Development 107, 107–112 (1989)
Printed in Great Britain © The Company of Biologists Limited 1989

Control of peripheral glial cell proliferation: enteric neurons exert an inhibitory influence on Schwann cell and enteric glial cell DNA synthesis in culture

P. A. ECCLESTON[1], P. G. C. BANNERMAN[1], D. E. PLEASURE[2], J. WINTER[3], R. MIRSKY[1] and K. R. JESSEN[1]

[1]*Department of Anatomy and Developmental Biology, University College London, Gower Street, London WC1E 6BT, UK*
[2]*Department of Neurology, University of Pennsylvania, Philadelphia, USA*
[3]*The Sandoz Institute for Medical Research, London WC1E 6BN, UK*

Summary

Neuronal membranes from rat dorsal root ganglia provide a mitogenic signal to cultured Schwann cells and it has been suggested this is an important factor in regulating Schwann cell numbers during development. In this study, the influence of enteric neurons on the DNA synthesis of both Schwann cells and enteric glia has been investigated as well as the effect of axonal membrane fractions (axolemma) on enteric glia.

The proliferation rate of rat Schwann cells and enteric glia was assessed in culture using [^{3}H]thymidine uptake and autoradiography in combination with immunolabelling to identify cell types. When purified rat Schwann cells were co-cultured with guinea pig enteric neurons, their DNA synthesis rate was reduced compared with control cultures of pure Schwann cells or Schwann cells not close to neurites or neuronal cell bodies. Nevertheless, in accordance with previous findings that sensory neurons stimulate Schwann cell division, these Schwann cells increased their DNA synthesis rate when in contact with neurites from purified guinea pig or adult rat dorsal root ganglion neurons and on exposure to bovine axolemmal fractions. The enteric neurons also suppressed the DNA synthesis of enteric glia in co-cultures of purified enteric neurons and enteric glia, while bovine axolemma stimulated their DNA synthesis.

These results indicate that a mitotic inhibitory signal is associated with enteric neurons and can exert its effect on both Schwann cells and enteric glia, and that enteric glia, like Schwann cells, are stimulated to divide by axolemmal fractions. It thus seems possible that during development glial cell numbers in the peripheral nervous system may be controlled by both positive and negative regulators of cell growth.

Key words: enteric glia, Schwann cells, axons, tissue culture, proliferation.

Introduction

Cessation of division is an important step in glial cell differentiation. In the peripheral nervous system (PNS), this differentiation gives rise to a cellular arrangement in the enteric nervous system (ENS) which is distinctly different from that in peripheral nerves. In peripheral nerves, Schwann cells ensheath and myelinate individual axons or ensheath groups of small axons. Enteric glial cells of the enteric nervous system support enteric neurons and axons in an organisation similar to that of astrocytes and neurons in the central nervous system (CNS) (Gabella, 1971, 1981).

In peripheral nerves, the axon has been implicated in control of glial cell numbers since the axonal membrane and axolemma fragments are potent mitogenic signals for Schwann cells (Salzer *et al.* 1980; De Vries, 1981; Pleasure *et al.* 1985). Thus, axonally associated signals, possibly together with soluble mitogens (Brockes *et al.* 1980; Pleasure *et al.* 1985; Eccleston *et al.* 1987), may be important for expanding Schwann cell populations during development and nerve regeneration. It is less clear, however, why Schwann cells do not divide in mature nerves. It seems likely, by analogy with other systems, that negative growth signals may be important (Braun *et al.* 1988; Sporn *et al.* 1987; Wang and Hsu, 1986).

Our preliminary results suggested that enteric neurons did not induce proliferation of enteric glial cells in explant cultures (Bannerman *et al.* 1987). Here we show that enteric neurons inhibit the DNA synthesis of both Schwann cells and enteric glia. Nevertheless we find

that, like Schwann cells, enteric glia increase their DNA synthesis in response to axolemma isolated from the CNS. Enteric neurons, under the culture conditions used, therefore differ from sensory neurons in their influence on glial cell proliferation. Thus the DNA synthesis of enteric glia and Schwann cells can be either stimulated or inhibited by neuron-associated factors. It is possible that, during development of the PNS, both types of signals may play a role in regulating glial cell numbers.

