Phosphotyrosine Phosphatases of the AtT-20 Murine Corticotroph Cell Line

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Somatostatin (somatotropin release inhibitory factor; SRIF) is a peptide-signaling molecule that activates a family of heterotrimeric guanine nucleotide binding protein (G-protein) coupled receptors (sst-1, sst-2, sst-3, sst-4). SRIF receptors control essential intracellular signaling events and, by reducing cyclic nucleotide levels, ion concentrations and protein phosphorylation, ultimately controlling cell proliferation and secretion. In the current study, we investigated the intracellular phosphatase activity present in the AtT-20 cell, as well as whether these enzymes were under direct SRIF receptor control. AtT-20 cells retain many of the properties of anterior pituitary corticotrophs, yet are an established cell line that expresses at least two SRIF receptor subtypes (sst-1 and sst-2). Both SRIF receptor subtypes potently suppress hormonally induced adrenocorticotropic hormone (ACTH) secretion from AtT-20 cells. Since intracellular protein kinase activation plays a crucial role in ACTH release from AtT-20 cells, identifying the corresponding protein phosphatases will provide valuable information on corticotroph function. The novel fluorescent phosphatase substrate, 6,8-difluoro-4-methylumbelliferyl phosphate (DiF-MUP) was employed to identify soluble or membrane-associated phosphatase activities. Treatment with a selective tyrosine phosphatase inhibitor (sodium vanadate; Na$_3$VO$_4$) attenuated DiF-MUP phosphatase activity by 75%, suggesting that the dominant activity detected in AtT-20 cells is a tyrosine phosphatase. Na$_3$VO$_4$ inhibition was potent as concentration response studies demonstrated an inhibitory concentration for 50% activity (IC$_{50}$) of 90 nM. A serine/threonine phosphatase inhibitor failed to decrease phosphatase activity, indicating that the dominant phosphatase activity present is due to protein.
tyrosine phosphatases. Immunoprecipitation studies, in conjunction with sodium dodecyl sulfate polyacrylamide electrophoresis (SDS-PAGE) and immunoblotting identified two intracellular phosphotyrosine phosphatases: Src homology phosphatase-1 (SHP-1) and Src homology phosphotyrosine phosphatase-2 (SHP-2). Immunoblotting with phospho-specific antisera confirmed the presence of SHP-1 and SHP-2, while also demonstrating that neither enzyme appears to be under SRIF control in AtT-20 cells. Together, these results show that AtT-20 murine corticotrophs contain sodium vanadate sensitive, soluble tyrosine phosphatase activity that is independent of SRIF control.
INTRODUCTION

Protein phosphatases are ubiquitous enzymes responsible for the dephosphorylation of intracellular proteins. These enzymes antagonize the effects of protein kinases, which phosphorylate proteins predominately on serine, threonine and tyrosine residues. Three major categories of protein phosphatases have been identified based on their preference for dephosphorylating the amino acid residues tyrosine, serine/threonine, or both tyrosine and threonine.

Protein tyrosine phosphatases (PTPs) are categorized into groups that either contain or lack a transmembrane domain, and are further subdivided into 17 groups (Andersen et al, 2001). PTPs, such as SHP-2 (SH2 containing protein tyrosine phosphatase 2), contain a src homology 2 (SH2) domain composed of approximately 100 amino acids, including a tyrosine residue that undergoes phosphorylation, followed by Glu-Glu-Ile, leading to specificity in signaling control. PTPs regulate phosphorylated tyrosine residues, thereby controlling cell growth and differentiation (reviewed in Tonks and Neel, 1996). A second type of PTP, SHP-1, is responsible for dephosphorylating signaling molecules such as p27 (Kip 1), leading to cell cycle arrest (Pages et al, 1999).

Serine/threonine phosphatases regulate diverse cellular mechanisms including glycogen metabolism, DNA replication, and exocytosis (Sim et al, 2003). Threonine/tyrosine phosphatases, known as dual specificity MAPK phosphatases (DS-MKPs) regulate the mitogen-activated protein kinases (MAPKs), which are critical protein kinases that regulate diverse physiological events, such as long-term potentiation, and inflammation (Wera and Hemmings, 1995; Farooq and Zhou, 2004). Dual-specificity phosphatases
(DSPs) aid in controlling the cell cycle by interacting with both MAPKs and stress activated kinases (Keyse, 1999).

