REPETITIVE PROPAGATION OF ACTION POTENTIALS DESTABILIZES THE STRUCTURE OF THE MYELIN SHEATH

A Dynamic X-ray Diffraction Study

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ABSTRACT Time courses of myelin lattice swelling in toad sciatic nerves preexposed to different treatments were determined by x-ray diffraction using a one-dimensional position-sensitive detector. In the nerves supramaximally stimulated for 1 h at 200 Hz, the subsequent process of myelin swelling occurred 45.0 ± 7.3 min ($n = 24$) sooner than in resting controls. Sciatic nerves incubated for 1 h in a Ringer's solution deprived of divalent cations (Ca^{++} and Mg^{++}) exhibited a kinetics of swelling similar to that shown by the stimulated nerves, that is, 52.5 ± 14.2 min ($n = 6$) sooner than controls preincubated for the same time in normal Ringer's solution (with divalent cations). The fact that both pretreatments supramaximal stimulation and removal of divalent cations from the perfusion solution produced a similar effect; namely, a decrease of the myelin lattice stability against swelling in distilled water, suggests that the repetitive propagation of action potentials could modify the ionic composition at either the intraperiod channel or the paranodal axoglial junction complexes.

INTRODUCTION

The swelling property of nerve myelin has been investigated by x-ray diffraction and electron microscopy since the 1950's (Finean and Millington 1957; Robertson 1958). Further studies performed extensively by several research groups have established that divalent cations play a significant role in maintaining the structure of the sheath (Joy and Finean 1963; Wolman and Wiener 1965; Worthington and Blaurock 1969; Melchior et al. 1979; Hollingshead et al., 1981). In previous reports from our laboratory (Padrón 1977, 1979; Padrón et al., 1979), it was proposed that the stability of the myelin sheath could be partially accounted for by Ca^{++}-mediated interactions between membranes at the intraperiod line. These ideas were reinforced by recent work of Ellisman et al. (1980) who, using electron probe wavelength spectroscopy, detected that Ca^{++} was much more concerntrated in both compact myelin and paranodal glial loops than in extracellular matrix, suggesting that these ions participate in the formation and stabilization of myelin membrane arrays.

The work cited above shows that divalent cations, at physiological concentration, play a fundamental role in maintaining the stability of the myelin structure. However, neither the nature nor the location on the membrane of the molecular components responsible for the stabilizing interactions are known. Concerning this question, Tabira et al. (1978) have shown intramyelin interlamellar junctions that, in the electron microscope, are visualized as a fusion of membranes at the intraperiod line and whose presence is characteristic of *Zonulae occludentes*. They inferred from their observations that these junctions were equivalent to an earlier observed structural complex, the radial component (Honjin, 1959; Peters, 1961; Honjin and Changue, 1964), which is topologically connected to the paranodal apparatus. In addition, freeze-fracture electron microscopy studies have revealed at the paranodal region a complex axoglial junction composed of particles contributed by both the axon and the Schwann cell paranodal loops (Schnapp et al. 1973, 1976; Livingston et al. 1973; Akert et al., 1974; Schnapp and Mugnaini, 1975; Ellisman, 1979).

In 1974, Müller-Mohnssen and co-workers reported that if a small surface (~1 μm^2) of the paranodal myelin is specifically damaged with a laser beam, the action potential propagation is blocked without changing the general passive electrical characteristics of the nerve fiber (i.e., rest potential, etc.). From these results Müller-Mohnssen et al. (1974) concluded that the integrity of the paranodal region was essential to the mechanism of generation and propagation of action potentials. Based on these observations, they proposed a model for the activation-inactivation process, which was named the synaptic-paranodal hypothesis (Müller-Mohnssen et al., 1975). In their model (which is similar to what occurs in a synapse) a suprathreshold

stimulus liberates ions that are necessary to produce an action potential from inside the myelin toward the nodal axolema membrane. Additionally, Ellisman et al. (1980), using electron probe wavelength spectroscopy, detected that sodium ions were five times more concentrated in compartments formed by the Schwann cell paranodal loops than in the extracellular matrix, suggesting that these structures are involved in providing an ionic source and/or sink, necessary for nerve impulse conduction.

