Cell–cell interactions modulate the responsiveness of PC12 cells to nerve growth factor

PATRICK DOHERTY, DEREK A. MANN and FRANK S. WALSH

Institute of Neurology, Queen Square, London WC1N 3BG, UK

Summary

The growth of PC12 cells on a collagen substratum or on monolayers of several non-neuronal cell types was studied by measuring nerve growth factor (NGF)-dependent increases in the expression of a 150×10^3 (Mr) neurofilament protein subunit and the membrane glycoprotein Thy-1. Both responses were found to be greatly suppressed in cultures of fibroblasts as compared to the C2 and G8-1 muscle cell lines and the C6 glioma cell line. This suppression was associated with an inhibition of NGF-dependent neuritic outgrowth from PC12 cells grown on fibroblast monolayers. There was no evidence that fibroblasts secrete soluble molecules that directly inhibit these responses or neutralize NGF. In addition, there was no difference in the neurofilament protein response from PC12 cells that had been treated with NGF prior to coculture, and the now primed PC12 cells readily extended axons over fibroblast monolayers. These data demonstrate that cell–cell and/or cell–matrix interactions can modulate biochemical responses to NGF and suggest that responsiveness of neuronal cells to environmental cues is not immutable. Control of the latter may be at the level of expression of receptor molecules for cell-surface- or matrix-associated macromolecules and a similar mechanism operating during development could play a role in growth cone guidance.

Key words: nerve growth factor, PC12 cells, neurite outgrowth, cell–cell interactions.

Introduction

Although little is known about the molecular nature of the components that operate to ensure correct innervation pattern formation during development, regulatory mechanisms have been suggested to include the availability of soluble neuronotrophic molecules such as nerve growth factor (NGF) (Thoenen & Barde, 1980), as well as spatiotemporal changes in the expression of cell surface and matrix components in apposition to the growth cone (Jacobson, 1978; Letourneau, 1982; Goodman et al. 1984; Edelman, 1984).

When bound to tissue culture plastic, relatively common extracellular matrix molecules such as fibronectin (FN) (Rogers et al. 1983), laminin (LN) (Manthorpe et al. 1983) and a LN–heparin sulphate proteoglycan complex (HeSPG) (Lander, Fujii & Reichardt, 1985) can all promote vigorous neurite outgrowth by both peripheral and central neurones. Two independent McAbs, CSAT and JG22, that react with a set of glycoproteins exhibiting functional properties of a LN and FN receptor (Horwitz et al. 1985) have been shown to inhibit neurite outgrowth on a variety of extracellular matrix substrata, including the LN–HeSPG complex (Tomaselli, Reichardt & Bixby, 1986). In the same study, both McAbs were reported to block neurite outgrowth on monolayer cultures of intact fibroblasts, but not astrocytes, Schwann cells or skeletal muscle. Both central (Noble, Fok-Seang & Cohen, 1984) and peripheral (Fallon, 1985) neurones show preferential growth on cultured astrocyte monolayers as compared with fibroblasts, even though cell surface LN was found in association with the latter and not the former (Fallon, 1985). These observations suggest the existence of a second and perhaps more specialized cell-membrane-associated recognition pathway operating independently of the LN/FN receptor and the LN–HeSPG complex. Consistent with this contention, it has
recently been shown that a cocktail of antibodies to various cell adhesion molecules, including the putative receptor for LN and FN as well as the neural cell adhesion molecule (N-CAM), are required to inhibit neurite outgrowth from chick ciliary ganglion neurones grown on muscle monolayers (Bixby, Pratt, Lilien & Reichardt, 1987).

The rat pheochromocytoma cell line, PC12 (Greene & Tischler, 1976), is useful for studies on the mechanism of neurite outgrowth as these cells do not require exogenous trophic support for survival and may undergo either generation (naïve cells) or regeneration (primed cells) of neurites in response to NGF (Greene, Burstein & Black, 1982; Greene, 1984). An additional advantage of PC12 cells is that, unlike most primary neurones that are available for culture, they may be used to study steps whereby a classical growth factor (NGF) stimulates neurotogenesis from cells previously unexposed to detectable levels of this factor.

