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Binding Characteristics of Ciliary Neurotrophic Factor to Sympathetic Neurons and Neuronal Cell Lines*

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Ciliary neurotrophic factor (CNTF) is a cytokine whose actions are largely restricted to the nervous system because of the predominant neuronal distribution of its receptor, CNTFRα. In this study, we sought to define the binding characteristics of CNTF to cultured sympathetic neurons and cell lines of neuronal origin. We report that ^{125}I-CNTF binds to cultured sympathetic neurons, MAH, PC12, and EW-1 cells via high and low affinity receptors that can be distinguished on the basis of their dissociation constants (K_{D1}~10^{-12} M and K_{D2}~10^{-9} M). Competition experiments showed that the IC_{50} for rat and human CNTF were, respectively, 65 pM and 5 nM for sympathetic neurons and 75 pM and 1.2 nM for EW-1 cells. Interestingly, leukemia inhibitory factor (LIF) did not compete for CNTF binding even at 100 nM concentration. The binding of ^{125}I-CNTF to sympathetic neurons involved all three components of the CNTF receptor complex, namely CNTFRα, LIFR, and gp130, as shown by cross-linking experiments. CNTF and LIF treatments down-regulated CNTF binding to sympathetic neurons and EW-1 cells, suggesting that heterologous ligands can regulate CNTF receptor levels, which may in turn modulate the efficacy of CNTF *in vitro* and *in vivo*.

Ciliary neurotrophic factor (CNTF)[1] was initially identified, purified, and molecularly cloned based on its ability to support the survival of parasympathetic neurons of the chick ciliary ganglion (Adler *et al.*, 1979; Lin *et al.*, 1989; Stöckli *et al.*, 1989). Subsequent studies have revealed that CNTF can also enhance the survival of sensory neurons (Skaper and Varon, 1986), motor neurons (Sendtner *et al.*, 1990; Arakawa *et al.*, 1990; Oppenheim *et al.*, 1991), cerebellar neurons (Lärkfors *et al.*, 1994), and hippocampal neurons (Ip *et al.*, 1991). CNTF inhibits proliferation and enhances cholinergic properties of neuronal precusors from the sympathetic ganglion (Ernsberger *et al.*, 1989) and stimulates cholinergic differentiation of mature sympathetic neurons (Saadat *et al.*, 1989). In addition, effects of CNTF on developing oligodendrocytes (Louis *et al.*, 1993), denervated (Helgren *et al.*, 1994) and intact (Gurney *et al.*, 1992; Forger *et al.*, 1993) skeletal muscles have also been documented.

Although CNTF does not contain a signal sequence which is typically associated with secreted proteins, Curtis *et al.* (1994) have demonstrated that CNTF can be retrogradely transported by adult sensory neurons from the periphery, suggesting a means by which CNTF gains access to neurons. Moreover, both sensory and motor neurons show greatly increased transport of CNTF following peripheral nerve lesion. Taken together, these observations suggest a possible role for CNTF in motor and sensory neurons after injury. Indeed, potent effects of CNTF toward motor neurons have been demonstrated *in vitro* (Arakawa *et al.*, 1990; Wong *et al.*, 1993) and confirmed *in vivo* using an axotomized-facial nerve paradigm (Sendtner *et al.*, 1990) or mouse mutants that exhibit neuromuscular deficits (Sendtner *et al.*, 1992; Mitsumoto *et al.*, 1994). These studies suggested therapeutic potential of CNTF in musculomotor diseases and have led to human clinical trials for amyotrophic lateral sclerosis (Barinaga, 1994).

