Induction of L1 mRNA in PC12 Cells by NGF Is Modulated by Cell–Cell Contact and Does Not Require the High-Affinity NGF Receptor

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We examined the effects of nerve growth factor (NGF) and cell–cell contact on expression of the neural cell adhesion molecule L1 in PC12 cells. After 7 d exposure to NGF, but not after exposure to EGF, FGF, TGFβ, or dibutyryl cAMP (dbcAMP), L1 mRNA levels increased fourfold. This increase was not blocked by K252a, an inhibitor of the high-affinity NGF receptor, although neurite extension was completely inhibited. L1 mRNA levels also increased in NGF-treated mutant PC12 cells (PC12m5) that lack the high-affinity NGF receptor. The effect of NGF on L1 mRNA was greatest in cells cultured at high density, but its effect on cells cultured at low density was augmented by antibody to L1 (to mimic L1 homophilic binding). Various extracellular matrix components had no differential effects on L1 mRNA levels in either the presence or absence of NGF. Together, these findings suggest that NGF regulates L1 expression by a mechanism that is independent of the high-affinity NGF receptor and that this regulation is modulated by cell–cell contact but not by cell–extracellular matrix interactions.

[Key words: neural cell adhesion molecule L1, PC12 cells, mRNA, differentiation, NGF, culture, K252a, cell–cell adhesion]

Neuronal gene programming is influenced by extracellular stimuli, including soluble factors and contacts with other cells or with extracellular matrix, which then act through intracellular signals including phosphorylation and second-messenger generation. Nerve growth factor (NGF) is one extracellular stimulus that plays essential roles in the development of both the CNS and PNS (Levi-Montaltini, 1987). Two receptors that bind NGF have been characterized: a high-affinity receptor, gp140/crk, the product of the proto-oncogene trk, and a low-affinity receptor, p75/LNGFR (Chao et al., 1991), and a low-affinity receptor, p75/LNGFR (Chao et al., 1991; Miura et al., 1991; Prince et al., 1991) indicates that these molecules are homologs.

McGuire et al. (1978) have reported that the NGF-induced increase in NILE is selectively inhibited by the RNA synthesis inhibitor camptothecin and, hence, appears to be mediated through a transcriptional pathway. By contrast, recent studies have suggested that mRNA levels for L1 and NILE are not altered by NGF treatment. The experiments described here test three aspects of this regulation: (1) Does NGF regulate the level of L1 mRNA in PC12 cells? (2) Are the mechanism(s) by which NGF regulates morphological differentiation in PC12 cells identical to those by which it regulates L1? (3) Does cell–cell or cell–extracellular matrix adhesion regulate L1 expression? The results indicate that NGF affects cell morphology and L1 gene expression by distinct mechanisms. The results further suggest that cell–cell contact, involving homophilic L1–L1 contact, but not cell–extracellular matrix contact, modulates the effect of NGF on L1 mRNA levels.

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Materials and Methods

Cell culture. Rat pheochromocytoma cells (PC12), originally developed by Greene and Tischler (1976), were the gift of Dr. K. Neet, Case
Western Reserve University. These cells were maintained in a humidified 10% CO2 atmosphere at 37°C in 85% Dulbecco's modified Eagle's medium containing 5% fetal calf serum, 10% donor horse serum, 100 U/ml penicillin G, 100 μg/ml streptomycin, and 250 ng/ml amphotericin B. At confluence, the cells were dislodged from the culture flask (Falcon, #3111, Bedford, MA) by shaking and trituration and 25% of these cells were used for new culture (data not shown).

