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Research Paper

A peptide disrupting the D2R-DAT interaction protects against dopamine neurotoxicity

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ABSTRACT

Dopamine reuptake from extracellular space to cytosol leads to accumulation of dopamine, which triggers neurotoxicity in dopaminergic neurons. Previous studies have shown that both dopamine D2 receptor (D2R) and dopamine transporter (DAT) are involved in dopamine neurotoxicity. However, blockade of either D2R or DAT causes side effects due to antagonism of other physiological functions of these two proteins. We previously found that DAT can form a protein complex with D2R and its cell surface expression is facilitated via D2R-DAT interaction, which regulates dopamine reuptake and intracellular dopamine levels. Here we found that an interfering peptide (DAT-S1) disrupting the D2R-DAT interaction protects neurons against dopamine neurotoxicity, and this effect is mediated by inhibiting DAT cell surface expression and inhibiting both caspase-3 and PARP-1 cleavage. This study demonstrates the role of the D2R-DAT complex in dopamine neurotoxicity and investigated the potential mechanisms, which might help better understand the mechanisms of dopamine neurotoxicity. The peptide may provide some insights to improve treatments for dopamine neurotoxicity and related diseases, such as Parkinson's disease, as well as methamphetamine- and 3,4-methsylenedioxy methamphetamine-induced neurotoxicity.

1. Introduction

Dopamine is an important neurotransmitter that is demonstrated to regulate various physiological functions, such as locomotor activity, emotion as well as cognition (Missale et al., 1998). Abnormalities in the dopamine system have been shown to be associated with several diseases, including addiction, schizophrenia, and Parkinson's disease (Missale et al., 1998). Dopamine is synthesized from its precursor, L-dihyroxyphenylalanine (L-DOPA). L-DOPA is produced from the amino acid tyrosine by the enzyme tyrosine hydroxylase (TH), and it is converted into dopamine by amino acid decarboxylase (AADC) in the cytoplasm of dopaminergic neurons (Axelrod and Weinshilboum, 1972).

Previous studies have shown that accumulation of dopamine in the neuronal cytosol outside the synaptic vesicles leads to deleterious neurotoxicity (Caudle et al., 2007; Chen et al., 2008b; Masoud et al., 2015; Mosharov et al., 2009). It has been demonstrated that injection of dopamine into the striatum causes damage to both presynaptic and postsynaptic neurons in the nigrostriatal pathway, and neuronal cell death resulting from apoptosis (Filloux and Townsend, 1993; Hattori et al., 1998). Using direct measurement of cytosolic dopamine in cultured neurons with intracellular path electrochemistry, Mosharov et al. demonstrated that increasing cytosolic dopamine levels enhanced dopamine neurotoxicity, whereas manipulations reducing the levels of cytosolic dopamine showed protective effects and improved cell survival (Mosharov et al., 2009). These results suggest that dopamine levels in cytosol are extremely associated with toxicity in dopaminergic neurons. Accumulative evidences demonstrated that dopamine neurotoxicity results from the production of reactive oxidative species (ROS) and activation of oxidative stress pathways. After trafficking to the cytoplasm, dopamine is auto-oxidized and converted to toxic substances such as superoxide radicals, dopamine quinones as well as hydrogen peroxide (Graham et al., 1978). Furthermore, dopamine-induced loss of dopaminergic cells was rescued by co-injection of antioxidants.

Dopamine transporters (DAT), expressed on the surface membrane of dopaminergic neurons, are responsible for dopamine reuptake into presynaptic dopaminergic neurons from extracellular area, which are extremely important for regulating and maintaining dopamine homeostasis. Extracellular dopamine levels significantly elevate in DAT knockout mice due to lack of reuptake (Giros et al., 1996; Jones et al., 1998). Besides controlling dopamine signaling in extracellular area, DAT also regulates intracellular dopamine levels. 95% decrease of the levels of stored dopamine is detected in DAT knockout mice (Sotnikova et al., 2005). Meanwhile, midbrain dopamine neurons decrease by 30–36% in transgenic mice overexpressing DAT in dopaminergic neurons, and

