Critical Role for STAT3 in Murine Pituitary Adrenocorticotropic Hormone Leukemia Inhibitory Factor Signaling*

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Leukemia inhibitory factor (LIF) is a pleiotropic neuroimmune cytokine that promotes corticotroph cell differentiation and induces proopiomelanocortin (POMC) mRNA expression and adrenocorticotropic hormone (ACTH) secretion. However, molecular mechanisms for this induction remain elusive. We therefore developed ACTH-secreting AtT20 transformants for wild-type or mutated STAT3, a cytokine signaling molecule, to address whether STAT3 is a determinant of LIF-mediated ACTH regulation. We show that these mutants act in a dominant negative manner by blocking endogenous STAT3 tyrosine phosphorylation or STAT3 DNA binding. Attenuation of STAT3 activity in the dominant negative AtT20 clones prevented LIF from promoting transcriptional activation of the POMC promoter (2.1-fold), whereas this LIF action was enhanced (7.7-fold; \( p < 0.05 \)) in wild-type STAT3-overexpressing clones in comparison to mock-transfected cells (4.5-fold). However, wild-type or dominant negative STAT3-overexpressing clones showed comparable (4-fold) POMC induction after treatment with cyclic adenosine monophosphate (cAMP), an alternate inducer of POMC transcription, indicating the STAT3 specificity for LIF signaling. Moreover, dominant negative inactivation of STAT3 activity resulted in abrogation of LIF-induced POMC mRNA levels and ACTH secretion, confirming the in vivo role of STAT3 in LIF-mediated corticotroph action. Chemical or molecular blockade of the mitogen-activated protein kinase pathway did not affect LIF-mediated corticotroph function. These results indicate that STAT3 is a critical intrapituitary component of the LIF-mediated neuroimmunoendocrine interface in corticotroph cells.

The coordinated neuroendocrine stress response comprises both classic endocrine as well as neuroimmune regulatory pathways (1). Pituitary corticotroph cell function is controlled by complex hypothalamic (2) and peripheral paracrine signals (3), all ultimately impacting on proopiomelanocortin (POMC)\(^1\)

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\(^1\) The abbreviations used are: POMC, proopiomelanocortin; ACTH, adrenocorticotropic hormone; CRE, cAMP response element; CREB protein, cAMP response element-binding protein; CRH, corticotropin releasing hormone; ERK, extracellular regulated kinase; HA, hemagglutinin; IL-6, interleukin 6; IL-11, interleukin 11; JAK, Janus kinase; gene expression and adrenocorticotropic hormone (ACTH) secretion. Several cytokines are both peripheral and central modulators of neuroendocrine function and serve as mediators of the immune neuroendocrine system interactions (1, 3–5). The pituitary is an abundant source of growth factors that regulate the hypothalamo-pituitary-adrenal axis in vivo and pituitary ACTH production in vitro (6–9). Leukemia inhibitory factor (LIF) is a pleiotropic neuroimmune cytokine whose gene expression has been demonstrated in human fetal, adult and murine pituitary cells (10, 11). LIF is a component of the interleukin-6 (IL-6) cytokine family, which also includes IL-6, IL-11, ciliary neurotrophic factor, oncostatin M, and cardiotrophin-1. Signaling initiated by these cytokines is transduced by activation of composite receptors that share a common transmembrane gp130 protein subunit (12–16). LIF ligand binding is associated with gp130 and LIF receptor subunit heterodimerization and subsequent activation of downstream tyrosine kinases (17–18). The cytokine receptors lack intrinsic kinase activity. Subsequent to LIF receptor dimerization, JAK2 is activated by tyrosine autophosphorylation (19). These events lead to tyrosine phosphorylation, auto- or hetero-dimerization, and nuclear translocation of STAT3 (signal transducer and activator of transcription) and, to a lesser extent, STAT1, which regulates immediate-response gene transcription (20–22).

In AtT20 murine corticotroph cells, LIF increases POMC mRNA levels and ACTH secretion, and synergizes with corticotropin releasing hormone (CRH) to trigger these effects (20). Recently, we delineated elements of the POMC promoter important for a component of LIF and CRH synergy on POMC transcription (23). However, identification of a specific LIF-induced transcription factor that activates POMC transcription has been elusive. We have recently shown that overexpression of SOCS-3, a cytokine-inducible signaling inhibitor related to JAB, SSI, or CIS (24, 25), inhibited LIF activation of POMC gene expression in vitro (26). As SOCS-3 was also potently induced by LIF, SOCS-3 was implicated as a negative intracellular regulator of pituitary LIF signaling. SOCS-3 overexpression in corticotroph cells abrogated LIF-induced gp130 and STAT3 phosphorylation, suggesting STAT3 as a critical regulator of pituitary LIF function.

