Nerve Growth Factor Enhances Expression of Neuron–Glia Cell Adhesion Molecule in PC12 Cells

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Abstract. The neuron–glia cell adhesion molecule (Ng-CAM) has been identified in mammalian brain tissue and PC12 pheochromocytoma cells as M_r 200,000 and M_r 230,000 species, respectively. When PC12 cells were treated with nerve growth factor (NGF), the amount of Ng-CAM at the cell surface was increased approximately threefold, whereas the amount of the neural cell adhesion molecule (N-CAM) remained unchanged. An NGF-inducible large external glycoprotein (NILE) has been previously identified by its enhanced expression in NGF-treated PC12 cells. Ng-CAM and NILE are similar in molecular weight, expression during development, and responsiveness to NGF in PC12 cells, suggesting that the two molecules are related. In addition, antibodies to Ng-CAM and NILE cross-reacted and the molecules had similar peptide maps after limited proteolysis. Moreover, antibodies to Ng-CAM inhibited fasciculation of neurites, a functional property shared with NILE. The results show that cell adhesion molecules can respond selectively to growth factors and suggest that NILE is, in fact, mammalian Ng-CAM.

It is becoming increasingly clear that interactions mediated by cell adhesion molecules (CAMs)[1] at the surface of cells are essential for regulative features of embryogenic and histogenetic development (14–16). The well-characterized primary CAMs, neural CAM (N-CAM) (30, 45) and the liver CAM (L-CAM) (21), are both expressed very early in embryogenesis on derivatives of all three germ layers in a spatiotemporal pattern that suggests a role for these molecules in inductive events (9, 18). So far, two CAMs have been identified on neuronal cells and their involvement in cell–cell adhesion and cell migration have been demonstrated in several assays. N-CAM is a homophilic ligand (29) i.e., N-CAM on one cell binds to N-CAM on another cell. Perturbation studies with specific antibodies to N-CAM have suggested roles for the molecule in neurite fasciculation (38), retinal layering (5), formation of retinotectal maps (19), and neuron–myotube interaction (27). A neuron–glia CAM (Ng-CAM) was originally identified by its involvement in neuron–glia adhesion (25). In contrast to the primary CAMs, Ng-CAM has been termed a secondary CAM inasmuch as it is not expressed on neurons until they become postmitotic and migrate from their proliferative zones (11, 28, 46). It is abundant on axons, particularly during development of fiber tracts (11). Ng-CAM is involved in migration of granule cell neurons along radial glial cells in the cerebellum (Hoffman, S., C.-M. Chuong, D. R. Friedlander, M. Grumet, and G. M. Edelman, manuscript in preparation) and in interactions among neurons (28); although not fully analyzed, its binding mechanism appears to be heterophilic.

Because changes in the expression of CAMs appear to play an essential role in embryogenesis (15, 16) it is important to gain understanding of the detailed mechanisms governing the regulation of CAM expression. It has already been shown that physiological alterations can lead to altered CAM expression: severing a peripheral nerve, for example, leads to increased N-CAM expression in the associated muscles (8, 37). This observation suggests that signals or factors from nerves might regulate the level of CAM expression and prompted us to search for similar phenomena in a defined cell line. It is known that altered expression of protein synthesis and cell function can be induced by growth factors (47): in the nervous system, nerve growth factor (NGF) is necessary for the survival and maintenance of sensory and sympathetic neurons (32). NGF, which induces the extension of neurites, also causes increased expression in rat PC12 pheochromocytoma cells of a large external glycoprotein called NILE (35). During mammalian development, NILE (M_r 200,000–230,000) (39, 40) first appears on postmitotic neurons (44) and antibodies to NILE inhibit fasciculation of neurites in cultures of rat brains (42). Because NGF alters protein expression in neurons, we examined its ability to alter the expression of N-

[1] *Abbreviations used in this paper:* CAM, cell adhesion molecule; L-CAM, liver cell adhesion molecule; N-CAM, neural cell adhesion molecule; Ng-CAM, neuron–glia cell adhesion molecule; NGF, nerve growth factor; NILE, nerve growth factor–inducible large external glycoprotein; NP-40, Nonidet P-40.

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CAM and Ng-CAM in PC12 cells.

We report here that NGF induces in PC12 cells an increase in expression of Ng-CAM but not of N-CAM. By using specific antibodies, it was found that both Ng-CAM and NILE were (*a*) induced by NGF, (*b*) similar in size and peptide maps, and (*c*) immunologically cross-reactive. Furthermore, perturbation experiments indicated that Ng-CAM is involved in neurite fasciculation, a functional property shared with NILE. These observations suggest that NILE is mammalian Ng-CAM.

