Cation Permeation through the
Voltage-dependent Potassium Channel
in the Squid Axon

Characteristics and Mechanisms

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ABSTRACT Characteristics of cation permeation through voltage-dependent delayed rectifier K channels in squid giant axons were examined. Axial wire voltage-clamp measurements and internal perfusion were used to determine conductance and permeability properties. These K channels exhibit conductance saturation and decline with increases in symmetrical K+ concentrations to 3 M. They also produce ion- and concentration-dependent current-voltage shapes. K channel permeability ratios obtained with substitutions of internal Rb+ or NH4+ for K+ are higher than for external substitution of these ions. Furthermore, conductance and permeability ratios of NH4+ or Rb+ to K+ are functions of ion concentration. Conductance measurements also reveal the presence of an anomalous mole fraction effect for NH4+, Rb+, or Ti+ to K+. Finally, internal Cs+ blocks these K channels in a voltage-dependent manner, with relief of block by elevations in external K+ but not external NH4+ or Cs+. Energy profiles for K+, NH4+, Rb+, Ti+, and Cs+ incorporating three barriers and two ion-binding sites are fitted to the data. The profiles are asymmetric with respect to the center of the electric field, have different binding energies and electrical positions for each ion, and (for K+) exhibit concentration-dependent barrier positions.

INTRODUCTION

There is evidence that K channels permit more than one ion within their permeation pathway at any given moment in time; i.e., they demonstrate multion behavior. For example, certain K channels exhibit positive coupling among permeating ions, as evidenced by high flux ratio exponents (Hodgkin and Keynes, 1955; Begenisich and De Weer, 1980). Others show steep voltage-dependent
block by ions such as Cs⁺, with relief of block by permeant ions placed on the opposite membrane surface (Bezanilla and Armstrong, 1972; French and Wells, 1977; Adelman and French, 1978; Armstrong and Taylor, 1980). Finally, some K channels demonstrate a nonmonotonic relationship between conductance or reversal potential and the mole fraction of two cations having different channel permeabilities (Hagiwara et al., 1977; Ashcroft and Stanfield, 1983).

All of these behaviors can be qualitatively accounted for by multi-occupancy energy barrier models developed from absolute reaction rate theory (Hille and Schwarz, 1978). In such models, a channel is represented by a free-energy profile consisting of a series of energy maxima and minima (barriers and wells) across which ions travel in single file. Ion movement is determined by a combination of chemical, electrostatic, and repulsive energies for each ion species, and requires the existence of vacant energy minima (i.e., it is not "knock-on" in nature).

Although it is well established that the delayed rectifier K channel of the squid giant axon is a multi-ion channel, many multi-occupancy characteristics predicted for such channels have not been observed in squid. For example, the conductance of a multi-ion channel in symmetrical K solutions should be a biphasic function of ion activity. At low activities, conductance should increase with ion activity when the channel is nearly empty. In the middle activity range, as exit from the pore becomes rate-limiting, conductance should saturate. Finally, at high activities, when most of the channels are full, transport should be limited by the requirement that a vacancy move from one side of the membrane to the other. In this case, conductance should vary inversely with ion activity since there would be almost no vacancies and new ones forming at the edge of the membrane would be filled by ions in the bathing solutions rather than by ions crossing the membrane (Hille and Schwarz, 1978). The particular pattern of conductance change as a function of activity should depend on each ion's specific energy profile. Therefore, conductance ratios \( g_x/g_K \), not only conductance, should depend on activity.

A second predicted behavior of multi-ion channels is that permeability ratios \( P_x/P_K \), like conductance, should depend on ion activity. In bionic cases, where only K⁺ is placed on one side of the membrane and only X⁺ is on the other, this ratio should depend on ion activity and the energy profile for each ion. At low activities, this ratio should depend on the highest barriers in the profiles. At intermediate activities, the depth of the energy wells should become important. The central barrier height alone should determine the permeability ratios at high ion activity levels (Hille and Schwarz, 1978). No observations of concentration-dependent permeability ratios have been reported for K channels.

