Ionic Selectivity, Saturation, and Block in a K^+-selective Channel from Sarcoplasmic Reticulum

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ABSTRACT The open-channel conductance properties of a voltage-gated channel from sarcoplasmic reticulum were studied in planar phospholipid membranes. The channel is ideally selective for K^+ over Cl^- and for K^+ over Ca^{++}. In symmetrical 1 M solutions, the single-channel conductance (in pmho) falls in the order: K^+ (214) > NH_4^+ (157) > Rb^+ (125) > Na^+ (72) > Li^+ (8.1) > Cs^+ (<3). In neutral bilayers, the channel conductance saturates with ion activity according to a rectangular hyperbolic relation, with half-saturation activities of 54 mM for K^+ and 34 mM for Na^+. Under symmetrical salt conditions, the K^+:Na^+ channel conductance ratio increases with salt activity, but the permeability ratio, measured by single-channel bi-ionic potentials, is constant between 20 mM and 2.5 M salt; the permeability ratio is equal to the conductance ratio in the limit of low-salt concentration. The channel conductance varies <5% in the voltage range −100 to +70 mV. The maximum conductance of K^+ and Na^+ is only weakly temperature dependent ($\Delta H\ddagger$ = 4.6 and 5.3 kcal/mol, respectively), but that of Li^+ varies strongly with temperature ($\Delta H\ddagger$ = 13 kcal/mol). The channel's K^+ conductance is blocked asymmetrically by Cs^+, and this block is competitive with K^+. The results are consistent with an Eyring-type model of the conduction process in which the ion must traverse three kinetic barriers as it permeates the channel. The data conform to Läuger's (1973. *Biochim. Biophys. Acta.* **311**:423–441) predictions for a "pure" single-ion channel.

A description of channel-mediated ion conductance requires the study of two separate processes: the formation of the conducting channel (the gating process) and the movement of current-carrying ions through the open channel (the conduction process). In the previous paper (Labarca et al., 1980) we described the gating properties of a K^+-selective channel from sarcoplasmic reticulum (SR) of mammalian skeletal muscle. We now wish to describe this channel's conduction process. Earlier work (Miller, 1978) showed that SR vesicles can be made to donate ion-conducting channels to a planar phospholipid bilayer membrane. These channels display selectivity for monovalent cations, but this selectivity was not quantified previously, and no attempt was made to develop a picture of the conduction process. We now intend to do

this. In particular, we are interested in finding a minimal model to explain several aspects of the conduction: the channel's ionic selectivity, the dependence of its conductance upon ion activity and voltage, and the blocking effect of Cs^+ ion. We will show that the results can be understood in terms of an Eyring-type single-ion channel mechanism.

MATERIALS AND METHODS

The preparation of lipids and SR vesicles has been described (Labarca et al., 1980), as has the basic planar bilayer setup. Most of these experiments are concerned with measuring current through single channels under a variety of conditions. This was done by allowing only a single SR vesicle to fuse with the planar bilayer in the presence of Ca^{++}, and then removing both the Ca^{++} and SR vesicles by extensive perfusion of the *cis* chamber with fresh buffer solution containing 0.1 mM EDTA. In experiments requiring an ion gradient across the membrane, the fresh solution had a composition different from the original. The unitary fluctuations in current due to the random opening and closing of individual channels were then recorded and analyzed by hand.

In most experiments, the composition of the planar bilayer was 95% phosphatidylethanolamine (PE)–5% phosphatidylcholine (PC) purified from egg yolk (Labarca et al., 1980). This lipid mixture was used for two reasons. First, to study the channel conductance at various ionic strengths, it is necessary to work with neutral membranes to minimize changes in surface potential. Though it has been shown that a negatively charged bilayer is required for massive fusion of SR vesicles (Miller and Racker, 1976), it is still possible to observe an occasional fusion event in neutral bilayers containing a high concentration of PE. Second, the channel fluctuations in egg lipids are much slower than those in the muscle or soy lipids used in previous work (Labarca et al., 1980); this facilitates the recording and accurate analysis of the channel fluctuations. The channel's gating characteristics in these lipids were similar to those described in lipids used previously, although the gating kinetics were slower in egg lipids (Labarca et al., 1980). Several experiments used charged lipids such as diphosphatidylglycerol (DPG) and soy asolectin as described previously (Labarca et al., 1980).

In some experiments, it was necessary to vary the temperature of the bilayer system. This was usually done by allowing the channels to be inserted at 25°–30°C, and then taking initial recordings of the fluctuations. Ice was then added to the bath feeding the water circulator, so that the temperature of the bilayer system dropped to about 10°C during the next 10 min. Bilayer temperature and channel fluctuations were recorded continuously so that the change in channel conductance with temperature could be determined.

It was often necessary to determine the zero-current ("crossover") potential of the channel under asymmetric ionic conditions. This was accomplished either by the macroscopic "tail-current" method or by the microscopic single-channel method. In the tail-current technique, channels were opened by applying a positive voltage until steady-state conductance was reached; the voltage was then shifted to various negative values at which the channels close, and the current relaxations were recorded. The voltage at which no time-dependent current is seen is the crossover potential of the channel. Alternatively, the crossover potential could be measured by determining the single-channel current-voltage relation directly and by interpolating to find the zero-current voltage. Potentials determined by these two methods agreed within 1 mV.

In experiments using bi-ionic conditions, i.e., with cations X^+ on the *trans* side of

the membrane and Y^+ on the *cis* side, at activities a_X and a_Y, respectively, the "permeability ratio," P_Y/P_X, is defined in terms of the crossover potential, *Vo*, as:

$$P_Y/P_X = (a_X/a_Y) \exp(-FVo/RT). \quad (1)$$

In all single-channel experiments used for quantitative work, the aqueous phase was composed of the Cl^- or $SO_4^=$ salts of various cations. Activities, when reported, were calculated from standard tables. The solutions also contained 1 mM morpholinopropane sulfonic acid (MOPS) and 0.1 mM EDTA and were adjusted to pH 7.0 with Tris base. Measurements were carried out at 20°C unless indicated otherwise.

RESULTS

Ionic Selectivity

Because many of the experiments to be reported here were performed in media containing Cl^-, and since it is known that SR vesicles do induce a Cl^- conductance in planar bilayers (Miller, 1978), it is desirable to know if any Cl^- current is carried by the channel under study. That this is not the case is suggested by the fact that the Cl^- conductance induced by SR does not display voltage dependence, inhibition at pH 5, sensitivity to sulfhydryl ligands, or modulation by proteolytic treatments (Miller, 1978; Miller and Rosenberg, 1979 *a* and *b*). To confirm this point directly, we measured channel crossover potentials in the presence of a KCl gradient across the bilayer, using the method of tail currents. This method eliminates the contribution to the total conductance of the background conductance in the system because the voltage- and time-dependent part of the conductance is mediated exclusively by the channel (Labarca et al., 1980). Fig. 1 shows the result of such an experiment in which a two-fold KCl activity gradient is imposed across the bilayer. The channels are opened by a 2-s pulse to +50 mV, and are then closed by pulses to various negative potentials. The crossover potential of the time-dependent part of the current is the zero-current voltage of the channel and is within 0.5 mV of −17.6 mV, the Nernst potential of K^+ in this experiment. In other words, the channel is ideally selective for K^+ over Cl^-; we have also observed this Nernstian behavior for K^+ over $SO_4^=$, NO_3^-, and glucuronate$^-$ (data not shown).

