Brain Research Bulletin 96 (2013) 28–38

Contents lists available at SciVerse ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Restoring long-term potentiation impaired by amyloid-beta oligomers: Comparison of an acetylcholinesterase inhibitior and selective neuronal nicotinic receptor agonists

Katja S. Kroker[a,*], Jens Moreth[a], Lothar Kussmaul[a], Georg Rast[b], Holger Rosenbrock[a]

[a] Boehringer Ingelheim Pharma GmbH & Co KG, Dept. of CNS Diseases Research, Birkendorfer Strasse 65, 88397 Biberach, Germany
[b] Boehringer Ingelheim Pharma GmbH & Co KG, Dept. of Drug Discovery Support, Birkendorfer Strasse 65, 88397 Biberach, Germany

ARTICLE INFO

Article history:
Received 16 January 2013
Received in revised form 12 April 2013
Accepted 15 April 2013
Available online 29 April 2013

Keywords:
LTP
Alzheimer's disease
Amyloid-β oligomer
Donepezil
Acetylcholinesterase inhibitor
Nicotinic acetylcholine receptor

ABSTRACT

As nicotinic acetylcholine receptor (nAChR) agonists directly address cholinergic neurotransmission with potential impact on glutamatergic function, they are considered as potential new symptomatic treatment options for Alzheimer's disease compared to the indirectly operating acetylcholinesterase inhibitors such as the current gold standard donepezil. In order to evaluate the therapeutic value of nAChR activation to ameliorate cognitive dysfunction, a direct comparison between α4β2, α7 nAChR agonists, and donepezil was performed on the level of an *ex vivo* experimental model of impaired memory formation. First, we demonstrated that amyloid beta $(A\beta)_{42}$ oligomers, which are believed to be the synaptotoxic Aβ-species causally involved in the pathophysiology of Alzheimer's disease, have a detrimental effect on long-term potentiation (LTP) in the CA1 region of rat hippocampal slices, a widely used cellular model of learning and memory. Second, we investigated the potential of donepezil, the α4β2 nAChR agonist TC-1827 and the α7 nAChR partial agonist SSR180711 to reverse $A\beta_{42}$ oligomer induced LTP impairment. Donepezil showed only a slight reversal of $A\beta_{42}$ oligomer induced impairment of early LTP, and had no effect on $A\beta_{42}$ oligomer induced impairment of late LTP. The same was demonstrated for the α4β2 nAChR agonist TC-1827. In contrast, the α7 nAChR partial agonist SSR180711 completely rescued early as well as late LTP impaired by $A\beta_{42}$ oligomers. As activating α7 nAChRs was found to be most efficacious in restoring $A\beta_{42}$ oligomer induced LTP deficits, targeting α7 nAChRs might represent a powerful alternative approach for symptomatic treatment of AD.

1. Introduction

The acetylcholinesterase (AChE) inhibitor donepezil is the current gold standard used for symptomatic treatment of mild-to-moderate Alzheimer's disease (AD) patients. However, experience with the therapeutic use of AChE inhibitors has raised concerns about the clinical relevance of the rather moderate efficacy and the emergence of tolerance with long-term administration (Courtney et al., 2004; Helou and Rhalimi, 2010; Hernandez et al., 2009; Kaduszkiewicz et al., 2005). Thus, an alternative approach targeting cholinergic neurotransmission by direct activation of nicotinic acetylcholine receptors (nAChRs) has come into focus for the treatment of AD and other cognitive disorders (Cincotta et al., 2008; Haydar and Dunlop, 2010; Terry and Decker, 2011). Different nAChR subtypes are expressed in the brain, of which homopentameric α7 and heteropentameric α4β2 nAChRs represent the predominant neuronal subtypes (Flores et al., 1992; Paterson and Nordberg, 2000; Tribollet et al., 2004). Activation of α4β2 or α7 nAChRs led to enhancement of synaptic plasticity *in vitro* (Biton et al., 2007; Bohme et al., 2004; McKay et al., 2007) and to memory improvement in several animal cognition tests (Bohme et al., 2004; Leiser et al., 2009; Levin, 2012) suggesting these receptors as attractive targets for treatment of cognitive deficits in patients (Geerts, 2012; Lendvai et al., 2013; Radek et al., 2010; Wallace and Bertrand, 2012).

In order to assess the potential therapeutic value of α4β2 and α7 nAChR activation as an alternative treatment for memory impairment mechanistically, in this study, we analyzed the effects of the AChE inhibitor donepezil, the selective α4β2 nAChR agonist TC-1827, and the selective α7 nAChR partial agonist SSR180711 on the level of an *ex vivo* experimental model of impaired memory formation. The results provide a more detailed insight into the potential clinical benefit of selective α4β2 and α7 nAChR agonists on the basis of long-term potentiation (LTP) modulation and hence could give a rationale for a benchmark of these approaches to enhance cognitive function in patients. LTP is a widely-used cellular experimental model of memory formation (Bliss and Collingridge, 1993), which can be distinguished into early and late forms of LTP being

* Corresponding author. Tel.: +49 7351 5492216; fax: +49 7351 5498928.
E-mail address: katja.kroker@boehringer-ingelheim.com (K.S. Kroker).

