Temperature Dependence of Na Currents in Rabbit and Frog Muscle Membranes

G. E. KIRSCH and J. S. SYKES

From the Department of Biological Sciences, Rutgers University, New Brunswick, New Jersey 08905

ABSTRACT The effect of temperature (0–22°C) on the kinetics of Na channel conductance was determined in voltage-clamped rabbit and frog skeletal muscle fibers using the triple-Vaseline-gap technique. The Hodgkin-Huxley model was used to extract kinetic parameters; the time course of the conductance change during step depolarization followed m^3h kinetics. Arrhenius plots of activation time constants (τ_m), determined at both moderate (−10 to −20 mV) and high (+100 mV) depolarizations, were linear in both types of muscle. In rabbit muscle, Arrhenius plots of the inactivation time constant (τ_h) were markedly nonlinear at +100 mV, but much less so at −20 mV. The reverse situation was found in frog muscle. The contrast between the highly nonlinear Arrhenius plot of τ_h at +100 mV in rabbit muscle, compared with that of frog muscle, was interpreted as revealing an intrinsic nonlinearity in the temperature dependence of mammalian muscle Na inactivation. These results are consistent with the notion that mammalian cell membranes undergo thermotropic membrane phase transitions that alter lipid-channel interactions in the 0–22°C range. Furthermore, the observation that Na channel activation appears to be resistant to this effect suggests that the gating mechanisms that govern activation and inactivation reside in physically distinct regions of the channel.

INTRODUCTION

In mammalian nerve and muscle membranes, the temperature sensitivity of several electrophysiological properties has been reported to undergo abrupt changes that are suggestive of membrane phase transitions. For example, in rabbit myelinated nerve (Chiu et al., 1979), a breakpoint in the temperature dependence of the time constant of Na channel inactivation (τ_h) occurs at ~6°C, such that τ_h is roughly 10 times more sensitive to temperature in the range 0–6°C than in the range 6–25°C. Similarly, in rat myelinated nerve (J. R. Schwarz, 1986), the temperature dependence of τ_h displayed about a twofold increase at temperatures below 10°C. However, in a study of K channel kinetics in rat

Address reprint requests to Dr. G. E. Kirsch, Dept. of Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, TX 77030. Dr. Sykes' present address is Cardiovascular Research Unit, Upjohn Company, Kalamazoo, MI 49001.

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muscle at temperatures of 1–37°C, Beam and Donaldson (1983) found that although no obvious breakpoints appeared in the Arrhenius plot of the time constant of K channel activation (τ_n), the plot became nonlinear at temperatures below ~12°C. These results show that in mammals the temperature dependence of Na and K channel gating exhibits a broad range of sensitivity, depending on the temperature range and the type of tissue examined. Hence, nerve Na channels appear to show the greatest and muscle K channels the least amount of anomalous behavior at low temperatures. If Arrhenius anomalies are indicative of temperature-dependent phase transitions in membrane lipids (Kumamoto et al., 1971), then the observed differences in temperature sensitivity suggest either that the K channels are more shielded from generalized membrane phase transitions than Na channels or that phase transitions do not occur in muscle membrane in this particular temperature range. In contrast, Na channel kinetics in ectothermic animals generally do not show breakpoints (Chiu et al., 1979; Kniffki et al., 1981; for an exception, see W. Schwarz, 1979).

In this article, experiments were performed on rabbit muscle to determine whether the temperature dependence of the muscle Na channels resembled that of mammalian nerve, and similar observations were made in frog muscle for comparison. A preliminary report of these data has been published (Kirsch and Sykes, 1986).

MATERIALS AND METHODS

Voltage Clamp

A triple-Vaseline-gap voltage clamp (Hille and Campbell, 1976) was used that incorporated modifications (Campbell, 1983) such as separate voltage-sensing and current-passing electrodes in the A-pool (to reduce series resistance). Membrane currents were measured from the voltage drop across a resistor in series with the E-pool. The operational amplifiers employed in the voltage-clamp circuit were individually balanced and tested using a passive electrical network. No rebalancing was done during the experiments. Instead, the voltage dependence of steady state Na inactivation was used as an acceptability criterion. The steady state inactivation curve in rabbit fibers followed a Boltzmann distribution with an average midpoint (measured at temperatures between 12 and 14°C) of -95.8 ± 1.8 mV. Data from fibers that varied by >1 SD (± 5.4 mV) were discarded.

