ELSEVIER

Neurobiology of Aging 31 (2010) 796–804

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Absence of α-synuclein affects dopamine metabolism and synaptic markers in the striatum of aging mice☆

Abdelmojib Al-Wandi[a], Natalia Ninkina[a,b], Steven Millership[a], Sally J.M. Williamson[c], Paul A. Jones[c,1], Vladimir L. Buchman[a,*]

[a] School of Biosciences, Cardiff University, Museum Avenue, Cardiff CF10 3US, United Kingdom
[b] Institute of Physiologically Active Compounds, Russian Academy of Sciences, 1 Severnyj Proezd, Chernogolovka, Russian Federation
[c] Astellas CNS Research Institute, University of Edinburgh, The Chancellor's Building, 49 Little France Crescent, Edinburgh EH16 4SB, United Kingdom

Received 21 April 2008; received in revised form 19 October 2008; accepted 8 November 2008
Available online 20 December 2008

Abstract

Despite numerous evidences for neurotoxicity of overexpressed α-synuclein, a protective function was suggested for endogenous α-synuclein and other members of the synuclein family. This protective role is most important for and evident in presynaptic terminals, where synucleins are normally accumulated. However, mice lacking synucleins display no adverse phenotype. In particular, no significant changes in striatal dopamine metabolism and only subtle deficit of dopaminergic neurons in the substantia nigra were found in juvenile or adult mice. To assess whether aging and synuclein deficiency may have additive detrimental effect on the nigrostriatal system, we studied dopaminergic neurons of the substantia nigra and their striatal synapses in 24–26-month-old α-synuclein and γ-synuclein null mutant mice. Significant ∼36% reduction of the striatal dopamine was found in aging α-synuclein, but not γ-synuclein null mutant mice when compared to age-matching wild type mice. This was accompanied by the reduction of TH-positive fibers in the striatum and decrease of striatal levels of TH and DAT. However, no progressive loss of TH-positive neurons was revealed in the substantia nigra of synuclein-deficient aging animals. Our results are consistent with a hypothesis that α-synuclein is important for normal function and integrity of synapses, and suggest that in the aging nervous system dysfunction of this protein could become a predisposition factor for the development of nigrostriatal pathology.

Keywords: Synuclein; Null mutant; Synucleinopathy; Parkinson's disease; Ageing; Dopaminergic neurons; Substantia nigra; Neuroprotection; Synaptoprotection

1. Introduction

The decade of intensive studies that followed the original demonstration of direct links between α-synuclein and Parkinson's disease (Polymeropoulos et al., 1997; Spillantini et al., 1997) revealed structural, functional and metabolic properties of this protein that might be responsible for its role in the development of Parkinson's and other neurodegenerative disorders, now known as synucleinopaties. Nevertheless, the question about exact mechanism of α-synuclein involvement in neurodegeneration is still wide open. Histopathological observations together with high propensity of α-synuclein to aggregate, which could be further increased by certain causative mutations, led to an intuitive hypothesis that aggregation into large insoluble filaments, which become major constituents of Lewy bodies and other inclusions, is the primary cause of neurodegenerative changes (Trojanowski et al., 1998; Spillantini and Goedert, 2000; Galvin et al., 2001). However, further clinical and experimental studies questioned the importance of the final products of α-synuclein aggregation, fibrils or filaments, for the cytotoxic effect of mutated or overexpressed α-synuclein (Mori et al., 1998; Saha et al., 2000; Volles et al., 2001; Gosavi et al., 2002; Volles and Lansbury, 2002). It has been suggested

☆ All animal work was carried in accordance with the United Kingdom Animals (Scientific Procedures) Act (1986).

* Corresponding author. Tel.: +44 29 20879068; fax: +44 29 20874116.
E-mail address: BuchmanVL@cardiff.ac.uk (V.L. Buchman).
[1] Present address: GE Healthcare, R & D, The Grove Centre, White Lion Road, Amersham, Buckinghamshire HP7 9LL, United Kingdom.

