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β-Nerve growth factor (βNGF) receptors on glial cells. Cell-cell interaction between neurones and Schwann cells in cultures of chick sensory ganglia

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Receptors for β-nerve growth factor (βNGF), so far regarded as specific cell surface markers of certain peripheral neurones, were found to be expressed on cultured non-neuronal cells of chick embryo dorsal root ganglia (drg) ($K_{d\beta NGF}$ = 2 x 10^{-9} M). Autoradiography revealed that binding of [^{125}I]βNGF was restricted to a subpopulation of the non-neuronal drg cells. Cultured embryonic skin fibroblasts, liver cells, gut cells, muscle fibroblasts, myoblasts, and myotubes, as well as macrophages and the cell lines 3T3, 3T3SV40, BHK, BHK Py, PCC3 and ND1, did not express receptors for βNGF. Non-neuronal drg cells obtained by a procedure designed for the preparation of pure Schwann cells, as well as RN6 Schwannoma cells, were βNGF receptor positive. The βNGF receptor-positive non-neuronal drg cells displayed behaviour typical of Schwann cells in their interaction with drg neurones in single cell, as well as explant cultures. Three stages of neurone-Schwann cell interaction were discernible: (1) association – neurites preferentially grew over βNGF receptor-positive non-neuronal cells; (2) cell division/alignment – βNGF receptor-positive non-neuronal cells were induced to proliferate and aligned and elongated along neurites; (3) ensheathment – the outline of βNGF receptor-positive non-neuronal cells and neurites merged. In drg cell cultures prepared from embryonic stages E6–E10, 25–40% of the non-neuronal cells were βNGF receptor-positive. Later in development, from E12 onward, ≤1% of the cultured non-neuronal cells expressed βNGF receptors.

Key words: cell-cell recognition/βNGF receptors/Schwann cells/sensory ganglia

Introduction

The only structurally well characterized protein known to affect peripheral nerve development is β-nerve growth factor (βNGF) (Greene and Shooter, 1980). In addition to its ability to induce neuronal sprouting, βNGF has a multitude of effects on neuronal cell physiology (Levi-Montalcini and Angeletti, 1968). All effects are initiated upon binding of this dimeric protein to cell surface receptors on the neurone (Banerjee *et al.*, 1973; Herrup and Shooter, 1973). The βNGF binding characteristics have been analysed in detail. One of the more interesting aspects of these studies was that βNGF binding to the neurones of sensory ganglia occurs with two affinities (K_d(I) = 2.3 x 10^{-11} M, K_d(II) = 1.7 x 10^{-9} M) which are not the consequence of a negative cooperative binding process (Sutter *et al.*, 1979a; Zimmermann *et al.*, 1978). A fractional occupancy of 8% of the high affinity binding sites is sufficient for the induction of neurite growth (Sutter *et al.*, 1979b). The role of the more abundant binding sites with lower affinity is not fully understood. Cell internalization of βNGF seems to be mediated by these sites (Sutter, 1981) and there is evidence to suggest that several membrane components participate in the generation of high affinity binding sites from the pool of low affinity binding sites in a cooperative process (Landreth and Shooter, 1980; Schechter and Bothwell, 1981). An unexpected result of the binding studies was that non-neuronal cells of sensory ganglia bind βNGF (Sutter *et al.*, 1979b). These cells had not been considered previously as targets of βNGF action.

In this work, we have visualized βNGF receptor binding to cultured embryonic sensory ganglia cells by autoradiographic means. The βNGF receptor-positive non-neuronal cells of the sensory ganglia were identified as Schwann cells on the basis of their characteristic interaction behaviour with neurites. The suitability of the βNGF receptor as a marker for Schwann cells and develomental aspects of βNGF receptor expression, as well as the potential role of βNGF receptors on Schwann cells in peripheral nerve development are discussed.

Results

βNGF receptors on non-neuronal cells

Sensory neurones from 8-day-old chick embryos bind βNGF with two affinities: K_d(I) = 2.3 x 10^{-11} M and K_d(II) = 1.7 x 10^{-9} M (Sutter *et al.*, 1979a; Zimmermann *et al.*, 1978). Cultured non-neuronal cells from the same stage of development also specifically bind βNGF (Sutter *et al.*, 1979b). In contrast to the binding characteristics on neurones, [^{125}I]βNGF binding to the non-neuronal cells appeared to be homogeneous. Figure 1 depicts the analysis of the binding data on non-neuronal cells according to Scatchard (1949).