Materials and Methods

Tissues and Cells

Tissues from the nervous system were dissected from 14-d chicken embryos (White Leghorn), neonatal NCS mice (Laboratory Animal Research Center, The Rockefeller University) and 16-d rat embryos (Taconic Farms Inc., Germantown, NY). PC12 cells (kindly provided by Dr. William B. Stallcup) were grown on plastic tissue culture dishes (Corning Glass Works, Corning, NY), coated with collagen (1) at initial concentrations of $\sim 1.5 \times 10^5$ cells/cm^2, and were passaged weekly. Cells were fed three times a week with RPMI 1640 medium, supplemented with 10% heat-inactivated horse serum and 5% heat-inactivated fetal calf serum, and maintained at 37°C in a 10% CO_2 incubator. Media and sera were obtained from Gibco, Grand Island, NY. For PC12 cell cultures grown in the presence of NGF (Collaborative Research, Lexington, MA), 250 ng/ml of 7 S NGF were used; no differences were observed when 50 ng/ml of 2.5 S NGF were used. For fasciculation studies, dorsal root ganglia from the lumbar region of embryonic day 10 chicken embryos were explanted on a collagen gel in plastic 35-mm dishes. Cultures were fed Dulbecco's modified Eagle's medium supplemented with 10% (vol/vol) heat-inactivated fetal calf serum and 10 ng/ml of 2.5 S NGF.

Antibodies

Rabbit antisera to rodent N-CAM (6), to chicken Ng-CAM (26), and to L-CAM (21), and IgG and Fab' fragments (4) were prepared as described. Affinity-purified antibodies to Ng-CAM were purified from rabbit anti-Ng-CAM IgG by immunoaffinity chromatography using chicken Ng-CAM coupled to Sepharose CL-2B (Pharmacia Fine Chemicals, Piscataway, NJ) (10). Anti-NILE antisera were generously provided by Dr. William B. Stallcup.

Iodination of Cell Surface Molecules

Cells or tissues were washed with phosphate-buffered saline (PBS) and then incubated with 50 IU/ml lactoperoxidase (Calbiochem-Behring Corp., La Jolla, CA), 1 mCi/ml Na[^{125}I] (New England Nuclear, Boston, MA) and H_2O_2 (34) at 37°C for 15 min. The material was washed three times with PBS containing 20 mM KI, and then extracted with hot SDS buffer and processed for immunoprecipitation with specific antibodies, as described below.

Metabolic Labeling

PC12 cells were washed three times with Hank's balanced salt solution and incubated for 20 h with 1 mCi/ml [^{3}H]leucine (New England Nuclear) in Eagle's minimal essential medium that lacked leucine, and that was supplemented with 10% dialyzed heat-inactivated fetal calf serum. After washing three times with Hank's balanced salt solution, the cells were extracted for immunoprecipitation.

Immunoprecipitation

Samples were solubilized by the addition of 5 vol of boiling SDS buffer (1% SDS/0.15 M NaCl/25 mM EDTA/20 mM Na_2HPO_4) followed by vigorous mixing. After addition of 20 vol of 1.25% Nonidet P-40 (NP-40)/0.15 M NaCl/25 mM EDTA/20 mM Na_2HPO_4 (pH 7.4), the samples were centrifuged at 100,000 *g* for 30 min. The extracts were incubated with specific antibodies bound to protein A-Sepharose (Pharmacia Fine Chemicals). The immunoprecipitates were washed (22) and solubilized in sample buffer (62.5 mM Tris [pH 6.8]/5% 2-mercaptoethanol/2% SDS/10% sucrose/0.0025% bromphenol blue) and resolved on SDS polyacrylamide gels (31). Gels were stained with Coomassie Brilliant Blue, destained, and impregnated with 1 M sodium salicylate in the case of ^{3}H label. Radiolabeled proteins were visualized by autoradiography at −70°C with Kodak XAR-5 X-ray film.

Immunoblots

Membranes prepared (29) from neonatal mice or embryonic chicken brains were extracted in PBS, 0.5% NP-40, 200 U/ml Trasylol (Mobay Chemical Corp., New York, NY) and centrifuged at 100,000 *g* for 1 h at 4°C. The extracts were boiled for 3 min in sample buffer, resolved on SDS PAGE, transferred to nitrocellulose paper, reacted sequentially with 50 μg of rabbit antibody to chicken Ng-CAM and 1×10^6 cpm of ^{125}I-labeled protein A, and labeled material was detected by autoradiography (48).

Peptide Maps

Bands on 6% acrylamide gels corresponding to Ng-CAM and NILE were excised and treated with 0.1 μg of papain or *Staphylococcus aureus* V8 protease, and the digestion products were resolved by SDS PAGE (15% acrylamide) by the method of Cleveland et al. (7).

