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Leptin But Not Ciliary Neurotrophic Factor (CNTF) Induces Phosphotyrosine Phosphatase-1B Expression in Human Neuronal Cells (SH-SY5Y): Putative Explanation of CNTF Efficacy in Leptin-Resistant State

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Growing evidences suggest that obesity is associated with hypothalamic leptin resistance, leading to the alteration of food intake control. Alternative treatment using ciliary neurotrophic factor (CNTF) has been suggested because CNTF exerts a leptin-like effect, even in leptin-resistant states, but the mechanisms by which CNTF maintains this effect are not yet understood. Both leptin and CNTF act in the hypothalamus through similar signaling pathways including janus kinase-2/signal transducer and activator of transcription (STAT)-3 pathway. To explore the differences and interactions between leptin and CNTF signaling pathways, differentiated human neuroblastoma cells (SH-SY5Y) were exposed to either leptin or CNTF and then challenged for each cytokine. Leptin pretreatment completely abolished leptin-dependent STAT-3 and ERK 1/2 phosphorylations without affecting CNTF action. The lack of cross-desensitization between leptin and CNTF signaling pathways occurred despite the induction of suppressor of cytokine signaling-3 in response to both cytokines. Interestingly, leptin as well as insulin induced the expression of phosphotyrosine phosphatase (PTP)-1B, whereas CNTF treatment did not affect its expression. In addition, acute leptin treatment but not CNTF induced PTP-1B expression in mouse hypothalamic arcuate nucleus. Furthermore, the overexpression of human PTP-1B in SH-SY5Y cells completely abolished leptin- and insulin-dependent janus kinase-2, STAT-3, and ERK 1/2 phosphorylations, but CNTF action was not altered. Collectively, our results suggest that PTP-1B constitutes a key divergent element between leptin/insulin and CNTF signaling pathways at the neuronal level, which may constitute a possible mechanism that explains the efficacy of CNTF in leptin-resistant states. (Endocrinology 150: 1182–1191, 2009)
high leptin plasma levels, suggesting a leptin resistance state (1, 5, 6). Furthermore, leptin resistance is frequently associated with other metabolic diseases such as type 2 diabetes (7, 8).

The mechanisms that cause the failure of elevated leptin levels to induce weight loss in obese humans or rodents have not yet been established and may involve multiple mechanisms such as an alteration of leptin signaling by the increased expression of suppressor of cytokine signaling (SOCS)-3 (7, 9) or as a defect in leptin transport to the central nervous system (10–12). Circulating leptin levels are positively correlated to the degree of adiposity; thus, whether the elevated leptin associated with obesity contributes to leptin resistance or is the consequence of obesity is, however, still a matter of speculation. Hence, overcoming hypothalamic leptin resistance may constitute a major step of reestablishing a normal control of food intake and energy expenditure in leptin-resistant obese.

The ciliary neurotrophic factor (CNTF), a cytokine from the IL-6 family, has shown leptin-like effects even in the peptin resistance state or animal models of obesity (13–15). CNTF clearly inhibits food intake, reduces body weight, and improves insulin sensitivity in normal and leptin-resistant mice (13, 15–17). CNTF acts on arcuate nucleus of the hypothalamus that expresses a tripartite complex receptor consisting of CNTF receptor-α, leukemia inhibitory receptor, and Glycoprotein 130 (16, 18). Similarly to leptin, CNTF binding to its receptor activates JAK-2/STAT-3 signaling pathway, which controls the expression of both anorexigenic and orexigenic neuropeptides. Despite the similarity between CNTF and leptin signaling pathways, it has been reported that the two cytokines initiate differential patterns of gene expression in the hypothalamus, suggesting distinct mechanisms of action (19, 20). Nevertheless, the divergence in early neuronal intracellular signaling steps between leptin and CNTF is still unknown, and its characterization is necessary for the understanding of the leptin resistance phenomenon and may identify new therapeutic targets. In the present work, we used a human differentiated neuroblastoma cell line (SH-SY5Y) that express both leptin and CNTF receptors in addition to insulin receptor (21–25). In this neuronal cell model, we show that in contrast to the cross-down-regulation between ObRb and insulin receptor (21, 26), the overexpression of leptin in the hypothalamus is associated with obesity contributes to leptin resistance or is the consequence of obesity is, however, still a matter of speculation. Hence, overcoming hypothalamic leptin resistance may constitute a major step of reestablishing a normal control of food intake and energy expenditure in leptin-resistant obese.

