Available online at www.sciencedirect.com

SCIENCE DIRECT

Molecular Brain Research 139 (2005) 137 – 152

MOLECULAR BRAIN RESEARCH

www.elsevier.com/locate/molbrainres

Research Report

Wild-type α-synuclein interacts with pro-apoptotic proteins PKCδ and BAD to protect dopaminergic neuronal cells against MPP^+-induced apoptotic cell death

Siddharth Kaul, Vellareddy Anantharam, Arthi Kanthasamy, Anumantha G. Kanthasamy*

Parkinson's Disorder Research Laboratory, Department of Biomedical Sciences, 2062, Veterinary Medicine Building, Iowa Sate University, Ames, IA 50011, USA

Accepted 12 May 2005
Available online 22 June 2005

Abstract

α-Synuclein is a pre-synaptic protein of unknown function that has been implicated in the pathogenesis of Parkinson's disease (PD). Recently, we demonstrated that 1-methyl-4-phenylpyridinium (MPP^+) induces caspase-3-dependent proteolytic activation of PKCδ, which subsequently contributes to neuronal apoptotic cell death in mesencephalic dopaminergic neuronal cells [50,96]. In the present study, we examined whether PKCδ interacts with α-synuclein to modulate MPP^+-induced dopaminergic degeneration. Over-expression of wild-type human α-synuclein in mesencephalic dopaminergic neuronal cells (N27 cells) attenuated MPP^+-induced (300 μM) cytotoxicity, release of mitochondrial cytochrome *c*, and subsequent caspase-3 activation, without affecting reactive oxygen species (ROS) generation. Wild-type α-synuclein over-expression also dramatically reduced MPP^+-induced caspase-3-mediated proteolytic cleavage of PKCδ, whereas over-expression of the mutant human $\alpha\text{-synuclein}^{A53T}$ did not alter the PKCδ cleavage under similar conditions. Immunoprecipitation-kinase assay revealed reduced PKCδ kinase activity in wild-type α-synuclein over-expressing cells in response to MPP^+ treatment. Wild-type α-synuclein over-expression also rescued mesencephalic dopaminergic neuronal cells from MPP^+-induced apoptotic cell death, while $\alpha\text{-synuclein}^{A53T}$ exacerbated the MPP^+-induced DNA fragmentation. Furthermore, co-immunoprecipitation studies revealed that α-synuclein interacts with the pro-apoptotic proteins PKCδ and BAD, but not with the anti-apoptotic protein Bcl-2 following MPP^+ treatment. We also observed that the interaction between PKCδ and α-synuclein does not involve direct phosphorylation. Together, our results demonstrate that wild-type α-synuclein interacts with the pro-apoptotic molecules BAD and PKCδ to protect dopaminergic neuronal cells against neurotoxic insults.

Theme: Disorders of the nervous system
Topic: Degenerative disease: Parkinson's

Keywords: Alpha-synuclein; Oxidative stress; PKC; Apoptosis; Protease; Protein interaction; Signal transduction; Neurotoxicity; Neurodegeneration; Parkinson's disease

1. Introduction

Parkinson's disease (PD) is characterized by a selective degeneration of dopaminergic neurons in the substantia nigra, resulting in irreversible motor dysfunction [86,101,103]. Lewy bodies, intraneuronal proteinaceous inclusions considered a pathological hallmark of PD, are predominantly composed of misfolded and aggregated α-synuclein protein [48,84,88]. The etiology of PD is still not fully understood, but genetic analyses, epidemiologic studies, neuropathologic investigations, and new experimental models of PD are providing important new insights into the pathogenesis of PD [23]. At least 10 distinct loci are responsible for the rare genetic forms of PD [22,91]. Among these loci are missense point mutations in the α-synuclein gene, A53T and A30P, linked to early-onset

* Corresponding author. Fax: +1 515 294 2315.
E-mail address: akanthas@iastate.edu (A.G. Kanthasamy).

0169-328X/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.molbrainres.2005.05.022

familial PD, supporting the role of α-synuclein in neurodegeneration [53,55,72]. The recent discovery that the triplication of the α-synuclein locus causes PD in some individuals, due to increased production of α-synuclein, clearly suggests that changes in the expression of this protein could be a risk factor for PD [82]. Furthermore, a new mutation, E46K, in the α-synuclein locus has also been linked to Parkinson and Lewy body dementia in some individuals [99].