Since the cloning of the CNTF-binding protein (hereon CNTFRα) the signal transduction pathway that mediates the effects of CNTF has been studied extensively. CNTFRα shows greatest homology to the α component of the IL-6 receptor (Davis *et al.*, 1990). Recent findings using MAH cells and other neuronal cell lines have demonstrated that CNTF, leukemia inhibitory factor (LIF), IL-6, and oncostatin M share common signaling pathways which involve gp130, the signal transducing component for the IL-6 receptor (Ip *et al.*, 1992); and all except IL-6 also share another receptor component LIFR (for review, see Ip and Yancopoulos, 1992). This family of cytokines has recently been shown to utilize the Jak/Tyk family of cytoplasmic tyrosine kinases (Stahl *et al.*, 1994). gp130 was first identified as a signal transducer for the IL-6 receptor and found to confer high affinity binding to the low affinity IL-6 receptor-α (Taga *et al.*, 1989; Hibi *et al.*, 1990). LIFR, on the other hand, was originally cloned as a LIF-binding protein (Gearing *et al.*, 1991). It has been suggested that gp130 and LIFR together form a functional LIF receptor complex (Gearing *et al.*, 1992; Ip *et al.*, 1992; Davis *et al.*, 1993a) and that addition of soluble CNTFRα to this complex is sufficient to convert a functional LIF receptor into a functional CNTF receptor (Ip *et al.*, 1992; Stahl *et al.*, 1993; Davis *et al.*, 1993b). Whereas the broad distribution of both LIFR and gp130 accounts for the very widespread actions of LIF in neural and non-neural tissues, the limited distribution of CNTFRα (predominantly to the nervous system) is consistent with the much more restricted actions of CNTF (Ip *et al.*, 1993).

While the therapeutic potential of CNTF is becoming apparent and its signal transduction pathway is being unraveled, the binding characteristics of CNTF to its receptor, the first step of the signal transduction cascade, need to be elucidated. Given the predominant neuronal distribution of CNTFRα, this study aimed to examine the binding and pharmacological characteristics of ^{125}I-CNTF to post-mitotic (sympathetic) neurons as well as cell lines of neuronal origin.

MATERIALS AND METHODS

Tissue Culture—Neonatal rat superior cervical ganglia (SCG) were dissociated and cultured as described previously (Kessler, 1984) in a medium consisting of Ham's nutrient mixture F12 with 10% heat-

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[1] The abbreviations used are: CNTF, ciliary neurotrophic factor; IL, interleukin; LIF, leukemia inhibitory factor; SCG, superior cervical ganglia; NGF, nerve growth factor; DSS, disuccinimidyl suberate.

inactivated fetal bovine serum (Hyclone), NGF (100 ng/ml), penicillin (50 units/ml), and streptomycin (50 μg/ml). Cultures were maintained at 37 °C in a humidified 95% air, 5% CO_2 atmosphere. Nonneuronal cells were eliminated by treatment with cytosine arabinoside (10 μM) on days 1 and 3 of culture. Cultures were fed three times/week and were routinely used for binding assays within 2 weeks. Ewing sarcoma (EW-1) and PC12 cells were maintained in RPMI 1640 culture medium supplemented with 10% fetal bovine serum and 2 mM glutamine. MAH cells were maintained in culture as described previously (Birren and Anderson, 1990). Binding assays were usually carried out on cells at 50–80% confluence.

Preparation of ^{125}I-CNTF—Recombinant rat CNTF was iodinated by a modification of the lactoperoxidase method (Marchalonis, 1969) as described previously (DiStefano *et al.*, 1992). The final reaction mixture was chromatographed through a Superdex-75 (Pharmacia Biotech Inc.) column to separate the labeled monomeric CNTF from dimeric and other multimeric derivatives. The percentage of incorporation was about 70–80%, as determined by thin layer chromatography. The specific activity was typically around 1000 Ci/mmol. In all our experiments, only the monomeric radioiodinated CNTF was used; it was stored at 4 °C and used within 1 week of preparation. The radiolabeled CNTF showed comparable biological activity (70–90%) to native CNTF in supporting the survival of chick ciliary neurons in culture.

Binding Assay and Cross-linking—Binding was performed directly on a monolayer of cells. Medium was removed, and cells were washed for 60 min with assay buffer consisting of phosphate-buffered saline (pH 7.4), 0.1 mM bacitracin, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, and 1 mg/ml bovine serum albumin. The cells were incubated in ^{125}I-CNTF for 2 h at room temperature, followed by two quick washes with assay buffer. Cells were lysed with phosphate-buffered saline containing 1% SDS and counted in a Packard Gamma Counter at 90–95% efficiency. Nonspecific binding was defined by the binding in the presence of 100–1000-fold excess of unlabeled CNTF. Specific binding of ^{125}I-CNTF to SCG, EW-1, MAH, and PC12 cells usually ranged from 70 to 95%. The optimal number of cells/well was determined by preliminary experiments to ensure that less than 10% of the ligand would be bound at equilibrium. Total number of cells/well was counted before assay for SCG neurons and estimated from the initial plating densities for cell lines.