Materials and Methods

Materials and chemicals

The human neuroblastoma cell line (SH-SY5Y) was obtained from Dr. B. Dufy (Unit Mixte de Recherche 5343, Centre National de la Recherche Scientifique, Bordeaux-II University, Bordeaux, France). All cell culture reagents were purchased from Invitrogen (Cergy-Pontoise, France). Antibodies directed toward STAT-3, phospho-STAT-3, ERK 1/2, phospho-ERK 1/2, JAK-2, and phospho-JAK-2 were from Cell Signaling (Ozyme, Saint Quentin-en-Yvelines, France). Antibodies against PTP-1B (sc-14021) were from Santa Cruz Biotechnology (Santa Cruz, CA). Insulin, all trans-retinoic acid, and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Nitrocellulose membranes were from Euromedex (Mundolsheim, France), enhanced chemiluminescence (ECL) detection reagents were from Amersham Biosciences (Buckinghamshire, UK). Recombinant ovine leptin was produced in Escherichia coli as previously described (27). Human CNTF was produced in our laboratory using synthetic DNA encoding human CNTF (C17S) and optimized for expression in E. coli that was purchased from Entelechon GmbH (Regensburg, Germany) and cloned into pMon3401 expression vector using NdeI and HindIII restriction sites. Similar steps were used for the production of human CNTF as those previously described for the production of ovine leptin (25). cDNA encoding human PTP-1B and mutant human PTP-1B (nonfunctional) were a generous gift from Dr. T. C. Meng (University of Taiwan).

Cell stimulation and immunoblotting

SH-SY5Y cells were seeded in six-well dishes and differentiated for 15 d with retinoic acid as previously described (21). Serum-starved cells were incubated in serum-free DMEM in presence or absence of insulin (100 nM), leptin (15 nM), or CNTF (1 nM) for 2, 4, or 16 h. Cells were then rinsed and stimulated for 10 min with insulin, leptin, or CNTF. After homonarial treatment, protein lysates were prepared and immunoblotting was performed. Briefly, proteins from whole-cell lysates (50–100 μg) were resolved by SDS-PAGE and electrotransferred to nitrocellulose. Membranes were then incubated with the appropriate primary and secondary antibodies, and targeted proteins were visualized by enhanced chemiluminescence reagents (ECL detection kit) followed by autoradiography.

Quantification of SOCS-3 and PTP-1B mRNA expression by quantitative RT-PCR

Total RNA from differentiated SH-SY5Y cells was extracted using RNA Insta Pure kit (Eurogentec, Angers, France) according to the manufacturer’s recommendations. One microgram of total denatured RNA was reverse transcribed, and the resulting cDNAs were submitted to quantitative PCR analysis as previously described (26). The PCR primer sequences used were as follows: SOCS-3 forward, 5'-GTCACCCCACAGCAAGTTT-3', SOCS-3 reverse, 5'-CTGAGGCTGAAGAAGTGG-3'; PTP-1B forward, 5'-CGACCAGTCAAGATCGCAGG-3', PTP-1B reverse, 5'-AACTCTTTGCGCTTCCA-3'; 18S forward, 5'-CGA-CAGATGACAGATGATAG-3'; 18S reverse, 5'-CTGGTGCT-TATCGGAATTAAC-3'.

Real-time PCR was carried out using the Roche LightCycler apparatus and the Fast Start DNA Master SYBER Green I kit (Roche Diagnostics, Mannheim, Germany). PCR amplification was performed in triplicate using the following conditions: initial activation of the hot start DNA polymerase for 15 min at 94°C followed by denaturation for 10 sec at 94°C, annealing for 10 sec at 48°C for SOCS-3 and at 57°C for PTP-1B, and extension for 10 sec at 72°C.
Forty cycles of PCR were programmed to ensure that the threshold crossing point (cycle number) was attained. Fluorescence emission was monitored continuously during cycling. At the completion of cycling, melting curve analysis was carried out to establish the specificity of the amplified product. The expression level of each mRNA and their estimated crossing point in each sample were determined relative to the standard preparation using the LightCycler computer software (Roche). A ratio of specific mRNA to 18S amplification was then calculated to correct for any difference in efficiency at reverse transcription.

