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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

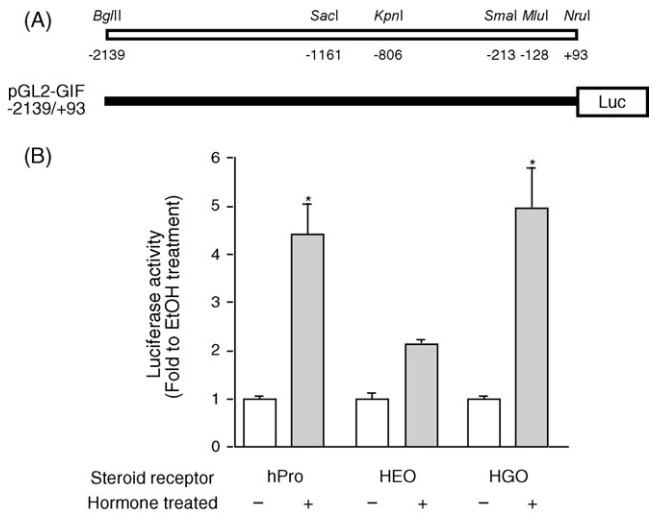

Fig. 1. Steroid hormone regulation of the GIF gene. (A) Schematic structure of the luciferase construct, pGL2-GIF−2139/+93. (B) The murine neuroblastoma NB41A3 cells were co-transfected with pGL2-GIF−2139/+93 and with one of the steroid receptor expression vectors shown at the bottom using the SuperFect Transfection Reagent. Transfected cells were then treated with 10^{-7} M of the corresponding steroid hormone (+) or with vehicle (ethanol) (−). Luciferase activity was measured using the Luciferase Assay System and normalized to protein concentration of cell lysates. Means ± SEM for three plates are shown as fold compared with no (vehicle) treatment. *ANOVA $p < 0.0001$ compared with no (vehicle) treatment. These experiments were repeated twice yielding reproducible results.

TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. One microgram of RNA was reverse transcribed to cDNA using the GeneAmp RNA PCR Core kit (Applied Biosystems) in a 20-μg reaction mixture containing 2.5 μm oligo $d(T)_{16}$, 4 μl 25 mM $MgCl_2$ solution, 1 μl 10× PCR buffer, 1 μl RNase inhibitor (1 U/μl), 1 μl MuLV reverse transcriptase (2.5 U/μl), 2 μl of each dNTP (10 mM) and DEPC-treated water. The reaction mixture was incubated at room temperature for 10 min and then annealed at 42 °C for 60 min. The cDNA was immediately used to amplify GIF and GAPDH (as internal control) cDNA using AmpliTaq Gold polymerase (Applied Biosystems). To quantify GIF mRNA levels by real-time PCR, sense primer 5′-ATCTGACTGCATCA GCGCCATCTA-3′ and antisense primer 5′-ATCAGCTGTGTGACGGATGACACT-3′ were employed. Real-time PCR was carried out with iCycler IQ System using iQ SYBR Green Supermix (Bio-Rad). Real-time PCR conditions were 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s, 60 °C for 10 s, 72 °C for 20 s, 1 cycle of 95 °C for 1 min, 55 °C for 1 min and finally cooling to 4 °C. The relative amount of GIF mRNA was normalized against GAPDH mRNA which was amplified by sense primer 5′-GTCTCCTGCGACTTCAAC-3′ and antisense primer 5′-TCATTGTCATACCAGGAAATGAGC-3′.

3. Results

3.1. Regulation of GIF gene transcription by steroids

The ability of steroid hormones to activate transcription of the GIF gene was evaluated in murine neuroblastoma NB41A3 cell line. Expression plasmids encoding estrogen (HEO), progesterone (hPro), or glucocorticoid (HGO) receptors along with a GIF promoter-reporter gene construct pGL2-GIF−2139/+93 were co-transfected into these cells. After treatment with the corresponding steroid hormone, cell extracts were prepared and assayed for luciferase activity. In the presence of the effector plasmid HGO, dexamethasone treatment increased the transcriptional activity of pGL2-GIF−2139/+93 by 5.0-fold compared with no glucocorticoid treatment (Fig. 1B). In addition, in the presence of hPro receptor, progesterone treatment increased the transcriptional activity of pGL2-GIF−2139/+93 by 4.4-fold compared with no progesterone treatment (Fig. 1B). On the other hand, in the presence of HEO, the effect of 17β-estradiol treatment on the transcriptional activity of pGL2-GIF−2139/+93 was not statistically significant compared with no estrogen treatment (Fig. 1B). As glucocorticoids and progesterone share a common consensus DNA recognition sequence [28,29], these observations indicate that a GRE/PRE in the GIF gene is responsive to hormonal manipulation.

