BRIEF COMMUNICATION

TEMPERATURE EFFECTS ON GATING CURRENTS IN THE SQUID GIANT AXON

FRANCISCO BEZANILLA, *Department of Physiology and Brain Research Institute, University of California School of Medicine, Los Angeles, California 90024,* AND
ROBERT E. TAYLOR, *Laboratory of Biophysics, National Institute of Neurological and Communicative Disorders and Stroke, National Insitutes of Health, Bethesda, Maryland 20014 U.S.A.*

ABSTRACT The effects of temperature (3°–26°C) on the nonlinear components of the displacement current were measured in internally perfused, voltage clamped squid axons. Steps of potential were applied from a holding potential of -70 mV (outside ground) to values from -130 to $+70$ mV and either the current or its integral (charge) was recorded as a function of time. For that component of the charge movement not linearly related to voltage, the total charge moved in a few milliseconds (about 1,500 electronic charges/μm^2) between saturation limits (e.g. -100 mV to $+50$ mV) showed an apparent increase of 13 ± 5% for a 10°C rise in temperature. Attempts to fit the falling phase of the gating current (or charge) with the sum of two exponentials showed temperature effects on both components but there was considerable scattering. At short times, records for current or charge made at 16°C, expanded by a factor α, superimposed on those made at 6°C for α about 1.6. For long times α was about 2.3.

In many excitable membranes, a component of the capacitative current has been found to be nonlinearly related to membrane voltage. In squid axon, a large fraction of this asymmetrical displacement current has been identified with the molecular rearrangements leading to the opening and closing of the sodium channels (Armstrong and Bezanilla, 1974; Keynes and Rojas, 1974; Meves, 1974), and for this reason they have been called gating currents. A comprehensive study and description of gating currents is expected to contribute to the understanding of gating phenomena in excitable membranes.

We report here the effect of temperature on gating currents studied in perfused and voltage clamped squid giant axons. The general technique has been described previously (Bezanilla and Armstrong, 1977). Two modifications were introduced in our experimental setup and recording technique. First, the command pulses feeding the voltage clamp apparatus were generated by a digital-to-analog converter connected to a Nova 3 digital computer (Data General Corp., Westboro, Mass.) and the data were

