OPTICAL RECORDING OF ACTION POTENTIAL PROPAGATION IN DEMYELINATED FROG NERVE

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ABSTRACT Conduction in focally demyelinated frog nerves has been measured optically using potential-sensitive dyes. Absorption changes were recorded with an array of photodiodes positioned in the image plane of a microscope. Both the amplitude and conduction velocity of the optical signals decreased in the demyelinated region. Conduction was improved after exposure to the potassium channel blocking agent 4-aminopyridine.

INTRODUCTION

In multiple sclerosis, a demyelinating disease of the central nervous sytem, periods of severe neurological deficits are often followed by periods of remission during which significant clinical recovery takes place. Demyelination is characterized physiologically by decreased conduction velocity, and increased refractory period, and can lead to conduction block. Two primary mechanisms have been advanced to account for the restoration of function in demyelinating disease. Remyelination has been shown to occur in the central nervous system, but is relatively rare when demyelination has been extensive (15). Another possibility is strongly suggested by the results of Bostock and Sears (1). From measurements of longitudinal currents over short segments of demyelinated ventral roots, these authors found that inward current, normally confined to nodes of Ranvier, spread into internodal regions leading to "continuous" conduction at very low velocity. This result suggests that sodium channels, localized at nodes in normal axons (16, 3, 4), migrate or are newly inserted into the internodal axolemma within several days after disruption of myelin. We have recently reported the application of patch clamp techniques to study ionic currents in isolated, single, demyelinated axons from frog sciatic nerve (5). Here we present still another probe of conduction in this preparation: optical recordings using potential-sensitive dyes. These signals have been shown to be precise indicators of changes in membrane potential in a number of preparations (7). Recently, Lev-Ram and Grinvald (12) have reported optical signals from myelinated axons in the rat optic nerve. These authors have also shown that the shape of the optical signal changes after application of an osmotic shock in a way that demonstrates a slowing of conduction velocity in many fibers due to demyelination.

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MATERIALS AND METHODS

Frogs (*Rana pipiens*) were anesthetized in tricaine methanesulfonate (3–5 g/liter). The sciatic nerve in one leg was surgically exposed and 2–4 μl of 1% lysolecithin in Ringer's solution was injected into the nerve bundle. The contralateral nerve served as a control. 5–15 d after surgery the nerves were excised and desheathed and conduction was tested. Nerves were soaked in dye (see below), dissolved in Ringer's solution for 1–2 h at room temperature, and were then mounted in a chamber fitted with pairs of platinum wires for external stimulation and recording. The optical recordings were made in the region between the stimulating and recording electrodes. While conduction was not monitored continuously during application of the dye, no large changes in action potentials were apparent after the staining period.

Optical measurements were made using a Leitz Ortholux II microscope in ordinary bright-field mode. The nerve was illuminated with light from a 12 V, 100 W tungsten-halogen lamp, which was passed through a heat filter and an interference filter. Köhler illumination was used. A 7 × 0.2 numerical aperture objective with a measured magnification of 7.5 was used to form an image of the nerve on a 12 × 12 photodiode array. Each pixel of the array is a square, 1.4 mm on a side; the centers of the pixels are separated by 1.5 mm. With a magnification of 7.5, the centers of adjacent elements are receiving light from points on the nerve that are separated by 200 μm. The outputs of each detector were amplified, and the amplifier outputs were multiplexed, digitized, and stored in a PDP 11/34 computer. Resting light intensities were measured with DC amplification at low gain and intensity changes with AC amplification at high gain. In the present experiments each of two analog-to-digital converter-multiplexor cards (ADAC, Inc., Woburn, MA) sampled 32 channels at a sampling interval of 13.25 μs and thus we sampled the output of each detector every 424 μs. Additional details of the apparatus have been described elsewhere (6, 9). For analysis of conduction velocity,