Materials and methods

Culture medium

Cultures were grown in Dulbecco's Modified Eagle's Medium (DME) supplemented with fetal calf serum (10%), glucose ($2\,mg\,ml^{-1}$), insulin ($5\,\mu g\,ml^{-1}$), penicillin ($100\,IU\,ml^{-1}$) and streptomycin ($100\,\mu g\,ml^{-1}$). Nerve growth factor ($10\,ng\,ml^{-1}$) was added to cultures of guinea pig dorsal root ganglia. Axolemma was prepared as described previously (Cullen *et al.* 1981; De Vries, 1981) and added to the culture medium at 35 or 70 μg per culture.

Enteric glial cells

Purified populations of enteric glial cells were prepared from explants of myenteric plexus as described previously (Jessen *et al.* 1983; Bannerman *et al.* 1988*b*). Essentially, segments of the myenteric plexus were dissected from the gut wall following enzyme treatment, explanted onto polylysine-coated glass coverslips and treated with cytosine arabinoside (AraC) for 4–5 days to suppress fibroblast growth. After a further 2–3 days without AraC, the central neuronal areas were removed and cultures treated for 1 h with anti-Thy1.1 and complement to kill any remaining neurons and fibroblasts. In most cultures, >95% of all cells were enteric glia.

Enteric neurons

Purified populations of enteric neurons were prepared from explants of myenteric plexus as described previously (Bannerman *et al.* 1988*b*). Briefly, explant cultures of the myenteric plexus were treated with AraC for a prolonged period to suppress the growth of fibroblasts and enteric glial cells. Any remaining glial cells were killed using the monoclonal antibody LB1 and complement. Cultures were 85–95% enteric neurons.

Schwann cells

Rat Schwann cells were purified from sciatic nerves by a modification of the method of Brockes *et al.* 1979 (Jessen *et al.* 1987*a*). They initially had a low proliferation rate and spindle-shaped morphology. With time in culture, and in the absence of any added mitogen, they gradually became more flattened in morphology and increased their proliferation rate. A similar increase in Schwann cell division rates after stimulation with mitogens has been described previously and is thought to be mediated via an autocrine mechanism (Porter *et al.* 1986, 1987). The change in morphology allowed us to choose cells with a higher division rate to study growth inhibition.

Guinea pig Schwann cells were purified from the sciatic nerves of 1- to 5-day-old Dunkin-Hartley guinea pigs as described previously (Eccleston *et al.* 1987). Once purified, Schwann cells were removed from the dishes using 0·25% trypsin diluted 1:25 in calcium- and magnesium-free DME.

Neurons from dorsal root ganglia of newborn guinea pig

Dorsal root ganglia were removed from newborn Dunkin-Hartley guinea pigs and desheathed. They were dissociated using 0·125% trypsin and 0·2% collagenase for 1 h as for guinea pig Schwann cells. AraC was included in the culture medium to remove dividing Schwann cells and fibroblasts.

Neurons from dorsal root ganglia of adult rats

Pure populations of adult rat dorsal root ganglion neurons were prepared as described previously (Wood *et al.* 1988). Briefly, dorsal root ganglia from rats over two months old were dissociated, enriched for neurons by a differential adhesion step and plated on laminin-coated coverslips. Further purification was achieved by including AraC in the culture medium.

Antibodies

Rabbit anti-Thy1.1 was a gift from Dr A. F. Williams and was used at a dilution of 1:100. Rabbit antiserum to S100 protein (DAKO immunoglobulins A/S) (dilution 1:400–1:800) was used as a specific glial cell marker in the PNS (Brockes *et al.* 1979; Mirsky *et al.* 1985; Bannerman *et al.* 1988*a*). The monoclonal antibody LB1, a gift from Dr J. Cohen, recognises a ganglioside on the surface of several cell types in the nervous system. In the ENS, it has been shown to bind specifically to enteric glial cells (Bannerman *et al.* 1988*a*). The monoclonal antibody, A5E3, is rat specific, recognising a cell surface glycoprotein on Schwann cells and fibroblasts (Mirsky *et al.* 1985). Tetramethyl rhodamine conjugated to goat anti-rabbit Ig (G anti-R Ig Rd) (Cooper Biomedical) was adsorbed with mouse Ig to remove cross-reacting antibodies and was used at a dilution of 1:100. Fluorescein conjugated to goat anti-mouse Ig (G anti-M Ig Fl) (Nordic Laboratories Ltd) was adsorbed with rabbit Ig and used at a dilution of 1:100.

