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Induction of myelination in the central nervous system by electrical activity

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ABSTRACT **The oligodendrocyte is the myelin-forming cell in the central nervous system. Despite the close interaction between axons and oligodendrocytes, there is little evidence that neurons influence myelinogenesis. On the contrary, newly differentiated oligodendrocytes, which mature in culture in the total absence of neurons, synthesize the myelin-specific constituents of oligodendrocytes differentiated *in vivo* and even form myelin-like figures. Neuronal electrical activity may be required, however, for the appropriate formation of the myelin sheath. To investigate the role of electrical activity on myelin formation, we have used highly specific neurotoxins, which can either block (tetrodotoxin) or increase (α-scorpion toxin) the firing of neurons. We show that myelination can be inhibited by blocking the action potential of neighboring axons or enhanced by increasing their electrical activity, clearly linking neuronal electrical activity to myelinogenesis.**

Oligodendrocytes, in the central nervous system (CNS), and Schwann cells, in the peripheral nervous system, have the unique ability to synthesize large amounts of membrane that wrap around axons and compact to form myelin. In the peripheral nervous system and the CNS, neuronal influences on myelin-forming cells appear, however, to be quite different. In the peripheral nervous system, axonal signals are mandatory at all the stages of Schwann cell precursor development into myelin-forming cells; for example, it has been shown that proliferation, survival, and differentiation of Schwann cell precursors does not occur in the absence of neurons (1, 2). Similarly, since the pioneer work of Aguayo and coworkers (3), it is now well established that the signal for nerve engulfment and ensheathment originates in axons with which mature Schwann cells interact (4–6). Myelin formation in the CNS, however, seems to be less axon-dependent, because oligodendrocyte progenitors *in vitro* proliferate and differentiate into postmitotic oligodendrocytes in neuron-free cultures (for review, see ref. 7). Moreover, newly differentiated oligodendrocytes, which mature in culture in the total absence of neurons, synthesize the myelin-specific constituents of oligodendrocytes differentiated *in vivo* (8, 9). Lastly, purified mature oligodendrocytes, when maintained in culture, extend at the tip of their processes large unfolded membranes, which can even wrap around themselves to form myelin-like figures (10, 11) or adhere to carbon fibers (12).

However, myelin formed *in vitro* in the absence of neurons, when analyzed by electron microscopy, is not as well compacted as when wrapped around axons (12, 13), suggesting a possible role for axons in myelin compaction. Other arguments speak in favor of a role for axons in oligodendrogenesis and myelin formation. It has been reported that, in mixed cultures, dorsal root ganglions exert a mitogenic effect on oligodendrocyte progenitors (14, 15), and that, *in vivo*, proliferation of such progenitors, in the optic nerve, depends on the electrical activity of retinal ganglion cells (16). Survival of newly differentiated oligodendrocytes has been proposed to depend on axonal signaling molecules (17, 18). The observation that, even in culture, oligodendrocytes myelinate only axons, not dendrites, suggests the existence, at the surface of axons, of a recognition signal that permits their ensheathment by the oligodendrocyte processes (13). Coculture experiments have also indicated that the presence of neurons result in the up-regulation of myelin-specific gene transcription by mature oligodendrocytes (19).

To determine whether the onset of myelination was the consequence only of oligodendrocyte maturation or depended on an axonal signal, we investigated the influence of axonal electrical activity on myelinogenesis. Indeed, action potentials have been demonstrated to play a key role during CNS development, particularly in the visual system (20, 21). Here we show that inhibition of electrical activity with the specific Na^+ channel blocker tetrodotoxin (TTX) prevents the initiation of myelinogenesis in a system of *in vitro* myelination using dissociated cultures from embryonic brain and *in vivo*, in the optic nerve. In addition, with K^+ that blocks action potentials by maintaining the cells in a depolarized state, or α-scorpion toxin (α-ScTX), which induces repetitive electrical activity by slowing Na^+ channel inactivation, we provide evidence that it is the action potential itself which is responsible for the onset of myelination.

MATERIALS AND METHODS

Animals. For *in vivo* experiments, outbred OF1 mice (Iffa Credo) were taken between postnatal day 3 (P3) and P8. For myelinating cultures, embryos were taken from pregnant mice, obtained from the same source, at 15 days of gestation.