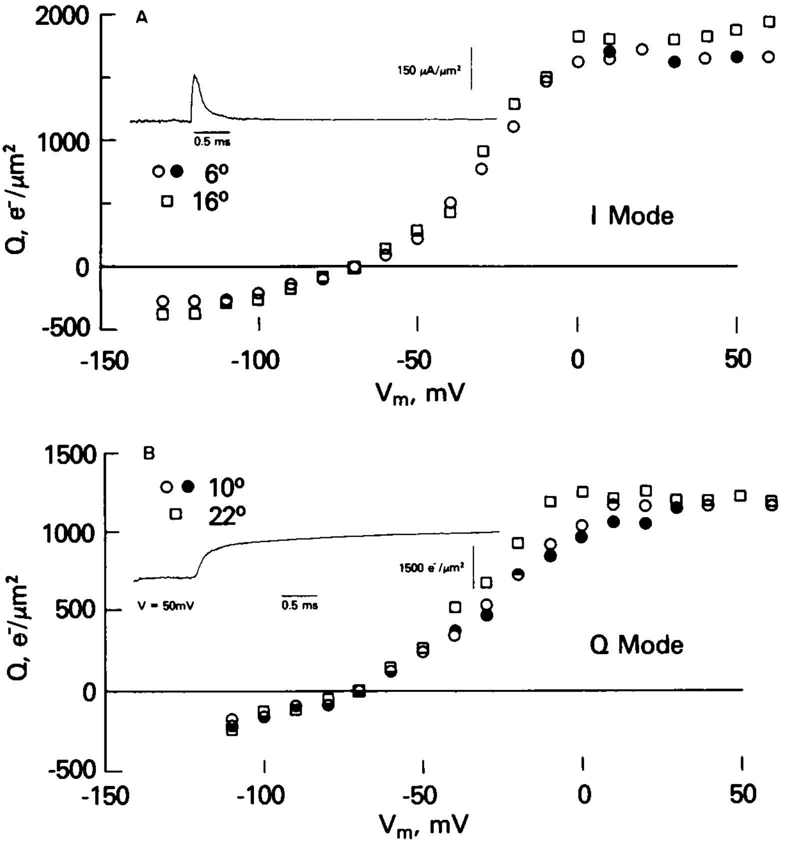

FIGURE 1 A. Total charge (in electronic charges/μm^2) moved in a few milliseconds obtained by integration of asymmetrical displacement current (*I* mode) after a step change in membrane potential from −70 mV to that indicated. *Inset* shows a representative current vs. time curve. B. Charge movement as in A but obtained by analog integration (*Q* mode). *Inset* shows representative curve of charge vs. time. Filled circles are recovery points.

acquired by a sample-and-hold and analog-to-digital converter connected directly to the same computer. All data acquired were stored in diskettes. The second modification was the acquisition, in some experiments, of the total asymmetric charge (*Q* mode) instead of the asymmetric current (*I* mode). This was done using the same P/4 pulse sequence described previously but the current was integrated with an analog integrator before subtraction and storage. This procedure is particularly useful in recording the charge at high temperatures when the rising phase and the early falling phase are too fast to be described accurately with a sampling rate of 10 µs/point.

The inset in Fig. 1 A shows a typical record of current vs. time and the inset in Fig. 1 B shows a record of charge vs. time, each for a step of potential from a holding potential (HP) of −70 to +50 mV at 6°C and sampling every 10 µs. To remove the components of charge movements linearly related to voltage the P/4 averaging technique was used (see Bezanilla and Armstrong, 1977). In this case subtracting pulses of

TABLE I

EFFECT OF TEMPERATURE ON GATING CURRENTS

Experiment	T_1	T_2	$\Delta Q_2/\Delta Q_1$	α_F	α_S	$(\tau_1/\tau_2)_F$	$(\tau_1/\tau_2)_S$	Mode
Jul. 29B	9	17	—			1.7–2	1.0–2.0	*I*
Aug. 3C	11	26	1.10			2–2.3	1.4–2.0	*I*
Aug. 31A	5	13	1.19			1.3–1.5	1.6	*I*
Sept. 3A	6	16	1.13	1.5	1.6–2	1.4–2.3	2.4	*I*
Aug. 11B	10	22	1.14					*Q*
Aug. 13A	10	22	1.18					*Q*
Aug. 13A	6	16	1.07	1.7	2.5			*Q*
Aug. 16B	10	22	1.07			2–2.3	1.4–2	*Q*
Aug. 22B	10	22	1.09			1.5–1.8		*Q*
Aug. 25C	6	16	1.19	1.4		1.4–1.7	1.0	*Q*

amplitude 120/4 mV were applied from a subtracting holding potential (SHP) of −150 mV, at 6°C, sampling every 10 μs. We have been unable to identify any artifactual basis for the initial rising phase either from the averaging procedure, the clamp time constant, or the effects of series resistance and must conclude for now that it is a property of the membrane.

One would not anticipate any effect of temperature on the total amount of charge (Q_T) moved between saturation limits unless the temperature affected the amount of charge available to move or changed the maximum displacement of the available charge within the membrane field. Fig. 1 A, the charge obtained by integrating current records (*I* mode), and Fig. 1 B, the charge measured directly by analog integration (*Q* mode), illustrate that Q_T increases by slightly >10% for a 10°C rise in temperature. The results of nine experiments are shown in Table I.

The effects of temperature on the time constants are much more difficult to describe because the curves are definitely not single exponentials and are not clearly sums of a few exponentials. An example of one attempt to fit data (5° and 13°C) with the sum of two exponentials (by computer, least squares program) is shown in Fig. 2. This figure illustrates the voltage dependence of the time constant and the decrease with temperature. Table I also shows a number of results of these attempts. The ratios of the τs for roughly 10°C temperature changes range from 1.6 to 2.0 for the fast and 1.5 to 2.0 for the slow.