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this is accompanied by evidence of oxidative stress (Masoud et al., 2015). These results indicate that DAT overloads dopamine to presynaptic dopaminergic neurons, and DAT-mediated dopamine reuptake is involved in dopamine neurotoxicity. In a previous study, Chen et al. showed that progressive cell loss and oxidative protein modifications are observed in GABAergic neurons expressing DAT in the striatum (Chen et al., 2008a). Thus, the function of DAT is important for regulation of dopamine neurotoxicity.

Previous studies indicate that D2-like receptor family is involved in dopamine neurotoxicity (Bozzi and Borrelli, 2006). D2Rs are located on the presynaptic neuron membrane and regulate dopamine release (Beaulieu and Gainetdinov, 2011), suggesting that it may be strongly associated with the dopamine neurotoxicity. Meanwhile, previous studies have also shown that D2R activation is related to dopamine neurotoxicity in some cell types (Coronas et al., 1997). Furthermore, studies using cultured primary cultured neurons also reported that D2R mediated dopamine toxicity in the formation of the huntingtin aggregates in Huntington's disease (Charvin et al., 2005). These suggest that D2R might contribute to the neurotoxicity of dopamine.

Although DAT inhibitors show some beneficial effects in clinical use, but due to completely blockade of all the physiological functions of DAT, there are many severe adverse effects that limit the therapeutic effects of DAT inhibitors, including dyskinesia, emesis, insomnia, sedation, constipation, dry mouth, light-headedness, hallucinations, nightmares, high abuse potential, and 'on-off' phenomena (Kintscher, 2012). In addition, there are very few DAT inhibitors showing beneficial effects on idiopathic PD Parkinsonism (Huot et al., 2015, 2016). These results make it urgent to find more novel mechanisms and drug targets for treatment of dopamine neurotoxicity with fewer adverse effects.

We previously found that DAT is regulated by the dopamine D2 receptor via a direct protein-protein interaction. This D2R-DAT interaction facilitates intracellular DAT recruitment to the cell membrane, thus enhancing dopamine reuptake. Mice injected with a peptide (DAT-S1) that specifically disrupts D2R-DAT interaction, showed a decrease in synaptosomal dopamine uptake (Lee et al., 2007). These data suggest that the DAT-S1 peptide is able to regulate DAT location on the cell membrane, and modulate dopamine reuptake, indicating the DAT-S1 peptide might be able to inhibit dopamine neurotoxicity. Here we report that the DAT-S1 peptide is able to prevent cell death when the cells are treated with a high concentration of dopamine. Furthermore, the DAT-S1 peptide disrupts D2R-DAT interaction in brain tissue, leading to reduced DAT expression on the cell membrane, and inhibiting PARP-1 and caspase-3 cleavage.

2. Materials and methods

2.1. Cell culture and treatment

SH-SY5Y cells (American Type Culture Collection (ATCC), Manassas, VA) were maintained as a monolayer in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, ON, Canada) with 10% fetal bovine serum (Gibco, ON, Canada), 100 U/mL penicillin (Sigma-Aldrich, Oakville, ON, Canada), and 100 U/mL streptomycin (Sigma-Aldrich, Oakville, ON, Canada). Cells were cultured in a humidified atmosphere of 5% CO_2, at 37 °C. All cells were cultured in 100-mm (diameter) cell culture plates (BD Biosciences, ON, Canada) until ~80% confluence and then seeded into 24-well plates (BD Bioscience, ON, Canada) to around ~90% confluence for 24–28 h prior to treatment. The medium was replaced by DMEM without fetal bovine serum 12 h before treatments.

2.2. Drugs

Dopamine was purchased from Sigma-Aldrich, dissolved in water to a stock concentration of 100 mM, and wrapped with foil to protect from light. DAT-S1 and DAT-S2 peptides were synthesized and purchased from Biomatik Corporation (Cambridge, USA), and dissolved in water to a stock concentration of 10 mM.

