

A Neuronal Cell Surface Heparan Sulfate Proteoglycan Is Required for Dorsal Root Ganglion Neuron Stimulation of Schwann Cell Proliferation

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ABSTRACT Axons of dorsal root ganglion neurons express on their surfaces one or more proteins which are mitogenic for Schwann cells (Salzer, J., R. P. Bunge, and L. Glaser, 1980, *J. Cell Biol.*, 84:767–778). Incubation of co-cultures of dorsal root ganglion neurons and Schwann cells with 4-methylumbelliferyl- β -D-xyloside, an inhibitor of proteoglycan biosynthesis, decreases the mitogenic response of the Schwann cell by over 95%. The effect of the β -D-xyloside has been localized to the neurons; pretreatment of neurons but not of Schwann cells with the inhibitor causes a marked reduction of the mitogenic response. In addition, Schwann cells treated with β -D-xyloside are still mitogenically responsive to soluble Schwann cell mitogens (cholera toxin and glial growth factor). Neurons treated with heparitinase and membrane vesicles prepared from heparitinase-treated neurons show diminished mitogenicity for Schwann cells, while other proteoglycan lyases have no effect. We conclude that a cell surface heparan sulfate proteoglycan is a component of the Schwann cell mitogen present on the surface of dorsal root ganglion neurons.

During embryonic development of the peripheral nerve trunks, Schwann cells proliferate along the axons which they will later ensheath and, in the case of larger axons, myelinate. This early proliferative response can be mimicked in vitro using cultures containing pure populations of embryonic rat dorsal root ganglion neurons and Schwann cells (Wood, 1976). The proliferative response of Schwann cells to axons can also be observed in serum-free medium; under these culture conditions Schwann cells relate to axons but do not ensheath or myelinate the nerve fibers (Moya et al., 1980). It has previously been shown that cell-cell contact is required for the mitogenic response of Schwann cells to neurons (Salzer et al., 1980a). Furthermore, neurite membrane fractions are mitogenic for Schwann cells, whereas conditioned medium from dorsal root ganglion cultures is not (Salzer et al., 1980b). One or more of the components on the neuron surface required for this response are proteins, since they are sensitive to treatment with trypsin (Salzer et al., 1980a). It has recently been reported that axolemmal preparations from brain (Cassel et al., 1982; DeVries et al., 1983; Sobue et al., 1983), as well as PC12 cells (a rat pheochromocytoma) (Ratner et al., 1984) provide mitogenic stimuli for Schwann cells. In none of these

cases has the mitogenic moiety been identified.

Specific inhibitors of macromolecular synthesis can provide additional insights into the chemical nature of the Schwann cell mitogen present on the surface of the dorsal root ganglion neurites, and thereby aid in the purification of these molecules.

In this paper, we describe the use of 4-methylumbelliferyl- β -D-xyloside, a competitive substrate for the galactosyl transferase required for the synthesis of the gal-gal-xylo oligosaccharide which links the carbohydrate chains of proteoglycans to the core proteins (Robinson et al., 1975; Galligani et al., 1975). It has been demonstrated that the effect of xylosides is specific for the β -D-xyloside anomeric linkage and glycone (Robinson et al., 1975; Galligani et al., 1975). In all systems studied, treatment of cells in culture with permeable β -D-xyloside results in accumulation of free glycosaminoglycan chains in the media (Galligani et al., 1975; Lohmander and Hascall, 1979; Thompson and Spooner, 1983; Kanwar et al., 1984), as well as inhibition of glycosaminoglycan addition to proteoglycan core proteins. Synthesis of all the xylose-linked proteoglycans is potentially inhibited by the addition of exogenous β -D-xyloside, including chondroitin sulfate, derma-

tan sulfate, and heparan sulfate, all of which have been localized to cell surfaces. For example, a chondroitin sulfate proteoglycan has been localized on fibroblast surfaces (Hedman et al., 1983), several cell surface-derived heparan sulfate proteoglycans have been isolated from various sources (Carlstedt et al., 1983, and references therein), and a dermatan sulfate proteoglycan has been isolated from rat ovarian granulosa cells (Yanagishita and Hascall, 1984). Cell-associated proteoglycans of neuronal origin have also been identified; these appear to be predominantly of the heparan sulfate class (Lander et al., 1982; Hampson et al., 1984; Maresh et al., 1984; Morris, 1984) and heterogeneous with respect to molecular size and glycosaminoglycan fine structure. One chondroitin sulfate/dermatan sulfate proteoglycan has also been identified (Aquino et al., 1984; Morris, 1984). We now present evidence that a surface-attached heparan sulfate proteoglycan is a component of the axonal mitogen for Schwann cells. Our evidence is based on use of 4-methylumbelliferyl- β -D-xyloside as an inhibitor of proteoglycan biosynthesis, and on specific digestion of the neuronal cell surface proteoglycans with hydrolytic enzymes.

MATERIALS AND METHODS

Cell Culture (Neurons): Embryonic day 15 rat fetuses were obtained from timed pregnancies from a local supplier. Spinal cords were removed from the embryos by the method described by Salzer et al. (1980b), so that dorsal root ganglia remain attached to the cord. Ganglia were then removed from the cords in sterile L 15 medium containing 10% fetal calf serum. Using this method, 800 dorsal root ganglia can easily be obtained from 20 spinal cords in 2 h. Ganglia are dissociated by incubation in 0.25% trypsin (Cooper Biomedical, Inc., Malvern, PA) in $\text{Ca}^{++}/\text{Mg}^{++}$ -free Hank's buffered salt solution (CMF)¹ for 30 min at 37°C. Several rinses in serum-containing medium (Dulbecco's modified Eagle's medium [DME] with 10% fetal bovine serum (KC Biological, Lenexa, KS), 6 mg/ml glucose, 2 mM glutamine, 10–20 units of nerve growth factor) were followed by trituration of ganglia through a narrow-bore glass pipette, which generates a single cell suspension. Cells were plated at five-ganglia equivalents in a single small drop onto the center of nitric acid-washed glass cover slips (22 mm) (Bellco Glass, Inc., Vineland, NJ), coated with ammoniated rat tail collagen. Alternatively, 40-ganglia equivalents of cells were plated in 3 ml of serum-containing medium onto collagen-coated tissue culture plastic lids from 35-mm dishes. In either case, cultures were re-fed the following day with antimetabolites (DME with 10% human placental serum, 6 mg/ml glucose, 2 mM glutamine, 10–20 units of nerve growth factor, 10^{-5} M uridine, 10^{-5} M fluorodeoxyuridine). 10^{-5} M cytosine arabinoside was added to this medium for 24 h in the case of the mass (40 ganglia/35-mm dish) cultures. This treatment was found to be essential in order to generate supporting cell-free cultures of neurons in these dense cultures. In both types of cultures, antimetabolic treatment was continued for 10 d, then cells were pulsed for 2–3 d with serum-containing medium, and returned to antimetabolites for an additional 1–2 wk. In the absence of nonneuronal cells, we have observed degeneration of neurons when cultures are maintained longer than 3–4 wk.

Schwann Cell Culture: All experiments described in this paper have been repeated on two populations of Schwann cells: those derived from dorsal root ganglia, which are prepared exactly as described in Ratner et al. (1984), and those derived from sciatic nerve, essentially as described by Brockes et al. (1979). Sciatic nerve-derived cells were transferred to cytosine arabinoside-containing medium (10^{-5} M) for 48 h prior to anti-thy 1.1 treatment and complement lysis of contaminating fibroblasts. We have found culture supernatant from the cell line ATCC TIB 103 (Lake et al., 1979) (American Type Culture Collection, Rockville, MD) to be an effective source of anti-thy 1.1. Suspensions of Schwann cells from either of these two sources were either replated onto glass multiwell Lab-Tek slides (Miles Laboratories, Inc., Elkhart, IN) and allowed to recover for 2 d prior to the mitogenicity assay (see below) or added to dissociated neurons on glass coverslips. The difference between the two Schwann cell populations was found to be solely quantitative; that is, the sciatic nerve cells were consistently more responsive to neurons or neuronal membranes than dorsal root ganglion-derived Schwann cells (e.g., contrast

Table II, in which dorsal root ganglion-derived Schwann cells were used, with Fig. 4, in which sciatic nerve-derived cells were used to do a similar experiment).