3.2. Functional localization of the glucocorticoid-responsive region in the GIF gene

To localize the glucocorticoid-responsive region in the GIF gene, serial 5′-deletion mutants of this gene were created and cloned in the 5′–3′-orientation upstream of the luciferase gene in the promoter-less vector pGL2-Basic (Fig. 2). NB41A3 cells were co-transfected with each of these five reporter plasmids and the effector glucocorticoid receptor expression vector HGO. After treatment with 10^{-7} M dexamethasone, cell extracts were prepared and assayed for luciferase activity. The longest construct, pGL2-GIF−1161/+93, showed glucocorticoid dependent increase in transcriptional activity with a 16.6-fold induction compared with that in cells transfected with HGO but not treated with the hormone (Fig. 2). The next 5′-deleted plasmid, pGL2-GIF−806/+93, showed a 9.1-fold increase in transcriptional activity compared with no hormone treatment (Fig. 2). pGL2-GIF−213/+93 and pGL2-GIF−128/+93 showed approximately 18.5-fold increases in transcriptional activity (Fig. 2). On the other hand, no glucocorticoid dependant increase in the transcriptional activity of pGL2-GIF−65/+93 was observed (Fig. 2). These results indicate that the region between nucleotides −128 and −66 of the GIF gene is important for the glucocorticoid response. Sequence analysis of this region revealed the presence of the putative GRE/PRE between bases −99 and −84 (Fig. 2, top panel).

3.3. Effect of the GRE/PRE region of the GIF gene on the TK promoter

The TK promoter is known to be a GRE-dependent promoter [30,31]. To confirm whether the GRE/PRE region in the GIF gene also stimulates transcriptional activity of the heterologous TK promoter, fragment −128 to −66 was inserted 5′ to the TK promoter in pCAT-TK yielding plasmid pCAT-GIF−128/−66-TK (Fig. 3A). This promoter-reporter gene construct was used along with the glucocorticoid receptor expression plasmid HGO (for dexamethasone treatment) or the progesterone receptor expression plasmid hPro (for progesterone treatment) to co-transfect NB41A3 cells. The TK promoter fused with the GRE/PRE region of the GIF gene in pCAT-GIF−128/−66-TK displayed a 1.8-fold increase in transcriptional activity with dexamethasone treatment (Fig. 3B). This induction achieved statistical significance. As expected, the TK promoter alone in pCAT-TK showed no significant increase in transcriptional activity with dexamethasone treatment compared with unliganded HGO (Fig. 3B). The progesterone responsiveness of this GRE/RE region was also assessed. pCAT-GIF−128/−66-TK displayed a statistically significant 1.6-fold increase in transcriptional activity with progesterone treatment (Fig. 3B). As expected, TK promoter alone in pCAT-TK showed no significant increase in transcriptional activity with progesterone treatment compared with unliganded hPro. These results indicate that the region between nucleotides −128 and −66 of the GIF gene effectively mediates activation of the TK promoter by glucocorticoid and progesterone (Fig. 3).