For cross-linking experiments, SCG neurons were incubated with 500 pM ^{125}I-CNTF for 2 h at room temperature. The receptor(s) was cross-linked to the ligand by the homobifunctional cross-linking agent DSS (150 μM, for 30 min, room temperature), followed by a wash with 50 mM Tris, 1 mM EDTA (pH = 7) to inactivate DSS. Protein lysates were boiled in sample buffer; equivalents of 6000–8000 neurons were loaded per lane and subjected to SDS-polyacrylamide gel electrophoresis on 7.5% acrylamide gels. The gels were dried and exposed to film. Trophic factors tested for their ability to compete with radiolabeled CNTF included rat CNTF, human CNTF, CNTF-myc (Squinto *et al.*, 1990), NGF, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), all at concentrations of 100 nM.

Growth Factors—Rat and human CNTF were the recombinant proteins expressed in *Escherichia coli* and purified as described (Masiakowski *et al.*, 1991). LIF and IL-6 were obtained from Upstate Biochemical, Inc. (UBI). NGF was purified from mouse salivary glands (Mobley *et al.*, 1976). Recombinant human BDNF and NT-3 were prepared, purified, and provided by Amgen-Regeneron Partners.

Data Analysis—Data from saturation curves were analyzed using a modification of the nonlinear least-squares regression program, LIGAND (Munson and Rodbard, 1980). Data from inhibition experiments were analyzed using GRAPHPAD (ISI).

RESULTS

In this study, cultured rat sympathetic neurons were used, for two reasons, as a model to study CNTF receptor binding. First, it is well established that homogeneous population of SCG neurons can be obtained by tissue culture techniques; thus specific binding reflects only ^{125}I-CNTF binding to sympathetic neurons and not to nonneuronal cells. Second, there is a well-defined physiological response of SCG neurons to CNTF, *i.e.* the stimulation of cholinergic phenotype and suppression of adrenergic phenotype (Saadat *et al.*, 1989). We compared the binding of ^{125}I-CNTF to SCG neurons with that of other adrenergic cells, namely, PC12 cells and MAH cells. EW-1 was also examined as an example of a responsive, but non-adrenergic cell type.

Kinetics—The time course of binding of ^{125}I-CNTF to cultured SCG neurons is shown in Fig. 1. The binding of ^{125}I-CNTF was rapid and reversible. At 22 °C, equilibrium was reached by 90 min and remained stable for up to 4 h. Since the ligand concentration did not change appreciably during the binding reaction (5.1% or less bound), our experiments were performed under pseudo-first-order conditions. Dissociation of the bound complex was initiated after 120 min of incubation with the addition of excess cold ligand (100 nM rat CNTF). The association rate constant (k_{+1}) for SCG neurons can be calculated from the pseudo-first-order equation: $k_{+1} = k_{obs} - k_{-1}/[L]$, where k_{obs} is the slope of a plot of $\ln(B_e/B_e\text{-}B)$ *versus* time (= 2.1×10^{-2} min^{-1}) and k_{-1} is the negative slope of a plot of $\ln(B/B_o)$ *versus* time (= 1.9×10^{-2} min^{-1}); thus, $k_{+1} = 2.0 \times 10^{-2}$ nM^{-1}min^{-1} at 100 pM ^{125}I-CNTF. The dissociation constant (K_D) for ^{125}I-CNTF in SCG neurons calculated from the ratio k_{-1}/k_{+1} was 950 pM.

