Veratridine Modification of the Purified Sodium Channel α-Polypeptide from Eel Electroplax

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ABSTRACT In the interest of continuing structure-function studies, highly purified sodium channel preparations from the eel electroplax were incorporated into planar lipid bilayers in the presence of veratridine. This lipoglycoprotein originates from muscle-derived tissue and consists of a single polypeptide. In this study it is shown to have properties analogous to sodium channels from another muscle tissue (Garber, S. S., and C. Miller. 1987. *Journal of General Physiology*. 89:459–480), which have an additional protein subunit. However, significant qualitative and quantitative differences were noted. Comparison of veratridine-modified with batrachotoxin-modified eel sodium channels revealed common properties. Tetrodotoxin blocked the channels in a voltage-dependent manner indistinguishable from that found for batrachotoxin-modified channels. Veratridine-modified channels exhibited a range of single-channel conductance and subconductance states. The selectivity of the veratridine-modified sodium channels for sodium vs. potassium ranged from 6–8 in reversal potential measurements, while conductance ratios ranged from 12–15. This is similar to BTX-modified eel channels, though the latter show a predominant single-channel conductance twice as large. In contrast to batrachotoxin-modified channels, the fractional open times of these channels had a shallow voltage dependence which, however, was similar to that of the slow interaction between veratridine and sodium channels in voltage-clamped biological membranes. Implications for sodium channel structure are discussed.

INTRODUCTION

Purification and reconstitution studies have provided a substantial body of information on the structure of the voltage-dependent sodium channel, demonstrating that it is an intricate transmembrane protein. This lipoglycoprotein consists of an α-polypeptide alone (electric organ of *Electrophorus electricus*), or in a complex with one (rat muscle) or two (rat brain) additional subunits (Catterall, 1988). Throughout

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these studies, toxins that modify channel properties have been used to identify the relevant peptides necessary for channel function, and to examine mechanisms of operation. While tracer uptake studies in vesicles have used activation by both veratridine and batrachotoxin to quantify the recovery of function (Tamkun et al., 1984; Tomiko et al., 1986; Duch and Levinson, 1987*a*), only batrachotoxin (BTX) has been used extensively in the examinations of the properties of purified single channels in planar bilayers (Hartshorne et al., 1985; Furman et al., 1986; Recio-Pinto et al., 1987*a*). The functional examinations of the purified proteins have demonstrated that the peptides from all examined tissues were sufficient to mediate appropriate sodium channel operation (sodium conductance and selectivity, voltage-dependent activation, and block by tetrodotoxin [TTX]). However, differences were found in some single-channel properties of these proteins, (see Table V of Recio-Pinto et al., 1987*a*).

It is not surprising that functional variations occur between sodium channels from several sources, as both the amino acid structures (Noda et al., 1985, 1986; Trimmer et al., 1988) and posttranslational processing (Thornhill and Levinson, 1987; James and Agnew, 1987; Catterall, 1988) vary between channels from different tissues. To quantitate the functional impact of this structural diversity, well characterized and highly purified systems must be studied.

To continue our initial characterization (Recio-Pinto et al., 1987*a*) of the properties of the highly purified α-polypeptide from the eel electroplax, we incorporated these preparations into planar lipid bilayers in the presence of veratridine, the most widely used sodium channel activator in vesicular uptake studies. While veratridine is believed to bind to the same site on the sodium channel as BTX (Catterall, 1980), its actions on macroscopic (Khodorov, 1985) and microscopic (Garber and Miller, 1987) sodium currents are substantially different. In this report we present our first study of purified electroplax sodium channel conductances and subconductances, voltage dependence of single-channel fractional open times, selectivity for sodium and potassium determined by reversal potential or conductance measurements, and the voltage dependence of TTX block after veratridine-modification. The structural significance of these results is considered. Preliminary reports of some of this work have been presented (Duch et al., 1988*b*, 1989).

MATERIALS AND METHODS

Preparation of Sodium Channel Material

The sodium channel from *Electrophorus electricus* was purified as previously described; the final reconstituted preparations contained only a single polypeptide with an apparent molecular weight of 260 kD (Duch and Levinson, 1987*a*). Reconstituted vesicles from four separate purifications were used.

Synaptosomal and P2 fractions from human and canine cortex were prepared with the methods of Cohen et al. (1977). When modified with BTX, the sodium channels from these preparations displayed typical behaviors (Krueger et al., 1983; Green et al., 1987; Duch et al., 1987, 1988*a*).

Planar Bilayers and Insertion of Na^+ Channels

Planar bilayer techniques were as previously described (Recio-Pinto et al., 1987*a*), unless otherwise noted. Planar bilayers were formed from neutral phospholipid solutions containing

(4:1) phosphatidylethanolamine and phosphatidylcholine in decane. Most experiments were conducted in symmetrical 500 mM NaCl, buffered with 10 mM HEPES at pH 7.4. Experiments were performed at room temperature (23–25°C); no corrections were made for results obtained at different temperatures.

Channel currents were recorded under voltage-clamp conditions using a standard current-to-voltage amplifier. The bilayer recording system was tested before each experiment by control measurements of the capacity and conductance of a membrane-equivalent circuit (1×10^{11} Ω, 311 pF capacitor). No drifts were noted, and test values were highly reproducible. A power spectrum was used to analyze the sources of noise in the system. The same control measurements were then repeated on the bilayer. Veratridine (50 μM, unless otherwise specified) was added to both chambers and stirred. The channel preparation was added to the *cis* chamber. After channel incorporation, a large dose of TTX (30–50 μM) was added to either the *cis* or *trans* chambers to block all measurable sodium channel activity on one side of the membrane, thus allowing the use of the electrophysiological sign convention.

