The anorexigenic cytokine ciliary neurotrophic factor stimulates POMC gene expression via receptors localized in the nucleus of arcuate neurons

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Ciliary neurotrophic factor (CNTF) is a 22-kDa cytokine expressed mainly in the nervous system by neuronal and glial cells. Originally, it was shown to promote the survival of ciliary ganglion neurons (6, 26). Today, we know that its spectrum of activities is much broader since it includes the differentiation and/or survival of a variety of neuronal cells such as motor neurons, oligodendrocytes, and astrocytes (29, 38, 50). In an initial clinical trial designed to test the efficacy of a CNTF analog to prevent motor neuron degeneration, some patients suffered substantial weight loss (39). Ever since then the mechanisms by which CNTF induces weight loss have been deciphered using animal models, and it has been shown that CNTF mimics the ability of leptin, an adipocyte-secreted cytokine, to decrease food intake and produce fat loss. More-
pathological states such as brain lesions or inflammation (23, 51, 62). However, the synthesis of CNTF is not limited to these conditions. Thus, we propose here that besides its extracellular action in pathological situations, endogenous CNTF may also act intracellularly to take part in the control of energy homeostasis.

We have shown previously that CNTF distribution shares similarities with that of its receptor subunits in the rat ARC. Indeed, a majority of neurons and astrocytes express both CNTF and CNTFRα, and both β-components of the receptor are ubiquitous in the rat ARC (57), suggesting the possibility of an intracellular effect of the cytokine, as previously envisaged in cell culture (40). The aim of the current work was to validate this hypothesis in vivo and to evaluate the impact of such a mechanism on the hypothalamic control of energy homeostasis. Here, we demonstrate that CNTF and its receptors interact in the nucleus of ARC cells, including anorexigenic POMC neurons. Moreover, CNTF stimulation of hypothalamic isolated nuclei increased Akt phosphorylation and POMC gene transcription. Taken together, these data indicate that intracellular CNTF may exert an endogenous anorexigenic action via the activation of receptors localized in the cell nucleus that leads to the stimulation of POMC gene expression. These data provide a novel plausible mechanism of CNTF action in the hypothalamic regulation of energy homeostasis.

MATERIALS AND METHODS

Animals and housing. All experiments were performed in agreement with European legal requirements (Decree 86/609/EEC) and approved by the local committee on Animal Welfare. Thirty 3-mo-old Wistar male rats (Rattus norvegicus) were used. They were housed under 12:12-h light-dark cycles (lights on 0700, lights off 1900), with access to food and water ad libitum. They were euthanized at the same time of the day (between 1400 and 1600).

SH-SY5Y cells. The human neuroblastoma cell line (SH-SY5Y) was obtained from Dr. B. Dufy (UMR 5543; Centre National de la Recherche Scientifique, Bordeaux-II University) and cultured according to the method of Benomar et al. (10).

Immunofluorescence. The immunohistochemical analyses were performed as described previously (57) in 10 rats. Hypothalamic floating sections (50 μm thick) were incubated with a combination of goat anti-CNTF (1:200; R & D Systems, Minneapolis, MN), goat anti-CNTFRα (1:200; Santa Cruz Biotechnology, Santa Cruz, CA), rabbit anti-LIFR (COOH terminus) (1:1,000; Santa Cruz Biotechnology), mouse anti-α-gliabial fibrillary protein (GFAP, 1:500; Sigma, St-Quentin-Fallavier, France), rat anti-anti-p130 (NH₂ terminus) (1:200; Santa Cruz Biotechnology), rabbit anti-JAK2 (1:100; Santa Cruz Biotechnology), rabbit anti-Akt (1:100; Cell Signaling Technology), mouse anti-neuronal nuclei (NeuN; 1:1,000; Millipore, Molsheim, France), and rabbit anti-POMC (1:5,000; Phoenix Pharmaceuticals, Burlingame, CA) antibodies. Primary antibodies were visualized by using FluoroProbes-488 (FP-488; Interchim, Montluçon, France) or cyanine-5 (Cy5; Jackson Immunoresearch Laboratories, West Grove, PA)-conjugated donkey anti-goat, rabbit, mouse, or sheep antibodies (1:400). The choice of antibodies was based on their cross-reactivity for the rat forms of our molecules of interest and on their previous characterization on nervous system sections (8, 17, 24, 35, 61). Moreover, their specificity was tested on Western blots and with several controls: incubation with blocking peptides (1 h at room temperature prior to addition to sections; Santa Cruz Biotechnology) and omission of the antibodies. In all cases, immunofluorescence was reduced to background levels. Moreover, the specificity of CNTF antibody was tested on brain sections coming from CNTF-knockout mice (generously given by Prof. R. Sendtner) (37). Some sections were counterstained with 4,6-diamidino-2-phenylindole (DAPI) or ethidium homodimer-2 (EtH-2; 2 μg/ml, 1 h; Invitrogen-Molecular Probes), a membrane-permeable fluorescent dye that binds to nucleic acids, allowing the visualization of the different cellular compartments.

