

## Neurotrophic cytokines and activin A differentially regulate the phenotype of cultured sympathetic neurons

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**ABSTRACT** A number of cytokines sharing limited sequence homology have been grouped as a family because of partially overlapping biological activities, receptor subunit promiscuity, and the prediction of a shared secondary structure. Since several of these cytokines regulate gene expression and cell number in the nervous and hematopoietic systems, this specific group is termed the neurotrophic cytokine family. Using a reverse transcription–polymerase chain reaction-based assay system for monitoring the expression of multiple phenotypic markers in cultured sympathetic neurons, we present further evidence that, in addition to cholinergic differentiation factor/leukemia inhibitory factor and ciliary neurotrophic factor, oncostatin M, growth promoting activity, interleukin 6, and interleukin 11 belong in this family. In addition, one member of the transforming growth factor  $\beta$  superfamily, activin A, shares a selective overlap with the neurotrophic family in the spectrum of neuropeptides that it induces in sympathetic neurons. The particular neuropeptides induced by activin A, however, demonstrate that the activity of this cytokine is distinct from that of the neurotrophic family. Twenty-six other cytokines and growth factors were without detectable activity in this assay.

Diffusible proteins can regulate neural development at a number of stages, from the initial proliferation and differentiation of progenitor cells, through the outgrowth of processes and formation of synapses, to the rearrangement of connections in postnatal life (1). Two of the families of proteins involved in these events include the neurotrophins and the neurotrophic cytokines. The prototype of the former group is nerve growth factor (NGF), and subsequent family members were cloned by utilizing the extensive homology between NGF and the second neurotrophin identified, brain-derived neurotrophic factor. In contrast, the neurotrophic family is emerging not through strong homologies among cytokine sequences, but rather by shared biological activities and predicted secondary structures (2, 3). Subsequent studies on the receptors for the neurotrophic cytokines have revealed not only structural homology among the receptors but the sharing of a common receptor transducing subunit and similar signal-transducing pathways (4–8).

Inaugural members of the neurotrophic family include a protein we termed the cholinergic differentiation factor (CDF; refs. 9–11), also widely known as leukemia inhibitory factor (LIF; ref. 12), and ciliary neurotrophic factor (CNTF; refs. 13–15). These two proteins display identical profiles of activity in the regulation of neuropeptide expression in cultured sympathetic neurons (16, 17) and in the support of motor neuron survival (18, 19). In addition, Bazan (2) predicted that CDF/LIF and CNTF would share a similar secondary structure with interleukin (IL)-6, oncostatin M (OSM), and granulocyte colony-stimulating factor (G-CSF). Similar predictions were made by Rose and Bruce (20). While

it was known that CDF/LIF and IL-6 elicited many of the same responses in nonneural cells (21), the linkage with OSM was not previously suspected. Further evidence for a close relationship between these proteins is the finding that OSM can displace CDF/LIF from its binding sites on M1 cells (22). Moreover, OSM was recently found to induce the same neuropeptide in a neuroblastoma line as CDF/LIF (23). Another candidate for membership in this family is IL-11, a cytokine that phosphorylates the same gp130 receptor subunit as do CDF/LIF, CNTF, and IL-6 (5, 24). Recently, growth-promoting activity (GPA), a protein that shares trophic activity for ciliary neurons with CNTF, was cloned from chicken and found to have a limited sequence homology with mammalian CNTF (25). Finally, extracts of rat foot pads (which contain sweat glands, the target of cholinergic sympathetic neurons) were found to contain a protein, termed the sweat gland factor (SGF), that exhibits both CDF/LIF and CNTF activities and is similar to, but distinct from, CNTF (26, 27). SGF has not as yet been sequenced. All of these proteins are therefore candidates for membership in the neurotrophic cytokine family.

Other cytokines may be involved in the development of the nervous system. IL-3 acts as a trophic factor for central cholinergic neurons (28), and IL-5, -7, -9, and -11 regulate differentiation of murine hippocampal progenitor cells (29). IL-1 and tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), two inflammatory cytokines released after injury, can induce NGF and CDF/LIF, which in turn have effects on neuronal survival and gene expression (30–32). Activin A, a member of the transforming growth factor  $\beta$  (TGF $\beta$ ) superfamily, stimulates expression of the neuropeptide somatostatin in cultured ciliary ganglion neurons (33). Accordingly, these cytokines are candidate neuronal differentiation factors. Nonetheless, there has been no systematic analysis of the effects of these cytokines on neuronal phenotype.

