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Sympathetic neurons can produce and respond to interleukin 6

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ABSTRACT **Neuronal expression of cytokines is an area of active investigation in the contexts of development, disease, and normal neural function. Although cultured rat sympathetic neurons respond very weakly to exogenous interleukin 6 (IL-6), we find that addition of soluble IL-6 receptor (sIL-6R) and IL-6 enhances neuronal survival in the absence of nerve growth factor. Neutralizing monoclonal antibodies against IL-6 block these effects. Addition of IL-6 and sIL-6R also induces a subset of neuropeptide and transmitter synthetic enzyme mRNAs identical to that demonstrated for leukemia inhibitory factor, ciliary neurotrophic factor, and oncostatin M. Both of these effects are duplicated by addition of a highly active fusion protein of sIL-6R and IL-6, covalently linked by a flexible peptide chain, which is designated H-IL-6. In addition, we show that sympathetic neurons produce IL-6. *In situ* hybridization indicates a neuronal localization of IL-6 mRNA in superior cervical ganglia, and bioactive IL-6 protein is detected in ganglion culture supernatants. Interestingly, the IL-6 produced by sympathetic neurons does not lead to survival of these cells in culture unless sIL-6R is added. Thus, sympathetic neurons can produce IL-6 and may respond to it in an autocrine/paracrine manner if sIL-6R is present. Moreover, the prior findings of sIL-6R in serum and inflammatory fluids now have added interest in the context of neuro–immune interactions.**

Interleukin 6 (IL-6) is a multifunctional cytokine that plays a central role in inflammatory responses and in the regulation of cells of the hematopoietic system (1). A number of studies have also provided evidence for the expression and action of IL-6 in the nervous system. IL-6 and IL-6 receptor (IL-6R) mRNAs are detected in discrete regions of the rat central nervous system (CNS), where both genes are developmentally regulated and localized in specific neuronal subpopulations (2–4). IL-6 has also been implicated in the regulation of neuronal survival (5–7) and in response of the CNS to trauma, inflammation, and degenerative disease (8–11).

IL-6 belongs to the neuropoietic cytokine family, which also includes ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), oncostatin M (OSM), interleukin 11 (IL-11), and cardiotrophin 1 (CT-1) (12–14). Overlapping biological activities and a similar secondary structure have been reported for these cytokines (12, 15–18). Moreover, these proteins utilize the same signal-transducing receptor subunit gp130. IL-6 binds to the specific IL-6R and subsequently induces homodimerization of gp130. In contrast, LIF, OSM, CNTF, and IL-11 induce heterodimerization of gp130 with LIF receptor, OSM receptor, or an as yet unidentified receptor subunit (19, 20). Homo- or heterodimerization of gp130 leads to activation of the Jak/STAT signaling pathway (21–24).

In addition to the membrane-bound IL-6R, a naturally occurring, soluble IL-6R (sIL-6R) can be generated by limited proteolysis of the membrane protein (25–27) or by differential splicing of the IL-6R pre-mRNA (28). The sIL-6R protein together with IL-6 can activate target cells that express gp130 on their cell surface but lack membrane-bound IL-6R (29, 30). This process has been called trans-signaling (29). In the absence of the sIL-6R such cells are not able to respond to IL-6. The potential biological importance of the sIL-6R is underlined by its presence in serum and a number of inflammatory fluids (29).

We have examined the responses of cultured sympathetic neurons to IL-6 and sIL-6R with regard to neuronal survival, differentiation, and neurotransmitter and neuropeptide expression. Although the majority of sympathetic neurons express norepinephrine as their neurotransmitter, a small percentage of neurons, particularly those that innervate the sweat glands, change transmitter and neuropeptide phenotype during normal development to produce acetylcholine and vasoactive intestinal peptide (VIP) (31, 32). Neuropoietic cytokines, including CNTF, LIF, OSM, IL-11, and CT-1, induce the same phenotypic changes of the neurotransmitter and neuropeptide expression pattern in sympathetic neurons *in vitro* as sweat glands do *in vivo* (31–33). In contrast, only minor effects have been reported for IL-6 on these neurons (33, 34). During development, sympathetic neurons acquire the ability to be supported *in vitro* by LIF and CNTF by postnatal day 6 (35). In contrast, maximal doses of IL-6 have no effect on the survival of such neurons (34). We have investigated whether sIL-6R can modulate the responsiveness of sympathetic neurons to IL-6. For this purpose we used a highly active fusion protein of sIL-6R and IL-6, covalently linked by a flexible peptide chain, which is designated H-IL-6.

