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NF-κB in mitochondria regulates PC12 cell apoptosis following lipopolysaccharide-induced injury*

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Abstract: Objective: To determine the relationship between nuclear transcription factor κB (NF-κB) expression in mitochondria and neuronal cell apoptosis after lipopolysaccharide (LPS)-induced injury. Methods: The effect of drug administration on PC12 cells was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and Hoechst 33342 staining. The morphology and function of mitochondria were investigated with electron microscopy and rhodamine 123, respectively. The activity of adenine nucleotide translocase 1 (ANT1), lipid peroxide, and anti-peroxidase enzymes was measured by enzyme-linked immunosorbent assay (ELISA). Relative expression levels of NF-κB were measured by reverse transcription-polymerase chain reaction (RT-PCR) and Western blotting. Results: Pyrrolidine dithiocarbamate (PDTC, an NF-κB inhibitor) and nerve growth factor (NGF) not only reduced apoptosis but also had a protective effect on mitochondrial structure and function in a PC12 cell LPS damage model. The subcellular distribution of NF-κB demonstrated that NGF, diterpene acid atractyloside (ATR, an ANT1 antagonist), and PDTC alleviated increases in mitochondrial NF-κB after LPS-induced injury. Conclusions: (1) NF-κB is activated in mitochondria via uptake of ANT1 during apoptosis following LPS-induced injury in neuronal cells; (2) NGF not only decreases the activity of NF-κB but also reduces ANT1 activity, which in turn decreases NF-κB levels in mitochondria and suppresses mitochondria-mediated apoptosis.

Key words: Nuclear transcription factor κB (NF-κB); Mitochondria; Apoptosis; Adenine nucleotide translocase 1 (ANT1); Lipopolysaccharide (LPS)

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1 Introduction

With the rapid growth of pollution, the incidence of cross infection has gradually increased, particularly in infections caused by Gram-negative bacteria. Since Kerr et al. (1972) discovered apoptosis, a large number of studies have indicated that apoptosis is associated with several diseases (Sugawara and Chan, 2003; Carrillo-de Sauvage et al., 2013), and many important characteristics of apoptosis have been closely associated with mitochondria. In normal eukaryotic cells, nuclear transcription factor κB (NF-κB) is massively distributed and participates in multiple biological functions, including inflammation, the immune response, cell proliferation, and apoptosis. When NF-κB is activated, it translocates from the cytoplasm into the nucleus, where it promotes specific inflammatory gene expression and induces inflammatory responses. Inflammatory reactions are

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accompanied by the release of reactive oxygen species (ROS) (Lu et al., 2014; Wani et al., 2014), which can damage the mitochondrial membrane. The NF-κB pathway may also activate the apoptosis pathway, but to date, relatively little research has addressed the effects of NF-κB in the mitochondrial on this pathway. Clarifying the relationship between NF-κB in mitochondrial and the mitochondrial apoptosis pathway may be helpful for curing infectious diseases.

Many reports have shown that nerve growth factor (NGF) is an essential neurotrophic substance for neuronal survival, differentiation, and function; however, the specific mechanisms by which NGF fulfills these functions require further research. This paper examines the role of NGF in protecting neuronal cells against lipopolysaccharide (LPS)-induced injury and seeks to clarify the mechanism of potential protective effects. NGF not only aided in the recovery of PC12 cells from mitochondrial damage due to LPS-induced injury but also reduced mitochondrial NF-κB levels by regulating the activity of adenine nucleotide translocase 1 (ANT1).

2 Materials and methods

2.1 Materials

NGF, LPS, pyrrolidine dithiocarbamate (PDTC), and diterpene acid atractyloside (ATR) were purchased from Sigma (Sigma-Aldrich, St. Louis, MO, USA). Rat ADP/ATP translocase 1 (SLC25A4) enzyme-linked immunosorbent assay (ELISA) kit was procured from Cusabio (Wuhan, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was obtained from the Shanghai Biotechnology Company (Shanghai, China). Dulbecco's minimum essential medium (DMEM) and horse serum were purchased from Gibco (Thermo Scientific, Waltham, MA, USA). Fetal calf serum was obtained from Evergreen (Hangzhou, China). Anti-NF-κB p65 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Phenylmethylsulfonyl fluoride (PMSF), nuclear protein extraction reagent, and a mitochondrial isolation kit were procured from Beyotime (Nanjing, China).

2.2 Cell culture and drug treatments

Well-differentiated PC12 cells were purchased from the Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China), maintained in DMEM, and supplemented with 5% heat-inactivated fetal calf serum and 10% heat-inactivated horse serum, which were changed in DMEM without NGF for 24 h before experiment. Cultured cells were divided into five groups: (1) a control group, which was not exposed to LPS, PDTC, or NGF; (2) an LPS damage group, which was treated with medium plus LPS; (3) an LPS+NGF group, which was treated with medium plus LPS and NGF; (4) an LPS+PDTC group, which was treated with medium plus LPS and PDTC; and (5) an LPS+ATR group, which was treated with medium plus LPS and ATR for 24 h.

2.2.1 MTT absorbance

In all experiments, 10 μl of MTT (5 mg/ml) was added to a 96-well plate before the end of culture. Cells were then incubated for an additional 4 h at 37 °C in 5% CO_2. The MTT product formazan was measured on a plate-reader by monitoring absorbance at 570 nm.