Quantitation of CAMs in PC12 Cells

Aliquots of extracts containing equal numbers of counts from [^{125}I] or [^{3}H]-leucine-labeled cells cultured in the presence or absence of NGF were incubated with an excess amount of anti-Ng-CAM or anti-N-CAM IgG and processed for immunoprecipitation as described above. Aliquots of the total extract were included in the gels, to be used in normalizing the amounts of CAMs to total labeled protein. Densitometric profiles of the autoradiographs were obtained with a Quick Scan scanner (Helena Laboratories, Beaumont, TX) and areas corresponding to Ng-CAM, N-CAM, and total protein were determined by weighing traces of the profiles. The increase in the amount of Ng-CAM or N-CAM due to treatment with NGF was expressed by the ratio

$$\left(\frac{\text{amount of CAM with NGF}}{\text{amount of total protein with NGF}}\right) \Big/ \left(\frac{\text{amount of CAM without NGF}}{\text{amount of total protein without NGF}}\right).$$

For immunoblots, values were normalized to total amounts of protein loaded per lane.

Immunofluorescent Staining

Live cultures were washed three times with medium and incubated for 30 min at room temperature in medium with 40 μg/ml of affinity-purified anti-Ng-CAM or non-immune IgG. After washing five times with Hank's balanced salt solution during a total of 5 min, cultures were fixed for 15 min with 3.5% (wt/vol) formaldehyde in 0.1 M sodium cacodylate, pH 7.4, and were sequentially incubated for 10 min in 100 mM phosphate buffer (pH 7.4)/0.1 M glycine, for 10 min in 0.1 M sodium phosphate/5% (vol/vol) goat serum, and for 30 min with a 1:100 dilution of fluorescein-conjugated goat anti-rabbit IgG (Miles Yeda, Rehovoth, Israel) in the same buffer. Cultures were washed five times in PBS, mounted in glycerol, and photographed. No staining was observed in cultures incubated with non-immune IgG.

Results

One of the main purposes of this work was to investigate the effect of NGF on the expression of CAMs. The rat cell line PC12 was ideally suited for this study because the effect of the growth factor on protein expression in these cells has previously been demonstrated and because protein expression can be studied independently from its effects on cell survival (23). Specific antibodies to mouse N-CAM and to chicken Ng-CAM were used to identify CAMs in mouse brain and PC12 cells. Anti-N-CAM Ig recognizes M_r 120,000, 140,000, and 180,000 components in the adult mouse and it strongly cross-reacts with rat N-CAM (24). In PC12 cells, it recognized M_r 140,000 and 180,000 molecules (Fig. 1*B*, lane *3*). Purified mouse N-CAM blocked immunoprecipitation by antibodies to N-CAM of PC12 material (Fig. 1*B*, lane *4*), confirming identification of these species as components of N-CAM in PC12 cells.

As shown previously (28), specific antibodies to chicken Ng-CAM recognized M_r 200,000, 135,000, and 80,000 components (Fig. 1*A*, lane *1*) in extracts of chicken brain. One major (M_r 200,000) component was found in extracts of

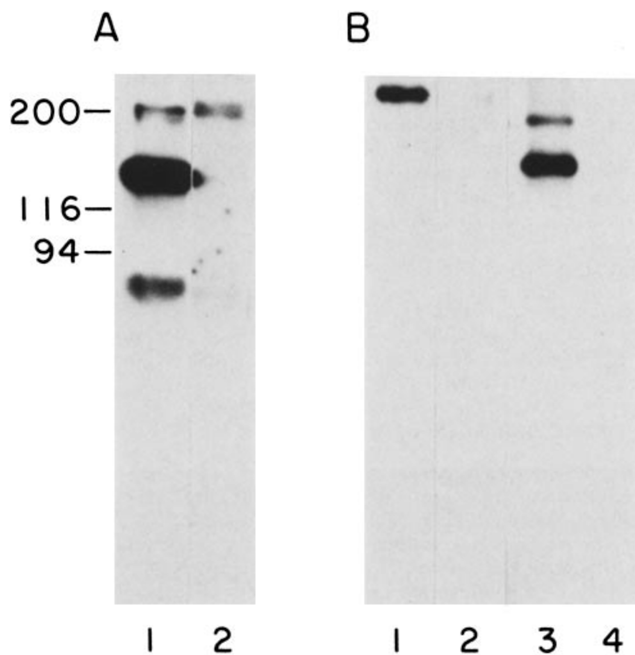

Figure 1. Detection of Ng-CAM and N-CAM in PC12 cells. (*A*) Extracts (100 μg of protein) of chicken (lane *1*) and mouse (lane *2*) brain were resolved by SDS PAGE (7.5% acrylamide), analyzed by immunoblotting using rabbit antibodies to purified chicken Ng-CAM and ^{125}I-protein A, and visualized by autoradiography. (*B*) ^{125}I-labeled cell surface proteins were extracted from PC12 cells with SDS buffer and immunoprecipitated with rabbit antibodies to chicken Ng-CAM (lanes *1* and *2*) or mouse N-CAM (lanes *3* and *4*). In lanes *2* and *4*, the antibodies were preincubated for 1 h with 5 μg of chicken Ng-CAM and 5 μg of mouse N-CAM, respectively. The immunoprecipitates were resolved on SDS PAGE (7.5% acrylamide) and visualized by autoradiography. The numbers on the left represent the molecular weight (× 10^{-3}) of standards that migrated to the indicated positions.

