SINGLE SODIUM CHANNELS FROM THE SQUID GIANT AXON

FRANCISCO BEZANILLA
Department of Physiology, Jerry Lewis Neuromuscular Research Center, Ahmanson Laboratory of Neurobiology, University of California at Los Angeles, Los Angeles, California 90024; and Marine Biological Laboratory, Woods Hole, Massachusetts 02543

ABSTRACT Since the work of A. L. Hodgkin and A. F. Huxley (1952. *J. Physiol.* [*Lond.*].117:500–544) the squid giant axon has been considered the classical preparation for the study of voltage-dependent sodium and potassium channels. In this preparation much data have been gathered on macroscopic and gating currents but no single sodium channel data have been available. This paper reports patch clamp recording of single sodium channel events from the cut-open squid axon. It is shown that the single channel conductance in the absence of external divalent ions is ~14 pS, similar to sodium channels recorded from other preparations, and that their kinetic properties are consistent with previous results on gating and macroscopic currents obtained from the perfused squid axon preparation.

INTRODUCTION

Sodium and potassium conductance changes underlie the generation and propagation of the nervous impulse (Hodgkin and Huxley, 1952). Today we know that these changes are produced by ionic channels whose probability of being open is controlled by the membrane potential. The sodium channel is a macromolecule with known primary structure (Noda et al., 1984) whose function has been studied with large populations of channels (macroscopic current) (Hodgkin and Huxley, 1952), charge movement related to voltage-dependent conformational changes (gating currents) (Armstrong, 1981; Keynes, 1983; Bezanilla, 1985) and single channel recordings (Sigworth and Neher, 1980; Aldrich et al., 1983; Horn and Vandenberg, 1984). The detailed modeling of sodium channel function requires these three types of measurements from the same preparation. The squid axon provides the best signal-to-noise ratio for recording macroscopic and gating currents. Conti and Neher (1980) were able to record single potassium channel events from the inside of the squid axon using bent pipettes in perfused axons, but the method had severe frequency response limitations. The high speed and apparently small single channel conductance of the sodium channels has prevented the recording of single Na^+ channel events in this preparation. The cut-open axon technique (Llano and Bezanilla, 1980) was developed to have free access to the internal surface of the axon, and a modification of the original method (Llano and Bezanilla, 1985) allowed us to reliably record single potassium channel events using the gigaohm seal technique (Hamill et al., 1981). The same technique has been used in the present study to record single sodium channel events. The results presented here have been communicated in abstract form (Bezanilla, 1987).

METHODS

Details of the technique will be published elsewhere (Llano, I., C. K. Webb, and F. Bezanilla, manuscript in preparation). The main procedure will be outlined below. A segment of axon dissected from the squid *Loligo pealei* was pinned to a Sylgard-covered coverslip and was cut open with microscissors while bathed in artificial seawater containing (in millimolar) 440 NaCl, 50 $MgCl_2$, 10 $CaCl_2$, 10 Tris, pH 7.6. This operation took ~3 min and was done at ~20°C. After the axoplasm was removed by forced solution circulation, the solution was changed to 540 mM NaCl and 10 mM Tris, pH 7.6. Patch pipettes were pulled from Corning 7052 glass (Garner Glass Co., Claremont, CA) with apertures of <1 μm and filled with (in millimolar) 35 Na glutamate, 125 *N*-methyl glucamine (NMG) glutamate, 20 NMG fluoride, 10 NaCl, 5 Tris EGTA, 10 Hepes, and 460 sucrose, pH 7.2, their resistance was ~10 MΩ. We did not explore in detail other types of glass but Corning 8161 was not as reliable as 7052. The patch pipette was approached to the internal surface under an inverted microscope at 350×. After the gigaseal was obtained, the patch was excised and the current was recorded with standard patch clamp equipment usually at 5°C. Pulses were generated by a D/A converter under computer control. Acquisition was normally done at 20 μs per point with a 16 bit A/D converter and with an analog bandwidth of 2 kHz. The hardware and software used for the interface is similar to the system described by Stimers et al. (1987).

