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Activation of the GDNF-inducible transcription factor (GIF) gene promoter by glucocorticoid and progesterone

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ABSTRACT

Steroid hormones, especially glucocorticoids, exert physiologic effects on dopaminergic neurotransmission and have been implicated in several dopamine-mediated neuropsychiatric conditions. D_2 dopamine receptor gene expression is regulated by the zinc finger-type nuclear protein GDNF-inducible transcription factor (GIF). In this study, we sought to investigate if steroids could regulate transcription of the GIF gene itself. Transient co-transfection of the D_2 expressing neuroblastoma cell line NB41A3 with GIF promoter-luciferase constructs along with expression vectors for steroid hormone receptors showed that activation of glucocorticoid receptors but not estrogen receptors up-regulates transcription from the GIF promoter 5.0-fold. Progesterone receptors, which share the same consensus DNA recognition sequence as glucocorticoid receptors, also activated the GIF promoter. Serial 5′-deletion mutants of the GIF gene upstream region localized the glucocorticoid-responsive segment between nucleotides −128 and −66 relative to the transcription start site. This region contains a putative glucocorticoid-responsive element/progesterone-responsive element (GRE/PRE). Additionally, this fragment of the GIF gene 5′-upstream region activated the heterologous herpes simplex virus thymidine kinase (TK) promoter, which is known to be glucocorticoid and progesterone responsive. Furthermore, glucocorticoid receptor activation up-regulated endogenous GIF gene mRNA expression in NB41A3 cells. These observations demonstrate a molecular basis for glucocorticoid and progesterone-induced up-regulation of GIF gene transcription and provide a mechanism for the modulation of dopamine-mediated behaviors by these hormones.

1. Introduction

Steroid hormone receptors are a class of cell-specific trans-acting transcription regulatory factors whose activity is controlled by binding to their cognate hormone. The hormone–receptor complex translocates from the cytoplasm to the nucleus and associates with promoter/enhancer elements of specific target genes resulting in activation of transcription [1,2]. These hormone-responsive elements (HREs) have an imperfect palindromic structure and can be classified into two main subgroups: the glucocorticoid-responsive element/progesterone-responsive element (GRE/PRE)-like group that mediates induction by glucocorticoids, progestins, androgens, and mineralocorticoids, and the estrogen-responsive element (ERE)-like group that mediates induction by estrogens, thyroid hormones, vitamin D_3, and retinoic acid [3].

Glucocorticoids represent an important class of homeostatic modulators and therapeutic agents [4]. Therefore, understanding their effects on a wide range of target genes has broad implications in basic biology and the treatment of disease. Glucocorticoid receptor complexes interact with GREs often located in the 5′-flanking regions of hormone-inducible genes such as mouse mammary tumor virus (MMTV) and are generally variations of the sequence AGA/GACANNNTGTT/CCT [1,5–7].

In the central nervous system, glucocorticoid receptors are present in large numbers of nerve and glial cell populations [8]. Several experimental paradigms have shown that glucocorticoids

Abbreviations: CAT, chloramphenicol acetyltransferase; ERE, estrogen-responsive element; HEO, human estrogen receptor; HGO, human glucocorticoid receptor; hPro, human progesterone receptor.

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modulate dopaminergic neurotransmission and are implicated in a range of neural functions including motor behavior, cognitive function and neuropsychiatric conditions including drug addiction [9–13]. Dopaminergic neurons express corticosteroid receptors [14], and dopamine-mediated behaviors are profoundly facilitated by glucocorticoids [15]. Acute administration of glucocorticoid impairs long-term memory retrieval, and dopamine D_2 receptor blockade is reportedly able to attenuate this effect [16]. Furthermore, increased glucocorticoid levels can induce behavioral changes similar to those attributed to enhanced dopaminergic activity. In humans, high levels of glucocorticoids can induce mood changes ranging from euphoria to psychosis [17]. Progesterone treatment also modulates the behavioral response to cocaine [18].

The D_2 dopamine receptor is under strong negative transcriptional control [19,20]. By screening a cDNA library constructed from the D_2 expressing neuroblastoma cell line NB41A3 with one of two principal negative control elements in the D_2 promoter as probe, we had identified a zinc finger-type transcription factor which we dubbed murine GIF, for GDNF-inducible transcription factor [21]. This designation was derived from the fact that GIF mRNA expression is induced in cultured cells following treatment with glial cell-derived neurotrophic factor (GDNF) [21]. GIF is expressed in the adult and developing brain in a specific distribution pattern including in basal ganglia structures [21]. In addition, its expression is robustly up-regulated following systemic administration of the glutamatergic agonist kainic acid in various brain regions in adult rats, including the caudate–putamen, nucleus accumbens and cerebral cortex [22]. In addition to its neurotrophic effects [23], GDNF plays a significant role in the biochemical and behavioral adaptations to drugs of abuse [24].

In the present investigation, we sought to link the biology of the GRE/PRE system, dopamine receptor expression and GDNF-inducible transcription factor by focusing on the transcriptional regulation of GIF itself. We report that glucocorticoid and progesterone receptor activation specifically up-regulates GIF transcription through an enhancer GRE/PRE element in its 5′-flanking region.