We show that primary cultures of neonatal rat sympathetic neurons survive in nerve growth factor (NGF)-free culture conditions after gp130 stimulation with sIL-6R. Furthermore, these neurons synthesize and secrete IL-6, which can be utilized only in the presence of exogenously supplied sIL-6R, pointing to a new mechanism of neuronal stimulation that depends on exogenously supplied sIL-6R.

MATERIALS AND METHODS

Chemicals. Recombinant human IL-6 was prepared as described (36). Soluble human IL-6R and H-IL-6 were ex-

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Abbreviations: CCK, cholecystokinin; ChAT, choline acetyltransferase; CT-1, cardiotrophin 1; CNTF, ciliary neurotrophic factor; DIG, digoxigenin; ENK, enkephalin; GLN, galanin; H-IL-6, interleukin-6–soluble interleukin-6 receptor fusion protein; IL-6, interleukin 6; IL-6R, IL-6 receptor; LIF, leukemia inhibitory factor; MTT, thiazolyl blue; NGF, nerve growth factor; NPY, neuropeptide Y; OSM, oncostatin M; RT-PCR, reverse transcriptase–PCR; SCG; superior cervical ganglia; sIL-6R, soluble IL-6R; SOM, somatostatin; SP, substance P; TH, tyrosine hydroxylase; VIP; vasoactive intestinal polypeptide.

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pressed in the methylotrophic yeast *Pichia pastoris* and purified as described (37, 38). NGF was isolated (39) with modifications as described (40). The NGF antibody (Ab) was obtained as described (40). The neutralizing IL-6 monoclonal antibody (mAb) was a gift of J. Brakenhoff (41).

IL-6 Bioassay. The hybridoma growth factor activity of IL-6 was measured in the mouse B9 assay as described (42). Briefly, B9 cells were washed three times in IL-6-free medium and plated at a concentration of 5,000 cells per well in 50 μl, and 50 μl of cytokines was added. After 68 hr at 37°C, the number of viable cells was measured by adding the tetrazolium salt thiazolyl blue (MTT) and incubating for a further 4 hr at 37°C. B9 cells were lysed by SDS and optical density was measured at 570 nm. The amount of IL-6 present in the sample was calculated by using an IL-6 standard curve generated by the same procedure. Human IL-6 produces half-maximal proliferation of B9 cells in this assay at 1 pg/ml.

PC12 and NIH 3T3 Cells. Rat pheochromocytoma PC12 cells and mouse fibroblast NIH 3T3 cells were maintained as monolayer cultures in Dulbecco's modified Eagle's medium (DMEM; GIBCO) containing 10% heat-inactivated fetal calf serum (Seromed) and 1% penicillin–streptomycin (GIBCO) solution in a 5% CO_2/95% air humidified atmosphere. The medium was changed every 3 days and cells were subcultured once a week. NIH 3T3 cells were stably transfected with the expression vector pBMGNeo, carrying cDNA encoding the extracellular domain of the rat gp80. Transcription of the transfected gene under the control of the mouse metallothionein promoter was induced by the addition of 10 mM $CdCl_2$ in serum-free medium for 18 hr. Supernatants of transfected cells were collected and purified by Mono Q Sepharose.

Primary Culture of Rat Sympathetic Neurons. Superior cervical ganglia (SCG) were dissected from neonatal Sprague–Dawley rats and decapsulated with forceps. Ganglia were dissociated first with 0.3% trypsin/0.3% collagenase/0.03% DNase I in L-15 medium (GIBCO) for 45 min at 37°C and then with 0.3% trypsin for 15 min at 37°C. Cells were dispersed by gently triturating through a fine-bore glass pipette and plated in collagen-coated 96-well plates in L-15 medium/2.5% adult rat serum (GIBCO) with supplements as described previously (43). To eliminate nonneuronal cells, cultures were treated with 10 μM cytosine arabinonucleoside (Sigma) on days 2 and 6. After 7 days in culture approximately 95% of the cells were neurons, according to morphological criteria. For reverse transcriptase (RT)-PCR, dissociated sympathetic neurons were seeded in 96-well plates at a density of 1 ganglion per well and kept at 37°C and 5% CO_2 in a humidified atmosphere (33, 44).