Microscopy and image acquisition. Immunofluorescence was examined by confocal microscopy (Zeiss MRC 1024ES; Zeiss Microscopy, Jena, Germany). Series of optical sections were collected through the z-axis at 0.4- or 0.2-μm intervals and averaged three times. Four lasers at 450, 488, 543, and 633 nm were used for the excitation of DAPI, FP-488, EtH-2, and Cy5, respectively. Images were taken through a ×40 or a ×63 oil immersion objective. The images used for three-dimensional cell reconstructions were scanned with a zoom of 3.5.

Image processing and analysis. CNTF and its receptor subunit immunofluorescence exhibited a punctiform-like pattern. To investigate the distribution of these punctuation through the ARC cells, 0.2-μm-thick focal planes of EtH-2-stained cells were acquired and processed for 3D reconstructions. Confocal image stacks were automatically converted to a format enabling their processing by programs developed using the Free-D software libraries (2). The segmentation was then performed as follows. The cell and nucleus contours were delineated manually on each EtH-2 colored focal plane, and a surface model of the plasmatic membrane (Fig. 3, gray) and the nuclear envelope (Fig. 3, red) was generated. A third model was created to point immunofluorescent spots (Fig. 3, green). The distribution of green spots was quantified in the cytoplasm and the nucleus on three different cells from three different sections and three different rats using ImageJ 1.36b software (National Institutes of Health).

Immunogold electron microscopy. Rats were perfused with a fixative solution (2% paraformaldehyde, 0.5% glutaraldehyde; Electron Microscopy Sciences, Washington, PA). Briefly, bilateral ARC were microdissected from hypothalamic slices, dehydrated, and embedded in LR White resin (Sigma). Ultrathin sections (70 nm) were cut and transferred to nickel grids with a formvar/carbon film for postembedding immunogold labeling. These sections were saturated and incubated with a combination of primary antibodies raised against CNTF (1:50), CNTFRα (1:100), LIFR (1:200), or gp130 (1:150) overnight at 4°C. The sections were then incubated with donkey anti-goat and/or rabbit anti-mouse immunoglobulin coupled to 10- or 6-nm colloidal gold particles, respectively (1:30; Aurion Costerwegs, Wageningen, The Netherlands). Sections were finally contrast-stained in a 5% ethidium homodimer-2 (EtH-2; 2 g/ml, 1 h; Invitrogen-Molecular Probes), a membrane-permeable fluorescent dye that binds to nucleic acids, allowing the visualization of the different cellular compartments.

Preparation of nuclear and cytosolic extracts. Hypothalamic samples from 10 rats were homogenized, and then nuclear, cytoplasmic, and membrane fractions were prepared as described previously (21). Briefly, whole hypothalami were homogenized individually in a nuclear homogenization buffer containing 20 mM Tris, pH 7.5, 10 mM NaCl, and 1 mM MgCl₂. Nonidet P-40 at the final concentration of 0.15%, 10 mM NaF, 20 mM β-glycerol phosphate, 1 mM PMSE, 1 mM NaVO₄, 2 μg/ml leupeptin, and 2 μg/ml aprotinin was then added. Homogenates were centrifuged for 5 min at 1,500 rpm. Supernatants were kept as cytosolic extracts, and nuclear pellets were resuspended in 0.5 volume of nuclear homogenization buffer and centrifuged at 1,500 rpm for 5 min twice again. Integrity of the nuclei was examined on a small part of the pellets using a toluidin blue staining.

