Laminin Promotes Neuritic Regeneration from Cultured Peripheral and Central Neurons

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ABSTRACT The ability of axons to grow through tissue in vivo during development or regeneration may be regulated by the availability of specific neurite-promoting macromolecules located within the extracellular matrix. We have used tissue culture methods to examine the relative ability of various extracellular matrix components to elicit neurite outgrowth from dissociated chick embryo parasympathetic (ciliary ganglion) neurons in serum-free monolayer culture. Purified laminin from both mouse and rat sources, as well as a partially purified polyornithine-binding neurite promoting factor (PNPF-1) from rat Schwannoma cells all stimulate neurite production from these neurons. Laminin and PNPF-1 are also potent stimulators of neurite growth from cultured neurons obtained from other peripheral as well as central neural tissues, specifically avian sympathetic and sensory ganglia and spinal cord, optic tectum, neural retina, and telencephalon, as well as from sensory ganglia of the neonatal mouse and hippocampal, septal, and striatal tissues of the fetal rat. A quantitative in vitro bioassay method using ciliary neurons was used to (a) measure and compare the specific neurite-promoting activities of these agents, (b) confirm that during the purification of laminin, the neurite-promoting activity co-purifies with the laminin protein, and (c) compare the influences of anti-laminin antibodies on the neurite-promoting activity of laminin and PNPF-1. We conclude that laminin and PNPF-1 are distinct macromolecules capable of expressing their neurite-promoting activities even when presented in nanogram amounts. This neurite-promoting bioassay currently represents the most sensitive test for the biological activity of laminin.

Neurons in the peripheral nervous system (PNS) can successfully regrow transected axons in vivo, often achieving substantial recovery of function (1), whereas central nervous system (CNS) neurons usually regenerate less well, if at all (2). Two requisites for such regeneration are that the injured neurons remain alive and that they are presented with a favorable microenvironment through which their regrowing neurites can advance. Implanted segments of peripheral nerve have been shown to provide a suitable microenvironment for axonal regeneration from both PNS and CNS neurons (3, 4). This axonal regrowth takes place upon cell surfaces or within the extracellular matrix (ECM) with which the advancing growth cone makes contact. ECM constitutes and particularly ECM glycoproteins may, therefore, constitute a family of contact-operating neurite-promoting agents.

A complementary approach to the study of axonal regeneration has been through the use of neuronal models in vitro. Neurons in culture also have two requirements for neuritic regrowth, namely, trophic factors that support the survival of neurons (5, 6), and substances that act specifically as neurite-promoting factors (7–13). It is reasonable to suppose that the latter agents may relate to the ECM constituents. The use of more highly adhesive substrata, such as tissue culture plastic (TCP) coated with polycationic substances (polylysine; polyornithine or PORN) provide for a rapid cell attachment even in the absence of added ECM constituents. Previous

Abbreviations used in this paper: CNS, central nervous system; DME, Dulbecco's Modified Minimal Essential Medium containing NaHCO₃ (to 26.4 mM), glutamine (to 2 mM), and penicillin (100 U/ml); ECM, extracellular matrix; PNPF, polyornithine-binding neurite-promoting factors; PNS, peripheral nervous system; PORN, polyornithine; PORN-TCP, PORN-coated TCP; TCP, tissue culture plastic.

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studies have demonstrated the presence, within culture media exposed to glial and other cells, of high molecular weight agents designated as polyornithine-binding neurite promoting factors or PNPF (9, 13–16). These agents have been shown to attach and confer to PORN-coated plastic surfaces neurite-promoting activity for dissociated PNS and CNS neurons (16–18). Fibronectin, a prominent component of the ECM, cell surfaces, and plasma, has been reported to stimulate neurite outgrowth from retinal cell reaggregate cultures (18). Very recently laminin, a glycoprotein component of the basal lamina (19), was reported to confer to tissue culture plastic surfaces a neurite-promoting activity for explanated human fetal sensory ganglia (20).

In this study we have examined the ability of several purified ECM components to confer neurite-promoting activity to plastic or PORN-coated plastic surfaces and we show that laminin from both rat and mouse sources is an extremely potent PNPF for both peripheral and central neurons. Fibronectin has a relatively weaker neurite-promoting activity using a peripheral neuron bioassay, while two proteoglycans tested lack detectable activity. Furthermore, we present evidence that rat laminin is distinct from partially purified PNPF derived from rat RN22 Schwannoma cells.

