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The effect of dopamine agonists: The expression of GDNF, NGF, and BDNF in cultured mouse astrocytes

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ABSTRACT

In Parkinson's disease, cell death is selectively induced in mesencephalic nigral dopaminergic neurons. At present, no disease modifying therapy or radical treatment has been found for this disease. Some dopamine agonists may have a neuroprotective action in cultured cells and animal models. In the present study, we examined stimulating effects of a non-ergoline D_2 dopamine agonist, ropinirole, on synthesis/secretion of neurotrophic factors, including NGF, BDNF, and GDNF, in cultured mouse astrocytes. These effects were compared with those of ergoline dopamine agonists, SKF-38393, a D_1 agonist, bromocriptine, D_2 agonist, and apomorphine, D_1/D_2 agonist. Ropinirole elevated GDNF levels to 4-fold, and NGF levels to 6.3-fold, compared with the control group. Of the dopamine agonists examined, ropinirole produced and secreted more GDNF than a 1.8-fold greater amount of apomorphine, a lesser amount of bromocriptine, or a 2.8-fold greater amount of SKF-38393, which served as the control group.

1. Introduction

Neurotrophic factor is an endogenous substance that promotes neuronal survival, differentiation, and function. Many neurotrophic factors are identified, including the neurotrophin family represented by nerve growth factor (NGF), the ciliary neurotrophic factor (CNTF) family, the fibroblast growth factor (FGF) family, the insulin-like growth factor (IGF) family, and the glial cell line-derived neurotrophic factor (GDNF). The action of these neurotrophic factors on the central nervous system is relevant to various neurodegenerative diseases. For example, BDNF and IGF-1, CNTF, neurotrophin-3 (NT-3) in amyotrophic lateral sclerosis, GDNF and BDNF in Parkinson's disease, NGF in Alzheimer's disease are well known [1,2].

Parkinson's disease is characterized by progressive death of dopaminergic neurons in the substantia nigra. GDNF markedly affects motor neurons and selectively protects the nigrostriatal dopaminergic pathway [3]. The protective effect of GDNF on motor neurons is several times greater than those of other currently known neurotrophic factors. In rats and monkeys lesioned by a neurotoxin, GDNF protects dopaminergic neurons of the ventral mesencephalon from cell death [3–5]. GDNF also acts directly on these dopaminergic neurons and promotes their function [3–5]. In an experiment using Parkinson's disease model monkeys (rhesus monkeys),induced by a neurotoxin,1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), GDNF administration for four weeks improved dyskinesia, rigidity, and postural instability significantly, and the efficacy was maintained for another four weeks [4]. This result strongly supports the possibility that GDNF can act as a curative drug for Parkinson's disease [4,6]. GDNF also helps protect neuronal lesions caused by 6-hydroxydopamine (6-OHDA) in the nigrostriatal dopaminergic pathway [7,8]. Based on a hypothesis that various neurotrophic factors including NGF, BDNF, GDNF, NT-3, CTNF as well as nuclear factor kappa B (NF-κB) strengthen neuronal cells and protect the cells from vulnerability, these neurotrophic factors have been used to delay the progression of neurodegeneration [1,2,6,9].

Symptoms of Parkinson's disease derive from neuronal depletion, and the symptoms are markedly relieved by administering a dopamine agonist to the brain. With regard to this symptom-relief therapy, particularly non-ergoline dopamine agonists are currently the first choice. If a symptomatic therapy also acts as neuronal cell protection therapy, a long-term therapy would be more promising. Recently, attention has focused on the application of dopamine agonists as neuromodulatory agents by partially stimulating neurotrophic factors in the brain. In this regard, we already reported that the stimulation of dopamine D_1 receptors may be required for GDNF

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synthesis and secretion, and concurrent stimulation of both dopamine D_1 and D_2 receptors would augment this effect further [10,11]. Höglinger and his colleagues reported experimental results that dopamine depletion in rodents decreases precursor cell proliferation in both the subependymal zone and the subgranular zone and that activation of D_2 receptors by ropinirole directly increases the proliferation of these precursors [12]. There is an open debate whether ropinirole might slow down the progression of Parkinson's disease.

In this study, we examined the ability of a non-ergoline dopamine agonist, ropinirole to produce neurotrophic factors, and compared the impact produced by ergoline dopamine agonists.

2. Methods

Whole brains of 8-day-old ICR mice were excised and cut into small pieces for culturing [11]. Cells were seeded on culture dishes containing serum DMEM with 10% FCS, at a density of 2×10^4 cells/cm^2, and cultured until they became confluent for several times. The passaged cells were exposed to FCS free DMEM for one week and then induced into a period of quiescence. Cultured mouse astrocytes were then exposed to four medicines (SKF-38393, apomorphine, bromocriptine, and ropinirole) at various concentrations for 24 h and neurotrophic factor levels in the culture medium were measured by enzyme-linked immunosorbent assay (ELISA). Each drug (10 mg/mL solution containing DMSO) produced a bell-shaped dose-response curve. The concentrations eliciting maximal stimulation of NGF and GDNF synthesis were used in subsequent studies of the time course of NGF, GDNF, and BDNF secretion. Maximally induced secretion of NGF and GDNF from astrocyte cultures was SKF-38393 at 309 μM, bromocriptine at 266 μM, apomorphine at 88 μM, and ropinirole at 360 μM. Levels of mRNA for each factor were measured by reverse transcriptase-polymerase chain reaction (RT-PCR). The passaged culture was a morphologically almost uniform glial cell population, with 97.5% of the cells positively stained for glial fibrillary acidic protein. SKF-38393, apomorphine and bromocriptine were purchased (Wako Pure Chemical Industries, Ltd. Osaka, Japan). Ropinirole was gifted by Glaxo SmithKline Co.(Tokyo, Japan).