Stimulation of isolated nuclei with CNTF or leptin. The nuclei were resuspended in a stimulation buffer containing 50 mM Tris-HCl, pH 7.4, 10 mM NaCl, and 1 mM MgCl₂. Nonidet P-40 at the final concentration of 0.15%, 10 mM NaF, 20 mM β-glycerol phosphate, 1 mM PMSE, 1 mM NaVO₄, 2 μg/ml leupeptin, and 2 μg/ml aprotinin was then added. Homogenates were centrifuged for 5 min at 1,500 rpm. Supernatants were kept as cytosolic extracts, and nuclear pellets were resuspended in 0.5 volume of nuclear homogenization buffer and centrifuged at 1,500 rpm for 5 min twice again. Integrity of the nuclei was examined on a small part of the pellets using a toluidin blue staining.

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7.5, 1 mM MgCl2, 2 mM EGTA, 10 mM NaF, 20 mM β-glycerolphosphate, 1 mM PMSF, 1 mM Na3VO4, 2 μg/ml leupeptin, and 2 μg/ml aprotinin before being incubated for 10 min at 37°C with CNTF (1 nM) or leptin (10 nM) in the presence or not of ATP (5 μM). Proteins (50–80 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).

**Immunoprecipitation.** Proteins (500 μg) were incubated overnight at 4°C with 10 μl of CNTFα, LIFR, or gp130 antibodies and lysate buffer containing 10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, and 0.5% Nonidet P-40 for the binding step. Then protein A (40 μl) was added to the preparation and incubated at 4°C for 2 h to capture the immune complex. After a spin at 13,000 rpm for 3 min, the pellets were resuspended in 500 μl of lysis buffer and centrifuged for 3 min at 13,000 rpm again. We performed this step three times before subjecting the proteins to SDS-PAGE and Western blot experiments using CNTFα, and gp130 antibodies. Positive control experiments were performed by immunoprecipitating our samples and blotting the membranes with the same antibodies. In parallel, negative control experiments were carried out by immunoprecipitating our samples with irrelevant antibodies raised against Lамper protein kinase and protein-tyrosine phosphatase 1B.

**Western blots.** Proteins (50 μg) were subjected to SDS-PAGE and Western blot analysis using the same antibodies as mentioned above in addition to mouse anti- phospho-tyrosine 100 (Millipore, Temecula, CA), rabbit anti-p44/42 ERK (Thr202/Tyr204), rabbit anti-44/42 ERK, rabbit anti-p-JAK2 (Tyr 1007/1008), rabbit anti-JAK2, rabbit anti-p- Akt (Ser473), rabbit anti-Akt, rabbit anti-p-STAT3 (Tyr705), and rabbit anti-STAT3 (Cell Signaling Technology). All Western blots were normalized to the corresponding total proteins. Blot quantification was performed by using Bio1D software (Villemour, Marne-la-Vallée, France).

**Nuclear run-on reaction.** Nuclei isolation from the hypothalamus of five rats was adapted from the previously published method (46). Briefly, hypothalami were isolated from five anesthetized Wistar rats. Hypothalamic samples (~70 mg) were rapidly homogenized in ice-cold buffer A (15 mM HEPES, pH 7.5, 60 mM KCl, 3 mg/ml BSA, 300 mM sucrose, 15 mM each of EDTA and EGTA, 1 mM dithiothreitol (DTT), 0.5 mM spermidine, 0.5 mM PMSF, and 2 μg/ml each of leupeptin and aprotinin). Following centrifugation at 700 g at 4°C for 10 min, the resulting pellet containing crude nuclei was suspended in ice-cold buffer B (similar to buffer A, except for 0.1 mM each of EDTA and EGTA and the addition of 5 mM Mg2OAc) and centrifuged in the same condition as described above. The final nuclei pellets were suspended in storage buffer (40% glycerol, 75 mM HEPES, pH 7.5, 60 mM KCl, 15 mM NaCl, 5 mM MgAc, 0.1 mM each of EDTA and EGTA, 1 mM DTT, 0.5 mM spermidine, and 2 μg/ml each of leupeptin and aprotinin), frozen immediately in liquid nitrogen, and stored at −80°C. To determine transcription of POMC gene in response to CNTF, a qRT-PCR based on the nuclear run-on technique was used as described previously (28). Briefly, frozen nuclei were thawed on ice, and equal volumes of nuclei and reaction buffer (20% glycerol, 100 mM KCl, 10 mM MgCl2, 4.5 mM DTT, 1.2 mM ATP, 0.6 mM each of CTP, TTP, and UTP, 80 U/ml RNase inhibitor, and finally, 1 nM of CNTF) were mixed and incubated at 37°C for 45 min. RNA was then extracted using Trizol (Invitrogen) according to the manufacturer’s recommendations.

