Experimental Neurology 252 (2014) 63–74

Contents lists available at ScienceDirect

Experimental Neurology

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

Alpha-synuclein interferes with cAMP/PKA-dependent upregulation of dopamine β-hydroxylase and is associated with abnormal adaptive responses to immobilization stress

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ARTICLE INFO

Article history:
Received 30 July 2013
Revised 21 October 2013
Accepted 10 November 2013
Available online 16 November 2013

Keywords:
α-Synuclein
Anxiety
cAMP
Dopamine β-hydroxylase (DBH)
Parkinson's disease
Stress

ABSTRACT

Parkinson's disease (PD) is clinically characterized not only by motor symptoms but also by non-motor symptoms, such as anxiety and mood changes. Based on our previous study showing that overexpression of wild-type or mutant α-synuclein (α-SYN) interferes with cAMP/PKA-dependent transcriptional activation in norepinephrine (NE)-producing cells, the effect of wild-type and mutant α-SYN on cAMP response element (CRE)-mediated regulation of the NE-synthesizing enzyme dopamine β-hydroxylase (DBH) was evaluated in this study. Overexpression of wild-type or mutant α-SYN interfered with CRE-mediated regulation of DBH transcription in NE-producing SK-N-BE(2) cells. Upon entering the nucleus, α-SYN interacted with the DBH promoter region encompassing the CRE, which interfered with forskolin-induced CREB binding to the CRE region. Interestingly, mutant A53T α-SYN showed much higher tendency to nuclear translocation and interaction with the DBH promoter region encompassing the CRE than wild type. In addition, A53T α-SYN expressing transgenic mice exhibited increased anxiety-like behaviors under normal conditions and abnormal regulation of DBH expression in response to immobilization stress with abnormal adaptive responses. These data provide an insight into the physiological function of α-SYN in NErgic neuronal cells, which further indicates that the α-SYN mutation may play a causative role in the generation of non-motor symptoms in PD.

Introduction

Parkinson's disease (PD) is one of the most common progressive neurodegenerative disorders, affecting approximately 1% of the elderly population (de Lau and Breteler, 2006). Clinical symptoms of PD involve motor dysfunction featuring slowness or lack of movement, rigidity, postural instability, and resting tremor (Jenner and Olanow, 2006; Olanow and Tatton, 1999). The pathogenesis of the movement dysfunction in PD involves loss of dopaminergic (DArgic) neurons in the substantia nigra pars compacta (SNpc).

Although PD is traditionally viewed as a motor disorder, evidence from numerous epidemiologic (Shiba et al., 2000) as well as case–control studies (Richard, 2005) suggest the occurrence of non-motor symptoms including hyposmia/anosmia, gastrointestinal disturbances, sleep abnormalities, autonomic dysfunction, anxiety, depression, and impaired cognition at premotor stages of PD (Braak et al., 2003; Langston, 2006). Among these, anxiety disorders frequently occur in association with PD (up to 40% of PD patients) and may be important causes of morbidity (Richard, 2005). While the pathophysiology of anxiety and depressive symptoms in PD patients is largely uncertain, loss of DArgic neurons in the nigrostriatal pathway is unlikely to be the cause. Such symptoms may instead be linked to other specific neurobiological processes that occur in PD.

The norepinephrinergic (NErgic) system in the brain plays an important role in attention, sleep/wakefulness, learning and memory, emotion, and central responses to stress (Sara, 2009). The central NErgic system has also been implicated in anxiety states and depression (Charney, 2003). Norepinephrine (NE) is synthesized by sequential enzymatic reaction of tyrosine. Dopamine β-hydroxylase (DBH) converts DA to NE, which is a hallmark protein of NErgic cells and is specifically expressed in NErgic neurons and chromaffin cells of the adrenal medulla. In the brain, NErgic neuronal activity originates from the locus coeruleus (LC) and is increased under stressful conditions by upregulation of enzymatic activity and transcription of tyrosine

Abbreviations: PD, Parkinson's disease; NErgic, norepinephrinergic; NE, norepinephrine; DBH, dopamine β-hydroxylase; LC, locus coeruleus; TH, tyrosine hydroxylase; cAMP, cyclic AMP; CRE, cAMP response element; α-SYN, alpha-synuclein; PBS, phosphate-buffered saline; ECL, enhanced chemiluminescence; HPLC-ECD, High-performance liquid chromatography-electrochemical detector; ChIP, Chromatin immunoprecipitation; IMO, immobilization stress.