Antibodies. Anti-myelin basic protein (MBP) mAb (mouse monoclonal IgG1, culture supernatant from clone M-010h; Euromedex, Souffelweyersheim, France) was diluted 1:60. For double immunolabeling, the following antibodies were used: mouse anti-MBP mAb, diluted 1:2 (IgG2a, culture supernatant from clone 1; Serotec); anti-myelin/oligodendrocyte glycoprotein (MOG) mAb (IgG1), diluted 1:20 [a culture supernatant from mouse 8–18C5 hybridoma, kindly provided by C. Linington, Max-Planck-Institut, Planegg Martinsried, Germany (22)]; and antimicrotubule associated protein 2

Abbreviations: CNS, central nervous system; TTX, tetrodotoxin; α-ScTX, α-scorpion toxin; P, postnatal day; MBP, myelin basic protein; MOG, myelin/oligodendrocyte glycoprotein; MAP-2, microtubule associated protein 2; PDGF-AA, platelet-derived growth factor AA; DIV, days *in vitro*.

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(MAP-2) antibody, mouse monoclonal IgG1, culture supernatant diluted 1:500 [a gift of A. Frankfurter, University of Virginia, Charlottesville, VA (23)]. Fluorescein-conjugated sheep anti-mouse IgG1 and Texas red-conjugated sheep anti-mouse IgG2a antibodies from Southern Biotechnology Associates were diluted 1:100.

Cell Cultures. Myelinating cultures were prepared as described (13). Briefly, E15 cerebral hemispheres were first mechanically dissociated and then treated with 0.05% trypsin (Seromed, Noisy le Grand, France). After washing, the cell suspension was passed gently through a nylon mesh (63-μm pores), pelleted, and resuspended in DMEM (Seromed) containing 10% fetal calf serum and 0.028% BSA (Miles). About 5.10^4 cells were plated on poly-L-lysine-coated (Sigma), 14-mm diameter glass coverslips deposited on the bottom of a 24-well plate (Costar). Cultures were maintained in Bottenstein and Sato medium (24), supplemented with 1% fetal calf serum, 1% penicillin-streptomycin (Seromed), 10 ng/ml recombinant platelet derived growth factor AA (PDGF-AA; Upstate Biotechnology, Lake Placid, NY).

Immunolabeling of Cells in Culture. Cultures were fixed in 4% paraformaldehyde in PBS for 20 min at room temperature. After washing and saturation in 4% dry skimmed milk in PBS, cells were incubated with the primary mAbs (anti-MBP, clone 1, and anti-MAP-2), which were diluted in DMEM containing 0.1% Triton X-100, for 25 min at room temperature. After washing excess antibodies, coverslips were incubated for 25 min with a mixture of anti-IgG1- and anti-IgG2a-conjugated antibodies. Negative controls were performed by incubating the cells with the second antibodies alone or by revealing each first mAb with a nonrelated conjugated antibody.

Intravitreous Injections. TTX (0.5 μl, 10^{-4} M; Sigma), vehicle (acetate buffer), or PBS was injected with a 5-μl Hamilton syringe through a 34-gauge needle, just posterior to the corneoscleral junction, into the vitreous humor of the right eye of cold-anesthetized P4 mice, as described (16). In preliminary experiments, the success of the injection was verified by adding the fluorescent dye 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindo-carbocyanine (Molecular Probes; 25 g/liter in dimethylformamide) to the TTX solution. In control animals receiving the solvent alone, dimethylformamide was found to dramatically decrease the immunostaining. Because dimethylformamide interferes either with immunodetection of myelin antigens or with the myelination process itself, possibly through a toxic effect on the retinal ganglion cells, the dye was not used systematically.

Immunostaining on Whole Mount Optic Nerves. Optic nerves were studied between P3 and P8. Mice were anesthetized then perfused intracardially with paraformaldehyde (4% in PBS). The optic nerves were removed, separated at the level of the chiasm, and post-fixed for 2 h in the same fixative before being processed for whole-mount immunohistochemistry. The nerves were incubated for 1 h in normal sheep serum diluted 1:1 in PBS containing 10% fetal calf serum (Flow Laboratories) to lower nonspecific staining, then overnight at 4°C with anti-MBP mAb (clone M-010h), or with a mixture of anti-MBP (clone 1) and anti-MOG antibodies. All antibodies were diluted in PBS containing 1% Triton X-100 and 0.2% gelatine. Excess first antibody was eliminated by washing in PBS. Specific binding of antibodies was revealed by a 1-h incubation at room temperature with fluorochrome-conjugated secondary antibodies diluted in the same medium. After elimination of excess second antibody, optic nerves were mounted in Vectashield (Vector Laboratories) and examined with a Leica fluorescent photomicroscope.

