SYMMETRY AND ASYMMETRY OF PERMEATION THROUGH TOXIN-MODIFIED Na^+ CHANNELS

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ABSTRACT Single Na^+ channels from rat skeletal muscle were inserted into planar lipid bilayers in the presence of either 200 nM batrachotoxin (BTX) or 50 μM veratridine (VT). These toxins, in addition to their ability to shift inactivation of voltage-gated Na^+ channels, may be used as probes of ion conduction in these channels. Channels modified by either of the toxins have qualitatively similar selectivity for the alkali cations ($Na^+ \sim Li^+ > K^+ > Rb^+ > Cs^+$). Biionic reversal potentials, for example, were concentration independent for all ions studied. Na^+/K^+ and Na^+/Rb^+ reversal potentials, however, were dependent on the orientation of the ionic species with respect to the intra- or extracellular face of the channel, whereas Na^+/Li^+ biionic reversal potentials were not orientation dependent. A simple, four-barrier, three-well, single-ion occupancy model was used to generate current-voltage relationships similar to those observed in symmetrical solutions of Na, K, or Li ions. The barrier profiles for Na and Li ions were symmetric, whereas that for K ions was asymmetric. This suggests the barrier to ion permeation for K ions may be different than that for Na and Li ions. With this model, these hypothetical energy barrier profiles could predict the orientation-dependent reversal potentials observed for Na^+/K^+ and Na^+/Rb^+. The energy barrier profiles, however, were not capable of describing biionic Na/Li ion permeation. Together these results support the hypothesis that Na ions have a different rate determining step for ion permeation than that of K and Rb ions.

INTRODUCTION

The mechanisms underlying the voltage-sensitive Na^+ currents have been under intense investigation ever since the work of Hodgkin and Huxley (1952) in the squid axon. We now know that these Na^+ currents, found in nerve, muscle, and other tissues, are mediated by Na^+ channel proteins. The amino acid sequence of Na^+ channels from eel electroplax, rat brain, and fruit fly have recently been determined (Noda et al., 1985, 1986; Salkoff et al., 1987). Even with extensive knowledge concerning this channel protein, apparently simple questions such as how ions pass through the channel remain subtle and elusive. This is primarily due to a paucity of available probes to selectively alter processes of ion selectivity. Recently, however, Garber and Miller (1987) have shown that batrachotoxin (BTX) and veratridine (VT), toxins whose abilities to alter the activation and inactivation processes of the voltage-gated Na^+ channel have been intensively studied, also alter the open channel properties of single Na^+ channels in subtly distinct ways and thus provide excellent probes of ion selectivity.

In a model of ion selectivity in voltage-gated Na^+ channels, Hille (1971, 1972, 1975, *a* and *b*) develops the concept of a physical constriction, or "selectivity filter" within the conduction pathway of the channel. This constriction excludes large ions from traversing the channel on the basis of size and distinguishes between small ions on the balance of energies required to dehydrate, bind, release, and rehydrate these ions as they pass through the channel. In this view, the selectivity filter of an ion channel is analogous to the active site of an enzyme. Thus, as an active site defines the major properties of an enzyme, a selectivity filter defines the major permeation properties of an ion channel. The model described by Hille allowed only a single ion to enter the channel pore at a time. While other workers (Cahalan and Begenisich, 1976; Begenisich and Cahalan, 1980, *a* and *b*) have presented more complicated models, for example, allowing two ions to occupy the channel, the concept of a localized selectivity filter has remained.

The description of ion channels as enzymes has been extremely useful in providing a conceptual framework in which to pursue the characterization of channel-ion interactions. For example, Michaelis-Menten kinetics and Eyring rate theory are often used to describe enzyme-substrate interactions. These tools have also been successfully applied to explaining channel-ion interactions, such as the saturation of conductance observed with increasing ionic concentration for many ion channels (Hille, 1984). Also in analogy to an enzymatic reaction, the rate-determining step in the selectivity of an ion channel is assumed to be the same for chemically similar ionic species (e.g., the alkali

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cations). Garber and Miller (1987), however, suggest that this may not be the case for permeation of Na and K ions through Na^+ channels and that the two ions have distinct rate-limiting steps.

In order to develop a quantitative physical model of the ion selectivity of these channels, I focus this report on the interactions of the alkali cations, Li^+, Na^+, K^+, and Rb^+ with single toxin-modified Na^+ channels as determined from current-voltage relationships, current saturation, biionic reversal potentials and ionic competition. I present the finding that biionic reversal potentials were dependent on the side of the channel on which the less permeant ions, K^+ and Rb^+, were located with respect to Na^+. The reversal potentials, however, remained independent of concentration. A simple, single ion occupancy model similar to that described by Hille (1975*a* and *b*) was sufficient to account for these observations and the current-voltage relationships observed in symmetrical solutions of Na, K, and Li ions. A preliminary report of some of this work has been presented (Garber, 1987).

MATERIALS AND METHODS

In all the experiments described here, currents through single channels were recorded by inserting vesicles of rat T-tubular membranes containing Na^+ channels into Montal-Mueller type planar lipid bilayers (in the presence of either 200 nM batrachotoxin [BTX] or 35–50 μM veratridine [VT] [Garber and Miller, 1987]). Bilayers were composed of egg phosphatidylethanolamine and brain phosphatidylcholine in a 4:1 ratio. Preparation of vesicles and insertion into bilayers has been described previously (Garber and Miller, 1987). The orientation of Na^+ channels in the bilayer was ensured by the addition of 1 μM tetrodotoxin (TTX) to one side of the bilayer chamber. All voltages are reported according to electrophysiological convention, with the TTX-sensitive side of the channel defined as zero voltage.

The aqueous solutions contained either Cl^- or acetate salts of alkali cations in addition to 5 or 10 mM MOPS and 1 mM EDTA, adjusted to pH 7.4 with the appropriate hydroxide salt. In all experiments the total concentration of the cation is reported, including contributions of the hydroxide salt.