We have developed enzyme-linked immunoadsorbent assays (ELISA) to index two independent and complementary NGF-dependent responses in PC12 cells. We have shown up to 30-fold increases in the expression of a neurofilament protein antigen, recognized by a McAb-coded RT97 (Wood & Anderson, 1981), to be associated with NGF-induced morphological differentiation of both naïve and primed PC12 cells (Doherty, Mann & Walsh, 1987). In contrast, the NGF induction of the Thy-1 glycoprotein (Morris, 1985), which can also readily be measured by ELISA assay, is a response that is not directly associated with morphological differentiation (Doherty & Walsh, 1987a).

In the present study, we have determined the ability of differing non-neuronal cellular monolayers to modulate both of these responses. The relationship between the induction of both antigens and the morphological differentiation status of individual PC12 cells has been evaluated. The results obtained suggest that, whereas PC12 cells may constitutively express receptors for the specialized adhesion molecules associated with muscle and glioma, the expression of receptors for common adhesive molecules such as FN may be controlled by extrinsic factors including target-derived growth factors. Similar control during development may be important in guiding axons to their target tissues.

**Materials and methods**

**Cell culture**

PC12 cells were grown in SATO media (Bottenstein, 1985) consisting of Dulbecco’s modified Eagle’s medium (DMEM) supplemented with (in mg l^{-1}) progesterone, 0.062; putrescine, 16.1; thyroxine, 0.4; selenium, 0.039; transferrin (human), 100; insulin (bovine pancreas), 10; triiodothyronine, 0.377. (All reagents from Sigma Chemical Company). The medium was further supplemented at 1 % (v/v) with Path-o-ocyte 4 (bovine serum albumin solution from Miles Chemical Company). For subculture of naïve cells and replating of primed cells (see below), cultures grown on collagen-coated tissue culture plates were washed once with versene solution (Gibco) followed by incubation for 5–7 min with 0.05 % (w/v) trypsin in the same buffer. Detached cells were centrifuged and resuspended by trituration with a pipette followed by a 19-gauge syringe needle. For priming, approximately 4×10^5 cells were plated onto a collagen-coated 100 mm tissue culture dish in media supplemented with 50 ng ml^{-1} NGF. The B-subunit of 7S NGF was a generous gift from Dr A. Leon (Fidia Research Laboratories, Abano Terme, Italy). Fresh NGF was added to cultures at 3-day intervals.

Monolayer cultures of G8-1 (Christian, Nelson, Peacock & Nirenberg, 1977) and C2 (Yaffe & Saxel, 1978) myotubes were established as previously described (Walsh & Phillips, 1981) in individual wells of 96-well microtitre plates. C6 glioma (Benda et al., 1986) and human skin fibroblasts were grown in microwells in DMEM supplemented with 10 % FCS until confluent.

Cocultures were established by seeding 20000 PC12 cells onto monolayers of the above cell types. For immunofluorescence, 50000 PC12 cells were seeded onto monolayers grown in a 35 mm culture dish. In some experiments, PC12 cells were seeded into individual wells on a collagen-coated microtitre plate. Cocultures were maintained for up to 7 days in serum-containing media (5 % horse serum and 2.5 % foetal calf serum) or alternatively for up to 3 days in defined media (see above). Monolayers were washed twice with DMEM and a further two times with the respective coculture media prior to the addition of PC12 cells.

**Conditioned media preparation**

SATO medium was conditioned over confluent cultures of skin fibroblasts and C2 myotubes for 1 or 4 days. The conditioned medium was collected, filtered through a 0.2-μm filter to remove debris and added directly to PC12 cell cultures. In addition, DMEM was conditioned over G8-1 myotubes and C6 glioma monolayers for 24 h, concentrated approximately 10-fold by ultrafiltration as described previously (Doherty, Dickson, Flanagan & Walsh, 1986a) and then added to PC12 cell cultures at up to 50 % (v/v).

