LASER TEMPERATURE-JUMP TECHNIQUE FOR RELAXATION STUDIES OF THE IONIC CONDUCTANCES IN MYELINATED NERVE FIBERS

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ABSTRACT A temperature-jump technique for single nodes of Ranvier has been developed using a pulsed laser system. The temperature perturbation was accomplished by firing the laser beam obtained from a neodymium rod through the solution surrounding a single node. The temperature step was achieved within 1 msec using the laser in the normal mode of operation. During the voltage-clamped steady-state current a temperature jump from 4°C increased the current to a new steady-state value within the time course of the *T*-jump. This finding suggests that the maximum potassium permeability P_K has a rapid relaxation time and that the steady-state value of n (the value of potassium permeability divided by its maximum value) is relatively independent of temperature. *T*-jumps applied during the voltage-clamped sodium currents showed that the sodium permeability changed with a relaxation time that was also shorter than the duration of the normal mode laser output. *T*-jumps observed during a hyperpolarization or at the resting potential showed no detectable conductance change. When a *T*-jump immediately preceded a voltage clamp pulse the technique was then used to investigate the effect of changes in the steady-state temperature on the ionic conductances. It was found that the magnitude of the change in membrane current due to a *T*-clamp was directly related to the level of cathodal polarization.

INTRODUCTION

The application of linear relaxation methods to a variety of chemical systems has led to considerable success in elucidating reaction mechanisms (Eigen and De-Maeyer, 1963). It has been shown that a small step perturbation in the temperature, pressure, or electric field of a complex chemical system leads to a change in the concentration of the product of a reaction which can be described by a sum of time-dependent exponential terms (Eigen, 1967, 1968). The number of terms required to fit the data indicate a minimum number of elementary steps for the reaction. The time constants constitute the relaxation spectra of the reaction which can be used to determine the forward and reverse rate constants and hence the equilibrium constant for each step of the process.

Relaxation theory has been used to interpret the voltage clamp experiment as a step perturbation in the electric field of the membrane (Moore and Jakobsson, 1971). Since the state of the nerve membrane at constant composition of the bathing medium is determined by the variables temperature, pressure, and electric field, a sudden change in one of the variables, such as the membrane potential, will define a new state to which the voltage-dependent conductances must relax. Similarly, the membrane conductance could be perturbed by a rapid change in the temperature (temperature jump).

Considering the potassium conductance, g_K, when $g_K = \overline{g_K}$ (the maximum potassium conductance) it is clear that a perturbation of the electric field would theoretically have no effect on the conductance since the conductance is at a maximum steady-state value. On the other hand, a temperature jump should displace the quasi equilibrium condition with a resulting relaxation time. Thus the reaction mechanism involving potassium ions and their respective sites can in principle be resolved in terms of elementary steps with the corresponding forward and reverse rate constants (Eigen and Hammes, 1963; Hammes, 1968).

Temperature jumps have usually been accomplished by the discharge of a capacitor through the system under investigation (Eigen and DeMaeyer, 1963; Owen et al., 1970); however, recent studies have proposed the use of laser to elicit temperature jumps (Hoffman et al., 1968; Rigler et al., 1970). This approach takes advantage of the extremely short duration (10 nsec–1 msec) of laser pulses which can be used to bring about step changes in the temperature of an absorbing medium. Many pertinent considerations are discussed in the recent paper by Caldin et al. (1971).

This paper describes the development of a laser-induced temperature-jump method for single myelinated nerve fibers. The method has been used two ways: (*a*) as a means to change the steady-state temperature rapidly and (*b*) as a relaxation method. In the laser steady-state temperature measurements the temperature jump precedes a voltage clamp pulse by a few milliseconds. Thus, the effect of temperature on the kinetic properties of the voltage-dependent conductances can be accurately measured since voltage clamp runs at two different temperatures can be made within milliseconds of each other without fiber deterioration.

The laser temperature-jump relaxation method has been used in conjunction with the voltage clamp technique by first turning on the ionic conductance system with a membrane potential step and then perturbing the temperature to determine how the activated ionic conductance relaxes from one quasi equilibrium value to another. These experiments have shown that for moderate cathodal polarizations a temperature jump from 4°C increased the potassium current to a new steady-state value within the time course of the *T*-jump. It was also found that the magnitude of the change in membrane current due to a *T*-jump was directly related to the level of depolarization.[1]

METHODS

Myelinated nerve fibers from the clawed toad (*Xenopus laevis*) were isolated and mounted in a Lucite chamber. The recording technique (Dodge and Frankenhaeuser, 1958) was identical with that previously described (Moore, 1967) with the exception that the model of operational amplifier used was a Philbrick/Nexus 1011 (Philbrick/Nexus Research, Boston, Mass.).

