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Mechanism of Nerve Growth Factor mRNA Regulation by Interleukin-1 and Basic Fibroblast Growth Factor in Primary Cultures of Rat Astrocytes

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SUMMARY

Neonatal rat cortical astrocytes in primary culture synthesize and secrete nerve growth factor (NGF). Interleukin-1β(IL-1) and basic fibroblast growth factor (bFGF) treatment of astrocytes increased NGF mRNA content by about 2-fold. The effect of these two factors was specific, because other growth factors, such as tumor necrosis factor-α, insulin-like growth factor-1, and epidermal growth factor, failed to change NGF mRNA content. The concentrations of IL-1 and bFGF causing half-maximal stimulation were 1 unit/ml and 1 ng/ml, respectively. The increase in NGF mRNA elicited by IL-1 and bFGF was maximal at 3 hr of incubation. In the presence of IL-1 this increase persisted for 36 hr, whereas in the presence of bFGF the initial increase in NGF mRNA was followed by a decrease to 50% of control levels after 24 hr of incubation. Readdition of bFGF after 24 hr of treatment gave a similar increase in NGF mRNA content, suggesting that the decrease at 24 hr was not due to receptor desensitization. The effect of IL-1 was reversible, because removal of IL-1 after 3 hr of incubation resulted in a decrease of NGF mRNA content to control levels by 6 hr, whereas a readdition of IL-1 at this time led to a 2–3-fold increase in NGF mRNA content after an additional 3 hr of treatment. This second increase in NGF mRNA was also maintained for several hours. The combined treatment of astrocytes with maximally effective doses of IL-1 and bFGF produced an additive increase in NGF mRNA content, suggesting that different mechanisms are operative. Treatment of astrocytes with cycloheximide increased (about 6-fold) NGF mRNA content, and this content failed to increase further with IL-1 or bFGF treatment. Experiments using actinomycin D indicated that IL-1 increased the stability of the NGF mRNA. bFGF treatment failed to change this parameter. Thus, IL-1 increases NGF mRNA content in astrocytes, at least in part, by stabilizing mRNA, whereas bFGF does not affect mRNA stability but may act at the level of NGF gene transcription.

NGF has a critical role in the development of peripheral sympathetic and sensory neurons (1–4) and is by far the most well characterized neurotrophic molecule, due in part to its great abundance in male mouse submaxillary glands, which has allowed the purification, the amino acid sequence determination, and the cloning of NGF (5–7). It is now well established that the CNS has the ability to produce NGF. Although NGF mRNA is widely distributed throughout the brain, the highest levels of NGF mRNA and NGF have been found in hippocampus and neocortex (8–10), the target areas innervated by magnocellular cholinergic neurons of the basal forebrain. An *in vivo* action of NGF on these central neurons has been shown by repeated intracerebroventricular administration of NGF to neonatal rats, which elevates ChAT activity in forebrain (11, 12). *In vitro*, NGF induces ChAT activity in cultured septal neurons prepared from embryonic rats (13, 14). Also, degeneration of cholinergic neurons in the medial septum after transection of the septo-hippocampal pathway can be prevented by chronic infusion of exogenous NGF (15–17).

In the peripheral nervous system as well as in the CNS, NGF is produced by the innervated target tissue, where it is taken up by nerve terminals and retrogradely transported to the cell soma to exert a neurotrophic action. In peripheral targets, several cell types produce NGF, including fibroblasts, epithelial cells, smooth muscle cells, and Schwann cells (18). Concerning the CNS, NGF mRNA has been detected in the brain of several species, but it is still unclear which cell type synthesizes NGF. *In situ* hybridization has shown NGF mRNA expression by neurons in the hippocampus (9, 10) and cortex (19). Cortical neurons in culture (20), as well as primary cultures of astrocytes

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ABBREVIATIONS: NGF, nerve growth factor; CNS, central nervous system; ChAT, choline acetyltransferase; IL-1, interleukin-1β; bFGF, basic fibroblast growth factor; EGF, epidermal growth factor; BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle medium; F12, Ham's nutrient mixture F-12; IL-3, interleukin-3; IGF-1, insulin-like growth factor-1; TNF, tumor necrosis factor α; PBS, phosphate-buffered saline; FBS, fetal bovine serum.

(20–23) and glia-derived cell lines (22, 24, 25), synthesize and secrete NGF.

Very little is known about the pharmacological regulation of NGF. Early studies using the C6 rat astrocytoma cell line demonstrated that β-adrenergic agonists increase NGF biosynthesis and secretion (24, 25). Subsequently, it was shown that catecholamines increase NGF mRNA content in these rat astrocytoma cells (26). Catecholamines have also been shown to increase NGF mRNA and NGF content in both mouse astroglial (27, 28) and fibroblast (28) cells. Recently, considerable attention has focused on a putative role of cytokines and other growth factors in NGF regulation. IL-1 has been shown to increase NGF mRNA content in fibroblasts (29) and in nonneuronal cells of the sciatic nerve (30), suggesting a role for IL-1 in cellular NGF regulation.

