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Expression of TrkA confers neuronlike responsiveness to nerve growth factor on an immortalized hypothalamic cell line

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ABSTRACT **The result of nerve growth factor (NGF) actions depends upon the cells in which it signals. To define how signaling is influenced by cellular context, it would be useful to examine cells committed to different fates or cells of a single type at different developmental stages. Interest in NGF actions on neurons of the central nervous system led us to examine GT1-1 cells, an immortalized hypothalamic cell line. GT1-1 cells demonstrated neuronal properties but were unresponsive to NGF and other neurotrophins. Through transfection, *trkA* expression conferred NGF signaling leading to enhanced neuronal differentiation, including dose-dependent induction of neurite outgrowth and a rapid transient increase in c-fos and NGFI-A mRNA. Under serum-free culture conditions, NGF also delayed cell death. These findings suggest that *trkA* transfection of neurons and neuronal precursors can be used to better define NGF signaling.**

Nerve growth factor (NGF) is the best-characterized member of a family of neurotrophic factors known as the neurotrophins (1). The other known neurotrophins are brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3), and neurotrophin 4/5 (NT4/5) (1, 2). *In vitro* and *in vivo* studies show that neurotrophins act to enhance the viability and differentiation of specific neurons of the peripheral and central nervous systems. Similar to several other growth factors in their actions, neurotrophins exert their effects by binding and activating receptor tyrosine kinases (2). These receptors are encoded by the *trk* gene family, which is known to include *trkA*, *trkB*, and *trkC* (2). NGF mediates its effects through TrkA (2). NGF binds to TrkA in high-affinity receptors and, by formation of TrkA dimers, induces their cross-phosphorylation and activation (2–4). Activated TrkA is critical for initiating NGF signal transduction. Another transmembrane glycoprotein, $p75^{NGFR}$, also binds NGF. Though there is evidence for a role of $p75^{NGFR}$ in NGF signaling (2), NGF-$p75^{NGFR}$ complexes may be neither necessary nor sufficient for NGF signal transduction.

The discovery that NGF acted via a receptor tyrosine kinase drew a parallel between NGF signaling and that due to mitogenic growth factors (2). Indeed, NGF can induce mitosis in certain cells. Following transfection of NIH 3T3 cells with *trkA*, NGF provoked rapid cell division and even morphologic transformation (5, 6). On the other hand, in PC12 cells, a neuronal cell line, NGF exerted actions similar to those seen in postmitotic neurons. These cells extend neurites and demonstrate a number of differentiated properties after NGF treatment (7). The consequences of NGF signaling through TrkA thus appear to be dependent on the cellular context in which signaling occurs. Defining the molecular basis of context is critical for understanding NGF actions. One view is that the NGF signal transduction mechanism is common to all cells; different responses to NGF would result from activating genetic and cellular programs that are characteristic of the responsive cells. Another view is that molecules participating in the signal transduction pathways differ between cells and that signaling events leading to cell division are thus different in key respects from those leading to differentiation or survival. Very little evidence exists to decide between these views.

To better define NGF signaling in neurons, we have taken advantage of the production of an immortalized hypothalamic neuronal cell line. Recently Mellon, Weiner, and colleagues (8) created transgenic mice in which a portion of the gonadotropin-releasing hormone (GnRH) promoter was used to drive expression of the oncogene encoding simian virus 40 (SV40) tumor (T) antigen. Three cell lines derived from one hypothalamic tumor (GT1 cells) had neuronal properties, expressed neuronal but not glial markers, and expressed the GnRH gene at high levels. Like GnRH-secreting neurons *in vivo*, these cells secreted GnRH in a pulsatile fashion (9). Unlike normal GnRH-secretory neurons, all GT1 cell lines continued to divide, and in one clone (GT1-1) only a small percentage demonstrated neurite-like process. We found that GT1-1 cells did not respond to NGF or other neurotrophins. However, stable transfection of these cells with *trkA* allowed for NGF signaling leading to enhanced neuronal differentiation. Thus NGF signaling could be imposed on previously unresponsive neuronal cells of the central nervous system.

