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L-DOPA modulates cell viability through the ERK-c-Jun system in PC12 and dopaminergic neuronal cells

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ABSTRACT

L-DOPA causes neurotoxicity by modulating the Epac-ERK system in PC12 cells. This study investigated the effects of a single treatment with L-DOPA and multiple treatments with L-DOPA (MT-LD) on ERK1/2 and JNK1/2-c-Jun systems. In PC12 cells, a toxic L-DOPA concentration (200 μM) induced sustained ERK1/2 and JNK1/2 phosphorylation that was inhibited by the Epac inhibitor brefeldin A, but not by the PKA inhibitor H89. This ERK1/2 and JNK1/2 phosphorylation was also inhibited by ERK1/2 (U0126) and JNK1/2 (SP600125) inhibitors, respectively, but sustained ERK1/2 phosphorylation was not affected by JNK1/2 phosphorylation. A non-toxic L-DOPA concentration (20 μM) induced c-Jun phosphorylation (Ser73) via transient ERK1/2 phosphorylation, whereas the toxic L-DOPA concentration induced c-Jun phosphorylation (Ser63) and c-Jun expression via Epac-sustained ERK1/2-JNK1/2 phosphorylation, which then enhanced cleaved caspase-3 expression. MT-LD (20 μM) initially enhanced c-Jun phosphorylation (Ser73) (for 1–4 days), but later (5–6 days) induced c-Jun phosphorylation (Ser63) and c-Jun expression. In the 6-hydroxydopamine-lesioned rat model of Parkinson's disease, L-DOPA administration (10 mg/kg) protected against neurotoxicity through c-Jun phosphorylation (Ser73) for 1–2 weeks. However, L-DOPA administration (10 or 30 mg/kg) showed neurotoxicity through c-Jun phosphorylation (Ser63) and c-Jun expression via ERK1/2 phosphorylation for 3–4 weeks. Thus, in PC12 cells, non-toxic L-DOPA treatment maintained cell survival through c-Jun phosphorylation (Ser73). By contrast, toxic L-DOPA treatment or MT-LD (20 μM) induced c-Jun phosphorylation (Ser63) and c-Jun expression via Epac-dependent sustained ERK1/2 and JNK1/2 phosphorylation, which subsequently led to cell death. These results were validated by those obtained after long-term L-DOPA administration in a rat model of Parkinson's disease. Our data indicate that L-DOPA causes neurotoxicity via the ERK1/2-c-Jun system in dopaminergic neuronal cells.

1. Introduction

L-3,4-dihydroxyphenylalanine (L-DOPA), the natural precursor of dopamine, is the most frequently prescribed drug for controlling the symptoms of Parkinson's disease (PD) (Marsden, 1994). However, chronic L-DOPA therapy accelerates the progression of PD (Maharaj et al., 2005). Low concentrations of L-DOPA exhibit neuroprotective effects against oxidative stress-associated cytotoxicity in dopaminergic neuronal and rat adrenal pheochromocytoma (PC12) cells (Koshimura et al., 2000; Shin et al., 2009). By contrast, high concentrations of L-DOPA can lead to cell death through oxidative stress-induced apoptosis mediated by generating reactive oxygen species in dopaminergic neuronal and PC12 cells (Cheng et al., 1996; Basma et al., 1995; Walkinshaw and Waters, 1995). The cellular responses of L-DOPA-induced oxidative stress in neuronal and non-neuronal cells affect various signaling pathways, such as those of p38 mitogen-activated protein kinase, c-Jun N-terminal kinase (JNK1/2) and extracellular-regulated kinase (ERK1/2), Bad and Bcl, and caspase cascades (Dérijard et al., 1994; Ichijo, 1999; Zha et al., 1996; Pedrosa and Soares-da-Silva, 2002).

Cyclic AMP (cAMP) protects both neuronal and PC12 cells from oxidative stress-induced cytotoxicity (Lambeng et al., 2001). By contrast, the intracellular levels of cAMP increased by treatment with toxic or high concentrations of L-DOPA (100–200 μM) or

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multiple treatments with a non-toxic concentration of L-DOPA (20 μM) (MT-LD) do not protect against L-DOPA-induced oxidative stress cell death (Jin et al., 2010; Park et al., 2014). Furthermore, the increased levels of cAMP induced by L-DOPA and forskolin have dual effects on neuronal cell viability in PC12 cells. Namely, cell survival is maintained by cAMP-dependent protein kinase A (PKA)-mediated transient ERK1/2 phosphorylation, whereas apoptotic cell death is mediated by the sustained ERK1/2-JNK1/2-caspase-3 pathway (Jin et al., 2010; Park et al., 2012). MT-LD (20 μM) also induces cytotoxicity through the PKA-ERK1/2-exchange protein activation by cAMP (Epac) pathway in PC12 cells and embryonic rat midbrain primary cells (Park et al., 2014).

Transcription factor c-Jun, a member of the transcription factor activator protein family, has been implicated in the regulation of cell differentiation and apoptosis in PC12 cells and sympathetic neurons (Leppä and Bohmann, 1999; Shaulian and Karin, 2002). Among the phosphorylation sites on c-Jun, phosphorylation at Ser63 and Ser73 is mediated primarily by JNK1/2 (Morton et al., 2003) and substantially enhances c-Jun activity (Pulverer et al., 1991), which functions as either a pro- or anti-apoptotic factor (Behrens et al., 1999; Wisdom et al., 1999). ERK1/2 also induces c-Jun expression and phosphorylation at Ser63 and Ser73 in PC12 cells (Leppä et al., 1998). In addition, c-Jun phosphorylation at Ser63, but not Ser73, mediates nitric oxide-induced apoptosis in neuroblastoma cells (Li et al., 2004). These observations suggest that the processes of neuronal cell viability induced by treatment with L-DOPA or MT-LD may involve the activation of c-Jun through the ERK1/2 and/or JNK1/2 signaling pathway.