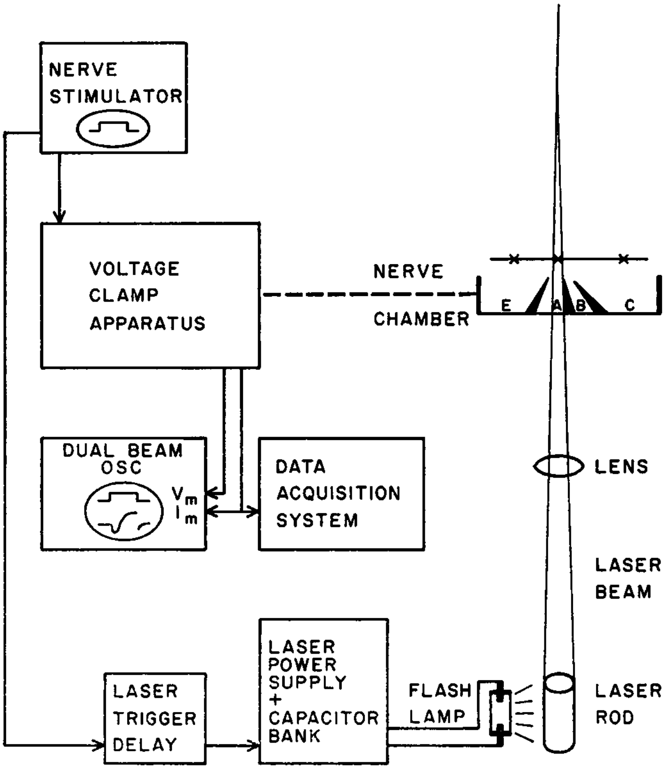

FIGURE 1 Schematic diagram of the laser temperature-jump system. The data acquisition system was an analogue to digital converter and paper tape unit. The single fiber preparation was connected to the electronic apparatus through calomel electrodes. The *x*'s shown above the nerve chamber represent the nodes of Ranvier. The chamber is diagrammed in side view with designations of the various compartments as in Dodge and Frankenhaeuser (1958). Only the properties of the node in pool *A* were measured.

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[1] Preliminary reports of portions of this work were given at the annual meeting of the Biophysical Society (Lindley and Moore, 1971), at the Cincinnati regional meeting of the American Chemical Society, and at the 25th International Congress of Physiological Sciences, Munich (Moore, Holt, and Lindley, 1971).

The experiments were done using a Lucite chamber similar to that described by Dodge and Frankenhaeuser (1958). The initial experiments were done with a pulsed laser beam fired into the side of the pool containing the central node of Ranvier (pool *A*, see Fig. 1; Dodge and Frankenhaeuser, 1958). The volume of this pool was less than 0.5 ml and was bounded on either side by glass cover slips through which the laser beam passed. Some light scattered by the Lucite struck the node as well as other portions of the fiber; however, much of the unabsorbed radiation passed through pool *A* and out of the chamber. Thus, the temperature change of the node was by conduction from the bulk solution under the nodal area. The bulk solution was directly heated by focusing the laser beam through a 7 diopter lens. The focal point was in front of the first cover slip.

The preliminary experiments done with the above system showed a marked effect of the laser pulse on the recording from single nodes. Large transient changes in the potential of pool *A* occurred with the firing of the laser pulse, often resulting in a single action potential. Electrical artifacts from the discharge of the laser pulse were eliminated by conventional shielding.

The above effects of the laser pulse were completely abolished when the laser beam was prevented from striking the preparation; however, some of the laser effects were present with dead fibers and were markedly enhanced when the laser beam was directed to the calomel electrode of pool *C* (see Fig. 1, Dodge and Frankenhaeuser, 1958). These findings suggest that most of the laser response observed was due to a photo-artifact on the electrode of pool *C*. The photo-artifact was present with both ruby and neodymium rods, i.e., radiation at 0.69 μ and 1.06 μ respectively. The photo-artifact was reduced almost completely by light shielding all the electrodes. The residual artifact seen in Fig. 2 was probably due to incomplete shielding of the electrodes.

In the experiments described in this paper the electrodes have been light shielded by placing a ¼ inch black Lucite partition between the electrodes and the nerve chamber. The calomel electrodes were connected to the pools of the Lucite chamber by small holes drilled in the black partition. The channels were packed with balsa wood which was saturated with a

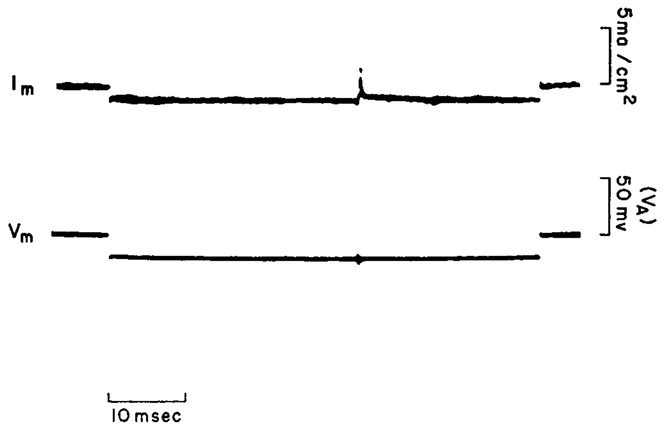

FIGURE 2 Effect of laser pulse on hyperpolarization of a single fiber. Note the laser artifacts on both the potential and current traces. In this and all subsequent figures the membrane current (I_m) calibration was calculated according to the relationship, $I_m = V_E/Z_{ED}A_N$, where V_E is the potential in pool *E*, Z_{ED} is the impedance pathway from pool *E* to axoplasm of the node in pool *A*, and A_N is the nodal area (Dodge and Frankenhaeuser, 1958). The value of the product $Z_{ED}A_N$ was 20 Ω cm². The holding level for the potential in this and subsequent experiments was about −70 mv.

1 M KCl solution. These KCl bridges provided no significant light pathway from the nerve chamber to the electrodes.

As illustrated in Fig. 1 a second modification required to demonstrate a laser-induced temperature jump was to direct the laser beam from underneath the nerve chamber through the solution of pool A surrounding the node. The beam was aligned in order that the node would be directly in its path. As before, the focal point was not in the solution but either above or below the preparation. A glass cover slip formed the floor of pool A through which the laser beam passed. In this configuration it was found unnecessary to use small volumes of solution in pool A. An alignment within 1 mm of the laser beam was required to elicit a temperature jump. Generally the beam was aligned with the nodal area in the center.

In all the experiments reported in this paper a Maser Optics pulsed laser system was used. The duration of the laser pulse was 0.5–1 msec with an energy output of about 1 joule per pulse. Unless otherwise indicated a neodymium rod was used.