Co-culture Experiments: $2-3 \times 10^4$ Schwann cells were added to dorsal root ganglion neurons on collagen-coated coverslips, which by the time of addition contained extensive neuritic outgrowth between neuronal somas in the original drop and also extending radially from the original drop. After 24 h of co-culture in normal serum-containing medium, Schwann cells settled onto neurons and began to proliferate. Cultures were then re-fed with serum-free medium (Bottenstein and Sato, 1979) for 3–4 d in order to expand populations of Schwann cells. At the end of this time, many neurites were sparsely covered with Schwann cells, and maximal proliferation of Schwann cells was occurring.

Mitogenicity Assays: Schwann cells plated onto Lab-Tek glass slides at a concentration of $1.5-2 \times 10^4$ cells/well (8-well slides, 0.83 cm² wells) were allowed to recover from trypsinization for 48 h, then soluble mitogens or membrane fractions were added for 16 h in DME, 10% fetal calf serum, penicillin (50 U/ml), streptomycin (50 $\mu\text{g}/\text{ml}$), and fungizone (2.5 $\mu\text{g}/\text{ml}$), all obtained from Gibco Laboratories, Grand Island, NY). Fungizone prevented contamination of wells by yeast which was a serious problem since we have stopped UV-irradiating membranes (Salzer et al., 1980b) prior to addition to assays, because we observed that UV treatment caused variable and often profound inactivation of the mitogenic activity of the membranes. Membranes were readded to wells for an additional 24 h in fresh medium containing 2 $\mu\text{Ci}/\text{ml}$ [³H]thymidine (6.7 Ci/mol, New England Nuclear, Boston, MA) and dialyzed fetal calf serum.

Fixation and Autoradiography: 24 h later, media was aspirated from the wells and replaced with 4% glutaraldehyde (EM grade, Sigma Chemical Co., St. Louis, MO) in Tris-buffered saline. After 1 h at room temperature, wells were washed four times over a 45-min period with water; the attached gasket was removed from the slides; and the slides were air dried, dipped with Kodak NTB-2 emulsion and exposed for 72 h. Slides were then developed and stained with toluidine blue as detailed in Salzer and Bunge (1980). 400–1,000 cells were counted per well to determine percent labeled nuclei, and duplicate samples were counted in each experiment. Coverslips containing co-cultures were fixed and washed as described for slides, then lifted out of Linbro wells using forceps, and dried in a laminar flow hood, mounted culture-side up onto a standard microscope slide in DPX mountant (Gallard-Schlesinger Chemical Mfg. Corp., Carle Place, NY) and allowed to dry overnight before dipping in emulsion. At least 600 cells were counted in duplicate cultures in these experiments.

Inhibitor Studies: α -Methyl xyloside, 4-methylumbelliferyl- β -D-xyloside, and 4-methylumbelliferyl- α -L-arabinoside (obtained from Sigma Chemical Co.) were dissolved at 100 mM in dimethyl sulfoxide. Stock solutions were stored in the refrigerator, from which they were added to medium, filter sterilized, and used as described in the text.

Enzyme Digestions: Chondroitinase ABC and heparitinase were obtained from Miles Laboratories Inc. and heparinase was obtained from either Miles Laboratories Inc. or Sigma Chemical Co. Enzymes were stored as suggested by the supplier. Chondroitinase digestions were carried out in CMF since this enzyme can be inhibited by Ca^{++} (Banerjee et al., 1977). In control experiments, treatment with the salt solution alone had no effect on mitogenicity of cultures (data not shown). Heparitinase and heparitinase digestions were carried out in serum-free medium, since these enzymes are activated and/or stabilized by Ca^{++} . Digestions of cell surface protein using 238 U/mg TPCK trypsin (Cooper Biomedical, Inc.) used 1 mg/ml of enzyme in CMF, and were stopped after 30 min by removal of enzyme solution followed by addition of CMF containing 10 mg/ml soybean trypsin inhibitor (Sigma Chemical Co.). This relatively high concentration of trypsin is necessary to completely destroy mitogenic activity (Salzer et al., 1980a).

[³⁵S]-H₂-SO₄ Labeling of Cultures: 4-wk-old dissociated dorsal root ganglion neuron cultures on plastic lids, which were observed using phase-contrast optics to be free of nonneuronal cells (<1 nonneuronal cells/5 10 \times fields) were labeled in serum-free medium (Bottenstein and Sato, 1979) made with SO₄-free DME (in which MgSO₄ is exchanged for MgCl₂), instead of normal DME, and containing 800 $\mu\text{Ci}/\text{ml}$ [³⁵S]-H₂SO₄, added from a 15-mCi/ml stock obtained from New England Nuclear. The final concentration of sulfate in this medium is 3.7 μM . Cultures were labeled for ~20 h, and incorporated in 1–2 $\times 10^4$ trichloroacetic acid-precipitable dpm/culture.

Precipitations with Trichloroacetic Acid: 5- μl aliquots of extracted cultures or membrane preparations were spotted onto Whatman No. 3 paper squares (Whatman Laboratory Products Inc., Clifton, NJ) and placed into a container of 10% trichloroacetic acid, heated at 100°C for 10 min, cooled on ice, rinsed with 10% trichloroacetic acid, acetone, and counted in 3a70 scintillation cocktail (Research Products International Corp., Mt. Prospect, IL).

Cell Extracts: After sulfate labeling, dorsal root ganglion cultures were washed 3 times with 1–2 ml ice cold CMF, scraped up from the tissue culture

¹ Abbreviations used in this paper: CMF, $\text{Ca}^{++}/\text{Mg}^{++}$ -free Hank's buffered salt solution; DME, Dulbecco's modified Eagle's medium.

plastic with a forceps (along with the collagen substrate) and placed into a microfuge tube with 200 μ l of extraction buffer (CMF, 1 mM CaCl₂, 1 mM MgCl₂, 1 mg/ml soybean trypsin inhibitor, 1 mM phenylmethylsulfonyl fluoride, 0.5% Triton X-100, 0.1% SDS). Cells were extracted on ice for 5 min, then centrifuged in the microfuge to remove intact nuclei and collagen. Supernatants were collected, radioactivity was determined by trichloroacetic acid precipitation, and supernatants were loaded onto polyacrylamide gels.

Membrane Preparation: Mass dorsal root ganglion cultures on collagen-coated plastic were rinsed 2–3 times with CMF, scraped from the plastic along with the collagen substrate, and placed into a 1-ml dounce homogenizer (Wheaton Scientific, Millville, NJ) containing 0.7 ml of ice cold buffer (0.25 M sucrose, 0.1 mM MgCl₂, 10 mM Tris-HCl, pH 7.4, 17 mU/ml aprotinin). The cells were disrupted with 50 strokes of the homogenizer, then centrifuged in a disposable borosilicate tube (10 \times 75-mm) for 10 min at 1,500 rpm in a tabletop clinical centrifuge at room temperature. The supernatant was carefully removed into a 0.65-ml nitrocellulose tube and placed into adaptors for an SW50.1 rotor and centrifuged at 45,000 rpm for 30 min at 4°C. The resulting pellet was resuspended in 100–300 μ l of the same buffer; the yield of membranes from 3–4-wk-old cultures varied with the percent surviving neurons, but was usually in the range of 100–300 μ g membrane protein/culture as assayed by the modification of the Lowry procedure for membrane proteins (Markwell et al., 1978).

PAGE and Fluorography: Gels were prepared as described (Laemmli, 1970). 6% resolving gels contained 2.5% stacking gels, and 15% and 10% gels had 5% stacking gels. Four times solubilization mix contained 40% glycerol, 20% β -mercaptoethanol, 20 mM Tris base, pH 8.0, 12% SDS. After electrophoresis at 50 mA/slab, gels were fixed in 10% acetic acid, 25% isopropanol and prepared for fluorography using EN³HANCE (New England Nuclear). Gels were dried and exposed to Kodak XAR film at –70°C.

Soluble Schwann Cell Mitogens: Cholera toxin (obtained from Sigma Chemical Co.) was solubilized and used within 2 mo of solubilization. Glial growth factor was prepared from lyophilized bovine pituitary glands (Pel-Freeze Biologicals, Rogers, AR) as described by Brookes et al. (1980). The crude CM-cellulose fraction was used in these experiments.

RESULTS

In initial experiments, Schwann cells and dissociated dorsal root ganglion neurons were co-cultured in serum-free media for several days (see Materials and Methods). These cultures, with vigorously proliferating populations of Schwann cells, were incubated for 72 h with 1 mM 4-methylumbelliferyl- β -D-xyloside. During the last 24 h, 2 μ Ci/ml [³H]thymidine were added to control and drug-treated cultures. Fig. 1 shows an example of such a culture, in which the percent of Schwann cells whose nuclei were labeled with [³H]thymidine (as assayed by autoradiography) decreased by 80% in the presence of the drug (Fig. 1B) as compared to the control (Fig. 1A), in which the percent labeled nuclei was 39.5%.

