SOLVENT SUBSTITUTION AS A PROBE OF CHANNEL GATING IN *MYXICOLA*

Effects of D_2O on Kinetic Properties of Drugs that Occlude Channels

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ABSTRACT The effects of solvent substitution on the steady-state and kinetic properties of drugs (gallamine triethiodide) and ions (nonyltriethylammonium and Ba^{++}) known to occlude Na^+ and K^+ channels have been examined and compared with the effects of D_2O on unmodified channels. In general, we observed large isotope effects on the kinetics of occlusion at temperatures of 5°C, but only minor effects at 15°C, consistent with processes involving significant solvent interaction. Steady-state behavior was not affected. In the case of gallamine, where a dual effect on I_{Na} is evident, although both processes were D_2O sensitive, only the occlusion phase had a significant temperature dependence.

INTRODUCTION

The effects of making an isotopic substitution in a chemical system can often provide considerable insight into the reaction processes occuring in it. In particular, we have previously shown that studies of the effects of heavy water (D_2O) substitution on the kinetics of ion channels at different temperatures can be used to distinguish those features of Na^+ and K^+ channel gating that may involve a significant interaction with the surrounding solvent from those that appear to be solvent independent (Schauf and Bullock, 1979). Furthermore, differential effects of solvent substitution on various components of channel gating (e.g., Na^+ activation vs. Na^+ inactivation) enable us to place constraints on the kinds of physical models and/or reaction sequences that may be capable of explaining existing voltage-clamp data (Schauf and Bullock, 1981).

Activation and inactivation of sodium channels in excitable membranes do not seem to be completely independent. Experimental evidence for this view has been obtained both from studies of the kinetics of sodium currents (Goldman and Schauf, 1972, 1973; Schauf et al., 1976*b*; Bezanilla and Armstrong, 1977) and from the time-course of membrane asymmetry currents and their variation with increasing pulse duration (Armstrong and Bezanilla, 1977; Bullock and Schauf, 1979). However, the nature of the "coupling" between activation and inactivation is unclear and may be more complicated than that expected if a channel is obliged to open before becoming inactivated (Horn et al., 1981). Nevertheless, a detailed kinetic model for inactivation has been developed based in part on the existence of a physiological "inactivating particle," which can enter and block the sodium channel from the interior of the cell (Armstrong and Bezanilla, 1977; Armstrong and Gilly, 1979).

As a result, there has been considerable interest in substances that can block sodium currents from the inside of a nerve fiber in ways consistent with a voltage- and/or time-dependent occlusion, and thus mimic physiological inactivation. Compounds with this effect include anesthetics (Strichartz, 1973; Cahalan, 1978; Cahalan and Almers, 1979*a*), methylstrychnine (Cahalan and Almers, 1979*b*), pancuronium (Yeh and Narahashi, 1977), polyglycyl arginine amide (Eaton et al., 1980), alkylguanidines (Kirsch et al., 1980; Morello et al., 1980), and gallamine triethiodide (Schauf and Smith, 1981). It might be expected that drugs that enter and occlude Na^+ channels would have kinetics that are strongly influenced by solvent changes. Thus, a comparison between solvent effects on drug interaction with the Na^+ channel and physiological inactivation might serve as a useful means of testing channel occlusion as a model for inactivation.

Voltage- and time-dependent occlusion can be observed in K^+ channels without the complication of a concommitant inactivation. Such effects are produced by Cs^+ (Adelman and French, 1978; Bezanilla and Armstrong, 1972), Ba^{++} (Armstrong and Taylor, 1980; Eaton and Brodwick, 1980), and tetraethylammonium and its various derivatives such as nonyltriethylammonium (Armstrong, 1966, 1969, 1971; Armstrong and Hille, 1972). Again, solvent substitution would be expected to have significant effects on the rate of block by such compounds.

The present study represents an initial attempt to evaluate the effects of D_2O substitution on occlusion of both Na^+ and K^+ channels by drugs and ions. It is hoped that

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such data can be ultimately used to more precisely define the molecular processes occurring not only during channel occlusion, but also under normal physiological conditions.

METHODS

Myxicola giant axons were dialyzed and voltage-clamped by methods that have been previously described (Bullock and Schauf, 1978). When potassium currents were measured, the internal solution was composed of 450 mM K^+ glutamate, 50 mM KF, 30 mM K_2HPO_4, and 1 mM Hepes (except for the Ba^{++} experiments; see below). For all the gallamine experiments the dialyzate contained 600 mM Cs^+ glutamate and 1 mM Hepes. The external solution was artificial seawater (ASW) containing 430 mM NaCl, 50 mM $MgCl_2$, 10 mM $CaCl_2$, and 20 mM Tris. Both external and internal solutions were adjusted to pH 7.3 ± 0.1. For experiments in high external $[K^+]$, the Na^+ concentration was reduced accordingly. In some experiments external $[Na^+]$ was reduced by equimolar substitution of Tris. Tetrodotoxin (10^{-6} M) was used when necessary to block Na^+ currents. Series resistance was routinely compensated and leakage and capacity currents eliminated by appropriate analog circuitry (Schauf and Bullock, 1980). Axons were recompensated after solvent substitution to allow for the lower equivalent conductivity of electrolyte solutions in D_2O (Swain and Evans, 1966). Temperature was 5.0 ± 0.5°C unless otherwise specified.

Deuterium oxide (99.8% D_2O) was obtained from Sigma Chemical Co., St. Louis, Mo. and redistilled before use. Ionic equilibria differ in D_2O because the self-ionization of D_2O is an order of magnitude smaller than for H_2O. As a consequence, pD does not equal pH, and pH readings for glass electrodes must be corrected via the expression $\mathrm{pD} = \mathrm{pH} + 0.41$ (Katz and Crespi, 1970). Thus, for example, an ASW solution in D_2O must show a pH of 6.9 to have a $\mathrm{pD} = 7.3$. It is also known that D_2O behaves as a stronger acid than H_2O, thus effectively raising the pK_a of titratable weak acids by ~0.5 pH unit (Covington and Jones, 1968). Fortunately, extensive data concerning the effect of pH changes on both the voltage-dependent ionic conductances and rate constants are already available in *Myxicola* (Schauf and Davis, 1976). Both the maximum sodium and potassium conductances ($\bar{g}_{Na}$ and $\bar{g}_K$) are reversibly depressed at low pH but the pK_a's are 4.8 and 4.4, respectively. The voltage dependence of g_{Na} and the time constants for Na^+ activation are also fairly insensitive to mild acidic conditions. In particular, at neutral pH and a membrane potential of 0 mV, a decrease of 0.5 pH unit causes no more than a 5% decrease in maximum sodium conductance, a shift of $g_{Na}(V)$ of 0.5 mV, and a shift in Na^+ kinetics of <2 mV. Such effects are negligible.

The effect of D_2O on drug occlusion of Na^+ channels was studied using gallamine triethiodide (Sigma Chemical Co.), a nondepolarizing neuromuscular blocking agent that, when applied internally, modifies Na^+ channels in a manner similar to pancuronium (Schauf and Smith, 1981). At concentrations of ≥0.1 mM, gallamine alters Na^+ channel kinetics and causes a striking increase in the magnitude of Na^+ tail currents after long duration pulses. However, at low concentrations such effects can take as long as an hour to reach a steady state similar to that seen after 5–10 min exposure to higher concentrations of gallamine. We feel this is primarily the result of the dialysis procedure. Although salt concentrations rapidly equilibrate (Bullock and Schauf, 1978), the dialysis tubing retards the movement of larger molecules such as gallamine (mol wt = 892). We found that dialysis with 5–10 mM gallamine produced an optimally rapid effect that would subsequently remain stable for the duration of our experiments.

Heavy water effects on K^+ channel occlusion were examined in tetrodotoxin (TTX)-treated axons, using both nonyltriethylammonium (C_9) and Ba^{++}. C_9 was synthesized for us by Polysciences Inc., Warrington, Pa., and was applied using the normal F^--containing dialyzate. Barium complexes with F^-, and thus in these studies only glutamate was used as the internal anion (Cl^- solutions could not be used because in *Myxicola* they produce a steady increase in leakage current and deterioration of both I_{Na} and I_K). Even so, Ba^{++} concentrations as high as those used by Armstrong and Taylor (1980) were required to demonstrate an effect. Either glutamate also complexes with Ba^{++} or the dialysis procedure failed to remove some internal constituent that buffers the divalent cation concentration. In any case, we do not know the true concentration of Ba^{++} near the interior of the cell membrane.

