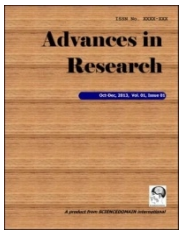

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TrkA Pathway(s) are Involves in the Regulation of TRPM2 and TRPM7 Expression in the Substantia Nigra of the Parkinson's Disease Rat Model Induced by 6-Hydroxydopamine

Yong-peng Yu[1,2*], An-mu Xie[3] and Xiao-han Yang[4]

[1]Department of Neurology, the Affiliated Wendeng Center Hospital of Weifang Medical College, Shandong Province 264400, P. R. China.
[2]Teaching and Research Division, the Medical College of Qingdao University, Shandong Province 266021, P. R. China.
[3]Department of Neurology, the Affiliated Hospital of Qingdao University Medical College, Shandong Province 266033, P. R. China.
[4]Institute of Brain Disorders and the Provincial Key Lab for Brain Disorders of Liaoning Province, Dalian Medical University, Liaoning 116044, P. R. China.

Authors' contributions

This work was carried out in collaboration between all authors. Author YPY designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author AMX managed the literature searches, analyses of the study performed the spectroscopy analysis and author XHY managed the experimental process and identified the species of plant. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Recently, it was demonstrated that the transient receptor potential melastatin 2 (TRPM2) and melastatin 7 (TRPM7) played a key role in ROS-induced neuronal death. Meanwhile, nerve growth factor (NGF), through activating tropomyosin-related kinase A (TrkA) pathway, is known to have survival and differentiation effects on neuronal cells. To

**Corresponding author: E-mail: yypeng6688@126.com;*

mediate these actions, NGF binds to the high affinity neurotrophin receptor TrkA to trigger the intracellular signaling cascades. Two kinases whose activities mediate these processes are phosphatidylinositol 3-kinase (PI-3K) and ras/mitogen-activated protein kinase (MAPK). In this study, the Parkinson's disease rat model induced by 6-hydroxydopamine (6-OHDA) was employed. TRPM7 and TRPM2 were found residing on dopaminergic neuronal body and process, and the effect of TrkA was concurrently observed on TRPM2 and TRPM7 in the cell body by immunohistochemistry staining. There was an increasing up-regulation of TRPM7 and TRPM2 expressions in the substantia nigra (SN) of the Parkinson's disease (PD) rat model at one week after 6-OHDA injection. The levels of TRPM2 and TRPM7 in the PD group were reversed by intracerebroventricular injection of NGF (500ng) 30 min before 6-OHDA injection, and the effect of NGF was completely abolished by co-injection of TrkA inhibitor K252a. In addition, when Wortmannin and U0126 were introduced to block PI-3K and MAPK pathways respectively, only PI-3K inhibitor wortmannin substantially abolished NGF effects. These results suggest that TrkA, after being activated by NGF, can inhibit up-regulation of TRPM2 and TRPM7 expressions in the SN neurons injured by 6-OHDA through PI-3K signal pathway. These findings open a new way for further investigation of the potential roles of TRPM2, TRPM7 and NGF in the pathogenesis of PD.

Keywords: TRPM2; TRPM7; 6-OHDA; Parkinson's disease.

1. INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disease which is characterized by the loss of dopamine neurons in the Substantia Nigra (SN). It has been generally regarded as the second most common neurodegenerative disorder after Alzheimer's disease (AD).The pathophysiological mechanisms responsible for this disorder still remains elusive [1].

Increasing evidence has accumulated that a number of ion channels belonging to the transient receptor potential (TRP) channel superfamily, which include TRP melastatin 2 (TRPM2) [2,3,4], TRPC3/4 [5], TRPC1/3/5/6 [6], TRPP2 [7], TRPV1 [8], and TRPA1 [9,10] channels, are sensitive to oxidative stress and may be involved in ROS-induced cell death [11]. In particular, TRPM2 channels are highly expressed in the brain including the cerebral cortex, cerebellum, striatum and hippocampus [2]. In addition, it was demonstrated that these channels were also expressed in the human SN [12]. Functional expression of native TRPM2 channels has been detected in the striatal [13,14], hippocampal [15,16] and cortical [2] neurons.TRPM7 would be activated when brain cells are deprived of oxygen and vital nutrients, triggering a lethal chain reaction [17]. TRPM7, as a calcium-permeable nonselective ion channel, has been shown to be a requirement for cell survival [18]. It has been proposed to be involved in Ca^{2+} influx and anoxic cell death in cortical neurons [19]. TRPM7 is implicated in various cell functions including cell growth, proliferation, embryonic development, anoxic neuronal death, and pathological response to vessel injury, and neurotransmitter release in sympathetic neurons [20]. Furthermore, in heterologous expression systems, genetic elimination of TRPM7 leads to immediate cell growth arrest and

death [21] and overexpression of TRPM7 also leads cells to die [22], which indicates that precise regulation of TRPM7 expression is required for cell survival in vivo.

