SOCS3 and SOCS6 are required for the risperidone-mediated inhibition of insulin and leptin signaling in neuroblastoma cells

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Abstract. Antipsychotic drugs are regularly used for the treatment of many types of psychiatric disorders. The administration of second-generation antipsychotics is often associated with weight gain and the development of diabetes mellitus; however, the molecular mechanisms underlying the effects of these drugs remain poorly understood. Leptin and insulin play key roles in the regulation of energy balance and glucose homeostasis, and resistance to the actions of these hormones can occur with obesity and inflammation, resulting in the pathogenesis of obesity and type 2 diabetes. In this study, the effects of risperidone on the insulin-induced protein kinase B (PKB) phosphorylation and leptin-stimulated signal transducer and activator of transcription 3 (STAT3) phosphorylation were investigated in the human SH-SY5Y neuroblastoma cell line. The treatment of these cells with risperidone induced the activation of extracellular signal-related kinase (ERK) by cellular cyclic adenosine 3-monophosphate (cAMP)-dependent protein kinase (also known as protein kinase A; PKA) and the mechanisms involved include the induction of suppressor of cytokine signaling 3 (SOCS3) and suppressor of cytokine signaling 6 (SOCS6) expression. The risperidone-induced ERK activation induced an upregulation of SOCS3 and SOCS6 mRNA expression levels. Taken together, these results suggest that risperidone modulates SOCS3 and SOCS6 expression through adenylate cyclase-mediated ERK activation, which, in turn, leads to an inhibition of insulin-induced PKB phosphorylation and leptin-stimulated STAT3 phosphorylation. Eventually, these effects result in excessive body weight gain due to the inhibition of both the leptin and insulin signaling pathways.

Introduction

Treatment with second-generation (atypical) antipsychotics has been associated with weight gain and the development of diabetes mellitus (1). Although atypical antipsychotic drugs are of great benefit to a wide variety of individuals with psychiatric disorders, particularly patients with schizophrenia (2), clinical observations indicate that these drugs can cause adverse metabolic effects (3), including an increased risk of obesity, diabetes and metabolic syndrome (4). However, the mechanisms underlying this process remain unclear. Dopamine D2 receptors are of key interest to the pathophysiology of schizophrenia (5), as all antipsychotics, typical as well as atypical, appear to be dopamine D2 receptor antagonists (6), particularly for postsynaptic receptors (7). Risperidone therapy is associated with modest weight gain (8). Claus et al (9) reported a mean weight gain of 2 kg after 12 weeks of treatment with risperidone with a mean final dose of 12 mg/day; similar gains were reported in the study by Owens (10).

Body weight is determined by the balance between energy intake and expenditure. Leptin and insulin are key hormones in the regulation of energy balance and glucose homeostasis (11,12). The actions of insulin are mediated through the insulin receptor (IR), which belongs to the tyrosine kinase receptor family. The binding of insulin to its receptor leads...
to a rapid autophosphorylation of the receptor followed by the tyrosine phosphorylation of IR substrate (IRS) proteins, which induce the activation of downstream signaling cascades, including phosphatidylinositol-3 kinase (PI3K) and protein kinase B/Akt (PKB/Akt) (13). Leptin is a major signaling molecule from the periphery that acts in the hypothalamus to regulate energy homeostasis and body adiposity (14,15). The effects of leptin involve the long isoform of the leptin receptor in the hypothalamus (16), where it influences pro-opiomelanocortin (POMC) neurons, which activate the secretion of an anorexic neuropeptide (α-melanocyte-stimulating hormone), and neuropeptide Y/agouti gene-related protein (NPY/AGRP) neurons, which inhibit the expression of an orexigenic neuropeptide (NPY). Leptin receptors belong to the cytokine receptor superfamily. The binding of leptin to its receptor activates Janus kinase 2 (JAK2), which, in turn, phosphorylates tyrosine residues in receptor tails, leading to the recruitment and activation of signaling molecules (16,17). Among these signaling molecules, signal transducer and activator of transcription 3 (STAT3) directly transmits the signals to the nucleus (18). The suppressor of cytokine signaling (SOCS) family of proteins was first discovered in 1997 (19) and is also referred to as JAK-binding proteins, signal transducer and activation of transcription (STAT)-induced STAT inhibitors, or cytokine-inducible Src homology-containing proteins family (20). SOCS proteins appear to be inducible negative regulators of cytokine signaling through the inhibition of the JAK/STAT pathway (20).