**Immunohistochemical analysis of neurofilament and Thy-1 levels**

Cultures were fixed by a 50 % medium exchange with 4 % paraformaldehyde in DMEM for 60 min at 20°C, followed by a total medium exchange for a further 60 min. Cells were permeabilized by treatment with methanol (−20°C) for a further 15 min. Cultures were then washed three times with phosphate-buffered saline (PBS) and the relative level of neurofilament protein antigen or Thy-1 protein determined essentially as previously described (Doherty, Dickson, Flanagan & Walsh, 1984a, b; Doherty & Walsh, 1987a; Doherty et al. 1987). Briefly, following incubation with PBS containing 0.5 % gelatin to block nonspecific protein-binding sites, cultures were incubated at 60 min at 20°C.
with a 1:1500 dilution of McAb RT97 ascites or alternatively a 1:50 dilution of McAb OX7 tissue culture supernatant. The latter was a generous gift from Dr Neil Barclay. Both reagents were in PBS containing 1 mg ml⁻¹ bovine serum albumin (BSA). Cultures were washed three times with PBS/BSA and incubated with a 1:1000 dilution of horseradish peroxidase-conjugated anti-mouse immunoglobulin for a further 60 min. Cultures were then washed four times with PBS, twice with distilled H₂O₂, and finally incubated with 50 μl of 0.2 % (w/v) o-phenylenediamine (OPD) and 0.02 % (v/v) H₂O in citrate buffer. After 10–20 min the conversion of OPD to its oxidized product was stopped by the addition of 50 μl of H₂SO₄ and optical density (O.D.) determined at 492 nm using a Flow Titretek Multiscan apparatus.

**Indirect immunofluorescence**

Indirect immunofluorescence on both live and fixed cultures was as previously described (Moore & Walsh, 1985; Doherty et al. 1987). The OX7 McAb was used at a 1:50 dilution and RT97 at a 1:1000 dilution.

**Results**

**NGF-induced neurofilament protein responses in cocultures**

McAb RT97 shows very little binding to undifferentiated PC12 cells, with up to 30-fold increases in binding associated with morphological differentiation induced by NGF (Doherty et al. 1987). Fig. 1 shows localization of McAb RT97 immunoreactivity in a typical culture of NGF-treated PC12 cells using indirect immunofluorescence. Staining is apparent, sparse in the cell body but intense along the full length of growing axons. Immunoreactivity can also be found associated with the growth cones. In NGF-treated PC12 cells, immunoblot analysis shows McAb RT97 to bind specifically to the 150×10⁵ (M₀) subunit of neurofilament protein (Doherty et al. 1987).

The effect of the cellular environment on this NGF-dependent response was determined by seeding PC12 cells onto confluent monolayer cultures of either C6 glioma, G8-1 skeletal myotubes or human skin fibroblasts. Fig. 2 shows the relative level of RT97 binding to cultures maintained in serum-containing growth medium for 7 days. In the absence of exogenous NGF, significant time-dependent increases in McAb RT97 binding are found only in C6 glioma cocultures and not muscle or fibroblast cocultures. In serum-containing, but not defined, media, C6 has previously been shown to secrete an NGF-like activity (Westerman & Unsicker, 1986). NGF-dependent increases in McAb RT97 binding to PC12 cells are apparent by day 5 in both muscle and glioma, but not the fibroblast cocultures. By day 7, there was a tenfold and fivefold greater binding of RT97 to NGF-treated PC12 cells grown on monolayers of glioma and muscle, respectively, as compared to fibroblasts.

Morphological differentiation induced by NGF occurs more rapidly in defined, as compared to serum-containing, media (P. Doherty, unpublished data). Fig. 3 shows the effect of differing concentrations of NGF on the binding of McAb RT97 to PC12 cells grown for 3 days on a collagen-coated substratum or alternatively on monolayer cultures of C2 muscle cells or skin fibroblasts. There were 3-5- and 3-0-fold greater neurofilament protein responses for cells grown on collagen or C2 muscle, respectively, as compared to fibroblast monolayers. An examination of the dose-response curve clearly shows responsiveness rather than sensitivity to NGF reduced in the fibroblast coculture.