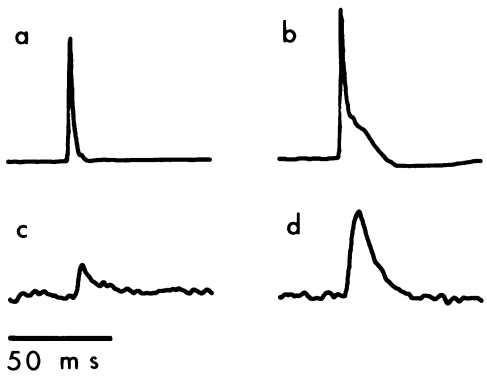

FIGURE 1 Optical signals from myelinated nerve. Records *a* and *b* represent longitudinal currents recorded from a normal segment of a focally demyelinated frog sciatic nerve. *c* and *d* show the change in transmitted light intensity in another normal region of the same nerve. (*a* and *c*) Ringer's solution. (*b* and *d*) After addition of 2 mM 4-AP. The optical signals represent averages of 64 sweeps. Dye, RH155. Incident light filtered at 705(±25) nm.

digitally filtered records were fitted by a third order polynomial in the region of peak amplitude, using two movable cursors. Closeness of the fit was insured by monitoring the procedure visually and the maximum was calculated (11, 13). This technique was effective in providing an objective means of reducing errors due to noise in the recording.

RESULTS AND DISCUSSION

Several dyes at a concentration of 1 mg/ml were screened using normal nerves. Following the suggestion of Lev-Ram and Grinvald (12), a pyrazo-oxonol dye (RH155, currently available from Nippon Kankoh-Shikiso Kenkyusho, Okayama, Japan, as NK 3041) (10) was found to have relatively large absorption changes. Nerves were oriented so that axons were parallel to rows of photodetectors in the diode array. The focally demyelinated zone was 2–3-mm long and was identified visually. This region also tended to be more highly stained by the dye and consequently the position of its image on the photodetector array could be identified by its lower resting intensity.

Fig. 1 illustrates the signals obtained from one photodiode element positioned over a normal segment of nerve. The top traces are records of longitudinal current and the lower records show changes in light intensity at 705 nm. Data on the left were taken in normal Ringer's solution while those on the right depict results after addition of 2.5 mM 4-aminopyridine (4-AP). This compound has been shown to block K^+ channels in nerve fibers (14, 21). Block of K^+ channels causes a 2–3-fold widening of the action potential in frog myelinated nerve fibers (18). The magnitude of the increase in amplitude of the optical signal, which represents the summed action potentials of many fibers (with temporal dispersion), is consistent with this increase in duration of action potentials, and their consequently greater degree of overlap. The increased separation between positive and negative phases of the time derivative of the action potential in 4-AP may be partially responsible for the small undershoot seen in some records