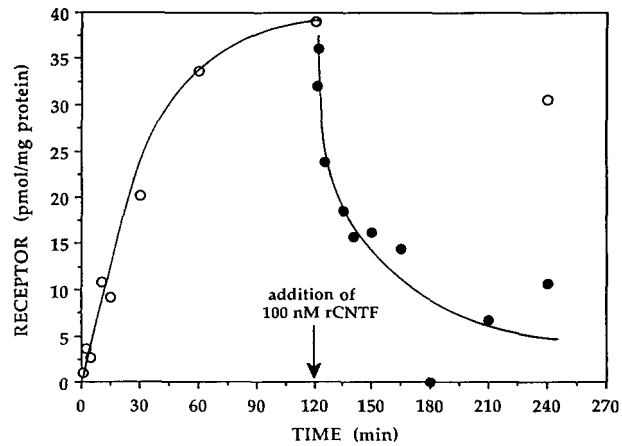

FIG. 1. **Time course of ^{125}I-CNTF binding in SCG neurons.** SCG cultures were incubated with 100 pM ^{125}I-CNTF and binding was brought to a steady state (120 min; *open circles*) before the addition of 100 nM unlabeled rat CNTF to all wells (*closed circles*). At the times indicated, the neurons in wells were washed and solubilized for counting of radioactivity. Values shown here are from a representative experiment, with points representing the mean of triplicate determinations.

Saturation Studies—Scatchard analysis of saturation binding data was carried out in SCG neurons (Fig. 2A), EW-1 (Fig. 2*B*), PC12 (Fig. 2*C*), as well as MAH (Fig. 2*D*) cells, using concentrations of ^{125}I-CNTF ranging from 20 pM to 10 nM. Binding of ^{125}I-CNTF to all the cell types studied was saturable and displayed high and low affinity receptor sites.

In SCG neurons, the biphasic Scatchard plot approached linearity at both extremes of ^{125}I-CNTF concentrations (Fig. 2*A*), suggesting the presence of two classes of receptors. Indeed, Scatchard analysis revealed two dissociation constants with values of 1.1 pM and 2.4 nM, and the number of low affinity binding sites is 87-fold higher than that of the high affinity sites (approximately 6.5×10^6/neuron and 7.5×10^4/neuron, respectively). As a result, the K_D derived from kinetic studies (950 pM) is much closer to the low affinity binding constant. Thus, the K_D derived from our kinetic studies is an approximation and mostly for the purpose of comparison; the K_D derived from Scatchard analysis is more accurate.

Scatchard plots of ^{125}I-CNTF binding to EW-1, PC12, and MAH cells also revealed two classes of receptors (Fig. 2, *B-D*). The high affinity receptors in these cell lines were 6–9-fold lower in affinity than those of SCG neurons (8.6 pM for EW-1, 9.3 pM for PC12, and 5.7 pM for MAH). With the exception of MAH cells (K_{D2} = 412 pM), the dissociation constants of the low affinity receptor were similar in all cells, 2.4, 1, and 3.4 nM in SCG neurons, EW-1 cells and PC12 cells, respectively (summarized in Table I). Primary sympathetic neurons, by far, con-

