NeuroToxicology 59 (2017) 49–55

Contents lists available at ScienceDirect

NeuroToxicology

Full Length Article

Nicotine suppresses the neurotoxicity by MPP$^+$/MPTP through activating α7nAChR/PI3K/Trx-1 and suppressing ER stress

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ARTICLE INFO

Article history:
Received 21 September 2016
Received in revised form 1 January 2017
Accepted 4 January 2017
Available online 9 January 2017

Keywords:
Parkinson's disease
Nicotine
MPP+/MPTP
Thioredoxin-1

ABSTRACT

Parkinson's disease (PD) is a neurodegenerative disease. Nicotine has been reported to have the role in preventing Parkinson's disease. However, its mechanism is still unclear. In present study we found that nicotine suppressed 1-methyl-4-phenylpyridinium ion(MPP$^+$) toxicity in PC12 cells by MTT assay. The expression of thioredoxin-1(Trx-1) was decreased by MPP$^+$, which was restored by nicotine. The nicotine suppressed expressions of Glucose-regulated protein 78(GRP78/Bip) and C/EBP homologous protein (CHOP) induced by MPP$^+$. The methyllycaconitine (MLA), the inhibitor of α7nAChR and LY294002, the inhibitor of phosphatidylinositol 3-kinase (PI3K) blocked the suppressions of above molecules, respectively. Consistently, pretreatment with nicotine ameliorated the motor ability, restored the declines of Trx-1 and tyrosine hydroxylase (TH), and suppressed the expressions of Bip and CHOP induced by 1-Methy-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice. Our results suggest that nicotine plays role in resisting MPP$^+$/MPTP neurotoxicity through activating the α7nAChR/PI3K/Trx-1 pathway and suppressing ER stress.

1. Introduction

Parkinson's disease (PD) is a common neurodegenerative disease characterized by the loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc), motor deficits and dementia (Hughes et al., 2002). It has been reported that the molecular basis of such a neurodegenerative disorder is related to mitochondrial dysfunction, endoplasmic reticulum(ER) stress, oxidative stress, and inflammation (Hauser and Hastings 2013; Taylor et al., 2013). 1-Methy-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) and metabolite 1-methyl-4-phenylpyridinium ion (MPP$^+$) induce dopaminergic neuron apoptosis *in vivo* or *in vitro* (Itano et al., 1994).

Nicotine, a potent agonist to nicotinic acetylcholine receptors (nAChRs), has a role in preventing Parkinson's disease (Quik et al., 2012). A recent clinical trial showed that symptoms in patients with PD could be improved and L-dopa treatment dose was reduced after received nicotine treatment (Villafane et al., 2007). Nicotine also ameliorates PD patients' cognitive impairment (Holmes et al., 2011). The observational study of dopamine transporter image showed that nicotine had the direct pharmacological role in decelerating neuronal loss (Itti et al., 2009). Nicotine has a protective role in suppressing dopaminergic toxicity induced by MPTP, attenuating the behavioral deficits and loss of the striatal dopaminergic neurons (Hu et al., 2007; Huang et al., 2009).

Thioredoxin-1 (Trx-1) is a 12 kDa multifunctional protein with active-site sequence: –Cys-Gly-Pro-Cys-. Accumulating evidences show that Trx-1 plays the role in resisting cellular damage and stressful perturbations (Bai et al., 2003; Luo et al., 2012). Trx-1 scavenges singlet oxygen and hydroxyl radicals (Das and Das, 2000), hydrogen peroxide, as well as protects cells from UV irradiation and ischemic damage (Nakamura et al., 1994; Takagi et al., 1999). Our previous study showed that Trx-1 expression was suppressed by MPP$^+$/MPTP, while overexpression of Trx-1 protected dopaminergic neurons by suppressing the expressions of glucose-regulated protein 78(GRP78/Bip), caspase-12, C/EBP

Abbreviations: PD, Parkinson's disease; MPP$^+$1, methyl-4-phenylpyridinium ion; Trx-1, thioredoxin-1; ER, endoplasmic reticulum; GRP78/Bip, glucose-regulated protein 78; CHOP, C/EBP homologous protein; TH, tyrosine hydroxylase; PI3K, phosphatidylinositol 3-kinase; DA, dopamine; SNc, substantia nigra pars compacta; NAChRs, nicotinic acetylcholine receptors; TH, Tyrosine hydroxylase; MPTP, 1-Methy-4-phenyl-1,2,3,6-tetrahydropyridine.

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http://dx.doi.org/10.1016/j.neuro.2017.01.002
0161-813X/

homologous protein (CHOP) induced by MPTP (Bai et al., 2007; Zeng et al., 2014).

However, whether nicotine induces Trx-1 or regulates ER stress has not been reported. The aim of this study is to examine whether nicotine protects dopaminergic neuron through inducing Trx-1 and suppressing ER stress.

2. Methods

2.1. Materials

MPP^+, MPTP-HCl, nicotine and methyllycaconitine (MLA) were from Sigma-Aldrich Corp. (St. Louis, MO, USA). LY294002 was purchased from Biosource International, Inc (Camarillo, CA, USA). Antibodies against GRP78/Bip, CHOP, tyrosine hydroxylase (TH), and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-mouse Trx-1 antibody was obtained from CTS (Danvers, MA, USA). PC12 cells were obtained from the Kunming Institute of Zoology (Kunming, China).