The culture medium was changed every 2–3 d. For total cellular RNA isolation, cells were plated on rat tail collagen (COL, 50 μg/ml), BSA (50 μg/ml), poly-L-lysine (PL, 50 μg/ml), laminin (LN, 50 μg/ml), or fibronectin (FN, 50 μg/ml)-coated 60 mm tissue culture dishes (Falcon, #3002, Bedford, MA) at 0–4 × 10^4 cells per cm2 in serum-free, chemically defined medium consisting of DMEM/F12 (1:1) (Sigma, St. Louis, MO) and 15 mM HEPES supplemented with progestosterone (62 ng/ml), pegasitene (16.1 μg/ml), selenium (39 ng/ml), insulin (10 μg/ml), and transferrin (100 μg/ml). In some experiments, cells were cultured in suspension at 80 rpm to prevent outgrowth of neurites and adhesion to the substrate. To obtain conditioned medium, fresh medium was added to near-confluent cultures of PC12 cells and then harvested 48 hr later, centrifuged to remove debris, and stored at −20°C. To test the effects of conditioned medium, PC12 cells were grown in fresh medium for 24 hr, before replacement by medium consisting of 50% conditioned medium and 50% fresh medium. To determine the role of gp140*, control PC12 cells and gp140*−null PC12n5 cells (kindly provided by University of Wisconsin) were pre-treated with 0.1% SDS, containing RPMI-1640 medium (Gibco, Grand Island, NY).

Isolation and cloning of a mouse LI segment. A partial LI cDNA was obtained by PCR using adult mouse brain cDNA as template. The upstream and downstream primers were 5′-AAGGATCCCGGAATTCCTGGCCAGACCA-3′ and 5′-CCCCGGTTACTGTGACCTGCGATAAAGG-3′, corresponding to nucleotides 70–95 and 1461–1486, respectively, of the mouse LI cDNA sequence of Moos et al. (1989). These primers were designed to enable cloning of IgG-like loops I to V of LI. The cloned LI cDNA fragments were converted to plasmid pATH20 for production of fusion protein.

Production of antibody to LI. Bacterial expression and purification of the fusion protein derived from pATH20 were carried out according to the method of Koerner et al. (1991). The fusion protein was electrophoretically eluted from a polyacrylamide gel after electrophoresis, mixed with Freund's adjuvant (Difco, Detroit, MI) in 10 mM phosphate-buffered saline containing 0.1% deoxycholate and protease inhibitors as described above and centrifuged for 1 hr at 10,000 × g at 4°C. The final supernatant was used as a crude membrane fraction. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis was performed on 6% polyacrylamide gels (Laemmli, 1970). In order to examine LI polypeptide levels in PC12 cells, proteins fractionated by SDS PAGE were transferred to Immobilon® P (Millipore, Bedford, MA) and processed for Western blot analysis with polyclonal LI antibody and biotinylated anti-rabbit IgG antibody using Vectastain® ABC reagent and diaminobenzidine as the peroxidase substrate for horseradish peroxidase-staining according to the manufacturer's instructions (Vector, Burlingame, CA).

Growth factors and other reagents. Growth factors and other factors were added to cultures at the time of plating and were replenished every 2–3 d. 2.5S NGF and FGF were purchased from Collaborative Biomedical Products (Bedford, MA). TGFβ and EGF were obtained from Genzyme (Cambridge, MA) and GIBCO/BRL (Grand Island, NY), respectively. All growth factors were diluted in PBS containing 100 μg/ml BSA. Controls received vehicle only. A 2 mm stock solution of K252a (Calbiochem, La Jolla, CA) was prepared in dimethylsulfoxide and stored at −20°C in the dark. N-(2-hydroxyethyl)adenosine 5′-cytidine monophosphate (dbcAMP) was obtained from Sigma (St. Louis, MO) and dissolved in culture medium immediately before use.

Statistical analysis. All data were represented as means ± SD from at least three independent experiments. Statistical analyses of all data were performed by ANOVA.

