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Type I interferons up-regulate the expression and signalling of p75 NTR/TrkA receptor complex in differentiated human SH-SY5Y neuroblastoma cells

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ABSTRACT

Both type I interferons (IFNs) and neurotrophins regulate neuroadaptive responses, but relatively little is known on the interaction between these two classes of regulatory proteins. Here we investigated the effect of IFN-β on the expression and functional activity of the common neurotrophin receptor p75NTR and the nerve growth factor (NGF) receptor TrkA. In differentiated human SH-SY5Y neuroblastoma cells prolonged exposure to IFN-β up-regulated p75NTR and TrkA levels, failed to affect the content of sortilin, a p75NTR co-receptor, and, consistent with our previous finding, down-regulated the brain-derived neurotrophic factor receptor TrkB. Quantitative real time RT-PCR indicated that IFN-β increased p75NTR and TrkA mRNA levels. In control and IFN-β treated cells proNGF failed to induce c-Jun N-terminal kinase and nuclear factor/kB activation, two p75NTR/sortilin signalling pathways mediating neuronal death. On the other hand, IFN-β treatment enhanced TrkA autophosphorylation and signalling induced by NGF and proNGF. Knockdown of p75NTR by siRNA reduced TrkA activation by proNGF and a subnanomolar concentration of NGF, whereas co-immunoprecipitation indicated close association of p75NTR and TrkA. Co-treatment with either NGF or proNGF reduced IFN-β pro-apoptotic and anti-neurotrophic effects. Similarly, in primary mouse hippocampal neurons IFN-β increased p75NTR and TrkA expression, down-regulated TrkB and enhanced NGF-induced phosphorylation of the pro-survival protein kinase Akt. The data demonstrate that in neuronal cells IFN-β differentially affects the expression and signalling of neurotrophin receptors and suggest that the up-regulation of the p75NTR/TrkA signalling complex may constitute a novel mechanism by which this cytokine selectively attenuates its pro-apoptotic effect in NGF-responsive cells.

1. Introduction

The type I interferons (IFNs) IFN-α and IFN-β are cytokines with pleiotropic actions widely used in the clinic for the treatment of several diseases, including chronic viral hepatitis, different types of malignancies and relapsing-remitting multiple sclerosis (MS) (Petska, 2007). Although IFN actions are mainly exerted on peripheral immune cells, these cytokines have also been shown to affect the central nervous system (CNS). Thus, IFN-β displays immunomodulatory effects on brain cells, regulating cytokine production, chemokine and cell adhesion molecules expression and growth factor release (Dhib-Jalbut and Marks, 2010). These effects are considered to contribute to IFN-β anti-inflammatory action in the CNS (Axtell and Steinman, 2008). Type I IFNs have also been shown to affect neurotransmission mediated by monoamines, glutamate and opioids and to alter the activity of the hypothalamus–pituitary–adrenocortical axis (Dafny and Yang, 2005).

Abbreviations: BDNF, brain-derived neurotrophic factor; CNS, central nervous system; DAPI, 4′,6-Diamidino-2phenylindole dihydrochloride; EAE, experimental allergic encephalomyelitis; ERK1/2, extracellular signal-regulated protein kinases 1 and 2; FCS, fetal calf serum; GAP-43, growth associated protein-43; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IFN, interferon; JNK, c-Jun N-terminal kinase; NGF, nerve growth factor; MAPK, mitogen-activated protein kinase; MS, multiple sclerosis; NF160/200, neurofilament of 160 and 200 kDa; NGS, normal goat serum; p75NTR, p75 NT receptor; PARP, poly (ADP-ribose) polymerase; PBS, phosphate buffered saline; PLC, phospholipase C; PMSF, phenylmethylsulphonyl fluoride; PI3K, phosphoinositide 3-kinase; RA, all-*trans*-retinoic acid; SDS, sodium dodecyl sulphate; siRNA, small interfering RNA; STAT, signal transducer and activator of transcription; TNF, tumor necrosis factor.

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Moreover, several clinical studies have reported that the therapeutic use of either IFN-α or IFN-β is often associated with the appearance of a variety of neuropsychiatric side effects, including anxiety, confusion, mania, psychosis, cognitive deficits, depression and suicidal behaviour (Raison et al., 2005). These observations indicate that, in addition to the favourable immunomodulatory actions, type I IFNs can alter neurotransmission and negatively affect brain functions, particularly following chronic treatments.

