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BRAIN RESEARCH

Research Report

A new alternative NF-KB Pathway mediated the neuroprotection of GDNF on 6-OHDA-induced DA neurons neurotoxicity

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ABSTRACT

Glial cell line-derived neurotrophic factor (GDNF) is a potent protective factor for dopaminergic (DA) neurons, but the signaling mechanisms underlying the effect of GDNF on these neurons remain obscure. Here, both our in vivo and in vitro studies demonstrate that the majority of DA neurons express the NF-κB-inducing kinase (NIK), which is the essential kinase for mediating activation of the new alternative NF-κB signaling pathway. Additionally, we also show that GDNF induced the time/dose-dependent phosphorylation of IκB kinase α (IKKα) and p100, facilitated the processing of p100 to p52 and accelerated the translocation of NF-κB dimmers into the nuclei of DA neurons. We furtherly found that the dimmer which translocate into the nucleus was RelA/p52 not RelB/p52. Meanwhile, the attenuation of 6-OHDA-induced DA neuronal apoptosis due to GDNF was reversed subsequent to the inhibition of p100 expression by RNAi while the neuroprotective effect of GDNF on injured DA neurons was strengthened by the overexpression of p100. Our data, therefore, indicate that a new alternative NF-κB signaling pathway, which was not the classic pathway but different from the non-canonical pathway, exists in DA neurons and mediates the neuroprotective effect of GDNF on these neurons.

1. Introduction

Glial cell line-derived neurotrophic factor (GDNF) was originally identified as an important factor for the survival and differentiation of ventral mesencephalic dopaminergic (DA) neurons (Lin et al., 1993). Subsequent reports further confirmed that GDNF could protect lesioned DA neurons in the substantia nigra pars compacta (SNc) of the midbrain (Cass, 1996; Winkler et al., 1996), and that degeneration of these neurons is the major pathological characteristic in Parkinson's disease patients. Therefore, the implication of GDNF as a potential therapeutic agent for Parkinson's disease underscores the significance of the wide range of GDNF signaling (Olson, 1997). Despite the extensive research on the biological effects

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Abbreviations: GDNF, glial cell line-derived neurotrophic factor; DA, dopaminergic; SNc, substantia nigra pars compacta; NIK, NF-κB-inducing kinase; I B, inhibitor of B; IKK, I B kinase; TH, tyrosine hydroxylase

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of GDNF on DA neurons, the mechanisms underlying the roles of GDNF remain obscure.

Recently the NF-κB pathway has drawn much attention for its pivotal role in neuronal survival and differentiation. NF-κB is a group of transcriptional factors that form dimers consisting of various combinations of members of the NF-κB/Rel family, including p105 (which is constitutively processed to p50), p100 (which is processed to p52), RelA, RelB, and c-Rel (Ghosh and Karin, 2002; Karin and Ben-Neriah, 2000; Siebenlist et al., 1994). These dimers are bound in the cytoplasm by the inhibitor of κB (I B), which prevents the nuclear translocation and transcriptional activation potential of the NF- κB complex (Baldwin, 1996; Ghosh et al., 1998; Siebenlist et al., 1994). Interestingly, both p105 and p100 exhibit inhibitory functions and therefore, are also categorized as IκB (Mercurio et al., 1993; Rice et al., 1992). Nowadays, NF- κB signaling is described as two separate and distinctive activation pathways (Ghosh and Karin, 2002). The "classical" or "canonical" pathway requires I B kinase (IKK) complex, which consists of two catalytic subunits, IKKα and IKKβ, as well as a regulatory subunit, IKKγ (Ghosh and Karin, 2002). Activation of the IKK complex results in the phosphorylation and ubiquitin-dependent degradation of IκB and the nuclear translocation of p50-containing dimers. The "alternative" or "non-canonical" pathway requires the NF- B-inducing kinase (NIK), which cooperates with IKKα to induce the processing of p100 to p52, resulting in the nuclear translocation of p52-containing complexes (Senftleben et al., 2001; Xiao et al., 2001a, 2001b). Our previous study revealed that p65/p52 heterodimers translocated from the cytoplasm into the nucleus of SNc neurons with GDNF administration into the SNc of early PD rat models (Cao et al., 2008).