Spranger *et al.* (31) and Čarman-Kržan *et al.* (23) have previously demonstrated that IL-1 increases NGF mRNA content and NGF secretion in primary cultures of rat cortical astrocytes. The rise in NGF mRNA content to a higher steady state preceded the increased secretion of NGF (23), indicating that in astrocytes the level of NGF mRNA determines, in large part, the magnitude of NGF secretion. In the present study, we have further investigated the effects of IL-1 on NGF mRNA content in astrocytes. We compared the effect of IL-1 with those of some other growth factor mitogens for astrocytes, such as bFGF, EGF, and IGF-1, and another cytokine, TNF. Of these factors, we found that bFGF is a potent inducer of NGF mRNA content, with small effects on NGF secretion, as reported previously (23). This observation prompted us to investigate whether IL-1 and bFGF have a similar or different mechanism of action in NGF mRNA regulation.

Experimental Procedures

Materials. Timed pregnant Sprague-Dawley rats were obtained from Zivic-Miller Laboratories. Nitex nylon screens were from Tetko. Dulbecco's PBS, DMEM/F12 (1:1), FBS, penicillin, and streptomycin were from GIBCO. Tissue culture dishes were from Nunc. IL-1 (human, recombinant, specific activity of $\geq 10^7$ units/mg), bFGF (bovine, recombinant), IGF-1 (human, recombinant), TNF (human, recombinant), and EGF (from mouse submaxillary glands) were from Boehringer Mannheim. Nylon membranes for RNA blotting were from Schleicher and Schuell. The SP6 polymerase was from BRL Life Technologies. [α-^{32}P]CTP, [α-^{32}P]dATP, and [α-^{32}P]dCTP (all >3000 Ci/mmol) were purchased from Amersham.

Preparation of astrocyte cultures. Primary cultures of astrocytes were prepared as previously described (23). Briefly, cerebral cortices from 2-day-old rat pups were rapidly dissected into sterile Leibovitz's L-15 medium. After the meninges were carefully removed, cells were mechanically dissociated through 75-μm sterile Nitex nylon screens into 10 ml of culture medium. The medium consisted of DMEM/F12 (1:1), 10% FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin. After trituration to obtain a homogeneous preparation, medium was added to give a concentration of 10^6 cells/ml, and cells were plated in 100-mm tissue culture dishes (10×10^6 cells/dish). Cells were grown for 7 days at 37° in a water-saturated air environment containing 6% CO_2. Culture medium was changed at 2 and 5 days after plating. Immunocytochemical staining for glial fibrillary acidic protein indicated that 90–95% of cells were astrocytes (23).

Treatment of cells. Astroglial cells, after 7 days in culture, were treated with IL-1, TNF, bFGF, IGF-1, or EGF. For treatments, culture medium was replaced with 5 ml of fresh culture medium. Cells were then treated with either vehicle (PBS containing 1 mg/ml BSA) (control cells) or growth factors in PBS/BSA. In experiments using cycloheximide or actinomycin D in combination with growth factors, these two drugs were added at a final concentration of 10 μg/ml. After incubation, cells were harvested in guanidine isothiocyanate for NGF mRNA determination. Samples were used the same day or were frozen at −70°.

Measurement of NGF mRNA. Total RNA was isolated by centrifugation through a cushion of cesium chloride, as described by Chirgwin *et al.* (32). An aliquot containing 30 μg of total RNA was applied to a 1.1% agarose gel containing 6% formaldehyde. After electrophoresis, RNA was transferred by the capillary blot procedure to a nylon membrane.

Preparation of the NGF probe and hybridization conditions were similar to those previously described (23, 26). Briefly, mouse clone pmBN-8B3, which contains a 543-base pair cDNA encoding mouse β-NGF (7), was subcloned into the *Pst*I site of the pGEM-3Z expression vector. Transcription with the SP6 polymerase generated the antisense RNA (cRNA) complementary to NGF mRNA. The *in vitro* transcription assay was performed according to the method of Melton *et al.* (33) and yielded a [^{32}P]cRNA probe with a specific activity of 8–9 × 10^8 cpm/μg of RNA. The blots were prehybridized for at least 4 hr at 65° in 50% formamide, 5× Denhardt's (50× Denhardt's is 1% BSA, 1% Ficoll, 1% polyvinylpyrrolidone), 750 mM NaCl, 0.2% sodium dodecyl sulfate, 25 mM EDTA, 12.5 mM Tris·HCl (pH 7.6), 0.1 mg/ml salmon sperm DNA. Blots were hybridized with the [^{32}P]cRNA probe, in fresh prehybridizing solution containing 1× Denhardt's, at 65° for 24 hr, followed by washing (four to six times) with 0.1× standard saline citrate (1× standard saline citrate is 0.15 M NaCl, 15 mM sodium citrate), 0.1% sodium dodecyl sulfate, at 65°, and were then exposed to Kodak X-OMAT film with intensifying screens at −70°. After exposure, the radioactivity was removed by washing of the blots at 65° in 5 mM Tris·HCl (pH 7.4), 0.2 mM EDTA, 0.1 mM sodium pyrophosphate, 0.02× Denhardt's. They were subsequently hybridized with the p1B15 cDNA clone, according to the procedure of Milner and Sutcliffe (34). This ^{32}P-nick translated p1B15 cDNA hybridizes to mRNA encoding cyclophilin, a stable structural protein (34, 35). The areas of the hybridized bands on the autoradiograph were quantitated by laser densitometry. The amount of NGF mRNA was expressed in arbitrary units, defined as the ratio between the densitometric area of the NGF mRNA hybridization band and that of the cyclophilin mRNA hybridization band. Thus, the measurement of NGF mRNA was corrected for any experimental variation, such as in recovery of mRNA and specific activity of the probes.