Muscle fiber fragments (4 mm in length) were isolated from the extensor digitorum longus muscles of male New Zealand White rabbits (2.5–4 kg) or from the semitendinosus muscle of *Rana pipiens* and mounted in the voltage-clamp chamber. Electrical connections were made to the pools of the chamber through Ag/AgCl pellets and 1 M KCl bridges. Potentials were not corrected for junction potentials. Series resistance in the membrane potential–measuring circuit was partially compensated (average compensation, 1.6 $\Omega \cdot cm^2$) by increasing positive feedback to a point just below the threshold of voltage-clamp oscillation. The holding potential was set at −100 mV. The voltage-clamp response time (10–90% rise time) was typically ~30 μs in rabbit and 20 μs in frog fibers. Good voltage control was critically dependent on fiber diameter; we found that only the largest fibers (~80–100 μm diam in rabbit muscle, measured in depolarizing solution) allowed good voltage control, as indicated by the lack of notches or secondary peaks at potentials near threshold. The lack of secondary peaks may also indicate that the fibers became function-

ally detubulated (Campbell, 1983) during the equilibration period (30–45 min) that followed the mounting of the fibers in the chamber.

Data Storage and Analysis

Membrane currents were low-pass-filtered at 12.5 kHz, sampled at 100 kHz with 12-bit resolution, and stored on magnetic disk for later analysis, by means of a computer (LSI 11/23, Digital Equipment Corp., Marlboro, MA). Command potentials were generated by a 12-bit digital-to-analog converter synchronously with sampling. Linear leakage currents were subtracted electronically from membrane current records. Additional leakage and capacitative current corrections were made on-line by subtraction of the currents elicited by test pulses preceded by prepulses (80 ms duration, 0 mV amplitude), which completely inactivated Na conductance at all temperatures. The residual currents were subtracted from test pulse currents evoked after hyperpolarizing prepulses (80 ms duration, −160 mV amplitude).

Na current kinetics were analyzed using a nonlinear least-squares algorithm (Hooke and Jeeves, 1961). The accuracy of the fit was checked visually by superimposing the calculated curves on the experimental data.

The Arrhenius relationship,

$$1/\tau = A \exp(-Ea/RT), \tag{1}$$

where A and Ea are constants, $R = 1.987$ cal/deg-mol, and T is in Kelvins, was used to determine energy of activation (Ea). For temperatures T_1 and T_2,

$$Ea = [RT_1T_2/(T_2 - T_1)] \ln(\tau_1/\tau_2), \tag{2}$$

where τ_1 and τ_2 are time constants obtained at T_1 and T_2 (Kniffki et al., 1981). The temperature coefficient, Q_{10}, was calculated from:

$$Q_{10} = \exp(10\ Ea/RT_1T_2). \tag{3}$$

Arrhenius data were also fitted to a nonlinear model (Duggleby, 1985) consisting of two straight line segments connected by a curved transition region and described by two different activation energies. Curve-fitting was performed using a nonlinear regression program (Duggleby, 1985). For each Arrhenius plot, a ratio-of-variance (F) test was performed using the sum of the squares of the residuals obtained by fitting both the nonlinear and linear models. A calculated value of F that exceeded $F(0.05)$ was taken to indicate that the nonlinear model provided a better fit; otherwise, a linear model was used.