Somatostatin (somatotropin release inhibitory factor; SRIF) was originally identified in the central nervous system as a hypothalamic inhibitor of growth hormone release (Brazeau et al, 1973). SRIF exists in two distinct forms, SRIF-14 and SRIF-28, both originating from the SRIF-prepropeptide. SRIF is distributed throughout the entire human body, with SRIF-28 predominately localized in the gastrointestinal tract and SRIF-14 localized to the nervous system (Reisine, 1995). SRIF peptides regulate a spectrum of endocrine effects, including thyroid-stimulating hormone (Singh et al, 2000), modulating gastric acid secretion (Larsson et al, 1979), and inhibiting pancreatic insulin and glucagon secretion (Strowski et al, 2000).

SRIF carries out its actions by interacting with a group of five highly homologous SRIF receptors subtypes (sst1 – sst5), including the sst2 receptor splice-variants (sst2A and sst2B). This receptor family exists in an overlapping expression pattern in many tissue types throughout the body (Reisine, 1995; Vanetti et al, 1992). The SRIF membrane receptors are seven transmembrane spanning proteins that couple to intracellular heterotrimeric guanine nucleotide-regulated proteins (G proteins) (Reisine, 1995). Through G protein activation and appropriate effector stimulation, SRIF receptors regulate cyclic nucleotide levels, membrane ion conductances, and cellular protein phosphorylation levels (Florio and Schettini, 1996).

AtT-20 cells are a transformed mouse corticotroph cell line which constitutively express a number of neuronal features, including cytoplasmic processes that resemble
neuronal growth cones, neurofilament polypeptides, as well as neuron-specific phosphoprotein synapsin 1 (Tooze et al, 1989). In addition, and of importance in the current study, the AtT-20 cells endogenously co-express the sst2A and sst3, but not sst1, SRIF receptor subtypes (Strowski et al, 2002; Cervia et al, 2003). AtT-20 cells retain the important property of primary corticotrophs, such as the hormonally regulated secretion of adrenocorticotropic hormone (ACTH) (Gumbiner and Kelly, 1981; reviewed in Antoni, 2000). Both corticotropin-releasing factor (CRF) and leukemia inhibitory factor (LIF) serve as potent ACTH secretagogues in corticotrophs and AtT-20 cells, making the AtT-20 cell an attractive model for studying ACTH stimulus-secretion coupling. (Vale et al, 1983; Auernhammer, 1998). SRIF treatment inhibits ACTH secretion by reducing intracellular 3', 5' cyclic adenosine monophosphate (cAMP) levels and suppressing calcium ion influx (Heisler et al, 1982; Richardson, 1983).

Mitogen-activated protein kinases (MAPKs) and signal transducers and activators of transcription (STATs) have also been implicated in ACTH secretion by AtT-20 cells. Phosphotyrosine phosphatases may dephosphorylate the activated kinases, leading to a halt in ACTH release. SRIF's ability to inhibit these intracellular signaling pathways may be due to the recruitment of protein phosphatases, capable of dephosphorylating the activated kinases. In murine NIH 3T3 cells, SRIF regulates dephosphorylation of the tyrosine residue of Raf-1, a mitogen-activated kinase kinase kinase (MAPKKK), by stimulating protein tyrosine phosphatase activity (Reardon et al, 1996). SRIF's inhibitory effects on AtT-20 cellular function, including the reduction of both cAMP and...
phosphorylated MAPK levels, led us to investigate and identify the possible protein phosphatase activity within this cell line.

