Molecular Motion Underlying Activation and Inactivation of Sodium Channels in Squid Giant Axons

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Summary. Measurements of the changes in birefringence associated with changes in membrane potential were made with internally perfused squid giant axons in low sodium solutions at 0-8°C. The time course of the birefringence changes share many properties of the 'gating' (polarization) currents previously studied in this nerve. Both can be demonstrated as an asymmetry in the response to voltage pulses symmetrical about the resting potential which is not present about a hyperpolarized holding potential. Both have a rapid relaxation, which precedes the sodium permeability change. Both exhibit an initial delay or rising phase. Both are reversibly blocked by perfusion with 30 mM colchicine; neither are altered by changes on sodium concentrations or 300 nM tetrodotoxin. The birefringence response has a decrease in the amplitude of the rapid relaxation associated with the appearance of a slow relaxation. This is similar to the immobilization of fast gating charges which parallels sodium current inactivation.

The amplitude of the birefringence and the gating current responses is consistent with a change in the alignment of several hundred peptide bonds per sodium channel.

Key Words birefringence · optical retardation · nerve impulse · action potential · sodium channels · excitability · colchicine

Introduction

There is a transient change in the birefringence of a nerve which is nearly coincidental with the passage of an action potential. Following its discovery (Cohen, Keynes & Hille, 1968; Cohen, Hille & Keynes, 1970) a voltage-clamp study was made, but the molecular origin(s) of the phenomena were not determined. The feeling was expressed that this was not a good prospect for obtained much direct information, at the molecular level, of conformational changes associated with activation and inactivation of sodium channels (Cohen, Hille, Keynes, Landowne & Rojas, 1971). Several recent experiments have led me to an alternative hypothesis, that the birefringence change permits a measurement of the amplitude and time course of the motion of or within the sodium channel molecules.

The sodium channel molecule is a large glycoprotein oriented in the nerve membrane with an external neurotoxin (tetrodotoxin or saxitoxin) binding site. The surface density of sodium channels in squid axons is 170-550 binding sites/μm² (Levinson & Meves, 1975; Strichartz, Rogart & Ritchie, 1979). The eel electroplaque sodium channel has been isolated, purified and reconstituted into lipid membranes where it behaves quantitatively and qualitatively as native sodium channels (Rosenberg, Tomika & Agnew, 1984). The sequence of its 1820 amino acids has been published (Noda et al., 1984).

For a protein without visible color the principle contribution to the index of refraction or the retardation of the passage of light is from the π-π* transition of the peptide bonds linking the amino acids. For visible and near infrared light polarized parallel to the transition dipole, about 15° from the N—C axis in the N—C≡O plane, the retardation of these bonds is proportional to their surface density (Cantor & Schimmel, 1980).

\[ R = S \cdot e^2/2\varepsilon_0 m \omega_0^2 = S \times 2.5 \times 10^{-15} \text{ pm.} \]

S is the number of peptide bonds/cm², \( \varepsilon_0 \) and \( m \) are the charge and mass of the electron, \( \varepsilon_0 \) is the permittivity of space and \( \omega_0 \) is the resonance frequency of this electronic oscillator, which is also responsible for the 190-nm absorption band. Cohen et al. (1970) estimated that the change in birefringence or the difference in retardation to light polarized in the two directions was about 0.15 pm. 60,000 peptide bonds/μm² rotating 90° from lying in the plane of the membrane to perpendicular to it, or a larger number rotating through a smaller angle, would produce a signal of this magnitude. Divided by the sodium channel density, this means the movement of a few hundred bonds/channel could be responsible for the birefringence change.

Electrical recording techniques have greatly in-
creased our understanding of the properties of sodium channels. Measurements of the asymmetry of displacement current transients have shown there is a voltage-dependent intramembranous movement of charge associated with the activation of sodium channels (the gating current) which is reduced or immobilized with a time course that parallels inactivation (Armstrong & Bezanilla, 1973, 1977; Almers, 1978). This movement precedes the sodium conductance change, so it would not have been described as ‘conductance-dependent’ had it been seen in the initial birefringence experiments. The published records were made at too slow a sweep speed to resolve this rapid movement. The investigators were influenced by the birefringence response to hyperpolarization, which clearly is not directly associated with the activation of sodium channels, and therefore did not study the events during depolarizing pulses in more detail.