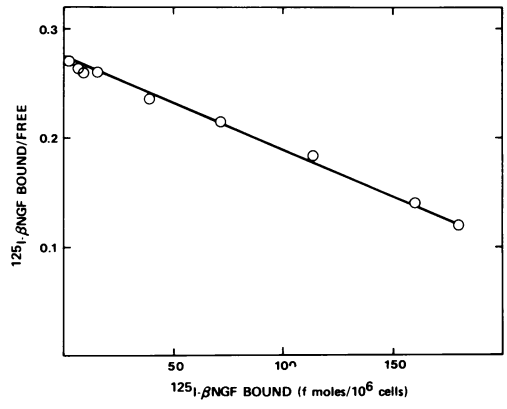

Fig. 1. Scatchard analysis of [^{125}I]βNGF binding on cultured non-neuronal chick sensory ganglia cells (E8). After 1 day in culture, the cells were dislodged from the culture plate by mechanical means. Binding assays were carried out as described (Sutter *et al.*, 1979a) with viable cells at a cell concentration of 5 x 10^5 cells/ml.

Short reports on this work have appeared in *Eur. J. Cell Biol.* (1981), **24, 27** and in Hucho,F. (ed.), *Neuroreceptors,* published by W. de Gruyter, Berlin-NY, p. 77.

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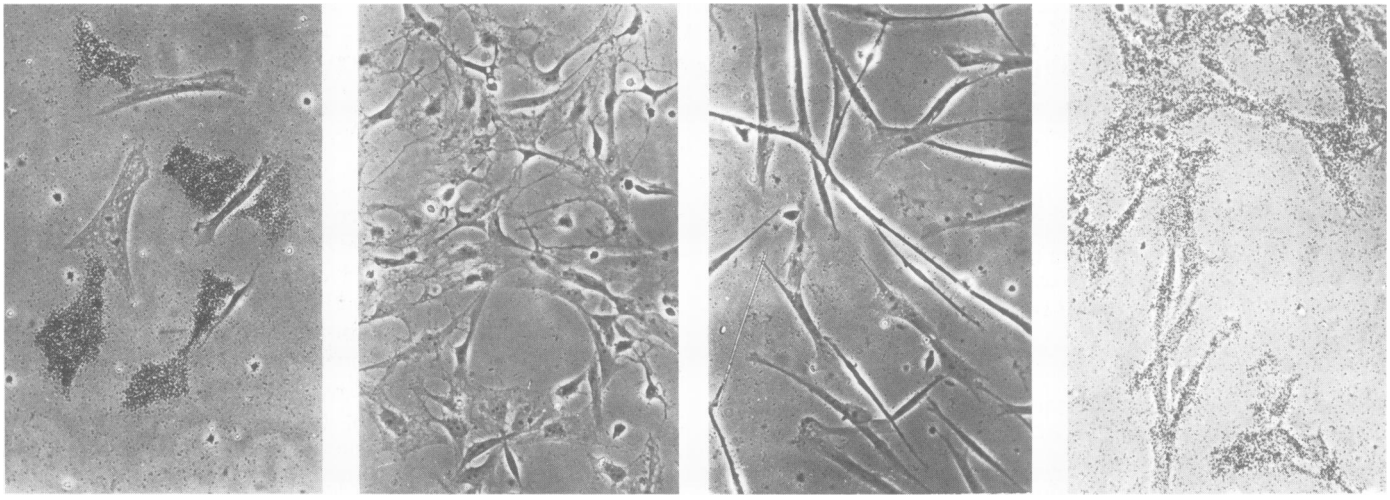


Fig. 2. [^{125}I]βNGF autoradiographs of non-neuronal cell cultures. [^{125}I]βNGF labelling was carried out as described in Materials in methods. **(a)** 1-day-old culture of separated non-neuronal chick sensory ganglia cells (E8) (x 180). **(b)** Same type of culture as **(a)** but [^{125}I]βNGF labelling was carried out in the presence of a 1000-fold excess of unlabelled βNGF. **(c)** Fusing myoblasts from E13 chick leg muscle. **(d)** RN6 Schwannoma cells (x 120).

From these data, an apparent dissociation constant (K_d) of 2×10^{-9} M was calculated. Autoradiographic techniques were used to investigate whether all or only a fraction of the non-neuronal cells expressed receptors for βNGF. Figure 2a shows an autoradiograph of a sensory ganglia cell culture from which the neuronal cells had been separated. Some, but not all, of the non-neuronal cells were labelled. Most of the labelled cells were of large polygonal morphology. βNGF binding to receptors on these cells was specific by the following criteria: [^{125}I]βNGF binding was displaced by βNGF only (Figure 2b) but not by proteins whether basic like βNGF, such as cytochrome c, or related to βNGF such as insulin and relaxin (Frazier *et al.*, 1972). Labelling of the non-neuronal cells was not abolished by preincubation of the cultures with deoxyglucose (10 mM) and azide (10 mM) for 45 min, nor by incubating and processing the cells at 4°C instead of 37°C (data not shown). Other embryonic cell types of ento-, meso- and ectodermal origin such as mouse and chick skin fibroblasts, liver cells, gut cells, chick muscle fibroblasts, myoblasts, myotubes (Figure 2c) and mouse macrophages were also analysed autoradiographically and by the radioreceptor binding assay and were found not to express receptors for βNGF. The same was true for several cell lines tested, namely the fibroblast lines 3T3, 3T3SV40, BHK, BHK Py, and the teratocarcinoma cell lines PCC3 and ND1. However, specific binding of [^{125}I]βNGF was observed on RN6 Schwannoma cells (Figure 2d). At embryonic day 8 (E8), 30% (±8%) of the non-neuronal cells from chick sensory ganglia were βNGF receptor-positive after 1 day in culture. No effect of βNGF on the survival and morphological differentiation of the cells was observed.