The question of the Ca^{++} permeability of the channel is an important one inasmuch as the SR functions as the major Ca^{++} regulatory system in skeletal muscle. Previous work (Miller, 1978) showed that Ca^{++} appears to block the K^+ conductance of the channel from the *cis* side of the bilayer, but not from the *trans* side. Therefore, to measure Ca^{++} permeability, it is necessary to set up an ion gradient with Ca^{++} at a high concentration only on the *trans* side. We have found that if Ca^{++} is the only small cation in the system, we fail to observe any channel activity, i.e., voltage-dependent conductance or channel fluctuations (data not shown). In an alternative attempt to observe Ca^{++} permeability, we studied the effect of Ca^{++} on the zero-current potential in the presence of K^+ on both sides of the membrane at various concentrations (Table I). With ~50 mM Ca^{++} on the *trans* side, the zero-current potentials of the channel are not different from those determined under identical ionic

conditions in the presence of valinomycin. It is necessary to carry out the experiment in this manner so that uncertainties due to Ca^{++} effects on K^+ activity coefficients and liquid junction potentials are eliminated. We can conclude from these results that Ca^{++} is not permeable to the channel, within experimental error. An upper limit on the ratio of Ca^{++} to K^+ permeability (using the Nernst-Planck electrodiffusion model) is 0.05.

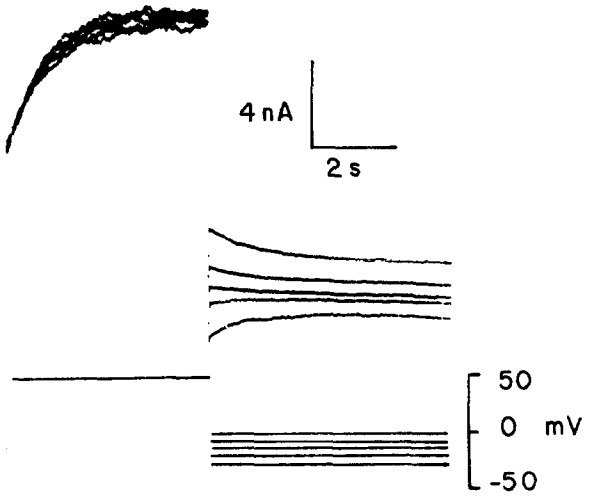


FIGURE 1. K^+ over Cl^- selectivity measured by tail currents. Massive insertion of SR vesicles was induced by the addition of SR (25 μg/ml) to the *cis* chamber in the presence of 1 mM $CaCl_2$, as described in Materials and Methods. The aqueous phase was 0.1 M KCl buffer, and the bilayer was composed of 70% washed asolectin–30% DPG. After the membrane conductance had reached 10 μmho/cm^2, fusion was stopped by the addition of 1.2 mM EDTA and perfusion of the *cis* chamber with fresh buffer. An aliquot of 3 M KCl was then added to the *cis* chamber to a final concentration of ~220 mM KCl. From a holding voltage of −40 mV, the pulse sequences shown in the lower part of the figure were applied and the current response (upper portion of figure) followed. After the recordings were made, aliquots of the *cis* and *trans* solutions were removed with the membrane in place for subsequent determination of K^+ concentration (using a K^+-specific membrane electrode). K^+ activities were calculated from standard tables. Crossover potential was determined by interpolation of the relaxation amplitudes; in this experiment it was −18 mV; the activity ratio was 2.03.

We would now like to learn about the channel's selectivity toward small monovalent cations. Fig. 2 shows single-channel conductance fluctuations in symmetrical solutions of 100 mM K^+, Na^+, Rb^+, Li^+, and Cs^+. Except for Cs^+, the fluctuations occur among well-defined unitary conductance levels, as previously demonstrated for K^+ current (Miller, 1978; Labarca et al., 1980). Under these conditions, the single-channel conductance is highest for K^+ (142 pmho) and lowest for Li^+ (5.4 pmho); Cs^+, which has been shown to block the

TABLE I

LACK OF Ca^{++} PERMEABILITY THROUGH SR CHANNEL

cis solution		*trans* solution		Zero-current potential	
$[K^+]$	$[Ca^{++}]$	$[K^+]$	$[Ca^{++}]$	Channel	Valinomycin
mM		*mM*		*mV*	
100	0.1	100	27	0 ± 0.5	0 ± 1
141	0.8	100	54	−7 ± 1	−6 ± 1
203	0.1	100	54	−15.3 ± 0.3	−16 ± 2
200	$<10^{-4}$	25	54	−45 ± 3	−41 ± 2

Asymmetric ionic conditions were set up across 95% PE–5% PC bilayers as indicated, and zero-current potentials were measured by either the tail-current or the single-channel method. These were compared with zero-current potentials with 1 μM valinomycin present, under identical ionic conditions; in some experiments, valinomycin was added to the same membrane used for determination of single-channel crossover potential. Each measurement represents the average of three to four determinations. We were unable to obtain any channel activity in membranes employing purely bi-ionic conditions; this is the reason for the presence of K^+ on both sides of the bilayer.

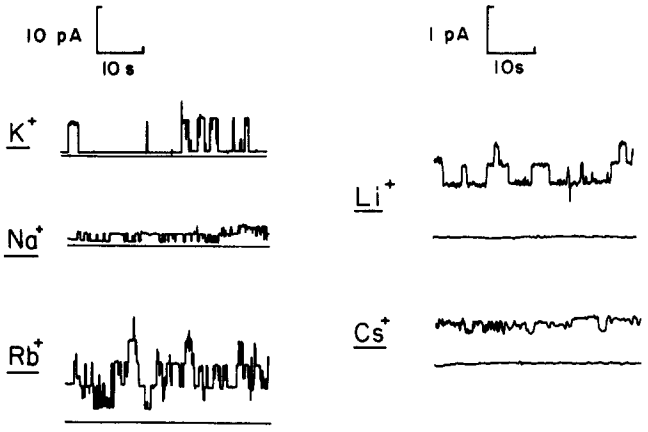


FIGURE 2. Single-channel conductance fluctuations with various cations. Channel fluctuations were recorded in bilayers of 90% egg PE–10% egg PA, with symmetrical solutions containing 100 mM concentrations of the cations indicated (sulfate salts). Each trace was taken from a separate bilayer. Channel conductances determined from compiled data were (in pmho): K^+, 142 ± 2; NH_4^+, 102 ± 1; Rb^+, 101 ± 1; Na^+, 41 ± 1; Li^+, 5.4 ± 0.2; Cs^+, <3 (mean ± SEM of 10–20 determinations). Holding voltage was −50 mV for all traces.

channel (Coronado and Miller, 1979), does not convincingly display discrete conductance fluctuations. Fig. 3 summarizes the selectivity of the channel conductance measured in neutral membranes, using 1 M salt solutions; we see that the conductance for K^+ is about 3 times that for Na^+ and about 30 times that for Li^+. Again, no fluctuations are observed in 1 M Cs^+ solutions.