MATERIALS AND METHODS

Reagents: Culture medium (DME-N1) was Dulbecco's Modified Minimal Essential Medium containing NaHCO₃ (to 26.4 mM), glutamine (to 2 mM), and penicillin (100 U/ml) (DME; Gibco Laboratories, Grand Island, NY) and containing the N1 supplement (21). Double-strength medium contained the N1 supplement at double its normal concentration. PORN (molecular weight = 3 x 10⁵; Sigma P6012, Sigma Chemical Co., St. Louis, MO), ovalbumin, and hyaluronic acid were obtained from Sigma Chemical Co. PNPF-1 was partially purified from serum-free RN2 Schwannom conditioned medium (33). Laminin was purified from rat yolk sac tumor or conditioned medium from mouse endodermal cell line PF HR-9 (22). Other ECM-related materials were fibronectin from rat plasma (23), a chondroitin sulfate proteoglycan from rat yolk sac tumor (24), and a heparan sulfate proteoglycan from a rat hepatoma (25). Heparin was kindly provided by Dr. Ulf Lindahl, Uppsala, Sweden. Protein was determined using the method of Bradford (26).

Neuronal Cell Dissociation: Dissociates from embryonic day 8 (E8) chick ciliary ganglia were prepared as described (27), except that the posthypoxic washes and trituration were carried out with 1% ovalbumin in DME and the cells were diluted in DME. The same modifications were applied to previously described procedures for the preparation of cell suspensions from E11 chick sympathetic ganglia (28), E8 chick and neonatal mouse dorsal root ganglia (28), E4 chick lumbar spinal cord (16), E8 chick telencephalon (21), optic lobe (29), and neural retina (17), and 18-d fetal rat hippocampus, septum, and striatum (30). All dissociated cell suspensions were diluted to 40,000 cells/ml, except spinal cord which was diluted to 20,000/ml.

Substratum Preparation: All experiments used 6-mm microwells in 96-well culture plates (Costar 3596; Belco Glass, Inc., Vineland, NJ). Each well was first incubated overnight at 25°C with 50 µl of DME solution (0.1 mg/ml in 15 mM HEPES buffer, pH 8.4; Sigma Chemical Co.), followed by 3 x 50-µl wash water. The PORN-treated wells then were supplied with 50 µl of the materials to be tested for neurite-promoting activity (seriously diluted in PBS, pH 7.0), incubated for 2 h at 37°C, and washed once with 100 µl of PBS containing 1% ovalbumin.

Culture Preparation and Analysis: The pretreated culture wells received 50 µl of double-strength culture medium followed by 50 µl of cell suspension in DME containing the required neuroneutrophic factor: 100 Trophic Units per milliliter of eye-derived ciliary neuroneutrophic factor for ciliary ganglia (31), 20 Biological Units per milliliter of 7S nerve growth factor (32) for E11 chick sympathetic ganglia, E8 chick and neonatal mouse dorsal root ganglia (28), or 25% (vol/vol in DME) rat astroglial conditioned medium, which had been depleted of endogenous PNPF activity by passage through a PM10 membrane (Amicon Corporation, Danvers, MA), for all of the CNS cultures (33, 34). It should be emphasized here that under all of the culture conditions reported in this study no neurons would survive without the addition of an adequate and appropriate supply of neuroneutrophic factors. After 24-h incubation (37°C, 5% CO₂-air), the cultures were fixed and neuronal numbers and percentages of neurons bearing neurites greater in length than one somal diameter were determined using phase contrast microscopy as described (13, 35, 36). For photomicrographs, the neurons were stained using silver nitrate and photographed using phase contrast optics, a procedure that enhances the visibility of neurites.

Immunocological Analysis: Rabbit antiserum to purified rat laminin was prepared and characterized as described (22). The antiserum was tested for its ability to block rat laminin, mouse laminin, or rat PNPF-I as follows. A constant amount (1 µg/ml, 50 µl) of laminin or PNPF-1 was presented for 2 h at 37°C to the PORN-treated wells followed by washes as indicated above. Then, 50 µl of serially diluted immune or preimmune serum were presented to wells (37) for 2 h at 37°C, and the wells were washed, seeded with ciliary ganglion neurons, and evaluated for their ability to elicit neurite production.