2. Materials and methods

2.1. Plasmid constructions

To obtain a genomic clone of GIF, a murine genomic library constructed in the bacterial artificial chromosome vector pBAC-Belo was screened with the murine GIF cDNA [21] as probe. The 11-kb BamHI–EcoRI fragment from one of the positive clones, which was analysed by restriction analysis, Southern blots and complete sequencing, was found to represent the transcribed region as well as 4.0-kb 5′-flanking region of the GIF gene. This fragment was subcloned into pBluescript SK(+) (Strategene, Cedar Creek, TX, USA), yielding pBS-GIF, for further characterization. pBS-GIF was digested with XbaI and NruI, and the 2.3-kb fragment that includes the 5′-flanking region was inserted into the XbaI–HincII sites of pUC19 yielding pUC-GIF−2159/+93. The latter plasmid was digested with BglII and HindIII, and the released fragment was inserted into the corresponding sites of pGL2-Basic (Promega, Madison, WI, USA) to yield pGL2-GIF−2139/+93. The same strategy was employed to generate pGL2-GIF−1161/+93, pGL2-GIF−806/+93, pGL2-GIF−213/+93 and pGL2-GIF−128/+93 from pUC-GIF−2159/+93. To construct pGL2-GIF−65/+93, a small 158 bp fragment of the GIF gene that excludes a putative GRE/PRE sequence (located between nucelotides −99 and −84) was generated by PCR using pGL2-GIF−128/+93 as template with sense primer GIF−65-F, 5′-GAGCTCTAGGCCCCGCCCTCTAC-3′ (−65 to −48, inserted SacI site underlined), and antisense primer GIF+93-R, 5′-AAGCTTCGAGCTGCCTGGCTGCTG-3′ (+93 to +76, inserted HindIII site underlined). The resultant fragment was ligated into pGEM-T Easy vector (Promega), yielding pGEMTe-GIF−65/+93. The latter plasmid was digested with SacI and HindIII, and the released fragment was inserted into the corresponding sites of pGL2-Basic (Promega, Madison, WI, USA) to yield pGL2-GIF−65/+93.

To fuse GIF regulatory elements to the heterologous herpes simplex virus thymidine kinase (TK) promoter, the promoter region was amplified by PCR using pRL-TK (Promega) as template with sense primer HSV_TK1-F, 5′-GTCGACAAGCTTAAATGAGTCTTCGGACCTCG-3′ (inserted SalI and HindIII sites underlined), and antisense primer HSV_TK1-R, 5′-TCTAGATTAAGCGGGTCGCTGCAGGG-3′ (inserted XbaI site underlined). The resultant fragment was ligated into pGEM-T Easy vector (Promega) yielding pGEMTe-HSV_TK1. The latter plasmid was digested with SalI and XbaI, and the released fragment was inserted into the corresponding sites of pCAT-Basic (Promega) to yield pCAT-TK. Complementary oligonucleotides spanning the 63 bp fragment of the GIF gene that includes a putative GRE/PRE consensus sequence (nucleotides −128 to −66) (Fig. 3) were synthesized (with HindIII sites inserted at both ends). After annealing, the double-stranded oligonucleotide was subcloned 5′ to the TK promoter at the HindIII site in pCAT-TK to yield pCAT-GIF−128/−66-TK.

The integrity of all constructs was verified by restriction analysis and partial sequencing.

2.2. Cell culture and transient expression assays

The murine neuroblastoma NB41A3 cell line was obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in Dulbecco's modified Eagle's medium (DMEM) without phenol-red (BioWhittaker, Walkersville, MD, USA) supplemented with 10% dextran-coated charcoal stripped fetal bovine serum (Hyclone, Logan, UT, USA) at 37 °C in humidified atmosphere containing 10% CO_2. Transfections were carried out using SuperFect Transfection Reagent (Qiagen Inc., Valencia, CA, USA) with serum free DMEM in 60 mm dishes. Three micrograms of the test pGL2 or pCAT plasmid and 2 μg of the steroid receptor expression plasmid (effector) were used. The effector plasmids (kindly provided by Professor Pierre Chambon, Strasbourg, France) contained the coding sequences for the human estrogen receptor (HEO), the human glucocorticoid receptor (HGO), or the human progesterone receptor (hPro) inserted into the eukaryotic expression vector pKCR2 downstream from the SV40 early promoter [25–27].

One hour after transfection, 10^{-7} M steroid hormones (17β-estradiol, dexamethasone or progesterone (all from RBI, Natick, MA, USA)) or vehicle (ethanol, 10 μl) were added to the corresponding dishes. The cells were harvested 48 h later and lysed by adding 250 μl of 1× lysis reagent (a component of the Luciferase Assay System, Promega) to the harvested cells followed by centrifugation. All plasmids used in transfections were purified by the Plasmid Midi Kit (Qiagen). Luciferase assay was carried out using the Luciferase Assay System (Promega). CAT assays were carried out using the CAT-enzyme linked immunosorbent assay (ELISA) kit (Roche Applied Science, Mannheim, Germany). Because β-galactosidase activity derived from pCMVβ plasmid (Clontech) used as internal control for transfection efficiency was affected by steroid treatment in our initial experiments, all luciferase and CAT assay results were normalized to the protein concentration of lysates measured by the BCA Protein Assay Reagent kit (Pierce, Rockford, IL, USA).

2.3. Quantitative real-time PCR

Total RNA from HGO-transfected cells, which were treated with 10^{-7} M dexamethasone (+) or vehicle (ethanol), was isolated by