α-Synuclein is an abundant pre-synaptic protein belonging to the family of proteins that include α-, β-, γ-synuclein, and synoretin [30,40,61]. Structurally, it is a 140 amino acid protein consisting of an acidic C-terminal region and a seven 11-mer repeat region in the N-terminal region which promotes binding of α-synuclein to phospholipids [11]. The physiological role of α-synuclein is still unclear, but it has been suggested to play a role in memory development, neurotransmitter release, and axonal transport [1,13,29,44]. Because of its open random coiled structure, α-synuclein has been suggested to also function as a chaperone [93] and shares physical and functional homology with the 14-3-3 protein chaperone family [69]. Abnormal accumulation and aggregation of α-synuclein has been implicated in the dysfunction, degeneration, and ultimate death of dopaminergic neurons in PD [84,92]. However, over-expression studies with the wild-type α-synuclein in cellular and animal models of PD have failed to yield any consistent information regarding the mechanism of its toxicity. Yet, human α-synuclein over-expression has been shown to decrease mitochondrial function, increase oxidative stress and enhance vulnerability to oxidative insult in models of dopaminergic toxicity [38,46,102]. Furthermore, studies in transgenic animals have shown that over-expression of wild-type α-synuclein can lead to formation of Lewy body like inclusions or extensive neurodegenerative processes [64] in the substantia nigra. In contrast, numerous recent studies have shown that over-expression of wild-type human α-synuclein confers resistance to oxidative insults and apoptotic cell death in some cellular models [2,35,59,80].

Accumulating evidence strongly suggests that the nigral striatal dopaminergic system is highly susceptible to oxidative stress, and the oxidative insult accelerates neuronal apoptosis in PD [3,32,43,89]. Major insights into the neurodegenerative PD process have been gained from the MPTP-induced models that faithfully replicate the salient Parkinsonian symptoms and pathology [7,54,65,83]. Recent studies have demonstrated that exposure to MPTP or its active metabolite MPP^+ induces many apoptotic events including ROS generation, cytochrome *c* release, caspase activation, and DNA fragmentation [50,81,90]. In addition, alterations in certain apoptotic cell death-related molecules such as Bcl-2, MAP kinases, and PARP [14,33,58,95] during MPP^+-induced dopaminergic degeneration have also been reported. Recently, we demonstrated that caspase-3-dependent proteolytic activation of PKCδ mediates and regulates oxidative stress-induced apoptotic cell death during exposure to various dopaminergic neurotoxins including MPP^+ [5,50,51]. In the present study, we attempt to identify the effect of wild-type human α-synuclein over-expression on the pro-apoptotic function of PKCδ in the MPP^+-induced rat mesencephalic dopaminergic clonal cell model (N27 cells). We have recently used this model in our laboratory, [50,96] as have others [73,79], to study cell death mechanisms associated with the nigral dopaminergic degeneration. Herein, we report that over-expression of human wild-type α-synuclein rescues rat dopaminergic mesencephalic cells from MPP^+-induced apoptotic cell death by attenuating the proteolytic activation of a novel protein kinase C isoform-δ (PKCδ), whereas the A53T α-synuclein mutant protein augments MPP^+-induced PKCδ activation and cellular apoptosis. We also demonstrate, for the first time to our knowledge, an enhanced physical association between α-synuclein and the pro-apoptotic molecules BAD and PKCδ during MPP^+-induced cell death in a dopaminergic model of neurodegeneration.

2. Materials and methods

2.1. Chemicals

MPP^+ (1-methyl-4-phenypyridinium), β-actin antibody (mouse monoclonal), histone H1, β-glycerophosphate, ATP, and protein A sepharose were purchased from Sigma-Aldrich (St. Louis, MO). Ac-DEVD-AMC (Acetyl-Asp-Glu-Val-Asp-7-Amino-4-methylcoumarin) was obtained from Bachem Biosciences (King of Prussia, PA); FITC-VAD-fmk was purchased from Promega (Madison, WI); PKCδ and the human α-synuclein antibodies were purchased from Santacruz labs (Santa Cruz, CA); the α-synuclein polyclonal antibody Syn-1, detecting both human and rat isoforms, was purchased from Transduction Labs (Lexington, KY, USA). Dihydroethidine (DhEt) and Hoechst 33342 were purchased from Molecular Probes (Eugene, OR). Cell Death Detection ELISA Plus Assay Kit was purchased from Roche Molecular Biochemicals (Indianapolis, IN). [γ-^{32P}]ATP was obtained from NEN (Boston, MA). RPMI 1640, fetal bovine serum, L-glutamine, penicillin, and streptomycin were purchased from Invitrogen (Gaithesburg, MD). Recombinant α-synuclein protein was obtained from rPeptide (Athens, GA) and recombinant PKCδ enzyme was bought from Prosci Incorporated (Poway, CA). The plasmid encoding wild-type human α-synuclein (α-SYN-pCEP4) was kindly provided by Dr. Eliezer Masliah, UCSD, San Diego. Dr. Henry L. Paulson, University of Iowa, Iowa City, kindly provided plasmid encoding the A53T α-synuclein mutant (α-SYNA53T). The immortalized rat dopaminergic mesencephalic clonal cell line (N27 cells) was a kind gift of Dr. Kedar N. Prasad, Univ. of Colorado Health Sciences Center (Denver, CO).