METHODS

Culture, Transfection, and Cloning of Cell Lines. GT1-1 cells were grown in Dulbecco's modified Eagle's (DME) medium containing 5% fetal calf serum and 5% horse serum. Transfection used Lipofectin reagent (BRL) with a plasmid containing the rat *trkA* cDNA under control of the murine sarcoma virus long terminal repeat (LTR) promoter and the neomycin-resistance gene under control of the SV40 promoter (pDM115; gift of M. Chao, Cornell University Medical College). Colonies resistant to G418 (BRL) at 300 μg/ml were screened for expression of *trkA* by immunoprecipitation. Cell lines were maintained in G418 at 300 μg/ml. When used, NGF (10) was added to the culture medium at time 0; cells were then fed with fresh medium containing NGF every 2 days. Purified BDNF and NT-3 were a gift (Franz Hefti, Genentech) and were used in the same manner.

RNA Isolation and Northern Blot Analysis. Total RNA was isolated from GT1-1 cells, the septal region of the rat brain, PC12 cells, or GT1-1-trk9 cells and analyzed on Northern blots (10). Blots were probed with ^{32}P-labeled cDNAs (10) for rat *trkA* (10), rat *trkB* (gift of L. Parada, NCI–Frederick Cancer Research Center), rat *trkC* (gift of L. Parada), rat

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Abbreviations: NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; NT-3, neurotrophin 3; GnRH, gonadotropin-releasing hormone; SV40, simian virus 40; T antigen, tumor antigen.

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p75^{NGFR} (gift of E. M. Shooter, Stanford Medical School), rat c-*fos* (gift of T. Curran, Roche Institute, Nutley, NJ), rat NGF-IA (gift of J. Milbrandt, Washington University, St. Louis), or 18S rRNA (10). Blots were washed under high stringency and exposed to x-ray films (10, 11) and quantitated (11).

Immunoprecipitation and Western Blot Analysis. Immunoprecipitation and Western blotting were performed essentially as described (12). After cell lysis, immunoprecipitation was with an anti-p75^{NGFR} antibody (anti-REX; gift of L. F. Reichardt, University of California, San Francisco), or an anti-Trk antibody (1088) overnight at 4°C. Antibody 1088 was made to a peptide corresponding to the C terminus (QALAQAPPVYLDVLG) of human TrkA. This sequence is identical to that for all rodent full-length Trk proteins. We showed that 1088 (*i*) immunoprecipitated and immunoblotted bands of the molecular masses of immature (110 kDa) and mature (140 kDa) TrkA in PC12 cells; and (*ii*) in PC12 cells immunoprecipitated a crosslinked complex (158 kDa) containing ^{125}I-labeled NGF (^{125}I-NGF) and a protein of the mass expected for TrkA. TrkAOUT is rabbit polyclonal antibody specific for the extracellular domain of TrkA (gift of D. Kaplan, NCI–Frederick Cancer Research Center). Immunoprecipitates were run on SDS/7.5% polyacrylamide gel electrophoresis, transferred to nitrocellulose, and immunoblotted (12). Tyrosine phosphorylation was detected by using 4G10, an anti-phosphotyrosine antibody (Upstate Biotechnology, Lake Placid, NY).

NGF Binding and Dissociation Kinetics. Binding studies were performed as described (4), using radiolabeled NGF (13). Cells plated 2 days before assay in six-well tissue culture plates (10^6 cells per well) were washed twice with ice-cold phosphate-buffered saline (PBS) and incubated at 4°C in 2 ml of binding buffer (PBS with bovine serum albumin at 1 mg/ml and glucose at 1 mg/ml) containing 100 pM ^{125}I-NGF for 4 h. After quick washing three times with ice-cold binding buffer, ^{125}I-NGF binding was measured (4). Dissociation of ^{125}I-NGF was studied by using the same protocol. After incubation with ^{125}I-NGF, cells were washed as above and incubated with 7 ml of ligand-free binding buffer for various periods at 4°C. After washing, bound ^{125}I-NGF was measured (4). Nonspecific binding was that present with 800 nM unlabeled NGF. Specific binding was 85% of total binding.

