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Neuropoietin Attenuates Adipogenesis and Induces Insulin Resistance in Adipocytes*

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Recent findings have implicated gp130 receptor ligands, particularly ciliary neurotrophic factor (CNTF), as potential anti-obesity therapeutics. Neuropoietin (NP) is a recently discovered cytokine in the gp130 family that shares functional and structural features with CNTF and signals via the CNTF receptor tripartite complex comprised of CNTFRα, LIF receptor, and gp130. NP plays a role in the development of the nervous system, but the effects of NP on adipocytes have not been previously examined. Because CNTF exerts anti-obesogenic effects in adipocytes and NP shares the same receptor complex, we investigated the effects of NP on adipocyte development and insulin action. Using cultured 3T3-L1 adipocytes, we observed that NP has the ability to block adipogenesis in a dose- and time-dependent manner. We also observed that cultured adipocytes, as well as murine adipose tissue, are highly responsive to acute NP treatment. Rodents injected with NP had a substantial increase in STAT3 tyrosine phosphorylation and ERK 1 and 2 activation. We also observed the induction of SOCS-3 mRNA in 3T3-L1 adipocytes following NP treatment. Unlike CNTF, our studies have revealed that NP also substantially attenuates insulin-stimulated glucose uptake in 3T3-L1 adipocytes. In addition, NP blocks insulin action in adipose tissue in vivo. These observations are supported by data demonstrating that NP impairs insulin signaling via decreased activation of both IRS-1 and Akt. In summary, we have observed that both adipocytes in vitro and in vivo are highly responsive to NP, and this cytokine has the ability to affect insulin signaling in fat cells. These novel observations suggest that NP, unlike CNTF, may not be a viable obesity therapeutic.

In recent years, gp130 receptor ligands, notably ciliary neurotrophic factor (CNTF), have been implicated as potential targets for obesity therapeutics (reviewed in Ref. 1). It is well known that obese individuals are usually leptin-resistant, despite increased production of endogenous leptin (2, 3). Hence, leptin is not a viable cytokine therapeutic. Therefore, the characterization of cytokine-mediated anti-obesity mechanisms that act independently of leptin may be utilized for the treatment of obesity. Because gp130 and leptin receptor (LRβ) possess substantial sequence homology (4, 5) and both activate the JAK/STAT pathway (6) and other signal transduction pathways that are important in energy balance (7–10), gp130 cytokines may provide a mechanism to evade leptin resistance. Also, recent studies have shown that CNTF can have anti-obesogenic effects in both high fat diet induced and db/db mice (11), indicating that the effects of CNTF are not dependent on a functional leptin receptor. In addition, leptin does not stimulate the tyrosine phosphorylation of gp130 to activate the JAK/STAT pathway (12). These data suggest that neither gp130 nor LRβ signal through each others ligand. Likewise, the efficacy of the gp130 cytokine as a therapeutic would not be altered because of the leptin-resistant phenotype.

CNTF was originally characterized through its ability to maintain the survival of parasympathetic and motor neurons (reviewed in Ref. 13). The anti-obesogenic properties of CNTF were discovered in clinical trials examining its effects on progression of the neurodegenerative disease amyotrophic lateral sclerosis (14). This observation led to many studies to understand the effects of CNTF on weight loss. Early studies revealed that the CNTF receptors and LRβ were colocalized in the hypothalamus, and CNTF administration activated or suppressed the same genes as leptin in the arcuate nucleus (15). These studies suggested similar roles for these cytokines in neuronal signaling pathways that affect satiety. Also, CNTF treatment of leptin-deficient ob/ob and leptin-resistant db/db mice as well as mice with diet-induced obesity and partial resistance to leptin was found to reduce adiposity, hyperphagia, and hyperinsulinemia (15). Subsequent studies revealed that Axokine, a human recombinant form of CNTF, also improved the phenotype of obese, leptin-resistant rodents (16). Collectively, these studies provide evidence to support the use of gp130 cytokines, specifically CNTF, as potential obesity therapeutics.