2.2. Cell culture

PC12 cells were cultured in RPMI 1640 medium (Invitrogen, Grand Island, NY, USA) supplemented with 10% heat-inactivated horse serum and 5% heat-inactivated fetal bovine serum and antibiotics (100 IU/ml penicillin and 100 mg/ml streptomycin) at 37 °C in a humid atmosphere containing 5% CO_2.

2.3. Cell viability

PC12 cells were seeded into a 96-well plate overnight and then were pretreated with nicotine or inhibitors for 30 min before MPP^+ treatment and then were incubated for 24 h after exposure to MPP^+. The 3-[4,5–dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay was used to measure cell viability according to the manufacturer's instructions. The mean OD of one group/mean OD of the control was used to calculate the viability.

2.4. Animal experiments

We used male C57BL/6 black mice, 8 weeks of age, in the experiments. Mice were housed in plastic cages and maintained on a 12 h light/dark cycle and had free access to food and water. The dose and time of MPTP-HCl, nicotine treatment were selected as reported previously (Liu et al., 2012; Luo et al., 2011).

Mice were divided into four groups (n = 5 per group). Mice of control group were administered saline only. Mice in the MPTP group received intraperitoneal injections of MPTP-HCl (20 mg/kg, twice daily for 7 days). For nicotine treatment group, mice were given intraperitoneal injections of nicotine (0.25 mg/kg), twice a day at 2 h interval for 7 days. For nicotine and MPTP-HCl treatment group, mice were given nicotine twice a day for 7 days and MPTP-HCl (20 mg/kg, twice daily for 7 days). The nicotine was injected 30 min before MPTP injection. Mice were sacrificed 2 h after the last treatment by cervical vertebra dislocation, and then heart perfusion was performed by using saline. The SNc was rapidly dissected out, frozen, and stored in a deep freezer at −80 °C until the assays. All procedures were performed in accordance with guidelines set for the use of experimental animals by the local committee on animal care and use (No. LA2008305).

2.5. Behavioral tests

Locomotor activity was measured in a plastic box (38.5 × 27.5 × 15 cm). Mice were divided into four groups (n = 5 per group). The locomotor activity was measured for 15 min after the last treatment. Limb impairment was assessed by a traction test, as described previously (Arai et al., 1990). Mice were hung from a horizontal wire by their forepaws. A mouse was scored as 3 for gripping the wire with both hind paws, 2 for gripping the wire with one hind paw, and 1 for not gripping the wire with either hind paw.

2.6. Western blot

Mice were sacrificed by cervical vertebra dislocation. The midbrain containing substantia nigra was taken quickly and homogenized in a lysis buffer (20 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% NP-40, 1 mM EDTA, 1 mM phenylmethanesulfonyl fluoride, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM Na_3VO_4, 1 mM β-glycerol phosphate, and 1 mg/ml leupeptin). Protein concentration was determined by using the Bio-Rad protein assay reagent (Hercules, CA, USA). Equal quantities of protein were separated by 12% (for GRP78/Bip, CHOP, and TH) or 15% (for Trx-1) SDS–PAGE and transferred to a polyvinylidene difluoride membrane (Millipore Corp., Billerica, MA, USA).The membrane was soaked in 10% skim milk (in phosphate buffered saline, pH 7.2, containing 0.1% Tween 20) or 3% bovine albumin V (in Tris-buffered saline, pH 7.2, containing 0.1% Tween 20) overnight at 4 °C and then incubated with primary antibodies (1:1000) followed by peroxidase-conjugated anti-mouse or anti-rabbit IgG (1:10,000) (KPL, Gaithersburg, MD, USA). The epitope was visualized by an ECL Western blot detection kit (Millipore Corp., Billerica, MA, USA). Densitometry analysis was performed using ImageJ software.

2.7. Data analysis

Data are expressed as means ± SD. Statistical analysis was performed using SPSS software. One-way ANOVA followed by a post hoc multiple comparison test was used to compare control and treated groups. P values less than 0.05 were considered statistically significant. All blots are representative of experiments that were performed at least three times.

3. Results

3.1. Nicotine increased cell viability of PC12 cell

To clarify the best concentration of nicotine used in present study, we used a range of different concentrations of nicotine (1 nM, 10 nM, 100 nM, 1000 nM) to treat the PC12 cells. After 24 h, nicotine significantly increased the viability of PC12 cells in a dose-dependent manner (Fig. 1A).The concentrations of 100 nM and 1000 nM showed similar effect on the cells and increased the cell viability about 25%. So we selected the concentration of 100 nM as the treatment in present study.

3.2. Nicotine protected PC12 cells from neurotoxicity induced by MPP^+

Our previous study showed that MPP^+ (0.3 mM) induced the neurotoxicity in PC12 cells. We examined the effect of nicotine on neurotoxicity induced by MPP^+ (0.3 mM). Nicotine significantly suppressed neurotoxicity induced by MPP^+ in PC12 cells (Fig. 1B).

3.3. Nicotine suppressed ER stress induced by MPP^+

It has been reported that ER stress is involved in the pathogenesis of PD (Levy et al., 2009).Thus, we further examined the effect of nicotine on MPP^+-induced ER stress. The expressions of GRP78/Bip (Fig. 1C) and CHOP (Fig. 1D) induced by MPP^+ were suppressed by nicotine, suggesting that nicotine suppresses ER