Electron Microscopy. Culture medium was removed and the cells were washed with 0.01 M PBS before being fixed in 2.5% glutaraldehyde in 0.1 M PBS for 2 h at 25°C. After three washes in PBS, cells were post-fixed in 2% osmium tetroxide for 30 min. After a brief passage in distilled water and dehydratation in a graded series of ethanol solutions, the cultures still on the coverslips were embedded in araldite. The blocks of araldite were separated from the coverslips by thermal shock with liquid nitrogen. Ultrathin sections were cut parallel to the surface of the cultures. Anesthetized 6-day-old mice were fixed by intracardiac perfusion of 1% glutaraldehyde and 1% paraformaldehyde in 0.1 M PBS. After perfusion, the optic nerves were removed, post-fixed for 1 h in the same fixative, processed, thin-sectioned, and stained by conventional techniques. Sections were examined with a JEOL 1200 EX electron microscope operated at 70 KV.

RESULTS

Effect of TTX on Myelination in Culture. The role of electrical activity on myelination was first studied *in vitro* with the selective Na^+ channel blocker TTX. Embryonic mouse brain hemispheres were dissociated at day 15 of gestation and maintained in culture in the presence of PDGF (10 ng/ml). Under these conditions, the first myelinated axons were seen after 13–15 days *in vitro* (DIV) (13). At the optical level, myelin sheaths wrapped around the neurites were easily recognizable by the bright double fluorescent line observed with an antibody against MBP, a major constituent of myelin (Fig. 1*A*). Electron microscopic examination of the cultures confirmed that the myelin was deposited around axons and was well compacted (see Fig. 3*A*). When cultures at 8 DIV, i.e., 5–7 days before the beginning of myelination, were treated with TTX for 2, 4, or 6 days, the number of myelinated fibers at 18–21 DIV was decreased by 83%, 87%, and 98% (Fig. 1*B*). Neither the number of oligodendrocytes (Fig. 1*C*) nor the number of neuronal cell bodies (Fig. 1*D*), up to 4 days in the presence of TTX, were affected by the treatment, and no evidence of oligodendroglial (data not shown) or neuronal suffering could be detected at the ultrastructural level by electron microscopy (see Fig. 3*B*). Neuronal loss of 42% was observed after 6 days of treatment. This might contribute in part to the nearly total absence of myelination observed (Fig. 1 *B* and *D*). An effect on the myelination process itself, however, must also have occurred since treatment with TTX for only 2 days at either 9 or 12 DIV resulted (at 21 DIV) in a 61% and 98% decrease in the number of myelinated fibers, respectively, without neuronal loss (Table 1). The inhibitory effect of TTX depended, therefore, on the developmental state of the oligodendrocytes and neurons. It was most pronounced when the toxin was added just before the onset of myelination and was only transient; indeed, when parallel cultures were examined at 28 DIV, the number of myelinated segments was similar in the TTX-treated and control cultures. Similarly, when TTX was added to the culture at 18–21 DIV and the preparations were analyzed at 28 DIV, no significant effect of the toxin was observed.

The effect of TTX on myelination could be due either to the change in the polarity of the axonal membrane or to the blockade of action potentials. To discriminate between these two possibilities, K^+ (15 mM) was added to the culture medium either alone or together with TTX. As shown in Table 1, K^+ was not only unable to counteract the effect of TTX, it also inhibited myelination itself, suggesting that the blockade of electrical activity, and not the changes in axonal membrane polarity, was responsible for the observed inhibition of myelination.

Effect of α-ScTX on Myelination in Culture. If blockade of the neuronal Na^+ channels inhibits myelination, stimulation of neuronal activity by opening the channels should increase myelination. This can be achieved with the highly selective Na^+ channel activator α-ScTX, which has been shown to dramatically increase the duration and frequency of spontaneous action potentials by slowing Na^+ channel inactivation without any effect on channel activation or resting membrane potential (26). Indeed, when cultures at 8 DIV were treated for 2 days with α-ScTX, the number of myelinated segments observed 10

