Biochimica et Biophysica Acta, 375 (1975) 115–123

BBA 76835

MEMBRANE CONDUCTANCE CHANGES IN SINGLE NODES OF RANVIER, MEASURED BY LASER-INDUCED TEMPERATURE-JUMP EXPERIMENTS

L. E. MOORE

Department of Physiology, School of Medicine, Case Western Reserve University, Cleveland, Ohio (U.S.A.)

(Received June 17th, 1974)

SUMMARY

Temperature-jump experiments on isolated myelinated nerve fibers were done using a pulsed laser system in the Q switched mode. Voltage-clamp and temperature perturbations were used to measure the relaxing ionic conductances of both the Na^+ and K^+ systems. It is shown that the *T* jump can be used to probe the K^+ and Na^+ conductances during non-steady state conditions and thereby elicit relaxation times for a variety of initial states. Temperature-induced K^+ conductance relaxation times were consistent with voltage-clamp measurements. The temperature-perturbation experiments were done as a combination of a temperature step and impulse change due to an adsorption of carbon black particles on the nerve. The experiments support the hypothesis that the relaxation times of the K^+ system are independent of the previous history of the axon. It is concluded that the K^+ conductance is at least a second-order system whose relaxation spectrum is composed of two exponential terms the magnitudes of which are markedly dependent on the initial conditions.

INTRODUCTION

Relaxation spectrometry provides a basic method for the kinetic analysis of membrane phenomena. The combination of the voltage clamp and temperature jump in the laser-induced temperature jump (*T* jump) has shown that the complex processes involved in the K^+ conductance of single nerve fibers can be separated into two parameters, one which is voltage independent and temperature dependent and another which is voltage dependent and temperature independent [1].

This paper describes a modification and extension of the combination voltage-clamp and *T*-jump methods to measure the relaxing ionic conductances of single nerve fibers. It is shown that the *T*-jump can be used to probe the K^+ and Na^+ conductances during non-steady state conditions and thus elicit relaxation times for

a variety of initial states. K^+ conductance relaxation times are demonstrated for different external K^+ concentrations.

METHODS

The basic temperature jump and voltage-clamp methods used in these experiments were similar to those described in an earlier paper [1]. All experiments were done with single fibers dissected from *Xenopus laevis*. The composition of the Ringers solution used was 114 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, and 1–2 mM Na_2HPO_4–NaH_2PO_4 buffer adjusted to pH 7.2. The high K^+ solution was 116.5 mM KCl, 1.8 mM $CaCl_2$ and contained the same phosphate buffer as the Ringer's solution.

As shown by others [2] a principal difficulty in the laser T jump is due to strong mechanical oscillations caused by the pressure shock initiated by the laser pulse. It has been shown [3] that this disturbance can be reduced by eliminating pressure reflections at the chamber walls. The T-jump chamber for single nerve fibers used in previous experiments was not a closed system but nevertheless showed this type of disturbance as manifested by the splashing of fluid out of the chamber pools when the laser light was directed through the glass cover slip of the chamber floor. In the present experiments it was found that passing the laser light through the top of the chamber considerably reduced the pressure artifacts. Therefore, the laser light in these experiments passed through an air–solution interface at the top of the chamber rather than an air–cover glass–solution interface from the bottom of the chamber as in previous experiments. Under these conditions it was found that successful T jumps could be done with the focal point of the focusing lens in the solution surrounding the nerve fiber.

A further difficulty with the laser-induced T jumps is the low signal-to-noise ratio due to the poor absorption of 1.06 μm laser light in aqueous solutions. This problem has been partially solved by shifting the laser light wavelength to a more favorable absorption region using the stimulated Raman conversion [4]. Similarly the addition of dyes could be expected to increase the size of the jump. In the experiments reported here the absorption of the laser light has been increased by the addition of carbon-black particles to the Ringer solution bathing the single node of Ranvier. Suspensions of carbon-black particles were made using India ink (A. W. Faber-Cartell Higgens No. 4425). One drop of India ink was placed in 50 ml of Ringer solution and thoroughly mixed before perfusing the single nodes. The presence of carbon-black particles had no effect on the voltage-clamp currents recorded from these fibers. The amount of energy per laser pulse required to observe a temperature response in the ionic conductance was greatly reduced by the carbon black and made possible the use of the T jump as a routine experimental procedure.