The unexpected effect of an inhibitor of proteoglycan biosynthesis on mitogenicity prompted us to undertake several control experiments to ascertain that the effect of the inhibitor was specific. The effects of α -methyl-D-xyloside and 4-methylumbelliferyl- α -L-arabinoside on Schwann cell proliferation was compared with that of 4-methylumbelliferyl- β -D-xyloside; α -methyl-D-xyloside has previously been shown to be ineffective as a substrate for galactosyl transferase (Robinson et al., 1975) and 4-methylumbelliferyl- β -D-arabinoside was included to control for the enhanced lipophilicity of the umbelliferyl moiety. Neither glycoside at 1 mM was able to substitute for 4-methylumbelliferyl- β -D-xyloside in inhibiting Schwann cell proliferation (Table I), suggesting that 4-methylumbelliferyl- β -D-xyloside is acting specifically in this system.

If β -xyloside is blocking proteoglycan assembly as described previously for other cell types, β -D-xyloside-linked glycosaminoglycans should accumulate in the culture media in the presence of the drug (Galligani et al., 1975; Lohmander and Hascall et al., 1979; Thompson and Spooner, 1983). Therefore cultures of dorsal root ganglion neurons, free of nonneu-

ronal cells, were labeled with [³⁵S]-SO₄, and the macromolecules present in the culture medium of control and xyloside-treated cultures were analyzed by PAGE followed by fluorography. As shown in Fig. 2, several [³⁵S]-SO₄-labeled high molecular weight bands can be observed in media from control cultures. These presumably represent neuronally derived secreted proteoglycans, since they are completely absent in 4-methylumbelliferyl- β -D-xyloside-treated cultures. In contrast, in inhibitor-treated cultures, considerable radioactivity now migrates near the dye front. Fig. 2B demonstrates that this material is sensitive to degradation by chondroitinase ABC. Some sensitivity to heparitinase is also observed although no glycosaminoglycan chains remain after chondroitinase ABC digestion. Since commercial heparitinase is contaminated by chondroitinase (Dr. A. Caplan, Case Western Reserve, Cleveland, Ohio, personal communication), we ascribe this degradation to contamination of the enzyme which is apparent at the high levels of enzyme used to ensure complete degradation of glycosaminoglycan chains.

We determined the dose of 4-methylumbelliferyl- β -D-xyloside necessary to inhibit Schwann cell proliferation (Fig. 3). Half-maximal inhibition of Schwann cell proliferation occurred at 0.7 mM 4-methylumbelliferyl- β -D-xyloside; 80–85% inhibition is routinely obtained using 1-mM concentrations of inhibitor.

To test whether the inhibitor is affecting the neuron, the Schwann cell, or both cell types, cultures of either neurons alone or Schwann cells alone were treated with 1 mM 4-methylumbelliferyl- β -D-xyloside or 1 mM α -methyl-D-xyloside, after which the two cell types were recombined in the presence or absence of inhibitor (Table II). Preincubation of neurons with 4-methylumbelliferyl- β -D-xyloside inhibits the mitogenic response by ~80% when the inhibitor is not present during the co-culture of the two cell types, and essentially totally when present throughout the experiment, suggesting that neurons are affected by treatment with the drug and that significant recovery of the mitogen(s) on the neuronal surface can take place during the 24-h co-culture. In contrast, preincubation of Schwann cells for 4 d with 4-methylumbelliferyl- β -D-xyloside did not prevent their mitogenic response when added back to neurons in the absence of inhibitor, suggesting that the inhibitor either has no effect on Schwann cells or that the recovery from the inhibitor can be extremely rapid.

Two types of experiments indicate that the Schwann cell response is not affected by treatment with 4-methylumbelliferyl- β -D-xyloside. First, protein synthesis in drug-treated Schwann cells is close to normal: 1 mM 4-methylumbelliferyl- β -D-xyloside-treated Schwann cells incorporated [³⁵S]methionine into trichloroacetic acid-precipitable material at 88% of control levels (quadruplicate cultures assayed, data not shown). Second, as shown in Table III, replated Schwann cells are competent to respond to soluble Schwann cell mitogens (cholera toxin and glial growth factor) (Raff et al., 1978) in the presence of the drug, indicating that 4-methylumbelliferyl- β -D-xyloside treatment does not prevent the Schwann cell from traversing the cell cycle. However, it is possible that the receptor for the neuronal mitogen which we presume exists on the Schwann cell surface might be specifically affected by the drug, especially since Schwann cells in culture have been shown to synthesize two proteoglycans, one of which appears to be associated with the cell surface and contains heparan sulfate (as well as chondroitin sulfate) chains (Mehta, H., C. Orphe, M. S. Todd, C. J. Cornbrooks, and D. J. Carey,

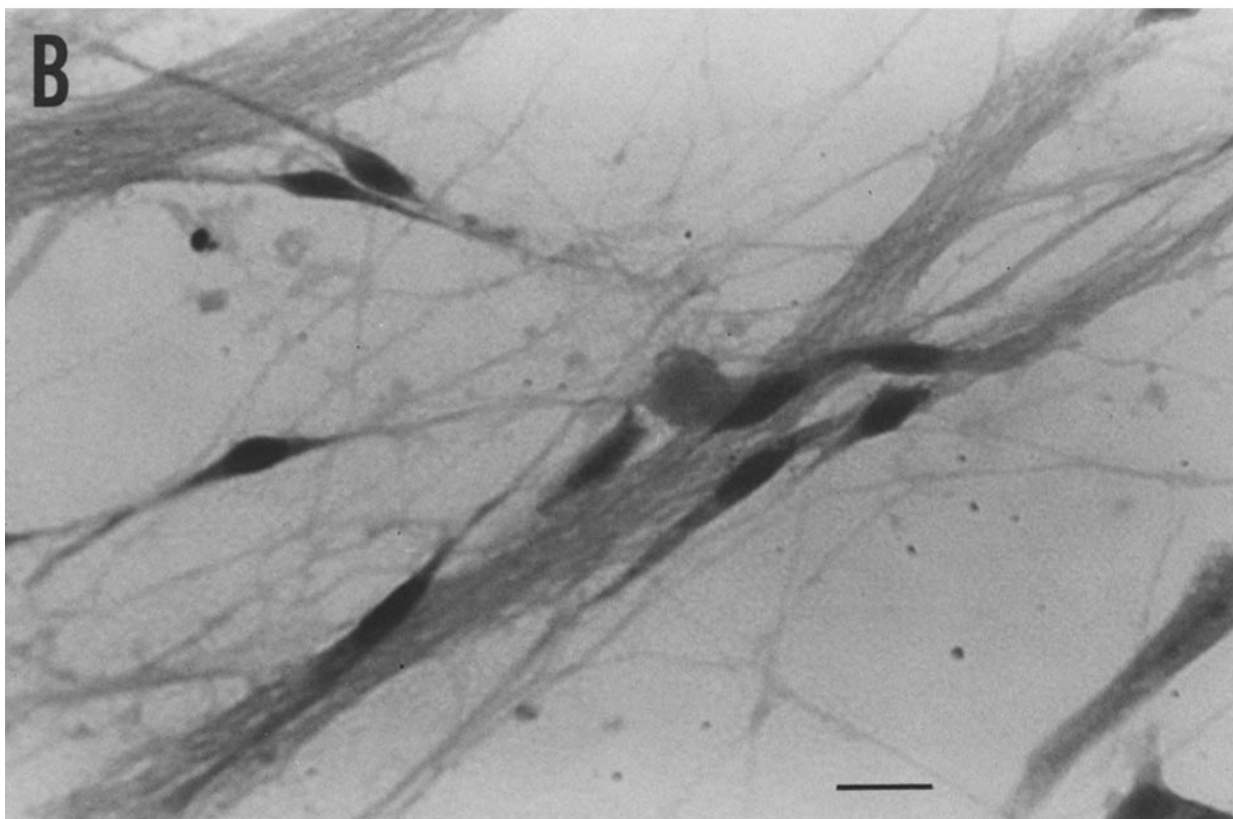
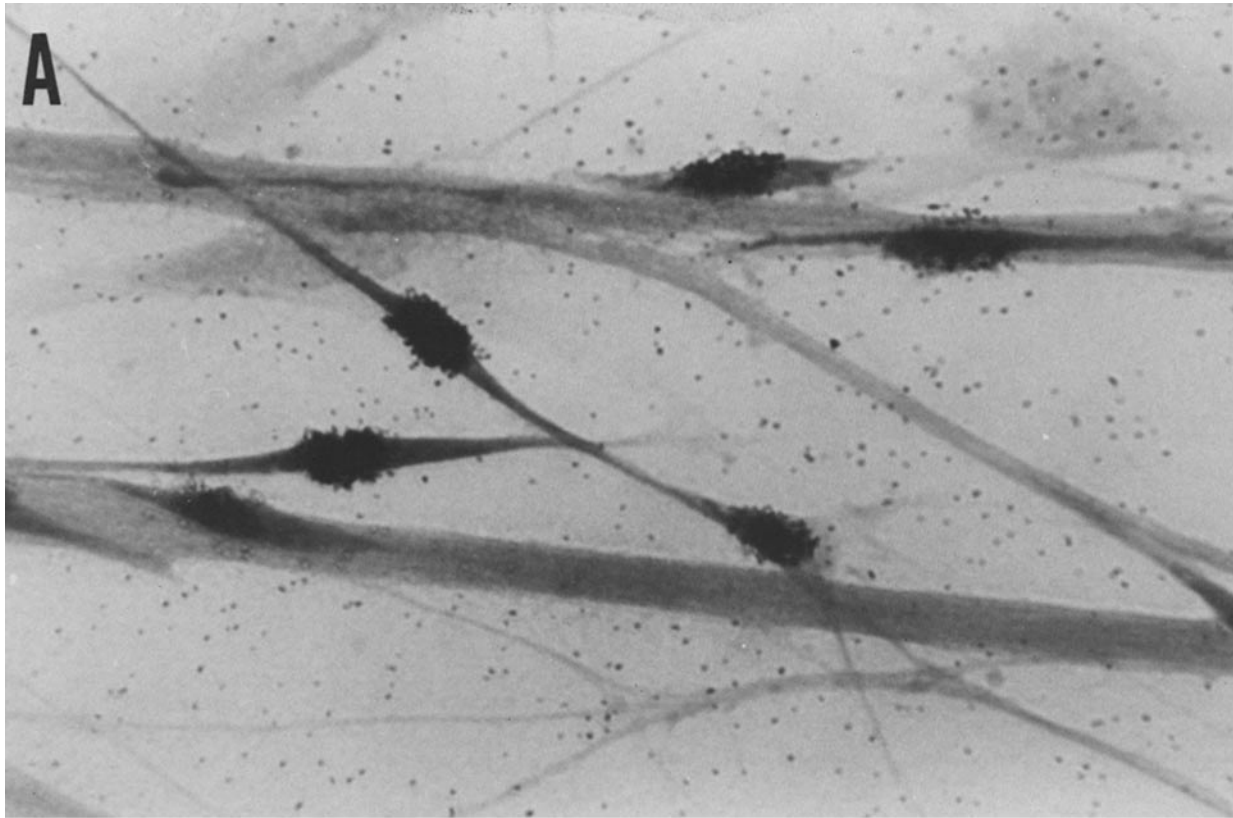