Statistical analysis. Statistical analysis was performed using the Student's *t* test or Duncan's multiple range test.

Results

In this study we have used primary astrocyte cell cultures as an *in vitro* model system to examine the regulation of NGF mRNA. After 7 days of growth in DMEM/F12 supplemented with 10% FBS, the cultures consisted of a layer of cells (80–90% confluent) of which 90–95% were astrocytes, by glial fibrillary acidic protein immunocytochemical staining (23). Several astroglial growth factors and cytokines were tested for an effect on NGF mRNA induction in these cultures. Astrocytes were incubated for 3 hr with IL-1 (10 units/ml) or TNF, bFGF, EGF, or IGF (each at 30 ng/ml). The concentration of each of these factors was chosen based on its ability to produce a pharmacological effect (36). As shown in Fig. 1, only IL-1 and bFGF significantly increased NGF mRNA content in astrocytes. There was a 2-fold increase in NGF mRNA content induced by these two factors. In contrast to NGF mRNA, p1B15 mRNA content was not affected by any of the treatments (data not shown). These results led us to perform a complete comparative study on NGF mRNA regulation in astrocytes by IL-1 and bFGF.

Time course of IL-1 and bFGF effects. The time course

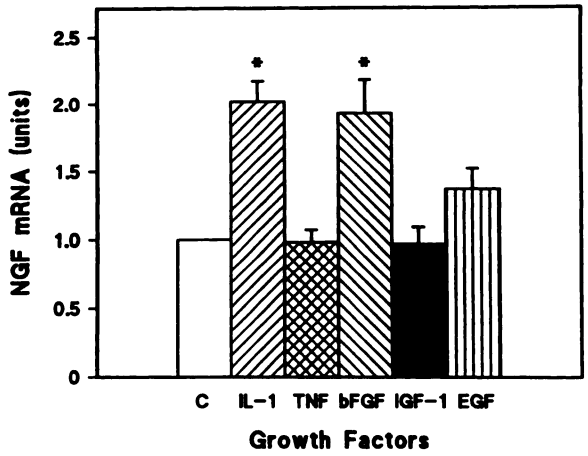

Fig. 1. Effect of IL-1, TNF, bFGF, IGF-1, and EGF on NGF mRNA content in rat astrocytes. Cells were treated with vehicle (*c*) or the different factors for 3 hr. NGF mRNA content was determined by Northern blot analysis, as described in Experimental Procedures. Values represent the mean ± standard error of three to six independent experiments. *, $p < 0.05$, Duncan's multiple range test.

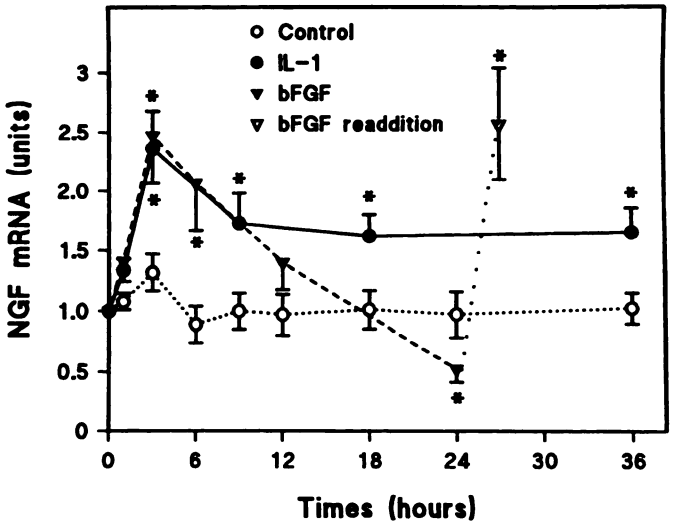

Fig. 2. Time-dependent effect of IL-1 or bFGF on NGF mRNA content in rat astrocytes. NGF mRNA content was determined by Northern blot analysis after different times of incubation with vehicle (○), IL-1 (10 units/ml) (●), bFGF (30 ng/ml) (▼), or a readdition of bFGF (30 ng/ml) (▽) for 3 hr after a prior 24 hr of bFGF treatment. Values represent the mean ± standard error of three to seven independent experiments. *, $p < 0.05$, Duncan's multiple range test.