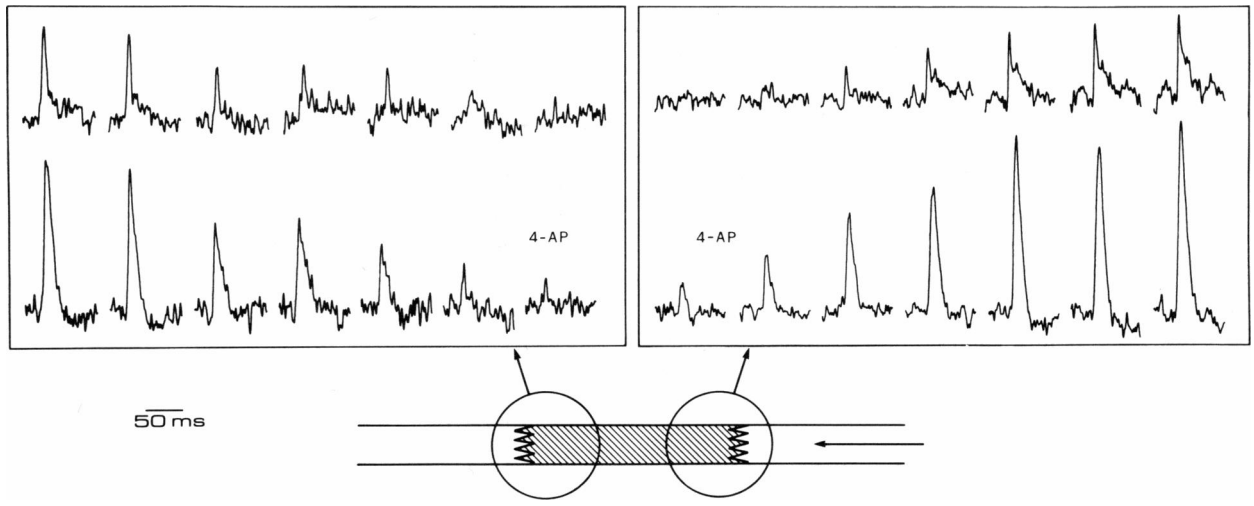

FIGURE 2 Intensity changes (ΔI) recorded from demyelinated nerve. The cross-hatched zone in the sketch represents the region of focal demyelination produced by injection of lysolecithin 11 d earlier. As suggested diagrammatically in the sketch, the borders of the demyelinated zone of the nerve bundle, judged by morphology under light microscopy, are not sharp. The transition from "normal" to "demyelinated" regions thus may not occur at just a single photodetector. Propagation is from right to left. The circles delineate the areas covered by the photodiode array. The magnification of the objective was 7.5 resulting in a spacing of optical elements at 200-μm lengths along the nerve, and a 705 (±25) nm interference filter was used. Each set of seven records represents ΔI signals from seven consecutive elements from one row of photodetectors. Within each rectangle, the top row of sweeps is from a nerve in normal Ringer's solution and the bottom row shows results after addition of 2.5 mM 4-AP. In this experiment the photodetector array was first focused on the region to the right. Records were taken first in normal Ringer's solution and again after adding 4-AP. The 4-AP was then washed off, the microscope stage was moved, and the sequence was repeated for the left region. Thus, the areas covered by each photodetector were identical for control and drug sweeps for a given nerve segment. Within each rectangle the amplifier gain was the same for all sweeps. The gain used in the left rectangle was 1.3× that used on the right. In this experiment the resting light transmission was too low to allow a calculation of the fractional change in intensity. All tracings are from AC-coupled recordings and thus differences in the DC light levels are not evident. Each sweep represents the average of 64 trials.

of longitudinal current (Fig. 1 *b*). The sharp repolarization of the optical signal in 4-AP (seen more clearly in Fig. 2) may result from the decrease in temporal dispersion that would be expected for a drug that improves conduction. Some optical records in 4-AP (Fig. 1 *d*, Fig. 2) appear to have after-hyperpolarizations. These are probably due to noise since if signals from a row of photodetectors are summed, the resultant trace has no afterpotential. If a similar summation is carried out on signals with no 4-AP present a small, positive afterpotential is just barely detectable above the noise. Optical signals decreased only slightly with repeated measurements. Thus, bleaching of the dye was slow. Electrical signals were also stable, suggesting little photodynamic damage.

Both in normal Ringer's and in 4-AP the optical signals have a slower time course than the electrode measurements. Several factors may contribute to this difference. First, the electrical signals have a component that is the time derivative of the potential change, which would tend to make them faster. Second, the electrical signals are expected to be proportional to the square of axon diameter and thus be dominated by the largest (and fastest) axons. The optical signals are expected to be linearly related to the fiber diameter and thus less dominated by larger axons. Third, the low pass filtering used for the optical measurements was more severe than the filtering of the electrode recordings.

In Fig. 2 we present results obtained with the photodiode array. In the sketch the cross-hatched zone represents the demyelinated region and the horizontal arrow gives the direction of propagation. The circles outline two fields of view covered by the photodetector array. Different segments of the nerve were observed by moving the preparation via the stage micrometer. Each set of records shows optical signals from seven consecutive photodetectors within one row on the array. The sweeps represent changes in light intensity at each detector. Within each rectangle the top set of records was taken with the nerve in normal