***In Situ* Hybridization.** A rat IL-6 cDNA clone (ATCC 37681, size 0.9 kb) was used for *in vitro* transcription and generation of a cRNA probe. Digoxigenin (DIG)-labeled single-stranded RNA probes, in sense or antisense orientation, were synthesized from IL-6 cDNA template by the appropriate bacterial RNA polymerase (T3 or T7 RNA polymerase) in the presence of DIG-UTP (Boehringer Mannheim), as described by the supplier. Probes were partly digested by alkaline hydrolysis to improve cell penetration and were stored at −20°C. Hybridization and immunological detection were performed as described (45). Sympathetic neuron cultures grown on collagen-coated 4-chamber slides (Nunc) were fixed for 15 min in 4% paraformaldehyde in PBS, permeabilized for 5 min by 0.2% Triton X-100 in PBS, and acetylated for 10 min (0.1 M triethanolamine·hydrochloride/0.25% acetic anhydride, pH 8.0). Prehybridization was carried out for 4–6 hr at room temperature in hybridization buffer consisting of 50% formamide, 5× SSC, 5× Denhardt's solution, 250 mg/ml tRNA, and 500 mg/ml herring sperm DNA. For hybridization, 100–200 ng of DIG-labeled cRNA probe per ml of hybridization solution was denatured for 5 min at 80°C. Hybridization proceeded for 16 hr at 37°C in a humid chamber. Slides were then dipped in 5× SSC and washed under high-stringency conditions for 60 min at 70°C in 0.2× SSC. After 5-min equilibration in buffer 1 (0.1 M maleic acid/0.15 M NaCl, pH 7.5) and 60-min blocking with buffer 1 containing 2% fetal calf serum and 0.3% Triton X-100, slides were incubated for 3–4 hr at room temperature with anti-DIG Ab conjugated to alkaline phosphatase (diluted 1:2000) in a humid chamber. Slides were rinsed twice with buffer 1 for 10 min and equilibrated for 5 min in buffer 2 (0.1 M Tris·HCl/0.1 M NaCl/5 mM $MgCl_2$, pH 9.5). To perform the color reaction, slides were incubated with substrate solution [45 μl of nitroblue tetrazolium (NBT; 100 mg/ml), 35 μl of 5-bromo-4-chloro-3-indolyl phosphate (50 mg/ml), and 24 μl of levamisole (100 mg/ml) per 10 ml of buffer 2] overnight in a humid chamber protected from light. This reaction was stopped by the addition of 10 mM Tris·HCl, pH 8.3/1 mM EDTA for 2 min. Slides were mounted in aqua PolyMount (Polysciences), coverslipped, and sealed with nail polish. Cells were examined with a phase-contrast microscope (Zeiss).

RT-PCR. Neuronal culture, preparation of RNA and cDNA, as well as the RT-PCR were done as previously described (33). Dissociated sympathetic neurons were seeded in 96-well plates in culture medium containing NGF, and aphidicolin (4 mg/ml) was added to eliminate nonneuronal cells. H-IL-6 and sIL-6R were added from the second day of culture and duplicate wells were prepared for each condition. Data were reproduced in two separate neuronal platings. Total RNA and cDNA synthesis as well as primer sequences and PCR conditions were as described previously. Samples from the PCR were analyzed on 2% agarose gels, and the products were visualized with ethidium bromide staining and UV illumination.

Soluble Rat IL-6R. A cDNA coding for a soluble rat IL-6R was prepared by introducing a translational stop codon upstream of the transmembrane domain of the rat IL-6R. The resulting sIL-6R cDNA was cloned in the eukaryotic expression vector pBMGNeo and used to stably transfect murine NIH 3T3 cells. Supernatants of transfected cells were collected and purified by Mono Q Sepharose. The resulting homogeneous protein was active in inducing the acute phase protein haptoglobin in HepG2 human hepatoma cells upon addition of rat or murine IL-6 (data not shown). In addition, a cDNA coding for the extracellular portion of the rat IL-6R without the signal peptide was cloned in the bacterial expression plasmid rRSET5d (46) and expressed in *Escherichia coli*. The band corresponding to the rat sIL-6R was cut out from a polyacrylamide gel and used to immunize rabbits. This antiserum recognizes a protein of 55 kDa corresponding to the soluble rat IL-6R secreted by the transfected NIH 3T3 cells.