Immunofluorescence

Schwann cells and enteric glia were identified using anti-S100. Rat Schwann cells in mixed cultures with enteric neurons were identified with anti-S100 alone or the rat-specific monoclonal antibody A5E3 in combination with anti-S100 in order to distinguish the rat Schwann cells ($S100^+$, $A5E3^+$) from any enteric glial cells present in the neuron cultures ($S100^+$, $A5E3^-$). Guinea pig dorsal root ganglion neurons were identified using anti-Thy1.1. For demonstration of S100, cultures were fixed in 4% paraformaldehyde in phosphate-buffered saline for 20 min followed by treatment with methanol at −20°C for 10 min. LB1, A5E3 and anti-Thy1.1 were applied to living cultures. The procedure used has been described previously (Jessen *et al.* 1987*b*).

Autoradiography

Cultures were incubated for 24 h with 0·1 μCi [^{3}H]thymidine ($5\,Ci\,mmol^{-1}$) (Amersham International, plc). They were then washed in several changes of Eagle's Minimal Essential Medium supplemented with Hepes buffer (0·015 M) and immunolabelled as described above. Coverslips were mounted cell side uppermost on gelatin-coated glass slides and processed for autoradiography as described previously (Eccleston *et al.* 1987). The percentage of Schwann cells or enteric glia with more than 5 silver grains over their nuclei was determined using a Zeiss microscope equipped with epi-fluorescence and phase-contrast optics. 200–500 cells per coverslip were scored.

Results

Influence of enteric neurons on enteric glial cell division in mixed explant cultures

In myenteric explant cultures, the neurons and a population of glial cells remain in the central explant area (Fig. 1). Other enteric glial cells migrate out and proliferate forming a glial cell carpet around the central area, over which fine neurites extend (Fig. 1B). The neurites remain on the surface of the glial cells and do not extend onto the glass coverslip (Jessen *et al.* 1983). Myenteric explant cultures and purified populations of glial cells initiated from the same animal were exposed to [^{3}H]thymidine for 24 h after 10 days in culture and labelled with anti-S100 to identify glial cells. [^{3}H]thymidine incorporation levels were assessed in three separate areas of the explant cultures. (1) In the outgrowth zone, excluding a narrow band of approximately 0·3 mm immediately surrounding the central neuronal area, the glial cells had a labelling index of 55·6 ± 1·0 (mean ± S.E.M.; $n = 6$). In parallel experiments, pure populations of enteric glial cells divided at a similar rate of 56·2 ± 2·1 (mean ± S.E.M.; $n = 8$). (2) In the area immediately surrounding the central neuronal area (an approximately 0·3 mm zone surrounding the explant area) S100-positive glial cells incorporated [^{3}H]thymidine at a greatly reduced rate of 13·7 ± 1·9 (mean ± S.E.M.; $n = 5$) compared with the outgrowth zone. (3) In the central explant area, almost no tritiated thymidine-labelled cells could be detected although many S100-positive enteric glial cells were present. The dense arrangement of glia in close apposition to neurons made cell identification and counting of individual cells impossible.

