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Molecular Characteristics and Functional Reconstitution of Muscle Voltage-Sensitive Sodium Channels

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Action potentials in mammalian nerve and muscle are generated by a stereotyped sequence of alterations in membrane conductance to monovalent cations [1,2]. The fundamental change that leads to the initiation of an action potential is a regenerative, but self-limited, increase in membrane conductance to sodium ions [3]. This time- and voltage-dependent conductance pathway is controlled by a unique transmembrane protein, the voltage-sensitive sodium channel [4].

Detailed kinetic analysis of currents flowing through the sodium channel began more than 30 years ago with the pioneering work of Hodgkin and Huxley [3]. Biochemical characterization of this channel protein was undertaken more recently but has moved forward rapidly with concurrent advances in general membrane biochemistry. It is now possible to isolate the sodium channel protein from nerve and muscle membranes [5–7], to examine its molecular properties [8–10], and to reconstitute it into defined lipid vesicles [11–13].

In this report, the molecular properties of the voltage-sensitive sodium channel purified from mammalian skeletal muscle will be reviewed and the evidence for its functional integrity considered. Although this discussion will concentrate on recent progress that has been made with reconstitution of the sodium channel from skeletal muscle, reviews of biochemical approaches to sodium channels in general [14] and in rat brain in particular [15] have appeared elsewhere.

NEUROTOXINS AND SODIUM CHANNELS

Since the sodium channel has no natural ligands and performs no function in the absence of an intact membrane, identifying the solubilized channel protein can be a limiting factor in biochemical studies. Work with the channel, therefore, has relied heavily on its interaction with specific neurotoxins to locate this protein after its

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removal from the membrane. One class of commonly used neurotoxins includes the small polar molecules tetrodotoxin (TTX) and saxitoxin (STX); these can be radiolabeled to high specific activity and have proven especially useful as reversible labels of the channel in biochemical studies [16]. A second group of nonpolar alkaloid neurotoxins, which includes batrachotoxin and veratridine, has been helpful in work with the reconstituted protein since these toxins can activate the channel without requiring a change in membrane potential [17]. Finally, a number of polypeptide neurotoxins from scorpions and from sea anemonae bind to the channel at one or more additional sites where they alter channel activation and inactivation and interact allosterically with the alkaloid neurotoxins [17]. All three groups of neurotoxins have been used extensively in biochemical studies of sodium channel proteins.

In its native membrane environment the voltage-sensitive sodium channel is characterized functionally on the basis of the ionic currents that it controls. The significance of results with the isolated channel protein depends heavily on the degree to which this protein retains these normal functional properties after purification. Although direct measurement of current gating by the purified channel is technically difficult, many of functional properties can be studied indirectly through the use of these neurotoxins. Initially, preservation of the specific binding sites for the various classes of toxins can be documented [11,18]. More importantly, these toxins can be used to open or close the purified channel after reconstitution into defined lipid vesicles so that channel function can be studied in the absence of a vesicle membrane potential [11–13].

ISOLATION OF MUSCLE SODIUM CHANNELS

The voltage-sensitive sodium channel, as identified by specific binding of tetrodotoxin and saxitoxin, has been isolated from rat and rabbit skeletal muscle by our group [6,19] and from rat brain and eel electroplax by others [5,7]. The muscle channels are most easily removed from the membrane with non-ionic detergents such as Nonidet P-40 (NP-40) or Triton X-100; ionic detergents such as CHAPS, deoxycholate, and cholate are effective in solubilizing the channel protein but are not as efficient in preserving its native conformation as measured by high affinity STX binding.

As was first demonstrated with the eel electroplax [20], phospholipid is required for sodium channel stability during purification [6]. When the channel is initially removed from the membrane, this phospholipid requirement can be satisfied by endogenous membrane lipid, but subsequent manipulation of the channel requires the addition of exogenous lipid to all buffers. For the muscle channel, phosphatidylcholine, mixed muscle phospholipids, or crude phospholipid extracted from soybean (asolectin) have proved to be effective protective agents. Ratios of detergent to phospholipid of 7:1 are sufficient to preserve channel function. In addition the high-affinity saxitoxin binding used to identify the channel protein in solution is temperature-labile after solubilization; all stages of channel purification must be carried out at 0–4°C.

