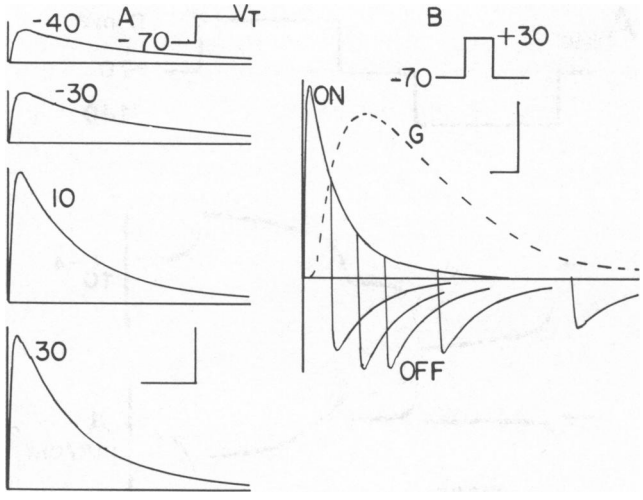

FIGURE 3 Computed asymmetry current, *I*. Time scales, 0.2 ms. Current scales, 50 $\mu A/cm^2$. *A*, ON currents for −70 mV to V_T. *B*, ON current during the control pulse, −70 mV to +30 mV, and OFF currents upon repolarization to −70 mV. Dashed curve shows the time course of the conductance during 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 $C = 4.8 \times 10^{-8}/cm^2$ 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.

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REFERENCES

1. Horn, R., J. Patlak, and C. F. Stevens. 1981. Sodium channels need not open before they become inactive. *Nature* (*Lond.*). 291:426–427.
2. Bean, B. P. 1981. Sodium channel inactivation in the crayfish giant axon. *Biophys. J.* 35:595–614.
3. Hodgkin, A. L., and A. F. Huxley. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* (*Lond.*). 117:500–544.
4. Goldman, L. 1975. Quantitative description of the sodium conductance of the giant axon of *Myxicola* in terms of a generalized second order variable. *Biophys. J.* 15:119–136.
5. Bezanilla, F., and C. M. Armstrong. 1977. Inactivation of the sodium channel. *J. Gen. Physiol.* 70:549–566.
6. Hoyt, R. C. 1982. Origin of the rising phase of gating currents. *Biophys. J.* 40:251–254.
7. Goldman, L., and R. Hahin. 1978. Initial conditions and the kinetics of the sodium conductance in *Myxicola* giant axons. II. Relaxation experiments. *J. Gen. Physiol.* 72:879–898.
8. Armstrong, C. M., and W. F. Gilly. 1979. Fast and slow steps in the activation of sodium channels. *J. Gen. Physiol.* 74:691–711.

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

axons had a fast phase sometimes followed by a rebound. With time or the application of various insults to the axon, these were irreversibly replaced with a slower state 2 response. All of their analysis was done on responses to hyperpolarizing pulses or to depolarizing pulses short compared to the rise time of the sodium current.

One of the most disappointing features of this early work was the absence of any component that could be linked through similarity of time course or voltage dependence or pharmacology to the sodium or potassium channels. I have found that colchicine reversibly and selectively blocks both the sodium current and the BRF response to a depolarizing voltage-clamp pulse (3). This encourages me to continue the analysis, looking for components of the BRF response associated with sodium channels.

METHODS

I have used the same methods described in references 1–3, mounting axons between crossed polars at 45°. The major technical improvements over the early work are the internal perfusion of the axons and lowering the temperature and the Na concentration of the external media. I have studied only the fast state 1 responses. I have looked for an asymmetry in the optical response, comparing the responses to symmetrical depolarizing and hyperpolarizing voltage-clamp pulses analogous to the "gating" or "asymmetry current" experiments (4–6).

RESULTS

When an axon membrane is depolarized from −70 mV to 0 mV there is a decrease in optical retardation by about one part in 10^4. Hyperpolarizing the membrane increases the BRF. Fig. 1 shows the combined average of 19 runs on three axons, a total of ~32,000 sweeps. The BRF response to the depolarizing pulse is smaller and has a different time course than the response to a symmetrical hyperpolarizing pulse. The asymmetry in the amplitude was described earlier (2). In Fig 1 *B* the BRF response to hyperpolarization has been inverted and scaled to match the amplitude of the response to depolarization to emphasize the difference in time course. The difference between the scaled responses is shown in the middle trace, just above the algebraic sum of the current records.

This asymmetry was generally seen in axons in state 1 when there was sufficient temporal resolution to see the rising phases of the depolarizing responses. This asymmetry was still present for larger pulses, with outward sodium currents, and in the presence of sufficient tetrodotoxin (TTX) to block the sodium current (not shown). Thus it is very unlikely to be a voltage-clamp artifact associated with the flow of ionic currents. The asymmetrical responses persisted after return from TTX; that is, TTX application of itself did not invariably induce the slower symmetrical state 2 response. The irreversible effects previously ascribed to TTX application (2, 7) probably are unrelated to a specific drug action.