There are other behaviors that might be seen in multi-ion channels with asymmetric permeation pathways (i.e., the barriers and wells are not placed symmetrically about the center of the membrane). The magnitude of both conductance and permeability might depend on whether ions traverse the channel across the highest or the lowest barrier first (Hille and Schwarz, 1978). If so, an asymmetric energy pathway could produce nonlinearities in instantaneous current-voltage \((I-V)\) curves, even for symmetrical concentrations of permeant cations. There would be a distinct curve for each ion type, depending
upon the specific series of barriers and wells encountered. Furthermore, permeability ratios might differ for ion substitutions on one side vs. the other side of the membrane.

We report here new experimental data on the permeability characteristics of delayed rectifier K channels of the squid giant axon. We have determined the conductance-activity relationship, the shapes of the I-V relationships in symmetrical permeant ion solutions, a dependence of permeability ratios on the compartment chosen for ion substitution, the effect of activity on conductance and permeability ratios, and the anomalous mole fraction effect. We have also extended prior work on the voltage-dependent block of these K channels by internal Cs⁺. Finally, we have used our experimental findings to explore some of the free parameters in a three-barrier, two-site rate model. Aspects of this work have been reported previously in abstract form (Oxford and Adams, 1981; May and Oxford, 1985, 1986).

METHODS

Single giant axons were isolated from *Loligo pealei* at the Marine Biological Laboratory, Woods Hole, MA. They were cleaned of most adhering tissue and the axoplasm was squeezed out with a tiny rubber roller. An artificial internal solution was then introduced into the axon via a micrometer syringe. Axons were mounted in a Macor (Corning Glass Works, Corning, NY) chamber, continuously perfused internally and externally, and voltage-clamped by the axial wire technique. Details of the procedures have been previously published (Wu and Narahashi, 1975; Oxford et al., 1978; Oxford, 1981). The current signals were digitized by a 12- or 14-bit analog-to-digital converter interfaced directly to a PDP-11/23 computer (Digital Equipment Corp., Marlboro, MA) for storage and analysis. The computer also allowed the generation of voltage-clamp pulses under program control from a digital-to-analog converter. Typically, voltage-clamp pulses were delivered in 10- or 20-mV increments from a holding potential of −80 mV. In experiments with external Rb⁺, the holding potential was placed at −100 mV to deactivate K channels, which open under these conditions (Matteson and Swenson, 1986). Leakage and linear capacitive currents were subtracted from total current records by use of an analog electronic bridge circuit and digitally using a −P/4 procedure. Series resistance compensation was employed as previously described (Oxford, 1981).

Solutions and Junction Potentials

Each experiment began with filtered seawater bathing the axon and a standard internal solution of 50 mM Na⁺, 350 mM K⁺, 320 mM glutamate−, 50 mM F−, 15 mM phosphate buffer, and 310 mM sucrose (pH 7.3). Many solution substitutions were made and are noted throughout the text and in the figures as external/ internal solutions. All external solutions contained 300 nM tetrodotoxin (TTX) to block Na channels and were buffered with 5 mM HEPES to pH 7.8. Internal solutions were buffered with either phosphate or MOPS to pH 7.3. Elevations in internal permeant cation concentration were accomplished with glutamate salts. The solution temperature was maintained at 10°C by a thermoelectric device in contact with the chamber and an electronic feedback circuit. For the purposes of analysis, concentrations were converted to activities according to Robinson and Stokes (1959) or Latimer (1952).

Junction potentials between the initial internal and external solutions were measured just before the axial electrode assembly was inserted into the axon and after withdrawal
at the end of the experiment. Changes in potential during the course of experiments were typically <3 mV. In separate measurements, we attempted to determine junction potentials for the wide array of external/internal solution combinations employed. This was accomplished with the electrodes used in axon experiments. For elevated ion concentrations, the values obtained were often unstable and may be in error by several millivolts. Nonetheless, the data presented have been corrected for junction potential offsets except as otherwise noted.

In the case of experiments with Ti+, the chamber and axon were thoroughly perfused with Cl−-free solutions for 30 min before the introduction of Ti+ to either solution. In addition, the electrodes were filled with KF or K-acetate rather than the usual KCl solutions. Although acetate or nitrate salts were used for external Ti solutions, 1 mM NaCl was also included to stabilize the Ag/AgCl electrode junction.

