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Stimulated neuronal expression of brain-derived neurotrophic factor by Neurotropin

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ABSTRACT

Expression of brain-derived neurotrophic factor (BDNF) was stimulated in human neuroblastoma SH-SY5Y cells by a nonprotein extract of inflamed rabbit skin inoculated with vaccinia virus (Neurotropin®), an analgesic widely used in Japan for treatment of disorders associated with chronic pain, with the optimal dosage at 10 mNU/mL. This stimulation was accompanied by activations of p42/44 MAP kinase, CREB and c-Fos expression. Inhibitors of MAP kinases or PI 3-kinase prevented the stimulatory action of Neurotropin, indicating that neuronal TrkB/CREB pathway mediates the action. Repetitive oral administration of Neurotropin (200 NU/kg/day, 3 months) prevented the age-dependent decline in hippocampal BDNF expression in Ts65Dn mice, a model of Down's syndrome. This effect was associated with the improvement of spatial cognition of the mice. These results open an intriguing new strategy in which Neurotropin may prove beneficial treatment for neurodegenerative disorders.

Introduction

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family and plays important roles in many developmentally regulated processes, such as cell survival, differentiation and synaptic plasticity of neurons as well as neurogenesis. Clinical and basic evidence supports the idea that abnormalities in brain neuronal regeneration assisted by BDNF are associated with a wide range of disorders such as neurodegenerative diseases (*e.g.* Alzheimer's disease, Parkinson's disease, Down's syndrome) and psychiatric or stress-related conditions (*e.g.* bipolar disorder, schizophrenia, post-traumatic stress disorder) (reviewed by Kozisek et al., 2008; Pezet and Malcangio, 2004).

Additionally, there is growing evidence using animal models to suggest roles for BDNF in chronic pain, which seem to be location dependent. In the dorsal root ganglion, spinal dorsal horn (reviewed by Pezet et al., 2002; Malcangio and Lessmann, 2003) and in supra-spinal neurons (Guo et al., 2006), an elevated expression of BDNF is believed to be crucial for establishing chronic pain (central sensitization). Meanwhile, BDNF has been reported to be reduced in higher centers such as the hippocampus and cortex (Duric and McCarson, 2005; 2007). Thus, the contribution of BDNF in chronic pain is still unclear.

Neurotropin is a nonprotein extract of inflamed rabbit skin inoculated with vaccinia virus, which has been reported to be effective on chronic pain conditions such as low back pain (Ono et al., 1982), neck–shoulder–arm syndrome (Ono et al., 1987), post-herpetic neuralgia (Yamamura et al., 1988), complex regional pain syndrome (Muneshige and Toda, 1996) and fibromyalgia (Nagaoka et al., 2004). Moreover, clinical effectiveness of Neurotropin has been reported for a wide variety of neuropathic symptoms associated with subacute myelo-opticoneuropathy (SMON) (Sobue et al., 1992), diabetic neuropathy (Orimo et al., 1989), infarction-associated ischemic brain damage (de Reuck et al., 1994) and Alzheimer's disease (Kimura et al., 1987). At least, the analgesic action of Neurotropin has been shown in rodent experiments to be mediated through activation of the descending monoaminergic pain regulatory systems in stress-related chronic pain conditions induced by the "specific alternation of rhythm in environmental temperature" (SART)

Abbreviations: BDNF, brain-derived neurotrophic factor; cAMP, cyclic AMP; CREB, cAMP responsive element binding protein; DMEM, Dulbecco's modified Eagle's medium; EIA, enzyme immunoassay; ELISA, enzyme-linked immuno-solvent assay; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HRP, horseradish peroxidase; MAP kinase, mitogen-activated protein kinase; MEK, MAP kinase kinase (MAPK/ERK kinase); NGF, nerve growth factor; NT-3, neurotrophin-3; PI 3-kinase, phosphatidylinositol 3-kinase; PVDF, polyvinylidene fluoride; RT-PCR, reverse transcription-polymerase chain reaction; Trk, tropomyosin-related kinase; WRAM, water radial-arm maze.

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(Kawamura et al., 1998; Ohara et al., 1991), peripheral inflammation (Miura et al., 2005), spinal nerve ligation (Suzuki et al., 2005), or chronic constriction injury (Toda et al., 1998; Saleh et al., 1998). However, precise molecular mechanisms underlying these various pharmacological actions of Neurotropin remain to be elucidated.

In the present study, we demonstrate that Neurotropin stimulates the expression of BDNF in the human neuroblastoma cell line, SH-SY5Y, and mouse hippocampus *in vivo*. Neurotropin clearly augmented the TrkB-dependent intracellular signaling pathways associated with activations of PI 3-kinase, MAP kinases and CREB, leading to increased BDNF expression. Furthermore, Neurotropin enhanced the expression of BDNF in the hippocampus of the Ts65Dn mice, a model of Down's syndrome, associated with age-dependant decline in hippocampal neurotrophin expressions (Hunter et al., 2004), along with functional recovery of cognition.

Results

Expression of BDNF in SH-SY5Y cells

Human neuroblastoma cell line SH-SY5Y expresses BDNF and its high-affinity receptor, TrkB (Kaplan et al., 1993). Incubation of the cells with Neurotropin resulted in enhanced expression of BDNF in a time-dependent fashion (Fig. 1A). The strength of BDNF induction was comparable to that by retinoic acid (1 μM) at 24 h. A similar enhancement was observed in expressions of other neurotrophins such as NGF and NT-3, but not for high-affinity neurotrophin receptors TrkA and TrkB (Fig. 1B). The effect on BDNF expression was dose-dependently observed with an optimal concentration at 10 mNU/mL (Fig. 1C). The effect was reduced at higher concentrations (50 and 250 mNU/mL), suggesting a bi-modal effect of Neurotropin on BDNF expression. No morphological change was observed in each Neurotropin-treated culture within 48 h (data not shown). Trypan blue extrusion assay indicates that there was no significant loss in cell viability even at the highest concentration of Neurotropin (250 mNU/mL).

