DEMYELINATION AS A TEST FOR A MOBILE NA CHANNEL MODULATOR IN FROG NODE OF RANVIER

PAMELA A. PAPPONE AND MICHAEL D. CAHALAN
Department of Physiology and Biophysics, University of California, Irvine, California 92717

ABSTRACT We found previously that the external surface of frog skeletal muscle fibers can be irreversibly modified by treatment with the amino group-specific reagent, trinitrobenzene sulfonic acid (TNBS). Reaction of the muscle membrane with TNBS permanently shifts the potential dependence of the sodium channel inactivation gating process, h_∞, to more hyperpolarized potentials. The experiments presented here show nearly identical effects on the sodium currents of voltage-clamped frog node of Ranvier in the presence of TNBS. In contrast to the results in muscle, in myelinated nerve the voltage dependence of sodium-channel inactivation returns rapidly to control values following a brief exposure to TNBS. We have used partial demyelination to test the hypothesis that recovery of the normal voltage dependence for h_∞ following TNBS treatment is due to lateral diffusion of reacted groups away from the sodium channels in the node. We find that increasing the membrane area exposed to TNBS by partial demyelination greatly slows reversal of the TNBS effects. This result suggests that a modifiable membrane component that affects sodium channel gating is mobile in the plane of the membrane and can rapidly diffuse between nodal and internodal regions.

INTRODUCTION

The fluid mosaic model of membrane structure predicts that the proteins and lipids of biological membranes are free to diffuse laterally in the plane of the membrane. Lateral mobility of lipids and membrane proteins, including ionic channels, has been demonstrated in a number of systems, most commonly in embryonic or transformed cells (Poo, 1981; Axelrod, 1983). Free mobility is not a universal characteristic of the membrane proteins of excitable cells, however. Both the inhomogeneous distribution of channel proteins in adult nerve and muscle cells and the direct measurement of lateral diffusion rates of sodium and acetylcholine-activated channels in muscle cells indicate that sodium, acetylcholine-activated, and possibly potassium channels have restricted mobility in these cells (Almers and Stirling, 1984; Stühmer and Almers, 1982; Almers et al., 1983; Tank et al., 1981). In myelinated nerve fibers, sodium channels are confined to the membrane of the node of Ranvier (Ritchie and Rogart, 1977; Chiu and Ritchie, 1980), where they are found in high density.

We found previously that membrane amino groups influence the gating of sodium channels in frog skeletal muscle membrane (Cahalan and Pappone, 1981). Reaction of the muscle membrane with the amino group-specific reagent trinitrobenzene sulfonic acid (TNBS) permanently shifts the potential dependence of the sodium-channel inactivation gating process to more hyperpolarized potentials. TNBS is a charged, membrane-impermeant reagent that reacts with normally positively charged titratable amino groups to produce neutral trinitrophenylated derivatives (Means and Feeney, 1971). TNBS reacts covalently with amino groups of lipid (e.g., phosphatidylethanolamine) and proteins (e.g., lysine).

Here, we show that TNBS similarly alters the inactivation gating of sodium channels in myelinated nerve fibers. However, the modification is reversible following short exposures to the reagent. We present evidence that the recovery from TNBS modification in nerve fibers may be due to the lateral diffusion of modified membrane components in the plane of the membrane. This result suggests that in spite of the apparent immobility of nodal sodium channels, membrane components that influence the gating properties of sodium channels may be free to diffuse between nodal to internodal membrane regions. A preliminary account of this work has appeared (Pappone and Cahalan, 1981).

METHODS

Preparation

Experiments were performed on single frog myelinated nerve or skeletal muscle fibers from *Rana catesbiana* or *Rana pipiens*. Fibers were voltage clamped using the Vaseline-gap method (Frankenhaeuser, 1957; Dodge and Frankenhaeuser, 1958; Hille, 1971; Hille and Campbell, 1976). Nerve fiber ends were cut in solutions of either 120 mM KF, 120 mM KCl, 120 mM CsF, or 80 mM K_2-EGTA, all containing 2 mM HEPES buffer, pH 7.2. Muscle fiber ends were cut in a solution containing 96 mM CsF, 24 mM NaF, and 2 mM HEPES, pH 7.2. External solutions bathing the nodal or muscle membrane were either normal Ringer's solution consisting of 115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, and 5 mM MOPS, pH 7.4, or high-pH Ringer's solution composed of 95 mM NaCl, 1.8 mM $CaCl_2$, 20 mM Na-borate buffer, pH 9.0. The mounted fibers were allowed to equilibrate for 30 min before starting the experiment.

Dr. Pappone's present address is the Department of Animal Physiology, University of California, Davis, CA 95616.

Muscle experiments were performed at 10°C or at room temperature, 20–24°C. Lower temperatures were favored in the muscle experiments to enhance survival, but the effects of TNBS on muscle were the same at 10°C or room temperature. Node experiments were performed at room temperature to avoid changes in temperature during solution changes. Solution changes were rapid, as judged by the rate of onset of block of potassium current by TEA, which occurred within 2 s. This is considerably faster than the rate of reaction of TNBS under our experimental conditions. Resting potentials were assumed to be −80 mV. Deviations in the true resting potential from this value will result in errors in the absolute membrane potentials reported here.

