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The NMDA receptor antagonist Radiprodil reverses the synaptotoxic effects of different amyloid-beta (Aβ) species on long-term potentiation (LTP)

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ABSTRACT

$A\beta_{1\text{-}42}$ is well accepted to be a primary early pathogenic agent in Alzheimer's disease (AD). However, other amyloid peptides are now gaining considerable attention as potential key participants in AD due to their proposed higher neuronal toxicity. Impairment of the glutamatergic system is also widely accepted to be associated with pathomechanisms underlying AD. There is ample evidence that $A\beta_{1\text{-}42}$ affects GLUN2B subunit containing N-methyl-D-aspartate receptor function and abolishes the induction of long term potentiation (LTP). In this study we show that different β-amyloid species, 1–42 $A\beta_{1\text{-}42}$ and 1–40 ($A\beta_{1\text{-}40}$) as well as post-translationally modified forms such as pyroglutamate-modified amyloid-(AβpE3) and nitrated Aβ (3NTyr10-Aβ), when applied for 90 min to murine hippocampal slices, concentration-dependently prevented the development of CA1-LTP after tetanic stimulation of the Schaffer collaterals with IC_{50}s of 2, 9, 2 and 35 nM, respectively whilst having no effect on baseline AMPA receptor mediated fEPSPs. $A\beta_{1\text{-}43}$ had no effect. Interestingly, the combination of all Aβ species did not result in any synergistic or additive inhibitory effect on LTP - the calculated pooled Aβ species IC_{50} was 20 nM.

A low concentration (10 nM) of the GLUN2B receptor antagonist Radiprodil restored LTP in the presence of $A\beta_{1\text{-}42}$, 3NTyr10-Aβ, $A\beta_{1\text{-}40}$, but not AβpE3.

In contrast to AMPA receptor mediated fEPSPs, all different β-amyloid species tested at 50 nM supressed baseline NMDA-EPSC amplitudes. Similarly, all different Aβ species tested decreased spine density. As with LTP, Radiprodil (10 nM) reversed the synaptic toxicity of Aβ species but not that of AβpE3.

These data do not support the enhanced toxic actions reported for some Aβ species such as AβpE3, nor synergistic toxicity of the combination of different Aβ species. However, whilst in our hands $A\beta pE_{3\text{-}42}$ was actually less toxic than $A\beta_{1\text{-}42}$, its effects were not reversed by Radiprodil indicating that the target receptors/subunits mediating such synaptotoxicity may differ between the different Aβ species tested.

1. Introduction

1.1. Pathophysiology of Alzheimer's disease

The pathophysiology of Alzheimer's disease (AD) is characterized by chronic, progressive neurodegeneration e.g. see (Danysz and Parsons, 2012). The precise aetiology of AD is still not fully clarified, but is known to be complex and multifactorial, with a notable overlap between familial (FAD) and sporadic (SAD) forms but also with different forms of dementia such as vascular dementia (Danysz and Parsons, 2012). 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

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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 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 peptides

One of the key histopathological hallmarks of the AD brain first described by Alois Alzheimer, is the presence of extracellular 'amyloid/senile plaques' around neurons and glia (Alzheimer, 1907). 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 both FAD and SAD. Aβ is produced by the enzymatic cleavage of amyloid precursor protein (APP) by β-secretase and γ-secretase complex (cuts in the middle of the membrane) whereas cleavage by α-secretase precludes Aβ formation. $A\beta_{1\text{-}42}$ has been reported to have higher tendency to aggregate than $A\beta_{1\text{-}40}$, and has therefore been ascribed to be the main pathogenic form of Aβ (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 actually have 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 cellular 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 $A\beta_{1\text{-}42}$ are mediated through perturbation of glutamatergic signalling (Parameshwaran et al., 2008; Danysz and Parsons, 2012). Strong support for the theory of $A\beta_{1\text{-}42}$ oligomer involvement in the early pathology of AD comes from studies showing that $A\beta_{1\text{-}42}$ oligomers negatively affect long-term potentiation (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. Post-translational modification of β-amyloid

More recently, biochemical studies have shown that Aβ peptides can undergo post-translational modifications in AD (Kummer and Heneka, 2014). In general, amino-/N-terminal processing is known to be an important post-translational modification affecting many proteins (Lai et al., 2015). Amongst the post-translationally modified species, pyroglutamate-modified Aβ (AβpE3) and nitrated Aβ (3NTyr10-Aβ) peptides have gained most attention as potential key participants in the pathology of AD due to their abundance in AD brain, high aggregation propensity, stability, cellular toxicity, and ability to cause severe neuron loss in transgenic mice (Frost et al., 2013; Jawhar et al., 2011; Kummer et al., 2011; Russo et al., 2002; Schilling et al., 2006). For several years it has been known that the enzyme glutaminyl cyclase (QC) catalyses the formation of AβpE3 (Cynis et al., 2006; Schilling et al., 2008). Its isoenzyme (isoQC) was more recently shown to contribute to aspects of inflammation by pGlu-modification and thereby stabilization of the monocyte chemoattractant protein CCL2 (Hofling et al., 2014).

1.5. 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 selective antagonists (GLUN2B specific antagonists/negative allosteric modulators (NAMs)) have been predicted to provide superior treatment potential for several CNS indications including neurological/neurodegenerative diseases such as neuropathic pain, Parkinson's disease, Huntington's disease and AD (Mony et al., 2009).

Neurotoxic signalling in AD 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 has been proposed to be, 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 also proposed for memantine (Parsons et al., 2007), it is likely that "synaptic noise" in Alzheimer's disease may be selectively decreased by GluN2B selective antagonists subsequent to preferential block of such "death receptors".

1.6. Radiprodil

We previously reported that Ro-25-6981 (a GluN2B NAM) delivered acutely *in vitro* at concentrations which still allow physiological synaptic activation, was able to prevent exogenous $A\beta_{1\text{-}42}$ 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).

2. Aims

As introduced above, post-translationally modified amyloid peptides are gaining considerable attention as potential key participants in the pathology of AD due to their abundance in AD brain, high aggregation propensity, stability, and cellular toxicity. As such, in the present study we tested different Aβ species ($A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, $A\beta_{1\text{-}43}$, pyroglutamate-modified amyloid (AβpE3) and nitrated Aβ (3NTyr10-Aβ)) on the induction of LTP as well another GLUN2B antagonist Radiprodil for its ability to restore LTP in the presence of