RESULTS AND DISCUSSION

Fig. 1 illustrates a series of voltage clamp records showing single sodium channel events and the average of a large number of similar records. The axon was bathed in a solution containing 540 mM Na in the nominal absence of calcium and magnesium. Divalents ions were eliminated

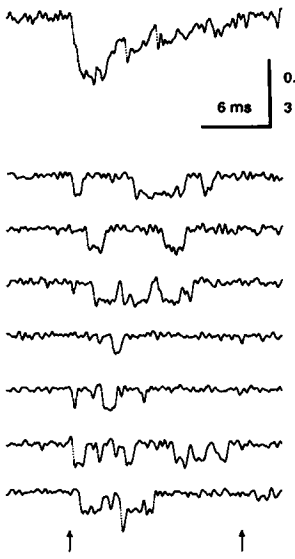

FIGURE 1 Single channel recordings (*lower traces*) and average current of 57 single traces (*top trace*) obtained from a cut-open squid axon. Holding potential was −50 mV and each pulse to −40 mV was preceded by a 50-ms prepulse to −90 mV. Interpulse interval was 1 s. Arrows indicate beginning and end of the pulse. The current calibration of 0.7 pA corresponds to the average current, and the calibration of 3 pA corresponds to the single channel traces.

because they block the squid axon sodium conductance (Taylor et al., 1976), making the single channel events too small to be clearly detected above the noise (Yamamoto et al., 1984). This patch seemed to contain two active sodium channels as can be evidenced in the bottom trace that has two levels of conductance. In most of the experiments one to four channels were present in the patch. This reflects a much lower density than expected from the macroscopic current measurements and most likely is the result of exposing the inside of the membrane to seawater containing calcium that activates proteases present in the axoplasm. In previous experiments done with loose seals with large pipettes (Levis et al., 1984) we observed that the channel density decreased progressively upon exposure of the internal side of the membrane to seawater but the kinetics of the sodium current remained unaltered. After formation of the loose seal with internal solution with low divalent cations, the isolated channel population remained constant for periods up to 5 h. Here the exposure to seawater was deliberately long (3–5 min) to decrease the channel density under the patch.

Resolvable events could be obtained in a narrow potential range (−50 to −20 mV) and an I-V curve is plotted in Fig. 2. The curve has been extrapolated to the calculated Nernst potential and gives a conductance of 14 pS. This conductance is much larger than previous estimates of conductance using noise analysis. Table I shows a comparison of single channel conductance of the sodium channel of the squid by several methods and different conditions. There are two reasons for the difference of noise and single channel results. The first is the large concentration of divalent ions used in the noise studies and the second is the recording bandwidth. The small value of 3.5 pS estimated with mean-variance analysis with patch pipettes in the cut-open axon (Llano and Bezanilla, 1984) is due to the high divalent ion concentration and limited bandwidth used in that study. The effect of frequency response limitation can be seen when comparing the single channel conductance estimated as 5.5 pS with noise analysis under the same divalent conditions with 20 kHz bandwidth (Levis et al., 1984). The relief of divalent block becomes apparent when the divalent ion concentration was reduced to 6 mM Ca and the estimated conductance was larger (8 pS). In the present study no divalent ions were added, therefore the apparent decrease of the conductance by divalent block is not present. The bandwidth used was only 2 kHz but in measuring the amplitude of single events one selects the open times that are long enough to be resolved to estimate the single channel current. When noise analysis is used the method will give an average of the single channel current for long well resolved events and for short attenuated events; this shows that estimates of single channel conductance obtained with noise analysis should be used with caution when the frequency response is limited.