In order to test this molecular motion hypothesis I have measured the birefringence response in voltage-clamped squid axons with sufficient temporal resolution to observe events with the time course of gating currents. By using low temperatures, low external sodium concentrations, internal perfusion and an improved internal electrode assembly, many of the problems of the earlier experiments have been avoided. An alkaloid has been found which reversibly reduces the ionic sodium current, the gating current and the birefringence response to a depolarizing voltage-clamp pulse (Landowne, Larsen & Taylor, 1982; Matsumoto, Ichikawa & Tasaki, 1984). The results presented here suggest that most of the birefringence response to modest depolarizing pulses is associated with the activation and inactivation of sodium channel molecules. Preliminary reports have appeared (Scruggs & Landowne, 1980; Landowne, 1984a, b).

Materials and Methods

15-mm segments of well-cleaned squid giant axons were cannulated, internally perfused and voltage-clamped using standard techniques. In order to measure and control the absolute potential across the membrane, the double spiral platinized-platinum internal electrode assembly used previously (Cohen et al., 1971) was replaced with a KCl-filled 80 μm glass tube with an electrically floating Pt wire inside to sense the voltage. A 50- to 60-μm bundle of 10-μm carbon fibers (about 20 fibers) was glued to the glass tube to provide current over a 5-mm length on both sides of the tip of the voltage electrode. Conducting silver paint made the electrical connection between the carbon fibers and a fine copper wire.

The axons were mounted in a 10 × 2 × 2 mm slot milled into a block of silver. The interior of this cavity was electroplated with gold and then platinized. Outside the slot the axon passed through airgaps and then rested on plastic bridges. In the central 4 mm the side walls were electrically isolated from the rest of the chamber, connected to a current-to-voltage converter (virtual ground) and used as the current-measuring electrodes. A second KCl-filled electrode sensed the bath potential through a hole in one of the walls. The floor of this guarded region was a 1-mm thick glass plate with a 5-mm f.l. cylindrical lens cemented to its lower surface. A removable coverslip formed the top of the chamber. With seawater in the chamber this combination focussed the light beam into a 1 × 4 mm rectangle at the top of the chamber without striking the walls.

The external solutions were a mixture of Na and TMA (tetramethylammonium) artificial seawaters referred to in the text by the percentage of Na. They contain (in mM) 500 Na or TMACl, 10 KCl, 50 CaCl₂ and 2 HEPES (N-2-hydroxyethylpiperazine-N’-2-ethanesulfonate) buffer, pH 7.4. They flowed continuously at about 0.1 ml/min through the chamber. The internal solution contained 400 K⁺, 320 glutamate, 50 F⁻, and 30 mM phosphate buffer, pH 7.4, flowing under a pressure of about 10 cm of water. A chilled water-methanol mixture cooled the block; the temperature was monitored with an indwelling thermometer. Cool dry flowing nitrogen kept the lens and coverslip from fogging.

The light source was a 12-V 100W tungsten-iodide bulb powered by a regulated DC supply. The beam was partially collimated and then passed through a Glans-Thompson crystal polarizer mounted at 45° to the long axis of the axon. No heat filter was used on the beam. The collimator was adjusted so a portion of the filament was focussed on the coverslip, thus the axon was critically illuminated to provide the greatest intensity. About 4 mW or 100 mW/cm² was measured at the coverslip.

After striking the axon the light was collected with a 10 × 0.4 n.a. microscope objective and passed through a second polarizer set perpendicular to the first one. At the image plane a ground glass plate was used to observe the axon and position stops just beyond its edges and over the image of the electrode. The plate was replaced with a YAG-444 photodiode (E.G. & G.) used in the photoconductive mode. This device is responsive from 400 to 1100 nm. The voltage changes developed across a 100 K load resistor were amplified and then digitized and stored with a Nicolet 1170 signal averager and an Apple II computer. With the amplifier bandwidth set to 30 kHz the system responded to a light pulse with an 8 μsec half time. A second channel of the averager was used to simultaneously record the current flowing through the central portion of the axon. Typically records were made by measuring the resting (DC) light intensity and then averaging 1024 changes in light intensity in response to a repeated voltage pulse impressed across the membrane. The results are expressed as the change in light intensity, ΔI/I₀, in parts per million (ppm).

In many experiments a secondary or ‘state 2’ response develops irreversibly. To assess the condition and stability of the axons, each experiment began with two or three control measurements. Except for the experiments designed to study the secondary response all of the results reported here come from bracketed experiments (control, test, recovery) made after this initial control period.

To measure the resting retardation the electrode assembly was removed and the axon was tied at both ends of the chamber so that it remained inflated with perfusion fluid. The axons were removed from the chamber and mounted between a pair of coverslips in 1 mm of seawater. This assembly was placed on a Zeiss polarization microscope with crossed polarizers and a rotating stage. Under 540-nm illumination the axons were rotated to an intensity minimum and then an additional 45° to the intensity...