Cation Selectivity Characteristics of the Reconstituted Voltage-Dependent Sodium Channel Purified from Rat Skeletal Muscle Sarcolemma*

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In this report, the alkali metal cation selectivity of the purified, voltage-dependent sodium channel from rat skeletal muscle is described. Isolated sodium channel protein (980–2840 pmol of saxitoxin binding/mg of protein) was reconstituted into egg phosphatidylcholine vesicles, and channels were subsequently activated by either batrachotoxin (5 × 10⁻⁹ M) or veratridine (5 × 10⁻⁹ M).

Activation of the reconstituted sodium channel by batrachotoxin permitted rapid specific influx of cations into channel-containing vesicles. Quenched flow kinetic techniques were adapted to allow resolution of the kinetics of cation movement. Uptake rates for ⁴⁺, ⁸⁺, and ³⁺ were measured directly and half-times for equilibration at 18 °C were determined to be 350 ms, 2.5 s, and 10 s, respectively, in this vesicle population. ²²⁺ equilibration occurred within the minimum quenching time of the apparatus (90 ms) but an upper limit of 50 ms at 18 °C could be assigned to its half-time. Based on this upper estimate for Na⁺, cation selectivity ratios of the batrachotoxin-activated channel were Na⁺ (1):K⁺ (0.14):Rb⁺ (0.02):Cs⁺ (0.005). Toxin-stimulated influx could be blocked by saxitoxin with a Kᵢ of ~5 × 10⁻⁹ M at 18 °C. Rates of cation movement through veratridine-activated channels were much slower, with half-times of 1.0, 1.2, 2.0, and 2.6 min at 36 °C for Na⁺, K⁺, Rb⁺, and Cs⁺, respectively.

The temperature dependences of batrachotoxin and veratridine-stimulated cation uptake were markedly different. The activation energies for ⁸⁺, ⁴⁺, and ³⁺ movement into batrachotoxin-activated vesicles were 7.6 and 6.1 kcal/mol, respectively, while comparable measurements for these two cations in veratridine-activated vesicles yielded activation energies of 31 kcal/mol. Measurements of cation exchange with batrachotoxin-activated channels may reflect characteristics of an open sodium channel while the process of channel opening itself may be rate-limiting when veratridine is used for activation.

The electrical signals or action potentials that characterize the surface membranes of nerve and muscle are usually produced by transient changes in membrane conductance to sodium and potassium ions (1). These time- and voltage-dependent ion conductances are controlled by intrinsic membrane proteins that span the bilayer and provide an aqueous pathway or channel for ion movement (2). The molecular characterization of these sodium and potassium channels has become an active topic of current neurochemical research.

The past several years have seen significant progress in the isolation and biochemical characterization of the voltage-dependent sodium channel. A sodium channel protein has been purified from eel electroplax (3), rat skeletal muscle sarcolemma (4), and rat brain synaptosomes (5). In each case, a large glycoprotein has been identified that exhibits anomalous migratory behavior on SDS-PAGE (5–7). In the two mammalian channel preparations, several smaller peptides are also thought to be components of the purified sodium channel (5, 7).

A number of investigators have studied the reconstitution of unpurified sodium channels or channel-containing membrane fragments into artificial liposomes (8–11). More recently, we reported the functional reconstitution of a purified sodium channel from rat sarcolemma into phosphatidylcholine vesicles (12). This purified channel protein retained its ability to gate ²²⁺ fluxes in response to activation by the alkaloid neurotoxins batrachotoxin and veratridine; these fluxes were specifically blocked by saxitoxin. Similar results have now been obtained with the sodium channel partially purified from rat brain synaptosomes (13).

Cation flux through opened sodium channels occurs very rapidly, and the rate of cation uptake into reconstituted vesicles through batrachotoxin-activated channels could not be resolved in our earlier studies (12). In this report, quenched flow kinetic techniques have been applied to the purified, reconstituted sarcolemmal sodium channel in order to measure the kinetics of uptake for various alkali metal cations. The sodium channel selectivity among Na⁺, K⁺, Rb⁺, and Cs⁺ has been determined following batrachotoxin or veratridine stimulation, and the activation energies for cation influx measured.