Although LIF action occurs mainly through the JAK-STAT pathway, other subcellular signaling pathways are activated by IL-6-related cytokines including phospholipase C, phosphoinositol 3-kinase, phosphotyrosine phosphatase D, pp120, the insulin receptor substrate 1, or several components of the mitogen-activated protein kinases cascade pathway, including SHC, GRB2, Raf-1, ERK1, and ERK2 (27–32). Activation of these widespread signal transducing molecules implies sub-

LIF, leukemia inhibitory factor; MAPK, mitogen-activated protein kinase; SIE, Sis-inducible element; SIF, Sis-inducible factor; SOCS, suppressor of cytokine signaling; STAT, signal transducer and activator of transcription.
stial convergence between cytokine-activated pathways and receptor tyrosine kinase-activated pathways. Nevertheless, JAK proteins appear to be key determinants for this convergence as their disruption results in failure of downstream phosphorylation events (31, 33). Furthermore, convergence between both pathways may also occur at the level of STAT proteins. Indeed, activation of Raf-1 by interferon γ and oncostatin M requires expression of the STAT1 transcription factor (34) and, reciprocally, ERK2 activity is required for stimulation of inter

To address molecular mechanisms responsible for LIF induction of the neuroendocrine stress response, we tested the involvement of STAT3 or MAPK in this LIF response. Inactivating STAT3 mutants include a phenylalanine substitution at a critical tyrosine carboxyl-terminal residue, Tyr-705 (STAT3F), and a mutant containing two alanine substitutions at positions important for STAT3 DNA binding (Glu-434 and Glu-435) (36). We therefore isolated stable mutant STAT3 AtT20 transformants and show that they behave as dominant negative inhibitors of endogenous corticotrop cell STAT3. Suppression of STAT3 activity is here shown to abrogate LIF action on POMC transcription and ACTH secretion. However, chemical or molecular blockade of the MAPK pathway did not impact LIF-induced POMC promoter activity. These results thus indicate the key role of STAT3 in the LIF-mediated neuroendocrine pituitary stress response.

MATERIALS AND METHODS

Cell Culture—AtT20 cells, obtained from the American Type Culture Collection (Rockville, MD), were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, 2 mM l-glutamine, 100 units/ml penicillin, and 0.25 µg/ml amphotericin B (Life Technology, Inc.).

Transformation of AtT20 Cells—To isolate stable transformants expressing wild-type or inactivated STAT3, pCAGGS-Neo-HA-Stat3WT, pCAGGS-Neo-HA-Stat3F, and pCAGGS-Neo-HA-Stat3D (36) were transfected into AtT20 cells by calcium precipitation and transformants selected with G418 (1 mg/ml). STAT3F presents a substitution of the glutamic acid residues 434 and 435 with two alanine residues and a mutant containing two alanine substitutions at positions important for STAT3 DNA binding (Glu-434 and Glu-435) (36). We therefore isolated stable mutant STAT3 AtT20 transformants and show that they behave as dominant negative inhibitors of endogenous corticotrop cell STAT3. Suppression of STAT3 activity is here shown to abrogate LIF action on POMC transcription and ACTH secretion. However, chemical or molecular blockade of the MAPK pathway did not impact LIF-induced POMC promoter activity. These results thus indicate the key role of STAT3 in the LIF-mediated neuroendocrine pituitary stress response.

