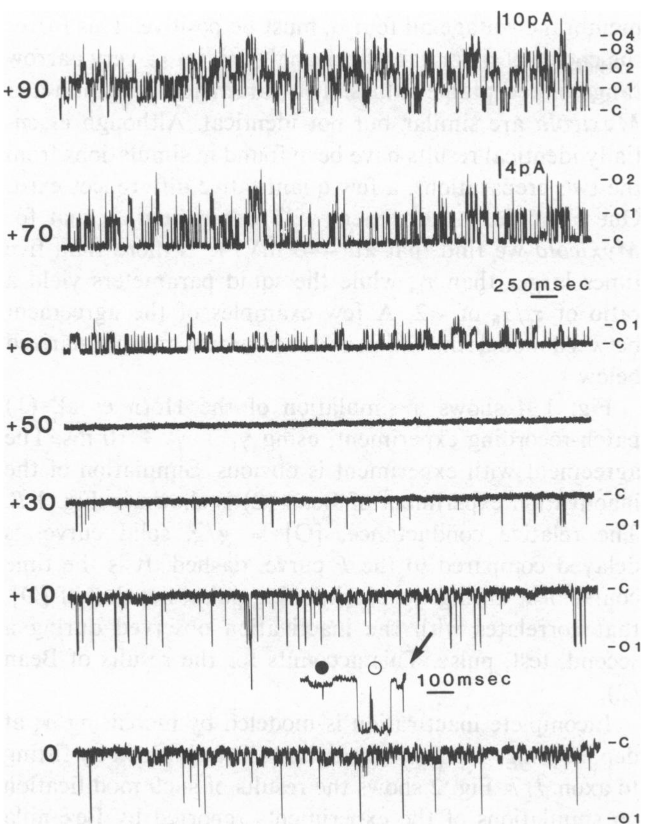

FIGURE 3 Single channel recordings from a cell attached patch on the somal region of a PN. The electrode contained high K^+ saline. Under this condition, channel activity was observed at zero applied polarization, and currents were inward. Currents mediated by both the large (○) and small (●) channel types could be identified. Note that the frequency of small channel openings decreased with depolarization while the frequency of large channel openings increased. Inset shows the currents at an expanded time base. The patch contained several channels of the larger type (single channel conductance = 250 pS).

(9) tissue. The Ca^{++} sensitivity of the K^+ channels in PN is presently under investigation.

I thank Vincent Dionne, Joseph Henry Steinbach, and George Siggins for helpful discussions and technical advice, Christi Franklin for technical assistance, and Nancy Callahan for secretarial skills.

This research was supported by grant NIAAA 03504 from the National Institutes of Health.

Received for publication 2 May 1983.

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A MODEL OF THE SODIUM CHANNEL

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We suggest that the opening and closing of sodium channels involves, at each channel site, two membrane components: (*a*) a single two-state gate system, G, with open (O) or closed (C) states; (*b*) a collection of coupled systems, E, each of which can exist in one of three states. The fraction of E systems in the "active" state (A) determines the probability, P_A, that the G system is in its (O) state.

$$C \underset{\gamma_2(A)}{\overset{\gamma_1(A)}{\rightleftharpoons}} O \tag{G}$$

$$R \underset{\alpha_2}{\overset{\alpha_1}{\rightleftharpoons}} A \underset{\alpha_4}{\overset{\alpha_3}{\rightleftharpoons}} I \tag{E}$$

$$P(A) = \gamma_1(A)/[\gamma_1(A) + \gamma_2(A)] \tag{I}$$

The rates, α_i, of the E systems are assumed to depend on the local electric field and thus on the membrane voltage, and are chosen to give the best fits to experimental clamp data. The rates of the G system are assumed to be independent of the voltage and to depend on the fraction of the E systems in the neighborhood of G that are in the (A) state. The form of the probability function P_A is not specified a priori, but is chosen to give best fits to experimental data. Scheme (E) has been restricted to three states since this is the maximum number that allows the α_i to be determined empirically from single step clamps with no ad hoc assumptions.

Simulations have revealed no clear discrepancies between prediction and experiment. Asymmetry currents

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Volume 45 January 1984 55–57

are ascribed to the field-dependent transitions between states R and A of (E), not to the field-independent transitions of the gates themselves, (G). While an E system must pass through (A) on its way from its resting state (R) to its inactive state (I), it is not necessary that an individual gate (G) open before the probability of its being open starts to decrease. This distinction between actual opening of a channel and the probability of its being open allows this model to simulate very closely the results reported by Horn et al. (1) and by Bean (2). The question they both pose, "must channels open before they inactivate?", is only valid if a state of a coupled system is itself assumed to represent the open channel. This is not true in our model, and the experimental results of both investigations lend us support; see discussion of Fig. 1.