All experiments involving D_2O substitution were done with bracketing controls in H_2O, and the data are generally presented in the form of ratios of time constants in D_2O to the average of those determined during the bracketing H_2O runs. Data are given as means ± standard errors. In general, four to six axons were examined in each part of this study, with data being obtained at 5–10 different membrane potentials in each axon. Since the D_2O effects were not appreciably voltage dependent, ratios were usually averaged over all voltages. Thus the tests of significance referred to in the text generally had *n*'s of 20–60.

RESULTS

Gallamine-modified Na^+ Channels in H_2O

Gallamine triethiodide does not affect g_K in *Myxicola* and does not alter the Na^+ channel when applied externally, even at high concentrations. However, when added internally, the decline in I_{Na} during step depolarizations is altered, and as the membrane potential is made more positive, the majority of Na^+ channels fail to inactivate (complete details are to be found in Schauf and Smith, 1981). Fig. 1 *A* shows that for depolarizations to potentials more negative than −10 mV, the sole effect of gallamine is to slow inactivation. However, inactivation is still complete, and thus for long pulses (15 ms; not illustrated) no Na^+ tail currents are observed. At potentials more positive than −10 mV, the rate of Na^+ inactivation in the presence of gallamine is biphasic, with an initial decrease that is voltage dependent and faster than inactivation in untreated axons (we have defined this time constant as τ^s_{h1}), followed by a slower decline (time constant τ^s_{h2}). This later decline is not only slower than normal inactivation, but seems nearly independent of membrane potential, and has a time constant comparable to that measured for gallamine-modified channels at potentials below −10 mV. As the potential increases, the fraction of channels showing a rapid shut-off increases and simultaneously the magnitude of the Na^+ tail conductance following 15-ms pulses increases by a comparable amount (open triangles in Fig. 1 *B*). At large positive potentials ~70–80% of Na^+ channels fail to inactivate. The gallamine-induced Na^+ tail currents exhibit a pronounced "hook," and decline more slowly than the Na^+ tail currents which are measured in control axons after short pulses (Fig. 1 *B*; *inset*).

At potentials positive to E_{Na}, a third effect of gallamine is seen. Outward Na^+ currents are inhibited more strongly by gallamine than expected from the voltage dependence of τ^s_{h1}. When E_{Na} is changed by increasing internal $[Na^+]$, $\tau^s_{h1}(V)$ is invariant but peak outward currents are more strongly inhibited than inward currents. Channels that

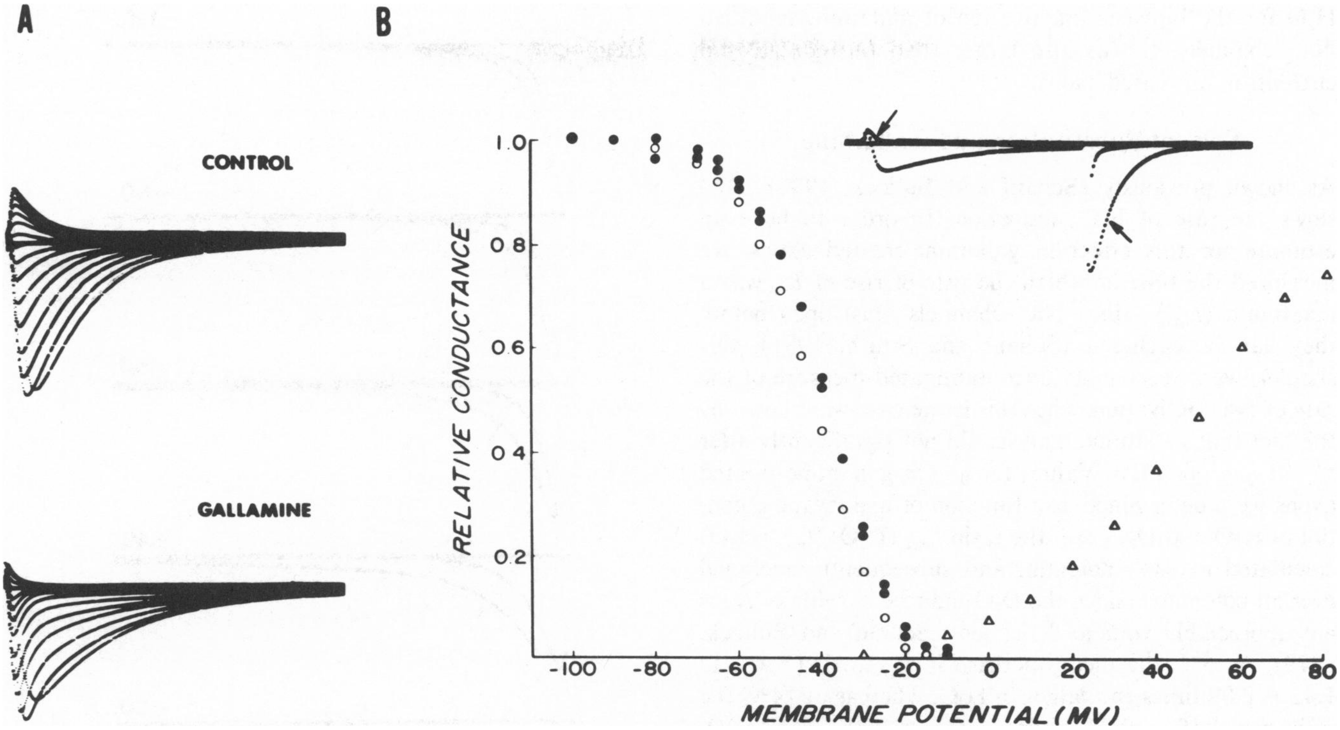

FIGURE 1 Summary of effects of internal gallamine on Na^+ currents in Cs^+-dialyzed *Myxicola* axons. Part *A* shows membrane currents for step depolarizations of 50–200 mV (10-mV increments) from a holding potential of −80 mV in a control and gallamine-dialyzed axon. Note the presence of three effects: for the two smallest pulses inactivation is slowed; for larger depolarizations inactivation is biphasic; for depolarizations more positive than E_{Na} outward currents are blocked. Calibrations are 0.75 mA/cm^2 and 2 ms. Temperature, 5°C. Part *B* is a composite illustrating the effects of gallamine on prepulse inactivation and on the degree to which Na^+ channels inactivate during maintained depolarizations. The filled and open circles in *B* were obtained before (●) and during (○) gallamine dialysis by varying the duration of a prepulse to the potential indicated on the abscissa and measuring the peak I_{Na} during a subsequent fixed pulse to +20 mV. The inset in the graph shows membrane Na^+ current in a gallamine-treated axon for 4-ms step depolarizations to −20 mV (unmarked) and +10 mV (arrows). Inactivation was not complete for the smaller pulse, and a small, fast tail is seen. The larger pulse would have resulted in complete inactivation in an untreated axon. Note the large, slow "gallamine tail" current following repolarization to −80 mV for the larger pulse. The open triangles plot the relative Na^+ conductance (compared with $\bar{g}_{Na}$) measured from the tail current immediately after a 16-ms depolarization to the potential indicated on the abscissa. Thus, they illustrate the fraction of Na^+ channels failing to inactivate.

have not become activated behave normally in the presence of gallamine as determined by measuring both steady-state prepulse inactivation (h_∞) curves (filled and open circles in Fig. 1 *B*), and the time constants for recovery from and development of inactivation at potentials more negative than −30 mV (not shown). Gallamine does not alter the time-course of Na^+ activation.

Thus gallamine has a dual effect on the Na^+ channel (Schauf and Smith, 1981). On the one hand it acts in a manner similar to pancuronium (Yeh and Narahashi, 1977), another neuromuscular blocking agent, by occluding Na^+ channels in a voltage-dependent fashion and preventing them from inactivating. On repolarization, the Na^+ channel cannot close until gallamine first dissociates, resulting in slow, hooked Na^+ tail currents. However, in contrast to pancuronium, gallamine and Na^+ are apparently able to compete for occupancy of a binding site. A second, possibly independent, effect of gallamine is to slow the rate of inactivation of nonoccluded Na^+ channels without preventing their ultimate inactivation.