RESULTS

Selected Extracellular Matrix-Related Materials Support Neuronal Maintenance and Stimulate Neurite Regeneration from Ciliated Ganglion Neurons

Neuronal Maintenance: Table I compares the effects, on ciliary ganglion neurons cultured in serum-free conditions, of rat RN22 Schwannoma-derived PNPF-1 and various ECM-related agents presupplied to either TCP or PORN-coated TCP (PORN-TCP) substrata. Despite the presence of an adequate supply of the ciliary neuroneutrophic factor in the medium, very few neurons could be maintained on TCP that had been unexposed (Table I, A), or exposed to most of the test agents (Table I, B and C). However, both fibronectin and laminin, particularly when presented to TCP at the highest concentration shown, 10 µg/ml, were able to sustain several-fold more neurons than unexposed TCP (Table I, D). In contrast, both untreated and treated PORN-TCP supported a relatively constant and higher neuronal number representing 60–85% of the number of neurons seeded (i.e., 1,000 neurons/well). The one exception to this was the heparin-exposed PORN-TCP wells (Table I, C) in which less than half of this number of neurons was sustained. No effort was made in this study to seek the explanations (neuron-substratum adhesive ness, neuronal survival regulation, toxicity, etc.) for such effects of different substrata on neuronal maintenance.

Neurite Regeneration: Partially purified PNPF-1 (13) was able to elicit a distinct but modest neurite growth on TCP, and this only at relatively high concentrations (10 µg/ml, Table I, B). However, on PORN-TCP, PNPF-1 recruited nearly all of the neurons present into neurite production even when presented in very low amounts. Neither of the proteoglycans nor hyaluronic acid (Table I, C) elicited neurite growth from ciliary ganglion neurons and in fact they appeared to inhibit the modest growth normally appearing on untreated PORN-TCP. Purified rat fibronectin conferred some neurite-promoting activity to TCP and to a lesser extent to PORN-TCP, particularly when presented at relatively high levels (10 µg/ml; Table I, D).

Rat and mouse laminin conferred to both TCP and PORN-TCP considerable neurite-promoting competence (Table I, D). This activity appeared to parallel their neuron-sustaining ability on the TCP substratum and both activities were essentially lost when the laminins were presented to TCP at 0.1 µg/ml. In contrast, even this low laminin concentration was able to confer to PORN-TCP substrata near maximal neurite-promoting activity.

Thus the PORN-TCP surface offered at least four advantages over TCP, namely (a) the PORN substratum sustained...
a relatively high and constant neuronal number, independent of further substratum pretreatments, (b) the neurite-promoting activity of materials used for subsequent treatments could be measured with greater sensitivity, and (c) neurite-promoting activity could be measured independent from cell-sustaining activity. In addition, (d) the distribution of the neurons over the culture surface was more uniform on PORN-TCP, making replicate diametral neuronal count values more consistent. For these reasons PORN-TCP was used to examine in more detail the laminin and PNPF-1 responses.

MORPHOLOGY OF CILIARY GANGLION NEURONS CULTURED IN SERUM-FREE MEDIUM ON PORN-TCP COATED WITH LAMININ OR PNPF-1: Some ciliary ganglion neurons exhibited a definite, although modest neuritic growth on PORN-TCP (Fig. 1 A) when cultured in serum-free medium, in contrast to our earlier studies (13, 14) in which serum was routinely used. These neurites seldom exceeded five somal diameters and were present on 10% or less of the neuronal population (cf. Table 1). However, when the PORN-TCP was exposed to either PNPF-1 or laminin, virtually all of the neurons exhibited neurite growth (Fig. 1, B and C). The morphology of the neurites appeared indistinguishable in the two cases; neurite length was usually greater than 20 somal diameters and sometimes extended over 1.0 mm.