Transient transfection

SH-SY5Y cells were seeded in 6-well plates and differentiated as previously described (21). Seventy percent confluent cells were then transfected using Exgen 500 (Euromedex) according to the manufacturer’s recommendations. Each well received 20 µl of Exgen 500 and 4 µg of plasmid DNA in 2ml of serum-free optiMEM medium (Invitrogen, Cergy-Pontoise, France). Cells were either not transfected or transfected with an expression vector encoding wild-type or mutant PTP-1B. After 3 h, cell medium was supplemented with 10% fetal calf serum. Twenty-four hours after transfection, SH-SY5Y cells were deprived for 16 h before stimulation with insulin (100 nM), serum. Twenty-four hours after transfection, SH-SY5Y cells were transfected with an expression vector encoding wild-type or mutant PTP-1B. After 3 h, cell medium was supplemented with 10% fetal calf serum. Twenty-four hours after transfection, SH-SY5Y cells were deprived for 16 h before stimulation with insulin (100 nM), serum. Twenty-four hours after transfection, SH-SY5Y cells were deprived for 16 h before stimulation with insulin (100 nM), serum.

Animal treatment

All experiments were performed in agreement with European legal requirements (Decree 86/609/EEC) and approved by the local committee on animal welfare. Fifteen 3-month-old male C57/BL6 mice were used in this study. Animals were housed under 12-h light, 12-h dark cycles (lights on at 0700 h) with access to food and water ad libitum. They were injected ip once daily on 4 successive days with either CNTF (0.3 mg/kg · d) or leptin (1 mg/kg · d). Control mice received a similar volume (depending on the weight) of saline solution only, according to the same schedule. Mice were killed 5 h after the last injection.

Fluorescent immunohistochemistry

The immunohistochemical protocol used in this study was modified from Vacher et al. (28). Mice were anesthetized by ip injection of pentobarbital (60 mg/kg of body weight) and transcardially perfused with PBS followed by 4% paraformaldehyde. Brains were removed and conserved in the same fixative solution at 4°C for 4 h and cryoprotected in 20% sucrose. Serial coronal 16-µm-thick sections were cut on a cryostat at −21°C (Microm; Microtech, Francheville, France). Sections were incubated in 50 mM NH4Cl for 20 min before blocking nonspecific immunoglobulin binding sites by a 2-h incubation in PBS containing 0.2% fish gelatin, 2% normal donkey serum, and 0.2% Triton X-100. They were then incubated with rabbit antiserum raised against PTP-1B (1:100; Santa Cruz Biotechnology) or SOCS-3 (1:100; Santa Cruz Biotechnology) for 48 h at 4°C. Their specificity was tested by omission of the antibodies. Primary antibodies were visualized by using Alexa-488 (Invitrogen-Molecular Probes, Paisley, UK) conjugated donkey antirabbit (1:400) for 1 h at room temperature. Sections were mounted with antifading mounting media (Vectashield; Vector Laboratories, Peterborough, UK).

FIG. 1. CNTF and leptin induce phosphorylation of STAT-3 and ERK in dose- and time-dependent manners. Differentiated SH-SY5Y cells were either subjected to increased concentrations of leptin (A and B) or CNTF (A and B) for 10 min or treated with leptin (15 nM) (C) or CNTF (1 nM) (D) at various times (from 10 to 960 min). Cell lysates were then collected and subjected to Western blot analysis. Membranes were probed sequentially with antiphospho-STAT-3 (p-STAT3) followed by anti-STAT-3 (t-STAT3) antibodies or antiphospho-ERK 1/2 (p-ERK 1/2) followed by anti-ERK 1/2 (t-ERK 1/2). The proteins on the blots were revealed by ECL. This is a representative experiment independently performed three times.
Confocal microscopy

Acquisition

Immunofluorescence was examined by confocal laser-scanning microscopy with a Zeiss LSM 510 system (Zeiss Microscopy, Jena, Germany). The argon laser was used to excite the fluorochrome at 488 nm. Optical sections were taken through the hypothalamic sample in the Z axis at 0.4-μm intervals. Each optical section was obtained by a sequential scanning and averaged three times.