of IL-1 (10 units/ml) and bFGF (30 ng/ml) effects on NGF mRNA is shown in Fig. 2. The NGF mRNA content in astrocytes was increased 2-fold by each factor after 3 hr of incubation. The increase in NGF mRNA induced by IL-1 remained significant up to 36 hr of incubation (Fig. 2). In additional experiments, the increased content of NGF mRNA was maintained even for 72 hr (1.7 ± 0.15 arbitrary units; $p < 0.05$, compared with control, Duncan's multiple range test). After 3 hr of treatment, some cultures were washed and incubated for various periods of time in the absence of IL-1. The results presented in Fig. 3 show that the IL-1 effect is reversible. In another batch of washed cells, at the time when NGF mRNA content returned to control levels, the readdition of IL-1 to these cells resulted in an increase in NGF mRNA to levels seen with the first IL-1 addition (Fig. 3). These results clearly

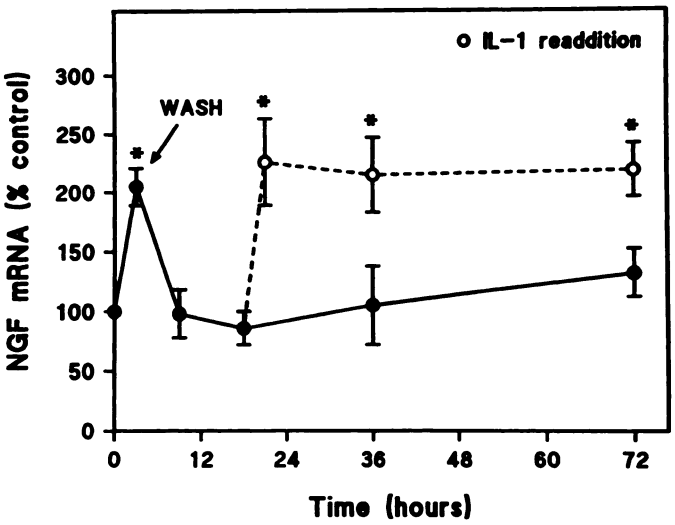

Fig. 3. Reversal effect of IL-1. Cells were treated with IL-1 (10 units/ml) for 3 hr, washed, and incubated for different times in fresh medium. At 18 hr (15 hr after washing), some washed cells were treated again with IL-1 (10 units/ml) and collected after different times of incubation. NGF mRNA was quantified by Northern blot analysis. Values are mean ± standard error of three or four independent experiments. *, $p < 0.05$, Duncan's multiple range test.

demonstrate that astrocytes respond to IL-1 stimulation even after previously being in contact with IL-1. The increased NGF mRNA content after IL-1 readdition was also maintained for several hours.

The time course of bFGF action indicated that, after the maximal increase at 3 hr, there was no effect of bFGF at 12 hr of treatment and by 24 hr NGF mRNA content decreased to 50% below control levels (Fig. 2). Addition of fresh bFGF at 12 hr in bFGF-treated cells failed to increase NGF mRNA content at later times (data not shown), whereas bFGF readdition at 24 hr induced a significant increase (2-fold), compared with control (Fig. 2).

Dose-dependent effect of IL-1 and bFGF on NGF mRNA content in astrocytes. NGF mRNA content was measured in astrocytes after treatment for 3 hr with different concentrations of IL-1 (0.1–30 units/ml) or bFGF (0.1–30 ng/ml) (Fig. 4). Maximal increases in NGF mRNA were obtained at about 10 units/ml (approximately 60 pM) and 30 ng/ml (1.7 nM), respectively, for IL-1 and bFGF. Concentrations giving half-maximal stimulation were 1 unit/ml and 1 ng/ml for IL-1 and bFGF, respectively.

Effect of IL-1 plus bFGF on astrocyte NGF mRNA content. Astrocytes were incubated with IL-1 plus bFGF, each at a concentration that induced a maximal effect, i.e., 10 units/ml and 30 ng/ml, respectively. Each factor alone increased NGF mRNA content by about 2-fold after 3 hr of treatment (Fig. 5). Treatment of cells with both IL-1 and bFGF resulted in an additive (4.3-fold) increase in NGF mRNA. It is interesting to note that the 50% decrease in NGF mRNA content seen after 24 hr of bFGF treatment does not affect the IL-1-stimulated NGF mRNA increase (Fig. 5). These results suggest that IL-1 and bFGF act by different mechanisms in inducing NGF mRNA in astrocytes *in vitro*.