MATERIALS AND METHODS

Materials used in the purification of sarcolemma and in the isolation of the sodium channel protein were reported previously (4, 7, 14). Chemicals used in the reconstitution were as detailed by Weigele and Barchi (12). Batrachotoxin was the gift of Dr. J. W. Daly of the National Institutes of Health. The isotopes ²²⁺, ⁸⁺, ⁴⁺, and ³⁺ were purchased from New England Nuclear Co. Dowex 50-X8

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The abbreviations used are: SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; NP-40, Nonidet P-40; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N',N'-tetraacetic acid.
Cation Selectivity of Reconstituted Sodium Channels

Phosphatidylcholine (Sigma Chemical Co., type V-E, 99% purity) was prepared in 10% NP-40 by stirring under argon. The purified sodium channel was previously described (12). Stock phosphatidylcholine (50 mg/ml) was prepared immediately prior to use and titrated to a 5:1 molar ratio. The channel protein was purified first on a guanidinium-Sepharose column and subsequently on a wheat germ agglutinin-Sepharose column. The column dimensions and conditions are detailed elsewhere (7).

Phosphatidylcholine in the guanidinium-Sepharose column (4) was synthesized by coupling 3,3-diaminodipropylamine (Eastman Kodak) to Affi-Gel 202 (Bio-Rad) to form an immobilized support with a large conjugation arm extending from the resin and subsequently converting the terminal primary amino group to a guanidinium function, followed by immobilization of 3,3-diaminodipropylamine on Affi-Gel 202 (Bio-Rad) to form an immobilized support with an atom extended spacer arm and subsequently converting the terminal primary amino group to a guanidinium function (20). Briefly, 2 ml of a 3,3-diaminodipropylamine solution; solid NaCl was then added to be used. Test reactions were used as described by Gutfreund (21) to show that the mixing time of the instrument was less than 4 ms. Furthermore, results were obtained from this instrument that were identical with those generated by conventional quenched flow instruments with motor enzyme reactions. Full details of the construction and performance of the instrument will be presented elsewhere (7).

For either single push or pulsing flow operation, the mixed solution containing vesicles and labeled cation was quenched by direct injection into a slurry of Dowex 50-W-X8 (2.5 ml) containing 0.25 mM bovine serum albumin buffer. The cation content of the vesicles was determined by liquid scintillation counting.

The overall quenching time for the quenched flow system including the Dowex resin step was determined as detailed under "Results" by measurement of the early linear time course of 3H uptake into batrachotoxin-activated vesicles. A quenching time of 200 ms or less (150 ms) was obtained. Attempts at stopping cation influx at faster times by quenching the reaction solution in 10–100% tetrodotoxin were unsuccessful.

RESULTS

In the experiments reported in this paper, the conditions for sodium channel reconstitution were kept as constant as possible (see "Materials and Methods"). We found previously that the results obtained from reconstitutions with our purest sodium channel preparations (2000–3000 pmol of saxitoxin binding/mg of protein, representing the product of a three-step purification including a final sucrose gradient) were comparable to those obtained with sodium channels carried through only two steps of purification and having a slightly lower specific activity (typically 1000–2200 pmol/mg) (12). For the studies reported here, we chose to optimize the number of experiments which could be carried out with a given preparation by using the larger quantities of channel protein available after the second column (wheat germ agglutinin-Sepharose) in our purification protocol (7).