Though all gp130 cytokines share the transmembrane protein gp130 as a transducer in their receptor complex (reviewed in Ref. 17), the other components of each respective complex are variable. Interleukin-6 (IL-6) and interleukin-11 (IL-11) first bind to IL-6 receptor α and IL-11 receptor α, respectively, and then recruit a gp130 homodimer complex for signaling. Interleukin-27 (IL-27) engages a gp130/WSX-1 heterodimeric receptor complex (18). Leukemia inhibitory factor (LIF) and
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oncostatin M (OSM) directly bind the gp130/LIF receptor (LIFR) complex to induce signal transduction; OSM can alternatively utilize a gp130/OSMR receptor (OSMR) complex. Cardiotrophin-1 (CT-1) recruits an α-receptor (19), which to date has not been characterized, and also signals via gp130/LIFR. CNTF, cardiotrophin-like cytokine (CLC), and neuropoietin (NP) all recruit CNTFRα and then induce the formation of a gp130/LIFR heterodimer for signal transduction (20–22). The requirement of NP to utilize CNTF signaling receptors was supported by studies which showed that NP did not signal in cells expressing only LIFR and gp130, but not CNTFRα (22).

CNTF-deficient mice develop normally and exhibit only a mild loss of motor neurons later in adulthood (23), whereas newborn mice lacking CNTFRα display a dramatic loss of motor neurons, fail to initiate the feeding process, and die shortly after birth (24). These studies provided evidence for the existence of an alternative ligand for the CNTF receptor complex, which led to the initial characterization of NP, a 22-kDa member of the gp130 cytokine family (22). Though not detected in adult tissues, NP is expressed in embryonic mouse neuroepithelia at a time during development when both CNTF and CLC are absent (22). NP, like CNTF, can also mediate motor neuron survival (22). Importantly, the actions of NP are dependent on the presence of the tripartite functional receptor complex for CNTF that includes CNTFRα, LIFR, and gp130 (20, 22). Because of the functional similarities between NP and CNTF and their utilization of the same tripartite receptor complex, we hypothesized that NP might mimic the anti-obesogenic properties of CNTF. We have previously shown that CNTF treatment induces STAT3 activation in adipocytes in vitro and in vivo and that CNTFRα is expressed in adipose tissue and regulated in obese/type II diabetic rats (25).

Because CNTF induces weight loss (14–16) and does not result in the development of insulin resistance in adipocytes (25) and NP utilizes the same tripartite receptor complex as CNTF (22), we examined the ability of NP to modulate adipocyte differentiation and insulin action. Although the roles of NP in the nervous system have been examined, the effects of NP on adipocytes have not been studied. Our results demonstrate that both 3T3-L1 adipocytes and rat adipose tissue are responsive to NP treatment. Our studies also indicate that NP specifically activates STAT3 Tyr705 in vivo and in vitro. We also observed the induction of SOCS-3 mRNA in 3T3-L1 adipocytes and MAPK (ERK 1 and 2) activation in vivo following NP treatment. In addition, our studies demonstrate that NP can inhibit adipogenesis and negatively affect insulin signaling in cultured adipocytes. Overall, our findings indicate that adipocytes are highly responsive to NP. However, unlike CNTF, our results indicate that NP is not a suitable anti-obesity therapeutic, because it inhibits insulin action in adipocytes.