Previous studies have proved that NGF could protect neurons from ischemia-induced necrosis or apoptosis [23] and dopaminergic neurons damage induced by rotenone toxicity [24]. The protective effects of NGF via its high affinity receptors TrkA and subsequently, activating appropriate signal transduction pathways. NGF increased TrkA phosphorylation in hippocampal neurons and provided protection that required phosphoinositol-3-phosphate-kinase (PI-3K) activity and Akt phosphorylation, whereas the mitogen-activated protein kinases (MAPK), extracellular-regulated kinases (ERk) 1/2 were not involved [25]. Among them, the PI-3K/Akt pathways have been reported to be involved in nigral DA neuronal neuroprotection mediated by estrogen and IGF-1 in a unilateral rat model of Parkinson's disease [26]. Remarkably, studies reveal that NGF can stabilize neuronal calcium homeostasis [27] and protect neurons against environmental insults [28]. Therefore, whether changes of TRPM2 and TRPM7 expressions are involved in 6-hydroxydopamine damage process and whether TrkA pathway(s) involves in the regulation of TRPM2 and TRPM7 expressions in this process are attractive. In the present study, we further explored whether TRPM7 and TRPM2 expressions was increased in 6-hydroxydopamine lesioned rat and whether K252a,Wortmannin and U0126, as inhibitors of trkA, PI-3K and ERK signal parthways respectively, affects the levels of TRPM2 and TRPM7.

2. MATERIALS AND METHODS

The study was approved by the animal study subcommittee of the local institutional review board and conformed to the guide for the care and use of laboratory animals. A total of 49 healthy, adult, male Wistar rats, weighing 210–240g, were housed at 21 Cº with a 12-hour day/night cycle and free access to food and water. All experimental procedures were performed in accordance with the Guidance Suggestions for the Care and Use of Laboratory Animals, formulated by the Ministry of Science and Technology of the People’s Republic of China. The study was approved by the animal study subcommittee of the local institutional review board. The gene-specific primers of TRPM2 and TRPM7 for PCR reactions were designed according to TRPM2 (Genbank NCBI_005119) and TRPM7 (Genbank NCBI_053705) mRNA sequences from rats in the.The following drugs were used: NGF (Sigma, USA), K252a (Fermentek, Australia), Wortmannin (Fermentek, Australia), U0126 (Sigma, USA). All drugs were dissolved in DMSO, except that of NGF in water.

2.1 Animal Grouping and Model Establishment

The animals were randomly assigned into seven groups (7 rats per group)as follows: the sham group, the NGF group (6-OHDA+NGF), the PD group (6-OHDA treatment), the K252a group (6-OHDA+K252a+NGF), the Wortmannin group (6-OHDA+Wortmannin+NGF), the U0126 group (6-OHDA+U0126+NGF) and the DMSO group (6-OHDA+DMSO+NGF). The experimental models were established by unilateral injection of 6-OHDA (Sigma, USA) into

the left medial forebrain bundle (MFB) as previously reported [29]. In short, for the rats in the PD group, the NGF group, K252a group, wortmannin group, U0126 group and the DMSO group, they were anesthetized with 6.5% chloral hydrate (35 mg/100 g) and given a unilateral stereotaxic injection of 18 μg 6-OHDA (4μg/μl) through two small burr holes in the skull into MFB at a flow rate of 0.5 μl/min using a micro-injection pump at the following coordinates: [2.5 μl at anteroposterior (AP)−4.4 mm, mediolateral (ML)+1.2 mm, and dorsoventral (DV)−7.8 mm, relative to bregma and dura; and 2 μl at AP−4.0 mm, ML+0.7 mm, and DV−8.0 mm] (Paxinos and Watson, 1986) into the left striatum. The rats in the sham group were injected with an equivalent dose of physiological saline using the same method.

2.2 Intracerebroventricular (ICV) Injection

The rats received ICV delivery of NGF solution (500 ng, 5μl) or a mixture of 5μl NGF and 0.5μl stock solution of K252a (final concentration 300nmol/l) or Wortmannin (2.5μg, 5μl saline/DMSO,1:1) or U0126 (5μg, 5μL) or 0.5μl DMSO (Sigma, USA) into the left cerebral ventricle 24h before 6-OHDA treatment. Injections were performed with a Hamilton syringe. The location of each injection was 2.5 mm posterior to bregma, 1.5mm lateral to midline, and 5.5mm deep to the skull surface.