Proliferation and cell-cell interaction behaviour of the βNGF receptor-positive non-neuronal cells

In cultures of non-neuronal sensory ganglia cells the percentage of βNGF receptor-positive cells rapidly decreased within a few days. At the end of the first week in culture only ≤3% of the cells expressed βNGF receptors. This decrease with time in culture was due to a lower mitotic activity of the βNGF receptor-positive non-neuronal cells as compared with the βNGF receptor-negative non-neuronal cells, rather than to a loss of receptors or cell death. In [^{3}H]thymidine labelling

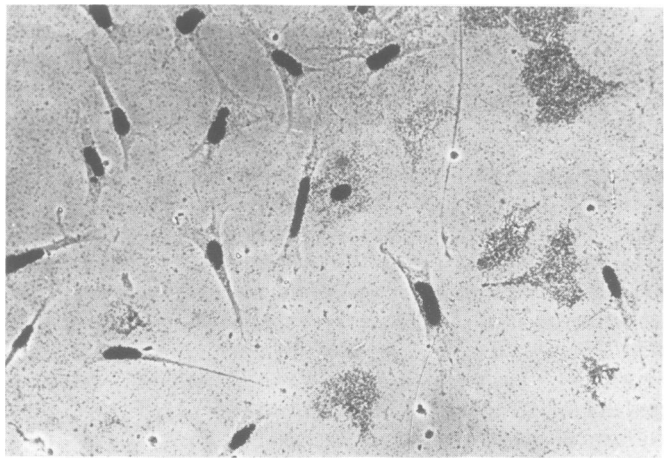
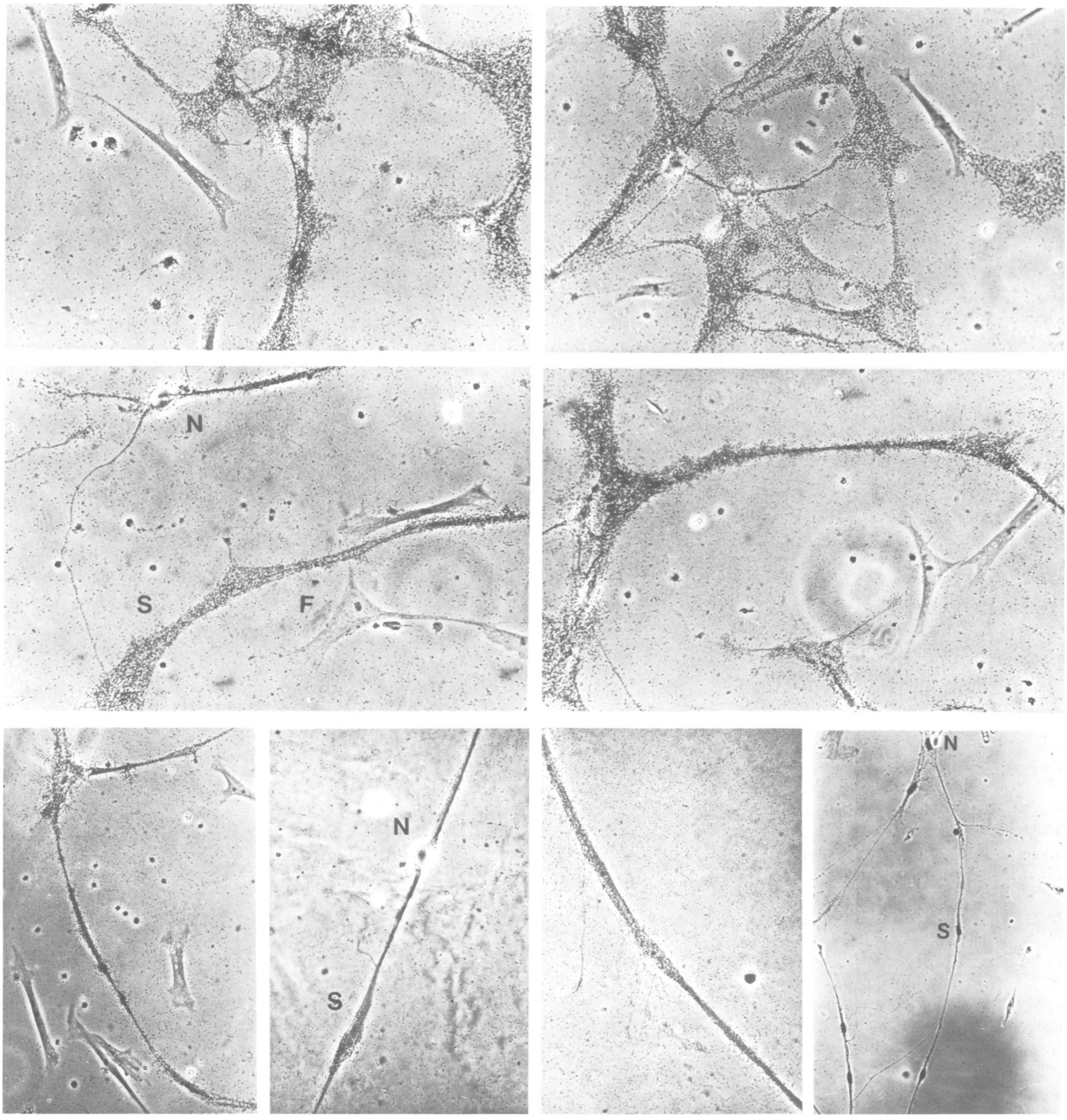