As was the case for BTX, channels modified by veratridine showed several conductance and subconductance levels. To characterize these levels in multi-channel membranes, the minimal number of transitions (eliminating matching opening and closing transitions) accounting for the difference between the background current level and the largest observed current levels were determined. Tabulated transitions from all potentials at which background was observed were then compared with each other. Corresponding transitions present at two or more potentials were counted as conductance levels. Membranes containing more than four channels (8 of a total 64) were not used because membrane currents rarely returned to background levels at the same potentials at which most channels were open simultaneously.

Examination of channels at all potentials was necessary because the voltage dependence of these channels varied, and some channels were observed only within certain potential ranges. Transition levels were also compared between potentials to ensure that only stable conductance levels were counted (each potential was held for at least 5 min). Although the contribution of single-channel substates to this analysis is minimized, it cannot be eliminated. Therefore this analysis does not attempt to assign conductance levels to individual channels, which cannot generally be done in multi-channel membranes.

The voltage dependence of channel fractional open times in multi-channel membranes was measured as previously described (Duch et al., 1988*a*). The results obtained from the descending and ascending pulse sequences were combined, normalized to the conductance at +90 mV, and averaged. For single-channel membranes, potentials were decremented or incremented sequentially in steps of 20 or 30 mV from an initial holding potential of +120 mV. Each potential was held for a minimum of 5 min, and f_o was measured by hand from chart records.

Reversal potential measurements were made under biionic conditions: 455 mM NaCl vs. 455 mM KCl, pH 7.4 with 10 mM HEPES. Ag-AgCl electrodes were zeroed in symmetrical solutions (either NaCl or KCl), before the solution in the *trans* chamber was replaced with the complementary salt. The electrode offset was again examined after each experiment by replacing the salt solution in the *trans* chamber with solution from the same stock used to fill the *cis* chamber. In almost all cases, the electrode offset was <1 mV, and was never more than 2 mV. For all experiments, the solution that was to face the extracellular side of the channel was placed in the *cis* chamber. After channel incorporation, 30–50 μM TTX was added to the *trans* chamber to completely block all channels incorporated in the opposite direction.

TTX block of single-channel membranes was measured as previously described (Levinson et al., 1986). To measure TTX block of sodium channels in membranes containing more than one channel, membrane potentials were decremented in 15-mV steps from a holding potential of either +120 or +90 mV to a holding potential of −75 mV, then incremented in 15-mV steps back to the original holding potential. Each potential was held for ~60 s. This sequence was repeated a minimum of three times during the control period. Current traces were recorded by computer and time-averaged after subtraction of the membrane capacitative transient. If at the end of the control period, total membrane current was significantly greater or smaller than during the first control sequence (indicating an increase or decrease in the number of channels present in the membrane), all measurements were repeated until at least three consecutive control sequences without further incorporation or loss of channels could be obtained. TTX (30–300 nM) was then added to the extracellular side of the channels, and the same potential sequences were repeated for at least three measurements. Membrane background conductance was calculated using the time-averaged current measurements at the most hyperpolarized potentials observed with TTX present, as no channels were open during these periods. This background conductance was subtracted from the calculated membrane conductances at all potentials during both the control and experimental periods. Channel fractional closed times (f_c) were calculated from the reduction in channel current

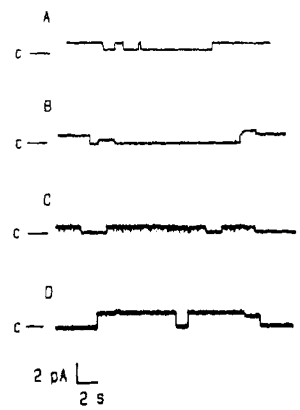

FIGURE 1. Veratridine-modified single-channel current transitions of purified eel electroplax sodium channels in symmetrical 500 mM NaCl at various membrane potentials. All traces are filtered at 50 Hz. (*A*) Single-channel trace for 10–13 pS channel. The channel was open >98% of the time at this potential, so TTX (300 nM) was added to see transitions. Holding potential was +60 mV. (*B*) Trace of large (12 pS) and small (4 pS) channels together in one membrane. Holding potential was +75 mV. (*C*) Single-channel trace for small channel (7 pS) with no larger channels in membrane. Holding potential was +80 mV. (*D*) Single-channel trace for transition between large channel and substate. The channel is maximally open with a conductance of 12 pS, then closes to a conductance of 8 pS before closing completely. Holding potential was +120 mV; TTX (300 nM) was present.

after TTX addition:

$$f_c = (g_c - g_{TTX})/g_c \tag{1}$$

where g_c is the total time-averaged channel conductance during the control experiments, and g_{TTX} is the time-averaged channel conductance remaining after TTX addition. $K_{1/2}$ of TTX block was calculated from the measured f_c as previously described (Levinson et al., 1986).

RESULTS

Single-Channel Conductance in Symmetrical Sodium Solutions

Almost all membranes examined in the presence of veratridine were multi-channel membranes, i.e., contained more than one sodium channel; only 2 of 62 bilayers with channel activity contained single channels. In 500 mM NaCl, the majority of channels exhibited conductances between 10 and 13 pS (Figs. 1 *A*, 2, and 3). However, smaller conductances were also observed regularly, usually in the presence of

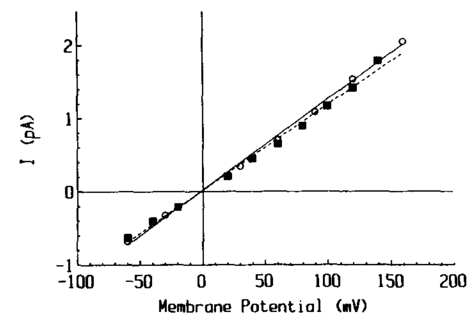

FIGURE 2. *I-V* curves of two membranes containing only a single sodium channel. Symmetrical 500 mM NaCl, 50 μM symmetrical veratridine. At potentials more negative than −60 mV, channels opened too infrequently for reliable measurement; error bars are within symbols. Data were fit by linear regression and not forced through 0; slope conductances were 11.7 (*squares*) and 12.4 pS (*circles*). The *I-V* curve with the squares was obtained from the same data used to plot the single-channel histogram in Fig. 3 *A*.

the larger conductances (Fig. 1 *B*), and rarely alone (Fig. 1 *C*). The 10–13 pS channels sometimes closed to substate levels with conductances similar to the smaller transitions (Fig. 1 *D*). The current-voltage relationships of the two single channel membrane recordings were linear and symmetrical (Fig. 2), with slope conductances of 11.7 and 12.4 pS. The same *I-V* relationship was observed for multi-channel membranes (Fig. 3 of Recio-Pinto et al., 1987*a*).