Purification of the muscle sodium channel is accomplished by sequential chromatography of the solubilized membrane proteins on an ion exchange resin and a lectin affinity matrix, followed by centrifugation on a sucrose density gradient [6,9]. For ion exchange chromatography a weak ion exchange resin is prepared that has a

guanidinium group at the terminus of a 20-atom hydrophilic spacer arm [6,9]; this resin retains the sodium channel while allowing >95% of the solubilized membrane protein to pass through unretarded. The peak of STX binding from this column is applied directly to a second column containing immobilized wheat germ agglutinin (WGA) [6,21]. Channel bound to the WGA is then eluted with N-acetylglucosamine, concentrated, and applied to a 5–15% sucrose density gradient. After centrifugation, fractionation of this gradient yields a single peak of specific STX binding that coincides with the major peak of protein on the gradient.

All stages of muscle membrane isolation and sodium channel purification are carried out in the presence of a panel of protease inhibitors that includes EGTA (0.5 mM), phenylmethylsulfonylfluoride (PMSF) (0.1 μM), iodoacetamide (1 mM), o-phenanthroline (1 mM), leupeptin (1.0 μg/ml), and pepstatin (0.1 μg/ml). This protocol has been applied successfully to rat and rabbit skeletal muscle channels [6,9,19]; preliminary studies suggest that it is applicable to human muscle as well (R. Roberts and R. Barchi, unpublished data).

For both the rabbit and the rat channel, specific activities in the purified material can approach 3,000 pmol STX bound per mg protein. If a molecular weight of 320,000 is assumed for the channel [22], a theoretical maximum specific activity of 3,130 pmol per mg can be expected. Thus, in the best preparations, channel purity probably exceeds 90%.

PHYSICAL PROPERTIES OF THE SOLUBILIZED SODIUM CHANNEL

The sodium channels from rat, rabbit, and human skeletal muscle appear very similar in their physical properties after solubilization (Table I). Chromatography on an analytical column of Sepharose 6B indicates an apparent Stokes' radius of approximately 8.6 nm for each in their mixed micellar form with detergent and phospholipid. This value is comparable to that reported for the channels from rat brain [23] and from eel electroplax [5,24].

TABLE I. Molecular Characteristics of Purified Sodium Channels

Source	Stokes radius (Å)	Apparent S value (H_2O)	Calculated $S_{20,w}$	Channel MW (calculated)	Subunits on SDS-PAGE
Eel electroplax	90[a] 85[b]	8	—		260,000[a] 270,000[b]
Rat muscle	86	9.1	9.8	314,000	230,000[c] (260,000)[d] 43,000 38,000
Rabbit muscle	86	8.6	8.8	295,000	260,000 38,000
Human muscle	85	8.3	9.1	287,000	
Rat brain	80	11.3	12	316,000	260,000 38,000 37,000

[a] See reference 5.
[b] See reference 24.
[c] Apparent molecular weight of large subunit after isolation of sarcolemma.
[d] True molecular weight of large subunit as determined by immunoblots with monoclonal antibodies [26].

Sedimentation measurements for muscle sodium channels in water yield apparent S values between 8.4 and 9.1 when compared to standard proteins of known $S_{20,w}$ [19,22]. These values will be in error, however, owing to the presence of lipid and detergent in the mixed micelle with the channel protein. An estimate of the true sedimentation coefficient of the solubilized protein can be made by comparing values obtained in water with those obtained relative to the same standard proteins in sucrose gradients prepared in D_2O. This approach is subject to some error since estimates must be made of the actual partial specific volume of the protein; nevertheless, comparison of the results obtained for each of the muscle sources is revealing. In each case, a value of 0.82–0.84 g/ml is found for the partial specific volume of the mixed micelle containing the channel and a calculated $S_{20,w}$ between 8.8 and 9.8 is obtained. Based on these values, the estimated molecular weight for the channels themselves is between 278,000 and 320,000 daltons [19,22]. Again, these values are comparable to those reported for the rat brain sodium channel [23].