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http://dx.doi.org/10.1016/j.expneurol.2013.11.009

hydroxylase (TH) and DBH (Sabban and Kvetnanský, 2001; Sabban and Serova, 2007; Wong and Tank, 2007). Regulation of DBH activity occurs at the transcriptional level; multiple intracellular effectors such as cyclic AMP (cAMP), glucocorticoids, calcium and related signaling molecules, and nerve growth factor are involved in its transcriptional regulation (Acheson et al., 1984; Kim et al., 1993, 1994; Kobayashi et al., 1989; McMahon and Sabban, 1992; Otten and Thoenen, 1976; Sabban et al., 1983). cAMP response element (CRE) is an essential positive element for both basal and stress response-inducible transcriptional regulations of the gene encoding DBH (Ishiguro et al., 1993; Lamouroux et al., 1993); therefore, interference with CRE-mediated DBH regulation may be involved in the etiology of anxious/depressive symptoms in PD (Lamouroux et al., 1993; Lieberman, 2006).

Several independent investigators have documented disturbances in central NErgic systems and early involvement of the LC in PD (Dickson et al., 2009; German et al., 1992; Marien et al., 2004; Schapira et al., 2006). Experimental lesions of the LC have also been shown to exacerbate PD pathology and behavioral symptoms in animal models (Marien et al., 1993; Mavridis et al., 1991; Srinivasan and Schmidt, 2003). Postmortem examinations indicate that the level of NE in the LC is reduced in PD, which is associated with a loss of pigmented neurons and the formation of Lewy body inclusions (Forno et al., 1993). Taken together, these results suggest that NE dysfunction occurs prior to significant loss of DArgic neurons and may be responsible for both motor and non-motor symptoms in PD patients.

The small (~14 kDa) acidic protein alpha-synuclein (α-SYN) is a major component of Lewy bodies and is suggested to be a genetic risk factor for PD. The A53T, A30P, and E46K mutations in the gene encoding α-SYN are associated with an autosomal dominant familial type of PD (Giasson et al., 2002; Krüger et al., 1998; Lee et al., 2002; Polymeropoulos et al., 1997; van der Putten et al., 2000; Zarranz et al., 2004). α-SYN is expressed predominantly in presynaptic terminals and plays a role in synaptic vesicle recycling, storage, and release of neurotransmitters for normal neurotransmission (Abeliovich et al., 2000; Yavich et al., 2004, 2006). Both missense and multiplication mutations of α-SYN affect DA transmission by interfering with the synthesis and vesicular storage of DA (Lotharius and Brundin, 2002). α-SYN interacts with TH and reduces TH activity, phosphorylation (Perez et al., 2002), and expression (Yu et al., 2004). More interestingly, our previous study revealed that overexpression of wild-type or mutant α-SYN interferes with CRE-mediated transcriptional regulation of TH (Kim et al., 2011). In addition, very recent evidence suggested that impairments in autonomic modulation of cardiac function were shown in mice overexpressing α-SYN (Fleming et al., 2013), which is also frequently observed in the synucleinopathy PD. Because significantly increased expression of α-SYN is observed in the brains of PD patients and aged populations (DeMarch et al., 2007), it is interesting to consider that elevation of α-SYN, especially its mutant form, caused by a specific disease or the normal aging process could be associated with mood changes by dysregulating NE neurotransmission.