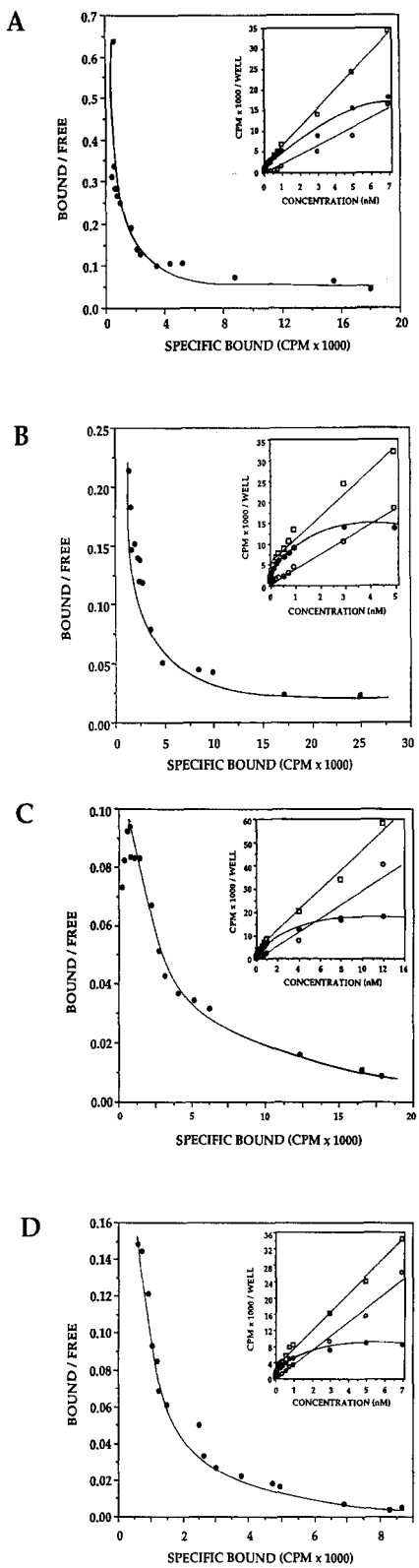

FIG. 2. **Saturation binding of ^{125}I-CNTF (*insets*) and Scatchard analysis in SCG neurons (*A*), EW-1 (*B*), PC12 (*C*), and MAH (*D*) cells.** Cultures were incubated with 15 concentrations of ^{125}I-CNTF (20 pM-10 nM). The specific binding (*closed circles*), defined as the difference between total (*open squares*) and nonspecific (*open circles*) binding, was saturable in all cell types examined. Values shown here are from a representative experiment, with points representing the mean of triplicate determinations.

TABLE I

Cell type	K_D	Receptors/cell	IC_{50}
SCG	1.1 pM	75,000	65 pM
	2.4 nM	6,500,000	
EW-1	8.6 pM	4,200	75 pM
	1.0 nM	22,000	
MAH	5.7 pM	1,500	ND[a]
	412 pM	103,000	
PC12	9.3 pM	18,000	ND[a]
	3.4 nM	361,000	

[a] ND, not determined.

tained the greatest number of both high and low affinity receptor/cell than any of the cell lines examined, most likely a result of their extensive neuritic network. The ratio of high to low affinity sites is similar in SCG neurons and MAH cells (1:87 and 1:69, respectively), but lower in PC12 and, particularly in EW-1 cells (1:20 and 1:5, respectively).

Pharmacological Properties—Inhibition of ^{125}I-CNTF binding (at 100 pM) to cultured SCG neurons (Fig. 3*A*) and EW-1 (Fig. 3*B*) cells was determined for 15 concentrations of rat and human CNTF. The IC_{50} values for rat and human CNTF were 65 pM and 5 nM, respectively, for SCG neurons and 75 pM and 1.2 nM for EW-1 cells (Table I). Human CNTF was, thus, less potent than rat CNTF in displacing ^{125}I-CNTF binding in both cell types. This is in good agreement with the difference in potency between rat and human CNTF as described previously (Panayotatos *et al.*, 1993). Interestingly, although LIF can elicit the same functional response in SCG neurons as CNTF (*i.e.* stimulation of choline acetyltransferase activity and decreased tyrosine hydroxylase activity), LIF did not compete for CNTF binding even at 100 nM concentration. The neurotrophins (such as NGF, BDNF, NT-3) and IL-6 did not compete for ^{125}I-CNTF binding in any cell type examined (Fig. 3*A*).

SCG Neurons Contain all Three Components of the CNTF Receptor Complex—In ^{125}I-CNTF cross-linking experiments in cultured SCG neurons, we observed three specific bands at molecular masses of approximately 100, 150, and 200 kDa, which corresponded to those predicted for the cross-linked products of ^{125}I-CNTF (22 kDa) with CNTFRα (80 kDa), gp130 (145 kDa), and LIFR (190 kDa), respectively (Fig. 4). The identities of the upper two cross-linked products containing LIFR and gp130 have been verified by immunoprecipitation with specific antibodies (Ip *et al.*, 1992; Stahl *et al.* 1993). Cross-linking to all three protein bands was displaced by rat CNTF, CNTF-myc, and human CNTF, but not by NGF, BDNF, or NT-3. This indicates the specificity of the binding and that binding of CNTF to SCG neurons recruits all three components of the receptor complex.

CNTF Treatment Down-regulates the Binding of ^{125}I-CNTF to SCG Neurons and EW-1 Cells—Cultures of SCG neurons and EW-1 cells were treated with CNTF or LIF (0.01–100 ng/ml) for 24 and 72 h, respectively, prior to ^{125}I-CNTF binding assay. We found that pretreatment with CNTF or LIF resulted in a concentration-dependent loss of specific ^{125}I-CNTF binding to SCG neurons (Fig. 5A), producing a 50% loss at approximately 0.5 ng/ml of CNTF or 5 ng/ml LIF. Similarly, pretreatment of EW-1 cells with CNTF resulted in a comparable loss of binding at approximately 5 ng/ml (Fig. 5*B*). IL-6 pretreatment had no effect on CNTF binding to either cell types. Our findings show that pretreatment with CNTF or LIF down-regulates CNTFRα in SCG neurons and EW-1 cells. This is consistent with the fact that CNTF and LIF share common receptor components, and further suggests that their signaling pathways converge down stream.