In all of the figures the current is expressed as V_E, the output of the clamping amplifier. This can be converted into membrane current (I_M) by the relation, $I_M = V_E/A_N Z_{ED}$ where A_N is the area of the node and Z_{ED} is the impedance from the current injecting point to the center of the axon at the node [5].

The laser used in these experiments was a Korad (Union Carbide) K-1 system with a neodymium rod and a flowing dye, passive Q switch attachment. The pulse duration used was 200–300 μs.

RESULTS

In a previous paper the hypothesis was put forward that the response of the steady state conductance to a temperature perturbation would not involve a change in the voltage-dependent component of the K^+ conductance. However, if the perturbation occurs before the conductance has reached a steady state then it should relax to a new steady state condition with a τ_n relaxation time determined by the membrane potential and final temperature. The relaxation time measured under these conditions is identical to that of the Hodgkin–Huxley formalism (τ_n). The two superimposed temperature-jump experiments shown in Fig. 1 illustrate this type of behavior. In both temperature jumps there are present short unresolvable relaxation times which are due to changes in the peak K^+ conductance, the leakage conductance, and possibly changes in the membrane capacitance.

These rapid relaxation have not been resolved for two reasons, (1) the frequency response of the node clamp is not sufficient for time constants below 50 μs and (2) the nerve membrane is irreversibly damaged when the laser pulse duration is less than 1 μs.

A complication in the use of carbon black to absorb the laser energy results from the adherence of carbon-black to the nerve itself. The effect of the increased accumulation of carbon black on the nerve leads to a greater temperature change in the nerve than occurs in the surrounding solution and a correspondingly rapid cooling of the nerve to the temperature of the solution. An estimate of the initial cooling time can be made by considering the nerve as a cylinder at an arbitrary temperature located in an infinite bath at some other temperature. The time constant for heat diffusion in this case is given by the expression $\tau = r^2/(2.4)^2\kappa$ [6] where r is the radius of the cylinder and κ is the thermal diffusivity of water in c.g.s. units. Using $r = 7$ μm and $\kappa = 0.0014$ cm^2/s leads to $\tau \approx 50\mu$s. Thus, if inhomogeneities in the tem-

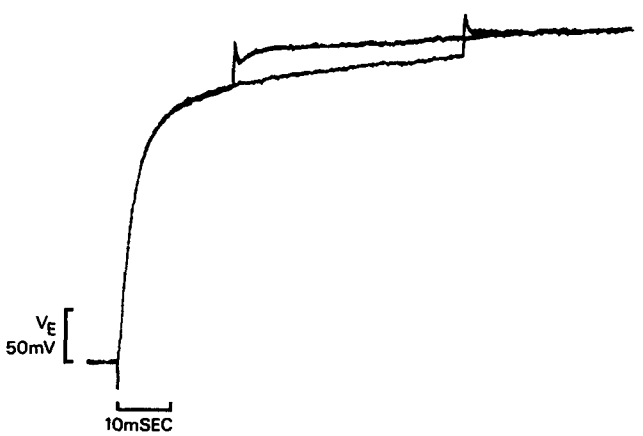

Fig. 1. Effect of T jump during rising phase of K^+ current. The laser pulse in this and all subsequent figures occurred at the break in the current record. The holding potential in this and the following experiments was about −60 mV and the temperture was 7–9 °C. The step potential in the two superimposed runs was a depolarization of 58 mV. The Na^+ transient is not faithfully reproduced in the photographic record. Unless otherwise stated the experiments were done in Ringer's solution (see text). Fiber 20.