In the current study we have characterized the endogenous protein phosphatase activity of the AtT-20 murine corticotroph. Using a fluorescent enzymatic (6,8-difluoro-4-methylumberiferyl phosphate; DiF-MUP) assay in conjunction with enzymatic phosphatase inhibitors, immunoprecipitation and immunoblotting we have established the presence of soluble phosphotyrosine phosphatase activity. Our results suggest the presence of a soluble tyrosine phosphatase activity that is independent of SRIF regulation.
MATERIALS AND METHODS

Cell culture

The AtT-20 cell line used in this study was a gift from Dr. Terry Reisine (Los Angeles, CA). Cell culture dishes and flasks were purchased from Corning (Corning, NY). Cell culture growth medium and supplements were purchased from Invitrogen (Carlsbad, CA). AtT-20 cells were cultured in Dulbecco's modified Eagle's medium, DMEM, (with GlutaMAX<sup>®</sup>, high glucose, 110mg/L sodium pyruvate and pyridoxine-HCL, Invitrogen, Carlsbad, CA) containing 100 U ml<sup>-1</sup> of penicillin, 100µg ml<sup>-1</sup> streptomycin, and 10% (v/v) fetal calf serum. Cultures were maintained in 5% CO<sub>2</sub> at 37°C. Cell monolayers were grown in T-75cm<sup>2</sup> flasks and passaged when 75% confluence was reached. Cell suspensions were transferred to a new T-75cm<sup>2</sup> flask at a 1:10 dilution. Cells were given 48 hours to recover prior to experimentation.

Tyrosine phosphatase assay

AtT-20 cultures were incubated in the presence or absence of 100nM SRIF-14 (Peninsula Labs, Belmont, CA) for 30 min. Cell cultures were washed in PBS and then lysed in a 50mM HEPES buffer containing 0.5% Triton X-100, 10% glycerol (GibcoBRL). 2.5µM pepstatin A, 12.5µM leupeptin, 12.5µM aprotinin, 12.5µM bacitracin and 200nM polymethylsulfonyl fluoride. Lysates were then centrifuged at 13,500 x g for 15 min at 6°C and the supernatant retained.

Protein phosphatase assays were performed in a clear bottom 96 well plate (Corning, Corning, NY). AtT-20 cell lysate was incubated with varying concentrations of the fluorescent phosphatase substrate, 6,8-difluoro-4-methylumbelliferyl phosphate.
(DiF-MUP; Gee et al., 1999; Molecular Probes, Eugene, OR) up to 30 min at 37°C with intermittent mixing in a CytoFluor Series 4000 fluorescent plate reader (Applied Biosystems, CA), with 360nm excitation and 460nm emission filters. Parallel assays were conducted in the presence of 10µM Na3VO4, an inhibitor of tyrosine phosphatase activity and a commercial 10 mM serine/threonine phosphatase inhibitor cocktail (Sigma, MO). The resulting data were analyzed with GraphPad Prism 4.0.

Immunoprecipitation

AtT-20 cells were cultured in T-75cm² flasks with DMEM and then incubated for 15 minutes with 100nM SRIF-14. Cells were washed with 5 mL PBS (with MgCl and CaCl, Invitrogen, Carlsbad, CA) and lysed with 500 µL of 50mM HEPES buffer. Supernatants were obtained and incubated overnight with phospho-SHP-1 or SHP-2 antibody (Cell Signaling, MA). Protein G agarose beads (450 mg/ml., Sigma, St. Louis, MO) were added and pellets were obtained and washed with 50 µL of 50mM HEPES buffer and resuspended in 1X NuPAGE LDS sample buffer.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting

AtT-20 cells were cultured in 12-well plates with medium (DMEM) for 48 hours and then incubated in the presence or absence of 100nM SRIF-14 for 20 min at 37°C. AtT-20 cells were then lysed using 1X NuPAGE LDS sample buffer. Cell lysates were sonicated and heated to 70°C for 10 min, resolved on NuPAGE 10% Bis-Tris gels (Invitrogen Life Technologies, CA) electroblotted onto polyvinylidifluoride (PVDF) membranes and treated for 45 min in a blocking buffer containing 5% (w/v) of Carnation non-fat milk.
dehydrated milk. Phospho-SHP-1 and SHP-2 were then immunodetected with a 1:1000 dilution of anti-phospho-SHP-1 and anti-SHP-2 antibodies, respectively. The PVDF membrane associated immunoreactivity were then washed and incubated for 1 hour in a 1:2000 dilution of a horseradish peroxidase-conjugated anti-rabbit secondary antibody and detected using ECL chemiluminescence (Amersham, NJ). The resulting autoradiograph was scanned and integrated pixel density was obtained using NIH Scion Imaging software.
RESULTS