Fig. 3. [^{125}I]βNGF/[^{3}H]thymidine double labelling of separated non-neuronal chick sensory ganglia cells (E8). 2 h after plating, 10 μCi [^{3}H]-thymidine/ml was added to the culture medium. 46 h later, cultures were autoradiographed following the [^{125}I]βNGF incubation (x 160).

experiments, the mitotic activity of the non-neuronal cells was monitored in the absence and presence of neurones. When non-neuronal cells were cultured in the presence of [^{3}H]-thymidine for 18 h following plating, ≤2% of the βNGF receptor-positive cells incorporated [^{3}H]thymidine, while 30% (±9%) of the βNGF receptor-negative cells had labelled nuclei. Twenty-four hours later, these values had increased to 65% (±5%) for the βNGF receptor-negative non-neuronal cells and to ≤10% for the βNGF receptor-positive cells (Figure 3). Different results were obtained when the non-neuronal cells were cultured together with the neurones. Under these conditions, the βNGF receptor-positive non-neuronal cells were induced to proliferate (Figure 6d). After 40 h culture in the presence of [^{3}H]thymidine, ≥90% of the βNGF receptor-positive non-neuronal cells in direct contact with the neurites of the sensory neurones, and 55% (±7%) of the ones distant from them had incorporated [^{3}H]thymidine into their DNA.

Aside from the stimulatory influence the neurones had on the cell division of the βNGF receptor-positive non-neuronal



Figs. 4–6. [^{125}I]βNGF autoradiographs of different stages of Schwann cell-neurite interaction in cultures of chick sensory ganglia cells (E8). **Fig. 4.** Stage I – association – 1-day-old cultures (x 250). **Fig. 5.** Stage II – alignment – 2-day-old cultures (x 250). **Fig. 6a–c.** Stage III – ensheathment – 4-day-old cultures (**a** x 120, **b, c** x 290). **(d)** Autoradiograph of the ensheathment stage following a 46 h [^{3}H]thymidine pulse (x 120). (N = neurone, S = Schwann cell).

cells, the two cell classes also showed a strong affinity for each other and displayed a very distinct and reproducible cell-cell interaction behaviour. Twenty-four hours after plating, dissociated ganglia cells in the presence of βNGF (10^{-10} M), the neurones had extended neurites which preferentially grew on βNGF receptor-positive non-neuronal cells. The neurites tended to avoid the βNGF receptor-negative non-neuronal cells or took the shortest path across them (Figure 4). On the second day in culture the non-neuronal cells expressing βNGF receptors had begun to divide, align and elongate along the neurites (Figure 5). Over the next few days the contact between neurites and non-neuronal cells increased, leading to a stage in which the presence of the βNGF receptor-positive non-neuronal cells was hardly discernible by phase contrast microscropy (Figure 6a). Only a bulging at the position of the nucleus betrayed the presence of the non-neuronal cells in this late association stage (Figure 6b, c). The whole cell was stretched out very thinly around the neurite, thereby barely in-

