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Short Communication

α-Synuclein enhances dopamine D2 receptor signaling

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Abbreviations:
CHO, Chinese hamster ovary
CRE, cAMP-responsive element
D2R, dopamine D2 receptor
LB, Lewy body
MAPK, mitogen-activated protein kinase
PD, Parkinson's disease
RIA, radioimmunoassay
SN, substantia nigra

ABSTRACT

Parkinson's disease (PD) is characterized by a selective loss of dopamine-producing neurons in the substantia nigra (SN), which in turn results in dopamine depletion in the striatum, and the presence of neuronal cytoplasmic inclusions known as Lewy bodies (LBs). α-Synuclein is a presynaptic protein that accumulates abnormally in LBs and is seen predominantly in cases of dementia with LBs. Although the central role of α-synuclein in neurodegeneration has been previously demonstrated by the discovery of missense α-synuclein mutations in familial PD, the specific mechanism by which α-synuclein contributes to these diseases remains unclear. In the present study, we examined whether α-synuclein affects the downstream signaling of dopamine D2 receptor (D2R). In CHO cells stably transfected with D2Rs, α-synuclein enhanced dopamine D2-agonist-mediated inhibition of adenylate cyclase, which consequently affected its downstream cAMP-responsive element (CRE)-mediated gene transcription, while C-terminal deletion mutant of α-synuclein did not. Our study suggests that the α-synuclein enhances the dopamine-mediated intracellular signaling pathways by D2R, thus provide a possible mechanism in presynaptic regulation of the synaptic homeostasis in the dopaminergic neurotransmission.

α-Synuclein is one member of the synuclein gene family, which is expressed abundantly in various regions of the brain, and is found predominantly in the presynaptic terminals (Goedert, 2001; Clayton and George, 1999; Sidhu et al., 2004a). Mutations in α-synuclein are linked in some familial cases of Parkinson's disease (PD). These cases tend to be characterized by the preferential and progressive destruction of nigrostriatal dopaminergic neurons (Polymeropoulos et al., 1997; Kruger et al., 1998). Moreover, studies have shown α-synuclein accumulation in dystrophic neurons and Lewy bodies (LBs) in idiopathic PD and other neurodegenerative disorders (Spillantini et al., 1997). These findings suggest that fibrillization and aggregation of α-synuclein may play a central role in neuronal dysfunction and cell death in PD.

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Structurally, α-synuclein is composed of three modular domains, including an amino-terminal lipid-binding α-helix, a β-amyloid-binding domain, which encodes the non-A β component of Alzheimer's diseased plaques (Ueda et al., 1993), and a carboxyl-terminal acidic tail. It has been reported that the C-terminal region, which represents a putative Ca^{2+}-binding region, shows significant differences in primary sequences among α-, β- and γ-synucleins (Kim et al., 2002). It has been also reported that α-synuclein could act as a molecular chaperone, and the deletion mutants of the C-terminal region could eliminate the chaperone activity leading to the facilitated aggregation of α-synuclein, indicating the importance of this carboxyl-terminal domain of α-synuclein as a crucial effector in its various physiological functions of α-synuclein (Kim et al., 2002; Ahn et al., 2006).

Although the overexpression of human α-synuclein induces dopaminergic nerve terminal degeneration in some neuronal cells (Dickson et al., 1999), the molecular mechanism by which α-synuclein contributes to the degeneration of these pathways remains enigmatic. It was suggested that the neurotoxic effects of α-synuclein might originate specifically in its interactions with dopamine in dopaminergic neurons (Conway et al., 2001), a finding consistent with earlier reports demonstrating that α-synuclein expression is toxic to dopaminergic neurons (Zhou et al., 2000).

However, although the normal cellular functions of α-synuclein are unknown, considerable evidence now suggests that α-synuclein may serve to integrate presynaptic signaling and membrane trafficking (Clayton and George, 1999; Davidson et al., 1998; Ahn et al., 2002; Jo et al., 2000; Sung et al., 2001), which can lead to a neuroprotective effect on the dopaminergic neurons (Manning-Boð et al., 2003; Sidhu et al., 2004b).

The dopamine D2R is known to be a principal subtype of dopamine receptor and represents as the presynaptic dopamine receptor (Picetti et al., 1997; Missale et al., 1998). The binding of dopamine to the D2R is crucial in the regulation of a myriad of physiological functions, the absence of D2R has been found to be associated with severe impairment of locomotor activity and abnormal pituitary development in dopamine D2R knock-out mice, demonstrating that the D2R plays a key role in dopaminergic nervous function (Picetti et al., 1997; Baik et al., 1995; Kelly et al., 1997; Usiello et al., 2000).