FIGURE 1 Treatment with 1 μ M 4-methylumbelliferyl- β -D-xyloside decreases the fraction of proliferating Schwann cells in neuron-Schwann cell cultures. (A) Bright field photomicrograph of toluidine blue-stained autoradiograph of a control neuron-Schwann cell culture. Neurite bundles are shown, with Schwann cell nuclei, both labeled and unlabeled, associated with fascicles. (B) Parallel culture treated for 72 h with 1 mM 4-methylumbelliferyl- β -D-xyloside before fixation and autoradiography. Schwann cells are associated with neurites as in the control, yet few labeled nuclei are observed. Bar, 10 μ m.

TABLE I. Effect of Xylosides on Schwann Cell Proliferation

Addition	Experiment		
	1	2	3
Control	39.5	19.6	52.0
4-Methylumbelliferyl- β -D-xyloside	2.2	0.3	12.5
α -Methyl-D-xyloside	ND	21.2	ND
4-Methylumbelliferyl- α -L-arabinoside	ND	ND	48.0

Schwann cells were co-cultured with dorsal root ganglion neurons as described in Materials and Methods. Where indicated, 1 mM inhibitors were added for 72 h, and [3 H]thymidine was present during the last 24 h. After fixation and autoradiography, the percentage of Schwann cell nuclei which has incorporated [3 H]thymidine was determined. ND, not done.

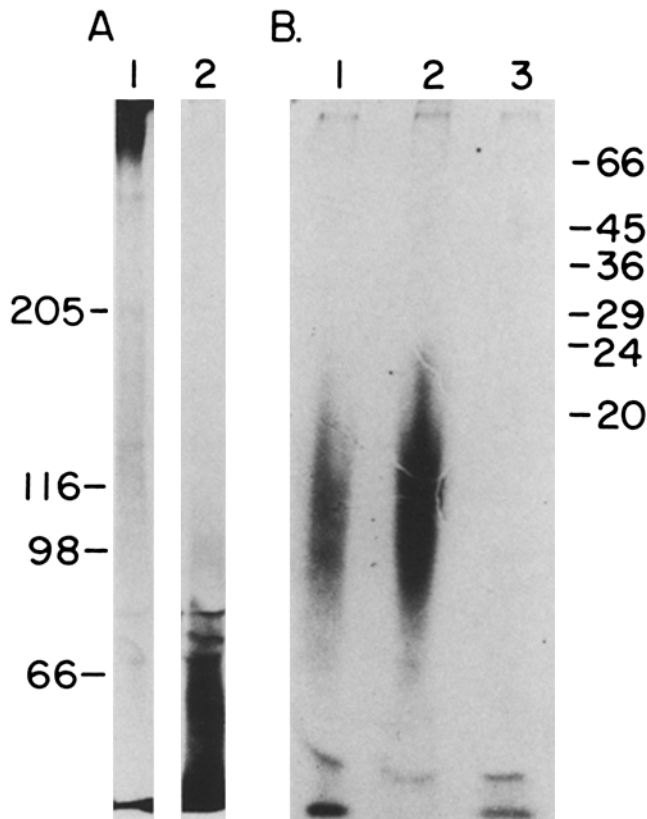


FIGURE 2. Analysis of secreted dorsal root ganglion neuronal proteoglycans. Neurons are metabolically labeled with [35 S]-SO $_4$ in serum-free, SO $_4$ -free medium, and 100- μ l aliquots of medium were analyzed by electrophoresis on a 6% polyacrylamide gel, followed by fluorography. (A) Lane 1, control medium. Lane 2, medium from a culture to which 1 mM 4-methylumbelliferyl- β -D-xyloside was added along with the isotope. Molecular masses in the left margin indicate the positions of migration of myosin (205 kD), β -galactosidase (116 kD), phosphorylase b (98 kD), and bovine serum albumin (66 kD). (B) Aliquots (10 μ l) of conditioned medium from β -D-xyloside-treated neurons were digested with glycosaminoglycan hydrolases (0.25 units) for 2.5 h, separated on 15% acrylamide gels, and subjected to fluorography. These concentrations of enzymes are 100 times those used to digest cell surfaces to ensure complete degradation. Lane 1, heparitinase digested. Lane 2, heparinase digested (same as control). Lane 3, chondroitinase ABC digested. Molecular masses in the right margin indicate the positions of migration of bovine serum albumin (66 kD), ovalbumin (45 kD), pepsin (36 kD), carbonic anhydrase (29 kD), trypsinogen (24 kD), and trypsin inhibitor (20 kD). Molecular masses refer to proteins and cannot be taken to directly correspond to the molecular mass of proteoglycans, which migrate abnormally in SDS gels due to their intrinsic negative charge.

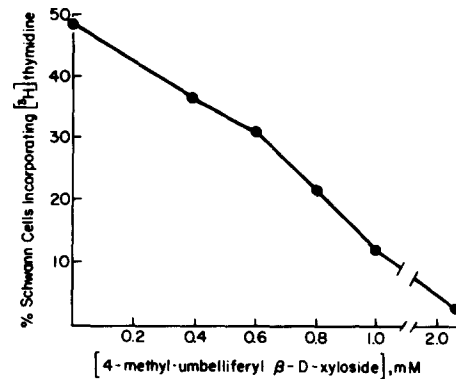


FIGURE 3. Dose-response relationship of inhibition of Schwann cell proliferation to increasing concentrations of 4-methylumbelliferyl- β -D-xyloside. Schwann cell-neuron cultures in serum-free medium were treated with 4-methylumbelliferyl- β -D-xyloside. After a total of 72 h in the presence of the drug, with 2 μ Ci/ml [3 H]thymidine present during the last 24 h, duplicate cultures were processed for autoradiography and labeled Schwann cell nuclei counted. Percent labeled Schwann cell nuclei in duplicate cultures varied by less than 5%.