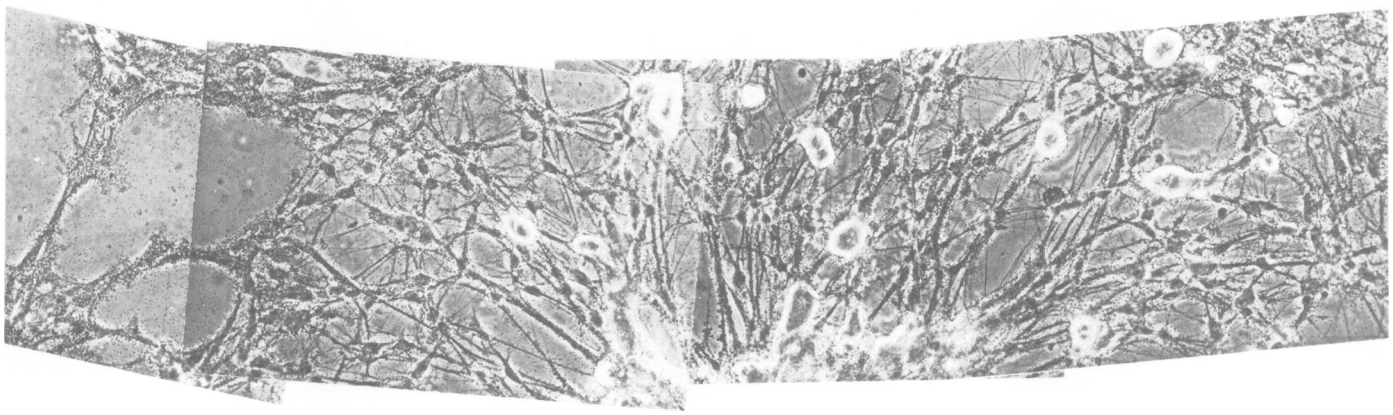


Fig. 7. Panoramic view of a [^{125}I]βNGF autoradiograph of a chick sensory ganglia explant (E8). Explant had been treated with ara-C and FUdR according to Wood (1976). [^{125}I]βNGF autoradiographs were taken following regrowth of neurites 4 days after ganglion excision (x 120).

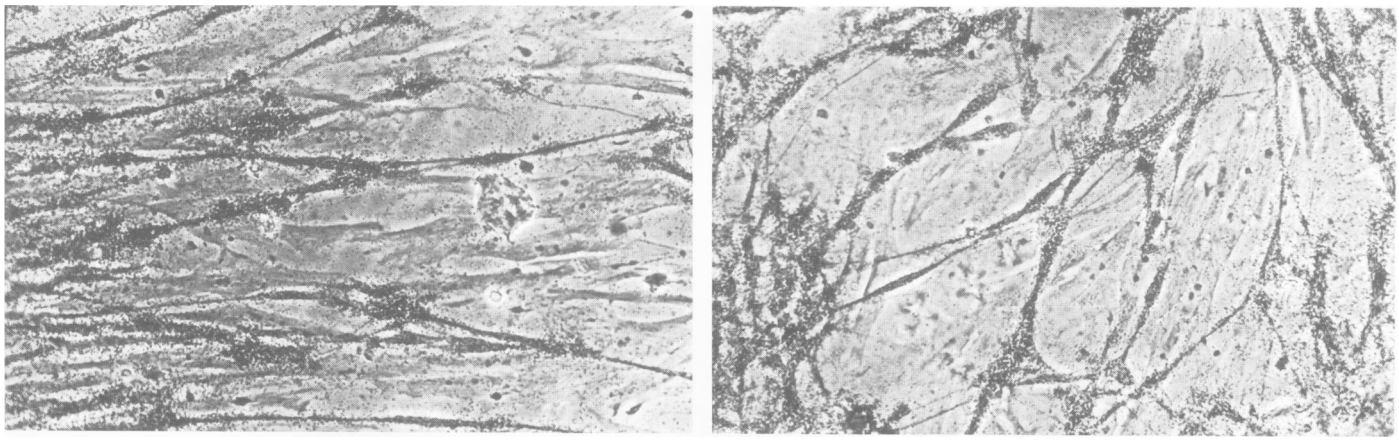


Fig. 8. [^{125}I]βNGF autoradiograph of the outgrowth region of a sensory ganglion explant (E8) after 3 days in culture (x 250).

creasing its diameter. The presence of the non-neuronal cell in close association with the neurite could be delineated by [^{3}H]-thymidine incubation of the culture at the beginning of day 2 in culture (Figure 6d). The βNGF receptor-positive cells thereby display a proliferative as well as a cell-cell interaction behaviour typical of Schwann cells.

βNGF receptors on non-neuronal cells in organotypic cultures and during development

Neurones, Schwann cells and fibroblasts are easily recognized in organotypic explant cultures from sensory ganglia. Neuronal cell bodies remain aggregated in the center of the ganglion and the growth and migration of fibroblasts and Schwann cells occur in two layers. Fibroblasts form a monolayer on the culture dish on which Schwann cells migrate in close association with outgrowing neurites. In addition, Schwann cells have been characterized operationally and functionally as a class of non-neuronal cells which survive in the ganglion following an anti-mitotic treatment with 5-fluorodeoxyuridine (FUdR) and cytosine-1-β-D-arabinofuranoside (ara-C) specially devised to remove the more rapidly dividing cells, namely fibroblasts and 'primary' Schwann cells. The remaining 'secondary' Schwann cells have been shown to retain their capacity to myelinate neurites (Wood, 1976). Using this procedure, explant cultures were obtained which had the capacity to regenerate neurites. The non-neuronal cells which survived the anti-mitotic treatment and migrated out in contact with the outgrowing neurites