EXPERIMENTAL PROCEDURES

Materials—Dulbecco’s Modified Eagle’s Medium (DMEM) was purchased from Sigma. Bovine and fetal bovine sera were purchased from Atlanta Biological. Mouse recombinant NP, mouse recombinant CT-1, mouse recombinant OSM, and rat recombinant CNTF were all purchased from R&D Systems. Mouse recombinant LIF was purchased from BD Transduction. GH (porcine) and insulin (bovine) were purchased from Sigma. STAT1 and STAT5A were polyclonal IgGs purchased from Santa Cruz Biotechnology. STAT3 was a monoclonal IgG purchased from BD Transduction. The phosphospecific monoclonal antibody for STAT3 (Tyr705) was purchased from BD Transduction. The highly phosphospecific polyclonal antibodies for IRS1 (Tyr524/532) and Akt (Ser473) were purchased from BIO-SOURCE and Cell Signaling, respectively. The ERK polyclonal antibody and the PPARγ monoclonal antibody were both purchased from Santa Cruz Biotechnology. The polyclonal active MAPK antibody was purchased from Promega. Adiponectin polyclonal antibody was purchased from Affinity Bioreagents. DNase I and TRIzol were both purchased from Invitrogen. Nitrocellulose and Zeta Probe-GT membranes were purchased from Bio-Rad. The BCA kit and the enhanced chemiluminescence kit were purchased from Pierce. Horseradish peroxidase-conjugated secondary antibodies were purchased from Jackson ImmunoResearch Laboratories.

Cell Culture—Murine 3T3-L1 preadipocytes were plated and grown to 2-day post-confluence in DMEM containing 10% bovine serum. Medium was changed every 48 h. Cells were induced to differentiate by changing the medium to DMEM containing 10% fetal bovine serum, 0.5 mM 3-isobutylmethylxanthine, 1 μM dexamethasone, and 1.7 μM insulin (MDI). After 48 h, this medium was replaced with DMEM supplemented with 10% fetal bovine serum, and cells were maintained in this medium until utilized for experimentation. In some experiments, cells were induced to differentiate in the presence of NP and treated at various times after the addition of the differentiation mixture.

Preparation of Whole Cell Extracts—Cell monolayers of 3T3-L1 adipocytes were harvested in a non-denaturing buffer containing 150 mM NaCl, 10 mM Tris, pH 7.4, 1 mM EGTA, 1 mM EDTA, 1% Triton X-100, 0.5% Igepal CA-630, 1 μM phenylmethylsulfonfyl fluoride, 1 μM pepstatin, 50 trypsin inhibitory milliunits of aprotinin, 10 μM leupeptin, and 2 mM sodium vanadate, and frozen. Next, the samples were thawed and centrifuged at 13,000 rpm at 4 °C for 10 min. Supernatants containing whole cell extracts were analyzed for protein content using a BCA kit according to the manufacturer’s instructions.

Preparation of Nuclear and Cytosolic Extracts—Untreated and NP-treated serum-deprived 3T3-L1 adipocytes were harvested in a nuclear homogenization buffer containing 20 mM Tris (pH 7.4), 10 mM NaCl, and 3 mM MgCl2. Igepal CA-630 was added to a final concentration of 0.15%, and cells were homogenized with 16 strokes in a Dounce homogenizer. The homogenates were centrifuged at 3500 rpm for 8 min. Supernatants were saved as cytosolic extract, and the nuclear pellets were resuspended in half the volume of nuclear homogenization buffer and were centrifuged as before. The pellet was then resuspended in an extraction buffer containing 20 mM HEPES (pH 7.9), 420 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, and 25% glycerol. Both cytosolic and nuclear samples were frozen until time for analysis and later thawed at room temperature. 200 units of DNase I were added to each nuclear sample, and the tubes were inverted and incubated an additional 10 min at room temperature. Finally, the samples were subjected to centrifugation at 13,000 rpm at 4 °C for 10 min. Cytosolic fractions
and supernatants containing nuclear extracts were analyzed for protein content using a BCA kit (Pierce).

**Rodent Adipose Tissue Isolation**—Animals were euthanized by cervical dislocation, and tissues were immediately removed and frozen in liquid nitrogen. Frozen tissues were homogenized in a buffer containing 150 mM NaCl, 10 mM Tris, pH 7.4, 1 mM EGTA, 1 mM EDTA, 1% Triton X-100, 0.5% Igepal CA-630, 1 mM phenylmethylsulfonyl fluoride, 1 mM pepstatin, and 50 trypsin inhibitory milliunits of aprotinin, 10 mM leupeptin, and 2 mM sodium vanadate. Homogenates were centrifuged for 10 min at 5,000 rpm to remove any debris and insoluble material and then analyzed for protein content. All animal studies were carried out with protocols which were reviewed and approved by institutional animal care and use committees.