The morphology of PC12 cells cultured on monolayers of C2 muscle cells and skin fibroblasts was highlighted by staining live cultures with the rat-

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**Fig. 1.** Anti-neurofilament immunostaining of PC12 cells. PC12 cells were primed with NGF (50 ng ml⁻¹) for 5 days, divested of their neuritic network and replated onto a collagen-coated 35 mm tissue culture plate in the presence of NGF (50 ng ml⁻¹) for a further 3-day period before being fixed and stained with RT97. Neurofilaments can be seen to be localized to axonal processes with immunoreactivity present at the growth cone. Scale bar is 40 μm.
specific McAb OX7 reactive with Thy-1 antigen. After 3 days of culture in SATO media, there is relatively little, if any, sign of neuritic outgrowth from cells grown in the absence of NGF (Fig. 4A, B, E, F). In the presence of NGF, there was a definite neuritic response from the vast majority of PC12 cells cultured on muscle cells (Fig. 4G, H). PC12 cells displayed relatively long and highly branched axonal processes with highly differentiated growth cones localized to the surface of the muscle. McAb RT97 binding was again localized to neurofilaments present within axonal processes (data not shown). In contrast, PC12 cells showed no sign of morphological differentiation when cultured in the presence of NGF on fibroblast monolayers (Fig. 4C, D).

**Effect of 'priming' on NGF-induced responses in coculture**

Cells primed by NGF over 5–7 days and subsequently divested of their neuritic network show a much more rapid formation of the network and accumulation of neurofilament protein antigen on re-exposure to NGF than naive cells (Doherty et al. 1987). Fig. 5 shows two examples of the neurofilament protein response for primed cells grown on monolayers of skin fibroblasts as compared with C6 glioma or fibroblasts compared with C2 muscle and collagen. In the former case, cultures were maintained in serum-containing media; in the latter, they were grown in defined media. In both examples, there was essentially no binding of McAb RT97 to PC12 cells in
Fig. 4. PC12 cells were grown for 3 days on monolayer cultures of skin fibroblasts and C2 muscle. PC12 cell morphology for the respective cultures was highlighted by staining live cultures with the OX7 McAb. The cultures were fixed with methanol and viewed under phase-contrast and epifluorescence optics. The micrographs show representative fields for PC12 cells grown in the absence of NGF on fibroblasts (A,B) and muscle (E,F), and in the presence of NGF on fibroblasts (C,D) and muscle (G,H). The scale bar represents 40 μm.
cultures grown in the absence of exogenous NGF. However, substantial NGF-dose-dependent increases in McAb RT97 binding were apparent after a 48 h period of coculture. In contrast to the results obtained with naïve cells, the nature of the non-neuronal cellular monolayer had very little effect on the relative increase in the expression of the neurofilament protein antigen induced by both submaximal and maximal concentrations of NGF. Again, in contrast to naïve cells, primed cells showed an extensive NGF-dependent neuritic outgrowth on fibroblast monolayers. This was clearly seen in cultures of both live unstained cells grown in microwells (Fig. 6A,C) and also low-density cell cultures stained with the McAb OX7 (Fig. 6B,D). On C2 monolayers, the morphological response was qualitatively similar for primed and naïve PC12 cells. There was essentially no PC12 cell differentiation in cultures grown in the absence of NGF (Fig. 6E,F) with an extensive neurite outgrowth apparent from the vast majority of cells grown in the presence of NGF (Fig. 6G,H). The morphological observation for cultures of naïve and primed PC12 cells further supports the contention that the relative expression of the neurofilament protein antigen recognized by RT97 is directly related to the degree of neuritic outgrowth induced by NGF.

