GATING CURRENTS

THE ROLE OF NONLINEAR CAPACITATIVE CURRENTS OF ELECTROSTRICTIVE ORIGIN

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ABSTRACT The nonlinear capacitative currents deriving from electrostrictive changes of membrane capacitance have been calculated under conditions similar to those employed by Armstrong and Bezanilla (1974) and Keynes and Rojas (1974) in their experiments on gating currents. For values of the parameter characterizing membrane electrostriction in the range suggested by optical retardation studies of Cohen et al. (1971), the nonlinear current of electrostrictive origin is comparable in magnitude and time-course, but is opposite in direction to the observed gating currents. Hence, the a priori neglect of electrostrictive currents is probably not justified. Conversely, if this current is, in fact, negligibly small in real situations, it follows that membrane compressibility must be significantly smaller than has been estimated.

INTRODUCTION

Two decades after Hodgkin and Huxley's prediction (1), gating currents were observed by Armstrong and Bezanilla (2) and Keynes and Rojas (3). Both groups employed essentially the same technique to isolate the rather small gating current, of order 30 $\mu A/cm^2$ at its peak, from the much larger ionic current and capacitative transients that follow application of a depolarizing or hyperpolarizing step in a voltage-clamp experiment. The experimental details and results are described more fully in subsequent papers (4, 5).

One may express the total membrane current as the sum of three contributions,

$$I_T = I_i + I_C + I_g, \tag{1}$$

where I_i is the ionic current, I_C is the capacitative transient current, and I_g is the transient gating current. To observe I_g in both refs. 4 and 5, the ionic current was greatly diminished by perfusing the axon with CsF and immersing it in a Na^+-free medium. In many of the experiments tetrodotoxin or saxitoxin was used to eliminate residual ionic currents through the sodium channels. The remaining ionic current is then a small "leakage" current. Although this leakage current has a nonlinear component (see Fig. 5, ref. 5), refs. 4 and 5 give convincing arguments that this current does not contribute to the observed nonlinear gating current.

Gating currents are, presumably, the result of a transient reorientation of membrane-bound gating particles that, by virtue of charged groups, respond to changes of

membrane potential. Since at normal resting potential the sodium gates are in their closed (or "nearly" closed, ref. 4) configuration, a hyperpolarizing voltage step should not result in a gating current, whereas an outward gating current should follow application of a depolarizing step. There should then be an analogous inward "turn-off" gating current when the membrane potential is returned to its resting value. Although the "turn-on" and turn-off currents need not be, and are not equal, their time integrals should be equal if, as hypothesized, both gating currents are due to intramembranous charge movement and do not contain ionic components.

Upon application of a voltage step, the charging or discharging of membrane capacitance results in a very large, exponentially decaying capacitative current. If it is assumed that the membrane capacitance (approx. 1 $\mu F/cm^2$) and its effective series resistance (about 7Ω-cm^2[6]) do not depend on membrane potential, the capacitative current should be of the same magnitude but of opposite sign for positive and negative pulses of equal magnitude. Hence, by adding the transient currents for equal but opposite voltage steps, from V_0 to $V_0 + \Delta V$ and $V_0 - \Delta V$, the linear capacitative current of Eq. 1 is cancelled, leaving only the nonlinear transient, which presumably results from the motion of gating particles. Because initially I_C is quite large, both refs. 4 and 5 used electronic gates to prevent amplifier saturation. Typically, the amplifiers were grounded at the initiation of a voltage step for a period of 20 μs in ref. 4 and for 50 μs in ref. 5.

These results provide rather conclusive evidence that the nonlinear currents observed are, indeed, associated with the activation and inactivation of sodium gates in the membrane. Both groups measured the total charge movement during turn-on and turn-off and find that, as expected, they are equal to within experimental precision (see Fig. 8, ref. 5, and Fig. 6, ref. 4). Moreover, the close association between gating current and sodium activation (7), the temperature dependence of the gating current, and its parallelism with respect to the "m" system of Hodgkin and Huxley clearly establish the correlation between gating action and the observed gating currents.

Both refs. 4 and 5 briefly refer to the fact that a voltage dependence of the membrane capacitance could also contribute to a nonlinear capacitative current. Both papers, however, dismiss this as a negligible effect. Furthermore, as Keynes and Rojas correctly point out, this nonlinear capacitative current arising from the electrostrictive change in membrane thickness will be of opposite sign to the observed gating current. They further contend that since the fractional change in membrane capacitance due to a 50 mV voltage pulse is probably of order 0.1% (8), this contribution would be insignificant in absolute magnitude.