TABLE II. β -D-Xylosides Block the Expression of the Dorsal Root Ganglion Surface Mitogen

Pretreatment 4 d		Co-culture 1 d	% [3 H]thymidine-labeled Schwann cells
Neurons	Schwann cells		
—	—	—	4.8 (4.0, 5.5)
α -D-xyI	—	—	5.1 (5.0, 5.1)
β -D-xyI	—	—	1.0 (1.0, 0.9)
β -D-xyI	—	β -D-xyI	0.2
—	β -D-xyI	—	6.0 (6.3, 5.6)

The cells indicated, either neurons or dorsal root ganglion-derived Schwann cells, were incubated with additions indicated for 4 d in serum-free medium. Schwann cells were isolated as described and held overnight following enzymatic dissociation in the continued presence of inhibitor prior to addition to neurons. The low labeling indices in this experiment are due to this protocol. Schwann cells and neurons were combined with additions as indicated and incubated for 24 additional h with 1 μ Ci/ml [3 H]thymidine, followed by fixation and autoradiography to determine the fraction of Schwann cells with labeled nuclei. Inhibitors were all added at 1 mM. β -D-xyI, 4-methylumbelliferyl- β -D-xyloside; α -D-xyI, α -methyl-D-xyloside. Numbers in parentheses are results from single cultures to show range of values.

TABLE III. Effect of 4-Methylumbelliferyl- β -D-Xyloside on the Response of Schwann Cells to Soluble Mitogens

Addition	% Labeled Cells	
	Exp. 1	Exp. 2
None	68.7	65.4
α -D-xyI	84.7	76.4
β -D-xyI	73.8	75.8

Schwann cells replated onto glass slides as described were incubated with 1 mM xyloside for 24 h, followed by the addition of 50 μ g/ml partially purified glial growth factor and 20 ng/ml cholera toxin. After 15 h, [3 H]thymidine was added for a further 24 h, cells were fixed and processed for autoradiography, and the fraction of labeled cells determined. Abbreviations as in Table I. The percent of labeled nuclei in the absence of added mitogen was 0.2% in experiment 1 and 0.7% in experiment 2.

manuscript submitted for publication). Therefore, we compared the time course of the effect of 4-methylumbelliferyl- β -D-xyloside treatment on neuron plus Schwann cell cultures with the time course of its effect on neurons pretreated for various lengths of time followed by co-culture with Schwann cells for 24 h. As shown in Fig. 4, 4-methylumbelliferyl- β -D-

xyloside treatment caused inhibition of Schwann cell proliferation with essentially the same kinetics and extent in both protocols. We therefore conclude that the major, if not the only, effect of 4-methylumbelliferyl- β -D-xyloside is on a proteoglycan on the neuronal surface. This experiment also allows us to conclude that the $t_{1/2}$ of the neuronal, xyloside-sensitive component related to the mitogenic activity is ~ 12 h.

If proteoglycans on the surface of dorsal root ganglion neurons are required in order to elicit a mitogenic response in Schwann cells, then removal of these molecules from the neuronal surface by treatment with appropriate hydrolytic enzymes should inhibit the mitogenic response. Heparan sulfate and chondroitin sulfate appear to be the major proteoglycan species in dorsal root ganglion cells, at least in the chick (Chernoff, 1984). We therefore treated co-cultures of Schwann cells and neurons with enzymes which degrade these molecules. As shown in Fig. 5A, incubation with chondroitinase ABC had no inhibitory effect on the mitogenic response, although in control experiments the enzyme was active in removing [35 S]-labeled proteoglycans from PC12 pheochromocytoma cells (data not shown). In contrast, treatment of the cultures with the combination of heparinase and heparitinase inhibited the mitogenic response (over 50%), without apparent alteration of cell morphology. In later experiments, digestion of the neuronal cell surface with heparitinase alone prior to the addition of Schwann cells was found to mimic the effect of the combination of the two enzymes on neuron and Schwann cells together (Fig. 5B). We conclude from these experiments that a neuronal cell surface heparan sulfate proteoglycan is required to elicit a mitogenic response in Schwann cells.

To ensure that our heparitinase preparation is not contaminated by protease, we digested a solubilized preparation of dorsal root ganglion membrane proteins with a 23-fold excess of enzyme (11.1 U/ml). Fig. 6 shows the effect of digestion of ~ 1 μ g of extracted protein by enzyme for 1 h at 37°C in tissue culture media. Although the enzyme is clearly active, as evidenced by removal of [35 S]-SO $_4$ from SO $_4$ -labeled material

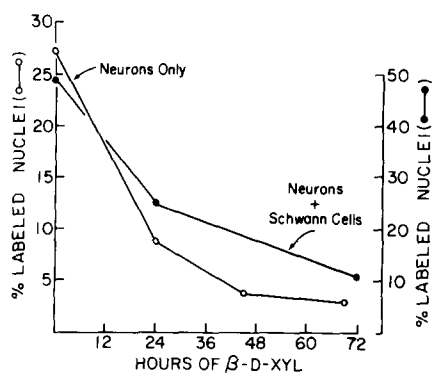


FIGURE 4 Kinetics of 1 mM 4-methylumbelliferyl- β -D-xyloside treatment. Cultures were treated with the drug for the number of hours denoted on the abscissa. The open circles show percent labeled Schwann cells for cultures in which sciatic nerve-derived Schwann cells, freshly scraped off plastic tissue culture dishes, were added to neurons for the final 24 h of the inhibitor treatment. Closed circles are cultures in which Schwann cells were present throughout inhibitor treatment. For all data points, 2 μ Ci/ml [3 H]-thymidine were added during the final 24 h of the experiment, after which cultures were fixed, processed for autoradiography, and stained, and labeled nuclei were counted.

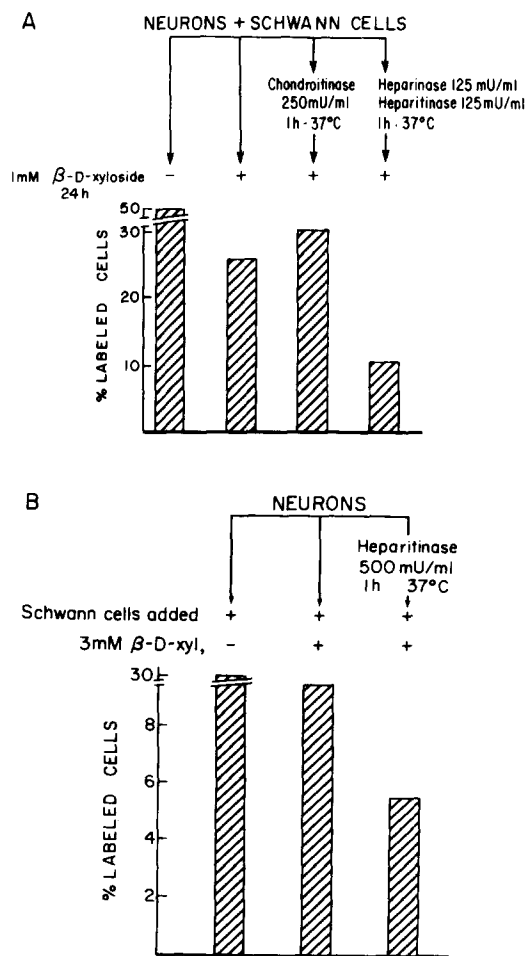


FIGURE 5 Reduction of mitogenic stimulation of Schwann cells by digestion of the neuronal surface with heparitinase. (A) Neuron-Schwann cell co-cultures were digested with either chondroitinase ABC or heparitinase plus heparinase under the indicated conditions. The cultures were then incubated for 12 h in the presence of 1 mM 4-methylumbelliferyl- β -D-xyloside to prevent resynthesis of proteoglycans and to allow any cells already in the S phase of the cell cycle to complete DNA synthesis, and then for an additional 12 h in the presence of inhibitor plus 2 μ Ci/ml [3 H]thymidine prior to fixation and processing for autoradiography. Labeled Schwann cell nuclei were counted as usual. Duplicate cultures were within 10% of the mean. (B) Cultures of neurons without Schwann cells were digested with heparitinase or heparitinase under the indicated conditions. After enzyme digestions, untreated sciatic nerve-derived Schwann cells were scraped off tissue culture plastic dishes and added to neurons in fresh medium. Addition of 4-methylumbelliferyl- β -D-xyloside and [3 H]thymidine were as described above, and were followed by autoradiography. Duplicate cultures were within 15% of the mean.

