

Ciliary Neurotrophic Factor and Leptin Induce Distinct Patterns of Immediate Early Gene Expression in the Brain

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Ciliary neurotrophic factor (CNTF) and leptin decrease food intake and body weight. Lipopolysaccharide (LPS) is a potent exogenous pyrogen and produces anorexia via cytokine production. CNTF-, leptin-, and LPS-induced cytokines all act on type I cytokine receptors. However, it is not known if these cytokines engage similar central nervous system (CNS) pathways to exert their effects. To assess mechanisms by which these cytokines act, we examined the patterns of immediate early gene expression (SOCS-3, *c-fos*, and *tis-11*) in the brain following intravenous administration. CNTF and LPS induced gene expression in circumventricular organs; ependymal cells of the ventricles, meninges, and choroid plexus; and the arcuate nucleus of the hypothalamus. CNTF administration also induced fever and cyclooxygenase-2 mRNA expression. In contrast, we found no evidence of leptin-induced inflammation. CNTF and leptin are being assessed as potential therapeutic anti-obesity agents, and both potently reduce food intake. Our findings support the hypothesis that CNTF and leptin engage distinct CNS sites and CNTF possesses inflammatory properties distinct from leptin. *Diabetes* 53:911–920, 2004

Leptin is fundamental in maintaining body weight and neuroendocrine homeostasis (1). The leptin receptor is a member of the class-I cytokine receptor family, and several isoforms have been identified (2). A long form is expressed in specific areas of the hypothalamus (2,3). Circulating leptin is thought to

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J.S.F. has received a research grant from Takeda Chemical. CNS, central nervous system; CNTF, ciliary neurotrophic factor; COX-2, cyclooxygenase-2; Fos-IR, Fos-like immunoreactivity; IL, interleukin; LPS, lipopolysaccharide; OVLT, organum vasculosum of the lamina terminalis; PFS, pyrogen-free saline; POMC, proopiomelanocortin; SFO, subfornical organ.

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regulate body weight, food intake, and energy expenditure by engaging these hypothalamic nuclei (1,4).

In addition to leptin, other cytokines may also regulate body weight, but the mechanisms by which this occurs are not well understood. For example, a leptin-like role has been suggested for ciliary neurotrophic factor (CNTF). CNTF administration to *ob/ob* and *db/db* and diet-induced obese mice attenuates the hyperphagia and obesity characteristic of these models (5,6,6a). Like the leptin receptor, CNTF receptors belong to the class-I cytokine receptor family and are widely expressed within the central nervous system (CNS) (7,8).

CNTF and leptin both inhibit food intake and decrease body weight, but the CNS pathways engaged by these cytokines have not been systematically compared. The cell groups activated by leptin have received considerable attention (1,3,4,9,9a). However, the extra hypothalamic pathways engaged by systemic CNTF administration remain largely unknown. To compare the CNS pathways engaged by these cytokines, we examined the expression of several immediate early genes. We also investigated the induction of fever and cyclooxygenase-2 (COX-2) mRNA in the brain, an obligate step in the production of fever (10,11).

RESEARCH DESIGN AND METHODS

Male Sprague-Dawley rats (250–350 g; Taconic) were housed singly in a light (12 h on/12 h off)- and temperature-controlled environment (21.5–22.5°C). Catheters were implanted into the femoral vein (12–14) and transmitters into the peritoneal cavity (Data Sciences International). Five days after surgery, rats were intravenously injected with recombinant human CNTF (250 µg/kg; R&D Systems) dissolved in PBS (Gibco BRL) containing BSA (25 mg/ml PBS/BSA; R&D Systems), followed by PBS. Controls received PBS/BSA alone followed by PBS (0.25 ml total vol/rat). Recombinant murine leptin (1.0 mg/kg; kindly provided by Eli Lilly) or lipopolysaccharide (LPS) (5 µg/kg; Lot 71H4033; Sigma) was dissolved in pyrogen-free saline (PFS; Sigma) followed by PFS or in PFS alone (0.25 ml total vol/rat). All injections were given between 10:00 A.M. and 12:00 P.M. The groups consisted of intravenous CNTF (1 h, *n* = 3; 2 h, *n* = 4; 6 h, *n* = 6), intravenous leptin (1 h, *n* = 3; 2 h, *n* = 3; 6 h, *n* = 4), intravenous LPS (1 h, *n* = 3), intravenous PBS/BSA (1 h, *n* = 3; 2 h, *n* = 3; 6 h, *n* = 6), and intravenous PFS (1 h, *n* = 3; 2 h, *n* = 3; 6 h, *n* = 4).

Ten mice (AKR/J; 20–25g) were put on a 23-h water-deprivation schedule. Then, mice were exposed on an alternating schedule to two different unsweetened Kool-Aid flavors (grape or cherry). The mice were divided into two groups: One group of mice (*n* = 4) served as a positive control for the conditioning procedures by receiving 0.15 mol/l LiCl i.p. at a volume equivalent to 2% of their total body weight paired with one kool-aid flavor (treatment-flavor), whereas the other flavor (vehicle-flavor) was paired with an equivalent volume of intraperitoneal saline. The second group of mice (*n* = 6) paired intraperitoneal CNTF at a dose of 10 µg/kg with one flavor (treatment-flavor) and intraperitoneal vehicle with the other flavor (vehicle-