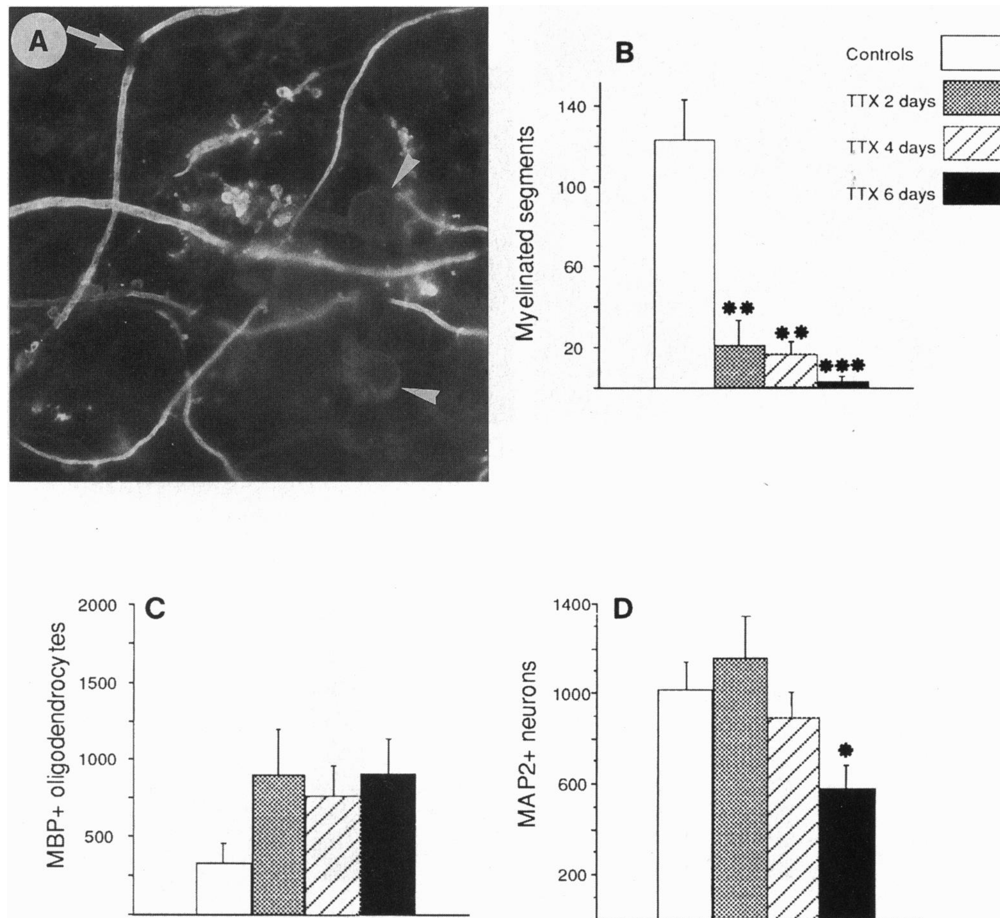

FIG. 1. The effect of TTX on the number of myelinated segments, oligodendrocytes, and neurons in myelinating cultures. Cocultures of neurons and oligodendrocyte progenitors were established from cerebral hemispheres of 15-day-old mouse fetuses. TTX (10^{-6} M) was added to the culture medium at 8 DIV and maintained for 2, 4, or 6 days, as indicated. Cultures at 18–21 DIV were doubly immunolabeled with a combination of anti-MBP and anti-MAP-2 antibodies. (*A*) A typical myelinated field of a control culture stained with anti-MBP antibody. Myelin deposited around axons appears as bright MBP-positive double lines, sometime interrupted at node of Ranvier (arrow), whereas the oligodendrocyte cell bodies (arrowheads) are faintly stained because MBP migrates out of the cell bodies of the myelin-forming oligodendrocytes. The total number of MBP-positive myelinated segments (*B*) or oligodendrocyte immunoreactive cell bodies (*C*) per coverslip was determined. In the same cultures, the number of neurons, MAP-2-positive cell bodies in *D*, was evaluated. Values are the mean ± SEM of eight different experiments. Significant differences between controls and TTX-treated samples are indicated as follows: *, $P < 0.05$; **, $P < 0.02$; ***, $P < 0.001$ (two-tailed unpaired Student's *t* test). (*A*, ×640.)

days later increased by a factor of 2.4, compared with control cultures, without a significant effect on the number of MBP-positive oligodendrocytes (Table 1). Longer exposures (4–6 days) to α-ScTX did not further increase the number of myelinated fibers. The effect of α-ScTX could not be attributed to an opening of astrocytic sodium channels as this toxin has no effect on cells that do not present spontaneous action potentials, whereas it induces a large increase in the duration of action potentials in spontaneously active cells. This property of α-ScTX was verified by measurement of toxin-induced $^{22}Na^+$ influx in cultures of either fetal mouse brain neurons or astrocytes. In neuronal cultures, α-ScTX (10^{-9} M) alone, was able to generate an increase in $^{22}Na^+$ influx, which was enhanced in the presence of veratridine, a Na^+ channel opener. In contrast, in cultured astrocytes, α-ScTX alone, even at 10^{-7} M, had no detectable effect on the $^{22}Na^+$ influx (data not shown).