In view of the great interest in and the fundamental importance of gating currents to an understanding of the functioning of excitable membranes, it seemed worthwhile to consider the electrostrictive contribution to the nonlinear capacitative current in some detail and establish that it is, indeed, negligibly small. This is all the more essential in view of the fact that the total charge movement associated with this current upon turn-on and turn-off will be the same, thus satisfying one of the criteria used to identify gating current.

If it is assumed that the membrane behaves like an elastically deformable dielectric, electrostriction will cause a reduction of membrane thickness with increasing voltage. Consequently, the membrane capacitance will exhibit a voltage dependence. If the fractional change in membrane thickness is small, the membrane capacitance depends on membrane potential according to the relation (9, 10)

$$C(V) = C_0(1 + \beta V^2) \tag{2}$$

where β is a parameter whose value depends on the dielectric constant of the membrane, its thickness T and Young's modulus.

In artificial membranes relatively large changes of membrane capacitance with voltage have been observed (10–13), but there is evidence that these changes are largely a consequence of extrusion of solvent from so-called lenses in the artificial lipid bilayer (14). Perhaps the most reliable data on the compressibility and the voltage dependent capacitance due to thickness changes are those of Wobschall (15) on bilayer membranes of cholesterol dissolved in decane. Wobschall separated capacitance changes due to solvent extrusion and membrane compression by a careful study of the frequency response, associating the latter with the high frequency plateau. From his results one obtains for the parameter β of Eq. 2 a value of about 0.07 V^{-2}.

Whether data on artificial membranes are really applicable to physiological membranes is, of course, debatable. The latter contain a substantial amount of proteinaceous material as well as lipid, with the proteins penetrating into and through the membrane (16). Therefore, although an ideal lipid bilayer may indeed by fairly stiff with a Young's modulus of about 2×10^7 N/m^2 (17), the same need not be true of the physiological membrane.

Unfortunately, there exist no direct measurements of the elastic properties of biological membranes. Indirect evidence suggests that the compressibility may be of the order 10^{-7} m^2/N (8). According to Cohen et al. (8) $\Delta T/T$, the fractional change in membrane thickness and, hence, in membrane capacitance resulting from a 50 mV depolarization, should be between 3×10^{-4} and 5×10^{-3}. These thickness changes are compatible with their data (8) on optical retardation, although induced birefringence could also account for the optical data. Cohen et al. suggest that the "fast phase" of the retardation response, with time constant $\tau_m \simeq 20$ μs at 14°C, may be connected with membrane thickness changes.

A fractional change of membrane capacitance of the magnitude given above corresponds to $\beta = 0.12$ V^{-2} and $\beta = 2.0$ V^{-2}. It is worth noting that the lower value is in rough agreement with that deduced from the results of Wobschall (15). In most of the calculations three values of β were employed, namely 2 V^{-2}, 0.4 V^{-2}, and 0.04 V^2, corresponding to $(\Delta T/T) = 5 \times 10^{-3}$, 10^{-3}, and 10^{-4} for a 50 mV change of membrane potential. These represent, perhaps, maximum, average, and quite conservative estimates for the parameter β.

We shall consider two extreme limits of membrane response, which we designate

A and B. In case A it is assumed that the membrane thickness responds instantaneously to changes in V_c, where V_c is the potential across the idealized membrane capacitance. In case B we follow the suggestion of ref. 5 and assume that the membrane thickness approaches a new value exponentially with a time constant τ_m, the time constant of the "fast phase" of the optical response (8). This time constant is assumed long compared to the time constant of the charging circuit of the membrane capacitance.

CASE A

Under the assumption that $I_i = 0$ the equivalent circuit for the membrane under voltage-clamp conditions is a simple series RC circuit, where C is given by Eq. 2 with $C_0 \simeq 1\,\mu\text{F/cm}^2$ and $R \simeq 7\Omega\text{-cm}^2$ (6). The voltage across the capacitance must then satisfy the equation

