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 × 10⁻⁵ M) or veratridine (5 × 10⁻⁴ 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 K⁺, ⁸⁶Rb⁺, and ¹³⁷Cs⁺ were measured directly and halftime for equilibration at 18 °C were determined to be 350 ms, 2.5 s, and 10 s, respectively, in this vesicle population. ²²Na⁺ 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 ⁸⁶Rb⁺ and ¹³⁷Cs⁺ 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.

As 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 electrophorus (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 ²²Na⁺ 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 by 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 Weigle and Barchi (12). Batrachotoxin was the gift of Dr. J. W. Daly of the National Institutes of Health. The isotopes ²²Na⁺, ⁸⁶Rb⁺, ⁸⁶K⁺, and ¹³⁷Cs⁺ were purchased from New England Nuclear Co. Dowex 50-X8.

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.

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Purification of the Sarcolemmal Sodium Channel—Sarcolemmal membranes were isolated from rat skeletal muscle of mixed fiber type using a procedure as described previously (14). Phosphatidylcholine (Sigma Chemical Co., type V-E, 99% purity) as well as sodium channel purification from the wheat germ column of the sodium channel purification procedure. The column dimensions and conditions are described elsewhere (7).

The guanidinium-Sepharose column described previously (4) was washed with 1500 ml of NaCl(0.2 M) and 0.01 M propylamine was prepared immediately prior to use and titrated to pH 7.4 at 0 °C with HCl. Affi-Gel 202 (45 ml of packed resin) was suspended in H2O to a volume of 75 ml and mixed with 75 ml of fresh 2 M 3,3-diaminodipropylamine solution; solid NaCl was then added to a final concentration of 0.1 M. The pH was maintained at 4.7 throughout the reaction period. The resin was washed with 1500 ml of NaCl (0.2 M) followed by 1500 ml of H2O on a siliconized sintered glass funnel, and rinsed twice in 0.1 M of an 0.75 M aqueous solution of O-methylisourea (pH 10.0) in water.

For either single push or pulsed 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; 200-400 mesh) in the Tris form. Under these conditions, the cation exchange resin rapidly sequestered residual cations in the extravesicular space. The vesicles were then rapidly separated from the Dowex by positive pressure and the washed twice with 0.6 ml aliquots of isotonic sucrose-bovine serum albumin buffer. The cation content of the vesicles was determined by liquid scintillation counting.

Cation Selectivity of Reconstituted Sodium Channels

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 reconstructions 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 step) were comparable to the sodium fluxes observed 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

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the presence of variable amounts of contaminating proteins. Variability in specific activity, the major protein components from the loss of high affinity saxitoxin binding rather than the 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 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 Mᵣ = 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 Mᵣ ∼ 38,000 and 45,000 (Fig. 1) (7). The Mᵣ = 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).

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The efficiency of each reconstitution was evaluated with a standard assay measuring the batrachotoxin-stimulated influx of "Na⁺ at 15 and 45 s as well as the control or leakage influx of "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 leak uptake 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).

**Fig. 1.** 7-20% SDS-PAGE of a sodium channel preparation prior to reconstitution (A) and following reconstitution into phosphatidylcholine vesicles (B). The specific activity of this preparation was ∼1,200 pmol of saxitoxin bound/mg of protein. Three major components are seen which are also present in the highest specific activity preparations analyzed: a large glycoprotein that runs anomalously in the high molecular weight region (1), a smaller band with Mᵣ = 40,000 (2), and an additional component often resolved as a doublet at Mᵣ = 38,000 (3A and 3B). For this gel, 200 ng of protein were labeled with ¹²⁵I-Bolton-Hunter reagent following denaturation in SDS using the method of Shing and Ruoho (37). Aliquots were subjected to electrophoresis as detailed elsewhere (7) and bands were visualized by autoradiography using a DuPont Cronex enhancing screen.

**Fig. 2.** Batrachotoxin activation ratio and control "Na⁺ influx at 15 s in representative sodium channel reconstitutions. A, the ratio of batrachotoxin (BTX)-stimulated uptake to control uptake of "Na⁺ at 15 s in 20 preparations is plotted against the specific activity of [³H]saxitoxin (STX) binding measured in that preparation prior to reconstitution. The stimulation ratio was variable and little correlation was found between stimulated uptake activity and specific activity over the range encountered in this study. The solid line indicates the least squares linear regression to the data (r = 0.10). B, the absolute level of control (unstimulated) "Na⁺ uptake at 15 s for 21 preparations versus the total concentration of protein present at the time of reconstitution. A weak correlation was found, suggesting that increasing nonspecific influx was associated with increasing protein concentration. The line indicates the least squares linear regression to the data points (r = 0.56).
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 $^{22}$Na could be resolved unequivocally using the quenched flow technique. $^{22}$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 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 $^{42}$K$^+$, the isotope with the fastest resolvable uptake rate. Extrapolation of corrected specific $^{42}$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 $^{22}$Na$^+$ equilibration under comparable conditions.