Time Course of Myelination in the Mouse Optic Nerve. To confirm the above *in vitro* findings, we analyzed the effect of electrical activity on induction of myelination in the optic nerve. First, the time course of myelination during postnatal development in the mouse optic nerve was determined using an mAb against MBP, which begins to be expressed by oligodendrocytes before myelination (27, 28). Nonmyelinating MBP-positive oligodendrocytes were first seen on the retinal portion of the optic nerve at P4. These cells had very rich arborizations, reminiscent of the typical "sun-like" mature oligodendrocytes observed in culture. Both the cell body and the processes were stained with the anti-MBP antibody (Fig. 2*B*) but were always MOG-negative. At P5, nonmyelinating MBP-positive oligodendrocytes were unevenly scattered along the optic nerve, including the chiasm. The first myelinating oligodendrocytes were first detected at P6. They could easily be distinguished by their morphology from nonmyelinating oligodendrocytes. They had a reduced number of processes, which were aligned along the neighboring axons to form myelin sheaths, recognizable by their characteristic strongly MBP-positive double outline, which contrasted with the weak labeling of the cell body (Fig. 2*A*). Myelin-forming cells were also MOG-positive, which, in addition to the morphological criteria, distinguished them unambiguously from nonmyelinating oligodendrocytes (data not shown). When examined at the ultrastructural level, the first myelin sheaths observed at P6 were poorly compacted (Fig. 3*C*).

Effect of Intravitreous Injection of TTX on Myelination in the Optic Nerve. When TTX was injected into the right intravitreous space at P4 and the optic nerve was examined at P6, there was no statistically significant modification in the