mouse brain (Fig. 1*A*, lane *2*). Anti–Ng-CAM IgG immunoprecipitated M_r 200,000 and 230,000 molecules, respectively, from detergent extracts of ^{125}I surface-labeled rat brain and dorsal root ganglia (data not shown). Immunoprecipitates of ^{125}I surface-labeled extracts of PC12 cells with anti–Ng-CAM IgG yielded a M_r 230,000 molecule (Fig. 1*B*, lane *1*). To confirm that the precipitation was due to authentic antibodies to Ng-CAM, the antibody was preincubated with an excess of unlabeled chicken Ng-CAM which strongly blocked immunoprecipitation of the PC12 molecule (Fig. 1*B*, lane *2*). The cross-species immunological reactivity (Fig. 1) and the ability of Fab′ fragments of the chicken antibody to inhibit adhesion between mouse neurons and glia (Grumet, M., and G. M. Edelman, unpublished observations) strongly suggest that the antigens recognized in mouse and PC12 cells are, in fact, rodent Ng-CAM.

Induction of Ng-CAM in PC12 Cells

To study the effect of NGF on expression of cell surface CAMs, PC12 cells were grown in the presence or absence of NGF and, after radioiodination at the cell surface, CAMs were quantitatively immunoprecipitated in the presence of excess antibody. While no differences were seen in the amounts of N-CAM on cells grown with or without NGF, cells grown in the presence of the growth factor expressed three to four times the amount of Ng-CAM on cells grown without NGF (Fig. 2; Table I). Using the same antisera, similar results were consistently obtained when the amounts of Ng-CAM and N-CAM were quantitatively measured in extracts of cells that had been metabolically labeled with [^{3}H]leucine, and in immunoblots of unlabeled extracts of PC12 cells (Table I).

To localize Ng-CAM in PC12 cells in the presence and absence of NGF, cultures were stained with affinity-purified antibodies to Ng-CAM. As shown previously (24), in response to NGF, PC12 cells extended neurites. Both cell bodies and neurites stained with antibodies to Ng-CAM (Fig. 3). As found previously for NILE (40), there was no apparent difference in the staining intensity for Ng-CAM cells grown in the presence or absence of NGF. However, it is difficult to quantitate data from such fluorescent staining, primarily because of the changes in cell shape. The fact that PC12 cells express neurites in response to NGF may be closely related to the overall biochemical increase in Ng-CAM that was detected (Fig. 1).

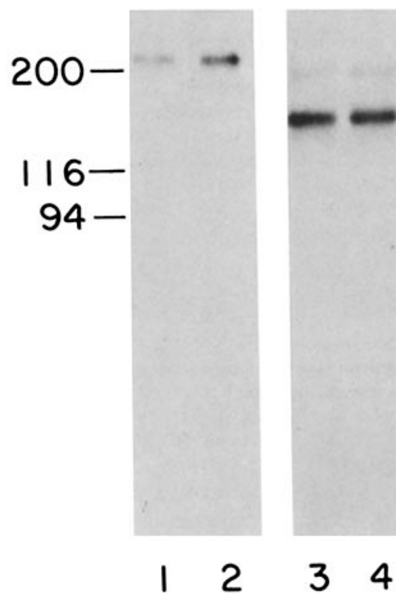

Figure 2. Effects of NGF on CAM expression. PC12 cells were grown in the absence (lanes *1* and *3*) or presence (lanes *2* and *4*) of NGF for 1 wk and were then labeled with ^{125}I. NP-40 extracts of cells containing equal amounts of counts were immunoprecipitated with specific antibodies to Ng-CAM (lanes *1* and *2*) and N-CAM (lanes *3* and *4*). The precipitated molecules were resolved on SDS PAGE (7.5% acrylamide) and visualized by autoradiography.

*Table I. Relative Amounts of Ng-CAM and N-CAM in NGF-treated and -untreated Cells**

Method of analysis	Ng-CAM	N-CAM
Immunoprecipitation of surface iodinated cells (n = 8)	2.9 ± 1.2‡	1.0 ± 0.2
Immunoprecipitation of metabolically labeled cells (n = 1)	4.3	1.2
Immunoblot of whole cell extracts (n = 2)	4.0 ± 0.1	1.4 ± 0.6

* The table shows the ratio of Ng-CAM or N-CAM in NGF-treated cells relative to untreated cells, as determined by three different methods. In each experiment, the amount of CAM was normalized to total protein and was expressed as the ratio in NGF-treated to -untreated cells, as described in Materials and Methods.
‡ Mean ± SEM. The number of determinations is shown in parenthesis.