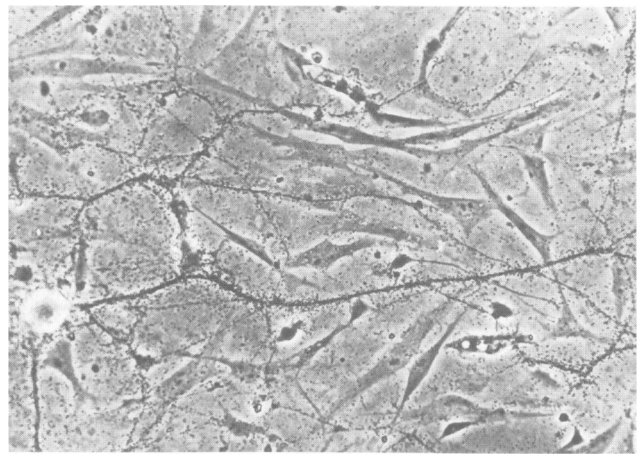


Fig. 9. [^{125}I]βNGF autoradiograph of a 1-day-old culture of chick sensory ganglia cells (E13) (x 160).

were βNGF receptor-positive (Figure 7). In control cultures which had not been treated with mitotic poisons non-neuronal cells expressing βNGF receptor were found aligned along neurites growing out on top of a dense fibroblastic cell layer (Figure 8). None of the cells of the fibroblast layer were βNGF receptor-positive. In these cultures the cells expressing

βNGF receptors were spindle shaped with the exception of the βNGF receptor-positive non-neuronal cells at the periphery of the neurite outgrowth; these had a flat extended morphology similar to that observed in stage II in single cell cultures.

The recovery of non-neuronal cells expressing receptors for βNGF in dissociates of sensory ganglia changed with developmental age. Between E6 and 10 of development, 25 – 40% of the non-neuronal cells in single cell cultures were βNGF receptor-positive and displayed behaviour typical of Schwann cells. At later embryonic stages, the percentage of non-neuronal cells expressing βNGF receptors decreased. At E12, 13, 14, and 15, ≤1% of the non-neuronal cells were βNGF receptor-positive and cell-cell interaction between neurones and non-neuronal cells was significantly reduced (Figure 9).

Discussion

Since βNGF action has so far only been discussed in the context of neuronal differentiation, it was somewhat surprising to find specific binding of βNGF (K_d = 2 x 10^{-9} M) to cultured non-neuronal sensory ganglia cells. The same K_d for βNGF was determined previously for one of the two binding sites for βNGF on sensory neurones. The autoradiographic analysis of the cellular distribution of βNGF receptors on the non-neuronal sensory ganglia cells revealed that only a subpopulation of these cells carried βNGF receptors. βNGF receptor expression is not a common characteristic of cultured embryonal cells. Cultured embryonic chick cells of ento-, meso- and ectodermal origin like skin fibroblasts, myoblasts or liver and gut cells, were all βNGF receptor-negative, as were several non-nervous system cell lines tested, e.g., 3T3, BHK and teratocarcinoma cells. RN6, a rat Schwannoma cell line was, however, βNGF receptor-positive.

In neurone-free cultures of embryonic chick sensory ganglia cells it was not possible to identify different cell types on the basis of their morphology as Schwann cells or fibroblasts. Cells with spindle shape morphology, described as typical for Schwann cells in rat sciatic nerve and 'secondary' Schwann cell cultures from rat sensory ganglia (Wood, 1976; Brockes *et al.*, 1979), were not observed. Nevertheless, it was shown that in neurone-free cultures, βNGF receptor-positive and βNGF receptor-negative cells displayed different morphological characteristics, the former being more often of large polygonal shape, whilst the latter were generally of fibroblastic morphology.

Several lines of evidence led to the conclusion that the βNGF receptor-positive non-neuronal cells were Schwann cells. [In this communication the Schwann cell type behaviour of the βNGF receptor-positive non-neuronal cells was studied. In using the term 'Schwann' cell we do not want to preclude that other glial cells ('satellite' cells) also carry βNGF receptors.] In the absence of neurones the mitotic activity of the βNGF receptor-positive non-neuronal cells was one order of magnitude lower than that of the fibroblastic βNGF receptor-negative cells. The presence of neurones/neurites led to a 10-fold increase in the mitotic indices of the βNGF receptor-positive non-neuronal cells. This neurone-induced proliferation is characteristic for Schwann cells and has been described by Aguayo *et al.* (1976) in grafting experiments with rat central and peripheral nerves, by Wood and Bunge (1975) and Wood (1976) with 'secondary' Schwann cells of rat spinal ganglia, by Brockes *et al.* (1977, 1979) with Schwann cells of rat sciatic nerve and by Skaper *et al.* (1980) with Schwann cells of rat spinal ganglia. The basal mitotic activity of βNGF receptor-positive non-neuronal cells of chick dorsal root ganglia in the absence of neurones was higher than that observed for 'secondary' rat Schwann cells (Salzer and Bunge, 1980). One reason for that may be procedural, namely the selection of more slowly dividing Schwann cells with the anti-mitotic treatment used to prepare pure rat Schwann cells (Wood, 1976). In the presence of neurones, the mitotic activity of the βNGF receptor-positive non-neuronal cells, in association with the neurites, corresponds to the values observed in the rat system with 'secondary' Schwann cells. The signal inducing Schwann cell proliferation has been described as a component of the neurite membrane (Salzer *et al.*, 1980). In our culture system, not only the βNGF receptor-positive cells in direct contact with neurones, but also those distant from neurones were induced to proliferate, though to a lesser degree. This does not necessarily imply that there is a long range effect of neurites on Schwann cell proliferation. The neuronal plating efficiency at optimal βNGF concentrations under the culture conditions used is <50%. Membrane fragments of decaying neurones could, therefore, be responsible for the apparent long range effect of neurones on the proliferative behaviour of the βNGF receptor-positive non-neuronal cells.