RESULTS

Isolation of Pituitary Transformants Stably Expressing Dominant Negative Forms of STAT3—The IL-6 family of cytokines activate the JAK-STAT pathway in several systems, and LIF is a powerful activator of the JAK-STAT pathway, in particular, in AtT20 cells. We therefore isolated pituitary transformants stably expressing wild-type or dominant negative forms of STAT3 to test whether STAT3 mediates LIF induction of corticotrop function in these cells. Plasmids encoding hemagglutinin peptide (HA)-tagged STAT3F (HA-STAT3F) and HA-tagged STAT3D (HA-STAT3D) as well as HA-tagged wild-type STAT3 (HA-STAT3wt) were introduced into AtT20 murine pituitary cells. These cells have been shown to be an excellent model for studying the role of cytokine action in the ACTH stress response (20, 23). Several different clones selected by G-418 resistance were established; each expressed HA-STAT3F, HA-STAT3D, or HA-STAT3wt. Expression levels of transfected STAT3F or D mutants or STAT3wt, respectively, were determined by immunoprecipitation of HA-STAT3 with anti-HA monoclonal antibody (12CA5) followed by immunoblot analysis with polyclonal anti-STAT3 antibody (Fig. 1). Three independent clones for STAT3wt transformants (clones 1, 2, and 3) and for each dominant negative form of STAT3F (clones 2, 4, and 5) or STAT3D (clones 2, 4, and 6), respectively, were selected. Each clone was shown to express similar levels of HA-STAT3 protein and was used in the ensuing experiments.

To confirm the dominant negative action of HA-STAT3F on endogenous STAT3 function, cell lysates were immunoprecipitated with STAT3 antibody (Santa Cruz) prebound to Sepharose-protein G beads (Sigma) or a polyclonal anti-STAT3 antibody (Santa Cruz) prebound to Sepharose A beads (Sigma). Immune complexes were collected by centrifugation, washed twice with a buffer containing 30 mM Hepes, 30 mM NaCl, and 0.1% Triton X-100, pH 7.4, and resuspended in 30 µl of SDS-sample buffer. Samples were separated on a 7.5% SDS-polyacrylamide gel and electroblotted on polyvinylidene difluoride membrane. The membrane was blocked for 16 h at 4 °C in 20 mM Tris, pH 7.4, 500 mM NaCl, and 0.1% Tween 20 supplemented with 3% bovine serum albumin, blotted for 3 h at room temperature with polyclonal anti-STAT3 or monoclonal anti-phosphotyrosine antibodies (Santa Cruz). Bound antibodies were detected with horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG followed by ECL detection (Amersham Pharmacia Biotech).

Electromobility Shift Assay—Whole cell extracts (20 µg) were preincubated in a final volume of 20 µl (10 mM Hepes, pH 7.9, 80 mM NaCl, 10 mM glycerol, 1 mM dithiothreitol, 1 mM EDTA) with 2 µg of poly(dI-dC)-poly(dI-dC) at room temperature for 15 min. A 32P-labeled double-stranded oligonucleotide SIE67 used as probe (40,000 cpm, 5 fmol/
This element is readily recognized by either STAT1 or STAT3 (35). After electrophoretic mobility shift assay with extracts derived from mock-transfected AtT20 cells treated for 15 min with 1 nM LIF, three specific complexes were visualized and were effectively competed by 100-fold molar excess of unlabeled SIE probe, but not by an exogenous double-stranded oligonucleotide (Fig. 2B). These three complexes correspond to the previously described SIF (Sis-inducible factor) A (STAT3 homodimer), SIF B (STAT1/STAT3 heterodimer), and SIF C (STAT1 homodimer) (19, 21). In the HA-STAT3wt transfected clone, 15 min of 1 nM LIF treatment induced the formation of a single specific complex. STAT3 was shown to be a component of this complex as addition of STAT3 antibody to cell extracts, prior to addition of the SIE probe, inhibited complex formation and induced a faint supershift. However, in the HA-STAT3D transfected clones, this complex was clearly not induced by LIF treatment. This abrogation of DNA binding demonstrated the dominant negative action of HA-STAT3D on endogenous STAT3 action in the HA-STAT3D transfected clones.

Dominant Negative HA-STAT3F and HA-STAT3D Block LIF-induced POMC Promoter Activity—As LIF activates rat POMC expression through the −706/+64 region of the POMC promoter (20), activity of this promoter was tested in the STAT3 dominant negative clones. Transient transfections of a POMC promoter-driven luciferase reporter, as well as of a vector encoding β-galactosidase (internal control), were performed in mock-transfected AtT20, and HA-STAT3wt, F, and D clones, respectively (Fig. 3A). Treatment with 1 nM LIF for 6 h of mock-transfected AtT20 caused a 4.5 ± 0.4-fold (p < 0.01) luciferase induction. Furthermore, similar LIF treatment of the HA-STAT3wt clones enhanced (p < 0.05) versus mock-transfected) this induction to 7.7 ± 0.8-fold. However, in the HA-STAT3F and D clones, LIF induction was significantly abrogated (p < 0.01) as compared with mock-transfected AtT20 or HA-STAT3wt. Indeed, the HA-STAT3F clones only showed 1.81 ± 0.1-fold induction (p < 0.01), and 2.55 ± 0.2-fold induction (p < 0.01) was observed in the HA-STAT3D clones. These results indicated the requirement for STAT3 in mediating LIF induction of POMC expression at the transcriptional level.