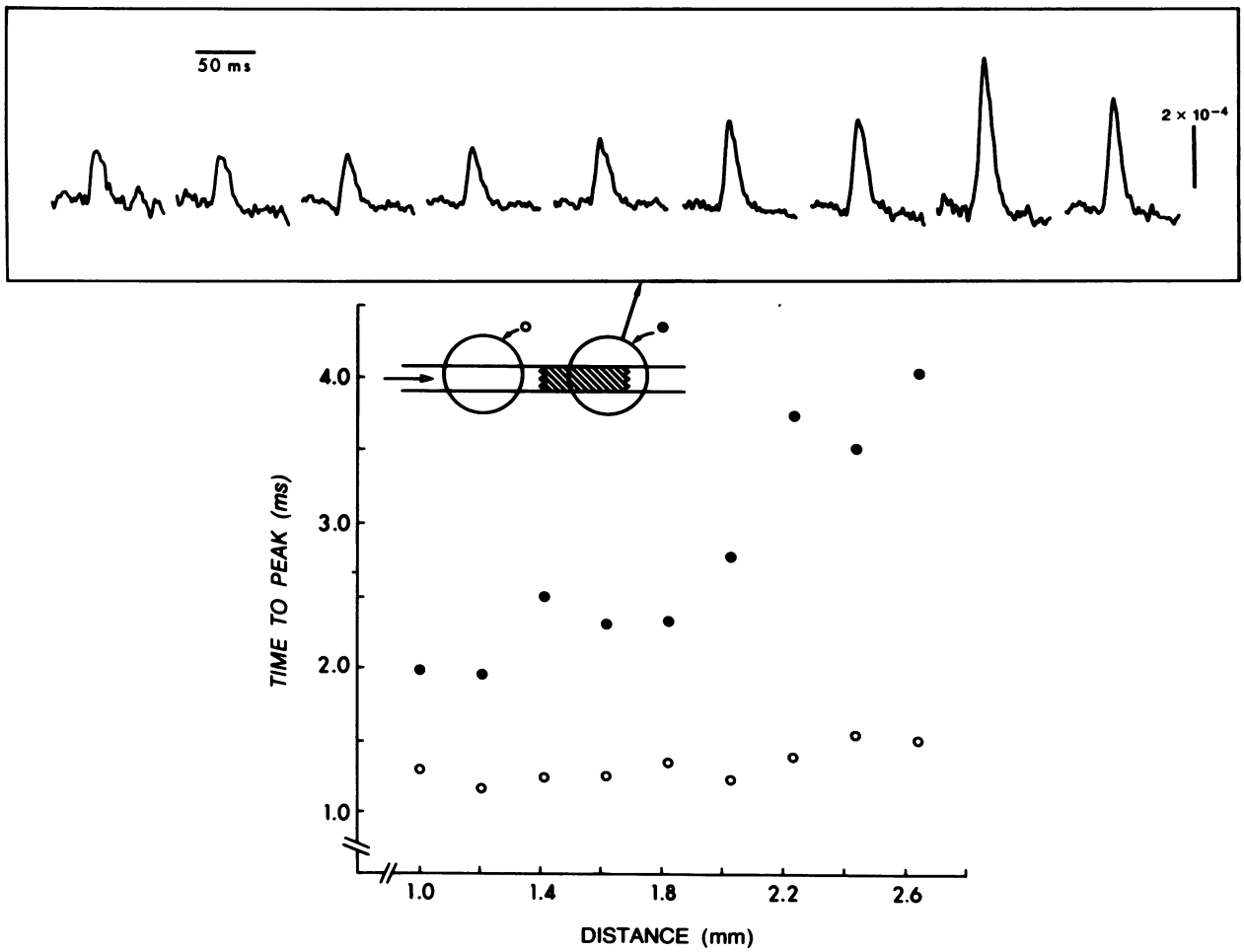

FIGURE 3 Conduction velocities measured optically. The sketch shows the position of the photodetector array relative to the demyelinated zone for two cases. Propagation from left to right. The top set of records represents the fractional change in intensity ($\Delta I/I$) recorded from nine consecutive elements in the array. 64 trials were averaged. The Ringer's solution contained 2.5 mM 4-AP. The graph gives conduction times measured as the time to peak optical signal, plotted versus the distance along the nerve. Conduction velocity is thus inversely proportional to the slope. Peaks were determined using fits of a third-order polynomial as described in the text. *Open circles*, are from a normal region of the nerve. *Solid circles*, are from the demyelinated nerve.