2.2.2 Hoechst 33342 staining

After experiment, to each well was added Hoechst 33342 (10 μg/ml) staining under dark for 10 min and then the cells were observed and photographed under a fluorescence microscope.

2.3 Mitochondrial membrane potential measurement

Mitochondrial transmembrane potential (MTP) was measured using rhodamine 123 staining, as the uptake of rhodamine 123 into mitochondria has been shown to be a function of MTP. Media were removed, and cells were subsequently exposed to rhodamine 123 for 30 min. Fluorescence was measured at an excitation wavelength of 488 nm and an emission wavelength of 525 nm. The cells in the 24-well plate were observed under the fluorescence microscope.

2.4 Electron microscopy

Cells were collected and fixed in 2.5% (0.025 g/ml) glutaraldehyde overnight at 4 °C. On the following day, cells were embedded and ultrathin-sliced according to standard procedures to study cell apoptosis and mitochondrial morphology under a transmission electron microscope.

2.5 ELISA

The standard curve was set, and we measured the expression of superoxide dismutase (SOD), malondialdelyde (MDA), glutathione peroxidase (GSH-PX), catalase (CAT), and ANT1 in each group by ELISA assay according to manufacturer's recommendations.

2.6 Reverse transcription-polymerase chain reaction (RT-PCR)

After treatment, cells were collected, and the relative expression of NF-κB was assessed using RT-PCR. The primer sequence for NF-κB was as follows: forward, 5'-CTAGCCCCAGGTCCTCCCC AATC-3'; and reverse, 5'-GGCCCCCAAGTCTTCA TCAGCATC-3', with a product length of 123 bp. The primer sequence for GAPDH was as follows: forward, 5'-GGTGGACCTCATGGCCTACAT-3'; and reverse, 5'-GCCTCTCTCTTGCTCTCAGTATCCT-3', with a product length of approximately 88 bp.

2.7 Western blot analysis

According to the data (Zamora et al., 2004), nuclear and cytoplasmic fractions were isolated using the nuclear and cytoplasmic extraction reagent kits, respectively. To isolate mitochondria and nuclei, cells were collected and resuspended at 4 °C in buffer and were centrifuged at 600*g* for 10 min at 4 °C. Supernatants were further centrifuged at 11000*g* for 10 min, and the pellet contained the mitochondria-enriched fraction. Extracts were separated with 12% SDS-PAGE and transferred onto polyvinylidene fluoride (PVDF) membranes. Subsequently, membranes were incubated overnight at 4 °C with anti-NF-κB p65 (1:1000, v/v). Immunoreactive proteins were visualized using the enhanced chemiluminescence (ECL).

2.8 Statistical analysis

All experiments were repeated three times. Results were expressed as the mean±standard deviation (SD). Statistical comparisons were performed using one-way analysis of variance (ANOVA). A P value of <0.05 was considered significant.

3 Results

3.1 Effects of NGF and PDTC on PC12 cells

MTT absorbance results indicated that a concentration of 100 nmol/L LPS was used for the damage model in the subsequent experiment. NGF and PDTC were able to alleviate damage on PC12 cells after LPS-induced injury (Figs. 1a and 1b).

3.2 Microscopic examination of morphology

The morphology under the microscope was the same as the results of MTT assay. The nucleus of normal PC12 cells was blue by Hoechst 33342 staining under the fluorescence microscope (Fig. 1c). However, some cells in LPS group were stained dark blue (Fig. 1d), which illustrated that the cells' nucleus had condensed, and had come into the apoptosis process. The apoptosis cells were significantly reduced in the NGF or PDTC group, which illustrated that NGF or PDTC could certainly reduce the damage of LPS on PC12 cells (Figs. 1e and 1f).

We additionally examined subcellular morphology under the transmission electron microscope. Normal PC12 cells were intact, and their cell membranes were smooth (Fig. 2a). Mitochondria were generally linear or granular and had an integral bilayer membrane structure (Fig. 2e). However, PC12 cells that had suffered LPS-induced injury were marked by cavities and strong condensation of heterochromatin congregated to the edge of the nuclear membrane, suggesting that cell apoptosis had occurred (Fig. 2b). Additionally, mitochondrial membranes were damaged, and cristae were characterized by edema, forming an even larger cavity (Fig. 2f). Cell morphology was much more integral and clear in the NGF and PDTC treatment groups than in the LPS group, and there was little evidence of apoptosis (Figs. 2c, 2d, 2g, and 2h).

3.3 Activity of peroxide/anti-peroxidase enzyme and ANT1

Compared with the normal group, levels of SOD (Fig. 3a), GSH-PX (Fig. 3b), and CAT (Fig. 3c) in the LPS-induced injury group were significantly lower. MDA (Fig. 3d) in the LPS group was significantly higher than that of the control group. However, activity in the NGF and PDTC groups was between that of the control and LPS groups. The above results demonstrated that NGF and PDTC balanced the activity of oxidize and antioxidant enzyme even after LPS-induced injury.

The ELISA assay indicated that expression of ANT1 in the LPS group was higher than that in the normal group, while in the NGF and ATR groups, ANT1 expression was significantly lower than that in the LPS group.