Effects of Temperature on Gallamine-modified Channels

In gallamine-modified Na^+ channels at 5°C, τ^s_{h1} varies from 0.44 ms at 0 mV to 0.15 ms at +100 mV, whereas values for τ^s_{h2} scatter between 2.7 and 3.2 ms with no resolvable voltage dependence (Schauf and Smith, 1981). The time constant for gallamine tail currents varies from 0.2 ms at −120 mV to 2.0 ms at 0 mV (Schauf and Smith, 1981). For $V > -10$ mV, where inactivation during a maintained depolarization is biphasic and good double exponential fits can be obtained, we found in the present study that the Q_{10}'s in H_2O ranged from 2.2 to 2.6 for both τ^s_{h1} and τ^s_{h2}. At voltages below −10 mV, a comparable Q_{10} was seen for τ^s_{h2}. These are similar to the Q_{10} of 2.56 ± 0.02 previously determined for inactivation during a maintained depolarization in untreated *Myxicola* axons (Schauf, 1973). In contrast, the average Q_{10} for gallamine tail currents in H_2O was 3.62 ± 0.48 (range 2.65–4.8). This was significantly larger ($P < 0.002$) than the Q_{10}'s in

H_2O for the biphasic inactivation of gallamine-modified Na^+ channels. It was also larger than the Q_{10} for tail currents in untreated axons.

Solvent Substitution and Gallamine

As shown previously (Schauf and Bullock, 1980), D_2O slows the rate of Na^+ activation. In order to have an estimate for this effect in gallamine-treated axons, we measured the time at which the rate of rise of I_{Na} was a maximum (t_{max}). Since Na^+ channels must open before they can be occluded (Schauf and Smith, 1981), this should give a reasonably uncontaminated measure of the rate of Na^+ activation. That this is the case was shown by the fact that gallamine dialysis did not significantly alter t_{max} at any potential. Values for t_{max} in gallamine-treated axons were determined as a function of membrane potential in H_2O and D_2O, and the ratio $t_{max}(D_2O)/t_{max}(H_2O)$ calculated at each potential and subsequently averaged over all potentials (since the D_2O-induced slowing of I_{Na} is not appreciably voltage dependent; Schauf and Bullock, 1979). At 5°C the mean increase in t_{max} in D_2O was to 1.42 ± 0.09 times the values in H_2O, whereas at 14°C the ratio was 1.10 ± 0.07, giving a Q_{10} of 0.75 (Table IV). These values are comparable to those previously obtained in untreated axons (Schauf and Bullock, 1980).

The effects of D_2O on the decline of I_{Na} during step depolarizations in gallamine-treated axons were then examined at 5 and at 13–14°C. For each of six axons, the decline in I_{Na} at voltages between -30 and $+50$ mV was fit by the sum of two exponentials both in D_2O and for the bracketing H_2O runs. At each voltage the time constants in D_2O were divided by the average of those obtained in H_2O. As in normal axons, there was no significant voltage dependence of the D_2O effects, and thus the time constant ratios were averaged over all membrane potentials examined. The rapid, voltage-dependent occlusion of Na^+ channels for $V > -10$ mV was slowed by D_2O substitution with an average $\tau^s_{h1}(D_2O)/\tau^s_{h1}(H_2O)$ ratio (Table IV) of 1.42 ± 0.15 at 5°C and 0.94 ± 0.43 at 13°C, giving a Q_{10} of 0.60. The voltage-independent, slow decline in I_{Na} that is seen both immediately after the rapid gallamine occlusion at $V > -10$ mV, and in isolation for $V < -10$ mV, was also slowed by D_2O. However, the ratio of $\tau^s_{h2}(D_2O)/\tau^s_{h2}(H_2O)$ averaged 2.00 ± 0.20 at 5°C and 1.88 ± 0.09 at 13°C (Table IV). Although the magnitude of the D_2O effect on τ^s_{h2} was larger at 5°C, its temperature dependence was lower ($Q_{10} = 0.92$). These differences between the D_2O effects on τ^s_{h1} and τ^s_{h2}, as well as between the results on normal and gallamine-modified channels, were all statistically significant ($P < 0.005$).

Gallamine-induced Na^+ tail currents following long pulses are also significantly slowed by D_2O substitution at all voltages (Fig. 2). The average ratio of tail time constants in gallamine-treated axons in D_2O compared with H_2O was 1.69 ± 0.08 at 5°C and 1.32 ± 0.06 at 13.5°C, giving a Q_{10} of 0.75 (Table IV). Again, there was no dependence of these ratios on membrane potential. This is a particularly interesting result because, as we show in the next section, Na^+ tail currents in untreated axons are not sensitive to D_2O substitution.

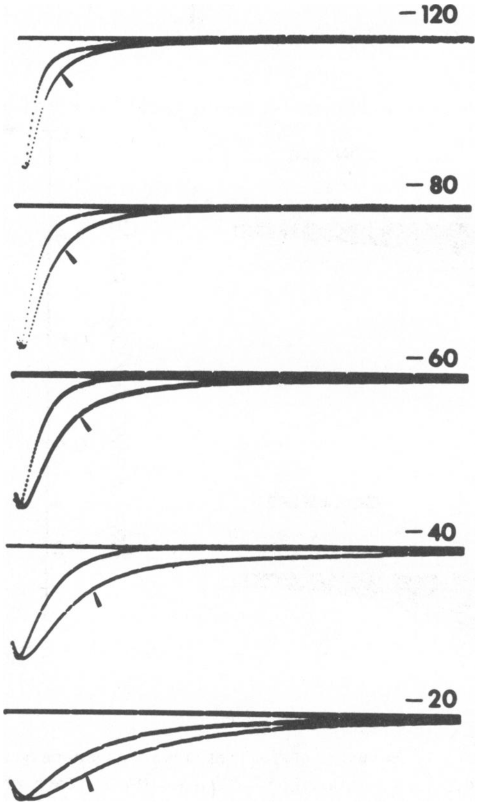

FIGURE 2 Tail currents in a gallamine-dialyzed axon after 16-ms depolarizations in H_2O and D_2O. The repolarization potential is given at the end of each record. The slower currents (arrows) were obtained in D_2O. Calibrations are 1.0 mA/cm² and 1 ms. Temperature, 5°C.

At $V > -10$ mV, gallamine causes a substantial fraction of Na^+ channels to fail to inactivate (Schauf and Smith, 1981). However, substitution of D_2O had no effect on the fraction of channels that remained conducting in the steady state at any membrane potential.

D_2O Insensitivity of Na^+ Tails in Untreated Axons

Fig. 3 shows sodium currents in a normal axon in H_2O and D_2O for a depolarizing pulse short enough so that repolarization occurred when g_{Na} was large. Since D_2O decreases maximum sodium conductance by ~25% (Schauf and Bullock, 1980) the currents in D_2O were scaled for comparison. In these experiments $[Na^+]$ was reduced to 86 mM (20% of normal) to eliminate possible artifacts from residual uncompensated series resistance (Schauf et al., 1977). The duration of the depolarizing pulse was made longer in D_2O because the rates of activation and inactivation are slowed, and we wished to record tail currents in both cases at comparable times after the

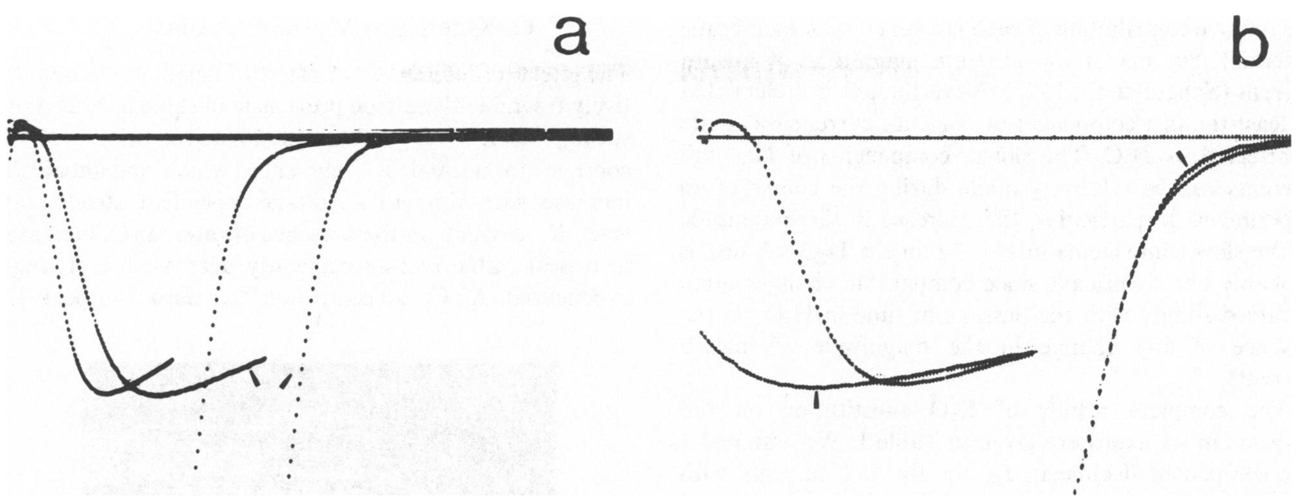

FIGURE 3 The effects of solvent substitution on sodium repolarization (tail) currents in *Myxicola*. The capacitative transient and linear leak current were removed by adding the response to an exactly matched hyperpolarizing pulse. In part *a*, currents are recorded in H_2O and D_2O during a depolarization to 0 mV, with the latter (indicated by the arrows) scaled so that the maximum inward currents appear equal. In part *b*, the D_2O record (arrows) has been shifted to the left so that the repolarization times coincide, and the time scale is expanded to aid visual comparison. The horizontal lines represent zero current. The vertical scale is 0.075 mA/cm^2. The time scale is 0.5 ms in part a and 0.25 ms in part b. The temperature was 5°C, and the holding potential was −80 mV.

occurrence of maximum inward current. In Fig. 3 the time to peak inward current was increased from 0.60 to 0.91 ms, and the time constant for inactivation of conducting channels (τ_h^s) was increased from 1.50 to 2.40 ms. In contrast, solvent substitution produced little or no change in the rate at which open channels closed after repolarization.

