Axolemma Is a Mitogen for Human Schwann Cells

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The mechanisms responsible for the induction of Schwann cell proliferation in peripheral nerves undergoing wallerian degeneration and segmental demyelination are not understood. To determine whether contact with axolemma stimulates mitosis of human Schwann cells, cultured Schwann cells from spinal roots obtained postmortem and from sural nerve biopsy specimens were incubated with axolemmal fractions prepared from human spinal cord or from adult rat central nervous system. Schwann cell proliferation was estimated by autoradiographic assay of tritiated thymidine incorporation. Schwann cell labeling indices after exposure to human or rat axolemmal fractions ranged from 26.7 to 59.9%; labeling indices of Schwann cells cultured without axolemmal fraction were 9.8 to 22.4%. The stimulation index, or ratio of Schwann cell labeling index with axolemmal fraction to that without axolemmal fraction, ranged from 1.97 to 3.40. This study demonstrates that both human and rat axolemma are capable of stimulating human Schwann cell replication in vitro.

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Schwann cell proliferation is rapid during normal peripheral nerve maturation [5, 6] and is a prominent feature of peripheral nerve regeneration after wallerian degeneration [4, 6, 11] or segmental demyelination [1]. The signals that trigger Schwann cell mitosis in developing nerve and after nerve injury are not known.

Recent studies have demonstrated that an axolemmal fraction prepared from either peripheral or central nervous system (CNS) is a potent mitogen for cultured neonatal rat Schwann cells [8, 12, 13]. This effect of axolemmal fraction is signal specific: plasma membrane fractions prepared from skeletal muscle or erythrocytes, or hepatic mitochondrial membranes, do not induce Schwann cell proliferation. The mitogenic effect of axolemmal fraction is also target specific: axolemmal fraction does not induce proliferation of cultured rat endoneurial or dermal fibroblasts or rat astroglia [12, 13]. In the present study we demonstrate that human Schwann cells cultured from the lumbar dorsal roots of a patient without neurological disease or from sural nerves of patients with polyneuropathies with various causes are stimulated to proliferate by incubation with axolemma prepared from either human or rat CNS.

Materials and Methods

Sural nerve fragments were obtained at the time of diagnostic biopsy from four patients (Table). Spinal roots were obtained 7 hours postmortem from a patient with scleroderma without neurological disease. Cultures were instituted by the method of Askanas and colleagues [3]. Epineural connective tissue was removed from the sural nerve specimens under a dissecting microscope, and the sural fascicles and nerve roots were cut into 1 mm^3 explants. These were attached to 22 mm^2 glass coverslips that had been coated with a 1:1 (v/v) mixture of chicken plasma (GIBCO) and chicken embryo extract (GIBCO). The coverslips were placed in dishes with 1.5 ml of a culture medium that contained Eagle's minimal essential medium with Earle's salts (GIBCO), 10% (v/v) fetal calf serum (GIBCO), penicillin 50 units/ml, and streptomycin 50 μg/ml. Outgrowths of Schwann cells from the explants were observed for 2 to 3 weeks in vitro. After this period, the explants were reexplanted on fresh coverslips that had been coated as before and were placed in dishes with fresh culture medium. Schwann cells growing out after the first to third reexplantation were used for the radioautographic studies (see the Table).

Axolemmal fraction was prepared from the lumbar spinal cords of a 52-year-old man (Table, patient 5) and a 53-year-old woman with diabetes mellitus, and from adult female Sprague-Dawley rat CNS white matter, by the method of DeVries with a minor modification [7, 12]. Human or rat axolemmal fraction was suspended in culture medium at a final concentration of 67 μg of axolemmal fraction protein per milliliter. After the explants were maintained for 2 days in 1.5 ml of the culture medium with or without axolemmal fraction, 1.2 μCi of tritiated thymidine (specific activity, 20 mCi/μmol; New England Nuclear) was added to each dish. Twenty-four hours later the medium was replaced with ice-cold 3% glutaraldehyde in 0.1 M sodium phosphate, pH 7.4,

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Mitogenic Response of Cultured Human Schwann Cells to Axolemma[a]

Patient No.	Sex, Age (yr)	Diagnosis	Culture Duration (wk) (Times Reexplanted)	% Labeling Index[b]		Stimulation Index
				No Axolemma	Axolemma	
1	M, 7	Chronic idiopathic polyneuropathy	7 (2) 9 (3)	16.2 ± 0.6 (2) 17.6 ± 3.4[c] (2)	43.0 ± 5.9 (3) 40.6 ± 3.1[c] (3)	2.65 2.31[c]
2	F, 59	Sarcoidosis, neuropathy	5 (2)	17.6 ± 4.9 (3)	59.9 ± 7.5 (2)	3.40
3	M, 62	Acquired amyloid polyneuropathy	5 (2)	22.4 ± 4.8 (4)	52.2 ± 4.1 (3)	2.33
4	F, 7	Chronic idiopathic polyneuropathy	4 (1)	19.0 ± 1.3 (3)	37.6 ± 4.3 (3)	1.97
5	M, 52	Scleroderma	3 (1)	9.8 ± 5.4 (2)	26.7 ± 6.2 (2)	2.72