Of the different characteristics of the non-neuronal cells expressing βNGF receptor their contact behaviour toward neurites impressed most. While non-neuronal cells lacking βNGF receptor did not exhibit any neurite oriented growth behaviour, the βNGF receptor-positive non-neuronal cells interacted with the neurites in a highly ordered fashion typical of Schwann cells. On the basis of the *in vitro* observations with dissociated ganglia cells (E8), the interaction between βNGF receptor-positive non-neuronal cells and neurones was divided into three stages: I, association; II, cell division/alignment; III, ensheathment. Stages I and II occur during the first days in culture. Stage III of neurone-glia cell interaction is increasingly represented along part, but not all, of the neurites after day 3 in culture. In agreement with these *in vitro* observations, *in vivo* studies have shown that at E10 chick sensory neurones can be seen wrapped completely by one or more overlapping expansions of a single Schwann cell (Pannese, 1969). Lodin *et al.* (1973) have observed the first myelin profiles in embryo extract containing cultures of sensory ganglia cells of chick embryos at the beginning of the second week in culture. Ultrastructural studies are under way to determine the degree of ensheathment obtained under the present culture conditions. From the report of Moya *et al.* (1980), one would expect that under our culture conditions ensheathment is not complete and that additions to the culture medium, such as embryo extract, will be necessary to obtain myelinization.

The characterization of βNGF receptor-positive non-neuronal ganglia cells as Schwann cells, on the basis of the autoradiographic analysis of single cell cultures, was confirmed in the experiments with organotypic explant cultures of sensory ganglia. The cellular organization of ganglionic explant cultures facilitated the identification of the different ganglionic cell types: neurones, Schwann cells and fibroblasts. In addition, the method of Wood (1976) allowed the preparation of explant cultures practically free of fibroblasts. Both 'primary' Schwann cells in untreated ganglia explant cultures and 'secondary' Schwann cells in ara-C/FUdR-treated ganglia explant cultures were found to be βNGF receptor-

positive. In contrast, no βNGF receptor-positive cells could be detected in the dense fibroblast layer of untreated explant cultures.

The variability in morphology of the βNGF receptor-positive cells in the four types of culture systems employed was remarkable and stresses again that cellular shape is an unreliable criterion for the identification of cultured cells. While βNGF receptor-positive cells displayed behaviour typical of Schwann cells in all culture systems studied, the 'characteristic' spindle shape morphology was only observed in single cell cultures at advanced stages of neurone-Schwann cell interaction and in ganglia explants not treated with mitotic inhibitors.

The representation of non-neuronal cells with βNGF receptor remains relatively constant between E6 and E10. A sharp drop in their relative number was observed in single cell cultures from E12 ganglia. It is not clear whether this reflects a developmentally regulated decrease of receptor-bearing cells or whether it is due to the increase in the number of capsule cells (fibroblasts) in conjunction with a poor recovery of viable Schwann cells which at E12 *in vivo* are engaged in the myelinization process. Similarly, a decrease in the number of neurite-associated βNGF receptor-bearing non-neuronal cells is observed in short term cultures of ganglion explants of later developmental stages (E14). However, in such explant cultures, a quantitation of βNGF receptor-positive non-neuronal cells might not be meaningful, since the cellular composition of the outgrowth region of the explants might not be representative for the whole ganglion. A drop in the percentage of βNGF receptor-bearing cells, as observed here for the non-neuronal ganglia cells, has also been described to occur around E16 for the neuronal cells (Rohrer and Barde, 1982). It is remarkable that with the loss of βNGF receptor-positive non-neuronal cells, the neurone-Schwann cell type interaction behaviour is also greatly reduced in single cell cultures of E12. Whether or not the βNGF receptor disappears from non-neuronal ganglia cells by E12, our findings show that between E6 and E10 the βNGF receptor delineates the Schwann cell population among the non-neuronal cells of the ganglion and should, therefore, be a useful cell surface marker for Schwann cells at a developmental stage when myelin-related markers, e.g., galactocerebroside, are not expressed in culture except in some late stages of ensheathment (A. Zimmermann, unpublished data).