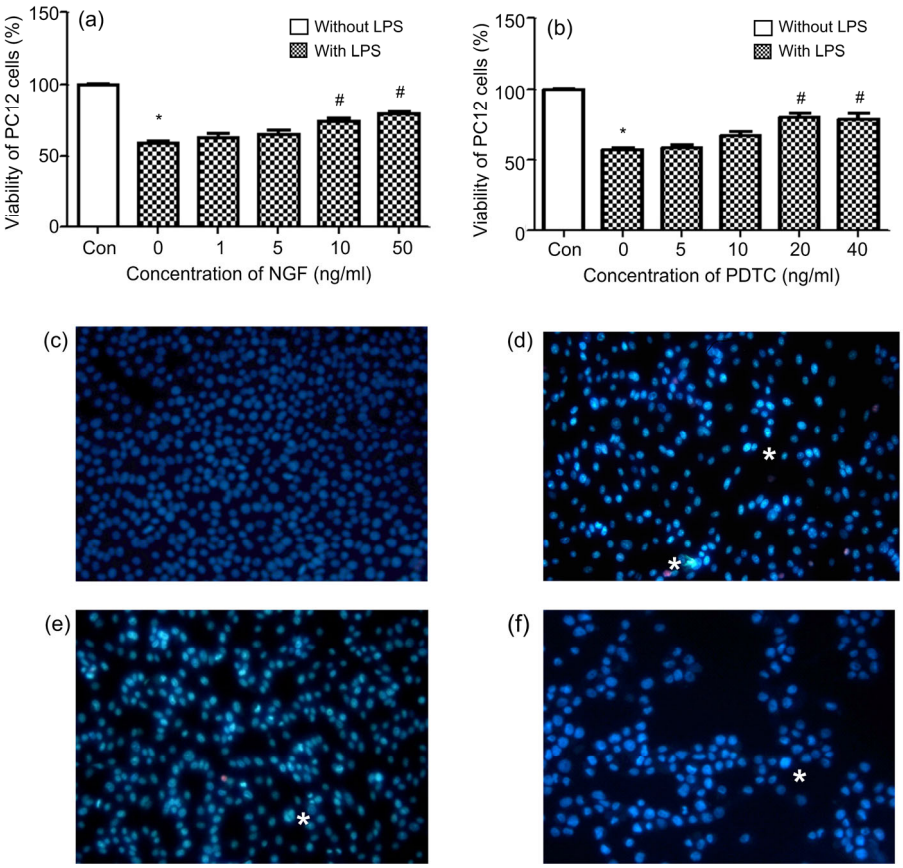

Fig. 1 Effects of NGF and PDTC on PC12 cells

(a) NGF alleviated PC12 cells from LPS injury. (b) PDTC alleviated PC12 cells from LPS injury. Data are expressed as mean±SD (n=6). * P<0.05, vs. the control; $^{\#}$ P<0.05, vs. the LPS injury group. (c–f) Cell morphology observation by fluorescence microscope after Hoechst 33342 staining (original magnification ×400). (c) Control: the nucleus of normal PC12 cells was blue by Hoechst 33342 staining under fluorescence microscope; (d) LPS: the cells' nucleus condensed and had come into the apoptosis process; (e) LPS+NGF and (f) LPS+PDTC: the apoptosis cells were significantly reduced in NGF (e) or PDTC (f) group. * represents apoptotic cell (Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

3.4 MTP observed by rhodamine 123 fluorescence

To further characterize changes in mitochondria following LPS-induced injury, MTP was monitored with a microplate reader using the fluorescent dye rhodamine 123. As shown in Fig. 4b, fluorescence in the injury group after LPS treatment was progressively lower than that in the normal group. The MTP also had the same changes as shown in Fig. 4e. The fluorescence of NGF or PDTC group was intermediate between the normal group and the injury group (Figs. 4c and 4d). The MTP of NGF or PDTC group also showed the identical result (Fig. 4e), suggesting that NGF and PDTC could reverse the mitochondrial membrane potential and maintain mitochondrial function in PC12 cells even after LPS-induced injury.

3.5 Expression of NF-κB (p65)

The RT-PCR assay showed that LPS damage to PC12 cells led to increased expression of NF-κB. However, relative expression of NF-κB was lower in the PDTC and NGF treatment groups (Fig. 5a). This suggested that these drugs inhibiting the NF-κB signaling pathway was a possible mechanism.

To further investigate changes in subcellular protein distribution within cells after LPS-induced injury, changes in NF-κB (p65) levels in the whole cell, nucleus, and mitochondria were analyzed in each group using Western blot. After treatment with LPS alone, NF-κB (p65) levels in whole PC12 cells as well as their nuclei and mitochondria were significantly higher than those in the normal group (Figs. 5b–5d). In addition, NF-κB (p65) levels in the NGF and