Chemical Modification

Solutions for chemical modification consisted of high pH Ringer's solution with Na-TNBS replacing an appropriate amount of NaCl. In most of the experiments 10 mM TNBS (Sigma Chemical Co., St. Louis, MO) was applied in pH 9 Ringer's solution to produce a rapid effect on sodium channel inactivation. At lower TNBS concentrations and lower pH, the rate of reaction is appreciably slower and can be followed by determining the voltage dependence of inactivation. The TNBS solutions were made up from crystalline TNBS immediately before use. TNBS reacts specifically and covalently with primary amino groups under these conditions (Means and Feeney, 1971). The reaction of TNBS with amino groups liberates bisulfite ions that bind to the trinitrophenyl group of the reaction product, producing a negative modified group. The binding of bisulfite is rapidly reversible (within 2–3 s) (Means et al., 1972) and bisulfite is therefore not present in our experiments after the TNBS solution is washed out.

Demyelination

Solutions for the demyelination treatment were composed of normal Ringer's solution to which 0.5 M urea or sucrose was added. Urea was more effective in demyelinating fibers than was sucrose. The nerve in the A pool was partially demyelinated by exposure to the hypertonic solution for 1–10 min. Repeated brief exposures were often effective in demyelinating without appearing to damage the fiber. The current during small voltage steps was monitored while the fiber was in the hypertonic solution, and an increase in the magnitude and duration of the capacity current transient was used as an assay for the effectiveness of the demyelination. Demyelination was considered to have occurred if on returning to isotonic Ringer's solution there was an increase in the magnitude of the delayed rectifier potassium currents accompanying the increase in membrane capacitance (see Results).

Data Collection and Analysis

Details of the methodology were as described previously for node (Cahalan and Hall, 1982; Cahalan and Pappone, 1983) and muscle (Cahalan and Pappone, 1981). Records of current were sampled at up to 100 kHz and stored on the disk of a minicomputer (Nova 3, Data General Corp., Westboro, MA). The computer was programmed to deliver two types of pulse protocols to the preparation for monitoring the level of steady-state sodium channel inactivation, h_∞. The first consisted of 100–200 ms conditioning prepulses, followed by a test pulse of −10 to −30 mV. The amplitude of the conditioning pulse was increased in 10-mV increments from −160 to −60 mV. Peak current amplitudes during the test pulses as a function of conditioning pulse potential were read automatically into a file. h_∞ was calculated and $\overline{V}_h$, the potential at which $h_\infty = 0.5$, determined by least-squares fitting of the h_∞ values between 0.1 and 0.9 with a logarithmic form of the Boltzmann equation

$$h_\infty = \frac{1}{1 + \exp\left[(V - \overline{V}_h)/k\right]}, \qquad (1)$$

where V is the membrane potential, and $\overline{V}_h$, the midpoint of the curve, and k, its steepness, were determined by the fit.

The second type of pulse protocol was used for rapid determinations of $\overline{V}_h$. 100–200 ms conditioning pulses of two amplitudes followed by a constant test pulse were applied to the preparation alternately. One pulse was negative enough to completely remove sodium current inactivation, and the other was chosen to give a value of h_∞ near 0.5. $\overline{V}_h$ was calculated from the peak currents during consecutive pairs of test pulses and Eq. 1 by assuming that k was equal to the average determined from complete h_∞-voltage relations measured before and after the rapid pulse train. k changed <10% in these experiments. This procedure was useful to follow $\overline{V}_h$ during solution changes.

RESULTS

Fig. 1 illustrates the effects of TNBS on the sodium current inactivation-voltage relation (h_∞) in nerve and muscle fibers. In both nerve and muscle fibers, h_∞ is rapidly shifted to more negative potentials in the presence of TNBS, as expected if TNBS acts by removing positive charge from the surface of the membrane. The rate and extent of TNBS action on sodium inactivation can be determined by following the midpoint of the h_∞-voltage