NGF-induced increases in Thy-1 antigen in coculture

In cultures of naïve PC12 cells, NGF can induce accumulation of the mRNA species encoding Thy-1 (Dickson et al. 1986). NGF-induced accumulation of the Thy-1 glycoprotein (Richter-Landsberg, Greene & Shelanski, 1985) can be conveniently measured by ELISA assay (Doherty & Walsh, 1987a). The OX7 McAb reacts specifically with rat Thy-1 (Mason & Williams, 1980) and we have taken advantage of this to quantify the relative expression of antigen in cultures of PC12 cells grown for 3 days on monolayers of human skin fibroblasts and C2 muscle cells. PC12 cells grown on a collagen substratum served as a positive control. Fig. 7 shows the relative level of expression of Thy-1 antigen as a function of NGF.

Fig. 5. PC12 cells were primed for 5 days with NGF (50 ng/ml) before being subcultured onto monolayers of C6 glioma and skin fibroblasts in one experiment or monolayers of C2 myotubes, skin fibroblasts and a collagen substratum in a second independent experiment. In the first experiment, cocultures were grown in serum-containing medium and in the second in defined medium. After 48 h the NGF-dependent increases in RT97 binding were determined as in Fig. 3. Each value is the mean ± S.E. of four independent determinations. In the first experiment, 100 arbitrary units = 0.263 O.D. units and in the second, 0.138 O.D. units.
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Fig. 6. PC12 cells were primed as in Fig. 5 and subcultured onto monolayers of fibroblasts or C2 muscle for 48 h. Phase-contrast micrographs show representative fields of live cocultures of PC12 cells grown in the absence (A) and presence (B) of NGF on fibroblast monolayers. Representative fields of these cocultures stained with OX7 McAb and fixed before viewing with epifluorescence optics are shown in B (—NGF) and D (+NGF). Phase-contrast and epifluorescent micrographs of OX7-stained PC12 cells grown on C2 monolayers are shown for cells grown in the absence (E,F) and presence (G,H) of NGF.
Fig. 7. Naïve PC12 cells were seeded onto a collagen substratum or fibroblast monolayer and grown for 3 days in media supplemented with up to 20 ng ml⁻¹ NGF. For each datum point, OX7 McAb binding was determined for three independent cocultures with nonspecific binding to sister monolayer cultures subtracted. The results show the mean ± S.E. of three independent determinations. Nonspecific binding of OX7 McAb to fibroblasts was measured as 0.06 ± 0.003 O.D. units, and this did not vary as a function of NGF concentration.

Concentration for PC12 cells grown on collagen as compared to skin fibroblasts.

As previously reported, NGF induces a two- to threefold increase in Thy-1 antigen when PC12 cells are grown on a collagen substratum. NGF is antimitotic for PC12 cells and this increased antigen expression reflects a response at the cellular level rather than an increase in cell number (Doherty & Walsh, 1987a). In the absence of NGF, there was no difference in Thy-1 expression for PC12 cells grown in coculture with fibroblasts as compared to those on a collagen substratum. This is consistent with the morphological observations that fibroblasts are not toxic to PC12 cells. However, a total suppression of the NGF-induced increase in Thy-1 was apparent in the fibroblast cocultures.

In the absence of NGF and after the same 3-day period of culture, there was a twofold increase in Thy-1 expression by PC12 cells grown on C2 muscle cell monolayers as compared to collagen (Fig. 8). The addition of a maximally active concentration of NGF to the muscle coculture increased the expression of Thy-1 by only a further 20%. The level of Thy-1 antigen expression in NGF-treated cultures did not differ for cells grown on collagen as compared to C2 muscle cell monolayers. These data suggest that coculture with C2 muscle cells can directly induce increased expression of Thy-1 at the cellular level and that this response is not additive with that induced by NGF.

Fig. 8. Naïve PC12 cells were seeded onto a collagen substratum or C2 monolayers and cultures grown in the presence and absence of NGF (11.5 ng ml⁻¹) for 3 days. OX7 binding was determined as in Fig. 7. The results show the absolute binding to PC12 cells with binding to the monolayer subtracted. The latter was measured as 0.06 ± 0.004 O.D. units. Bars show ±1 S.E.

