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Prostate stem cell antigen interacts with nicotinic acetylcholine receptors and is affected in Alzheimer's disease

Majbrit M. Jensen [a], Maria Arvaniti [a], Jens D. Mikkelsen [a], Dominik Michalski [b], Lars H. Pinborg [a,c], Wolfgang Härtig [d], Morten S. Thomsen [a,*]

[a] Neurobiology Research Unit, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark
[b] Department of Neurology, University of Leipzig, Leipzig, Germany
[c] Epilepsy Clinic, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark
[d] Paul Flechsig Institute for Brain Research, University of Leipzig, Leipzig, Germany

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disorder involving impaired cholinergic neurotransmission and dysregulation of nicotinic acetylcholine receptors (nAChRs). Ly-6/neurotoxin (Lynx) proteins have been shown to modulate cognition and neural plasticity by binding to nAChR subtypes and modulating their function. Hence, changes in nAChR regulatory proteins such as Lynx proteins could underlie the dysregulation of nAChRs in AD. Using Western blotting, we detected bands corresponding to the Lynx proteins prostate stem cell antigen (PSCA) and Lypd6 in human cortex indicating that both proteins are present in the human brain. We further showed that PSCA forms stable complexes with the α4 nAChR subunit and decreases nicotine-induced extracellular-signal regulated kinase phosphorylation in PC12 cells. In addition, we analyzed protein levels of PSCA and Lypd6 in postmortem tissue of medial frontal gyrus from AD patients and found significantly increased PSCA levels (approximately 70%). In contrast, no changes in Lypd6 levels were detected. In concordance with our findings in AD patients, PSCA levels were increased in the frontal cortex of triple transgenic mice with an AD-like pathology harboring human transgenes that cause both age-dependent β-amyloidosis and tauopathy, whereas Tg2576 mice, which display β-amyloidosis only, had unchanged PSCA levels compared to wild-type animals. These findings identify PSCA as a nAChR-binding protein in the human brain that is affected in AD, suggesting that PSCA-nAChR interactions may be involved in the cognitive dysfunction observed in AD.

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia in the elderly individuals. Characteristics of AD consist of accumulation of β-amyloid (Aβ) plaques and neurofibrillary tangles of hyperphosphorylated tau proteins (Querfurth and LaFerla, 2010). Another neuropathologic feature of AD is the selective degeneration of cholinergic neurons in the basal forebrain, which is associated with the cognitive decline in AD (reviewed in Auld et al., 2002).

The most abundant nicotinic acetylcholine receptors (nAChRs) in the brain, α7 and α4β2, are involved in cognitive performance, such as attention and memory function, as well as synaptic plasticity (reviewed in Sarter et al., 2009; Thomsen et al., 2010). Accordingly, they have been widely investigated for their involvement in the pathology of AD. Genetic analyses have demonstrated associations between single-nucleotide polymorphisms in the α7 nAChR gene and AD (Carson et al., 2008b) as well as the delusional symptoms in AD (Carson et al., 2008a). Single-nucleotide polymorphisms in the genes of α4 (Dorszewska et al., 2005; Kawamata and Shimohama, 2002) and β2 nAChR subunits (Cook et al., 2004; Laumet et al., 2010) have also shown to be associated with AD. Recent studies agree that α4β2 nAChR levels are decreased in AD. Thus, reduced [^{3}H]-nicotine binding was observed postmortem in frontal cortex of AD patients (Marutle et al., 2013), and binding of the α4β2 nAChR radioligand [^{18}F]-2FA-85380, by the use of PET, was decreased in several brain regions of AD patients and demonstrated significant correlations with both Aβ levels in the medial prefrontal cortex and cognitive performance of the AD patients (Okada et al., 2013). In contrast, there is currently no consensus on whether α7 nAChR levels in the brain are changed in AD, because binding

* Corresponding author at: Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark. Tel.: +4535331717.
E-mail address: morten.s.thomsen@sund.ku.dk (M.S. Thomsen).

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studies have reported either downregulation or no change (Court et al., 2001; Davies and Feisullin, 1981; Hellstrom-Lindahl et al., 1999; Marutle et al., 2013).

$A\beta_{1-42}$ has been shown to bind both α7 and α4β2 nAChRs, the former with picomolar affinity, whereas its affinity for α4β2 was 5000 times lower (Wang et al., 2000). Furthermore, $A\beta_{1-42}$ is able to modulate the function of both α7 and α4β2 nAChRs with dose-dependent effects (reviewed in Buckingham et al., 2009; Jurgensen and Ferreira, 2010).

In addition to $A\beta_{1-42}$, several members of Ly-6/neurotoxins (Lynx) protein superfamily have been shown to modulate the function of nAChRs (reviewed in Miwa et al., 2011; Thomsen and Mikkelsen, 2012). Therefore, alterations in α7 and α4β2 nAChRs may be secondary to changes in Lynx protein levels.

The Lynx superfamily has structural similarities to snake venom toxins such as α-bungarotoxin and are characterized by a "three-fingered" loop motif (Tsetlin, 1999). The Lynx proteins Lynx1 and Lynx2 have been shown to form stable complexes with and negatively regulate both α7 and α4β2 nAChRs (Ibanez-Tallon et al., 2002; Miwa et al., 2006; Tekinay et al., 2009). In addition, mice with genetic deletions of Lynx1 or Lynx2 displayed increased associative learning and elevated anxiety-like behavior, respectively (Miwa et al., 2006; Tekinay et al., 2009). Moreover, it was recently shown that Lynx1 through a nAChR-dependent action had a crucial role in the loss of synaptic plasticity observed in adult visual cortex (Morishita et al., 2010). Furthermore, transgenic overexpression of the Lynx protein Lypd6 in mice resulted in increased pre-pulse inhibition indicating possible involvement in attention (Darvas et al., 2009). Thus, Lynx proteins are suggested to be involved in cognitive function.

