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Dopamine D3 receptor-modulated neuroprotective effects of lisuride

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ABSTRACT

Dopamine (DA) contributes to the regulation of voluntary movement, and a deficiency in DAergic neurons leads to movement disorders. The objective of this study was to examine the neuroprotective effect of DA D2-like receptor agonist, lisuride, and the role of DA receptors in this protection. Treatment with lisuride alleviated loss of tyrosine hydroxylase (TH) both direct and intraperitoneal injection in 6-hydroxydopamine (6-OHDA) mouse model. Similar results were obtained in primary neuronal cultures treated with lisuride. Lisuride protected TH expression against 6-OHDA-induced cytotoxicity in a concentration-dependent manner. Then, we evaluated the role of DA D2 and D3 receptor in neuroprotective effect of lisuride. Treatment of neuronal cultures with L-741,626, a DA D2 receptor-selective antagonist, did not alter neuroprotective effect of lisuride. However, protective effect of lisuride on TH expression was abolished when cells were treated with GR103691, a D3 receptor selective antagonist. Furthermore, whether lisuride can alleviate mitochondrial damage of DAergic neurons induced by 6-OHDA, we investigated the expression of the mitochondrial regulatory protein, paraplegin, and changes in mitochondria morphology. Treatment with lisuride countered a 6-OHDA-induced reduction in paraplegin and TH expression, and co-treatment with GR103691 blocked this effect of lisuride. Transmission electron microscopy confirmed the lisuride mitigation of 6-OHDA-induced damage to the mitochondrial membrane and cristae. These results suggest that the DA D3 receptor mediates the neuroprotective effects of lisuride by preventing mitochondrial damage.

1. Introduction

Dopamine (DA) neurons are located in the substantia nigra (SN), ventral tegmental area (VTA), and retrorubral field (Nair-Roberts et al., 2008). DAergic neurons in the SN play important roles in normal motor function. A loss of DAergic neurons in this region is the main underlying cause of Parkinson's disease (PD), the second most common neurodegenerative disease (Nutt and Wooten, 2005).

DA D2-like receptor agonists have been used to alleviate movement disorders in PD patients. Ergot derived DA D2-like receptor agonist lisuride has shown a locomotor stimulatory effect (Wachtel, 1991). Under experimental conditions, treatment with lisuride can stimulate DAergic neurons in a dose-dependent manner (Schechter, 1984). In the 6-hydroxydopamine (6-OHDA)-induced DAergic neuronal deficiency rat model, lisuride can stimulate locomotor activity and a DA D2-like receptor antagonist can effectively abolish such increase in locomotor activity (Horowski and Wachtel, 1976). Clinically, lisuride has been used in combination with levodopa (L-DOPA) to postpone L-DOPA-induced dyskinesia and decrease L-DOPA treatment dosages (Allain et al., 2000). Interestingly, although lisurideis considered asa DA D2-like receptor agonist, it has higher affinity for DA D3 receptor than other DA D2-like receptor agonists (Gille et al., 2002; Horowski and Wachtel, 1976; Schechter, 1984).

Mitochondria are essential for cell survival and normal physiological functions. They provide cells with ATP, the energy source. Disturbanceof ATP production is related to abnormal cell function and cell death (Yumnam et al., 2014). Postmortem studies of PD patients have demonstrated morphologically abnormal and dysfunctional mitochondria. These studies suggest that mitochondrial dysfunction might be the cause of sporadic form of PD (Smigrodzki et al., 2004; Trimmer et al., 2000).

Recent studies have shown the importance of paraplegin, ATPase associated with various cellular activity protease, for

Abbreviations: 6-OHDA, 6-hydroxydopamine; DAergic, dopaminergic; E, embryonic day; L-DOPA, levodopa; PD, Parkinson's disease; SN, substantia nigra; TEM, transmission electron microscopy; TH, tyrosine hydroxylase; VTA, ventral tegmental area.

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mitochondrial normal function (Casari et al., 1998). Paraplegin is connected with mitochondrial normal function. Deficiency of paraplegin can cause hereditary spastic paraplegia, a neurodegenerative disease (Kara et al., 2016). In experimental conditions, deficiency of paraplegin can decrease mitochondrial complex I activity (Atorino et al., 2003). Paraplegin also has functions in regulating mitochondrial permeability transition pore (Shanmughapriya et al., 2015).

The objective of this study was to determine the neuroprotective effect of lisuride against 6-OHDA-induced damage in the SN, the role of DA receptors in such protective effect and their associations with mitochondria structure and function.

2. Materials and methods

2.1. Animals

Colonies of ICR mice were maintained in specific pathogen-free conditions in a controlled room with 12/12-h light and dark cycles. Female mice were housed with a male mouse for15–17 h. They were examined for the presence of vaginal plugs at 7:00 a.m. The presence of a plug indicated conception and the day of plug discovery was designated as embryonic day 0 (E0). Animal experimental protocols were approved and followed by the Animal Ethics Committee of Gyeong Sang National University (Approval Number: GNU-LA-10). This research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as stated in the European Community guidelines (EEC Directive of 1986; 86/609/EEC) and the US guidelines (NIH publication #85-23, revised in 1985).