A key feature of human insulin and leptin resistance involves a defect in the ability of insulin to stimulate PKB phosphorylation and leptin-induced STAT3 phosphorylation. Thus, in this study, we investigated whether risperidone exerts direct biological effects on the insulin-induced PKB activation and leptin-stimulated STAT3 phosphorylation in cultured SH-SY5Y cells. Our results demonstrated that risperidone reduced both insulin-mediated PKB activation and leptin-induced STAT3 phosphorylation. Furthermore, risperidone significantly increased the mRNA levels of SOCS3 and SOCS6 in an extracellular signal-related kinase (ERK)1/2-dependent manner. Taken together, these results clearly indicate that the actions of risperidone are mediated through the induction of SOCS3 and SOCS6 expression, which, in turn, inhibit insulin-induced PKB phosphorylation. Moreover, the induction of SOCS3 expression leads to a modulation of leptin-stimulated STAT3 phosphorylation; this results in the inhibition of the leptin and insulin signaling pathways. These effects of risperidone may be responsible for the antipsychotic drug-induced weight gain observed in patients with psychiatric disorders.

Materials and methods

Antibodies and reagents. The following antibodies were used: anti-PKB, anti-p-PKB (Ser473), anti-STAT3, anti-p-STAT3 (Tyr705), anti-ERK1/2 and anti-p-ERK1/2 antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). Risperidone was obtained as a gift from Johnson & Johnson (New Brunswick, NJ, USA). Insulin and forskolin were from Sigma-Aldrich (St. Louis, MO, USA). Recombinant human leptin was purchased from R&D Systems (Minneapolis, MN, USA). The MEK inhibitor, U0126, and the cellular cyclic adenosine 3-monophosphate (cAMP)-dependent protein kinase (also known as protein kinase A; PKA) inhibitor, H89, were obtained from Calbiochem (La Jolla, CA, USA).

Cell culture. The human SH-SY5Y neuroblastoma cells line were used for all the experiments. The cells were maintained at 37°C 5% CO₂ in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 g/ml streptomycin. For the treatment of the SH-SY5Y cells with risperidone and forskolin, the cells were starved for 24 h followed by treatment of the cells with 100 nM risperidone or 10 μM forskolin for the indicated periods of time (0, 2, 6, and 12 h). The control sample was the untreated cells.

Western blot analysis. After the treatment of the cells with the different reagents as described in the figure legends, the cells were washed twice in ice-cold PBS and lysed at 4°C in the lysis buffer, containing 50 mM Tris-HCl (pH 7.5), 25 mM NaF, 40 mM β-glycerol phosphate (pH 7.5), 120 mM NaCl and 1% NP-40, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM sodium vanadate and 1 mM benzamidine. The protein concentration of the samples was determined by the Bradford protein assay with bovine serum albumin as a standard. The cell lyses were analyzed by 10% SDS-polyacrylamide gel electrophoresis and blotted onto polyvinylidene difluoride membranes. After blocking with 5% skim milk in Tris-buffered saline (TBS) containing 0.02% Tween-20, the membranes were probed with the corresponding antibodies and visualized by enhanced chemiluminescence, according to the manufacturer's instructions (GE Healthcare, Pittsburgh, PA, USA).

Reverse transcription (RT)-PCR. Total cellular RNA was isolated from the SH-SY5Y cells using PureHelix RNA Extraction Solution (NanoHelix Co., Ltd., Boston, MA, USA) following the manufacturer's instructions. cDNA was synthesized from 2 µg of RNA using oligo(dT) primer and a first-strand cDNA synthesis kit (Promega, Madison, WI, USA). The housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was amplified as a control for RNA loading and variations in cDNA synthesis efficiency. The following primer sets were designed for the amplification of human SOCS1, SOCS3, SOCS6 and SOCS7. The following primers were used: SOCS1 Forward, CAC GCA CTT CCG CAC ATT and reverse, ACG AGC TAC CGG AGG CAG; SOCS3 forward, GAG TAC CAC CTG AGT CTC CA and reverse, GAC CTC TCT CTC TTC CAC CT; SOCS6, forward, AAA TGT CTT TTT CTC CGG TC and reverse, AAT TCA TTG GCC CCC AAT AC; and SOCS7, forward, GCG GAA TTC ATG GGT GAT GTT and reverse, TAT GGA TCC GC CTC ATT AGT AGC.

