Comments on the Measurement of Gating Currents in the Frequency Domain

Dear Sir:

We would like to offer some comments about the determination of gating currents in the frequency domain with special reference to the paper of Takashima (1978).

Takashima has done experiments to determine the voltage dependence of the admittance in squid axon membranes under conditions where the ionic currents are suppressed with the view of comparing these results with the gating currents that have been measured in the time domain (e.g., Armstrong and Bezanilla, 1974; Keynes and Rojas, 1974; Meves, 1974).

In the frequency domain, measurements interpretable as admittance determinations must be done in the steady state with respect to the relaxation times of the voltage-dependent parameters of the system. For this reason Takashima perfused the axons with pronase to eliminate the changes produced by inactivation.

Gating currents are the result of charge movements within the membrane that are nonlinearly related to voltage. To compare the measurements in the time domain with those in the frequency domain we have made a simple model of the voltage dependence of the charge movement which fits the main features of the gating current data and allows us to predict the results to be expected in the frequency domain.

For a simple system of charges (or dipoles) moving between two stable states with a first order transition $Q_T - Q \rightleftharpoons Q$, the rate equation is

$$\frac{dQ}{dt} = \frac{Q_\infty(V) - Q}{\tau(V)}, \tag{1}$$

where Q is the charge in one of the states, and V is membrane potential. Using a single barrier model for the forward and backward rates, we obtain

$$Q_\infty(V) = \frac{Q_T}{1 + \exp\left(\frac{-e\alpha(V - V_0)}{kT}\right)} \tag{2}$$

and

$$\tau(V) = \frac{A}{\cosh\left(\frac{e\alpha(V - V_0)}{2kT}\right)}, \tag{3}$$

where Q_T is the total charge available to move, V_0 is the membrane potential at which half the Q_T is in each state, e is the electronic charge, k is the Boltzman constant, and T is the absolute temperature. α is an experimentally determined parameter which in this model would be the valence times the fraction of the total field through which the charge moves.

To predict the admittance (Y_g) produced by this charge movement when the membrane potential is suddenly changed to V, Eqs. 2 and 3 were substituted in Eq. 1 and Eq. 1 was linearized, assuming small perturbations around an operating point (V, Q) following a procedure similar to one described by Chandler et al. (1962). This procedure is valid only for times after Q has reached its steady-state value.

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The linearized equation was Laplace transformed to get the operational impedance. The final result for the admittance is:

$$Y_g(\omega) = \frac{\omega^2 R_g C_g^2}{1 + (\omega R_g C_g)^2} + j\frac{\omega C_g}{1 + (\omega R_g C_g)^2}$$

$$\text{with } j = \sqrt{-1}, \quad \omega = 2\pi f$$

$$R_g = \frac{4AkT}{e\alpha Q_T}\cosh\left(\frac{e\alpha(V - V_0)}{2kT}\right)$$

$$C_g = \frac{Q_T e\alpha/4kT}{\cosh^2\left(\dfrac{e\alpha(V - V_0)}{2kT}\right)}. \tag{4}$$

Eq. 4 can be represented as an equivalent circuit consisting of a resistor (R_g) in series with a capacitor (C_g), both of which are independent of frequency. This equation can also be represented by a conductance ($Re(Y_g)$) in parallel with a capacitor ($Im(Y_g)/\omega$). The admittance given by Eq. 4 is the component contributed by the gating current and is in parallel with the leakage conductance and capacitance of the rest of the membrane. Takashima's "conductivity" is defined as the real part of the total admittance and his "capacity" is defined as the imaginary part of the total admittance divided by ω. We have computed the real and imaginary part of the admittance contributed by the gating current (Eq. 4) for $Q_T = 1{,}000$ electronic charges/μm^2, $V_0 = -20$ mV, A = 200 μs, and $\alpha = 2$, which are representative values for a healthy axon. At any frequency the maximum change in capacity produced by the charge movement occurs at $V = V_0$. At $V = V_0$ the capacity change is a maximum of 0.33 μF/cm^2 at zero frequency and decreases to zero as the frequency goes to infinity.

With regard to the "conductivity," the predicted increase due to charge movement is maximum at $V = V_0$ at any frequency, and it increases with frequency from zero to 1.64 mS/cm^2 as frequency becomes infinite.

Fig. 1 shows the results of computations at 1,500 Hz for the total "conductivity" and total "capacity" as a function of voltage for the charge movement parameters given above (e.g., Bezanilla and Taylor, 1978) and assuming a membrane leakage of 0.1 mS/cm^2 and a membrane capacitance of 0.8 μF/cm^2. The graph shows that the maximum change in capacity is 0.07 μF/cm^2. This contrasts with the expectation expressed by Takashima (1978) of 0.3–0.4 μF/cm^2, but it is not clear that it is inconsistent with his experimental results which have a SD $\simeq$0.07 μF/cm^2. Fig. 1 also shows the predicted voltage dependence of the "conductivity" at 1,500 Hz with a maximum change at $V = V_0$ of 1.28 mS/cm^2. This value is larger than the standard deviation shown in the results presented by Takashima. The expected

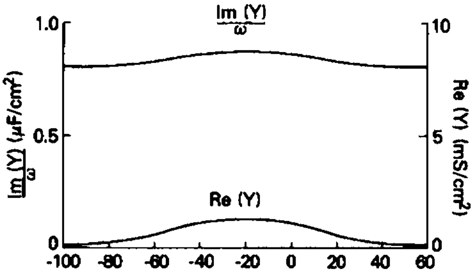

FIGURE 1 Computed "capacity" (imaginary part of admittance [Y] divided by $\omega = 2\pi f$) and "conductivity" (real part of admittance) vs. absolute membrane potential for $Q_T = 1{,}000\, e^-/\mu\text{m}^2$, $\alpha = 2$, τ_{max} = A = 0.2 ms, $C = 0.8\ \mu\text{F/cm}^2$, $G = 0.1$ mS/cm^2, $V_0 = -20$ mV, $T = 10$°C, and $f = 1{,}500$ Hz.

inrease in conductivity in the neighborhood of −20 mV could be obscured by nonlinear leakage or some residual potassium conductance not blocked by 15 mM tetraethylammonium ions inside.

The voltage-independent component of his conductivity is $\simeq$3.5–4 mS/cm^2 (at 1,500 Hz), which is quite different from the value of 0.1 mS/cm^2 that we have used for Fig. 1. We feel that the value of 0.1 mS/cm^2 is an upper bound for the resting conductance of axons perfused with impermeant ions used for measurements of gating currents. One example can be seen in Fig. 14 of Armstrong and Bezanilla (1974) where they show single sweeps without averaging. Our own experience (unpublished) is that a healthy axon has a resting leakage much less than 0.1 mS/cm^2. It is well known that axons treated with pronase are very fragile and become leaky very easily, and that may be the explanation for the figure of 3.5–4 mS/cm^2 reported by Takashima.

We do not fully understand the discrepancy between Takashima's results and our expectations. It would be highly desirable to have measurements of resting conductance using conventional pulse techniques and a determination of gating currents in the time domain in the same axon before and after the frequency domain determinations.

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