Recently, single sodium channels from the retinal nerve of the squid have been incorporated in planar bilayers (Latorre et al., 1987) and observed in presence of batrachotoxin (BTX). The single channel conductance is 18 pS in absence of divalent ions and 2 pS with 10 Ca and 50 Mg. This result compares very well with the single channel conductance of BTX-treated channels observed in the cut-open axon with divalent ions present (Llano and Bezanilla, 1986) (see Table I). The value of 14 pS reported

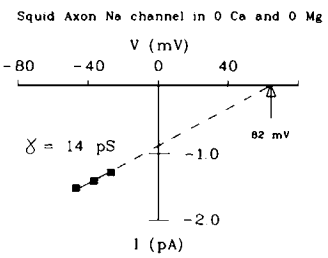

FIGURE 2 Single channel current amplitudes for three different voltages. The curve has been extrapolated to the calculated reversal potential with the known internal (45 mM) and external (540 mM) sodium concentrations. Experimental voltages have been corrected for liquid junction potentials.

TABLE I

Preparation	Ionic conditions	Type of measurement	T	γ
			°C	pS
Perfused axon*	10 Ca, 50 Mg	Noise 1 kHz	6	4
Cut-open axon‡	10 Ca, 50 Mg	Noise, 2 kHz	5	3.5
Cut-open axon§	10 Ca, 50 Mg	Noise, 20 kHz	5	5.5
Cut-open axon§	6.0 Ca, 0 Mg	Noise, 20 kHz	5	8
Cut-open axon‖	11 Ca, 55 Mg	Noise, 5 kHz	5	4.4
Cut-open axon¶	10 Ca, 50 Mg, BTX	Single channel	5	1.5
Cut-open axon	0.0 Ca, 0 Mg	Single channel	5	14
Retinal nerve**	0.0 Ca, 0 Mg, BTX	Single channel	22	18
Retinal nerve**	10 Ca, 50 Mg, BTX	Single channel	22	2

*Conti et al., 1975.
‡Llano and Bezanilla, 1984.
§Levis et al., 1984.
‖Bekkers et al., 1986.
¶Llano and Bezanilla, 1986.
**Latore et al., 1987.