Quantification

All quantification steps were performed with ImageJ 1.36b software (National Institutes of Health, Bethesda, MD). The differences in level expression of PTP-1B or SOCS-3 in the arcuate nucleus of control and treated mice were assessed by comparing the fluorescence intensities emitted by Alexa-488. The integrated fluorescence density level quantifies the sum of the values of the pixels within the reproductive contours of a whole arcuate nucleus. All measurements were performed in defined-size outline drawn and reused for every condition examined and averaged for each animal from 15 different focal planes homogeneously distributed through three hypothalamic sections.

Statistical analysis

Statistical analysis was performed using ANOVA (Statview Software program, version 5; ASAP Software, St. Ouen, France) to detect significant intergroup differences. Data are presented as means ± SEM, and P < 0.05 was considered statistically significant.

Results

Leptin and CNTF increase STAT-3 and ERK phosphorylations in dose- and time-dependent manners

To examine the effects of leptin and CNTF on STAT-3 and ERK 1/2 phosphorylations, SH-SYSY cells were treated for 10 min with increasing concentrations of leptin or CNTF. Cell lysates were subjected to Western blot analysis using adequate antibodies. Leptin and CNTF induced a dose-dependent increase in STAT-3 (Fig. 1A) and ERK 1/2 (Fig. 1B) phosphorylation levels. These phosphorylations were detectable already with 15 or 1 nM of leptin or CNTF, respectively (Fig. 1, A and B).

To establish the kinetics of STAT-3 and ERK 1/2 phosphorylations in response to leptin or CNTF, SH-SYSY cells were treated with leptin (15 nM) or CNTF (1 nM) for various periods of times. STAT-3 and ERK 1/2 phosphorylations were estimated by Western blot. Leptin (Fig. 1C) and CNTF (Fig 1D) induced the phosphorylation of STAT-3 from 10 min of incubation, which was maintained until 30 min and then gradually declined. The same pattern of phosphorylation was observed for ERK 1/2 with a maximum phosphorylation level at 10 min (Fig. 1, C and D) in response to both cytokines.

Chronic leptin treatment affects leptin-induced phosphorylation of STAT-3 and ERK but not CNTF action and vice versa

To investigate the cross-regulation between leptin and CNTF signaling pathways in SH-SYSY cells, we examined the impact of chronic leptin or CNTF treatment on STAT-3 and ERK 1/2 phosphorylations in response to both cytokines. Cells were pretreated with or without leptin (15 nM) or CNTF (1 nM) for various periods of time (2, 4, and 16 h) and then acutely stimulated for 10 min with 15 nM leptin or 1 nM CNTF. Phosphorylation of STAT-3 and ERK 1/2 was estimated by Western blot using adequate antibodies. The results were normalized to either total STAT-3 or total ERK 1/2.

Cells overexposed to leptin had significantly decreased lepin-dependent phosphorylation of STAT-3 (Fig. 2A) and ERK 1/2

![Image](https://example.com/image.png)

**FIG. 2.** Chronic effect of leptin or CNTF on leptin or CNTF-induced STAT-3 and ERK phosphorylations in SH-SYSY neuronal cells. Serum-deprived SH-SYSY cells were incubated with 15 nM leptin or 1 nM CNTF for a various period of time (2, 4, and 16 h) and then stimulated for 10 min without or with 15 nM leptin (A and B) or 1 nM CNTF (C and D). Cell lysates were then subjected to Western blot analysis. Membranes were probed sequentially with antiphospho-STAT-3 (p-STAT3) followed by anti-STAT-3 (t-STAT3) antibodies or antiphospho-ERK 1/2 (p-ERK 1/2) followed by anti-ERK 1/2 (t-ERK 1/2). The proteins on the blots were revealed by ECL and bands quantified by densitometry (using Bio-1D software, Vilber Lourma, France). The results were expressed as the ratio of p-STAT3 to t-STAT3 or p-ERK to t-ERK and presented as the means ± SEM (n = 3). * * * * * P < 0.05, P < 0.001, and P < 0.0001, respectively, compared with the control.
In M CNTF. Then STAT-3 and ERK 1/2 phosphorylations or CNTF (1 nM) for a various period of time (2, 4, and 16 h) cells. Cells were pretreated with or without insulin (100 nM) and insulin, another anorexic hormone, signaling in SH-SY5Y phosphorylation of ERK but not STAT-3 phosphorylation. Chronic insulin treatment affects CNTF-induced and B).