Immunological data also support a strong similarity among sodium channel proteins. Monoclonal and polyclonal antibodies have been prepared against the purified rat muscle sodium channel [25,26]. Using immunocytochemical techniques, we find that polyclonal antiserum against the rat channel cross-reacts with the surface membrane of human, mouse, and guinea pig skeletal muscle, indicating shared immunogenic determinants between these species [25]. This antiserum also reacts with sodium channels at the rat peripheral nerve node of Ranvier, underscoring the similarity between nerve and muscle sodium channels. A number of the monoclonal antibodies prepared against the rat muscle channel show cross-reactivity with the rabbit muscle channel and with preparations from rat brain, further supporting the concept of close structural similarity among these channel proteins [26].

SUBUNIT COMPOSITION

Both the rabbit and the rat muscle sodium channels contain a large glycoprotein subunit with anomalous migratory characteristics on SDS-PAGE as well as one or more smaller subunits [9,19]. In highly purified preparations of rabbit sodium channel, a diffuse band is seen that migrates with an apparent molecular weight of ~260,000; a single second subunit appears at 38,000 MW on these gels (Fig. 1). These two bands are progressively enriched during the purification process, and codistribute with the peak of STX binding on the final sucrose gradient. In our purest preparations of rabbit sodium channel, these two proteins account for more than 90% of the total protein detectable on the gels [19].

The purified rat sodium channel exhibits more complex behavior on SDS-PAGE. In channel preparations obtained from isolated sarcolemma, a large glycoprotein subunit is found that runs as a very diffuse band between ~160,000 and 220,000 MW on 7–15% gradient SDS-PAGE [9]. In addition, two smaller bands are seen. One is comparable in size to the rabbit small subuinit, with a MW of 38,000, while the second is about 43,000 MW. The apparent MW of the large glycoprotein in both rat and rabbit channels varies considerably depending on the acrylamide concentration used for electrophoretic analysis. Ferguson plots of relative mobility vs. gel concentation suggest a free solution mobility for this component of the rat channel that is nearly twice that seen with standard proteins [9].

Several monoclonal antibodies raised against the purified channel protein have been used to trace the large glycoprotein of the rat channel during the purification of

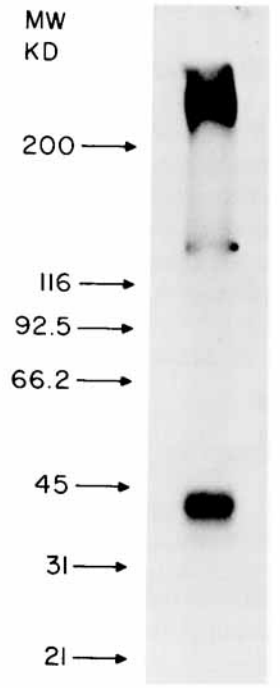

Fig. 1. Subunit composition of the purified sodium channel from rabbit skeletal muscle. The purified channel contains a large glycoprotein with an apparent MW of about 260,000 and a smaller component of 38,000 MW. Proteins were labeled with Bolton-Hunter reagent prior to electrophoresis, separated on a 7–20% gradient SDS-polyacrylamide gel, and visualized by autoradiography [9,19].

sarcolemma and the subsequent solubilization and purification of the sodium channel itself [26]. On blot transfers from 12% acrylamide-0.075% bis-acrylamide SDS-PAGE gels these antibodies recognize a diffuse, high molecular weight component between 160,000 and 200,000 MW among proteins from sarcolemmal membranes that have been prepared in the presence of a full panel of protease inhibitors; this component is detected throughout the various stages of the channel purification and corresponds to the large subunit of the purified channel. If muscle is homogenized in the presence of the same protease inhibitors, but the membranes are immediately solubilized by the inclusion of 1% NP-40, these same antibodies identify only a broad band centered at 260,000 MW [26]. This higher molecular weight band corresponds in location to the large glycoprotein in the purified rabbit channel. These antibody binding data suggest that the large subunit in the rat channel is also ~260,000 daltons in its native form but that modification by an endogenous protease or glycosidase occurs during preparation of sarcolemmal membranes in spite of the presence of protease inhibitors, producing a component that runs below 200,000 MW.