Sodium tail currents in *Myxicola* cannot be described by a single exponential (Schauf et al., 1977; Goldman and Hahin, 1978). Both fast and slow components exist, and

TABLE I
EFFECTS OF D_2O ON REPOLARIZATION CURRENTS IN MYXICOLA*

V_H	$\tau^f_{H_2O}$	$\tau^s_{H_2O}$	I^s_0/I^f_0 [H_2O]	$\tau^f_{D_2O}$	$\tau^s_{D_2O}$	I^s_0/I^f_0 [D_2O]	$t_{pk}^{D_2O}/t_{pk}^{H_2O}$
mV	*μs*	*μs*		*μs*	*μs*		
−80(2°C)	195	—	—	195(1.0)	—	—	1.69
−80(2°C)	115	1,110	0.03	107(0.93)	1,020(0.92)	0.08	1.52
−80(2°C)	118	850	0.03	125(1.06)	720(0.85)	0.08	1.30
−80(2°C)	137	820	0.17	155(1.13)	560(0.68)	0.08	1.41
−80(5°C)	90	370	0.17	92(1.08)	330(0.97)	0.15	1.55
	80	310	0.18				
−100(5°C)	82	340	0.16	86(1.13)	320(1.08)	0.14	1.50
	71	250	0.17				
−60(5°C)	120	380	0.28	97(0.92)	320(0.89)	0.23	1.66
	90	340	0.15				
−80(5°C)	104	630	0.10	90(0.92)	450(0.69)	0.10	1.36
	92	680	0.05				
−100(5°C)	84	650	0.09	87(0.98)	550(0.96)	0.08	1.48
	94	500	0.09				
−60(5°C)	122	700	0.13	119(1.07)	490(0.62)	0.11	1.52
	100	890	0.03				

*The holding potential is given in column 1. The numbers in parentheses in columns 5 and 6 are the ratios of the time constants in D_2O to those in H_2O as calculated from the data in columns 2, 3, 5, and 6. In the first four experiments there were no bracketing data in H_2O, whereas in the remainder the bracketing H_2O data were used to calculate the D_2O effect. Data from six axons is included. Columns 4 and 7 contain values for the relative coefficients (expressed as a ratio) of the slow and fast components in H_2O and D_2O, respectively. The last column gives the ratios of time to peak inward current in D_2O compared with H_2O and is a measure of the basic solvent effect.

A two-exponential fit to the data for 2 ms following repolarization was used because three exponentials gave no better fit, and because the lability of the slow components was sufficient to render any more detailed analysis suspect.

the relative contribution of each is a function of membrane potential, but not of the absolute magnitude of inward current (Schauf et al., 1977). Nevertheless, it is clear that at least the fast component of Na^+ tail current was quite unaffected by D_2O. The slower components of Na^+ tail currents can be relatively labile during the course of an experiment. In particular, the decrease in the magnitude of the slow components in Fig. 3 *b* in the D_2O solution is probably not significant since comparable changes often occurred simply with the passage of time in H_2O (in the absence of any change in the magnitude of inward current).

The complete results of D_2O substitution on tail currents in six axons are given in Table I. We assumed a two-component decline in I_{Na} for the tail currents with time constants τ^f and τ^s. The value of Na^+ tail currents extrapolated to $t = 0$ for the fast and slow components are termed I_0^f and I_0^s. The ratio of time to peak I_{Na} in D_2O to that in H_2O averaged over all voltages was 1.50 ± 0.04 at 5°C, whereas the ratio of the time constants for the fast component of the Na^+ tail current was 1.01 ± 0.03. As can be seen from the tabulated data, there was some tendency for the slow component to become faster as the experiment progressed. If anything, D_2O accelerated the slow component(s).

It should, of course, be noted that the isotope effects on Na^+ activation and deactivation are measured at very different membrane potentials. Unfortunately, there is little choice since measurements of activation require depolarizations to potentials of at least -35 mV, but in this range the repolarization currents are in part governed by the kinetics of inactivation (Schauf et al., 1977; Goldman and Hahin, 1978). Our previous data concerning the effects of isotope substitution on Na^+ activation kinetics suggest that the D_2O effect may increase, rather than decrease, very slightly as the membrane potential becomes more negative over the range $+60$ to -60 mV (Schauf and Bullock, 1979). Furthermore, the voltage dependence of the steady-state conductance was not detectibly altered by D_2O over the same voltage range. Thus, a voltage-dependent D_2O effect that was smaller at -80 mV does not seem a likely explanation for the insensitivity of repolarization currents to solvent substitution.

The measurement of Na^+ repolarization currents is, of course, relatively difficult. Tail currents are sensitive to series resistance compensation and the presence of any spatial nonuniformity. We minimized such problems by the use of low sodium solutions and by verifying that the kinetics did not change when the absolute magnitude of membrane current is varied (Schauf et al., 1977). Still, some uncertainty remains. However, these considerations are not relevant here, since the experimental observation is that D_2O was without a measurable effect on repolarization currents, even though activation was significantly delayed.

C_9 Kinetics in Myxicola Axons

The effects of internal C_9 in *Myxicola* giant axons qualitatively resemble those seen previously in squid axons (Armstrong, 1969, 1971) and are summarized in Fig. 4. In contrast to normal K^+ currents, which monotonically increase with time to a voltage-dependent steady-state level, K^+ currents in the presence of internal C_9 increased to a peak value and subsequently decreased as a single exponential. As C_9 concentration increased, the peak K^+

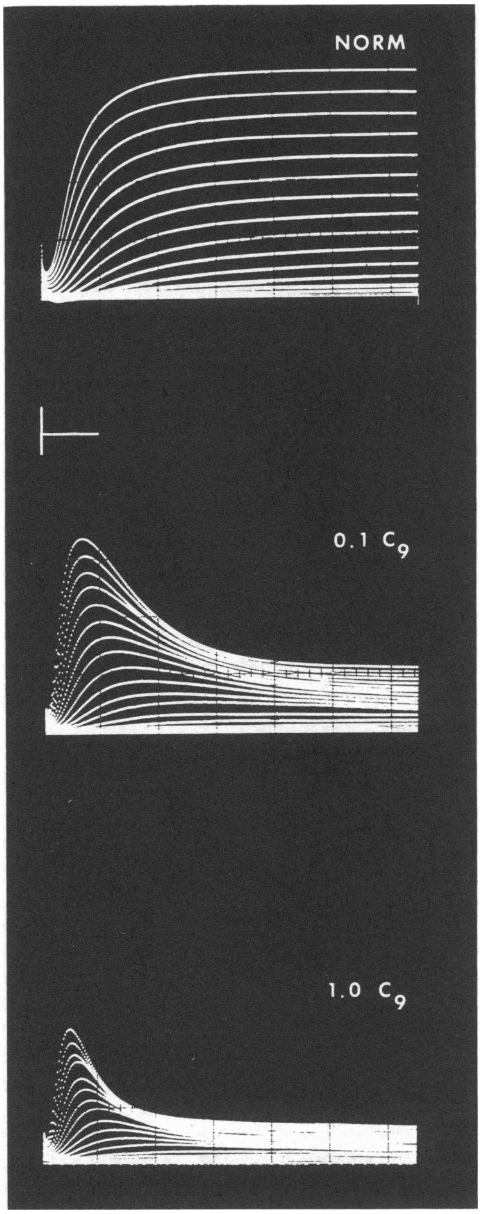

FIGURE 4 Effects of C_9 on K^+ currents in *Myxicola*. Membrane currents are shown for step depolarizations of 30–180 mV (10-mV increments; holding potential, -80 mV) in an axon bathed in K^+-free ASW plus 10^{-6} M TTX. Top to bottom are control records, and records with 0.1 mM C_9 and 1.0 mM C_9 dialysis. Calibrations are 3 ms and 0.75 mA/cm^2. Temperature, 5°C.

currents decreased, and inactivation was accelerated and became more complete. However, C_9 did not affect the rate of activation of K^+ channels. In K^+-free ASW the fraction of K^+ current blocked at equilibrium $[f(V)]$ increased with increasing C_9 concentrations and averaged 0.70 ± 0.02 with 0.1 mM C_9. However, $f(V)$ was not voltage dependent over the range of -10 to $+100$ mV where reliable measurements could be obtained. Values for the time constant for the decline in I_K to its equilibrium value $[\tau_{C_9}]$ averaged 17.4 ± 1.6 ms at 0 mV and 6.9 ± 0.7 ms at $+100$ mV (Table II). There was little or no effect of external K^+ concentration (0–200 mM) on the rate or steady-state level of C_9 block in *Myxicola*.