0361-9230/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.brainresbull.2013.04.006

protein-synthesis independent and protein-synthesis dependent, respectively (Kroker et al., 2011a; Reymann and Frey, 2007). In previous studies, we demonstrated that donepezil, TC-1827, and SSR180711 can improve early and late LTP in naïve hippocampal slices to different extents (Kroker et al., 2011b, 2012). Therefore, in this study, we aimed at analyzing the effects of these compounds in a more disease-related context. In particular, we assessed their potential to restore hippocampal LTP impaired by amyloid beta $(A\beta)_{42}$ oligomers, which are believed to be the synaptotoxic Aβ-species causally involved in the pathophysiology of AD (Ferreira and Klein, 2011; Haass and Selkoe, 2007; Klein, 2013). We first established a concentration-response relation of $A\beta_{42}$ oligomers induced impairment of early as well as late LTP, which, to our knowledge, has not been performed so far. Then, we investigated the potential of donepezil, the α4β2 nAChR agonist TC-1827, and the α7 nAChR partial agonist SSR180711 to ameliorate $A\beta_{42}$ oligomer induced early and late LTP impairment. Therefore, this is the first study performing a comprehensive evaluation regarding comparison of α4β2 and α7 nAChR activation and AChE inhibition on the level of an *ex vivo* experimental model of impaired memory formation.

2. Experimental procedures

2.1. Preparation of brain slices

Procedures involving animals and their care were conducted in conformity with institutional and European Union guidelines (EEC Council Directive 86/609) and were approved by the Ethics Committee of the responsible regional council (Tübingen). Male Wistar rats (Janvier, Le Genest Saint Isle, France) aged seven weeks were shortly anaesthetized with isoflurane, sacrificed by decapitation and transverse hippocampal slices were prepared according to Kroker et al. (2011c). Briefly, the brains were quickly removed and immersed in ice-cold ACSF and transverse hippocampal brain slices (400 μm thickness) were cut using a Vibratome. Slices were allowed to recover in a holding chamber for at least one hour, then they were transferred to integrated brain slice chambers, where they were superfused (flow rate of 2.5 mL min^{-1}, 25 ± 0.2 °C) with ACSF. Prior to doing any electrophysiological recordings, the slices were allowed to equilibrate for at least 30 min.

2.2. Multi-slice recording

Field excitatory postsynaptic potentials (fEPSPs) were recorded using the semi-automated SliceMaster multi-slice recording system (Scientifica Limited, East Sussex, UK). For the present study, an optimized system described by Kroker et al. (2011c) was used. fEPSPs were elicited in the CA1 region by stimulation of the Schaffer collateral–commissural fibres in the stratum radiatum using glass electrodes with broken tips (filled with ACSF). For recording, glass electrodes (2–6 MΩ, filled with ACSF) were placed in the apical dendritic layer. The amplitudes of fEPSPs were used as the parameter of interest (Collingridge et al., 1983). To generate fEPSPs at a constant sub-threshold stimulus, as in previous studies (Seabrook et al., 1997), the stimulus strength of the pulses was adjusted to 30% of the fEPSP maximum and this voltage was used for the experiment. During baseline recording each slice was stimulated every 30 s for at least one hour. Early LTP was induced by weak high frequency stimulation (HFS) made up of 20 pulses at the frequency of 100 Hz (Bashir et al., 1991; Kroker et al., 2011a), whereas late LTP was induced by repeated strong HFS consisting of 100 pulses at the frequency of 100 Hz, repeated two times in five minute intervals (Kroker et al., 2011a; Lu et al., 1999).

2.3. Data acquisition, software and analysis

A modular electrophysiology system, supplied by npi electronic GmbH (Tamm, Germany), conducted the low noise recordings of extracellular signals. AC coupled signals were amplified 1000-fold and internally filtered with a five kHz low-pass filter as well as a three Hz high-pass filter. In our study, a customized system (programmable pattern generator) was used allowing simultaneous stimulation of all slices either with a single stimulus or a specific stimulus pattern (Kroker et al., 2011c). For data acquisition and analysis, the software Notocord® was used.

Data are shown as mean percent (±S.E.M.) of the baseline fEPSP amplitude. Data were analyzed using one-way ANOVA (post hoc test: Bonferroni-Test) to compare multiple conditions. Histograms show the mean amplitude (±S.E.M.) of fEPSPs measured between 25 and 35 min after weak or repeated strong HFS according to Jia et al. (2010) and Kroker et al. (2011a).