**Gel Electrophoresis and Western Blot Analysis**—Proteins were separated in 7.5% polyacrylamide (acrylamide from National Diagnostics) gels containing SDS according to Laemmli (26) and transferred to nitrocellulose membrane in 25 mM Tris, 192 mM glycine, and 20% methanol. Following transfer, the membrane was blocked overnight in 4% milk at 4 °C. Results were visualized with horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescence.

**RNA Analysis**—Total RNA was isolated from cell monolayers with TRIzol according to the manufacturer’s instructions with minor modifications. For Northern blot analysis, 20 μg of total RNA was denatured in formamide and electrophoresed through a formaldehyde/agarose gel. The RNA was transferred to Zeta Probe-GT, cross-linked, hybridized, and washed as previously described (27). Probes were labeled by random priming using Klenow fragment and [α-32P]dATP.

**Determination of [3H]-labeled 2-Deoxyglucose Uptake**—The assay of 2- [3H]deoxyglucose was performed as previously described (28). Prior to the assay, mature 3T3-L1 adipocytes were serum-deprived for 2 h and then incubated in the presence or absence of insulin (50 nm) for 10 min and the presence or absence of NP for 6 h. Glucose uptake was initiated by addition of 2- [3H]deoxyglucose at a concentration of 0.1 mM 2-deoxyglucose in 1 μCi of 2-[3H]deoxyglucose in Krebs-Ringer-Hepes buffer and incubated for 3 min at room temperature. Glucose uptake is reported as [3H]radioactivity, corrected for nonspecific diffusion (5 μM cytochalasin B) and normalized to total protein content as determined by BCA analysis. Nonspecific uptake and absorption was always less than 10% of the total uptake. Uptake measurements were performed in triplicate under conditions where hexose uptake was linear.

**RESULTS**

To determine if adipocytes were responsive to NP, we examined the ability of this gp130 cytokine to activate STAT proteins in adipocytes. Fully differentiated 3T3-L1 adipocytes were treated with several different gp130 cytokines including CT-1, LIF, OSM, and NP for 10 min. As shown in Fig. 1, NP-activated STAT3 Tyr705 phosphorylation in a manner equivalent to CT-1, LIF, and OSM. Because growth hormone (GH) is not a gp130 cytokine and is not a potent activator of STAT3 tyrosine phosphorylation in these cells (29), this growth factor was used as a negative control. The levels of STAT3 protein were equivalent in each sample.

To investigate the specificity of NP action in adipocytes, serum-deprived fully differentiated 3T3-L1 adipocytes were treated with various doses of NP (0.12, 0.25, 0.5, 1.2, and 2.5 nM) or insulin for 10 min. Although we detected low levels of STAT3 activation with 0.25 and 0.5 nM NP, we observed substantial activation with 1, 2, and 2.5 nM NP treatments (Fig. 2A). These results indicate that NP activated STAT3 Tyr705 in a dose-dependent manner, with maximal activation at 1 nM. Because insulin induces the serine, but not the tyrosine, phosphorylation of STAT3 (29, 30), this peptide hormone was used as a negative control. ERK (MAPK) protein levels are shown to demonstrate even loading.

Previous studies have demonstrated that, in addition to STAT3, STATs 1, 5A, 5B, and 6 are expressed in both cultured and native adipocytes (31). Therefore, we examined the ability
of NP to activate other adipocyte-expressed STATs. Fully differentiated 3T3-L1 adipocytes were treated with 1 nM NP for different times, ranging from 0.3 to 5 h. The cells were homogenized and fractionated into nuclear and cytosolic extracts. The activation of STATs was observed by examining the presence of these proteins in the nucleus. Based on the results in Fig. 2B, NP did not activate STATs 1 and 5A, as the levels of these STATs in the nucleus of NP-treated cells were similar to untreated control cells. In contrast, we observed a robust time-dependent activation and nuclear translocation of STAT3.

