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Involvement of GluN2B subunit containing N-methyl-D-aspartate (NMDA) receptors in mediating the acute and chronic synaptotoxic effects of oligomeric amyloid-beta (Aβ) in murine models of Alzheimer's disease (AD)

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ABSTRACT

To elucidate whether a permanent reduction of the GluN2B subunit affects the pathology of Alzheimer's disease (AD), we cross-bred mice heterozygous for GluN2B receptors in the forebrain (hetGluN2B) with a mouse model for AD carrying a mutated amyloid precursor protein with the Swedish and Arctic mutation (mAPP) resulting in a hetGluN2B/mAPP transgenic. By means of voltage-sensitive dye imaging (VSDI) in the di-synaptic hippocampal pathway and the recording of field excitatory postsynaptic potentials (fEPSPs), hippocampal slices of all genotypes (WT, hetGluN2B, mAPP and hetGluN2B/mAPP, age 9–18 months) were tested for spatiotemporal activity propagation and long-term potentiation (LTP) induction. CA1-LTP induced by high frequency stimulation (HFS; 100 Hz/1s) was not different in all genotypes. $A\beta_{1-42}$ (50 nM)-application reduced potentiation of fEPSP in WT and hetGluN2B/mAPP mice, LTP in mAPP and hetGluN2B mice was not affected. For VSDI a fast depolarization signal was evoked in the granule cell layer and propagation was analysed in hippocampal CA3 and CA1 region before and after theta stimulation (100pulses/5 Hz). LTP was not significantly different between all genotypes. In mAPP mice θ-stim produced an epileptiform activity reflected in a pronounced prolongation of the FDS compared to the other genotypes. In slices of hetGluN2B/mAPP and GluN2B mice, however, these parameters were similar to WT mice indicating a reversal effect of the attenuated GluN2B expression.

The induction of a hetGluN2B mutation in the mAPP reversed some pathophysiological changes on hippocampal LTP and provide further evidence for the involvement of the glutamatergic system in AD and emphasize the GluN2B subunit as a potential target for AD treatment.

1. Introduction

1.1. Pathophysiology of Alzheimer's disease

The pathophysiology of Alzheimer's disease (AD) is characterized by chronic, progressive neurodegeneration. The precise aetiology of AD is still not fully clarified, but is known to be complex and multifactorial, with a notable overlap between familial and non-familial forms but also with different forms of dementia such as vascular dementia. The neurodegeneration seen in AD involves early synaptotoxicity and loss of neuropil, neurotransmitter disturbances, accumulation of extracellular β-amyloid (Aβ) deposits (amyloid/senile plaques) and intracellular neurofibrils (neurofibrillary tangles, NFTs), gliosis and only at later stages overt loss of neurons and associated brain atrophy (Bell and Claudio Cuello, 2006; Citron, 2010; Heininger, 1999; Yankner, 1996). At early stages of the disease, the entorhinal cortex and hippocampus are

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particularly affected and this is associated with deficits in cognition/memory (Braak et al., 1993). Over the course of AD, up to 80% of neurons in the hippocampus die, and the progressive symptoms of AD manifest themselves as cognitive disturbances, reduced ability to cope with everyday life, and worsening of clinical global impression score (Morris and Kopelman, 1986).

1.2. β-amyloid

As described by Alois Alzheimer himself (Alzheimer, 1907), one of the key histopathological hallmarks of the AD brain is the presence of extracellular 'amyloid/senile plaques' around neurons and glia. Such amyloid plaques are insoluble, quasi-crystalline deposits (Lesne et al., 2006), the main component of which is Aβ - a peptide (most commonly 40–42 amino acids in length) that is formed by enzymatic cleavage of the transmembrane amyloid precursor protein (APP) (Citron, 2010; Hardy and Higgins, 1992). Due to its neurotoxic effects and accumulation in AD, Aβ is believed to be a crucial pathogenic factor in disease development, both in familial and non-familial forms. Aβ is produced by the enzymatic cleavage of APP by β-secretase (extracellular cleavage) and γ-secretase (cuts in the middle of the membrane) whereas cleavage by α-secretase precludes formation of Aβ. The 42 amino acid form, $A\beta_{1-42}$, has a higher tendency to aggregate than $A\beta_{1-40}$ and has been ascribed to be the main pathogenic form of this peptide (Citron, 2010). Aβ is continually released from neurons and glial cells into the extracellular environment where, at low concentrations and possibly in monomeric form it may play a physiological role (Puzzo et al., 2008).

1.3. Soluble β-amyloid oligomers

More recent evidence indicates that soluble oligomeric forms of Aβ, rather than the insoluble deposits, are primarily responsible for both the neurodegeneration and especially the impairment of synaptic function in AD (Barghorn et al., 2005; Demuro et al., 2010; Ferreira et al., 2007, 2011; Ferreira and Klein, 2011; Lacor et al., 2007; Wilcox et al., 2011; Xia, 2010).