FIG. 3. Chronic effect of insulin or CNTF on CNTF- or insulin-induced STAT-3 and ERK phosphorylations in SH-SY5Y neuronal cells. Serum-deprived SH-SY5Y cells were incubated with 100 nM insulin or 1 nM CNTF for a various period of time (2, 4, and 16 h) and then stimulated for 10 min without or with 100 nM insulin (A and B) or 1 nM CNTF (C and D). Cell lysates were then subjected to Western blot analysis. Membranes were probed sequentially with antiphospho-STAT-3 (p-STAT3) antibodies or antiphospho-ERK 1/2 (p-ERK 1/2) followed by anti-ERK 1/2 (t-ERK 1/2). The proteins on the blots were revealed by ECL and bands quantified by densitometry (using Bio-1D software). The results were expressed as the ratio of p-STAT3 to t-STAT3 or p-ERK to t-ERK and presented as the means ± SEM (n = 3). * * * and ****, P < 0.05, P < 0.001, and P < 0.0001, respectively, compared with the control.

(Fig. 2B) in a time-dependent manner. In contrast, CNTF-dependent phosphorylation of STAT-3 and ERK 1/2 was not affected (Fig. 2, C and D). When cells were overexposed to CNTF, STAT-3 (Fig 2C), and ERK 1/2 (Fig. 2D), phosphorylations in response to CNTF were diminished in a time-dependent manner but leptin effect was not altered (Fig. 2, A and B).

Chronic insulin treatment affects CNTF-induced phosphorylation of ERK but not STAT-3 phosphorylation

Next we examined the possible cross talk between CNTF and insulin, another anorexic hormone, signaling in SH-SY5Y cells. Cells were pretreated with or without insulin (100 nM) or CNTF (1 nM) for a various period of time (2, 4, and 16 h) and then acutely stimulated for 10 min with 100 nM insulin or 1 nM CNTF. Then STAT-3 and ERK 1/2 phosphorylations were estimated as described above. Cell overexposed to insulin had significantly reduced insulin-dependent STAT-3 and ERK 1/2 phosphorylations in a time-dependent manner (Fig. 3, A and B). Insulin pretreatment did not alter CNTF-dependent STAT-3 phosphorylation (Fig. 3C) but reduced CNTF-dependent ERK 1/2 phosphorylation (Fig. 3D). When cells were overexposed to CNTF, insulin-dependent phosphorylation of ERK 1/2 was significantly altered in a time-dependent manner (Fig 3B) but not STAT-3 phosphorylation (Fig. 3A). In summary these results clearly indicate a cross talk between insulin and CNTF to desensitize their signaling pathways at the level of ERK 1/2 but not at the level of STAT-3.

Chronic effect of leptin, insulin, or CNTF pretreatments on SOCS-3 and PTP-1B mRNA expression in SH-SY5 cells

To understand the lack of cross-regulation between CNTF and leptin or insulin signaling pathways in SH-SY5Y cells, we analyzed the impact of insulin, leptin, or CNTF on the induction of the common negative regulators: SOCS-3 and PTP-1B. Cells were incubated with or without insulin (100 nM), leptin (15 nM), or CNTF (1 nM) for 4 h. Incubation of 4 h was chosen according to the results obtained in Figs. 2 and 3, because leptin, CNTF, or insulin led to dramatic down-regulation of their signals after 4 h of treatment. Then SOCS-3 and PTP-1B mRNA expressions were measured using quantitative RT-PCR. Insulin, leptin, or CNTF treatment significantly increased SOCS-3 expression by more than 5-fold (Fig. 4A). Insulin and leptin in contrast to CNTF had also significantly increased PTP-1B expression (Fig. 4B).