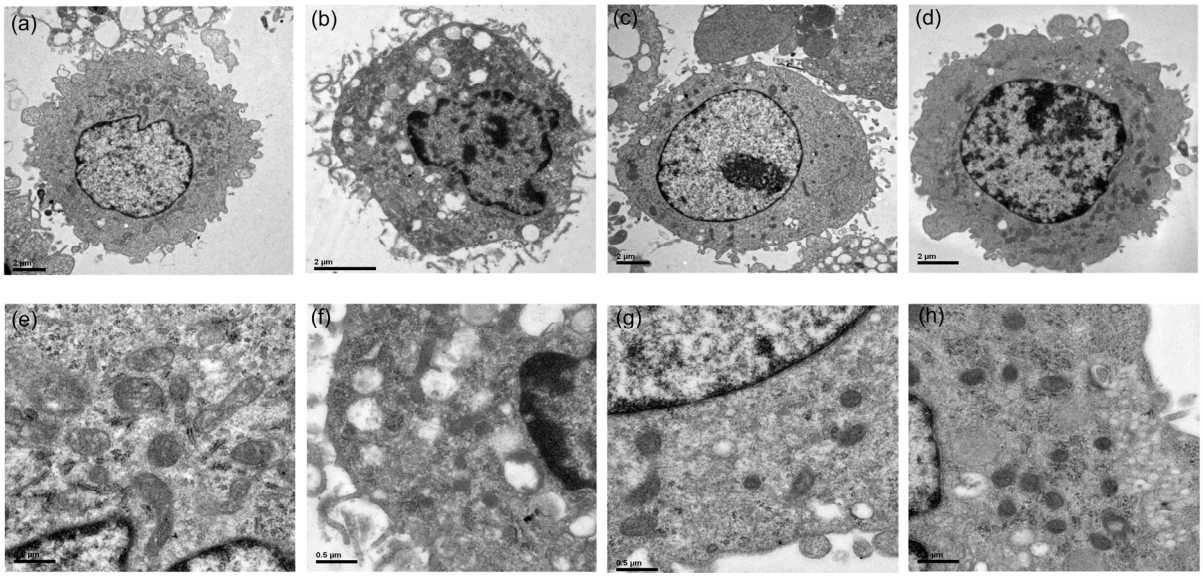

Fig. 2 Subcellular morphology observed under transmission electron microscope

(a) The normal group of PC12 cells showed clear intact nucleus. (b) LPS-induced apoptosis: PC12 cells' nucleonic heterochromatin condensed. (c, d) In the presence of NGF (c) or PDTC (d), PC12 cells maintained relatively normal nuclear morphology. (e) Normal mitochondria of PC12 cells. (f) LPS-induced apoptosis: PC12 cells' mitochondrial membrane appeared with cavity and its matrix disappeared. (g, h) In the presence of NGF (g) or PDTC (h), PC12 cells maintained relatively normal mitochondrial morphology

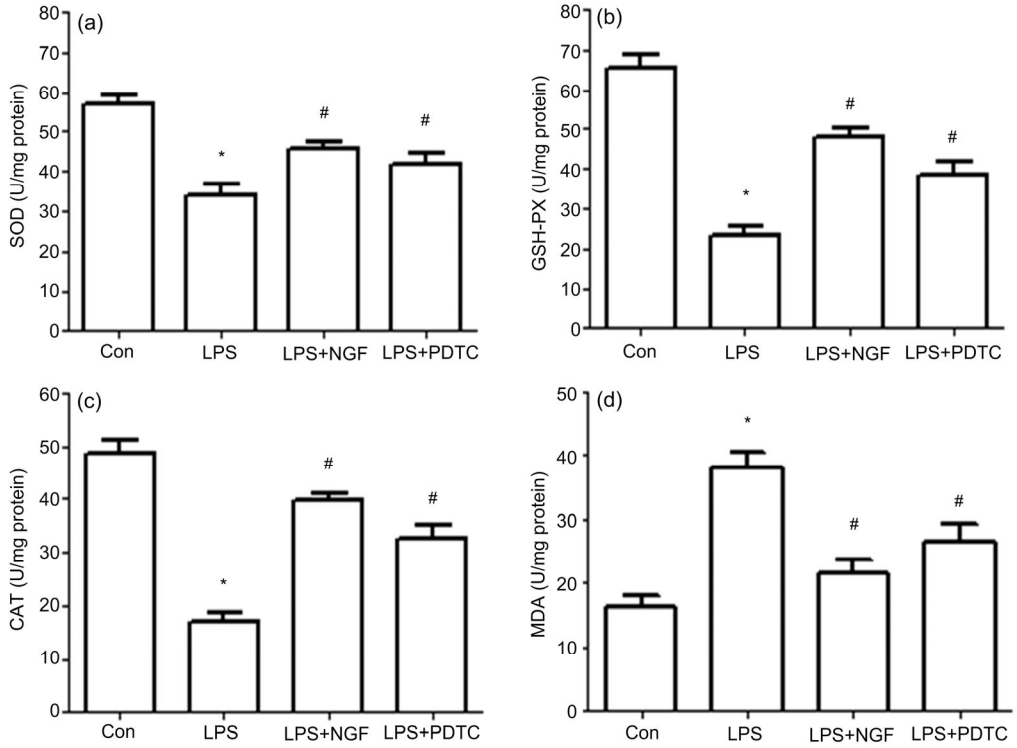

Fig. 3 Effects of NGF and PDTC on PC12 cells by determining the activity of oxidation and anti-oxidation enzymes

(a) SOD; (b) GSH-PX; (c) CAT; (d) MDA. Con: control; LPS: 100 nmol/L LPS. Data are expressed as mean±SD (n=6). * P<0.05, vs. the control; # P<0.05, vs. the LPS injury group