Conductance-Activity Relation

In symmetrical solutions of permeant cations, the channel conductance reaches a maximum value as ion activity is increased. The conductance follows a simple saturation curve of the form shown for Na^+ and K^+ in Fig. 4:

$$\gamma_i = \gamma_i^m a_i / K_i / (1 + a_i / K_i), \quad (2)$$

where a_i is the activity, K_i the apparent dissociation constant, and γ_i^m the maximum conductance of ion i. Eadie-Hofstee plots of these data (not shown) are linear down to 20 mM, the lowest concentration used in these experiments. The K^+ conductance is half saturated at 54 mM, and the Na^+ conductance at 34 mM. This relatively high affinity saturation allows us to investigate the

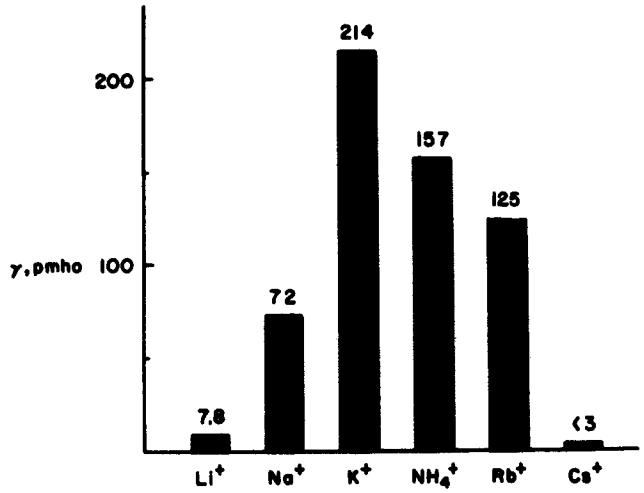


FIGURE 3. Maximum single-channel conductance in neutral membranes. Channel conductances were determined as in Fig. 2, except that the solutions contained 1 M concentrations of the chloride salts of the various cations, and the bilayers were composed of 95% PE–5% PC. The use of 1 M salt ensures that the measured conductances are within 15% of the maximum channel conductance (see Fig. 4).

channel at ion activities up to 50 times the half-saturation activity. We see that even at very high salt activity, there is no indication of a decrease in conductance, as there is, for instance, with the gramicidin channel (Hladky and Haydon, 1972; Eisenman et al., 1978). The significance of this result is that the channel behaves as though it can be occupied by no more than one ion at a time. We should emphasize that these measurements were made in neutral bilayers, so that changes in surface potential with ionic strength would not occur.

Selectivity Determined by Conductance or Permeability

A comparison of the channel conductances for two ions is only one assay of a channel's selectivity. An alternative measurement is the permeability ratio

determined under bi-ionic conditions, e.g., with K^+ on one side of the bilayer and Na^+ at the same concentration on the other side (Fig. 5). When we measured both the K^+/Na^+ conductance ratio and the permeability ratio, $P_K/$

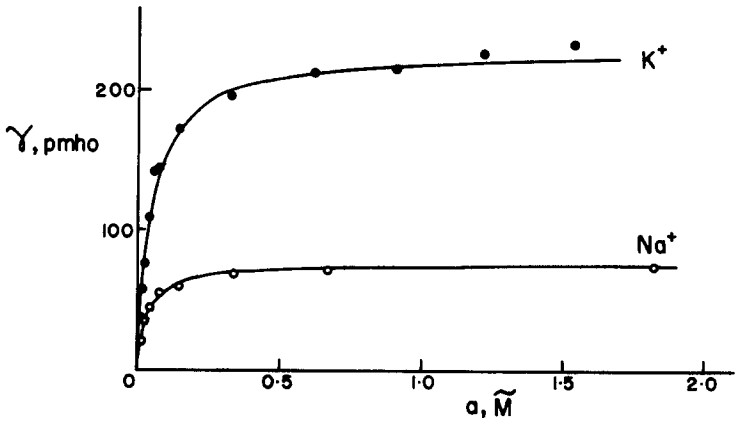


FIGURE 4. Conductance-activity relation in neutral membranes. Channel conductances were measured as in Fig. 3, with either NaCl or KCl buffers of various salt concentrations. Each point represents the mean ± SEM of 20–100 determinations; the width of the points is larger than the SEM. Salt activity was calculated from Robinson and Stokes (1955), and curves were drawn according to Eq. 1. The apparent dissociation constants and maximum conductances are given in Table II.

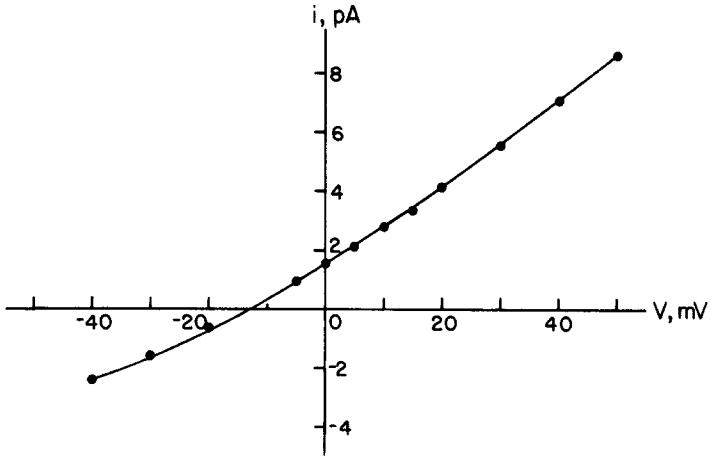


FIGURE 5. Current-voltage curve under bi-ionic conditions. The current-voltage curve for the open channel was measured with 0.5 M salts of KCl and NaCl on the *cis* and *trans* sides of the bilayer, respectively, under the conditions of Fig. 4. Fusion of a single SR vesicle was allowed to occur in symmetrical NaCl buffer, and immediately after fusion the *cis* chamber was extensively perfused with KCl buffer to set up the bi-ionic conditions and to remove vesicles. Each point represents at least five determinations. In this experiment, the crossover potential was −13 mV, which corresponds to $P_K/P_{Na} = 1.8$, when activity coefficient differences are considered.

P_{Na}, as a function of salt activity, we found that these two indicators of selectivity behave differently (Fig. 6). The conductance ratio varies from a value of 2.0 at low concentration to about 3.0 at high concentration; the reason for this variation is that apparent dissociation constants for K^+ and Na^+ differ by a factor of 1.5 (Fig. 4). The striking result shown in Fig. 6 is that the permeability ratio is constant over the entire range of salt concentration and that its value is numerically equal to the conductance ratio at low salt, i.e., 2.0. This behavior is expected of a channel that can accommodate at most one ion at a time (see below).