The present study, therefore, investigated whether the cytotoxicity induced by a single treatment with a high concentration of L-DOPA or MT-LD was mediated by the ERK-c-Jun system in PC12 cells and embryonic rat midbrain primary cells. In addition, the effects of long-term L-DOPA administration on ERK1/2 and c-Jun phosphorylation in the midbrains of 6-hydroxydopamine (6-OHDA)-lesioned rats, used as a model of PD, were investigated to evaluate the mechanisms of L-DOPA-induced neurotoxicity.

2. Materials and methods

2.1. Chemicals

L-DOPA, H89, U0126, SP600125, and brefeldin A were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Primary antibodies against ERK1/2, phospho-ERK1/2 (Thr42/44), c-Jun, phospho-c-Jun (Ser63 and Ser73), JNK1/2, phospho-JNK1/2 (Thr183/185), cleaved caspase-3 (Asp175), and β-actin were purchased from Cell Signaling Technology Inc. (Beverly, MA, USA). All sera, antibiotics, RPMI 1640, Neurobasal medium, B-27, fetal bovine serum, and donor horse serum for cell culture were obtained from Gibco BRL Division (Grand Island, NY, USA). All other chemicals were reagent grade.

2.2. Experimental animals

The rats used in primary cultures of embryonic rat midbrain cells (Sprague–Dawley, embryonic day 18) and the rats used in PD model (Sprague–Dawley, male, 200–250 g) were purchased from Samtako (Osan, Korea). The rats were housed in standard conditions of temperature (22 ± 2 °C), humidity (60 ± 5%), and illumination (12 h light–dark cycle), and were provided with ad libitum access to standard rat food and water. The protocols for the animal-related experiments were approved by the Animal Ethics Committee of Chungbuk National University (approval no. CBNUA-709-141-01), and the experiments were performed in accordance with the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals*.

2.3. Cell cultures

PC12 cells were grown in RPMI 1640 medium supplemented with 5% heat-inactivated fetal bovine serum, 10% heat-inactivated horse serum, 100 units/ml penicillin, and 100 μg/ml streptomycin (Tischler et al., 1983). These cells were placed in a humidified atmosphere with 5% CO_2 and 95% air at 37 °C.

2.4. Embryonic rat midbrain primary cell cultures

Primary cultures of embryonic rat midbrain cells from embryonic day 18 fetuses were performed as described previously (Studer, 2001). Fetal rat midbrains were dissected and incubated for 15 min in a solution of 2 mg/ml trypsin in Ca^{2+}- and Mg^{2+}-free Hank's balanced salt solution (HBSS). Cells were rinsed three times in HBSS and dissociated in poly-D-lysine-coated 6-well plastic culture plates (initial plating densities of 0.5×10^6 cells/ml). At the time of plating, each well contained Neurobasal medium supplemented with B-27. The cells were incubated at 37 °C in a humidified atmosphere with 5% CO_2, and the medium was changed every other day until the cells differentiated 3–5 days after plating.

2.5. Treatment with L-DOPA and MT-LD

The PC12 cells (1×10^6 cells/ml) and embryonic rat midbrain primary cells (0.5×10^6 cells/ml) were plated in 60 mm plastic culture plates (Nunc, Roskilde, Denmark). The culture media were changed every day, with fresh media added before L-DOPA addition. The cells were pretreated with H89 (10 μM), brefeldin A (2 μM), U0126 (10 μM), or SP600125 (10 μM) for 1 h, and then treated with L-DOPA (20–200 μM) once at the indicated times (6–24 h). The cells were also treated multiple times with the non-toxic concentration of 20 μM L-DOPA (MT-LD) once daily for 30 min to 1 h for 6 days after changing the culture media.

2.6. Western blotting of PC12 and embryonic rat midbrain primary cells

The phosphorylation and/or expression of ERK1/2 at Thr202/Tyr204, c-Jun at Ser63 and Ser73, JNK1/2 at Thr183/Thr185, and cleaved caspase-3 at Asp175, ERK1/2, JNK1/2, c-Jun, and β-actin were examined using western blot analysis. PC12 cells (1×10^6 cells/ml) and embryonic rat midbrain primary cells (0.5×10^6 cells/ml) were collected and homogenized in sodium dodecyl sulfate lysis buffer at 4 °C. After the addition of sample loading buffer, proteins in samples (1–10 μg) were separated using 10–15% sodium dodecyl sulfate-poly acrylamide gel electrophoresis. Proteins were transferred to polyvinylidene difluoride membranes, and 15 V was applied to the membranes for 1 h. The blots were blocked for 1 h at room temperature in fresh blocking buffer (Tris-buffered saline, TBS, containing 5% bovine serum albumin, BSA) and incubated overnight at 4 °C using primary antibodies diluted 1:1000 in TBS-T with 5% BSA. The blot was then incubated for 1 h at room temperature using secondary antibodies (1:5000 in TBS with 5% BSA), according to standard procedures. The blots were washed, and the transferred proteins were incubated with ECL substrate solution (Amersham Pharmacia Biotech, Inc., Piscataway, NJ, USA) for 5 min, according to the manufacturer's instructions, and visualized with radiographic film.

2.7. Preparation of the 6-OHDA-lesioned rat model of PD

The rats were anaesthetized intraperitoneally (i.p.) with Zoletil 50 (100 mg/kg; Virbac, Carros, France) and mounted on a stereotaxic