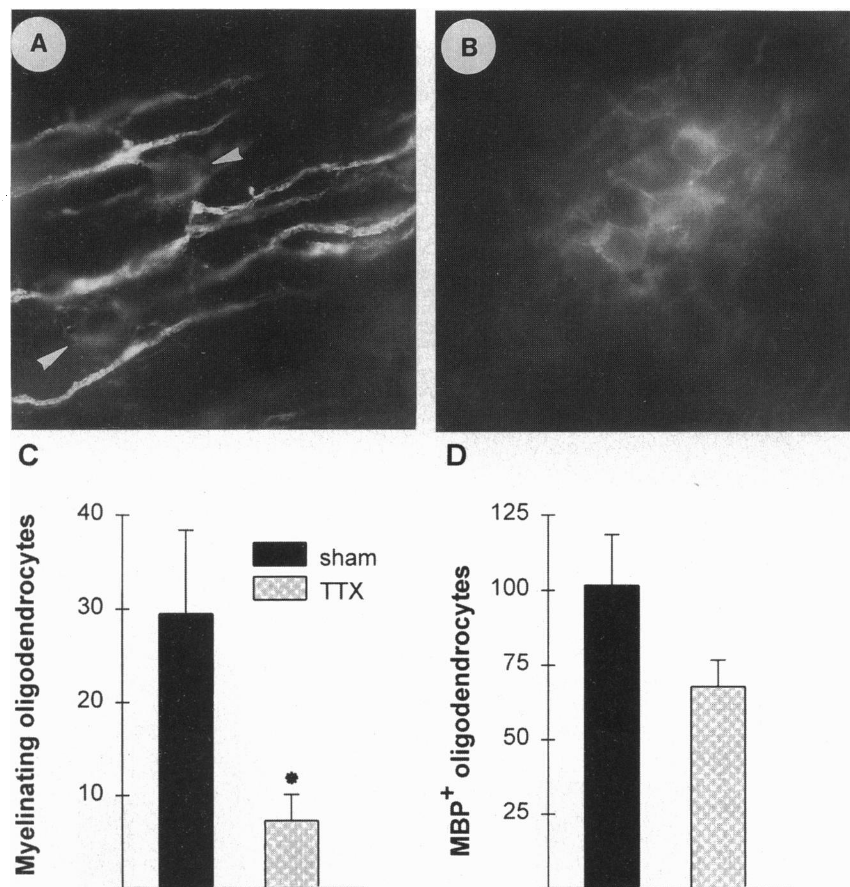

FIG. 2. The effect of an intraocular injection of TTX on the onset of myelination in the developing optic nerve in mice. Typical myelin-forming (A) and nonmyelinating (B) MBP-positive oligodendrocytes as seen by epifluorescence microscopy in whole mounts of mouse optic nerve at P6 (*A*) and P4 (*B*). The numbers of myelinating (*C*) and nonmyelinating (*D*) MBP-positive oligodendrocytes per optic nerve in TTX-treated or sham-injected mice are shown. The total number of nonmyelinating and of myelin-forming MBP-positive cells was determined in each optic nerve from the emergence of the optic canal to the optic chiasm (*C* and *D*). The TTX-treated and sham-injected control optic nerves were always from littermates. Results obtained in 12 sham and 10 TTX-injected animals (4 separate experiments) are expressed as the mean ± SEM, and significant differences between controls and TTX-treated samples are indicated as follows: *, $P < 0.05$ (two-tailed unpaired Student's *t* test). Arrowheads in *A* point to the cell bodies of myelinating oligodendrocytes. (*A* and *B*, ×530).

total number of MBP-positive oligodendrocytes, whereas myelinating oligodendrocytes were decreased by 75% (Fig. 2 *C* and *D*), suggesting that electrical activity affected the onset of myelination. This effect of TTX could not be attributed to axon damage, as shown by electron microscopic examination of the injected optic nerve (Fig. 3*D*). Furthermore, in the optic nerve of TTX-treated animals, very rare myelin sheaths were observed. These ensheathments had the normal morphology of newly formed uncompacted myelin (Fig. 3, compare *C* and *E*). Injection of TTX at P5 did not significantly decrease the percentage of myelinating oligodendrocytes at P7. This suggests that electrical activity affects the myelination process only within a narrow time frame.

DISCUSSION

To examine the role of neuronal electrical activity on myelination, we have used neurotoxins highly specific for voltage-sensitive Na^+ channels. Our results show that myelination can either be inhibited by TTX, a blocker of Na^+ channels, or enhanced by α-ScTX, which slows inactivation of these channels.

The inhibitory effect of TTX on myelination could be in part explained, however, by an indirect effect on the proliferation

Table 1. The effect of TTX, α-ScTX, and K^+ on myelination in cultures

Factor added	Time added, DIV	Myelinated segments, % controls	MBP-positive cells, % controls
TTX (10^{-6} M) (n = 3)	9	39.4 ± 16.2	92.6 ± 17.3
TTX (10^{-6} M) (n = 3)	12	2.4 ± 1.1	159.6 ± 13.8
α-ScTX (10^{-8} M) (n = 5)	8	241 ± 44	119.8 ± 22.9
K^+ (15 mM) (n = 3)	12	2.9 ± 1.4	80.1 ± 11.8
TTX + K^+ (15 mM) (n = 3)	12	1.2 ± 0.6	93.8 ± 15.3

Myelinating cultures were as in Fig. 1. At various times after seeding, TTX, α-ScTX (toxin II from *Androctonus australis* Hector), K^+, or TTX plus K^+ were added to the culture media. Treatment lasted 2 days, after which excess reagents were thoroughly eliminated by washing as described (25). The total number of myelinated segments and of MBP-expressing oligodendrocytes per coverslip was evaluated at 21 DIV after immunolabeling with the anti-MBP antibody. Results are expressed as percent of values observed in control cultures (mean ± SEM).