The saxitoxin-binding activity of the pooled fractions from...
the wheat germ agglutinin-Sepharose column ranged from 980 to 2,840 pmol/mg of protein in the 40 preparations used to generate the results reported below. The mean specific activity of the preparations used was 1,245 pmol/mg of protein (~40% purity based on theoretical maximal binding of 3,180 pmol/mg for a protein of $M_r = 314,000$ (23)). In spite of the variability in specific activity, the major protein components of the material used for reconstitution were the same and included a large glycoprotein which ran anomalously in the high molecular weight region of our 7-20% SDS-PAGE and two smaller peptides of $M_r \sim 38,000$ and 45,000 (Fig. 1) (7). The $M_r = 38,000$ component often appeared as two closely spaced bands. Despite the wide range of specific activities obtained in different preparations, SDS-PAGE showed very similar gel patterns, suggesting that the variability resulted from the loss of high affinity saxitoxin binding rather than the presence of variable amounts of contaminating proteins. We have previously shown that purified sodium channel protein loses its capacity for high affinity saxitoxin binding with time although its apparent subunit composition shows no change (7).

The average concentration of sodium channels in preparations prior to reconstitution was 38.6 ± 12.2 pmol of saxitoxin-binding sites/ml and the average protein concentration was 0.031 ± 0.013 mg/ml. All reconstitutions were carried out with egg phosphatidylcholine at a concentration of 5 mg/ml; the average protein:lipid ratio for the studies reported here was therefore 0.006 mg of protein/mg of phosphatidylcholine. Forty to 50% of both the saxitoxin-binding sites and the total protein was typically recovered in the reconstituted vesicles.

The efficiency of each reconstitution was evaluated with a standard assay measuring the batrachotoxin-stimulated influx of $^{22}$Na$^+$ at 15 and 45 s as well as the control or leakage influx of $^{22}$Na$^+$ at these time points in the absence of batrachotoxin. The total batrachotoxin-stimulated sodium uptake at 15 s ranged between 1.3 and 5.9 times the nonspecific or control uptake at this time point (mean = 2.8 ± 1.2). Considerable random scatter in this ratio was seen among preparations although the value obtained in any given preparation showed little variability over a 24-h period. We found little correlation between the magnitude of the stimulated/control uptake ratio and either the specific activity of saxitoxin binding (Fig. 2A) or the concentration of binding sites/ml in the reconstitution solution over the narrow range of values used for this study, suggesting that other undefined factors were of more importance in determining the final activity of a given reconstitution. No systematic attempt was made to vary the sodium channel protein concentration over a wider range where such a correlation with channel concentration might be expected to be resolved. The nonactivated or leakage uptake was also compared to both sodium channel concentration and total protein concentration. Again, no relationship was seen between the magnitude of the leakage and the concentration of saxitoxin-binding sites, but there was a weak correlation between increasing concentration of total protein and increasing nonspecific leakage flux (Fig. 2B).

Quenched Flow Measurements—Quenched flow techniques were used to study the kinetics of cation influx into batrachotoxin-activated vesicles. Typically, 8–10 time points were used to define an uptake curve and 2–3 measurements were made at each time point with activated and control vesicles. Control values were subtracted from the total uptake to give the specific uptake. Examples of uptake curves for $^4$K$^+$ and $^{86}$Rb$^+$ are shown in Fig. 3. In general, the control (nonsaturated) influx measured with this rapid technique was much...
lower than that seen using the manual technique at 15 and 45 s, relatively long times compared to the rate of cation equilibration.

The kinetics of uptake for each of the alkali metal cations except \(^{22}\text{Na}^+\) could be resolved unequivocally using the quenched flow technique. \(^{22}\text{Na}^+\) uptake was essentially complete at the earliest time point, taken with 8 ms between mixing of vesicles and isotope and the start of the Dowex quench (Fig. 4). The actual elapsed time, however, must include the time between the injection of the mixed solution into the Dowex resin and the binding of all extravascular \(\text{Na}^+\) to the resin, since this interval will allow additional isotope movement into activated vesicles. This quenching time for the system was determined by careful measurement of the early, linear phase of uptake for \(^{42}\text{K}^+\), the isotope with the fastest resolvable uptake rate. Extrapolation of corrected specific \(^{42}\text{K}^+\) uptake values to base-line (Fig. 4) yielded an effective quenching time of 90 ms. Using this value for the quenching time, an upper limit of 50 ms could be set for the actual half-time for \(^{22}\text{Na}^+\) equilibration under comparable conditions.