We have designed a reverse transcription (RT)-PCR assay to analyze the effects of soluble factors on the expression of neuropeptides and neurotransmitter synthesizing enzymes in cultured sympathetic neurons. In this assay, CDF/LIF and CNTF induce an identical set of neuropeptide and neurotransmitter enzyme mRNAs (34). We here present the results of testing the effects of 33 different recombinant cytokines and growth factors on neuronal gene expression. These

Abbreviations: CCK, cholecystokinin; CDF/LIF, cholinergic differentiation factor/leukemia inhibitory factor; CGRP, calcitonin gene-related peptide; ChAT, choline acetyltransferase; CNTF, ciliary neurotrophic factor; DYN, dynorphin; ENK, enkephalin; FGF, fibroblast growth factor; GAD65, 65-kDa glutamic acid decarboxylase; GAD67, 67-kDa glutamic acid decarboxylase; G-CSF, granulocyte colony-stimulating factor; GPA, growth-promoting activity; GRO $\alpha$ , growth-related cytokine  $\alpha$ ; IL, interleukin; M-CSF, macrophage colony-stimulating factor; MIP1 $\alpha$ , macrophage inflammatory protein 1 $\alpha$ ; NGF, nerve growth factor; NPY, neuropeptide Y; OSM, oncostatin M; rh-, recombinant human; RT, reverse transcription; SGF, sweat gland factor; SOM, somatostatin; SP, substance P; TGF $\alpha$  and - $\beta$ , transforming growth factor  $\alpha$  and  $\beta$ ; TH, tyrosine hydroxylase; TNF $\alpha$ , tumor necrosis factor  $\alpha$ ; TPH, tryptophan hydroxylase; VIP, vasoactive intestinal polypeptide.

results support the hypothesis that GPA and OSM belong in the neurotrophic family. In addition, weak activities of IL-6 and IL-11 can also be detected in this assay. The pattern of neuronal gene expression induced by activin A, however, sets it apart from this family.

## MATERIALS AND METHODS

**Animals and Reagents.** Neonatal Sprague-Dawley rats were purchased from Simonsen Laboratories (Gilroy, CA). Most of the tissue culture reagents, Superscript reverse transcriptase II, and the 1-kb DNA marker were purchased from GIBCO/BRL. NGF, glycogen, dATP, dCTP, dGTP, dTTP, acidic fibroblast growth factor (FGF) and basic FGF were purchased from Boehringer Mannheim. RNase inhibitor (RNasin) and *Taq* DNA polymerase were purchased from Promega. Oligo(dT) was obtained from Pharmacia. Oligonucleotide primers were synthesized in the Biopolymer Synthesis and Analysis Resource Center at Caltech. The recombinant proteins murine IL-1, murine TNF- $\alpha$ , human activin A, human growth-related cytokine  $\alpha$  (GRO $\alpha$ ), and rat CNTF were gifts from Genentech. Chick GPA was kindly provided by T. Finn and Rae Nishi of Oregon Health Sciences University (Portland). Human recombinant activin A was also obtained from Jim Smith at the National Institute for Medical Research (London). Human macrophage inflammatory protein 1 $\alpha$  (MIP1 $\alpha$ ) was provided by Mary Freshney and Gerry Graham at the Beatson Institute for Cancer Research (Glasgow, U.K.). The recombinant proteins murine IL-3, -4, -6, -7, and -9, human IL-8, and macrophage colony stimulating factor (M-CSF) were purchased from R & D Systems. The recombinant protein human IL-10 was supplied from Genzyme. The recombinant protein human IL-11 was a gift from Yu-Chung Yang at Indiana University. The recombinant protein murine IL-12 was provided by Stanley Wolf from Genetics Institute (Cambridge, MA). The recombinant protein human OSM was provided by David Gearing at Immunex. Other cytokines and growth factors used in this study were recombinant human (rh) proteins provided by James Miller and colleagues at Amgen. All other reagents were purchased from Sigma.