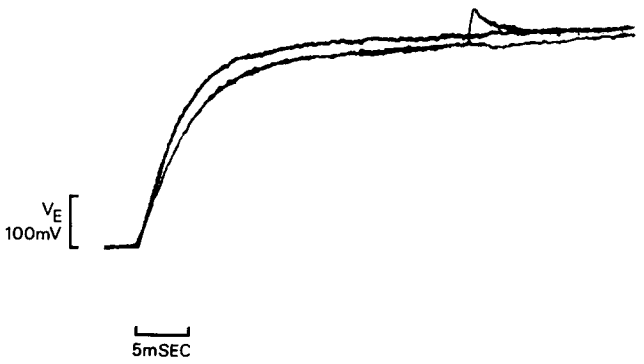

Fig. 2. Impulse response of K^+ current. The lower curve is the control voltage-clamped current for a cathodal polarization of 58 mV. The continuous upper record is the outward current recorded after a *T* jump which occurred 2 ms before the step change in membrane potential and thus is the current at a new steady-state temperature. The third superimposed voltage-clamp recording is shown by the lower curve until the break in the current occurs, which then shows an impulse relaxation to the new steady state value at elevated temperature. Fiber 21.

perature do occur the temperature profile with time can be approximated by an impulse perturbation superimposed on a step function. The two temperature jump experiments of Fig. 2 illustrate the nature of this type of perturbation. The upper continuous curve is the response to a steady-state temperature change in the solution and nerve as a result of a temperature jump preceding the voltage-clamp pulse. The *T* jump applied during the rising phase of the outward current shows an impulse response of the steady state conductance which relaxes to the same final steady state condition as the first run at the elevated temperature. The time constant of the impulse response is similar to the τ_n measured by a voltage-clamp perturbation. It is thus possible to apply a temperature pulse to the nerve and elicit the slower relaxation times for the K^+ conductance near its steady state value. The more rapid relaxation times are undoubtedly present in the response but are not measured due to the additional

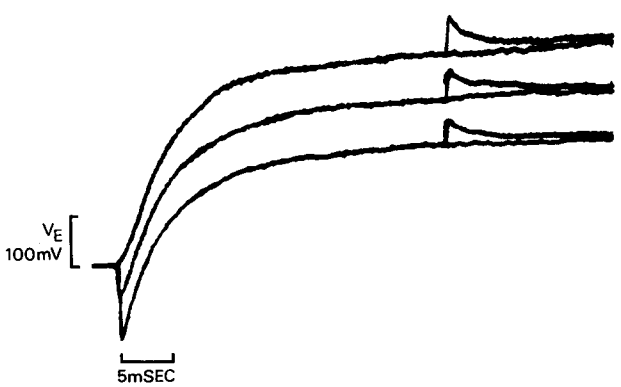

Fig. 3. Response of K^+ current to temperature pulses. The cathodal polarizations of the three pairs of superimposed voltage clamp runs from top to bottom were 58, 49 and 41 mV. Fiber 21.

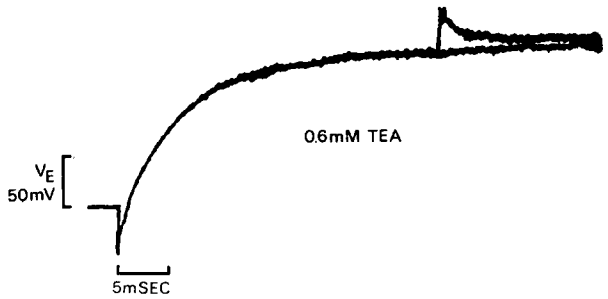

Fig. 4. *T*-jump experiment in tetraethylammonium chloride. The cathodal step polarization was 49 mV. Fiber 21.

cooling time constant and the limited frequency response of the voltage-clamp system. Fig. 3 illustrates the same experiment for three voltage-clamp runs.

Since *T* jumps during the steady state showed extremely rapid relaxation times this method provides a way of measuring the kinetic behavior of agents which interact with the specific K^+ channels. One such compound is tetraethylammonium chloride, which decreases the K^+ conductance when applied in the external solution. The experiments shown in Fig. 4 show that despite a considerable reduction in the steady-state current by tetraethylammonium chloride the relaxation response to the *T* jump is similar to the corresponding control run of Fig. 3. The conclusion of this and other experiments at different tetraethylammonium chloride concentrations is that the additional relaxation time for tetraethylammonium ions binding to the membrane is less than 50 μs.