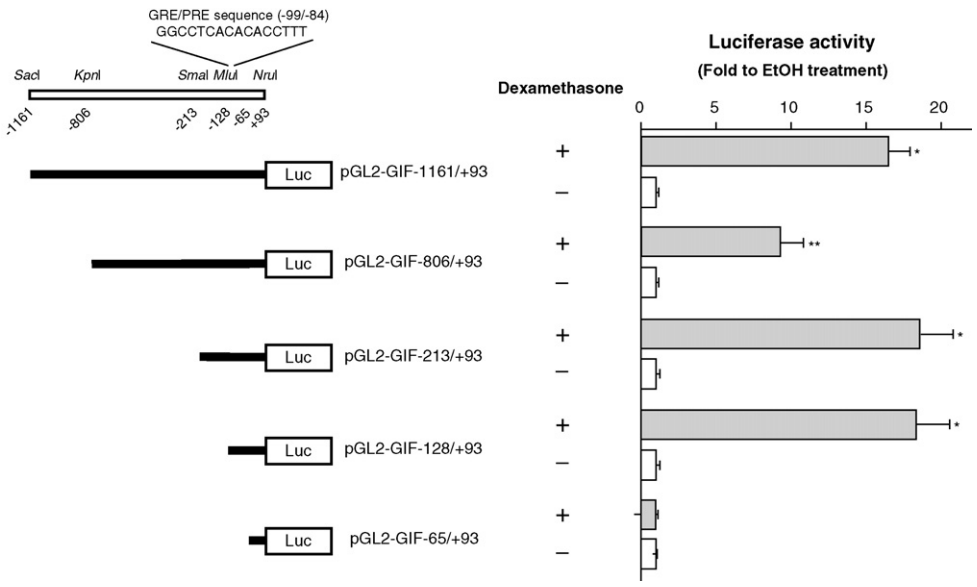

Fig. 2. Functional localization of the glucocorticoid-responsive region using serial 5′-deletion mutants of the GIF promoter. NB41A3 cells were co-transfected with the designated luciferase constructs and with the glucocorticoid receptor expression plasmid, HGO using the SuperFect Transfection Reagent. Transfected cells were then treated with 10^{-7} M dexamethasone (+) or with vehicle (ethanol) (−). Luciferase activity was measured using the Luciferase Assay System and normalized to protein concentration of cell lysates. Means ± SEM for three plates are shown as fold compared with no (vehicle) treatment. *ANOVA $p < 0.0001$, **ANOVA $p < 0.0004$ compared with no (vehicle) treatment. These experiments were repeated twice yielding reproducible results. The GRE/PRE sequence in this gene is shown in the gene organization diagram in the top panel.