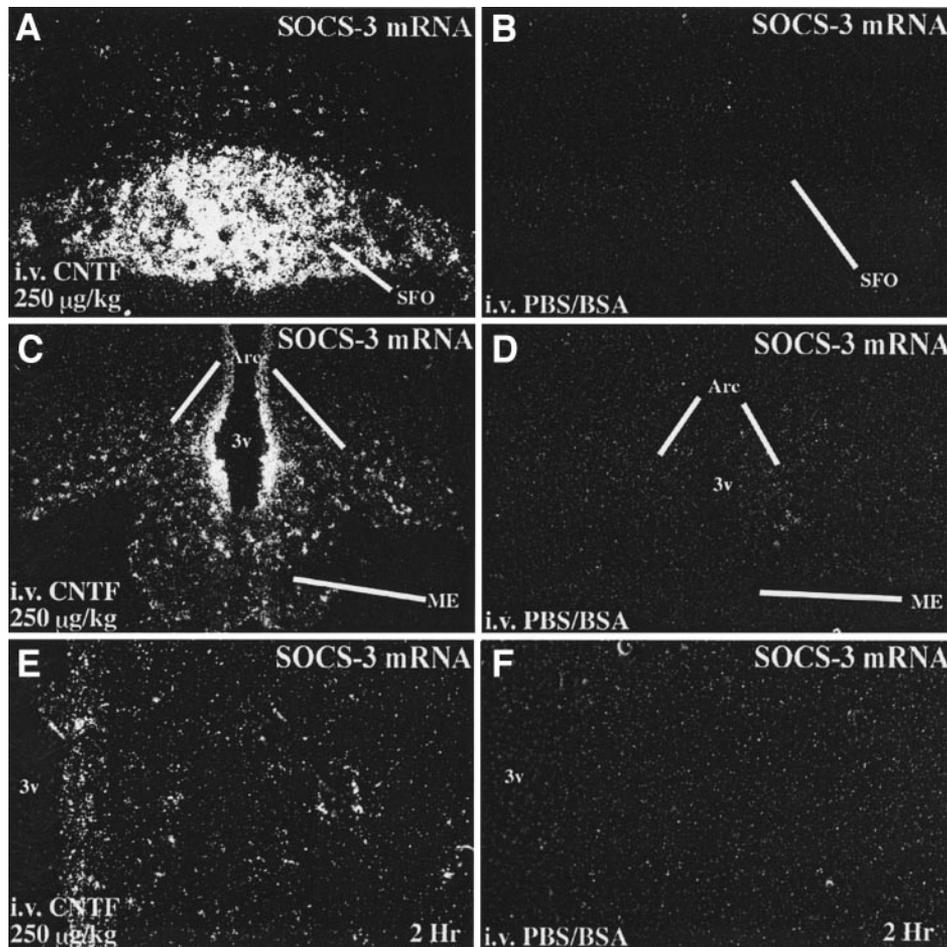


FIG. 1. Photomicrographs demonstrating the distribution of SOCS-3 mRNA 1 or 2 h after intravenous CNTF (*A*, *C*, and *E*) or intravenous PBS/BSA (*B*, *D*, and *F*). One hour following CNTF, intense hybridization is observed in the SFO, the area dorsal to it (*A*), the arcuate nucleus of the hypothalamus (Arc), median eminence (ME), and the ependymal lining of the third ventricle (3v) (*C*). One hour following PBS/BSA, the SFO (*B*) and the Arc (*D*) reveal hybridization only slightly above background. Two hours following CNTF, hybridization is still seen in the ependymal and, in neurons, lateral to the third ventricle (*E*). The corresponding area 2-h after PBS/BSA reveals no hybridization (*F*).

flavor). The flavor (grape or cherry) serving as the treatment-flavor and that serving as the vehicle-flavor were counterbalanced across subjects. Over a 10-day period, water-deprived mice were exposed to each flavor two times followed by the appropriate treatment (LiCl or CNTF for the treatment-flavor and NaCl or BSA for the vehicle-flavor). On day 10, mice were given access to both flavors, and intake was measured after 1 h. The preference for the treatment-flavor, as compared with the vehicle-flavor, was calculated for both groups of mice.

The temperature data are reported as the change in temperature from the average baseline over the hour preceding injection. For analysis, the data are presented as means \pm SE. The effects of CNTF, leptin, and vehicle injections on temperature were compared by ANOVA and Fisher's protected least significant difference test, and $P < 0.05$ was considered significant. Conditioned taste aversion data were compared using the Student's *t* test to determine whether the values deviated from the expected 0.5 preference ratio.

In situ hybridization histochemistry and immunohistochemistry. One, 2, or 6 h following CNTF, leptin, LPS, or vehicle administration, rats were perfused with 10% neutral-buffered formalin (Sigma). The brains were removed, postfixed, and placed in a 20% sucrose solution in diethyl pyrocarbonate-treated PBS. The brains were then sectioned on a freezing microtome (30 μ m; 1:5 series). Sections were mounted on slides and processed for in situ hybridization histochemistry (13,14). Slides were then dipped in NTB2 emulsion (Kodak), developed, fixed, counterstained, dehydrated, and coverslipped.

Sense (579-bp) and antisense (577-bp) rat TIS-11 (15) cRNA probes were generated by digesting the plasmids with *Eco*RI or *Nsp*I, respectively, and subjected to in vitro transcription with T7 or T3 RNA polymerase (Promega), respectively. Sense and antisense mouse *c-fos* cRNA probes were generated by digesting the plasmid (kind gift of Michael Greenberg [16]) with *Nco*I or *Bgl*II, respectively, and subjected to in vitro transcription with T7 or SP6 RNA polymerase (Promega), respectively. The 450-bp sense and antisense mouse

SOCS-3 cRNA probes were generated as previously described (17). Sense (1,662-bp) and antisense (576-bp) rat COX-2 cRNA probes (18) were generated by digesting with *Sac*I or *Eco*RI, respectively, and subjected to in vitro transcription with T3 or T7 RNA polymerase, respectively.

A series of sections from the 2-h CNTF group were processed for Fos immunohistochemistry (12–14) using a rabbit primary antiserum (Ab-5; Oncogene) (1:150,000). Photomicrographs were produced using a digital camera (Kodak DCS 460) mounted on the microscope (Zeiss Axioplan) and an Apple Macintosh Power PC computer. Adobe Photoshop was used to combine photomicrographs into plates. Only the sharpness, contrast, and brightness were adjusted.