Conductance amplitude histograms were constructed for individual membranes,

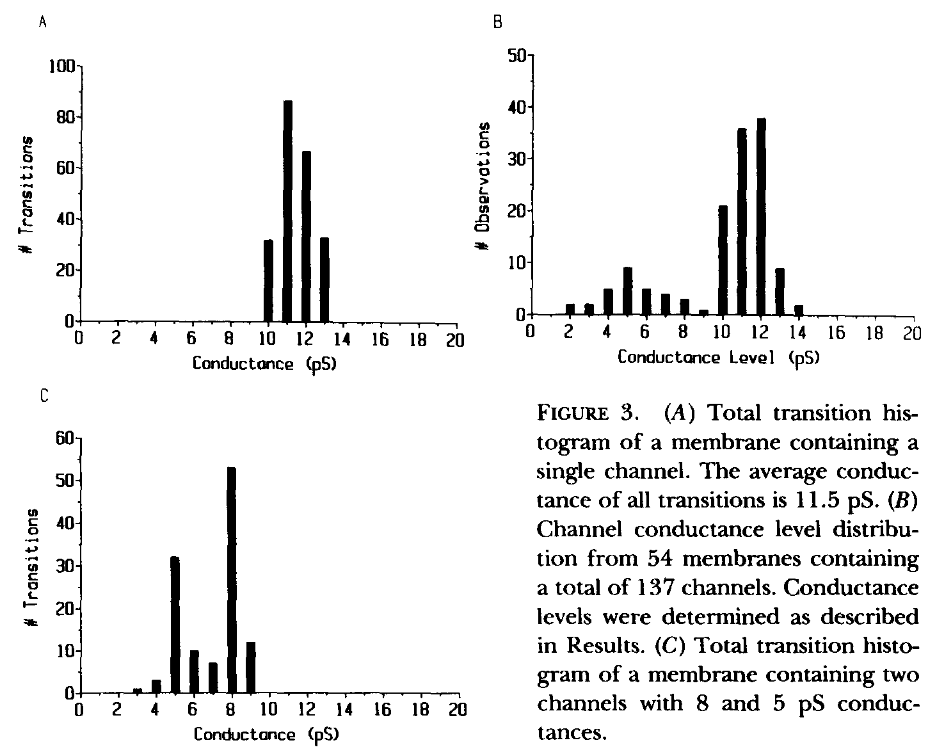

FIGURE 3. (*A*) Total transition histogram of a membrane containing a single channel. The average conductance of all transitions is 11.5 pS. (*B*) Channel conductance level distribution from 54 membranes containing a total of 137 channels. Conductance levels were determined as described in Results. (*C*) Total transition histogram of a membrane containing two channels with 8 and 5 pS conductances.

combining all transitions from all observed potentials. Such an analysis for the 11.7-pS channel in Fig. 2 is given in Fig. 3 *A*. Examination of the histograms from multichannel membranes, however, indicated the consistent presence of two conductance peaks: one at 11–12 pS and the second at ~5 pS. To further examine and quantify these conductance populations, conductance level histograms were constructed as described in Methods. The results from all membranes containing four or fewer channels (54 membranes, 137 channels) were combined (Fig. 3 *B* and Table I). The 10–13-pS veratridine-modified sodium channel represented the predominant conductance population, constituting 77% of all determined conductance levels, with 23% being 9 pS or less. The average of the conductance levels in the

TABLE I

Conductance Distributions of Channels (Membranes with <5 Channels)

Preparation	Specific activity	Fraction of total
1*	2,084	
>10 pS		1.00 (5)
<10 pS		0
2	1,607	
>10 pS		0.75 (18)
<10 pS		0.25 (6)
3	1,402	
>10 pS		0.76 (61)
<10 pS		0.24 (19)
4	662	
>10 pS		0.79 (22)
<10 pS		0.21 (6)
Average	1,439 ± 591	
<10 pS		0.77 (106)
>10 pS		0.23 (31)

The number of channels contributing to each fraction is given in parentheses; a total of 54 membranes were included.

*Only two membranes were includes with this preparation; membranes with smaller subconductance levels were recorded, but contained more than four channels and were excluded from the analysis. Specific activities are picomoles TTX bound/milligram protein for each preparation, determined after purification and reconstitution into vesicles.

larger peak (Fig. 3 *B*) was 11.4 ± 0.92 pS (SD); the smaller conductance peak average 5.4 ± 1.74 pS. The spread in the smaller peak in the present study, however, was almost twice as high as in the larger peak. This spread appears to represent a true variability in single-channel conductances, rather than a resolution problem, as shown in Fig. 3 *C*. This amplitude histogram was obtained from a membrane containing two clearly distinguishable channels with conductances of 8 and 5 pS. Despite combining transitions for both channels from all potentials, the spread of the resulting histogram is comparable to that previously reported for single BTX-modified sodium channels examined at only a single potential (Andersen et al., 1986; Green et al., 1987).

It was further examined (Table I) whether the distribution of conductance levels was consistent among all reconstituted purifications, or if some variable in experimental procedures correlated with the observed diversity of channel conductances (e.g., the use of frozen or fresh liposomal preparation, variable degeneration of channels during purification and reconstitution, etc.). No significant differences in the proportion of channel conductances among reconstituted preparations were found. This distribution was also the same when all fresh preparations (78% and 22%, respectively) were compared with all frozen preparations (77% and 23%).