QUANTITATION OF THE NEURITE-PROMOTING ACTIVITY OF LAMININ: We have previously developed a quantitative bioassay for the RN22 Schwannoma–derived...
PNPF and have used this short-term assay to monitor its purification (13). Here we have used a similar assay to (a) estimate the specific neurite-promoting activity (units of activity per milligram of protein) of rat and mouse laminin, (b) compare the specific activities of the laminins with that of the partially purified rat PNPF-1, and (c) confirm that during the purification of laminin the neurite-promoting activity copurifies with the laminin protein. We have also used the assay to show that anti-laminin antibodies inhibit quantitatively the neurite-promoting activities of laminin.

Fig. 2 shows the relationship between neurite promotion and dosages of purified rat laminin, mouse laminin, or partially purified rat PNPF-1 using ciliary ganglionic neurons in serum-free culture medium on PORN-TCP. Both laminins and PNPF-1 can recruit up to nearly 100% of the ciliary ganglion neurons into neuritic production when used for PORN-TCP pretreatment at concentrations of 1 μg/ml. This recruitment decreases as the pretreatment concentration is decreased. The limits of reliable detection (i.e., ~20% neuritic growth) are reached when the laminins and PNPF-1 are presented to the wells at 25 and 200 ng/ml, respectively. The half-maximal responses (i.e., those elicited by 1 neurite-promoting unit or NPU per ml) are reached when 50 ng/ml of laminin or 300 ng/ml of PNPF-1 is presented to the PORN-TCP well. Thus it is possible to derive a specific activity (NPU/mg protein) for the rat laminin, mouse laminin, and rat PNPF-1 of, respectively, about 20,000, 20,000, and 3,300 NPU/mg of protein.

Co-purification of Rat Laminin Antigen and Rat Laminin-induced Neurite-Promoting Activity

The quantitative bioassay shown in Fig. 2 was used to monitor neurite-promoting activity in sequential fractions obtained during the course of laminin purification from rat yolk sac tumor (22). Table II compares specific activities measured in such fractions using an enzyme-linked immunosorbent assay and the neurite promotion bioassay. The specific activity for neurite promotion increased progressively with the progression of laminin purification, thereby providing evidence that laminin itself, rather than a nonlaminin contaminant, is responsible for the neuritic effect. The ratio of the two data columns (i.e., U/mg laminin) decreased from ~85,000 in the 3.5 M NaCl fraction to 34,000 in the final product. This decrease could be due to a loss of the neurite-promoting activity of laminin incurred during the purification procedure. Three different rat laminin preparations purified to homogeneity by electrophoretic criteria have yielded specific activities of 18,000, 20,000, and 38,300 NPU/mg.

Effects of Anti-Laminin Antibodies on the Neurite-Promoting Activity of Laminin and PNPF-1: We next studied the ability of rabbit anti-rat laminin antibodies to block the neurite-promoting activity of rat and mouse laminin and rat PNPF-1. Each neurite-promoting agent was first presented to the PORN-TCP wells at 1 μg/ml concentrations (i.e., enough to assure a maximal neurite response—see Fig. 2) followed by a second 2-h incubation with serial dilutions of anti-rat laminin antiserum (50 μl/well). The wells were then washed and examined for their retained ability to promote neuritic growth. The results are shown in Fig. 3.

Despite the similarity of their biological activities the laminin and PNPF-1 are not identical molecules. Anti-rat laminin serum blocks the neurite-promoting activity of purified rat and mouse laminin but not that of rat PNPF-1. This particular antiserum was also capable of blocking the neurite-promoting activity of crude conditioned medium from the mouse cell line PF-HR9, which contains laminin (38), but incapable of blocking a similar activity in crude Schwannoma-conditioned medium as well as in conditioned media from purified cell cultures of rat astroglia (39), oligodendroglia (40), C-6 glioma (33), and mouse Schwann cells (41), all of which contain PNPF (15) (data not shown). Rabbit antiserum to rat fibronectin and normal rabbit serum did not affect the activity of either the laminins or PNPF-1 (data not shown).