Tyrosine Phosphatase Activity in AtT-20 cell lysate

To identify a possible role of SRIF-14 in controlling AtT-20 cellular phosphatase activity, experiments using increasing concentrations of the fluorescent substrate DiF-MUP were performed with AtT-20 cell lysates prepared from untreated and treated (100 nM SRIF-14) cultured cells. DiF-MUP hydrolysis provides a fluorescent measure of protein phosphatase activity (Gee et al., 1999). Cleavage of the phosphate group results in the product 6,8-difluoro-4-methylumbelliferyl, a fluorescent molecule capable of emitting high levels of fluorescence.

![Chemical structure of 6,8-difluoro-4-methylumbelliferyl phosphate (DiF-MUP). Gee et al., 1999; Molecular Probes, Eugene, OR.](image)

A concentration-response relationship for DiF-MUP was established by incubating increasing concentrations of DiF-MUP with a soluble fraction from the AtT-20 cell lysate (Figure 2). Non-linear regression analysis of the fluorescence results revealed an approximate effective concentration for 50% activity (EC50) of approximately 50 µM. However, as shown in Figure 2, it should be noted that the lysate enzyme saturation could not be achieved, even at the highest DiF-MUP concentrations used in this study. In addition, the concentration-response curve indicates that no
significant difference exists between SRIF-14 treated and non-treated samples (Figure 2). Experiments performed with AtT-20 cell membranes prepared from cultured cells demonstrated similar results, indicating that SRIF-14 had no significant effect on membrane protein phosphatase activity (data not shown).

![Tyrosine Phosphatase Activity in AtT-20 Cell Lysate](image)

**Figure 2.** Tyrosine phosphatase activity in AtT-20 cell lysate. Cell monolayers were incubated in the presence or absence of 100 nM SRIF-14 for 15 min at 37°C. A soluble cell lysate was prepared by lysis of the AtT-20 cell monolayers, and fluorescent activity was assessed in a 96-well using a Cytofluor 4000 fluorescent plate reader (Applied Biosystems). Assays were conducted in triplicate and the data mean± SEM of twelve separate experiments are shown.

AtT-20 lysate tyrosine phosphatase inhibition by sodium vanadate (Na3VO4)

DiF-MUP provides a convenient and robust fluorescent measure of protein phosphatase activity, which when combined with an appropriate enzyme inhibitor, can help characterize and define the type of phosphatase activity being studied (Gee et al., 1999). For example, phosphotyrosine phosphatases are exquisitely sensitive to the potent
tyrosine phosphatase inhibitor sodium vanadate, Na$_3$V$_2$O$_4$ (Swarup et al, 1982).

Vanadate-mediated enzyme inhibition serves as a readily available marker for establishing the amount of tyrosine phosphatase activity present in a heterogeneous cell lysate. To establish the level of tyrosine phosphatase activity in the AtT-20 cell lysates, approximately 50 µg of AtT-20 cell lysate was incubated for 15 min with 50 µM DiFMUP in the presence or absence of 10 µM Na$_3$VO$_4$. DiFMUP activity was reduced in AtT-20 cell lysate samples approximately 75% by Na$_3$VO$_4$ (Figure 3; * p < 0.05 vehicle control).

To determine the potency of Na$_3$VO$_4$ on tyrosine phosphatase activity, increasing concentrations of sodium vanadate were added to cell lysates. The DiFMUP activity was measured using a CytoFluor 4000 plate reader and data were analyzed in GraphPad Prism 4.0. One way ANOVA followed by Dunn’s non-parametric post hoc test, * p < 0.05 compared vehicle to Na$_3$VO$_4$ treated AtT-20 cells.

