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Therapeutic significance of NR2B-containing NMDA receptors and mGluR5 metabotropic glutamate receptors in mediating the synaptotoxic effects of β-amyloid oligomers on long-term potentiation (LTP) in murine hippocampal slices

Gerhard Rammes [a,b], Anne Hasenjäger [d], Kamila Sroka-Saidi [d], Jan M. Deussing [c], Chris G. Parsons [d,*]

[a] Dept. of Anesthesiology, Technische Universität München, D-81675 Munich, Germany
[b] Neuronal Network Dynamics, Max Planck Institute of Psychiatry, Kraepelin Straße 2, D-80804 Munich, Germany
[c] Molecular Neurogenetics, Max Planck Institute of Psychiatry, Kraepelin Straße 2, D-80804 Munich, Germany
[d] Dept. of In vitro Pharmacology, R&D CNS, Merz Pharmaceuticals GmbH, D-60318 Frankfurt am Main, Germany

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ABSTRACT

Soluble amyloid beta (Aβ) oligomers are widely accepted to be neurotoxic and lead to the memory loss and neuronal death observed in Alzheimer's disease (AD). Ample evidence suggests that impairment in glutamatergic signalling is associated with AD pathology. In particular, $A\beta_{1-42}$ is thought to affect N-methyl-D-aspartate (NMDA) receptor function and abolish the induction of long-term potentiation (LTP), which is regarded to be a phenomenon relevant to memory formation. The involvement of glutamatergic signalling in the pathology of AD is underscored by the therapeutic success of memantine, an uncompetitive NMDA receptor antagonist, used to treat patients with moderate to severe AD. In this study we show that $A\beta_{1-42}$ oligomers applied to acute murine hippocampal slices prevented, in a concentration-dependent manner, the development of CA1-LTP after tetanic stimulation of the Schaffer collaterals with a half maximal inhibitory concentration of around 2 nM (before oligomerization). The highest concentration of $A\beta_{1-42}$ oligomers (50 nM before oligomerization) completely blocked LTP ($105 \pm 1\%$ potentiation versus $141 \pm 3\%$ in control) whereas scrambled $A\beta_{1-42}$ (50 nM) was without effect ($144 \pm 10\%$ potentiation).

Pre-incubation with memantine (1 μM) restored LTP in the presence of $A\beta_{1-42}$ (50 nM; $135 \pm 5\%$ potentiation). NMDA receptors containing the NR2B subunit have been proposed to play a particularly important role in excitotoxicity, functioning as extracellular "death receptors". The metabotropic glutamate receptor 5 (mGluR5) is mechanistically coupled to postsynaptic NMDA receptors. As such, allosteric sites on both receptors offer alternative means to modulate NMDA receptor function. We therefore tested low concentrations (each 300 nM) of allosteric antagonists of NR2B (Ro 25-6981, [R-(R*,S*)]-α-(4-Hydroxyphenyl)-β-methyl-4(phenylmethyl)-1-piperidine propanol hydrochloride) and mGluR5 receptors (MPEP, 2-methyl-6-(phenylethynyl)-pyridine). Both compounds restored LTP in the presence of $A\beta_{1-42}$ oligomers (50 nM, fEPSPs were potentiated to $129 \pm 13\%$ and $133 \pm 7\%$ respectively). Finally, we demonstrated that slices from mice heterozygous for NR2B receptor) in the forebrain are not susceptible to the toxic effects of $A\beta_{1-42}$ oligomers but express normal LTP ($138 \pm 6\%$). These experiments demonstrate that glutamate receptor antagonists delivered at concentrations which still allow physiological activities *in vitro*, are able to prevent $A\beta_{1-42}$ oligomer-induced synaptic toxicity and further support the glutamatergic system as a target for the development of improved symptomatic/neuroprotective treatments for AD.

1. Introduction

Alzheimer's disease (AD) is the most common of the age related dementias, currently affecting 10% of the population above 65 years and nearly 50% of those above 85 years (Evans et al., 1989; Kukull and Bowen, 2002). Accumulation of amyloid beta peptide (Aβ) in the brains of AD patients has long been suggested to have

* Corresponding author. Dept. of In vitro Pharmacology, Preclinical R & D CNS, Merz Pharmaceuticals, Eckenheimer Landstr. 100, 60318 Frankfurt, Germany. Tel.: +49 69 1503 368; fax: +49 69 1503 188.
E-mail address: christopher.parsons@merz.de (C.G. Parsons).