FITTING MODEL TO EXPERIMENT AND A SAMPLING OF RESULTS

The model has been fitted to the Hodgkin and Huxley (3) axon #17 and to four *Myxicola* axons from Goldman (4), yielding, for each preparation, a P_A curve and the α_i rates as functions of membrane voltage. These parameters have then been used to simulate a wide variety of experiments. A crucial step in the fitting process is the determination of $\bar{g}$, where the conductance g is given by $g = [O] \times \bar{g}$. The criterion for this choice is the requirement that at each membrane voltage all four α_i must be positive. This forces the choice of $\bar{g}$, for each fiber analyzed, into a very narrow range of acceptable values. The parameters for squid and *Myxicola* are similar but not identical. Although essentially identical results have been found in simulations from the two preparations, a few quantitative differences exist. One example, in agreement with experiment, is that for *Myxicola* we find that at -40 mV, τ_c is more than five times larger than τ_h, while the squid parameters yield a ratio of τ_c/τ_h of <2. A few examples of the agreement between model simulation and experiment are summarized below.

Fig. 1 *A* shows a simulation of the Horn et al. (1) patch-recording experiment, using $\gamma_1 + \gamma_2 = 10$ ms. The agreement with experiment is obvious. Simulation of the inactivation experiment of Bean (2) is shown in Fig. 1 *B*. The relative conductance, $[O] = g/\bar{g}$, solid curve, is delayed compared to the *P* curve, dashed. It is the time course of *P* during the conditioning pulse, not that of [O], that correlates with the inactivation observed during a second, test, pulse. This accounts for the results of Bean (2).

Incomplete inactivation is modeled by increasing α_4 at depolarizing voltages, above the values obtained by fitting to axon #17. Fig. 2 shows the results of such modification on simulations of the experiments reported by Bezanilla and Armstrong (5). Note that this agreement with experiment is obtained without postulating the existence of

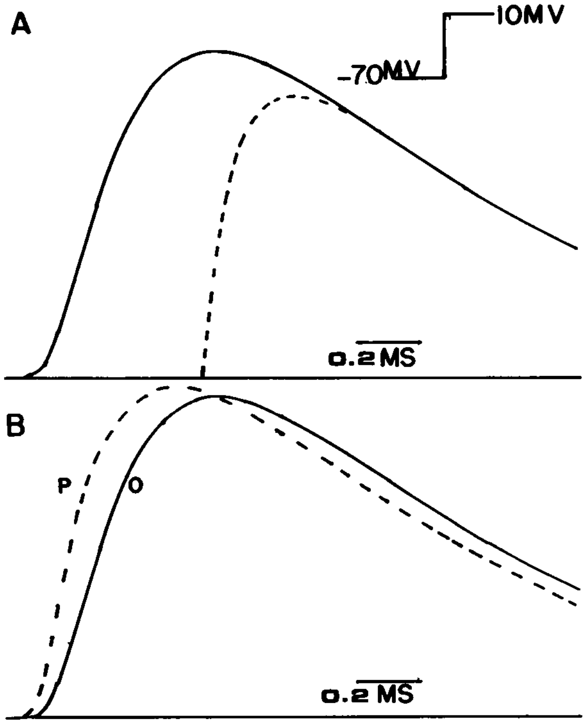

FIGURE 1 Simulated behavior of single channels. *A*, the solid curve shows the calculated fraction open, *O*, averaged over all channels. The dashed curve shows the result for those channels that do not open before 0.62 ms. *B*, the solid curve, *O*, is the same as in *A*. The dashed curve gives the time variation of P_A.