Single Channels from Dog and Human Brain Synaptosomes

For comparative purposes, single channels from synaptosomal fractions of human and canine cortex were incorporated into planar lipid bilayers in the presence of

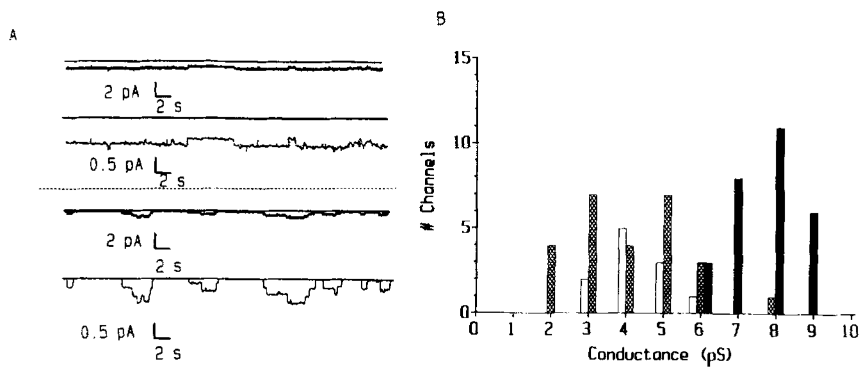

FIGURE 4. Channels from dog and human brain synaptosomes in the presence of veratridine. (*A*) Veratridine-modified dog channels blocked by TTX. Channel transitions from a membrane containing three channels, before (*top two traces*; same recording filtered at 50 Hz and 10 Hz) and after (*bottom two traces*; same recording filtered at 50 and 10 Hz) 100 nM TTX addition; symmetrical 500 mM NaCl, 50 μM symmetrical veratridine, +60 mV. The time-averaged channel conductance before TTX was added was 9.4 pS (312 s recording time); after TTX addition, it decreased to 3.0 pS (192 s recording time). Maximal channel currents before and after TTX addition were unchanged (0.8 pA), demonstrating that the number of the channels in the membrane remained constant. The three channels had measured conductances of 4, 4, and 5 pS. (*B*) Distribution of channel conductances. Veratridine was used in various concentrations between 1 and 100 μM. Filled bars represent channels from human brain preparations; checked bars are channels from dog brain P2 preparations; open bars are channels from dog brain synaptosomes.

veratridine (Fig. 4). In contrast to the purified eel preparations, not all channel transitions recorded with these unpurified preparations could be attributed with certainty to voltage-dependent sodium channels. However, conditions were chosen that favored the observation of Na^+ channels (i.e., symmetrical Na^+ solutions with no other cations present). Channels could usually be recorded only for several minutes before the membrane recordings became noisy, probably reflecting the activation or incorporation of additional channels and synaptosomal components into the bilayer. Consequently, except for rare occasions (Fig. 4*A*), it was not possible to test whether the channels could be blocked by TTX, a sodium channel–specific blocker.

The conductance level distribution was determined (Fig. 4 *B*) as described above. The synaptosomal preparations yielded distributions that were different both from each other and from the eel electroplax preparation. Most significantly, no conductances of 10–13 pS (corresponding to the purified eel veratridine-modified sodium channel) were found with either preparation (at least 37 channels in 20 membranes from 4 different preparations with dog brain; at least 28 channels in 12 membranes from 2 different preparations with human). Instead, the predominant conductances were between 3 and 5 pS (dog brain), and 7–9 pS (human brain). The inability to obtain long-duration channel recordings from the unpurified synaptosomal prepa-

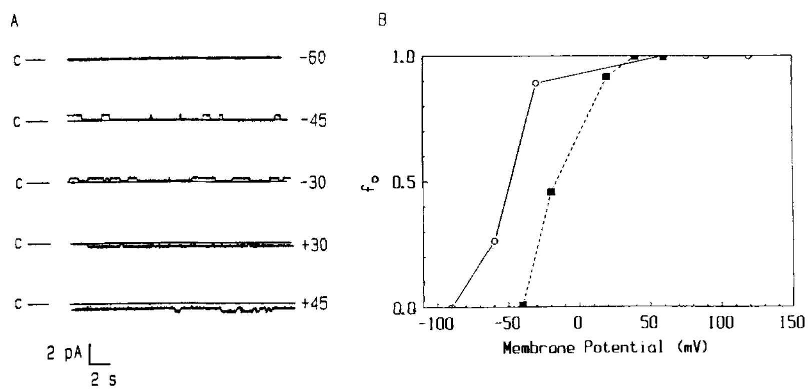

FIGURE 5. Voltage dependence of fractional open time of veratridine-modified eel sodium channels. (*A*) Current traces of a membrane at different potentials; solid line represents background current level. Channel open times and the number of channels open both increase with depolarized potentials. All traces filtered at 50 Hz. (*B*) Voltage dependence of fractional open times for two single-channel membranes. Fractional open time was measured by hand from current traces. The two channels were both open almost all the time at depolarized potentials, but the voltage at which the channel was open half of the time, V_o, was different for each (−48 vs. −20 mV).

rations in the presence of veratridine, in contrast to BTX (Duch et al., 1988*a*), precluded their further characterization at this time.