Since we have previously demonstrated (Padrón, 1977; 1979; Padrón et al., 1979) that the stability of the myelin sheath against swelling strongly depends on the presence of divalent cations, and inferring from the synaptic-paranodal hypothesis of Müller-Mohnssen et al. (1975) that the ionic content within the myelin should vary with nerve stimulation, we have decided to investigate the effect of the action-potential propagation on subsequent myelin sheath stability in distilled water.

The results presented here show that the process of myelin lattice swelling in distilled water is significantly accelerated in sciatic nerves prestimulated supramaximally as compared with resting nerves.

MATERIALS AND METHODS

Nerve Specimens

All the experiments were performed with recently captured toads (*Bufo marinus*) that were maintained for not longer than 2 wk in special tanks, with fresh running water.

The toads were double pithed by means of a steel needle. The two sciatic nerves of an animal were dissected at room temperature (21°C) and transferred to a petri dish containing a normal physiological solution (NaCl, 101.8 mM; KCl, 2.7 mM; $MgCl_2$, 2.1 mM; $CaCl_2$, 1.9 mM; $NaHCO_3$, 2.0 mM; NaH_2PO_4, 0.36 mM). Under the microscope both nerves were desheathed by careful removal of the epineurium. 40 mm of identical anatomical regions (crural) were separated from the rest of the sciatic nerve by tying off the extremities with surgical silk. The process of dissection and removal of the epineural sheaths from the sciatic nerves of both legs was completed in <45 min. Each experiment was performed with a pair of such nerve specimens from the same toad.

Nerve Holder

To perform the electrophysiological and the x-ray diffraction measurements simultaneously, we modified the perfusion holder used in previous experiments (Padrón, 1979). The holder contained two separate perfusion systems, one for the experimental, the other for the control nerve specimen. Each independent system consisted of a 1.5-mm diameter quartz capillary that was connected to two small wet chambers, each provided with one pair of platinum wire electrodes. The sciatic nerve was stimulated in one of the chambers, the compound action potential was registered in the other chamber. The distance between the two pairs of electrodes was 30 mm. The holder also contained a thermistor probe, located very close to the stimulated region and in contact with the perfusion solution, by means of which a continuous record of the temperature in the vicinity of the nerve was obtained. The possibility of measuring this parameter assured that the changes detected in the stability properties of the sheath, are not due to significant temperature variations of the nerve specimen as a consequence of the action potential propagation.

X-ray Techniques

The x-ray source was a rotating-anode generator (Marconi Elliott Avionic Systems, Borehamwood, England, model GX6) with copper target and microfocus setting (0.1 × 1 mm), operated at 30 kV and 30 mA. Linear collimation and focusing was achieved using a nickel-coated bent glass mirror. The beam issued from the mirror was limited, perpendicularly to its face, to 0.15 mm width by two slits. The x-ray diffraction patterns were recorded with a position-sensitive detector, 60 mm effective length and 10 mm aperture width (Gabriel and Dupont, 1972), and stored in the magnetic tape unit of an on-line computer (Multi-20, Intertechnique, Plaisir, France). When required, the computer-stored diffraction patterns were displayed on a cathode ray tube and photographed on 35-mm films. Periodicity and intensity measurements were obtained from print enlargements of the films. The periods measured from the positions of the reflections were accurate to within 1%. For the purpose of the present work the intensities of the reflections were determined from peak heights.

By means of this experimental setup any changes in the structure of the myelin sheath, produced by the repetitive propagation of action potentials, can be detected by x-ray diffraction.