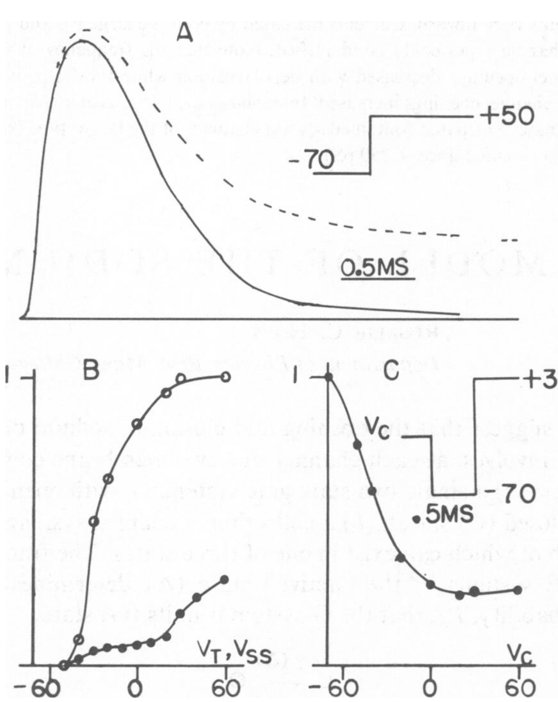

FIGURE 2 Simulation of incomplete inactivation. *A*, the effect on conductance of increasing α_4 from 0.365 ms (—), to 0.715 ms (---). *B*, relative conductance for clamps from -70 mV to V_T (o), and peak repolarization currents from V_{SS} to -70 mV (•). *C*, double-pulse inactivation curve.

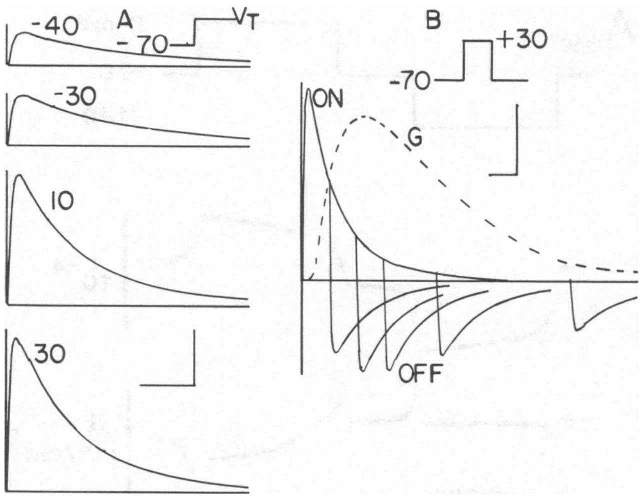

FIGURE 3 Computed asymmetry current, *I*. Time scales, 0.2 ms. Current scales, 50 μA/cm^2. *A*, ON currents for −70 mV to V_T. *B*, ON current during the control pulse, −70 mV to +30 mV, and OFF currents upon repolarization to −70 mV. Dashed curve shows the time course of the conductance during the control pulse.

additional "active" states in (E) nor of additional open states in (G).

We assume that asymmetry currents are given by $I_A = -CR$, where *C* is a physical scaling constant. Some results are shown in Fig. 3, with $C = 4.8 \times 10^{-8}/\text{cm}^2$ and a membrane relaxation constant, Hoyt (6), of $\tau_R = 0.012$ ms. The ON and OFF currents show a two-time constant behavior during their falling phase, as observed experimentally. The relation of conductance and ON current shown in (*b*) and the inactivation of OFF currents also agree with experiment. The expected charge movement is $Q = C(1 - R_\infty)$, where R_∞ is the equilibrium value of R. When plotted against voltage the midpoint is shifted by ~ -20 mV and the slope at midpoint is steeper than in many experimental curves. However, similar changes have been experimentally produced by altering the environment, holding potential, etc.

So far only two model results have been found to be directly contrary to experiment: (*a*) The absence of a third, very fast, time constant in repolarization currents, reported by Goldman and Hahin (7); and (*b*) no disappearance of the initial rising phase of asymmetry currents with long depolarizations, reported by Armstrong and Gilly (8). If confirmed by other experimenters, these results may require modifications of the model, such as the presence of additional states in E. However the basic premise of separation of excitation and actual opening or closing of gates may still be valid.

Received for publication 22 April 1983.

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OPTICAL STUDIES OF SODIUM CHANNELS

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If there are structural rearrangements of sodium channels associated with "opening" and "closing," they should be optically detectable. In the absence of specific data on optical properties of sodium channels, the simplest and most sensitive optical measurement is birefringence (BRF). BRF measures the difference between the number and strength of molecular dipoles in two orthogonal directions. Sodium channels are vectorially oriented in nerve membranes. If a change in transmembrane potential moved a net dipole from a direction in the plane of the membrane to a direction more parallel to the applied electrical field, there would be a concomitant change in BRF.

Cohen, Hille and Keynes (1) found changes in axon BRF with a time course very similar to action potentials. They found a decrease in optical retardation with a radially oriented optical axis in a thin cylinder immediately surrounding the axoplasm. Cohen et al. (2) analyzed this retardation change using the voltage-clamp technique. They concluded that there is more than one source of retardation change, defining a fast phase (or state 1), a slow phase (or state 2) and a rebound response. Fresh