from parallel cultures, no change in mobility or any of the many methionine-labeled proteins present in this preparation were detected after electrophoresis on 10% polyacrylamide gels followed by autoradiography. In addition, the defined N2 medium used in the heparitinase digestion of dorsal root ganglia cultures contains a total of 15.1 μ g/ml of protein, mostly bovine serum albumin, which would act as an inhibitor of nonspecific protease. Though we cannot rule out an effect of a very specific protease on the mitogen itself, this experiment indicates that the heparitinase preparation is not contaminated by a general protease; this is an important control since it has been established that the neuronal mitogen

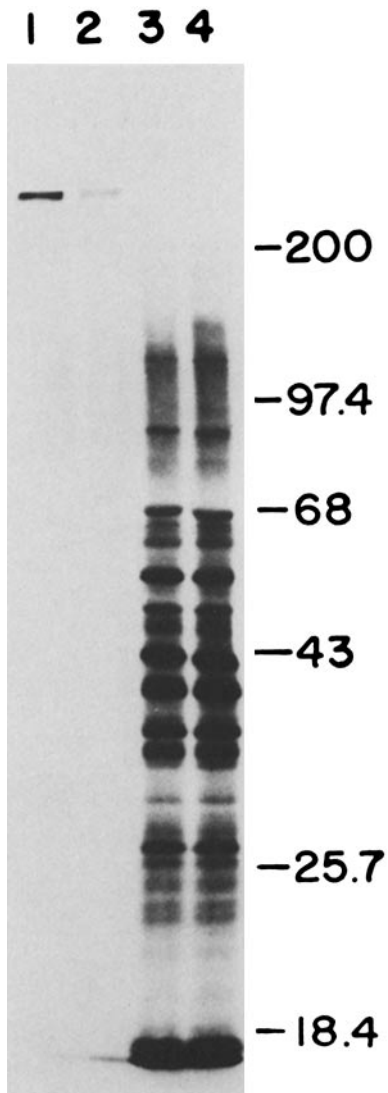


FIGURE 6 Heparitinase does not have significant proteolytic activity. A proteoglycan-enriched fraction was prepared by extracting membranes from dorsal root ganglia neurites with 35 mM octylglucoside, 0.5 M NaCl, 0.15 mM EGTA in homogenization buffer. This extract was passed through a Biogel HTP column and the effluent, after dialysis, was digested for 1 h at 37°C with 300 mU of heparitinase in 27 μ l, followed by PAGE in 10% gels. Lanes 1 and 2, [³⁵S]-SO₄-labeled cells. Lanes 3 and 4, [³⁵S]methionine-labeled cells. Lanes 2 and 4 are heparitinase treated and lanes 1 and 3 are controls. Note that heparitinase degrades the proteoglycan (lane 2) while leaving protein intact (lane 4). For details see text.

is protease sensitive (Salzer et al., 1980*a, b*; Cassel et al., 1982; Ratner et al., 1984), although high concentrations of protease are required to degrade the mitogen(s).

Previous work from this laboratory (Salzer et al., 1980*b*; Cassel et al., 1982; Ratner et al., 1984) as well as from another (Hanson and Partlow, 1980) has demonstrated that membranes prepared from dorsal root ganglion neurons are mitogenic for Schwann cells. The notion that a neuronal cell surface heparan sulfate proteoglycan is required for the mitogenic response would be strengthened if membranes prepared from heparitinase-treated neurons or from neurons grown in the presence of 4-methylumbelliferyl- β -D-xyloside elicited a diminished response when added to replated Schwann cells. The data in Fig. 7*A* show dose-response curves

for membranes prepared from cultures treated with heparitinase immediately before membrane preparation. We observe a 30–60% reduction in mitogenicity of membranes prepared from heparitinase-treated neurons; the mitogenicity of the membrane fractions derived from these cultures is reduced to levels similar to those obtained by trypsin treatment. We presume that at least a portion of the residual activity is derived from a pool of intracellular membranes, inaccessible to the enzymes. The variation in the reduction of mitogenicity of treated membranes appears to depend on the density of the neurons and neuritic outgrowth in the starting cultures, although this has not been studied systematically.

Five separate experiments have been carried out to compare the mitogenicity of control membranes to that of membranes from cells grown in the presence of 1 mM 4-methylumbelliferyl- β -D-xyloside. Dose-response curves for all five experiments show a similar trend: membranes from treated cultures are not as mitogenic as control membranes, yet they retain a significant amount of activity based on amount of protein added to Schwann cells. A typical experiment is shown in Fig. 7*B*; at maximal stimulation, 66% of the activity remains in membranes from neurons grown for 5 d in 1 mM 4-methylumbelliferyl- β -D-xyloside; 37.4% remains in membranes

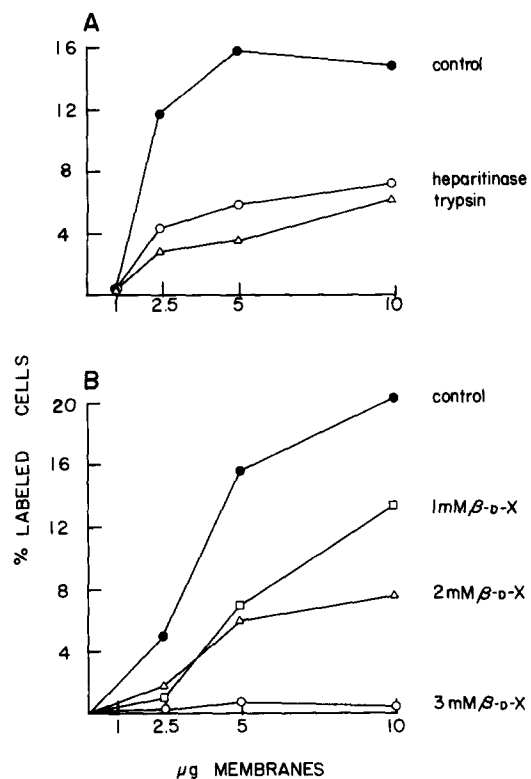


FIGURE 7 Membranes prepared from heparitinase-digested neurons or from neurons grown in 4-methylumbelliferyl- β -D-xyloside show diminished mitogenicity for Schwann cells. (A) Mass cultures of dorsal root ganglion neurons were used as a source of membranes. These cultures were digested with either trypsin or heparitinase as described, membranes prepared, and added to Schwann cells replated onto glass slides. A second addition of membranes was made after 16 h, in the presence of [³H]thymidine. After 24 h, cultures were fixed, processed for autoradiography, and labeled Schwann cells counted. (B) Mass cultures of neurons were grown for 5 d in the indicated concentration of 4-methylumbelliferyl- β -D-xyloside. Membranes were then prepared and added to Schwann cells as described above.

from neurons grown in 2 mM inhibitor. At these concentrations, remaining mitogenicity of intact neurons for Schwann cells would be at most 25% at 1 mM xyloside and 10% at 2 mM xyloside after 3 d. A possible explanation for these experimental observations is that the core protein of the proteoglycan (or a linked protein) is not reaching the cell surface. To test this hypothesis, membranes were prepared from neurites alone (essentially cell surface membrane) or from cultures containing neurites as well as cell bodies (which contain all the neuronal Golgi apparatus and endoplasmic reticulum). Table IV shows that while neurites of control cultures are even more mitogenic than total cultures on a protein basis, neurites from 4-methylumbelliferyl- β -D-xyloside-treated cultures are only 18.6% as mitogenic as control membranes. This value agrees well with the percent inhibition obtained in xyloside-treated neuron-Schwann cell co-cultures.