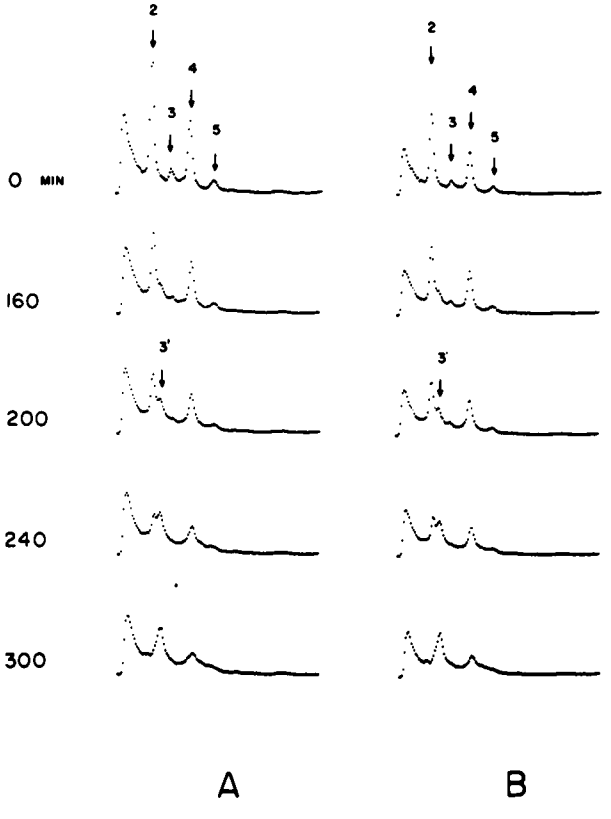

FIGURE 1 Comparison of the kinetics of myelin swelling in distilled water between the two sciatic nerves from a single toad. *A* and *B* refer to the two independent perfusion systems of the specimen holder (see Materials and Methods). Each pattern was collected during 5 min. The times indicated in this and in Figs. 3 and 4 represent the elapsed time at the onset of each data collection period. The two initial spectra at zero time are very similar and were recorded after 1 h incubation in normal Ringer's solution (with divalent cations). To aid in identifying the sequence of the spectra, reflections 2 to 5 from the native structure and the third-order reflection from the swollen lattice (3') are indicated by arrows. Observe that the two myelins arrive simultaneously (≃240 min) at the characteristic pattern equal intensities (see text).

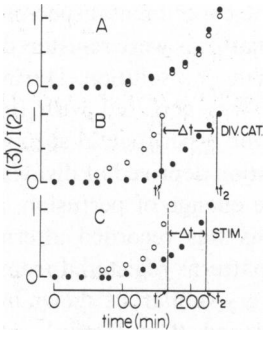

FIGURE 2 Measurement, on the serial x-ray diffraction spectra shown in Figs. 1, 3, and 4, of the relative amounts of native and swollen myelin lattices. The ordinates represent the ratio between the intensity of the third-order reflection from the swollen lattice [I(3')] to that of the second order reflection from the native structure [I(2)]. The abcissa is the elapsed time after the onset of the perfusion with distilled water. t_1 and t_2 represent, respectively, the time at which the experimental and the control myelin reached the pattern equal intensities. ●, control nerve; ○, experimental nerve; DIV. CAT., divalent cation; STIM., stimulated. (*A*) Control experiment shown in Fig. 1. Both nerves were preincubated in normal Ringer's solution for 1 hr and then perfused continuously with distilled water. The two swelling processes are almost identical. (*B*) Experiment shown in Fig. 3. The control nerve (●) was preincubated for 1 h in normal Ringer's solution; the experimental (○) was treated for the same time in Ringer's solution without divalent cations. The kinetics of the two swelling processes was clearly different. Δt (t_2 minus t_1) is about 73 min. (*C*) Experiment shown in Fig. 4. Both nerves were preincubated for 1 h in normal Ringer's solution. During the preincubation the experimental (○) was stimulated supramaximally at 200 Hz, whereas the control (●) was maintained at rest. The kinetics of swelling of the two nerves was also significantly different. Δt is ~60 min.

RESULTS

Kinetics of Myelin Swelling in Distilled Water

Control Experiments. The sciatic nerves from both legs of a toad were dissected as indicated above and each was mounted in one of the independent perfusion channels of the nerve holder (see Materials and Methods). One of the nerves was arbitrarily selected as the experimental specimen, the other was the control. Sequential x-ray diffraction patterns of 5 min duration were recorded alternately from the specimens during the first hour of perfusion with normal Ringer's solution. No significant changes in the spectra were detected during this time. Immediately after the first hour acquisition, the perfusion was changed to distilled water and sequential 5-min counting x-ray diffraction patterns were registered alternately from both nerves during the swelling. This experiment was repeated nine times; in all cases the process was found to be similar to that previously described (Padrón et al. 1979). The time course differed significantly from one animal to another, but it was nearly identical between the same anatomical regions from the two legs of a given toad.