BRF responses with symmetrical time courses could be obtained by selecting a holding potential sufficiently hyperpolarized so that the depolarizing pulse did not open

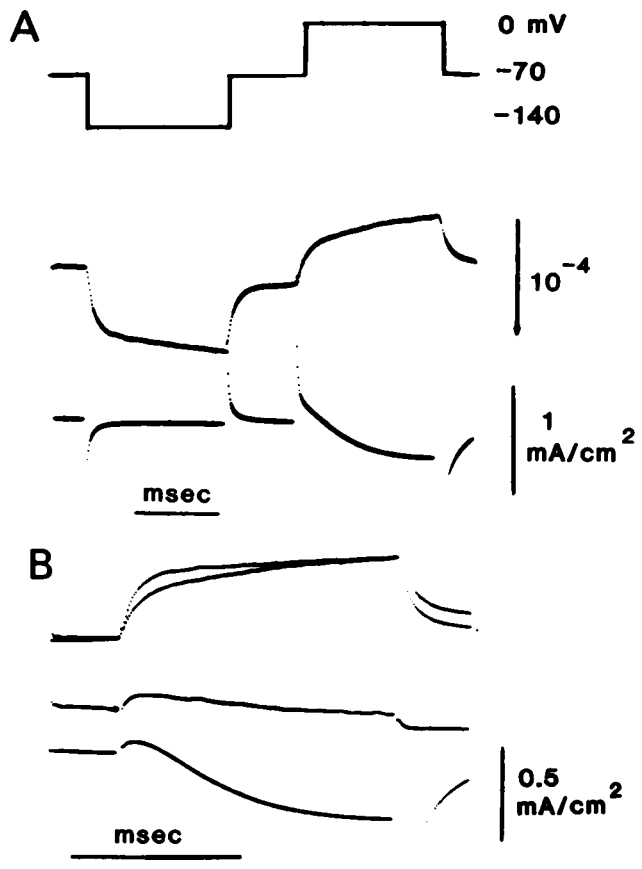

FIGURE 1 Asymmetry of the birefringence response. *A*, *Upper trace*, membrane potential; *middle trace*, birefringence; *lower trace*, membrane current. The length of the arrow represents a change in light intensity of 10^{-4}, increased light is a downward deflection. *B*, *Upper traces*, attempt to scale and superimpose the two BRF changes from *A*; *middle trace*, the difference between the two upper traces; *lower trace*, difference between the two current records of *A*. Axons 80–44, 80–45, and 80–46. Temp. 3°–4°C. 10% Na seawater//280 K, 10 TEA perfusion fluid. Optical record filtered at 30 kHz.

sodium channels (Fig. 2). When the holding potential was −160 mV, the time course of the BRF response to an 80-mV step was the same for steps to −80 mV or to −240 mV. These both had the same time course as the response

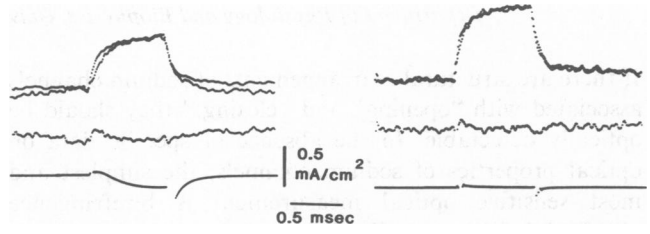

FIGURE 2 The effect of the holding potential on the asymmetry of the birefringence response. *Left traces* show responses to symmetrical 80-mV pulses from a −80 mV holding potential. *Right traces* show response to 80-mV pulses from a −160 mV holding potential. Same data format as in Fig. 1 *B*. Axons 82–11 and 82–12. Temp. 0°–1°C. 10% Na seawater/ /400 K perfusion fluid. Optical record filtered at 30 kHz.