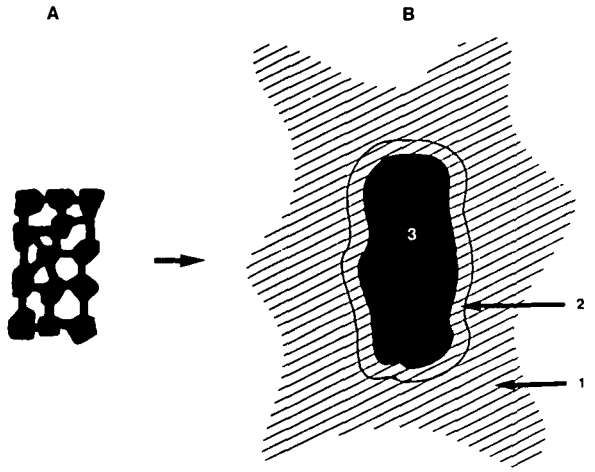

Fig. 1. Diagrammatic representation of areas of enteric plexus explant cultures. A shows the isolated plexus after dissection and attachment to a culture substratum. Within 24 h the net-like meshwork is filled in by migration of glial cells and/or neurons. B shows an explant after approximately one week in culture. The neurons remain in the central explant area (3) together with a population of glial cells. Glial cells migrate out from the explant area, proliferate and form a carpet over which axons are able to grow (1). The labelling index of enteric glia in the outgrowth zone (1) was compared to that in the central explant area (3) and in a zone 0·3 mm in width immediately surrounding the explant area (2).

Thus enteric glia in these experiments do not show a mitogenic response when they are exposed to neuronal membranes. Moreover, the results suggest that under some circumstances enteric neurons can exert an inhibitory influence on the division rate of enteric glial cells. This is in contrast to results obtained with Schwann cells in contact with dorsal root ganglion neurons or axolemmal fragments.

Influence of axolemma on enteric glial cell division

The reason for the failure of enteric glia to show a mitogenic response to enteric neurons could be that these neurons do not express the mitogenic molecule found in association with other neuronal membranes. Alternatively, enteric glia might lack receptors for the neuronal mitogen. To test the latter possibility, purified populations of guinea pig enteric glial cells were exposed to 35 or 70 μg of bovine axolemma for 24 h or 4 days. In all cases, axolemma increased the labelling indices of the enteric glial cells (Table 1). Controls were done using short-term cultures of rat Schwann cells to show that the axolemma preparations were active, and guinea pig Schwann cells to show that the bovine axonal membrane preparation was able to cross-react with cells from guinea pig. In both cases, Schwann cell DNA synthesis was increased by the axolemma (Table 1). In addition, like short-term cultures, dividing rat Schwann cells from long-term secondary cultures (see Methods) showed increased [^{3}H]thymidine incorporation rates in response to axolemma. Control cultures had a labelling index of 12·4 ± 1·0 whereas those exposed to axolemma had a labelling index of 24·0 ± 3·0 (mean ± S.E.M.; $n = 6$).

Table 1. *Axolemma increases peripheral glial cell DNA synthesis*

	Labelling index
Enteric glia:	
Control	39·4 ± 1·9 ($n = 17$)
Axolemma (35 μg) 1 day	68·8 ± 2·8 ($n = 11$)
Axolemma (35 μg) 4 day	72·0 ± 4·5 ($n = 10$)
Axolemma (70 μg) 1 day	71·8 ± 1·7 ($n = 7$)
Rat Schwann cells:	
Control	3·2 ± 0·8 ($n = 6$)
Axolemma (35 μg) 1 day	15·5 ± 2·0 ($n = 6$)
Guinea pig Schwann cells:	
Control	22·1 ± 1·4 ($n = 5$)
Axolemma (35 μg) 1 day	48·0 ± 4·3 ($n = 4$)

Guinea pig enteric glial cells and Schwann cells and rat Schwann cells were purified as described in Materials and methods. Axolemma (35 or 70 μg/culture) was added to test cultures for 24 h or 4 days. To remove unbound axolemma cultures were washed with medium. After 4 days [^{3}H]thymidine (0·1 μCi) was added to all cultures including controls. The proportion of S100-positive glial cells with more than 5 silver grains over their nuclei was determined by counting 200–500 cells per coverslip. Results are mean ± S.E.M.

Influence of enteric neurons on glial cell division in co-cultures of purified populations

Since enteric glia, like Schwann cells, could clearly respond to the axolemmal mitogen by an increase in proliferation rate, the alternative possibility, i.e. that enteric neurons lacked the mitogen or even exerted an inhibitory effect on glial division, was explored further.