RESULTS

Gene expression patterns following CNTF. Rats received CNTF (250 μ g/kg) or vehicle intravenously and were perfused 1, 2, or 6 h later. At the 1-h time point, intense SOCS-3 mRNA hybridization was observed in the circumventricular organs, including the core of the organum vasculosum of the lamina terminalis (OVLT), median eminence, subfornical organ (SFO), and area postrema (Fig. 1). The hybridization in the OVLT was associated with cells within the brain parenchyma and cells surrounding blood vessels. The pattern in the SFO was widespread in both the core and periphery. There was also intense hybridization in the rostral and middle regions of the arcuate nucleus of the hypothalamus adjacent to the

median eminence. However, the hybridization in the caudal arcuate nucleus was less intense. Hybridization was observed in the median preoptic nucleus, ventromedial preoptic nucleus, nucleus of the solitary tract, and the paraventricular nucleus of the hypothalamus. Intense SOCS-3 hybridization was also observed in ependymal cells of the ventricles (Fig. 1C), cerebral aqueduct, and central canal. Hybridization was also observed in the meninges, the choroid plexus, and cells of the perivascular space associated with blood vessels throughout the brain.

Two hours after CNTF administration, a similar, but reduced, pattern of SOCS-3 mRNA was seen. Specifically, there was less hybridization observed over the ependymal cells. Distinct from the 1-h group, hybridization was also observed over large cells (presumed neurons) lateral to the ependymal cells of the third ventricles. Hybridization associated with blood vessels was still present at this time period.

In the control rats, scattered hybridization of SOCS-3 mRNA that was slightly higher than background was observed in cells of the median eminence, arcuate nucleus of the hypothalamus, and area postrema. Scattered non-neuronal hybridization was observed in the SFO, meninges, and choroid plexus. Hybridization at all sites was only slightly above background and well below what was observed in the CNTF-treated rats (Fig. 1).

The *c-fos* mRNA hybridization pattern 1 h following CNTF administration was very similar to the SOCS-3 mRNA pattern. Specifically, intense hybridization was observed in the circumventricular organs and ependymal cells. Less intense hybridization was observed in cells associated with blood vessels throughout the brain. Control animals displayed hybridization of *c-fos* mRNA in cells scattered throughout the brain. However, very little hybridization was seen in cells associated with barrier regions of the brain.

One series of sections from each animal from the 2-h time point was examined using immunohistochemistry to investigate the patterns of CNTF-induced Fos-like immunoreactivity (Fos-IR). The patterns of Fos-IR mirrored the pattern of Fos mRNA 1 h after CNTF administration. Fos-IR distinct from controls was observed in the meninges and in a very large number of cells lining the ventricular system (Fig. 2A, E, and F). Cells expressing Fos-IR were observed in the OVLT (Fig. 2A and B), ventral medial preoptic area, dorsal lateral division of the bed nucleus, core of the SFO (Fig. 2C and D), median eminence (Fig. 2E and G), arcuate nucleus of the hypothalamus (Fig. 2E), central nucleus of the amygdala, external lateral parabrachial nucleus, ventrolateral periaqueductal gray, nucleus of the solitary tract (Fig. 2F and H), and area postrema (Fig. 2F). Within the SFO, the Fos-IR was most intense in the core of the nucleus with fewer cells in the periphery of the structure. Notably, little Fos-IR was observed in the paraventricular hypothalamic nucleus. Within the arcuate nucleus, Fos-immunoreactive neurons were observed throughout the medial to lateral extent of the nucleus (Fig. 2E). The arcuate nucleus, median eminence, and nucleus of the solitary tract contained two types of immunoreactive cells, one with round nuclei as seen throughout the brain (presumed neurons). In addition, a distinct nuclear staining pattern with thinner nuclear staining was also

observed (Fig. 2H). The PBS/BSA controls displayed only scattered Fos-immunoreactive neurons.

The hybridization pattern of TIS-11 mRNA was similar to that of the SOCS-3 and *c-fos* mRNA pattern. One hour following intravenous CNTF, intense hybridization of TIS-11 mRNA was observed in cells of the OVLT (Fig. 3A), SFO, area postrema (Fig. 3E), arcuate nucleus of the hypothalamus, and median eminence. Hybridization was also observed in neurons of the paraventricular nucleus of the hypothalamus. Notably, nonneuronal cell hybridization was observed in the meninges, choroid plexus, perivascular regions throughout the brain (Fig. 3C), and ependymal cells. Two hours following CNTF, hybridization of TIS-11 mRNA in most areas decreased, including the meninges, choroid plexus, perivascular space, ependymal cells, circumventricular organs, and arcuate nucleus. In the PBS/BSA controls, only scattered hybridization was observed in the meninges, choroid plexus, circumventricular organs, and arcuate nucleus (Fig. 3B, D, and F).

LPS and interleukin (IL)-1 induce COX-2 expression in barrier cells of the brain (10,11). Therefore, we investigated the distribution of COX-2 mRNA 2 h after administration of CNTF or leptin. Two hours after CNTF, hybridization of COX-2 mRNA was associated with blood vessels throughout the brain (Fig. 3G). Hybridization was also observed in the meninges, area postrema, OVLT, and SFO. Two hours following leptin administration, hybridization of COX-2 mRNA was not different from that of controls. Notably, leptin did not induce COX-2 mRNA associated with blood vessels. In all groups, hybridization was observed in neurons throughout the brain, including the hippocampus and cortex (19).

Gene expression patterns following leptin. We have previously reported patterns of Fos and SOCS-3 after leptin administration (12–14,17). Similar to those studies, when animals were given an intravenous bolus of leptin (1.0 mg/kg) 1 h before perfusion, the distribution of SOCS-3 mRNA was similar to the distribution of long-form leptin receptor (Ob-Rb) mRNA (1,4,20–22). Briefly, SOCS-3 hybridization was observed in neurons in the retrochiasmatic area, arcuate nucleus (Fig. 4A), ventromedial nucleus, ventral premammillary nucleus, and dorso-medial nucleus of the hypothalamus. Also, moderate hybridization was observed in a small number of cells in the paraventricular nucleus of the hypothalamus, posterior hypothalamus, and dorsal raphe nucleus. Scattered, less intense hybridization was observed in the lateral hypothalamic area and nucleus of the solitary tract. Control animals demonstrated much lower levels of SOCS-3 mRNA in these sites.