NGF Crosslinking and Immunoprecipitation. Cells were suspended in binding buffer (5×10^6 cells per ml), incubated at 4°C for 4 h with 100 pM ^{125}I-NGF, and treated at 4°C for 30 min with bis(sulfosuccinimidyl) suberate (BS^3; Pierce) at 0.8 mM final concentration for crosslinking to TrkA (14), or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC; Pierce) at 2 mM for crosslinking to p75^{NGFR} (14). Nonspecific binding and crosslinking were measured by adding 800 nM unlabeled NGF to the incubation mixture. Cells were washed in binding buffer and lysed in lysis buffer (12), all at 4°C. In some cases, cells were washed in ligand-free binding buffer for 1 h at 4°C prior to crosslinking. Lysates were immunoprecipitated with either 1088 or anti-REX and were analyzed on gradient SDS/PAGE (5.0% acrylamide with 0.1% bisacrylamide to 12.0% acrylamide with 0.5% bisacrylamide). A PhosphorImager (Molecular Dynamics) was used to quantitate results.

Immunocytochemistry and Cell Counts. Neuron-specific enolase (NSE) expression was assessed in cells grown in plastic tissue culture dishes, washed two times with PBS, fixed for 15 min with 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4), and then stained as described (10, 11) with an antibody to NSE (Polyscience). To assess cell growth, cells were plated in six-well plastic tissue culture dishes (4×10^5 cells per well). At intervals, cells were washed once with PBS, incubated in 0.05% trypsin in PBS for 5 min, and resuspended in culture medium. Cells excluding trypan blue were counted in a hemocytometer. For experiments in serum-free medium, cells were washed three times with serum-free DME H-21 medium and cultured in the same medium. Cell survival was quantitated according to Rukenstein *et al.* (15).

RESULTS

Examining NGF Responsiveness and Receptors in Parental GT1-1 Cells. We first determined whether GT1-1 cells would respond to NGF. Neither NGF, BDNF, nor NT-3 induced neurite outgrowth, suggesting that GT1-1 cells did not express neurotrophin receptors. No *trkA*, *trkB*, or *trkC* mRNA was detected by Northern blotting (Fig. 1*A*). Also, no Trk protein was detected by immunoprecipitation (Fig. 1*C*). A low level of p75^{NGFR} mRNA was present (Fig. 1*A*); there was 5.7% ± 0.2% (mean ± SEM; $n = 3$ determinations) of the level in PC12 cells. Little or no p75^{NGFR} protein was detected by immunoprecipitation (Fig. 1*B*). Because there was some specific NGF binding to GT1-1 cells (see below), we pursued further the identity of the receptors present by using ^{125}I-NGF binding followed by crosslinking and immunoprecipitation. NGF was specifically crosslinked to both p75^{NGFR} and TrkA; crosslinking to TrkA was only 0.59% ± 0.01% ($n = 3$) of that in PC12 cells. These data indicated that TrkA was present but at a low level, apparently below that required for signaling. This was confirmed when NGF treatment failed to induce tyrosine phosphorylation of TrkA in GT1-1 cells.