Ringer's solution and the lower set after addition of 2 mM 4-AP. The transition from normal to demyelinated nerve is marked by a decrease in amplitude of the optical signals. Part of this decrease is due to a lower resting light intensity in the demyelinated zone due to higher dye binding in this region. It is possible that the lower resting intensity in the demyelinated region results from additional dye binding to myelin debris, vesicles, or other nonexcitable tissues. If this nonspecific binding is the only reason for the smaller signals in the demyelinated region, then the fractional intensity change ($\Delta I/I$) should be constant in the two regions. However, in data plotted as the fractional change in intensity this decrease in amplitude, while smaller, persists (see Fig. 3). The remaining decrease in amplitude must be due to other factors. The higher capacitance and leakage conductance of demyelinated axons acts to decrease action potential amplitudes and would lower optical signals. It is also possible that in the demyelinated zone less dye is bound to the axon membrane itself. On the other hand the increase in axon membrane area exposed to dye would be expected to increase optical signals. While the information provided by signal amplitudes in these experiments on intact nerve bundles is thus clearly limited, some conclusions can be reached. The fact that the optical signals "re-emerge" at the distal region of the demyelinated zone (Fig. 2, *top left*) and are again large, suggest that the progressive decrease in amplitude does not simply represent conduction block in increasing numbers of fibers. Finally, from the records on the right we can observe the improved penetration of activity into the demyelinated zone after exposure to 4-AP that has been described electrophysiologically (2).

Fig. 3 shows the data from an experiment demonstrating a change in conduction velocities in the demyelinated zone. The optical signals shown at the top are normalized by the resting light intensities, and include the transition zone from demyelinated to intact nerve. 4-AP had been added to the bath and signals were considerable in the demyelinated region. The graph shows conduction times to the peak of the optical signal for a normal segment (*open circles*) and for the demyelinated area (*solid circles*). Conduction velocities measured from least squares fits were 5.4 m/s (intact) and 0.8 m/s (demyelinated). The former value is only approximate but it is clear that conduction was slowed in the demyelinated region.

Two lines of evidence suggest that the optical absorption signals that we recorded in intact preparations originate in myelinated fibers. At the magnification used the length of nerve that could be monitored by the photodetector array was 2.2 mm. In intact regions of nerve there was generally no detectable difference in conduction time across this length. From the sampling time used (0.42 ms) we calculated a minimum conduction velocity of 5.2 m/s. This is about ten times faster than that of the largest "C" fibers in the frog, but is appropriate for myelinated fibers (8). Second, the optical signals were elicited at low to moderate stimulus strengths, levels well below those generally required for the stimulation of small unmyelinated axons. Longitudinal currents recorded during these experiments showed no contribution from small "C" fibers. As mentioned earlier, Lev-Ram and Grinvald (12) have recently reported optical signals from rat optic nerve, a preparation that is virtually 100% myelinated (20).

In addition to a fast signal similar to that described here, with certain styryl dyes Lev-Ram and Grinvald (12) found a much slower signal that they attributed to a glial response. Neither we nor Lev-Ram and Grinvald have detected slow signals with the dyes we have used. However, in cerebellar slices, slow signals were detected with RH155 (17).

The present experiments show that optical techniques can be used to follow conduction in demyelinated nerve. This method may be of value in the central nervous system, in regions not readily monitored by electrophysiological means. Our results with 4-AP suggest that optical measurements may provide a useful means of assessing the mode of action of drugs that improve conduction in demyelinated axons. Also, they provide signals directly proportional to membrane potential. This adds a third parameter to those already measured in demyelinated axons: longitudinal currents (1, 19) and ionic current (5). A measurement of the time course of the potential change should be of value in the development of models to describe conduction in normal and demyelinated nerve. Work is in progress to extend this technique to single isolated demyelinated fibers.

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REFERENCES

1. Bostock, H., and T. A. Sears. 1978. The internodal axon membrane: electrical excitability and continuous conduction in segmental demyelination. *J. Physiol. (Lond.).* 280:273–301.
2. Bostock, H., T. A. Sears, and R. M. Sherratt. 1981. The effects of 4-aminopyridine and tetraethylammonium ions on normal and demyelinated mammalian nerve fibers. *J. Physiol. (Lond.).* 313:301–315.
3. Chiu, S. Y., and J. M. Ritchie. 1981. Evidence for the presence of potassium channels in the paranodal region of acutely demyelinated mammalian single nerve fibres. *J. Physiol. (Lond.).* 313:415–437.
4. Chiu, S. Y., and J. M. Ritchie. 1982. Evidence for the presence of potassium channels in the internode of frog myelinated nerve fibres. *J. Physiol. (Lond.).* 322:485–501.
5. Chiu, S. Y., P. Shrager, and J. M. Ritchie. 1985. Loose patch clamp recording of ionic currents in demyelinated frog nerve fibers. *Brain Res.* 359:338–342.