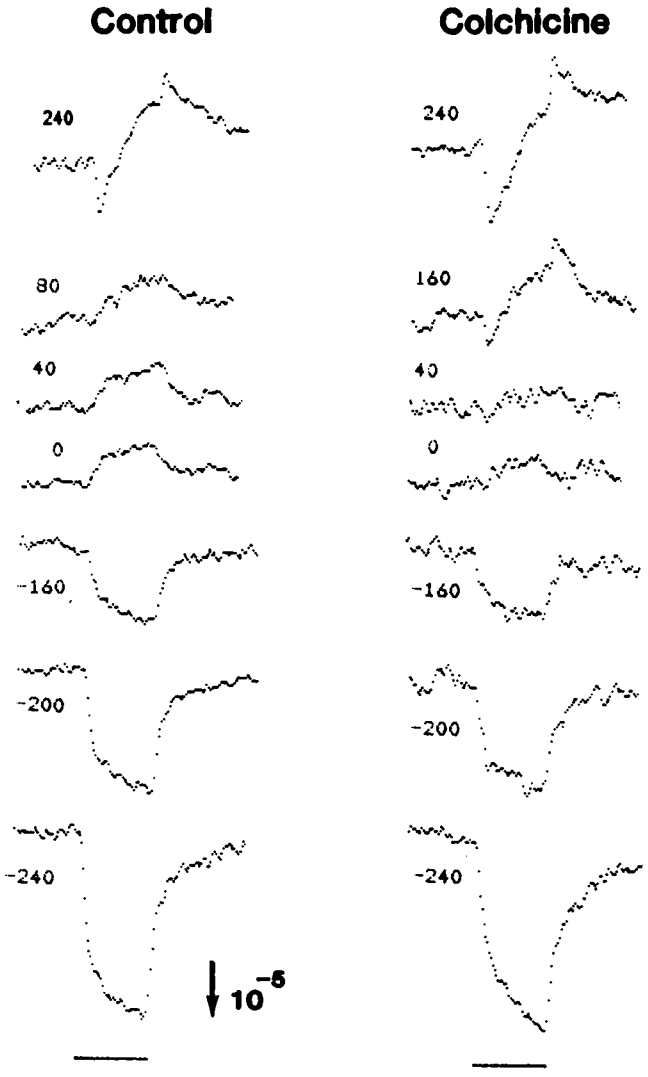

FIGURE 3 Variation of the birefringence response with pulse amplitude in the absence and presence of 30 mM colchicine. During the 0.75 ms indicated by the bars at the bottom of the figure the membrane potential was pulsed from −80 mV to the value marked by each trace. Axon 82–28. Temp. 0°C. 10% Na sea water//400 K perfusion fluid. Optical records filtered at 20 kHz.

to a pulse from −80 mV to −160 mV. The amplitudes of the responses to hyperpolarizing pulses were ~30% larger from the −160 mV holding potential. The amplitude of the response to a pulse from −80 to −160 mV was 1.06 ± 0.08 (n = 6) times as large as to a pulse from −160 to −80 mV.

Fig. 3 shows BRF responses to a wide range of pulses. The amplitude of the response measured near the beginning of the pulse could be described as proportional to the square of the difference between the voltage and +60 mV. Notice the downward deflection at +240 mV. During the depolarizing pulses the retardation increases, so that at longer times there is a different BRF-voltage relationship.

Colchicine reversibly blocks the sodium currents and selectively alters the BRF response to depolarizing pulses. The effect of colchicine is different at the various potentials. It appears as if an upward component has been subtracted from the control records to produce those in colchicine. While these records are not adequate for meaningful subtraction, qualitatively, it looks as though the amount of BRF change removed is very small for hyperpolarizations; it increases and reaches a maximum for depolarizations of ~+40 mV and then remains constant up to +240 mV.

CONCLUSIONS

There is an asymmetry in the time course of the BRF response that is reminiscent of the sodium channel gating currents or the asymmetry of the capacity charging transients. This BRF asymmetry is not seen when the holding potential is made more negative and the pulses no longer open sodium channels. The BRF-voltage relationship is time-dependent, more complex than previously described (2), and suggests there is more than one component in the BRF response. One component is reversibly blocked by colchicine, which also reversibly blocks sodium channels.

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REFERENCES

1. Cohen, L. B., B. Hille, and R. D.Keynes. 1970. Changes in axon birefringence during the action potential. *J. Physiol.* (*Lond.*). 211:495–515.
2. Cohen, L. B., B. Hille, R. D.Keynes, D. Landowne, and E. Rojas. 1971. Analysis of the potential-dependent changes in optical retardation in the squid giant axon. *J. Physiol.* (*Lond.*). 218:205–237.
3. Landowne, D., J. B. Larsen, and K. T. Taylor. 1983. Colchicine alters the nerve birefringence response. *Science* (*Wash., DC*). 220:953–954.
4. Armstrong, C. M., and F. Bezanilla. 1973. Currents related to movement of the gating particles of the sodium channels. *Nature* (*Lond.*). 242:459–461.
5. Keynes, R. D., and E. Rojas. 1973. Characteristics of the sodium gating currents in the squid giant axon. *J. Physiol.* (*Lond.*). 233:28–30P.
6. Meves, H. 1974. The effect of holding potential on the asymmetry currents in squid giant axons. *J. Physiol.* (*Lond.*). 243:847–867.
7. Cohen, L. B., and D. Landowne. 1971. Optical studies of action potentials. *In* Biophysics and Physiology of Excitable Membranes. W. J. Adelman, editor. Van Nostrand Reinhold, New York. 247–263.