Temperature Measurement and Control

The muscle chamber was mounted in an aluminum housing whose base was in contact with a thermoelectric Peltier device (model 806-1036, Cambion Corp., Cambridge, MA). An insulated cover was placed over the chamber and the housing assembly to minimize temperature changes caused by convection loss. The rate of temperature change in the muscle chamber was adjusted to $<1\,^{\circ}$C/min by controlling the current flow through the thermoelectric device. The temperature was measured using a thermocouple probe (150 μm diam; model IT-23, Sensortek, Inc., Clifton, NJ), placed 1.5 mm downstream from the muscle fiber in the A-pool of the muscle chamber. The amplified output of the probe was sampled ~100 ms before sampling the membrane current and stored with the membrane current records. Tests for temperature gradients in the muscle chamber were conducted with a second probe mounted 1.5 mm upstream from the fiber. With the

solution flow rates adjusted to ~0.4 ml/min, no detectable temperature difference (± 0.1°C) between the two probes was found.

Solutions

Before the isolation of single rabbit muscle fibers, muscles were stored for up to 6 h at 5°C in a standard Tyrode's solution containing (mM): 149 Na^+, 5 K^+, 2 Ca^{2+}, 1 Mg^{2+}, 146 Cl^-, 12 HCO_3^-, 1 HPO_4^{2-}, and 11 glucose. The solution was continuously oxygenated with a mixture of 95% O_2 and 5% CO_2 and maintained at pH 7.2. Frog muscles were stored up to 24 h in unoxygenated Ringer's solution at 5°C.

External solutions used during voltage-clamp experiments in rabbit muscle consisted of a physiological solution containing (mM): 149 Na^+, 5 K^+, 2 Ca^{2+}, 1 Mg^{2+}, 160 Cl^-, and 5 buffer (4-morpholinepropanesulfonic acid [MOPS]). The solution was titrated to pH 7.4 with tetramethylammonium hydroxide (TMA-OH). The internal solution consisted of (mM): 133 Cs^+, 27 Na^+, 160 F^-, and 5 MOPS. The pH was adjusted to 7.4 with CsOH. Cs^+ blocked K^+ channels and F^- prevented muscle contraction during depolarization.

The frog muscle external solution consisted of a low-Na Ringer's (in order to facilitate measurement of outward currents) containing (mM): 40 Na^+, 80 TMA^+, 2.5 K^+, 4 Ca^{2+}, 130.5 Cl^-, and 5 MOPS. The pH was adjusted to 7.4 with TMA-OH. The internal solution contained: 115 Cs^+, 5 Na^+, 120 F^-, and 5 MOPS. The pH was adjusted to 7.4 with CsOH.

The effect of temperature on the pH of the internal and external solutions was negligible. Cooling from 20 to 0°C raised the pH 0.27 units.

RESULTS

The time course of Na currents was analyzed by fitting the experimental records to the Hodgkin-Huxley (1952) model:

$$I_{Na}(t) = I_{Na}(0)[1 - \exp(-t/\tau_m)]^3[\exp(-t/\tau_h)], \quad (4)$$

where $I_{Na}(0)$ is the maximum current obtained by extrapolation to $t = 0$, τ_m is the time constant of activation, and τ_h is the time constant of inactivation. The implicit assumptions of this model are that activation (m) and inactivation (h) are independent gating reactions whose time courses can be determined from the rising and falling phases of the Na current during step depolarization. These assumptions have been challenged on the basis of more recent experimental results (e.g., Bezanilla and Armstrong, 1977; Aldrich et al., 1983). Nonetheless, the Hodgkin-Huxley terminology provides a convenient empirical description of the time course of Na conductance; hence, the conventional terminology will be used without implying a specific physical interpretation.

To compare our results with those obtained in rabbit nerve (Chiu et al., 1979), a test pulse potential of −20 mV was used in the rabbit muscle experiments. A comparable level of activation in frog muscle was obtained by using a test pulse potential of −10 mV. In addition, test pulse potentials of +100 mV were used to determine whether the temperature sensitivity is altered by membrane potential (Anderson et al., 1977). At both of these membrane potentials, the time constants are related to the Hodgkin-Huxley rate constants of channel gating: $\alpha_m = 1/\tau_m$ and $\beta_h = 1/\tau_h$, since β_m and α_h are negligible (Pappone, 1980; Kirsch and Anderson, 1986). In this model, $I_{Na}(0)$ is proportional to the number of activatable channels multiplied by the single channel conductance and the driving

force. However, the significance of $I_{Na}(0)$ extrapolated from experimental records is doubtful (French and Horn, 1983), since activation and inactivation are partially coupled (Bezanilla and Armstrong, 1977). Therefore, only the kinetic parameters will be considered below.