Neurotrophins, comprising nerve growth factor (NGF), brain-derived growth factor (BDNF), NT3 and NT4, are a family of structurally related polypeptides that regulate the generation, survival and a variety of other cellular processes in neuronal and non-neuronal populations (Huang and Reichardt, 2001). Neurotrophins are produced as pre-pro-proteins, which are then cleaved to the mature forms, and act on two structurally different receptors: the p75NTR receptor, which belongs to the tumor necrosis factor (TNF) receptor superfamily, and the Trk tyrosine kinase receptors (Huang and Reichardt, 2003). All neurotrophins are capable of binding with equal affinity to p75NTR, whereas NGF displays higher selectivity for TrkA, BDNF and NT4 for TrkB and NT3 for TrkC. Neurotrophin activation of Trks is commonly associated with neuronal survival, neurite outgrowth and synaptic plasticity (Huang and Reichardt, 2003). On the other hand, p75NTR can be activated by neurotrophins and proneurotrophins either individually or by forming a complex with other receptor molecules. Several studies have demonstrated that p75NTR acts as a co-receptor for the Trks and augments their signalling by enhancing the affinity and specificity for the cognate neurotrophin (Huang and Reichardt, 2003). Conversely, in Trk-deficient cells activation of p75NTR has been found to induce cell death through apoptosis (Nykjaer et al., 2005). Of particular importance for the pro-apoptotic action of p75NTR is its ability to form a receptor complex with sortilin, a member of the Vps 10p-domain receptor family (Nykjaer et al., 2004). The resulting p75NTR/sortilin heterodimer has been demonstrated to mediate the pro-apoptotic effects of the pro-neurotrophins proNGF and proBDNF (Nykjaer et al., 2004; Nykjaer and Willnow, 2012). There is also evidence that p75NTR can form receptor complexes with membrane proteins or membrane-associated proteins other than Trks and sortilin, including NogoR, Lingo-1, Neurophilin and Ephrin-A, to modulate axonal growth and guidance (Nykjaer et al., 2005; Teng et al., 2010).

Although both type I IFNs and neurotrophins have been shown to modulate neuroadaptive responses, relatively few studies have investigated the interaction between these different classes of regulatory molecules. In cultured mouse astrocytes, Awatsuji et al. (1995) found that either IFN-β or IFN-γ suppressed the synthesis of NGF, whereas Boutros et al. (1997) reported that IFN-β behaved as a potent promoter of NGF production. An increased secretion of NGF by human brain microvascular endothelial cells was observed following exposure to lymphocytes treated with IFN-β or obtained from MS patients treated with IFN-β (Biernacki et al., 2005). We have recently observed that in differentiated SH-SY5Y neuroblastoma cells, a cell line commonly used as a model of human neuronal cells (Xie et al., 2010), prolonged exposure to IFN-β inhibits the expression and signalling of TrkB, the tyrosine kinase receptor of BDNF, and impairs BDNF neurotrophic activity (Dedoni et al., 2012), a major promoter of neuronal survival and synaptic plasticity and a critical regulator of affective and cognitive functions (Hu and Russek, 2008). However, it is not known whether type I IFNs may also alter the expression and/or function of other receptor components of the neurotrophin system.

In the present study, we used neuronally differentiated SH-SY5Y cells and primary mouse hippocampal neurons to investigate whether prolonged exposure to IFN-β affected the expression and function of p75NTR and the co-receptors TrkA and sortilin.

2. Materials and methods

2.1. Materials

Recombinant human IFN-β1b (32×10^6 UI/mg protein) and IFN-α2a (270×10^6 UI/mg protein) were obtained from Schering (Milano, Italy) and Roche (Milano, Italy), respectively. Recombinant mouse IFN-β was obtained from ProSpec-Tany TechnoGene Ltd (Rehovot, Israel). Cleavage-resistant mutated form of proNGF (K103A and R104A) was obtained from Alomone Labs (Jerusalem, Israel). Recombinant human β-NGF and tumor necrosis factor (TNF)-α was obtained from ImmunoTools (Friesoythe, Germany). K252a was from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All-*trans*-retinoic acid (RA), tyrphostin AG879 (AG879) and the other reagents were from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Cell culture

SH-SY5Y cells were obtained from the European Cell Culture Collection (Salisbury, UK) and grown in Ham's F12/MEM medium (1:1) supplemented with 2 mM L-glutamine, 1% non-essential amino acids, 10% fetal calf serum (FCS) and 100 U/ml penicillin-100 μg/ml streptomycin (Invitrogen/Life Technologies, Monza, Italy) at 37 °C in a humidified atmosphere of 5% CO_2 in air. Cells were differentiated by exposure for 6–8 days to RA dissolved in dimethyl sulfoxide. The final RA concentration (10 μM) was obtained by dilution of stock solution in the cell culture medium. The medium was renewed every 48 h and during the RA treatment care was taken to avoid cell culture exposure to light.