Voltage Dependence of Channel Fractional Open Times

The fractional open time, f_o, of the veratridine-modified eel channels showed voltage dependence, with higher positive potentials increasing both single-channel open times and the number of channels open at any time (Fig. 5 *A*). However, no activation gating similar to that described for BTX-modified channels in bilayers was observed with these pulse protocols in the voltage range between ±140 mV membrane potential (compare with Recio-Pinto et al., 1987*a*). Channels differed in the voltage dependence of their fractional open times (Fig. 5 *B*). The two single channels examined had midpoint potentials (V_o) for f_o of ~ −45 and −20 mV. The fractional open time for these channels increased at more positive potentials due to an

TABLE II

Averaged Open and Closed Times for Single Channels

V_m	Membrane 1		Membrane 2	
	τ_o	τ_c	τ_o	τ_c
	s		*s*	
+120	—	—	154.7	0.2
+90	—	—	235.6	0.2
+60	66.0 ± 20.7	0.5 ± 2.2	150.9	0.3
+40	74.7 ± 41.5	0.6 ± 3.4	—	—
+20	29.4 ± 12.8	3.0 ± 2.5	—	—
−20	5.3	63.2	—	—
−30	—	—	21.7 ± 9.0	2.01 ± 1.0
−40	2.4	215.4	—	—
−60	—	—	9.7 ± 4.5	20.9 ± 13.6

τ_o is the average time that a channel remained open; τ_c is the average time between channel openings. Values are the average and SEM of one to seven determinations. Where no standard errors are given, only one or two measurements were possible.

increase in the length of time that they remained open (τ_o), and a concomitant decrease in the amount of time that a channel remained closed (τ_c) at the same potentials (Table II). The voltage dependence of f_o for these channels did not appear to change significantly with time. Individual channels in multi-channel membranes could also differ in V_o.

The overall voltage dependence of sodium channel conductances was examined by combining the results from 10 separate membranes (Fig. 6). Channel fractional open time, as reflected in these normalized membrane conductances, increased at all potentials more depolarized than −60 mV. In most membranes, only rare and brief channel openings were observed at potentials more negative than −60 mV. In addition, channel fractional open time continued to increase for some channels at potentials more depolarized than +100 mV.

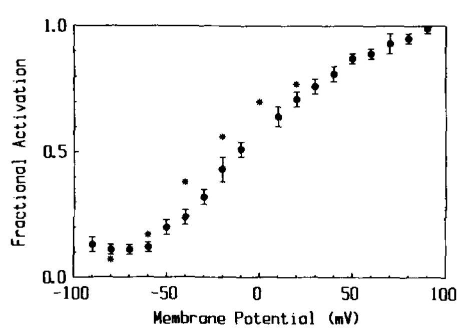

FIGURE 6. Voltage dependence of veratridine-modified sodium channel conductance. Time-averaged sodium channel conductance was normalized to the channel conductance at +90 mV, and used as a measure of channel fractional activation. All points are the average of 10 membranes containing at least 35 channels; error bars are ±SEM. The membrane capacitative transient at potentials more negative than −60 mV is larger than at low negative and positive potentials, therefore it could not be completely compensated for during the measurement of the time-averaged membrane conductance. This resulted in the calculation of a small, persistent conductance at these potentials. Inspection of the chart records reveal that sodium channels remain closed almost all the time at these potentials. Data from Rando (1989) were normalized to the average conductance at +40 mV in this graph and plotted for comparative purposes (*).

Single-Channel Conductance in Symmetrical Potassium Solutions

To determine selectivity, measurements of sodium channel conductance to potassium were carried out in symmetrical (*cis* and *trans*) 455 mM KCl solutions with either 50 μM veratridine (added *cis* and *trans*) or 1 μM BTX (*trans* only) present. The purified eel channels recorded had significantly lower conductances than in symmetrical NaCl solutions (Fig. 7 *A*). In the presence of either BTX or veratridine, sodium channels exhibited a range of single-channel conductances. Conductance level histograms were constructed for all experiments (Fig. 7 *B*). The major peak for the BTX-modified channels (1.6–1.9 pS) had an average conductance of 1.7 pS. The major conductance peak for veratridine-modified channels was about half of that

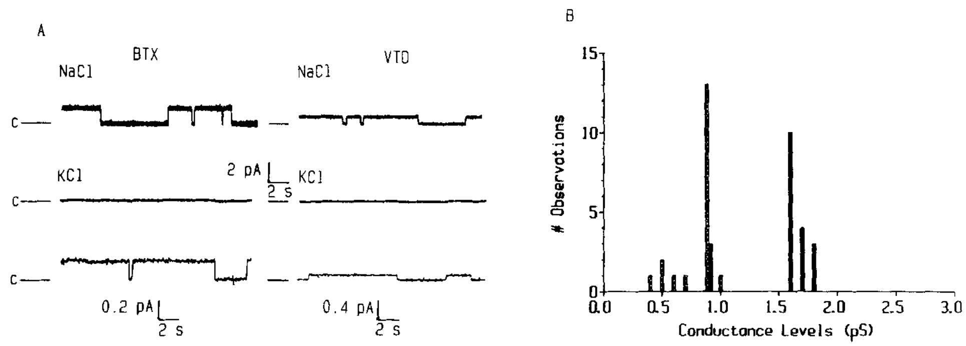

FIGURE 7. Eel electroplax sodium channels in symmetrical potassium solutions. (*A*) Single-channel traces of BTX (*left column*) and veratridine-modified (*right column*) sodium channels in either symmetrical 500 mM NaCl (*top trace of each column*) or symmetrical 455 mM KCl (*middle and bottom traces*). Submaximal quantities of TTX were added to the membranes when BTX was present. The middle two traces were recorded with the same gain and filtering (50 Hz) as the top two traces. The bottom two traces are the same records as the middle, except at higher resolution and filtering (4 Hz). (*B*) Distribution of single-channel conductance levels of BTX and veratridine-modified sodium channels in symmetrical 455 mM KCl. Channel conductances were measured as described in the text. Four membranes containing at least 22 channels were recorded in the presence of BTX; six membranes and at least 19 channels were recorded with veratridine.

observed with BTX: 0.9–1 pS, with an average of 0.9 pS. Smaller conductance channels were also recorded with both BTX and veratridine.

The single-channel conductance ratios for Na^+/K^+ determined using all measured conductance levels were 14.6 (BTX) and 12.2 (veratridine). Comparing the major conductance peaks alone, g_{Na}/g_K was 14.7 for BTX and 12.6 for veratridine.