For this purpose, we employed purified populations of guinea pig enteric glia or rat Schwann cells and purified explant cultures of enteric neurons from which essentially all glial cells had been removed. When purified enteric glial cells were added back to the neuronal cultures a slight inhibition of glial cell proliferation was detected. Pure enteric glial cell controls had a labelling index of 47·2 ± 3·2 (mean ± S.E.M.; $n = 3$) and enteric glial cells in the neuronal area of the mixed cultures had a labelling index of 33·0 ± 2·6 (mean ± S.E.M.; $n = 5$). In similar experiments, enteric neurons inhibited the DNA synthesis of rat Schwann cells, only Schwann cells in the neuronal area of the cultures being affected. For these experiments dividing long-term secondary Schwann cell cultures were used. Cultured alone these cells had a labelling index of 31·9 ± 3·5 (mean ± S.E.M.; $n = 6$). In co-cultures with enteric neurons, those Schwann cells that were in the neuronal area had a labelling index of 8·5 ± 0·7 (mean ± S.E.M.; $n = 9$) whereas those Schwann cells distant from the neuronal explant area synthesised DNA at a similar rate to Schwann cell controls, with a labelling index of 28·8 ± 2·0 (mean ± S.E.M.; $n = 5$) ($P < 0·001$ for Schwann cells in the neuronal area compared with Schwann cells distant from neurons). These results are summarised in Table 2.

These results indicated that a mitotic inhibitory signal was associated with guinea pig enteric neurons. To test whether this was a general feature of guinea pig neurons or, alternatively, whether the inhibitory effect was specifically associated with enteric neurons, purified rat Schwann cells were added to purified cultures of guinea pig dorsal root ganglion neurons. Schwann cells in control cultures, where no neurons were present, had a labelling index of 4·8 ± 1·1 (mean ± S.E.M.; $n = 3$). In the neuronal area of mixed cultures, Schwann cells had a labelling index of 23·8 ± 2·5 (mean ± S.E.M.; $n = 4$) and in areas where no neurons were identified the Schwann cell labelling index was 5·2 ± 1·1 (mean ± S.E.M.; $n = 3$).

Table 2. *Enteric neurons inhibit peripheral glial cell DNA synthesis*

	labelling index	% inhibition
Enteric glia alone	47·2 ± 3·2 ($n = 3$)	
Enteric glia and neurons	33·0 ± 2·6 ($n = 5$)	30·1
Schwann cells alone	31·9 ± 3·5 ($n = 6$)	
Schwann cells and neurons	8·5 ± 0·7 ($n = 9$)	73·4

Guinea pig enteric neurons were purified and guinea pig enteric glia or rat Schwann cells added to them as described in Materials and methods. After 4 days – 1 wk, [^{3}H]thymidine (0·1 μCi) was added to the mixed co-cultures or to control cultures of pure enteric glia or Schwann cells. The proportion of glial cells with more than 5 silver grains over their nuclei was determined. Cells in the neural area of co-cultures were compared with pure glial cell populations. Results are mean ± S.E.M.

The mitogenic effect seen with guinea pig dorsal root ganglion neurons indicated that an inhibitory effect on glial DNA synthesis might be a particular feature of enteric neurons. In the guinea pig, the enteric plexuses are, however, relatively well developed at birth and three to four weeks of culturing are required for depletion of glial cells. The enteric neurons have therefore also had a considerable period to mature *in vitro* by the time they are re-exposed to glial cells. To test the possibility that an inhibitory effect of cultured neurons on glial DNA synthesis was a feature of mature neuronal membranes, purified Schwann cells were added to cultures of adult rat dorsal root ganglion neurons that had been maintained for 2–4 weeks in culture. Schwann cells in control cultures grown on polylysine in the absence of neurons had a labelling index of 2·5 ± 0·7 (mean ± S.E.M.; $n = 3$). In mixed cultures, Schwann cells had a labelling index of 12·3 ± 0·6 (mean ± S.E.M., $n = 3$). Since the neurons were grown on a laminin-coated substratum, controls were done where the neurons were gently removed from the coverslip with a jet of medium. Schwann cells grown on this surface synthesised DNA essentially at the same rate as those on polylysine, having a labelling index of 2·5 ± 0·7 (mean ± S.E.M., $n = 2$). The results shown are from one experiment using one batch of neurons and Schwann cells. Similar results were obtained in two further experiments where the Schwann cells had higher labelling indices.