Support for the hypothesis that the protein moiety of the proteoglycan (or another protein) is required for mitogenicity comes from the fact that exogenous heparan sulfate, obtained either from Miles Laboratories Inc. or from Dr. A. Linker (Linker and Hovingh, 1973) and obtained from bovine lung,

TABLE IV. Effect of 4-Methylumbelliferyl- β -D-Xyloside on the Mitogenicity of Neuronal Cell Bodies and Neurites

		% labeled cells	% control
Control	Cell bodies + neurites	29.6	
	Neurites only	36.2	
Xyloside treated	Cell bodies + neurites	25.0	84.5
	Neurites only	5.5	18.6

6 wk prior to use, dissociated and embryonic dorsal root ganglia were plated onto collagen substrata in drops containing 20 ganglia—equivalents of cells in a 2-mm drop. Antimitotic treatment was as described in Materials and Methods. 5 d prior to use, half of the cultures were treated with 1 mM 4-methylumbelliferyl- β -D-xyloside. Membranes were prepared from cultures containing neuronal somas and neurites, or from cultures from which cell bodies were removed using a razor blade fragment. Membranes were added to replated Schwann cells at 10 μ g/well of Schwann cells in duplicate. The percent labeled Schwann cells in the absence of membranes was 1%.

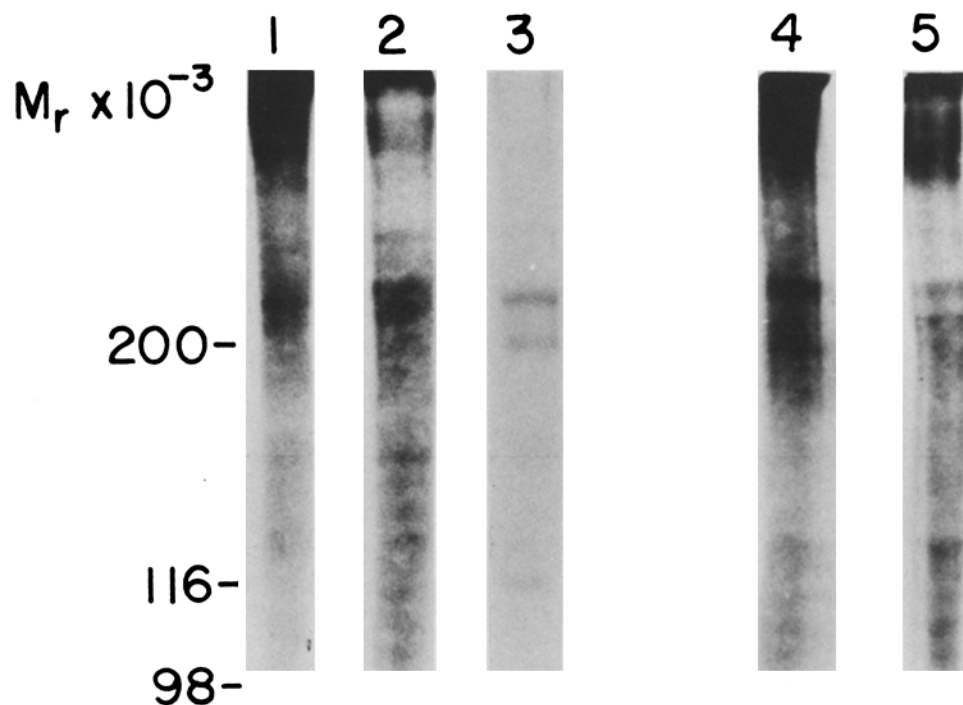


FIGURE 8 $[^{35}\text{S}]\text{-SO}_4$ -labeled components of dorsal root ganglion neurons: sensitivity to 4-methylumbelliferyl- β -D-xyloside and heparitinase and localization in membranes. Fluorogram of 6% SDS polyacrylamide gel. Lane 1, cell extract of control neurons. Lane 2, cell extract of neurons digested with heparitinase. Lane 3, cell extract of 1 mM 4-methylumbelliferyl- β -D-xyloside-treated neurons. Lane 4, membrane fraction prepared from control neurons. Lane 5, membrane fraction prepared from heparitinase-digested neurons. Each lane contains the total protein isolated from one mass culture of neurons.

is not able to stimulate replated Schwann cells to proliferate when added at concentrations up to 100 μ g/ml (data not shown). (Heparin and chondroitin sulfate are similarly inactive.) Similarly, the addition of heparan sulfate, heparin, or chondroitin sulfate to co-cultures of Schwann cells and neurons fails to show any inhibition of the mitogenicity induced in the Schwann cell population by the presence of the neurons. These concentrations of polysaccharides are adequate to inhibit cell-to-substratum adhesion in another system (Cole et al., 1985). These negative results are open to multiple interpretations; the added polysaccharides may have the wrong structure, thus concentration may be wrong or they may not have adequate access to the surface of the Schwann cell. Alternatively, these experiments may indicate that the protein moiety of the proteoglycan (or of another protein) is required to elicit a mitogenic response.

We considered whether we could detect a xyloside-sensitive, heparitinase-sensitive moiety on the surface of dorsal root ganglion neurons which is present in mitogenic membrane fractions, since identification of such a molecule will facilitate its isolation. Therefore, cultures of neurons were prepared (see Materials and Methods), solubilized, and subjected to PAGE. Fig. 8 shows that $[^{35}\text{S}]\text{-SO}_4$ incorporation by these embryonic neurons leads to labeling of xyloside-insensitive components (sulfated proteins and glycoproteins) as well as xyloside-sensitive components (proteoglycans). When compared to protein standards, major neuronal xyloside-sensitive components show low mobility, i.e., apparent high molecular weight. To determine whether any of the xyloside-sensitive molecules showed homology with the proteoglycan involved in the mitogenic response, companion cultures were sulfate labeled, then digested with heparinase or heparitinase before preparation of cell extracts. No sulfate-labeled material was removed by heparinase (data not shown), but heparitinase significantly diminished labeling of material near the top of the 6% resolving gel. This material is surface membrane associated, as demonstrated by the fact that it is sensitive to enzyme digestion of the intact cell, and also since it is enriched

in membranes prepared from neurons (Fig. 8). Therefore, dorsal root ganglion neurons contain one or more heparan sulfate proteoglycans on their cell surfaces; however, we have not yet demonstrated that the material observed by fluorography of [³⁵S]-SO₄-labeled material on polyacrylamide gels is related to the mitogen.

DISCUSSION

Two independent approaches indicate that a neuronal cell surface proteoglycan, probably heparan sulfate, is required to elicit a mitogenic response in Schwann cells. This conclusion is based on the use of an inhibitor of proteoglycan synthesis as well as an enzymatic digestion of intact neurons. Though each of these approaches has potential pitfalls, the use of both reinforces the conclusion that a proteoglycan is required for this mitogenic effect. In parallel studies (Ratner, N., S. Porter, A. Elbein, R. P. Bunge, and L. Glaser, manuscript in preparation), we have shown that inhibitors which modify the maturation (trimming) of asparagine-linked oligosaccharides on glycoproteins do not affect the mitogenic interaction between neurons and Schwann cells.

Cell surface heparan sulfate proteoglycans have been described on many cell types (Kawakami and Terayama, 1981; Carlstedt et al., 1983; Rapraeger and Bernfield, 1983; Yanagishita and Hascall, 1984) and, although the function of these cell surface molecules is still largely unknown, correlative evidence suggests that proteoglycans can be involved with growth control. An intact basal lamina, of which a major component is heparan sulfate proteoglycan, is required for proliferation of epithelial cells of which the corneal epithelium is an example (Gospodarowitz et al., 1982). Many cells in tissue culture proliferate in the absence of exogenous growth factors when in contact with an extracellular matrix (Gospodarowitz et al., 1978). Heparin itself, a soluble glycosaminoglycan as opposed to a basal lamina component, acts synergistically with endothelial growth factor (Thornton et al., 1983; Maciag et al., 1984) in stimulating survival and proliferation of human endothelial cells, while heparin inhibits the growth of smooth muscle cells *in vitro* and *in vivo* (Cochran et al., 1984). It is nevertheless surprising that a cell surface heparan sulfate proteoglycan is important in growth control in the neuron-Schwann cell system.

In several control experiments that have been carried out to substantiate that β -D-xyloside is only acting as an inhibitor of proteoglycan biosynthesis in this system, we observed the following. (a) The effect of the inhibitor is specific for the xylose (β -D-arabinoside will not substitute for β -D-xyloside) as well as the anomeric linkage (only the β -xyloside inhibits Schwann cell proliferation). (b) Schwann cells are capable of responding to mitogens other than neurons in the presence of the drug (cholera toxin and glial growth factor stimulate Schwann cell proliferation in the absence of β -D-xyloside). (c) Schwann cells treated with 4-methylumbelliferyl- β -D-xyloside are capable of responding to intact neurons within 24 h after withdrawal of the drug. (d) Synthesis of bulk protein by Schwann cells treated with β -D-xyloside as assayed by trichloroacetic acid-precipitable [³⁵S]methionine-labeled protein is 88% of control values. (e) Finally, in the presence of the inhibitor, synthesis of the normal neuronal proteoglycans, both cell associated and secreted, is halted, and small chondroitinase ABC-sensitive material and free glycosaminoglycan chains are secreted into the culture medium.