Results

Nerve growth factor induces LI mRNA

To characterize the molecular mechanisms underlying the regulation of LI expression, the kinetics of expression of LI mRNA in PC12 cells following treatment with NGF were determined. PC12 cells were exposed to 50 ng/ml NGF, and total cellular RNA was isolated 0–7 d later. Northern blot analysis revealed that LI mRNA specific activity increased fourfold during 7 d in culture (Figs. 1A,B). This change was gradual and peaked at 5 d of treatment. In untreated control cultures, no significant changes in LI mRNA were observed. In these experiments, a single 60 kb LI mRNA was detected, as reported previously (Tacke et al., 1987; Miura et al., 1991). Roughly equal amounts of RNA were found to have been loaded when each of the lanes was probed for GPDH (Fig. 1B, lower bands) and there was no obvious effect of NGF treatment on the GPDH/total RNA ratio. To compare the time course of LI mRNA induction by NGF with the well-known morphological differentiation of these cells caused by NGF, the cultures were photographed before RNA harvest. NGF-induced neurites were readily visible after 1 d of treatment and were prominent after 5 d (Fig. 1C). Thus, the time course of NGF-induced morphological differentiation paralleled that of LI mRNA induction. Similar results were obtained when PC12 cells were cultured in serum-containing medium (data not...
Figure 1. Time course of NGF-induced changes in L1 mRNA levels. A, PC12 cells were cultured for various time intervals in hormone-supplemented chemical-defined medium with (●) or without (○) 50 ng/ml NGF and L1 mRNA levels were determined as described in Materials and Methods. L1 mRNA levels (means ± SD from four independent experiments) are expressed as the percentage of the relative intensity obtained at 0 d. Statistical differences were analyzed as compared with growth without NGF; a, p < 0.05; b, p < 0.01. B, Autoradiographs of typical Northern blots are presented in the upper panel (+NGF) and the lower panel (-NGF). C, Phase contrast micrographs illustrating typical PC12 cell morphologies at day 0, 1, and 5 following exposure to NGF (50 ng/ml). Scale bar, 25 μm. D, Western blot analysis of L1 in PC12 cells. Lane 1, PC12 cells grown for 5 d; lane 2, NGF treated PC12 cells. L1 (upper band) is indicated by a large arrow; a small arrow shows nonspecific signal (lower band). Apparent molecular weights (in kDa), determined from markers run in parallel, are indicated to the right.

To confirm that L1 polypeptide levels also increased after treatment with NGF, Western blot analysis was performed. The L1 polypeptide level had clearly increased 5 d after treatment with NGF (Fig. 1D). These results suggest that the NGF-induced increase in L1 occurs via increased transcription or stability of L1 mRNA.

To further examine whether the morphological changes induced by NGF correlate with L1 mRNA expression, varying concentrations of NGF were used to treat cell cultures. PC12 cells treated with 3 ng/ml NGF had neurites at least twice greater in length than that of the cell body at day 5 (Fig. 2). The length and density of neurites of PC12 cells appeared to be greater following treatment with a high dose (100 ng/ml) of NGF than with lower doses of NGF (Fig. 2B), while the L1 mRNA level induced by 100 ng/ml of NGF was not significantly higher than levels seen at 3 ng/ml. In summary, these experiments demonstrated that NGF induced L1 mRNA expression in PC12 cells with a time course coincident with morphological differentiation, although the dose dependencies of morphological differentiation and of L1 mRNA induction appeared to differ slightly.

L1 mRNA is specifically induced by NGF
To determine whether the induction of L1 was specific to NGF, other growth factors and agents were examined for their ability to induce L1 mRNA levels. This question is of particular interest because agents such as FGF and dbcAMP have been shown to
NGF regulation of L1 mRNA is not associated with gp140α
NGF selectively binds to and activates gp140α, a transmembrane protein tyrosine kinase (Chao, 1992). To examine whether
NGF effects on L1 expression are mediated via gp140α, we tested the effect of K252a, an inhibitor of the tyrosine kinase activity of gp140α (Berg et al., 1992). K252a (200 nM) did not inhibit NGF induction of L1 mRNA (Table 1), although this concentration completely inhibited the extension of neurites by these PC12 cells (Fig. 4). This result suggests that gp140α may not mediate the effects of NGF on expression of L1 mRNA. To further test this idea, we determined the effect of NGF on L1 mRNA levels in PC12nnr5 cells, which specifically lack gp140α. As shown in Figure 5, Northern blot analysis of RNA isolated from PC12 and PC12nnr5 cells after treatment with 0, 50, or 200 ng/ml NGF, revealed that L1 mRNA was increased 1.9-fold in PC12nnr5 cells treated with 200 ng/ml, although this treatment did not induce neurite outgrowth in PC12nnr5 cells. Together, these findings indicate that induction of L1 mRNA in PC12 cells by NGF does not require the high-affinity NGF receptor.