**Neuronal Culture.** Dissociated sympathetic neurons were prepared and cultured in serum-free medium as described previously (34–36). For reproducibility, neurons were seeded in 96-well plates (Falcon), at a density of one ganglion per well. Cultures were maintained for 7 days, and half of the medium was changed every 36 hr in an effort to ensure that cytokines maintained activity. The antimetabolic agent aphidicolin was added to the cultures at the concentration of 4  $\mu$ g/ml, effectively eliminating nonneuronal cells. After 7 days, each well contained approximately 3000 neurons, and more than 95% of the surviving cells were neurons, as judged by phase-contrast microscopy (36). Cytokines and growth factors were added from the second day of culture, and duplicate wells were prepared for each condition. All results reported here were reproduced in at least two separate neuronal platings.

**Preparation of RNA and cDNA.** Total RNA preparation and cDNA synthesis from cultured neurons were as described previously (34). Briefly, 160  $\mu$ l of lysis buffer was added to each well. The lysate was transferred to a 1.7-ml Eppendorf tube and swirled vigorously to shear DNA, and then one round of acidic phenol/chloroform extraction was performed to deplete DNA and proteins. RNA was coprecipitated with 30  $\mu$ g of glycogen in a 50% isopropyl alcohol solution and stored at  $-20^{\circ}\text{C}$  overnight.

To produce cDNA from the cultured neurons, the total RNA was centrifuged, dried, and directly dissolved in 10  $\mu$ l of 13 mM methylmercury hydroxide for 10 min, and an additional 2  $\mu$ l of 75 mM 2-mercaptoethanol was added for 5

min. Oligo(dT)-primed RT was done in a final volume of 20  $\mu$ l, containing the RT buffer (34), 100 units of reverse transcriptase, and 20 units of RNasin, for 1 hr at  $37^{\circ}\text{C}$ .

**PCR.** The sequences of the primers and conditions used in the PCR for each neuronal gene analyzed were described in a previous report (34). Amplification of cDNA was performed in a thermal cycler (MJ Research, Watertown, MA). Each tube contained a final volume of 20  $\mu$ l, consisting of 1  $\mu$ l of cDNA, 1 $\times$  PCR buffer (Promega), 0.5 unit of *Taq* DNA polymerase, 0.25 mM dNTP, and one set of primers (200 nM). After the reaction, 8  $\mu$ l of each PCR sample was analyzed on a 2% agarose gel, and the products were visualized after ethidium bromide staining and UV illumination. The appropriate amplification cycle schedule for each neuropeptide gene was determined empirically, to allow a minimal, yet detectable, signal for control samples (neurons grown without cytokines or growth factors). Cytokines were deemed to have regulatory effects on specific genes if the intensities of the PCR products were about 3-fold different from those of negative controls, as judged by eye.

## RESULTS

**Screening of 33 Cytokines and Growth Factors for Effects on Neuronal Gene Expression.** In the first screen for effects of cytokines and growth factors on neuronal phenotype, two concentrations (10 ng/ml and 100 ng/ml) of each recombinant protein were applied to neonatal rat sympathetic neurons in culture for 6 days. The expression pattern of different neuromodulators in the presence of cytokines and growth factors was elucidated by the RT-PCR assay and the results are summarized in Table 1. Twenty-six factors showed no effect on the intensity of the PCR products for any of the six genes analyzed, in at least two independent experiments; these factors were not analyzed further. In addition to reporting our findings with CDF/LIF and CNTF, we report here in detail for the first time the effects of GPA, OSM, IL-6, IL-11, and activin A on neuronal gene expression. Some of the inductive effects of CDF/LIF, CNTF, and OSM have been demonstrated previously (16, 17, 23, 34, 37).