If the *T* jump response measured is principally due to changes in the K^+ conductance it would be possible to reverse the direction of the temperature-induced

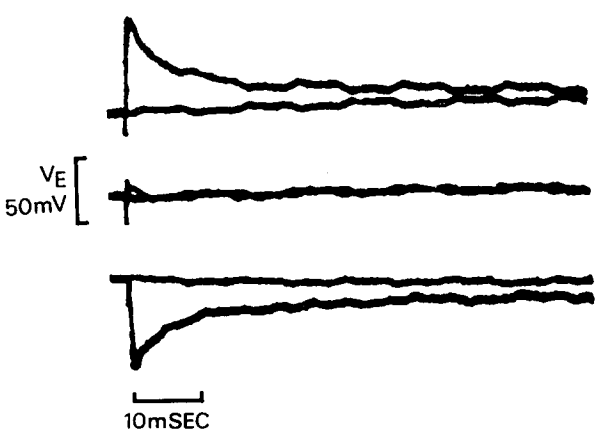

Fig. 5. *T*-jump experiments in high K^+ solution. Three pairs of superimposed records of voltage-clamp currents are shown for cathodal steps of 63, 45, and 18 mV from top to bottom. The lower mark of the V_E calibration scale represents 50 mV rather than 0 mV as in all previous records. These records show only the last 70 ms of a 100-ms pulse. Note that the response to the temperature jump is an increase in the inward current in the lower pair of records, no change in current for the second depolarization, and an increase in the outward current for the upper pair of records. Fiber 30.

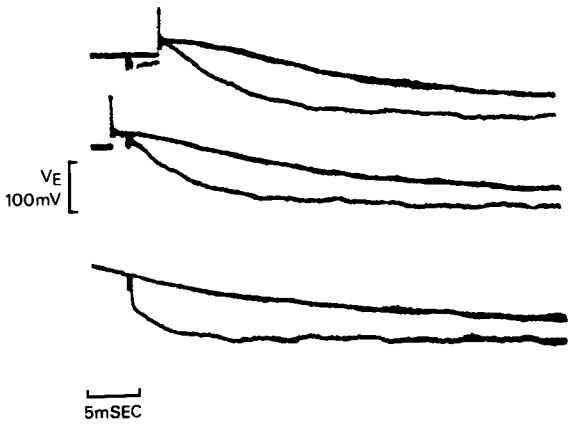

Fig. 6. Delayed inward K^+ currents in high K^+ solution during *T*-jump experiments. Three pairs of superimposed records of voltage-clamp currents measured in isotonic KCl are shown for a single cathodal step of 18 mV. The two upper pairs show the resting current level before the voltage-clamp step or *T* jump. The *T* jump precedes the voltage step in the upper record and follows it in the middle record. The lower record is a continuation of the upper records. As before the *T* jump occurs at the discontinuity in the current which occurred 7 ms after the beginning of the voltage step. Note that in these records the response to the *T* jump is always an increase in the inward current. The location of the calibration scales in this figure is arbitrary. Fiber 30.

K^+ current by changing the external K^+ concentration and stepping the potential to either side of the K^+equilibrium potential (E_K). The temperature-jump experiments in high external K^+ solution of Fig. 5 show a reversal in current direction about the steady current reversal potential. The relaxation spectra are of the impulse response type and clearly demonstrate that the principle effect of the temperature perturbation is on K^+ conductance. The lack of response at the reversal potential indicates there are no other conductance changes induced by a temperature jump applied when the current has nearly reached a steady state.

Experiments with a high external K^+ concentration allow a further test of the hypothesis that the slow relaxation times (ms) induced by a temperature jump before the voltage-induced K^+ conductance (*n* system) is in the steady state are similar to the voltage-clamp time constants (τ_n). The experiments illustrated in Fig. 1 show that the slow ms time constant is independent of the time during a voltage-clamp pulse at which the *T* jump is applied, however, as the steady state is approached, the magnitude of the τ_n component diminishes. Temperature impulses applied during the time course of the inward K^+ currents of Figs 6 and 7 show dramatic differences in response depending on the time of the temperature perturbation. This finding is consistent with the previous result if one takes into consideration the impulse response characteristics of a system in a transient state. The total increase in the conductance during the temperature impulse is proportional to the value of the conductance. At short times after the voltage step the conductance is low and the time course of the response to the temperature perturbation is mainly determined by the relaxation of the *n* system to the voltage step and new steady-state temperature. There is no peaking in conductance because the temperature impulse did not increase the conductance above its