Fig. 1 shows a series of 5-min counting x-ray diffraction patterns recorded from a pair of sciatic nerves during the swelling with distilled water. During the first hour after the onset of the perfusion the spectra remained basically unchanged (Fig. 1, 0 min). At about 2.5 h a new set of reflections from a 231-Å period structure became visible (Fig. 1, 160 min). The intensity of these new reflections increased as those of the 173 Å native pattern decreased. This is shown in Fig. 2*A*, where the ratio of the intensity of the third-order reflection from the swollen myelin to that of the second-order from the native structure has been plotted as a function of the time of perfusion with distilled water. These two reflections attained a similar intensity at about the same time (~4 h) in both the experimental and the control nerve specimens. This kind of characteristic pattern was called "equal intensities." (Fig. 1, 240 min). This spectrum is very easy to detect; therefore, it can be used as a reference point in the swelling process.

One way to compare the progress of myelin swelling

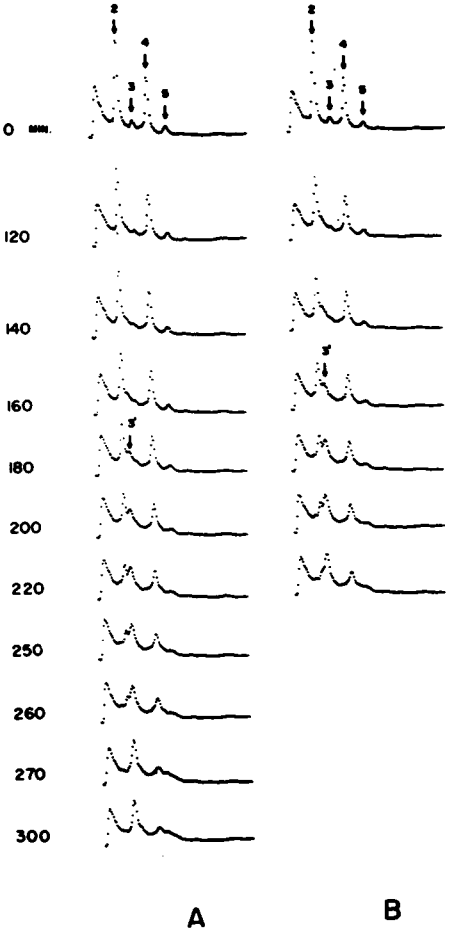

FIGURE 3 Kinetics of swelling in distilled water of *A*, myelin from a sciatic nerve preincubated for 1 h in normal Ringer's solution (with divalent cations). *B*, myelin from the other sciatic nerve from the same toad after 1 h preincubation in Ringer's solution without divalent cations. Observe that in the control myelin (*A*), the pattern equal intensities is reached later than 220 min, while in the myelin exposed to solutions free of divalent cations (*B*) a similar spectrum is observed at ~180 min.

between the two nerves is by measuring the time difference at which the specimens reached the characteristic pattern equal intensities. This time difference is called the Δt for a particular pair of specimens: if the experimental sciatic nerve achieves this characteristic pattern more rapidly than the control nerve, Δt is defined as positive. The mean value of Δt determined for nine different pairs of control sciatics was -2.0 min (± 2.1 min SEM). This result indicates that there is no difference in myelin swelling behavior between an experimental sciatic nerve and its control if treated identically. This is represented in Fig. 5*A*.

B. Effect of Perfusing a Sciatic Nerve with a Ca^{++}- and Mg^{++}-free Ringer's solution on the Subsequent Myelin Swelling in Distilled Water

A pair of sciatic nerves dissected from the same animal were mounted in the holder, one of them served as control and the other as the experimental specimen. For a control, 5-min diffraction patterns were registered from both specimens in normal Ringer's solution. During the next hour, the control nerve was perfused with the same Ringer's solution, whereas the experimental specimen was perfused with a similar solution deprived of divalent cations. Simultaneously with the change of perfusion, a series of 5-min diffraction patterns was recorded alternately from both nerves. The final patterns registered from the two myelins were found to be very similar, as shown in Fig. 3 (compare the patterns in *A* and *B* at 0 min). This indicates the removal of Ca^{++} and Mg^{++} from the perfusion Ringer's solution does not change the structure of the myelin sheath, at least at 35-Å resolution.