We have tried a different procedure to describe the effects of temperature on the time-course of the gating current or charge. Because the average time constant ratios for the fast and slow components shown in Table I were in the same range, one might think that the time expansion applied to the curves recorded at the higher temperatures might superimpose on those for low temperature. This is clearly not the case, as illustrated in Fig. 3 A for the curve at 6°C compared to that at 16°C. The time expansion required to superimpose the early parts of the curves was 1.45 and the later parts 2.0. These values are typical as shown in Table I and Fig. 3 B. In Fig. 3 B, the time expansion factor (α) needed to fit four different portions of the curves is plotted against the membrane potential during the pulse. Because of noise the results are

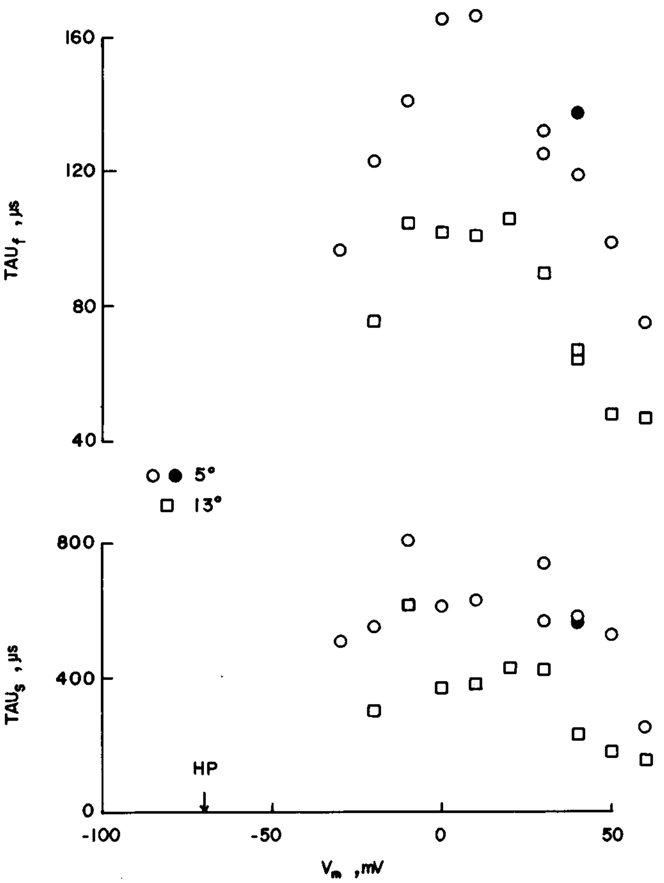

FIGURE 2 Time constants of fast and slow components of the falling phase of gating currents for pulses to different potentials obtained by least squares computer program for fitting the sum of two exponentials. Filled circles are recovery points.

difficult to determine at potentials more negative than −50 mV, but to the extent that this was possible it appeared that the compressions seen at +50 mV in Fig. 3 B occur also for the more negative potentials.

The results indicate that the total charge available to move with a potential step is not very dependent on temperature. The increase of 13% with a 10°C change could be the result of some extra charge being freed by the increased thermal motion brought about by the increase in temperature. These results are different from those reported by Kimura and Meves (1977). This may be due to a difference in the analysis procedure.

The kinetics of gating currents is strongly dependent on temperature but the equivalent Q_{10} is not as large as the values for m kinetics (2.7–3.5) as reported by Hodgkin et al. (1952). It is clear that the gating current transient is not a single process because time scaling reveals different temperature coefficients for different portions of the