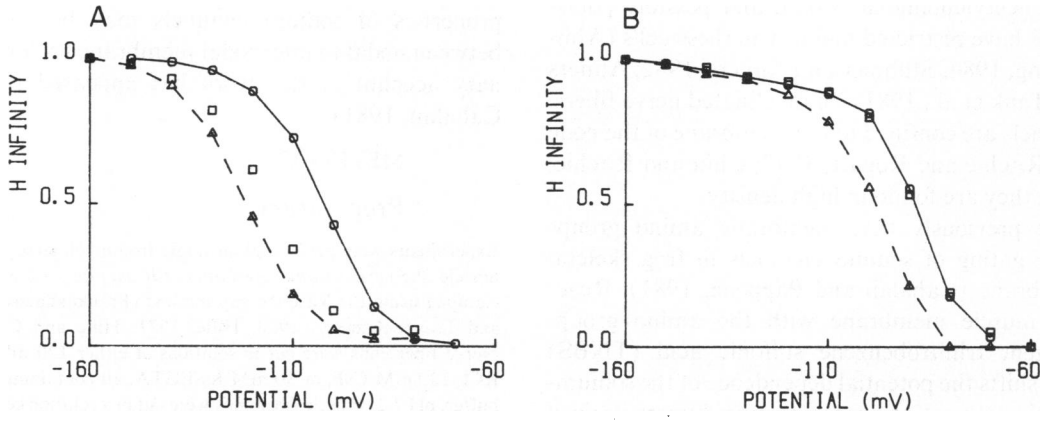

FIGURE 1 The potential dependence of steady-state sodium current inactivation, h_∞, before (○), during (△), and after (□) exposure to 10 mM TNBS, all at pH 9.0. h_∞ calculated from the peak sodium current during a test pulse to −10 mV (*A*) or −20 mV (*B*) following conditioning pulses to the potentials shown. (*A*) Muscle fiber, 3-min exposure to TNBS. 200-ms conditioning pulse, holding potential −110 mV. 10°C. (*B*) Nerve fiber, 4-min exposure to TNBS. Ends cut in 120 mM CsF, 50-ms conditioning pulse, holding potential −120 mV. Room temperature.

relation, $\overline{V}_h$. As we found previously in muscle fibers (Cahalan and Pappone, 1981), the effects of TNBS on the sodium channel are selective for the inactivation process in nerve, and the kinetics and potential dependence of activation are little affected by the modification. In 18 experiments on myelinated nerve fibers, 2–10 min exposures to 10 mM TNBS at pH 9 resulted in an average change in $\overline{V}_h$ of -12.2 ± 1.0 mV ($\pm$ SE of mean) relative to the control value at pH 9. This average shift of $\overline{V}_h$ produced by TNBS in nodes of Ranvier was approximately two-thirds of the shift determined in experiments on muscle fibers (Cahalan and Pappone, 1981). In the muscle fiber, the effect of TNBS on sodium inactivation is permanent, either at 10°C or room temperature, even following brief exposure (~2 min) to the reagent. In the nerve, however, the TNBS effect is completely reversed shortly after washout. Fig. 2 shows the time course of these effects in muscle (*A*) and nerve (*B*, *C*) fibers. In the muscle, washout of the TNBS results in only a very slight recovery of $\overline{V}_h$ toward control values. This small reversible component is present in all our experiments and probably reflects reversible binding of TNBS and/or reaction products to the membrane (Means et al., 1972). Aside from the minor initial change in $\overline{V}_h$, there is no further recovery from the TNBS-induced shift in the muscle fiber, and $\overline{V}_h$ remains shifted for the remaining 45 min of the experiment. In contrast, recovery from the TNBS-induced shift in the node of Ranvier is complete within 2–3 min following a brief exposure to the reagent (Fig. 2 *B*). Repeated short-duration treatments with TNBS resulted in similar shifts in $\overline{V}_h$ during each exposure, with $\overline{V}_h$ returning rapidly to control values following each washout.

Since sodium channels of frog nerve and muscle cells are so similar in their kinetic and pharmacological properties as to suggest they may represent the same molecular entity (Campbell and Hille, 1976), and because the effects seen in the presence of TNBS are similar in the two preparations, differing only in the magnitude of the h_∞ shift, it seems unlikely that the difference in reversibility of the TNBS effects is due to differences in the chemical reaction of TNBS with nerve and muscle cell membranes. Morphological differences between the nerve and muscle preparations could account for the difference in recovery from TNBS effects in myelinated nerve and muscle if modified membrane components can diffuse laterally in the plane of the membrane away from the sodium channels at the node of Ranvier. In the muscle fibers, the entire 100 μm length of fiber in the recording pool (pool A) is exposed to TNBS. In the nerve, only the membrane of the fiber at the node of Ranvier is accessible to the external solution, the remainder of the nerve membrane being protected from exposure to the solution by the myelin sheath, as diagrammed in Fig. 3 *A*. If the modified membrane components were free to diffuse in the plane of the membrane, the different geometries of the two preparations would be expected to result in very different rates of recovery from the TNBS effects.