Discussion

All axons so far tested from the central and peripheral nervous systems have had a stimulatory effect on Schwann cell DNA synthesis (De Vries *et al.* 1982; Mason *et al.* 1989; Salzer *et al.* 1980; Pleasure *et al.* 1985). The present work shows that the enteric neuron has an inhibitory influence on the DNA synthesis rates of both Schwann cells and enteric glia in culture. This is in contrast to the axons of sensory neurons and axolemmal fragments, which stimulate division of peripheral glia. A heparan sulfate proteoglycan is required for the proliferation of Schwann cells along the neurite membrane (Ratner *et al.* 1985, 1988). Several growth factors, including fibroblast growth factors, bind to heparin with high affinity (Lobb *et al.* 1986; Rybak *et al.* 1988), and it seems probable that the axonal mitogen is a proteoglycan–growth factor complex (Ratner *et al.* 1988). Enteric neurites might lack the heparan sulphate proteoglycan associated with their plasma membrane. If so they would not bind the mitogenic growth factor and thus not stimulate glial cell division. Furthermore, it is possible that the mitogenic effect of dorsal root ganglion neurites is due to binding of a factor or factors that also can act as soluble mitogens, e.g. glial growth factor. The presence of receptors for the neurite-associated mitogen on enteric glia would then be consistent with our previous observations that guinea

pig enteric glia and Schwann cells respond to the same range of soluble and extracellular-matrix-derived mitogens (Eccleston *et al.* 1987). They both respond to fibroblast growth factor and glial growth factor but not to epidermal growth factor. In addition, laminin, fibronectin and a basement membrane-like extracellular matrix stimulate the proliferation of both enteric glial cells and Schwann cells and both divide in response to cAMP and cholera toxin (Eccleston *et al.* 1987).

In development, it is conceivable that the same axon might sequentially act, first as a mitogen to expand cell numbers and then in a more mature state, as an inhibitor of cell division when the correct number of glial cells has been attained. In culture, a bound growth factor might mask any inhibitory effect that the axon would otherwise exert on peripheral glial cells. Thus, the results presented here might not simply indicate that enteric and sensory neurons differ in their control of glial cell proliferation, but could be revealing an inhibitory signal common to all neurons under the correct conditions. Indeed, a precedent for this exists in the CNS where both axolemmal fragments and granule neurons have been shown to inhibit the proliferation of astrocytes in culture (Sobue and Pleasure, 1984; Hatten, 1987).

Cerebellar granule neurons inhibit astrocyte division in the CNS via a non-diffusible neuronal membrane molecule (Hatten, 1985, 1987; Edmondson *et al.* 1988). We were unable to use the method of Hatten (1987), where fixed neurons were added to glial cells to determine whether the inhibitory molecule was membrane bound, since sufficient numbers of enteric neurons could not be purified by present methods. Attempts to use neurons, fixed directly on coverslips, using the same procedure as described for experiments reported here using unfixed neurons have so far failed since the neurons fixed using a mild treatment with paraformaldehyde began to disintegrate before the experimental procedure was complete. However, only glial cells close to the neuronal cell area showed a reduced DNA synthesis rate. Those in the periphery of the cultures had labelling indices that were the same as controls where no neurons were present. This indicates that a membrane-bound growth inhibitor is likely to be involved in the inhibition of glial cell DNA synthesis by enteric neurons.

When cultures of Schwann cells or enteric glia were added to pure populations of enteric neurons glial cell division was inhibited. In explant cultures of myenteric plexus that contained both enteric neurons and enteric glia, the results were somewhat different. The inhibition of glial cell DNA synthesis was only detectable close to the neuronal cell bodies i.e. in the central neuronal area of the cultures and in a zone approximately 1 mm wide surrounding the neuronal area. If a membrane-associated molecule is involved in mitotic inhibition, these results indicate that it may be present at a higher concentration over the cell bodies than in the neurites. Alternatively, the very fine network of neurites growing over the glial carpet may not provide a sufficient area of contact for inhibition to be initiated.