Laminin and PNPF-1 Stimulate Neuritic Regeneration from Both Peripheral and Central Neurons in Culture

It has been previously demonstrated that a variety of PNS

![Figure 2](image-url)
produced little, if any, neuritic growth on PORN-TCP, and identified as such by the use of tetanus toxin binding (30, 34), the neurons from both of these tissue sources, previously cortex (telencephalon) and 18-d fetal rat hippocampal neu-

grows near the perimeter of the neuronal soma (Fig. 4 D). Note that the activity of rat laminin was more readily inhibited than that of mouse laminin activity and that PNPF-1 activity was not blocked by laminin antisera. The reciprocal of the serum dilution is shown.

neurons (13, 14, 15) as well as some spinal cord (16) and retinal neurons (17) will increase neurite production on PORN-TCP in response to pretreatment of PORN-TCP with PNPF-containing RN22 Schwannoma-conditioned media. We have extended these observations, using partially purified PNPF-1, to a variety of avian and rodent peripheral and intrinsic central neurons and we have compared the response of all of these test neurons to that produced using purified rat laminin.

Fig. 4 illustrates the morphologies of E11 chick sympathetic and neonatal mouse dorsal root ganglionic neurons cultured on untreated PORN-TCP (Figs. 4, A and D), laminin-treated PORN-TCP (Fig. 4, B and E) and PNPF-1-treated PORN-TCP (Fig. 4, C and F). Both neuronal types exhibited dramatic neurite production in response to both laminin and PNPF-1 in a manner very similar to that already presented for ciliary ganglion neurons as in Fig. 1. Mouse sensory neurons appeared to produce some neurites on untreated PORN-TCP (Figs. 4, A and D), laminin-bearing neurons was always <10%, with the one exception of mouse sensory neurons. As noted above, however, (i.e., Fig. 4 D), this growth was greatly restricted in length and frequency. Both laminin and PNPF-1 presented to the substrate at 1 μg/ml, elicited considerable neuritic extension from all of the neuronal types tested. It remains to be determined whether each of these neuronal types exhibits a dose-response to laminin and PNPF-1 that is similar to that previously shown for ciliary ganglion neurons (cf. Fig. 2).

DISCUSSION

The data presented here demonstrate that laminin is an extremely potent neurite-promoting agent in vitro. Laminin can exert its action when anchored to either polynornithine or TCP substrata, although it is orders-of-magnitude less effective with plastic. Laminin stimulates neuritic outgrowth from a variety of avian and rodent neurons derived from both peripheral and central neural tissues. Neurite promotion can be achieved at half-maximal levels by substratum pretreatments with 50 ng/ml or less of either rat or mouse laminin. Assuming a molecular weight of 10⁶ for laminin (42), this represents a concentration of 4 × 10⁻¹⁴ M. It should be pointed out that the 50 ng/ml of laminin that elicits a half-maximal neurite response is an operational value and does not necessarily reflect the amount of laminin to which the neurons actually respond. Each culture well receives (e.g. for half-maximal effects) 0.05 ml (or 2.5 ng) of laminin, and possibly only a very small fraction of this will eventually be available to the neurons. This is because (a) the culture well bottom on which the neurons attach represents only 25% of the total inside well area exposed to the laminin, (b) all of the laminin presented may not bind to the PORN-TCP surface, and (c) being seeded at a low cell density, the neurons or their neurites or growth cones will have an opportunity to contact only a fraction of the total area of the laminin-bound culture surface. Thus, the amount of laminin to which the neurons actually respond by neuritic production may be far lower than the amount originally presented to the culture well. Irrespective of these considerations, the bioassay for neurite-promoting activity constitutes a more sensitive test for the biological activity of laminin than previous assays that measure its effects on the adhesion or differentiation of other types of cells (43-47).

The attribution of neurite-promoting activity to laminin is supported by (a) the high potency of the laminin preparations, (b) the progression of specific neurite-promoting activity during the purification process which increases along with that of laminin immunoreactivity, and (c) the blocking effect of anti-laminin antibodies. That the activity is associated with laminin itself is also supported by the fact that laminins prepared from different sources using different methods are active. We used both mouse and rat laminin and, after this work was completed, a report by Baron-van Evercooren et al.
(20) appeared attributing neurite-promoting activity to laminin prepared from a mouse tumor different from the one used here. Moreover, we have recently observed (48) that neurite-promoting activity is also conferred to PORN-TCP by fragments of human laminin, obtained from peptic digests of human placenta and purified on the basis of affinity for an immobilized monoclonal antibody to human laminin.