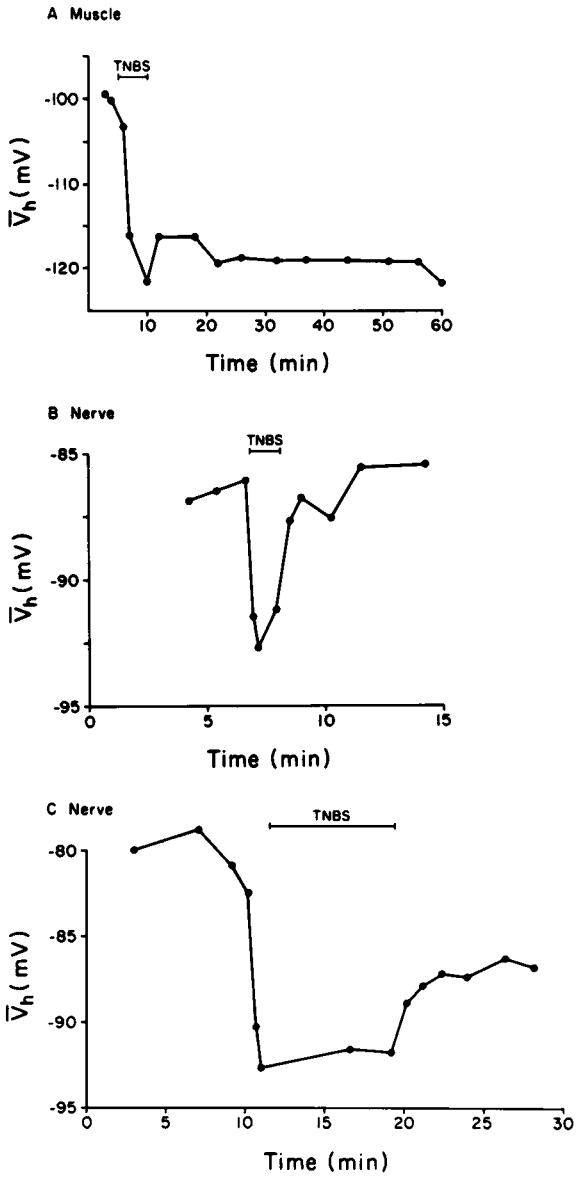

FIGURE 2 The midpoint of the h_∞-potential relation, $\overline{V}_h$, during the course of experiments on muscle (*A*) and nerve (*B*, *C*) fibers. $\overline{V}_h$ was determined as described in the methods. Fibers were exposed to 10 mM TNBS for the times shown. (*A*) Same muscle fiber as Fig. 1. (*B*) Nerve fiber, ends cut in 120 mM CsF, holding potential -110 mV. Similar results were obtained from fibers cut in K_2-EGTA or KCl. (*C*) Nerve fiber, ends cut in 80 mM K_2-EGTA, holding potential -110 mV.

If one assumes for simplicity that exposure to TNBS resulted in the idealized concentration profile of modified membrane components shown in Fig. 3 *B* for $t = 0$, the concentration profile along the fiber as a function of time following washout of the reagent is given by (Crank, 1975)

$$C(x, t) = \frac{1}{2} C_0 \left\{ \operatorname{erf} \frac{h - x}{2\sqrt{Dt}} + \operatorname{erf} \frac{h + x}{2\sqrt{Dt}} \right\}, \qquad (2)$$

where $C(x,t)$ is the concentration of modified components

in the membrane, x is the distance from the center of the treated region of membrane, t is the time following washout of the reagent, C_0 is the initial concentration between $x = -h$ and $x = +h$ at $t = 0$, D is the diffusion coefficient, and $2h$ is the length of fiber exposed to the reagent. Because the rate of diffusion from the center of the treated area depends on the square of the length of the modified membrane, diffusion over a 1 μm distance like the nodal gap could be quite rapid in the nerve, while the same process would be 10,000 times slower in a 100 μm length like the exposed region of the muscle fiber. For a membrane component with a lateral diffusion coefficient of 10^{-8} cm^2/s, the fastest rate of lateral mobility of lipids measured in cell membranes (Poo, 1981), the time to reach half the initial concentration at the center of a 100-μm gap would be 190 min. The same process would take less than a second in a 1-μm gap like that at the node of Ranvier.

In this analysis we have considered the diffusion of modified amino groups away from the nodal region. One might also consider diffusion into the node of unmodified components. However, we cannot say whether sodium inactivation is altered by TNBS due to the presence of the trinitrophenyl reaction product, or the absence of charge in the unmodified component. Therefore, for simplicity, we consider diffusion of the TNBS-modified components out of the node.

According to this lateral diffusion model, increasing the duration of exposure of the nerve membrane to TNBS would increase the length of nerve containing modified membrane components (increasing h in Eq. 2), since significant diffusion of modified components into the internode could occur during the course of exposure to the reagent. This would have the effect of slowing the rate of recovery from TNBS, but would not be expected to alter the magnitude of the shift in $\overline{V}_h$. In accordance with the lateral diffusion hypothesis, longer duration exposures of the nerve to TNBS did not increase the magnitude of the shift in $\overline{V}_h$, but did result in a less rapid and complete recovery of $\overline{V}_h$ following washout, as shown in Fig. 2 *C*. If the nerve were in fact a perfect cylinder as depicted in Fig. 3, the rate of recovery due to lateral diffusion should be simply related to the duration of the preceding exposure to TNBS. However, the nodal region of axon is surrounded by paranodal swelling of the axon (Berthold, 1978). This deviation from cylindrical geometry would be expected to speed the rate of recovery relative to that in a cylinder. Uncertainties as to the exact morphology of the node preclude a quantitative assessment of this effect.