**GPA Induces the Same Set of Neuropeptides and Neurotransmitter Synthetic Enzymes as CNTF.** GPA was purified from chick and has the same ciliary neurotrophic activity as mammalian CNTF. Moreover, the amino acid sequence of GPA shows a 50% identity to the sequences for human, rat, and rabbit CNTF (25). In contrast to CNTF, however, GPA has been shown to be secreted from cells in a transient transfection experiment (25). We tested whether GPA has the same effects as CNTF on cultured sympathetic neurons, and the results are illustrated in Fig. 1. The  $\beta$ -actin band was used to monitor the amount of mRNA present in each sample, and duplicate samples were prepared for each condition. GPA induces mRNAs for the neuropeptides substance P (SP), enkephalin (ENK), vasoactive intestinal polypeptide (VIP), cholecystokinin (CCK), and somatostatin (SOM), as well as the enzyme for synthesizing acetylcholine, choline acetyltransferase (ChAT), in a dose-dependent manner. This pattern of neuropeptide induction is very similar to that of CNTF and CDF/LIF. We also analyzed expression of the mRNAs for the enzymes synthesizing  $\gamma$ -aminobutyric acid (glutamic acid decarboxylase; GAD65 and GAD67 for the 65- and 67-kDa forms), the enzyme for synthesizing catecholamines (tyrosine hydroxylase; TH), the enzyme for synthesizing serotonin (tryptophan hydroxylase; TPH), and the neuropeptides calcitonin gene-related peptide (CGRP), dynorphin (DYN), and neuropeptide Y (NPY). As with CNTF, GPA has no detectable inductive effect on these genes at the mRNA level (data not shown). This identity in the expression patterns of 13 neuropeptide and neurotransmitter mRNAs

Table 1. Regulation of gene expression in sympathetic neurons

Cytokines	CCK	ChAT	ENK	SOM	SP	VIP	CGRP, DYN, NPY	GAD, TH, TPH
<b>Neuroipoietic</b>								
CDF/LIF, CNTF, GPA	++	+	++	++	+++	+	-	-
OSM	+	+	+	+	++	+	-	-
IL-6, IL-11	-	-	-	-	+	-	-	-
G-CSF	-	-	-	-	-	-	-	-
<b>Other</b>								
Activin A	+	+	-	++	-	-	++	-
IL-1 $\alpha$ , -2, -3, -4, -5, -7, -8, -9, -10, -12, IFN- $\gamma$ , GM-CSF, M-CSF, EPO, TGF $\alpha$ , TGF $\beta$ , EGF, IGF-1, TNF $\alpha$ , SCF, MIP1 $\alpha$ , GRO $\alpha$ , PDGF, acidic FGF, basic FGF	-	-	-	-	-	-	ND	ND

A + indicates that there is induction effect; a - indicates that there is no induction effect; ND, not determined. Cytokines not previously defined: IFN- $\gamma$ , interferon  $\gamma$ ; EPO, erythropoietin; TGF $\alpha$  and - $\beta$ , transforming growth factor  $\alpha$  and  $\beta$ ; EGF, epidermal growth factor; IGF-1, insulin-like growth factor 1; SCF, stem cell factor; PDGF, platelet-derived growth factor. Neuropeptides and enzymes in column heads are defined in the text.

strengthens the idea that GPA and CNTF share many biological activities.

**OSM, IL-6, and IL-11 Have Activity in the Sympathetic Neuron Assay.** OSM and CDF/LIF have the same biological activities in several assay systems, including triggering differentiation of M1 leukemia cells and the induction of the acute phase response in hepatocytes (38, 39). Recently, Gearing and colleague (22) provided evidence that OSM binds to the CDF/LIF receptor with high affinity. To test the effects of OSM on sympathetic neurons, various concentrations of human OSM were applied and the expression of neuronal genes was analyzed. For comparison, CDF/LIF at 1.6 ng/ml was included in sister cultures. The data in Fig. 2 illustrate that OSM induces mRNAs for SP, ENK, ChAT, and CCK, but only at concentrations (>25 ng/ml) higher than required for similar inductive effects by CDF/LIF. OSM induces a low level of mRNA for SOM, even at 400 ng/ml. It is possible that this quantitative difference is due to the species difference between OSM (human) and the target neurons (rat). Reduced responsiveness of rat cells to OSM was also demonstrated in an acute phase protein assay (39). Like GPA, OSM does not induce the expression of the mRNAs for GAD65, GAD67, TH, TPH, CGRP, DYN, and NPY (data not shown).