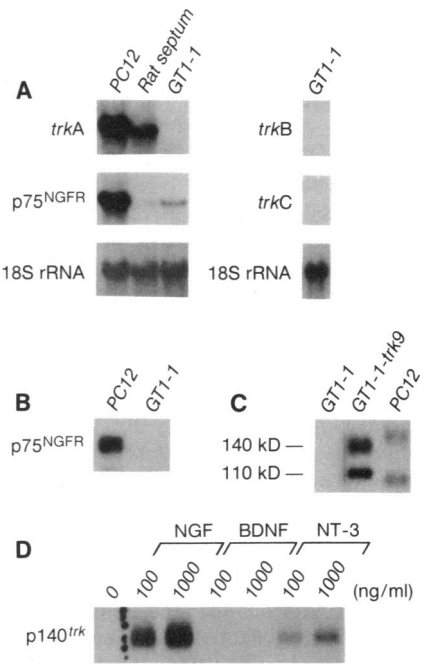

FIG. 1. Characterization of GT1-1 and GT1-trk9 cells. (*A*) Northern blot of 20 μg of total RNA from PC12 cells, septal region of the rat brain, and GT1-1 cells. The blot was probed with cDNAs for rat *trkA*, *trkB*, or *trkC*, then with cDNA for rat p75^{NGFR}, and finally with a cDNA for 18S rRNA. (*B*) Immunoblot for p75^{NGFR}. Equal amounts of protein from PC12 and GT1-1 cells were immunoprecipitated with anti-REX and the blot was probed with the same antibody. (*C*) Immunoblot for TrkA. Equal amounts of protein from GT1-1, GT1-trk9, and PC12 cells were immunoprecipitated with 1088 and the blot was probed with the same antibody. (*D*) GT1-trk9 cells were untreated (lane labeled 0) or treated with NGF, BDNF, or NT-3 for 5 min. Equal amounts of protein were immunoprecipitated with 1088 and the blot was probed with an anti-phosphotyrosine antibody (4G10). The signal with NT-3 at 100 ng/ml was 9% of that for the same concentration of NGF.

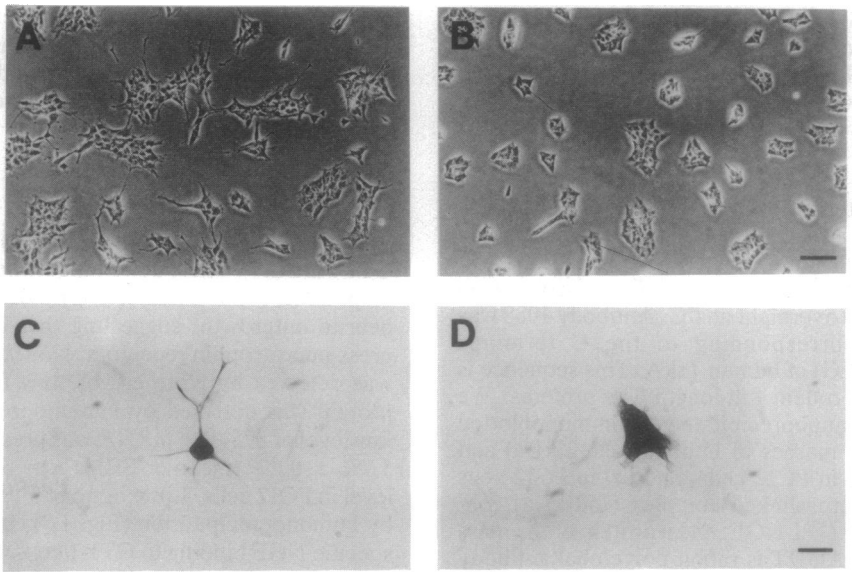

FIG. 2. NGF induces neurite outgrowth in GT1-trk9 cells. (*A* and *C*) Cells (5×10^4 per ml) cultured with NGF at 100 ng/ml for 24 h. (*B* and *D*) Cells cultured without NGF. (Bars: *A* and *B* = 120 μm; *C* and *D* = 30 μm.)

BDNF and NT-3 also failed to induce phosphorylation of Trk proteins (data not shown).

