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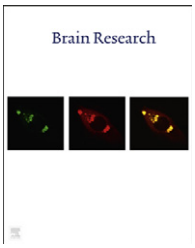

Research Report

Neuroprotective cyclopentenone prostaglandins up-regulate neurotrophic factors in C6 glioma cells

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ABSTRACT

In a previous study, we developed newly synthesized arylthio derivatives of cyclopentenone prostaglandins (GIF-0642, GIF-0643, GIF-0644, GIF-0745 and GIF-0747), which are neuroprotective against both manganese toxicity in PC12 cells and glutamate toxicity in HT22 cells. In the present study, we showed that these compounds and their lead compound, NEPP11, are potent inducers of glial cell line-derived neurotrophic factor (GDNF) expression in C6 glioma cells and primary astrocytes. These neuroprotective cyclopentenone prostaglandins also induced the gene expression of nerve growth factor and, to a lesser extent, brain-derived neurotrophic factor. The induction of GDNF mRNA was transcription-dependent, and the overexpression of dominant-negative Nrf2 attenuated the ability of the (arylthio)cyclopentenone prostaglandins to stimulate GDNF gene expression. These results suggest that (arylthio)cyclopentenone prostaglandins increase GDNF gene expression partly via the Keap1/Nrf2 pathway. A growing number of reports demonstrate the importance of increasing the amounts of neurotrophic factors, especially GDNF, in neuropathological states. Although the precise mechanisms by which the GIF compounds inhibit cell death are under investigation, an increase in neurotrophic factors may contribute to the diverse pharmacological properties of (arylthio)cyclopentenone prostaglandins *in vivo* and will make them potentially valuable in the treatment of neurodegenerative disorders.

Abbreviations: BDNF, brain-derived neurotrphic factor; GDNF, glial cell line-derived neurotrophic factor; NEPPs, neurite outgrowth-promoting prostaglandins; NGF, nerve growth factor; PAGE, polyacrylamide gel electrophoresis; PPARγ, peroxisome proliferator-activated receptor-γ; SDS, sodium dodecyl sulfate

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1. Introduction

Parkinson's disease is a progressive neurodegenerative disorder characterized by tremor, bradykinesia, akinesia, and rigidity due to the selective loss of dopaminergic neurons in the nigrostriatal pathway. Current medical and surgical therapies offer symptomatic relief but do not provide a cure. Neurotrophic factors promote the survival, differentiation and maintenance of neurons in the developing and adult nervous systems, making them potential therapeutic candidates for Parkinson's disease (Peterson and Nutt, 2008). Currently, the most potent neurotrophic factor for dopaminergic neurons is glial cell line-derived neurotrophic factor (GDNF) (Lin et al., 1993). Experimental studies suggest that GDNF has the ability to protect, both *in vitro* and *in vivo*, degenerating dopaminergic neurons in neurotoxin (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and 6-hydroxydopamine)-induced animal models of Parkinson's disease (Lapchak et al., 1997). If toxin-induced cell death in dopaminergic neurons shares any mechanisms with the pathogenesis of Parkinson's disease, then the actions of trophic factors could slow or stop the neurodegeneration of the dopaminergic neurons in Parkinson's disease. Another therapeutic benefit of trophic agents is the restoration of function in neurons that are losing their dopaminergic phenotype, including the capacity to synthesize and release dopamine (Xiao et al., 2002). However, clinical trials of GDNF-infusion remain inconclusive (Gill et al., 2003; Lang et al., 2006). A major problem in clinical use of neurotrophic factors involves the methods of delivery of the potential therapeutic agents to dopaminergic nerve terminals and cell bodies, as trophic factors generally do not readily penetrate the blood brain barrier. In this context, increasing the brain levels of neurotrophic factors by small molecules is feasible.

