Electrophysiological and Ca^{2+} imaging experiments were performed in coronal MS/DB slices of p17–21 mice of either sex (C57B6, BAC-vesicular glutamate transporter 2 (VGLUT2)::Cre (Vglut2-Cre) (Borgius et al., 2010) or glutamic acid decarboxylase 2 $(GAD2)^{tm2(cre)Zjh}$/J (GAD-Cre) (Jackson Labs, Bar Harbor, ME, USA)). These respective Cre mice lines were used for the identification of glutamatergic and GABAergic neurons while ChAT+ neurons were identified post hoc using immunohistochemistry. Unless otherwise noted, Vglut2-Cre mice were crossed with Z/EG reporter mice to drive the expression of GFP in Vglut2+ neurons (Borgius et al., 2010) while GAD2-Cre mice were crossed with B6; 129S6-Gt(ROSA)26Sortm9(CAG-tdTomato)Hze/J (Jackson Labs). All procedures followed Karolinska Institute or Uppsala University guidelines for the care and use of laboratory animals. In some experiments using C57B6 mice, the cytoplasm and organelles were sucked into the recording pipette tip after patch-clamp recordings to use subsequent single cell polymerase chain reaction (PCR) as an alternative method to identify glutamatergic, GABAergic, or cholinergic cells. Ca^{2+} imaging was performed using epifluorescence and electroporation of the Ca^{2+} indicator Oregon Green BAPTA 1 (Molecular Probes, Eugene, OR, USA) (Nevian and Helmchen, 2007). In Ca^{2+} imaging experiments, firing synchrony between neuron pairs were assessed by cross-correlation (Trappenberg, 2002). Student *t* test was used for statistical analysis. Data are stated as value ± standard error of the mean.

2.1. Electrophysiology

Mice were decapitated and the brain rapidly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF, in mM: KCl 2.49, NaH_2PO_4 1.43, $NaHCO_3$ 26, glucose 10, sucrose 252, $CaCl_2$ 1, $MgCl_2$ 4). The brain was then trimmed and coronal 350-μm thick MS/DB slices were obtained using a vibratome (Leica VT1000S, Wetzlar, Germany). Slices were kept in a submerged holding chamber containing ACSF (in mM: NaCl 124, KCl 3.5, NaH_2PO_4 1.25, $MgCl_2$ 1.5, $CaCl_2$ 1.5, $NaHCO_3$ 26, glucose 10), constantly bubbled with 95% O_2 and 5% CO_2, incubated at 35 °C for 1 hour and then allowed to cool to room temperature. For recordings, slices were transferred to a submerged recording chamber (28 °C) under an upright microscope equipped with infrared and DIC (differential interference contrast) optics and perfused with oxygenated ACSF at a rate of approximately 1 mL per minute. In experiments with BAC-Vglut2::Cre//Z/EG mice MS/DB glutamatergic neurons were identified by green fluorescence after blue excitation (GFP [green fluorescent protein]) (Borgius et al.,