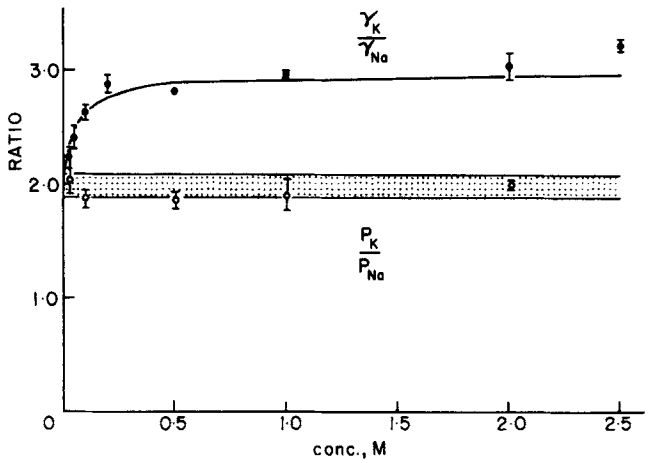


FIGURE 6. Concentration dependence of channel selectivity. The channel selectivity was studied as a function of salt concentration with two different types of measurements. The channel conductance for K^+ or Na^+ under symmetrical conditions was measured as in Fig. 4, and the ratios were calculated at the same concentrations (filled circles). Alternatively, the permeability ratio was measured under bi-ionic conditions as in Fig. 5 (open circles). Each permeability ratio represents the mean ± SEM of three determinations, each on a different membrane. The continuous upper curve is drawn as in Fig. 4, with the ratio of K^+ and Na^+ conductances and with correction for activity coefficient differences at equal concentrations of NaCl and KCl. The stippled region represents the range of permeability ratio predicted by a single-ion model, given the errors of the parameters describing the conductance-activity relation (Table II).

In Table II the selectivity parameters of a series of monovalent cations are compared. We report the ionic selectivity of three separate measurable quantities: the maximum conductance, the ion-channel dissociation constant (half-saturation activity), and the permeability. As in Fig. 6, we see that the maximum conductances of the different ions are not strictly proportional to the permeability ratios; the maximum conductance of NH_4^+, for example, is lower than that of K^+, and the NH_4^+/K^+ permeability ratio is greater than unity. We should further notice that the permeability and binding sequences are roughly opposite; Li^+ displays high affinity and low permeability, whereas

the situation is reversed for K^+. Finally, it is important to realize that for Li^+, Na^+, K^+, and Rb^+, the quantity $(\gamma_K^m/\gamma_X^m)(K_X/K_K)(P_X/P_K)$ is equal to unity within experimental error. As we shall discuss below, this condition is required of all single-ion channels. The condition does not hold for NH_4^+. We have not included results with Cs^+ in Table II because this ion acts as a strong blocker of the channel (see below).

Current-Voltage Relation of the Open Channel

Information about channel conductance mechanisms may be obtained by studying the current-voltage relation of the open channel. This relation is not expected to be linear; depending on the free energy profile of the ion moving through the channel, the curve may be either sublinear or hyperlinear. For simple models of the conduction mechanism, departures from linearity are typically in the range of 5 to 20% between zero voltage and 100 mV (Läuger, 1973). Fig. 7 shows that the channel conductance measured at saturating ion concentration is nearly independent of voltage, i.e., the current-voltage relation is only slightly hyperlinear in the range of −100 to +70 mV. This result allows us to place a lower limit on the number of energy barriers that must be crossed by the conducting ion (see below).

TABLE II
CHANNEL SELECTIVITY PARAMETERS

Ion (X)	γ^m	K	P_X/P_K	$(\gamma_K^m/\gamma_X^m)\ (K_X/K_K)\ (P_X/P_K)$
	pmho	*mM*		
Li^+	7.7 ± 0.3	19 ± 4	0.13 ± 0.01	1.4 ± 0.5
Na^+	77 ± 1	34 ± 1	0.51 ± 0.01	1.0 ± 0.1
K^+	240 ± 3	54 ± 2	(1)	(1)
Rb^+	135 ± 5	46 ± 4	0.88 ± 0.04	1.3 ± 0.3
NH_4^+	168 ± 2	47 ± 2	1.27 ± 0.05	1.6 ± 0.2

Single-channel conductance was measured in neutral membranes, using symmetrical solutions of the chloride salts of the indicated cations under the conditions of Fig. 4, to obtain the maximum channel conductance, γ^m, and the apparent dissociation constant, K. Permeability ratio was determined with K^+ always on the *cis* side of the membrane under bi-ionic conditions, as in Fig. 5. We checked to be sure that permeability ratios were independent of salt concentration in the range 0.1–0.5 M, and most measurements were made with 0.2 M salt. γ^m and K experimental errors were estimated by "rocking" the line of a double-reciprocal plot through the points by eye. Permeability ratio errors represent the SEM of three to five determinations on separate bilayers. Conductance was measured between −50 and + 50 mV to approximate the zero-voltage conductance.

Effect of Temperature

For a channel with a conductance as high as that studied here, the energy barriers over which an ion moves in its journey across the membrane must be quite low, and one might expect that the channel conductance would be only weakly affected by temperature. Fig. 8 shows that this is the case for K^+; the

apparent activation enthalpy is 4.6 kcal/mol, not very far from its value for conductivity in aqueous solution (Robinson and Stokes, 1955). This is also true of Na^+, whose activation enthalpy is only slightly higher: 5.3 kcal/mol. However, the Li^+ conductance is extraordinarily sensitive to temperature, with an apparent activation enthalpy of 13 kcal/mol, equivalent to $Q_{10} = 2.3$. This value is far higher than the activation enthalpy for aqueous conductivity of all the alkalai metal cations: 4.3–4.5 kcal/mol (Robinson and Stokes, 1955). It is important to realize that the measurements in Fig. 8 were made under saturating conditions, at salt concentrations at least 20 times higher than the half-saturation concentration. These determinations represent good approximations of the channel's maximum conductance for each of the three ions.

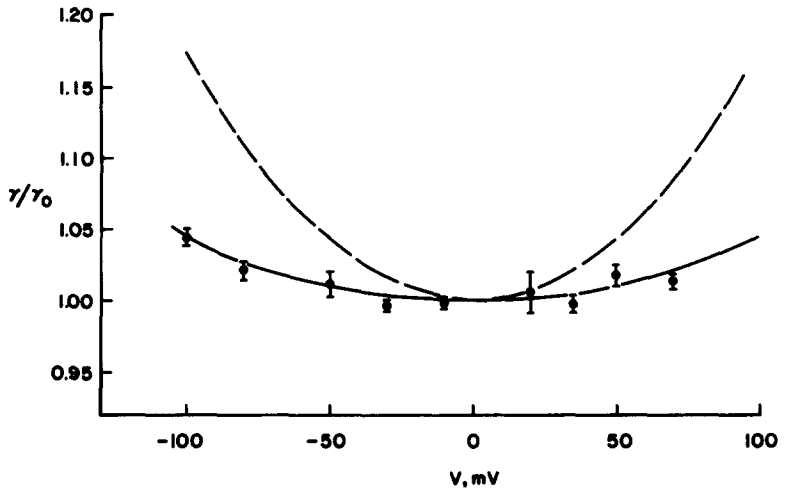


FIGURE 7. Conductance-voltage relation for K^+ conductance. Channel K^+ conductance was measured as a function of applied voltage with 300 mM KCl buffer but otherwise under the conditions of Fig. 4. The conductance γ is normalized to its value measured between −20 and +20 mV, which is taken as its zero-voltage value, γ_0, and is 176 pmho in this experiment. All data were taken from the same membrane to reduce variation as much as possible; each point represents the mean ± SEM of 5–15 channel fluctuations. The broken curve is drawn according to a symmetrical two-barrier model, Eq. 9. The continuous curve is drawn for a three-barrier model with symmetrically placed peaks 38% in from each side of the membrane and with the central peak 0.1 kcal/mol lower than the two outer peaks, following Eq. 11. (A similar curve drawn with all peaks of equal height falls slightly below the point at −100 mV.)