Effect of IL-1 or bFGF in cycloheximide-treated astrocytes. Cycloheximide (10 μg/ml), an inhibitor of protein synthesis that has been reported to increase several mRNAs by an effect on mRNA stabilization (37, 38), was used to determine

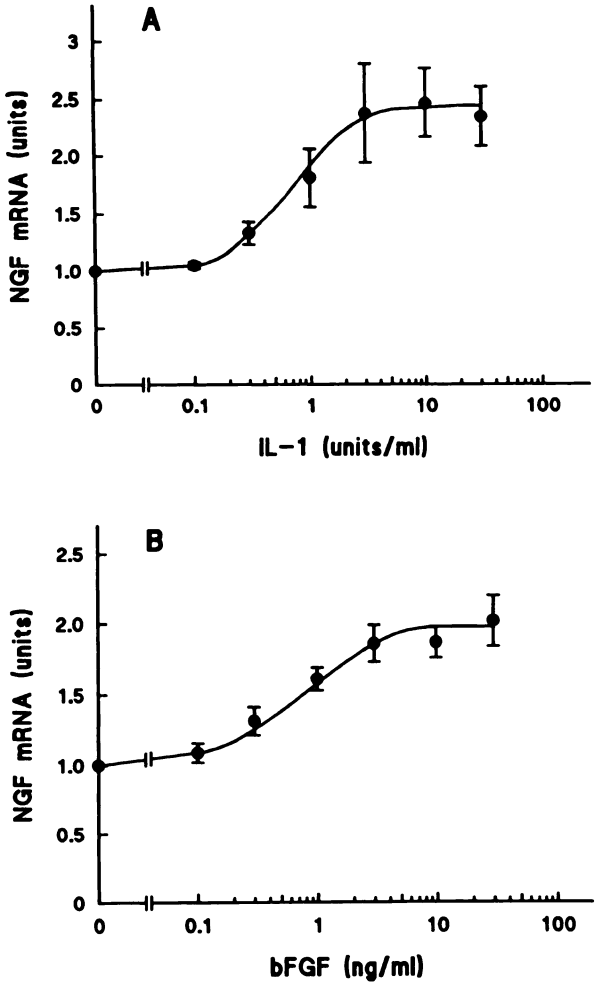

Fig. 4. Dose-dependent effect of IL-1 and bFGF on NGF mRNA content in astrocytes. Cells were treated for 3 hr with different concentrations of IL-1 (A) or bFGF (B). NGF mRNA content was determined by Northern blot analysis, as described in Experimental Procedures. Values are mean ± standard error of three to six independent experiments.

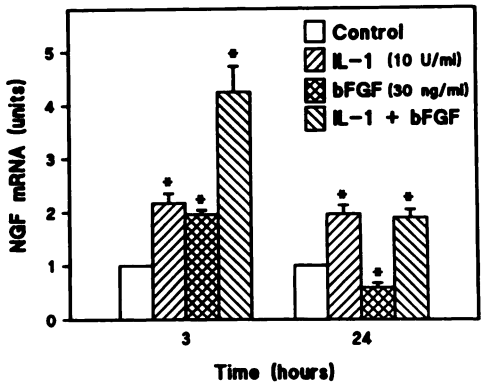

Fig. 5. Additive effect of IL-1 and bFGF. Cells were treated with maximal concentrations of IL-1, bFGF, or IL-1 plus bFGF, as indicated, for 3 or 24 hr. NGF mRNA content was determined using Northern blot analysis. Values are mean ± standard error of three or four independent experiments. *, $p < 0.05$, Duncan's multiple range test.

whether IL-1 or bFGF could increase NGF mRNA content in the absence of protein synthesis. A typical Northern blot of such an experiment is shown in Fig. 6. Cycloheximide treatment of cells increased NGF mRNA by 6-fold [compare Fig. 6, *lane 1* (1.0 arbitrary unit) and *lane 2* (6.0 units)]. Increased content

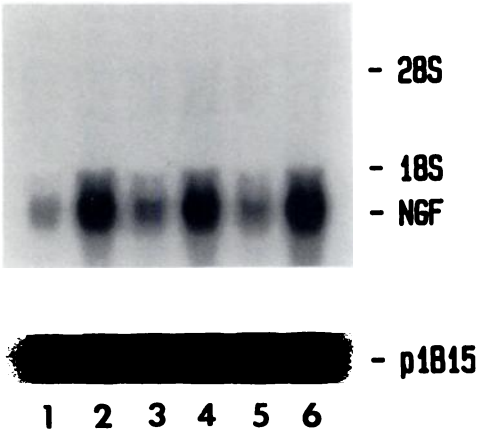

Fig. 6. Effect of cycloheximide, in combination with IL-1 or bFGF, on NGF mRNA content in rat astrocytes. Cells were treated for 3 hr with vehicle, IL-1 (10 units/ml), or bFGF (30 ng/ml), in the absence or presence of cycloheximide (10 μg/ml). *Lane 1*, control; *lane 2*, cycloheximide; *lane 3*, IL-1; *lane 4*, IL-1 plus cycloheximide; *lane 5*, bFGF; *lane 6*, bFGF plus cycloheximide. The electrophoretic positions of the 28 S and 18 S ribosomal, NGF, and p1B15 mRNAs are indicated.

of NGF mRNA was seen in cells treated with IL-1 [Fig. 6, *lane 3* (2.0 arbitrary units)] or bFGF [Fig. 6, *lane 5* (1.7 arbitrary units)] in the absence of cycloheximide. When cells were treated with these factors in the presence of cycloheximide, IL-1 [Fig. 6, *lane 4* (5.5 arbitrary units)] and bFGF [Fig. 6, *lane 6* (5.8 arbitrary units)] failed to increase NGF mRNA content above that seen with cycloheximide alone [Fig. 6, *lane 2* (6.0 arbitrary units)]. These results imply that IL-1 and bFGF do not act directly on NGF gene transcription, although an indirect effect through stimulation of early inducible genes cannot be ruled out by these experiments.