In experiments in which a small conditioning depolarization of variable duration precedes a fixed test pulse to $+160$ mV, the maximum current during the test step decreased exponentially with increasing pulse duration after an initial delay (cf. Armstrong, 1969). This allowed us to define the fraction of g_K that is inactivated at potentials more negative than 0 mV in a way analogous to the definition of prepulse inactivation of the sodium conductance. Values for what we will call τ_{C9}^{p} averaged 45 ms at -40 mV, 34 ms at -20 mV, 17 ms at 0 mV, and 13.5 ms at $+20$ mV. These time constants were comparable to the values of τ_{C_9} measured during maintained depolarizations at the same potentials. A similar protocol in which a pulse to $+160$ mV (lasting long enough to allow complete K^+ inactivation) was followed by a return to the holding potential of -80 mV, then after a variable interval by a fixed test pulse, allowed calculation of the recovery time constant (τ_{C9}^{re}). This averaged 180 ms at -80 mV.

Temperature and C_9 Kinetics

Because they have not been previously reported in detail, the effects of temperature on C_9 kinetics have been provided along with the solvent substitution data in Table II. With increasing temperature τ_{C_9} decreased with a Q_{10} of 3.02 ± 0.10 (compare column 2 with column 5) in axons in which K^+ activation itself had a Q_{10} of 2.40 ± 0.08. Thus, the temperature dependence of C_9 block was greater than the temperature dependence of $g_K(t)$. The time constant for recovery from C_9 inactivation had a Q_{10} of 1.70 (not shown), significantly less than the foregoing. Steady-state inhibition of g_k decreased substantially at higher temperatures (Fig. 5), with the average steady-state K^+ current being 30% of maximum at 5°C, and 55% of maximum at 14°C. This implies a larger activation energy for inactivation by C_9 than for recovery.

Solvent Substitution and C_9 Kinetics

The effects of D_2O substitution on a C_9-treated axon are shown in Fig. 5. At 5°C, D_2O decreased the rate of K^+ inactivation at all voltages. At 14°C, however, D_2O had no appreciable effect on τ_{C_9}. At neither temperature did D_2O substitution change the steady-state level of C_9 block. These results are presented quantitatively in Table II for three axons at two different temperatures. Only kinetic data are given since, as we noted, $f(V)$ was unchanged by D_2O. At 5°C, τ_{C_9} is increased by D_2O, and the degree of slowing was independent of membrane potential. On the average (including data at all voltages) τ_{C_9} was increased by 74 ± 7%. At 14°C, the D_2O induced slowing averaged 14 ± 5% in all axons, yielding a Q_{10} of 0.61 for the solvent substitution effect.

The time constant for the decrease in peak potassium current as a function of prepulse duration ($\tau_{C_9}^{p}$) was also examined in D_2O (three axons; data not shown). D_2O substitution increased $\tau_{C_9}^{p}$ by 120 ± 17% at 5°C, a significantly greater effect than seen for maintained depolarizations. In a single axon the time constant of recovery from C_9 block was increased by 64% at 5°C and was unaffected by D_2O at 14°C.

In the C_9-treated axons, $I_K(t)$ records were differentiated to determine the time at which $dI_K(t)/dt$ was maximum, and thus provide a measurement of the rate of K^+ activation. In these axons D_2O slowed K^+ activation by 46 ± 5% (comparable to that seen previously; Schauf and Bullock, 1980), which is significantly less than the D_2O

TABLE II
EFFECTS OF SOLVENT SUBSTITUTION ON C_9 KINETICS*

V_M	$\tau_{C_9}^{H_2O}$	$\tau_{C_9}^{D_2O}$	D_2O/H_2O ratio	$\tau_{C_9}^{H_2O}$	$\tau_{C_9}^{D_2O}$	D_2O/H_2O ratio
mV	*5°C*	*5°C*		*14°C*	*14°C*	
0	17.4 ± 1.6	30.0 ± 1.9	1.72 ± 0.18	6.2 ± 0.4	7.1 ± 0.5	1.14 ± 0.08
20	14.0 ± 0.09	24.5 ± 2.4	1.75 ± 0.14	5.0 ± 0.6	5.0 ± 0.4	0.98 ± 0.08
40	11.5 ± 1.0	20.0 ± 2.1	1.74 ± 0.11	3.9 ± 0.5	4.1 ± 0.7	1.04 ± 0.08
60	9.9 ± 0.8	17.0 ± 0.9	1.72 ± 0.17	3.4 ± 0.3	3.8 ± 0.5	1.12 ± 0.07
80	7.9 ± 0.08	13.4 ± 1.4	1.70 ± 0.14	2.7 ± 0.4	3.0 ± 0.4	1.10 ± 0.07
100	6.9 ± 0.7	11.7 ± 1.1	1.70 ± 0.21	2.6 ± 0.4	2.7 ± 0.3	1.05 ± 0.08

*Only data for τ_{C9} are shown because D_2O did not change $f(V)$. These data are averages from three axons in which τ_{C9} was determined at both 5 and 14°C. The overall averages referred to in the text include not only these data but also data at other voltages and from axons examined at only a single temperature.

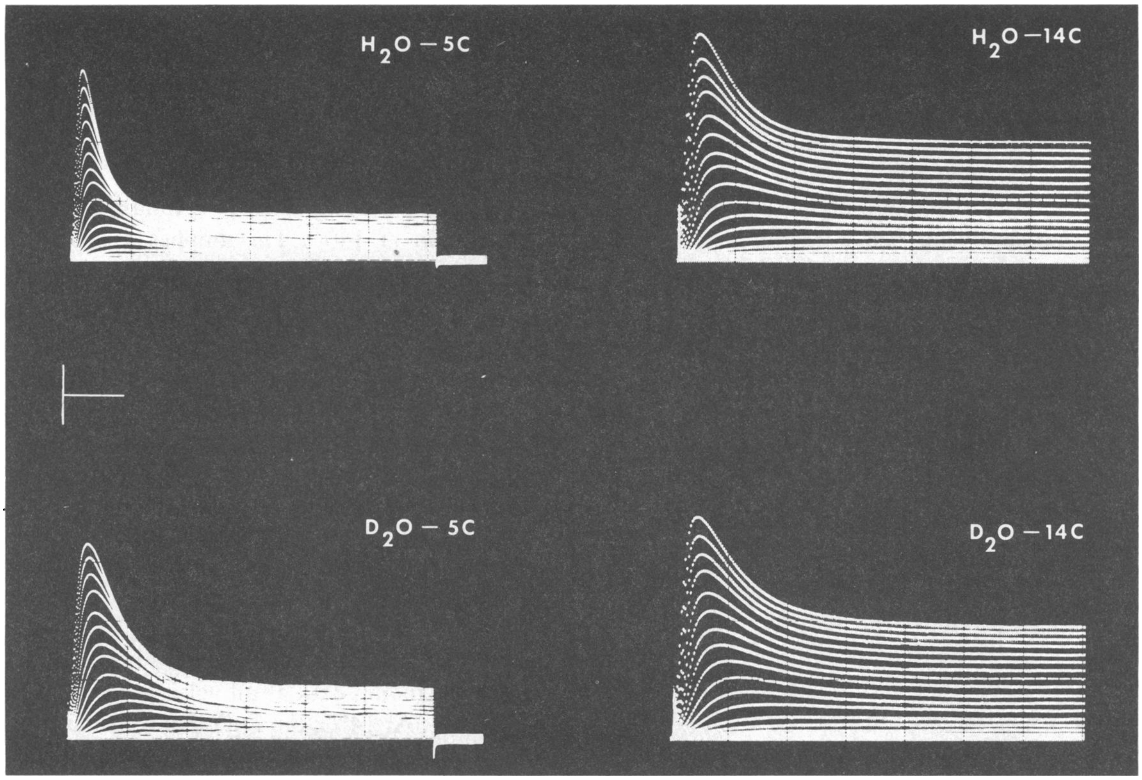

FIGURE 5 Effects of solvent substitution on C_9 kinetics (0.1 mM). Membrane currents are shown for step depolarizations of 30–180 mV (10-mV increments; holding potential, −80 mV) at 5°C (left-hand records) and 14°C (right-hand records in H_2O (upper records) and D_2O (lower records). Calibration is 10 ms for the 5°C records, 2.5 ms for the 14°C records.

effect on τ_{C_9} ($P < 0.01$). The Q_{10} for D_2O slowing of K^+ activation was 0.75, compared with the Q_{10} of 0.61 for τ_{C_9} ($P < 0.007$).