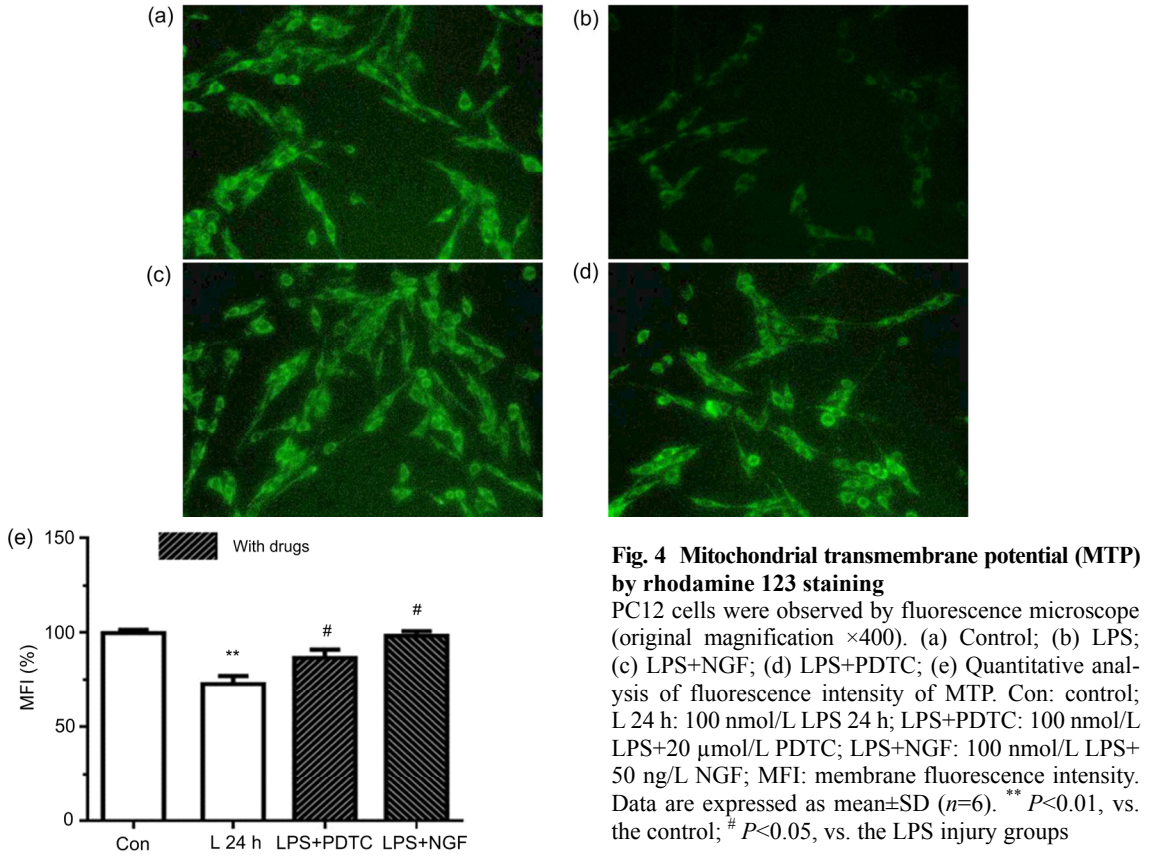

Fig. 4 Mitochondrial transmembrane potential (MTP) by rhodamine 123 staining

PC12 cells were observed by fluorescence microscope (original magnification ×400). (a) Control; (b) LPS; (c) LPS+NGF; (d) LPS+PDTC; (e) Quantitative analysis of fluorescence intensity of MTP. Con: control; L 24 h: 100 nmol/L LPS 24 h; LPS+PDTC: 100 nmol/L LPS+20 μmol/L PDTC; LPS+NGF: 100 nmol/L LPS+50 ng/L NGF; MFI: membrane fluorescence intensity. Data are expressed as mean±SD (n=6). ** P<0.01, vs. the control; $^{\#}$ P<0.05, vs. the LPS injury groups