The time course for equilibration of \(^{42}\text{K}^+\), \(^{86}\text{Rb}^+\), and \(^{137}\text{Cs}^+\) into batrachotoxin-activated vesicles was clearly resolved using the quenched flow technique, and the half-times for vesicle equilibration were directly measured (Fig. 5). Half-times for these cations were sufficiently slow that the quenching time of the apparatus was of significance only for \(^{42}\text{K}^+\); for that cation, a small correction to the measured half-time was necessary. The half-time for \(^{42}\text{K}^+\) uptake, calculated either from initial rate data as shown in Fig. 4 or from the complete influx as in Fig. 5, was approximately 350 ms, while that for \(^{86}\text{Rb}^+\) and \(^{137}\text{Cs}^+\) was 2.5 and 10 s, respectively. The wide spread of values for these four alkali metal cations indicates significant cation selectivity in the purified, reconstituted sodium channel. Using an upper limit for the \(^{22}\text{Na}^+\) half-time of 50 ms, the calculated ion selectivity ratios were (Na\(^+\)) 1:(K\(^+\)) 0.14:(Rb\(^+\)) 0.02:(Cs\(^+\)) 0.005 (Table I).

Reproducibility of time courses for uptake of a given cation was good from reconstitution to reconstitution. For example, points shown for \(^{42}\text{K}^+\) uptake on Fig. 4 were derived from three separate reconstitutions, yet all points fall along the same time course. Similar reproducibility was seen with \(^{137}\text{Cs}^+\) and \(^{86}\text{Rb}^+\), suggesting that the size distribution of vesicles containing active sodium channels was fairly constant from preparation to preparation. The larger standard deviations seen with \(^{22}\text{Na}^+\) measurements may reflect the more significant contribution of variability in quenching time to measured uptake because of the rapid influx of this cation into activated vesicles.

Veratridine-stimulated Cation Influx—Veratridine-stimulated influx was measured for \(^{22}\text{Na}^+\), \(^{42}\text{K}^+\), \(^{86}\text{Rb}^+\), and \(^{137}\text{Cs}^+\).
in reconstituted vesicles that were identical in all characteristics with those used for batrachotoxin studies (Fig. 6). In all cases, cation influx occurred much more slowly for a given cation than in parallel measurements in batrachotoxin-stimulated vesicles (Fig. 5). Although measurements of veratridine-stimulated uptake could be made in the quenched flow device using a double push mode to retain the sample after mixing for longer intervals prior to quenching, time courses were slow so that most assays were carried out manually. The half-time for $^{22}\text{Na}^+$ uptake at $36^\circ\text{C}$ was approximately 1 min, while those measured for $^{42}\text{K}^+$, $^{86}\text{Rb}^+$, and $^{137}\text{Cs}^+$ were 2.2, 2.0, and 2.6 min, respectively (Fig. 6). With each cation, however, the total space accessible through veratridine-activated channels was approximately the same as that accessible through batrachotoxin-activated vesicles in a given preparation.

Control influx was nonlinear over these long time intervals, with the most rapid nonspecific influx occurring during the first 15 s (12). The average ratio of total stimulated influx to control influx at the time of peak stimulated influx for these four cations was 1.7.

**Temperature Dependence of Specific Cation Influx—Batrachotoxin- and veratridine-activated cation influxes differed markedly in their temperature dependence. With veratridine-activated vesicles, specific $^{22}\text{Na}^+$-activated influx was barely detectable at temperatures below 20 $^\circ\text{C}$ but increased sharply with increasing temperatures. A typical experiment illustrating this point is shown in Fig. 7A. In other preparations, the maximal space accessible through activated sodium channels was approximately the same for all cations in any given preparation. Maximal $^{22}\text{Na}^+$ uptake was already observed at the shortest interval shown on this graph (O). All measurements shown were made at 18 $^\circ\text{C}$.