Single channel records were stored and analyzed as described in Garber and Miller (1987). For figures which compare observed data and theoretical predictions of current-voltage relationships, current values at specific voltages taken from ramp data are plotted as individual points to allow for comparison with data taken at maintained holding voltages and theoretically derived points.

Recorded values of the reversal potentials were corrected for liquid junction potential asymmetries using the Henderson equation (Robinson and Stokes, 1968), and were within the range of 3–6 mV. The measured reversal potentials were independent of the anion present. The voltage ramp technique allows the direct determination of the zero-current voltage to within ~5 mV.

Energy barrier profiles for permeation of the alkali cations through both BTX- and VT-modified Na^+ channels presented here are modified from the four-barrier, three-well model for a single ion occupancy channel presented by Hille (1975, *a* and *b*) and more recently altered by Worley et al. (1988). Although other profiles were capable of describing a given set of experimental data, a four-barrier model was the most satisfactory. The four-barrier energy profile, such as the one shown in Fig. 1 *A* for Na^+ ion conduction, spans the region of the channel given by the electrical distance (δ) over which the voltage drop occurs, with the left side corresponding to the intracellular, and the right to the extracellular side of the channel. The positions of the barriers (with the peaks placed symmetrically in between wells) along the electrical distance are considered fixed. No voltage-dependent blockers were studied here to determine barrier positions. Thus, the positions of the barriers have been chosen to allow direct comparison with the work of Worley et al. (1988) on BTX-modified Na^+ channels from rat brain. Moving the position of a peak or a well $\pm 0.04\ \delta$ along the voltage drop resulted in little or no change in the predicted current-voltage relationship. The rate constant, k_i, over each barrier of energy G_i^* is given by

$$k_i = V \exp(-G_i^*/RT).$$

The value of the preexponential factor, V, is arbitrary as long as it is the same for all ions. It is assigned the value of $kT/h = \sim 10^{+13}\ (s^{-1})$ (where k is Boltzman's constant, T is the temperature in Kelvin, and h is Planck's constant). Four linear flux equations (for each ion) were then solved for the currents going across the barrier profile as a function of voltage in a manner described previously (Begenisich and Cahalan, 1980, *a* and *b*),

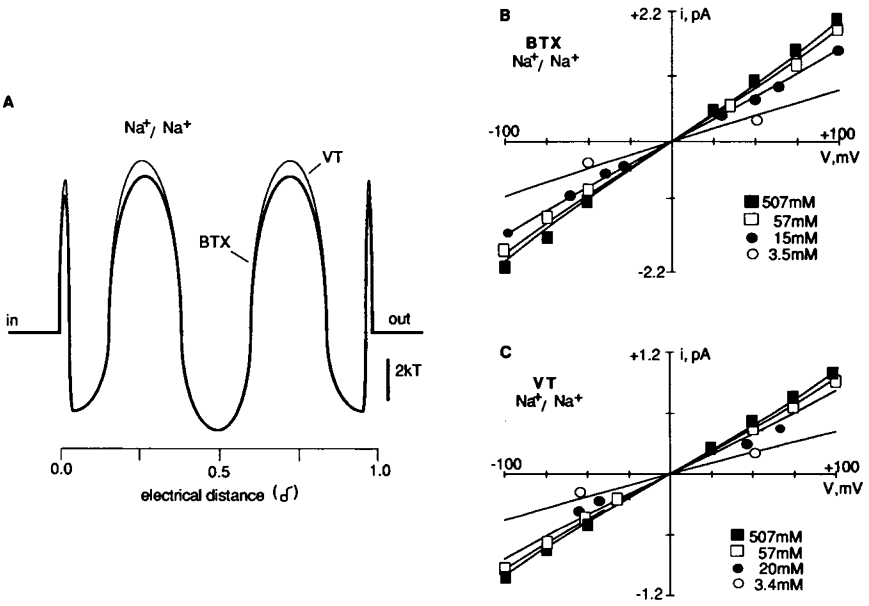

FIGURE 1 (*A*) Hypothetical symmetric energy barrier profiles for Na^+ ion conduction in BTX-(*thick lines*) and VT-modified Na^+ channels (*thin lines*). The difference in Na ion conduction for the two toxin-modified Na^+ channels is in the heights of the barriers. The heights of the barriers, in units of kT, and the positions of the peaks and wells along the electrical distance (δ) are given in Table I. (*B* and *C*) Current-voltage relationships for single BTX- (*B*) and VT-modified (*C*) Na^+ channels in symmetrical solutions containing Na ions at concentrations of 3–507 mM. The solid lines indicate the predicted current-voltage relationship from the hypothetical energy barrier shown in *A*. The solid symbols are data compiled from several experiments (≥ 3) as described in Methods. Standard errors of data points are smaller than symbols. Note the current scales are different for each toxin-modified Na^+ channel.

using a computer program (Cukierman et al., 1985) generously provided by Dr. C. Miller.

RESULTS

Garber and Miller (1987) showed at the single channel level that the presence of the toxins BTX and VT results in different amplitude Na current flowing through Na^+ channels. To account for the observed differences, I compare below data concerning the ion conductance and ion selectivity for the group IA cations, Li^+, Na^+, K^+, and Rb^+ in these modified channels to the predictions of a simple single ion occupancy model.

Ionic Conductance

Na and Li Ions. The distinction in the conduction of Na ions through BTX- and VT-modified Na^+ channels is in the maximum single-channel conductance. This conductance differs by a factor of ~2, being 21 and 10 pS, respectively. The apparent binding of Na ions within the pore, however, is 6–7 mM regardless of the toxin present (Garber and Miller, 1987). It was possible to account for the effects of BTX and VT on Na ion conduction in a simple manner, using a single ion occupancy energy profile having four barriers and three wells to describe the path of Na ions traversing the channel pore (as described in the Methods). Using such a simple model the depths of the hypothetical energy wells within the pore in the presence of either toxin did not differ, as the binding of ions is independent of the toxin present (Lauger, 1973; Hille, 1975*a*). The difference in the maximum single channel conductance could be attained by increasing only the peak energy required for a Na ion to pass over barriers in a VT-modified Na^+ channel, as compared with those of a BTX-modified channel (Fig 1 *A*). The essentially linear I/V relationships resulting from these barrier profiles provided an adequate description of the observed I/V data (Fig. 1, *B* and *C*).