The pattern of *c-fos* mRNA after leptin was similar to previous studies describing the patterns of leptin-induced Fos-IR and SOCS-3 mRNA (12–14,17,23,24). Thus, we will highlight only the differences between the SOCS-3 mRNA pattern and the *c-fos* mRNA pattern following leptin. One major difference was observed in the arcuate nucleus of the hypothalamus. The hybridization of SOCS-3 mRNA was observed throughout the arcuate nucleus (Fig. 4A). In contrast, *c-fos* mRNA hybridization was only observed in the lateral regions of the arcuate nucleus. The medial regions of the arcuate nucleus, along the third ventricle, contained little *c-fos* mRNA (Fig. 4B). Notably, TIS-11

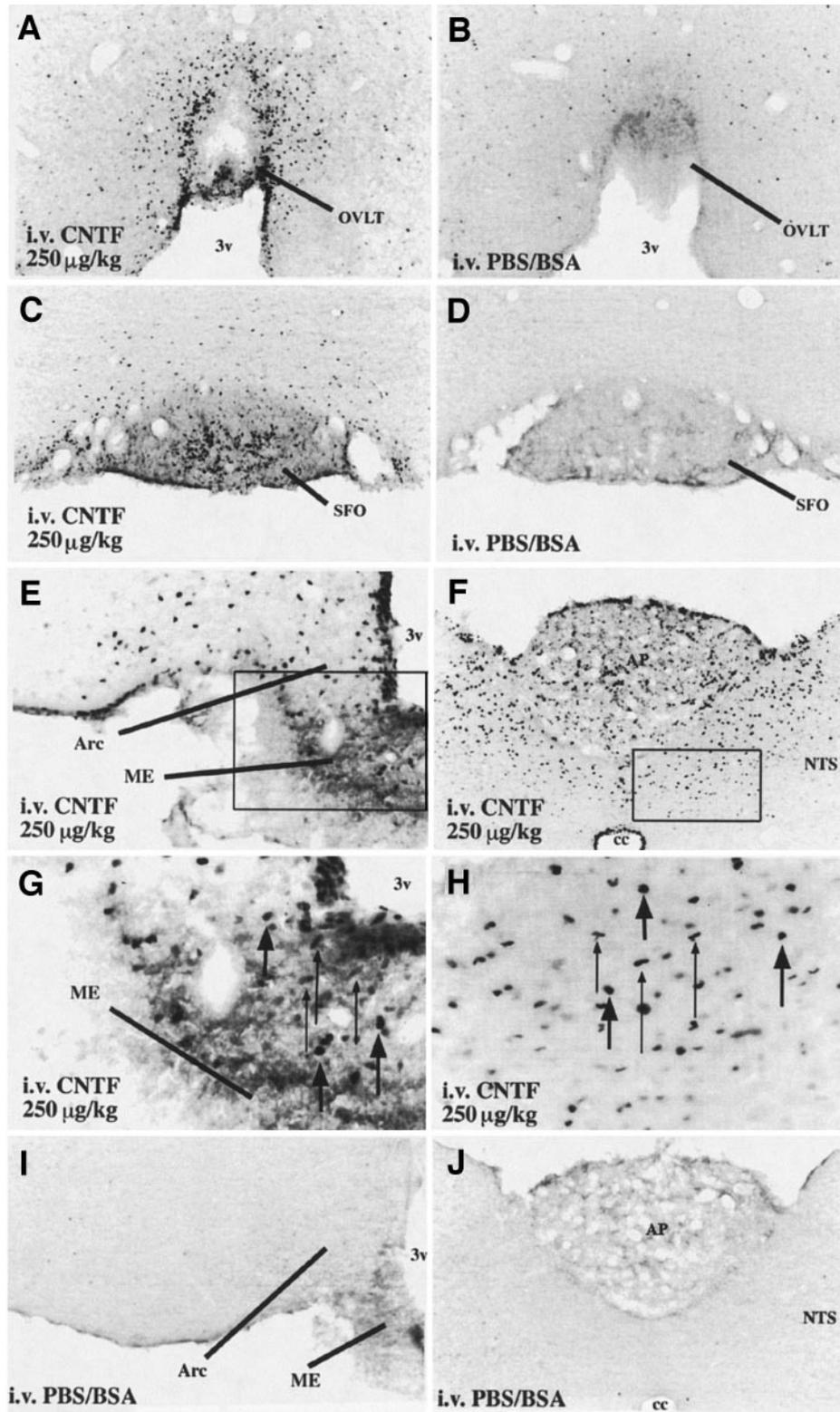


FIG. 2. Photomicrographs demonstrating the distribution of Fos-IR in the rat brain 2 h following intravenous CNTF (A, C, and E–H) or intravenous PBS/BSA (B, D, I, and J). Following CNTF, Fos-IR is observed in OVLt (A) and in the core of the SFO (C). Fos-IR is also observed in the arcuate nucleus (Arc), the median eminence (ME), and the ependymal of the third ventricle (3v; A and E). The boxes in E and F are magnified in G and H to show different shapes of nuclei. F, H, and J: The distribution of Fos-IR in the area postrema (AP) nucleus of the solitary tract (NTS). Note the Fos-IR in cells lining the ependymal wall of the central canal (cc). Following PBS/BSA, little FOS-IR is observed (B, D, I, and J).

mRNA expression after leptin administration was not distinct from controls except in the median eminence and the arcuate nucleus. The hybridization intensity in the arcuate nucleus was only slightly above control levels.