Ba^{++} Kinetics in Myxicola

Fig. 6 illustrates the interaction of internal Ba^{++} with K^+ channels in *Myxicola* axons. In contrast to C_9-modified axons, for voltages more negative than +20 mV, little effect of Ba^{++} was seen in 50 or 100 mM external K^+. However, for $V > +20$ mV, K^+ currents inactivated in the presence of Ba^{++}, and furthermore, unlike C_9, the steady-state level of K^+ inactivation was strongly voltage dependent. In fact, the steady-state level of Ba^{++} block increased sufficiently rapidly with voltage that the current-voltage relation exhibited a negative slope conductance. The Ba^{++} block was antagonized by increasing external $[K^+]$. In K^+-free solutions the steady-state $I(V)$ curve was decreased at most voltages and peaked at +25 mV, whereas at $[K^+]_o = 100$ mM there was no change until $V \geq 35$ mV and the peak was at +45 mV. The rate of decline of I_K in Ba^{++}-treated axons also was decreased with increasing $[K_o^+]$. These effects qualitatively resemble those reported previously in squid axons (Armstrong and Taylor, 1980; Eaton and Brodwick, 1980). The results of a more quantitative analysis of such data are given in Table III along with the solvent substitution data for several different membrane potentials. Internal Ba^{++} only slightly delays K^+ activation. As in the C_9 experiments, the time of maximum $dI_K(t)/dt$ was determined after Ba^{++} dialysis as a function of membrane potential for untreated axons. In general, a 10% slowing was observed in the preence of Ba^{++}. At +100 mV the respective values were 0.86 ± 0.05 and 0.99 ± 0.08 ms. These values were unaffected by

TABLE III
EFFECTS OF SOLVENT SUBSTITUTION ON Ba^{++} KINETICS IN MYXICOLA

V_M	$F(V)$*	$\tau_{Ba}^{H_2O}$	$\tau_{Ba}^{D_2O}$	Ratio‡
mV		*ms*	*ms*	
Experiments with $[K^+]_0 = 0$				
+20	0.47 ± 0.05	25.2 ± 2.1	37.2 ± 4.4	1.48
+40	0.65 ± 0.04	18.0 ± 1.1	28.0 ± 3.6	1.56
+60	0.77 ± 0.03	15.5 ± 0.9	26.6 ± 3.5	1.72
+80	0.82 ± 0.03	12.2 ± 1.1	18.9 ± 2.5	1.55
+100	0.90 ± 0.02	10.1 ± 0.4	16.0 ± 2.9	1.58
Experiments with $[K^+]_0$ = f215mM				
+20	<0.02	NR	NR	—
+40	0.02 ± 0.02	NR	NR	—
+60	0.20 ± 0.05	33.0 ± 4.7	37.0 ± 4.9	1.12
+80	0.47 ± 0.06	23.0 ± 5.6	24.0 ± 4.4	1.04
+100	0.65 ± 0.07	16.5 ± 4.4	19.7 ± 3.9	1.19

*Values of $f(V)$ in H_2O are given. Those in D_2O were not significantly different.

‡Ratio of τ_{Ba} in D_2O to that in H_2O.

NR, not resolvable.

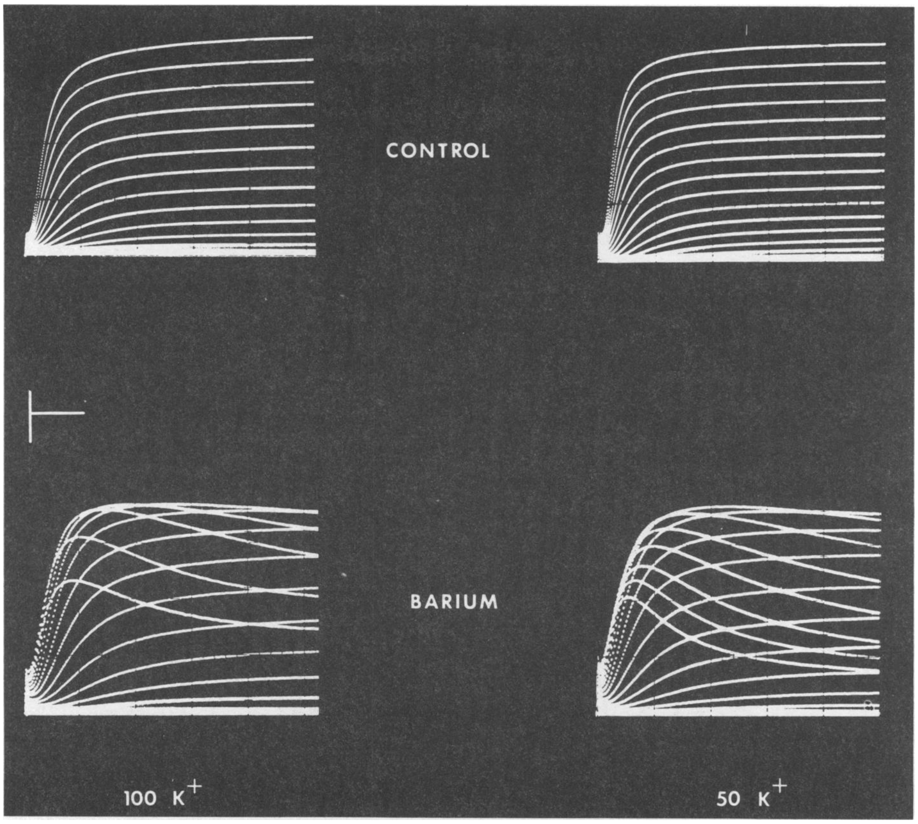

FIGURE 6 Effects of barium on K^+ currents in *Myxicola*. Membrane currents are shown for depolarizations of 30–180 mV (10-mV increments; holding potential, −80 mV) before and during dialysis with 5 mM Ba^{++}, and for two different external K^+ concentrations. Calibrations are 0.75 mA/cm^2 (upper records) or 0.4 mA/cm^2 (lower Ba^{++} records) and 3 ms. Temperature, 5°C.

$[K_o^+]$. As noted for squid axons (Armstrong and Taylor, 1980), recovery from Ba^{++} block was very slow. Approximately 1 min was needed for full recovery between pulses in K_o^+-free ASW.

Solvent Substitution and Ba^{++} Kinetics

In axons bathed in K^+-free ASW, substitution of D_2O slowed the rate of K^+ inactivation in the presence of internal Ba^{++} (Table III). The ratio of $\tau_{Ba}(D_2O)/\tau_{Ba}(H_2O)$ was voltage independent in K^+-free ASW and averaged 1.56 ± 0.14 at 5°C. The isotope effect on K^+ activation in the same axons was determined by measuring the time of maximum $dI_K(t)/dt$ and the ratio $t_{max}(D_2O)/t_{max}(H_2O)$ averaged 1.51 ± 0.13.

At higher $[K_o^+]$ the behavior was very different, however. Although the ratio $t_{max}(D_2O)/t_{max}(H_2O)$ describing the isotope effect on K^+ activation was unchanged, averaging 1.56 ± 0.14 in 215 mM K_o^+, the ratio $\tau_{Ba}(D_2O)/\tau_{Ba}(H_2O)$ was only 1.12 ± 0.08. Thus, increased external K^+ seems to antagonize not only the rate and degree of Ba^{++} block, but also the magnitude of the isotope effect itself. Data at higher temperatures unfortunately were not available.