Based on reports demonstrating that 1) NE deficiency and non-motor symptoms including anxiety and depression are clinically observed in the very early stages of PD, 2) abnormal regulation of DBH expression is implicated in the etiology of anxious/depressive symptoms, and 3) α-SYN is upregulated in PD as well as in the aging process and interferes with cAMP/PKA-dependent CREB signaling in catecholaminergic neurons, this study addressed the possibility that the α-SYN mutation plays a causative role in the pathogenesis of non-motor symptoms in PD. We showed that overexpression of wild-type or mutant α-SYN interfered with CRE-mediated regulation of DBH transcription in NE-producing SK-N-BE(2) cells. In addition, mice expressing A53T mutant α-SYN exhibit heightened depression/anxiety-like behaviors and abnormal responses to immobilization stress.

Materials and methods

Cell culture

Human neuroblastoma SK-N-BE(2) cells (ATCC, CRL-2271) were maintained in DMEM containing 10% fetal bovine serum (Gibco Invitrogen, Grand Island, NY), 100 IU/l penicillin, and 10 μg/ml streptomycin at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. Cells were placed on polystyrene culture plates or dishes at 1.0×10^5 cells/well in 24-well plates, 5.0×10^5 cells/well in 6-well plates, 1.0×10^6 cells/60-mm culture dish, or 3.0×10^6 cells/100-mm culture dish. After 24 h incubation, cells were fed with fresh medium for subsequent experiments.

Transient transfection

Human wild-type or mutant (A30P or A53T) α-SYN expression constructs (pcDNA3.1) and flag-tagged human wild-type or mutant (A30P or A53T) α-SYN expression constructs (pCMV) were transiently transfected into SK-N-BE(2) cells for 24 h using polyethylenimine reagents (Polyscience, Warrington, PA) according to the manufacturer's instructions.

Reporter gene assay

A dual-luciferase reporter assay system (Promega, Madison, WI) was used to determine DBH promoter activity. Cells were plated at a density of 1.0×10^5 cells/well in 24-well culture plates and transiently transfected with 0.1 μg of wild-type or mutant (A30P or A53T) α-SYN constructs, 0.5 μg of pDBH-luciferase plasmids, and 0.05 μg of pRL-TK renilla luciferase plasmid using polyethylenimine reagents (Polyscience, Warrington, PA) according to the manufacturer's instructions on the following day. After treatment with forskolin, cells were washed with PBS and lysed with passive lysis buffer (Promega, Madison, WI), and the firefly and renilla luciferase activities in the cell lysates were determined using a microplate luminometer (Microlumat Plus LB 96 V, BERTHOLD). Luciferase activities were presented in relative luciferase units (RLUs) compared with control cells. Data were expressed as mean ± SEM of firefly luciferase activity normalized against that of renilla luciferase activity (pRL-TK control vector).

Immunoblot analysis

For immunoblot analysis, cells were washed with phosphate-buffered saline (PBS) and lysed with RIPA buffer (150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 2 mM EDTA, 50 mM Tris-HCl, pH 7.5). The supernatants were collected after centrifugation at 13,200 rpm for 10 min. Equal amounts of protein were separated on 10% SDS polyacrylamide gel and trans-blotted onto polyvinylidene difluoride-nitrocellulose filters (Millipore Corporation, Billerica, MA). Membranes were incubated with appropriate primary antibodies and then incubated with horseradish peroxide-conjugated secondary antibodies. Specific bands were visualized using the enhanced chemiluminescence (ECL) detection kit (Amersham Bioscience, Piscataway, NJ).

High-performance liquid chromatography-electrochemical detector (HPLC-ECD) analysis

Catecholamines were measured by HPLC-ECD. Small amount of cell was washed with PBS and lysed with RIPA buffer. Protein concentration was determined using Bradford reagent (Bio-Rad; Hercules, CA). Cells were washed with PBS and lysed 2 volumes of ice-cold 0.1 M perchloric acid during overnight at −80 ºC. After centrifugation at 15,000 ×*g* for 30 min at 4 °C, the supernatant was directly injected onto the HPLC column-Nova-Pak® C18 reversed-phase column (150 mm × 3.9 mm