The single channel I/V relationship of Li^+, like that of Na^+ is essentially linear (over a voltage range of -100 to $+100$ mV) and is also smaller through Na^+ channels modified by VT rather than BTX (Garber and Miller, 1987). The I/V relationships in Fig. 2 were used to derive energy barrier profiles for Li ion conduction through each of the modified channels. In a manner similar to the description of Na ion conduction, it was possible to account for the lower conductance of Li ions through VT-modified Na^+ channels (Fig. 2) by increasing barrier heights without altering the well depths of the energy barrier profile as compared with those for BTX-modified Na^+ channels. Fig. 2 compares the observed and predicted current-voltage (I/V) relationships with equivalent solutions of Li^+ (57–507 mM) on either side of the channel. Deviation between the observed and predicted I/V relationships in VT-modified Na^+ channels became apparent at 57 mM. It was not possible to alter the barrier profile for Li ion conduction without introducing other deviations from observed data. The barrier profiles, on the whole however, provided a good description of the data. The energy values for the profiles describing Na and Li ion conduction for both BTX- and VT-modified Na^+ channel are given in Table I. The values of both the energy peaks and wells for Na ion conduction presented here are lower than those suggested by Worley and colleagues (1988) for BTX-modified Na^+ channels from rat brain. This reflects the apparent higher affinity and lower single channel conductance of Na^+ in the muscle channel.

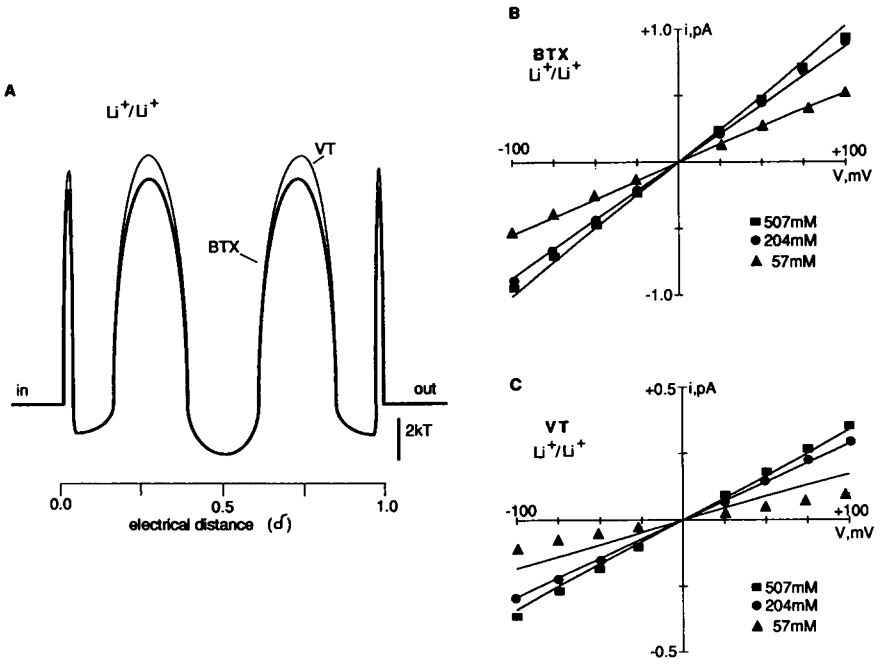

FIGURE 2 (*A*) Hypothetical symmetric energy barrier profiles for Li ion conduction through BTX- (*thick lines*) and VT-modified Na^+ channels (*thin lines*). Like conduction of Na ions, the difference between Li ion conduction in the two modified Na^+ channels is ascribed to the difference in the barrier heights. (*B* and *C*) Current-voltage relationships for single BTX-(*B*) and VT-modified (*C*) Na^+ channels in symmetrical solutions containing 57, 204, or 507 mM Li ions. The solid symbols are data complied from several experiments (≥3) as described in Methods. Standard errors of data points are smaller than symbols. Note the current scales are different for each toxin-modified Na^+ channel.

TABLE I
HYPOTHETICAL ENERGY BARRIER PROFILES: PARAMETERS FOR A FOUR-BARRIER, THREE-WELL MODEL

Position	δ	Na		Li		K	
		BTX	VT	BTX	VT	BTX	VT
Peak 1	0.04	7	7.7	10.5	11.6	7	7
Well 1	0.08	−4	−4	−1.4	−1.4	−3	−3
Peak 2	0.30	8	8.7	11	12.1	8	8
Well 2	0.52	−5	−5	−2.4	−2.4	−3*	−3*
Peak 3	0.74	8	8.7	11	11	9.7	9.9
Well 3	0.96	−4	−4	−1.4	−1.4	−4.6	−4.4
Peak 4	0.98	7	7.7	10.5	10.5	10.5	10.7

Parameters for free energy profiles depicted in Figs. 1–4 and 6. The values of free energy are given in units of kT, at the given electrical distance, δ, with δ = 0.0 corresponding to the cytosolic side of the membrane. *Parameter may be varied from −3 to 8 energy units without significantly affecting the fit to the data.

K Ions. K ion conduction, in contrast to that of Na ions, is independent of the toxin modifying the channel (Garber and Miller, 1987). Furthermore, unlike the linear current-voltage relationship observed in symmetrical Na^+, the current-voltage relationship in symmetrical 57, 207, or 507 mM K^+ decreases in slope at positive voltages, as compared with that at negative voltages (Fig. 3). The asymmetry in the current-voltage relationship was mimicked by an energy profile of four different barrier heights, as illustrated in Fig. 3. The energy values of the barriers were adjusted to make it more difficult for a K ion inside the pore to exit toward the extracellular side, rather than toward the cytosolic side of the channel. Interestingly, the energy value of the central well had little effect on the shape of I-V relationships in experiments in which intra- and extracellular solutions contained the same concentration of K ions. This reflects the importance of the barriers at the entrances of the pore and the deep well toward the extracellular side of the pore.