On plain TCP, both fibronectin and laminin had to be presented at relatively high concentrations (1–10 µg/ml) in order to elicit even a modest neurite-promoting effect (Table 1). On PORN-TCP, however, fibronectin treatment elicited very little neurite growth, and this only at concentrations 1,000-fold higher than the laminin concentrations needed for the same effect. To promote neurite regeneration in the...
present bioassay, an agent must be endowed with both neurite-promoting competence and adequate affinity for the PORN-TCP substratum. Thus, the relatively low activity of fibronectin may derive from insufficient binding to polyornithine rather than from actual lack of neurite-promoting capabilities. Conversely, laminin may be a weaker neurite promoter on TCP because it does not allow adequate adhesion of the neurons. The PORN-TCP allows efficient adhesion of the neurons, and laminin, which binds to polyornithine, can then promote neurite regeneration.

Our results regarding the relative inefficiency of fibronectin to promote neurite growth are in agreement with previous reports. Baron-van Evercooren et al. (20) observed neurite-promoting activity from human fetal sensory ganglionic ex-
beensuggestedtobeglycoproteinslikelamininandfibronec-
othercell-conditionedmedia(8-10,13,14,17,18).PNPF have
producedneuritepromotion,butourresultsobtainedwithfibro-
stratumpretreatment.No attemptwasmade inthisstudyto
growtheffectsrequired10tag/mloffibronectinduringsub-
when fibronectinisbound toplasticatpH 7 thanatpH 6.
reaggregateculturesofchickembryoretinalcells.Theyfurther
plasticorpolylysinesubstrata,elicitsneuriteoutgrowthfrom
proteoglycanfrom a ratyolksactumor (24)forneurite-
activity.We testeda heparansulfateproteoglycanpurified
frombovinecornealendothelialcellscontainsa polylysine-
Landeretal.(49)have reportedthatconditionedmedium
bylamininantiserum(Fig.3)andthusconstitutesa distinct
PNPF-1-inducedneurite-promotingactivityisnotblocked
effectiveon severalganglionic(14,15),spinalcord(15,16),
in,orproteoglycans(17,36,49).Likelaminin,PNPF are
seen. 1983.
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plants on plastic substrata treated with 40 µg/ml fibronectin.
Akers et al. (18) have reported that fibronectin, bound to
plastic or polyllysine substrata, elicits neurite outgrowth from
reaggregate cultures of chick embryo retinal cells. They further
report that less fibronectin-induced neurite outgrowth occurs
when fibronectin is bound to plastic at pH 7 than at pH 6.
However, even in optimal conditions, half-maximal neurite
growth effects required 10 µg/ml of fibronectin during sub-
stratum pretreatment. No attempt was made in this study to
examine the pH dependency of fibronectin- or laminin-induc-
ed neurite promotion, but our results obtained with fibro-
nectin coating at pH 7 were similar to Akers et al. (18).
PNPF have been repeatedly observed in glial, heart, and
other cell-conditioned media (8-10, 13, 14, 17, 18). PNPF have
been suggested to be glycoproteins like laminin and fibronec-
tin, or proteoglycans (17, 36, 49). Like laminin, PNPF are
effective on several ganglionic (14, 15), spinal cord (15, 16),
and retinal (17) neurons in monolayer cultures. Nevertheless,
PNPF-1-induced neurite-promoting activity is not blocked by
laminin antiserum (Fig. 3) and thus constitutes a distinct
category of substratum-binding neurite-promoting agents.
Lander et al. (49) have reported that conditioned medium
from bovine corneal endothelial cells contains a polyllysine-
binding neurite-promoting factor for sympathetic neurons
that apparently requires heparan sulfate proteoglycan for its
activity. We tested a heparan sulfate proteoglycan purified
from a rat hepatoma (25) as well as a chondroitin sulfate
proteoglycan from a rat yolk sac tumor (24) for neurite-
promoting activity and found them both to be inactive. It
remains to be determined whether the proteoglycan studied
by Lander et al. (49) does have inherent neurite-promoting
activity or whether it serves as a carrier for a separate neurite-
promoting agent, which itself may or may not be identical to
laminin or PNPF-1.
The potent activity of laminin and PNPF-1 as neurite-
promoting factors in vitro has considerable implications in
vivo. Laminin is a constituent of basal lamina, the ECM
structure observed at the interfaces between mesodermal and
ectodermal tissues (42, 50). Basal lamina is particularly abund-
ant in peripheral nerve (51), but is restricted to perivascular
spaces and meninges in central nervous tissue (52). Laminin
(and PNPF) can be produced and secreted by Schwann cells
(13, 53, 54) and PNPF is produced by CNS glia in culture
(15). Neuroblastoma cells, a PNS-derived neuronal line, have
also been reported to produce laminin in vitro and to deposit
laminin on the substratum occupied by their neuritic exten-
sions (55). Axonal regeneration occurs readily in peripheral
nerve, and the properties and organization of its Schwann
cells may be important contributors to axonal regrowth (56).
In contrast, in the adult mammal, axons in central nervous
tissue usually fail to regenerate (1, 2). Firm evidence has been
recently produced that central axons will regenerate as readily
as peripheral ones if provided with peripheral nerve as the
regeneration microenvironment (3, 4, 57). It is an attractive
speculation, therefore, that the restricted availability of lami-
in (and/or PNPF) may be important in the regulation of
axonal regeneration in the CNS in vivo. Newly developed
culture systems for central neurons (30, 34) and the availabil-
ity of new models for in vivo regeneration studies (58-60)
should make it possible to submit this speculation to experi-
mental testing.