Decreased temperature resulted in a marked prolongation of both the activa-

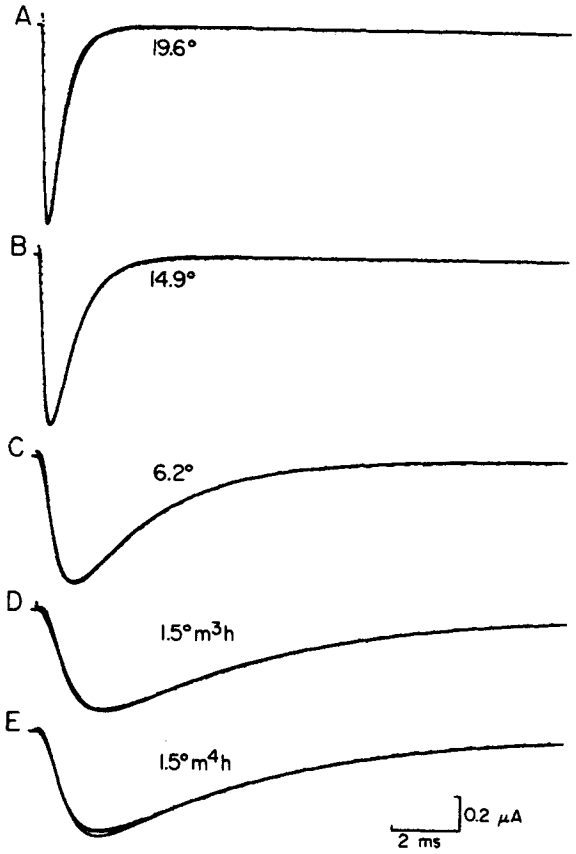

FIGURE 1. Effects of temperature on Na currents in rabbit skeletal muscle. Test pulse potential, −20 mV amplitude and 15 ms duration. Holding potential, −100 mV. Prepulse, −160 mV amplitude and 80 ms duration. Currents were sampled at 100 kHz. The smooth curve superimposed on data points represents the integrated Hodgkin-Huxley current equation with parameters (see text) chosen to fit the data. Panels *A–D* were fitted according to m^3h kinetics. Panel *E* is a replotting of the data of *D* with an m^4h kinetic model. Fiber AU16C.

tion and inactivation phases of the currents, as shown in Fig. 1, which illustrates typical Na currents in rabbit muscle evoked by a test pulse potential of −20 mV during cooling from 19.6 to 1.5°C. The data points of the current records and the solid line (calculated by fitting the parameters of Eq. 4 to the data) were plotted together in order to show the good superposition that was typically

observed. The activation term in Eq. 4 raised to the third power provided the most accurate fit to the sigmoidal onset of the current at temperatures greater than ~8°C. At lower temperatures (e.g., 6.2 and 1.5°C, Fig. 1, *C* and *D*), the slight delay between the initial data points and the fitted curve suggests that a higher power of m might have given a better fit. However, the magnitude of the delay varied among different fibers; several fibers showed a much smaller delay than that illustrated. Fig. 1*E* shows the result of fitting m^4 kinetics to the data of Fig. 1*D*. Although the calculated curve provides a better fit to the initial points, the peak Na current is not well fitted. Hence, we used m^3 kinetics even though they tend to overestimate τ_m at low temperatures and thus overestimate the effect of cooling on activation. The decay of Na current (inactivation) followed a monophasic time course. Thus, the apparent kinetics of activation and inactivation could be described empirically by the time constants τ_m and τ_h.