Cs^+ Blocking

As reported in Fig. 2, we were unable to discern any single-channel conductance fluctuations using symmetrical Cs^+ solutions, and, indeed, under these conditions the macroscopic conductance induced by massive fusion of SR vesicles is not voltage dependent.[1] We have shown previously that Cs^+ actually

[1] Coronado, R., R. L. Rosenberg, and C. Miller. Unpublished observations.

blocks the channel's K^+ conductance (Coronado and Miller, 1979), and we explained this effect on the basis of a single-site model in which Cs^+ binds to a site 38% of the way through the electric field from the *cis* side. The binding of Cs^+ to this site was assumed to prevent the permeation of K^+, as would be consistent with a single-ion model of the channel.

One important question previously left unanswered is whether Cs^+ and K^+ compete for the same site. Fig. 9 shows that the blocking effect of Cs^+, studied

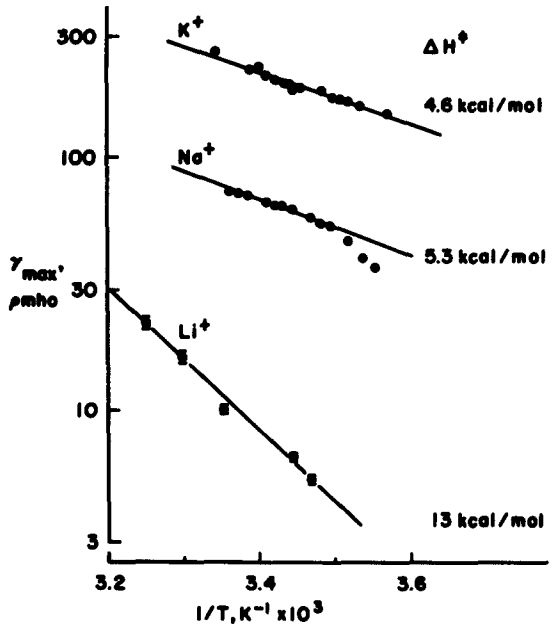
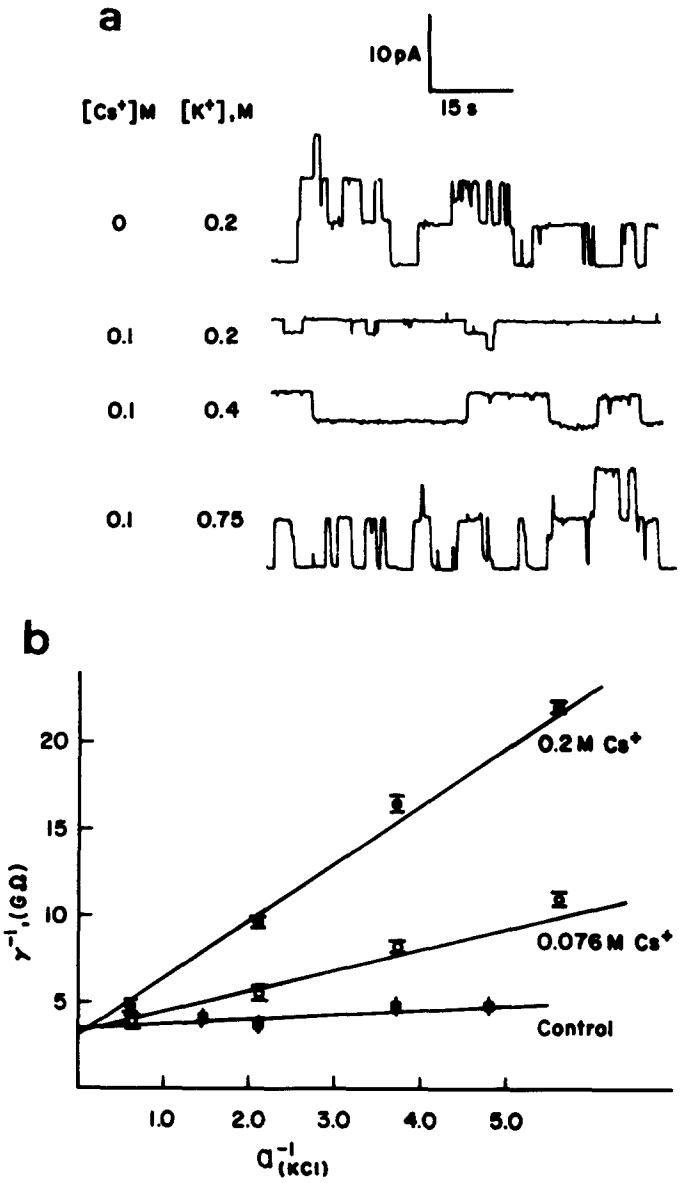


FIGURE 8. Temperature dependence of maximum channel conductance. Channel conductances were measured as a function of temperature in buffers of 0.5 M KCl, NaCl, or LiCl at an applied voltage of −50 mV. Membranes were composed of 70% PE–10% PC–20% DPG. Each point represents the mean ± SEM of 4–10 determinations; for K^+ and Na^+, the error bars are smaller than the size of the points. Lines through the points were drawn by eye, and the apparent activation enthalpy, $\Delta H\ddagger$, is indicated for each ion. The departure of the Na^+ conductance from a linear relation below 12°C was reproducibly observed, and these points were deliberately ignored for the determination of $\Delta H\ddagger$.

now in neutral bilayers, is indeed competitive with K^+. Fig. 9 *a* displays channel fluctuations at a fixed Cs^+ concentration (0.1 M) as K^+ concentration is varied. In the presence of both Cs^+ and K^+, the channel conductance is smaller than with K^+ alone. This effect has been explained by assuming that the measured channel conductance is a time-averaged value established by the rapid binding and dissociation of Cs^+ from the blocking site (Coronado and Miller, 1979). Increasing the K^+ concentration in the range 0.2 to 1.0 M

removes the Cs^+ block and gradually increases the channel conductance; note that in this range of K^+ concentration without Cs^+ present, the conductance is virtually independent of K^+ concentration because the channel is nearly saturated above 200 mM K^+ (Fig. 4). In Figure 9 *b*, the results of variations of K^+ and Cs^+ on channel conductance are summarized. The figure shows in double-reciprocal form the channel conductance against K^+ activity at two Cs^+ activities. The maximum channel conductance is independent of Cs^+, but



the apparent dissociation constant of K^+ varies with Cs^+, as would be expected for a single-site competitive blocking scheme. The "true" dissociation constant for Cs^+, determined by extrapolating the apparent constants to zero K^+, is 15 mM at +30 mV.

DISCUSSION

The results of this study show that the voltage-gated channel from sarcoplasmic reticulum is absolutely specific for small monovalent cations, with K^+ being the most conductive. Neither anions nor divalent cations such as Ca^{++} permeate the channel. This lack of Ca^{++} permeability, taken together with the previous finding that divalent cations produce a severe block of the K^+ conductance (Miller, 1978), argues against identifying this channel as the Ca^{++} release mechanism of the SR. The channel conductance saturates in a simple fashion as the activity of permeant ion is increased, and the current-voltage relation is nearly linear at both high and low concentrations of permeating ion. Cs^+ blocks the channel asymmetrically in a voltage-dependent manner (Coronado and Miller, 1979), and this block is relieved by increasing K^+ concentration.