Aβ oligomers are now believed to impair neuronal function and cognition, even before the appearance of overt toxicity (Lesne et al., 2006). However, the exact pathogenic role of deposits vs. soluble forms and, in the latter case especially the major oligomeric species of Aβ involved (e.g. dimer, trimer or dodecamer), is still controversial (Bao et al., 2012; Barghorn et al., 2005; Selkoe, 2008). There is a large body of evidence showing that the pathologic actions of $A\beta_{1-42}$ are mediated through perturbation of glutamatergic signalling (Danysz and Parsons, 2012; Hu et al., 2011; Parameshwaran et al., 2008). Strong support for the theory of $A\beta_{1-42}$ oligomer involvement in the early pathology of AD comes from studies showing that $A\beta_{1-42}$ oligomers negatively affect LTP in the hippocampus (Walsh et al., 2002; Oddo et al., 2003; Townsend et al., 2006; Rammes et al., 2011), a phenomenon thought to underlie the synaptic plasticity necessary for memory formation and learning (Granger and Nicoll, 2014; Macdougall and Fine, 2014). Furthermore, cognitive performance in rodents is strongly attenuated after intra-cerebral/hippocampal administration of Aβ peptides associating the earliest amyloid toxicity to soluble species in the absence of plaques (for review see Chambon et al., 2011).

1.4. Interactions between β-amyloid and GluN2B containing NMDA receptors

Functional NMDA channels are heteromeric tetramers of GluN1 and GluN2A-D subunits. GluN2B-containing NMDA receptors account for about 50% of all NMDA receptors (Chazot and Stephenson, 1997). Of the different subtypes of NMDA receptors, GluN2A and GluN2B types are the most prominent in the forebrain (Yashiro and Philpot, 2008). GluN2B specific antagonists/negative allosteric modulators (NAMs) are expected to provide superior treatment potential for several CNS indications including neurological/neurodegenerative diseases such as neuropathic pain, Parkinson's disease, Huntington's disease and Alzheimer's disease (Mony et al., 2009).

Neurotoxic signalling in Alzheimer's disease has been hypothesized to start with stimulation of extra synaptic GluN2B-subunit-containing NMDA receptors – so called "death receptors" (Amadoro et al., 2006; Hardingham et al., 2002). This is at least partially due to direct interactions of β-amyloid (Aβ) with these postsynaptic NMDA receptors (Albrecht et al., 2009; Martinez-Coria et al., 2010; Renner et al., 2010; Wilcox et al., 2011). As proposed for memantine (Parsons et al., 2007), it is likely that "synaptic noise" in Alzheimer's disease may be selectively decreased by GluN2B antagonists subsequent to preferential block of such "death receptors".

We previously reported that Ro-25-6981 (a negative allosteric modulator of GluN2B-containing NMDA receptors – GluN2B NAM) delivered acutely *in vitro* at concentrations which still allow physiological synaptic activation, was able to prevent exogenous $A\beta_{1-42}$ oligomer-induced synaptic toxicity (Rammes et al., 2011). Others confirmed that Ro-25-6981 reversed Aβ induced changes in LTP *in vivo* and extended these findings to positive effects on synaptic markers, CREB dephosphorylation and nuclear accumulation of Jacob (Ronicke et al., 2011). In our previous study, heterozygous but not homozygous knockout of GluN2B subunits had the same beneficial effects against acute bath application of oligomeric $A\beta_{1-42}$ (Rammes et al., 2011). Such findings further champion the glutamatergic system as an attractive target for the development of improved symptomatic/neuroprotective treatments for AD.

The aim of this study was to elucidate whether similar permanent reduction of the GluN2B subunits affects AD-like pathology in double transgenic animal models. To this means, we crossbred two transgenic mouse lines, one with the Arc and Swe mutation in human APP (mAPP), serving as an AD model, and a second one with a conditional, i.e. forebrain-restricted, heterozygous knockout of GluN2B (hetGluN2B) resulting in altered GluN2B-related physiology/pathophysiology. Double transgenic mice (hetGluN2B/mAPP) were used to address the question whether the pathological effects of chronically overexpressed Aβ can be prevented by a permanent reduction in GluN2B expression/activity. hetGluN2B mice were used to analyse the effects of down regulation of GluN2B expression *per se* and mAPP animals functioned as positive controls for chronic Aβ-induced changes.

Initially, we studied synaptic plasticity in the CA1 region of the hippocampus of all four genotypes to detect potential alteration in the sensitivity of LTP induction due to the molecular pathogenesis of AD. Then, we investigated the effects of bath-applied $A\beta_{1-42}$ in all genotypes to investigate whether attenuation of GluN2B activity can reverse acute $A\beta_{1-42}$-mediated synaptotoxic effects. Last, we used VSDI techniques in hippocampal slices to monitor the spatiotemporal dynamics of neuronal populations and LTP in CA3 and CA1 following theta frequency stimulation (θ-stim) in the granule cell layer (GCL). We analysed the propagation of the fast depolarization-mediated signal (FDS) upon a single stimulus before θ-stim to characterize basal synaptic transmission between WT and transgenic animals and after θ-stim to study differences in LTP magnitude and signal propagation. As with fEPSP recordings, we predicted alterations in LTP and signal propagation in mAPP mice that could be reversed by heterozygous knockout of GluN2B.