Current-voltage curves for the peak conductance veratridine and BTX-modified channels are shown in Fig. 8 *A*. The *I-V* curves are linear and symmetrical, with no detectable rectification. Corresponding curves for the smaller conductances are shown in Fig. 8 *B*, and are also linear and symmetrical.

Reversal Potential Measurements

In an alternative selectivity measurement, channels were incorporated into membranes with 455 mM NaCl on one side and 455 mM KCl on the other. TTX (30–50 μM) was added to either the NaCl or the KCl side for examination of channel selectivity with NaCl present only on the cytoplasmic or extracellular side of the channel, respectively. As was observed with the conductance levels in symmetrical salt solutions, there was a heterogeneity of reversal potentials, even for channels within the same membrane. For each potential, therefore, all channel transitions were averaged and used to determine the membrane reversal potential.

When sodium and potassium were present in their physiological orientation (Na on the extracellular side of the channel, K on the intracellular side), the reversal

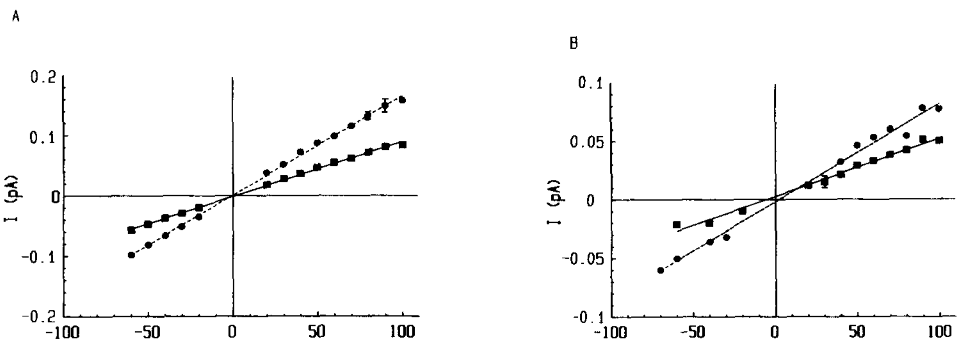

FIGURE 8. Current-voltage relationships for BTX- and veratridine-modified channels in symmetrical KCl solutions. (*A*) *I-V* relationships for channel transitions in larger peaks of Fig. 7 *B*. Data were fit by linear regression; fits were not forced through 0. The slope conductance for the BTX channels was 1.6 pS (r = 0.9993), and for veratridine, 0.9 pS (r = 0.9992). Error bars are within symbols. (*B*) *I-V* relationships for channel transitions in smaller peaks of Fig. 7 *B*. The curves were fit by linear regression; the relationships are linear and symmetrical. BTX channels (*filled circles*) had a slope conductance of 0.8 pS (r = 0.9953), and veratridine channels (*open circles*) had a slope conductance of 0.5 pS (r = 0.9942). Error bars are within symbols.

potential measurements ranged from +40 to +52 mV, with an average of 46.2 ± 1.0 mV (SEM; 10 membranes). This represented an average permeability ratio for sodium to potassium (P_{Na}/P_K) of 6.3 ± 0.25 (SEM). Under reverse conditions, a much wider range of reversal potentials was recorded: −33 to −62 mV, with an average of −51.8 ± 3.2 mV (SEM, n = 9 membranes). The averaged P_{Na}/P_K was 8.3 ± 0.9. The averaged currents for all membranes are shown in Fig. 9 *A*. Although the permeability of the channel under reverse ionic conditions was greater than that measured with physiological salt orientation (sodium outside, potassium inside), the slope conductance of sodium in the region where sodium current predominates was almost twice as high with the physiological salt orientation (9 ps between −50 and 0 mV) than under the reverse conditions (5 pS between +50 and 0 mV). The increased conductance (physiological vs. reverse orientation) near the resting potential, rather than decreased selectivity near the reversal potential, could be of functional significance in the generation of action potentials since at threshold membrane potentials the balance of Na^+ and K^+ conductances becomes critical in deciding whether or not an action potential is fired.

The shape of the current-voltage relationship depended on the orientation of the ions relative to the channel (Fig. 9 *A*). With ions in the physiological orientation, the relationship shows a marked rectification at positive potentials. Under reverse conditions, however, the relationship is almost linear, but rectification still appears to occur at higher positive potentials. This rectification was especially evident in membranes with low permeability ratios (Fig. 9 *B*). Under reverse conditions, the shape of the *I-V* curve at potentials more negative than the reversal potential could not be

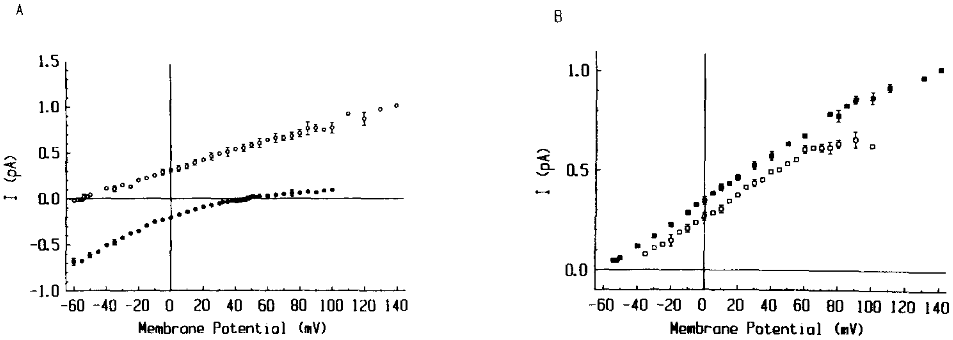

FIGURE 9. Reversal potential measurements of veratridine-modified eel electroplax sodium channels under biionic conditions, 455 nM NaCl vs. 455 mM KCl. (*A*) *I-V* relationships for channels with sodium in the physiological orientation (facing the TTX-binding site, filled circles are average of 2–11 membranes), and in the reverse orientation (facing the cytoplasmic side of the channel, open circles are average of 2–9 membranes). Error bars are ±SEM; some error bars fit within symbols. The reversal potential measured under physiological conditions was +48 mV; under reverse physiological conditions it was −56 mV. (*B*) *I-V* curves under reverse physiological ionic conditions. Open squares contain measurements from four experiments with $P_{Na}/P_K < 8$ (average of 6.5); filled squares are average of five membranes with $P_{Na}/P_K > 8$ (average of 10.4). Only points with at least two determinations are shown; error bars indicate SEM. Points without error bars had only two determinations.

determined, since the veratridine-modified sodium channels were closed almost all the time at these potentials.