The single channel conductance in symmetrical solutions at low concentrations of K^+ (≤50 mM) for either modified Na^+ channel was difficult to resolve from baseline noise. Therefore it was not possible to measure the affinity of K ions directly by measuring single channel conductance at increasingly lower concentrations of K^+. The greater depth of the outermost well for K ion conduction (Fig. 3) as compared with the central well of the Na ion energy profile (Fig. 1) suggests, however, that the binding of K ions within the channel is tighter than that for Na ions.

Biionic Permeability Measurements

Na/K Ions and Na/Rb Ions. In a single ion occupancy model, such as the one considered here, the biionic permeability ratio is a measure of the relative ability of an ion to enter the channel pore (Lauger, 1973; Hille, 1975*a*; Armstrong, 1975). The distinct I/V relationships in symmetrical solutions of either Na or K ions suggested that the biionic Na^+/K^+ permeability ratios depend on the orientation of K ions (intra- or extracellular) with respect to the channel. This was found to be the case.

The I/V relationship observed with Na ions only on the extracellular side and only K ions facing the intracellular side of a BTX-modified Na^+ channel gave a reversal potential that differs considerably from that determined with the ions in the opposite configuration (i.e., with K ions extracellularly and Na ions intracellularly) (Fig. 4). The reversal potential measurements are −55 mV ± 2 mV and +35 ± 5 mV (at 207 mM), respectively (Fig. 4 *A*). Biionic Na^+/K^+ reversal potentials measured in VT-modified Na^+ channels show a similar, though reduced, difference

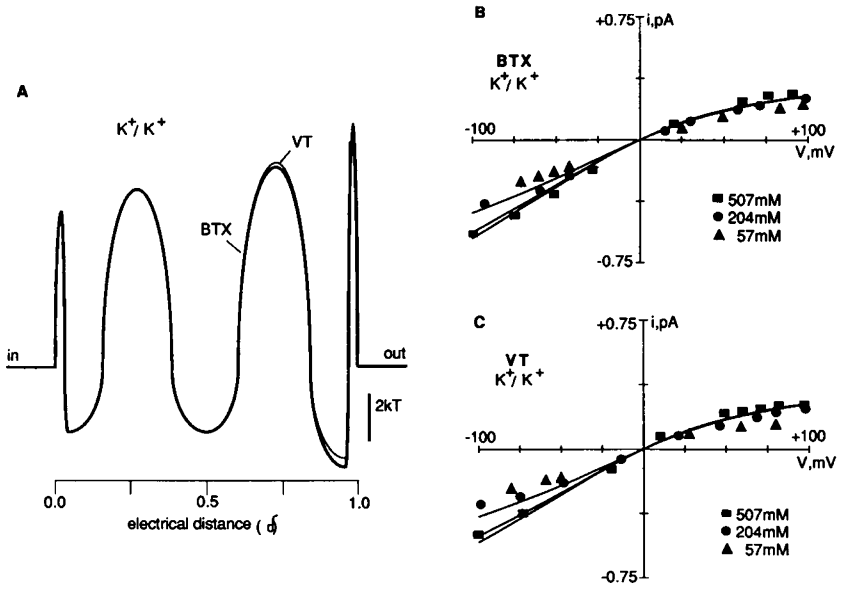

FIGURE 3 (*A*) Asymmetric hypothetical energy barrier profiles for K ion conduction through BTX- (*thick lines*) and VT-modified Na^+ channels (*thin lines*) are very similar. (*B* and *C*) Current-voltage relationships for single BTX- (*B*) and VT-modified (*C*) Na^+ channels in symmetrical solutions containing K ions at three concentrations (57, 204, or 507 mM). The solid symbols are data complied from several experiments (≥3) as described in Methods. Standard errors of data points are smaller than symbols. Note the current scales are the same for each toxin-modified Na^+ channel.

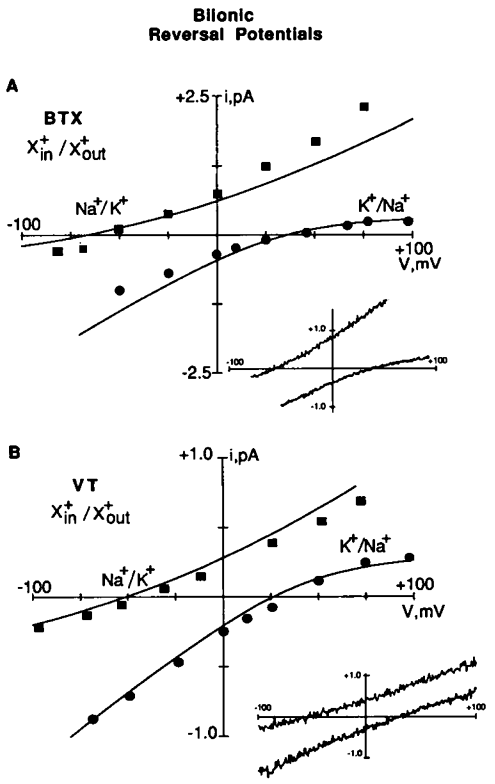

FIGURE 4 Current-voltage relationships for (*A*) BTX- or (*B*) VT-modified Na^+ channels under biionic conditions with 207 mM Na^+ containing solutions on one side and 207 mM K^+ containing solutions on the other (as indicated). Examples of voltage ramps under the given conditions are shown in the insets. The solid lines are the predictions from the energy barrier profiles from Na and K ions shown in Figs. 1*A* and 3*A*. Solid symbols are data compiled from several experiments (≥3) as described in Methods. Standard errors of data points are smaller than symbols. Note the current scales are different for each toxin-modified Na^+ channel.

when K ions were facing the intracellular side of the channel versus that when on the extracellular side: -39 ± 1 mV and $+33 \pm 4$ mV, respectively, at 207 mM (Fig. 4 *B*). The asymmetry of the Na^+/K^+ reversal potentials measured in the presence of VT was also apparent from experimentally determined biionic permeability ratios, as presented in Table II. Na^+/K^+ reversal potentials for Na^+ channels modified by either toxin are independent of concentration (Table II), as would be expected in a single ion occupacy permeation model.