Patterns of gene expression following LPS. CNTF and leptin both act on cytokine receptors; therefore, we compared the patterns of SOCS-3 and TIS-11 mRNA induced by both with that induced by LPS. LPS initiates a stereotyped

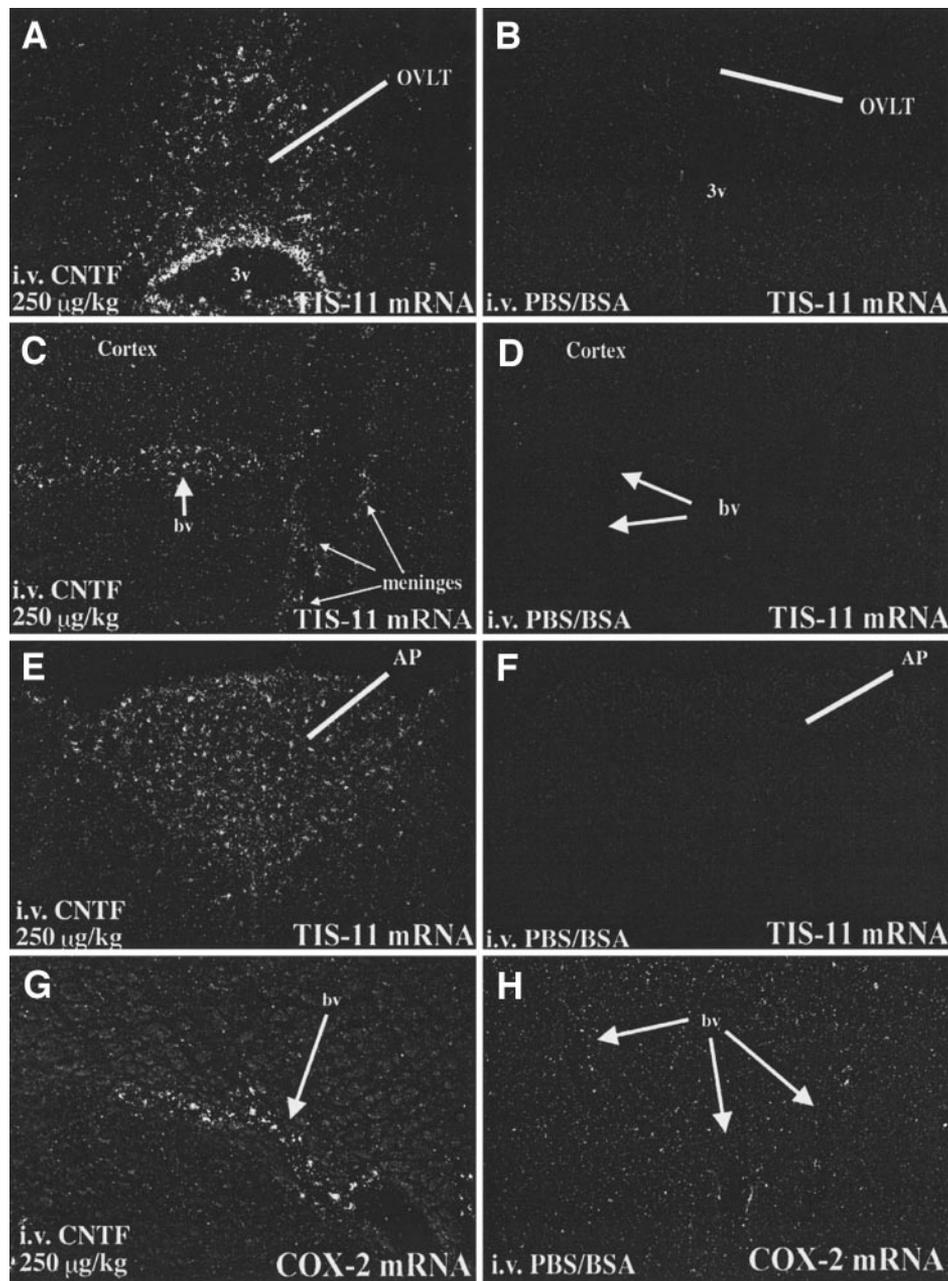


FIG. 3. Photomicrographs demonstrating the distribution of TIS-11 (A–F) or COX-2 (G–H) mRNA 1 h following intravenous CNTF (A, C, E, and G) or intravenous PBS/BSA (B, D, F, and H). Following CNTF, TIS-11 hybridization is observed in the organum vasculosum or the lamina terminalis (OVLT) (A), in meninges (thin arrows) and areas associated with blood vessels (bv; thick arrows) in the cortex (C), and in the area postrema (AP; E). Scant TIS-11 hybridization is observed following intravenous PBS/BSA. COX-2 mRNA is associated with blood vessels in the brain stem (white arrows) following CNTF (G). 3v, third ventricle.

host response, including fever and anorexia, that is mediated by inflammatory cytokines (10,11). One hour before perfusion, rats received an intravenous bolus of LPS (5 $\mu\text{g}/\text{kg}$). The pattern of SOCS-3 mRNA was similar to that described previously (25). Specifically, hybridization was observed in the meninges and perivascular cells throughout the brain. There was scattered hybridization in the SFO and choroid plexus. In all cases, including controls, low levels of hybridization were observed in the arcuate nucleus of the hypothalamus and median eminence.

In contrast to leptin, the induction of TIS-11 mRNA by LPS was striking. Intense neuronal hybridization was observed in the SFO (Fig. 5A), paraventricular nucleus of

the hypothalamus, area postrema (Fig. 5E), and neurons scattered throughout the entire brain. Intense nonneuronal hybridization was observed in the meninges, median eminence, choroid plexus, and perivascular regions (Fig. 5A and C) throughout the entire brain. Control animals showed no hybridization above background in these sites (Fig. 5B, D, and F).