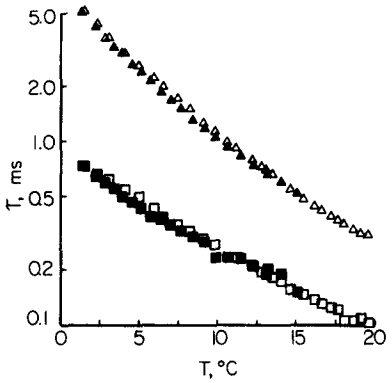

FIGURE 2. Temperature dependence of Na current kinetics in a typical rabbit fiber. Ordinate: time constant (milliseconds) of Na activation (τ_m, squares) and inactivation (τ_h, triangles), log scale. Abscissa: temperature (°C). Filled and open symbols represent data obtained during cooling and heating, respectively. Fiber AU14E.

Fig. 2 shows the variation of τ_m (squares) and τ_h (triangles) as a function of temperature in a typical experiment. Data obtained during cooling and heating, represented by filled and open symbols, respectively, were superimposable. Shifting the temperature from 10 to 20°C caused a threefold decrease in both τ_m and τ_h, which is comparable to the effect of temperature on gating in squid axon and frog myelinated nerve (Hodgkin and Huxley, 1952; Frankenhaeuser and Moore, 1963). In contrast, a shift from 0 to 10°C, which caused a threefold decrease in τ_m, resulted in a fivefold decrease in τ_h. Thus, inactivation appears to be more sensitive to low temperatures than activation. In an Arrhenius plot, altered temperature sensitivity would appear as a change in slope. Thus, τ_m should be described by a linear plot, whereas τ_h should be nonlinear, with a steeper slope in the low temperature region of the plot.

As shown in Fig. 3 (squares), the Arrhenius plot of pooled τ_m values in rabbit

fibers (test pulse potential, −20 mV; n = 10 fibers) was linear. The average slope of the individual plots for each fiber was 17.5 kcal/mol, which is equivalent to a Q_{10} of ~3.0.

The determination of the temperature dependence of τ_m (Fig. 3, triangles) at a test potential of +100 mV was limited by the response time of the voltage clamp to temperatures below ~9°C. Nonetheless, within the temperature range 0.3–8.5°C, the Arrhenius plot appeared to be linear and could be described by Ea = 12.7 kcal/mol. Thus, for τ_m determined at either moderate or strong depolarizations, we found no evidence for increased temperature sensitivity at low temperatures.

The temperature dependence of τ_m in frog muscle was similar to that found

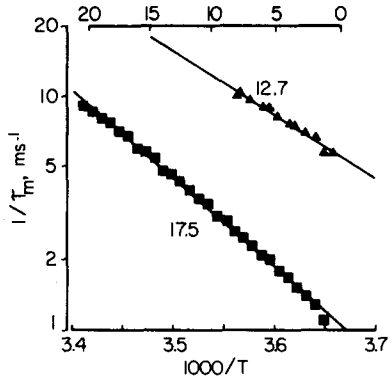

FIGURE 3. Arrhenius plot of mean τ_m in rabbit muscle. Data were obtained at membrane potentials of −20 mV (squares, average of 10 fibers) and +100 mV (triangles, average of 4 fibers) during cycles of both heating (open symbols) and cooling (filled symbols). Ordinate: 1/time constant (per millisecond) plotted on a logarithmic scale. Abscissa, lower scale: 1,000 × (1/K). Upper scale: °C. For purposes of clarity, the total number of data points was reduced by calculating the mean in each of 28 temperature bins. The standard error bars are smaller than the symbols and hence are not shown. Curve-fitting was performed by a nonlinear least-squares regression program (Duggleby, 1985). Each line segment is labeled with the apparent activation energy (kilocalories per mole) calculated from the slope.

in rabbit muscle. As shown in Fig. 4, the pooled data (n = 6) obtained at test pulse potentials of both −10 and +100 mV could be described by linear Arrhenius plots with apparent activation energies of 16.0 and 13.2 kcal/mol at −10 and +100 mV, respectively.