The current-voltage relationships shown by the solid lines in Fig. 4 have been generated using the energy barrier profiles described above for Na and K ions. It was not necessary to alter either the relative heights or positions of the barriers for either ion, suggesting that the selectivity differences between BTX- and VT-modified Na^+ channels may be primarily attributed to Na ion conduction. The combination of these two profiles resulted in asymmetric Na^+/K^+ permeation ratios for these two ions, close to that observed experimentally in both BTX- and VT-modified Na^+ channels (Table II).

TABLE II
PERMEABILITY RATIOS: Na^+/X^+ BIIONIC CONDITIONS

	BTX				VT			
$[X^+]$: *mM*	X^+, in		X^+, out		X^+, in		X^+, out	
	exp	th	exp	th	exp	th	exp	th
K^+: 57	3.3	3.3	9.2	11.4	3.2	2.3	4.2	6.0
207	3.9	3.3	8.5	11.4	3.1	2.3	3.2	6.0
507	4.4	3.3	8.2	11.4	3.6	2.3	4.6	6.0
Rb^+: 207	4.7	—	31.9	—	4.7	—	14.1	—
Li^+: 57	1.1	>26	1.0	>26	*	>39	1.1	>39
207	*	>26	1.0	>26	1.0	>39	1.1	>39
507	1.1	>26	*	>26	1.2	>39	0.8	>39

Experimentally observed biionic Na^+/X^+ permeability ratios (*exp*), as determined in methods, are similar to permeability ratios (*th*) determined from the hypothetical energy barriers for Na, K, and Li ions given in Table 1. (Hypothetical energy barrier profiles were not derived for Rb ions.) X^+, *in* or X^+, *out* indicates the ion under consideration, and whether it faces the intra- or extracellular side of the Na^+ channel. Uncertainty surrounding value of experimentally derived permeability ratios is 20% or less. *Data not available for comparison.

The pattern of asymmetric biionic reversal potentials was further exaggerated when K ions are replaced by Rb ions, for both modified Na^+ channels (Fig. 5). The Rb^+/Na^+ reversal potentials were -89 ± 4 mV and $+40 \pm 4$ mV in the presence of BTX, and -68 ± 6 mV and $+40 \pm$

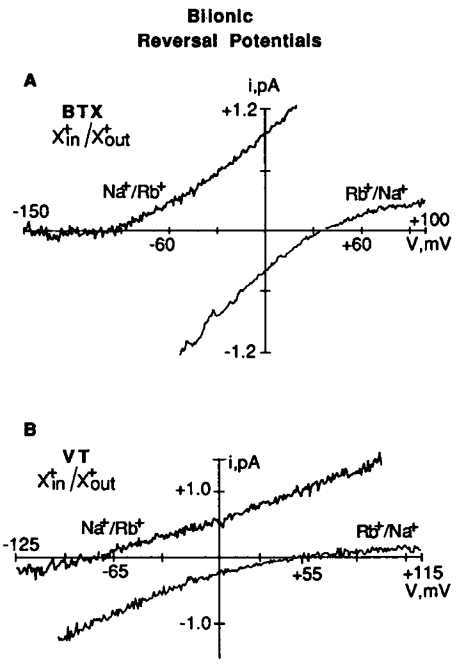

FIGURE 5 Current-voltage relationships for (*A*) BTX- or (*B*) VT-modified Na^+ channels under biionic conditions with 207 mM Na^+-containing solutions on one side and 207 mM Rb^+-containing solutions on the other (as indicated in the figure). Solid symbols are data compiled from several experiments (≥3) as described in Methods. Standard errors of data points are smaller than symbols. Note the current scales are the same for each toxin-modified Na^+ channel.

4 mV in the presence of VT, with Rb ions located extra- and intracellularly, respectively. It was very difficult to directly measure the I/V relationships in symmetrical Rb^+ solutions for either modified Na^+ channel as the small currents recorded were difficult to resolve from background noise. The extreme asymmetry in the Na^+/Rb^+ reversal potentials, however, suggests a similarity of Rb ions to K ions in this system. This implies that the I/V relationship in symmetrical Rb^+ solutions, as in K^+ solutions, may be independent of the toxin present, and it may show a greater rectification than that measured in symmetrical K^+ solutions.

Na/Li Ions. The biionic permeability ratio for Na^+/Li^+, unlike those for Na^+/K^+ and Na^+/Rb^+, did not depend on the orientation of Li ions with respect to the Na^+ channel (Table II). The permeability ratios for both modified Na^+ channels was similar to those measured for Na^+/Li^+ in other preparations, whch range from 1 to 1.6 (Hille, 1972; Khodorov and Revenko, 1979; Moczydlowski et al., 1984; Recio-Pinto et al., 1987). The I/V relationships under biionic Na^+/Li^+ solutions are essentially linear from -100 to $+100$ mV (see Garber and Miller, 1987). Combining the profiles described in Fig. 2 for Li ions and those for Na ion conduction, for either modified Na^+ channel, resulted in a reversal potential distant from 0 mV (Table II). (The large values of the permeation ratios predicted from these profiles result from the significantly higher peaks in the Li ion profile. Note also that the well depths of the Li ion profile are higher than those of the Na ion profile.) These theoretical predictions were in direct conflict with the observed Na^+/Li^+ permeability ratios. It was not possible to resolve this conflict using the existing data and assuming that the selection of Li ions over Na ions occurs in a simple manner within the voltage drop across the pore.

Ion Competition Experiments

Na vs. K Ions. Whereas biionic permeability experiments provide information concerning the entry of a single ionic species into a channel pore, the following experiments address the relative ability of an ionic species to compete for occupancy within the pore. Fig. 6 shows the effect of symmetrical additions of K^+ on the current-voltage relationship with 57 mM symmetrical Na^+, for both BTX- and VT-modified Na^+ channels, as reported by Garber and Miller (1987). The increase in the concentration of K^+ results in a striking reduction of current flowing at positive voltages. This pattern of rectification generated from the energy profiles for Na^+ and K^+ described in Figs. 1 and 3, is shown in Fig. 6. These results imply that K ions moving outward were better at occupying, rather than moving through, the pore, particularly at more depolarized voltages. This is consistent with the I/V relationships, in symmetrical K^+ solutions, shown in Fig. 3.