As outlined in the introduction, there are results raising the possibility that several other cytokines may belong to the neuroipoietic family. These include G-CSF, IL-6, and IL-11. These proteins were tested in the sympathetic neuron assay,

along with CDF/LIF for comparison, and the results are illustrated in Fig. 3. G-CSF has no detectable activity on the expression of any of the genes analyzed. IL-6 and IL-11, in contrast, induce preprotachykinin mRNA (which produces the SP neuropeptide). This effect is weak and is observed only at concentrations of >100 ng/ml. Among the several genes influenced by CDF/LIF and CNTF, SP is the one that displays the most striking response (>100-fold increase; ref. 16). Therefore, rather than displaying more specificity than CDF/LIF and CNTF, it could be that IL-6 and IL-11 are such weak inducers that only an increase in SP can be observed.

**Activin A Induces a Different Set of Neuronal Genes.** Activin A is a member of the TGF $\beta$  superfamily and has effects on erythroid differentiation, embryonic mesoderm induction and axis formation, and sexual development (40). Although this protein does not belong in the neuroipoietic cytokine family on the basis of structure, it does have effects on neuronal gene expression. Since it was recently demonstrated that activin A can induce SOM in ciliary ganglion neurons (33), it was of interest to determine whether activin A has similar effects on sympathetic neurons. In fact, activin A elicits an induction pattern of mRNAs for neuropeptides and neurotransmitter synthetic enzymes that is distinct from that of CNTF and CDF/LIF. As illustrated in Fig. 4, CDF/LIF induces mRNAs for SP, ENK, and VIP but does not alter the expression of CGRP and DYN mRNAs. Activin A, in contrast, does not alter the expression of SP, ENK, and VIP mRNAs, even at the concentration of 400 ng/ml, but it does have potent effects on CGRP and DYN mRNAs (Fig. 4).

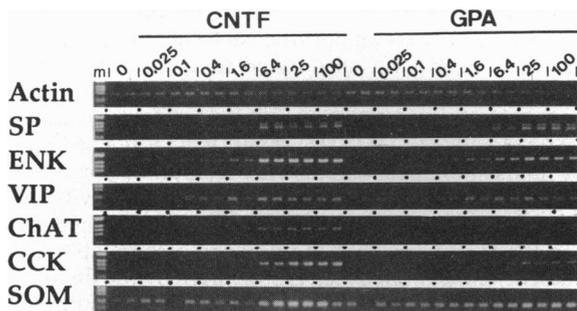


FIG. 1. Rat CNTF and chicken GPA induce mRNAs for SP, ENK, VIP, ChAT, CCK, and SOM with a similar dose dependency. Various concentrations (ng/ml) of rat CNTF and chicken GPA were added to cultured sympathetic neurons from day 2 to day 7. Duplicate samples were run for each concentration. The relative abundance of each mRNA was analyzed by RT-PCR. The expression of  $\beta$ -actin was used to monitor the amount of RNA in each sample. The DNA molecular weight standard (m lane) is a 1-kb DNA marker.

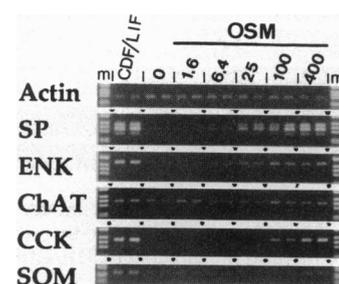


FIG. 2. OSM induces mRNAs for SP, ENK, ChAT, CCK, and SOM, but only at high concentrations. Various concentrations (ng/ml) of rhOSM, or rhCDF/LIF at 1.6 ng/ml, were added to cultured sympathetic neurons from day 2 to day 7 and RT-PCR was used to monitor mRNA expression of neuropeptides and transmitter biosynthetic enzymes. Duplicate samples were prepared for each condition. Induction of mRNAs for SP and ENK occurs at 25 ng/ml. At 100 ng/ml, rhOSM begins to induce mRNAs for ChAT and CCK. Induction of SOM was just visible at 400 ng/ml.