ANALYSIS

Voltage clamp currents collected from an analogue-digital converter were analyzed on a PDP-11 computer (Digital Equipment Corp., Maynard, Mass. The data were corrected for leakage current I_l by assuming a constant leakage conductance which was determined by a hyperpolarizing pulse of 10–30 mv. The total ionic current I_i was assumed to fit an expression of the following form, $I_i = \sum_i A_i e^{-t/\tau_i}$. This type of analysis is more general than the Hodgkin and Huxley (1952) formalism and was used to allow an interpretation (Moore and Jakobsson, 1971) of the data in terms of linear relaxation theory (Eigen and DeMaeyer, 1963). The sodium and potassium currents were analytically separated by assuming that the slowest single exponential term of the total current was characterized by the time constant τ_n for the potassium current. The positive outward currents were then used to determine the best γ and τ_n of the equation (Moore, 1971),

$$I_K = A_0 (1 - e^{-t/\tau_n})^\gamma \tag{1}$$

where A_0 is the steady-state current.

The sodium current I_{Na} was thus the total current minus the potassium current as given by equation 1. The sodium currents were analyzed by successively subtracting or peeling off the slowest exponential term. The individual terms of the equation

$$I_{Na} = A_m e^{-t/\tau_1} + A_h e^{-t/\tau_2} \tag{2}$$

were determined from the data by the least squares method.

The sodium current given by equation 2 was subtracted from the leakage-corrected total current and the remaining outward current analyzed by the above peeling method with the equation,

$$I_K = A_0 + A_k e^{-t/\tau_3} + A_n e^{-t/\tau_4}.$$

As shown in Table I, the total current was given by

$$I_i = I_l + A_m e^{-t/\tau_1} + A_n e^{-t/\tau_2} + A_0 + A_k e^{-t/\tau_3} + A_n e^{-t/\tau_4}.$$

TABLE I

ANALYSIS OF VOLTAGE CLAMP CURRENTS

V_m*	Temperature‡	I_{Na}				I_K				
		A_m§	τ_1^{-1}	A_h§	τ_2^{-1}	A_0§	A_k§	τ_3^{-1}	A_n§	τ_4^{-1}
			msec^{-1}		*msec*$^{-1}$			*msec*$^{-1}$		*msec*$^{-1}$
53	T_1	456	−1.178	−417	−0.045	1	—	—	—	—
53	T_2	815	−1.375	−471	−0.058	−11	—	—	—	—
53	T_1	548	−1.102	−416	−0.047	4	—	—	—	—
63	T_1	—	—	−342	−0.041	0	—	—	—	—
63	T_2	—	—	−392	−0.058	31	—	—	—	—
63	T_1	—	—	−341	−0.041	0	—	—	—	—
74	T_1	—	—	−279	−0.054	31	—	—	—	—
74	T_2	—	—	−304	−0.075	70	—	—	—	—
74	T_1	—	—	−283	−0.056	47	—	—	—	—
97	T_1	—	—	−120	−0.155	187	126	−0.940	−310	−0.032
97	T_2	—	—	−44	−0.364	304	—	—	−302	−0.032
97	T_1	—	—	−131	−0.190	187	142	0.092	−323	−0.032
118	T_2	−102	−0.350	108	−0.079	344	82	−0.148	−428	−0.038
118	T_1	19	−0.755	−95	−0.48	234	103	−0.107	−339	−0.032
118	T_1	—	—	−102	−0.46	226	116	−0.104	−341	−0.035
128	T_1	−41	−0.434	38	−0.107	289	77	−0.109	−369	−0.035
128	T_2	−49	−1.190	42	−0.104	328	120	−0.145	−440	−0.041
128	T_1	−69	−0.550	46	−0.172	297	85	−0.101	−384	−0.036
140	T_1	−38	−0.697	81	−0.098	351	71	−0.130	−464	−0.032
140	T_2	−86	−0.689	119	−0.158	383	120	−0.131	−509	−0.050
140	T_1	−132	−0.410	162	−0.195	351	87	−0.116	−472	−0.035
152	T_1	−50	−0.564	127	−0.119	398	114	−0.208	−508	−0.051
152	T_2	−173	−0.599	232	−0.231	414	98	−0.223	−517	−0.060
152	T_1	−79	−0.796	132	−0.148	398	98	−0.188	−500	−0.050

* V_m is the displacement of the membrane potential from the holding value, $E_H = -94$ mv.

‡ T_1 was the initial temperature at 4°C and T_2 was the elevated steady-state temperature due to the laser pulse which preceded the voltage clamp step.

§ The units of the A's were in millivolts reflecting the potential, V_E, which is proportional to the membrane current.

RESULTS

Steady-State Temperature Measurements

The laser-induced temperature jump is well demonstrated by the experiment illustrated in Fig. 3. In this voltage clamp run the laser was fired before the cathodal polarization of nerve. Thus, the temperature was changed to a new steady-state

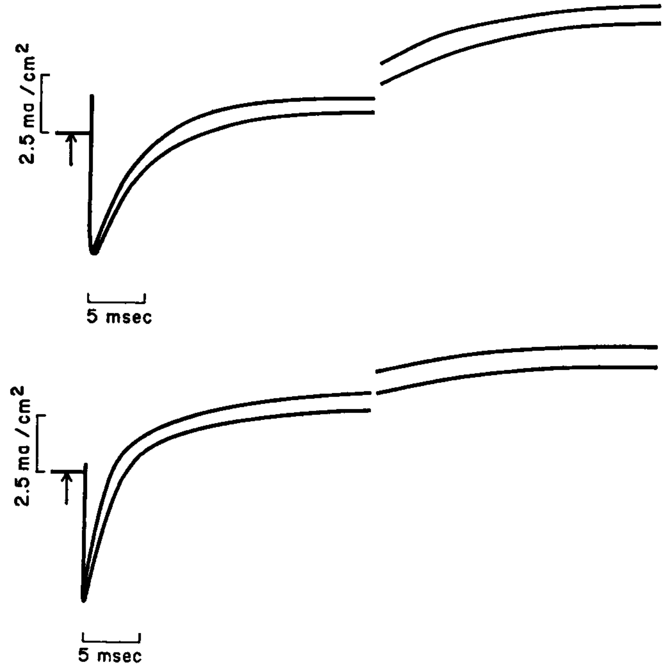

FIGURE 3 Effect of a steady-state temperature change on the voltage clamp currents. Tracings of the original records are superimposed where the upper trace of each pair represents the voltage clamp current after firing the laser at the time indicated by the arrow. Each pair of records shows a sequential double-pulse experiment where the second pulse begins at the break in the curves. The sequential cathodal depolarizations were 32 and 52 mv for the upper pair and 48 and 52 mv for the lower pair of currents. Note that in this and subsequent figures the capacitative current was not traced.