We thank Drs. Michael Brennan and Ake Oldberg for the proteo-
glycan samples.

This work was supported by grant NS-16349 (SV) from the Na-
tional Institute for Neurological and Communicative Diseases and
Stroke and grant AM 30051 (EE) from the National Institute of
Arthritis and Metabolic Diseases and grant CA 28896 (ER) and
Cancer Center Support Grant CA 30199 from the National Cancer
Institute, Dept. of Health and Human Services.

Received for publication 29 March 1983, and in revised form 8 July 1983.

TABLE III
Induction of Neurite Elongation from Cultured Peripheral and
cNS Neurons by PORN-TCP-Bound Laminin and PNPF-1

| Neuronal source | No. of neurons* (range) | Percent with neurites on PORN-TCP pretreated with: |
|-----------------|------------------------|--------------------------------------------------|
|                 |                       | Saline (control) | Laminin (1 µg/ml) | PNPF-1 (1 µg/ml) |
| PNS ganglia     |                        |                  |                    |                  |
| E8 chick ciliary| 700-840                | 6                | 97                 | 97               |
| E8 chick dorsal  | 910-1,070              | 2                | 59                 | 82               |
| root            |                        |                  |                    |                  |
| E11 chick sympa-
thetic              | 830-970                | 1                | 70                 | 70               |
| Neonatal mouse  | 580-600                | 45               | 93                 | 83               |
| dorsal root     |                        |                  |                    |                  |
| CNS tissue      |                        |                  |                    |                  |
| E4 chick lumbar | 450-470                | 3                | 49                 | 83               |
| spinal cord     |                        |                  |                    |                  |
| E8 chick telence-
cephalon          | 1,440-1,550            | 7                | 52                 | 69               |
| E8 chick optic-
tectum          | 1,440-1,750            | 4                | 46                 | 45               |
| E8 chick neural | 1,060-1,210            | 2                | 43                 | 81               |
| retina          |                        |                  |                    |                  |
| E18 rat hippo-
campus        | 1,110-1,390            | 8                | 80                 | 85               |
| E18 rat septum  | 800-870                | 4                | 76                 | 77               |
| E18 rat corpus  | 980-1,160              | 4                | 73                 | 76               |
| striatum        |                        |                  |                    |                  |

* This represents the number of neurons surviving per well after 24 h of
culture. The number of cells originally seeded was 2,000 for each of the
PNS sources (40-50% of which were neurons) and 1,000 cells for E4 cord.
The number of cells seeded per well for the other CNS sources was 2,000
but the proportion of neurons present in these dissociates was not deter-
mined.

This work was supported by grant NS-16349 (SV) from the Na-
tional Institute for Neurological and Communicative Diseases and
Stroke and grant AM 30051 (EE) from the National Institute of
Arthritis and Metabolic Diseases and grant CA 28896 (ER) and
Cancer Center Support Grant CA 30199 from the National Cancer
Institute, Dept. of Health and Human Services.
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