**Figure 3.** Sodium vanadate attenuates protein phosphatases activity in AtT-20 cell lysate. AtT-20 cell monolayers were prepared in cell lysis buffer and lysates were incubated 15 minutes with 50 µM DiFMUP in the presence or absence of 10 µM Na$_3$VO$_4$. Fluorescence was measured using a CytoFluor 4000 plate reader and data were analyzed in GraphPad Prism 4.0. One way ANOVA followed by Dunn’s non-parametric post hoc test, * p < 0.05 compared vehicle to Na$_3$VO$_4$ treated AtT-20 cells.
found in the cell lysate exhibits a potent sensitivity to Na₃V₀₄ as non-linear regression analysis of the DIF-MUP inhibition revealed a 50% inhibitory concentration (IC₅₀) of approximately 90 nM (Figure 4).

Na₃V₀₄ inhibition of Tyrosine Phosphatase Activity

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<td>[Na₃V₀₄ M]</td>
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<td>10⁻⁷</td>
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Figure 4. Sodium vanadate inhibition of DIF-MUP activity in AtT-20 cell lysate. AtT-20 cell lysates were prepared in the cell lysis buffer and incubated 15 minutes with 50µM DIF-MUP and Na₃V₀₄ at concentrations ranging from 10⁻⁹M to 10⁻⁶M. The IC₅₀ value in control cell lysates was 90nM.

AtT-20 cell lysate phosphatase inhibition with serine/threonine cocktail inhibitor

To determine if DIF-MUP hydrolysis is also sensitive to serine/threonine phosphatase activity, AtT-20 cell lysates were co-incubated with 50 µM DIF-MUP and 10 mM serine/threonine phosphatase cocktail inhibitor for 15 minutes. Statistical analysis of the inhibition data (using Dunn's non-parametric post-hoc test) shows no significant difference between the cocktail inhibitor treated and non-treated samples, indicating that serine/threonine phosphatases do not play a significant role in DIF-MUP hydrolysis under these experimental conditions (Figure 5).
### Figure 5. Phosphatase inhibitor cocktail effect on DiF-MUP activity in AtT-20 cell lysate.

AtT-20 cell lysates were prepared in the cell lysis buffer and incubated 15 minutes with 50µM DiF-MUP and 10mM of the phosphatase inhibitor cocktail. Fluorescence was measured using a Cytofluor 4000 plate reader and data were analyzed in GraphPad Prism 4.0. One-way ANOVA, followed by Dunn's non-parametric post hoc test, shows no significant difference.

#### Identification of SHP-2 and Phospho-SHIP1 in AtT-20 cells

The tyrosine phosphatase SHP-2 has been implicated in SRIF actions in several cellular models. For example in S49 cells, the endogenously expressed sst2 receptor couples an increase in protein tyrosine phosphatase activity to a suppression of MAP kinase activity (Dent et al, 1997). SRIF-14 treated and non-treated AtT-20 cell monolayers were lysed with 1X SDS and proteins were separated using gel electrophoresis. SHP-2 phosphatase (72 kDa) was detected using an anti-SHP-2 polyclonal antibody. A paired student T-test was performed demonstrating that the SRIF-14 treated sample is not significantly different from the control sample.
The SHP-1 phosphatase plays a role in regulating intracellular signaling events leading to cell cycle arrest and cell growth inhibition (Ferjoux et al., 2003). A polyclonal anti-Phospho-SHP1 antibody identified the tyrosine phosphatase (140 kDa) in SDS-PAGE resolved and electroblotted AtT-20 cell lysates. SRIF-14 did not appear to control SHP-1 phosphorylation as no statistically significant difference was observed on the percent integrated density values obtained from the control and SRIF-14 treated samples. (p > 0.05; paired Student t-test)
Figure 7. Detection of Phospho-SHP1 in AtT-20 cells. AtT-20 cell lysates were separated by SDS-PAGE, electroblotted and incubated with anti-Phospho-SHP1 primary antibody. Images were quantified using NIH Scion Image. The results represent the mean ±SEM of three separate experiments. The paired student T-test shows no significant difference between the vehicle and SRIF-14 treated samples. The inset shows a representative autoradiograph.
SRIF regulates multiple intracellular signaling pathways such as intracellular ion concentrations, cyclic nucleotide levels and protein phosphorylation (Blake et al., 2004; Olias et al., 2004). Protein phosphorylation controlled by SRIF includes the activation of phosphoprotein phosphatases (Olias et al., 2004) that are responsible for regulating crucial cellular events, including cell growth and proliferation, differentiation, and cell cycle progression.