Since α-synuclein is supposed to modulate the neuronal homeostasis of presynaptic dopaminergic neurons, we investigated how α-synuclein and the C-terminal region of α-synuclein affects the dopamine D2R-mediated signal pathway, through such mechanisms as the inhibition of adenylate cyclase and cAMP-mediated gene transcription, by overexpressing α-synuclein and an deletion mutants of the C-terminal region of α-synuclein (Syn 1–95), in CHO cells which stably expressed two isoforms of dopamine D2Rs, D2L (long) and D2S (short), respectively (Choi et al., 1999; Kim et al., 2004). The generation of the CHO cell lines stably expressing the D2L and D2S dopamine receptors has been described previously (Choi et al., 1999; Kim et al., 2004). Two isoforms of D2Rs, D2L and D2S differ by the insertion of a 29 amino acid specific to D2L within the putative third intracellular loop of the receptor (Montmayeur et al., 1991).

The CHO cells expressing dopamine D2L receptor (CHOD2L) or D2S receptors (CHOD2S) were grown to confluence in 48-well plates. Transient transfections were performed using the liposome-mediated transfection reagent, FuGENE 6 (Roche, Indianapolis, IN, USA). In brief, 70%–80% confluent monolayers incubated were incubated in 48-well plates at 37 °C with transfection mixture, which contained pCH110 carrying the β-galactosidase gene, and α-synuclein or deletion mutants of the C-terminal region of α-synuclein plasmid DNA. The CHOD2L or CHOD2S cells transfected with either WT or mutant α-synuclein were recruited with ice-cold PBS containing 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 mM PMSF, homogenized with probe type sonicator on ice, and followed by centrifugation at 13,000×*g* for 10 min at 4 °C. Cytosolic protein (about 100 μg) was separated on a 15% SDS-PAGE, blotted onto prewetted polyvinylidene difluoride (PVDF) membrane. Primary antibodies used were a rabbit polyclonal anti-pan synuclein (1:1000; Chemicon, Temecula, CA). Specific bands were detected by enhanced chemiluminescence (Pierce ECL; Pierce biotechnology Inc., Rockford, IL) and analyzed by LAS3000 image analysis system (Fuji, Tokyo, Japan) (Fig. 1C). Assays were performed 48 h after transfection. In order to perform the assays, medium was removed and the cells were replaced with F-12 medium supplemented with 0.5% bovine serum albumin (BSA) containing 0.5 mM 3-isobutyl-1-methyl-xanthine for 30 min. Forskolin (10 μM) was added for 10 min and then incubated with appropriate concentrations of dopamine for a further 30 min at 37 °C. The medium was then discarded completely, and the cells were frozen at −70 °C for 30 min and thawed at room temperature for 15–20 min, repeated three times. Subsequently, the cells were detached from the plates with 50 mM HCl (1 ml) solution per well, transferred to a 1.5-ml Eppendorf tube, followed by 10 min of centrifugation at 1900×*g*. The cAMP concentration was measured using the cAMP-^{125}I radioimmunoassay (RIA) Kit (BTI Inc.), according to the manufacturer's instructions.

For CRE-mediated luciferase reporter gene assays, CHOD2L and CHOD2S cells were transfected with mixture containing pCRE-luc (stratagene), pCH110 carrying the β-galactosidase gene and α-synuclein or the C-terminal deletion mutant of α-synuclein plasmid DNA. After 6 h, the transfection mixture was replaced with fresh growth medium. Assays were performed 48 h after transfection. Forskolin (10 μM) was added for 30 min and then incubated with appropriate concentrations of dopamine for a further 6 h at 37 °C. CHOD2L and CHOD2S cells were assayed for luciferase activity using the luciferase assay system (Promega), and luminescence was measured using a 96-well luminometer (Microlumat; EG and Berthold, Bad Wilbad, Germany). The expression of the reporter gene was normalized using β-galactosidase activity. The mean values of the data obtained were fitted to a sigmoid curve with a variable slope factor using the nonlinear squares regression in a GRAPHPAD PRISM. IC_{50} (nM) values were described as the mean±SE. All of the cAMP assay or luciferase reporter gene activity assays were performed in at least four iterations.

As shown in Fig. 1, the overexpression of α-synuclein in CHOD2L cells, interestingly, enhanced D2R-mediated inhibition of cAMP accumulation, while the overexpression of the C-terminal deletion mutant of α-synuclein (Syn 1–95) attenuated that effect. Indeed, the co-expression of α-synuclein ameliorated the inhibitory effect of IC_{50} in forskolin-stimulated