**Quantitative RT-PCR.** Nascent RNA from the nuclear run-on reactions was reverse transcribed using the Super-Script II RNAse H-system (Invitrogen), as described previously (9). The resulting cDNAs were submitted to quantitative PCR. The PCR primer sequences used were as follows: POMC forward 5′-AGGTGAGGAGGATGACG-3′, reverse 5′-CGTCTAGGAGGTCCTACAG-3′; NPY forward 5′-ATGCTAGTAACAAACG-3′, reverse 5′-ATGATGGTGCCAGA-3′; and 18S forward 5′-TCCCGGAAGTCTCCACAG-3′, reverse 5′-CTTC-
nuclear distribution of CNTF and its receptor subunits was observed throughout the hypothalamus. In addition, JAK2, a signaling molecule associated with CNTF receptor subunits, was also present in the cell nucleus, although its main location was cytoplasmic and close to the inner cell membrane (Fig. 3, E and G). Finally, Akt, a downstream signaling element, exhibited a nuclear localization that reached 63 ± 22% in the ARC cells (Fig. 3, F and G).

**CNTF and its receptor subunits may interact in the cell nucleus of rat ARC cells.** Previous in vitro studies have demonstrated that the activation of CNTF receptor required the heteromerization of its subunits. To check the possibility that these molecules form a CNTF tripartite complex in the ARC cells, we performed multiple immunogold stainings followed by a transmission electron microscopy analysis. Four pairs of proteins were tested, according to the host species of precharacterized antibodies (see MATERIALS AND METHODS): CNTF/LIFR, CNTF/gp130, CNTFRα/LIFR, and CNTFRα/gp130. For each group of particles, deposits were performed to eval-

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**Fig. 1.** Ciliary neurotrophic factor (CNTF) is present in proopiomelanocortin (POMC) neurons of the rat arcuate nucleus. A–H: POMC and CNTF were detected by multiple immunofluorescence followed by a confocal laser scanning. Sections were counterstained with 4,6-diamidino-2-phenylindole (DAPI). I and J: detection of CNTF immunofluorescence in wild-type (WT) and CNTF-knockout mice (CNTF–/–). A–D: 0.4-μm-thick focal planes. E–H: 1.2-μm-thick stacks of high-magnification scan (×63). I and J: epifluorescence observation. Scale bars = 30 (A–D), 10 (E–H), and 50 μm (I and J).