SRIF receptor subtypes are well documented in the AtT-20 cell, controlling cyclic nucleotide metabolism (Strowski et al., 2002; Cervia et al., 2003) and hormonal secretion (Strowski et al., 2002). In the AtT-20 cell model, SRIF inhibits ACTH release via both cAMP independent and dependent mechanisms, suggesting that multiple intracellular events may be under SRIF control (Litvin et al., 1986; Reisine et al., 1988; McFerran and Guild, 1996). However, whether SRIF events outside of intracellular ion concentrations or cyclic nucleotide metabolism are affected remains unknown. In the current study, we sought to establish and identify the protein phosphatase family of enzymes in the AtT-20 cell, which may serve to inactivate the protein kinases responsible for ACTH secretion.

Within the AtT-20 cell, we have identified at least two cytosolic protein tyrosine phosphatases, SHP-1 and SHP-2. We used the fluorescent substrate difluoro-4-methylumberiferyll phosphate (DiF-MUP) in combination with sodium vanadate to measure the relative fluorescence of PTPs upon SRIF-treatment, as a means of indirectly determining phosphatase activity. Our results demonstrate that basal phosphatase activity
was not affected by SRIF incubation, suggesting that within AtT-20 cells, PTPs, including SHP-1 and SHP-2, act independently of SRIF activation.

Both SHP-1 and SHP-2 have been identified in previous studies, but this experiment provides the first indication of the presence of these protein tyrosine phosphatases within a corticotroph cell line, such as the AtT-20 cell. SHP-1 plays a largely inhibitory role within a cell, negatively regulating both cytokine and growth receptors, as well as intracellular signaling molecules such as Janus activating kinase 2 (Jak2) (Tonks and Neel, 2001; Wu et al, 2000). In the AtT-20 cell, SHP-1 function may dephosphorylate both growth and cytokine receptors, the CRF- and LIF-receptors, respectively. The negative regulation of both G protein coupled receptors and an interleukin-6 cytokine receptor would prove important in the overall control of ACTH secretion.

SHP-2 function in a cell demonstrates both negative and positive regulatory roles. SHP-2 activity involves inactivation and dephosphorylation of both JAK and STAT in rat brain microglial cells, but it functions as a positive regulator of receptor tyrosine kinase signaling (Kim et al, 2003; Keyse, 2000). Within the AtT-20 cell, SHP-2 may co-regulate ACTH secretion with SHP-1 via a cytokine-induced pathway leading to the activation of JAK-STAT signaling proteins. In rat pancreatic cancer cells, both SHP-1 and SHP-2 have been found to work in conjunction with one another by coupling to ssh2 (Ferjoux et al, 2003), indicating a potential mechanism for SRIF control of these PTPs. Our results indicate that within the AtT-20 cell, both SHP-1 and SHP-2 are independent.
of SRIF function, suggesting that neither PTP is docked to the sst, receptor under basal conditions.

AtT-20 cells endogenously express the SRIF receptor subtypes sst₂ and sst₅; however, sst, has been reported to be the dominant receptor subtype in the rodent pituitary corticotroph (Patel et al., 1996). Studies have shown that SHP-1 is constitutively associated with sst₅, rather than sst, and displays antiproliferative effects in CHO (Chinese hamster ovarian) cells when the stimulated sst₅ receptor activates the tyrosine phosphatase (Lopez et al., 1997). In contrast, CHO cells transfected with sst, do not recruit either protein tyrosine phosphatases or serine/threonine phosphatase (Buscail et al., 1995). Provided with this information, we can speculate that the AtT-20 cell responds to SRIF-14 via sst, rather than sst, to regulate the activity of PTPs. The expression profile of sst₂₅ in the AtT-20 may explain the inability of SRIF-14 to significantly increase PTP levels. A novel approach to clarify the SRIF receptor pharmacology may involve profiling sst₅ selective agonist-induced PTP expression. Delineating SRIF’s intracellular effects via different receptors could serve to answer the question of why multiple receptor subtypes are co-expressed in individual cells and tissues, a question that may have significant therapeutic implications for hormone secretion from SRIF sensitive tissues (Blake et al., 2004; van der Hooe et al., 2005).