Effect of IL-1 or bFGF in actinomycin D-treated astrocytes. We used actinomycin D (10 μg/ml) as a blocker of transcription to examine the effect of IL-1 (10 units/ml) and bFGF (30 ng/ml) on NGF mRNA stability. The disappearance of NGF mRNA in the presence of actinomycin D was measured at various times in 1) astrocytes treated with vehicle, IL-1, or bFGF added simultaneously with actinomycin D and 2) astrocytes pretreated with IL-1 or bFGF for 3 hr before incubation with actinomycin D in the continued presence or absence of these factors. A Northern blot of an actinomycin D/IL-1 experiment is shown in Fig. 7, whereas Fig. 8 summarizes the decline with time of NGF mRNA under the same experimental conditions. It is evident from these experiments that IL-1 treatment decreases the rate of decline of the NGF mRNA, i.e., after actinomycin D addition, the NGF mRNA content was higher in the IL-1-treated cells than in vehicle-treated control cells (Figs. 7 and 8). Similar results were found when the cells were pretreated with IL-1 before addition of actinomycin D, as when IL-1 and actinomycin D were added at the same time (Figs. 7 and 8, no pretreatment versus IL-1 pretreatment). In contrast, addition of bFGF either before or simultaneously with actinomycin D failed to slow the rate of NGF mRNA decline, compared with control cells (Fig. 8). From the decline of NGF mRNA shown in Fig. 8, we estimated the rate of disappearance of NGF mRNA induced by actinomycin D in the presence of IL-1 or bFGF (Table 1). IL-1 treatment slowed the rate of NGF mRNA decline by 30%, whereas bFGF failed to change this rate. As a consequence of the slower decline of NGF mRNA with time, the half-life of NGF mRNA was significantly in-

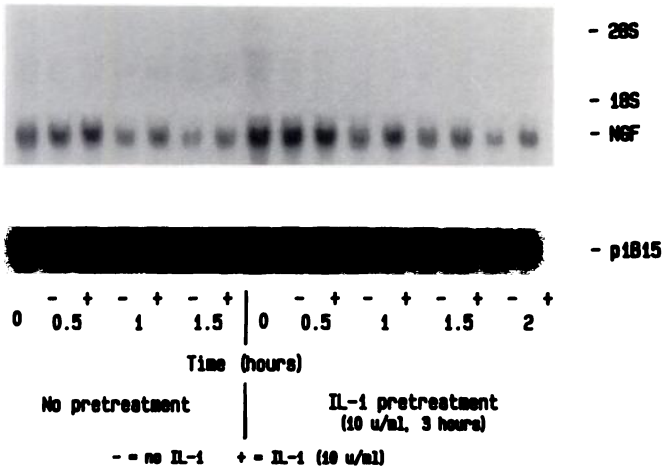

Fig. 7. Effect of IL-1 on NGF mRNA content in astrocytes treated with actinomycin D. Cells were treated with actinomycin D (10 μg/ml), in the absence (−) or presence (+) of IL-1 (10 units/ml), for different times under two different conditions, i.e., with no pretreatment or after a prior 3-hr IL-1 (10 units/ml) pretreatment. At the indicated times, cells were collected and RNA was isolated. The RNA was electrophoresed, blotted, and hybridized with the indicated probes, as described in Experimental Procedures. A representative autoradiograph of a Northern blot is shown. The electrophoretic positions of the 28 S and 18 S ribosomal, NGF, and p1B15 mRNAs are indicated.