2.1. ELISA for NGF, GDNF, and BDNF

Our two-site ELISA system for NGF, GDNF, and BDNF was employed [11]. The bound biotinylated antibody was quantified with streptavidin-linked horseradish peroxidase (HRP). The culture medium was directly applied to the wells for ELISA. Measurement sensitivities were 2.5, 5, and 5 pg/mL for NGF, GDNF and BDNF respectively.

2.2. RT-PCR

Semiquantitative RT-PCR analysis was performed to measure the levels of mRNA expression against GAPDH [10]. Total RNA was extracted from astrocytes with TRIzol reagent (Gibco BRL, Life Technologies, Inc.) then treated with DNase I (Gibco BRL) to avoid the possible contamination of genomic DNA. First-strand cDNA was synthesized from RNA by using random hexamers and AMV reverse transcriptase (Promega, Madison, WI). Aliquots of cDNA corresponding to 80 ng of RNA for NGF, GDNF and BDNF, and 40 ng for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were amplified in 25 μl of PCR cocktail containing each specific primer (200 nM) and Taq DNA polymerase (TaKaRa, Kyoto, Japan). The sets of primers for NGF, GDNF, BDNF and GAPDH were identical to those described previously [10,11,13–15]. A linear correlation between intensity and cycle number was found in 30–34 cycles for NGF and BDNF, 32–35 cycles for GDNF, and 23–25 cycles for GAPDH. The intensity of the bands of NGF and GDNF relative to the intensity of untreated control was standardized with respect to the intensity of the GAPDH band.

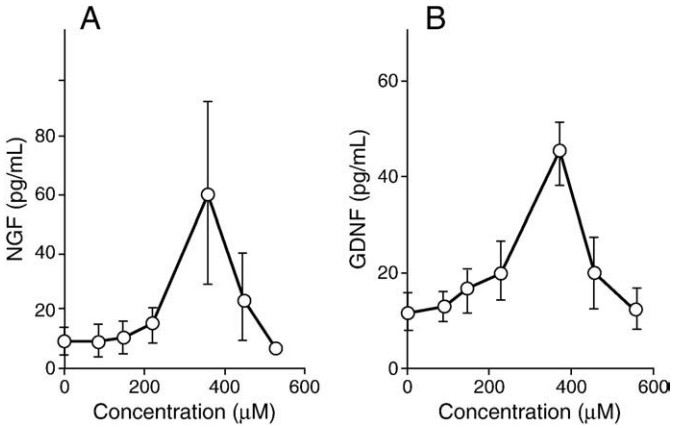

Fig. 1. Effects of various concentrations of ropinirole on stimulation of NGF, GDNF, and BDNF secretion. Results are means ± S.D. Cultured astroglial cells were exposed to various concentrations of ropinirole for 24 h and NGF, GDNF, and BDNF levels in the culture medium were measured by ELISA.

2.3. Statistics

Data are expressed as means ± S.D. Differences were analyzed by Student's *t*-test and 2-way ANOVA. *P*-values < 0.05 were considered significant.

3. Results

Various concentrations of medicines were added to cultured astroglial cells, and cultured for 24 h. The secreted amounts of NGF, GDNF and BDNF in the culture medium showed bell-type dose-response curves, indicating that ropinirole at 360 μM induced the maximal secretion of neurotrophic factors in the cultured supernatant. The following experiments were then performed (Fig. 1).

At 24 h, the NGF secretion level elevated to 6.3-fold, compared with the control group. The GDNF secretion level had increased 3-fold at 8 h, and 4-fold at 24 h. BDNF secretion was not markedly enhanced (data not shown)(Fig. 2).

Immediately after ropinirole was added to cultured astroglial cells, NGF and GDNF mRNA began to be expressed. They reached their maximum secretion levels at 6 h in NGF and 3 h in GDNF. On the other hand, BDNF mRNA was not significantly enhanced (Fig. 3).

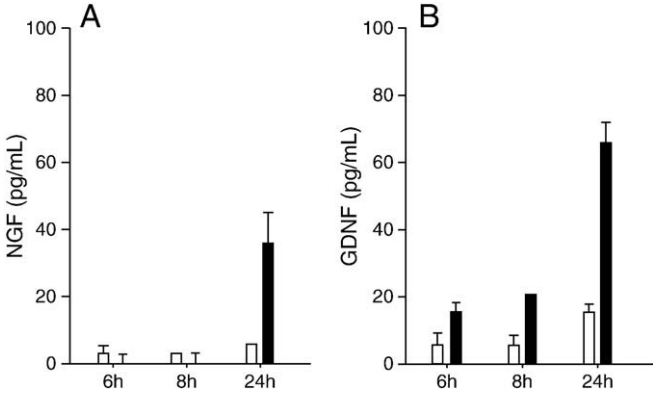

Fig. 2. Induction of neurotrophic factor secretion (NGF, GDNF, and BDNF) by ropinirole at 360 μM. Levels of NGF, GDNF, and BDNF in the culture medium were measured by ELISA. Results are means ± S.D. Closed columns, ropinirole treated; open columns, untreated controls. Student's *t*-test was used to determine significant differences from controls ($^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.005$, $^{****}P<0.001$).