We failed to detect BDNF in culture supernatants since the amount of secreted free BDNF was lower than the detection limit (3.9 pg/mL) of our ELISA method (data not shown). This is thought to be due to immediate sequestration of free BDNF by TrkB as previously reported (Balkowiec and Katz, 2000). Alternatively, we measured intracellular BDNF levels. Consistent with the elevated mRNA expression, intracellular expression of mature BDNF was enhanced by Neurotropin at concentrations greater than 10 mNU/mL (Fig. 1D).

Mechanism for BDNF upregulation

BDNF expression is known to be upregulated through phosphorylation of the cyclic AMP responsive element binding protein (CREB).

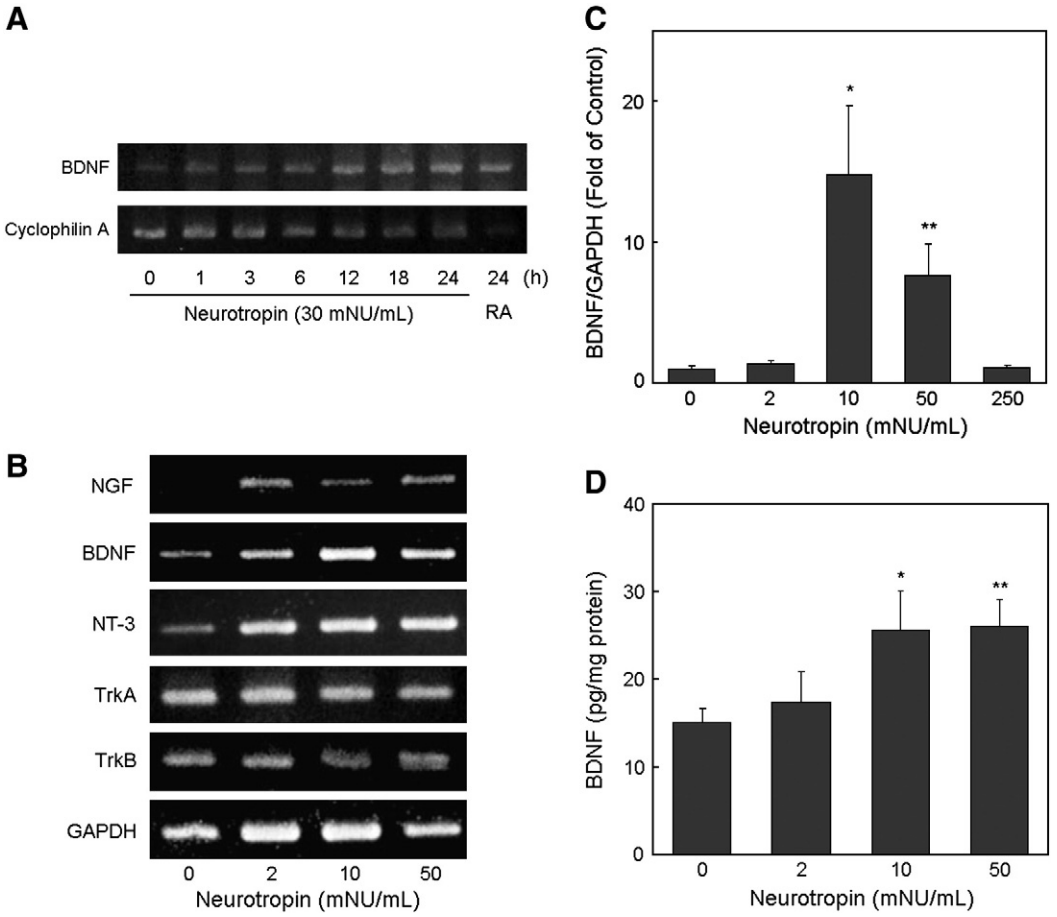

Fig. 1. Neurotropin upregulated BDNF expression in SH-SY5Y cells. A. Time dependency of BDNF expression. Cells (5×10^5 cells) were treated with Neurotropin (30 mNU/mL) or retinoic acid (RA, 1 μM) for the indicated period of time (0, 1, 3, 6, 12, 18, and 24 h) in serum-free DMEM. BDNF expression was measured by semi-quantitative RT-PCR as described in Experimental methods. B, C. Dose dependency of neurotrophin expressions by Neurotropin. Cells (approx. 5×10^5 cells) were incubated for 24 h in serum-free DMEM at indicated concentrations of Neurotropin (0, 2, 10, 50 and 250 mNU/mL). Then the extracted total RNA was subjected to semi-quantitative (B) and quantitative (C) RT-PCR. For quantitative assay, the data represent averaged ratio of copy numbers of BDNF to GAPDH transcripts of eight experiments expressed as fold increase over control treatment (= 1.0), with the standard errors (SEs) indicated by error bars. The indicated conditions are statistically different from the saline-treated controls (*P<0.05; **P<0.01). D. Intracellular BDNF expression. Cells (1×10^6 cells) were incubated with indicated concentrations of Neurotropin (0, 2, 10, 50 mNU/mL) for 24 h. Cells were lysed and the resultant extract was subjected to ELISA assay and protein quantitation as described in Experimental methods. Results are expressed as means and standard deviations (SDs) of triplicate measurements. The indicated conditions are statistically different from the untreated control (*P<0.05; **P<0.01).