In searching for a model of the conduction process, we must realize at once that the phenomena of saturation and block and the quantitative discrepancy between the selectivity measured by conductance and by zero-current potential are direct demonstrations that the "independence principle" (Hodgkin and Huxley, 1952) does not hold here. The behavior of a given permeating ion depends upon the channel's occupancy by other ions.

In spite of the failure of the independence principle to explain the results, they are understandable in terms of a remarkably simple conduction model. A recent and fruitful approach to channel-mediated ion transport has been to represent the process as the movement of an ion over a sequence of kinetic barriers and to apply Eyring rate theory to derive the fluxes (see Hille [1975 *b*] for a lucid exposition). In this approach, the "reaction coordinate" is a

FIGURE 9. (*opposite*) Competitive block of K^+ conductance by Cs^+. Channels were incorporated into 95% PE–5% PC bilayers as in Fig. 4, and channel conductance was measured at +30 mV with various concentrations of KCl and CsCl, always under symmetrical conditions. Temperature was 25°C. (*a*) Channel fluctuations at constant Cs^+ and increasing K^+ concentrations, as indicated on the figure. (*b*) Data obtained as in *a* at two Cs^+ concentrations (indicated on graph) and plotted in double-reciprocal form against K^+ activity. The control had no Cs^+ present. Each point (mean ± SEM of 8–15 determinations) was obtained from a separate membrane. That the Cs^+ block is competitively relieved by K^+ was confirmed by calculating the "true" dissociation constant for Cs^+ from each of the two sets of data from determinations in which Cs^+ was present. The slope of the line for a competitive scheme is given by slope $= \left(1 + \frac{a_{Cs}}{K_{Cs}}\right)\left(\frac{K_K}{\gamma^m}\right)$, where the parameters are as described in the text. Using the value of K_K/γ^m from the control curve with no Cs^+, we obtain K_{Cs} = 14 ± 3 mM and 16 ± 2 mM at 0.2 M and 0.076 M Cs^+, respectively. In these experiments, γ_m = 275 ± 5 pmho, and K_K = 60 ± 3 mM.

distance in space (more properly an electrical distance representing the movement of the ion down the electric field within the membrane). Such a model involving three barriers and two wells is shown in Fig. 10. The rate constants over the barriers are voltage dependent since the permeating ion's energy will depend on the distance the ion has fallen through the electric field. This voltage dependence may be written explicitly (Eyring et al., 1949; Läuger, 1973; Hille, 1975 *a* and *b*).

To explain the results here, we need only to make the additional assumption that the channel can be occupied by at most one ion at a time. Läuger (1973) has treated the general case of a single-ion channel with an arbitrary number of barriers and wells of arbitrary heights and depths located at arbitrary positions. He showed that for such a case, the channel conductance saturates with ion activity according to Eq. 2. Let us consider such a channel with n barriers and $(n-1)$ wells, with the ith peak and well free energies p_i and w_i, respectively, taking the external solution as the zero of energy (1 M standard state). Then, Läuger's treatment leads to particularly simple expressions for the dissociation constant, K_X, and maximum channel conductance, γ_X^m, measured at zero voltage, for ion X:

$$K_X \propto 1 \Big/ \sum_{i=1}^{n-1} \exp(-w_i/RT) \tag{3}$$

$$\gamma_X^m \propto \sum_{i=1}^{n} \exp(-p_i/RT) \Big/ \sum_{i=1}^{n-1} \exp(-w_i/RT). \tag{4}$$

Let us further accept Hille's "peak energy offset condition," i.e., that all the peak heights (relative to the outside solution) for a given ion differ from those of a second ion species by exactly the same free energy, (Hille, 1975 *a* and *b*):

$$\Delta p = p_i^Y - p_i^X, \tag{5}$$

where the subscript indicates the peak number and the superscripts indicate the ionic species. Using this condition as applied to Läuger's approach, it can be easily shown that the permeability ratio measured under bi-ionic conditions is independent of ion concentration and is given by:

$$P_Y/P_X = \exp(-\Delta p/RT). \tag{6}$$

This result has been illustrated for particularly simple cases by Hille (1975 *b*) and Armstrong (1975). The implication of Eq. 6 is a profound one: that for any single-ion channel satisfying the peak-energy offset condition, the selectivity measured by zero-current potential depends only on the heights of the energy peaks relative to the outside solution and is totally independent of the depths of the "binding sites," i.e., the energy wells. Likewise, the measured dissociation constant depends only on the well energies and is independent of peak heights, according to Eq. 3, whereas the maximum channel conductance depends upon both the peak and well energies, as in Eq. 4. (Läuger [1973] showed that the bi-ionic potential for a single-ion channel depends only on peak heights, even without assuming the peak energy offset condition; but in this general case, Eq. 6 obviously does not hold.)

We are now in a position to derive a general condition that must hold for all single-ion channels satisfying Eq. 5. We call this the "conductance-affinity-permeability" condition, and it follows immediately from Eqs. 3, 4, and 6, as applied to two ions, X and Y:

$$(\gamma_Y^m/\gamma_X^m)\cdot(K_X/K_Y)\cdot(P_X/P_Y) = 1, \tag{7}$$

or stated in terms of the measurable selectivity ratios: (maximum conductance ratio)·(binding affinity ratio)/(permeability ratio) = 1. It further follows from Eqs. 2 and 7 that the permeability ratio (which is concentration independent) must be equal to the conductance ratio measured in the limit of zero ion concentration, i.e., that:

$$P_X/P_Y = \gamma_X^m K_Y/\gamma_Y^m K_X = \lim_{c\to 0}(\gamma_X/\gamma_Y). \tag{8}$$

Given these results of previous theoretical work, we assert that, for K^+ and Na^+ conduction at least, the SR channel operates as a single-ion channel. The three strong requirements of such a model, simple saturation, concentration-independent permeability ratio, and the conductance-affinity-permeability condition, are all satisfied. This conclusion is probably also valid for Li^+ and Rb^+ (Table II), although we have not made extensive measurements of the concentration dependence of permeability ratios for these ions. The NH_4^+ ion clearly violates Eq. 7 (Table II). We do not know whether this is because of a violation of the single-ion condition or of the peak energy offset condition; we suggest the latter as the more likely possibility since NH_4^+ follows a simple saturation scheme very precisely (data not shown) and since this cation, unlike the alkalai metal cations, forms hydrogen bonds with a fixed geometry.

This channel is not highly selective for K^+ over Na^+, but it is instructive to analyze the existing selectivity in terms of the free energy profile of Fig. 10. The permeability ratio, 2.0, is only slightly higher than the single-ion conductivity ratio in aqueous solution, 1.5, and is equivalent to peak heights which are 0.42 kcal/mol higher for Na^+ than for K^+. Furthermore, the dissociation constants determined from the conductance-activity relation (Fig. 4) show that Na^+ binds to the channel more strongly than does K^+ by a factor of 1.5 (0.24 kcal/mol). Indeed, this tighter binding of Na^+ shows up as an enhancement of the K^+/Na^+ conductance ratio over the two-fold permeability ratio; Na^+, besides being only half as permeable as K^+, has a 50% greater thermodynamic difficulty getting out of the saturated channel, and so the ratio of maximum conductances is 3 rather than 2.