2.3 Tissue Processing

The rats were euthanized by decapitation under anesthesia of sodium pentobarbital (40mg/kg) at the seventh day starting on the first day of the experiment. The brain was rapidly removed and the ventral half of midbrain around 4.8mm posterior to the Bregma was used as the substantia nigra part to be analyzed. These samples were immediately frozen in liquid nitrogen, and stored at −80ºC until used for analysis. Tissue samples were homogenized using a tissue homogenizer immersed in ice water in 2.0 ml PBS (0.1M, pH7.5) containing 10mM Hepes, 2.0mM ethylene diamine tetraacetic acid (EDTA), 2.0mM EGTA, 0.6mM MgSO4, 4.6mM KCl, pepstatin A, leupeptin, aprotinin, and phenyl methylsulfonyl fluoride (PMSF). Samples were centrifuged at 1000×g for 10 min/4ºC to remove cell debris and the collected supernatant was centrifuged at 10,000×g for 10 min/4ºC. Supernatant was used for analysis.

2.4 Immunohistochemistry

The rats were anesthetized with 10% chloral hydrate and the perfusion fixed with 4% paraformaldehyde (PF) in a phosphate buffer saline (PBS; pH 7.2) by intracardiac method. Brains were removed from the cranium, post-fixed in the perfusion fixative for 2 days then stored in the PBS until sectioning. Serial coronal sections (5μm thick) were cut at the level of the substantia nigra (SN) using a rotary microtome. Sections were incubated with the rabbit-anti-rat primary antibodies at 37ºC for 1 h. Primary antibodies employed were anti-TRPM2, TRPM7 (1:100, Boster inc., WuHan, China). Subsequently, sections were incubated

successively with freshly prepared biotinylated goat-anti-rabbit IgG (1:200) and avidin-biotin complex for 30 min at room temperature respectively. Finally they were incubated with 0.02 % diaminobenzidine (DAB) and 0.003% hydrogen peroxide in 50mM Tris-HCl buffer (pH7.6) for 3 min. After the DAB reaction, the tissues were rinsed with PBS; the sections were mounted on gelatine-coated slides and coverslipped with mounting medium. Control sections were incubated with the secondary antibody to take the place of antibody and no immunoreactivity was observed in these controls. Every fourth section in the SN was selected from the region spanning from Bregma –4.0 to –4.4. The mean optical density (OD) of TRPM2 and TRPM7-immunoreactive (ir) positive cells in the SN was detected by a bright-field microscope and an image analyzer.

2.5 Western Blot Assay

Midbrain tissues or cultures of rats were harvested, cleaned of connective tissue and blood, and frozen in liquid nitrogen. The segments were pulverized in a matching bullet tube and pestle, homogenized in RIPA buffer containing 50mM Tris. HCl, pH 8.0, with 150mM sodium chloride, 1.0% Non-idet P-40, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate with protease inhibitor cocktail and incubated on ice for 15 min. After centrifugation (15 min at 15,000g), a portion of the supernatant was used for total protein quantification by DC Protein Assay. Equal amounts of sample protein were mixed with LDS sample buffer and sample reducing agent and heated at 70ºC for 10 min. Whole isolated protein were separated by 7.5% sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to a polyvinylidene difluoride membrane. The membranes were then incubated with polyclonal antibody against TRPM2,TRPM7 (1:100 dilution, Boster inc., WuHan, China) or monoclonal antibody against β-actin (1:1000 dilution, Boster inc., WuHan, China) at 4 Cº overnight. Proteins recognized by the antibody were revealed by an ECL kit, following the manufacturer's instructions (Boster inc., Wuhan, China).

2.6 RNA Extraction and Reverse Transcriptase-PCR

Total RNA of midbrain tissues or cultures was isolated using RNA Slov Reagent (Boster inc., Wuhan, China) following the manufacturer's instructions. Reverse transcription was performed using 2ul of total RNA (3.6μg/ml) A standardized semi-quantitative PCR method was used based on amplification of the target gene TRMP7 and a constitutively expressed gene, β-actin. First strand cDNA was synthesized using Revert AidTM First Strand cDNA Synthesis Kit (K1621, China, Boster inc.,) and used as a template for PCR reactions with gene-specific primers for TRPM7 (NC_000021.9), TRPM2 (NG_021363.1), β-actin (Table 1). The PCR reaction was conducted with 25 pmol forward and reverse primers, 10% of the cDNA reaction mixture using BioRT Two Step RT-PCR kit (Boster inc., Wuhan, China). The mixture was preheated to 95 Cº for 3 min, then subjected to 30 cycles of a 60s denaturing phase at 94 Cº; a 40s annealing phase at 55 Cº, and a 60s extension phase at 72 Cº, followed by final extension at 72 Cº for 10 min. Amplificated products of TRPM7 and β-actin were electrophoretically separated by 1.5% agarose gels containing ethidium bromide.

Electrophoreses images were photographed with Kodak Digital Science Scanner system and analysis was processed using this system.