volume before the voltage clamp currents were initiated. This technique allows voltage clamp runs to be taken first at the bath temperature T_1, then with the laser firing at a new temperature T_2, and a few seconds later, a second control run at T_1. In Figs. 3 and 4 two major effects of an increased temperature on the current are seen to be an increased steady-state current, indicating an increase in the maximal steady-state conductance at this potential, and an increase in the rate of the conductance changes.

The return of the steady-state current to its original value at T_1 required a period greater than 500–1000 msec. This is the time required to dissipate the heat in the nodal area and equilibrate the system to its original temperature. The temperature equilibration is partly accomplished by the adjacent solution in pool *A* as well as the Lucite partitions of the nerve chamber.

One complication of these experiments was the occurrence of pressure changes in pool *A* during the laser firing. These pressure changes were frequently large enough to cause some splashing of the fluid out of the pool. The splashing was minimized by conducting the experiments near 4°C (Hoffman et al., 1968). Pro-

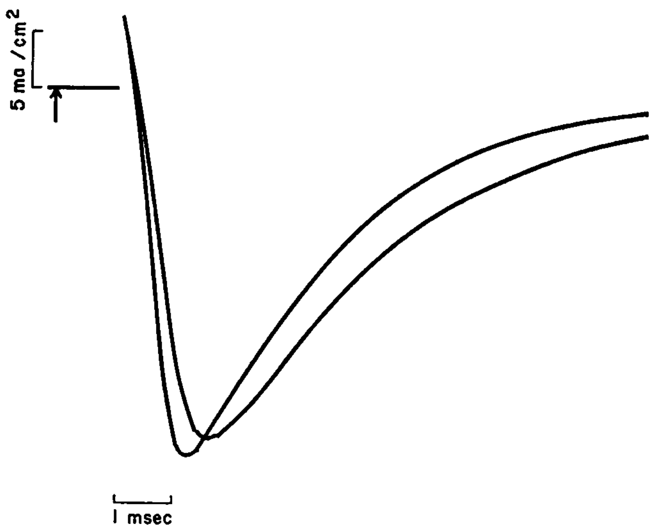

FIGURE 4 Effect of laser pulse on initial transient current. Tracings of the original records were superimposed where the more rapidly changing curve was recorded after the laser pulse. The time of laser firing is indicated by the arrow. A cathodal depolarization of 40 mv occurred at the break in the resting current.

vided the splashing was kept at a minimal level the results were reproducible. This result suggests that the effect of the laser light on the nerve membrane was not deleterious.

The finding that the temperature-sensitive steady-state currents were maintained for hundreds of milliseconds means that the nerve fiber and much of its surrounding area was heated. If only the fiber were heated the surrounding solution would cool the preparation more rapidly because of the small volume of the fiber. Confirmation of these conclusions comes from the additional result that firing the laser with a ruby rod showed qualitatively the same results as a neodymium rod. Since radiation from a ruby rod at 0.69 μ does not absorb in H_2O as well as 1.06 μ from a neodymium rod, it is apparent that the initial absorption of energy is by the nerve chamber and petroleum jelly seals, as well as the nerve fiber and surrounding solution.

The effects of changes in the temperature of only the nodal area (Frankenhaeuser and Moore, 1963) have been shown to be similar to those obtained by altering the temperature of the entire preparation (Moore, 1971). It is therefore assumed that a laser-induced temperature jump of only the nodal area is a valid method of changing the temperature.

The analysis of the data from steady-state temperature measurements in Table I support the finding of Moore (1971) that the temperature dependence of the time constants is relatively independent of the membrane potential. Comparison of these measurements with those of Frankenhaeuser and Moore (1963) indicate a temperature jump of about 2–3°C for these experiments.

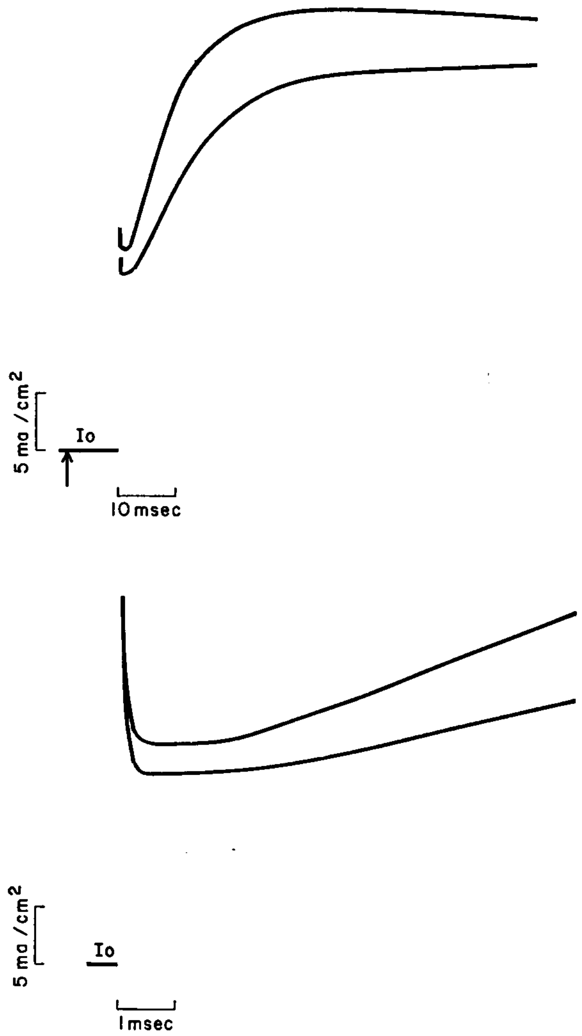

FIGURE 5 Effect of temperature on the delayed steady-state currents. Tracings of the same currents are superimposed for two time scales. The upper trace of each pair was recorded after the laser fired. The time of the laser firing is indicated by the arrow. A cathodal depolarization of 127 mv occurred at the break in the resting current.