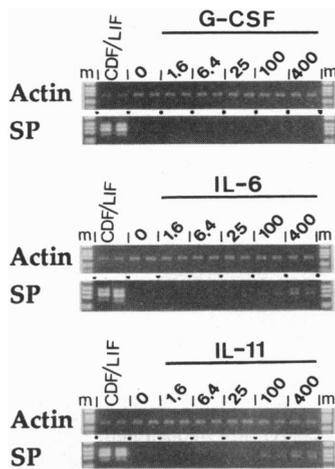


FIG. 3. High concentrations of IL-6 and IL-11 induce expression of mRNA for SP, while G-CSF has no effect on the neuronal genes tested. Various concentrations (ng/ml) of rhIL-11, rhG-CSF, recombinant murine IL-6, or rhCDF/LIF at 1.6 ng/ml were added to neuronal cultures and the effects were analyzed by RT-PCR. Duplicate samples were prepared for each condition. IL-6 and IL-11 begin to show induction of SP at 100 ng/ml, but they do not induce other neuromodulators.

Moreover, while CDF/LIF down-regulates the expression of mRNA for TH and NPY, activin A induces higher levels of NPY mRNA, while TH mRNA levels appear to be unaltered. Activin A and CDF/LIF do have some similarities in action; both induce ChAT, SOM, and CCK mRNAs, and both have no effect on GAD67, GAD65, and TPH mRNAs.

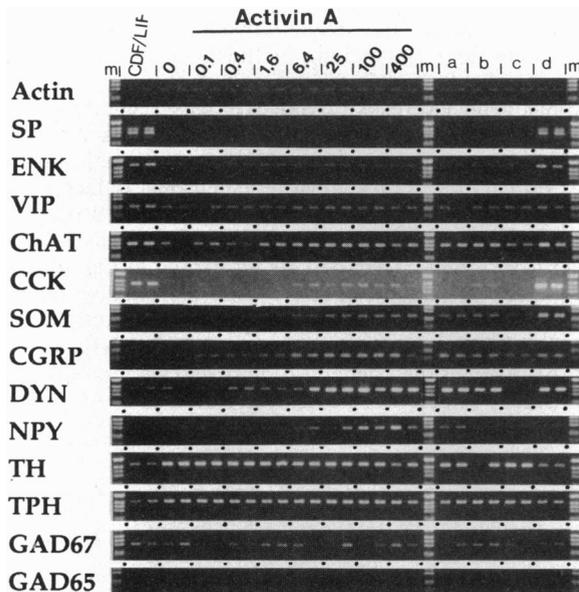


FIG. 4. Recombinant human activin A induces a novel set of neuronal genes; this induction is not blocked by recombinant human inhibin A and is additive to the effects of CDF/LIF. rhCDF/LIF at 1.6 ng/ml or activin A at various concentrations (ng/ml) was applied to neurons and the effects were analyzed by RT-PCR. For the blocking experiment, activin A at 25 ng/ml and inhibin A at 200 ng/ml were added to the cultures and the results are shown in lane b. The effects of activin A alone at 25 ng/ml are shown in lane a, and inhibin A alone at 200 ng/ml in lane c. To observe additive effects, CDF/LIF at 1.6 ng/ml and activin A at 25 ng/ml were added together (lane d). Duplicate samples were prepared for each condition. Although inhibin A inhibits NPY induction by activin A in this set of samples, this result was not observed consistently, and no other inhibitory effects were seen.

Two proteins, inhibin and follistatin, inhibit the action of activin A. We tested one of them, inhibin A, at the concentration (200 ng/ml) that was shown previously to inhibit induction of erythroid differentiation by activin A at 25 ng/ml (33). In the sympathetic neuron assay inhibin has no effect by itself on the neuronal genes tested (Fig. 4, lane c), nor does it block the effects of activin A when these two proteins are added together (Fig. 4, lane b). This lack of effect suggests that these neurons have the type of activin A receptor that does not recognize inhibin A. When activin A and CDF/LIF are added together in the assay, each at a concentration sufficient to generate maximal inductive effects in this assay, additive effects are observed (Fig. 4, lane d). This implies that activin A and CDF/LIF use different mechanisms to regulate neuronal gene expression.