The time course for equilibration of $^{42}$K$^+$, $^{86}$Rb$^+$, and $^{137}$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 $^{42}$K$^+$; for that cation, a small correction to the measured half-time was necessary. The half-time for $^{42}$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 $^{86}$Rb$^+$ and $^{137}$Cs$^+$ were 25 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 $^{22}$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 $^{42}$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 $^{137}$Cs$^+$ and $^{86}$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 $^{22}$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 $^{22}$Na$^+$, $^{42}$K$^+$, $^{86}$Rb$^+$, and $^{137}$Cs$^+$.
uptake was already observed at the shortest interval shown on this graph (0). The maximal space accessible through activated sodium channels was approximately the same as that accessible through veratridine-activated channels (A), 36Rb+ (C), and 137Cs+ (O). The maximal space accessible through activated sodium channels was already observed at the shortest interval shown on this graph (O). All measurements shown were made at 18 °C.

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

| Cation | Batrachotoxin | Veratridine |
|--------|--------------|-------------|
| 22Na+  | t₁/₂: 50 ms  | t₁/₂: 1.00  | t₁/₂: 0.005 | Ratio: 1.00 | Ratio: 2.6 |
| 42K+   | t₁/₂: 350 ms | t₁/₂: 1.20  | t₁/₂: 0.50  | Ratio: 0.83 | Ratio: 0.38 |
| 36Rb+  | t₁/₂: 2.5 s  | t₁/₂: 2.00  | t₁/₂: 0.50  | Ratio: 0.83 | Ratio: 0.38 |
| 137Cs+ | t₁/₂: 10 s   | t₁/₂: 2.60  | t₁/₂: 1.00  | Ratio: 0.38 | Ratio: 1.00 |

* 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 at least three control measurements in duplicate. Time courses with batrachotoxin were carried out at 18 °C; those with veratridine were performed at 36 °C.

Temperature Dependence of Specific Cation Influx—Batrachotoxin- and veratridine-activated cation influxes differed markedly in their temperature dependence. With veratridine-activated vesicles, specific 22Na+-activated influx was barely detectable at temperatures below 20 °C but increased sharply with increasing temperatures. A typical experiment illustrating this point is shown in Fig. 7A. In other preparations, control influx at the time of peak stimulated influx for these four cations was 1.7.

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.

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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 reliable measurements could be made. Similar studies were carried 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 temperature dependent. $^{22}$Na influx 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 therefore 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 Iionic 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, $^{42}$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 monovalent cations in the solution. In a control experiment, reconstitution 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 equilibrium 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 Influx—We have previously shown using manual measuring techniques with 15-s time resolution that batrachotoxin-stimulated $^{22}$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 $K_d$ for equilibrium binding of this toxin to the channel. This discrepancy was explained by the rapidly reversible nature of saxitoxin 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-examined here under conditions in which the initial rate of cation uptake could be resolved.

The influx of $^{86}$Rb was quantitated in batrachotoxin-stimulated 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 $^{86}$Rb$^+$ influx was 5–10 nM (Fig. 9) at 18 $^\circ$C, approximately the same value as the $K_a$ for saxitoxin binding in these vesicles at this temperature measured by equilibrium binding of [3H]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 $K_a$ for saxitoxin inhibition of cation influx corresponds to the $K_a$ measured for [3H]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 Rb$^+$ and Cs$^+$ influx. Cation selectivity for the batrachotoxin-activated channel, based on an upper limit estimate of the half-time for $^{22}$Na$^+$ influx, was 1:0.14:0.02: 0.005 for Na$^+$, K$^+$, Rb$^+$, and Cs$^+$, respectively. If the actual value for Na$^+$ equilibration 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 (28) than those opened by depolarization. Cation selectivity values based on isotopic flux measurements through batrachotoxin-opened 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 ($Q_0$ ~3.0) and maximal sodium channel conductance ($Q_0$ = 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 $^{22}$Na$^+$ or $^4$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 Cs$^+$ and Rb$^+$ data, but the fact that the ratio of the equilibration rates for these ions to the rate for 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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