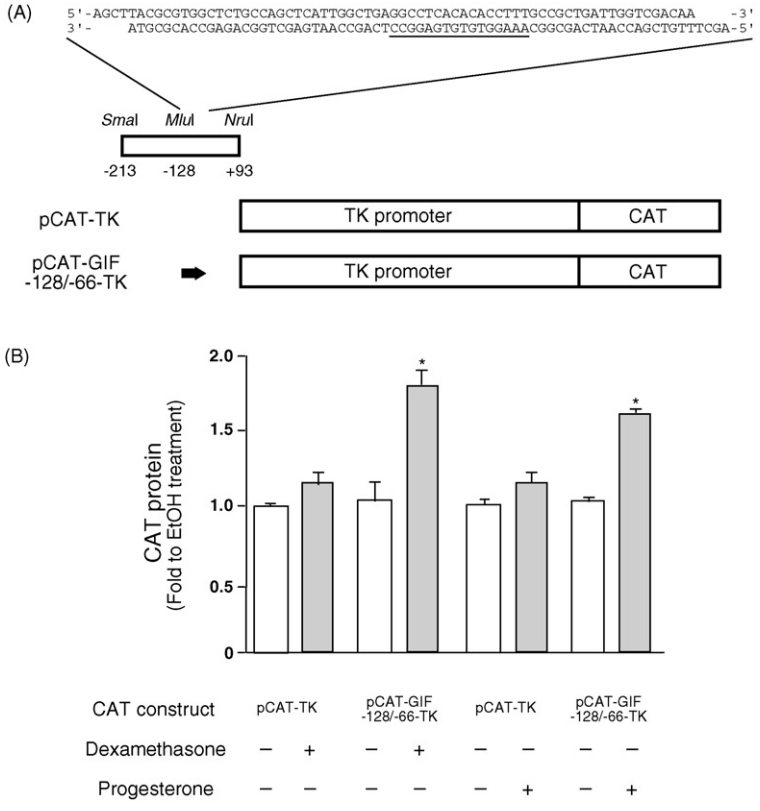

Fig. 3. Glucocorticoid or progesterone responsiveness of the TK promoter by the GRE/PRE sequence of the GIF gene. (A) Schematic structures of CAT constructs. The sequence between −128 and −66 is shown above the gene organization line, and underlined letters in the sequence indicate the GRE/PRE sequence. (B) NB41A3 cells were co-transfected with the designated CAT constructs and the glucocorticoid receptor expression vector, HGO (for dexamethasone treatment) or the progesterone receptor expression vector, hPro (for progesterone treatment) using the SuperFect Transfection Reagent. Transfected cells were then treated with 10^{-7} M dexamethasone (+), progesterone (+), or with vehicle (ethanol) (−). CAT activity was measured using the CAT-ELISA kit and normalized to protein concentration of cell lysates. Means ± SEM for three plates are shown as fold compared with no (vehicle) treatment. *ANOVA $p < 0.001$ compared with no (vehicle) treatment. These experiments were repeated twice yielding reproducible results.

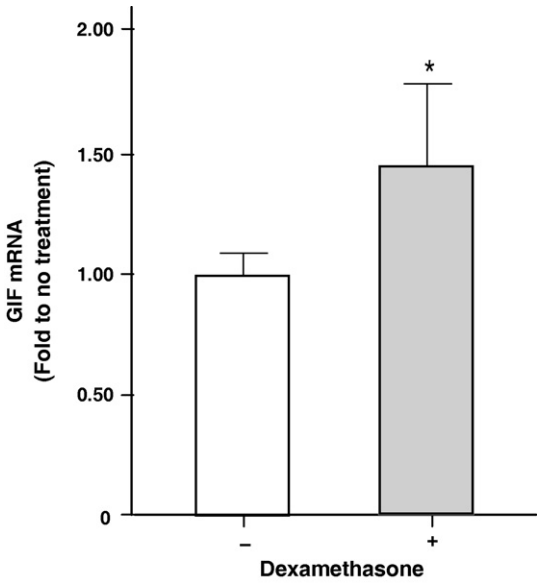

Fig. 4. Activation of endogenous GIF gene transcription by glucocorticoid detected by quantitative real-time PCR. NB41A3 cells were transfected with the glucocorticoid receptor expression vector, HGO, and treated with dexamethasone (+) or ethanol (−). Total RNA was subjected to real-time PCR. The relative quantity of GIF mRNA was normalized against that of GAPDH. Means ± SEM for three samples are shown as fold compared with no (vehicle) treatment. *ANOVA $p < 0.05$ compared with no (vehicle) treatment. These experiments were repeated twice yielding reproducible results.

3.4. Effect of glucocorticoid treatment on endogenous GIF gene transcription

To confirm the physiological significant of the above findings, the ability of glucocorticoid to up-regulate transcription from the endogenous GIF promoter was studied using quantitative real-time PCR. NB41A3 cells were transfected with glucocorticoid receptor and treated with either dexamethasone or vehicle. The relative quantity of GIF mRNA was approximately 1.5-fold higher following dexamethasone treatment compared to vehicle treatment (Fig. 4). This finding shows that endogenous GIF gene transcription is positively regulated by glucocorticoid.

4. Discussion

The present investigation provides the first evidence for the transcriptional activation of the GIF gene by steroid hormones. Transient co-transfection experiments in the D_2 expressing murine neuroblastoma cell line NB41A3 using reporter luciferase constructs along with steroid receptor expression vectors revealed the strongest induction of the GIF gene with dexamethasone treatment and a slightly lesser induction with progesterone treatment (Fig. 1). On the other hand, no significant transcriptional regulation was detected with activation of estrogen receptors (Fig. 1). These findings point to molecular regulation of the GIF gene by glucocorticoid and progesterone. Additionally, the physiological relevance of the glucocorticoid effect was confirmed by demonstrating induction of the endogenous GIF promoter in NB41A3 cells following glucocorticoid receptor activation (Fig. 4).