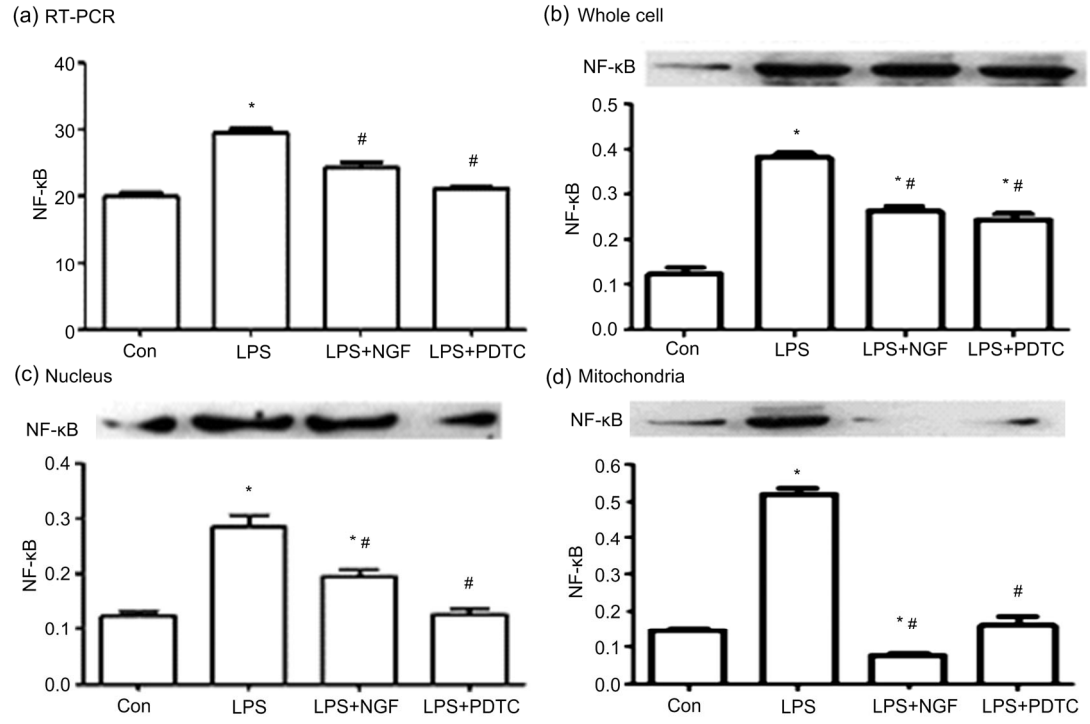

Fig. 5 Expression of NF-κB by RT-PCR and Western blotting

(a) The expression of NF-κB by RT-PCR; (b) The expression of NF-κB by Western blotting in whole cell; (c) The expression of NF-κB by Western blotting in nucleus; (d) The expression of NF-κB by Western blotting in mitochondria. Con: control. Data are expressed as mean±SD (n=3). * P<0.05, vs. the control; $^{\#}$ P<0.05, vs. the LPS injury group

PDTC groups were lower in the whole cell, nuclei and mitochondria than in the injury group. Furthermore, NF-κB (p65) levels in the nucleus were lower in the PDTC group than in the NGF group; NF-κB (p65) levels in mitochondria were slightly higher in the PDTC group than in the NGF group.

Finally, changes in NF-κB (p65) protein in the whole cell, nucleus, and mitochondria in the LPS and ATR (which is an ANT1 antagonist) groups were analyzed by Western blotting (Figs. 6b–6d). Results indicated that NF-κB (p65) levels in whole PC12 cells as well as their nuclei and mitochondria were significantly higher in the LPS group than in the normal group. Whole-cell NF-κB (p65) levels in the ATR group in whole cells were only slightly different from those in the LPS group. However, mitochondrial NF-κB (p65) level was lower than that in the LPS group, but the level in the nucleus was higher than that in the LPS group.

4 Discussion

Central nervous infection with Gram-negative bacteria is a serious disease with high mortality. LPS, which is the main component of the outer membrane of Gram-negative bacteria, stimulates the organism to produce cell cytokines, such as nitric oxide (NO) and tumor necrosis factor (TNF), resulting in severe inflammatory injury (Que et al., 2016; Xu et al., 2016). PC12 cells can differentiate into neuron-like cells after treatment with NGF. These cells can release neurotransmitters and exhibit properties similar to neurons, providing a model to study neuronal pharmacology. In line with references (Ye et al., 2013; Bayunova et al., 2015), we conducted experiments on a PC12 cell model of LPS-induced injury to study infection in the central nervous system (Mao et al., 2011). MTT results indicated that the injury was concentration-dependent. Additionally, morphological

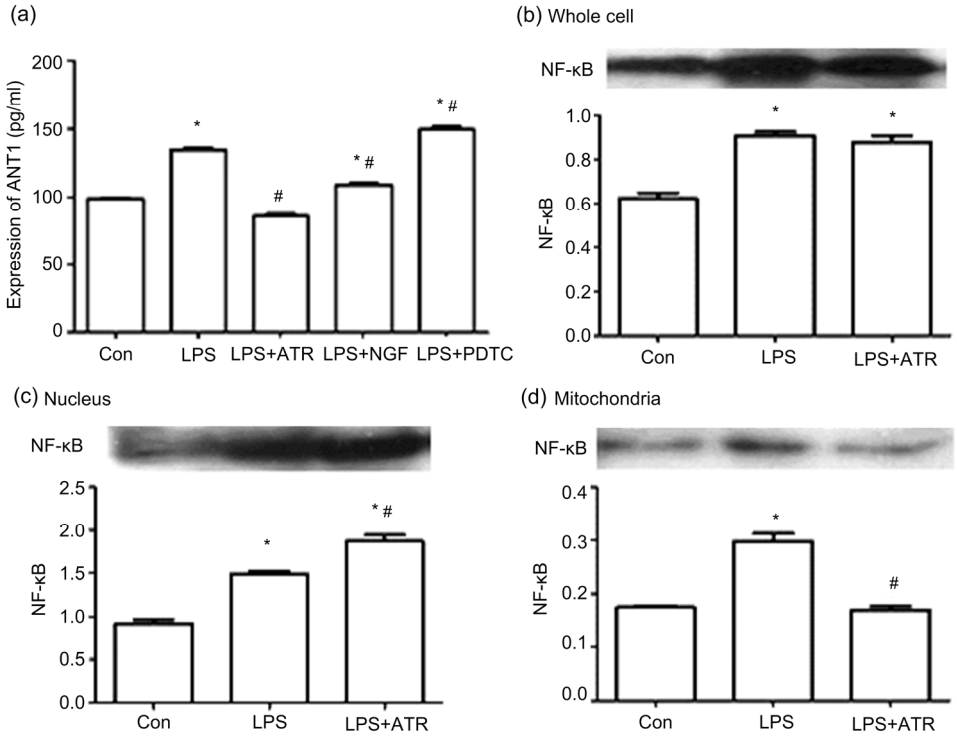

Fig. 6 Effect of ATR on ANT1 and NF-κB activation in PC12 cells

(a) The effects of ATR, NGF, and PDTC on PC12 cells by determining the activity of ANT1 by ELISA; (b) The effect of ATR on LPS-induced NF-κB (p65) activation in the whole cells by Western blotting; (c) The effect of ATR on LPS-induced NF-κB (p65) activation in the nucleus by Western blotting; (d) The effect of ATR on LPS-induced NF-κB (p65) activation in the mitochondria by Western blotting. Con: control. Data are expressed as mean±SD (n=3). * P<0.05, vs. the control; $^{\#}$ P<0.05, vs. the LPS injury group

examination of cells also confirmed that LPS induced neuronal apoptosis.