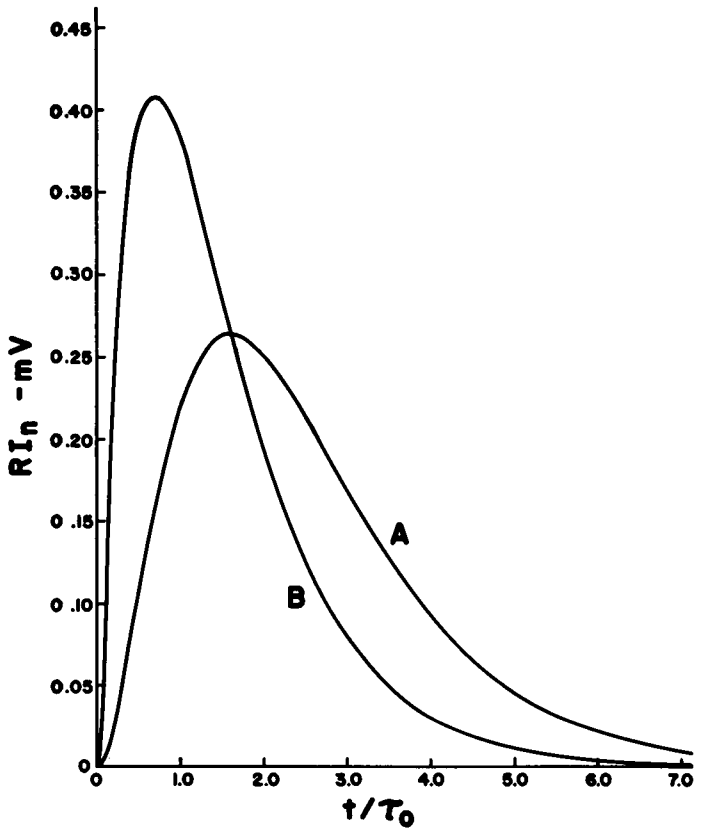

FIGURE 1 Electrostrictive nonlinear capacitative current for symmetrical voltage pulses of ±70 mV from a resting potential of −70 mV. A, turn-on current; B, turn-off current. Abscissa is in units of t/τ_0, where $\tau_0 = RC_0$; ordinate in units of RI_n, where R is the effective membrane series resistance. Curves are for $\beta = 0.4\,\text{V}^{-2}$.

$$V = V_c + R(\mathrm{d}/\mathrm{d}t)(CV_c) = V_c + RC_0(1 + 3\beta V_c^2)(\mathrm{d}V_c/\mathrm{d}t), \qquad (3)$$

where V is the potential set by the voltage clamp. The membrane current is then given by

$$I = (V - V_c/R). \qquad (4)$$

We have integrated the nonlinear differential Eq. 3 numerically,[1] using for β the three values given above and for symmetrical voltage steps of ±70 mV from a resting potential of −70 mV as well as voltage steps of ±80 mV from a resting potential of −60 mV; these voltage conditions correspond to two of the experimental procedures employed by Armstrong and Bezanilla and appear to be fairly typical for these kinds of measurements. The algebraic sum of the capacitative currents for these steps, i.e. the sum of the nonlinear contributions, is shown in Figs. 1 and 2 for $\beta = 0.4\ \mathrm{V}^{-2}$. The time scale is in units of $\tau_0 = RC_0$, and the ordinate is RI_n, where I_n is the nonlinear current, rather than I_n, to facilitate the use of these curves for other choices of R and C_0. Curves for the other two values of β ($\beta = 2.0\ \mathrm{V}^{-2}$ and $\beta = 0.04\ \mathrm{V}^{-2}$ scale almost exactly (within 5%) with β and are, therefore, not displayed.

The curves exhibit some noteworthy features. First, the nonlinear current does not reach its peak at $t = 0$, the time when the capacitative current is at maximum. Instead, I_n rises fairly rapidly from zero to its maximum value, reached near $t = 1.6\ \tau_0$, and then decays roughly exponentially with a time constant of about $1.5\ \tau_0$. Thus, the nonlinear current is at its peak when the linear capacitative current has already dropped to about one-fifth of its initial value; at $t = 4\tau_0$, i.e. after four time constants of the charging circuit, I_n is still more than one-third of its maximum value. Second, I_n at its peak is by no means negligible compared to the observed gating currents, even when $\beta = 0.04\ \mathrm{V}^{-2}$, the conservative estimate for this parameter. For $\beta = 0.4\ \mathrm{V}^{-2}$, the "average" estimate, I_n has a peak value of 37 $\mu\mathrm{A/cm}^2$ (with $R = 7\Omega\text{-cm}^2$) and is still about 12.5 $\mu\mathrm{A/cm}^2$ at $t = 4\tau_0$. These currents should be compared with those observed by Armstrong and Bezanilla, which have peak values that range between about 20 and 40 $\mu\mathrm{A/cm}^2$. Although the use of the 20-μs blanking at the beginning of each voltage step would, of course, obscure much of this nonlinear electrostrictive current the "window" was opened at about $t = 3\tau_0$, at which time $I_n = 24\ \mu\mathrm{A/cm}^2$, still a very substantial contribution. Only if the parameter $\beta = 0.04\ \mathrm{V}^{-2}$, the conservative estimate, is this nonlinear current relatively insignificant, although even then it still constitutes at its peak about 10% of the observed nonlinear transient gating current.

