Neurobiology of Aging xxx (2014) 1–10

Contents lists available at ScienceDirect

Neurobiology of Aging

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

Rescue of impaired late–phase long-term depression in a tau transgenic mouse model

Tariq Ahmed [a], David Blum [b,c,d], Sylvie Burnouf [b,c,e], Dominique Demeyer [b,c], Valérie Buée-Scherrer [b,c,d], Rudi D'Hooge [a], Luc Buée [b,c,d], Detlef Balschun [a,*]

[a] Laboratory of Biological Psychology, University of Leuven, Leuven, Belgium
[b] Université Lille-Nord de France, UDSL, Lille, France
[c] Inserm UMR837, Jean-Pierre Aubert Research Centre, Lille, France
[d] CHRU-Lille, Lille, France
[e] Max-Planck Institute for Biology of Ageing, Köln, Germany

ARTICLE INFO

Article history:
Received 2 July 2013
Received in revised form 1 September 2014
Accepted 17 September 2014

Keywords:
Long-term depression
LTD
Synaptic plasticity
Alzheimer's disease
Tauopathy
Glycogen synthase kinase-3
Protein-phosphatase 2A
Sodium selenate
SB216763
Okadaic acid
Hippocampus
CA1-region

ABSTRACT

Cognitive decline, the hallmark of Alzheimer's disease, and accompanying neuropsychiatric symptoms share dysfunctions of synaptic processes as a common cellular pathomechanism. Long-term potentiation has proven to be a sensitive tool for the "diagnosis" of such synaptic dysfunctions. Much less, however, is known about how long-term depression (LTD), an alternative mechanism for the storage of memory, is affected by Alzheimer's disease progression. Here, we demonstrate that impaired late LTD (>3 hours) in THY-Tau22 mice can be rescued by either inhibition of glycogen synthase kinase-3 (GSK3β) activity or by application of the protein-phosphatase 2A agonist selenate. In line with these findings, we observed increased phosphorylation of GSK3β at Y216 and reduced total phosphatase activity in biochemical assays of hippocampal tissue of THY-Tau22 mice. Interestingly, LTD induction and pharmacologic inhibition of GSK3β appeared to downregulate GSK3ß activity via a marked upregulation of phosphorylation at the inhibitory Ser9 residue. Our results point to alterations in phosphorylation and/or dephosphorylation homeostasis as key mechanisms underlying the deficits in LTD and hippocampus-dependent learning found in THY-Tau22 mice.

1. Introduction

It is well known that aging and the progression of neurodegenerative diseases like Alzheimer's disease (AD) are characterized by the deterioration of cognitive functions, in particular of declarative forms of memory (Di et al., 2007; Glodzik et al., 2011; Hornberger and Piguet, 2012; McKhann et al., 1984; Nestor et al., 2006; Sydow et al., 2011; Van der Jeugd et al., 2011, 2012). Less known is that in most AD patients and some AD mouse models, the decline in cognition is accompanied by neuropsychiatric symptoms at some stage of the disease (Alexander et al., 2011; Lyketsos et al., 2011; Price et al., 2012; Van Der Jeugd et al., 2013). Since cognitive decline and psychiatric symptoms are due to dysfunctions of synaptic processes (Hoover et al., 2010; Moechars et al., 1999; Rowan et al., 2003; Ting et al., 2007; Van Spronsen and Hoogenraad, 2010), it is tempting to use readouts of synaptic function as early markers for the onset of AD pathology.

It is widely believed that synaptic function bidirectionally adapts to the recent history of activation by plastic changes in synaptic transmission. A robust sustained increase in synaptic transmission is referred to as long-term potentiation (LTP) and a lasting decrease is referred to as long-term depression (LTD). Both LTP and LTD are considered as models for memory storage at the cellular level and can be artificially induced by certain protocols of electrical stimulation (see Bliss and Collingridge, 1993; Citri and Malenka, 2008; Collingridge et al., 2010 for further details).