CNTF-induced temperature responses and conditioned taste aversion. To assess the pyrogenic properties of CNTF and leptin, body temperatures were recorded for 6 h following intravenous CNTF (250 $\mu\text{g}/\text{kg}$), leptin (1.0 mg/kg), or vehicle administration. We found a significant rise in body temperature (CNTF versus PBS/BSA; Fig. 6A)

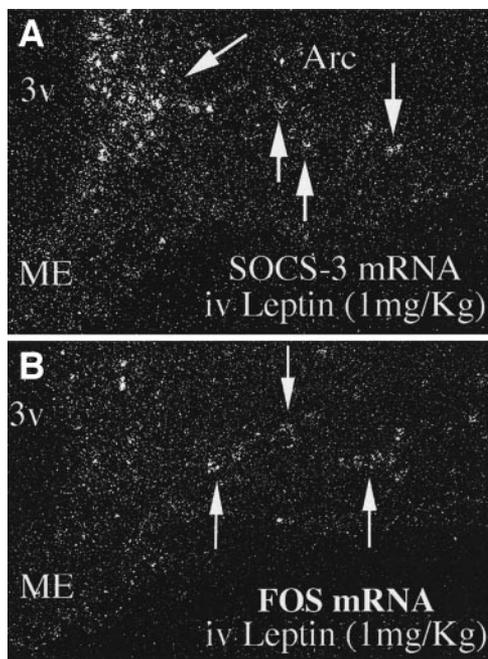


FIG. 4. Photomicrographs demonstrating the distribution of SOCS-3 (A) and Fos mRNA (B) following intravenous leptin in the arcuate nucleus of the hypothalamus (Arc). Vertical arrows mark hybridization that is common to both SOCS-3 and Fos mRNAs. The diagonal arrow in A marks hybridization unique to SOCS-3. Note that leptin induces SOCS-3 mRNA in both the medial and lateral regions of the arcuate. In contrast, leptin induces Fos mRNA only in the lateral regions of the arcuate nucleus. 3v, third ventricle; ME, median eminence.

90–270 min following the injection. For example, the mean temperature at 210 min following CNTF injection was 37.58°C (0.55°C above baseline). In contrast, the mean temperature for rats 210 min following vehicle administration was 37.07°C. Notably, lower doses of CNTF (10 µg/kg) did not induce significant elevations in body temperature. We found no significant change in body temperature following leptin or vehicle administration (leptin versus PFS; Fig. 6B).

The CNTF-induced increase in body temperature that was observed previously (26–29) and in the current studies may suggest that CNTF may produce anorexia by producing nonspecific aversive effects. We tested this hypothesis in mice by assessing the ability of CNTF to produce a conditioned taste aversion (Fig. 6C). As expected, mice consumed significantly less of the LiCl-paired flavor [$t(3) = 6.89$, $P < 0.005$], indicating that they had learned a conditioned taste aversion to LiCl. In contrast, mice showed no preference between the CNTF and vehicle-paired flavors [$t(5) = 0.21$, $P > 0.05$]. Consequently, this dose of CNTF that decreases food intake in mice, unlike LiCl, does not condition a taste aversion.

DISCUSSION

CNTF and leptin engage cytokine receptors and decrease food intake (1,2,5,9a,30). In the current study, we found that the patterns of gene expression induced in the brain by these two molecules are distinct. In addition, we found that LPS also induces Tis-11 and SOCS-3 mRNA expression throughout the CNS, including cells in circumventricular organs and in cells lining blood vessels. Our findings support the hypothesis that CNTF and leptin act

on distinct CNS sites and confirms previous findings that CNTF possesses inflammatory properties distinct from leptin.

SOCS-3 is an inhibitor of JAK-STAT signaling that is induced by activation of type I cytokine receptors (31–33). For example, systemic leptin, CNTF, and LPS induce SOCS-3 mRNA expression in the rat and mouse brain (14,17,25,34). Presumably, SOCS-3 mRNA expression is induced by leptin-, CNTF-, or LPS-induced cytokines (e.g., IL-6) in cells containing cytokine receptors. Although the function of the *tis-11* gene product is unclear, TIS-11 mRNA has been used as a marker for activation following CNTF administration (5). TIS-11 mRNA has been shown to be rapidly induced in human neutrophils (35). Notably, we found that LPS markedly induces TIS-11 mRNA in a pattern similar to the LPS-induced COX-2 mRNA. In agreement, we found LPS induces SOCS-3 mRNA in cerebral blood vessels. Taken together, these findings suggest that TIS-11 and SOCS-3 are both useful marker of cells responding to cytokines.

Previous studies in mice and rats have shown that doses of CNTF and leptin that decrease food intake also induce TIS-11 and SOCS-3 mRNA and phosphorylated STAT3 (pSTAT) in the brain (5,9a,17,34). To determine whether CNTF and leptin target different areas of the brain, we used those markers in conjunction with the expression of Fos-IR, which has been widely used as a marker of neuronal activation (36). Absence of Fos-IR, however, does not exclude the participation of a nuclear group, as Fos may not be expressed in all activated neurons, and inhibited neurons would likely not express Fos (36). In addition, Fos expression does not imply direct activation by a particular stimulus (e.g., leptin or CNTF), as Fos-IR likely results from indirect stimulation as well (e.g., synaptic inputs, changes in blood glucose).

In contrast to CNTF, leptin did not induce gene expression in cells associated with blood vessels. In addition, unlike CNTF and LPS, we found little or no hybridization of TIS-11 in the brain following leptin. However, leptin did induce SOCS-3 mRNA in cells inside the blood-brain barrier, with unremarkable expression in barrier regions. This includes neurons, since leptin receptors are expressed by proopiomelanocortin (POMC) and neuropeptide Y neurons and leptin induces SOCS-3 in neuropeptide Y and POMC neurons (3,4,14). Our results suggest that the use of SOCS-3 and Fos in combination allows one to predict that leptin is directly and differentially acting on hypothalamic neurons, resulting in activation of one population and direct inhibition of another (14) (Fig. 4). These observations are consistent with the recent observations of Cowley et al. (37), who found that leptin directly depolarizes POMC neurons and hyperpolarizes a non-POMC population within the arcuate.