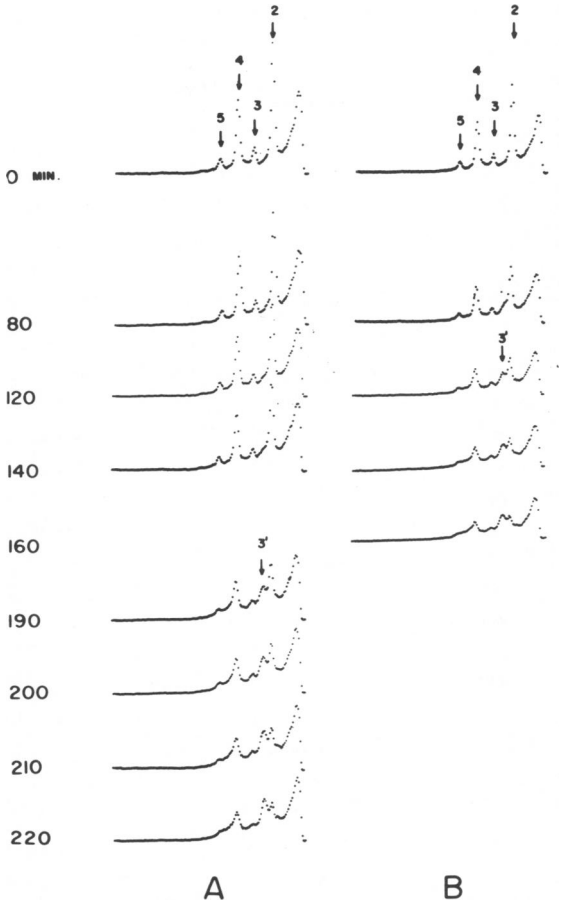

FIGURE 4 Kinetics of swelling in distilled water of myelin from *A*, a sciatic nerve preincubated for 1 h in normal Ringer's solution (with divalent cations), compared with *B*, the other sciatic nerve from the same toad after 1 h of supramaximal stimulation at 200 Hz. Observe that despite the two nerves having been exposed for 1 h to the same solution (with divalent cations), the repetitive propagation of action potentials accelerates significantly the rate of subsequent myelin swelling in distilled water (*A*, 220 min; *B*, 160 min).

After this treatment was completed, the two compartments of the dual perfusion system were simultaneously connected to the distilled water reservoir and the kinetics of myelin swelling were followed by x-ray diffraction. Except for the difference in time course, the swelling process was similar in both nerves. The specimen preincubated with the solution deprived of divalent cations shows a significant acceleration in the kinetics of swelling relative to the nerve that was maintained in contact with the normal Ringer's solution (with divalent cations). As in section A, the ratio between the third-order reflection of the swollen lattice and the second-order reflection of the native lattice for a pair of nerves, is plotted as a function of time in Fig. 2*B*. For this experiment the value of Δt (see previous section) was ~73 min, but varied between 40 and 100 min depending on the animal. A similar experiment was repeated in six pairs of sciatic nerves; the mean Δt was 52.5 min (± 14.2 min SEM). This value is represented in Fig. 5*B*.

C. Effect of the Repetitive Propagation of Action Potentials on the Kinetics of Myelin Lattice Swelling in Distilled Water

For controls, 5-min diffraction patterns were recorded from a freshly dissected pair of sciatic nerves in normal Ringer's solution. After this acquisition was completed the experimental specimen was supramaximally stimulated for 1 h at 200 Hz, while the control nerve was maintained at rest in normal Ringer's solution. At the end of the stimulation period, x-ray patterns were recorded from both specimens. In agreement with previous observations (Padrón and Mateu, 1980), we find that this treatment did not produce significant alterations of diffraction orders 1–5 (compare Figs. 4 *A* and *B*, spectra at 0 min). This result indicates that, at low resolution (~35 Å), no change in myelin structure is caused by the repetitive propagation of action potentials.