We have also used enzymes which specifically degrade glycosaminoglycan chains to arrive at a similar conclusion, that is, that a specific proteoglycan is required for the mitogenic response of Schwann cells to neurons to occur. Neither active preparations of chondroitinase ABC nor heparinase reduced the mitogenicity of dorsal root ganglion neurons, but heparitinase pretreatment of neurons causes them to be much less effective than untreated controls in stimulating Schwann cell division, and heparitinase digestion of neurons prior to isolation of membranes causes these membranes to be as inactive as membranes isolated from trypsin-treated neurons in mitogenic activity. These observations substantiate the hypothesis that a proteoglycan is involved in signal transduction and, in addition, suggest that the active proteoglycan is a heparan sulfate proteoglycan. Some caution must be applied to interpretation of this result since it is based solely on the specificity of the enzyme. The neuronal proteoglycan may contain some linkages sensitive to heparitinase, which are necessary to maintain the molecules' activity, and also contain other types of sugar chains.

It is also necessary to ask whether it has been absolutely established that the proteoglycan is a cell surface molecule, and not secreted from the neuron and reattached to the collagen, either directly or as part of the extracellular matrix. Several pieces of information argue against these interpretations. (a) Cultures of neurons, without supporting Schwann cells, do not contain a visible extracellular matrix (Bunge et al., 1982). (b) Even when many neurons are present in a culture, Schwann cells not in direct contact with neurons are not stimulated to divide (Wood and Bunge, 1975; Salzer et al., 1980a). (c) Membrane preparations in which collagen is depleted by at least 75% (Salzer et al., 1980b) are enriched in mitogenic activity. (d) Finally, PC12-derived membrane fractions, obtained from PC12 cells grown in suspension and therefore free of collagen, contain a molecule mitogenic for Schwann cells (Ratner et al., 1984); PC12 cell-stimulated Schwann cell proliferation is reduced by 50–60% in the presence of 1 mM 4-methylumbelliferyl- β -D-xyloside (Ratner et al., 1985), providing some evidence that similar mitogenic molecules are present in PC12 cells and dorsal root ganglion neurons.

Our evidence strongly supports the hypothesis that a cell surface heparan sulfate proteoglycan is required for neurons to stimulate Schwann cell division; however, until such a molecule is isolated and tested directly for mitogenicity, we cannot distinguish whether this proteoglycan is itself mitogenic for Schwann cells (and, if so, whether the mitogenic activity resides in the core protein or the glycosaminoglycan chain[s]) or whether the proteoglycan is a co-factor for a mitogenic activity residing in another neuronal surface molecule. In fact, we do not know whether the core protein of the relevant heparan sulfate proteoglycan is inserted into the membrane after treatment with β -D-xyloside; studies of the cellular location of proteoglycan core proteins of cell surface proteoglycans following treatment with β -D-xylosides have not been reported. In experiments using the glycoprotein inhibitor tunicamycin, which generates core proteins without mature N-linked carbohydrate chains, it appears that each oligosaccharide-free core protein behaves differently with respect to arrival at its proper destination (Olden et al., 1985). If the core protein of the mitogen is present on the cell surface, it is unable itself to elicit a mitogenic response in Schwann cells. In addition, a population of mitogenic molecules ap-

pears to be retained in intracellular membranes before arrival at the cell surface (Table IV). These molecules may lack sufficient glycosaminoglycan side chains to reach the cell surface, yet retain enough side chains to enable proper folding and therefore mitogenicity, either directly or as a co-factor for another cell surface molecule.

In this model, (a) treatment of the neuronal membrane with heparitinase must abolish mitogenicity by removal of all the glycosaminoglycan chains on surface molecules, since these are rendered nonmitogenic; and (b) increasing concentrations of β -D-xyloside inhibit mitogenicity of membrane fragments by progressive decrease in the leakiness of the competitive inhibitor; at 1 mM β -D-xyloside most proteoglycans may retain one or more glycosaminoglycan side chains, and therefore activity, whereas at 3 mM β -D-xyloside most proteoglycans may be naked core proteins, and therefore be nonmitogenic.

The cell division initiated in Schwann cells by neurons must involve an initial adhesion step; Schwann cells must contact neurons in order to be stimulated to divide and recontact neurons following progress through the cell cycle. It is therefore of some interest to note that when the progress of Schwann cells through the cell cycle has been halted by the addition of 4-methylumbelliferyl- β -D-xyloside, these cells still relate normally to axons (Fig. 1). Even at the growing tips of the axons, where the effects of the inhibitor would be expected to be maximized due to insertion of newly synthesized protein (Pfenninger and Maylie-Pfenninger, 1981a, b) and lipid (Pfenninger and Johnson, 1983), and presumably also proteoglycans, no effect is observed on the relationship of the Schwann cell to the neurons. This finding should be compared to previous observations by Salzer et al. (1980a) who showed that Schwann cells failed to adhere to or align with neurites previously fixed with glutaraldehyde or digested with trypsin. This data implies that a trypsin-sensitive (but not β -D-xyloside-sensitive) molecule is the adhesion-mediating factor, and that long-term neuron-Schwann cell interaction in nondividing Schwann cells is not stabilized by β -D-xyloside-sensitive components. However, we cannot rule out the possibility that (a) the core protein itself is responsible for cell-cell interaction, or that (b) since xyloside treatment is always leaky due to competition of native core proteins with the drug, the relatively few native proteoglycan molecules present in treated cells suffice to maintain adhesion between neurons and Schwann cells, yet are insufficient in number to serve as mitogenic signals. Sobue and Pleasure (1985) have correlated binding of axolemmal fragments to Schwann cells with the mitogenic potential of the membranes; we can now show that, although adhesion is required for mitogenicity, the two are distinguishable events.

The cell adhesion molecule Ng-CAM is a candidate molecule for maintenance of neuron-Schwann cell interaction. This molecule (Grumet et al., 1984) may be present on Schwann cells as well as neurons (Faissner et al., 1984; Grumet and Edelman, 1984). Antibodies against the 135-kD component of this molecule inhibit the interaction of neurons with central nervous system glia (Grumet et al., 1984).

Since a proteoglycan is so strongly implicated as a component of the mitogenic signal, it is of some interest that the extracellular matrix component, laminin, can bind to heparin (Shakashita et al., 1980; Kleinman et al., 1984) and is expressed on Schwann cell surfaces (Cornbrooks et al., 1983). In defined media, laminin is expressed as discrete patches on

the Schwann cell surface (Cornbrooks et al., 1983), although no basal lamina is formed under these conditions (Moya et al., 1980). It is conceivable that the binding of heparin sulfate on the neuronal surface to laminin on the Schwann cell might result in regulation of the mitogenic activity. Laminin has been shown to mediate Schwann cell-substratum interaction; anti-laminin antibodies cause detachment of Schwann cells from the substratum (McGarvey et al., 1984). However, since neither 4-methylumbelliferyl- β -D-xyloside treatment nor heparitinase digestion of neuronal surfaces decreases adhesion between Schwann cell and neuron, a laminin-heparan sulfate interaction is probably not the mediator of intracellular adhesion in this system.

The involvement of a proteoglycan in the mitogenic response suggests a possible mechanism of regulation of the mitogen, since proteoglycans can be degraded by extracellular heparitinases (Lark and Culp, 1983). Kalderon (1984) has suggested that plasminogen, activated by plasminogen activator released from Schwann cells, might degrade a neuronal cell adhesion molecule, clipping from the neuronal surface a fragment mitogenic for Schwann cells. From previous experiments (Salzer et al., 1980a), we know that if this mechanism is correct, the fragment must have a very short half-life or a very low concentration. We speculate that heparan sulfate chains which are required for mitogenicity can be clipped from the neuronal surface by heparitinases and in this way inactivate the mitogen, providing a mechanism for regulating Schwann cell-neuron interaction.

In conclusion, our results demonstrate that cell surface heparan sulfate is a specific component required for the mitogenic response of Schwann cells to neurons, suggesting a unique biological function for heparan sulfate proteoglycans in the nervous system.

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