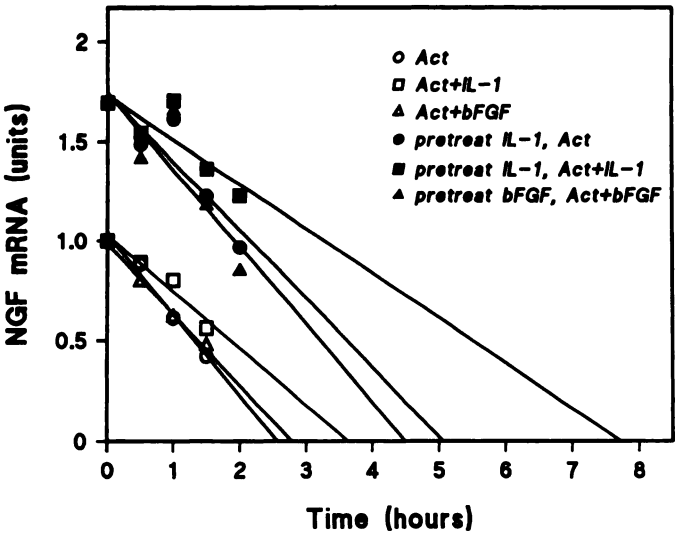

Fig. 8. Quantitation of the effect of IL-1 and bFGF on NGF mRNA stability. Astrocytes were treated with actinomycin D (10 μg/ml) in the absence (*Act*) and presence of IL-1 (*Act+IL-1*) or bFGF (*Act+bFGF*). Other cells were pretreated with IL-1 or bFGF before the addition of actinomycin D plus control solvent (*pretreat IL-1, Act*), actinomycin D plus IL-1 (*pretreat IL-1, Act+IL-1*), or actinomycin D plus bFGF (*pretreat bFGF, Act+bFGF*). The data for the experimental condition of pretreatment with bFGF followed by addition of actinomycin D only were similar to those for pretreatment with IL-1 followed by actinomycin D only (i.e., *pretreat IL-1, Act*) and, therefore, were not included for the sake of clarity. The concentrations of IL-1 and bFGF used were 10 units/ml and 30 ng/ml, respectively. NGF mRNA and p1B15 mRNA were quantitated at the indicated times, as described in Fig. 7 and Experimental Procedures. The data presented are from a typical experiment, and the average decline rates of NGF mRNA from five to eight such experiments are presented in Table 1.

TABLE 1

Effect of IL-1 and bFGF on rate of decline of NGF mRNA in astrocytes treated with actinomycin D

NGF mRNA decline rates were calculated from results of experiments identical to those shown in Figs. 7 and 8, but using five time determinations (0, 0.5, 1, 1.5, and 2 hr) in each experiment. Values are mean ± standard error of five to eight independent experiments. Values in parentheses show percentage of change.

Experimental conditions	Decline with time
	units/hr
Control	0.54 ± 0.05
IL-1	0.37 ± 0.04* (−31)
bFGF	0.50 ± 0.04 (−7)

*$p < 0.05$, Student's t test.

creased, from 77 ± 7 min to 120 ± 12 min, by IL-1 treatment. The NGF mRNA half-life did not change with bFGF treatment (83 ± 7 min).

Discussion

The results presented here confirm that the cytokine IL-1, a major mediator of inflammatory and immune reactions, is an agent that putatively regulates NGF mRNA in primary cultures of astrocytes. The results also show that bFGF, another trophic factor involved in CNS development and function (36, 39), regulates NGF mRNA content in astrocytes. The increase in NGF mRNA induced by these two factors was time and dose dependent, reversible, and additive, with maximal stimulation (2-fold) seen after 3 hr of treatment. The additivity of IL-1 and bFGF effects suggests that the mechanisms of action of these two factors are different. However, it should be noted that astroglia are heterogeneous with respect to the expression of neurotransmitter receptors (40, 41) and their roles in neuronal development and brain injury (41, 42). Thus, the selective activation of different astroglial subpopulations by IL-1 and bFGF may explain, in part, the additivity of their effects.

Spranger *et al.* (31) have recently reported a 6-fold increase of NGF mRNA content in astrocytes after IL-1 or bFGF treatment for 6 hr. In contrast to our studies, they used replated astrocytes and treatment under low serum concentration conditions. Evidence in our laboratory indicates a lower basal content and a larger IL-1 induction of NGF mRNA in astrocytes cultured under serum-free conditions. In addition, Spranger *et al.* (31) quantified the NGF mRNA content using a recovery and calibration standard, whereas we determined the ratio between NGF and cyclophilin mRNA hybridization in each blot. These methodological variations may explain the differences between their and our results.

In our study, a major difference between IL-1 and bFGF in NGF mRNA regulation appeared to be the time course of action of these two factors. Whereas the IL-1 effect was maintained for a long period of time, the increase of NGF mRNA content due to bFGF treatment was significant for only a short period of time (6 hr), followed by a decrease to 50% of control levels by 24 hr. The fact that readdition of fresh bFGF at 12 hr failed to increase NGF mRNA suggests a transient desensitization to the action of bFGF. However, readdition of bFGF at 24 hr induced an increase in NGF mRNA content, indicating that by this time there was no desensitization of the cellular processes mediating the NGF mRNA increase elicited by bFGF. The transient increase of NGF mRNA content by bFGF treatment was followed by a 50% increase in NGF secretion between 6

and 9 hr of treatment, with a return to control levels by 14 hr.[1] We found previously that IL-1 enhanced cellular NGF content as well as NGF secretion from astrocytes (23). The increase in NGF secretion was observed after 14 hr of incubation and reached a higher constant level by 38 hr (300% increase). The earlier (at 3 hr) and maintained increase of NGF mRNA content stimulated by IL-1 is, therefore, responsible for the prolonged stimulation of NGF secretion. Because NGF is not stored to a significant extent in astrocytes and is constitutively secreted (23), these results indicate that the effect of IL-1 on astrocyte NGF secretion is probably due to increased NGF synthesis, resulting from the higher content of NGF mRNA induced by this agent.