0197-4580/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.neurobiolaging.2008.11.001

that although enhanced aggregation is a prerequisite for α-synuclein cytotoxicity, the toxic effect should be attributed not to fibrils but to soluble intermediates of aggregation process, oligomers or protofibrils (reviewed in Dev et al., 2003; Volles and Lansbury, 2003). Moreover, a swing of aggregation kinetics in favour of the final steps of the process could reduce intracellular concentration of these toxic α-synuclein species by precipitating and trapping them within the insoluble deposits. Aggregation products could be cleared up to a certain extent, by intracellular systems (e.g. autophagosome and lysosome) that are different from systems metabolising soluble proteins (e.g. proteasome), which increases the ability of cells to combat the increased production of potentially toxic proteins like α-synuclein (Ciechanover and Brundin, 2003; Webb et al., 2003; Cuervo et al., 2004; Lee et al., 2004; Rideout et al., 2004). Although toxicity of aggregation intermediates is currently widely accepted as the major step in the development of α-synuclein-induced pathology, other mechanisms may also play an important role in this process. One significant consequence of aggregation or aberrant intracellular trafficking of α-synuclein is depletion of its pool at the intracellular sites where this protein is normally localised, primarily in the presynaptic terminals. The outcome of the resulting loss-of-function is not apparent because above all the function itself is still not defined. However, recent studies clearly demonstrated the importance of α-synuclein in protection from at least some types of synaptic dysfunction and consequent neurodegeneration (Chandra et al., 2005). This function of α-synuclein could be revealed only in a case when certain other protective mechanisms fail, suggesting an auxiliary role of this protein in neuroprotection. Studies of various tissue culture models also demonstrated that α-synuclein could act as a protective factor but only in particular conditions and types of cells (reviewed in Dev et al., 2003). Moreover, animals lacking functional α-synuclein gene as the result of either spontaneous deletion or targeted inactivation do not show any signs of degeneration in their nervous system (Abeliovich et al., 2000; Specht and Schoepfer, 2001; Cabin et al., 2002; Dauer et al., 2002; Schluter et al., 2003). A simple functional compensation by other members of the family, β-synuclein and γ-synuclein, could not explain this lack of pathological changes because neither double nor triple synuclein adult null-mutant mice develop adverse phenotype (Chandra et al., 2004; Robertson et al., 2004; Papachroni et al., 2005 and our unpublished observations). However, no studies of the nervous system of aging synuclein null mutant mice have been carried out so far. Aging is associated with functional decline of many protective systems within cells. In particular, age-related changes in the nigrostriatal system could predispose to the development of Parkinson's disease (Phinney et al., 2006; Chu and Kordower, 2007; Collier et al., 2007). It is feasible that the absence of auxiliary protective factors like synucleins might exacerbate pathological changes related to the senescence of the nervous system. This is consistent with clinical data suggesting that aging is the major risk factor for developing idiopathic synucleinopathies.

We studied the nigrostriatal system of aging synuclein null mutant mice and demonstrated that the absence of α-synuclein but not γ-synuclein leads to the substantial decline of dopamine level and decrease of expression of certain synaptic markers in the striatum without reduction of the number of dopaminergic neurons in the substantia nigra pars compacta (SNpc).

2. Methods

2.1. Animals

Generation and maintenance of colonies of α-synuclein and γ-synuclein null mutant mice on pure C57Bl6J genetic background was described previously (Ninkina et al., 2003; Robertson et al., 2004). Experimental cohorts for this study were formed from litters produced by intercrossing heterozygous animals. After weaning and genotyping the null mutant and wild type male littermates were housed one per cage with free access to water and food until they reach age of 24–26 months. Animals (for simplicity refer in the manuscript as 2-year-old mice) were sacrificed by cervical dislocation, and tissues were collected and coded. Therefore, individuals that performed all further analyses were unaware of the sample genotype. Animal performance in an accelerating rotarod test was assessed as described previously (Robertson et al., 2004).

2.2. Neuronal cell counts

For quantification of neurons, brains of age-matching wild type, α-synuclein and γ-synuclein null mutant mice were collected, fixed, processed, embedded and stained simultaneously. 8-μm-thick sections were cut using a HM 310 microtome (Microm International) and mounted onto poly-L-lysine coated slides. The borders of the substantia nigra and ventral tegmental area (VTA) on histological sections were outlined using distribution atlas of tyrosine hydroxylase (TH)-positive cells (Hokfelt et al., 1984). The number of dopamine positive neurons in these two brain regions of 2-year-old animals was assessed by stereological counting of cells stained for TH on serial histological sections as described previously (Robertson et al., 2004). Briefly, the first section for counting was randomly chosen from the first 10 sections that included SNpc/VTA region. Starting from this section, every TH-positive cell with a clearly seen nucleus was counted on the every tenth section through the whole region. The Axiovision imaging program (Carl Zeiss Vision) was employed to measure diameters of 50 nuclei of dopaminergic neurons in the SNpc and 50 nuclei of dopaminergic neurons in the VTA of every mouse brain included in this study. The nuclei were chosen randomly and the distance measured as the horizontal length as they appeared on screen. A mean was calculated for each animal and used for Abercrombie's correction (Abercrombie, 1946) to obtain an actual number of TH positive cells in the structure.