Special Precautions

In experiments requiring comparisons of conductance at a constant number of open channels, "instantaneous" I-V measurements were made. We have assumed that macroscopic K+ conductance reflects properties at the single channel level. K channels were opened with a voltage prepulse followed by steps to various potentials. The current response at the instant of voltage change was sampled at either 20- or 50-μs intervals. In theory, this measurement reflects the current response to a change in driving force in a fixed population of open channels before gating changes have occurred. Values for "instantaneous" currents in all experiments were obtained by fitting an exponential to the current response (tail current) and extrapolating the fit to the precise moment of test voltage change. The resulting current values at voltages near the apparent reversal potential were used to compute the slope conductance (dI/dV) by linear regression. Instantaneous current measurements were also used to determine the biionic zero-current reversal potential. This potential, at which there is no net current, was determined in symmetrical K solutions and after substitutions of test cations internally or externally. The change in reversal potential with test ion substitution was used to compute the permeability ratio from Eq. 1 (derived from the Goldman-Hodgkin-Katz formulation [Hille, 1971]).

\[ P_X/P_K = \frac{[K^+]/[X^+]}{\exp(\Delta E_{rev}/RT)}. \]  

Several procedures were used to reduce periaxonal ion accumulation, a problem inherent to axons with Schwann cell coverings (Adelman et al., 1973). Accumulation leads to a time-dependent variation in ion concentrations near the membrane surface. These concentrations vary as a function of the integral of the prepulse current, complicating comparisons of conductance or permeability changes with assumed concentrations. Therefore, to minimize the problem, brief prepulses to positive voltages (opening all available channels) or prepulses to the reversal potential (opening fewer channels and resulting in no net current) were used (Clay, 1985). When brief positive prepulses were used, the resulting slope conductance was scaled to the maximum conductance as determined from a sufficiently long pulse to the prepulse potential, ensuring that a set number of channels was opened. This protocol was primarily used for conductance comparisons. When reversal potential prepulses were used, the pulse was long, ~10 ms, to compensate for possible variations in activation rates. Reversal potential prepulses were used in permeability ratio calculations since they resulted in the least ion accumulation/depletion phenomena. In addition, most experiments employed symmetrical cation concentrations, which dramatically reduce accumulation.
On extremely rare occasions (twice), we observed an unusual current pattern for depolarizations to $-20$ mV, where very small currents at the beginning of the step would be outward and then reverse direction, becoming inward after 1 or 2 ms (e.g., Fig. 1, middle panel). We believe this anomaly reflects an error in our analog capacitance compensation in those instances. The possibility also exists that it could reflect $K^+$ accumulation during the step and subsequent shift in $E_K$. However, since we worked in symmetrical $K^+$ concentrations and the currents in question were very small, this explanation seems unlikely. Nevertheless, the appearance of such behaviors does not alter our conclusions from the permeability experiments as they relied exclusively on instantaneous methods that avoided this time and voltage range.

Energy Barrier Modeling

Fits to the instantaneous $I-V$ data were obtained with a three-barrier, two-site model of the permeation pathway in which the rate constants governing ion transitions over the barriers are derived from absolute reaction rate theory. Extensive descriptions of the theory and parameters involved can be found in Hille and Schwarz (1978). We label the energy levels of the model according to the following convention: $G_1$, $G_2$, $G_3$, and $G_4$ represent the sequence of binding sites (wells) from external to internal surface (inclusive), while $G_{12}$, $G_{23}$, and $G_{34}$ represent the external, central, and internal barriers, respectively (see Fig. 13). It was assumed that the energy profiles for various ions were different and unaffected by other ions within the channel except by mutual repulsion. Further, the repulsive energy of each ion was assumed to drop linearly between wells and was included in calculations as a factor multiplying the rate constants. Each ion was permitted to move only from one well or bathing compartment to the next adjacent unoccupied well or compartment in single-file progression. Any detectable changes in flux were assumed to result from alterations in rates or probabilities of transit through open channels and not from changes in channel numbers. It was not assumed that the electric field was constant across the membrane and therefore electrical distances do not correspond to physical distances (Hille, 1975a). The barrier peaks were not assumed to be positioned midway between energy minima. Trial-and-error adjustment of the free parameters included both magnitudes and positions of barriers and wells, and the ionic repulsion factor. Our calculations employed a matrix inversion method (Begenisich and Cahalan, 1980) to solve nine simultaneous differential equations, given the activities of the permeants. The unidirectional flux of each ion was calculated as occupancy probability times the corresponding rate constant. Finally, net flux was determined as the difference in unidirectional fluxes across any barrier. The resultant $I-V$ fits were vertically scaled to match experimental macroscopic current magnitudes. The program for this model was written in FORTRAN and modified from a version kindly provided by Dr. Peter Hess of the Department of Physiology at Yale University.