Because various members of the gp130 cytokine family are known to induce Suppressors of Cytokine Signaling (SOCS)-3 (32), we examined the ability of NP to induce SOCS-3 expression in fully differentiated 3T3-L1 adipocytes. As shown in Fig. 3, NP treatment resulted in a transient induction of SOCS-3 mRNA that was detectable at 30 min, and maximum expression occurred after a 1-h treatment. Both aP2 and β-actin mRNA levels were unaffected by the NP treatments. Ribosomal RNAs are shown to demonstrate even loading of total RNA.

The data in Figs. 1–3 clearly indicated that NP activates STAT3 and SOCS-3 in adipocytes in vitro; hence, we wanted to determine if adipocytes in vivo were responsive to NP. Sprague-Dawley rats were injected with either NP (0.20 μg/g animal) or vehicle (saline) and then sacrificed 15 min following the injection. As shown in Fig. 4, NP injection induced the activation of STAT3 Tyr705 in both the epididymal and retroperitoneal adipose tissues, but not in skeletal muscle. NP also induced the activation of MAPK (ERK 1 and 2) in adipose tissue, but not skeletal muscle. The inability of NP to induce STAT3 tyrosine phosphorylation in skeletal muscle was not caused by decreased levels of STAT3 expression.

Because gp130 cytokines have been reported to have differential effects on adipogenesis (33–35), we investigated the ability of NP to modulate adipocyte differentiation. As shown in Fig. 5A, 3T3-L1 preadipocytes were induced to differentiate in the presence or absence of exogenously added NP, and whole cell extracts were harvested at the indicated time points. Adipogenesis was assessed by examining the induction of PPARγ and adiponectin. NP attenuated adipogenesis, as evidenced by the decrease in expression of both PPARγ and adiponectin at 72 h and 96 h after the induction of differentiation. We also examined the ability of NP to modulate adipogenesis in the absence of insulin. The preadipocytes were induced to differentiate with normal differentiation medium (MDI) or medium minus insulin (MD) and harvested 96-h postinduction. As shown in Fig. 5B, NP was capable of blocking the induction of both PPARγ and adiponectin without insulin present in the induction mixture. Because cells treated with MD medium exhibited less differentiation, we used a greater protein concentration than in the normally differentiated cells. To compare the effect of NP to other gp130 cytokines, 3T3-L1 preadipocytes were induced to differentiate in the presence of NP, as well as CNTF, CT-1, OSM, and GH. As shown in Fig. 5C, both NP and OSM greatly attenuated adipogenesis as shown by the decreased expression of PPARγ and adiponectin, whereas CNTF and CT-1 had no effect on adipocyte marker expression and was similar to untreated cells. We have previously shown that CT-1 (33) and CNTF (25) do not inhibit adipogenesis. These results also confirm recent studies showing that OSM can inhibit murine (35) and human (36) adipocyte differentiation. It is also known that GH does not affect the adipogenesis of 3T3-L1 cells (37). STAT3 expression does not change during adipogenesis of murine (38) or human (39) cells and was used as a loading control.

To demonstrate the specificity of the ability of NP to negatively affect adipogenesis, we investigated whether the attenuation was dose- and time-dependent. In Fig. 6A, 3T3-L1 preadipocytes were induced to differentiate in the presence of NP at the various doses indicated and harvested 4 days after the induction of adipogenesis. Cells were treated every 24 h with a fresh bolus of NP. Although there were no changes in protein expression at 0.25 nM NP, we observed a decrease in PPARγ and adiponectin expression at 0.5 nM, with the greatest attenuation of adiponectin at 1 nM and PPARγ at 2 nM. In Fig. 6B, 3T3-L1 preadipocytes were induced to differentiate in the presence of NP at different times post the induction of adipogenesis. Cells were treated every 24 h with a fresh bolus of NP after their
initial treatment. Our data indicated that a decrease in expression of adipogenic markers occurred when NP was added between 0- and 48-h postinduction. When NP was added 72- or 96-h postinduction, we did not observe a change in PPARγ or adiponectin levels. The efficacy of NP was assessed by examining the activation of STAT3 Tyr705, and STAT3 protein levels were observed to demonstrate even loading in both experiments.