NF-κB is a central molecule for mediating immune and inflammatory responses; it is also a vital transcription regulator to induce cell apoptosis while LPS damages endometrial epithelial cells and myocardial cells (Long et al., 2017; Lyu et al., 2017). Under normal conditions, NF-κB remains inactive by combining with its inhibitor (I-κB) to form protein compounds in the cytoplasm. In response to various harmful stimuli, such as ischemia and infection, I-κB proteins phosphorylate and degrade, causing NF-κB to separate from these compounds, becoming activated and entering the nucleus to adjust the expression of inflammation genes (Xu et al., 2009; Hernandez-Esquivel et al., 2014). PDTC (Hirata et al., 2007) is a specific NF-κB inhibitor that retards the activation of NF-κB signaling in multiple ways.

We found that apoptosis occurred in LPS-damaged cells. In the PDTC group, there was minimal cell apoptosis, which indicated that inhibiting activation of NF-κB could mitigate LPS damage. RT-PCR results showed that LPS damage could result in NF-κB increases and that PDTC could decrease the relative expression of NF-κB and protect PC12 cells from LPS damage. This finding confirmed that LPS damage was generated by activation of the NF-κB signaling pathway.

With the excitation of various external and internal inflammation factors, mitochondria generate oxidative stress and initiate the mitochondrial apoptotic pathway and autophagy (Song et al., 2014, 2015). The activity of specific enzymes can thus serve as a measure of mitochondrial damage. After oxidation, fatty acids are decomposed into a series of complex compounds such as MDA. Accordingly, the level of MDA was used to express lipid oxidation and mitochondrial metabolizing function. SOD also plays a very important role in the balance between oxidation and anti-oxidation in the organism (Amigoni et al., 2013; Ghavami et al., 2014). Here, measures of SOD, GSH-PX, and CAT activity in each group showed that anti-peroxidative stress to cells in the NGF and PDTC groups was lower than that in the normal group but much higher than that in the LPS damage group. However, the activity of MDA was in contrast to that of the antioxidant. The results above suggested that PC12 cells' mitochondria were injured and released oxides in response to LPS-induced damage, and that injury was repaired by NGF or PDTC.

In response to various types of insult, the multiple conductive ion proteins situated between the external and inner membranes of mitochondria open and induce further change in the mitochondrial membrane potential ($\Delta\varnothing_m$) (Cardoso et al., 2013). Normally, the mitochondrial membrane potential results from an asymmetrical distribution of electrons on the two sides of the inner membrane; a decrease in the mitochondrial membrane potential is considered to be an irreversible event in the early stages of apoptosis. Furthermore, the mitochondrial membrane potential plays a key role in mitochondrial-dependent apoptosis, and is much more sensitive than in other types of apoptosis. Thus, the effect of NF-κB activity on the mitochondrial apoptotic pathway (Sapsrithong et al., 2012) after LPS-induced injury should be further studied through observation of conditions in each experimental group. In the current study, the mitochondrial fluorescence was measured by staining with rhodamine 123. Results indicated that fluorescence in the damage group was much lower than that in the normal group. In addition, the PDTC and NGF groups were obviously able to mitigate the collapse of the mitochondrial membrane potential.

Then, we wanted to know the reasons for the recovery effects of NGF and PDTC in a future experiment. The mitochondrial membrane plays a key role in mitochondrial-dependent apoptosis (Cheng et al., 2014). Thus, the effect of NF-κB activity on the mitochondrial apoptotic pathway after LPS-induced injury should be further studied through observation of mitochondrial conditions in each experimental group. Electron microscopy was additionally used to examine subcellular structure and mitochondrial morphology in each group. The results indicated that PC12 cell morphology was intact in the normal group, and the mitochondrial membrane was integral. However, vacuoles appeared in the mitochondria of PC12 cells in the injury group, and cell apoptosis occurred. The cells of the PDTC and NGF groups, in contrast, maintained their normal structure.

The above results suggested that mitochondrial structure was destroyed by LPS-induced injury; subsequently, the mitochondria collapsed, anti-oxidant activity was reduced, and cells ultimately underwent apoptosis. Moreover, these changes coincided with

activation of the NF-κB pathway and exchange of NF-κB between the nucleus and mitochondria. The transmission of messages from the mitochondria to the nucleus is particularly interesting.

Previous studies have reported that NF-κB exists in mitochondria and plays an important role in cell growth and apoptosis (Christensen et al., 2013). Western blot results in our study showed that NF-κB (p65) exists in the mitochondria of PC12 cells. Moreover, the expression of NF-κB in whole cells, nuclei, and mitochondria was obviously higher in the LPS group than in the normal group and distinctly lower in the NGF and PDTC groups. The subcellular distribution of NF-κB coupled with changes in mitochondrial membrane morphology and enzymes clearly suggested that apoptosis signals were transmitted between the mitochondria and nucleus by altering the balance of NF-κB between the mitochondria and nucleus. Associations between NF-κB levels in the mitochondria and nucleus require further investigation.