### Table I

**Rates of cation uptake in batrachotoxin- and veratridine-activated channels**

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*Half-time for vesicle filling. Values represent the average of influx curves on at least two different reconstitutions, each consisting of 5-10 time points in triplicate and at least three control measurements in duplicate. Time courses with batrachotoxin were carried out at 18 $^\circ\text{C}$, those with veratridine were performed at 36 $^\circ\text{C}$.

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measurements were focused on early time points at each 
temperature to allow quantitation of the rate of vesicle filling 
during the early, linear phase of uptake and these values were 
used to construct Arrhenius plots for stimulated cation influx. 
An activation energy of 23.6 kcal/mol was determined for 
veratridine-activated $^{22}$Na influx. The Arrhenius plot was 
linear over the temperature range of 15-36 °C in which reli-
able measurements could be made. Similar studies were car-
ried out with $^{86}$Rb (Fig. 7B) and $^{137}$Cs*. In both cases, linear 
Arrhenius plots were obtained, with high activation energies 
(31 kcal/mol) (Table II).

Batrachotoxin-stimulated cation influx was much less tem-
perature dependent. $^{22}$Na* fluxes could easily be detected 
throughout the temperature range of 5-36 °C, but the rapid 
initial rate precluded accurate quantitation. For comparison 
with veratridine data, initial rate measurements were there-
fore made with $^{86}$Rb* and $^{137}$Cs* in batrachotoxin-activated 
vesicles using the quenched flow apparatus. Uptake rates for 
these cations could be readily resolved within the temperature 
range studied. Arrhenius plots of the data appeared linear 
throughout this range without evidence of a break point, 
although the limited number of temperatures studied restricts 
our resolution (Fig. 7B). Calculated activation energies for 
$^{86}$Rb* and $^{137}$Cs* were much lower (7.6 and 6.1 kcal/mol, 
respectively) in batrachotoxin-activated than in veratridine-
activated vesicles (Table II).

Flux Measurements in Other Ionic Environments—For 
most flux measurements in this study, solutions inside and 
outside the vesicles contained 100 mM NaCl and 20 mM 
potassium phosphate (pH 7.4). Under these conditions, 
measurements with tracer amounts (<10 μM) of $^{22}$Na*, $^{41}$K*, $^{86}$Rb*, 
or $^{137}$Cs* initially added only to the external solution represent 
exchange measurements in which the measured rate of isotope 
movement was assumed to be independent of other monova-
 lent cations in the solution. In a control experiment, reconsti-
truction of a preparation of sodium channels was carried out 
either in 100 mM NaCl, or 100 mM RbCl, and the influx of 
$^{86}$Rb* was measured in each case. Identical values for the half-
time of $^{86}$Rb* equilibration were obtained in the two solutions 
(Fig. 8A).

It has been reported that an external cation-binding site 
must be saturated in order for veratridine to activate sodium 
channels in some excitable cells (24). In an attempt to address 
this question, vesicles containing the purified channel from 
a single preparation were reconstituted in solutions containing 
sodium concentrations between 20 and 100 mM while total 
ionic strength was maintained constant by the reciprocal 
addition of choline. $^{22}$Na* influx measurements were then 
carried out with each set of vesicles activated with veratridine. 
In each case, the half-time for $^{22}$Na* influx was the same and 
showed no dependence on sodium concentration within this 
range (Fig. 8B).

**Saxitoxin Inhibition of Batrachotoxin-activated Cation In-
flux**—We have previously shown using manual measur-
ing techniques with 15-s time resolution that batrachotoxin-stim-
ulated $^{22}$Na* influx could be inhibited by saxitoxin (12). Under 
those conditions, the concentration of saxitoxin required for 
complete inhibition of cation flux was well above the $K_0$ for 
equilibrium binding of this toxin to the channel. This discrep-
ancy was explained by the rapidly reversible nature of saxi-
toxin binding, the very short period required for filling of a 
given vesicle (typically tens of milliseconds), and the relatively 
long influx period permitted by the assay method being used. 
Saxitoxin inhibition of activated cation influx was re-exam-
ined here under conditions in which the initial rate of cation 
uptake could be resolved.