To confirm that the HA-STAT3F and D clones were functionally viable, and yet failed to respond specifically to LIF because of lack of inducible STAT3, cells were also treated with dibutyryl cAMP, which stimulates POMC expression through an AP-1 like element in the first exon of the POMC gene (37), and whose recognition motif is also present within the POMC promoter fragment (−706/+64) (Fig. 3B). 5 mM dibutyryl cAMP treatment for 6 h similarly stimulated POMC transcription in both mock-transfected AtT20 cells (4.1 ± 0.2), and in the HA-STAT3wt clones (4.3 ± 0.1) as well as in the mutant HA-STAT3F and D clones (3.9 ± 0.2 and 4 ± 0.2, respectively).
Fig. 2. HA-STAT3F and HA-STAT3D act in a dominant negative manner. 

A, AtT20 clones transfected with HA-STAT3wt, HA-STAT3F, or HA-STAT3D were serum-starved for 16 h and treated with 1 nM LIF or vehicle (C) for 5 and 10 min. Cells were lysed and STAT3 proteins immunoprecipitated with polyclonal anti-STAT3 antibody, separated on an SDS-7.5% acrylamide electrophoresis gel, and the tyrosine-phosphorylated form of STAT3 was identified with a monoclonal anti-phosphotyrosine antibody (upper panel). To confirm equal protein loading in each lane, the membrane was stripped and rebotted with a polyclonal anti-STAT3 antibody (lower panel). B and C, gel-shift analysis with whole cell extracts (20 μg) derived from mock-transfected AtT20 (B) or HA-STAT3wt or HA-STAT3D clones (C) treated with 1 nM LIF or vehicle for 15 and 30 min. 32P-Labeled SIE oligonucleotide was used as the probe with AtT20 nuclear extracts (B, lanes 1–5), HA-STAT3wt nuclear extract (C, lanes 1–6), or HA-STAT3D nuclear extract (C, lanes 7–12). 100-fold molar excess of unlabeled SIE or unlabeled AP-1 oligonucleotides were used as competitors (B, lanes 4 and 5, respectively; and C, lanes 4 and 10, and lanes 5 and 11, respectively). Anti-STAT3 antibody was added to the nuclear extracts before addition of SIE probe (C, lanes 6 and 12).
Role of MAPK in LIF Induction of POMC Promoter Activity—
The IL-6 cytokine family, which transduces through the gp130 subunit, may also activate Ras-MAPK pathways (27–32). We therefore tested whether LIF induces MAPK activation in AtT20 cells. Using LIF-activated AtT20 cell extracts in a Western blotting experiment using an anti-tyrosine-phosphorylated form of MAPK antibody, treatment for 10 min with 1 nM LIF was shown to induce MAPK phosphorylation 2-fold (data not shown). To test whether the Ras-MAPK pathway might be a functional component of LIF function in AtT20 cells, we blocked MAPK by either using a chemical inhibitor of mitogen-activated protein kinase kinase (MAPKKK), PD 98059, which inhibits MAPK activation and subsequent phosphorylation of MAPK substrates, or, a cDNA encoding a dominant negative form of MAPK (TAYF). Pretreatment of AtT20 cells for 1 h with PD 98059 (5–100 μM) did not affect either LIF-induced POMC promoter activity (Table I) or LIF-induced STAT3 binding to the SIE probe (data not shown). Cotransfection of various concentrations of a dominant negative form of MAPK (T183A/ Y185F) with the POMC promoter luciferase construct also did not alter LIF-induced POMC promoter activity (Table II). Thus, MAPK does not appear to be implicated in LIF corticotroph function despite the weak induction of ERK2 tyrosine phosphorylation by LIF in these cells.