Levels of L1 mRNA are increased by cell–cell contact
To determine whether L1 mRNA expression is influenced by cell–cell contact, PC12 cells were plated at cell densities ranging from 0.5 to 4 × 10⁴ cells/cm² and cultured for 5 d in the absence of NGF. Within this range of cell densities, L1 mRNA levels did not increase in proportion to the increases in cell density in the absence of NGF (Fig. 6). In the presence of NGF, however, PC12 cells grown at high cell density (4 × 10⁴ cells/cm²) expressed twofold higher levels of L1 mRNA than cells grown at low cell density (5 × 10³ cells/cm²) (Fig. 6). Moreover, L1 mRNA levels in NGF-treated PC12 cells at high cell density were approximately eightfold greater than in untreated cells (Fig. 6). To test whether this effect resulted from cell–cell contact or accumulation of soluble factors, PC12 cells at low cell density were cultured in the presence or absence of medium that had been conditioned by cells growing at high cell density. As shown in Figure 7, the conditioned medium did not enhance the effect of NGF on L1 mRNA in cells at low density. To examine further whether cell–cell contact modulates NGF-induction of L1 mRNA, we tested whether homophilic L1 to L1 binding could play a role in cell–cell contact-mediated regulation of L1 mRNA expression. PC12 cells maintained at low cell density (5 × 10³ cells/cm²) with or without NGF were treated with antibody to L1 to mimic homophilic L1–L1 interaction (Schuch et al., 1989; Atashi et al., 1992; Itoh et al., 1992; von Bohlen und Halbach et al., 1992). Treatment with antibody to L1 alone did not increase L1 mRNA above the basal level, but the combination of NGF and antibody to L1 significantly increased (40%) L1 mRNA levels above the level induced by NGF alone (data not shown). In contrast, treatment with antibodies to N-CAM had no effect on NGF induction of L1 mRNA. These observations suggest that homophilic L1–L1 binding may be one of the mechanisms by which cell–cell contact influences the ability of NGF to increase L1 mRNA levels.

Cell–extracellular matrix contact does not regulate expression of L1 mRNA
To assess whether cell–extracellular matrix contact could also influence L1 mRNA levels, PC12 cells were plated on different substrates (BSA, PL, COL, LN, and FN) in the presence or absence of NGF and L1 mRNA expression was assessed. In the absence of NGF, no increase in L1 mRNA was observed when PC12 cells were cultured for 5 d on any of the extracellular matrix components. When cultured with NGF, the increase in

![Figure 2. The effect of various doses of NGF on expression of L1 mRNA. L1 mRNA levels were determined as described in Materials and Methods. The upper panel shows L1 mRNA levels in PC12 cells cultured for 5 d in the presence of 0–100 ng/ml NGF. L1 mRNA levels (means ± SD from five independent experiments) are expressed as a percentage of the 0 ng/ml NGF-treated culture. The bottom panel shows phase contrast micrographs of PC12 cell morphology after 5 d treatment with NGF (A, 0 ng/ml; B, 1 ng/ml; C, 3 ng/ml; D, 100 ng/ml). Scale bar, 50 μm.](image-url)