Table 1. The gene-specific primers for TRPM2 and TRPM7

PCR primers	Forward	Reverse
TRPM2	5'-AGGGATCCAGGCTTTCCTAA-3'	5'GCCATCGGGATCGTAGAATA-3'
TRPM7	5'-CTGAAGAGGAATGACTACAC-3'	5'-ACAGGGAAAAAGAGAGGGAG-3'
β-actin	5'-CCTTCCTGGGCATGGAGTCCTG-3'	5'-GGAGCAATGATCTTGATCTTC-3'

2.7 Statistical Analysis

All data were expressed as the mean±SEM, and statistical significance was determined by one-way ANOVA analysis of variance combined with either Dunnett's test for multiple comparisons between different treatment groups. Statistical significance was set at $p<0.05$.

3. RESULTS

3.1 TRPM2 and TRPM7 Expressions in Substantia Nigra were Up-regulated after 6-OHDA Treatment

Semi-quantitative RT-PCR and Western blot assay were carried out to examine the time course of TRPM2 and TRPM7 expressions in the SN of rats at one week after 6-OHDA injection. It showed that TRPM2 and TRPM7 expressions in the PD group were significantly higher than that in the sham group. Following normalization to the β-actin signal, TRPM2 (1.95-fold, $p< 0.05$) and TRPM7 (1.4- fold, $p< 0.05$) mRNA in the PD group were higher than that in the sham group (Fig. 1). Consistent with mRNA changes, the proteins of TRPM2 (2.35-fold, $p< 0.05$) and TRPM7 (3.10-fold, $p< 0.05$) were increased at the same time respectively, as compared with the sham group. In parallel with the RT-PCR and western blot analysis, immunohistochemical analysis of the SN regions using anti-TRPM2 and TRPM7 antibodies also showed that TRPM2 (1.67-fold, $p< 0.05$) and TRPM7 (2.40-fold, $p< 0.05$) were significantly increased in the PD group (Fig. 1).

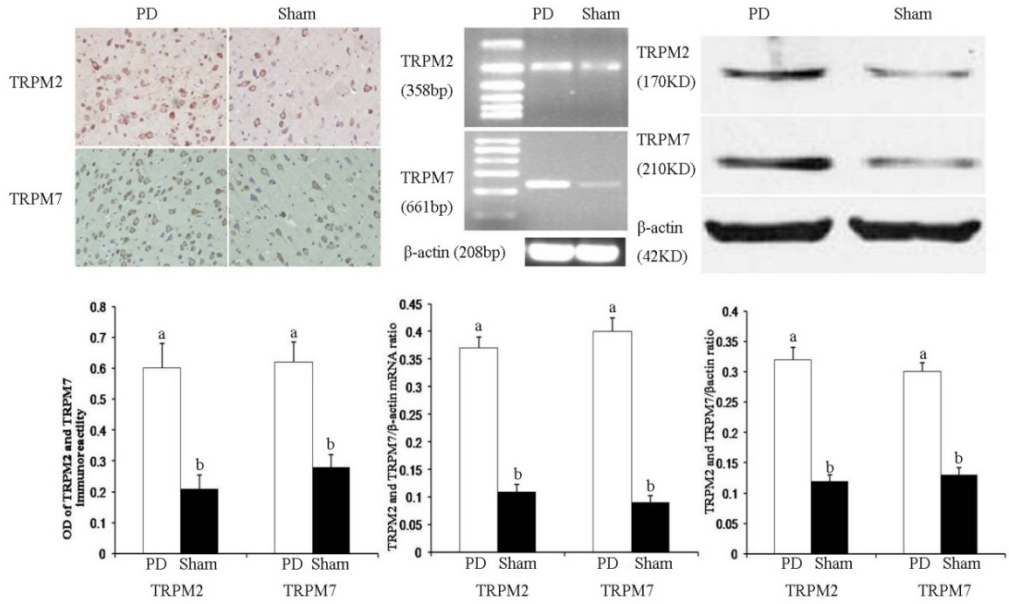

Fig. 1. Immunohistochemical (Left), Western blot (Middile) and RT-PCR (Right) staining analysis showing the expressions of TRPM2 and TRMP7 in the SN of rats at one week after 6-OHDA injection. Results were expressed as relative intensity in density of TRPM7 band as compared to that of β-actin. The levels of TRPM2 and TRMP7 were increased in the PD group compared to the sham group ($p<0.05$). Data are presented as means±SEM. Columns relevant to each parameter bearing different superscripts (a, b) differ significantly

3.2 TRPM2 and TRPM7 Expressions in Substantia Nigra in 6-OHDA Lesioned Rat after NGF Treatment

To address the possible role of NGF in regulation of TRPM2 and TRPM7 expressions, the PD group was injected intracerebroventricularly with NGF 60 min followed by 6-OHDA injection and TRPM2 and TRPM7 expressions in ipsilateral SN were detected at one week after 6-OHDA injection. As shown in Fig. 2, ICV injection of NGF markedly decreased TRPM2 and TRPM7 expressions.