Large glycoprotein subunits have also been identified in each of the other sodium channels for which purification has been reported (Table I). In the rat brain [10] and eel preparation [8,24], this component has a MW of ~260,000, comparable to that obtained here for the rabbit channel and suggested for the rat channel by antibody binding [19,26]. The mammalian channels all contain at least one smaller

component of 38,000 MW; no small subunits are seen in the channel preparations from eel electroplax.

The significance of the small subunits seen in the mammalian channel preparations remains to be defined. In spite of the presence of protease inhibitors in the muscle and brain preparations, it is possible that the 38,000 MW component is the result of proteolytic cleavage of the larger protein by endogenous enzymes. However, of twelve monoclonal antibodies that react with the large subunit, we have not found any that also cross-react with this smaller component as might occur if the latter was derived from the former [26]. Alternatively, the small protein may represent a component of the channel found only in higher species, or may be an associated protein derived from the cellular cytoskeletal system that assists in anchoring the sodium channel in mammalian excitable membranes. These points remain to be resolved.

RECONSTITUTION OF THE PURIFIED CHANNEL PROTEIN

The rabbit and the rat skeletal muscle sodium channels have been functionally reconstituted following purification [11,12,19]. In both cases, the channel protein isolated in the presence of NP-40 was reconstituted into egg phosphatidyl choline (PC) vesicles using BioBeads SM-2 to remove the detergent. This procedure results in the production of a bimodal population of vesicles; about half of the lipid is associated with vesicles 200–300 Å in diameter, while the rest is found in vesicles larger than 1,000 Å [11,13]. Freeze-fracture electron micrographs of vesicles containing the reconstituted rat muscle channel reveal intramembranous particles of ~95 Å diameter that are absent from control vesicles formed without the sodium channel [11].