**Fig. 2.** CNTF receptor subunits are present in POMC neurons of the rat arcuate nucleus. POMC and CNTF receptor-α (CNTFRα; A–C), leukemia inhibitory factor receptor (LIFR; D–F), and glycoprotein 130 kDa (gp130; G–I) were detected by multiple immunofluorescence followed by a confocal laser scanning. Sections were counterstained with DAPI; 0.4-μm-thick focal planes. Scale bar = 15 μm.
uate particle size segregation. Diameter means ± SE were calculated, and particle diameters were plotted in a frequency distribution (Fig. 4A). The difference was highly significant [5.579 ± 0.17 (n = 72) vs. 9.522 ± 0.1 (n = 84), P = 0.0001; 2-tailed Student’s t-test]. Despite the use of a postembedding method chosen to facilitate the accessibility of the antibodies to the deep cellular compartments such as the cell nucleus, the tissue integrity was preserved, and the main organelles, including rough endoplasmic reticulum, mitochondria, nuclear envelope, heterochromatin, and euchromatin, were easily identified. The same four pairs of proteins, as mentioned above, were tested. Omission of the primary antibody validated the specificity of the immunolabeling. CNTF and its receptor subunits were observed in both the cytoplasm and the nucleus of the ARC cells. The nuclear localization represented 57 ± 11, 65 ± 16, 72 ± 12, and 52 ± 7% of the total CNTF, CNTFRα, LIFR, and gp130 immunostaining, respectively (Fig. 4B). The concomitant detection of CNTF and its receptor subunits confirmed the proximity of CNTF and LIFR (Fig. 4C), CNTF and gp130 (Fig. 4D), CNTFRα and LIFR (Fig. 4E), and CNTFRα and gp130 (Fig. 4F) in the cell nucleus. The cytoplasmic compartment also contained CNTF and its receptor subunits. This staining was particularly intense near the rough endoplasmic reticulum. Moreover, single or double staining of CNTF and its receptor subunits was also evidenced in nuclear pores (not shown). Finally, it is of note that an extracellular staining was observed for CNTF, CNTFRα, and gp130. This soluble fraction was estimated at 17, 12, and 5% for CNTF, CNTFRα, and gp130, respectively (Fig. 4B).

We next performed a Western blot analysis from fractionated hypothalamic samples. These biochemical data corroborated the common nuclear location of CNTF and its receptor subunits in ARC cells. These experiments showed a mixed distribution of CNTF, CNTFRα, and gp130 between the cytoplasmic (Fig. 5A, lane C) and the nuclear (Fig. 5A, lane N) compartments and revealed that LIFR and gp130 were also
The fact that CNTFRα was not detected in the membrane fraction, although functional after an intraperitoneal injection of CNTF (34, 57), may be due to its labile glycosyl-phosphatidylinositol anchor to the cell membrane (14). Moreover, JAK2 and Akt were detected in both cytoplasmic and nuclear fractions (Fig. 5A). The quantitative variability of cellular distributions between the three-dimensional reconstructions and the Western blot may be due to the fact that the analyses were performed on ARC cells and on whole hypothalamic extracts (which contain heterogeneous cell populations), respectively. The purity of the different fractions was verified by detecting different cytoplasmic or nuclear proteins, such as β-tubulin and GFAP (cytoplasmic) and NeuN (nuclear). Moreover, the examination of cresyl violet stained fractions under a light microscope confirmed the purity and the integrity of the isolated nuclei. The possibility for CNTF receptor subunits to interact in the cell nucleus was assessed by coimmunoprecipitations from hypothalamic nuclear fractions. As shown in Fig. 5B, gp130 was found to coimmunoprecipitate with CNTFRα and LIFR, and CNTFRα coimmunoprecipitated with LIFR and gp130. The immunoprecipitation with irrelevant antibodies raised against Lammer protein kinase and protein-tyrosine phosphatase 1B confirmed that the immunoprecipitated complexes were specific (not shown).

CNTF stimulates POMC transcription in isolated hypothalamic nuclei. Because nuclear CNTF was found in ARC POMC neurons, we assessed its capability to modulate POMC transcription in isolated nuclei from hypothalamus. A 45-min incubation with CNTF (1 nM) increased the level of nascent POMC-RNA significantly (P < 0.05, n = 5; Fig. 6). By contrast, CNTF did not alter the transcription of NPY gene, and leptin treatment had no significant effect on the transcription level of our genes of interest (Fig. 6).
CNTF stimulates signaling pathways in isolated hypothalamic nuclei. We next investigated the effects of nuclear CNTF on the activation of signaling pathways after the incubation of hypothalamic nuclear extracts from 10 different rats with CNTF. Western blot analysis using anti-p-JAK2 and anti-p-Akt antibodies showed that a 10-min incubation with CNTF (1 nM) increased their phosphorylation levels (Fig. 7A). The band intensity analysis of the blots (after normalization with total JAK2 or total Akt) evidenced a significant increase of the JAK2 (P < 0.0005) and Akt (P < 0.005) phosphorylation levels compared with control conditions (Fig. 7B). It is of note that phosphorylation of these proteins was not affected by leptin (10 nM) and was prevented in the absence of ATP. Moreover, CNTF did not induce the phosphorylation of ERK42/44 in nuclear extracts, but it induced that of STAT3 in two out of 10 animals (data not shown).