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[1] Eq. 3 has an analytic solution for a step voltage from V_i to V_f, namely,

$$\frac{t}{RC_0} + (1 + 3\beta V_f^2)\log[(V_f - V_c)/(V_f - V_i)] + \frac{3\beta}{2}(V_c - V_i)(2V_f + V_c + V_i) = 0.$$

To obtain $V_c(t)$ it is still necessary to resort to numerical calculation to find the root of this equation for a specified value of t. We have verified our numerical integration for several values of t using the above solution.

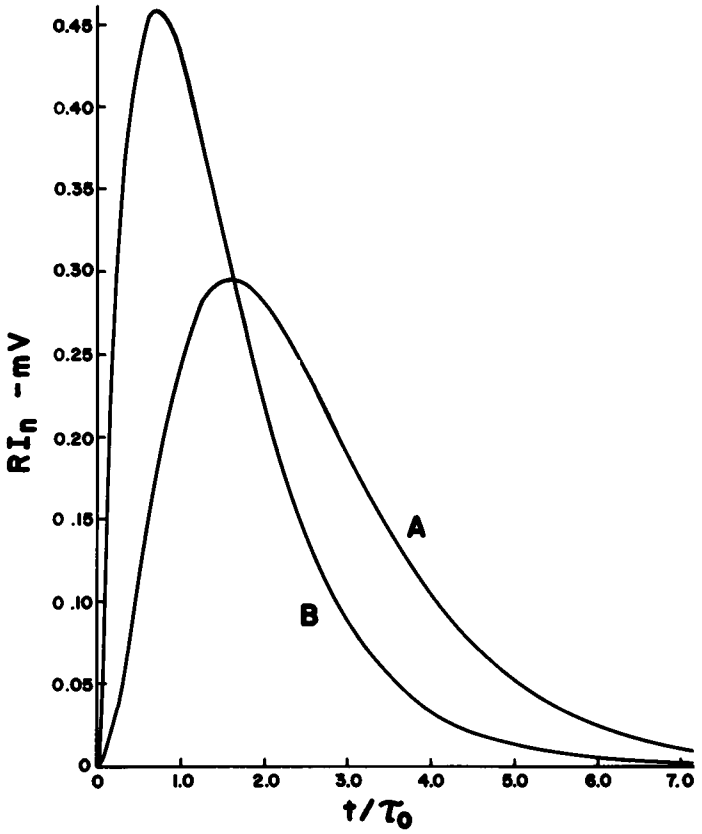

FIGURE 2 Electrostrictive nonlinear capacitative current for symmetrical voltage pulses of 80 mV from a resting potential of −60 mV. A, turn-on current; B, turn-off current. Abscissa and ordinate units as in Fig. 1. $\beta = 0.4\ V^{-2}$.

The experimental results of refs. 4 and 5 indicate that the turn-off gating current has a significantly different temporal pattern than the turn-on current. The turn-off current appears to be rather larger initially but then decays somewhat more rapidly than the turn-on gating current. In Fig. 1 we show the corresponding electrostrictive nonlinear turn-off current, i.e. the sum of capacitative currents due to voltage steps from 0 to −70 mV and from −140 to −70 mV. In close analogy with the observed gating currents, the calculated turn-off current reaches a substantially larger peak than the turn-on current, which also appears much earlier, at $t = 0.7\tau_0$, and then decays toward zero more rapidly than the turn-on current, as it must to conserve total charge movement. The same pattern obtains also for the situation depicted in Fig. 2, for which the resting potential is −60 mV and the potential steps are 80 mV.