By considering each of the conducting ions in a similar manner, we notice that the channel displays two independent selectivity sequences, one for permeability and one for binding affinity. In these sequences lie clues to the chemical nature of the wells (binding sites) and the peaks (transition sites) of Fig. 10. The permeability follows Eisenman's (1961 and 1969) sequence IIIa: NH_4^+ (1.27) > K^+ (1) > Rb^+ (0.88) > Na^+ (0.51) > Li^+ (0.13). Such a selectivity pattern could be interpreted as indicating a transition state in which the ion is fleetingly associated with an anionic site of low electric field, such as a carboxylate group near electron-withdrawing residues. In contrast,

the binding affinity sequence, as measured by dissociation constants, is ordered according to a "high field strength" sequence, XI of Eisenman: Li^+ (2.8) > Na^+ (1.6) > Rb^+ (1.2) ~ NH_4^+ (1.2) ~ K^+ (1).

The question arises: how many barriers does the channel contain? We cannot answer this question, but the results do allow an estimate of the minimum number of barriers in the channel. Let us first consider a two-barrier model, with a single binding site, such as that treated by Hille (1975 *b*). We assume that each barrier is symmetrically shaped, i.e., that the peak is halfway between the wells. As will all single-ion channels, this model satisfies the basic requirements of saturation and selectivity observed here with the SR channel. However, we know that this model cannot be an adequate representation of the conduction process. The reason for our certainty on this point is that the two-barrier model necessarily predicts a strongly hyperlinear current-voltage relation for the maximum conductance. We have found that, under saturating conditions, the current-voltage relation of the SR channel is only slightly hyperlinear (<5%) in the range −100 to +70 mV (Fig. 7). It is easily shown that there is no combination of the two adjustable parameters in the equations for the two-barrier saturated current-voltage curve that will give less than a 15% departure from linearity in this voltage range. For instance, for a symmetrical channel with two barriers, the maximum channel conductance, γ^m, normalized to its zero-voltage value, γ_0^m, is given by:

$$\gamma^m/\gamma_0^m = (\sinh[\Psi/2])/(\Psi/2), \tag{9}$$

where $\Psi = FV/2RT$, and V is voltage (Läuger, 1973). The graph of Eq. 9 in Fig. 7 illustrates the impossibility of fitting a two-barrier model to our data. Therefore, we are forced to reject the two-barrier model and to consider the three-barrier model shown in Fig. 10.[2]

For a three-barrier model, similar application of Läuger's approach to a symmetrical channel gives:

$$\gamma^m/\gamma_0^m = \frac{(2 + \exp[p_c - p_o])\sinh\Psi}{\Psi\{\cosh([\delta - 1]\Psi) + \cosh([3\delta - 1]\Psi) + \exp(p_c - p_o)\cosh(2\delta\Psi)\}}, \tag{10}$$

where p_c and p_o are the peak heights of the central and outer barriers, and δ is the position of the wells. For such a model, it is easy to adjust parameters to generate very nearly linear current-voltage curves over the voltage range of the measurements. One such set is drawn in Fig. 10 for K^+ conduction. We choose to place the *cis*-facing well 38% in from the *cis* side in accordance with

[2] We offer one reservation with regard to our claim that a two-barrier model cannot predict a nearly linear current-voltage relation. We are considering here only symmetrical barriers, but if we relax this restriction, allowing the peak to be placed asymmetrically between the wells, it is possible to generate nearly linear current-voltage curves with only two barriers. However, this can be achieved only under a very restricted set of parameters (well position, barrier asymmetry factors, barrier heights). Therefore, we consider this model more, rather than less, complicated than a symmetrical three-barrier model.

the "effective valence" of the Cs^+ blocking reaction (Coronado and Miller, 1979). The *trans*-facing well is placed arbitrarily 38% of the way in from the *trans* side. With the wells and barriers as shown in the figure, the current-voltage curve is linear within 1% in the range −80 to +80 mV, and the apparent dissociation constant for K^+ is 54 mM.

Whereas a symmetrical free energy profile such as that shown in Fig. 10 is adequate to explain the behavior of the conducting ions, this is clearly not the case for Cs^+, which displays highly asymmetric effects on the channel. The Cs^+ block would be explained by entry of Cs^+ into the *cis*-facing well; Cs^+ would then be unable to move farther into the channel because of an impossibly high central barrier for this ion, perhaps due to a narrow "selectivity filter" through which the large (3 Å in diameter) dehydrated ion could not squeeze. We also postulate that Cs^+ would not enter the channel from the *trans* side because there is no blocking effect from this side of the bilayer (Coronado and Miller, 1979). We emphasize that Cs^+ is postulated to gain access only to the *cis*-facing well—not to both wells—from the *cis* side since the Cs^+ blocking constant is very precisely exponentially dependent on voltage in the range −50 to +50 mV (Coronado and Miller, 1979). If Cs^+ were able to enter both wells, the blocking constant would vary with voltage in a double-exponential fashion, as can be shown by extending the treatment of Woodhull (1973) to a two-site scheme. Finally, we note that in the present model of the SR channel, we identify the Cs^+ blocking site as the first energy well for K^+ movement through the channel, since K^+ competes with Cs^+ in the blocking reaction.

It is worthwhile to perform several speculative calculations from absolute rate theory to obtain a picture of the selectivity of the channel's maximum conductance and of the nature of the transition state of the rate-limiting step for ion permeation. We consider the model of Fig. 10, with equal peaks and wells of free energies p and w. We ask: given the values of the measured maximum single-channel conductances for the various ions, what are the calculated magnitudes of the Gibbs free energies of activation of the maximum conductance for the different ions, i.e., what are the magnitudes of the barrier heights? For a channel with n equal barriers and $(n-1)$ equal wells, it can be straightforwardly shown using Läuger's (1973) general results that the maximum channel conductance, γ^m, and the dissociation constant, K, measured at zero voltage, can be expressed in terms of the peak and well free energies:

$$\gamma^m = \frac{\epsilon^2}{n(n-1)h} \exp(-[p-w]/RT) \quad \text{and} \tag{11}$$

$$K = \frac{\exp(w/RT)}{n-1}, \tag{12}$$

where h is Planck's constant, and ϵ is the electronic charge. The coefficient ϵ^2/h has a value of $\sim 3.9 \times 10^{-5}$ mho. For the model of Fig. 10, we can calculate the peak-height and well-depth free energies for the permeant cations (Table III).