The influx of $^{86}$Rb* was quantitated in batrachotoxin-stimu-
lated vesicles, and the inhibition of this rate was measured 
as a function of saxitoxin concentration. Experiments were
carried out either with inward-facing channels blocked by saturating concentrations of saxitoxin within the vesicles, in which case complete block of initial influx was obtained with titration of external sites, or with no internal saxitoxin where a maximal inhibition of 50–70% was obtained with titration of outward-facing channels as previously reported (12). In either case, the apparent \( K_i \) for saxitoxin inhibition of \( {}^{86}\text{Rb}^+ \) influx was 5–10 nM (Fig. 9) at 18 °C, approximately the same value as the \( K_d \) for saxitoxin binding in these vesicles at this temperature measured by equilibrium binding of [\( ^{3}\text{H}\)]saxitoxin (12).

**Discussion**

The sodium channel purified from rat skeletal muscle sarcolemma has been reconstituted into egg phosphatidylcholine vesicles. This purified channel retained the ability to specifically gate cation fluxes in response to activation by batrachotoxin and veratridine at concentrations comparable to those active on the channel in situ (12). These cation fluxes were specifically blocked by saxitoxin, and we show here that the \( K_i \) for saxitoxin inhibition of cation influx corresponds to the \( K_d \) measured for [\( ^{3}\text{H}\)]saxitoxin binding to the channel using equilibrium binding techniques. In vesicles containing the purified sarcolemmal sodium channel, cation influx stimulated by batrachotoxin occurred too rapidly for kinetic resolution using manual assay techniques. The application of quenched flow methodology to this system now allows the influx kinetics for various alkali metal cations to be resolved and the ion selectivity characteristics of the purified channel to be studied. Similar quenched flow techniques have been used by others in the study of rapid ion fluxes in vesicles containing the acetylcholine receptor protein from eel (25).

Although the vesicle phospholipid composition was held constant in these experiments, the range observed in the magnitude of specific toxin-stimulated uptake was large and not directly correlated with the saxitoxin-binding capacity of a given preparation. We have not yet been able to define the various factors which govern the successful incorporation of functional channels into these vesicles, although a systematic investigation is in progress. Regardless, successful reconstitution with measurable batrachotoxin-stimulated specific influx was obtained in 40 of 42 consecutive attempts using the methods given here. Leakage (control) influx did increase with increasing protein concentration present during the reconstitution; however, these studies do not allow us to differentiate between leakage due to incompetent sodium channels and leakage contributed by the presence of a contaminant polypeptide.

Physiological studies suggest that batrachotoxin opens the sodium channel by shifting its activation curve far toward hyperpolarizing voltages and by eliminating sodium inactivation (26, 27). Since the ionic conditions used here result in zero membrane potential, we expect that functioning channels will be open most of the time in the presence of batrachotoxin. Cation flux stimulated by batrachotoxin should then approximate cation movement through an open channel. The rapid rates measured for the alkali metal cations in our batrachotoxin-activated vesicles using the quenched flow method support this interpretation, as do the low activation energies determined for \( \text{Rb}^+ \) and \( \text{Cs}^+ \) influx. Cation selectivity for the batrachotoxin-activated channel, based on an upper limit estimate of the half-time for \( ^{22}\text{Na}^+ \) influx, was 1:0.14:0.02: 0.005 for \( \text{Na}^+ \), \( \text{K}^+ \), \( \text{Rb}^+ \), and \( \text{Cs}^+ \), respectively. If the actual value for \( \text{Na}^+ \) influx was in fact more rapid than this upper limit, the channel selectivity ratios would be even higher than those indicated here.