induce morphological differentiation of PC12 cells (Gunning et al., 1981; Rydel and Greene, 1987). Treatment for 5 d with FGF, EGF, TGFβ, or dbcAMP did not cause any significant change in L1 mRNA expression in PC12 cells (Fig. 3), although FGF and dbcAMP promoted neurite outgrowth as effectively as NGF (data not shown). These results indicated that, although the neurite extension induced by FGF and dbcAMP was morphologically indistinguishable from that induced by NGF, L1 mRNA was induced only by NGF treatment. Thus, L1 mRNA is specifically induced by NGF treatment and is not an obligatory consequence of morphological differentiation of the cells.
L1 mRNA (approximately threefold) was similar on all substrates (Fig. 8). Furthermore, L1 mRNA levels also increased approximately three- to fourfold following exposure to NGF when PC12 cells were grown in suspension, to prevent cell–substrate adhesion and neurite extension (data not shown). In conclusion, these results imply that, for the substrates tested, cell–substrate adhesion does not significantly influence the expression of L1 mRNA.

Discussion

In the present study, we have established that L1 mRNA levels in PC12 cells are specifically enhanced by NGF, leading to increased expression of L1 at the cell surface. Strikingly, our findings suggest that the effect of NGF on L1 expression is not mediated by the high-affinity NGF receptor, gp140*.

These NGF-induced changes in L1 expression were facilitated by cell–cell interactions, but were not affected by the interactions of PC12 cells with several extracellular matrix components.

Table 1. Effect of K252a on L1 mRNA levels in PC12 cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>L1 mRNA (%) of control</th>
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<tbody>
<tr>
<td>None</td>
<td></td>
<td>100 ± 12</td>
</tr>
<tr>
<td>NGF</td>
<td>4 nM</td>
<td>343 ± 36*</td>
</tr>
<tr>
<td>NGF + K252a</td>
<td>4 nM + 200 nM</td>
<td>345 ± 55*</td>
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The levels of L1 mRNA were determined by Northern blot analysis (see Materials and Methods). The values represent the means ± SD of three or more determinations.

* p < 0.01, None versus NGF
* Not significant, NGF versus NGF + K252a.

NGF influences both the levels of protein and mRNA for several neuron-related proteins in PC12 cells including NCAM, Thy-1, and neurofilaments (Doherty et al., 1987; Prentice et al., 1987; Lindenbaum et al., 1988). In contrast, recent studies suggest that mRNA levels for L1 do not change following exposure to NGF in PC12 cells, although L1 polypeptide levels increase (Sajovic et al., 1987; Prince et al., 1989; Miura et al., 1991). We have reexamined this potential discrepancy by determining the kinetics of expression of L1 mRNA and polypeptide in the continual presence of NGF using Northern and Western blot analyses, respectively. The level of L1 mRNA increased slowly over the 5 d period of NGF treatment and by then the amount of L1 protein was sufficiently higher than in nontreated cells to be readily detectable in Western blotting. After 5 d treatment with NGF, the level of L1 mRNA declined slightly; previous studies determined this level at 7 d, so that the difference between NGF-treated and untreated cultures was not as large as the maximal difference we saw. This lesser difference may have been difficult to detect without accurate internal controls for RNA loading, such as were performed in our study. The timing of the increase in L1 mRNA suggests that L1, like NCAM (Prentice et al., 1987), is a member of the class of late genes that are induced only after several hours to days of NGF treatment.

Several of the studies reported here bear on the mechanism by which NGF affects L1 expression. The high-affinity NGF receptor has recently been identified as gp140* (Kaplan et al., 1991a,b). NGF binds directly to homodimer gp140*, activating its tyrosine kinase activity (Jiug et al., 1992). gp140* is phosphorylated in a dose-dependent manner in the range of 0.1–100 ng/ml NGF. Increasing the concentration of NGF effectively induces not only the phosphorylation of gp140*, but also neuronal
Figure 4. Effect of K252a on morphology of NGF-treated PC12 cells. PC12 cells were cultured for 5 d in the absence (A) and the presence of NGF (50 ng/ml) (B) or in the presence of NGF plus K252a (200 nM) (C). Scale bar, 50 μm.