The effects of steady-state temperature jumps on the transient currents are illustrated in Figs. 3, 4, and 6. At low depolarizations an increased temperature increased the inward transient current; however, at large depolarizations the inward transient current is either unchanged or decreased in magnitude.

The steady-state temperature effects on the delayed current are illustrated in Figs. 5 and 6. The voltage clamp runs in Fig. 5 taken near the sodium equilibrium potential showed that an increased temperature not only increased the voltage-dependent conductance but possibly increased the leakage conductance. One inter-

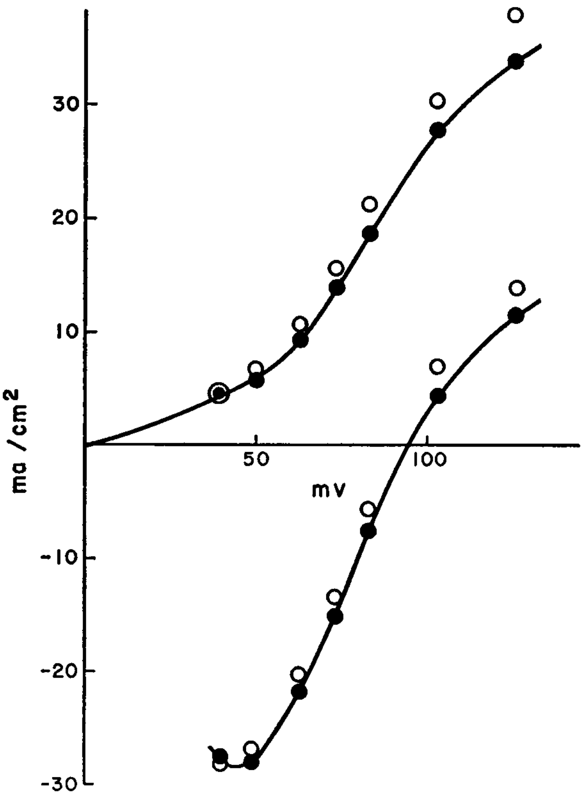

Figure 6 Current voltage relationships for the transient and steady-state currents. The solid circles represent the control runs before the laser pulse was fired. The open circles represent the currents after the laser pulse, thus at a higher steady-state temperature. The time of the laser pulse is the same as in Fig. 5.

pretation of the decrease in the transient current with increased temperature is that the increased leakage conductance is greater than the increased peak sodium conductance.

The observation that there was a greater effect of a temperature jump on the steady-state currents at high depolarizations compared to low depolarizations is consistent with a constant identical increase in the steady-state conductance at all polarizations. Such a change in the conductance would show a greater difference in currents for a large compared to a small driving force.

Temperature-Jump Relaxation Experiments

Like the steady-state measurements the relaxation experiments indicate that the voltage-dependent ionic conductance must be turned on for significant temperature effects to be observed. A striking finding of these studies was that, at moderate depolarizations and apart from the photo-artifact, a temperature jump shifted the

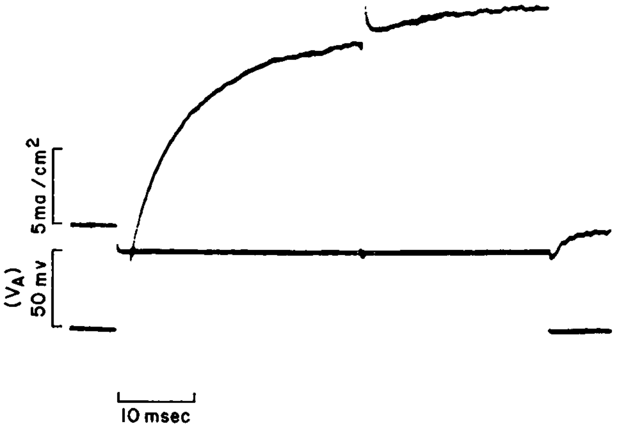

FIGURE 7 Effect of laser pulse during the steady-state current. The laser pulse was fired at the break in the steady-state current. The upper trace is the membrane current. The lower trace is the membrane potential.

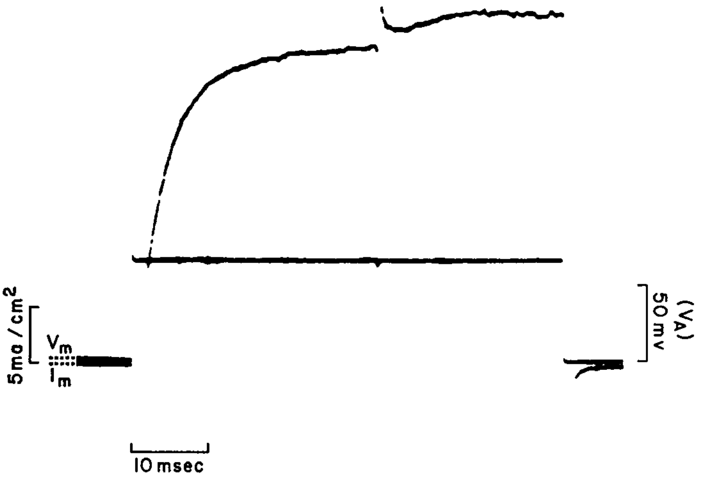

FIGURE 8 Effect of laser pulse at high depolarizations. The laser firing occurred at the break in the steady-state current. The resting values of the membrane potential V_m and the membrane current I_m are indicated by the upper and lower dashed lines, respectively.

potassium current from one steady-state value to another. This result is illustrated in Fig. 7 where the laser pulse was fired during the turning on of the potassium current. The transition period was approximately the time course of the laser pulse which indicates that the relaxation time of the steady-state potassium conductance is of the order of a millisecond or less. It is therefore imperative to use a shorter laser pulse to determine these relaxation times.