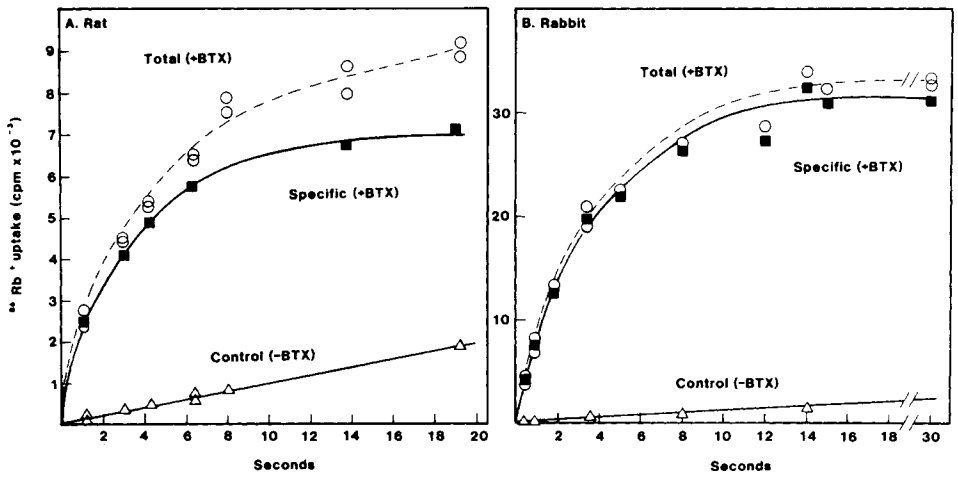

Fig. 2. Purified muscle sodium channels reconstituted into egg phosphatidyl choline (PC) vesicles gate cation fluxes in response to pharmacological activation. Vesicles containing purified rat channel protein (A) or rabbit channel protein (B) were equilibrated for 45 min with 1.5 μM batrachotoxin (○) or with buffer alone (△). Influx was initiated by rapidly mixing the activated vesicles with buffer containing $^{86}Rb^+$ and was terminated by injection of the mixture into a slurry of Dowex resin [12]. Rapid specific influx of $^{86}Rb^+$ (■; total influx with BTX minus control influx in the absence of BTX) was seen with both preparations. This influx was blocked ~50% by externally applied tetrodotoxin, and 100% when tetrodotoxin was present on both the inside and outside of the vesicle.

Measurements with [^{3}H]-saxitoxin indicate that about 50% of the high-affinity toxin binding sites are exposed on the external surface of the reconstituted vesicles. The remainder of these binding sites are evident only after disruption of vesicle integrity with detergents. Since the saxitoxin binding site is known to be on the extracellular side of the channel [16], these results imply that channel insertion during reconstitution is a random process. Similar results have been reported for the reconstituted sodium channel from rat brain [13].

Channel-containing vesicles that have been exposed to batrachotoxin, an alkaloid neurotoxin capable of opening the channel in the absence of a membrane potential [17], exhibit a very rapid influx of labelled cations that is not present in the absence of this toxin or in vesicles that do not contain sodium channels (Fig. 2) [11]. This influx is inhibited 50–70% by externally applied saxitoxin or tetrodotoxin. When the vesicles are formed with saxitoxin trapped inside, 100% of the remaining batrachotoxin-activated influx can be blocked by the subsequent application of external saxitoxin or tetrodotoxin. It appears that sodium channels inserted into the vesicle membranes either in an inward or outward facing direction can be activated by batrachotoxin, and that all the activated channels can be blocked by saxitoxin or tetrodotoxin if these agents are present on both sides of the membrane.

Batrachotoxin and veratridine activate the reconstituted channel in a dose-dependent manner (Table II). Our data suggest that both bind to a single class of sites with an apparent K_d of 1.5 μM and 35 μM, respectively [11]. Saxitoxin blocks the stimulated cation influx by binding to a separate site with a K_d of $\sim$ 1 nM [12]. These values are all comparable to those measured with sodium channels in situ. Lidocaine and benzocaine, local anesthetics that inhibit sodium channel function in intact nerve and muscle, are also effective in blocking activation of the purified channel by batrachotoxin [27]. Flux studies with the reconstituted rat brain sodium channel have yielded comparable results with some of these toxins (Table II) [13].

TABLE II. Properties of Reconstituted Sodium Channels

Source	Rat muscle (sarcolemma)	Rabbit muscle (t-tubules)	Rat brain (synaptosomes)
Lipid	Egg PC	Egg PC	Egg PC
Channel orientation	50–70% outward	50% outward	50% outward
Channel activators	Batrachotoxin Veratridine Aconitine	Batrachotoxin Veratridine	Veratridine
Inhibitors of stimulated influx	Saxitoxin Tetrodotoxin Lidocaine Benzocaine	Saxitoxin Tetrodotoxin	Saxitoxin Tetrodotoxin Tetracaine
Cation selectivity	$Na^+ > K^+ > Rb^+ > Cs^+$ (Batrachotoxin and veratridine activation)	$Na^+ > K^+ > Rb^+ > Cs^+$ (Batrachotoxin and veratridine activation)	$Na^+ > Rb^+ > Cs^+$ (Veratridine activation)

QUENCHED FLOW MEASUREMENTS OF CATION SELECTIVITY

Due to the small size of the vesicles containing the reconstituted sodium channel, the cation fluxes stimulated by channel activators equilibrate very rapidly. In order to resolve the kinetics of cation influx, we adapted a quenched flow system to measure isotope uptake into activated vesicles [12]. This device is capable of mixing 20 to 50 μl of activated vesicles with an equivalent volume of buffer containing a labeled cation within 4 msec. After a variable holding period, cation uptake is quenched by injection of the mixture into a slurry of Dowex resin in the Tris form. The dead-time for the device operating in this mode has been determined to be 90 msec; after that interval, increments as small as 5 msec can be resolved.

