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Distinct effects of p75 in mediating actions of neurotrophins on basal forebrain oligodendrocytes

Yangzhou Du, Tanya Z. Fischer, Patricia Clinton-Luke, Lauren D. Lercher, and Cheryl F. Dreyfus*

Department of Neuroscience and Cell Biology, UMDNJ/Robert Wood Johnson Medical School, Piscataway, NJ 08854, USA

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Previous studies indicate that brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) and neurotrophin-3 (NT-3) increase myelin basic protein, (MBP) in differentiating basal forebrain (BF) oligodendrocytes (OLGs) (Du, Y., Fischer, T.Z., Lee, L.N., Lercher, L.D., Dreyfus, C. F., 2003. Regionally specific effects of BDNF on oligodendrocytes. Dev. Neurosci. 25, 116–126). While receptors, trk and p75, are expressed by subsets of oligodendrocytes (Du, Y., Fischer, T.Z., Lee, L.N., Lercher, L.D., Dreyfus, C. F., 2003. Regionally specific effects of BDNF on oligodendrocytes. Dev. Neurosci. 25, 116–126), those responsible for affecting differentiation have not been defined. In contrast, studies of peripheral Schwann cells reported that myelination is enhanced by BDNF working through p75, and diminished by trkC mediated processes (Cosgaya, J.M., Chan, J.R., Shooter, E.M., 2002. The neurotrophin receptor p75NTR as a positive modulator of myelination. Science 298, 1245–1248). To define receptors affecting central oligodendrocyte MBP, p75 knockout animals, p75 blocking antibodies, and an inhibitor of neurotrophin binding to p75, PD90780, were utilized. While p75 was implicated in the actions of NGF and NT-3, it did not affect actions of BDNF. On the other hand, K252a, an inhibitor of trk receptors, abolished the effects of the neurotrophins, including BDNF. All neurotrophins activated their respective trk receptors.

Keywords: Glia; Neurotrophins; NGF; p75; NT-3; MBP

Introduction

Oligodendrocytes, the myelinating cells of the central nervous system (CNS), arise from progenitors in the subventricular zone and undergo a well-regulated process of proliferation, migration, and differentiation (Miller, 1996). Various factors regulate these events. For instance, two of the neurotrophins, NGF and NT-3, have been shown to regulate oligodendrocyte development in vitro and in vivo. NT-3, in the presence or absence of Platelet-Derived Growth Factor (PDGF), promotes the proliferation and survival of oligodendrocytes (Barres et al., 1993; Cohen et al., 1996; Kumar et al., 1998). At low concentrations, NGF enhances oligodendrocyte survival (Cohen et al., 1996), fiber regeneration and proliferation (Althaus et al., 1992), while at high concentrations (100 ng/ml), it elicits apoptosis of mature cortical oligodendrocytes (Casaccia-Bonnefil et al., 1996). These studies are complemented by examination of knockout mice that exhibit reduced numbers of oligodendrocyte progenitors in the absence of NT-3 or trkC (Kahn et al., 1999). In addition, effects on oligodendrocyte differentiation, as defined by the elevation of myelin basic protein (MBP) in postmitotic cells, has been found to be influenced by NGF and NT-3 in oligodendrocytes derived from rat BF (Du et al., 2003).

In contrast to the effects of NGF and NT-3, effects of BDNF have been more poorly understood. Several reports have indicated that under injury conditions BDNF may affect oligodendrocytes. For example, transplantation of BDNF-expressing fibroblasts into injured spinal cord induces oligodendrocyte myelination of regenerating axons (McTigue et al., 1998), and intrathecal administration of BDNF promotes the recovery of MBP in the compressed spinal cord (Ikeda et al., 2002). In the developing animal, intraparenchymal injection of BDNF reduces effects of ibotenate in white matter of postnatal animals (Husson et al., 2005) and reduced immunoreactivity was found for MBP in the brain of BDNF−/− postnatal animals (Djalali et al., 2005). Moreover, BDNF knockout mice exhibit decreased myelination of the optic nerve (Cellerino et al., 1997).

While these in vivo studies do not indicate that BDNF acts directly on oligodendrocytes, recent studies reveal that BDNF can enhance the levels of MBP in cultured oligodendrocytes derived from rat basal forebrain (BF). BDNF also increases the numbers of MBP+ cells, without affecting total cell number (Du et al., 2003), suggesting that BDNF can promote the progression of oligodendrocyte lineage.

The receptors mediating these effects have not been clearly identified. There are two types of neurotrophin receptors, the p75 and tyrosine receptor kinase (trk) receptors. While p75 is the "common" receptor that can bind all neurotrophins with similar

* Corresponding author. Fax: +1 732 235 4990.
E-mail address: dreyfus@umdnj.edu (C.F. Dreyfus).
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affinity, NGF binds most selectively to trkA, BDNF and NT-4 to trkB, and NT-3 to trkC (Dechant et al., 1994). Oligodendrocytes express both trk and p75 receptors. For example, in culture trkA, trkC and truncated trkB are reported on some oligodendrocyte populations (Cohen et al., 1999), while full-length trkB is associated with those of the BF (Du et al., 2003). p75 is present on developing and mature oligodendrocytes in culture (Du et al., 2003) and in the normal brain (Cohen et al., 1996; Althaus et al., 1997) as well as in the demyelinating state of multiple sclerosis (Chang et al., 2000).

Work on differentiation of Schwann cells has reported that neurotrophins influence myelination in this peripheral population. Schwann cells express p75 and trkC, but only low levels of full-length trkB (Cosgaya et al., 2002). Interestingly, while NT-3 decreases myelination through the mediation of trkC, BDNF enhances myelination through the mediation of p75 (Cosgaya et al., 2002). In the current study, we examined receptors mediating the effects of NGF, BDNF and NT-3 on MBP expression of oligodendrocytes. We suggest that the effects of NGF and NT-3 involve trk as well as p75 receptors. However, p75 does not influence the effects of BDNF.