***trkA* Transfection Confers NGF Responsivity.** The rat *trkA* gene was introduced into GT1-1 cells by using a vector containing the neomycin-resistance gene. G418-resistant colonies expressing TrkA were selected by immunoprecipitation for TrkA and by response to NGF. Several lines were produced; one, GT1-trk9, expressed a relatively high level of TrkA and was further characterized. Fig. 1*C* shows that 1088 detected Trk proteins of 110 and 140 kDa in GT1-trk9 cells. These same bands were detected by TrkAOUT, a TrkA-specific antibody. Bands of similar molecular mass were detected in PC12 cells. These bands probably represent different glycosylated TrkA derivatives, as shown previously (16). The 110-kDa band may correspond to a high-mannose form and the 140-kDa band to the mature protein. The 140-kDa band in GT1-trk9 cells was 1.8-fold the amount of the band in PC12 cells. NGF induced tyrosine phosphorylation of the 140-kDa species (Fig. 1*D*). NT-3 also induced TrkA tyrosine phosphorylation, but far less potently than NGF. BDNF was inactive even at 1000 ng/ml (Fig. 1*D*). Thus, the pattern of neurotrophin specificity for TrkA activation in GT1-trk9 cells was similar to that seen in PC12 cells (6). $p75^{NGFR}$ mRNA was present in GT1-trk9 cells at the same level as in GT1-1 cells, and little or no $p75^{NGFR}$ protein was seen by immunoprecipitation (data not shown).

NGF Induces Neurite Outgrowth from GT1-trk9 Cells. To examine the consequences of TrkA activation, GT1-trk9 cells were treated with NGF. Untreated cells displayed a morphology similar to untransfected GT1-1 cells. Approximately 5.2% ± 0.4% (n = 14) had neurites longer than one cell body (Fig. 2 *B* and *D*). After treatment with NGF (100 ng/ml) for 24 h, 15% ± 1% (n = 6) of cells extended neurites longer than one cell body (Fig. 2 *A* and *C*). By 2 days, 42.4% ± 5.6% (n = 3) of cells had neurites. After 4 days, cells were present in clumps, 72.8% ± 1.7% (n = 2) of which extended neurites. Neurite outgrowth was dose dependent; the EC_{50} was 11 ng/ml, similar to the value for PC12 cells (6). If TrkA activation is linked to neurite growth, NT-3 and BDNF responses should parallel TrkA tyrosine phosphorylation. NT-3 induced neurites at 100 ng/ml, and at 1 μg/ml the number was similar to that seen with NGF at ≈5 ng/ml. BDNF did not induce neurites, even at concentrations as high as 1 μg/ml. The data are consistent with NGF acting through TrkA to induce neurite outgrowth.

NGF Regulates Gene Expression in GT1-trk9 Cells. NGF induces expression of a number of the immediate early genes, including c-*fos* (17) and *NGFI-A*. We examined early responses to NGF in GT1-trk9 cells. NGF treatment produced an abrupt large increase in c-fos and NGFI-A mRNA levels. NGF effects were evident by 15 min and were increased further by 30 min. For both c-fos and NGFI-A, the mRNA level fell to near baseline levels after 2 h (Fig. 3*A*). The pattern of responses in GT1-trk9 cells was similar to that seen in PC12 cells (18).

GT1-1 cells express and secrete GnRH. To investigate long-term NGF actions on gene expression, we examined the effect of NGF on GnRH mRNA levels in these cells (Fig. 3*B*). Remarkably, GnRH expression was inhibited ≈37-fold (n = 2) after 1 day of NGF treatment; GnRH mRNA levels were depressed for several days. As indicated, T-antigen expression in these cells is controlled by a portion of the rat GnRH promoter. NGF decreased T-antigen mRNA 2.6-fold (n = 2); nevertheless, this species was readily detected even after several days (Fig. 3*B*).