Using this device, we have examined the rates of equilibration of four monovalent cations into vesicles containing the purified sodium channel from either rat or rabbit skeletal muscle [12,19]. After activation by batrachotoxin, uptake of Cs^+ proceeds rather slowly; at 22°C, the average half-time for vesicle equilibration is 10 sec. At the same temperature Rb^+ moves inward more rapdily, with an average half-time of 2.5 sec. Equilibration of K^+ is tenfold more rapid, with a half-time of 350 ms. Na^+ uptake is so fast that only the very last stages of vesicle filling are resolved; the process is virtually complete by 90 msec, and an upper limit of 50 msec can be placed on the half-time for this cation (Table III).

Based on the half-times for vesicle equilibration with each of these cations, a cation selectivity sequence and relative selectivity ratios can be calculated (Table III). These ratios confirm that the purified, reconstituted channel from both rat and rabbit muscle retain their selectivity for sodium over other cations [12,19]. These relative selectivity ratios are similar to those measured for the toxin-activated channel in its native membrane environment [28,29].

Measurements of cation uptake have also been made in vesicles activated with veratridine [12,19]. With this toxin, half-times for vesicle equilibration are several orders of magnitude slower than comparable measurements made with batrachotoxin-activated vesicles (Table III). It is necessary to raise the assay temperature to 37°C in order to produce influx rates sufficiently rapid to measure with adequate signal-to-noise ratio above the temperature-insensitive, nonspecific background influx. Under these conditions, the veratridine-activated channel shows much less apparent discrimination between cations; the half-times for Na^+ and K^+ are indistinguishable, while the slowest cation, Cs^+, requires less than tenfold longer periods for equilibration.

TABLE III. Cation Selectivity in the Reconstituted Sodium Channel

A. Rabbit T-tubular sodium channel

	Batrachotoxin							Veratridine							
Sequence	Na^+	>	K^+	>	Rb^+	>	Cs^+	Na^+	≈	K^+	>	Rb^+	>	Cs^+
T½	< 50 msec		390 msec		2.6 sec		6.5 sec	1.1 min		1.2 min		2.7 min		3.6 min
Ratio	1	:	0.13	:	0.019	:	.008	1	:	0.92	:	0.41	:	0.31

B. Rat sarcolemmal sodium channel

	Batrachotoxin							Veratridine							
Sequence	Na^+	>	K^+	>	Rb^+	>	Cs^+	Na^+	>	K^+	>	Rb^+	>	Cs^+
T½	< 50 msec		350 msec		2.5 sec		10 sec	1.0 min		1.2 min		2.0 min		2.6 min
Ratio	1	:	0.14	:	0.02	:	0.005	1	:	0.83	:	0.50	:	0.38

In order to shed more light on the difference in cation uptake rates through batrachotoxin- and veratridine-activated channels, measurements of the temperature dependence of cation influx have been made [12]. Rb^+ and Cs^+ influx following batrachotoxin activation show little temperature dependence; the a Q_{10} for cation influx is about 1.3 and the activation energy, calculated from Arrhenius plots, is ~ 7 kcal/mol for both cations. Measurements for the same cations after veratridine activation indicate a marked dependence on temperature; Q_{10} is measured to be about 3.0, with calculated activation energies of 31 kcal/mol for both cations.

Clearly the rate-limiting process for cation influx through batrachotoxin- and veratridine-activated channels is not the same. Electrophysiological [30] and recent patch clamp [31] data suggest that batrachotoxin-activated channels are open most of the time at 0 mV. Influx measurements in batrachotoxin-activated vesicles probably reflect the relative ease of cation movement through an open channel. Veratridine-activated channels, on the other hand, may flicker infrequently between the closed and opened state, spending much of their time closed [32]. The measured rates and temperature dependence of cation influx with this activator may reflect the probability of channel opening in a given vesicle rather than the actual cation selectivity of the open channel.