Over the past decade, LTP has developed into a prime tool for the detection of synaptic deficits in AD mouse models. This is because it has been proven to be a sensitive indicator for early-onset pathology, and its mechanisms are well explored which facilitates causal conclusions (Hoover et al., 2010; Moechars et al., 1999; Rowan

* Corresponding authors at: Laboratory of Biological Psychology, University of Leuven, KU Leuven, Tiensestraat 102, box 3714, B-3000 Leuven, Belgium. Tel.: +3216325816; fax: +3216326099.
E-mail address: Detlef.Balschun@ppw.kuleuven.be (D. Balschun).

0197-4580/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2014.09.015

et al., 2003; Ting et al., 2007). Impairments of LTP in brain regions such as the hippocampus and neocortex were described to precede neurodegenerative changes that are typical for clinical AD (Hoover et al., 2010; Moechars et al., 1999; Rowan et al., 2003; Ting et al., 2007). Although LTP has been extensively studied in animal models of AD, LTD, the physiological counterpart of LTP, has been largely neglected although recent evidence indicates that LTD is crucial for some types of hippocampus-dependent learning (Brigman et al., 2010; Collingridge et al., 2010; Goh and Manahan-Vaughan, 2012; Kemp and Manahan-Vaughan, 2007; Morice et al., 2007; Zeng et al., 2001). Therefore, LTD as a complementary mechanism for memory storage is likely to be affected by the progression of neurodegenerative diseases, both in human subjects and in mouse models.

Most studies that documented LTD changes in AD animal models focused on amyloid beta (Aß)-related pathology, examined only early phases and reported almost unanimously a strengthening of LTD (Chakroborty et al., 2012; Chang et al., 2006; Cheng et al., 2009; Hsieh et al., 2006; Kim et al., 2001; Li et al., 2009; Shankar et al., 2008; Ting et al., 2007). Recently, we described for the first time an impairment of LTD in a tauopathy mouse model (THY-Tau22 mice) (Van der Jeugd et al., 2011), which was previously found to have normal LTP (Schindowski et al., 2006). The impairment pertained particularly to the late phase of depression and paralleled the progression of tauopathy and memory impairments (Belarbi et al., 2011; Van Der Jeugd et al., 2011).

LTD is dependent on activation of glycogen synthase kinase-3 (GSK3β) (Peineau et al., 2007, 2009) and regulated by serine and/or threonine phosphatases (Winder and Sweatt, 2001). Both modulate the phosphorylation state of the microtubule associated protein tau and tau hyperphosphorylation has been identified as one of the most critical molecular events in tauopathies and the progression of AD (Alonso et al., 2001; Braak et al., 1998; Brion et al., 1985; Buee et al., 2000; Mandelkow et al., 1995; Sergeant et al., 2008; Takashima, 2012; Van der Jeugd et al., 2012). In tauopathies, active GSK3β is closely associated with neurofibrillary tangles (NFTs) (Kremer et al., 2011; Leroy et al., 2007), and there is a concomitant reduction in total serine and/or threonine phosphatase activity (Sontag et al., 2004). These events might be instrumental in the development of tau pathology and subsequent memory impairments, because reducing GSK3β activity or promoting protein-phosphatase 2A (PP2A) activity were reported to be beneficial in tau mouse models (Bhat et al., 2004; Corcoran et al., 2010; van Eersel et al., 2010).

Here, we report the apparent paradox that inhibition of GSK3β deteriorates LTD under physiological control conditions but rescues an impaired late LTD (L-LTD) in THY-Tau22 mice. The LTD deficit in THY-Tau22 mice was also rescued by activation of the PP2A complex by selenate application (Corcoran et al., 2010; van Eersel et al., 2010). Thus, normalizing the phosphorylation and/or dephosphorylation imbalance in tau phosphorylation reinstates L-LTD, a functional marker that is susceptible to early synaptic deficits in tauopathies.