[a]All results were obtained using Schwann cells cultured from sural nerve biopsy specimens with the exception of results for patient 5; in that case, lumbar dorsal roots obtained 7 hours postmortem were employed. Human axolemma was used in all cases.
[b]All values expressed ± standard deviation; number of determinations given in parentheses.
[c]Schwann cells were also exposed to rat central nervous system axolemma.

for 10 minutes. After overnight washing with 140 mM of sodium chloride, 5 mM sodium phosphate, pH 7.4 (phosphate-buffered saline), the coverslips were air dried, mounted on glass slides, and dipped into Kodak NTB-2 photographic emulsion in the dark. After 5 days at 5°C, the slides were developed, fixed, washed, dried, and stained with 0.3% toluidine blue in phosphate-buffered saline for 20 seconds.

Schwann cells were distinguished from fibroblasts in the toluidine blue–stained preparations by their long, spindle shape, darker cytoplasm, and narrow, elongated nuclei [2, 3, 9]. In contrast, fibroblast-like cells were flat, were polymorphous in outline, and had lightly stained cytoplasm and round nuclei (see Fig 1).

Under the conditions used in this study, nuclei were either completely blackened by confluent silver grains (scored "positive") or contained at most a few scattered silver grains (scored "negative"). A minimum of 400 Schwann cell nuclei were scored on each slide. Quantitative evaluation was performed in each case by two independent observers. The percentage-of-labeling index was calculated as the ratio of labeled Schwann cell nuclei to total Schwann cell nuclei counted. The stimulation index was expressed as the ratio of the Schwann cell labeling index with axolemmal fraction to the Schwann cell labeling index without axolemmal fraction.

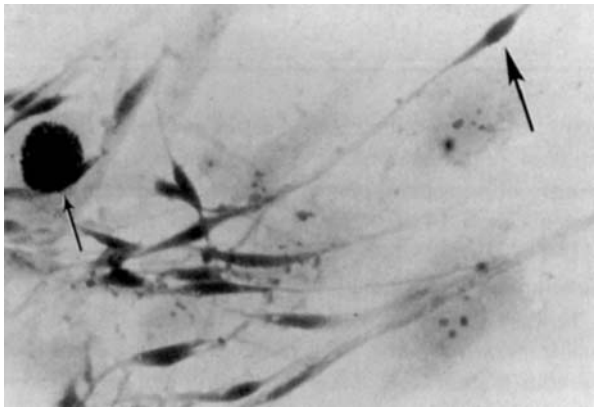

Fig 1. Photomicrograph of cultured human Schwann cells and fibroblast-like cells after exposure to tritiated thymidine. Schwann cells are spindle shaped, with an elongated nucleus and darkly stained cytoplasm. Fibroblast-like cells are flat, are irregular in outline, and have a round nucleus and lightly stained cytoplasm. In this field dense clusters of silver grains demonstrate that one Schwann cell (large arrow) *and one fibroblast-like cell* (small arrow) *have incorporated tritiated thymidine into the nucleus. (×480 before 30% reduction.)*

Results

Figure 1 shows Schwann cells and fibroblast-like cells in the outgrowth from an explant. In this field, one Schwann cell and one fibroblast-like cell incorporate tritiated thymidine. Schwann cells are readily distinguished from fibroblast-like cells by their characteristic morphological features (see Materials and Methods section).

Although scattered Schwann cell nuclei in cultures not treated with axolemmal fraction incorporated tritiated thymidine (Fig 2), many more Schwann cell nuclei were labeled in parallel cultures to which axolemmal fraction had been added (Fig 3). Percentage-of-labeling indices of Schwann cell nuclei in axolemmal fraction–treated cultures ranged from 26.7 to 59.9, markedly greater than the 9.8 to 22.4 labeling indices of Schwann cell nuclei in the simultaneous nontreated cultures (see the Table). Schwann cell stimulation indices ranged from 1.97 to 3.40 (see the Table).

Schwann cells from one patient were tested with rat axolemmal fraction as well as with human axolemmal fraction (Table, patient 1). The stimulation index obtained with the rat fraction was similar to that obtained with the human fraction.

