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Interferon-alpha inhibits glucocorticoid receptor-mediated gene transcription via STAT5 activation in mouse HT22 cells

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ABSTRACT

Interferon (IFN)-alpha is an innate immune cytokine that induces significant depressive symptoms in clinical populations. A number of mechanisms have been considered regarding the relationship between IFN-alpha and depression, including the effects of IFN-alpha on the hypothalamic–pituitary–adrenal (HPA) axis. Here, we examined the impact of mouse interferon (mIFN)-alpha and its signaling pathways on the functioning of the glucocorticoid receptor (GR), which plays a key role in HPA axis regulation. mIFN-alpha treatment (100–1000 IU/ml) of HT22 mouse hippocampal cells for 24 h was found to significantly inhibit dexamethasone (DEX)-induced GR-mediated MMTV-luciferase activity and significantly decrease DEX-induced GR-binding to its DNA response element. Of note, mIFN-alpha treatment for 24 h had no effect on DEX-induced GR translocation or GR protein expression. Inhibition of DEX-induced GR function by mIFN-alpha was significantly reversed by pharmacological inhibition of janus kinase/signal transducer and activator of transcription (Jak-STAT) signaling pathways, but not by inhibition of p38 mitogen-activated protein kinase. Moreover, pretreatment of cells with siRNA targeted to STAT5, but not STAT1 or STAT2, significantly attenuated IFN-alpha inhibition of DEX-induced MMTV-luciferase activity. Immunoprecipitation experiments revealed nuclear co-immunoprecipitation of activated STAT5 and GR following IFN-alpha plus DEX treatment. Taken together, these results indicate that negative regulation of GR function by IFN-alpha in hippocampal HT22 cells is mediated by activation of Jak/STAT signaling pathways leading to nuclear STAT5-GR protein–protein interactions. Given the role of GR in depressive disorders, IFN-alpha effects on GR function in cells of hippocampal origin may contribute to HPA axis alterations and depressive symptoms in IFN-alpha-treated patients.

1. Introduction

Interferon (IFN)-alpha is a cytokine of the innate immune response that has both antiviral and antiproliferative activities (Abbas and Lichtman, 2003). Accordingly, IFN-alpha has become a mainstay in the treatment of certain cancers such as malignant melanoma and viral infections including hepatitis C (Heathcote, 2007). Despite its therapeutic efficacy, IFN-alpha is well-known to induce the development of depressive symptoms in 20–50% of patients, depending on the dose (Raison et al., 2005).

A number of mechanisms have been considered regarding the relationship between IFN-alpha and depression including the effects of IFN-alpha on the hypothalamic–pituitary–adrenal (HPA) axis. For example, one study found that patients who developed major depression during IFN-alpha therapy were more likely to exhibit exaggerated HPA axis responses to the first IFN-alpha injection (Capuron et al., 2003). Given the role of corticotropin releasing hormone (CRH) in the regulation of HPA axis outflow, these results suggest that increased sensitivity of CRH pathways to IFN-alpha may represent a vulnerability factor for the development of IFN-alpha-induced depression. Of note, hypersecretion of CRH is believed to play a central role in the development of depression, and IFN-alpha has been shown to stimulate CRH expression in the hypothalamus as well as the amygdala of laboratory animals (Raber et al., 1997). IFN-alpha has also been shown to lead to flattening of the diurnal cortisol slope and increased evening cortisol concentrations, both of which were correlated with increased IFN-alpha-induced depressive symptoms (Raison et al., 2008).