Effects of conditioned media from non-neuronal cells on neurofilament and Thy-1 responses

SATO medium conditioned over skin fibroblasts and C2 myotubes for 1 or 4 days was added at up to 50 % (v/v) to naïve PC12 cells grown on a collagen-coated substratum. After three days, the binding of RT97 and Thy-1 was determined from cells grown in the presence and absence of NGF (50 ng ml⁻¹). The conditioned medium was without effect on both the basal and the NGF-induced increases in neurofilament protein and Thy-1 glycoprotein expression (data not shown). Similarly, DMEM conditioned over G8-1 myoblasts and myotubes or C6 glioma for 24 h and concentrated up to tenfold was found to be without effect on the binding of RT97 to naïve and primed PC12 cells grown for 3 days on collagen. The medium conditioned with G8-1 has previously been shown to contain an activity that increases survival of spinal

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containing naive PC12 cells grown on a collagen cell-HA, 0-45\(\mu\)m diameter) were carefully placed from other homogenous cell populations.

To simplify interpretation, we have studied differentiation of the naive PC12 cells and the increased expression of neurofilament protein induced by exogenously added NGF.

**Discussion**

The polypeptide hormone, NGF, has been shown to have wide-ranging and diverse effects throughout the peripheral and central nervous system, and has more recently been implicated as a modulator of the immune system (Levi-Montalcini & Calissano, 1986). In the present study, we have determined the effect of the cellular environment of three potentially related NGF responses, namely (1) the induction of neurofilament protein, (2) Thy-1 antigen and (3) neurite outgrowth. To simplify interpretation, we have studied differentiation of a well-characterized NGF-responsive cell line cultured on monolayers derived from other homogenous cell populations.

NGF-induced neurite outgrowth is closely associated with the accumulation of neurofilament protein antigen recognized by the McAb RT97. The logic, justification and advantages of primarily quantifying the neurofilament protein response rather than conventional morphological indices of neuronal differentiation have been outlined (Doherty et al. 1984a,b; Doherty et al. 1987; Doherty & Walsh, 1987). The most conspicuous observation of the present study was the relative failure of NGF to induce an increased expression of the 150\(\times\)10\(^3\) (Mr) neurofilament subunit when naive PC12 cells were cultured on a fibroblast monolayer. This was associated with a total suppression of neurite outgrowth. The differential neurofilament protein response was made relative to C6 glioma and two independent muscle cell lines under two differing culture conditions (serum-containing and defined media). In the case of C6 glioma, the release of endogenous trophic molecules may partially account for a tenfold greater neurofilament protein response from PC12 cells grown over 7 days in growth medium containing NGF and serum. However, there was no evidence for the release of similar molecules from G8-1 or C2 muscle cultures, with no induction of neurofilament protein or morphological differentiation of PC12 cells apparent in cocultures maintained in the absence of exogenous NGF. Primed PC12 cells readily extended axons over the fibroblast monolayers and medium conditioned over fibroblasts was ineffective in modulating NGF-dependent responses from PC12 cells grown on a collagen substratum. Thus, the relative inhibition of NGF responses from naïve PC12 cells cannot be attributed to an inherent disability of fibroblasts to support neurite outgrowth, or to the release of soluble components that are toxic to PC12 cells. Similarly, the evidence is not consistent with fibroblasts directly neutralizing NGF.