Discussion

The magnitude of the mitogenic effect of human CNS axolemmal fraction on cultured human Schwann cells (stimulation index of 1.97 to 3.40 after 3 days' expo-

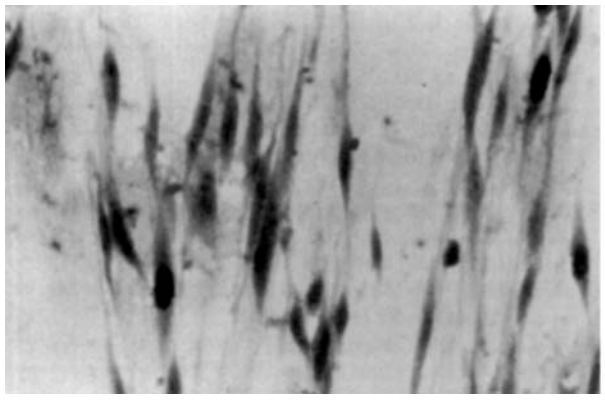

Fig 2. Photomicrograph of Schwann cell–rich portion of outgrowth from an explant from patient 3 without axolemma. Four Schwann cell nuclei are covered by silver grains. (Toluidine blue stain; ×480 before 30% reduction.)

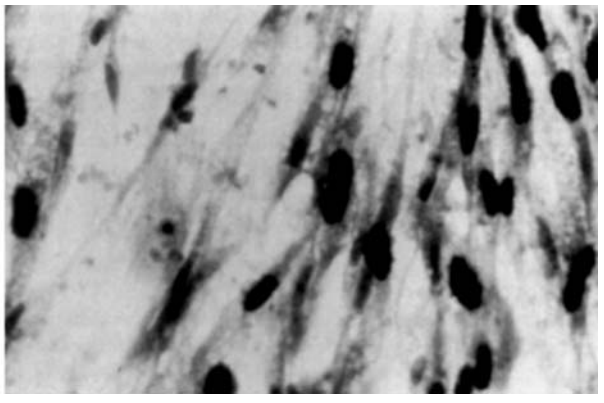

Fig 3. Photomicrograph of Schwann cell–rich portion of outgrowth from an explant from patient 3 after treatment with human central nervous system axolemma. More than half the Schwann cell nuclei are covered by silver grains. (Toluidine blue stain; ×480 before 30% reduction.)

sure to axolemmal fraction) was considerably smaller than that observed previously when cultured neonatal rat Schwann cells were treated with rat CNS axolemmal fraction (stimulation index of 14 or more after 2 days' exposure to axolemmal fraction) [12, 13]. This discrepancy may reflect a difference in proliferative potential between Schwann cells of newborn rat and those from human children and adults. Alternately, it may be a consequence of the selection of differing populations of Schwann cells by the enzymatic dissociation–differential adhesion procedure employed for the rat and the explantation-reexplantation procedure [3] used for the human specimens.

We found that cultured human Schwann cells respond to the mitogenic signal of rat CNS axolemmal fraction as well as to human CNS axolemmal fraction. This lack of a species barrier in the interaction of Schwann cells with axonal plasma membrane is consistent with previous reports that bovine as well as rat axolemmal fractions stimulate rat Schwann cell proliferation [8].

Axonal disintegration is a prominent early feature of wallerian degeneration [1]. It seems likely that Schwann cells in such degenerating nerves come in contact with exposed axonal plasma membranes during this process [4, 6, 11]. This consideration, and the observation that the proliferation of Schwann cells in the distal stumps of transected mouse sciatic nerves [4] is similar in magnitude and time course to that induced by the addition of axolemmal fraction to cultured rat Schwann cells [12], suggest that Schwann cell proliferation during wallerian degeneration is induced by the same mechanism as the Schwann cell proliferation observed in vitro upon addition of axolemmal fraction to the medium. Proliferation of Schwann cells in the distal segment of unmyelinated autonomic nerves after a crush injury proximally is considerably less prominent than that observed in the distal segment of cut or crushed myelinated nerves [10], however. The reasons for this difference in the mitogenic response of Schwann cells to wallerian degeneration of myelinated and unmyelinated nerves are not known.

Increased numbers of Schwann cells are also present in nerves that have undergone segmental demyelination, particularly when several cycles of demyelination and remyelination have occurred. Excess Schwann cells in such nerves surround axons in a circumferential pattern to form "onion bulbs" [1]. It seems possible that Schwann cell proliferation in these nerves is a consequence of the contact of Schwann cells with the exposed axolemma of demyelinated axons.

We have shown that the rate of proliferation of cultured human Schwann cells is considerably accelerated by addition of fragments of human axonal plasma membrane to the medium. This phenomenon was observed with Schwann cells from both spinal roots and sural nerves, from both children and adults, and from patients both with and without acquired neuropathies. Further studies are needed to determine whether Schwann cells derived from patients with the various genetic polyneuropathies will also be stimulated to proliferate by fragments of axonal plasma membrane.

Because very limited numbers of Schwann cells can be cultured from human sural nerve biopsy specimens, it has been difficult to initiate biochemical investigations of Schwann cell metabolism in the genetic and acquired demyelinative and dysmyelinative polyneuropathies. Larger numbers of Schwann cells can be obtained by stimulation of Schwann cell proliferation with axolemmal fragments [12], and this technique should facilitate future biochemical studies of cultured Schwann cells derived from patients with these diseases.

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