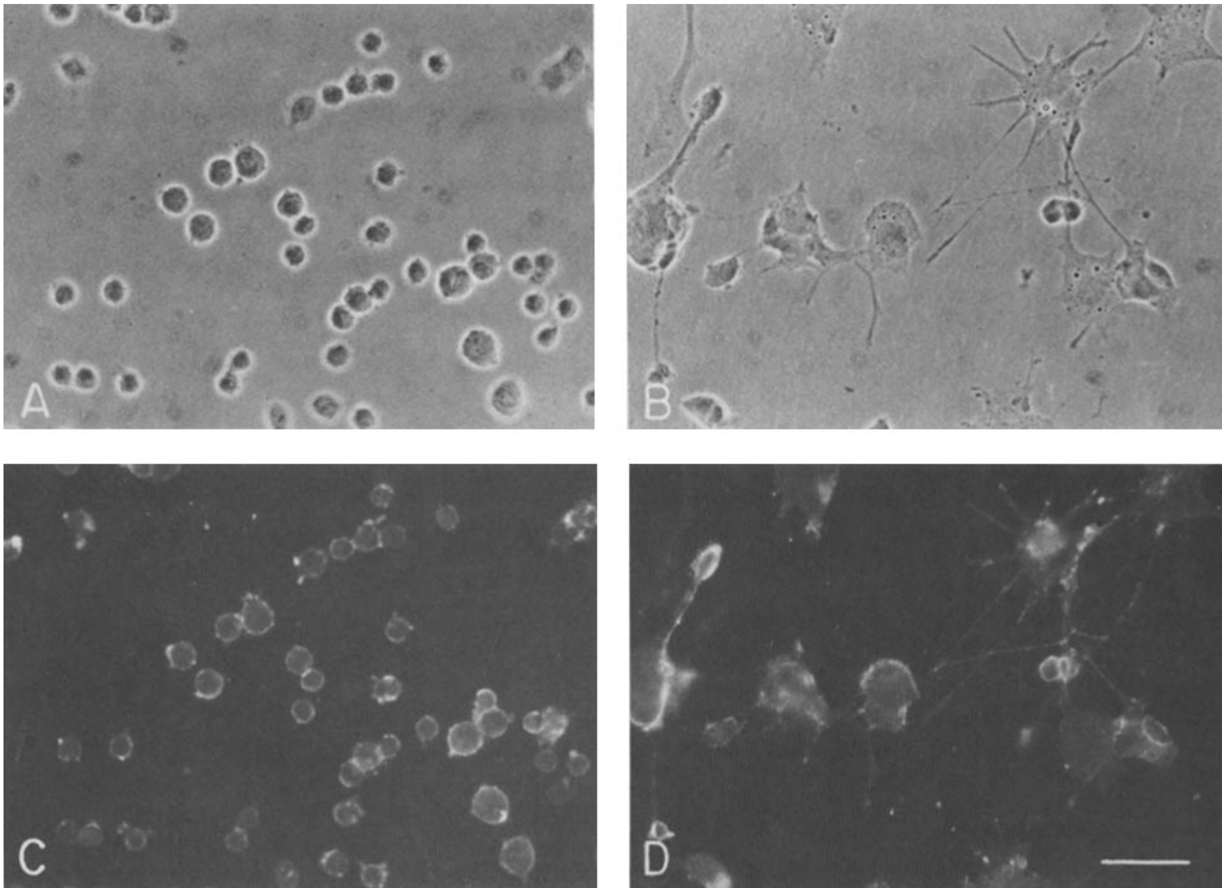

Figure 3. Immunofluorescent staining of PC12 cells with anti–Ng-CAM IgG. PC12 cells were grown for 1 wk in the absence (*A* and *C*) or presence (*B* and *D*) of NGF and stained with affinity-purified anti–Ng-CAM IgG. *A* and *B* are phase-contrast micrographs of the fields shown in *C* and *D*. Bar, 50 μm.

The validity of this interpretation, however, will require a comparative study of biosynthesis, membrane insertion, and turnover rates of Ng-CAM and other molecules in PC12 cells.

Relationship between Ng-CAM and NILE

The observations that NGF induces increased expression of Ng-CAM and NILE in PC12 cells, and that both molecules have the same molecular weight, suggested that the molecules are related. To compare Ng-CAM and NILE, extracts of PC12 cell plasma membranes labeled with ^{125}I were depleted of Ng-CAM by repeated immunoprecipitation with specific anti-Ng-CAM antibodies (Fig. 4*A*, lanes *4–6*); the depleted extract was then immunoprecipitated with antibodies to NILE. The results indicated that depletion of Ng-CAM was accompanied by removal of NILE (Fig. 4*A*, lanes *7* and *8*), while the amount of N-CAM that remained in the extracts was not altered by this treatment (Fig. 4*A*, lane *9*). This experiment suggested that, in addition to the similarities mentioned, NILE is immunologically related to Ng-CAM.

In a complementary experiment, we found that anti-NILE antibodies recognized Ng-CAM. Ng-CAM was immunoaffinity purified from PC12 cells that had been labeled at their surface with ^{125}I using immobilized anti–Ng-CAM antibodies. The Ng-CAM was then immunoprecipitated with specific antibodies to Ng-CAM, NILE, and L-CAM. The M_r 230,000 Ng-CAM component was recognized both by antibodies to Ng-CAM and to NILE but not by antibodies to L-CAM (Fig. 4*B*).