As has been suggested by others (see Introduction), differential growth may simply be explained by muscle serving as a more adhesive substratum for PC12 growth cones than fibroblasts. Candidate adhesive molecules present on the former, but not the latter, have been shown to include N-CAM. Antibodies to N-CAM have previously been reported to disrupt adhesion of nerve to muscle (Rutishauser, Grumet & Edelman, 1983); however, others failed to find inhibition of neurite outgrowth by the sole addition of N-CAM antibodies (Tomaselli et al. 1986). Inhibition of chick ciliary ganglion neurone outgrowth on muscle monolayers was only seen when a combination of antibodies against specialized adhesive molecules including N-CAM, as well as antibodies to the putative receptor for the common adhesive molecules FN and LN are added to cultures (Bixby et al. 1987). One possibility is that naïve PC12 cells constitutively express receptors molecules for specialized adhesion molecules, but not for common adhesive molecules such as FN. In support, we have previously shown naïve PC12 cells to express N-CAM at their cell surface, together with a very rapid increase in this expression in response to NGF treatment (Prentice et al. 1987). It has also been observed that naïve PC12 cells show poor adhesion to fibronectin and that the latter does not support initial neurite outgrowth from these cells (P. Doherty & F. S. Walsh, unpublished data; Fujii, Massoglia, Savion & Gospodarowicz, 1982; Vlodavsky et al. 1982). An alternative possibility that we do not exclude is that, in addition to expressing adhesive molecules that promote neurite outgrowth, fibroblasts and possibly other cell types may express a second class of cell-surface- or matrix-associated proteins that can inhibit neurite outgrowth.

In the present study, we have shown that coculture of PC12 cells with C2 muscle cells can directly induce an increased expression of the Thy-1 antigen independently of overt morphological differentiation. We have also found that activation of protein kinase C by phorbol esters can also mimic the transcription-dependent induction of Thy-1 by NGF independently of morphological differentiation (Doherty & Walsh, 1987b). The demonstration that induction of Thy-1

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neurones (Doherty, Dickson, Flanigan, Kennedy & Walsh, 1986b).

To determine further if fibroblasts directly neutralize NGF or secrete neurotoxic components, confluent monolayers stabilized on cellulose membranes (Millipore, 0.45\mu m diameter) were carefully placed into individual wells of a 24-well tissue culture dish containing naïve PC12 cells grown on a collagen substratum. Under these conditions and over a 5-day period, fibroblasts failed to inhibit both the morphological differentiation of the naïve PC12 cells and the increased expression of neurofilament protein induced by exogenously added NGF.
is also suppressed in PC12–fibroblast cocultures suggests that, rather than solely altering growth cone stability, the modulatory interactions between PC12 and other cell types may also be operative at the much earlier level of transduction of the NGF signal.

In contrast to naïve cells, primed PC12 cells show no initial difference in their preference for substrate. Extensive NGF-dependent axonal outgrowth was readily apparent in fibroblast cocultures, with immunostaining used to highlight extensive differentiation from individual PC12 cells. Morphological differentiation was again associated with an induction of the neurofilament protein antigen and there was little difference in the magnitude of this response and its sensitivity to NGF for primed cells grown on fibroblasts as compared with C6 glioma, C2 muscle or collagen substratum. There are several possibilities that could account for the differing behaviour of naïve and primed PC12 cells. The most likely is that NGF can induce an increased expression of receptors for common adhesive molecules such as FN; however, we cannot yet exclude the possibility that NGF may induce a down-regulation of receptors for inhibitory molecules. Both possibilities are currently under investigation. During development, neurones have to make critical decisions as to when to enter a 'common' or 'public' pathway, and similar choices as to when to exit that pathway. Changes in the level of the expression of receptor molecules for common adhesive molecules may be of crucial importance in determining such choices.

In conclusion, the results of the present study have shown PC12 cell responsiveness to NGF to be critically dependent on the nature of the local micro-environment. Responsiveness, in terms of neurite outgrowth, to the cues present in the micro-environment is not immutable and can in itself be modified by pre-exposure to NGF. The novel demonstration that responses other than overt morphological differentiation can also be influenced by the microenvironment suggests that control may be exerted at the level of transduction of the NGF signal. Although we cannot exclude the existence of a short-range or highly labile soluble molecule controlling the above responses, it appears more probable that control is mediated via direct cell–cell and/or cell–matrix interactions. We are currently using the quantitative bioassays to determine the nature of the molecules on the muscle and fibroblast membranes that modulate neurite outgrowth from PC12 cells.

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References


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