Regulation of BDNF release in dopaminergic neurons

전홍성

Department of Biological Science, Chosun University

375 Seosuk-Dong, Gwangju 501-759.

Tel. (062)230-6609, FAX (062)230-6619

Abstract

The major pathological lesion in Parkinson's disease(PD) is selective degeneration and loss of pigmented dopaminergic neurons in substantia nigra (SN). Although the initial cause and subsequent molecular signaling mechanisms leading to the dopaminergic cell death underlying the PD process is elusive, the potent neurotrophic factors (NTFs), brain derived neurotrophic factor (BDNF) and glial cell line derived neurotrophic factor (GDNF), are known to exert dopaminergic neuroprotection both *in vivo* and *in vitro* models of PD employing the neurotoxin, MPTP. BDNF and its receptor, trkB are expressed in SN dopaminergic neurons and their innervation target. Thus, neurotrophins may have autocrine, paracrine and retrograde transport effects on the SN dopaminergic neurons. This study determined the BDNF secretion from SN dopaminergic neurons by ELISA. Regulation of BDNF synthesis/release and changes in signaling pathways are monitored in the presence of free radical donor, NO donor and mitochondrial inhibitors. Also, this study shows that BDNF is able to promote survival and phenotypic differentiation of SN dopaminergic neurons in culture and protect them against MPTP-induced neurotoxicity via MAP kinase pathway.

Introduction

The coexpression of BDNF and trkB in SN dopaminergic neurons and other neurons of CNS suggested that BDNF acts in a autocrine and/or paracrine mode. BDNF has been known to exert both neurotrophic and neuroprotective effects on dopaminergic neurons of SN. It increased the survival of dopaminergic neurons in fetal mesencephaliccultures, enhanced high affinity dopamine uptake, tyrosine hydroxylase(TH) activity, dopamine contents, neurite outgrowth and protected against the neurotoxic effects of MPP^+, the active metabolite of MPTP.[1,2] Several *in vivo* studies, delivering BDNF by direct infusion or transplantation of genetically modified cells, confirmed some functional roles of BDNF, such as, the activation of the dopaminergic system,

amelioration of Parkisonian symptoms in the 6-OHDA lesion model and increased neuronal survival against MPP^+ lesions.[3,4] However, the definitive molecular mechanisms for neuroprotective role of BDNF have yet to be firmly established in primary mesencephalic cultures and animal models of Parkinson's disease. Addressing molecular mechanisms of BDNF action in both primary embryonic mesencephalic cultures and *in vivo* animal models has been technically difficult because dopaminergic neurons in SN are relatively rare and present with many heterogeneous cell populations in midbrain. Thus, we have developed and characterized a dopaminergic neuronal cell line of embryonic SN origin that is more accessible to molecular analysis and studied as an *in vitro* model system for the regulation of both production and autocrine action of BDNF in dopaminergic neurons.[5] Our dopaminergic cell line, SN4741 expressed both BDNF and trkB. In the SN4741 cells, a low level of trkB activation was constitutively observed at over 60-70% confluence, induced by the secreted BDNF. Pharmacological treatment of mitochondrial inhibitors and oxidative stress supressed BDNF synthesis and release in the dopaminergic cells. In SN4741 cells, BDNF protects against MPP^+-induced neurotoxicity by activating, through the MAP kinase pathway, genes coding for proteins that protect against neuronal death.

Materials and Methods

The dopaminergic cell line SN4741 was cultured at 33℃ with 5% CO_2 in RF medium.[5] To evaluate the effect of BDNF release, conditioned media were collected after 24h treatment of SN4741 with several chemicals and stored at -80℃ until assayed by ELISA. In a single experiment, each treatment was performed in quadruplicate. BDNF amount was quantified using BDNF E_{max} Immunoassay system(Promega, Madison, WI) and the automatic ELISA plate reader.

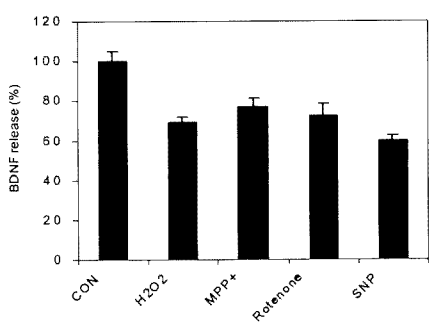

Figure 1. Regulation of BDNF release in the dopaminergic cells by oxidative stressors, and mitochondrial inhibitors. The response of dopaminergic cells to various chemicals for 24h was quantified by BDNF ELISA assay. All chemicals tested had inhibition effect on BDNF release by 30-40%.

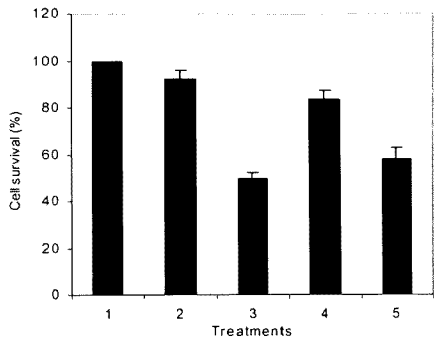

Figure 2. The neuroprotective effect of BDNF and its inhibition by MEK-specific inhibitor, PD98059. 1) no treatment, 2)+PD98059(25μM), 3)+MPP^+, 4)+MPP^+ and BDNF (50ng/ml), 5) +MPP^+, BDNF and PD98059. Data are the mean ±SEM of three independent experiments performed in triplicate.