RESULTS

We first asked whether IL-6 and sIL-6R can promote the survival of dissociated sympathetic neurons in the absence of NGF. For this purpose, we produced a fusion protein (H-IL-6) consisting of human IL-6 and part of the human sIL-6R. This protein is 100- to 1000-fold more effective than IL-6 and sIL6R added as separate proteins (38). While no neurons survive in the absence of NGF in control cultures, addition of H-IL-6 promotes long-term neuronal survival, at a level of 62% of that seen with NGF (Fig. 1*A*). The effect of H-IL-6 is dose dependent, with an optimal concentration of 10 ng/ml (Fig. 1*B*). Interestingly, no inhibition of survival by doses up to 1000 ng/ml was seen (data not shown).

The survival activities of NGF and H-IL-6 are blocked by their respective neutralizing mAbs. The NGF response is inhibited by the anti-NGF mAb 23c4 (40) (Fig. 1*C*) but not by anti-IL-6 mAbs (41) (data not shown). Similarly, the H-IL-6 response is abrogated by anti-IL-6 but not by anti-NGF mAbs

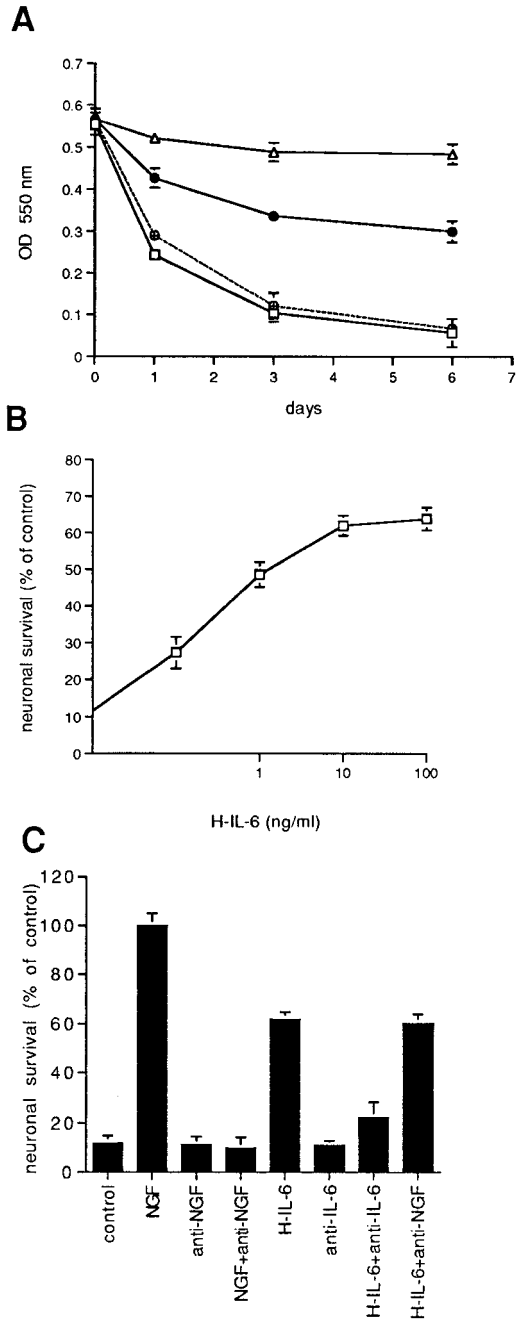

FIG. 1. The IL-6–sIL-6R fusion protein H-IL-6 induces survival of sympathetic neurons. (*A*) The time course of sympathetic neuron survival after treatment with 100 ng/ml NGF (△), 10 ng/ml H-IL-6 (●), 10 ng/ml IL-6 (⊕), or medium alone (□) is shown. Neuronal survival was determined after 1, 3, and 6 days of culture by the MTT assay. (*B*) Dose–response of sympathetic neurons treated with H-IL-6 for 5 days. Data are derived from three independent experiments (n = 3). (*C*) Independence of the responses to NGF and IL-6/sIL-6R. Dissociated sympathetic neurons were plated into medium containing growth factors and/or neutralizing Abs as indicated for 6 days (100 ng/ml NGF, 200 mg/ml anti-NGF Ab 23c4, 10 ng/ml H-IL-6, 10 mg/ml anti-IL-6 Ab 16). Cultures were analyzed by the MTT assay. In *B* and *C*, the OD obtained in the MTT assay for NGF-treated cells was set at 100% and the OD measured for neurons treated with H-IL-6 was expressed as percentage of this value. The data are derived from two independent experiments (n = 3).