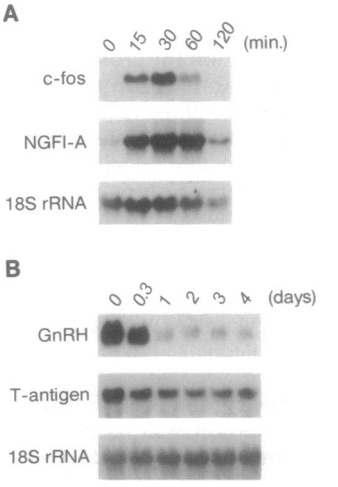

FIG. 3. Gene expression regulated by NGF. NGF (100 ng/ml) was added to GT1-trk9 cells. Northern blots of total RNA (20 μg) from cells treated for the times indicated are shown. (*A*) Immediate early gene expression. (*B*) Long-term gene expression. Filters were hybridized with the ^{32}P-labeled cDNA probes indicated. An 18S rRNA cDNA probe controlled for RNA loading.

NGF Induces Differentiation in Dividing Cells. We characterized NGF actions on GT1-trk9 cell growth for several days. NGF slowed growth initially (Fig. 4*A*). The number of cells in NGF-treated cultures increased by 40% after 3 days, while the number of untreated cells doubled. In longer-term cultures the doubling time for NGF-treated cells reverted to that seen with untreated cells. Continued cell division in the presence of NGF suggested that GT1-trk9 cells could respond to NGF by differentiating while maintaining the ability to undergo mitosis. To test this idea, cells were placed in low-density cultures (5×10^3 cells per ml) and individual cells were observed serially. We examined 41 individual cells with well-developed neurites. Over 4 days, 24/41 were observed to retract their neurites, divide, and then reextend neurites. These data show that NGF induced differentiation in cells destined to divide.

NGF Delays Cell Death Caused by Serum Deprivation. NGF prevents death of PC12 cells in serum-free medium (15). We tested whether GT1-trk9 cells would behave similarly. In the absence of serum, 90% of the cells died within 3 days (Fig. 4*B*). Membrane fragments and debris were seen in these cultures. In serum-free medium plus NGF, cell number increased during the first 2 days with no increase in membrane fragments or debris. However, NGF effects were transient. By 6 days the number of surviving cells declined to 20% of that at day 0. Cell death was not due to failure of TrkA expression, since immunoprecipitation continued to show TrkA at day 4 (data not shown).