Since the enteric neuron and its neurites do not stimulate enteric glial cell proliferation the mechanism of generation of glial cells in development of the ENS may be different from that for Schwann cells, where *in vitro* evidence suggests that the axon plays an important role in expanding cell populations (Salzer and Bunge, 1980; Salzer *et al.* 1980; Wood, 1976). The present work suggests that the generation of adequate numbers of enteric glia depends more on soluble mitogenic growth factors than on growth signals associated with neuronal membranes.

This work was supported by the Medical Research Council of Great Britain, Action Research for the Crippled Child and the Multiple Sclerosis Society of Great Britain. We thank Drs A. F. Williams and J. Cohen for their gifts of anti-Thy1.1 and LB1 respectively and M. Hardy for preparation of axolemma.

References

Bannerman, P. G. C., Mirsky, R. & Jessen, K. R. (1987). Analysis of enteric neurons, glia and their interactions using explant cultures of the myenteric plexus. *Dev. Neurosci.* **9**, 201–227.

Bannerman, P. G. C., Mirsky, R. & Jessen, K. R. (1988*a*). Antigenic markers and laminin expression in cultured enteric neural cells. *Brain Res.* **440**, 87–98.

Bannerman, P. G. C., Mirsky, R. & Jessen, K. R. (1988*b*). Establishment and properties of separate cultures of enteric neurons and enteric glia. *Brain Res.* **440**, 99–108.

Braun, L., Mead, J. E., Panzica, M., Mikumo, R., Bell, G. I. & Fausto, N. (1988). Transforming growth factor mRNA increases during liver regeneration: A possible paracrine mechanism of growth regulation. *Proc. natn. Acad. Sci. U.S.A.* **85**, 1539–1543.

Brockes, J. P., Lemke, G. E. & Balzer, D. R. (1980). Purification and preliminary characterization of glial growth factor from bovine pituitary. *J. biol. Chem.* **255**, 8374–8377.

Brockes, J. P., Fields, K. L. & Raff, M. C. (1979). Studies on cultured Schwann cells. 1. Establishment of purified populations from cultures of peripheral nerve. *Brain Res.* **165**, 105–118.

Cullen, M., De Vries, G. & Webster, H. (1981). Freeze fracture of isolated myelin and axolemma membrane fractions. *Brain Res.* **229**, 311–322.

De Vries, G. H. (1981). Isolation of axolemma enriched fractions from mammalian CNS. In (ed. N. Marks and R. Rodnight) Research Methods in Neurochemistry, vol. 5, Plenum Press, New York, pp. 3–38.

De Vries, G. H., Salzer, J. L. & Bunge, R. P. (1982). Axolemma-enriched fractions isolated from PNS and CNS are mitogenic for cultured Schwann cells. *Dev. Brain Res.* **3**, 295–299.

Eccleston, P. A., Jessen, K. R. & Mirsky, R. (1987). Control of peripheral glial cell proliferation: A comparison of the division rates of enteric glia and Schwann cells and their response to mitogens. *Devl Biol.* **124**, 409–417.

Edmondson, J. C., Liem, R. K. H., Kuster, J. E. & Hatten, M. E. (1988). Astrotactin: A novel neuronal cell surface antigen that mediates neuron-astroglial interactions in cerebellar micro cultures. *J. Cell Biol.* **106**, 505–517.

Gabella, G. (1971). Glial cells in the myenteric plexus. *Z. Naturforsch.* **26b**, 244–245.

Gabella, G. (1981). Ultrastructure of the nerve plexuses of the mammalian intestine: The enteric glial cells. *Neuroscience* **6**, 425–436.

Hatten, M. E. (1985). Neuronal regulation of astroglial morphology and proliferation in vivo. *J. Cell Biol.* **100**, 384–396.

Hatten, M. E. (1987). Neuronal regulation of astroglial cell proliferation is membrane mediated. *J. Cell Biol.* **104**, 1353–1360.

JESSEN, K. R., SAFFREY, M. J. & BURNSTOCK, G. (1983). The enteric nervous system in tissue culture. 1. Cell types and their interactions in explants of the myenteric plexus and submucous plexuses from guinea pig, rabbit and rat. *Brain Res.* **262**, 17–35.