Adenine nucleotide translocase (ANT) (Gavaldà-Navarro et al., 2014), a type of transport protein on the inner mitochondrial membrane, is an essential protein for maintaining the MTP and regulating energy generation and consumption. Isomers of ANT play different roles in the course of apoptosis. As ANT1 activity increases, the MTP collapses, leading to mitochondrial matrix swelling and cell apoptosis (Mariappan et al., 2010). Recent research has reported that overexpression of ANT1 inducts NF-κB-IκB complexes into mitochondria. The mitochondrial apoptosis process is activated in HeLa cells in response to widespread activation of NF-κB in the mitochondria; however, studies of central nervous system infection remain few. ATR is a specific inhibitor of ANT1 that can block the ADP/ATP transfer function of ANT1 in mitochondria and maintain inactivation (Zhivotovsky et al., 2009). ELISA results demonstrated that LPS damage could induce overexpression of ANT1; ANT1 expression was decreased in the ATR and NGF groups, with slightly higher expression in the NGF group than in the ATR group. This result indicated that ANT1 and NF-κB influenced each other after LPS-induced injury within PC12 cells.

Additional Western blot results showed that the expression of NF-κB in whole cells, mitochondria, and nuclei was significantly higher in the LPS-induced injury group than in the normal group and that the whole-cell expression of NF-κB (p65) was nearly identical in the ATR and LPS groups. However, mitochondrial expression of NF-κB (p65) was significantly lower in the ATR group than in the LPS-induced injury group, approximating the expression in the normal group. The expression of NF-κB (p65) in nuclei was higher in the ATR group than in the LPS-induced injury group. This result implied that ATR (an ANT1 inhibitor) did not decrease total NF-κB levels after LPS-induced injury, but that it could block the transmission of NF-κB from the nucleus to the mitochondria by ANT1. The relatively smaller amount of activated NF-κB in the mitochondria was sufficient to maintain the mitochondrial membrane and protect PC12 cells from mitochondria-dependent apoptosis. NGF is the first neurotrophic factor to be discovered and acts as a kind of neural cell growth regulating factor: it has an important role in the regulation of central and peripheral neuronal development, differentiation, and regeneration. Results of an MTT cell viability assay and cell morphology showed that NGF significantly rescued PC12 cells from LPS damage. RT-PCR results showed that relative NF-κB expression levels in NGF group cells were significantly lower than those in the LPS group and slightly higher than those in both the normal and PDTC groups. These findings indicated that the effect of NGF was correlated with the degree of NF-κB activation, but the holding back NF-κB effect was less than that in the PDTC group. However, another interesting and important issue is why NGF had a greater recovery effect than PDTC on cellular morphology after LPS-induced injury and how NGF affected cellular vitality, morphology, and various antioxidant enzymes. This present study found that NGF not only decreased the total quantity of NF-κB in whole cells but also reduced ANT1 activity, which in turn decreased expression of NF-κB in the mitochondria and restrained mitochondrially mediated apoptosis. Our results provide the first demonstration that the mechanism of NGF effects consists of decreasing mitochondrial expression of ANT1 and NF-κB, thus preventing mitochondrial injury. These findings may deepen a novel discovery that NGF protects PC12 cells from apoptosis due to LPS-induced damage.

5 Conclusions

The current study demonstrates first and foremost that the NF-κB pathway is activated after LPS-induced injury to neuronal cells and that both PDTC and NGF can repair damage to PC12 cells, particularly to mitochondrial morphology and function. Second, using a specific inhibitor of ANT1 (ATR) and an NF-κB inhibitor (PDTC), we have demonstrated that ANT1 activity is increased in mitochondria, causing the downstream compound NF-κB to be separated in the mitochondria; these changes are the mechanism by which LPS-induced injury to neuronal cells causes cell apoptosis. Finally, NGF decreased the activity of not only NF-κB but also ANT1; this decrease, in turn, reduced NF-κB levels in the mitochondria and inhibited mitochondrially mediated apoptosis.

Compliance with ethics guidelines

Ying SONG, Zhuo-chao WU, Wei DING, Yun BEI, and Zhi-yun LIN declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要

题　目：线粒体中的 NF-κB 调节脂多糖诱导损伤后 PC12 细胞的凋亡

目　的：探讨线粒体内核转录因子（NF-κB）的表达与脂多糖（LPS）诱导损伤后神经细胞的凋亡之间的关系。

创新点：（1）LPS 诱导后，线粒体中 NF-κB 的增加导致细胞凋亡；（2）腺嘌呤核苷酸转位酶 1（ANT1）活性决定线粒体中 NF-κB 的水平。

方　法：通过 MTT 和 Hoechst 33342 染色来测定药物对 PC12 细胞的作用；用电子显微镜和罗丹明 123（rhodamine 123）检测线粒体的形态和功能；通过酶联免疫吸附测定（ELISA）测量 ANT1、脂质过氧化物和抗过氧化物酶的活性；反转录聚合酶链反应（RT-PCR）和蛋白质印迹法（Western blot）检测 NF-κB 的相对表达水平。

结　论：（1）在 LPS 诱导的神经元细胞损伤后的细胞凋亡期间，NF-κB 通过摄取 ANT1 在线粒体中被激活；（2）神经生长因子（NGF）不仅降低 NF-κB 活性，还降低 ANT1 活性，进而使得线粒体中 NF-κB 的表达水平下降，并抑制线粒体介导的细胞凋亡。

关键词：核转录因子（NF-κB）；线粒体；细胞凋亡；腺嘌呤核苷酸转位酶 1（ANT1）；脂多糖（LPS）