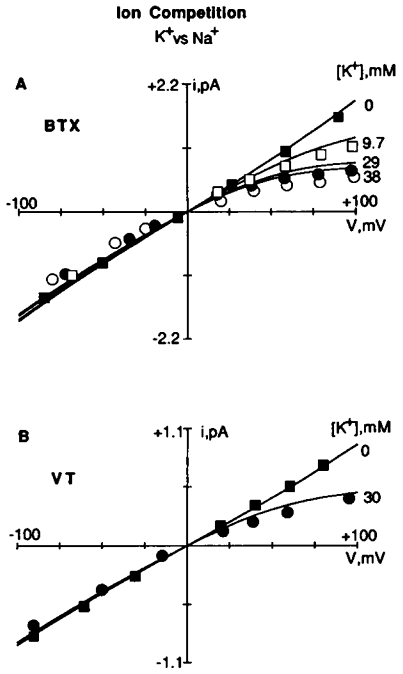

FIGURE 6 The asymmetric block of Na^+ currents by increasing amounts of K^+ made by symmetrical additions to each side of the bilayer in (*A*) BTX- and (*B*) VT-modified Na^+ channels. Similar data generated with symmetrical additions of 5 and 20 mM K^+ containing solutions to bilayers containing BTX-modified channels, and 10, 20, 40, and 50 mM K^+ containing solutions to bilayers containing VT-modified channels, were not shown for clarity. Solid lines describing the open channel current-voltage relationships are predicted from the hypothetical energy barriers for each ion shown in Figs. 1*A* and 3*A*. The solid symbols are data compiled from several experiments ($\geq$3) as described in Methods. Standard errors of data points are smaller than symbols. Note the current scales are different for each toxin-modified Na^+ channel.

The relationship of the apparent inhibition constant for K^+ (K_I[app]) to voltage, plotted in Fig. 7, provides a sensitive comparison of the observed data and theoretical predictions. The zero-voltage inhibition constant for K ions in VT-modified Na^+ channels was larger than that of BTX-modified channels for both observed and theoretically derived values. The relationship of the observed K_I[app] to voltage, derived from BTX- and VT-modified Na^+ channels, give an *e*-fold increase in affinity per 26 and 64 mV, respectively. The theoretical predictions, however, give an *e*-fold increase in affinity per 50 mV, independent of the toxin used to modify the Na^+ channel. The deviations between the results derived from observed data and theoretical predictions suggest that there may be ion–ion or ion–protein interactions involved in Na and K ion competition for occupancy of the Na^+ channel pore.

DISCUSSION

This report considers the ionic selectivity of voltage-gated Na^+ channels using the steroidal toxin, BTX, and alkaloid toxin, VT, as molecular probes. The work of Garber and

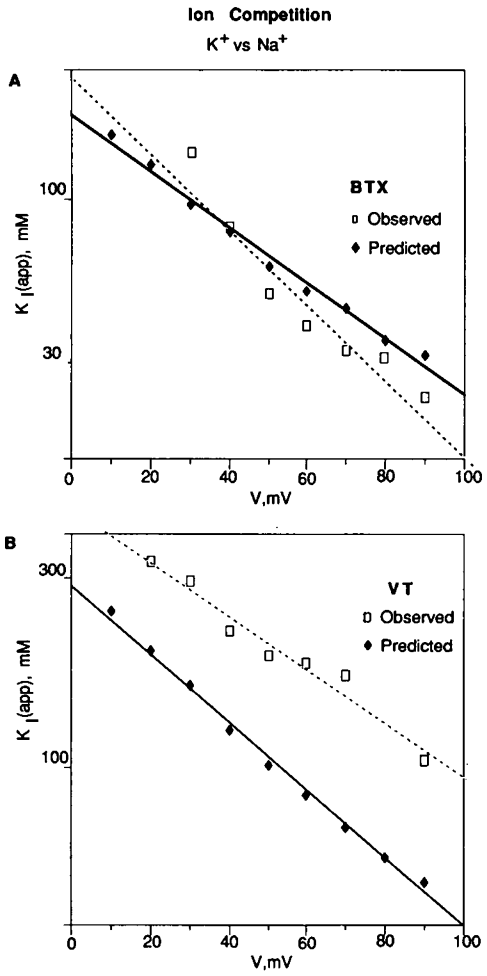

FIGURE 7 The voltage dependence of the block of Na^+ current by K ions in (*A*) BTX- and (*B*) VT-modified Na^+ channels (*open symbols, dashed lines*). The apparent inhibition constants of Na^+ (K_I[app]) by K^+ at applied voltages were determined from plots of reciprocal current vs. [K^+]. Solid symbols lines in *A* and *B* show the voltage-dependence predicted of K_I[app] (calculated in a similar manner from the current value of the theoretical curves in Fig. 6 at indicated voltages) from the hypothetical energy barriers shown for Na and K ions in Figs 1*A* and 3*A*. Straight lines were the best fit of a simple regression analysis. (The fit is slightly different than that reported by Garber and Miller [1987].) The slopes of these lines give an *e*-fold increase in affinity of K^+ block of Na^+ current per 26 and 64 mV for the observed data from BTX- and VT-modified Na^+ channels, respectively, and 50 mV for the theoretical predictions of both BTX- and VT-modified channels. The zero-voltage inhibition constant of K^+ was 245 and 460 mM from the experimental data and 182 and 293 mM from the theoretical predictions (BTX- and VT-modified channels, respectively).