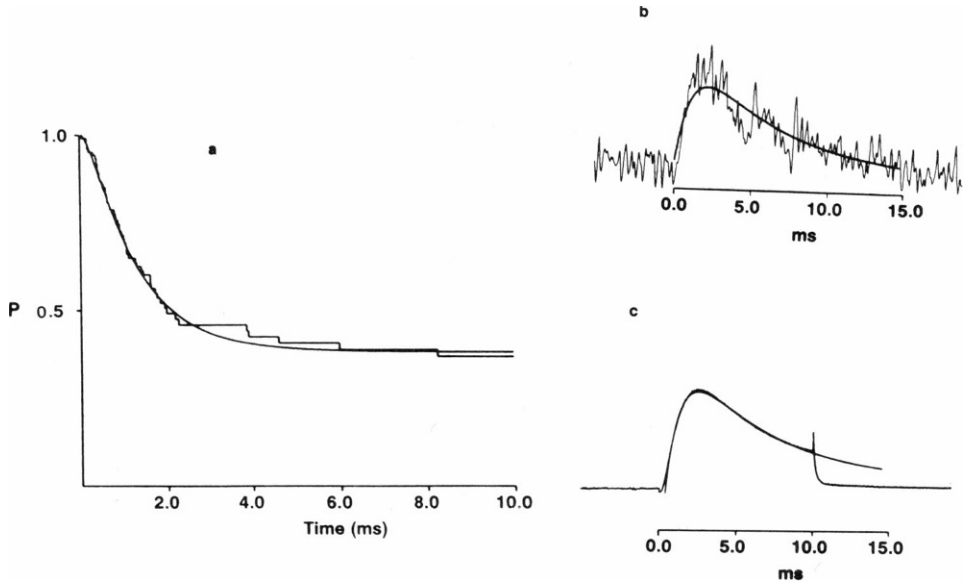

FIGURE 3 *a* is the first latency distribution function estimated from latency of first openings for a series of single channel currents recorded at −40 mV. The function was calculated according to the equation $P = P_\infty + [\tau_1 \exp(-t/\tau_1) - \tau_2 \exp(-t/\tau_2)]/(\tau_2 - \tau_1)$, where P is the probability that an individual channel has not yet opened for the first time at t ms after the onset of the depolarizing pulse and P_∞ is P at $t = \infty$. The time constants estimated were 1.1 and 0.21 ms and $P_\infty = 0.38$. This patch contained two channels and the latency function was estimated from the square root of the experimental first latency function. *b* is the average current for the same group of records used to estimate the first latency function. The time constant of the exponentials fitted were 1 ms for rising, and 5.9 ms for the falling phase. *c* is a sodium current recorded from a perfused axon bathed in a low sodium solution containing 50 mM Mg and 10 mM Ca. The time constants of the fitted exponentials were 1 ms for rising and 5.5 ms for the falling phase. The potential during the pulse was −20 mV. Inward current has been plotted up in parts *b* and *c*.

here was obtained at 5 C and it is not directly comparable with the value of the single-channel conductance of the squid retinal nerve sodium channel incorporated in bilayer in presence of BTX.

Fig. 1 shows that a single channel appears to open more than once during the depolarizing pulse (recall that this patch contained only two channels). This result is different to the very brief openings reported by Aldrich et al. (1983) and Aldrich and Stevens (1987) in neuroblastoma cells but is similar to the results of Horn and Vandenberg (1984) in GH3 cells. The first latency distribution function was estimated from single-channel records as described by Aldrich et al. (1983) and the result is plotted in Fig. 3 *a*; the two time constants were 0.21 and 1.1 ms. Fig. 3 *b* shows the average current along with a fit of two exponentials. The decaying exponential that fits the macroscopic inactivation is much slower than the slow component of the first latency indicating that at this potential the observed inactivation is not the result of late openings as observed in neuroblastoma. This result is consistent with previous measurements of macroscopic and gating currents in squid axon that show that the inactivation step is slow and partially coupled to the activation sequence (Armstrong and Bezanilla, 1977). The present result is based on the estimate of two channels in the patch; if this number was an underestimate, the values of the time constants would also be underestimated.

Fig. 3 *c* shows a sodium current recorded from a perfused axon in the presence of 10 Ca and 50 Mg. The potential of the pulse is 20 mV more positive than the pulse potential of the averaged single-channel current obtained in zero divalent conditions (Fig. 3 *b*). The time course of both currents compared very well, and the shift in the voltage axis is to be expected because of the absence of divalent ions.

The understanding of channel function at the molecular level requires not only knowledge of the structure of the channel but also a detailed modeling of its physical states. The combination of all types of electrophysiological measurements will help in obtaining more reliable estimates of the voltage-dependent parameters used in modeling sodium channel gating. The results presented here show that it is now possible to record the three electrophysiological expressions of sodium channels from the same preparation eliminating some of the ambiguities inherent in comparing results from sodium channels with different biological preparations.

We thank Dr. R. A. Levis for constant encouragement and for lending a low noise patch clamp, and Dr. C. Vandenberg for critical comments on the manuscript.

This work was supported by U.S. Public Health Service grant GM30376.

Received for publication 6 July 1987and in final form 21 August 1987.

REFERENCES

Aldrich, R. W., D. P. Corey, and C. F. A. Stevens. 1983. Reinterpretation of mammalian sodium channel gating based on single channel recording. *Nature (Lond.).* 306:436–441.

Aldrich, R. W., and C. F. Stevens. 1987. Voltage-dependent gating of single sodium channels from mammalian neuroblastoma cells. *J. Neurosci.* 7(2):418–431.

Armstrong, C. M. 1981. Sodium channels and gating currents. *Physiol. Rev.* 61:644–683.

Armstrong, C. M. and F. Bezanilla. 1977. Inactivation of the sodium channel. II. Gating current experiments. *J. Gen. Physiol.* 70:567–590.