Further biochemical data suggesting the identity of the two molecules was obtained by peptide mapping following limited proteolysis (7). Extracts of PC12 cell plasma membranes labeled with ^{125}I were incubated with antibodies to Ng-CAM, NILE, and N-CAM, and the immunoprecipitates were separated by SDS PAGE. The M_r 230,000 components of Ng-CAM and NILE and the M_r 140,000 species of N-CAM were digested either with *S. aureus* V8 protease or with papain. For each enzyme, the resulting patterns of proteolytic cleavage products of Ng-CAM and NILE were very similar, and clearly different from those of N-CAM (Fig. 5). Taken together, these biochemical and immunological experiments strongly suggest that Ng-CAM and NILE from PC12 cells are very similar or identical.

Role of Ng-CAM in Fasciculation

The role of Ng-CAM in neuron–neuron adhesion (28) and its preferential location on axonal membranes (11) suggested the possibility of a role for this molecule in neurite fasciculation. To test this possibility, we examined fasciculation in chicken embryo dorsal root ganglia, both because our Ng-CAM antibodies were prepared against the chicken protein, and because we have previously used this assay to study fasciculation (38). Dorsal root ganglia from 10-d embryonic chickens were grown

for 1 to 2 d in vitro in the presence of equivalent amounts of non-immune or anti–Ng-CAM Fab′ fragments. Fasciculation of neurites occurred in cultures grown with control antibodies (Fig. 6*A*) but was strongly inhibited by anti–Ng-CAM antibodies (Fig. 6*B*). Antibodies to Ng-CAM caused a 50% reduction in the mean fascicle diameter; the magnitude of the inhibition was similar to that caused by antibodies against N-CAM, although the relative potency of antibodies to Ng-CAM was much greater than antibodies to N-CAM (Hoffman, S., C.-M. Chuong, D. R. Friedlander, M. Grumet, and G. M. Edelman, manuscript in preparation).

Control experiments were performed to verify that the lower level of fasciculation observed in the presence of antibodies to Ng-CAM was not an indirect result of increased fiber-to-substrate adhesion mediated by antibodies bound to the substrate. Cultures were grown on dishes that had been preincubated with antibodies and then washed to remove unbound molecules. When compared with cultures grown on untreated dishes, no difference in the degree of fasciculation was observed. This result suggests that antibodies to Ng-CAM inhibit fasciculation primarily by interfering with fiber–fiber interaction. Consistent with this result is the observation that antibodies to other molecules that are richly expressed on dorsal root ganglia neurites do not inhibit fasciculation (3). The fact that both Ng-CAM (Fig. 6) and NILE (42) are involved in neurite fasciculation gives further support to the interpretation of the immunological and biochemical data suggesting that NILE is mammalian Ng-CAM.

Discussion

The major conclusions of the present study are that NGF selectively increases the expression of Ng-CAM on the surface of PC12 cells and that NILE glycoprotein is Ng-CAM. Using specific antibodies to chicken Ng-CAM that cross-reacted with

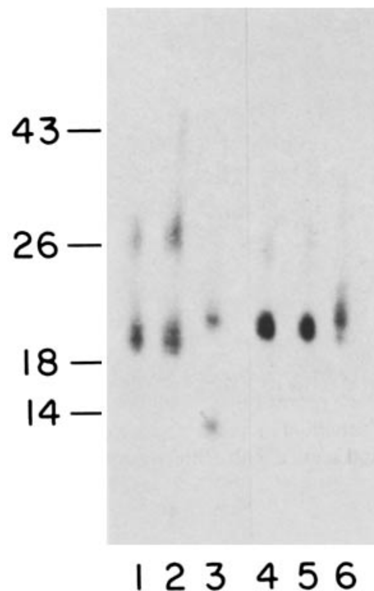

Figure 5. Comparison of peptide fragments of Ng-CAM and NILE. Ng-CAM (lanes *1* and *4*), NILE (lanes *2* and *5*), and N-CAM (lanes *3* and *6*) were immunoaffinity-purified from extracts of PC12 cells that had been labeled at their surfaces with ^{125}I. The purified molecules were then resolved on SDS PAGE, excised from the gel, digested with papain (lanes *1–3*) or V8 protease (lanes *4–6*), resolved on SDS PAGE (7), and visualized by autoradiography.