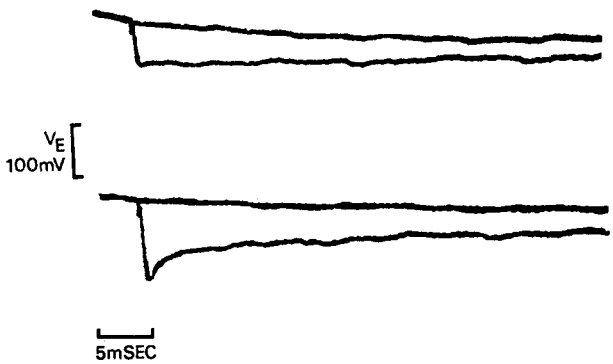

Fig. 7. Response of delayed inward K^+ current in high K^+ solution to temperature perturbation. These records are a continuation in time of those in Fig. 6. The *T* jump of the upper pair occurred 25 ms after the voltage step. In the lower pair the *T* jump occurred 50 ms after the voltage step. The location of the calibration scales is arbitrary in this figure. Fiber 30.

final steady-state value. At later times in the voltage-clamp pulse the temperature impulse does increase the conductance above its steady-state value and the corresponding peaking is observed since the conductance must relax to its lower final value. As the conductance increases during the voltage-clamp step, the temperature pulse response is greater. This has the effect of increasing the rate of rise in the response of the conductance to the *T* jump as a function of the duration of the voltage-clamp pulse.

The application of the temperature-perturbation method to systems in a transient state can be extended to studies of the Na^+ permeability. A temperature jump applied during the peak of the inward Na^+ current is illustrated in Fig. 8. The increased current seen after the temperature perturbation has a time course similar to that observed with voltage-clamp steps.

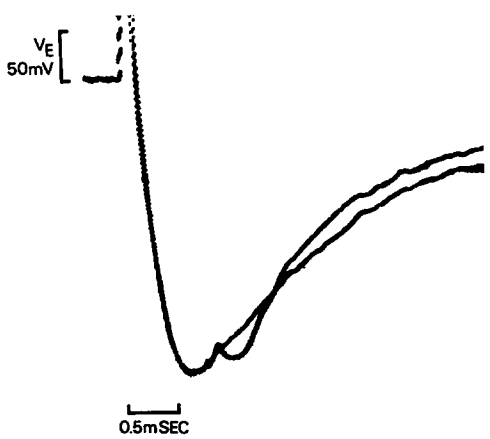

Fig. 8. Response of Na^+ current to temperature perturbation. The *T* jump occurred 0.7 ms after the cathodal step of 25 mV. The control record is the typical continuous inward current. Fiber 20.

DISCUSSION

These experiments have shown that for non-steady state conditions it is possible to detect a temperature-induced relaxation time. The measurements were done using a temperature perturbation whose profile with time was an impulse superimposed on a step change due to a concentration of carbon-black particles on the nerve itself. The treatment of the temperature pulse as an impulse function is only valid for small axons and relaxation times greater than 50 μs. Shorter relaxation times could be resolved if the exponential fall of the temperature was considered.

These experiments support the hypothesis that the relaxation time of the K^+ system is independent of the previous history of the K^+ conductance. However, the magnitude of a particular relaxing component, such as the process described by τ_n, is markedly dependent on the initial condition of the membrane and therefore difficult to resolve as the conductance approaches its final steady-state value.

Consideration of the K^+ system as observed in a typical voltage-clamp experiment suggests a process consisting of multiple relaxation times. In the Hodgkin–Huxley formalism these multiple time constants are expressed through a single exponential function raised to a power greater than one. This is equivalent to a sequential unimolecular model having dependent time constants [7].