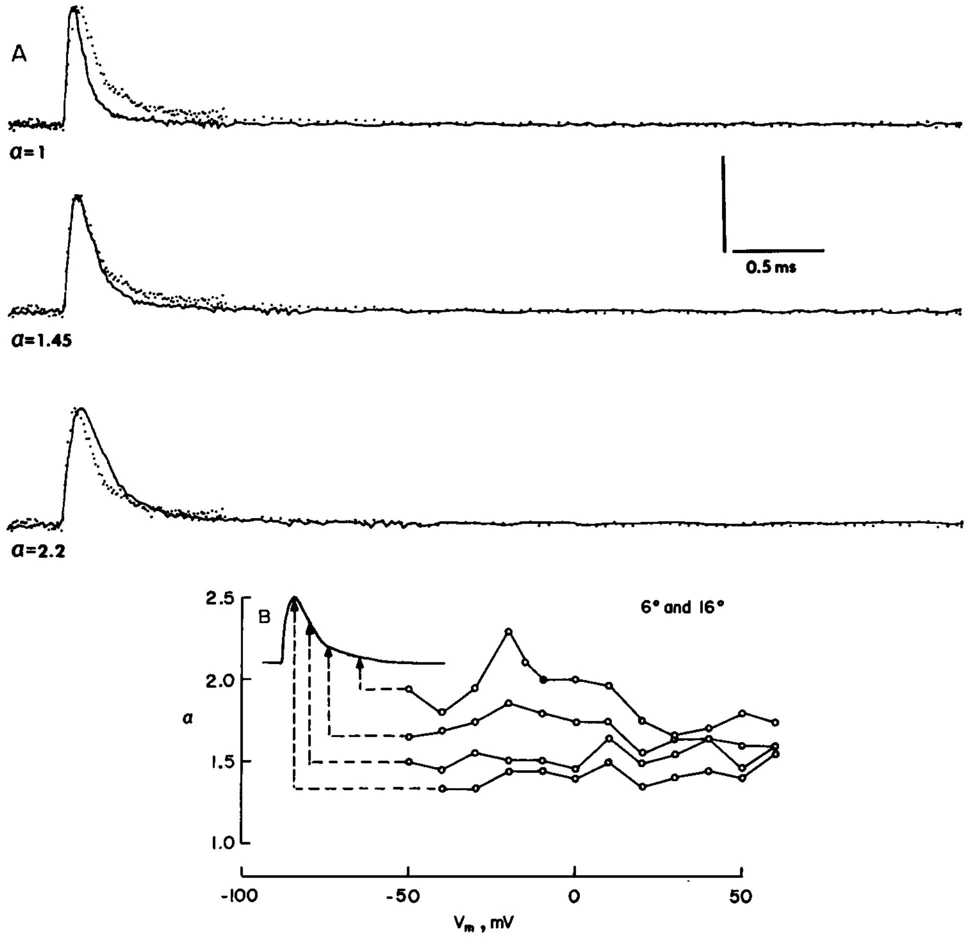

FIGURE 3 A. Time-course of gating current at 6° and 16°C (step from -70 mV to 0 mV; SHP = -150 mV). Top curve: as recorded. Middle curve: record for 16°C after time expansion by factor $\alpha = 1.45$ superimposes at short times on record for 6°C. Bottom: $\alpha = 2.2$ needed to superimpose record for 16°C on that for 6°C at long times. Dotted curve: 6°C, vertical line = 100 μA/cm^2. Solid curve: 16°C, vertical line = 199 μA/cm^2. B. Time expansion factor (α) required to fit any of four positions of gating current curve obtained at 16°C to that obtained at 6°C as a function of potential during the step. The arrows indicate the centers of the regions over which the traces were made to coincide.

transient. This can be interpreted as a single charged group (or dipole) moving in discrete steps or as a system of charged groups (or dipoles) moving with different kinetics. In either case, the high temperature dependence of the kinetics indicates that the charged groups or dipoles do not move continuously across the membrane field, but rather they have to overcome a finite number of barriers between stable positions. For a single barrier of height $\Delta G = \Delta H - T\Delta S$, where ΔH and ΔS are independent of temperature, the enthalpy contribution (ΔH) would be in the neighborhood of 6 kcal/mol for the fast process and 13 kcal/mol for the slow. To obtain a time constant of the order of 150 μs (see Fig. 2) for the fast process, one would require an entropy barrier ($T\Delta S$) of some 18 kcal/mol, but 13 kcal/mol is sufficient to give the slow process a time constant of 500 μs with very little entropy change.

The experimental work presented here was done at the Marine Biological Laboratory, Woods Hole, Mass., using space and equipment provided by Dr. C. M. Armstrong, and equipment loaned to us by Dr. M. Cahalan.

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