Miller (1987) has shown, on the single channel level, that VT-modified channels are less selective than BTX-modified channels. Yet, BTX- and VT-modified channels retain the same relative ion selectivity of voltage-gated Na^+ channels ($Na^+ \sim Li^+ > K^+ > Rb^+ > Cs^+$) . This earlier study, together with much previous work (Khodorov and Revenko, 1979; Naumov et al., 1979; Huang et al., 1979; Quant and Narahashi, 1982; Leibowitz et al., 1986; Barnes and Hille, 1987), indicates that these toxins decrease the relative permeability of voltage-gated Na^+ channels to inorganic cations. These changes in selectivity are in addition to the drastic changes observed in the activation and inactivation properties which occur after the binding of either VT or BTX to the Na^+ channel. This body of work, together with selectivity measurements on unmodified Na^+ channels (Hille, 1971), suggest that there is a spectrum in the ability of the voltage-gated Na^+ channel to conduct ions; with unmodified channels being the most selective, followed by BTX- and VT-modified channels, respectively.

The relationships of current to voltage under symmetrical ionic solutions, biionic, and competitive ionic conditions for Na^+ and K^+ presented here and by Garber and Miller (1987) indicate that K ions are more likely to occupy the pore under physiological ionic conditions (with intracellular K ions) and under depolarizing potentials. K ions also enter the channel more easily from the cytosolic than from the extracellular side of the channel. This picture suggests that the interaction of K ions with the lumen of the channel may have a minor physiological role, as a "permeant blocker," by providing a check on inward Na^+ current elicited during an action potential in addition to preventing extracellular K ions from leaking into a cell through open Na^+ channels. Worley and colleagues (1988) have developed a similar view of K ion conduction in BTX-modified Na^+ channels from rat brain by considering the reduction of Na^+ current in the presence of K ions (Krueger et al., 1983).

Ion Selectivity in Toxin-modified Na^+ Channels

Several groups have reported the Na^+/K^+ permeation ratio in BTX-modified Na^+ channels in several different preparations to be 4–5/1 (Khodorov and Revenko, 1979; Mozhayeva et al., 1982; Green et al., 1987; Recio-Pinto et al., 1987) whereas others have reported higher values (~10/1, when corrected for liquid junction potentials [Krueger et al., 1983; Moczydlowski et al., 1984]). In the past, these apparently incompatible results were ascribed to species or tissue differences. These results, however, are predicted by the energy barrier profiles of Na and K ions presented in this report. The measurements of Khodorov and Revenko, and Mozhayeva et al. were made from complete replacement of external Na^+ ions in voltage-clamped nodes of Ranvier from frog. The physiological composition of the internal K^+ solution was retained. The measurements of Green et al., with Na^+ channels from dog brain, and Recio-Pinto et al., in eel electroplax, were also made under relatively physiological conditions (with predominantly Na ions in the external solution and K ions in the internal solutions) in planar lipid bilayers. Using the ionic conditions described by Green et al. and Recio-Pinto

et al., the Na^+/K^+ permeation ratio of 4.5/1 predicted by the profiles described in Figs. 1 and 3 for Na and K ions is in excellent agreement.

Less physiological ionic compositions (with predominantly Na^+ containing solutions inside and K^+ solutions outside), were used by Krueger et al. (1983) and Moczydlowski et al. (1984) to measure permeation ratios of BTX-modified Na^+ channels from rat brain and rat muscle in planar lipid bilayers. The Na^+/K^+ permeation ratios predicted by the energy profiles described here are 10.5/1 when using the ionic conditions of either Krueger et al. or Moczydlowski et al. Again, the values of the predicted permeation ratio are in good agreement with the experimental observations. This agreement indicates that the apparently conflicting values of the reported Na^+/K^+ permeation ratios in BTX-modified Na^+ channels are most likely due to the ionic conditions under which the measurements were made, rather than the nature of the preparation itself.

The permeation profiles presented in this report indicate that the differences in ion selectivity between the toxin-modified Na^+ channels themselves are primarily due to differences in the conduction of Na ions. The toxins appear to bind and alter a portion of the Na^+ channel which is a rate-determining step for Na^+ permeation that is distinct from the rate-determining step for K^+ (and presumably Rb^+, too) permeation. Unfortunately, the time resolution of the planar bilayer system does not presently allow for the direct comparison of native Na^+ channels to toxin-treated ones.

The structural differences induced in the presence of either of these toxins may be quite small and subtle, because even minute movements (<0.1 nm) in an ion-liganding region could be expected to result in profound differences in the interaction of the pore and the conducting ion (Armstrong, 1975; Hille, 1975*a*). Certainly, a comparison of the energy barrier profiles of Na and K ions presented here suggest that a small change in the energy involved in conduction (less than that of a hydrogen bond) can lead to a distinct outcome. An analogous example is that of hemoglobin, where the difference in its ability to bind and release oxygen is the result of a shift of ~0.1 nm in the protein structure. A shift of such a magnitude, due to the binding of a steroidal or alkaloid toxin, could certainly result in major changes in the activation and ion selection processes of the Na^+ channel. Such a result would not be surprising in a protein as large and complex as the Na^+ channel (Noda et al., 1985).

Single Ion Occupancy as First Approximation

The first well-developed model of ion permeation through the Na^+ channel (Hille, 1971, 1972, 1975*a* and *b*) has also been successfully applied, with some modifications, to describe Ca^{2+} block of both native and BTX-modified Na^+ channels (Yamamoto et al., 1984; Worley et al., 1988). The results presented here show that such a simple, albeit crude, single ion occupancy model may approximate the way that Na, K, and, presumably, Rb ions permeate and conduct through toxin-modified Na^+ channels. In fact, the rectifying I/V relationship in symmetrical K^+ solutions, the biionic reversal potential measurements and the block of Na current by K ions described here fit the general predictions made by Worley and colleagues (1988) on BTX-modified Na^+ channels from rat brain. The differences in the energy barrier profiles appear to be primarily due to the greater single channel conductances and lower Na ion affinity described for Na^+ channels from brain (Krueger et al., 1983; Andersen et al., 1986).

