Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Research Report

Neurogranin binds α-synuclein in the human superior temporal cortex and interaction is decreased in Parkinson's disease

Andrew O. Koob[a,b,*], Gideon M. Shaked[a], Andreas Bender[c], Alejandro Bisquertt[a], Edward Rockenstein[a], Eliezer Masliah[a,d,**]

[a]Departments of Neurosciences, 9500 Gilman Drive, University of California, San Diego, La Jolla, CA 92093-0624, United States
[b]Departments of Psychiatry, 9500 Gilman Drive, University of California, San Diego, La Jolla, CA 92093-0624, United States
[c]Department of Neurology, University of Munich, Klinikum der Universität München—Großhadern, 81377 München, Germany
[d]Departments of Pathology, 9500 Gilman Drive, University of California, San Diego, La Jolla, CA 92093-0624, United States

ARTICLE INFO

Article history:
Accepted 8 October 2014
Available online 19 October 2014

Keywords:
Parkinson's disease
Dementia with Lewy bodies
Neurogranin
α-Synuclein
Calmodulin
LTP

ABSTRACT

Neurogranin is a calmodulin binding protein that has been implicated in learning and memory, long-term potentiation and synaptic plasticity. Neurons expressing neurogranin in the cortex degenerate in late stages of Parkinson's disease with widespread α-synuclein pathology. While analyzing neurogranin gene expression levels through rtPCR in brains of mouse models overexpressing human α-synuclein, we found levels were elevated 2.5 times when compared to nontransgenic animals. Immunohistochemistry in the cortex revealed colocalization between α-synuclein and neurogranin in mouse transgenics when compared to control mice. Coimmunoprecipitation studies in the superior temporal cortex in humans confirmed interaction between α-synuclein and neurogranin, and decreased interaction between α-synuclein and neurogranin was noticed in patients diagnosed with Parkinson's disease when compared to normal control brains. Additionally, phosphorylated neurogranin levels were also decreased in the human superior temporal cortex in patients diagnosed with Parkinson's disease and patients diagnosed with dementia with Lewy bodies. Here, we show for the first time that neurogranin binds to α-synuclein in the human cortex, and this interaction decreases in Parkinson's disease along with the phosphorylation of neurogranin, a molecular process thought to be involved in learning and memory.

*Corresponding author at: Biology Department, University of Wisconsin—River Falls, 410 S. 3rd St., River Falls, WI 54022, United States. Fax: +1 715 425 0738.
**Corresponding author at: Department of Neurosciences, University of California, San Diego, La Jolla, CA 92093-0624, United States. Fax: +1 858 534 6232.
E-mail addresses: andrew.koob@uwrf.edu (A.O. Koob), emasliah@ucsd.edu (E. Masliah).

http://dx.doi.org/10.1016/j.brainres.2014.10.013
0006-8993/

1. Introduction

In Parkinson's disease (PD), the traditional emphasis on research has been the degeneration of the substantia nigra and the motor deficits which arise as a result of the depletion of dopaminergic neurons in this region. However, as PD progresses, the onset of Parkinson's disease dementia (PDD) and cell loss in the cortex and hippocampus is likely (McKeith et al., 2005). Dementia with Lewy bodies (DLB) is diagnosed when dementia occurs concurrently or before parkinsonism while PDD is described as when dementia occurs more than a year after a diagnosis of PD (McKeith et al., 2005). PD, PDD and DLB are all characterized by α-synuclein (α-syn) accumulation extracellularly in cortical and subcortical regions. It is believed that α-syn protein functions normally in cell body and presynaptic terminal (Maroteaux et al., 1988), but is capable from moving from cell to cell as it begins to aggregate (Lee et al., 2010).

In cortical regions in PD and DLB, it is not understood what makes specific cells pregnable to disease. Typical corticalbasal atrophy occurs in parasagittal posterior frontal and parietal regions with more generalized degeneration of the inferior frontal and temporal lobes after onset of dementia (McKeith et al., 2005). Swollen neurons are found throughout affected cortical areas, but are most frequent in layers III, IV and VI (Rebeiz et al., 1967). Neurogranin (Ng) expression is prominent in neurons in layers IV and VI of the cortex: Areas which project to and receive projections from the thalamus (Gerendasy and Sutcliffe, 1997; Neuner-Jehle et al., 1996; Represa et al., 1990; Watson et al., 1992, 1994). Ng is also found in the pyramidal neurons of the hippocampus. These areas of the cortex and hippocampus are damaged in later stages of Parkinson's disease during the onset of dementia (McKeith et al., 2005).