(Fig. 1*C*). These results suggest that the responses to NGF and H-IL-6 do not depend on the release of the other protein.

We also determined whether H-IL-6 can regulate neuronal gene expression as well as survival. A semiquantitative RT-PCR assay using primers specific for cholecystokinin (CCK), choline acetyltransferase (ChAT), enkephalin (ENK), galanin (GLN), neuropeptide Y (NPY), somatostatin (SOM), substance P (SP), tyrosine hydroxylase (TH), vasoactive intestinal polypeptide (VIP), and β-actin was used for this purpose (13, 33). The expression of β-actin is used to monitor the amount of RNA in each sample. Addition of H-IL-6 induces an expression pattern of mRNAs for neuropeptide and transmitter biosynthetic enzymes very similar to that previously demonstrated for LIF, CNTF, OSM, and CT-1 (47, 48) (Fig. 2). Induction of mRNAs for ChAT, ENK, VIP, GLN, and SP are clear at 1 ng/ml H-IL-6. At 10 ng/ml, H-IL-6 begins to induce mRNAs for CCK and SOM. As with LIF and CNTF, H-IL-6 does not induce, but rather suppresses expression of NPY and TH. The effect of H-IL-6 on VIP expression is complex; VIP mRNA is induced by low concentrations of H-IL-6 but is inhibited by high concentrations of H-IL-6 (Fig. 2). This pattern was also observed for LIF (48). Two RNA species are induced for SP, as was observed for LIF and CT-1 previously (48). The apparent induction of GLN by sIL-6R shown in Fig. 2 was not observed in other experiments.

Because rat pheochromocytoma (PC12) cells synthesize and secrete biologically active IL-6 (P.M., unpublished observation), we tested primary sympathetic neurons for this capacity. Using both a species-specific ELISA and a biological assay (42), we found that the medium from NGF- and H-IL-6-supported neuronal cultures contained 200–600 pg/ml IL-6 after 5 days (ELISA data in pg/ml IL-6, n = 3: 72 ± 9 background; 216 ± 18 neurons plus NGF; 533 ± 123 neurons plus H-IL-6) (B9 assay data in pg/ml IL-6, n = 3: 70 ± 8 background; 571 ± 101 neurons plus NGF). The medium from neurons grown in H-IL-6 cannot be tested in the B9 assay because human IL-6 stimulates B9 cell proliferation, whereas the ELISA is species-specific for rat IL-6.

Despite the fact that very significant levels of IL-6 are found in the culture medium, the neurons do not survive in the absence of NGF. This result, coupled with our finding that addition of the IL-6 fusion protein containing the IL-6R does promote survival, suggests that the neurons are not expressing

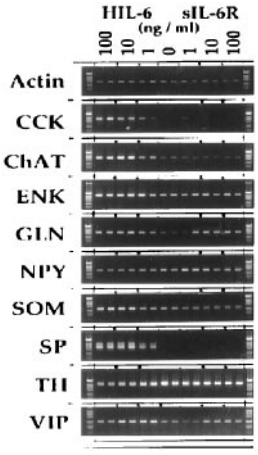

FIG. 2. The IL-6–sIL-6R fusion protein H-IL-6 induces the cholinergic phenotype in sympathetic neurons. Various concentrations of recombinant H-IL-6 or sIL-6R were added to neuronal cultures, and the effects on neuronal gene expression were analyzed by RT-PCR. The expression of β-actin is used to monitor the amount of RNA in each sample. H-IL-6 induces mRNAs for substance P (SP), cholecystokinin (CCK), choline acetyltransferase (ChAT), enkephalin (ENK), galanin (GLN), and somatostatin (SOM), whereas sIL-6R alone has no effect on these neuronal genes (the effect of the latter on GLN expression was not reproducible). Neuropeptide Y (NPY) and tyrosine hydroxylase (TH) expression were inhibited by H-IL-6, whereas vasoactive intestinal polypeptide (VIP) expression was induced by low concentrations of H-IL-6 and inhibited by high concentrations.

sufficient IL-6Rs for IL-6 autocrine/paracrine-driven survival. To test this hypothesis further, we purified rat sIL-6R from stably transfected NIH 3T3 cells. The dominant protein in the supernatant (Fig. 3*A*, lane 1) was purified by ion-exchange chromatography (Fig. 3*A*, lane 2). Its identity as sIL-6R was verified by immunoblotting (Fig. 3*B*). Using the recombinant sIL-6R, we increased the number of IL-6Rs on the neurons by addition of the purified protein. As shown in Fig. 3*C*, 10 ng/ml sIL-6R promotes survival of the neurons, even in the absence of exogenous IL-6. In fact, the effect of purified recombinant rat sIL-6R is similar to that of the fusion protein H-IL-6 (Fig. 3*C*).