The time course of the delayed current in Fig. 7 after the temperature jump is consistent with the hypothesis that the usual voltage-dependent potassium conduct-

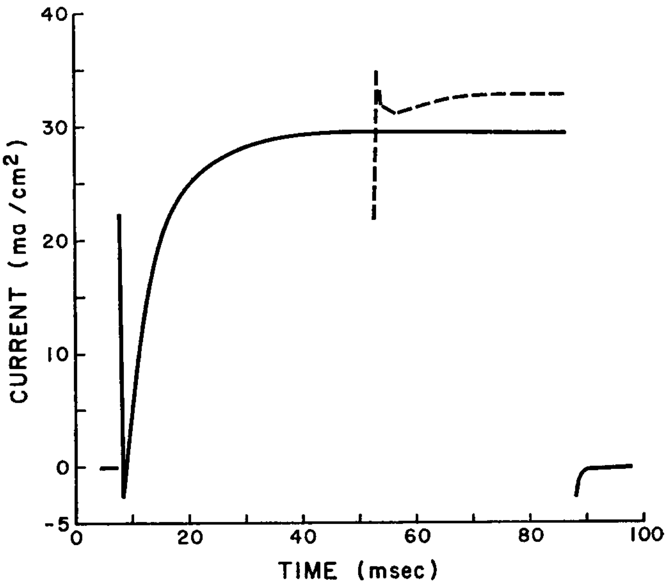

FIGURE 9 Temperature jump during the steady-state current. The dashed line indicates the response due to the laser pulse. The tracing includes part of the capacitative current as observed on the original record. A cathodal polarization of 90 mv occurred at the break in the resting current.

ance is relaxing to its steady-state value with the τ_n relaxation time characteristic for the temperature and electric field across the membrane. These results are consistent with the steady-state temperature measurements of Frankenhaeuser and Moore (1963) and Moore (1971) which showed that n_∞ of the Hodgkin-Huxley formulation (Hodgkin and Huxley, 1952) was relatively independent of temperature (see Discussion).

Figs. 8 and 9 illustrate that at very high depolarizations a T-jump applied during the steady-state current did show a marked slow relaxation time in addition to the previously observed rapid shift in the potassium current.

The temperature jumps during the time course of the sodium inward current shown in Fig. 10 also showed sudden displacement of the current during the temperature jump. The kinetics of the sodium conductance are too rapid to permit any comment about relaxation times with the normal mode laser output.

DISCUSSION

From a slightly generalized point of view the kernel of the Hodgkin-Huxley analysis rests in the demonstration that all one needs to know about the history of the nerve can be summarized in the present values of three internal state variables designated m, n, and h (see Hodgkin and Huxley, 1952) which moreover change according to rather simple dynamics. One might question the exact forms chosen for the under-

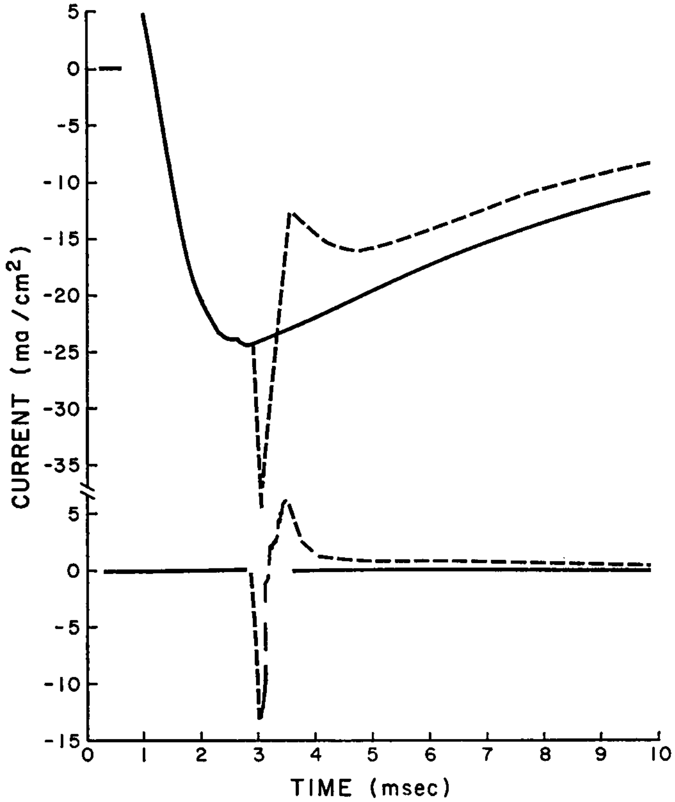

FIGURE 10 Temperature jump during the initial transient current. The dashed line of the upper pair of traced curves indicates the effect of the laser pulse on the transient current at a cathodal polarization of 50 mv. The dashed line of the lower curve is a tracing of the laser artifact at the holding potential.

lying dynamics and the output mapping from the state variables to the membrane current; however, the success of this model serves to show that a small number of processes not directly accessible to experiment is involved in the determination of the membrane conductance. The existence of these degrees of freedom suggests immediately the correspondence with relaxation phenomena.

The current across the nerve membrane at any moment is determined by the driving force and the membrane permeabilities. The permeabilities depend by some simple output mapping only on the state of the membrane, given by the values of a small number of internal variables. Each of these variables possesses a definite equilibrium or steady-state value dependent on electric field, temperature, and perhaps pressure and calcium concentration.