Because other gp130 cytokines have been reported to have effects on insulin signaling, we examined how NP affects insulin action. As shown in Fig. 7A, mature 3T3-L1 adipocytes were pretreated with NP for the times indicated, and then exposed to an acute 10-min treatment of insulin. As expected, a 10-min insulin treatment resulted in the activation of IRS-1 Tyr896 and Akt Ser473. However, pretreatment with NP prior to insulin stimulation resulted in a decrease in IRS-1 Tyr896 signaling at 10 min, and we observed this inhibition with each NP pretreatment. Although we did not see a decrease in Akt Ser473 signaling after the 10-min NP pretreatment, we observed a significant attenuation in Akt activation after 2 h with NP pretreatment. The efficacy of the NP was demonstrated by confirming the activation of STAT3 Tyr705. A longer exposure of the film revealed lower levels of STAT3 phosphorylation following longer NP pretreatment. ERK (MAPK) protein levels were examined to demonstrate even loading.
Because NP negatively affects insulin signaling in adipocytes, we examined the effects of NP on insulin signaling in preadipocytes. As shown in Fig. 7B, confluent 3T3-L1 preadipocytes were pretreated with NP (+) for the times indicated, and then exposed to an acute 10-min treatment of insulin. Although a 10-min insulin treatment resulted in the activation of IRS-1 Tyr896, pretreatment with NP for various times prior to insulin stimulation resulted in a decrease in IRS-1 Tyr896 phosphorylation. The efficacy of NP was demonstrated by observing the activation of STAT3 Tyr705. ERK (MAPK) protein levels were observed to demonstrate even loading.

Because we observed a decrease in insulin signaling with NP pretreatment, we examined the ability of NP to regulate insulin-stimulated glucose uptake in 3T3-L1 adipocytes. As shown in Fig. 7C, a 10-min treatment with insulin resulted in a nearly 5-fold increase in glucose uptake as compared with the vehicle-treated cells. However, following a 6-h pretreatment with NP, we observed a substantial decrease in insulin-stimulated glucose uptake compared with controls. The lack of insulin-stimulated uptake was not due to a significant increase in basal glucose uptake.

The data in Fig. 7 clearly indicated that NP negatively affects insulin signaling in adipocytes in vitro; therefore, we examined the ability of NP to attenuate insulin signaling in adipose tissue in vivo. 7-week old male C57B1/6J mice were given an intraperitoneal injection of either vehicle (saline) control, or insulin (5 units/kg), with or without a pre-injection of NP (0.20 µg/g animal). In each individual experiment, two animals were used for each condition. The mice were treated with insulin alone or following either a 30- or 90-min NP pretreatment. After the mice were injected with insulin for 15 min, they were sacrificed, and epididymal fat pads (A) and retroperitoneal fat pads (B) were immediately removed and frozen in liquid nitrogen. 250 µg (epididymal) or 150 µg (retroperitoneal) of each tissue extract were separated by SDS-PAGE, transferred to nitrocellulose, and subjected to Western blot analysis. Each figure represents an experiment independently performed twice on separate groups of mice.
DISCUSSION