An increase in NGF mRNA content induced by IL-1 or bFGF treatment could be due to enhanced gene transcription and/or stabilization of the mRNA. Treatment of astrocytes with cycloheximide increased NGF mRNA content, an effect perhaps due to mRNA stabilization, as seen in other mRNA studies (37, 38). Because the effect of IL-1 treatment on NGF mRNA content was not additive with that of cycloheximide, this factor may be acting similarly to cycloheximide by stabilizing NGF mRNA. However, the cycloheximide results do not rule out the possibility that IL-1 and bFGF act by inducing the expression of an early inducible gene, such as c-*fos*, the protein product of which then activates NGF gene transcription. This latter action would be prevented in the presence of cycloheximide. Indeed, in some cell types, IL-1 induces c-*fos* gene transcription (43). The transcription factor AP1 consensus sequence for Fos and Jun proteins is present in the promoter region of the NGF gene (44), and such consensus sequences might be operative in NGF transcriptional activation, as shown by Mocchetti *et al.* (45) and by Hengerer *et al.* (44). However, in order to implicate early inducible genes in astroglial NGF mRNA expression, it is essential to document that in astrocytes IL-1 and bFGF can induce Fos and Jun proteins, bringing about the activation of the AP1 consensus sequence.

More direct evidence that IL-1, but not bFGF, acts by increasing NGF mRNA stability comes from the actinomycin D experiments. Actinomycin D inhibits gene transcription and, therefore, changes in mRNA levels after treatment with this drug reflect the stability of the mRNA species under study. It should be kept in mind, though, that inhibition of gene transcription with actinomycin D may not give a completely accurate estimation of mRNA stability, in contrast to the pulse/chase method of measuring mRNA turnover (46), because it would prevent the synthesis of proteins from short-lived mRNAs that may control mRNA degradation. Despite this reservation, we found that IL-1 reduced the NGF mRNA decline with time after actinomycin D addition, indicating an increase in NGF mRNA stability. In contrast, bFGF failed to change the rate of decline of NGF mRNA after actinomycin D, suggesting that gene transcription is required for its action. The fact that IL-1 slows the disappearance of NGF mRNA either with or without a 3-hr pretreatment argues against an involvement of gene transcription in the mRNA-stabilizing action of IL-1 and suggests that IL-1 is regulating, at a post-transcriptional level, the activity of an mRNA-destabilizing process. Such an action of IL-1 might be mediated by inhibition of the synthesis (similar to the action of cycloheximide) or by post-translational modification, such as phosphorylation, of a critical protein involved in the degradation of NGF mRNA. Further support for an action of IL-1 on NGF mRNA stability is the finding by Lindholm *et al.* (29) that IL-1 increases NGF mRNA content in fibroblasts by an effect on NGF mRNA stabilization.

It has been demonstrated that the stabilization of mRNAs with short half-lives is due to the presence of a repetitive AUUUA sequence in the 3′ untranslated region of the mRNAs (47, 48). The binding of uncharacterized proteins to this consensus sequence (48) is thought to confer instability to cytoplasmic mRNA. In two different species, mouse and human, the 3′ untranslated region of the NGF mRNA contains two AUUUA sequences (7). A comparison of these two 3′ untranslated mRNA regions indicated a very high percentage of homology, suggesting that this region of the mRNA might be an important determinant of NGF mRNA stability. At this time, one can only speculate that the interaction of an unknown protein with the 3′ untranslated region of the NGF mRNA, or the cellular content of such a protein, may be regulated by IL-1 in astrocytes. Further studies are needed to test the validity of such an hypothesis.

Recent evidence indicates that different cytokines, such as IL-1 and IL-3, may play important roles in the CNS response to trauma. The presence of IL-1 and IL-3 and of their receptors in the brain supports such a possibility (49). For instance, it has been reported that IL-3 has trophic effects on cholinergic neurons in mouse and rat brain (50). *In vitro* IL-3 promotes axonal growth and increases ChAT activity, whereas *in vivo* IL-3 can prevent neuronal cell death. Several neurobiological effects of IL-1 have been described, including induction of fever, slow-wave sleep, increases of adrenocorticotropic hormone and neuropeptide release, and decrease of appetite (51). IL-1 is produced by astrocytes and microglial cells, and the IL-1 content increases significantly after brain injury (52). When the brain is injured, astrocytes proliferate in proximity to the injury (53). Signals operative in such proliferation may include growth factors that can also regulate astrocyte metabolism. Activated microglia and inflammatory cells at the site of injury secrete, among other factors, IL-1, which may regulate astrocyte proliferation and/or metabolism. In support of this, our study shows that IL-1 increases NGF mRNA content in primary cultures of astrocytes. The stabilization of NGF mRNA by IL-1 appears to be one mechanism whereby the *in vitro* rate of NGF secretion can be increased (23).

Because NGF exerts trophic effects on brain cholinergic neurons (4, 11–17), which appear to undergo degeneration in Alzheimer's disease, the IL-1-induced increase of astrocyte NGF mRNA content and NGF secretion (23, 31) (present results) may deserve particular attention for devising new therapeutic strategies.

[1]M. Čarman-Kržan and B. C. Wise, unpublished observations.

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