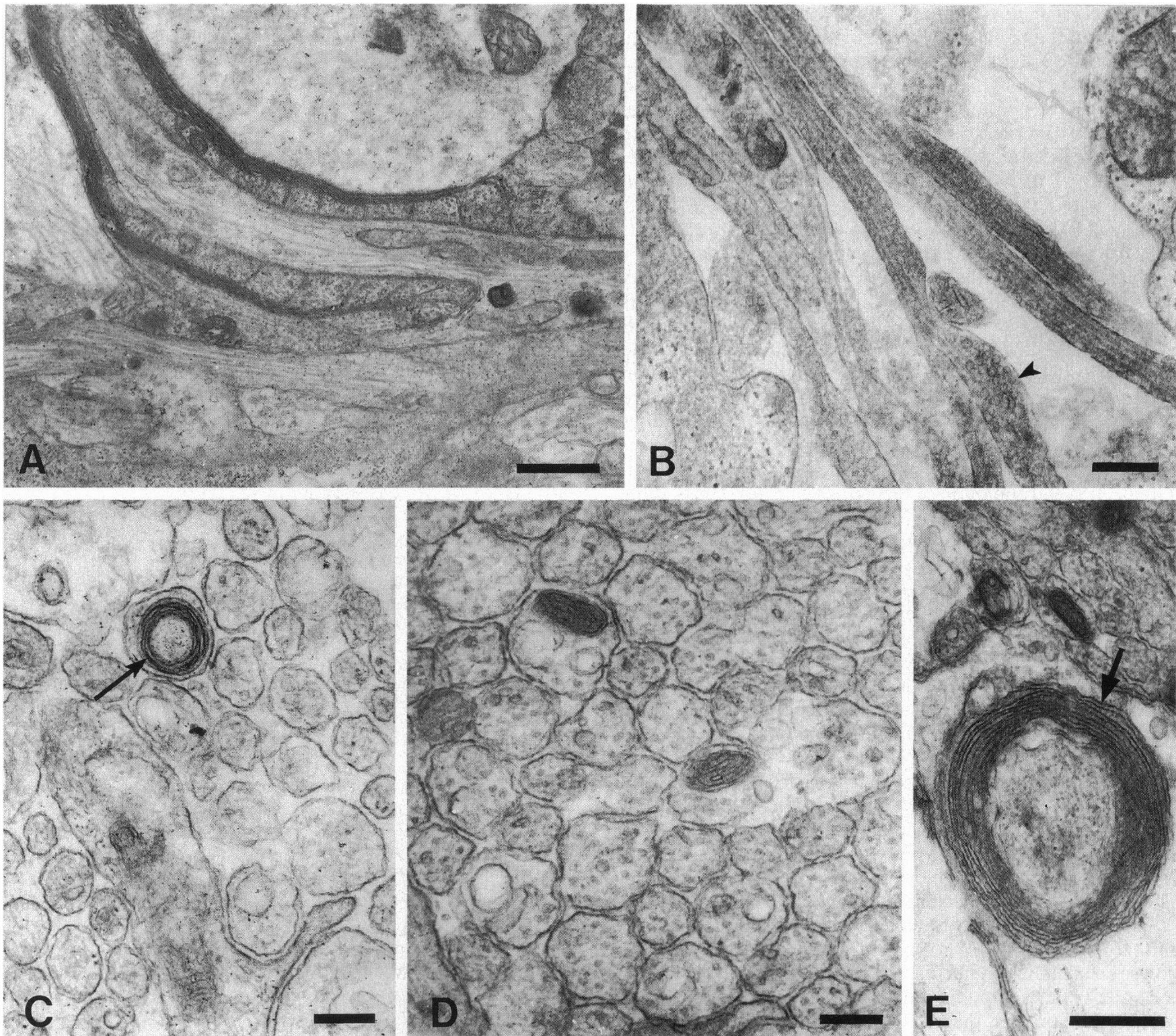

FIG. 3. Transmission electron micrographs of control (*A* and *C*) and TTX-treated (*B*, *D*, and *E*) myelinating cultures at 21 DIV (*A* and *B*) and 6-day-old mouse optic nerve (*C–E*). (*A*) The paranodal region of a longitudinally sectioned myelinated axon from a control culture illustrates the region where the myelin sheath ends and become progressively thinner as the lamellae terminates. (*B*) TTX (10^{-6} M) treatment for 4 days did not cause apparent morphological damage to the axons. The arrowhead points to a varicosity containing small vesicles. (*C–E*) Transverse sections of P6 optic nerves either sham- (*C*) or TTX-injected at P4. In the control nerve, the myelination process had just started, as illustrated by an axon surrounded by few layers of uncompacted myelin (arrow in *C*). In the TTX-treated nerve, axons retain a normal morphology and show no evidence for degeneration (*D*). The number of myelinated axons was markedly reduced. However, in the rare case when a myelinated axon (arrow) was observed (*E*), the myelin sheath appeared similar to the control. (Bars: *B–D*, 200 nm; *A* and *E*, 500 nm.)

of oligodendrocyte progenitors (16). This is unlikely to be the case *in vitro*, as this latter effect can be counteracted by adding PDGF-AA to the culture medium (16). Indeed, in TTX-treated cultures supplemented with PDGF, the number of oligodendrocytes was similar to the number of controls. Under these experimental conditions, TTX induced a dramatic inhibition of the number of myelinated segments. In the optic nerve, oligodendrocyte progenitors that are prevented from proliferating by TTX treatment will differentiate prematurely into oligodendrocytes. However, it has previously been shown (9, 27) that it takes 3–5 days for a newly differentiated oligodendrocyte to mature into an MBP- expressing cell and an additional 1–2 days before it starts to deposit myelin (27–28). As our experimental protocol took place over a 2-day period, premature differentiation of oligodendrocyte progenitors is unlikely to have interfered with the results.