Immediately after stimulation, the experimental and the control nerves were perfused with distilled water, and the kinetics of myelin lattice swelling was determined by x-ray diffraction as indicated above. Fig. 4 shows a series of 5-min diffraction patterns recorded alternately from the

prestimulated (Fig. 4 *B*) and from the resting control nerve (Fig. 4 *A*). The patterns show that, in the former specimen (experimental), the third-order reflection for the swollen lattice attained an intensity similar to that of the second-order for the native lattice (equal intensities) at 160 min. (Fig. 4 *B*), while for the control nerve a similar pattern was achieved much later (220 min, Fig. 4 *A*). This experiment was repeated 24 times. In all cases the prestimulated sciatic nerve was found to swell more rapidly than the corresponding resting control. The Δt for the 24 pairs of sciatic nerves ranged between 30 and 120 min and the mean value was 45.0 min (±7.3 min SEM). This result is shown in Fig. 5 *C*.

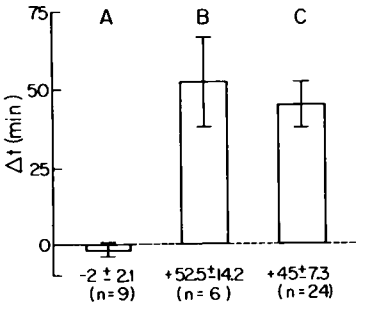

FIGURE 5 Bar diagram showing the difference in time of swelling with distilled water, between two sciatic nerves from the same animal subjected to different treatments. The mean Δt (as defined in the text) and the standard error of the mean are shown for the three experimental situations considered in the present work. (*A*) Control experiment. Pairs of sciatic nerves preincubated in normal Ringer's solution for 1 h and immediately swollen in distilled water (nine experiments). (*B*) Effect of preincubating a sciatic for 1 h in Ringer's solution without divalent cations, on the subsequent kinetics of myelin swelling (six experiments). (*C*) Effect of the repetitive propagation of action potentials on the subsequent kinetics of myelin swelling (24 experiments). The test of statistical significance indicates that the values of Δt corresponding to experimental situations named in *B* and *C* are different from that of the control experiment named in *A*, at a significant level of confidence >99%. Also there is not a significant difference between Δt for experiments *B* and *C*.

DISCUSSION

In agreement with the results from several laboratories. (Joy and Finean, 1963; Wolman and Wiener, 1965; Worthington and Blaurock, 1969; Melchior et al. 1979), in our previous reports (Padrón, 1977, 1979; Padrón et al., 1979) we suggested that divalent cations participate in the membrane interactions that maintain the structural stability of the myelin sheath. The experimental evidence that allowed us to reach such interpretation was obtained from sciatic nerves perfused with hypotonic solutions at very low ionic strength. In the current work, the experimental data have been collected from nerve specimens treated with normal solutions at physiological osmolarity and ionic strength and we have arrived at a similar conclusion. Myelin swelling in nerves pretreated for 1 h in Ringer's solution without divalent cations occurs ~1 h sooner than in nerves perfused with solutions where Ca^{++} and Mg^{++} are present in normal amounts. This indicates that the ionic content of the sheath may be modified following the concentration gradient, and agrees with previous results (Briceño et al. 1977; Padrón 1977; Briceño, 1978), which indicated that the Δt (see text) between an experimental sciatic nerve and its control depends on the preincubation time of the former in Ringer's solution without divalent cations. In other words, myelin swells sooner when Ca^{++} and Mg^{++} are washed out for longer times. It has been pointed out by other groups (Schnapp and Mugnaini, 1975) that the structural stability of myelin may be dependent on the specialized junctions at the periphery of the sheath. These contacts, which include paranodal tight junctions and paranodal axoglial junctions, may constrain the unwinding of the spirally wrapped myelin during swelling. Breaking of the axoglial junctions after exposure to reduced calcium levels has been observed in myelinated cultures of sensory ganglia using light and electron microscopy. (Blank et al. 1974). Also it is likely that the stability of the arrays of myelin membrane pairs occurs through interactions between apposed external membrane surfaces in which divalent cations participate (Worthington and Blaurock, 1969; Padrón et al. 1979; Ellisman et al., 1980). Therefore, the stability of the myelin sheath depends both on stable packing of membrane pairs in the internode and maintaining intermembrane contacts at the paranodal region, and divalent cations seem to be involved at both loci.