Results and Discussion

The quantitative amount of BDNF produced in the dopaminergic neurons of SN region (B6CBA/J6 female mice, 12 weeks old) were measured by ELISA and compared with the BDNF release in 24hr by dopaminergic cell line, SN4741 (4×10^4 cells). Basal BDNF release from SN tissue sample was about 130pg/mg protein and the BDNF release from SN4741 cells for the first 24h culture was about 230pg/mg protein. To investigate the intracellular mechanisms that may regulate BDNF expression and release, several chemicals were used to stimulate or inhibit specific intracellular signaling pathways. Figure 1 shows that free radical donor (H_2O_2), mitochondrial inhibitor(MPP^+, rotenone)[6] and NO donor (SNP)[7] inhibit the BDNF synthesis/release by 30-40%. Figure 2 reveals that the MAP kinase kinase (MEK)-specific inhibitor, PD98059, significantly abolished the neuroprotective effect exerted by BDNF. When SN4741cultures were treated with BDNF (50ng/ml) for 1 day before and after exposure to MPP^+, the loss of the SN4741 cells was significantly reduced to less than 23%. To test whether the activation of the MAP kinase(p44/42) is involved in the neuroprotective role of BDNF, we measured the SN4741 cell death in the presence of PD98059 during neuroprotection by BDNF against MPP^+-induced neurotoxicity. The presence of PD98059 in the SN4741 cultures did not affect the survival of the cells, excluding toxic or nonspecific effect of the MEK inhibitor on the SN4741 cells. Of great interest, in the SN dopaminergic cell line SN4741, the presence of PD98059 abolished the neuroprotective effect of BDNF. The activation of the MAP kinase pathway appears to be an essential step in suppression of free radical formation and in blocking cell death.[8] Moreover, in other neuronal cultures BDNF was shown to activate the MAP kinase

pathway through trkB.[9,10] These data provide support for the hypothesized role of the activation of MAP kinase pathway during the neuroprotection exerted by BDNF against MPP^+-induced neurotoxicity.

References

1. Knusel, B., Winslow, J.W., Rosenthal, A., Burton, L.E., Seid, D.P., Nikolics, K. and Hefti, F., "Promotion of central cholinergic and dopaminergic neurons differentiation by brain-derived neurotrophic factor but not neurotrophin-3." (1991), Proc. Natl. Acad. Sci. USA, 88, 961-965.
2. Hyman, C., Juhasz, M., Jackson, C., Wright, P., IP, N.Y. and Landsay, R.M., "Overlapping and distinct actions of neurotrophins BDNF, NT-3 and NT4/5 on cultured dopaminergic and GABAergic neurons of the ventral mesencephalon." (1994), J. Neurosci. 14, 335-347.
3. Altar, C.A., Boylan, C.B., Fritsche, M., Jones, B., Jackson, C., Wiegand, S.J., Lindsay, R.M. and Hyman, C., "Efficacy of brain-derived neurotrophic factor and neurotrophin-3 on neurochemical and behavioral deficits associated with partial nigrostriatal dopamine lesions." (1994), J. Neurochem., 63, 1021-1032.
4. Frim, D.M., Uhler, T.A., Galpern, W.R., Beal, M.F., Breakefield, X.O. and Isacson, O., " Implanted fibroblasts genetically engineered to produce BDNF prevent MPP^+ toxicity to dopaminergic neurons." (1994), Proc. Natl. Acad. Sci. USA, 91, 5104-5108.
5. Son, J., Chun, H., Joh, T., Cho, S., Conti, B., Lee, J., "Neuroprotection and neuronal differentiation studies using substantia nigra dopaminergic cells derived from transgenic mouse embryos." (1999), J. Neurosci., 19, 10-20.
6. Lander, H.M., "An essential role for free radicals and derived species in signal transduction." (1997), FASEB J., 11, 118-124.
7. Przedborski, S., Jackson-Lewis, V., Yokoyama, R., Shibata, T., Dawson, V. and Dawson, T.M., "Role of neuronal nitric oxide in MPTP-induced dopaminergic neurotoxicity." (1996), Proc. Natl. Acad. Sci. USA, 93, 4565-4571.
8. Dugan, L.L., Creedon, D.J., Hohnson, E.M. and Holtzman, D.M., "Rapid suppression of free radical formation by NGF involves the MAP kinase pathway." (1997), Proc. Natl. Acad. Sci. USA, 94, 4086-4091.
9. Kishino, A. and Nakayama, C., "Enhancement of BDNF and activated-ERK immuno-reactivity in spinal motor neurons after peripheral administration of BDNF." (2003), Brain Res., 964, 56-66.
10. Purcell, A., Sharma, S., Bagnall, M., Sutton, M. and Carew, T., "Activation of a Tyrosine Kinase-MAPK Cascade Enhances the Induction of Long-Term Synaptic Facilitation and Long-Term Memory in Aplysia." (2003), Neuron, 37, 473-484.