## DISCUSSION

The utility of an RT-PCR-based assay for screening factors that may affect neuronal gene expression is borne out by the present results. Thirty-three cytokines and growth factors were tested for their effects on the expression of a variety of neuropeptides and transmitter-synthesizing enzymes that are known to be present in the peripheral nervous system. Active factors can be quickly identified, and dose-response experiments allow a qualitative assessment of which genes respond to each factor. As illustrated by the results presented here, assays of a smaller subset of neuronal genes could have led to conclusions entirely different from those we derive from the larger data set. Although this method is qualitative, comparison of dose-response results obtained with known cytokines allows an accurate analysis of the effects of novel factors.

As summarized in Table 1, most of the factors tested did not alter the expression of phenotypic markers in cultured sympathetic neurons. We cannot rule out, however, the possibility that some of these cytokines might have been active had we been able to screen the recombinant rat proteins. Further studies using neurons and cytokines derived from the same species are necessary to clarify this uncertainty. Indeed, Mehler and colleagues (29) have provided evidence that IL-5, -7, -9, and -11 are survival and/or differentiation factors for murine hippocampal progenitor neurons. Another example is FGF. Both acidic FGF and basic FGF induce the cholinergic phenotype in cultured chick sympathetic neurons (41), but we were not able to observe any change of expression in rat sympathetic neurons in several trials. Other differences in responsiveness to factors have been observed between rat and chicken sympathetic neurons (e.g., ref. 42).

CNTF was identified and cloned from human, rat, and rabbit by its ability to support the survival of ciliary neurons *in vitro*, and later it was also found to maintain the survival of motor neurons. It does not contain a signal peptide for secretion, however, and it is not detected in the medium of transfected cells (14, 15). Moreover, CNTF is not found in tissue fluids or extracellular matrix preparations, except after axonal injury (43). A protein with CNTF-like activity (SGF) is found in the soluble fraction of sweat gland homogenates, raising the possibility that there exists a secretable CNTF homologue (26, 27). This idea is supported by the finding that the chick expresses a secretable protein that displays 50% amino acid sequence identity with CNTF (25). Our laboratory has recently cloned a partial cDNA for a different CNTF sequence homologue from chicken, however, and this molecule appears to be the chicken version of mammalian CNTF (44). Therefore, chicken GPA is also likely to be a family member. The present finding that GPA possesses an activity on sympathetic neurons identical to that of CNTF and CDF/LIF further solidifies the position of GPA in this family.

The present results also provide further support for OSM as a neuropoietic cytokine. Although OSM shows only a modest sequence homology with CDF/LIF, it can displace the latter from its receptor on M1 cells, and if this is also the case for the sympathetic neurons, the mechanism for how these two cytokines can regulate the same set of neuronal genes is straightforward. We provide evidence that IL-6 and IL-11 weakly induce expression of SP, and not the other genes tested. Since it appears that IL-6, IL-11, CNTF, CDF/LIF, and OSM use the same receptor subunit as a signal transducer, gp130, the relatively weak effects observed here for IL-6 and IL-11 may indicate very low levels of the ligand-binding subunits for the latter two cytokines in sympathetic neurons.

The observation that activin A induces a distinctive subset of neuronal genes is important for two reasons. First, it demonstrates that the various neuropeptide genes assayed can, in fact, be regulated independently of each other. That is, the observations that CCK, ENK, SP, VIP, and ChAT are all induced together by CDF/LIF, CNTF, GPA, and OSM is not simply due to a mandatory coordinate regulation of these neuropeptide genes. The activin A results thus serve to further link the neuropoietic group together. The lack of induction of SP by activin A is particularly striking, given the extraordinarily strong induction of this neuropeptide by all of the neuropoietic cytokines. The second point of interest in the activin A findings is that the particular set of neuropeptides induced by this factor closely resembles the neuropeptide phenotype of the cholinergic sympathetic neurons that innervate the sweat gland in the rat foot pad. The presence of CGRP induction and the absence of SP induction are important because cholinergic sympathetic neurons express CGRP, but not SP, in rats (45). On this basis, activin A is a legitimate candidate, along with SGF, for the target-derived neuronal differentiation factor that induces sympathetic neurons innervating sweat glands to become cholinergic. It will therefore be of interest to determine if activin A is expressed in the sweat glands during normal development.

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