Armstrong and Bezanilla also employed a somewhat modified technique, the "divided pulse" method, to exhibit the true activation gating current for depolarizing pulses, undiminished by the current which they attribute to the change of sodium gates from "nearly closed" to "fully closed" conformations after the application of a hyper-

polarizing voltage step. In this method the depolarizing pulse from $V = -60$ mV to $V = +20$ mV was followed by a change in membrane potential to the hyperpolarizing value of -170 mV for a period of 200 ms. A further hyperpolarizing pulse of -20 mV (the depolarizing pulse height divided by four) was then applied, and the resulting capacitative current was first multiplied by a factor of four before addition to the capacitative current after depolarization by 80 mV. With this divided pulse technique, these authors observed substantially faster rise times and larger gating currents.

For comparison we have evaluated the electrostrictive nonlinear capacitative currents under the same voltage-clamp conditions as employed by Armstrong and Bezanilla, using Eq. 3. The calculations were performed only for $\beta = 0.4\ V^{-2}$ and the resulting curves of turn-on and turn-off currents are shown in Fig. 3, with a different ordinate scale from that of Fig. 2. Comparison of the curves of Figs. 2 and 3 reveals that I_n on turn-on rises more rapidly than before, reaching its maximum near $t = 1.1\ \tau_0$, and the maximum value of I_n is greatly enhanced, by a factor of about 3.5.

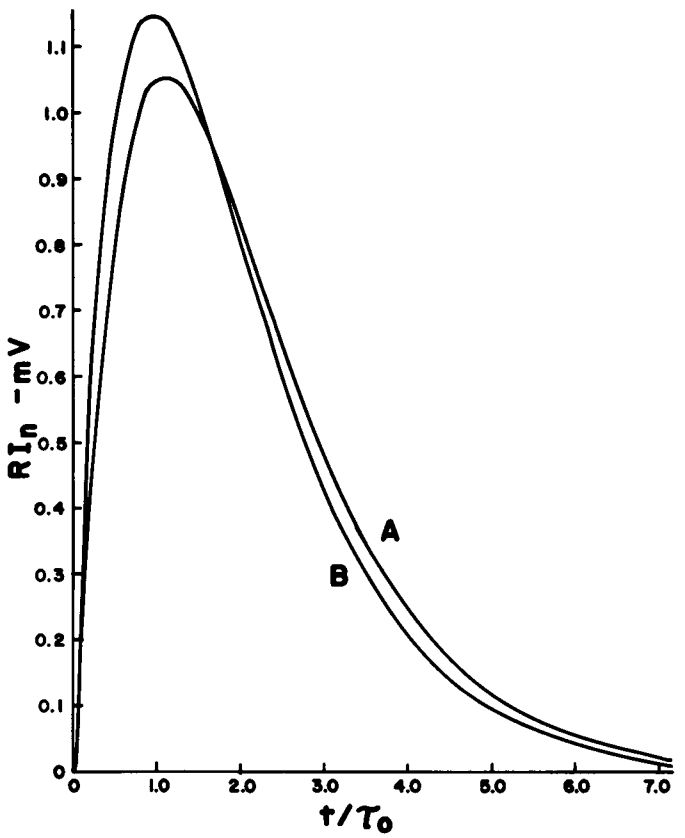

FIGURE 3 Electrostrictive nonlinear capacitative current in the "divided pulse" method. Depolarizing pulse of +80 mV from a resting potential of −60 mV; hyperpolarizing pulse of −20 mV from a resting potential of −170 mV. A, turn-on current; B, turn-off current. Abscissa and ordinate units as in Figs. 1 and 2; note, however, change in ordinate scale. $\beta = 0.4\ V^{-2}$.

In this mode of operation, however, the difference between turn-on and turn-off currents is much reduced from that which we found for symmetrical depolarizing and hyperpolarizating pulses.

Thus, in case *A* the electrostrictive nonlinear capacitative current appears to mimic the temporal behavior of the observed gating current for turn-on and turn-off under both the symmetrical and divided pulse conditions. Moreover, with reasonable values of the parameter characterizing the dependence of membrane thickness on potential, this current is of the same order of magnitude as the observed gating current. Lest the reader draw the wrong inference from these results, we emphasize that the direction of the electrostrictive current is opposite to that of the observed gating current so that its presence would only serve to enhance the true gating current. It is, however, clearly incorrect to assume, a priori, that the electrostrictive effect can be ignored.