The neurotoxins probably did not influence the process of myelination by a direct effect on oligodendrocytes, but rather by modulating neuronal activity. It has indeed been clearly established that TTX-sensitive Na^+ channels are not expressed on mature oligodendrocytes (29). It has been demonstrated that neurons in primary cultures, however, express voltage-dependent Na^+ channel after 4 days in culture (30). Thus, the effect of TTX on myelination *in vitro* and the inverse effect of α-ScTX strongly suggest that neuronal Na^+ channels, and hence electrical activity, are involved in the process. These observations are consistent with previous reports, in more physiological circumstances, showing that myelination of the optic nerve is delayed in animals reared in the dark (31), and highly decreased in the naturally blind cape mole rat[‖], whereas premature eye opening accelerates myelination in the optic nerve (32).

‖Omlin, F. X., Proceedings of the 1st European Meeting on Glial Cell Function, March 24–27, 1994, Heidelberg, p. 146 (abstr.).

During normal development, electrical activity seems to affect myelination only within a narrow time frame. In the optic nerve, the effect of TTX was more pronounced when the injection was at P4 and the nerves examined at P6, as compared with the P5–P7 time interval. Similarly, in culture, no effect of the toxin was observed when TTX was added to the culture medium 1 week after the onset of myelination. Furthermore, the effect of TTX seems to be only transient, because in cultures treated for only 2 days and examined at 28 DIV, instead of 21 DIV, the number of myelinated segments was similar to control values. This suggests that removal of TTX from the medium and subsequent recovery of electrical activity in the neurons (25), allows myelination to proceed. It is possible, however, that in the sustained absence of electrical activity, myelination would not occur at all. Indeed, in the naturally blind cape mole rat, a high number of axons remain unmyelinated[||], suggesting that when retinal ganglion cells are not electrically active, myelination is not just delayed, it does not take place. These observations, however, are in contradiction with data recently reported showing no decrease in myelination in a group of three rats pups given, for 9 days, daily intraocular injections of TTX (33).

These findings suggest that spontaneous neuronal activity, dependent upon functional voltage-sensitive Na^+ channels, plays a major role in the initiation of myelination and raises the question of the nature of the communication between neurons and oligodendrocytes. K^+ released by electrically active axons is probably not the intermediary, because exogenous K^+ added to the medium did not counteract the effect of TTX (Table 1). This result indicates that permanent depolarization of the neuronal cell membrane was unable to mimic the effect of spontaneous action potentials. The observation that K^+ alone inhibits myelination to the same extent as TTX supports the hypothesis that it is the electrical influx itself that induces the onset of myelination. Indeed, maintaining the culture for 2 days in the presence of 20 mM K^+ caused a sustained depolarization of the neurons, thus blocking the action potentials.

The nature of the molecular mechanisms that are responsible for the initiation of myelinogenesis and are induced by the electrical activity are unknown. One possibility is active secretion of a signaling molecule. In mature neurons, exocytosis of synaptic vesicles is restricted to axon terminals. Earlier in development, however, the exo-endocytosis of synaptic vesicles takes place in all cellular compartments and, in particular, along the axons (34). The depolarization-mediated liberation of axonal factors could, therefore, act either directly on the oligodendrocytes to activate the myelination program or indirectly, via astrocytes, as has been proposed for PDGF (16). Alternatively, neuronal activity might induce the expression of signaling molecules on the axonal surface that trigger the myelination process by contact with the oligodendrocyte. It has been shown that mobilization of the polysialic neural cell adhesion molecule or the neural recognition molecule L1 on the cell surface of neurons requires electrical activity (35, 36). Consistent with the existence at the axonal surface of signaling molecules interacting with oligodendrocytes are the observations that (*i*) even in culture, only axons (and not dendrites) are myelinated, suggesting the existence of an axonal surface recognition signal (13); and (*ii*) an mAb recognizing a specific axolemmal antigen has been shown to inhibit myelination (37).

Whatever the mechanism, our findings suggest that, in the CNS, axonal electrical activity induces the onset of the myelination process by oligodendrocytes. Our findings may also have important implications for the understanding of the physiopathology of demyelinating diseases. In multiple sclerosis, it is now well established that in recent plaques there are attempts to remyelinate that fail (38), despite the presence, within the plaques, of mature oligodendrocytes (39, 40). This inability to remyelinate may be the consequence of a conduction block along demyelinated axons.

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