In contrast, τ_h showed a statistically significant, nonlinear temperature dependence. The data were fitted empirically by two straight lines (Duggleby, 1985). The effect was particularly clear in frog muscle at a test pulse potential of −10 mV and in rabbit muscle at +100 mV. Pooled Arrhenius data for frog (Fig. 5) show that, at −10 mV (squares), the temperature dependence of τ_h was markedly nonlinear (the difference in the slopes was 9.5 kcal/mol), such that Q_{10}

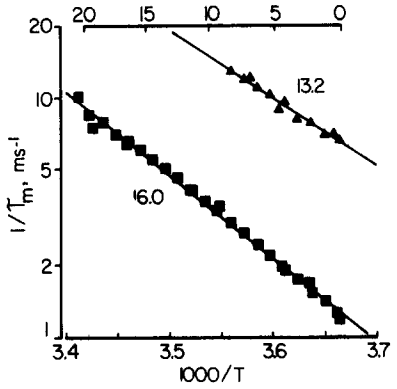

FIGURE 4. Arrhenius plot of mean τ_m in frog muscle. Time constants were determined in six fibers at test pulse potentials of −10 (squares) and +100 mV (triangles). See legend of Fig. 3 for details.

values of 1.7 and 3.4, respectively, were required to fit the high and low temperature ranges. Strong depolarization, however, tended to linearize (slope change, 7.4 kcal/mol) the frog muscle τ_h Arrhenius plot (Fig. 5, triangles), mainly by increasing the apparent temperature sensitivity at high temperatures, such that, at +100 mV, Q_{10} values of 3.0 and 5.3, respectively, were required to fit the data in the high and low temperature ranges.

A quantitatively different temperature response was obtained in rabbit muscle, as illustrated in Fig. 6. Arrhenius plots of pooled τ_h data obtained at test pulse potentials of both −20 (squares) and +100 (triangles) mV showed increased slopes at lower temperatures. Unlike in frog muscle, however, strong depolarization increased the degree of curvature of the Arrhenius plot. In rabbit fibers at low temperatures, the apparent activation energy was greater at test pulse potentials of +100 mV than −20 mV and the τ_h values obtained at the two test

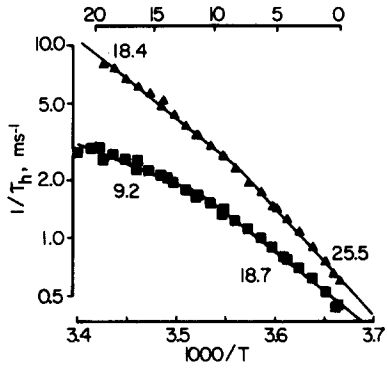

FIGURE 5. Arrhenius plot of mean τ_h in frog muscle. Data were obtained at test pulse potentials of −10 (squares) and +100 (triangles) mV. See legend of Fig. 3 for details.

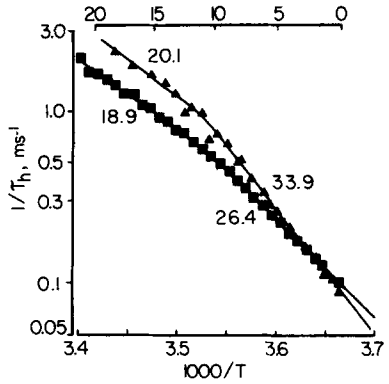

FIGURE 6. Arrhenius plot of mean τ_h in rabbit muscle. Data were obtained at membrane potentials of −20 (squares) and +100 mV (triangles). See legend of Fig. 3 for details.

potentials converged. The data obtained at +100 mV showed a temperature-sensitive change in slope of 13.8 kcal/mol, whereas a slope change of only 7.5 kcal/mol was observed at −20 mV. The temperatures at which the straight lines intersected were 11.5 and 10.2°C, respectively, for the −20 and +100 mV Arrhenius plots.