2.3. Propidium iodide (PI) and Hoechst33342 staining

Cultured SH-SY5Y cells or primary cultured neurons were gently rinsed with phosphate-buffered saline (PBS) (pre-warmed in 37 °C) twice, incubated with 50 μg/mL PI (Invitrogen, Carlsbad, CA) alone or double-stained with both PI and Hoechst33342 (20 μg/mL) (Invitrogen, Carlsbad, CA) for 30 min, and then rinsed three times with PBS. Fluorescent intensity was measured with a plate reader (Victor 3; Pekin-Elmer, Waltham, MA). The level of cell death was defined as the ratio of PI-labeled vs. Hoechst33342. The fraction of dead cells was normalized to the cell toxicity that occurred in control group. For immunostaining in primary cultured neurons, the images were captured at 10× magnification on an Olympus FV10i confocal microscope.

2.4. Protein extraction

Striatal tissues were homogenized in ice-cold buffer containing (in mmol/L): 50 Tris-Cl, pH 7.4, 150 NaCl, 2 EDTA, 1 PMSF plus 1% Igepal CA-630, 0.5–1% sodium deoxycholate, 1% Triton X-100 and protease inhibitor mixture (5 μL/100 mg of tissue; Sigma-Aldrich, Okaville, ON, Canada) on ice and shaken at 4 °C for 1 h. Striatal tissues dissolved in the lysis buffer was centrifuged at 12,000*g* for 10 min at 4 °C to yield the total protein extract in the supernatant. The protein concentration was measured with BCA protein assay kit (Pierce Protein Biology, ON, Canada). Equal amounts of samples (50–100 μg) were denatured and subjected to 10% SDS-PAGE and Western blot analyses.

2.5. Gel electrophoresis and Western blot analyses

Samples were separated using SDS-PAGE with 10% separating gel and 5% stacking gel, and transferred to a nitrocellulose membrane after gel electrophoresis. After blocking for 1 h with 5% fat-free milk powder in TBST (10 mM Tris, 150 mM NaCl, 0.05% Tween-20, pH 7.4), blots were incubated overnight at 4 °C with primary antibodies, including 1:100 anti-D2R (Santa Cruz Biotechnology, Dallas, Texas), 1:200 anti-DAT (Santa Cruz Biotechnology, Dallas, Texas), 1:10,000 anti-α-Tubulin (Sigma), 1:200 anti-caspase-3 (Santa Cruz Biotechnology, Dallas, Texas), and 1:200 anti-PARP-1 (Santa Cruz Biotechnology, Dallas, Texas). After rinses, blots were incubated with HRP-conjugated secondary antibodies (Sigma-Aldrich, Okaville, ON, Canada) for 2 h at room temperature. Immunoactivity was visualized with ECL Western blot detection reagents (GE Healthcare). Data representative of three experimental repeats are shown.

2.6. Co-immunoprecipitation analyses

Co-immunoprecipitation and Western Blot analyses were performed as previously described (Su et al., 2014). For co-immunoprecipitation experiments, 500–700 μg solubilized protein extracted from mouse striatal tissue was incubated in the presence of protein A/G plus agarose (Santa Cruz Biotechnology) for 12 h. Pellets were washed, boiled for 5 min in SDS sample buffer (Bio-Rad) and subjected to SDS-PAGE. 10–50 μg of tissue extracted protein was used as control in each experiment. Beads were washed, boiled for 5 min in SDS sample buffer and subjected to SDS-PAGE. The intensity of protein level was quantified by densitometry (software: Image J, NIH).

2.7. Acute striatal slices

Acute striatal slices (350 μm-thick) were dissected and prepared from CD-1 mice with a McIlwain tissue chopper (Mickle Laboratory Engineering, Gomshall, United Kingdom), and remained in ice-cold artificial cerebrospinal fluid (aCSF) containing 126 mM NaCl, 2.5 mM KCl,