3.3 Effect of K252a on TRPM2 and TRPM7 Expressions in the Substantia Nigra in 6-OHDA Lesioned Rat after NGF Treatment

To explore whether trkA was necessary for the effect of 6-OHDA treatment on the expressions of TRPM2 and TRPM7, we used a trkA inhibitor K252a in the present study. The PD group rats were ICV injected with DMSO+NGF (vehicle) or K252a+NGF just before 6-OHDA injection. As shown in Figs. 3 and 4, low levels of TRPM2 and TRPM7-ir were observed in the PD group rats treated with DMSO in the SN regions. However, TRPM2 and

TRPM7 were significantly increased in the K252a group (treatment with 6-OHDA+K252a+NGF). In parallel with the immunohistochemical analysis, western blot analysis of the SN regions using anti-TRPM2 and TRPM7 antibodies also showed that K252a treatment increased the levels of TRPM2 and TRPM7 expressions compared to DMSO treated rats (Figs. 3 and 4). Our results indicated that the effect of NGF on the expressions of TRPM2 and TRPM7 could be inhibited by the trkA inhibitor K252a in the 6-OHDA lesioned rat.

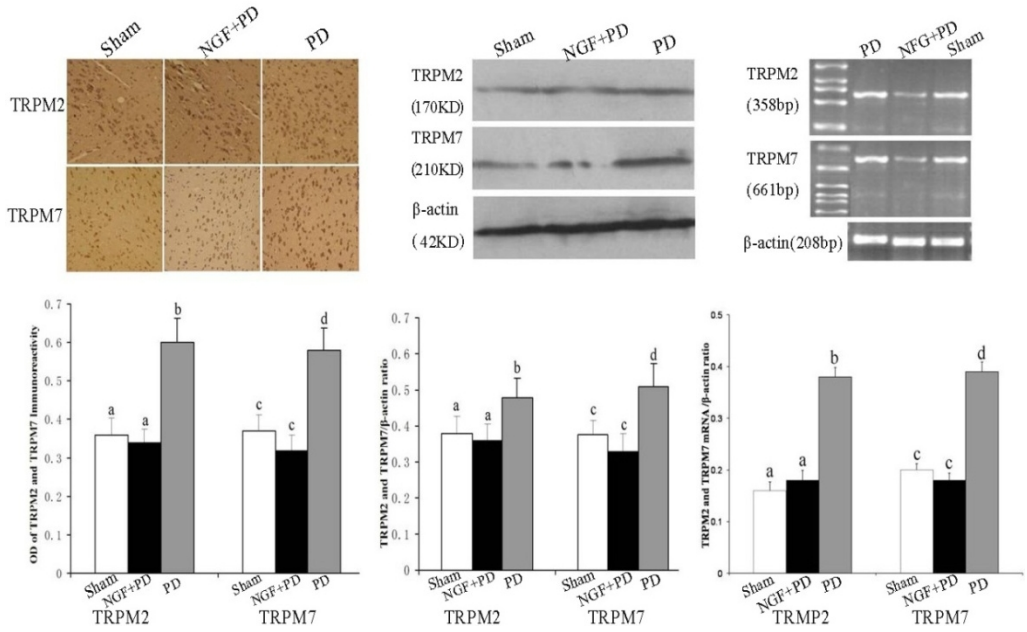

Fig. 2. Western blot, RT-PCR and immunohistochemical staining analysis showing the effects of NGF on the expressions of TRPM2 and TRMP7 in the SN of rats at one week after 6-OHDA injection. The levels of TRPM2 and TRMP7 were increased in the PD group compared to the sham group ($p<0.05$). However, NGF treatment inhibited the increase of TRPM2 and TRMP7 expressions in 6-OHDA lesioned rat. Data are presented as means±SEM. Columns relevant to each parameter bearing different superscripts (a, b, c or d) differ significantly ($p<0.05$)

3.4 Effect of Wortmannin and U0126 on TRPM2 and TRPM7 Expressions in the Substantia Nigra in 6-OHDA Lesioned Rat after NGF Treatment