The interaction of membrane potential and temperature with τ_h in rabbit muscle are illustrated in Fig. 7, which shows that at a constant temperature of either 22 (squares) or 14°C (diamonds), τ_h was markedly voltage dependent, such that the apparent rate of decay increased 2.5–2.6-fold at +100 compared with −20 mV. However, when the temperature was held at 0°C, τ_h (triangles) at the two test potentials was nearly the same, which suggests that at low temperatures the rate-determining step for Na inactivation is no longer voltage dependent. Similar results were obtained in the other rabbit fibers examined. The average τ_h at 0°C was 9.4 ± 1.3 and 10.6 ± 0.7 ms at test pulse potentials of −20 and +100 mV, respectively.

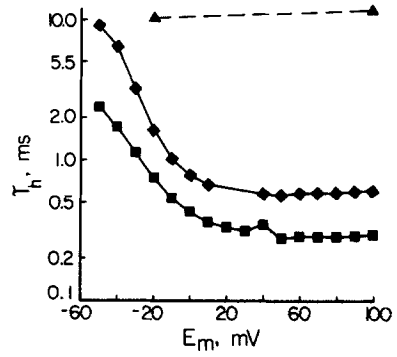

FIGURE 7. Voltage dependence of τ_h in rabbit muscle. Data were obtained at constant temperatures of 22 (squares), 14 (diamonds), and 0°C (triangles). Fiber MR22A.

Temperature also affected the voltage dependence of steady state inactivation. In agreement with Chiu et al. (1979), we found that in rabbit muscle, the midpoint of the inactivation curve was shifted reversibly by -9.2 ± 2.7 mV ($n = 3$) upon cooling from 22 to 0°C. However, this effect would not be expected to alter significantly the apparent temperature dependence of τ_h, particularly at test pulse potentials where τ_h is voltage independent.

DISCUSSION

The nonlinearity of the τ_h Arrhenius plot observed in rabbit muscle was similar to that seen in rat nerve Na channels (J. R. Schwarz, 1986) and to that of τ_n in rat muscle K channels (Beam and Donaldson 1983), namely, a two- to threefold steeper temperature dependence at temperatures near 0 than at temperatures near 20°C.

The temperature dependence of Na channel kinetics that we observed in frog muscle is in some ways similar to that reported by W. Schwarz (1979), who found that the Arrhenius plot of τ_h at −30 mV could be fitted by two straight lines, intersecting at 5–8°C, with slopes of 3–4 and 30–50 kcal/mol, respectively, at temperatures above and below the critical temperature. Similarly, we found that the τ_h plot obtained at −10 mV could be fitted by lines intersecting at 6°C, with slopes of 9 and 19 kcal/mol, respectively, at the high and low temperature extremes. Given the variability of the low temperature slopes reported by W. Schwarz (part of which may be due to the use of several different species of frogs), the two sets of data are similar in this temperature range. Furthermore, although the slopes obtained at high temperatures appear to be different, our τ_h data obtained at a potential of +100 mV clearly show that the slope of the Arrhenius plot is dependent on the test pulse potential: strong depolarization tends to linearize the Arrhenius plot by increasing the slope at high temperatures. Thus, the difference between our data, obtained at −10 mV, and those obtained by W. Schwarz (1979) at −30 mV can be reconciled by the effect of the test pulse potential.

A more serious discrepancy is our observation of a linear temperature dependence of τ_m, which is clearly different from the results reported by W. Schwarz (1979), who found that the Arrhenius plot of τ_m closely resembled that of τ_h. We have no ready explanation for this difference; however, it should be noted that more recent reports (Romey et al., 1980; Vijverberg et al., 1983) have shown that τ_m in frog nerve has a linear temperature dependence.