While SRIF has been shown to have no direct effect on basal tyrosine phosphatase activity in AtT-20 cells, SRIF’s effects on stimulated AtT-20 cells has not been examined. Clearly, future experiment measuring SRIF’s actions on tyrosine phosphatase activity in LIF-stimulated AtT-20 cells may demonstrate an increase in
activity. Leukemia inhibitory factor (LIF), has been shown to increase ACTH secretion in AtT-20 cells (Auernhammer et al, 1996) through the JAK-STAT intracellular signaling pathway. Studies have shown that endogenously expressed LIF receptors stimulate Janus-activated kinases (JAK), a family of tyrosine kinases located within the AtT-20 cell membrane. (Stahl et al, 1994). Following activation, signal transduction and activation of transcription (STAT) proteins are phosphorylated on tyrosine residues and translocate to the nucleus to promote the expression of proopiomelanocortin (POMC), the precursor to ACTH. SRIF may recruit protein tyrosine phosphatases capable of dephosphorylating STAT3 on tyrosine residues, preventing nuclear localization and subsequent POMC gene expression. Recruitment of PTPs, such as SHP-1 and SHP-2, could occur by association with sst3, based on the ability these phosphatases to interact with the SRIF receptor subtype.

The importance of delineating the regulatory mechanism of ACTH secretion from the murine corticotroph may provide a model relevant in understanding the aberrant secretory responses in Cushing's syndrome. Cushing's syndrome is a hormonal disorder caused by the hyperstimulation of the adrenal gland by ACTH, allowing dangerous levels of cortisol to be released. This can be caused by pituitary adenomas and impose detrimental effects on a patient, including high blood pressure, high blood glucose levels and obesity. Currently, research is being conducted on the SRIF pharmacological analogue, SOM230, and its ability to inhibit ACTH secretion from both human and mouse corticotroph adenomas (Hofland et al, 2005). Uncovering a potential role of protein tyrosine phosphatases in ACTH secretion could assist in understanding the
mechanism of SOM230 inhibition, solidifying its role as a potential therapeutic agent for Cushing's syndrome.
CONCLUSIONS

In summary, the activation of protein kinases within the AtT-20 cell regulates the cellular processes involved in ACTH secretion. SRIF has been shown to inhibit the secretion of ACTH in AtT-20 cells by regulating cyclic nucleotide levels, ion conductance, and protein phosphorylation. While the protein kinases involved in AtT-20 cellular mechanisms have been well characterized, protein phosphatases responsible for antagonizing protein kinase effects have yet to be determined.

Our results demonstrate that AtT-20 cells contain a soluble protein phosphatase activity capable of hydrolyzing the fluorescent substrate DiF-MUP. This protein phosphatase activity is independent of SRIF control, but is exquisitely sensitive to sodium vanadate, a potent tyrosine phosphatase inhibitor. The sensitivity of the enzymatic activity to sodium vanadate indicates that protein tyrosine phosphatases are present within the AtT-20 cell. Further inspection of this activity led to the identification of the protein tyrosine phosphatases SHP-2 and SHP-1 within the murine corticotroph, however neither phosphatase was under the control of SRIF.

AtT-20 cells endogenously co-express three SRIF receptors, sst2A, sst2B, and sst. Future studies, with receptor-selective agonists, should be used to delineate each receptor's influence on SRIF's effects on tyrosine phosphatase activity in AtT-20 cells. Further investigation of tyrosine phosphatase recruitment in AtT-20 cells stimulated by LIF should be performed in order to develop a possible regulatory mechanism of ACTH secretion. This information could lead to a better understanding of how ACTH secretion
controlled in AtT-20 cells and provide possible therapeutic implications in diseases such as Cushing's syndrome.


Reisine, T., Wang, H.L., Guild, S. Somatostatin inhibits cAMP-dependent and c-AMP-


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