Bekkers, J. M., N. G. Greef, and R. D. Keynes. 1986. The conductance and density of sodium channels in the cut-open squid giant axon. *J. Physiol. (Lond.).* 377:463–486.

Bezanilla, F. 1985. Gating of sodium and potassium channels. *J. Membr. Biol.* 88:97–111.

Bezanilla, F. 1987. Single sodium channels recorded from the cut-open squid giant axon. *Biophys. J.* 51:195a. (Abstr.)

Conti, F., L. J. De Felice, and E. Wanke. 1975. Potassium and sodium ion current noise in the membrane of the squid axon. *J. Physiol. (Lond.).* 248:45–82.

Conti, F., and E. Neher. 1980. Single channel recording of K^+ currents in squid axons. *Nature (Lond.).* 285:140–143.

Hamill, O. P., A. Marty, E. Neher, B. Sackmann, and F. J. Sigworth. 1981. Improved patch clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch. Eur. J. Physiol.* 391;85–100.

Hodgkin, A. L., and A. F. Huxley. 1952. A quantitative description of the membrane current and its application to conduction and excitation in nerve. *J. Physiol. (Lond.). 117:500–544.*

Horn, R., and C. A. Vandenberg. 1984. Statistical Properties of single sodium channels. *J. Gen. Physiol.* 84:505–534.

Keynes, R. D. 1984. Voltage-gated ion channels in the nerve membrane. *Proc. R. Soc. Lond. B. Biol. Sci.* 220:1–30.

Latorre, R., A. Oberhauser, M. Condrescu, R. DiPolo, and F. Bezanilla. 1987. Incorporation of sodium channels from squid optic nerve into planar lipid bilayers. *Biophys. J.* 51:195a. (Abstr.)

Levis, R. A., F. Bezanilla, and R. M. Torres. 1984. Estimate of the squid axon sodium channel conductance with improved frequency resolution. *Biophys. J.* 45:11a. (Abstr.)

Llano, I., and F. Bezanilla. 1980. Current recorded from a cut-open squid giant axon under voltage clamp. *Proc. Natl. Acad. Sci. USA.*77:7484–7486.

Llano, I., and F. Bezanilla. 1984. Analysis of sodium current fluctuations in the cut-open squid giant axon. *J. Gen. Physiol.* 83:133–142.

Llano, I., and F. Bezanilla. 1985. Two types of potassium channels in the cut-open squid giant axon. *Biophys. J.* 47:221a. (Abstr).

Llano, I., and F. Bezanilla. 1986. Batrachotoxin-modified single sodium channels in squid axon. *Biophys. J.* 49:43a. (Abstr.)

Noda, M., S. Shimizu, T. Tanabe, T. Takai, T. Kayano, T. Ikeda, H. Takahashi, H. Nakayama, Y. Kanaoka, M. Minamino, K. Kangawa, H. Matsuo, M. A. Raftery, T. Hirose, S. Inayama, H. Hayashida, T. Miyata, and S. Numa. 1984. Primary structure of *Electrophorus electricus* sodium channel deduced from cDNA sequence. *Nature (Lond.).* 312:121–127.

Sigworth, F. J., and E. Neher. 1980. Single Na^+ channel currents observed in cultured rat muscle cells. *Nature (Lond.).* 287:447–449.

Stimers, J. R., F. Bezanilla, and R. E. Taylor. 1987. Sodium channel gating currents. Origin of the rising phase. *J. Gen. Physiol.* 89:521–540.

Taylor, R. E., C. M. Armstrong, and F. Bezanilla. 1976. Block of sodium channels by external calcium ions. *Biophys. J.* 16:27a. (Abstr.)

Yamamoto, D., J. Z. Yeh, and T. Narahashi. 1984. Voltage-dependent calcium block of normal and tetramethrin-modified single sodium channels. *Biophys. J.* 45:337–344.