SINGLE-CHANNEL MEASUREMENTS

We have shown that purified, reconstituted muscle sodium channels retain the capacity to gate cation fluxes in response to pharmacological activation and that these fluxes can be specifically inhibited by appropriate neurotoxins. The purified channel still displays excellent cation selectivity. The next major feature that must be sought in the reconstituted channel is voltage activation of ionic currents. This is a more difficult issue to deal with since the time resolution of the quenched flow system is not sufficient to resolve events on the scale of the single-channel openings that occur in response to voltage [33]. We have approached this question using patch clamp of large liposomes.

Early work with single-channel recordings of purified sodium channels reconstituted into liposomes was hampered by artifacts produced by the detergents used in the channel purification and liposome preparation. Nonionic detergents usually used for sodium channel purification, such as NP-40, Triton X-100, and Lubrol PX, generated channel-like artifacts in patch-clamped lipid vesicles without protein, presumably because of traces of detergent that remain after BioBead treatment. These artifacts have been seen with various batches and brands of these detergents and do not seem to be an isolated problem with a single supplier (R. Furman, unpublished data). Bile salts, however, do not introduce these spurious channels.

The sarcolemmal sodium channel can be solubilized in deoxycholate and similar ionic detergents, but the channel is much less stable than in the nonionic detergents. A purification scheme has been developed that utilizes the zwitterionic detergent CHAPS; channel protein purified with this detergent appears comparable on gel electrophoresis to that obtained with NP-40, but specific saxitoxin binding activity is not well preserved and resultant specific activities are not as high as with our usual purification procedure. Presumably some sodium channel protein is denatured or undergoes an unfavorable conformational change during the purification. Approaches to improving the stability of the channel after solubilization in CHAPS are now being explored.

In spite of these problems we have observed single-channel activity in purified channel prepared in CHAPS and reconstituted into phosphatidylserine/phosphatidylethanolamine vesicles. Several classes of channel activity have been seen repeatedly in inside-out patches pulled from liposomes enlarged by freezing and thawing [34].

One class of channels appears to be voltage- and neurotoxin-insensitive and may represent channels damaged during purification. Generally these are seen as random channel openings of 1–50-msec duration with a conductance of ~ 15 pS in symmetrical solutions containing 100 mM NaCl at 22°C. Some of these channels appear to have a subconductance state of 8–10 pS. Other channels with the same 15-pS conductance alternate between low and high noise levels during the channel-open interval. Occasionally, a conductance event of 30–40 pS is seen in these patches.

A second and more interesting class of channel events shows voltage-dependent behavior in the presence of batrachotoxin. These channels typically exhibit only irregular bursts of openings in the absence of batrachotoxin but convert to continuous opening flickers within seconds of application of the toxin. With 100 mM NaCl on both sides of the membrane, these toxin-activated channels demonstrate voltage-dependent activation (Fig. 3). At large hyperpolarizing voltages the channel is mostly closed, showing occasional discrete opening events. With progressive depolarization, channel openings become more frequent and the open time increases. At more depolarized voltages the channel is mostly open with brief transitions to the closed state. The voltage dependence of channel opening appears shallower than that seen with the channel in situ. It remains to be determined whether this difference reflects alterations in the channel or changes in the membrane environment itself.

Freeze-thaw appears to reorient the channels such that the tetrodotoxin binding site is on the outside of the vesicles, resulting in an "inside-out" orientation of patches on the electrode. Because of the resultant inaccessibility of the tetrodotoxin binding site after the patch is formed, it has not yet been possible to document TTX sensitivity in the channels that have shown this voltage-dependent activation.

Preliminary results with the patch-clamped, reconstituted channel are encouraging. Our experience suggests that the major limitation is purifying the channel in the presence of acceptable detergents without producing channel damage rather than in the technical aspects of single channel measurements with the reconstituted protein.