Because of recent studies that implicate gp130 cytokines, specifically CNTF, as potential therapeutic targets for obesity (22), we investigated the actions of NP in adipocytes both in vitro and in vivo. Even though sequence analysis suggests that the np gene has evolved into a pseudogene in humans (22), we hypothesized that NP, due to similar functional roles with CNTF, could induce anti-obesogenic effects and be considered a potential therapeutic treatment. Although NP signals via the same tripartite receptor complex as CNTF, our findings demonstrate both similar and opposing roles for NP compared with CNTF. In adipocytes, both NP and CNTF activate STAT3 Tyr705 in vivo and in vitro and induce the nuclear translocation of STAT3 but not STAT1 and 5A in vitro. Our studies also indicate that NP treatment, like CNTF, resulted in ERK 1 and 2 activation in vivo (6). As shown in Fig. 3, NP and CNTF (33) are also alike in their ability to induce SOCS-3 mRNA in 3T3-L1 adipocytes. SOCS-3 proteins have been shown to play a role in both leptin resistance (40) and insulin resistance (41). Interestingly, SOCS-3 is induced by all known gp130 cytokines (32, 33, 42–47), as well as additional hormones, including insulin (48), growth hormone (49), and leptin (50). However, many inducers of SOCS-3, including CNTF, do not induce insulin resistance. It is striking that so many cytokines, in addition to NP and CNTF result in the activation of STAT3, ERK 1 and 2 and SOCS-3, yet the action of these cytokines have different responses despite the activation of the same signaling proteins.

Although our observations indicated that NP induced the same signaling proteins as CNTF in adipocytes, we observed different effects of NP, as compared with CNTF, on both adipogenesis and insulin action. Our results demonstrated that NP can inhibit adipogenesis in a dose- and time-dependent manner in 3T3-L1 cells, whereas CNTF does not attenuate adipogenesis in this model system (25). The different effects do not appear to be due to altered levels of STAT3 activation, as equivalent levels of NP and CNTF results in similar activation of STAT3 (data not shown). CT-1, LIF, and OSM activate STAT3 in a manner similar to CNTF and CT-1, yet, neither CNTF nor CT-1 attenuate adipogenesis (25, 33). Of all the gp130 cytokines, NP is the most structurally and functionally related to CNTF and CT-1, yet, neither CNTF nor CT-1 attenuate adipogenesis (25, 33). These observations suggest the specificity of these gp130 cytokines to modulate adipocyte differentiation involves more than their ability to activate STAT3 and ERK and induce SOCS-3 expression and indicate that NP possesses unique signaling abilities that differ from CNTF despite use of the same receptor complex.

Despite the presence of CNTFRA in skeletal muscle (25, 51), our results demonstrate that acute NP treatment failed to activate STAT3 Tyr705 in skeletal muscle in vivo, in contrast to CNTF which induced STAT3 Tyr705 phosphorylation in the skeletal muscle of rodents (25). In addition, CNTF has been shown to increase fat oxidation and reduce insulin resistance via an AMP-activated protein kinase (AMPK)-dependent mechanism in skeletal muscle (11). It is possible that NP could also mediate signal transduction in skeletal muscle via other signaling pathways that are independent of the JAK/STAT pathway, as we did not test this hypothesis in our experiments. Nonetheless, our studies indicate that NP and CNTF have very divergent actions not only in adipose tissue, but also in skeletal muscle.

Importantly, our studies indicate that NP treatment not only attenuates insulin signaling in vitro, as demonstrated by decreased activation of both IRS-1 Tyr896 and Akt Ser473, but also in vivo, as evidenced by the reduction in Akt activation. In comparison, CNTF treatment was shown to act synergistically with insulin to increase the activation of both IRS-1 Tyr896 and Akt Ser473 in cultured adipocytes (25) and in vivo studies demonstrated that CNTF could improve insulin sensitivity in diet-induced and genetic models of obesity (11). In addition to our findings that NP negatively regulates insulin signaling proteins, we demonstrated that NP treatment impaired insulin-stimulated glucose uptake in cultured adipocytes. Because CNTF treatment of cultured adipocytes did not result in insulin resistance (25), these results clearly indicate significant and opposing roles between NP and CNTF.

This represents the first study to examine the role of NP in adipocytes. Interestingly, we observed distinct responses between NP and CNTF in adipocytes and skeletal muscle despite studies which showed that both of these cytokines act through the same signaling receptor complex (20, 22). In summary, our findings indicate that NP can affect adipogenesis and insulin sensitivity in fat cells both in vitro and in vivo. Based on our present data, NP, unlike CNTF, is not a suitable anti-obesity therapeutic because it inhibits insulin action and negatively affects glucose uptake in adipocytes.

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