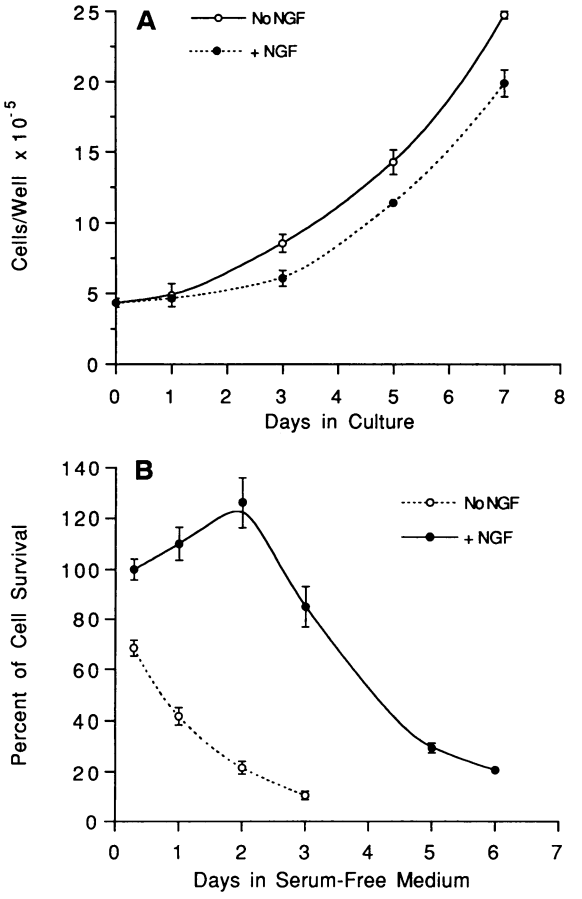

FIG. 4. NGF effects on growth of GT1-trk9 cells in serum-containing medium and on survival in serum-free medium. (*A*) Cells were counted after being grown in serum-containing medium in the absence (○) and presence (●) of NGF (100 ng/ml) for the periods indicated. Each data point is mean ± SEM; $n = 3$. (*B*) Cells maintained in serum-containing medium were washed three times with serum-free medium and fed with either serum-free medium (○) or serum-free medium plus NGF (100 ng/ml) (●). The number of surviving cells was determined. Each value is mean ± SEM; $n = 3$.

NGF Binding and Crosslinking Studies. NGF signaling is mediated through high-affinity receptors (2). The response of GT1-trk9 cells to NGF at low nanomolar concentrations suggested that high-affinity receptors mediated NGF actions. In other cells, NGF high-affinity receptors have been defined by the slow rate for NGF dissociation, leading to their designation as "slow" receptors (19). We investigated NGF binding to GT1-trk9 cells; ^{125}I-NGF (100 pM) was incubated with cells at 4°C for 4 h, and total binding was measured. To measure binding to "fast" and "slow" receptors, cells were then rapidly washed at 4°C in binding buffer prior to measuring radioactivity. The kinetics of dissociation were complex, reflecting at least two different rates of dissociation. In the fastest component, at least 20% of ^{125}I-NGF binding dissociated by 15 min. As is characteristic of PC12 cells (14), the slowly dissociating component, which accounted for 70% of bound ^{125}I-NGF, persisted essentially indefinitely at 4°C (69% ± 1% at 2 h; 69.8% ± 3% at 4 h; both $n = 6$). As indicated, we also observed ^{125}I-NGF binding to GT1-1 cells. There was 16.7% ± 1.5% ($n = 3$) of that found in GT1-trk9 cells. Only 33% ± 2% ($n = 3$) of this binding remained after a 1-h wash.

To demonstrate that NGF was bound to TrkA in "slow" receptors, crosslinking studies were carried out with ^{125}I-NGF. Crosslinked proteins were immunoprecipitated with 1088 (Fig. 5). A radiolabeled band was observed at 158 kDa. The mass of this band is consistent with a complex containing 140-kDa TrkA and an NGF monomer (13 kDa). After a 1-h wash, the signal in this band was 74% ± 8% ($n = 2$) of that seen with no wash. This result is evidence that NGF was bound to TrkA in "slow" receptors.

DISCUSSION

The result of TrkA tyrosine kinase activation depends critically on the cells in which the receptor acts. NGF actions range from cellular transformation (5, 6) to inhibition of growth with neuronal differentiation (7). Defining how cellular context influences TrkA signaling is critical for elucidating NGF actions. Very few cell lines with neuronal properties are available for such studies. Our search led to GT1-1 cells, which show evidence of neuronal differentiation. After parental GT1-1 cells were shown to be NGF unresponsive, we asked (*i*) whether *trkA* transfection would result in NGF signaling and (*ii*) what the consequences of NGF actions would be. We found that *trkA* transfection did

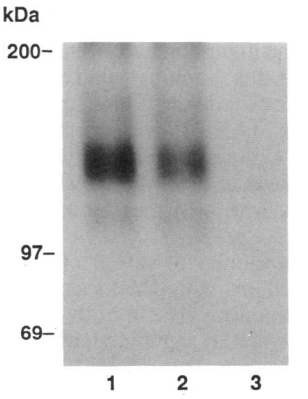

FIG. 5. ^{125}I-NGF was crosslinked to TrkA in GT1-trk9 cells. Lane 1, ^{125}I-NGF crosslinked to TrkA after 4-h incubation; lane 2, ^{125}I-NGF crosslinked to TrkA after 4-h incubation followed by a 1-h wash at 4°C; lane 3, binding and crosslinking carried out in the presence of 800 nM unlabeled NGF.

lead to NGF signaling and that the principal outcome was enhanced neuronal differentiation. These data indicate that GT1-trk9 cells can be used to study NGF signaling and suggest that *trkA* transfection can be used to further explore signaling in other neurons and neuronal precursors.