Though these values are not unreasonable for activation energies for a

diffusion process, they should not be taken as "hard" calculations, since they are based upon an idealized model of the channel and upon absolute rate theory. It is nevertheless instructive to compare the calculated free energies of activation (barrier heights, $p - w$) with the measured enthalpies of activation (Fig. 8). The measured $\Delta H\ddagger$ for K^+ and Na^+ are 4.6 and 5.3 kcal/mol, respectively, and we can say that these are consistent with the calculated barrier heights. We certainly do not expect strict equality here because the calculations are inherently uncertain; the point to be taken is that the

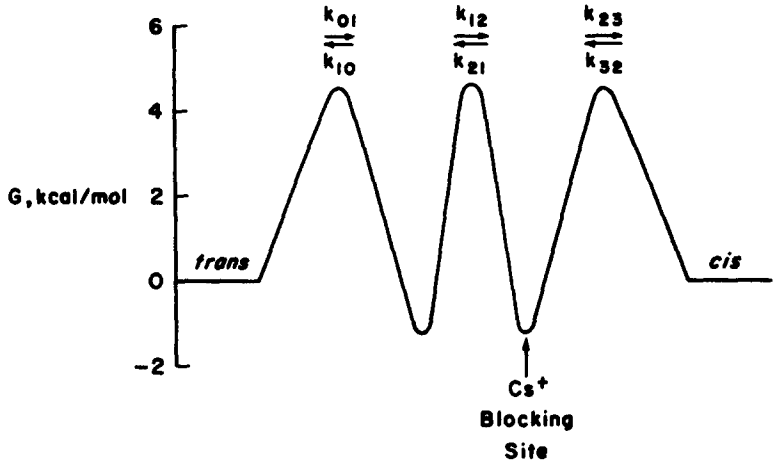


FIGURE 10. Three-barrier model for the SR K^+ channel. This diagram represents the Eyring-type free energy profile that must be negotiated by a K^+ ion during its movement through the channel at zero voltage. It is drawn in the spirit of Hille's (1975 *a*) model of Na^+ conduction in the axon Na^+ channel, likewise with the restriction that at most one ion at a time may occupy the channel. The profile is adjusted to be consistent with the results presented here: the approximate independence of conductance of voltage, a Cs^+ blocking site 38% of the way in from the *cis* side, an apparent K^+ dissociation constant of 54 mM, and maximum K^+ conductance of 240 pmho. There are many other combinations of peak heights, well depths, and positions that would be consistent with our data; this profile is presented for its simplicity and symmetry.

calculated difference in $(p - w)$ parallels the measured difference in $\Delta H\ddagger$ for the two ions.

However, when we make a similar comparison for Li^+ and K^+, a truly remarkable discrepancy is revealed. Whereas the calculated values of $(p - w)$ require that the barriers be about 2 kcal/mol higher for Li^+ than for K^+, the $\Delta H\ddagger$ for Li^+ is a full 8.5 kcal/mol higher than that for K^+ (Fig. 8). This difference is manifested in the strong temperature dependence of the maximum channel conductance for Li^+. If we had ignored the absolute rate calculations, assigning barrier heights only on the basis of the measured $\Delta H\ddagger$, we would have predicted the Li^+ conductance to be 2 million times lower than the K^+ conductance. But these conductances differ only by a factor of

30. A consideration of only the enthalpy contributions to the free energy profile for ion conduction leads us to a prediction of the selectivity that is five orders of magnitudes in error.

We are inclined to conclude, then, that there are substantial entropy contributions favoring the conduction of Li^+ through the channel over the movement of K^+ or Na^+. The above discrepancy would be removed if Li^+ is entropically favored over K^+ in the transition state by 20–25 cal/mol-K. This calculated value is somewhat dependent on the number of barriers assumed and the particular free energy profile, but the general conclusion holds that there is a substantially favorable entropic factor and an unfavorable enthalpic factor for the conduction of Li^+ compared with K^+. It is important to realize

TABLE III
FREE ENERGY PROFILES CALCULATED FROM THREE-BARRIER MODEL

Ion	Free energy			$\Delta H‡$	$\Delta S‡$
	p	w	$(p - w)$		
	kcal/mol			*kcal/mol*	*cal/mol-K*
Li^+	6.0	−1.9	7.9	13.0	+17
Na^+	5.0	−1.6	6.6	5.3	−4
K^+	4.6	−1.3	5.9	4.6	−4
Rb^+	4.9	−1.4	6.3	—	—
NH_4^+	4.8	−1.4	6.1	—	—

Absolute rate theory was applied to a symmetrical three-barrier model (Fig. 9) to calculate the heights of the peaks, p, and of the wells, w, at zero voltage, using the values of γ^m and K in Eq. 11 and 12. The ion at 1 M activity in the aqueous solution is taken as the zero of free energy. The total barrier height, $(p - w)$, was also calculated and is compared with the measured $\Delta H‡$ of the maximum conductance (Fig. 8). The activation entropy for the maximum conductance, $\Delta S‡$, was then calculated from $(p - w)$ and $\Delta H‡$. The absolute values of $\Delta S‡$ are not significant, but the differences in $\Delta S‡$ among the ions are meaningful. The calculated peak energy for NH_4^+ is higher than that for K^+; this is clearly incorrect since $P_{NH_4}/P_K > 1$ (Table II). This disagreement is a consequence of the departure of NH_4^+ from the "conductance-affinity-permeability" relation (see Table II).

that the crucial step in conduction here is not the entry of the ion into the channel since these measurements were done under saturating conditions in which the channel is almost always loaded with an ion: the conduction is limited by the movement of the ion through and out of the channel. It is tempting to attribute a large excess entropy of activation for Li^+ to a partial dehydration of the ion in a rate-limiting transition state. If the channel were mostly water filled, but if such a dehydration were rate-limiting at one of the peaks in the free energy profile, then an entropy difference of 20 cal/mol-K between Li^+ and K^+ would not be outrageous, since the entropies of hydration of the two ions differ by 16 cal/mol-K (Burgess, 1978). Much more work needs to be carried out before this could be taken as more than merely a suggestion.

In spite of the complications in the particular mechanistic interpretation of these results, we should not lose sight of the overall simplicity of the behavior

described here. The SR channel appears to conduct ions more simply even than gramicidin, a "model" channel composed of only 30 uncharged amino acids (Urry, 1971). Gramicidin does not act as a single-ion channel but displays double-saturation phenomena, multi-site interactions, and concentration-dependent permeability ratios (Hladky and Haydon, 1972; Myers and Haydon, 1972; Neher, 1975; Eisenman et al., 1978). The uncomplicated behavior of the SR channel is surprising, considering the channel's origin in a highly evolved membrane and since the channel protein is large and complex enough to display sensitivity to sulfhydryl ligands, proteolytic enzymes, and Cs^+, as well as extensive conformational changes driven by the intramembrane electric field (Miller and Rosenberg, 1979 *a* and *b*; Coronado and Miller, 1979; Labarca et al., 1980).

Nature has not provided physiologists with an abundance of single-ion channels to study. Both well-described nerve channels, the Na^+ and K^+ channels of axons, behave like multi-ion channels (Cahalan and Beginisich, 1976; Hille and Schwarz, 1978). Only one channel studied by eletrophysiological means, the acetylcholine-gated channel of muscle endplate, has so far remained describable in terms of a single-ion scheme (Adams, 1979; Lewis and Stevens, 1979; Horn and Brodwick, 1980).

The SR K^+ channel has now been shown to behave in this simple manner, at least when studied in an artificial bilayer of known and controllable composition. It will eventually be important to develop methods for carrying out similar studies on channels which have been studied electrophysiologically to enable us to investigate the fidelity of the planar bilayer system and, more importantly, to attack the biochemical problems that are unapproachable in the channel's native membrane.

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