The lateral-diffusion hypothesis was tested further by increasing the length of axon membrane exposed to TNBS through partial demyelination. If lateral diffusion of chemically modified membrane constituents underlies the recovery from TNBS in the node, then the recovery should be slower when an increased length of fiber is exposed to TNBS. Since the time for recovery through lateral diffusion will be proportional to the square of the length of fiber

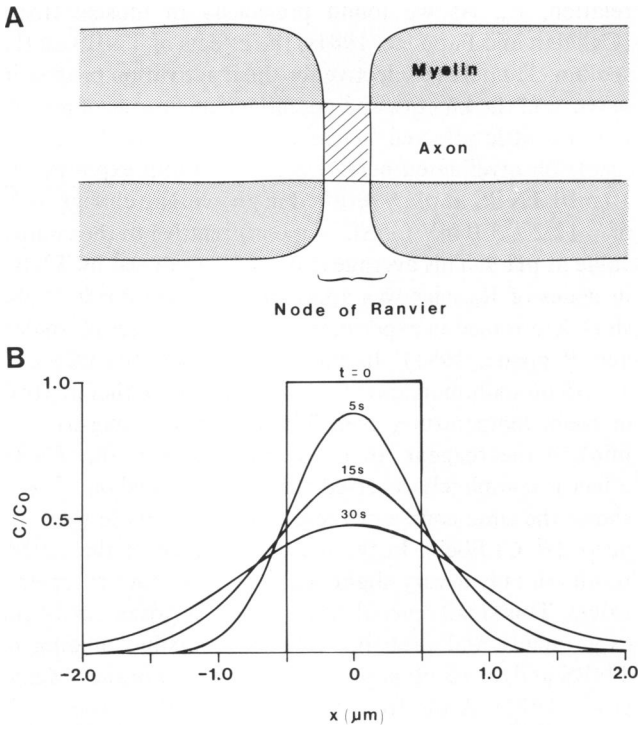

FIGURE 3 (*A*) Diagram of the nerve preparation. The hatched area represents the fiber membrane exposed to the TNBS solution. Dimensions have been distorted for illustration purposes. In an actual nerve the length of the nodal gap is ~1/10 the diameter of the fiber, instead of 1/2 the diameter as in this diagram. (*B*) Curves showing the theoretical concentration profiles as a function of time predicted by Eq. 2 for the starting distribution depicted in part A. Parameters used in the calculations were: $D = 10^{-10}$ cm^2/s, $h = 0.5$ μm. The predicted concentration profiles are for $t = 0$, 5, 15, and 30 s.

reacted with TNBS (Eq. 2), small changes in the length of fiber exposed at the node should have large effects on the recovery rates. If recovery occurs through some other process, such as a reversible chemical reaction, changing the geometry through demyelination would be expected to have little effect on the recovery rate.

Axons were partially demyelinated by treatment with hypertonic (0.5 M) urea or sucrose solutions. Fig. 4 shows membrane currents recorded in a nerve before and after urea treatment. In agreement with previous studies (Chiu and Ritchie, 1982), the magnitude of both the capacity current transient and the potassium currents are increased, whereas the sodium current is little changed by demyelination. The nodal capacity measured from the integral of the capacity current transient increased as much as 16-fold following urea treatment, and the potassium current magnitude was up to six times larger than the control. In six fibers the sodium currents were 0.98 ± 0.08 (mean ± SEM) of control values, while potassium currents increased by a factor of 1.83 ± 0.23 following partial demyelination. On the average, the apparent membrane capacity increased from 6.2 ± 0.9 pF to 26.4 ± 7.0 pF (± SEM, $n = 9$), indicating that the exposed membrane area