CRH hypersecretion in major depression and flattening of the cortisol slope is believed to be related in part to impaired negative feedback regulation of the HPA axis by glucocorticoids, an effect that may be mediated by decreased glucocorticoid receptor (GR) function (Nemeroff, 1996; Raison and Miller, 2003). Indeed, depressed patients have been shown to exhibit decreased GR function both in vivo and in vitro as manifested by failure of the synthetic glucocorticoid dexamethasone (DEX) to suppress cortisol secretion during the DEX suppression test (DST) and DEX–CRH test (Holsboer, 2000; Ising et al., 2005) and reduced sensitivity of peripheral blood mononuclear cells to the in vitro inhibitory effects of DEX on mitogen-in-

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duced lymphocyte proliferation (Pariante, 2004). In addition, flattening of the cortisol slope has been associated with non-suppression of cortisol during the DST in patients with metastatic breast cancer (Spiegel et al., 2006). Of relevance to IFN-alpha, a recent report demonstrated that IFN-alpha treatment of several cell lines for 72 h was associated with decreased GR mRNA and protein expression (Cai et al., 2005). In addition, plasma levels of IFN-alpha have been associated with decreased GR binding affinity and decreased sensitivity to cortisol in peripheral blood monocytes of patients with acquired immunodeficiency syndrome (AIDS), although no direct effect of IFN-alpha on GR function was observed in this study (Norbiato et al., 1996). Finally, IFN-alpha is known to stimulate p38 mitogen-activated protein kinase (MAPK) and signal transducer and activator of transcription (STAT) pathways (Platanias, 2005), and both p38 MAPK and STAT induction have been shown to inhibit GR function (Biola et al., 2001; Stocklin et al., 1996; Wang et al., 2004). Taken together, these data suggest that one mechanism by which IFN-alpha may contribute to depression is through disruption of GR function and its role in the regulation of HPA axis responses.

The current study was designed to further explore the effects of IFN-alpha on GR function as well as the signaling pathways involved. For these studies, mouse HT22 cells, a hippocampus-derived cell line, and mouse IFN-alpha (mIFN-alpha) were used. Hippocampal GR has been shown to be essential for maintenance of normal HPA axis function and have been implicated in the underlying HPA axis pathophysiology of major depression (De Kloet et al., 1998). Moreover, administration of mIFN-alpha to mice has been shown to activate IFN-alpha signaling molecules in multiple brain regions including neurons in the hippocampus (Wang et al., 2008).

2. Materials and methods

2.1. Cells and reagents

Mouse hippocampal HT22 cells, kindly provided by Dr. Y. Sagara (University of California, San Diego, CA), were grown at 37 °C and 5% CO_2 in DMEM supplemented with 10% heat-inactivated (56 °C, 30 min) fetal bovine serum (Hyclone, Logan, UT), 50 U/ml penicillin, and 50 mg/ml streptomycin. GR-green fluorescent protein (GFP) chimera was a gift from Dr. Carmine Pariante, Institute of Psychiatry, London, UK. Pharmacologic reagents included recombinant type-I mouse IFN-alpha (mIFN-alpha) (PBL Biomedical Laboratories, Piscataway, NJ), DEX (Sigma–Aldrich, St. Louis, MO), Janus kinase (Jak) inhibitor I (Calbiochem, San Diego, CA) and SB203580 (Calbiochem, San Diego, CA).

2.2. Transfection

HT22 cells were either transiently or stably transfected with the mouse mammary tumor virus (MMTV)-luciferase reporter gene construct containing multiple glucocorticoid receptor binding sites [(glucocorticoid response elements (GREs)] upstream of the promoter region. Transfections were accomplished using Lipofectamine 2000 (Invitrogen Life Technologies, Carlsbad, CA). Stably transfected HT22 cells were produced by treatment for 2 weeks with G418 and cloned by limiting dilution. Clones were then screened for DEX-induced MMTV-luciferase activity. Transient transfections with the MMTV-luciferase reporter or GR-GFP were performed in serum-free medium. Stripped fetal bovine serum (FBS) was added to wells 5 h after transfection.

2.3. Luciferase assay

HT22 cells were seeded into 12-well plates and grown for 20–24 h until 70–80% confluent. After drug treatments, HT22 cells were washed once with cold 1× phosphate buffered saline (PBS), and lysed using a passive lysis buffer (see below). Cells were then centrifuged at 10,000 rpm for 15 s at room temperature (RT) to remove cellular debris. Luciferase activity was measured using a microplate luminometer (Luminoscan Ascent, Thermo Labsystems, Helsinki, Finland) and luciferase substrate (Promega, Madison, WI). All activity values reported are the means of treatments from three independent experiments represented as fold change relative to the vehicle control.