To explore which trkA-mediated signaling pathways were necessary for the effect of 6-OHDA on TRPM2 and TRPM7 expressions, we used Wortmannin, a P-I3K inhibitor, and U0126, an ERK inhibitor, in the present study. The PD group rats were ICV injected with a mixture of 5μl NGF and DMSO (vehicle) or Wortmannin, or U0126 just before 6-OHDA treatment. No differences in density of TRPM2 and TRPM7 expressions were shown between U0126 and DMSO treatment (Figs. 3 and 4). However, TRPM2 and TRPM7 were significantly increased

in the Wortmannin group (treatment with 6-OHDA+Wortmannin+NGF) (Figs. 3 and 4). In parallel with the immunohistochemical and Western blot analysis of the midbrain homogenates using anti-TRPM2 and TRPM7 antibodies also showed that U0126 treatment scarcely changed the levels of TRPM2 and TRPM7 compared to DMSO treatment. However, administration of Wortmannin significantly increased the expression levels of TRPM2 and TRPM7 (Figs. 3 and 4). Our results indicated that the expressions of TRPM2 and TRPM7 could be upregulated by a PI3K inhibitor (Wortmannin) but not by an ERK inhibitor (U0126) in the 6-OHDA lesioned rat. K252a also did blocked NGF-mediated inhibitory effect, but DMSO did not.

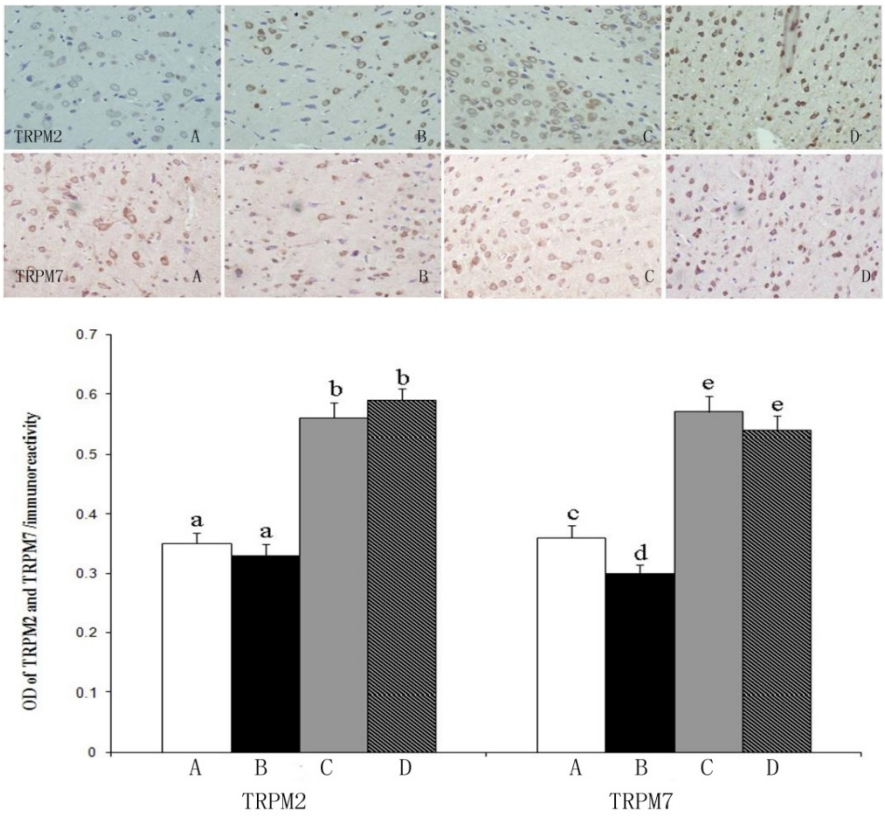

Fig. 3. (A)6-OHDA+DMSO+NGF; (B) 6-OHDA+U0126+NGF; (C) 6-OHDA+K252a+NGF (D) 6-OHDA+Wortmannin+NGF; Graphic representation of the expressions of TRPM2 and TRMP7 analyzed by immunohistochemical staining in the SN treated with DMSO, K252a, Wortmannin, or U0126. Note that treatment with K252a or Wortmannin in 6-OHDA lesioned rat significantly increased TRPM2 and TRMP7 expressions compared to DMSO and U0126 treatments. There was no difference in the levels of TRPM2 and TRMP7 between the DMSO group and U0126 group. Values are mean±SD.[b,e]Significant difference at $p<0.05$ compared to the DMSO and U0126 groups in the SN, respectively. Data are presented as means±SEM. Columns relevant to each parameter bearing different superscripts (a, b, c, d or e) differ significantly ($p<0.05$)