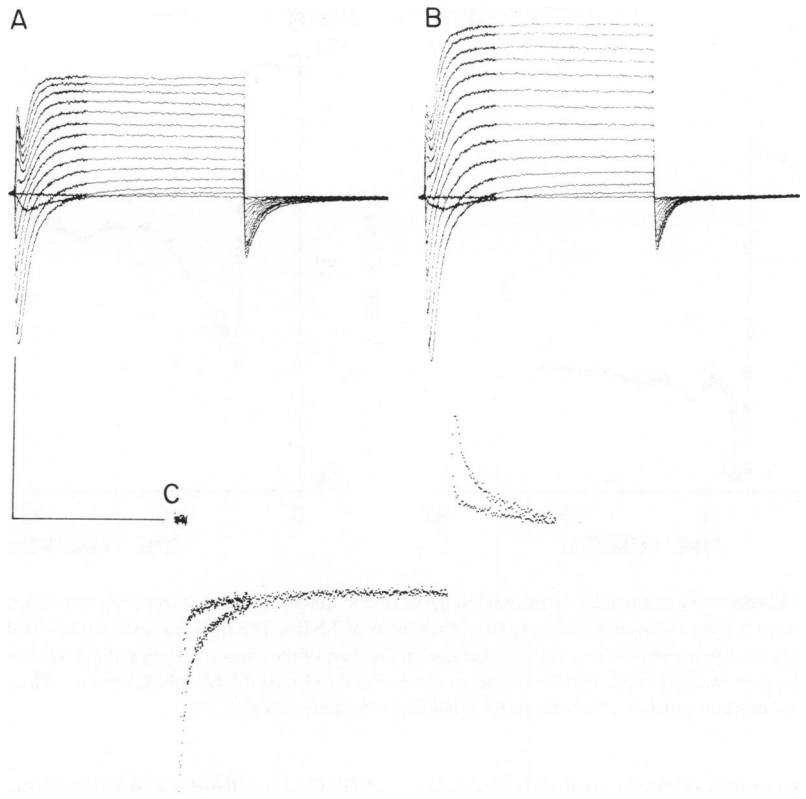

FIGURE 4 (*A*) Membrane currents in a normal nerve fiber during depolarization from −60 mV to +135 mV in increments of 15 mV. Fiber ends cut in KCl, holding potential −90 mV. (*B*) Membrane currents in the same nerve fiber for the same pulse sequence following partial demyelination produced by a 3-min exposure to Ringer's solution + 0.5 M sucrose. Current records in both *A* and *B* had the linear components of the leak and capacity current transient electronically subtracted. (*C*) Superimposed membrane currents in another fiber recorded without leak and capacity compensation during a pulse to −150 mV before and after urea treatment. Record showing the larger capacity transient is that following urea treatment. The membrane capacity measured from the integral of the current transient increased from 2.8 pF to 6.5 pF, while the leakage current remained the same. Records filtered with 80 μs time constant. Fiber ends cut in 120 mM KCl, holding potential −90 mV. Time scale bar = 5 ms for part *A* and *B*; 2.5 ms for part *C*. Current amplitude scale bar = 30 nA for part *A*, *B*; 6 nA for part *C*.

was increased at least fourfold. This is a minimal estimate of the increase in membrane area because the control measurement of nodal capacity includes the stray capacitance of the preparation. The smaller increase in potassium current compared to membrane capacity suggests that there is a lower density of potassium channels beneath the myelin than at the node in frog nerve. Insofar as urea treatment had no effect on the gating of sodium or potassium channels, we conclude that urea treatment increases the nodal capacity and potassium current through demyelination without changing the properties of the nodal membrane.

Increasing the membrane area exposed to TNBS by partial demyelination slows the recovery from TNBS as predicted by the lateral diffusion hypothesis. Nodal sodium currents remained sensitive to TNBS following partial demyelination and $\overline{V}_h$ was shifted by an average of −9.5 ± 1.6 mV (± SEM, n = 4) in the presence of TNBS before and −10.5 ± 1.1 mV, n = 8, after demyelination in these fibers. However, recovery from the effects of TNBS is greatly slowed in demyelinated fibers, as shown in Fig. 5. $\overline{V}_h$ remained shifted for up to 30 min after washout of TNBS in demyelinated fibers following brief (<3 min) exposure to the reagent. Most of our demyelinated fibers gave results like those shown in Fig. 5 *A*; that is, there was no recovery in $\overline{V}_h$ beyond the initial washout effect. However, several demyelinated fibers showed a very slow rate of recovery, as in Fig. 5 *B*, which continued for the lifetime of the preparation. Two types of control experiments indicate that the slow recovery in demyelinated fibers is not due to loading of TNBS in a space from which washout is restricted following demyelination: (*a*) The rate of washout does not depend on the concentration of TNBS, even for concentrations low enough to produce only a minimal effect, and (*b*) The onset of TEA block of potassium currents and recovery from the block following washout are rapid in demyelinated fibers. The profound effect of demyelination on the rate of recovery from TNBS is strong evidence for the lateral diffusion hypothesis.

We can use the rate of recovery from TNBS following a brief exposure to estimate what the mobility of the modified membrane group would need to be for consistency with the lateral-diffusion hypothesis. Assuming that only the 1 μm length of fiber exposed to TNBS contains