Consequently, if one suddenly changes the electric field or the temperature, the system should relax along internal reaction coordinates to a new "equilibrium." The time constants for this relaxation then carry kinetic information in their depend-

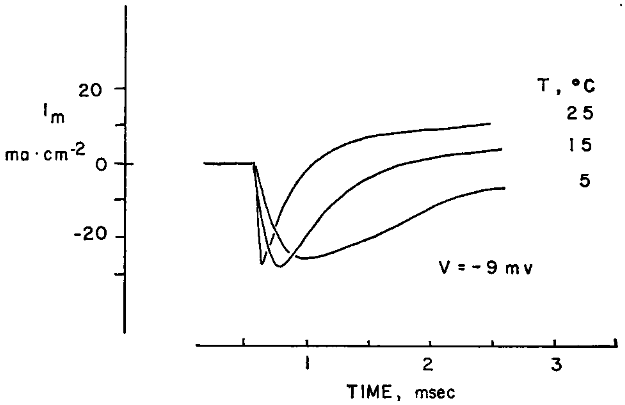

FIGURE 11 Computation of the steady-state temperature effect on the voltage clamp currents. Three voltage clamp currents were computed at 5, 15, and 25°C. The voltage clamp occurred at the break in the horizontal line. All potentials in the figures illustrating the computations are given as the internal minus the external potential. See text for details.

ence on the composition variables. From this point of view, the Hodgkin-Huxley-Katz experiments were relaxation experiments—electric field jumps (Moore and Jakobsson, 1971).

The sensitivity of the ionic conductances to temperature is illustrated in Fig. 11 where the Frankenhaeuser-Huxley equations (Frankenhaeuser and Huxley, 1964) were used to compute membrane currents for three different temperatures at voltage clamp steps from -70 to -9 mv. Given such steady-state temperature dependence, changing the temperature suddenly at a constant electric field ought to produce a relaxation which could be analyzed into a spectrum of time constants which in fact ought to be identical with the voltage clamp—determined time constants for the same final conditions of membrane potential and temperature.

At first thought assertion of identity between V-jump and T-jump time constants may seem without foundation; however, for times greater than those involved in, e.g., equipartition of energy, the system ought to be without memory given the specification of the proper number of internal state variables. The system "knows" only that it does not have the configuration required for equilibrium under the constraints (constant T, p, E); it is "ignorant" of why it does not, whether it was formerly at a different temperature or at a different electric field. Thus as long as the conditions are close enough to equilibrium, the time constants of the relaxation spectrum will be essentially the same for all modes of perturbation for the same set of final constraints. The magnitudes of departure from equilibrium along the various coordinates will differ for different perturbations. Thus the relative prominence of the various components of the sum of exponentials will vary and cause differing time courses. Nonetheless, the set of time constants will be the same. These statements are of course subject to some second-order qualification: for example, in an actual T-jump there may in fact be some pressure variation. The essential identity

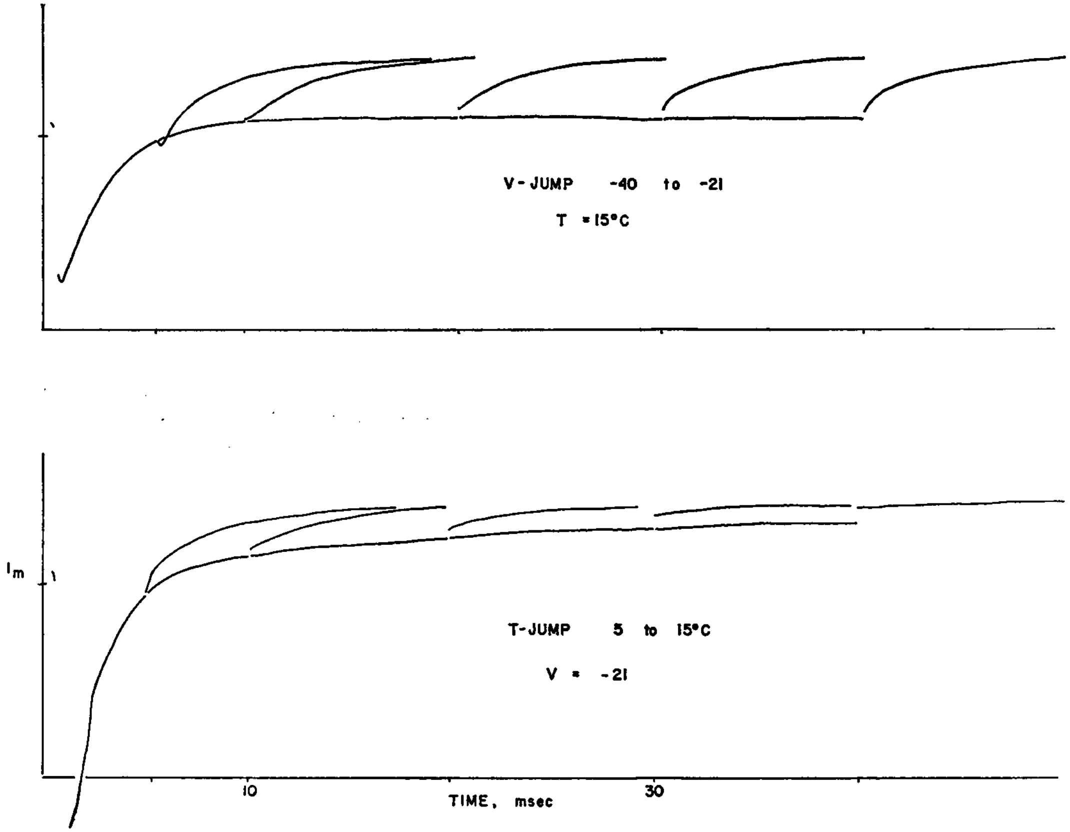

FIGURE 12 Computation of double-pulsed voltage clamp currents and temperature jumps during the delayed current. The upper record illustrates the effect of a second voltage step to −21 mv at increasing time intervals from the initial voltage step to −40 mv. The lower record demonstrates the effect of a 10°C temperature jump for a cathodal polarization to −21 mv at increasing time intervals from the onset of the voltage clamp. Abscissa, time; ordinate, membrane current where the single cross mark of each set of computations represents zero current. For calibration the peak inward current of the upper curve is 12 ma/cm^2. Note that the final voltage and temperature are identical for both sets of computations.

of the spectra, however, is a good assumption and has indeed been in general use for some years among workers using relaxation methods to study chemical kinetics.