DISCUSSION

Anorexigenic properties of CNTF have conferred to this cytokine a promising therapeutic potential in the treatment of obesity. Besides, several studies have tended to show that endogenous CNTF can also represent a new modulator of energy homeostasis. Indeed, a null mutation in CNTF gene has been associated with a significant increase in body mass in humans (25, 42), and variants in CNTF or CNTFRα gene in humans have been associated with a lower age at the onset of eating disorders (22). Furthermore, hypothalamic neurons controlling food intake represent a significant source of CNTF that varies in inverse ratio with body weight in rats fed a hyperca-
compared with control condition with paired Student evidence of CNTF in the nucleus of ARC neurons was unex-
rons and glial cells throughout the brain (3–5, 27, 35), the
been described previously in the nuclear compartment of neu-
bind to the cell nucleus (47). However, although CNTF has
mode, and most of them have been reported to translocate and
raised against different domains of the protein, and this com-
parative study confirmed CNTF to be present not only in the
cytosol but also in the nucleus of ARC cells. The mecha-
nisms underlying the nuclear translocation of CNTF have not
been elucidated yet. CNTF gene can be cotranscribed in mouse
and human with that encoding zinc finger protein 91, a nuclear
proliferative and antiapoptotic factor (Entrez Gene: ZFP91-
 CTNF transcription unit) (56), but no nuclear targeting signal
(NTS) was found for CNTF. Nevertheless, alternative NTS-
 independent pathways exist for nuclear transport (reviewed in
Ref. 59). In vitro studies have indicated that CNTF could reach
the nucleus by a facilitated transport mechanism in oocytes and
astrocytes (4, 5). Moreover, the fact that we evidenced both
CNTF and LIFR passing through the nuclear envelope by the
same pore could suggest a receptor-mediated translocation, as
demonstrated previously for IL-1, another signal peptide lacking
cytokine (13).

Our data indicate that CNTF receptor subunits are present
not only at the membrane and in the cytoplasm but in the
nucleus of ARC cells as well. This observation is in accord
with several studies showing that intracrine factors can usually
act both at the membrane and in the nucleus (47). Furthermore,
CNTF receptor subunits may associate in the cell nucleus, as
demonstrated using transmission electron microscopy and by
coimmunoprecipitation from nuclear extracts. To our knowl-
edge, among the different CNTF receptor subunits, LIFR is the
only one that has been described previously in cell nucleus,
notably in neurons (17, 20).

Among the different cell types constituting the ARC,
POMC-expressing neurons appeared as a privileged place for
nuclear CNTF to activate its receptor complex. Indeed, all the
partners are present in the nucleus of these neurons, and the
stimulation of nuclear extract with CNTF induced a specific
increase in POMC gene expression. The important transcrip-
tional activity of the activated CNTF receptor complex is
illustrated by the high degree of colocalization between these
proteins and HP1, a positive regulator of active transcription in
euchromatin (33). By comparison, NPY-nascent RNA levels
were not affected by CNTF. In the hypothalamus, POMC
neurons play a crucial role in the regulation of energy homeo-
static by controlling food intake, energy expenditure, and