Both systemic CNTF and leptin reduce food intake, raising the possibility that both are potential pharmaceutical agents for modifying food intake and body weight. Specifically, administration of CNTF to *ob/ob*, *db/db*, and diet-induced obese mice dose dependently decreased food intake and body weight (1, 10, and 50 µg/mouse; ~25 and 250 µg/kg and 1.25 mg/kg in the *db/db* mice) (5). Modified versions of CNTF have been shown to induce food intake and body weight at doses of 100 µg · kg⁻¹ · day⁻¹ for

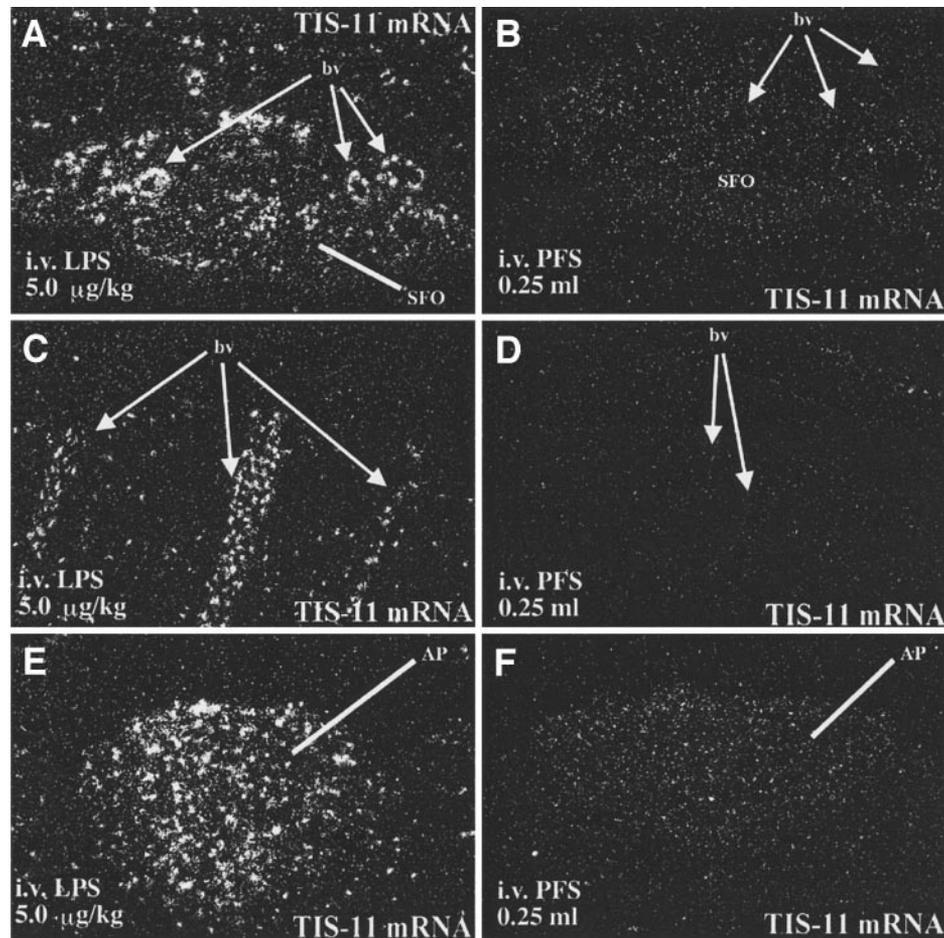


FIG. 5. Photomicrographs demonstrating the distribution of TIS-11 mRNA following intravenous LPS (A, C, and E) or intravenous PFS (B, D, and F). Following LPS, TIS-11 mRNA is strongly induced in cells associated with blood vessels (bv; white arrows) in the SFO (A), areas associated with blood vessels (bv; white arrows) (C), and neurons in the area postrema (AP) (E). Following PFS, the SFO (B), blood vessels (D), and AP (F) contain no hybridization for TIS-11 mRNA.

diet-induced obese mice. A dose of $300 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ CNTF was needed to reduce food intake and body weight to an extent similar to leptin (1.0 mg/kg) in *ob/ob* mice (6). Additionally, both CNTF and leptin induced phospho-STAT immunoreactivity in the arcuate nucleus of the hypothalamus, suggesting that both cytokines act within this nucleus. This was observed in lean animals following both cytokines. Interestingly, this was only observed following CNTF in diet-induced obese mice and mice made obese using gold-thioglucose (6,9a). These findings led to the hypothesis that CNTF may be effective in reducing body weight in leptin-resistant states, including diet-induced obesity. Moreover, the effects may be long lasting, with no rebound weight gain (6,9a).

Previously, CNTF has been shown to cause fever, weight loss, and anorexia in animals (26,27), and humans that receive CNTF report side effects consistent with an immune response, including nausea, anorexia, and weight loss (28,29). Based on the findings discussed above, we chose a dose of CNTF that was likely to reduce food intake and body weight to an extent similar to a leptin dose (1.0 mg/kg) that was previously used by us and other groups (6,12–14,38).

Our present results are consistent with the hypothesis that CNTF administration ($250 \mu\text{g/kg}$) at high doses is

pyrogenic. However, it is noteworthy that a lower dose ($10 \mu\text{g/kg}$) did not significantly raise body temperature or induce a conditioned taste aversion. Lambert et al. (6) argued that the pyrogenic and cachectic effects of CNTF are dose dependent and that doses of $100 \mu\text{g/kg}$ reduced food intake and body weight without increasing corticosterone levels or loss of lean body mass. However, $1,000 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ greatly increased weight loss, including lean mass and increased plasma corticosterone. Lambert et al. did not report the effects of the $300\text{-}\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ dose on these parameters. They did report that a dose of $500 \mu\text{g/kg}$ induced a conditioned taste aversion.

Similar to Anderson et al. (9a), we found that leptin and CNTF induce distinct patterns of gene expression throughout the CNS, suggesting that CNTF and leptin may engage distinct neuronal pathways to mediate their effects. This may be due to the fact that the expression patterns of each of the receptors are largely distinct (7,22,39,40). We also found that CNTF is inducing gene expression in several nonneuronal cell groups. Another possibility to be considered is that CNTF may induce the expression of cytokines of central origin that may exert secondary effects.