Block of Channels by TTX

For the two single-channel membranes previously described, TTX was added and the f_o before and after TTX addition was measured directly from chart records. $K_{1/2}$ of TTX block was calculated for each observed potential. This method for determining $K_{1/2}$ was not possible for all membranes, however, since the total number of channels present could not always be determined accurately (see above). For these experiments (four membranes), block was estimated from the decrease in measured membrane conductance after TTX addition, as described in Methods.

In all cases TTX blocked channels (Fig. 10 *A*) in a voltage-dependent manner (Fig.

10 *B*) and the fraction of the membrane potential sensed, *a*, was calculated (Levinson et al., 1986). The values obtained by averaging the individual linear regression fits from all experiments are (at 0 mV) $K_{1/2}$ = 17.5 ± 4.1 nM (SEM, *n* = 6), and *a* = 0.53 ± 0.09 (SEM, *n* = 6).

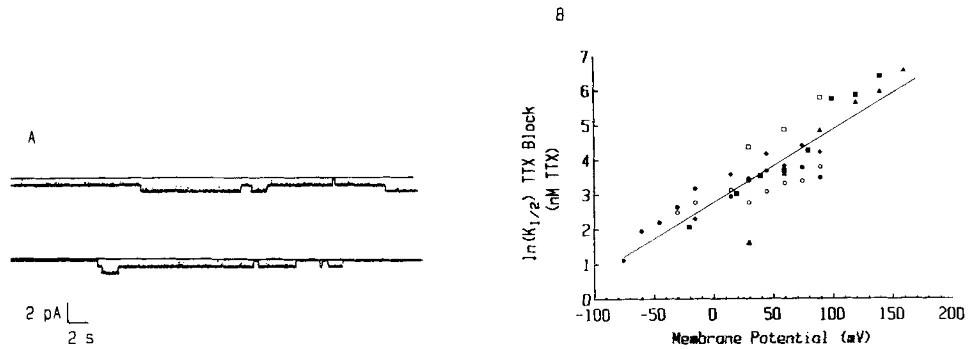

FIGURE 10. Block of eel sodium channels by TTX. (*A*) Traces of a membrane with incorporated sodium channels, before (*top trace*) and after (*bottom trace*) the addition of 30 nM TTX. Holding potential was +60 mV; traces were filtered at 50 Hz. (*B*) Voltage dependence of block by TTX in symmetrical 500 mM NaCl solutions. The $K_{1/2}$ was calculated as described in text. Symbols represent individual determinations from six different membranes. The curve is a linear-regression fit to all data points; *r*= 0.884. The $K_{1/2}$ at 0 membrane potential was 16.1 nM; the fraction of the applied potential that affects TTX block, *a*, was 0.54. Temperature, 23–25°C.

DISCUSSION

In our first report on the properties of the purified sodium channel α-polypeptide from eel electroplax (Recio-Pinto et al., 1987*a*), BTX was used to probe the molecular properties of this reconstituted protein. We compared their characteristics with those of unpurified eel channels examined under the same conditions and concluded that the purified α-polypeptide alone was sufficient to demonstrate the characteristic properties of these channels (sodium conductance and selectivity, voltage-activated gating, block by TTX). However, certain properties of both purified and unpurified eel channels were notably different from those observed with other preparations in the bilayer. In the present study, these molecular properties were further examined using another alkaloid toxin, veratridine.

Single-Channel Conductance

The primary single-channel conductance observed in our experiments averaged 11–12 pS. This is comparable with the 10-pS conductance reported for rat muscle channels in bilayers in the presence of veratridine (Garber and Miller, 1987). While BTX-modified sodium channels for eel, dog, and human had similar conductances, veratridine-modified channels from these same tissues did not. Conductances >9 pS were not found in either the dog or human preparations in the presence of veratridine. On the other hand, preliminary reports from another laboratory indicate that 10 pS is the primary channel conductance in purified rat brain preparations (Corbett et al., 1987), although smaller conducting channels were also found (Corbett and Krueger, 1988). It is not clear if these differences in channel conductances observed with human and dog brain preparations, and those found with eel, rat muscle, and rat brain, are due to tissue and/or species differences, the presence of sodium channel subtypes (Moczydlowski et al., 1986; Noda et al., 1986; Gordon et al., 1987, 1988), or to differences in the preparation of sodium channels from each source. Tissue-specific differences between the properties of veratridine-modified channels in muscle and brain have been noted previously by Rando (1989). Therefore, veratridine may be able to distinguish between different channel types while BTX cannot.

In addition to the primary 11–12 pS conductance level, channels with significantly lower maximal conductances, as well as single-channel subconductance states, were present in the eel preparations. Sodium channel subconductances and/or smaller conductance channels have been reported in a range of tissues and with several methods (Corbett et al., 1986; Duch et al., 1987; Green et al., 1987; Nagy, 1987; Barnes and Hille, 1988; Duch et al., 1988*a*; Patlak, 1988; Meves and Nagy, 1989).

These observed subconductance states could arise from modified current pathways, or result from the unresolved rapid opening and closing of the channel. The extent of the observed inhomogeneity in conductance levels observed in our eel preparations was not seen in sodium channels from human synaptosomal fractions. Examination of unpurified human sodium channels with the same bilayer system indicated that out of 259 membranes with channels, <1% contained channels with conductances outside the principal 26-pS peak (unpublished data).