The aim of our present study was to further confirm whether the new alternative NF-κB signaling pathway could be activated by GDNF and whether this signaling pathway was involved in mediating the neuroprotective effect of GDNF on the SNc DA neurons. Our results showed that GDNF could activate the new alternative NF-κB signaling pathway in DA neurons, and that this pathway could mediate the neuroprotective effect of GDNF in these neurons.

2. Results

2.1. *The existence of the new alternative NF-κB pathway in the DA neurons*

To confirm whether a new alternative NF-κB pathway exists in DA neurons, immunocytochemistry was employed to detect the expression of NIK and IκB in the cytoplasm of MN9D cells. The results showed that NIK-positive and IκB-positive cells were deeply stained in the cytoplasm (Fig. 1). Furthermore, immunofluorescence assays revealed that NIK and IκB respectively co-expressed with TH in the same neurons in the SNc of the rats. (Fig. 2).

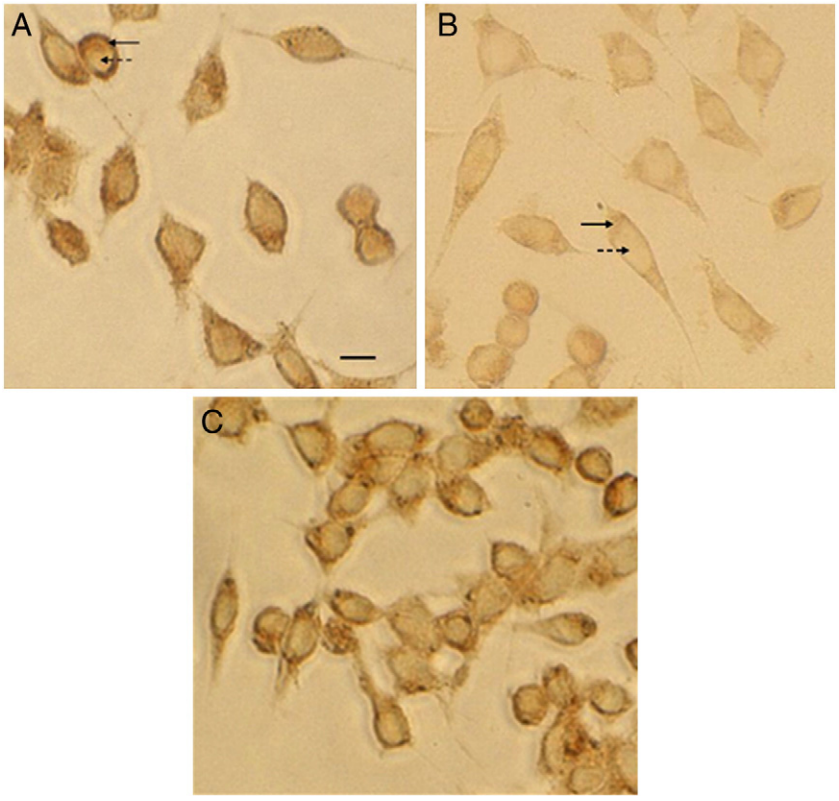

Fig. 1 – There was a non-canonical NF-κB pathway existed in the MN9D cells. (A) The image showed the MN9D cells labeled with antibody against the NIK. Notice that the cells deeply stained in cytoplasm. (B) The image showed the MN9D cells labeled with antibody against the IκB. Notice that the cells lightly stained in cytoplasm. Scale bar=10 μm. (C) The control group with secondary antibodies alone. Scale bar=10 μm. Solid arrow indicated the cytoplasm,dash arrow indicated the nucleus.