2.3. Primary cultures of neurons from mouse hippocampus

CD-1 mice were obtained from Harlan (S. Pietro al Natisone, Udine, Italy). One day old mice of both sexes were used to prepare primary cultures of hippocampal neurons by using trypsin digestion as previously described (Olianas et al., 2008). Briefly, the brains were collected in ice-cold Neurobasal A medium and, with the aid of a dissection microscope, the hippocampus was isolated from a coronal brain slice obtained by making a transverse cut through the median eminence and a second cut through the midbrain. The tissue was triturated and dissociated by incubation in Neurobasal A medium containing 0.05% trypsin for 30 min at 30 °C and treatment with 50 μg/ml of Dnase I (Sigma) for 5 min. Following aspiration through fire-polished Pasteur pipettes, cells were collected by centrifugation and resuspended in Neurobasal A medium containing B27 serum-free supplement, 0.5 mM L-glutamine, 50 μM β-mercaptoethanol and penicillin-streptomycin (Invitrogen). Cells were plated on six-well plates pre-coated with 0.01% poly-L-lysine (Sigma) at a density of $0.8–1.0 \times 10^6$ cells/well. The medium was removed 4–6 h later. Cultures were used 8–10 days after plating and contained 95% neuronal cells as assessed by immunofluorescence staining of neurofilament 160/200 (NF160/200) and glial fibrillary acidic protein. Seven separate culture preparations were used. Experiments were performed according to the principles of laboratory animal care (Law on animal experiments in Italy, D.L. 116/92) and were approved by the institutional ethical committee.

2.4. Biotinylation of surface proteins

Surface biotinylation of cell proteins was performed as previously described (Olianas et al., 2011), with some modifications. RA-differentiated SH-SY5Y cells were grown in 100 mm plates and treated at confluency with either vehicle or IFN-β (30 ng/ml) for 24 h in medium containing 1% FCS. Thereafter the cells were washed three times with ice-cold phosphate buffered saline (PBS) (pH 8.0) and incubated for 45 min at 4 °C without or with the cell impermeable biotinylating agent sulfosuccinimidyl-6-(biotin-amido)hexanoate (sulpho-NHS-LC-biotin) (0.50 mg/ml) (Pierce, Rockford, IL, USA). Thereafter, the medium was aspirated and the cells washed three times with ice-cold PBS containing 20 mM glycine. Cells were then solubilized by incubation for 60 min at 4 °C in a lysis buffer containing PBS, 0.1% SDS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 2 mM EDTA, 2 mM EGTA, 4 mM sodium pyrophosphate, 2 mM sodium orthovanadate, 10 mM sodium fluoride, 20 nM okadaic acid, 0.5% phosphatase inhibitor cocktail 3, 1% protease inhibitor cocktail and 1 mM phenylmethylsulphonyl fluoride (PMSF) (RIPA buffer) supplemented with 1% Triton X 100. Cell extracts were centrifuged at $14,000 \times g$ for 10 min at 4 °C and the supernatants incubated overnight with streptavidin-conjugated agarose beads with continuous rotation at 4 °C. The samples were then centrifuged to obtain a supernatant and a pellet fraction containing the plasma membrane-associated proteins. The agarose beads were washed three times with ice-cold Tris buffer containing 50 mM Tris–HCl (pH 7.5), 2.5 mM EDTA, 150 mM NaCl and 1% Triton X 100, followed by two washes with 50 mM Tris–HCl (pH 7.5), 2.5 mM EDTA, 500 mM NaCl and 0.1% Triton X 100 and one final wash with 50 mM Tris–HCl (pH 7.5). The pellet was then mixed with sample buffer and incubated 2 min at 100 °C. The proteins were then analysed by Western blot.

2.5. Plasma membrane isolation

RA-differentiated SH-SY5Y cells grown in 100 mm plates were treated at confluency with either vehicle or IFN-β (30 ng/ml) for 24 h in 1% FCS. Thereafter, the cells were washed three times with ice-cold PBS and then scraped in 0.25 M sucrose in Tris/EDTA buffer containing 10 mM Tris–HCl (pH 7.4), 1 mM EDTA, 4 mM sodium