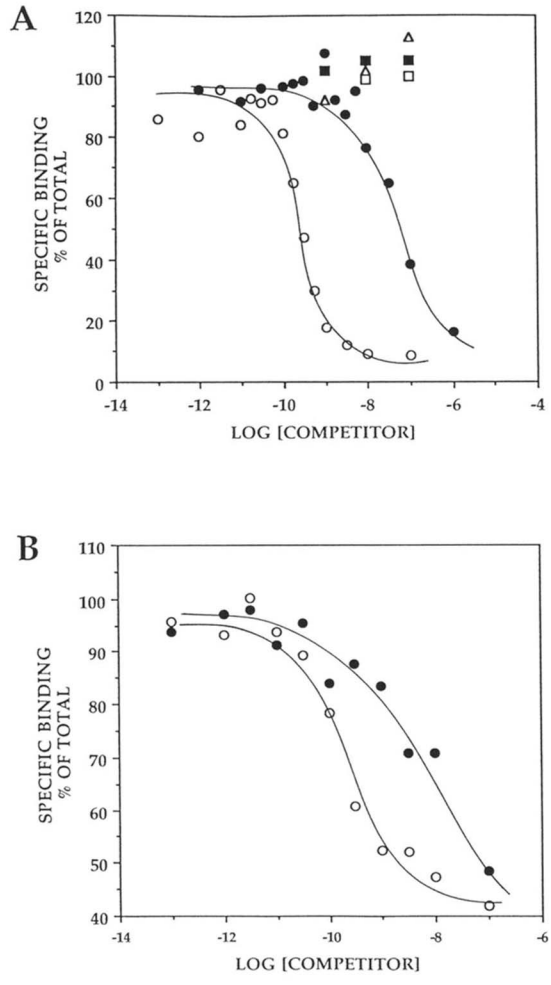

FIG. 3. **Pharmacological characterization of ^{125}I-CNTF binding in SCG neurons (*A*) and EW-1 cells (*B*).** Cultures were incubated with 200 pM ^{125}I-CNTF in the presence of 15 concentrations of unlabeled rat CNTF (*open circles*), human CNTF (*closed circles*), LIF (*open squares*), IL-6 (*closed squares*), or BDNF (*triangles*). Values shown here are from a representative experiment, with points representing the mean of triplicate determinations.

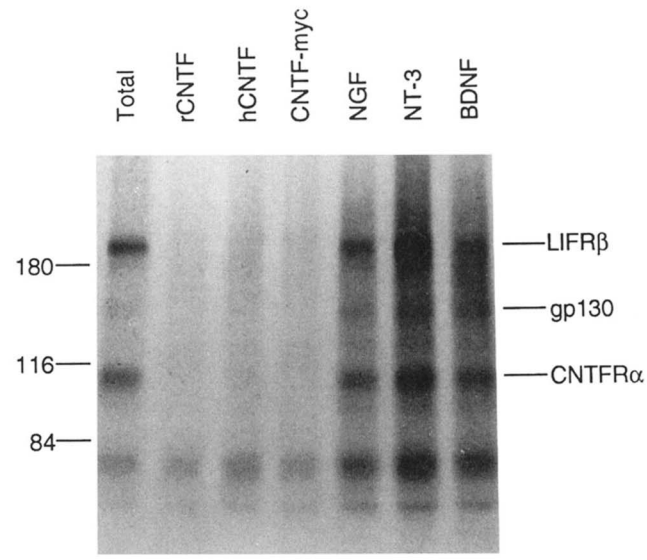

FIG. 4. **Cross-linking of ^{125}I-CNTF to SCG neurons.** SCG cultures were incubated with 500 pM ^{125}I-CNTF in the presence of rat CNTF, human CNTF, CNTF-myc, NGF, NT-3, or BDNF (all at 100 nM). The receptor was cross-linked to the ligand by DSS (150 μM), solubilized, and the cell lysates fractionated by SDS gel electrophoresis. Each lane was loaded with pooled lysate of three culture wells of approximately equal number of SCG neurons.