In voltage clamp studies, sodium channels opened by batrachotoxin have been shown to have a lower maximal conductance (26) and a lower cation selectivity (29) than those opened by depolarization. Cation selectivity values based on isotopic flux measurements through batrachotoxin-stimulated channels in tissue-cultured nerve and muscle also suggest that this toxin alters to a variable degree the ion selectivity of the channel (24, 29). The cation selectivity demonstrated here for the purified, reconstituted sarcolemmal sodium channel falls in the range of those reported in the literature for batrachotoxin-activated channels in situ; although apparent selectivity is greater than in many batrachotoxin studies, it is less than that expected for the voltage-activated sarcolemmal sodium channel in its active state (30, 31). Cation influx through veratridine-activated channels occurred on a much slower time scale than that through batrachotoxin-activated channels in vesicles containing the same purified sarcolemmal sodium channel. Since veratridine is known to be only a partial agonist for channel opening (32), and voltage clamp studies suggest that veratridine-modified channels activate 1000-fold more slowly than unmodified channels (33), one interpretation of these slow rates would be that the channel is opened only for brief periods in any given time interval. If batrachotoxin-activated channels are assumed to be opened 100% of the time, veratridine-activated channels would be opened 0.5% of the time if, for example, the relative rates of batrachotoxin- and veratridine-activated \( K^+ \) equilibration were explained on this basis. For veratridine-activated channels, the rate-limiting step might then be channel opening rather than the rate of ion movement through an open channel. This hypothesis is supported by the much higher activation energies measured for influx of the alkali metal cations through veratridine-activated vesicles (~30 kcal/mol) as compared to batrachotoxin-activated vesicles (~7 kcal/mol), corresponding to \( Q_0 \) values of >3 and ~1.8, respectively. These values may be compared to those for sodium channel activation (\( Q_0 \sim 3.0 \)) and maximal sodium channel conductance (\( Q_0 \sim 1.5 \)) measured physiologically using voltage clamp techniques in intact nerve and muscle (34, 35).

Although the selectivity sequence for veratridine-activated channels is the same as for those activated with batrachotoxin, the apparent relative selectivity ratio between cations is much lower. A similar observation has been reported for unpurified sodium channels inserted into soybean phospholipid vesicles by freeze-thaw cycles and activated by grayanotoxin I (36). With that preparation, low apparent cation selectivity was also associated with slow rates of cation equilibration. These results may be due in part to veratridine-induced changes in channel structure leading to a modification in selectivity, and it seems probable that at least some of the difference must be ascribed to such a change. However, other factors must be considered in light of the small internal volume of the vesicles under study and the rapid equilibration time for these vesicles through opened sodium channels. Thus, if veratridine produces infrequent channel openings, but channels once activated remain open for several hundred milliseconds, most vesicles would fill with either \( ^{22}\text{Na}^+ \) or \( ^{4}\text{K}^+ \) during a single open channel event. The very similar time course for uptake of these two cations in veratridine-activated vesicles may therefore reflect the probability of channel opening rather than the true relative cation selectivity of the open channel. These considerations could also contribute to the veratridine-stimulated \( \text{Cs}^+ \) and \( \text{Rb}^+ \) data, but the fact that the ratio of the equilibration rates for these ions to the rate for \( \text{Na}^+ \) is lower for batrachotoxin stimulation than for veratridine suggests that veratridine activation itself may cause a further reduction in channel cation selectivity.
We have previously shown that the purified, reconstituted sarcolemmal sodium channel retained the capacity to gate sodium fluxes in response to activation by batrachotoxin and veratridine and also contained the receptor site that allowed these fluxes to be specifically blocked by saxitoxin. We can now state that the purified channel exhibits selectivity among four alkali metal cations which is comparable to that found for the native channel in situ under similar conditions of activation. Demonstration of voltage-dependent activation in the purified channel remains a major goal of future research.

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