The discovery of βNGF receptors on Schwann cells appears to be fortunate in view of the scarcity of specific cell surface markers for this cell type (Brockes *et al.*, 1977; Mirsky *et al.*, 1980). The question remains, however, as to whether all neural crest-derived cells express βNGF receptors early in development. In fact, βNGF receptors have been reported on human melanoma cells (Fabricant *et al.*, 1977) and, while this work was in progress, Rohrer and Sommer (1982) have found βNGF receptors on cells expressing neuronal as well as glial properties in ciliary ganglia of chick embryos. As differentiation proceeds, βNGF receptor expression may become restricted. The restriction of originally common cellular traits marking steps in development is not an uncommon observation (Caplan and Ordahl, 1978; Zimmermann *et al.*, 1979; De Vitry *et al.*, 1980). Though the functions of the majority of identified developmentally regulated cell surface markers is unknown, it appears reasonable to assume that at least some take part in the stage-specific intercellular communication.

At the moment, one can only speculate about a possible function of βNGF receptors on Schwann cells. Does βNGF play a role for Schwann cell physiology and differentiation as it clearly does for sympathetic and sensory neurones? So far, no difference in survival or morphological differentiation of βNGF receptor-positive cells has been observed when non-neuronal sensory ganglia cells are grown in the presence or absence of βNGF. It has been suggested that Schwann cells might produce βNGF (Varon *et al.*, 1974). βNGF receptors on Schwann cells could function as feed-back receptors monitoring the βNGF concentration in the intercellular space. There is good evidence that the target tissues of βNGF-dependent neurones produce βNGF (Ebendahl *et al.*, 1980) and growth cones of sensory neurones show positive chemotaxis in a βNGF gradient (Letourneau, 1978; Gunderson and Barrett, 1980). Were Schwann cells to provide a specific βNGF uptake system, they could stabilize βNGF gradients between neurones and their target organs. Preliminary data suggest that non-neuronal sensory ganglia cells internalize βNGF in an energy-dependent process (A. Sutter, unpublished data). In view of the fact that both neurones and Schwann cells express receptors for βNGF, it could be speculated that βNGF – a stable dimer under physiological conditions (Bothwell and Shooter, 1977) composed of two identical protomers each with a receptor binding domain (Zimmermann *et al.*, 1981) – might serve as a mediator of neurone-Schwann cell contact by binding to receptors on both cell types. Independent of whether such a mechanism might play a role in cell-cell recognition, it is known that βNGF affects cell-cell adhesion, increasing it for some cell types and decreasing it for others (Schubert and Whitlock, 1977).

Materials and methods

Cells

Chick sensory ganglia were dissociated and cultured as described previously (Sutter *et al.*, 1979a, 1979b) in modified F12 medium and 5% fetal calf serum on polylysine (Sigma) coated polystyrene slides (Lux) in the presence or absence of βNGF (10^{-10} M). For the separation of the neuronal and non-neuronal sensory ganglia cells, the published preplating procedure was used (Sutter *et al.*, 1979b). Muscle cells were grown on collagen-coated polystyrene slides. Ganglia explant cultures were grown on collagen-coated polystyrene slides in the presence of βNGF (5×10^{-10} M). For the elimination of fibroblasts in explant cultures, the procedure of Wood (1976) was followed. RN6 cells were a gift from W. Wechsler, University of Düsseldorf. BHK, BHKPy, 3T3 and 3T3SV40 cells were obtained from K. Lange and K. Keller at this Institute, PCC3 and ND1 teratocarcinoma cells were donated by P. Goodfellow (ICRF, London). Primary tissues were dissociated using trypsin/DNase as described (Zimmermann *et al.*, 1976). Culture conditions were the same as for sensory ganglia cells. Variations in cell counts from autoradiographs are given as S.D. of at least four different experiments.

Autoradiography

Dissociated sensory ganglia cells or ganglia explant cultures grown on polylysine or collagen-coated polystyrene slides were washed free of serum and βNGF and incubated at 37°C for 20 min with 5×10^{-10} M [^{125}I]βNGF (40–60 c.p.m./pg) in phosphate-buffered saline containing 1 mg/ml bovine serum albumin (PBS-BSA). Following the incubation with [^{125}I]βNGF, the slides were washed 10 times in PBS-BSA. They were fixed for 30 min at room temperature in 1% paraformaldehyde/2% glutaraldehyde, washed free of fixative, dipped into NTB-2 emulsion (Kodak) diluted 1:1 (v/v) with distilled water and exposed for 6 days at 4°C. For [^{3}H]thymidine labelling, 10 μCi/ml [^{3}H]thymidine (Amersham) were added to the growth medium.

Proteins

βNGF was purified from saliva of NMRI mice following the procedure of Burton *et al.* (1978) and radioiodinated with Na ^{125}I (Amersham) as described by Sutter *et al.* (1979a). Insulin was obtained from Hoechst, cytochrome c from Sigma and relaxin was a gift from F. Greenwood, University of Hawaii.

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