DISCUSSION

In parallel with the discovery and complete characterization of a number of neurotrophic factors has been the molecular characterization of their receptors (for review, see Davis and Yancopoulos, 1993; Ip and Yancopoulos, 1993). The biological effects and signal transduction pathways for one of these factors, CNTF, have been extensively studied, and binding properties of CNTF to its receptor have been reported in transformed hematopoietic cell lines (Gearing *et al.*, 1994). Considering the predominant neuronal distribution of CNTFRα, knowledge of CNTF binding characteristics in neurons is important for understanding the physiological role of CNTF *in vivo*. In this study, we sought to define the binding characteristics of ^{125}I-CNTF to primary sympathetic neurons and to cell lines of neuronal origin. We report that ^{125}I-CNTF binds to cultured SCG neurons, EW-1, MAH, and PC12 cells via two classes (high and low affinity) of receptors which can be distinguished on the basis of their dissociation constants (K_{D1}~10^{-12} M and K_{D2}~10^{-9} M). A hallmark of neurotrophic factors is their potency in producing biological effects; the high affinity binding constant of CNTF is in good agreement with the observed potency of its biological effects on chick ciliary ganglion neurons (EC_{50} = ~2 pM; Masiakowski *et al.*, 1991) and rat sympathetic neurons (EC_{50} = ~20 pM; Sadaat *et al.*, 1989). It has been shown that the three CNTF receptor components (CNTFRα, LIFR, and gp130) are initially unassociated on the cell surface and are brought together in a step-wise fashion upon CNTF binding (Stahl and Yancopoulos 1993). The non-signal transducing binding of CNTF to CNTFRα is most likely low affinity, whereas high affinity binding probably ensues when the tripartite receptor complex is formed. Interestingly, PC12 cells, which lack LIFR,[2] confer both high and low affinity binding, suggesting that CNTF·CNTFRα complex binds to gp130 with high affinity with or without further heterodimerizing with LIFR. This is in contrast to the findings in transformed hematopoietic cells where LIFR, but not gp130, was shown to be critical for generating high affinity CNTF binding (Gearing *et al.*, 1994).

CNTF and LIF have been shown to elicit identical functional responses in SCG neurons (Kotzbauer *et al.*, 1994), and they both induce *tis11* and c-*fos* gene expression and the phosphorylation of LIFR and gp130 in MAH and EW-1 cells (Ip *et al.*, 1993; Stahl *et al.*, 1993). Similarly, retrograde transport of ^{125}I-CNTF in lesioned sciatic nerve was inhibited by excess unlabeled CNTF as well as LIF (Curtis *et al.*, 1994). In the present study, however, we found that the 2 cytokines have different binding characteristics. LIF, even at 100 nM concentration, did not compete for ^{125}I-CNTF binding in SCG neurons. While CNTF and LIF share common signal transduction components (LIFR and gp130), CNTFRα is unique for CNTF. Thus, although LIF does not bind to CNTFRα, it can deplete LIFR and gp130 and, in turn, should block high affinity CNTF binding. However, because the concentration of ^{125}I-CNTF used in our competition experiments (100–300 pM) labeled both high and low affinity binding sites, and since low affinity sites were in great excess, changes in high affinity binding would most likely be obscured.

In addition to the classical physiologic responses that neurotrophic factors are known to evoke, they also regulate and modulate the functional properties of their own receptors. They may increase or decrease the number of their receptors or

[2] N. Y. Ip, N. Stahl, and G. D. Yancopoulos, unpublished data.