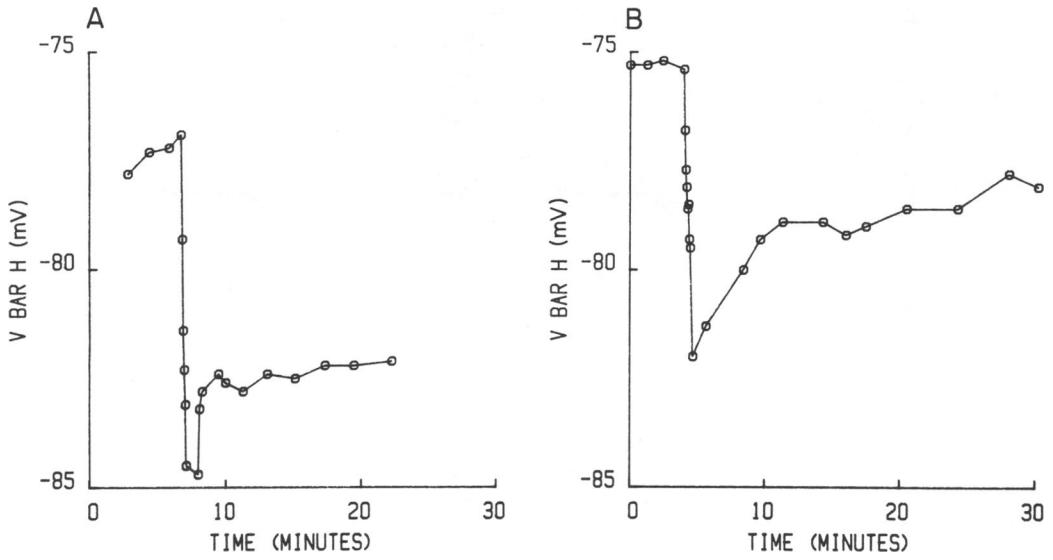

FIGURE 5 The effect of TNBS on $\overline{V}_h$ in partially demyelinated nerve fibers. Before these measurements were taken, the fibers were exposed repeatedly to 0.5 M urea. (*A*) A fiber showing no recovery from the effects of TNBS. The fiber was exposed to 10 mM TNBS at pH 9 for 67 s at the time shown. The urea treatment resulted in a 3.9-fold increase in the membrane capacity. Ends cut in CsF, holding potential −110 mV. (*B*) A fiber showing partial slow recovery from TNBS. The nerve was exposed to 1 mM TNBS, pH 9, for 136 s. The urea treatment resulted in a 2.3-fold increase in the membrane capacity. Ends cut in KCl, holding potential −90 mV.

modified membrane components initially, and that there is a linear relation between the concentration of modified membrane components and $\overline{V}_h$, then from the measured time for half-recovery following brief exposure to TNBS, 34 ± 10 s (± SEM, n = 7), Eq. 2 predicts a diffusion coefficient for the group of 8×10^{-12} cm^2/s. The fastest recovery rate measured, 8 s, would be consistent with a diffusion coefficient of 3×10^{-10} cm^2/s. Our value for the diffusion coefficient is only a rough estimate, and may represent a lower limit, since the calculation neglects the time for solution changes, the diffusion of modified membrane components occurring during the exposure to TNBS, and the swelling of the paranodal axon. Diffusion into the internode of reacted components during exposure to TNBS would tend to fill the paranode with reacted species delaying complete recovery upon washout. To gain a qualitative assessment of this effect, we considered the spread of a rapidly diffusing component ($D = 10^{-8}$ cm^2/s), assuming also that reaction with TNBS is rapid. During a 2-min exposure, typical of the brief exposures used in experiments from which the above estimate of a diffusion coefficient was obtained, a substance with a diffusion coefficient of 10^{-8} cm^2/s would spread in either direction ~10 μm, the calculated distance for the concentration to be half that at the node. Diffusion during the exposure to TNBS would thus increase the distance for diffusion away from the node during the washout by a factor of 20 and result in an underestimate of the diffusion coefficient by a factor of 400. Considering the potential inaccuracies and assumptions involved in these calculations of the diffusion coefficient, our estimate of an average diffusion coefficient of 8×10^{-12} cm^2/s could be consistent with lateral diffusion of lipid or protein (Poo, 1981; Axelrod, 1983; McCloskey and Poo, 1984).

DISCUSSION

We have shown that reaction with TNBS alters the inactivation gating process in myelinated nerve sodium channels. The effects of TNBS treatment on nodal sodium channels are similar to those previously seen in skeletal muscle fibers (Cahalan and Pappone, 1981), in that the potential dependence of inactivation is shifted to more negative potentials in a manner consistent with an increase in the negative surface potential. In addition, there was little effect of TNBS on sodium channel activation in the node, as was found in muscle. However, unlike the permanent modification seen in muscle fibers, the effects of TNBS on sodium currents are reversible in nerve fibers following a short exposure to the reagent. In contrast to these results on sodium channels, we have found that TNBS irreversibly affects the gating of delayed rectifier potassium channels in myelinated nerve (Cahalan and Pappone, 1983).

In this paper, we have explored the possibility that recovery from TNBS modification in nerve fibers is due to the lateral diffusion of reacted membrane components away from the sodium channels at the node. We find that the differences in recovery from TNBS treatment in normal nerve, demyelinated nerve, and muscle fibers can be explained by the lateral diffusion of TNBS-modified membrane components. As predicted by this hypothesis, increasing either the duration of exposure of the nodal membrane to TNBS or increasing the length of nerve fiber

reacted with the reagent slows recovery from the TNBS effects. These results suggest that the membrane of myelinated nerve fibers contains both mobile and immobile constituents, as has been demonstrated in other cell types. If this hypothesis is correct, our data indicate that mobile, TNBS-reactive components of the nerve membrane can influence sodium-channel gating. Such interactions between sodium channels and other membrane constituents could be important in determining and regulating the firing properties of axons. Our data do not address the question of what type of membrane component is reacting with TNBS. TNBS reacts with amino groups of both lipids and proteins, and could alter sodium inactivation by altering local surface charge or through steric or hydrophobic influences on the gating process. Because of the uncertanties involved in the calculated diffusion coefficient we cannot distinguish whether the mobile group is a protein or a lipid.