Overexpression of PTP-1B differentially regulates insulin, leptin, and CNTF signaling pathways in SH-SY5Y cells

To investigate whether PTP-1B affects leptin, insulin, and CNTF signaling in SH-SY5Y cells, we analyzed the effects of PTP-1B overexpression on the activation of two major signaling pathways: JAK-2/STAT-3 and ERK 1/2. Cells were transiently transfected with an expression vector encoding human wild-type PTP-1B cDNA (wt-PTP-1B) or its nonactive mutant PTP-1B (mut-PTP-1B). The total cell lysates from untransfected or transfected cells were immunoblotted with PTP-1B antibodies to ensure the overexpression of wt-PTP-1B and mut-PTP-1B in SH-SY5Y cells (Fig. 5). Cells were then stimulated for 10 min with insulin, leptin, or CNTF and JAK-2, STAT-3, and ERK 1/2 phosphorylations estimated by Western blot. In untransfected cells or cells transfected with mut-PTP-1B cDNA, insulin, leptin, and CNTF significantly activated JAK-2, STAT-3, and ERK 1/2 phosphorylations (Fig. 6, A, C, D, F, G, and H). Furthermore, the effect of CNTF on JAK-2/STAT-3 signaling pathway was more predominant that those of leptin and insulin. In contrast, in cells transfected with wt-PTP-1B cDNA (Fig. 6, B, E, and I) both leptin and...
insulin effects on JAK-2, STAT-3 and ERK 1/2 phosphorylations were totally abolished, whereas CNTF action was fully preserved.

**Effect of chronic leptin or CNTF treatment on PTP-1B and SOCS-3 expression on mouse hypothalamic arcuate nucleus**

To investigate whether the differential effect of leptin and CNTF on PTP-1B expression could be extrapolated in vivo, mice were treated for 4 successive days by leptin or CNTF, and then PTP-1B and SOCS-3 expression was determined by immunohistochemistry in mice arcuate nucleus. Leptin clearly induced both SOCS-3 and PTP-1B expression (Fig. 7), whereas CNTF was able to induce SOCS-3 but not PTP-1B (Fig. 7). We even observed a reduced amount of PTP-1B in mice treated with CNTF compared with animals treated with saline (Fig. 7).

**Discussion**

Despite the recruitment of signaling pathways similar to those of leptin, CNTF anorectic effect was maintained in leptin resistance state. As suggested by Anderson *et al.* (16), one possible explanation is that CNTF and leptin do not act on the same neuronal populations in the hypothalamus. Indeed CNTF and leptin mainly activate STAT-3 phosphorylation, respectively, in the ventromedial and the lateral parts of hypothalamic arcuate nucleus. Nevertheless, CNTF and leptin similarly target hypothalamic proopiomelanocortin neurons, indicating that both cytokines induce proopiomelanocortin expression through a STAT-3-dependent mechanism (17). We first showed that both leptin and CNTF activate STAT-3 and ERK 1/2 phosphorylation with a higher efficiency of CNTF compared with leptin in SH-SY5Y cells. The effect of leptin is significant from 15 nM, which is in good agreement with previous data obtained on the same cells, whereas the effect of leptin on STAT-3 phosphorylation, on cell proliferation or dephosphorylation of H9270-protein was observed only at 100 nM (23, 25). It is noteworthy that the common concentrations of leptin used in cell models or even in vivo for acute treatment are 50- to 100-fold higher than circulating leptin. This could be attributed to the sensitivity of Western blot technique used for measuring changes in protein phosphorylation levels. In contrast and as previously reported on SH-SY5Y cells, CNTF is efficient at 1 nM (29).

Despite the common signaling pathways shared by these cytokines, they probably mechanistically diverge on signaling components that are not yet identified. In the present study, we attempted to address this issue, and we show that the overexposure of SH-SY5Y human neuronal cells to leptin does not affect CNTF-dependent STAT-3 or ERK 1/2 phosphorylation and vice versa. Similar results were obtained when
cells were overexposed to insulin, another anorectic hormone, except that CNTF-dependent ERK 1/2 phosphorylation was attenuated, indicating a partial cross-desensitization of ERK 1/2 signaling pathway in response to insulin or CNTF. However, the mechanism underlying this phenomenon is still unknown.