DISCUSSION

We have previously shown that LIF stimulated POMC promoter activity, POMC mRNA expression, and ACTH secretion (20). These results now demonstrate that disruption of STAT3 expression in AtT20 corticotroph cells significantly abrogates these LIF responses. We used two forms of mutated STAT3, which were both expected to behave as signaling inhibitors via different mechanisms (36). Both mutants of STAT3, STAT3F and STAT3D with a phenylalanine substitution at tyrosine 705 and STAT3D mutated at positions important for DNA binding, acted in a dominant negative manner in these cells. Endogenous STAT3 was not tyrosine-phosphorylated in HA-STAT3F clones, nor did STAT3 bind the SIE probe from the c-Fos promoter in the HA-STAT3D clones. This dominant negative action implies that the mutants compete with endogenous STAT3 protein for recruitment to activated gp130-JAK complex in the HA-STAT3F/D clones. In the HA-STAT3D clones, endogenous homodimericSTAT3 DNA binding is abrogated. This result is also consistent with previous studies using similar STAT3 mutants and showing STAT3 involvement in IL-6-induced growth arrest and terminal differentiation in M1 cells or in LIF-induced ES cell differentiation (36, 38). Furthermore, these HA-STAT3F and D AtT20 transformants were shown, in a separate study, to inhibit LIF-induced SOCS-3 mRNA expression.

In the DNA binding assay showing disrupted endogenous STAT3 DNA binding activity in the HA-STAT3D clones, a single complex was consistently observed in the assay using HA-STAT3D extracts, and was identified as a STAT3 homodimeric because addition of STAT3 antibody blocked its formation and also caused a band supershift. This complex was identified as SIF A (Sis-inducible factor) and is composed of a STAT3 homodimer, as distinct from SIF B and C composed.
respectively, of STAT1 and 3 heterodimer or of STAT1 homodimer (40). SIF B and C were not observed in the electrophoretic mobility shift assay using HA-STAT3wt extracts, whereas both these complexes were evident using mock-transfected AtT20 cell extracts. This may be explained by the strong overexpression of HA-STAT3wt competing with endogenous STAT1 for SIE binding. Furthermore, SIF C (STAT1 homodimer), which was evident in AtT20 extracts, was not regulated by LIF treatment. This observation also points to the major role of STAT3 for LIF signaling in these cells as compared with other STAT family members.

Using the rat POMC promoter (−706/+64), we previously
TABLE I
Blockade of MAPK pathway does not alter LIF-induced POMC promoter activity

Rat POMC promoter-luciferase transiently transfected AtT20 cells were pretreated for 1 h with 0–100 μM PD 98059, and subsequently treated for 6 h with 1 μM LIF. Luciferase activity was measured as described under “Materials and Methods.” The results are representative of three separated experiments and are expressed as -fold induction above control.

| µg  | -fold induction |
|-----|----------------|
| 0   | 5.95 ± 0.95    |
| 5   | 6.07 ± 0.88    |
| 10  | 5.97 ± 0.86    |
| 20  | 6.97 ± 0.63    |
| 50  | 7.27 ± 0.44    |
| 100 | 7.77 ± 0.84    |

* All effects are nonsignificant.

TABLE II
Blockade of MAPK pathway does not alter LIF-induced POMC promoter activity

Dominant negative MAPK (TAYF) was transiently co-transfected into AtT20 cells with rat-POMC promoter-luciferase. Cells were then treated for 6 h with 1 μM LIF, and luciferase activity measured as described under “Materials and Methods.”

| µg  | -fold induction |
|-----|----------------|
| Mock| 3.86 ± 0.34    |
| 0.1 | 4.19 ± 0.46    |
| 0.5 | 4.22 ± 0.56    |
| TAYF| 3.44 ± 0.21    |
| 0.1 | 3.78 ± 0.13    |
| 0.5 | 3.99 ± 0.17    |

* All effects are nonsignificant.

showed that LIF stimulated basal POMC expression and potentially enhanced CRH action on rat POMC transcription (20). A common POMC promoter element, which does not contain a STAT-binding site, also mediated a component of the synergy between both ACTH-inducing peptides. Moreover, disruption of this element (−173/−160), within the full-length POMC promoter (−706/−64), only partially blocked LIF and CRH-mediated effects (23). Thus, additional mechanisms may also be involved in this synergistic induction of POMC expression. The effects of STAT3 disruption in AtT20 cells also support this hypothesis, as LIF stimulation of POMC-promoter activity was reduced from 4.5-fold in mock-transfected or 7.7-fold in HA-STAT3wt clones to 1.8- or 2.5-fold in HA-STATF or D clones, respectively. Furthermore, overexpression of STAT3wt in HA-STAT3wt clones induced LIF action in comparison with mock-transfected AtT20 cells (7.7-fold versus 4.5-fold, respectively; p < 0.05). This observation also further confirmed the STAT3 specificity of LIF action, as was the demonstration that, in the STAT3 dominant negative clones, POMC transcription was briskly induced via an alternate pathway. CRH-like receptor binding ligands induce cAMP and activate CREB protein, which also induces POMC transcription (37, 41–42). We therefore stimulated the STAT3 dominant negative clones, as well as HA-STAT3wt and mock-transfected AtT20 cells with dibutyryl cAMP to increase steady state levels of cAMP, and showed that POMC was effectively induced in all clones.