Illustrated in Figs. 12 and 3 and seemingly contrary to these expectations, computation of the membrane current during a voltage clamp and temperature jump show that when the membrane current has reached a steady state after a voltage step, a sudden change in temperature causes only instantaneous shifts due to changes in scale factors.

Actually, this result is to be expected and rests on the following property of the Frankenhaeuser-Huxley analysis. The dynamics of the state variables are taken as first-order differential equations; thus two parameters are involved for each variable,

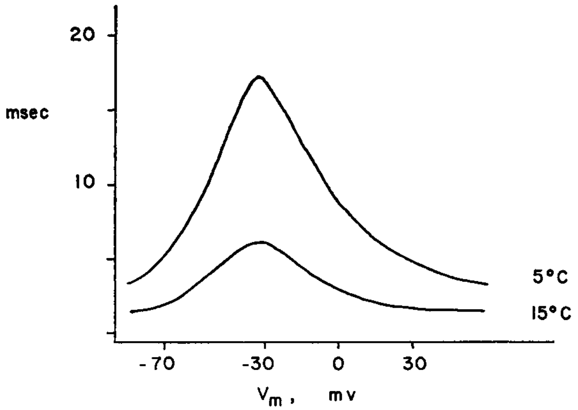

FIGURE 13

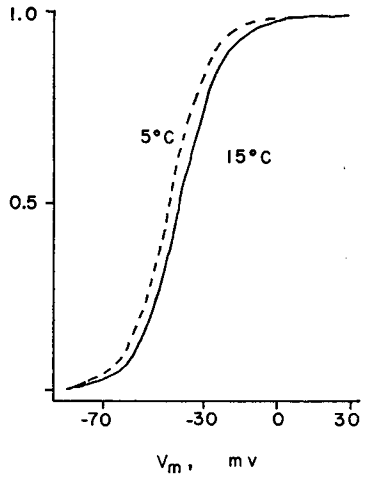

FIGURE 14

FIGURE 13 Computation of the voltage dependence of τ_n. Abscissa, membrane potential; ordinate, msec.

FIGURE 14 Computation of the voltage dependence of n_∞. The dashed line indicates the shift in n_∞ assuming a difference in the temperature dependence of α_n and β_n. The energy of activation for α_n was 9 kcal/mole-degree and for β_n, 16 kcal/mole-degree. See text for details.

the steady-state value n_∞ and the time constant τ_n. However, experimentally one determines the permeability, e.g., $P_K = P'_K n^2$. For this determination, τ_n can be unambiguously evaluated, but P'_K and n_∞ are bound together. In the Frankenhaeuser and Moore (1963) temperature investigations the temperature dependence was determined in regions where n_∞ was believed to be either 0 or 1. One then makes the assumption that the temperature coefficient of τ is independent of the potential and uses these values for computing changes in τ at all levels. Fig. 13 shows computed values of τ_n at two different temperatures.

The experimental finding that τ_n had almost identical temperature dependence at both extremes of n_∞ means that the curve representing the dependence of n_∞ on the membrane potential is essentially unchanged by changing the temperature.

Fig. 14 shows the shift one might have anticipated for n_∞. In fact, no shift was observed, and the curves for different temperatures were superimposed. Thus, changes in the steady-state magnitude of the currents at different temperatures reside only in changes in the "scale factors" such as P'_K. These factors must change rather more rapidly than the state variables or they would complicate the ordinary voltage clamp kinetics. For lack of information it has been assumed for the computations that P'_K changes instantaneously, although as a matter of fact temperature-jump techniques can be applied to a direct study of these faster processes.

These considerations have a number of implications:

(*a*) a temperature-jump at steady-state voltage clamp should be a useful way to study the kinetics of the scale factors;

(*b*) a temperature jump will not be an effective way to study the ordinary m, n, and h kinetics except in combination with voltage clamp steps;

(*c*) the separation of the permeabilities into scale factors and state variables has some more independent physical significance than one might have expected; and

(*d*) the possibility that n_∞ vs. voltage curve is temperature insensitive must put powerful constraints on models.

The T-jump shown in Figs. 8 and 9 at high depolarizations showed a prominent long relaxation time. In order to observe clearly the T-jump relaxation time in the steady-state current it was necessary to have a large potassium conductance and a maximum energy pulse from the laser. In general no long relaxation times of the steady-state current were observed for low potassium currents and smaller T-jumps.

A further complication in comparing the experimental results with the Frankenhaeuser-Huxley model is the effect of increased temperature on the leakage conductance. A temperature jump preceding a voltage clamp run at the sodium equilibrium potential is shown in Fig. 5. The leakage current as represented by the delay in the potassium current was clearly increased by an increased temperature.

In summary, the temperature jump relaxation experiments have shown that the laser pulse perturbs the peak conductance represented by $\overline{P_K}$, the leakage conductance, and at high depolarizations the steady-state condition of the potassium gating mechanism represented by the voltage-dependent variable n. The rapid and unresolved relaxation times indicate that the limiting conductance of the membrane involves fast processes, possibly relaxations of transition assemblies from one substate to another (Lindley, 1968). The slower relaxation times probably represent the voltage-dependent process normally elicited by a voltage clamp experiment.

In the present work we have established what is, to our knowledge, the first experimental justification of the separation of permeability into scale factor and normalized variable terms, a feature of the Hodgkin-Huxley analysis which heretofore was simply one of convenience. We have in addition established an upper limit for the relaxation time of the scale factors.

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