In 1935, Schmitt et al. first studied the structure of myelin by x-ray techniques. The aim of their investigations was to obtain the diffraction patterns from stimulated and resting nerves in order to detect structural changes between these two states. Although it was reported that the patterns from the stimulated sciatic nerves show significant differences as compared with the control, an interpretation of these results was not given, since the classic techniques available at that time were not entirely adequate in providing a nonambiguous explanation for the observed differences. Recently, we published (Padrón and Mateu, 1980) the results of an experiment similar to that performed by Schmitt and co-workers. In our communication it was pointed out that (at 15 Å resolution) the structural changes produced in the internodal myelin by supramaximal stimulation of sciatic nerves are very small and poorly significant statistically. The present work is our second attempt to detect, at a molecular level, changes in myelin structure coupled with physiology. Our experiments have shown that the stimulation of the Ranvier node and the subsequent nerve impulse propagation destabilizes the internodal myelin and that this destabilization is similar to that produced when divalent cations are removed from the sheath.

The present results have clearly demonstrated that one of the structural characteristics of myelin, namely, its apparent stability, is significant modified by the repetitive propagation of action potentials. Experiments are in progress to elucidate whether the electrical stimulation is

affecting the structure and properties of the intraperiod channel or those of the axoglial tight junction at the paradonal region.

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REFERENCES

Akert, K., C. Sandri, R. B. Livingston, and H. Moor. 1974. Extracellular space and junctional complexes at the node of Ranvier. *Actual. Neurophysiol.* 10:9–22.

Blank, W. F., M. B. Bunge, and R. P. Bunge. 1974. The sensitivity of the myelin sheath, particulary the Schwann cell-axolemmal junction, to lowered calcium levels in cultured sensory ganglia. *Brain Res.* 67:503–518.

Briceño, J. 1978. Influencia del Calcio en la estabilidad estructural de un sistema cristalino unidimensional. Mat. Sc. Ing. Thesis. Universidad Simón Bolívar. Caracas, Venezuela.

Briceño, J., R. Padrón, and L. Mateu. 1977. Modificación de la estabilidad estructural de la capa de mielina en condiciones de fuerza iónica fisiológica: estudio por difractometría dinámica de rayos-X. *Acta Cient. Venez.* 28(1):7.

Ellisman, M. H. 1979. Molecular specializations of the axon membrane at nodes of Ranvier are not dependent upon myelination. *J. Neurocytol.* 8:719–735.

Ellisman, M. H., P. L. Friedman, and W. J. Hamilton. 1980. The localization of sodium and calcium to Schwann cell paranodal loops at nodes of Ranvier and of calcium to compact myelin. *J. Neurocytol.* 9:185–205.

Finean, J. B., and P. F. Millington. 1957. Effects of ionic strength of immersion medium on the structure of peripheral nerve myelin. *J. Biophys. Biochem. Cytol.* 3:89–94.

Gabriel, A., and Y. Dupont. 1972. A position sensitive proportional detector for X-ray crystallography. *Rev. Sci. Instrum.* 43:1600–1602.

Hollingshead, C. J., D. L. D. Caspar, V. Melchior, and D. A. Kirschner. 1981. Compaction and particle segregation in myelin membrane arrays. *J. Cell Biol.* 89:631–644.

Honjin, R. 1959. Electron microscopic studies on the myelinated nerve fibers in the central nervous system. *Acta Anat. Nippo.* 34:43–44.

Honjin, R., and G. W. Changue. 1964. Electron microscopy of nerve fibers VIII. Again on the radial component in the myelin sheath. *Okajimas Folia Anat. Jpn.* 39:251–261.