Statistical analysis. The quantification of the western blot analysis results was carried out using the Tina version 2.1 program (Raytest Isotopenmegerate). Briefly, the relative intensity (area density) of the bands of interest was quantified using a densitometer. The background value from a blank band was subtracted. The results were calculated as the ratio change using a Student's t-test (SPSS version 12.0 software, SPSS Inc., Chicago, IL, USA). A value
insulin and leptin signaling pathways and may be an important effect of risperidone on insulin-mediated PKB activity in SH-SY5Y cells. (A) SH-SY5Y cells were serum-starved for 24 h and pre-treated with 100 nM risperidone for the indicated periods of time followed by 100 nM insulin stimulation for 15 min. Cells were harvested and analyzed by western blotting with anti-phospho-Ser473 (S473) PKB antibody or anti-PKB antibody. (B) Relative density was obtained by densitometry of the corresponding immunoblot data. Statistical differences of PKB phosphorylation were determined by normalizing values for total PKB at each lane. The results are the means ± standard deviation (SD) of 3 independent experiments. *p<0.05; **p<0.01. α indicates antibody.

Leptin-mediated STAT3 activation is blocked by risperidone in SH-SY5Y cells. The adipocyte-derived hormone, leptin, acts as a satiety signal in hypothalamic nuclei and regulates energy homeostasis and body weight (22,23). To evaluate the effects of risperidone on insulin signaling and the inhibition of STAT3 phosphorylation by risperidone in the SH-SY5Y cells following treatment with risperidone, beginning at 2 h post-treatment and continuing until 12 h post-treatment (Fig. 1A). Statistical analyses of the 3 independent experiments clearly indicated that insulin-mediated PKB activation was inhibited by risperidone in the SH-SY5Y cells (Fig. 1B).

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Upregulation of SOCS3 and SOC6 mRNA levels following treatment with risperidone and forskolin in SH-SY5Y cells. The elevation of intracellular cAMP-induced SOCS3 expression and the inhibition of STAT3 phosphorylation on the tyrosine residue in endothelial cells have been described previously (26). Furthermore, SOCS3 and other SOCS proteins (SOCS1, SOCS6 and SOCS7) appear to modulate insulin-mediated PKB signaling by several mechanisms (27-30). Thus, in this study, the mRNA levels of SOCS proteins were examined in the SH-SY5Y cells. Following the administration of risperidone to the cells, the mRNA levels of SOCS3 and SOCS6, but not those of SOCS1 and SOCS7, gradually increased in time-dependent manner (Fig. 4A). To further
confirm that this action is dependent on adenylate cyclase activity, the cells were treated with forskolin. The expression of SOCS3 and SOCS6 was elevated at 2 h post-treatment and gradually decreased thereafter (Fig. 4B). This indicates that the risperidone-mediated induction of SOCS3 and SOCS6 is modulated by adenylate cyclase.

Risperidone-mediated ERK phosphorylation is dependent on PKA activity. cAMP and PKA are evolutionary conserved molecules with a well-established position in the complex network of signal transduction pathways (31). Intracellular cAMP can activate the mitogen-activated protein kinase (MAPK)/ERK cascade through either Ras or Rap1 activation in several cell types (32). Therefore, the effects of either risperidone or forskolin on ERK1/2 activity in the SH-SY5Y cells were monitored. Western blot analyses with phospho-specific anti-ERK antibodies revealed that ERK1/2 activation occurred in the risperidone- or forskolin-treated cells (Fig. 5A), suggesting that adenylate cyclase activity is important for risperidone-mediated ERK1/2 activation. To further determine the possible involvement of PKA in these events, H89, a specific PKA inhibitor, was utilized. Risperidone- or forskolin-mediated ERK1/2 activation was completely blocked in the SH-SY5Y cells treated with H89 (Fig. 5B), indicating that
PKA activity is required for risperidone- or forskolin-mediated ERK1/2 activation.

Upregulation of SOCS3 and SOCS6 by treatment with risperidone is blocked by the MEK inhibitor, U0126. Sands et al (26) reported that the elevation of intracellular cAMP promotes the phosphorylation of ERK1/2, which, in turn, leads to the induction of SOCS3 protein levels in vascular endothelial cells. To further investigate the possibility that cAMP-induced MAPK/ERK activation is required for SOCS3 induction, the MEK inhibitor, U0126, was employed. RT-PCR using SOCS3- and SOCS6-specific primers revealed that the risperidone- or forskolin-mediated upregulation of SOCS3 and SOCS6 is dependent on MEK1/2 activity (Fig. 6).

Discussion

As an important side-effect of antipsychotic medication, weight gain can be a serious health issue in patients with schizophrenia and other psychoses (8) and may have adverse implications with adherence to long-term antipsychotic therapy. Excessive weight gain may also lead to other adverse health effects, including type 2 diabetes, hyperlipidemia and cardiovascular disease (33). Risperidone and olanzapine, two widely used atypical antipsychotics, have similar efficacy in the treatment of patients with schizophrenia (34,35). However, weight gain occurs to varying extents depending on the particular atypical antipsychotic drug (34,35).