Prostate stem cell antigen (PSCA), another Lynx protein, was found to suppress nicotine-induced Ca^{2+} influx via activation of α7 nAChRs, when retrovirally expressed in dissociated avian ciliary ganglion neurons (Hruska et al., 2009). In contrast, nicotine-induced Ca^{2+} currents were enhanced in dissociated murine trigeminal ganglia neurons from transgenic mice overexpressing Lypd6, suggesting that Lypd6 is a positive modulator of nAChR function (Darvas et al., 2009).

Because nAChRs and Lynx proteins can bind directly to each other to affect cognitive function and synaptic plasticity, it is pertinent to examine the involvement of Lynx proteins in AD. The purpose of this study was to (1) investigate PSCA and Lypd6 in the human brain in relation to expression and complex formations with nAChR subtypes; (2) examine the ability of PSCA to modulate a nicotine-mediated response in PC12 cells; and (3) reveal whether the levels of the two Lynx proteins were changed in AD in both human AD patients and transgenic AD mouse models.

2. Methods

2.1. Human tissue

Temporal cortical tissue was obtained from anterior temporal lobectomies in two patients (1 female, aged 30 years and 1 male, aged 57 years) with medically intractable temporal lobe epilepsy with hippocampal onset. Written informed consent was obtained from both patients before surgery. The study was approved by the Ethical Committee in the Capital Region of Denmark (H-2-2011-104) and performed in accordance with the Declaration of Helsinki. The tissue was dissected and immediately frozen on dry ice and stored at −80 °C until use. The neuropathologic examinations of the neocortex from both patients were normal.

Postmortem brain tissue from medial frontal gyrus of 7 AD subjects and 8 nondemented (non-AD) control subjects (see Table 1) were obtained from the Netherland's Brain Bank, Amsterdam, the Netherlands. Autopsies were performed on donors from whom written informed consent had been obtained either from the donor or direct next of kin. All AD subjects were confirmed by standard clinical (Dubois et al., 2007; McKhann et al., 1984) and neuropathologic (Braak and Braak, 1991, 1995) diagnosis criteria.

Table 1
Clinicopathologic data of the human brain material

Diagnosis	Age	Gender	pH	PMD (h:min)	Braak stage
Nondemented control	60	F	6.27	06:50	1
Nondemented control	60	F	6.80	07:30	1
Nondemented control	62	M	6.36	07:20	1
Nondemented control	78	M	6.52	<17:40	1
Nondemented control	87	M	7.11	08:00	1
Nondemented control	87	F	6.91	08:00	2
Nondemented control	97	F	—	10:00	2
Nondemented control	90	F	6.54	06:10	3
Alzheimer's disease	67	F	6.73	03:30	5
Alzheimer's disease	58	M	6.29	05:15	6
Alzheimer's disease	58	M	6.42	06:25	6
Alzheimer's disease	59	M	6.26	05:05	6
Alzheimer's disease	62	M	6.31	04:15	6
Alzheimer's disease	62	F	6.53	04:25	6
Alzheimer's disease	62	F	6.06	04:45	6

Key: F, female; M, male; PMD, postmortem delay.

2.2. Animals

Triple transgenic mice (3×Tg-AD), expressing mutant human transgenes (human amyloid precursor protein [APP] 695 with the Swedish double mutation K670N/M671L [hAPPSwe], tau P301L) mutation, and the presenilin-1 mutant M146V knock-in construct (Oddo et al., 2003), were studied in two different age groups: 6-month-old mice (n = 8) and 19- to 21-month-old mice (n = 8) with age-matched wild-type (WT) mice (Sv129/B6; n = 7–8) as controls. The mice were bred at the Medizinisch-Experimentelles Zentrum at Leipzig University. At the day of experiment, the mice were decapitated, frontal cortex and hippocampus dissected, and the tissue was immediately frozen on dry ice and stored at −80 °C until use.

Twelve male Tg2576 mice expressing the hAPPSwe mutations (Hsiao et al., 1996) and their WT littermates (n = 15) bred on an inbred 129S6 background were obtained from Taconic (Ry, Denmark). At the age of 9 months, in an unrelated set of experiments, the mice received bilateral intracerebroventricular phosphate-buffered saline (PBS) injections (Laursen et al., 2014) followed by electrophysiological testing. Mice were euthanized at the age of 15 months, and one hemisphere was dissected, snap frozen, and kept at −80 °C until further analysis. Frontal cortex was later separated from the frozen sample and used for Western blot analyses as described in the following.

Experiments with 3×Tg-AD were approved by the Animal Care and Use Committee of the University of Leipzig and local authorities (Regierungspräsidium Leipzig; T40/13) and conformed to the European Communities Council Directive (86/609/EEC). Experiments with Tg2576 mice were approved by the Danish National Committee for Ethics in Animal Experimentation.

2.3. Tissue fractionation

To fraction tissue into membrane and soluble fractions, human temporal cortical tissue from neurosurgery (150–170 mg, n = 2) was homogenized 2 × 30 seconds in Buffer1 (0.5 M NaCl, 50 mM Tris–HCl, 10 mM $MgCl_2$, 2 mM ethylenediaminetetraacetic acid (EDTA), 10 μL/mL protease inhibitor cocktail [Sigma-Aldrich, Brøndby, Denmark], pH 7.3) using a PT1200C polytron blender (Kinematica, Luzern, Switzerland) and centrifuged for 30 minutes