Ng is a postnatally expressed protein and a member of the calpactin family involved in calcium signaling (Represa et al., 1990). It is located in soma and dendritic spines and traditionally thought to bind calmodulin (CaM) in the absence of calcium (Baudier et al., 1991; Hoffman et al., 2014b; Prichard et al., 1999). CaM has been shown in vitro to bind to the N-terminus of α-syn (Gruschus et al., 2013; Martinez et al., 2003). Ng is also a specific substrate for the γ-isoform of protein kinase C (PKC), and is phosphorylated at Ser36 location in the IQ domain when calcium is available to CaM, and is closely associated with cell membranes (Baudier et al., 1991; Dominguez-Gonzalez et al., 2007; Liu et al., 1990). Because of site proximity, Ng-CaM binding and Ng phosphorylation by protein kinase C appear to be mutually exclusive (Baudier et al., 1991). Ng-CaM binding can accelerate CaM dissociation from calcium in postsynaptic neurons (Kubota et al., 2007; Kumar et al., 2013). Additionally, recent studies have shown that Ng bound to CaM can lower the threshold for long-term potentiation (LTP) (Zhong and Gerges, 2012).

Ng knockout mice look similar to nontransgenic mice phenotypically but exhibit difficulties in learning and memory tasks while also developing emotional disruptions (Miyakawa et al., 2001). Decreases in Ng expression levels was asseverated in the hippocampus of amyloid precursor protein (APP) transgenic mice and blocking phosphorylation of Ng can prevent learning indicating a role for Ng in dementia (Alexander et al., 1987; Liu et al., 1990). Additionally, Ng expression has been affected differentially in Alzheimer's and Frontal Temporal dementia (Chang et al., 1997). Ng levels are increased in the cerebral spinal fluid in Alzheimer's disease (Thorsell et al., 2010). A genetic variant upstream of the Ng gene (*NRGN*) has also been shown in patients diagnosed with schizophrenia, further revealing a role for Ng in cognition (Ohi et al., 2013; Stefansson et al., 2009; Steinberg et al., 2011; Walton et al., 2013).

The degeneration of cells in areas that express Ng in Parkinson's disease dementia indicate that Ng may be involved in pathways which lead to dementia in the brain. Additionally, Ng and α-syn both appear to interact with CaM in calcium signalling pathways, suggesting Ng likely contributes a role to α-syn molecular functionality.

2. Results

2.1. *Ng expression in Tg mice and colocalization with α-syn*

A 2.5-fold increase of Ng gene expression in Tg mice over-expressing human α-syn versus nonTg mice was shown by quantitative real time PCR (Fig. 2; 2.58±1.21 vs. 0.98±0.45 relative expression, respectively; $p=0.008$; 2-sided *t*-test) (Fig. 1).

In nonTg mice, in areas of the cortex dorsal to the hippocampus, Ng immunohistochemistry was observed through confocal microscopy most closely associated with the membrane in the cell body and dendrite (Fig. 2A). α-syn immunohistochemistry in nonTg mice was relegated to pixelated synaptic expression (Fig. 2B). When merged, no colocalization was seen in cell bodies (Fig. 2C). A higher level magnification of Ng expressing cells with α-syn expression can be seen in Fig. 2D.

Ng immunohistochemistry in Tg mice was also widespread in the dendrites and the cytosol of cell bodies in areas dorsal to the hippocampus in Tg mice (Fig. 2E). However, in these mice, immunohistochemistry for α-syn revealed large cell body inclusions (Fig. 2F). In Tg mice, colocalization of Ng and α-syn was widespread throughout the cortex (Fig. 2G and Fig. 3H).

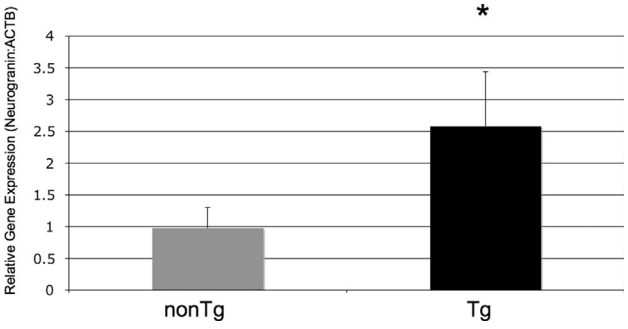

Fig. 1 – Neurogranin expression levels. Neurongranin RNA levels in the brains of Tg mice over expressing human α-syn. Expression increased 2.5 times in Tg mice when compared to normal controls (Fig. 2; 2.58±1.21 vs. 0.98±0.45 relative expression, respectively; $p=0.008$; 2-sided *t*-test).