Cyclopentenone prostaglandins, the A and J series of prostaglandins, are naturally occurring eicosanoids that exhibit unique biological activities in a variety of cultured cell types. Unlike typical prostaglandins, cyclopentenone prostaglandins permeate cells and exhibit anti-neoplastic, anti-viral and anti-inflammatory properties (Rossi et al., 2000; Straus and Glass, 2001). Furthermore, some cyclopentenone prostaglandins have been found to exert dual effects on cell survival and apoptosis (Musiek et al., 2005). 15d-PGJ_2, a natural ligand for peroxisome proliferator-activated receptor gamma (PPARγ), protects cultured cortical neurons, mouse hippocampal HT22 cells and rat pheochromocytoma PC12 cells from oxidative stress (Aoun et al., 2003; Forman et al., 1995; Lim et al., 2004; Saito et al., 2007), however, it also induces apoptosis in SH-SY5Y and PC12 cells at relatively high concentrations (Hirata et al., 2004; Kondo et al., 2002). Neurite outgrowth-promoting prostaglandins, referred as to NEPPs, are synthetic cyclopentenone prostaglandins originally designed on the basis of the chemical structure of Δ^{12}-prostaglandin J_2. NEPPs not only promote the neurite outgrowth from PC12 cells and dorsal root ganglia explants in the presence of nerve growth factor (NGF) but also prevent the glutamate-induced death of HT22 cells (Satoh et al., 2000, 2001). NEPP11 (12-iso-15-deoxy-13,14-dihydro-Δ^7-PGA_1 methyl ester) is the most potent NEPP compound *in vitro*, and can also protect the brain *in vivo*, as observed in a focal ischemia model of mice with permanent middle cerebral artery occlusion (Satoh et al., 2001). Multiple mechanisms, including inhibition of the NF-κB pathway and transcriptional activation of the PPARγ pathway, have been proposed to explain the diversity in the actions of cyclopentenone prostaglandins (Forman et al., 1995; Rossi et al., 1997, 2000; Straus et al., 2000). In addition, it has been reported that the modification of the Keap1 by 15d-PGJ_2 and NEPP11 activates Nrf2-dependent transcription of heme oxygenase-1 (HO-1) and γ-glutamyl cysteine lygase (γ-GCL), which act as cellular antioxidants (Levonen et al., 2004; Satoh et al., 2006). We previously reported that newly synthesized (arylthio)cyclopentenone prostaglandins, arylthio derivatives of NEPPs, suppress manganese-induced apoptosis in PC12 cells by inhibiting caspase activation and cytochrome *c* release from mitochondria and inhibit glutamate-induced cell death in HT22 cells through a PPARγ-dependent pathway (Shibata et al., 2009a,b). In this study, we examined the effects of these (arylthio)cyclopentenone compounds on the expression of GDNF of particular relevance to Parkinson's disease. We also examined the expression of other neurotrophic factors that promote neuronal survival, differentiation and synaptic function including NGF and brain-derived neurotrphic factor (BDNF), the neurotrophin family, neurturin (NTN), a member of the GDNF family, and mesencephalic astrocytes-derived neurotrophic factor (MANF), an invertebrate neurotrophic factor supporting dopaminergic neurons (Petrova et al., 2003).

2. Results

2.1. *Effect of (arylthio)cyclopentenone prostaglandins on the gene expression of various neurotrophic factors in C6 cells*

RT-PCR analysis was used to assess the effects of the (arylthio)-cyclopentenone derivatives (GIF compounds) GIF-0642, 0643, 0644, 0745 and 0747 in C6 cells, a rat astrocytic tumor cell line, on the expression of various neurotrophic factors, including GDNF and NTN (the GDNF family), NGF and BDNF (the neurotrophin family) and MANF. The expression of GDNF and NGF mRNA was strongly induced in cells treated with 10 μM or 20 μM GIF compounds for 3 h, whereas the expression of MANF and NTN mRNA was unchanged (Fig. 1A). The expression of BDNF mRNA was moderately increased. The results indicate that the (arylthio)cyclopentenone prostaglandins are

Fig. 1 – (Arylthio)cyclopentenone prostaglandins induce the expression of GDNF, NGF and BDNF mRNA. (A) Effects of the (arylthio)cyclopentenone derivatives on the mRNA expression of various neurotrophic factors in C6 glioma cells. The cells were treated with GIF-0642 at 10 μM, or GIF-0643, GIF-0644, GIF-0745, and GIF-0747 at 20 μM for 3 h. (B) Induction of GDNF mRNA expression by GIF-0642 in C6 glioma cells. The cells were treated with 5, 10 μM, or 20 μM GIF-0642 for 3 h or with 5 μM GIF-0642 for various periods. (C) Induction of GDNF mRNA expression by GIF-0643 in cultured rat astrocytes. Astrocytes were exposed to GIF-0643 for 3 h. Total RNA was isolated using Trizol reagent, and the mRNA levels of the neurotrophic factors were analyzed by semi-quantitative RT-PCR, as described in Section 4. The data are expressed as the mean ± SD (n=3–4). $*p<0.05$; $**p<0.01$.