The finding that medium from sympathetic neuronal cultures contains IL-6 is subject to the caveat that small numbers of nonneuronal cells present may be responsible for the cytokine production. To examine this issue, we used *in situ* hybridization to localize the expression of IL-6 mRNA in the cultures. Although no signal was detected by using an IL-6 sense probe, neurons identified as very large spherical cells with long processes are clearly stained with an IL-6 antisense probe (Fig. 4). IL-6 mRNA was also detected in neurons by *in situ* hybridization on sections of adult rat SCG (49).

DISCUSSION

The major findings of this study are as follows: (*i*) cultured rat sympathetic neurons express IL-6 mRNA and secrete biologically active IL-6; (*ii*) although these neurons do not express IL-6Rs in sufficient quantity to allow the endogenous IL-6 to drive autocrine/paracrine-mediated survival, addition of sIL-6R does support survival using autocrine/paracrine IL-6; and (*iii*) exogenous IL-6 regulates the same set of neuronal genes controlled by the other members of the neuropoietic cytokine family.

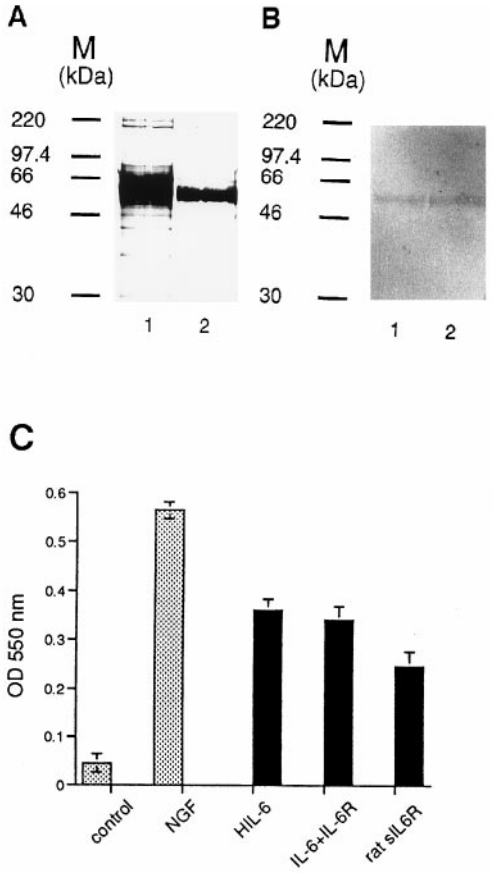

FIG. 3. Sympathetic neuron survival is stimulated by sIL-6R and endogenous IL-6. (*A*) Silver-stained protein gel after SDS/PAGE of supernatants of transfected cells (lane 1) and purified sIL-6R (lane 2). (*B*) Western blot analysis of the gel shown in *A*, using a rabbit antiserum raised against recombinant rat sIL-6R. (*C*) Neurons were plated in medium (control), 100 ng/ml NGF, 10 ng/ml H-IL-6, 100 ng/ml human IL-6 + 1 mg/ml human sIL-6R, or 10 ng/ml rat sIL-6R. Six days after treatment, neuronal survival was determined by the MTT assay.

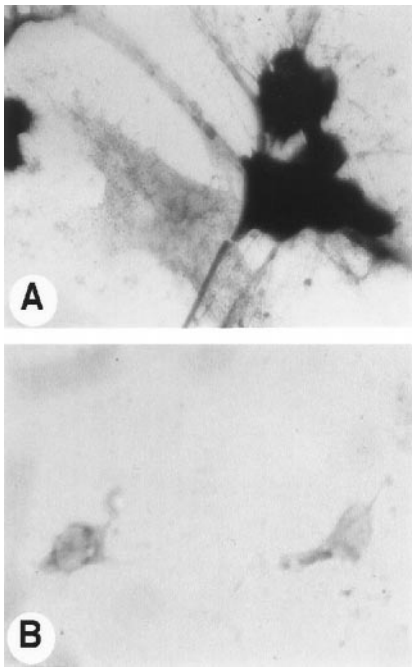

FIG. 4. Expression of IL-6 mRNA by sympathetic neurons. Neuronal localization of IL-6 mRNA in primary sympathetic neuron cultures, using *in situ* hybridization and DIG-labeled cRNA probes for IL-6 antisense (*A*) and IL-6 sense (*B*), is shown. Only the very large, spherical cells with long processes were stained above background. (×200.)