Several of the results support the idea that NGF might affect morphological differentiation and L1 mRNA levels via distinct mechanisms. The observation that NGF increased L1 mRNA expression in suspension-cultured PC12 cells, which do not extend neurites, suggests that NGF does not affect L1 gene expression by the same mechanism that mediates its effects on morphological differentiation of PC12 cells, namely binding to gp140<sup>trk</sup>. This suggestion was strongly supported by the finding that K252a, a specific inhibitor of gp140<sup>trk</sup> tyrosine kinase activity, which completely blocks NGF-induced neuronal differentiation (Koizumi et al., 1988; Berg et al., 1992), has no effect on NGF induction of L1 mRNA. Most important, L1 mRNA was induced by NGF in PC12nnr5 cells, which lack gp140<sup>trk</sup>, although the dose of NGF required was higher in PC12nnr5 cells. Higher doses may have been required because the level of low-affinity NGF receptor in these cells is only 20–25% that of the parental PC12 line (Green et al., 1986). An even more intriguing possibility relates to recent observations that the high- and low-affinity NGF receptors interact (Hempstead et al., 1991; Verdi et al., 1994). These observations raise the possibility that binding of NGF to a heterodimer of high- and low-affinity receptors triggers dual responses, one involving the gp140<sup>trk</sup> tyrosine kinase and one proceeding from the p75<sup>LNGFR</sup> low-affinity receptor. In this view, the absence of these interactions in PC12nnr5 cells would contribute to the requirement for a higher dose of NGF. These findings are also consistent with the observation that L1

Figure 5. Effect of NGF on L1 mRNA in PC12nnr5 cells. A, PC12 and PC12nnr5 cells were cultured for 5 d in the presence of NGF (0, 50, and 200 ng/ml). The results (means ± SD from three to five independent experiments) were expressed as the percentage of untreated cultures. Statistical differences were analyzed as compared with growth without NGF in PC12 cells: a, not significant; d, p < 0.05; and in PC12nnr5 cells: b, not significant; c, p < 0.01. B, Autoradiographs of typical Northern blots showing L1 mRNA (upper band) and GPDH mRNA (lower band).
expression in Schwann cells is increased by NGF even though these cells express only the low-affinity NGF receptor, p75NGFR (Heumann et al., 1987; Seilheimer and Schachner, 1987; Johnson et al., 1988).

Taken together, these results strongly indicate that NGF induces L1 mRNA by a novel mechanism. By implication, it appears that the effect of NGF on L1 expression is mediated by the low-affinity NGF receptor (p75NGFR), although we have no evidence that this is the case. The observation that NGF binding to p75NGFR does not induce neurite outgrowth (Green et al., 1986; Loeb et al., 1991) is consistent with this suggestion, but further studies will be necessary to obtain a more complete understanding of the mechanisms involved.

The present study also indicated that the NGF-induced increase in L1 mRNA in PC12 cells is modulated by cell-cell interactions. The primary evidence supporting this conclusion was the observation that NGF induction of L1 mRNA was cell density-dependent when PC12 cells were cultured at different densities in the presence of NGF (Fig. 6). The cell density-dependence could be due to cell-cell contacts or to accumulation of autocrine factors. For example, an autocrine factor and a serum factor appear to be responsible for the cell-density-dependent