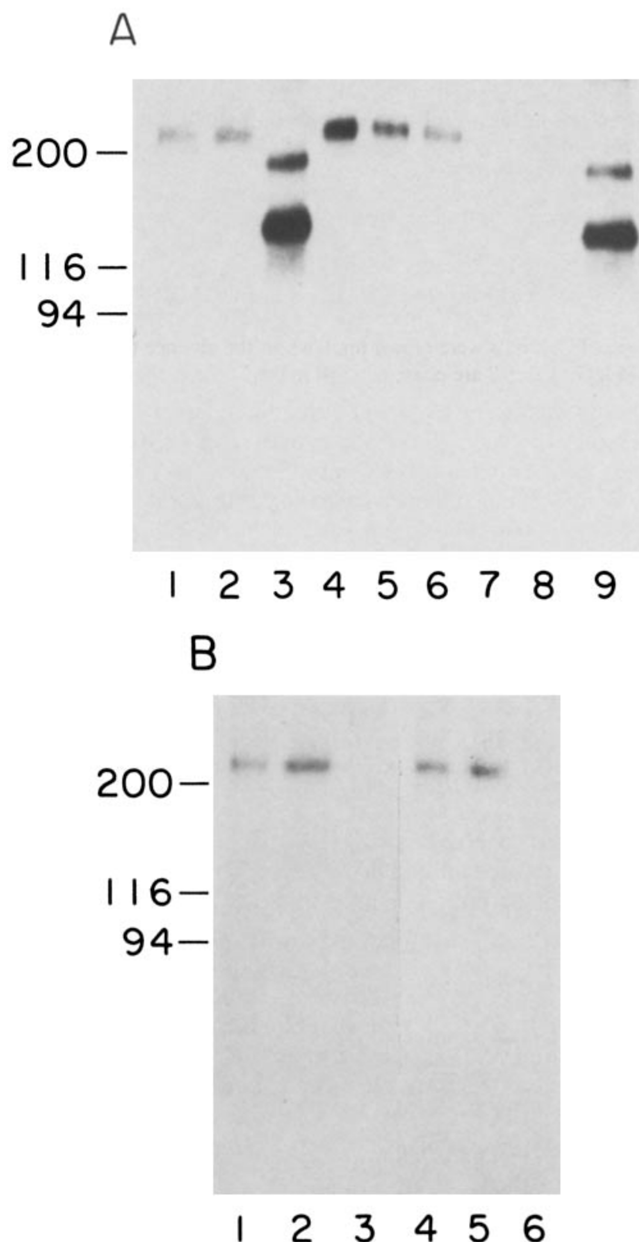

Figure 4. Immunological cross-reactivity between Ng-CAM and NILE. (*A*) Extracts of PC12 cells labeled at their surfaces with ^{125}I were immunoprecipitated with 50 μg of anti–Ng-CAM IgG (lanes *1* and *7*), 10 μl of anti-NILE antisera (lanes *2* and *8*), or 50 μg of anti–N-CAM IgG (lanes *3* and *9*). The immunoprecipitations were performed on untreated extracts (lanes *1–3*) or on extracts that had been depleted of Ng-CAM (lanes *7–9*) by three sequential precipitations with 250 μg of anti–Ng-CAM IgG. Ng-CAM precipitated in the three sequential steps are shown in lanes *4*, *5*, and *6*, respectively. (*B*) Ng-CAM was purified from ^{125}I-labeled PC12 cells by immunoprecipitation with polyclonal antibodies to Ng-CAM that were covalently linked to Sepharose CL-2B beads (10); material was eluted with 50 mM diethylamine, pH 11.5, and the pH was neutralized (30). The untreated extract (lanes *1–3*) or the purified Ng-CAM (lanes *4–6*) was then immunoprecipitated with anti–Ng-CAM IgG (lanes *1* and *4*), anti-NILE antisera (lanes *2* and *5*), and anti-L-CAM IgG (lanes *3* and *6*); material that bound was analyzed on SDS PAGE (6% acrylamide) and visualized by autoradiography.

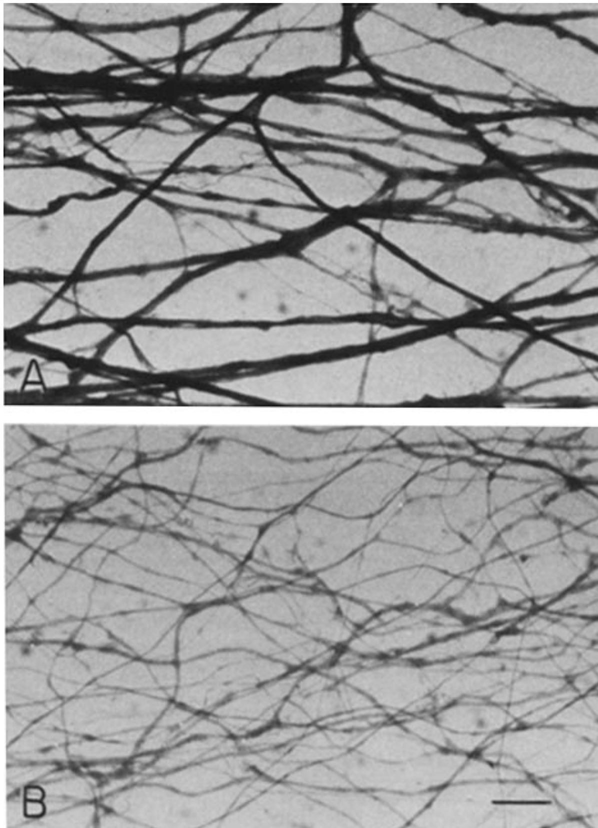

Figure 6. Inhibition of fasciculation by antibodies to Ng-CAM. Dorsal root ganglia from the lumbar region of 10-d-old chick embryos were explanted on a collagen gel and cultured in the presence of 10 μg/ml non-immune (*A*) or anti-Ng-CAM (*B*) Fab′ fragments. After 2 d, cultures were fixed, stained with toluidine blue, and air dried to facilitate visualization. Bar, 10 μm.