A noteworthy site of overlap of leptin- and CNTF-induced gene expression is the arcuate nucleus, through which leptin exerts many of its effects (9a). It is notewor-

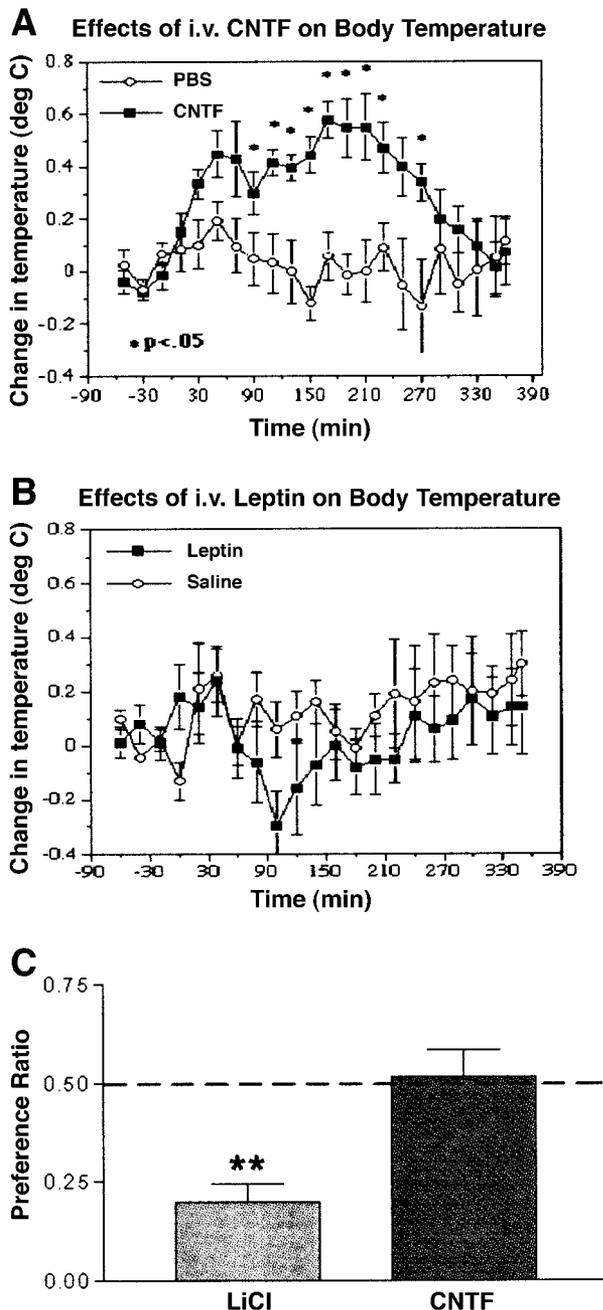


FIG. 6. *A*: Graph showing the change in body temperature following intravenous CNTF (250 µg/kg; ■) and intravenous PBS/BSA (○). * $P < 0.05$ vs. PBS/BSA group. *B*: Graph showing the lack of change in body temperature following intravenous leptin (1 mg/kg; ■) and intravenous pathogen-free saline (○). Values are means \pm SE; $n = 6$ /group in *A* and $n = 4$ /group in *B*. Data were analyzed by ANOVA, and the differences between treatment groups were assessed by Fisher's protected least significant difference test. *C*: Bar graph comparing the preference ratio of the LiCl- or CNTF-paired flavor over total fluid intake. Ratio of 0.5 indicates no preference. * $P < 0.005$, comparing the ratio with the 0.5 null hypothesis.

thy that inflammatory and cachectic signals also engage central melanocortin pathways originating in the arcuate nucleus to induce anorexia. Specifically, it is known that LPS or cytokines induce gene expression in the arcuate nucleus (41,42), including POMC neurons (43). Moreover, it is becoming clear that sickness-induced anorexia may act through central melanocortin circuits, including can-

cer-induced anorexia. LPS-induced anorexia is also attenuated by central antagonism of melanocortin receptors (44–48). Thus, POMC neurons in the arcuate nucleus respond to cytokines. However, it is also noteworthy that Anderson et al. found that CNTF derivatives were effective at inhibiting food intake in mice treated with gold-thioglucose, which presumably would destroy at least a subset of POMC neurons in the arcuate nucleus (48a). Thus, while both leptin and CNTF act directly on cells in the arcuate, it is likely that CNTF does not require an intact arcuate nucleus to mediate its effects.

To investigate potential inflammatory properties of CNTF and leptin, we assessed the induction of COX-2 mRNA, an obligate step in fever production (10,11). We observed CNTF-induced COX-2 mRNA in blood vessels throughout the brain. In contrast, leptin did not induce COX-2 expression. We examined the expression of COX-2 only at the 2-h time point for both leptin and CNTF. Thus, it is possible that leptin does induce COX-2 expression at other time points. Moreover, it is not clear how CNTF induces COX-2 expression, which is more characteristic of cytokines that activate nuclear factor κ B signaling pathways (11). It is noteworthy that neither leptin nor CNTF induced COX-2 expression, as assessed by RT-PCR in whole hypothalamus (49) or COX-2 immunoreactivity in the brain (6). However, our observation of the induction of COX-2 mRNA by CNTF is consistent with the observation that indomethacin blocks CNTF-induced fever (26). Moreover, our results suggest that CNTF induces the expression of Tis-11, SOCS-3, and COX-2 mRNA similar to that induced by LPS or IL-1 administration (10,11,25). Taken together, it is plausible that the relatively high dose of CNTF that we used underlies the induction of COX-2 and body temperature responses.

In contrast to CNTF, leptin is normally found in the circulation, albeit at lower levels than what was used in this study. Despite the high dose of leptin used, we did not observe an increase in body temperature. This is in contrast with the results of Luheshi et al. (50), who found that leptin administration induces fever in rats. The differences between the two studies are unclear, although the source and the species of the recombinant proteins used is always a potential confounding issue. Nonetheless, even at a dose of 5.0 mg/kg, our leptin preparation did not induce a significant change in body temperature. In addition, unlike LPS, IL-1, or CNTF, which induce COX-2 mRNA at febrile doses, leptin did not induce COX-2 mRNA in the brain. Thus, our results suggest that intravenous leptin is not inflammatory. Moreover, we suggest that the anorexia and weight loss observed following CNTF could be due in part to activation of distinct CNS pathways.

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