The abrogation of LIF effects by STAT3 disruption differed quantitatively on POMC promoter activity, and on POMC mRNA expression or ACTH secretion; LIF effects on endogenous POMC expression and ACTH secretion were not as marked as those observed in the POMC promoter studies. This could be explained by the sensitivity of the luciferase assay for studying POMC promoter activity, especially in the mock-transfected AtT20 and HA-STAT3wt cells, than the in vivo assays. We utilized a 770-base pair fragment of the POMC promoter to study LIF action on luciferase reporter expression. However, the POMC promoter containing potential LIF-regulated elements may be longer in vivo, and thus may require transcription factors other than STAT3 (20).

Disrupting STAT3 signal transduction by either blocking tyrosine phosphorylation (HA-STAT3F clones) or preventing DNA binding (HA-STAT3D) resulted in decreasing LIF effects. These results also imply that the DNA-binding event of STAT3 is indispensable for LIF action, and thus render it unlikely that STAT3 tyrosine phosphorylation serves as the sole link between the JAK-STAT and known or unknown pathways (e.g. CREB) involved in POMC transcriptional regulation.

Furthermore, we examined whether other LIF-induced pathways might be implicated in LIF corticotroph function. LIF weakly induced MAPK phosphorylation (2-fold) (data not shown). The magnitude of LIF activation of MAPK differs in various cell lines but is generally low, and less efficient than oncostatin M (29). The MAPK pathway might be a component of LIF signaling for several reasons. Our results show that LIF weakly induces MAPK phosphorylation in AtT20 cells; JAK2 has been shown to activate both STAT and Ras-dependent -independent MAPK pathways (33), and STAT3 contains a serine phosphorylation site corresponding to a consensus site for MAPK serine phosphorylation activity (35). Nevertheless, neither coexpression of a dominant negative form of MAPK nor pretreatment with a specific inhibitor of MAPKK abrogated POMC promoter activity. We therefore conclude that, even if LIF weakly stimulates MAPK phosphorylation, it is not a primary mechanism by which LIF activates corticotroph function.

STAT3 involvement in LIF promotion of the stress response in AtT20 cells was not previously considered likely, mainly because no apparent STAT3 element has been identified in the POMC promoter. The −173/−160 element of POMC promoter shown to mediate LIF and CRH transcriptional synergy does not contain an evident STAT3 binding motif. Nevertheless, we now show that STAT3 disruption significantly blocks LIF transcriptional activation of POMC and ACTH secretion. Direct interactions between STAT3 and POMC promoter remain, however, to be elucidated. STAT3 might interact directly with the POMC promoter by binding to a non-classical STAT binding site. In HepG2 cells, STAT3 and a CRE-like transcription factor interaction were stimulated by interleukin-6 and shown to bind to a JRE-IL6 response element, which differed from a classical high affinity STAT binding site but which contained a low affinity STAT-binding site overlapping with an Ets-like and a CRE-like site (43). Interactions between STAT3 and CRE-like binding proteins on the POMC promoter will be important to explore. Indeed, CRH stimulates POMC transcriptional activation by activating a CRE binding protein (37). Furthermore, interaction between STAT and other transcription factors have been reported elsewhere (44, 45).

The dominant negative forms STAT3 transfectants are useful for studying molecular mechanisms by which LIF specifically determines corticotroph cell activation in the hypothalamo-pituitary-adrenal axis. The use of a pituitary cell line lacking endogenous STAT3 was elusive mainly because the POMC gene is specifically and abundantly expressed in the pituitary corticotroph cell, which contains tissue-specific POMC promoter elements (39). Thus, STAT3 appears to be a critical mediator for the LIF-mediated neuroimmunoendocrine interface in corticotroph cells.
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