The data of Cole and Moore [8] and Moore [9] suggest that the magnitudes of the more rapid relaxing processes are dependent on the initial conditions, namely the preceding membrane potential and the external Ca^{2+} concentration. As a first approximation to the voltage-clamp relaxation spectra, a two-time constant model for the K^+ conductance has been applied to T jump data [1]. This analysis showed that both of the time constants were temperature dependent (see Table I of ref. 1). The analysis has been extended in experiments on the effect of prepulses and external Ca^{2+} concentrations on the delay of the K^+ currents measured at the Na^+-equilibrium potential (Moore, L. E. unpublished). These experiments showed that for a single voltage-clamp step at the Na^+-equilibrium potential, the translation of the current versus time seen with changes in the holding potential or external Ca^{2+} concentration can be described by varying only the coefficients of two exponential terms.

Further support for multiple time constants in the voltage-relaxation spectra of the K^+ system is found from voltage-clamp data on single fibers in solutions containing a high K^+ concentration. The experiments of Frankenhauser [10] show that the delay in the K^+ current is equally pronounced for small or large voltage steps. An extrapolation of these measurements to small signals does not suggest a limiting single relaxation response. A single time constant for small signals would be expected if the K^+ conductance was described by the Hodgkin–Huxley formalism [11].

It is therefore concluded that the K^+ conductance is at least a second-order system whose relaxation spectrum is composed of two exponential terms the magnitudes of which are markedly dependent on the initial conditions. Only one time constant was observed in the T jump experiments because all of the perturbations were done at large depolarizations. At large depolarizations it has been shown that small signal voltage steps lead to a single observable relaxation time [12] as would be expected if the magnitude of the more rapid component is small.

The analysis of large pulse voltage clamp data in terms of linear relaxation theory is dependent on the assumption that the time constants and their number

are independent of the previous history of the axon membrane. The number of exponential terms describing the Na^+ conductance is invariant for a large number of experimental conditions, however, the delay in the steady state current is markedly dependent on the preceding potential [8]. Despite the apparent change in number of relaxation times, the principal time constant, τ_n, does remain invariant for different initial conditions. The experimental results of Moore [12] suggest that the apparent change in the number of relaxation times is an alteration in the magnitude of the rapid relaxing processes such that the rapid components are experimentally unresolvable for initial conditions when the membrane is depolarized.

The conclusion that relaxation times of the K^+ conductance depend on the step potential and not the holding potential means that the linear relaxation theory can be used to analyze voltage-clamp data at all potentials. The recent data of Brismar and Frankenhauser [13] on the dependence of steady state currents on the external Ca^{2+} concentration suggests that a relaxation model similar to that previously described for the Na^+ system [14] would fit the kinetics of K^+ conductance.

ACKNOWLEDGEMENT

This work was supported by a grant from the U.S. Public Health Service (NB08409).

REFERENCES

1 Moore. L. E., Holt, Jr, J. P , and Lindley, B. D. (1972) Biophys. J. 12, 157–174
2 Hoffman, H., Yeager, E. and Stuehr, J. (1968) Rev. Sci. Instrum. 39, 649–653
3 Koffer, H. (1971) Ber. Bun. Ges. 75, 1245–1247
4 Beitz, J. V., Flynn, G. W., Turner, D. H. and Sutin, N. (1970) J. Am. Chem. Soc. 92, 4130–4132
5 Dodge, F. A. and Frankenhauser, B (1958) J. Physiol. 143, 76–90
6 Jost, N. (1960) Diffusion pp. 45–46, Academic Press, New York
7 Armstrong, C. (1969) J. Gen. Physiol. 54, 553–575
8 Cole, K. S. and Moore, J. W. (1960) Biophys. J 1, 1–14
9 Moore, L. E. (1971) Am. J. Physiol. 221, 131–137
10 Frankenhaeuser, B. (1962) J. Physiol. 160, 40–45
11 Mauro, A., Conti, F., Dodge, F. and Schor, R. (1970) J. Gen. Physiol. 55, 497–523
12 Moore, L. E. (1967) J. Physiol. 193, 433–442
13 Brismar, T. and Frankenhaeuser, B. (1972) Acta Physiol. Scand. 85, 237–241
14 Moore, L. E. and Jacobsson, E. (1971) J. Theor. Biol. 33, 77–89