CASE B

Here we assume that the membrane capacitance is charged (or discharged) in a time much shorter than that required for a change in membrane thickness. In this case the nonlinear electrostrictive current is easily derived under the assumption that

$$C(t) = C_f - (C_f - C_i)\exp(-t/\tau_m) \tag{5}$$

where C_i and C_f are the initial and final values of membrane capacitance as determined by application of Eq. 2. For symmetrical depolarizing and hyperpolarizing pulses of height, ΔV, the nonlinear current, is then given by

$$I_n = (6\beta C_0 V_i \Delta V^2/\tau_m)\exp(-t/\tau_m) \tag{6}$$

For V_i = 60 mV and ΔV = 80 mV, and taking C_0 = 1 μF/cm^2, one obtains $I_n = 2.3 \times 10^{-3}(\beta/\tau_m)\exp(-t/\tau_m)\mu$A/cm^2. We now follow the suggestions of Cohen et al. (8) and associate τ_m with the time constant of the "fast phase" of the optical retardation response. Armstrong and Bezanilla performed their experiments at a temperature of 2°C; Cohen et al. did not extend their measurements to such low temperature, but judging from their results (see Fig. 13, ref. 8) $\tau_m(2°C) \simeq 200\,\mu$s. Hence, under these conditions $I_n = 11.5\ \beta \exp(-t/\tau_m)\,\mu$A/cm^2. For β = 0.4 V^{-2}, I_n, though smaller than in case *A*, is still of significant magnitude and, of course, persists for a fairly long time. At higher temperatures, such as were employed by Keynes and Rojas, τ_m is greatly reduced (its value at 7°C is about 70 μs) and the initial value of I_n is correspondingly increased; I_n decays, however, also more rapidly.

One can again calculate the change that would arise as a result of the use of the divided pulse technique. As in case *A*, the nonlinear electrostrictive current is enhanced by the divided pulse method. The nonlinear current calculated for a depolarizing pulse of 80 mV from a resting potential of −60 mV, followed by a hyperpolarizing pulse of −20 mV from a resting potential of −170 mV, is 2.4 times as large as that obtained if symmetrical 80-mV pulses are used starting from a resting potential of −60 mV.

As in case A, the electrostrictive nonlinear capacitative current is opposite in direction to the observed gating currents.

CONCLUSION

We have calculated the nonlinear capacitative current that derives from the electrostrictive change in membrane thickness with membrane voltage. These currents were calculated in two extreme limits, A corresponding to instantaneous adjustment of membrane thickness to the voltage across the membrane capacitance, and B corresponding to a dynamic response of the membrane that is slow compared to the time constant of the electrical circuit. For physically and possibly physiologically reasonable values of the parameter characterizing the membrane thickness voltage relation, we find that in both limits the nonlinear current of electrostrictive origin is of the same order of magnitude as the gating current observed by Armstrong and Bezanilla and Keynes and Rojas but is, as already pointed out by Keynes and Rojas, of opposite sign to the gating current. Consideration of the electrostrictive current will, therefore, tend to enhance the magnitude of the actual gating current and may modify its time-course somewhat. In any event, if electrostrictive capacitance changes of magnitudes suggested by Cohen et al. (8) do indeed occur, they may well contribute significantly to the nonlinear capacitative currents, contrary to previous expectations. In the absence of conclusive evidence to the contrary, the neglect of electrostrictive effects in physiological membranes, particularly as they influence gating currents, does not appear to be justified.

On the other hand, the results of Armstrong and Bezanilla strongly support the belief that the observed nonlinear currents are true gating currents, unperturbed by extraneous contributions. First, they found that internal perfusion with 10 mM $ZnCl_2$, which reversibly quenches the sodium ion current (18), similarly quenches gating current reversibly. Second, they depolarized the membrane to +56 mV for an extended period. They then returned the membrane potential to −70 mV and tested for I_{Na} and I_g. The results show that immediately after the return to −70 mV both I_{Na} and I_g were negligibly small, recovering in parallel over several minutes. Last, I_{Na} and gating currents were diminished by a positive prepulse that inactivates the sodium conductance. Only in the case of extended depolarization was there any evidence of an inward turn-on current that might, conceivably, be associated with the electrostrictive effect. It is, however, difficult, though perhaps not impossible, to explain why perfusion with $ZnZl_2$ should alter the mechanical properties of the membrane so as to eliminate electrostriction. Hence, one possible corollary of the calculations presented here is that they, in conjunction with the results of Armstrong and Bezanilla, indicate the absence of electrostrictive effects. If that is the correct interpretation of these results, it also suggests that the compressibility of physiological membranes is considerably less than has been estimated previously, and effectively eliminates electrostriction as a viable interpretation of the optical retardation data of Cohen et al.

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