Results

The p75 receptor mediates effects of NGF and NT-3, but does not participate in the effects of BDNF on MBP expression in oligodendrocytes

Immunocytochemical and Western blot analysis have revealed that the trkA, full-length trkB, full-length trkC and the p75 receptor are present in subsets of mature oligodendrocytes (Du et al., 2003). In the PNS, neurotrophins influence differentiation of the myelinating Schwann cells. However, while NT-3 inhibits this process, BDNF enhances it. While effects of NT-3 are mediated through the trkC receptor, p75 is responsible for BDNF actions (Cosgaya et al., 2002).

To evaluate the receptors responsible for neurotrophin action in BF oligodendrocytes, we began by examining the role played by p75. We took 3 approaches and employed immunocytochemically detected myelin basic protein to evaluate effects of neurotrophins on oligodendrocytes. As indicated in Fig. 1, this method easily permits the evaluation of numbers of MBP-positive cells as well as more immature myelin basic protein-negative cells in cultures grown under varying conditions.

Initial studies examined effects of reducing p75 by using p75 knockout mice. Other work decreased p75 actions by blocking binding of neurotrophins to this receptor. Dissociated cell cultures of P1 mouse basal forebrain were treated with concentrations of neurotrophins found to be optimal in dose response studies published previously (Du et al., 2005; NGF, 1 ng/ml, BDNF, 10 ng/ml or NT-3, 1 ng/ml). MBP+ cell numbers were examined after 5 days. The response of cells from $p75^{+/+}$ and $p75^{-/-}$ littermates was compared. All three neurotrophins increased MBP+ cell number in the presence of p75. However, effects of NGF and NT-3 were partially reduced in the $p75^{-/-}$ cultures. On the other hand, effects of BDNF were not affected by the loss of the p75 receptor (Fig. 2).

The mouse cultures are a mixture of glial cells and contain astrocytes and microglia, in addition to cells of the oligodendrocyte lineage. To further evaluate the role of p75 in a more enriched

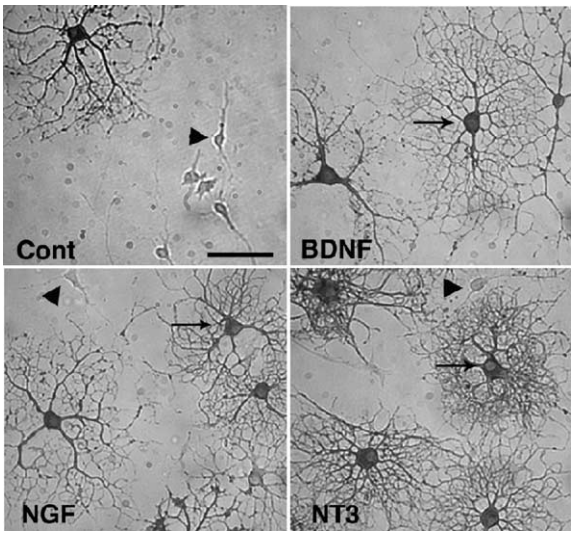

Fig. 1. Myelin basic protein can be clearly detected in subpopulations of cultured oligodendrocytes using immunocytochemical techniques. Both MBP-positive (arrows) and MBP-negative (arrowheads) cells are apparent when grown in SFM and treated with NGF (1 ng/ml), BDNF (10 ng/ml), or NT-3 (1 ng/ml). Marker = 100 μm.

population, we grew cultures of oligodendrocyte lineage cells derived from P1 rat BF. Postmitotic cells, 3 days in serum-free medium were grown for 2 additional days in the presence or absence of optimal concentrations of NGF, BDNF and NT-3. Effects on numbers of MBP+ cells were monitored. Anti-p75 blocking antibody was utilized to inhibit the p75 receptor. This p75 antibody partially reduced elevations in MBP+ cell numbers elicited by NGF and NT-3, but had no effect on increases elicited by BDNF (Fig. 3).

To confirm this observation, effects of PD90780 were evaluated. This agent binds to a p75 binding site on the neurotrophins and inhibits their ability to bind to p75 (Spiegel et al., 1995; Mount et al., 1998). PD90780 inhibited effects of NGF and NT-3 on MBP+ cells, but did not influence actions of BDNF (Fig. 4). These studies indicate that while p75 contributes to increases in MBP+ cells in response to NGF and NT-3, it does not affect response to BDNF.

K252a blocks the effects of neurotrophins on MBP expression of oligodendrocytes

To examine the influence of trk receptors, K252a (100 nM,) the trk tyrosine kinase inhibitor, was applied at concentrations shown by others to inhibit trk receptors (Knusel and Hefti, 1992). Cultured postmitotic oligodendrocytes derived from P1 rat BF were treated with neurotrophins, as above, for 2 days. K252a was added 1 h before neurotrophin treatment. K252a totally abolished the effects of NGF, BDNF, and NT-3 to increase MBP-expressing cell numbers (Figs. 5A, B), suggesting that trk receptors are responsible for neurotrophin actions on MBP expression of oligodendrocytes.

Others have reported that another growth factor, PDGF, can increase levels of MBP mRNA in postmitotic oligodendrocytes (Grinspan et al., 1993). To determine that K252a was acting specifically on the neurotrophin receptors, parallel studies evaluated effects of PDGF in the presence of K252a (Figs. 5C, D). While effects of NGF, BDNF and NT-3 were abolished by K252a,