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a causative role in AD pathology (Hardy and Selkoe, 2002). Although the Aβ-derived fibrillar plaques, which are a characteristic feature of AD brains, were initially thought to be a primary toxic locus (Pike et al., 1993; Lorenzo and Yankner, 1994), more recent evidence indicates that it is rather the soluble oligomeric form of $A\beta_{1-42}$ that leads to memory impairment (Lambert et al., 1998) and that the Aβ-derived fibrillar plaques are somewhat less noxious "rubbish bins" (Walsh et al., 2002). There is a large body of evidence that the pathologic actions of $A\beta_{1-42}$ soluble oligomers are mediated through perturbation of glutamatergic signalling, affecting in particular N-methyl-D-aspartate (NMDA) and possibly metabotropic glutamate receptors.

$A\beta_{1-42}$ has been shown to co-immunoprecipitate with the NR1 and NR2A subunits of the NMDA receptor (Venkitaramani et al., 2007) and its oligomers bind to excitatory neuronal synapses expressing NR1 and NR2B receptors but not to inhibitory synapses expressing gamma-aminobutyric acid (GABA) receptors (Lacor et al., 2007). The question whether $A\beta_{1-42}$ oligomers bind directly to NMDA receptors or somewhere in their vicinity has, thus far, not been completely resolved. It has been speculated that $A\beta_{1-42}$ forms clusters in the postsynaptic membrane, interacting with scaffolding proteins such as PSD-95 and, in this manner, perturbs glutamate receptor function, through e.g. the recruitment of NMDA and metabotropic mGluR5 glutamate receptors (Lacor et al., 2004, 2007, 2008). On the other hand, pre-incubation of neurons with NMDA receptor specific antibodies largely attenuated $A\beta_{1-42}$ oligomer binding as well as the induction of reactive oxygen species (ROS), supporting a direct interaction of $A\beta_{1-42}$ oligomers with NMDA receptors (Klein et al., 2007). However, $A\beta_{1-42}$ oligomer binding to neuronal cultures was not displaced by any of the classical NMDA ligands, suggesting either a novel binding site or non-direct interaction (Von Euler et al., 2008).

In addition to direct effects on postsynaptic glutamate receptors, $A\beta_{1-42}$ has also been shown to indirectly render neurons more susceptible to excitotoxicity (Mattson et al., 1993) by enhancing glutamate release by microglia (Noda et al., 1999) and inhibiting glutamate uptake by astrocytes (Harris et al., 1996). This is in strong contrast to the action of the soluble amyloid precursor protein (APP) fragment, which stabilizes neuronal Ca^{2+} (Mattson et al., 1993) and stimulates aspartate uptake by astrocytes via the aspartate/glutamate transporter (Masliah et al., 1998; Li et al., 2009).

Strong support for the theory of $A\beta_{1-42}$ oligomer involvement in the pathology of AD comes from studies showing that $A\beta_{1-42}$ oligomers negatively affect long-term potentiation (LTP) in the hippocampus (Walsh et al., 2002; Oddo et al., 2003; Townsend et al., 2006), a phenomenon thought to underlie the synaptic plasticity necessary for memory formation and learning (Malenka and Bear, 2004). Intriguingly, it has also been demonstrated that $A\beta_{1-42}$ at picomolar concentrations (as found in healthy brains) actually has a positive effect on synaptic plasticity (Puzzo et al., 2008). This beneficial function of $A\beta_{1-42}$ is hypothesized to be mediated through actions at presynaptic alpha7 nicotinic acetylcholine receptors (α7-nAchR). It has therefore become increasingly clear that targeting all species of Aβ with e.g. secretase inhibitors bears the risk of counteracting some possible positive physiological functions of Aβ in the attempt to provide more effective therapies for AD.

It has recently been reported that the detrimental effects of nM concentrations of $A\beta_{1-42}$ oligomers on LTP can be reversed by therapeutically-relevant concentrations of memantine (1 μM) (Albrecht et al., 2008; Klyubin et al., 2009; Martinez-Coria et al., 2010). This is in line with considerable previously published preclinical evidence suggesting that memantine is both neuroprotective and provides symptomatic benefit in AD by this same mechanism of action (MoA) i.e. moderate affinity, uncompetitive, voltage-dependent NMDA receptor antagonism (Parsons et al., 2007).