Other interpretations of our data are possible. For instance, the reaction of TNBS with components of the nerve membrane could be slower than its reaction in muscle, and speeded up by pretreatment with the hypertonic solutions used to demyelinate. The rapidity of the onset of TNBS action argues against such effects being caused by limited access to the nodal membrane. Since both urea and sucrose demyelination treatments produced similar results, the mechanism of acceleration of the reaction would have to be shared by these two chemically distinct solutions. We have interpreted our data in terms of a lateral diffusion of modified membrane components because this seems the simplest and most straightforward hypothesis consistent with all our results.

Supported by fellowships from the National Institutes of Health (NIH) and the American Heart Association to P. A. Pappone, and by NIH grant number NS14609 and a Research Career Development Award to M. D. Cahalan.

Received for publication 20 April 1984 and in final form 6 July 1984.

REFERENCES

Almers, W., P. R. Stanfield, and W. Stühmer. 1983. Lateral distribution of sodium and potassium channels in frog skeletal muscle: measurements with a patch-clamp technique. *J. Physiol.* (*Lond.*). 336:261–284.

Almers, W., and C. Stirling. 1984. Distribution of transport proteins over animal cell membranes. *J. Membr. Biol.* 77:169–186.

Axelrod, D. 1983. Lateral motion of membrane proteins and biological function. *J. Membr. Biol.* 75:1–10.

Berthold, C.-H. 1978. Morphology of normal peripheral axons. *In* Physiology and Pathobiology of Axons. S. G. Waxman, editor. Raven Press, New York. 3–63.

Cahalan, M. D., and J. Hall. 1982. Alamethicin channels incorporated into frog node of Ranvier: calcium-induced inactivation and membrane surface charges. *J. Gen. Physiol.* 79:411–436.

Cahalan, M. D., and P. A. Pappone. 1981. Chemical modification of sodium channel surface charges in frog skeletal muscle by trinitrobenzene sulfonic acid. *J. Physiol.* (*Lond.*). 321:127–139.

Cahalan, M. D., and P. A. Pappone. 1983. Chemical modification of potassium channel gating in frog myelinated nerve by trinitrobenzene sulfonic acid. *J. Physiol.* (*Lond.*). 342:119–143.

Campbell, D. T., and B. Hille. 1976. Kinetic and pharmacological properties of the sodium channel of frog skeletal muscle. *J. Gen. Physiol.* 67:309–323.

Chiu, S. Y., and J. M. Ritchie. 1980. Potassium channels in nodal and internodal axonal membrane of mammalian myelinated fibers. *Nature* (*Lond.*). 284:170–171.

Chiu, S. Y., and J. M. Ritchie. 1982. Evidence for the presence of potassium channels in the internode of frog myelinated nerve fibers. *J. Physiol.* (*Lond.*). 322:485–501.

Crank, J. 1975. The Mathematics of Diffusion. Clarendon Press, Oxford, England. 15.

Dodge, F. A., and B. Frankenhaeuser. 1958. Membrane currents in isolated frog nerve fibers under voltage clamp conditions. *J. Physiol.* (*Lond.*). 143:76–90.

Frankenhaeuser, B. 1957. A method for recording resting and action potentials in the isolated myelinated nerve fiber of the frog. *J. Physiol.* (*Lond.*). 135:550–559.

Hille, B. 1971. The permeability of the sodium channel to organic cations in myelinated nerve. *J. Gen. Physiol.* 58:599–619.

Hille, B., and D. T. Campbell. 1976. An improved vaseline gap voltage clamp for skeletal muscle fibers. *J. Gen. Physiol.* 67:265–293.

Hille, B., A. M. Woodhull, and B. I. Shapiro. 1975. Negative surface charge near sodium channels of nerve: divalent ions, monovalent ions, and pH. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 270:301–318.

McCloskey, M., and M.-m. Poo. 1984. Protein diffusion in cell membranes: some biological implications. *Int. Rev. Cytol.* 87:19–81.

Means, G. E., W. I. Congdon, and M. L. Bender. 1972. Reactions of 2,4,6-trinitrobenzenesulfonate ion with amines and hydroxide ion. *Biochemistry.* 11:3564–3571.

Means, G. E., and R. E. Feeney. 1971. Chemical Modification of Proteins. Holden-Day, Inc., San Francisco, CA.

Pappone, P. A., and M. D. Cahalan. 1981. Partial demyelination as a test for lateral diffusion of surface charges in the frog node of Ranvier. *Soc. Neurosci. Abstr.* VII 8.12:16.

Poo, M.-m. 1981. In situ electrophoresis of membrane components. *Annu. Rev. Biophys. Bioeng.* 10:245–276.

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.

Stühmer, W., and W. Almers. 1982. Photobleaching through glass micropipettes: sodium channels without lateral mobility in the sarcolemma of frog skeletal muscle. *Proc. Natl. Acad. Sci. USA.* 79:946–950.

Tank, D., E. S. Wu, and W. W. Webb. 1981. Enhanced mobility of acetylcholine receptor and membrane probes in muscle membrane blebs. *Biophys. J.* 33(2, Pt.2):74*a*. (Abstr.)