6. Cohen, L. B., and S. L. Lesher. 1986. Optical monitoring of membrane potential: methods of multisite optical measurement. *In* Optical Methods in Cell Physiology. P. DeWeer and B. M. Salzberg, editors. John Wiley & Son, Inc., New York. 72–99.
7. Cohen, L. B., and B. M. Salzberg. 1978. Optical measurement of membrane potential. *Rev. Physiol. Biochem. Pharmacol.* 83:36–77.
8. Erlanger, J., and H. S. Gasser. 1937. Electrical Signs of Nervous Activity. University of Pennsylvania Press, Philadelphia. 1–33.
9. Grinvald, A., L. B. Cohen, S. Lesher, and M. Boyle. 1981. Simultaneous optical monitoring of activity of many neurons in invertebrate ganglia using a 124 element photodiode array. *J. Neurophysiol. (Bethesda).* 45:829–840.
10. Grinvald, A., R. Hildesheim, R. Gupta, and L. B. Cohen. 1980. Better fluorescent probes for optical measurement of changes in membrane potential. *Biol. Bull. (Woods Hole).* 159:484.
11. Hille, B. 1971. The permeability of the sodium channel to organic cations in myelinated nerve. *J. Gen. Physiol.* 58:599–619.
12. Lev-Ram, V., and A. Grinvald. 1986. Ca^{2+} and K^{+}-dependent communication between central nervous system myelinated axons and oligodendrocytes revealed by voltage-sensitive dyes. *Proc. Natl. Acad. Sci. USA.* 83:6651–6655.
13. Lo, M. -V. C., and P. Shrager. 1981. Block and inactivation of sodium channels in nerve by amino acid derivatives. I. Dependence on voltage and sodium concentration. *Biophys. J.* 35:31–43.
14. Meves, H., and Y. Pichon. 1975. Effects of 4-aminopyridine on the potassium current in internally perfused giant axons of the squid. *J. Physiol. (Lond.).* 251:60.
15. Raine, C. S. 1978. Pathology of demyelination. *In* Physiology and Pathobiology of Axons. S. G. Waxman, editor. Raven Press, New York. 283–312.
16. Ritchie, J. M., and R. B. Rogart. 1977. The density of sodium channels in mammalian myelinated nerve fibers and the nature of the axonal membrane under the myelin sheath. *Proc. Natl. Acad. Sci. USA.* 74:211–215.
17. Salzberg, B. M., A. L. Obaid, and A. Konnerth. 1986. Selective binding of potentiometric probes allows optical recording of electrical activity from different cell types in elasmobranch cerebellar slices in vitro. *Biophys. J.* 49 (, Pt.):365*a*. (Abstr.)
18. Schmidt, H., and R. Stampfli. 1966. Die Wirkung von Tetraathylammoniumchlorid auf den einzelnen Ranvierschen Schnurring. *Pfluegers Arch. Eur. J. Physiol.* 287:311–325.
19. Smith, K. J., H. Bostock, and S. M. Hall. 1982. Saltatory conduction precedes remyelination in axons demyelinated with lysophosphatidyl choline. *J. Neurol. Sci.* 54:13–31.
20. Waxman, S. G., J. A. Black, and R. E. Foster. 1983. Ontogenesis of the axolemma and axoglial relationships in myelinated fibers: electrophysiological and freeze-fracture correlates in membrane plasticity. *Int. Rev. Neurobiol.* 24:433–484.
21. Yeh, J. Z., G. S. Oxford, C. H. Wu, and T. Narahashi. 1976. Interactions of aminopyridines with potassium channels of squid axon membranes. *Biophys. J.* 16:77–81.