Table II. Similarities between Ng-CAM and NILE

	Ng-CAM	NILE
Molecular properties		
Central nervous system	M_r 200,000 (Fig. 1 *A*)	M_r 200,000 (40)
PC12 cells or peripheral nervous system	M_r 230,000 (Fig. 1 *B*)	M_r 230,000 (35, 40)
Phosphorylation and glycosylation	+ (28)	+ (35, 39, 40)
Distribution		
First appearance on postmitotic mononeurons	+ (11, 28, 46)	+ (44)
Preferential expression on axons in fiber tracts	+ (11)	+ (44)
Central glia	− (26)	− (43, 44)
Schwann cells	+ (*)	+ (43, 44)
Function in vitro (perturbation by antibodies)		
Neuron–glia adhesion	+ (28)	ND
Neuron–neuron adhesion	+ (28)	ND
Neurite fasciculation	+ (Fig. 6)	+ (42)
Granule cell migration in cerebellum	+ (‡)	ND

Numbers in parentheses are references. ND, not determined.

* Daniloff, J. K., G. Levi, M. Grumet, F. Rieger, and G. M. Edelman, manuscript in preparation.

‡ Hoffman, S., C.-M. Chuong, D. R. Friedlander, M. Grumet, and G. M. Edelman, manuscript in preparation.

the rodent molecules, it was shown that NGF stimulated the expression of Ng-CAM in PC12 cells by a factor of approximately three. A similar level of stimulation has been observed for NILE (39). The following molecular properties indicate that Ng-CAM and NILE are very similar or identical: (*a*) the molecular weight of both molecules in PC12 cells and in rodent neural tissues is the same; (*b*) purified Ng-CAM is recognized by antibodies to NILE; (*c*) when Ng-CAM is depleted from extracts of PC12 cells by anti–Ng-CAM, NILE is also depleted; and (*d*) the patterns of proteolytic cleavage products of both molecules are very similar.

In addition to these properties of Ng-CAM and NILE, several anatomical and functional characteristics are also similar and are summarized in Table II. In the central nervous system, both Ng-CAM (11, 28, 46) and NILE (44) are first expressed on postmitotic neurons and are more abundant on axons than on cell bodies. Neither Ng-CAM (26) nor NILE (43, 44) are expressed in astrocytes, but both are expressed on neurons and Schwann cells in the peripheral nervous system (Daniloff, J. K., G. Levi, M. Grumet, F. Rieger, and G. M. Edelman, manuscript in preparation; see references 43 and 44). Perturbation experiments using specific monoclonal and polyclonal antibodies to Ng-CAM provide compelling evidence that the molecule is involved in neuron–glia and neuron–neuron adhesion (summarized in Table II). In the one case that has so far been tested (neurite fasciculation), both Ng-CAM (Fig. 6) and NILE (42) have been found to be involved. A third molecule, L1 antigen (33, 41), also shares most of these properties, and we have previously suggested that it is rodent Ng-CAM (28).

For interpreting the role of CAMs in regulating neural morphogenesis in terms of surface modulation (13, 15), the responses to local signals and to changes in overall tissue architecture are critical elements. Previous studies in this (37) and other (8) laboratories have indicated that disturbance of neuromuscular connection leads to increased expression of N-CAM in muscle. This observation suggests that local signals from the neuron that have not yet been identified, are involved in modulating the amount of muscle N-CAM. The present observation that expression of Ng-CAM but not of N-CAM is enhanced by NGF is the first direct demonstration that CAMs can respond selectively to growth factors. The cellular and molecular aspects of the action of NGF have been studied (32, 47), and this knowledge should be useful in determining the mechanisms by which this growth factor selectively enhances CAM expression.

Another instance of altered CAM expression is the decrease in the amount of N-CAM at the surface of retinal cells following transformation by Rous sarcoma virus (2). Recent studies with cDNA probes for CAMs (12, 36) indicate that changes in the levels of N-CAM mRNA underlie the observed changes in N-CAM expression in transformed cells (Brackenbury, R., and G. M. Edelman, unpublished observations). Similar studies using genetic probes for CAMs and studies with in vitro systems that exhibit changes similar to those observed in vivo (20) should help to determine the mechanisms by which NGF controls the expression of Ng-CAM. The fact that selective CAM expression can be induced by a growth factor provides a strong impetus to search for other growth factors and signals that modulate CAM expression as

well as for those aspects of tissue architecture that lead to the production of such signals (17).

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