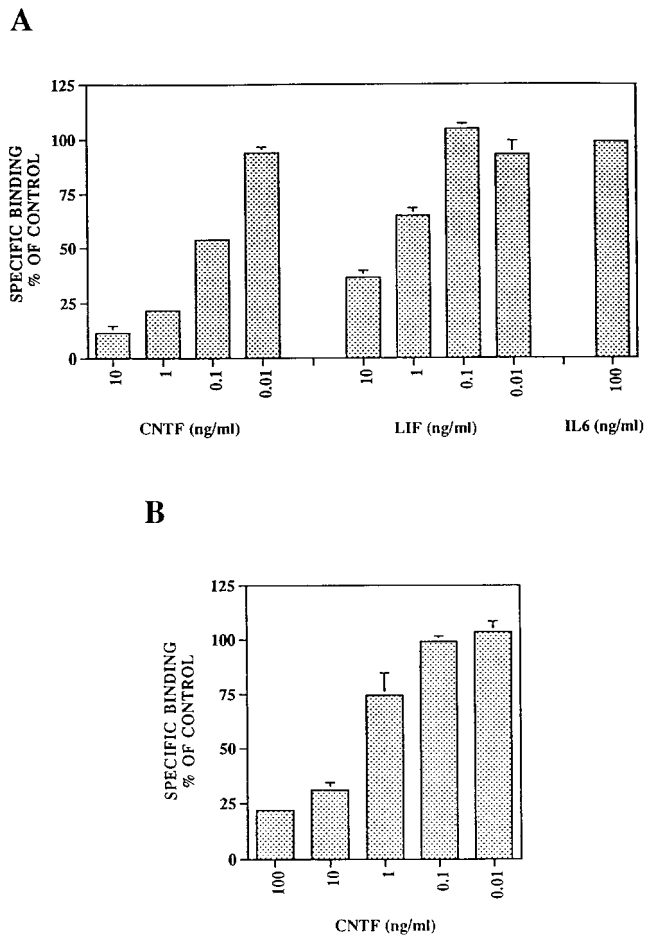

FIG. 5. **Regulation of ^{125}I-CNTF binding.** SCG neurons (*A*) and EW-1 cell (*B*) were pretreated with CNTF, LIF, or IL-6 for 24 and 72 h, respectively, prior to ^{125}I-CNTF binding assay. Values shown here are from a representative experiment, with points representing the mean of triplicate determinations ± S.E.

change the efficiency of binding to their receptors. For instance, intrathecal infusion of NGF to dorsal root ganglia resulted in an up-regulation of low affinity NGF receptor mRNA levels (Verge *et al.*, 1992). Our findings showed that CNTF binding to SCG neurons was down-regulated by prior treatment with either CNTF or LIF. It is most likely that these decreases in specific binding were due to down-regulation of receptors, and not simply competition by any residual CNTF left over from treatment because similar down-regulation was also evident after LIF treatment. As LIF and CNTF share common receptor signaling subunits, gp130 and LIFR, all downstream signals produced by these two ligands are identical. However, CNTF is the only cytokine that binds to CNTFRα (for review, see Stahl and Yancopoulos, 1993). LIF *does not* bind to nor require CNTFRα for signal transduction, but was able to down-regulate CNTF binding in sympathetic neurons; this strongly suggests that such down-regulation is a specific event and not due to competition by any residual CNTF left over from treatment. Moreover, results from kinetic studies have shown that CNTF dissociates from its receptor to an almost base-line level within 20 min following a cold chase (Fig. 1) and within 40 min even without cold chase (data not shown). Since the cultures were washed for 60 min prior to binding, it is unlikely that there was sufficient CNTF left behind to alter the binding results. Taken together, the data represented in Fig. 5 were not the result of receptor saturation, but rather, specific down-regulation of CNTF binding after LIF or CNTF treatment.

Unlike the competition experiments, where the ability of LIF to compete for CNTFRα sites was specifically measured, the regulation experiments might reflect the binding of LIF to any of the three components in the CNTFR complex. In this case, LIF binds to LIFR which then heterodimerizes with gp130 and initiates signal transduction; upon activation of their specific receptor components, the signal transduction pathways of CNTF and LIF converge downstream in the neuron. It is possible that SCG neurons cannot distinguish whether the signal was elicited by CNTF or LIF; thus, although LIF does not compete for CNTF binding, it can indirectly down-regulate CNTFRα. This may have functional consequences *in vivo* under pathological and pharmacological conditions. If LIF is present in the vicinity where CNTF is "released" or administered *in vivo*, it may cause an indirect down-regulation of CNTF receptors and, in turn, may alter the efficacy of CNTF. Thus, binding characteristics of CNTF and regulation of its receptor complex provide crucial information to the use of CNTF as a therapeutic agent.

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