Voltage Dependence of Veratridine-modified Conductances

The averaged voltage dependence of channel fractional open time in multi-channel membranes for veratridine-modified electroplax sodium channels observed in these experiments is similar to that reported for the slowly developing veratridine activation of voltage-clamped frog node of Ranvier preparations (Fig. 6 and Rando, 1989). This may be an indication that the underlying processes are similar in both preparations. In contrast, no fast sodium channel activation gating with a voltage dependence analogous to that reported for veratridine-modified channels in muscle (Sutro, 1986), nerve (Rando, 1989), or for BTX-modified sodium channels in bilayers (Krueger et al., 1983; Recio-Pinto et al., 1987*a*) was observed in the voltage range examined in these experiments under our steady-state conditions (±140 mV).

It has been proposed that the slow interaction between veratridine and the sodium channel arises either from binding of the toxin to spontaneously opening normal channels (Sutro, 1986; Barnes and Hille, 1988), or from veratridine modification of fast inactivated sodium channels (Rando, 1989). In previous studies, purified, reconstituted eel sodium channels have been shown to spontaneously open in the absence of any toxins (Duch and Levinson, 1987*b*). If veratridine modification of channels occurs through the open-channel pathway, the voltage dependence of the fractional open time may be reflective of the population kinetics of channels opening spontaneously.

Reversal Potential Measurements and Conductance Ratios

Values for the selectivity of veratridine-modified sodium channels depended on how selectivity was defined, and therefore on the method of measurement. In symmetrical salt solutions, the conductance ratio (g_{Na}/g_K) was 12. Channel permeability determined by reversal potential measurements under biionic conditions differed according to the orientation of sodium and potassium in relation to the channel. With sodium on the extracellular side of the channel (physiological orientation), $P_{Na}/P_K = 6$; in the reverse physiological orientation (sodium on the cytoplasmic side of the channel), $P_{Na}/P_K = 8$. These permeability values are similar to those found for BTX-modified channels in this and our previous report (Levinson et al., 1986). BTX- and veratridine-modified rat muscle sodium channels also yielded similar P_{Na}/P_K when reversal potentials were measured with sodium and potassium in their physiological orientation (Garber, 1987, 1988).

The conductance ratios were determined with salt concentrations at which channel conductances would approach saturation (Recio-Pinto et al., 1987*a*). Under these conditions, and in a simple one-site pore, the ratio of exit rate constants would dominate the conductance ratio (Läuger, 1973). However, the permeability ratio would be proportional to the ratio of entry rate constants. Consequently, these results are consistent with a stronger binding of potassium rather than sodium inside the pore.

Several significant differences between the selectivity properties of eel electroplax sodium channels and those of rat muscle channels (Garber and Miller, 1987; Garber, 1987, 1988) were found. First, the single-channel conductance of the rat muscle channels in symmetrical potassium was independent of the modifying toxin (BTX or veratridine; Garber and Miller, 1987). Second, while the eel sodium channel had a linear *I-V* relationship in symmetrical potassium solutions (Fig. 8), the rat muscle channel showed rectification at positive membrane potentials (Garber and Miller, 1987). Similar to eel channels, BTX-modified rat brain channels showed no rectification under these same conditions (Hartshorne et al., 1985). Third, reversal potentials determined under biionic conditions were asymmetrical in BTX-modified rat muscle sodium channels, but little asymmetry was found with veratridine as the modifier (Garber, 1987, 1988). A similar, but reduced, asymmetry has also been reported for BTX-modified human brain sodium channels (Duch et al., 1988*a*). As discussed above, eel sodium channels had reversal potential asymmetries in the presence of veratridine.

Based on the selectivity properties of rat muscle sodium channels, it has been proposed that the channel's selectivity filter is not the rate-determining step for sodium conduction in the channel (Garber and Miller, 1987; Garber, 1988). Our results with eel sodium channels, on the other hand, do not require such a model and can be explained with simpler interpretations (Hille, 1975). This difference between electroplax and rat muscle sodium channels, if not explicable by procedural differences, could represent a significant difference in conduction pathways. Such differences would argue against the general applicability of conductance models among channels from various sources, especially when toxins that modify that pathway are present. It will be of interest to examine the structural sources of these functional differences: amino acid structure, higher (secondary and tertiary) protein structures, posttranslational modifications, protein subunits, etc.

Voltage Dependence of TTX Block

TTX block of veratridine-modified eel sodium channels was not experimentally distinguishable from TTX block of BTX-modified eel sodium channels (Levinson et al., 1986). The $K_{1/2}$ at 0 mV was 16 nM with veratridine and 17 nM with BTX (Levinson et al., 1986); while the fraction of the applied voltage that affected block (a) was 0.54 and 0.49, respectively. However, this finding contrasts with that of Rando and Strichartz (1985) that voltage-clamped veratridine-modified sodium channels in the frog node of Ranvier do not show voltage-dependent TTX block. It is possible that the voltage-dependent block only manifests itself when channels are open for long periods (steady state), so that binding equilibrium of the toxin can take place.

The values for the voltage dependence of TTX block found in eel are similar to those reported for guanidinium toxin binding to BTX-modified sodium channels from several different tissues, including TTX-insensitive channels (Guo et al., 1987; also see Table V of Recio-Pinto et al., 1987*a*). This marked similarity in channels from so many sources, in contrast to other noted differences in channel properties, is an indication that the structures involved in the voltage dependence of TTX block are highly conserved in all sodium channel proteins.

In summary, veratridine modification of the highly purified sodium channel α-polypeptide from eel electroplax was used to continue the characterization of the single-channel properties of this protein. The dependence of these properties on alkaloid toxin modification and sodium channel origin was discussed. Current studies of the effects of sialic acid removal on eel sodium channel function (Recio-Pinto et al., 1987*b*) in conjunction with future studies, including the examination of the properties of purified α-polypeptides in the absence of toxins, will help to correlate this observed functional variability between channels with their structural differences.

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