2.2. Primary cell culture and drug treatments

Primary cultures were prepared as described previously (Brewer and Torricelli, 2007) with minor modifications. Briefly, brains were obtained from E12 mice and placed in Hanks balanced salt solution (Gibco). Neurons were separated from debris and oligodendrocytes via a density gradient. Collected neurons were plated at a density of 100,000 cells/cm^2 in 0.1 mg/ml poly-L-lysine-coated wells in Neurobasal (Gibco) containing B27 supplement, basic fibroblast growth factor, L-glutamic acid, and gentamicin. Cells were subcultured at 37 °C in a humidified air atmosphere containing 5% CO_2 for 7–14 days. To evaluate the neuroprotective effect of lisuride against 6-OHDA (10 μM, Sigma-Aldrich, USA), lisuride malate (Tocris, UK) was co-administered to primary cultures. Moreover, D2 receptor selective antagonist L-741626 (1 and 10 μM, Tocris, UK) or D3 receptor selective antagonist GR103691 (1 and 10 μM, Tocris, UK) was co-treated with lisuride malate to estimate the role of DA receptor in the protective effect of lisuride. Samples were collected for analysis at72 h after treatments.

2.3. Unilateral 6-OHDA stereotaxic surgery and drug treatments

Four mice in each group were anaesthetized with zoletile (10 mg/kg) and xylazine (5 mg/kg), placed in a stereotaxic frame, and unilaterally administered with 5 μl 6-OHDA (0.4 mg/kg, body weight in saline containing 0.1% ascorbic acid) in the SN (3.0 mm posterior to bregma, 1.4 mm lateral to midline, 4.0 mm ventral to the skull surface) at coordinates according to a mouse brain atlas (Paxinos and Franklin, 2001). To evaluate the direct neuroprotective effect of lisurde, 5 μl lisuride maleate (3 mg/kg body weight) was slowly injected into the SN over 5 min (rate, 1 μl/min)using a Hamilton syringe. To estimate the neuroprotective effect of lisuride based on administration routes, 50 mg/kg bodyweight lisuride was injected in intraperitoneal (I.P.) route. At 30 min after lisuride injection, 1 μl (0.08 mg/kg, body weight in saline containing 0.1% ascorbic acid) or 5 μl 6-OHDA (0.4 mg/kg, body weight in saline containing 0.1% ascorbic acid) was administered in the SN lesionunilaterally. After which the syringe was kept in place for 5 min following the injection. The skin was then sutured and mice were returned to their cages following recovery from anesthesia.

2.4. Sample preparation and immunohistochemistry

Seven days after the surgery, animals were deeply anesthetized and sacrificed by intracardiac perfusion with 4% paraformaldehyde (pH 7.4). The brains were post-fixed for 48 h and embedded in paraffin wax. Tissue samples were serially sectioned at a thickness of 10 μm with a rotary microtome for immunohistochemical analysis. Non-specificstaining was blocked with 3% fetal bovine serum in phosphate-buffered saline for 1 h. Slides were incubated with a rabbit monoclonal primary antibody against tyrosine hydroxlyase (TH; diluted 1:1000; Pelfreez, USA), a mouse monoclonal antibody against paraplegin (1:100; Novus, USA), and a rabbit monoclonal antibody against cleaved form caspase-3 (1:1000; Cellsignaling; USA)at 4 °C overnight. Streptavidin Alexa fluor 594 conjugated anti-mouse IgG (1:400, Invitrogen, USA), and Streptavidin Alexa fluor 488 conjugated anti-rabbit IgG (1:400, Invitrogen, USA)were used as secondary antibodies for fluorescence image acquisition. DAB substrate kit (Vector Laboratories, USA) was used to stain 3,3'-diaminobenzidine tetrahydrochloride (DAB). All images were captured using microscopy Leica DM600B microscope. Images were analyzed using ImageJ software. Statistical analysis was done using Instat® software (Graphpad, San Diego, CA).

2.5. Western blot

E12 mouse primary cultures were treated with 6-OHDA (10 μM) followed by treatment withDA receptor agonist and antagonists for 72 h. Cultured cells were washed with cold phosphate-buffered saline. Total cell lysates were prepared using lysis buffer (1 M Tris–HCl [pH 8.0], 5 M NaCl, 1% NaN_3, 10% SDS, 10% NP-40, and 0.5% $C_{24}H_{39}NaO_4$). An equal amount of sample was loaded onto 10% polyacrylamide gels. Following electrophoresis, proteins were transferred to polyvinylidene membranes. Non-specific binding was blocked with 5% skim milk in 0.1% Tween for 30 min. The membranes were incubated with anti-TH (1:1000; Millipore, USA), anti-paraplegin (1:1000; Novus, USA),anti-cleaved form caspase-3 (1:1000; Cellsignaling, USA) and anti-actin (1:1000; Cell Signaling, USA) antibodies at 4 °C overnight. After washing five times, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies. Proteins were detected using enhanced chemiluminescence reagent (Anigen, Seoul, Korea). Densitometry was performed using ImageJ software. Statistical analysis was done using Instat® software (Graphpad, San Diego, CA). β-actin served as an internal control.

2.6. Transmission electron microscopy

Brain samples were collectedat 24 h after 6-OHDA intracranial injection. Samples were fixed in cold (4 °C) 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.4. After rinsing samples withphosphate-buffered saline in the ice for 5 min, post-fixing was performed using 1% osmium tetroxide. After post-fixation, samples were dehydrated in an ethanol series and propylene oxide. They were then embedded with Poly/Bed 812 kit (TED PELLA, INC) and incubated at 60 °C for 72 h. Ultra-thin sections (60–90 nm) through the seminal vesicle were cut with an ultramicrotome (Leica EM UC6). Sections were placed on 300 and 200 mesh copper grids and double-stained with uranyl acetate and lead citrate. Finally, all