A variety of hormones (i.e., leptin and insulin) and growth
factors (i.e., leukemia inhibitory factor and brain-derived neu-
rotrrophic factor) have been shown to operate in an intracrine
mode, and most of them have been reported to translocate and
bind to the cell nucleus (47). However, although CNTF has
been described previously in the nuclear compartment of neu-
rons and glial cells throughout the brain (3–5, 27, 35), the
evidence of CNTF in the nucleus of ARC neurons was unex-
pected. This result was validated not only by the diversity of
the approaches used in our study (immunohisto/cytochemistry,
3D reconstructions, and Western blot from fractionated cells)
but also by the implementation of several control experiments
(omission of the primary antibody, competition with blocking
peptide, and use of CNTF-knockout mice). Moreover, we have
tested several antibodies obtained from different species and
raised against different domains of the protein, and this com-
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were not affected by CNTF. In the hypothalamus, POMC
neurons play a crucial role in the regulation of energy homeo-
static by controlling food intake, energy expenditure, and
glucose metabolism (45). They integrate nervous and circulat-
LORIC Diet, suggesting a protective action of the cytokine in a
fraction of individuals against diet-induced weight gain (57).
However, how hypothalamic CNTF contributes to the control
of energy balance is still an open question since it lacks a signal
peptide and thus may not be released by the classical exocytose
pathways (49). In the current study, we show for the first time
that CNTF can exert a direct intracrine action by activating its
receptor in the cell nucleus of ARC cells. This leads to the
stimulation of POMC expression and may account for the
anorexigenic effect of exogenous CNTF. Indeed, CNTF injec-
tion induces an increase in POMC expression (1), and the
ablation of gp130 in POMC neurons prevents CNTF from
activating STAT3 phosphorylation in these neurons and from
inhibiting food intake (32). Thus, here we demonstrate that
endogenous CNTF could take part in the control of energy
homeostasis by regulating POMC gene expression via the

Fig. 7. CNTF stimulates Akt signaling in hypothalamic isolated nuclei. Isolated
nuclei were obtained from individual fresh rat hypothalami and incubated in
the presence or not of ATP (5 μM), CNTF (1 nM), and leptin (10 nM) for 10
min at 37°C. They were then subjected to SDS-PAGE and Western blot
analysis. A: montage of nitrocellulose membranes blotted with an anti-phospho
(p)-JAK2 or an anti-p-Akt before being stripped and blotted a 2nd time with an
anti-total (tot) JAK2 or anti-tot Akt. B: quantification of the blots. The data are
presented as the %control condition ± SE. *P < 0.05 and **P < 0.01
compared with control condition with paired Student t-test; n = 10 rats.
activation of its receptor subunits in the cell nucleus of these neurons.

The signaling cascades involved in such a regulation need to be elucidated. According to our results, we can assume that an Akt-dependent pathway mediates the effect of nuclear CNTF on POMC transcription. Indeed, we observed JAK2 and Akt in the nucleus of ARC cells, and the stimulation of nuclear extracts with CNTF induced the phosphorylation of both proteins. This hypothesis is reinforced by the fact that Akt can be activated directly in the cell nucleus in different cellular models (60). Moreover, POMC gene transcription is known to be regulated by signaling pathways, including JAK2 and Akt (7, 31). The role of STAT3 cannot be totally excluded since CNTF induced its phosphorylation in two out of 10 animals. By contrast, leptin was unable to induce Akt and JAK2 phosphorylation in isolated nuclei, indicating a specific effect of CNTF. Moreover, the presence of CNTF-Rα and/or LIFR in the nucleus seems to be necessary to activate these signaling pathways since in the SH-SY5Y neuroblastoma cell line, which exhibits none of these subunits in the nucleus, CNTF failed to activate the JAK2/Akt signaling pathways in isolated nuclei.

The impact of our data may not be restricted to the comprehension of the central control of energy homeostasis but extended to therapeutic applications. Indeed, the use of a cell penetrating modified CNTF (48, 58), which crosses the cell membrane and translocates to the nucleus, can retain all or part of the CNTF activities without inducing the side effects usually caused by the cytokine (i.e., fever, cough, asthenia) (12).

In conclusion, our results indicate that endogenous CNTF could activate its receptor complex in the nucleus of ARC cells, including POMC neurons. This mechanism, which is not shared by leptin, may contribute to the regulation of POMC gene expression and thus to the control of energy homeostasis. The involvement of such a process in the protective action of endogenous CNTF against diet-induced weight gain deserves further investigation. Nevertheless, these data could influence future drug discovery efforts for the development of new therapeutic targets against obesity.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

O.C., A.A., D.C., and J.D. performed the experiments; O.C. analyzed the data; O.C. interpreted the results of the experiments; O.C. and C.-M.V. prepared the figures; A.G., M.T., and C.-M.V. did the conception and design of the research; C.-M.V. drafted the manuscript.

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