Risperidone is associated with modest weight gain that is not related to the dose used. The majority of studies have reported a mean weight gain of approximately 2-2.5 kg over treatment periods ranging from 8 weeks to 1 year (9,10,36). The action of risperidone is mediated through an inhibition of the post-synaptic dopamine D2 receptors (7). Insulin and leptin are major peripheral signals acting in the hypothalamus to regulate energy homoeostasis and body adiposity (11). IRs and (long isoform) leptin receptors share a number of signaling cascades, such as PI3K/PKB and JAK2/STAT3 (16,22). Studies have demonstrated that atypical antipsychotic drugs exhibit inverse agonist activity at dopamine D2 receptors (37) and stimulate cAMP formation (24). Furthermore, the inhibition of dopamine D2 receptors can activate adenyl cyclase, increase the levels of cAMP, and activate PKA (38,39) and ERK; in addition, the cAMP response element-binding protein (CREB) can be activated by risperidone in a PKA-dependent manner (40).

To the best of our knowledge, this study provides the first evidence that risperidone regulates both insulin and leptin signaling in the human SH-SY5Y neuroblastoma cell line.
Treatment of the cells with risperidone markedly inhibited insulin-induced PKB activation, as well as leptin-mediated STAT3 phosphorylation (Figs. 1 and 2). These events appear to occur through adenylate cyclase downstream of the dopamine D2 receptor. It has been reported that forskolin inhibits the interleukin (IL)-6-stimulated phosphorylation of STAT3 in human aortic endothelial cells (26). Similarly, the current findings provide evidence that pre-treatment with forskolin leads to an inhibition of insulin-induced PKB phosphorylation and leptin-stimulated STAT3 phosphorylation at 24 h post-treatment (Fig. 3A and B). Moreover, the degree of inhibition by forskolin treatment of insulin- and leptin-mediated signaling was less effective than risperidone treatment, suggesting that the activation of adenylate cyclase is part of risperidone-mediated signaling events.

Specific members of the SOCS protein family are thought to play a role in the development of leptin and insulin resistance (41). It has been shown that SOCS3 is important in the development of leptin resistance (42), and that the inhibition of insulin is mediated by several SOCS proteins, including SOCS1, SOCS3, SOCS6 and SOCS7 (27-30). It has been suggested that the elevation of intracellular cAMP induces SOCS3 protein expression, leading to the inhibition of leptin and the IL-6-stimulated phosphorylation of STAT3 in human aortic endothelial cells (26). In this study, we found that cAMP-induced SOCS3 protein expression is mediated by ERK1/2. Another study found that the elevation of intracellular SOCS3 levels blocks insulin signaling through the ubiquitin-mediated degradation of IRS1 and IRS2 (43). Similarly, in this study, SOCS3 expression was enhanced by stimulation of the cells with risperidone or forskolin (Fig. 4A and B). SOCS6 expression was elevated, whereas the expression of SOCS1 and SOCS7 was unaltered (Fig. 4A). The SOCS6 protein has been shown to be a key factor in the inhibition of insulin-dependent PKB activation and IR-mediated IRS1 phosphorylation (28). cAMP-stimulated ERK signaling occurs through several mechanisms and is a cell type-specific event (32), as cAMP can activate ERK1/2 in either a PKA-dependent or PKA-independent manner (44-46). In the current system using SH-SY5Y cells, the effects of risperidone on ERK1/2 were dependent on PKA activity (Fig. 5B). These results were confirmed by pre-treatment of the cells with the MEK inhibitor, U0126, which clearly indicates that the risperidone-induced SOCS3 and SOCS6 protein expression is dependent on ERK1/2 activity (Fig. 6). Thus, these data provide clear evidence that the action of risperidone may be an important mechanism underlying the attenuation of insulin-induced PKB phosphorylation and leptin-stimulated STAT3 phosphorylation.

In conclusion, this study demonstrates that the action of risperidone in the human SH-SY5Y neuroblastoma cell line involves the induction of both SOCS3 and SOCS6 proteins. A possible mechanism underlying risperidone-induced insulin and leptin resistance is suggested (Fig. 7). The administration of risperidone triggers the accumulation of cAMP, which leads to the enhancement of the expression of both SOCS3 and SOCS6 in a PKA/ERK1/2 signaling-dependent manner. SOCS3 and SOCS6 block insulin-stimulated PKB activity and SOCS3 inhibits the leptin-stimulated phosphorylation of STAT3. Based on the present data and previously published findings, it is possible to delineate a tentative model in which risperidone, through the cAMP/PKA/ERK pathway, induces the expression of SOCS3 and SOCS6 proteins. These findings suggest that this may be an important mechanism underlying the risperidone-induced insulin and leptin resistance, which leads to weight gain in schizophrenic patients.

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References