Joy, R. T., and J. B. Finean. 1963. A comparison of the effects of freezing and of treatment with hypertonic solutions on the structure of nerve myelin. *J. Ultrastruct. Res.* 8:264–282.

Landon, D. N., S. T. Hall. 1976. The myelinated nerve fibers. *In* The Peripheral Nerve.. D. N. Landon, editor. Chapman and Hall, London. 1–105.

Livingston, R. B., K. Pfenninger, H. Moor, and K. Akert. 1973. Specialized paranodal and interparadonal glial-axonal junctions in the peripheral and central nervous system: a freeze-etching study. *Brain Res.* 58:1–24.

Melchior, V., C. J. Hollingshead, and D. L. D. Caspar. 1979. Divalent cations cooperatively stabilize close membrane contacts in myelin. *Biochim. Biophys. Acta.* 554:204–226.

Müller-Mohnssen, H., A. Tippe, F. Hillenkamp, and E. Unsöld. 1974. Is the rise of the action potential of the Ranvier node controlled by a paranodal organ? *Nature (Lond.).* 61:369–370.

Müller-Mohnssen, H., A. Tippe, F. Hillenkamp, and E. Unsöld. 1975. Über die Bedeutung paranodaler Strukturen für die Impuls regeneration am Ranvier Schen Schnürring. *Z. Naturfosch.* 30C:271–277.

Padrón, R. 1977. La estabilidad estructural de la mielina: estudio por difracción de rayos-X. M.Sc. Thesis. Centro de Estudios Avanzados. Instituto Venezolano de Investigaciones Científicas. Caracas, Venezuela.

Padrón, R. 1979. Alteración de la estabilidad estructural de la mielina: estudio por difracción de rayos-X. Ph.D. Thesis. Centro de Estudios Avanzados. Instituto Venezolano de Investigaciones Científicas. Caracas, Venezuela.

Padrón, R., and L. Mateu. 1980. The effect of the repetitive propagation of action potentials on the structure of toad sciatic nerve myelin membranes: an x-ray diffraction study at 11-Å resolution. *J. Neurosci. Res.* 5(6):611–620.

Padrón, R., L. Mateu, and D. A. Kirschner. 1979. X-ray diffraction study of the kinetics of myelin lattice swelling. Effect of divalent cations. *Biophys. J.* 28:231–239.

Peters, A. 1961. A radial component of central myelin sheaths. *J. Biophys. Biochem. Cytol. 11:733–735.*

Robertson, J. D. 1958. Structural alterations in nerve fibers produced by hypotonic and hypertonic solutions. *J. Biophys. Biochem. Cytol.* 4:349–364.

Schmitt, F. O., R. S. Bear, and G. L. Clark. 1935. X-ray diffraction studies on nerve. *Radiology.* 25:131–151.

Schnapp, B., and E. Mugnaini. 1975. The myelin sheath: electron microscopic studies with thin sections and freeze-fracture. *In* Golgi Centennial Symposium: Perspectives in Neurobiology. M. Santini, editor. Raven Press, New York. 209–233.

Schnapp, B., C. Peracchia, and E. Mugnaini. 1973. Freeze-fracture of Ranvier nodes. *J. Cell Bio.* 59:360a.

Schnapp, B., C. Peracchia, and E. Mugnaini. 1976. The paranodal axoglial junction in the central nervous system studied with thin sections and freeze-fracture. *Neuroscience.* 1:181–190.

Tabira, T., M. J. Cullen, P. J. Reier, and H. de F. Webster. 1978. An experimental analysis of interlamellar tight junctions in amphibian and mammalian C.N.S. myelin. *J. Neurocytol.* 7:489–503.

Tippe, A., and H. Müller-Mohnssen. 1975. Further experimental evidence for the synapse hypothesis of Na^+ current activation and inactivation at the Ranvier node. *Naturwissenschften.* 62:490–491.

Wolman, M., and H. Wiener. 1965. Structure of myelin sheath as a function of concentration of ions. *Biochim. Biophys. Acta.* 102:269–279.

Worthington, C. R. and A. E. Blaurock. 1969. A low-angle X-ray diffraction study of the swelling behavior of peripheral nerve myelin. *Biochim. Biophys. Acta.* 173:427–435.