The aim of the present study was to verify this result using an $A\beta_{1-42}$ oligomer preparation more closely resembling physiological/pathophysiological conditions. In our previous experiments, we prepared $A\beta_{1-42}$ oligomers according to the method described by Barghorn and colleagues, which utilizes sodium dodecyl sulphate (SDS) (Barghorn et al., 2005) to stabilize $A\beta_{1-42}$ "globulomers". Since it has been suggested that SDS itself could be responsible for such toxic effects (Klein et al., 2007) and SDS is certainly not present in the AD brain, we decided to use an alternative protocol to prepare $A\beta_{1-42}$ oligomers that, we believe, results in more pathophysiologically relevant $A\beta_{1-42}$ oligomers (see Methods). Further, we wished to evaluate different mechanisms of modulating glutamatergic transmission i.e. using the NR2B receptor specific allosteric antagonist Ro 25-6981 ((R*,S*)]-α-(4-Hydroxyphenyl)-β-methyl-4-(phenylmethyl)-1-piperidine propanol hydrochloride) (Fischer et al., 1997) and MPEP (2-methyl-6-(phenylethynyl)-pyridine), a specific allosteric antagonist against mGluR5 receptors (Gasparini et al., 1999) for their ability to ameliorate $A\beta_{1-42}$ oligomer-induced LTP deficits in an attempt to determine the potential therapeutic value of these allosteric sites on glutamate receptors as alternative targets in the treatment of AD. Finally, we wished to confirm if the effects of NR2B allosteric antagonists could be further validated using genetic manipulation of the NR2B receptor.

2. Methods

2.1. $A\beta_{1-42}$ oligomer preparation

$A\beta_{1-42}$ (order number H-1368; Bachem, CH-Bubendorf) or scrambled $A\beta_{1-42}$ (Covance, rPEP-598P) were suspended in 100% HFIP (Sigma Aldrich) to 1 mg/ml and shaken at 37 °C for 1.5 h. This solution was aliquoted to 50 μg portions and then HFIP was removed by evaporation using a Speedvac for approximately 30 min, and when completely dry, the peptides were stored at −20 °C. The $A\beta_{1-42}$ and scrambled $A\beta_{1-42}$ were dissolved in dry DMSO (Sigma Aldrich) to a concentration of 10 μM or 100 μM with the aid of an ultrasonic water bath. This solution was further diluted using Ringer solution to a concentration of 1, 10, 50 nM, 100 nM, 500 nM or 1 μM $A\beta_{1-42}$. The final concentration of DMSO in the Ringer solution for the LTP experiments was negligible (<=0.1%) and had no effect on LTP alone (data not shown).

2.2. Punctate binding

$A\beta_{1-42}$ at 500 nM, 100 nM, 50 nM, 10 nM, 5 nM and 1 nM was applied to E18 primary hippocampal neurons (DIV17), plated in Poly-D-Lysine coated 96 well plates. Treated cells were rinsed and fixed with 4% PFA, permeabilized with 0.1% Triton/PBS and after blocking incubated with ACU954 (Shughrue et al., 2010) and MAP2 (Cell Signalling Technology, Boston, MA, USA) antibody overnight, followed by an incubation with DAPI, anti-mouse Cy5 and anti-rabbit Alexa 586 (Invitrogen, Darmstadt, Germany) secondary antibodies. After washing, plates were analyzed using Operetta HCS-Imaging and Harmony Software (Perkin Elmer, Germany).

2.3. Brain slice preparation

Saggital hippocampal slices (350 μM thick) were obtained from adult (2 month) C57BL/6 male mice that were anaesthetized with isoflurane before decapitation. The experimental protocols were approved by the ethical committee on animal care and use of the government of Bavaria, Germany. The brain was rapidly removed, and slices were prepared in ice-cold Ringer solution using a vibroslicer. All slices were placed in a holding chamber for at least 60 min and were then transferred to an immersion superfusing chamber for extracellular recordings. The flow rate of the solution through the chamber was 1.5 ml/min. The composition of the Ringer solution was 124 mM NaCl, 3 mM KCl, 26 mM $NaHCO_3$, 2 mM $CaCl_2$, 1 mM $MgSO_4$, 10 mM D-glucose, and 1.25 mM NaH_2PO_4, bubbled with a 95% O_2–5% CO_2 mixture, and had a final pH of 7.3. Extracellular recordings were made using glass microelectrodes (2–3 MΩ) filled with Ringer solution. All experiments were performed at room temperature. $A\beta_{1-42}$ stock solution in DMSO was added to the bath solution and resulted in a final $A\beta_{1-42}$ concentration of 1, 10 and 50 nM.

2.4. Electrophysiological recordings

fEPSPs were evoked by stimulating the Schaffer collateral commissural pathway (Sccp) in the dendritic region of hippocampal CA1, by test stimuli (4–5 V, 20 μs) delivered via two bipolar tungsten electrodes (at 0.033 Hz per electrode) insulated to the tip (tip diameter 50 μm). For the induction of LTP, high-frequency