In cells overexposed to leptin, SOCS-3 expression levels were significantly increased and accompanied with altered leptin-dependent STAT-3 phosphorylation but maintained CNTF action. The induction of SOCS-3 expression has been reported in several leptin-resistant models such as in diet-induced obesity (30), whereas overexpression of SOCS-3 inhibits leptin signaling through SH2 binding to tyrosine residues of ObRb leading to the abolition of STAT-3 activation (31–33). Furthermore, the conditional neuronal-specific SOCS-3 knockout conferred resistance to diet-induced obesity with elevated leptin sensitivity (34). Here we show that cell overexposure to CNTF induced SOCS-3 expression without altering leptin or insulin-dependent STAT-3 phosphorylation. Thus, SOCS-3 expression does not lead to a cross-desensitization phenomenon between CNTF and leptin signaling pathways in SH-SY5Y human neuronal cells. This is in good agreement with previous reports, indicating that GH pretreatment of adipocytes did not affect STAT-3 phosphorylation induced by cardiotropin-1a or CNTF but completely abolished GH-dependent STAT-5 phosphorylation (35). Our finding could, at least partially, explain the anorexic effect of CNTF in a leptin-resistant environment with leptin-induced GH-dependent STAT-5 phosphorylation induced by cardiotropin-1a or CNTF but completely abolished GH-dependent STAT-5 phosphorylation (35). Our finding could, at least partially, explain the anorexic effect of CNTF in a leptin-resistant environment with leptin-induced GH-dependent STAT-5 phosphorylation (35).
leptin signaling in CHO cells expressing both ObRb and prolactin receptors (36). It has been also reported that SOCS-3 blocks insulin signaling by ubiquitin-mediated degradation of insulin receptor substrate-1 and insulin receptor substrate-2 (37), and SOCS-3 inhibits IL-1 signaling by targeting the TNF receptor-associated factor-6/TGFβ-activated kinase complex through the ubiquitination of TNF receptor-associated factor-6. We may suggest that similar mechanisms could affect leptin and CNTF signaling pathways.

SOCS-3 expression is similarly induced by leptin and CNTF and because it is not involved in a cross-desensitization process between these two cytokines, we focused on PTP-1B, the other major negative regulator involved in leptin resistance (38, 39). Indeed, previous studies have evidenced a resistance to diet-induced obesity and increased leptin signaling in PTP-1B-deficient mice (40). Likewise more recent studies reported hypersensitivity to leptin in neuron-specific PTP-1B knockout mice (41). In addition, age-dependent leptin resistance has been reported to be associated with an increased hypothalamic PTP-1B expression (42). Here the overexposure of SH-SY5Y human neuronal cells to leptin or insulin, but not CNTF, induced the expression of PTP-1B (43). Thus, PTP-1B may constitute a potential signaling component involved in the divergence between leptin and CNTF signaling pathways at the neuronal level. This hypothesis was corroborated by the fact that the transient overexpression of PTP-1B completely abolished leptin and insulin signaling pathways without altering CNTF action in SH-SY5Y human neuronal cells. Thus, we may not suggest, as for SOCS-3, that PTP-1B inhibits only the stimulating cytokine but not CNTF. The mechanisms underlying the selective effect of PTP-1B are still unclear.

Taken together, these data indicate that overexposure to leptin induces both SOCS-3 and PTP-1B, whereas CNTF induces only SOCS-3, and overexpression of PTP-1B in SH-SY5Y human neuronal cells alters leptin and insulin but not CNTF signaling pathways. This may constitute a major step in the understanding of the mechanisms involved in the efficacy of CNTF to reduce body weight in a variety of rodent models of obesity/type 2 diabetes and even in humans exhibiting leptin resistance. In conclusion, PTP-1B is clearly a divergent component between leptin and insulin vs. CNTF signaling, and understanding the mechanism involved in its inefficacy to inhibit CNTF signaling may

FIG. 7. Effect of leptin or CNTF chronic treatment on PTP-1B and SOCS-3 expression in mouse hypothalamic arcuate nucleus. Mice were treated for 4 d with saline, leptin (1 mg/kg · d), or CNTF (0.3 mg/kg · d) and using adequate antibodies SOCS-3 (A–C) and PTP-1B (D–F) were detected and signal quantified using ImageJ software 1.36b (National Institutes of Health). The results of quantification are reported in G for SOCS-3 and H for PTP-1B. The results in G and H are expressed as mean ± SEM where ** indicates P < 0.005 (n = 5).
reveal new therapeutic targets for the treatment of leptin resistance.

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Disclosure Statement: Y.B., F.B., C.-M.V., B.D., J.D., and M.T. have nothing to declare. A.G. is a retired professor at The Hebrew University.

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