There were both similarities and differences in the responses of PC12 and GT1-trk9 cells to NGF. In both, NGF treatment leads to rapid TrkA activation, enhances neurite outgrowth, and induces immediate early genes. This suggests that the signal transduction pathways leading to these responses are the same or very similar. On the other hand, PC12 cells cease dividing in response to NGF (7). This was not true for GT1-trk9 cells. However, given that these cells were immortalized by SV40 T antigen, we expected NGF to have little or no effect on cell growth. Transient slowing of growth during the first few days may have been due to NGF-mediated down-regulation of T-antigen expression. One clear difference between PC12 and GT1-trk9 cells was the inability of NGF to prevent the death of GT1-trk9 cells in serum-free medium. Whereas PC12 cells live for long periods under these conditions (7), GT1-trk9 cells began to die by day 3 and most were dead by day 6. Cell death must have resulted from an event(s) that distinguishes signaling in these two cell types. Whether the difference arises at the level of signaling molecules or the programs activated by the NGF signaling cascade is uncertain, but the question can now be addressed by using GT1-trk9 cells. Recently, it was shown that NT-3 did not induce neurites in PC12 cells (6). In contrast, we found that NT-3 did induce outgrowth in GT1-trk9 cells, suggesting the existence of yet additional differences in signaling in these cells. That different responses to neurotrophins can be recorded in different neuronal cell lines points again to the importance of cellular context for their actions.

NGF was able to induce neurite outgrowth from GT1-trk9 cells that later divided. This confirms that neuronal morphological differentiation is possible in mitotically active cells (20). A question that arises is whether NGF could act on mitotically active neural cells under physiological conditions. Our data suggest that if TrkA were present, NGF would act. Earlier data showing NGF actions on both the number and differentiation of adrenal chromaffin cells are consistent with our findings (21, 22). By examining neurons at different stages of differentiation, it may be possible to discover neurotrophin signaling mechanisms leading to mitogenesis, differentiation, and survival.

In enhancing the differentiation of GT1-trk9 cells, our studies suggest that activation of TrkA could be used to augment active endogenous cellular programs. Such activation might be particularly useful if differentiation of neural tumors could be augmented. It is interesting to note that an association has been found between expression of *trkA* and favorable outcome in human neuroblastoma (23). Analysis of 5-year survival rates demonstrated that a very favorable outcome was associated with a high level of TrkA expression (86% survival vs. 14%). It was speculated that TrkA may have promoted cellular differentiation leading to a more benign tumor. The data presented herein raise the possibility that gene therapy with *trkA* may be used to modulate neural tumor biology.

Our studies are similar to earlier ones on PC12nnr5 cells, a mutant PC12 cell line which lacks significant levels of TrkA and is unresponsive to NGF. In both lines, introduction of a *trkA* cDNA allows NGF signaling leading to neuronal differentiation (24). As was true in PC12nnr5 cells (24), TrkA expression in GT1-1 cells produced receptors that transduced signaling at NGF concentrations in the low nanomolar range. Consistent with what has been seen in both neuronal and nonneuronal cells (4, 19, 25), we found that introduction of *trkA* created "slow" (i.e., high-affinity) NGF receptors. Controversy remains regarding whether $p75^{NGFR}$ contributes to the creation of these receptors in neurons (2). Due to low expression of the $p75^{NGFR}$ gene, a contribution of $p75^{NGFR}$ to "slow" receptors in GT1-trk9 cells is a possibility. Nevertheless, "slow" NGF receptors were created with a ratio of TrkA to $p75^{NGFR}$ gene expression greatly in excess of that present in most neurons studied to date.

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