In general, changes in the slope of an Arrhenius plot indicate a change in the rate-determining step of a reaction. Because of the complexity of Na channel gating, however, the time constants determined from macroscopic Na currents may not be directly related to the rates of activation and inactivation in single channels (Aldrich et al., 1983; Horn, 1984). Consequently, Arrhenius plots of time constants must be interpreted with caution. In particular, at moderate depolarizations (i.e., −30–0 mV), τ_h may be contaminated by slow rate constants associated with the activation mechanism (Aldrich et al., 1983). To avoid this problem, we extended our observations to strong depolarizations (+100 mV), where τ_h gives a good approximation of the rate of inactivation. Thus, in both

frog and rabbit muscle, the shape of the τ_h Arrhenius plot measured at moderate depolarizations may be determined in part by changes in the amount of overlap between the kinetics of Na activation and inactivation rather than by an intrinsically nonlinear temperature dependence of inactivation. According to this view, if inactivation has a steeper temperature dependence than activation, τ_h could be determined by slow components of activation at high temperatures and by a slow rate of inactivation at low temperatures. This interpretation is supported in frog muscle by the observation that a test pulse potential of +100 mV tended to linearize the Arrhenius plot of τ_h and to increase the slope. However, in rabbit muscle, additional factors must be operating to retard the onset of inactivation at low temperatures, since the nonlinearity of the temperature dependence of τ_h was even more pronounced at +100 than at −20 mV.

There are several plausible explanations for the nonlinearity observed in the τ_h Arrhenius plot in rabbit muscle. One possibility is the occurrence of thermotropic phase transitions that result in lipid-phase separations within the membrane (Sandermann, 1978). Such changes would affect membrane-bound proteins excluded from the solid-phase lipids by altering the composition and physical properties (e.g., "microviscosity") of the lipids that remain in the immediate vicinity of the proteins. Typically, this effect appears in an Arrhenius plot as a sudden change of slope, referred to as a breakpoint (Kumamoto et al., 1971). The plot can be fitted by two straight lines intersecting at the critical temperature of the phase transition. In rabbit muscle, the Arrhenius plot of τ_h at +100 mV showed a slope change of 13.8 kcal/mol at a critical temperature of 10.2°C, which may indeed be a breakpoint in the temperature dependence of Na inactivation. However, it is clear that this effect was not as great as that obtained in rabbit nerve (Chiu et al., 1979), where a slope change of 37 kcal/mol was obtained at a critical temperature of 6°C. The main reason for this difference in temperature sensitivities between nerve and muscle membranes appears to be due to a difference in activation energies measured at temperatures between 0 and 6°C (52.9 kcal/mol in rabbit nerve [Chiu et al., 1979] vs. 33.9 kcal/mol in rabbit muscle); the activation energies measured in the range 15–20°C are nearly the same (18.4 kcal/mol in nerve [Chiu et al., 1979] and 20.1 kcal/mol in muscle).

W. Schwarz (1986) has suggested that some of the apparent nonlinearity in temperature dependence may arise artifactually from uncompensated series resistance. This is unlikely as an explanation of the present results since the frog muscle experiments were performed in low-Na (40 mM) Ringer's, and, in rabbit muscle, nonlinear behavior was clearly observed even at a test pulse potential of +100 mV, where inactivation gating is no longer voltage sensitive.

In summary, the inactivation process in both frog and rabbit muscle appears to have a steeper temperature dependence than does activation. Therefore, at moderate depolarizations, the curvature of the τ_h Arrhenius plot may be due in part to a temperature-dependent change in the amount of overlap between the activation and inactivation time courses rather than to a change in the physical properties of the membrane. Only in rabbit muscle was the temperature dependence of τ_h at strong depolarizations also markedly nonlinear. Since τ_h at this potential is unlikely to be affected by slow components of activation, the temper-

ature dependence of inactivation in rabbit muscle appears to be nonlinear, a result that is consistent with the notion that alterations in membrane fluidity affect the temperature dependence of the mammalian Na channel. Furthermore, Na channel activation appears to be much less sensitive to changes in temperature. Thus, although the activation and inactivation mechanisms appear to be functionally coupled, such that most channels reach the inactivated state by first passing through the open state (Bezanilla and Armstrong, 1977; Aldrich and Stevens, 1984), the differences in their temperature dependence suggest that the gating mechanisms that govern the rates of the two processes reside in physically distinct regions of the channel.

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