DISCUSSION

The overall results are summarized in Table IV. The effects of solvent substitution on various parameters

TABLE IV
SUMMARY OF ISOTOPE EFFECTS ON MYXICOLA

Parameter	D_2O/H_2O ratio at 5°C	D_2O/H_2O ratio at 13–14°C	Q_{10}
Untreated axons			
Na^+ Activation	1.47 ± 0.02	1.18 ± 0.03	0.72
τ_h^s	1.52 ± 0.03	1.37 ± 0.03	0.84
τ_h^p	2.61 ± 0.21	1.43 ± 0.05	0.47
Na^+ tails	No effect	No effect	—
Gating current	No effect	No effect	—
K^+ activation	1.40 ± 0.03	1.17 ± 0.02	0.79
Gallamine-treated axons			
Na^+ activation	1.42 ± 0.09	1.10 ± 0.07	0.75
$\tau_{h_1}^h$	1.42 ± 0.15	0.94 ± 0.43	0.60
$\tau_{h_2}^s$	2.00 ± 0.20	1.88 ± 0.09	0.92
Na^+ tails	1.69 ± 0.08	1.32 ± 0.06	0.75
C_9-treated axons			
K^+ activation	1.46 ± 0.05	1.16 ± 0.08	0.75
τ_{C9}	1.74 ± 0.07	1.14 ± 0.05	0.61
τ_{C9}^p	2.20 ± 0.17	—	—
$f(V)$	No effect	No effect	—
Ba^{++}-treated axons			
K^+ activation	1.51 ± 0.13	—	—
$\tau_{Ba}(0\ K^+)$	1.56 ± 0.14	—	—
$\tau_{Ba}(215\ K^+)$	1.12 ± 0.08	—	—
$f(V)$	No effect	—	—

describing channel gating are given at a temperature of 5°C along with the Q_{10} derived from the temperature dependence of the isotope effects. This is done both for untreated axons (data taken from Schauf and Bullock, 1980, 1981), and axons in which gating has been modified by the agents examined in this study. It is noteworthy that measurements of solvent effects on Na^+ and K^+ activation were remarkably consistent over the course of this and prior studies.

The framework for interpretation of isotope effects is detailed elsewhere (Schauf and Bullock, 1979). Briefly, hydrogen atoms of membrane constituents may in the first instance exchange with solvent deuterium and change the rate or equilibrium constants for reactions involving bonds to the substituted atoms or adjacent bonds (primary and secondary isotope effects). The ratio of the rate constant of an unsubstituted system (K) to that of the deuterium-substituted system (K^*) is generally given by: $K/K^* = I \exp(\Delta E_0/RT)$, where ΔE_0 is the isotopically induced difference in the separation of the zero-point energies of products and reactants and I is a term (between 1 and 2) involving the ratio of the masses and moments of inertia of both systems (Melander, 1960; Laidler, 1969; Thornton and Thornton, 1970). The general characteristic of these effects is that an appreciable temperature dependence is only evident if the kinetic effects themselves are very large. For example, a K/K^* ratio of 10 (typical of a deuterium-hydrogen exchange in a strong covalent bond) gives a Q_{10} of ~0.9.

However, important effects are also associated with the behavior of liquid H_2O and D_2O as solvents. The viscosity, melting point, and heat capacity are all significantly higher in D_2O as a result of more extensive intermolecular hydrogen bonding. It is significant that solvent structure breaks down more rapidly with temperature in D_2O than in H_2O (Heppolette and Robertson, 1960). The temperature dependence of solvent-dependent properties is experimentally observed to be much greater than that predicted from the K/K^* ratio assuming a hydrogen-deuterium exchange. At 5°C D_2O produces an ~50% slowing of the ionic current kinetics in normal *Myxicola* axons, but the effects disappear with increasing temperature with Q_{10}'s of 0.6–0.8 (Schauf and Bullock, 1980). A primary or secondary isotope effect with this temperature dependence should yield a K/K^* ratio of the order of 1,000. Thus we have suggested that changes in structural properties of the solvent may be the most important factor determining the magnitude of these kinetic effects.

The two phases of Na^+ inactivation in the presence of gallamine, probably representing a voltage-dependent occlusion combined with some other alteration in channel kinetics, are affected very differently by D_2O. The fast decline (τ^s_{h1}) is moderately slowed by D_2O at 5°C, but this effect disappears almost completely at 14°C ($Q_{10} = 0.6$), which suggests that changes in solvent structure may be most important for occlusion. The slower phase of Na^+ inactivation (τ^s_{h2}) is more strongly affected by D_2O at 5°C, and this marked slowing persists at high temperatures. The temperature dependence of the D_2O effect on τ^s_{h2} could therefore be explained by a primary or secondary isotope effect on membrane macromolecules in which solvent interaction plays no significant role.

Tail currents in gallamine-treated axons are slowed by D_2O in a way consistent with a moderate solvent effect (Q_{10} of 0.75). In contrast, Na^+ tail currents in untreated axons are insensitive to solvent substitution, despite the fact that Na^+ activation and inactivation are markedly slowed by D_2O. Thus, although the creation of an open channel normally involves appreciable changes in solvent order, deactivation is solvent independent. This differential effect may well be an important clue to understanding the underlying mechanisms of channel gating. At a minimum, the rate constant(s) for the transition from closed to open channels need to be D_2O sensitive, whereas those for the transition from open to closed channels need not be. This would be possible if only the last of a sequence of steps were reversed, and this particular step was solvent insensitive. However, such an interpretation is complicated by the fact that D_2O slows activation of Na^+ channels with no effect on the intramembrane charge movement which is presumably associated with transitions among preceding closed states (Schauf and Bullock, 1979, 1981). Perhaps the process by which a channel closes upon repolarization is not simply the reverse of that by which it opened.

Nonyltriethylammonium produces K^+ inactivation in *Myxicola* axons in much the same fashion as in squid axons and frog node (Armstrong, 1969, 1971; Armstrong and Hille, 1972), except that the voltage dependence of τ_{C_9} in *Myxicola* is only about half as large. Inactivation by C_9 seems to require opening of the K^+ channel, and recovery is delayed by hyperpolarization. Interestingly, recovery from C_9 block at −80 mV seems to have a lower activation energy than the initial binding step. Substitution of D_2O has the same sort of effect on C_9 block during step depolarizations as it does on the rapid phase of Na^+ inactivation in gallamine-treated axons. The increase in τ_{C_9} produced by D_2O at 5°C is moderate and disappears almost completely with increasing temperature, which suggests a strong solvent interaction. It is well established that in contrast to kinetic effects, under equilibrium conditions free energy differences are less sensitive to solvation because of the high degree of entropy-enthalpy compensation present in such systems. This is particularly true of hydrophobic bond strengths, which differ very little in H_2O and D_2O (Kresheck et al., 1973; BenNaim et al., 1973; Oakenfull and Fenwick, 1975). Such an insensitivity was observed when D_2O effects on the steady-state properties of the Na^+ channel were examined (Schauf and Bullock, 1979), and thus it is not surprising that the steady-state binding of C_9 was also D_2O insensitive.

Armstrong (1969) has suggested that the tetraalkylammonium (TAA) binding site contains a hydrophobic component because of the alkyl chain length dependence of the binding constant. In the present study C_9 binding was found to decrease with increasing temperature, corresponding to an enthalpy change of approximately -8 kcal/mol. Hydrophobic bonding contributes positive terms to the enthalpy, so if present it is evidently overwhelmed by other forces. According to the studies of Kirshnan and Friedman (1969), the structural contribution to the enthalpy of desolvation of alkyl groups ranges from $+2.5$ kcal/mol for ethyl to $+8.5$ kcal/mol for amyl. Thus if the hydrophobic and negative contributions are simply additive, the temperature dependence of TAA derivatives of differing chain lengths may be quite different. Such experiments may be useful in further characterizing the TAA binding site.

Barium effects in *Myxicola* again differ from those seen in squid axons only in the magnitude of the voltage dependence of τ_{Ba}. Nevertheless, the voltage dependence of the Ba^{++} effect was sufficient to produce the same sort of crossover of K^+ currents with increasing steady-state block seen with increasing depolarizations in squid axons. Solvent substitution slows the rate of Ba^{++} inactivation at 5°C, but we were unable to obtain data on temperature dependence. The effect of Ba^{++} is decreased by external potassium, and it is interesting that the D_2O effect is also antagonized. Perhaps more than a simple competition for binding sites is involved.

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REFERENCES

Adelman, W. J., and R. J. French. 1978. Blocking of the squid axon potassium channel by external cesium ions. *J. Physiol. (Lond.).* 276:13–25.

Armstrong, C. M. 1966. Time course of TEA⁺-induced anomalous rectification in squid giant axon. *J. Gen. Physiol.* 50:491–503.