2.4. Nuclear, cytosolic, and whole cell extracts

Cell monolayers were rinsed with 1× PBS and then harvested in a nuclear homogenization buffer (NHB) containing 20 mM Tris (pH 7.4), 10 mM NaCl, 3 mM $MgCl_2$, 5 μM dithiothreitol, 1 μM phenylmethylsulfonyl fluoride, 1 μM pepstatin, 50 trypsin-inhibitory mU of aprotinin, 10 μM leupeptin, and 2 mM sodium vanadate. Igepal CA-630 (Nonidet P-40) was added to a final concentration of 0.15%, and cells were homogenized with 16 strokes in a Dounce homogenizer. The homogenates were centrifuged at 3500 rpm for 5 min. Supernatants were saved as cytosolic extract, and the nuclear pellets were resuspended in 0.5 volumes of NHB and were centrifuged as before. The pellet of intact nuclei was resuspended again in one-half of the original volume of NHB and centrifuged again. The majority of the pellet (intact nuclei) was resuspended in an extraction buffer containing 20 mM HEPES (pH 7.9), 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM ethylenediaminetetraacetic acid (EDTA), 5 μM dithiothreitol, 1 μM phenylmethylsulfonyl fluoride, 1 μM pepstatin, 50 trypsin-inhibitory mU of aprotinin, 10 μM leupeptin, 2 mM sodium vanadate, and 25% glycerol or immunoprecipitation (IP) buffer containing 10 mM Tris (pH 7.4), 150 mM NaCl, 1 mM ethylene glycol tetraacetic acid, 1 mM EDTA, 1% Triton X-100, 1 μM phenylmethylsulfonyl fluoride, 1 μM pepstatin, 50 trypsin inhibitory mU of aprotinin, 10 μM leupeptin, and 2 mM sodium vanadate. Nuclei were extracted for 30 min on ice. The samples were then subjected to centrifugation at 10,000 rpm at 4 °C for 10 min. These supernatants contained nuclear protein. To generate whole cell extracts, cells were harvested in 1× PBS and lysed in radioimmunoprecipitation assay (RIPA) buffer [50 mM Tris–HCl (pH 7.4), 1 mM EDTA, 150 mM NaCl, and 1% NP-40 containing protease inhibitors (1 mM PMSF, 1 μg/ml aprotinin, 1 μg/ml pepstatin, and 1 μg/ml leupeptin]. After 30 min incubation on ice, cell lysates were centrifuged at 13,000*g* for 10 min at 4 °C, and the supernatant was collected. For normalization of sample loading for relevant assays, protein concentrations were determined using a commercial bicinchoninic acid (BCA) assay (Pierce, Rockford, IL) with bovine serum albumin as the protein standard.

2.5. Western blot analysis

Fifty micrograms cytosolic protein or whole cell protein were mixed with sodium dodecyl sulfate (SDS) buffer and subjected to SDS–polyacrylamide gel electrophoresis (PAGE). For Western blot analysis of nuclear extracts, 25 μg nuclear protein was mixed with SDS buffer before SDS–PAGE. Separated proteins were then electrophoretically transferred onto a nitrocellulose membrane. The membrane was blocked for 1 h in a 5% milk/tris-buffer saline Tween 20 solution, and then incubated overnight in the presence of the primary antibody (1:1000 dilution) raised against GR (M-20; Santa Cruz Biotechnology, Santa Cruz, CA) or phospho (p-)STAT5a/b (Tyr694; Cell Signaling, Danvers, MA). The washed membrane was subsequently incubated with the secondary antibody (1:2000 dilution) for 1 h. The membrane was washed again and visualized using a commercially available chemoluminescence kit from Amersham Biosciences Corp. (Piscataway, NJ).