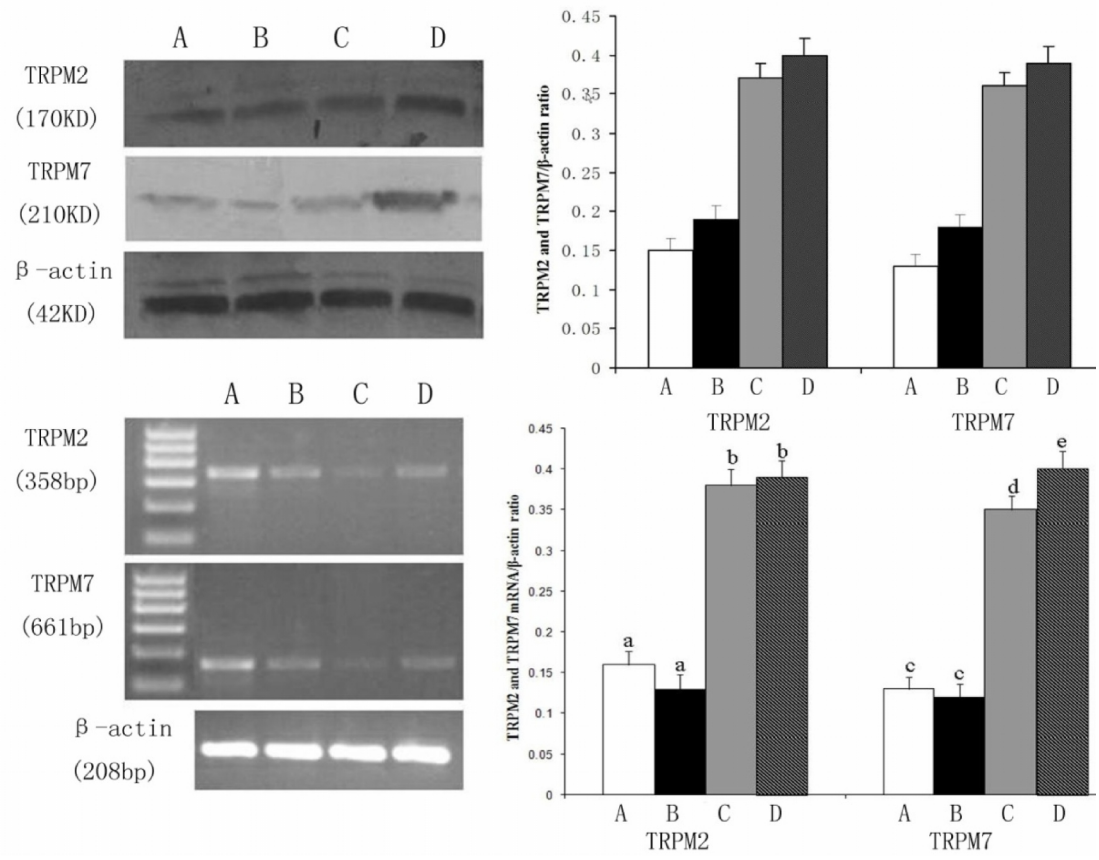

Fig. 4. (A)6-OHDA+DMSO+NGF; (B) 6-OHDA+U0126+NGF; (C) 6-OHDA+K252a+NGF (D) 6-OHDA+Wortmannin+NGF; Graphic representation of the expressions of TRPM2 and TRMP7 analyzed by Western blot and RT-PCR in the SN treated with DMSO, K252a, Wortmannin, or U0126. Note that treatment with K252a or Wortmannin in 6-OHDA lesioned rat significantly increased TRPM2 and TRMP7 expressions compared to DMSO and U0126 treatments. There was no difference in the levels of TRPM2 and TRMP7 between the DMSO group and U0126 group. Values are mean±SD.[b,d,e]Significant difference at $p<0.05$ compared to the DMSO and U0126 groups in the SN, respectively. Data are presented as means±SEM. Columns relevant to each parameter bearing different superscripts (a, b, c, d or e) differ significantly ($p<0.05$)

4. DISCUSSION

Although both NGF, TRPM7 and TRPM2 are known to participate in the pathophysiological processes of center nervous system disease, NGF was involved in rotenone toxicity induced dopaminergic neurons damage and expression of TRPM2 channels has been detected in wide spread of the brain including the striatal [14,15], hippocampal [16,17,18], cortical [30]

neurons and SN [31]. TRPM7 are also widely expressed in the nervous system [32]. Although the specific physiological function of TRPM7 in neurons is unknown at present, the neurotoxic role of TRPM7 in anoxic neuronal death [33] as well as an additional function of TRPM7, namely of mediating neuronal response to extracellular low divalent ions has been well demonstrated in previous studies [34]. TRPM2 and TRPM7 channels are physiologically important in oxidative stress-induced cell death [35]. Previous study indicated that the NGF significantly reduced rotenone toxicity on TH-positive neurons in midbrain neuronal cultures. The protective effect of NGF was completely abolished by inhibiting the microtubule-associated protein kinase kinase (MEK) and partially reversed by blocking PI-3K [24]. In view of the fact that SN neurons are especially vulnerable to oxidative stress, and previous study has shown that SN tissues express a measurable density of TRPM2 [14], NGF and TrkA [36]. These TRPM channels may also play a role in other neurodegenerative. Up till now, the relationship between them during PD is still poorly understood. In the present study, by using 6-OHDA induced experimental PD models, we firstly examined the change of TRPM2 and TRPM7 expressions in the SN of 6-OHDA lesioned rats, and then explored the possible role of TrkA pathway(s), activated by NGF, in regulation of TRPM2 and TRPM7 expressions. It showed that NGF, by activating TrkA receptors, prevented 6-OHDA-induced high expressions of TRPM2 and TRPM7 through PI-3K signal pathway.