Armstrong, C. M. 1969. Inactivation of the potassium conductance and related phenomena caused by quaternary ammonium ion injection in squid axons. *J. Gen. Physiol.* 54:553–574.

Armstrong, C. M. 1971. Interaction of tetraethylammonium ion derivatives with the potassium channels of giant axons. *J. Gen. Physiol.* 58:413–437.

Armstrong, C. M., and F. Bezanilla. 1977. Inactivation of the sodium channel. II. Gating current experiments. *J. Gen. Physiol.* 70:567–590.

Armstrong, C. M., and W. F. Gilly. 1979. Fast and slow steps in the activation of sodium channels. *J. Gen. Physiol.* 74:691–711.

Armstrong, C. M., and B. Hille. 1972. The inner quaternary ammonium ion receptor in potassium channels of the node of Ranvier. *J. Gen. Physiol.* 59:388–400.

Armstrong, C. M., and S. R. Taylor. 1980. Interaction of barium ions with potassium channels in squid giant axons. *Biophys. J.* 30:473–488.

Ben-Naim, A., J. Wilf and M. Yaacobi. 1973. Hydrophobic interaction in light and heavy water. *J. Phys. Chem.* 77:95–102.

Bezanilla, F., and C. M. Armstrong. 1972. Negative conductance caused by the entry of sodium and cesium ions into the potassium channels of squid giant axons. *J. Gen. Physiol.* 60:588–608.

Bezanilla, F., and C. M. Armstrong. 1977. Inactivation of the sodium channel. I. Sodium current experiments. *J. Gen. Physiol.* 70:549–566.

Bullock, J. O., and C. L. Schauf. 1978. Combined voltage-clamp and dialysis of *Myxicola* axons: behavior of membrane asymmetry currents. *J. Physiol. (Lond.).* 278:309–324.

Bullock, J. O., and C. L. Schauf. 1979. Immobilization of intramembrane charge in *Myxicola* giant axons. *J. Physiol. (Lond.).* 286:157–172.

Cahalan, M. D. 1978. Local anesthetic block of sodium channels in normal and pronase-treated squid giant axons. *Biophys. J.* 23:285–311.

Cahalan, M. D., and W. Almers. 1979 *a*. Interactions between quaternary lidocaine, the sodium channel gates, and tetrodotoxin. *Biophys. J.* 27:39–56.

Cahalan, M. D., and W. Almers. 1979 *b*. Block of sodium conductance and gating current in squid giant axons poisoned with quaternary strychnine. *Biophys. J.* 27:57–74.

Covington, A. K., and S. J. Jones. 1968. Hydrogen Bonded Solvent Systems. Taylor and Francis Publishers. London.

Eaton, D. C., and M. S. Brodwick. 1980. Effects of barium on the potassim conductance of squid axon. *J. Gen. Physiol.* 75:727–750.

Eaton, D. C., M. S. Brodwick, G. S. Oxford, and B. Rudy. 1980. Arginine-specific reagents remove sodium channel inactivation. *Nature (Lond.)* 271:473–476.

Goldman, L., and R. Hahin. 1978. Initial conditions and the kinetics of the sodium conductance in *Myxicola* giant axons. II. Relaxation experiments. *J. Gen. Physiol.* 72:879–898.

Goldman, L., and C. L. Schauf. 1972. Inactivation of the sodium current in *Myxicola* giant axons. Evidence for coupling to the activation process. *J. Gen. Physiol.* 59:659–675.

Goldman, L., and C. L. Schauf. 1973. Quantitative description of sodium and potassium currents and computed action potentials in *Myxicola* giant axons. *J. Gen. Physiol.* 61:261–284.

Heppolotte, R. L., and R. E. Robertson. 1960. The temperature dependence of the solvent isotope effect. *J. Am. Chem. Soc.* 83:1834–1838.

Horn, R., J. Patlak, and C. F. Stevens. 1981. Sodium channels need not open before they inactivate. *Nature (Lond.)* 291:426–427.

Katz, J. L., and H. L. Crespi. 1970. Isotope effects in biological systems. *In* Isotope Effects in Chemical Reactions. C. J. Collins and N. S. Bowman, editors. Van Nostrand-Reinhold, New York. 286–263.

Kirsch, G. E., J. Z. Yeh, J. M. Farley, and T. Narahashi. 1980. Interaction of *n*-alklyguanidine with the sodium channel of squid axon membrane. *J. Gen. Physiol.* 76:315–336.

Kresheck, G. C., H. Schneider, and H. A. Scheraga. 1965. The effect of D_2O on the thermal stability of proteins. Thermodynamics parameters for the transfer of model compounds from H_2O to D_2O. *J. Phys. Chem.* 69:3132–3144.

Krishnan, C. V., and H. L. Friedman. 1969. Solvation enthalpies of various nonelectrolytes in water, propylene carbonate, and dimethyl sulfoxide. *J. Phys. Chem.* 73:1572–1580.

Laidler, K. J. 1969. Theories of Chemical Reaction Rates. McGraw-Hill, New York.

Melander, L. 1960. Isotope Effects on Reaction Rates. Ronald Press, New York.

Morello, R., T. Begenisich, W. Trzos, and J. K. Reed. 1980. Interaction of nonylguanidine with the sodium channel. *Biophys. J.* 31:435–440.

Oakenfull, D., and D. E. Fenwick. 1975. Hydrophobic interaction in deuterium oxide. *Aust. J. Chem.* 28:715–720.

Schauf, C. L. 1973. Temperature dependence of the ionic current kinetics of *Myxicola* giant axons. *J. Physiol. (Lond.).* 235:197–205.

Schauf, C. L. 1976. Comparison of two-pulse sodium inactivation with reactivation in *Myxicola* giant axons. *Biophys. J.* 16:245–250.

Schauf, C. L., and J. O. Bullock. 1979. Modification of sodium channel gating in *Myxicola* giant axons by deuterium oxide, temperature and internal cations. *Biophys. J.* 27:193–208.

Schauf, C. L., and J. O. Bullock. 1980. Solvent substitution as a probe of channel gating in *Myxicola:* differential effects of D_2O on some components of membrane conductance. *Biophys. J.* 30:295–306.

Schauf, C. L., and J. O. Bullock. 1981. Isotope effects on ionic currents and intramembrane charge movements in *Myxicola* axons: implications for models of sodium channel gating. *In* Advances in Physiological Sciences. J. Salanki, editor. Pergamon Press, Akademiai Kiado, Budapest, Hungary. 4:51–66.

Schauf, C. L., and F. A. Davis. 1976. Sensitivity of the sodium and potassium channels of *Myxicola* giant axons to changes in external pH. *J. Gen. Physiol.* 67:185–195.

Schauf, C. L., J. O. Bullock, and T. L. Pencek. 1977. Characteristics of sodium tail currents in *Myxicola* axons: comparison with membrane asymmetry currents. *Biophys. J.* 19:7–28.

Schauf, C. L., and K. J. Smith. 1982. Gallamine triethiodide-induced modifications of the sodium conductance in *Myxicola* giant axons. *J. Physiol. (Lond.).* In press.

Schauf, C. L., T. L. Pencek, and F. A. Davis. 1976 *a*. Slow sodium inactivation in *Myxicola* axons: evidence for a second inactive state. *Biophys. J.* 16:771–778.

Schauf, C. L., T. L. Pencek, and F. A. Davis. 1976 *b*. Activation-inactivation coupling in *Myxicola* axons injected with tetraethylammonium. *Biophys. J.* 16:985–990.

Smith, K. J., and C. L. Schauf. Effects of gallamine triethiodide on membrane currents in amphibian and mammalian peripheral nerve. *J. Pharmacol. Exp. Ther.* 217:719–726.

Strichartz, G. R. 1973. The inhibition of sodium current in myelinated nerve by quaternary derivatives of lidocaine. *J. Gen. Physiol.* 62:37–57.

Swain, C. G., and D. F. Evans. 1966. Conductances of ions in light and heavy water at 25°C. *J. Am. Chem. Soc.* 88:383–390.

Thornton, E. L., and E. R. Thornton. 1970. Origin and interpretation of isotope effects. *In* Isotope Effects in Chemical Reactions. C. J. Collins and N. S. Bowman, editors. Van Nostrand-Reinhold Company, New York. 286–363.

Yeh, J., and C. M. Armstrong. 1978. Immobilization of gating charge by a substance that simulates inactivation. *Nature (Lond.).* 273:387–389.

Yeh, J. Z., and T. Narahashi. 1977. Kinetic analysis of pancuronium interaction with sodium channels in squid axon membranes. *J. Gen. Physiol.* 69:293–323.