JESSEN, K. R., MIRSKY, R. & MORGAN, L. (1987*a*). Axonal control of Schwann cell phenotype: an immunohistochemical study of the glycolipid galactocerebroside in transected and regenerating nerves. *J. Neurosci.* **7**, 3362–3369.

JESSEN, K. R., MIRSKY, R. & MORGAN, L. (1987*b*). Myelinated, but not unmyelinated axons reversibly down-regulate N-CAM in Schwann cells. *J. Neurocytol.* **16**, 681–688.

LOBB, R. R., HARPER, J. W. & FETT, J. W. (1986). Purification of heparin-binding growth factors. *Anal. Biochem.* **154**, 1–14.

MASON, P. W., BIGBEE, J. W. & DE VRIES, G. H. (1989). Cerebellar granule cells contain a membrane mitogen for cultured Schwann cells. *J. Cell Biol.* **108**, 607–611.

MIRSKY, R., GAVRILOVIC, J., BANNERMAN, P. G. C., WINTER, J. & JESSEN, K. R. (1985). Characterization of a plasma membrane protein shared by non-myelin forming PNS and CNS glia, a subpopulation of PNS neurons, perineural cells and smooth muscle. *Cell Tissue Res.* **240**, 723–733.

PLEASURE, D., KREIDER, B., SHUMAN, S. & SOBUE, G. (1985). Tissue culture studies of Schwann cell proliferation and differentiation. *Dev. Neurosci.* **7**, 364–373.

PORTER, S., CLARKE, B., GLASER, L. & BUNGE, R. P. (1986). Schwann cells stimulated to proliferate in the absence of neurons retain full functional capability. *J. Neurosci.* **6**, 3070–3078.

PORTER, S., GLASER, L. & BUNGE, R. P. (1987). Release of autocrine growth factor by primary and immortalized Schwann cells. *Proc. natn. Acad. Sci. U.S.A.* **84**, 7768–7772.

RATNER, N., BUNGE, R. P. & GLASER, L. (1985). A neuronal heparan sulfate proteoglycan is required for dorsal root ganglion neuron stimulation of Schwann cell proliferation. *J. Cell Biol.* **101**, 744–754.

RATNER, N., HONG, D., LIEBERMAN, M. A., BUNGE, R. P. & GLASER, L. (1988). The neuronal cell-surface molecule mitogenic for Schwann cells is a heparin-binding protein. *Proc. natn. Acad. Sci. U.S.A.* **85**, 6992–6996.

RYBAK, S. M., LOBB, R. R. & FETT, J. W. (1988). Comparison of the effects of class 1 and class 2 heparin-binding growth factors on protein synthesis and actin mRNA expression in BALB/c-3T3 cells. *J. cell Physiol.* **136**, 312–318.

SALZER, J. L. & BUNGE, R. P. (1980). Studies of Schwann cell proliferation. 1. An analysis of proliferation during development, Wallerian degeneration and direct injury. *J. Cell Biol.* **84**, 739–752.

SALZER, J. L., WILLIAMS, A., GLASER, L. & BUNGE, R. P. (1980). Studies on Schwann cell proliferation. II. Characterization of the stimulation and specificity of the response to a neurite membrane fraction. *J. Cell Biol.* **84**, 753–766.

SOBUE, G. & PLEASURE, D. (1984). Astroglial proliferation and phenotype are modulated by neuronal plasma membrane. *Brain Res.* **324**, 175–179.

SPORN, M. B., ROBERTS, A. B., WAKEFIELD, L. M. & DE CROMBRUGGHE, B. D. (1987). Some recent advances in the chemistry and biology of transforming growth factor-beta. *J. Cell Biol.* **105**, 1039–1045.

WANG, J. L. & HSU, Y-M. (1986). Negative regulators of cell growth. *Trends in Biochem. Sci.* **11**, 24–26.

WOOD, P. M. (1976). Separation of functional Schwann cells and neurons from normal peripheral nerve tissue. *Brain Res.* **115**, 316–375.

WOOD, J. N., WINTER, J., JAMES, I. F., RANG, H. P., YEATS, J. & BEVAN, S. (1988). Capsaicin-induced ion fluxes in dorsal root ganglion cells in culture. *J. Neurosci.* **8**, 3208–3220.

(*Accepted 26 June 1989*)