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**Figure 6.** Effects of cell density on L1 mRNA levels in PC12 cells. A, PC12 cells were cultured for 5 d at various densities (0.5-4 × 10^4 cells/cm^2) in the presence (left) or absence (right) of NGF (50 ng/ml). The results (means ± SD from four independent experiments) were expressed as the fold increase relative to the values measured in cells cultured at 0.5 × 10^3 cells/cm^2 in the absence of NGF. Statistical differences were analyzed as compared with growth at 0.5 × 10^3 cells/cm^2 in the absence of NGF (a, no significance), and with growth at 0.5 × 10^3 cells/cm^2 in the presence of NGF (b, p < 0.05; c, p < 0.01). B, Autoradiographs of typical Northern blots showing L1 mRNA (upper band) and GPDH mRNA (lower band). RNA was extracted from cells cultured in the absence (lanes 1-4) or presence (lanes 5-8) of NGF at densities of 0.5 × 10^3 cells/cm^2 (lanes 1 and 5), 1 × 10^3 cells/cm^2 (lanes 2 and 6), 2 × 10^3 cells/cm^2 (lanes 3 and 7), and 4 × 10^3 cells/cm^2 (lanes 4 and 8).

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**Figure 7.** Effect of PC12 cell-conditioned medium on NGF stimulation of L1 mRNA levels in PC12 cells. The figure shows autoradiographs of typical Northern blots with L1 mRNA (upper band) and GPDH mRNA (lower band). Lanes contained RNA from cells cultured without additions (lane 1), with conditioned medium alone (lane 2), 50 ng/ml NGF plus conditioned medium (lane 3), or 50 ng/ml NGF alone (lane 4).

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**Figure 8.** Effects of various extracellular matrix components on NGF induction of L1 mRNA. A, PC12 cells were cultured on collagen (COL, 50 μg/ml), BSA (50 μg/ml), poly-L-lysine (PL, 50 μg/ml), laminin (LN, 50 μg/ml), or fibronectin (FN, 50 μg/ml) for 5 d in the presence (right) or absence (left) of NGF (50 ng/ml). L1 mRNA levels (means ± SD from four independent experiments) were expressed as the ratio of intensity values of L1 and GPDH.
expression of NCAM in N2a cells (Roubin et al., 1990). In the present experiments, however, conditioned serum-containing medium did not enhance NGF stimulation of L1 mRNA levels, suggesting that density-dependent effects on NGF induction of L1 mRNA are not mediated by autocrine factors and serum, but by cell–cell contact. Additional supporting evidence came from the observation that antibodies to L1 (presumed to mimic L1–L1 homophilic binding) enhanced the effect of NGF on PC12 cells cultured at low cell density. Previous studies have shown that homophilic interactions between L1 molecules trigger changes in intracellular calcium and phospholipid hydrolysis (Schuch et al., 1989; von Bohlen and Halbach et al., 1992; Itoh et al., 1992), which could lead to alterations in L1 gene expression. Finally, in contrast to the effect of cell–cell contact on L1 mRNA expression, our measurements of L1 mRNA levels in PC12 cells cultured on substrata coated with different extracellular matrix components suggested that the effect of NGF on the increase in L1 mRNA expression is not dramatically influenced by cell–substrate interactions.

These findings have some implications for the roles of NGF and L1 during neuronal recovery after injury in the PNS. L1 is known to increase at cell contact sites between growth cones and fasciculating axons upon transecting or crushing the sciatic nerve (Martini and Schachner, 1988). In addition, L1 expression in the PNS is regulated by NGF (Seilheimer and Schachner, 1987). The present results suggest that NGF produced by nerve injuries could stimulate L1 expression via the low-affinity NGF receptor at nerve injury sites. This expression could be further enhanced by L1 interactions between cells of the PNS. These mechanisms may serve to promote the axonal reextension during regeneration.

In summary, the present studies show that NGF stimulates L1 mRNA accumulation in PC12 cells and that this induction is modulated by cell–cell contact, including L1 homophilic binding, but not by cell–substrate interaction. A particularly interesting issue raised in this study is the suggestion, from several lines of evidence, that the effect of NGF on L1 expression is not mediated by the high-affinity NGF receptor. By implication, the low-affinity NGF receptor, which has no known physiological function, may be involved. Testing this possibility may open new avenues for investigating the functions of NGF in neuronal differentiation and regeneration.

References
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