One of the expectations of this simple model is a saturation of the single channel conductance according to a simple Langmuir relationship, with increasing Na ion concentration. This was observed by Garber and Miller (1987) in BTX- and VT-modified Na^+ channels reconstituted into planar bilayers from rat muscle. If multiple ion occupancy is a significant factor in ion conductance, then the single channel conductance may be expected to reach a plateau before decreasing at very high concentrations (Hille and Schwartz, 1978). This has been observed for K^+ channels from squid axon (Wagoner and Oxford, 1987). The single channel conductance measured for toxin-modified Na^+ channels up to ~500 nM does not deviate appreciably from a simple Langmuir. At very high concentrations (>1 M Na) the single channel conductance exhibits a gradual increase (Andersen et al., 1986; Moczydlowski et al., 1984). This may be an indication that the channel can be multiply-occupied at very high, unphysiological ionic strengths (see: Hille and Schwartz, 1978; Miller and Garber, 1988).

Another expectation of an ion channel that can be occupied by only a single ion at a time is concentration-independent permeability ratios. The results presented here show that on the single channel level, permeability ratios for Na^+/K^+ and Na^+/Li^+ of toxin-modified Na^+ channels are not concentration-dependent. Na^+/K^+ permeability ratios are instead dependent on the relative permeability of the electrolytes in solutions facing the intra- and extracellular sides of the Na^+ channel. (Orientation dependence of the Na^+/Li^+ permeability ratio is difficult to discern only because it hovers around 0 mV.) Furthermore these results may be explained by barrier profiles among which the profile of one of the permeant ions is asymmetric. This resolution is similar to the one obtained by Busath and Begenisich (1982) when they showed that the concentration-dependent reversal potentials measured in squid axons by Begenisich and Cahalan (1980*a* and *b*) could be explained using a single rather than multiple ion occupancy model. Orientation-dependent reversal potentials have also been observed for K^+ currents in squid axon and in node of Ranvier (Wagoner and Oxford, 1987; Plant, 1987).

How Good an Approximation?

The single ion occupancy model can provide a good approximation of the conduction and permeation of Na^+ and K^+ ions under simple ionic conditions. Deviations from the observed data and theoretical predictions, however, became substantial when both Na and K ions were in competition for occupancy of the pore. For both BTX- and VT-modified Na^+ channels, the observed K_I[app] was higher than the predicted value, indicating that more K ions than expected for independently acting ions are needed to drive a Na ion out of the pore. These deviations suggest that electrostatic interactions may be involved in "trapping" Na ions within the pore, preventing K ion occupancy.

Garber and Miller (1987) have suggested that the rate-determining step of K ion conduction is distinct from that for Na ions. These differences in the rate determining steps for these ions appear to result from the dramatic differences in their energy barrier profiles, presented here for both Na and K ions. This is reinforced by the asymmetry of the biionic reversal potentials. The asymmetric reversal potentials of Rb^+/Na^+ suggest that the rate-determining step for Rb^+ may be similar to that of K^+.

That the movement of such closely related ions as Na^+ and K^+ may be restricted at different sites within a channel pore is an unexpected outcome. The studies of permeation in K^+ channels by Wagoner and Oxford (1987) and Plant (1987) suggests that this may not be an unusual phenomena. In addition, studies of the L-type Ca^{2+} channel from guinea pig heart have shown that the permeation, and thus the rate-determining steps, of mono- and divalent cations is distinct (Hess et al., 1987; Lansman et al., 1987). Several rate-determining steps have also been suggested to be involved in permeation of different cations through the gramicidin channel (Eisenman et al., 1978).

Unlike that of Na and K ions, the permeation and conductance of Li ions cannot be easily accounted for with the simple model presented here. An energy barrier profile, developed under the assumptions of this simple model, could not be found which fits both the I-V relationship measured under symmetrical Li^+ conditions and the Na^+/Li^+ permeation ratio of 0 mV. The apparently anomalous permeation of Li ions provides one example at which simple permeation models become inappropriate.

Discrepancies between experimentally and theoretically derived peredictions presented here could have been reduced somewhat by manipulating barrier profiles for individual ionic compositions. Such manipulation, however, to account for specific ionic conditions would have defied the goal of a general description of ion selectivity in toxin-modified Na^+ channels. Further levels of complexity, such as the fluctuations of energy barriers or multiple occupancy under certain ionic conditions, may be needed to attain this goal (Lauger et al., 1980; Eisenman and Horn, 1983). Whereas the concentration-independence of Na^+/Li^+ permeation ratios suggest that only a single ion may occupy the pore at any one time, the possibility of multiple ion occupancy can be tested by looking for anomalous mole fractions effects (Neher, 1975; Eisenman and Horn, 1983). Preliminary results with K ions, however, did not reveal any mole fraction anomalies (Garber, S. S., unpublished observations).

The available data concerning Li ions, however, suggests that the rate-determining steps in conduction are similar to those of Na ions. Both I-V relationships are linear and the single channel conductances saturate with increasing concentration. Measurements of the biionic reversal potential also suggest that the two ions enter the voltage drop across the pore with equivalent ease and suggest that only a single ion enters the pore at any one time. The most apparent distinction between the ions is the lower single channel conductance in symmetrical Li^+ solutions and the lower affinity of Li ions within the channel. Whereas the experiments described here have considered the relative abilities of Li and Na ions to enter and exit the pore, it would be of interest to determine the ability of Li ions to compete with Na ions for occupancy of the channel pore.

The simple barrier and well model presented in this report does allow the development of general characteristics of cation permeation through the pore of a toxin-modified Na^+ channel, despite the observed deviations. The difference in the single channel Na^+ conduction between BTX- and VT-modified channels can be ascribed to an increase in the peak heights, not the well depths, of the permeation profiles. The discrimination of Na and K ions occurs as a result of distinct rate-determining barriers within the lumen of the channel, due in part to the asymmetry of K ion conduction. The permeation of Li ions, however, remains anomalous.

It is appealing to imagine that there are physical structures that correspond to rate-determining barriers of conduction and permeation for each of the alkali cations. It will remain difficult, however, to attach physical significance to a theoretical model until detailed structural information of the channel is known. It is not yet possible to relate the functional asymmetry of K ion permeation with a physical structure within the channel lumen. Until then, little information seems to be gained by using increasingly complex permeation models.

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