2. Methods

2.1. Animals

Male THY-Tau22 mice of C57/Bl6J background (10–12 month old) and littermate wild-type (WT) animals were generated by overexpression of human 4-repeat tau mutated at sites G272 V and P301 S under the control of Thy1.2 promoter (Schindowski et al., 2006). All animals were kept in standard animal cages under conventional laboratory conditions (12 hour/12 hour light-dark cycle, 22 °C), with ad libitum access to food and water. They were maintained and experiments were conducted in accordance with university regulations and the European Community Council Directive (86/609/EC). Phenotypic traits of the THY-Tau22 mouse line have been described previously (Belarbi et al., 2009, 2011; Schindowski et al., 2006).

2.2. Electrophysiological recordings

Long-term electrophysiological recordings were performed as reported previously (Ahmed et al., 2011; Van der Jeugd et al., 2011). In brief, male Thy-Tau22 mice were killed by cervical dislocation, the hippocampus was rapidly dissected out and transferred into ice-cold (4 °C) artificial cerebrospinal fluid (ACSF), oxygen saturated with carbogen (95% O_2/5% CO_2). ACSF consisted of (in mM): 124 NaCl, 4.9 KCl, 24.6 $NaHCO_3$, 1.20, KH_2PO_4, 2.0 $CaCl_2$, 2.0 $MgCl_2$, 10.0 glucose, pH 7.4. Transverse slices (400-μm thick) were prepared from the dorsal area of the right hippocampus with a tissue chopper and placed into a submerged-type chamber, where they were kept at 33 °C and continuously perfused with ACSF at a flow rate of 2.5 mL/min. After 90 minutes incubation, 1 slice was arbitrarily selected, and a tungsten electrode was placed in the stratum radiatum of area CA1. For recording of field excitatory postsynaptic potentials, a glass electrode (filled with ACSF, 3–7 MΩ) was lowered into the stratum radiatum about 200 μm from the stimulation electrode. The time course of the field EPSP was measured as the descending slope function for all sets of experiments. After input/output curves had been established, the stimulation strength was adjusted to elicit a field excitatory postsynaptic potential-slope of 35% of maximum and was then kept constant throughout the experiment. During baseline recording, 3 single stimuli (0.1 ms pulse width; 10 seconds interval) were measured every 5 minutes and averaged. For LTD induction, a low-frequency stimulation (LFS) at 2 Hz (0.2 ms pulse width) was applied for 10 minutes and repeated 3 times with a 10-minute interval between completion of 1 LFS train and the start of the next. Immediately after each conditioning stimulus, evoked responses were monitored at 2, 5, and 8 minutes and then subsequently every 5 minutes until the end of experiment at 240 minutes.

2.3. Drug application

All compounds tested were dissolved according to suppliers instructions. The following compounds were purchased from Abcam PLC (Cambridge, UK): NMDA receptor (NMDAR)-specific competitive antagonist D-AP5 [D(-)-2-amino-5-phosphopentanoic acid], L-type voltage dependent calcium channel antagonist Nifedipine (1,4-Dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylic acid dimethyl ester), selective GSK3ß inhibitor SB216763 (3-(2,4-Dichlorophenyl)-4-(1-methyl-1H-indol-3-yl)-1H-pyrrole-2,5-dione, and the protein-phosphatase 2A specific inhibitor, okadaic acid (9,10-Deepithio-9,10-didehydroacanthifolicin). Lithium chloride and sodium selenate (Na_2SeO_4)decahydrate were purchased as ultra-pure salts from Sigma-Aldrich LTD (Steinheim, Germany). Stock solution (1 M) of each was prepared and diluted in ACSF as required. All experiments were interleaved between genotypes or drugs and vehicles. Drugs were added from 30 minutes before until 60 minutes after LFS-application (see figures for details).

2.4. Phosphatase assay

Phosphatase activity was measured using the Ser/Thr phosphatase assay system from Promega, USA (#V2460), which is based on the detection of free phosphate generated by dephosphorylation of a synthetic phosphopeptide (RRA(pT)VA) and measures the absorbance of a molybdate:malachite green:phosphate complex at 620 nm. In our study, phosphatase 2A activity was preferentially measured in the