CONCLUSIONS

The voltage-sensitive sodium channel controls a complex relationship between membrane potential, time, and ionic currents. It contains binding sites for a variety of neurotoxins, some of which are allosterically coupled. Kinetic schemes for channel activity based on biophysical measurements suggests the presence of multiple intermediate states, presumably reflecting different channel configurations. In spite of the obvious complexity of channel function, the purified protein may be composed in its most primitve form of a single very large subunit. In higher animals one or more smaller subunits may be present, but the binding sites for tetrodotoxin and for the polypeptide neurotoxins still are found on the large subunit [34,35]. Unraveling the mechanisms through which a single polypeptide chain carries out the multiple complex and interrelated functions identified with the sodium channel will be a major task.

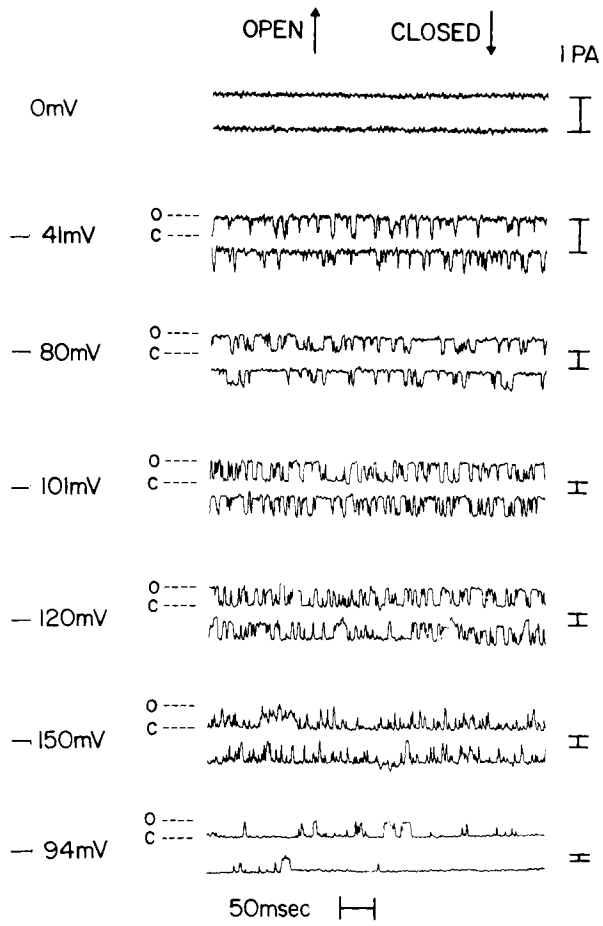

Fig. 3. Voltage-sensitive single-channel conductance events after batrachotoxin activation, measured using patch-clamp techniques, with purified rat sodium channels reconstituted into phosphatidyl serine/phosphatidyl ethanolamine (PS/PE) vesicles. Batrachotoxin was used to remove channel inactivation in order to facilitate detection of channels; this toxin also shifts the voltage-dependence of channel activation toward more hyperpolarized potentials [30]. Single-channel events with a conductance of 15–18 pS are seen. The channel is mostly closed at hyperpolarizing potentials; the frequency and duration of channel openings increases with depolarization. At depolarized potentials, the channel is mostly open with brief transitions to the closed state. These recordings were made with 100 mM NaCl on both sides of the patch. The channel was oriented as in an inside-out cellular patch. The sign of the potential across the patch is given in terms of the normal cell membrane potential.

The purified mammalian sodium channel proteins retain most of their normal functional properties. They gate ionic fluxes in response to pharmacologic activators, and effectively select among monovalent cations. Preliminary data suggest that they also contain the machinery for voltage-dependent activation. These findings support the impression that the purified protein does indeed represent the entire channel rather than a single isolated component. Comparison of channel proteins from different

sources underscores their strong similarities and suggests a high degree of conservation of protein structure during evolution. The major challenge for future work on the sodium channel will be to correlate emerging structural information on the channel protein with various aspects of its complex time and voltage dependent function.

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