Localization of the glucocorticoid-responsive region in the GIF gene was undertaken using serial 5′-deletion mutants in co-transfection experiments in NB41A3 cells. Removal of the fragment between nucleotides −128 and −66 resulted in loss of the trans-activation of the GIF gene by glucocorticoid (Fig. 2) suggesting the presence of a functional glucocorticoid-responsive sequence in this region. Indeed, analysis of this region revealed the presence of a GRE/PRE sequence between nucleotides −99 and −84. The construct that deleted this element, pGL2-GIF−65/+93 did not respond to glucocorticoid stimulation. Further inspection of the sequence between nucleotides −2139 and +93 in the GIF gene revealed three additional elements that correspond to the estrogen-responsive elements at positions −1996, −1842 and −1033. However, these elements were clearly not functional as EREs in the GIF gene, since transcriptional regulation of this gene was not affected significantly by estrogen (Fig. 1). pGL2-GIF−2139/+93, which contains 2232 bp BglII–NruI fragment of the GIF gene showed moderate transcriptional activity with 5.0-fold induction compared with no glucocorticoid treatment (Fig. 1). On the other hand, pGL2-GIF−1161/+93, which includes the 1254 bp SacI–NruI fragment, showed relatively strong transcriptional activity with 16.6-fold induction compared with no glucocorticoid treatment (Fig. 2). Thus, pGL2-GIF−1161/+93 showed 3.3-fold higher glucocorticoid-induced activity than pGL2-GIF−2139/+93, suggesting that the 978 bp BglII–SacI region contains negative regulatory element(s) for glucocorticoid responsiveness. Furthermore, pGL2-GIF−806/+93, which contains 899 bp KpnI–NruI fragment showed 1.8-fold lower glucocorticoid-induced activity than pGL2-GIF−1161/+93 (Fig. 2), suggesting that the 594 bp SacI–KpnI region contains positive regulatory element(s). Further deletion in the 5′ end of the GIF gene from pGL2-GIF−806/+93 (pGL2-GIF−213/+93) resulted in significant increase in glucocorticoid-induced activity reaching 2.0-fold compared to pGL2-mGIF−806/+93 (Fig. 2), suggesting the presence of negative regulatory element(s) between −806 and −214. Putative transcription factor binding sites in the negative regions (nucleotides −2139 to −1162 and −806 to −214) include Sp1 and AP2, and in the positive region (nucleotides −1161 to −807) include an AP2 binding site. These regions remain to be investigated.

To determine whether the putative GRE/PRE region of the GIF gene stimulates transcriptional activity of the heterologous TK promoter, this fragment was fused with the TK promoter in reporter pCAT-TK construct. These transfection experiments in NB41A3 cells indicated that the TK promoter along with the GRE/PRE sequence containing fragment, but not the TK promoter alone, was transcriptionally activated by glucocorticoid (Fig. 3). Thus, this GRE/PRE region of the GIF gene could confer glucocorticoid responsiveness to the heterologous TK promoter. In addition, the TK promoter along with the GRE/PRE sequence was also transcriptionally activated by progesterone (Fig. 3). It is known that glucocorticoid and progesterone receptors have identical base contact points in the GRE/PRE sequence, localized within two consecutive major grooves, binding to the same face of the DNA [28]. Our findings suggest that there are likely additional molecular mechanisms that distinguish the glucocorticoid response from the progesterone response at their DNA recognition site such as recruitment of distinct coactivator complexes [32].

GIF protein was originally isolated as an inhibitory transcription factor against the D_2 dopamine receptor gene promoter [21]. The results of the present experiments indicate that transcription of the GIF gene can be activated by glucocorticoid. Thus, increased amount of GIF protein by glucocorticoid is expected to have an inhibitory effect on transcription of the D_2 dopamine receptor gene. *In vivo*, chronic glucocorticoid administration to adrenelectomized rats resulted in down-regulation of striatal D_2 receptor mRNA expression [10]. Although such *in vivo* regulation is often subject to complex and indirect effects such as changes in striatal dopamine release or effects through other neurotransmitter systems, transcriptional regulation of the D_2 gene may also occur under these circumstances.

In conclusion, the forgoing data define a molecular mechanism by which glucocorticoids and progesterones can directly modulate the GIF gene. Further elucidation of the mechanisms involved in GIF gene regulation by these hormones could lead to

molecular interpretation of clinical phenomena and help design more rational therapeutic strategies for certain neuropsychiatric conditions.

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