Much remains to be learned regarding IL-6 expression and function in the nervous system (50, 51). Astrocytes and microglia can be stimulated in culture to produce IL-6 (52, 53), raising the possibility that this cytokine may function in the response to injury and/or infection. A variety of neurons have been shown to respond to IL-6 addition in culture (5) and *in vivo* (54). Where is IL-6 made in the normal, undisturbed brain? Although IL-6 mRNA has been localized to certain neurons in the brain and peripheral nervous system *in vivo* (49, 55, 56), there was no evidence that neurons can produce IL-6 protein. We demonstrate that neuron-alone cultures secrete biologically active IL-6, and the neuronal localization of this cytokine is supported by our finding that the neurons express IL-6 mRNA.

These neurons also possess the downstream signaling pathways for IL-6 responsivity, as indicated by the effect of the IL-6 cytokine-receptor fusion protein (H-IL-6) on neuronal survival and neuropeptide induction. Moreover, when neuronal IL-6 receptor levels are increased by the addition of sIL-6R, these cells can also utilize their endogenous IL-6 for enhanced survival in the absence of NGF (Fig. 3). Because sympathetic neurons also express IL-6 mRNA *in vivo*, these findings raise the possibility that IL-6 could be acting as an autocrine/paracrine factor for survival or changes in gene expression *in vivo* as well. Studies on IL-6 knockout mice have not yet reported on possible changes in sympathetic neuron survival or function (57, 58).

Are receptor levels high enough to respond to IL-6 at concentrations likely to occur *in vivo*? For instance, IL-6 is found in peripheral tissues (1, 59) and is induced after injury

to peripheral nerve (60–63). It would be of interest to know if IL-6R is also induced in neurons under such circumstances. It has recently been shown that the soluble form of the IL-6R is generated by limited proteolysis and that the protease involved is a membrane-associated metalloprotease (25–27), which is regulated by protein kinase C (25, 64). Thus, stimuli that lead to activation of protein kinase C could cause release of sIL-6R. It remains to be determined which cells release sIL-6R or whether levels of circulating sIL-6R (70 ng/ml; ref. 65) are sufficient to cause effects in neurons *in vivo*.

The observation that IL-6 can act as a cholinergic neuronal differentiation factor (Fig. 2; ref. 66) raises the possibility that this cytokine could be involved in the phenotypic switch driven by sweat glands during normal sympathetic neuron development (32). Analysis of IL-6 knockout mice could be informative in this regard.

The lack of an autocrine/paracrine effect of endogenous IL-6 in sympathetic neurons is reminiscent of an earlier study in which we stably transfected human hepatoma cells (HepG2) with human IL-6 cDNA (67). Transfected cells that secrete biologically active human IL-6 are not stimulated to produce IL-6-responsive acute-phase proteins. When recombinant human sIL-6R is added to these cells, however, acute-phase protein synthesis is induced. We speculated that binding of IL-6 to IL-6R in the endoplasmic reticulum leads to the down-regulation of endogenous IL-6R, and sIL-6R compensates for the loss of endogenous IL-6R. Similarly, we recently found that human lung fibroblasts synthesize and secrete IL-6 that does not lead to autostimulation of the cells, although the cells can be stimulated by recombinant human sIL-6R (68). It is also worth noting that another member of this cytokine family, LIF, is produced by cultured sympathetic neurons, acts in an autocrine/paracrine manner in this system, and is expressed by these neurons *in vivo* (69).

The present results with the IL-6R fusion protein have potential therapeutic implications. This fusion protein containing human IL-6 and human sIL-6R connected by a flexible protein linker has 100- to 1000-fold higher specific activity than the components IL-6 and sIL-6R added separately (38). Moreover, the rat sIL-6R/IL-6 complex is considerably more active in promoting sympathetic neuronal survival than LIF or CNTF (35). It will therefore be of interest to test such constructs in neurodegenerative disease and injury models.

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