2.4. $A\beta_{42}$ oligomer (ADDLs) preparation and application

$A\beta_{42}$ oligomers were prepared according to Lambert et al. (1998) with slight modifications (Moreth et al., 2013). Briefly, $A\beta_{42}$ (purchased from Bachem) was solubilized in 1,1,1,3,3,3-hexafluoro-2-propanol at a final concentration of 1 mM followed by sonication (10 min). Then, it was snap-frozen in liquid nitrogen and lyophilized. The lyophilized $A\beta_{42}$ was resolubilized in 10 mM NaOH to a concentration of 2 mM and brought up in ice-cold Ham's F12 medium (w/o phenol red) to a final concentration of 100 μM (pH 7.4 at 4 °C for at least 14 h). The samples were centrifuged at 15,000 × *g* for ten minutes at 4 °C. The supernatant containing $A\beta_{42}$ oligomers (=ADDLs) was then diluted in the respective assay buffers and used immediately. Quality tests using atomic force microscopy, electron microscopy and dynamic light scattering revealed that the $A\beta_{42}$ oligomers were globular aggregates with a mean size of 6 ± 2 nm in height and that they were stable in ACSF for at least one hour at room temperature (Moreth et al., 2013). $A\beta_{42}$ oligomer concentrations were calculated based on the monomeric Aβ concentration used. $A\beta_{42}$ oligomers were applied 30 min before LTP stimulation and remained in the superfusion buffer until 30 min after LTP stimulation. For rescue experiments, a combination of $A\beta_{42}$ oligomers and the respective compound was applied 30 min before LTP stimulation and remained in the superfusion buffer until 30 min after LTP stimulation.

2.5. Drug solutions and application

The following drugs were used: MK-801 ((5S,10R)-((−)-5-methyl-10,11-dihydro-5H-dibenzo-[a,d]-cyclo-hepten-5,10-imine hydrogen maleate), nifedipine (1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylic acid dimethyl ester) and donepezil hydrochloride monohydrate ((RS)-2-[(1-benzyl-4-piperidyl)methyl]-5,6-dimethoxy-2,3-dihydroinden-1-one) were purchased from Sigma–Aldrich Corporation (St. Louis, USA). TC-1827 ((S)-N-methyl-5-(5-pyrimidinyl)-4-penten-2-amine hemigalactarate) and SSR180711 (1,4-diazabicyclo[3.2.2] nonane-4-carboxylic acid, 4-bromophenyl ester) were synthesized in the Department of Medicinical Chemistry at Boehringer Ingelheim Pharma GmbH & Co. KG.

The compounds were prepared as stock solutions and diluted in ACSF immediately before application. Nifedipine and SSR180711 were prepared in DMSO (the final concentration of DMSO was 0.1%). All compounds, except MK-801, were applied 30 min before LTP stimulation and remained until 30 min after LTP stimulation. MK-801 was used in such a way that, corresponding to Frankiewicz et al. (1996), full blockade of NMDA receptors can be expected: MK-801 was present immediately after preparing slices, thereafter for an average of 6.8 ± 0.4 h prior to LTP stimulation and remained until 30 min after LTP stimulation (Kroker et al., 2011a).

3. Results

3.1. Effects of $A\beta_{42}$ oligomers on early and late LTP

The effects of different concentrations of $A\beta_{42}$ oligomers on early and late LTP are shown in Fig. 1. Early LTP was decreased by $A\beta_{42}$ oligomers (1–1000 nM tested) in a concentration dependent manner with a complete blockade at 100–1000 nM (Fig. 1A) (0.5 h after weak HFS: control: 204 ± 11%, n = 10; 1 nM $A\beta_{42}$: 175 ± 15%, n = 5; 10 nM $A\beta_{42}$: 142 ± 6%, n = 5; 100 nM $A\beta_{42}$: 99 ± 2%, n = 5; 1000 nM $A\beta_{42}$: 104 ± 2%, n = 5). $A\beta_{42}$ oligomers (1–1000 nM tested) also decreased late LTP in a concentration dependent manner with a maximum effect at 100–1000 nM (Fig. 1B) (0.5 h after repeated strong HFS; control: 309 ± 13%, n = 10; 1 nM $A\beta_{42}$: 313 ± 15%, n = 5; 10 nM $A\beta_{42}$ oligomers: 264 ± 11%, n = 6; 100 nM $A\beta_{42}$: 199 ± 15%, n = 5; 1000 nM $A\beta_{42}$: 211 ± 16%, n = 5). At 100–1000 nM $A\beta_{42}$ oligomers concentration, late LTP was not completely abolished, but reduced by approximately 50%. None of the concentrations of $A\beta_{42}$ oligomers affected basal fEPSPs.

Furthermore, the signalling pathways putatively being impaired by $A\beta_{42}$ oligomers in late LTP were investigated (Fig. 2). To analyze the potential effects of $A\beta_{42}$ oligomers on NMDA receptor signalling, 10 μM of the NMDA receptor antagonist MK-801 was applied in addition to 100 nM $A\beta_{42}$ oligomers. This concentration of MK-801 was reported to be sufficient to block the NMDA receptor dependent part of late LTP (Kroker et al., 2011a). As shown in Fig. 2A, MK-801 had no further effect on $A\beta_{42}$ oligomer mediated impairment of late LTP (0.5 h after weak HFS; control: 292 ± 9%, n = 10; 100 nM $A\beta_{42}$: 187 ± 14%, n = 5; 100 nM $A\beta_{42}$ + 10 μM MK-801: 209 ± 11%, n = 5). Basal fEPSPs were not affected by the mixture of $A\beta_{42}$ oligomers and MK-801. To analyze the role of