Recently, TRPM7 is revealed to reside in the rat hippocampal neurons [32], but no evidence in substantia nigra. Previous work has revealed a significant up-regulation of TRPM7 expression in rat cortex and hippocampus tissues following cerebral ischemia [33]. In this study, we detected the expressions of TRPM2 and TRPM7 in the SN of 6-OHDA lesioned rat. The results showed that, TRPM2 and TRPM7 mRNA and protein in ipsilateral SN in 6-OHDA lesioned rats had significantly increased than that in normal rat and both were down-regulated by NGF treatment. TRPM2 channels are sensitive to oxidative stress, and their activation can lead to cell death. Thus, by considering all of above, it is possible for TRPM2 and TRPM7, due to up-regulated expressions, contributes to oxidative stress mediated neuronal damage during 6-OHDA treatment. It is speculated that Ca^{2+} might play a role in the neuronal pathology seen in PD. Then there is a hypothesis that the primary factor driving neurodegenerative changes in PD is the metabolic stress created by sustained Ca^{2+} entry, particularly in the face of genetic or environmental factors [37]. TRPM subfamilies are a diverse group of voltage-independent Ca^{2+}-permeable cation channels that are expressed in mammalian cells [38]. It is speculated that there might be another mechanism of TRPM2 and TRPM7 contributes to Ca^{2+} influx which resulted from increased TRPM2 and TRPM7 as well as its activity facilitated induced by 6-OHDA. Regarding that TRPM2 and TRPM7 are involved in neuronal death, reduction or blockade of TRPM2 and TRPM7 levels might be a method of reducing cell death and PD treatment. Thus finding out the regulator(s) of TRPM2 and TRPM7 expressions in 6-OHDA lesioned rats is especially significant.

The lipid kinase PI-3K has been linked to a number of cellular processes including transcription, cell growth, proliferation, survival, apoptosis and glucose metabolism. PI-3K signaling pathway has been reported to contribute to the neuroprotective response of many

growth factors. NGF, as a neurotrophic factor firstly found, supports the survival and maintenance of many neuronal populations of central and peripheral nervous system, and its induction in response to brain ischemic injury is regarded as a normal protective mechanism. In this work, vivo experiments showed that NGF significantly decreased TRPM2 and TRPM7 expressions in the 6-OHDA lesioned rat. Thus, it can conclude that NGF is an important regulator of TRPM2 and TRPM7 expressions and, which might be new targets of NGF during 6-OHDA damage period. In addition, K252a, a kinase inhibitor that prevents the actions of NGF by inhibiting the TrkA signal substantially abolished the effect of NGF, which suggests that the negative regulation of NGF on TRPM2 and TRPM7 expressions in the 6-OHDA lesioned rat was mediated by its high-affinity receptor-TrkA which was observed co-localization with TRPM2 and TRPM7 in the SN. Further more, blocking one of the TrkA pathways by PI-3K inhibitor wortmannin can also negatively regulates TRPM2 and TRPM7 expressions. However, blocking ras/MAPK pathway with MEK inhibitor U0126 did not.

Moreover, all inhibitors have no effects on TRPM2 and TRPM7 expressions in the absence of NGF, which suggest that only the PI-3K pathway is activated by NGF, it negatively regulates TRPM2 and TRPM7 expressions and that it is NGF downstream PI-3K pathway responsible for regulating TRPM2 and TRPM7 expressions. It may be another mechanism for PI-3K pathway involved in NGF-dependent protective effect on neurons against 6-OHDA by down-regulating TRPM2 and TRPM7 expressions. Despite there is no direct findings for ras/MAPK or PLC-pathways in regulation of TRPM2 and TRPM7 expressions, we cannot rule out they may serve specific regulation in TRPM2 and TRPM7 channels activity. A latest study has reported that PLC-pathway downstream of TrkA triggered by NGF potently reduces TRPM7-like current in hippocampal neurons [39]. So we speculate that NGF might dually regulate the expressions and channel activity of TRPM2 and TRPM7 through activating different TrkA signal pathways.

5. CONCLUSION

Only PI-3K pathway was responsible for NGF-mediated down-regulation of TRPM2 and TRPM7 expressions in the 6-OHDA lesioned rat. TRPM2 and TRPM7 channels might be involved in the development of PD. Given the close relationship between TRPM2, TRPM7 and oxidative stress and calcium channels, further studies are needed to determine the roles of these channels in PD process.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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