RESULTS

$K$ Channel Conductance vs. Ion Activity

The effects of symmetrical changes in permeant ion activity on $K$ channel currents and conductance were examined using both steady state and instantaneous measurements. After voltage-clamping each axon, compensating for linear capacitance and series resistance, the standard internal solution was exchanged for a solution containing (millimoles per liter): $500$ K$^+$, 420 glutamate$^-$, 50 F$^-$,
15 phosphate buffer. The external filtered seawater was also exchanged at this time for 500 K⁺, 40 Mg²⁺, 10 Ca²⁺, 600 Cl⁻. These two solutions were used for every conductance vs. activity experiment and data obtained during their use served to normalize conductances among all axons. After families of currents and instantaneous I-V measurements were obtained, solutions were switched to lower concentrations, down to 100 K⁺/100 K⁺, or to higher concentrations, up to 3,000 K⁺/3,000 K⁺. Recovery in 500/500 solutions was always attempted and was usually successful. The internal/external osmolality ratio was maintained at ~1.08 throughout these experiments. At low concentrations, the external osmolality was kept constant with Na⁺ and the internal osmolality was kept constant with sucrose, glycine, or urea. No obvious differences were seen among the internal nonelectrolytes.

When the concentration of K⁺ was increased symmetrically from 100 to 500 mM, the amplitude of the currents in response to voltage steps from -60 to +100 mV increased (Fig. 1). Increases in K⁺ concentration to 1 M further increased the current magnitude. However, when the [K⁺] exceeded 1 M, the currents declined, as illustrated for another axon in Fig. 2. The changes in current magnitude in response to changes in concentration were reversible, as seen from the recovery records in this figure. The slope of the instantaneous I-V relationship also demonstrated an increase and decrease as a function of concentration, indicating a rise and a fall in conductance (Fig. 3). This pattern of conductance rise below 1 M and fall above was seen in all 33 axons studied (although the degree of change was variable) (Fig. 4A). The averaged, normalized slope conductance as a function of the symmetrical K⁺ concentration (Fig. 4B) doubled for the 10-fold increase in concentration from 0.1 to 1 M.

Similar increases in conductance were seen (Fig. 4A) with symmetrical elevation of Rb⁺ (open triangles) or NH₄⁺ (open circles) to 2 M. Most axons were unstable in symmetrical solutions of either of these two ions above 1 M and thus we were unable to reliably determine whether Rb⁺ or NH₄⁺ conductance declines at the higher concentrations.

![Figure 1](image-url)
The conductance changes seen in symmetrical $K^+$ are not due to coincidental increases in osmolality or ionic strength alone. Control experiments were performed in which the osmolality was increased symmetrically with the nonelectrolytes glycine or urea. In general, conductance decreased when osmolality was increased above that in 500/500 solutions (Fig. 5, open symbols). If the observed conductance changes in symmetrical $K^+$ are corrected for the drop in conductance owing only to increases in osmolality, the pattern of conductance change (Fig. 5, dashed line) as a function of $K^+$ concentration remains essentially unchanged. No significant differences were seen in control experiments employing glycine vs. urea. Sucrose was not used, because of its high viscosity at...
concentrations above 500 mM. Although experiments with elevated concentrations of nonelectrolytes led to increases in leak conductance, exposure to these substances was kept brief and no permanent loss of functioning channels occurred, as evidenced by good recovery in solutions of normal osmolality.

Control experiments were also completed to assess the effect of increases in external ionic strength on conductance using two prepulse protocols as described in the Methods (Fig. 6). As the internal K\(^+\) concentration was increased from

![Graph A](image1.png)

**Figure 4.** Slope conductance (see Methods) as a function of symmetrical ion concentration or activity. (A) Conductance in symmetrical K\(^+\) (filled circles), NH\(_4\)\(^+\) (open circles), or Rb\(^+\) (open triangles) normalized to the conductance for each axon in 500 mM symmetrical concentrations of that ion. In the case of K\(^+\), 33 axons are represented; 13 axons were exposed to increasing concentrations from 0.1 to 0.5 M, while 25 axons were studied at concentrations of 0.5 to 1.0, 1.5, 2.0, 2.5, or 3.0 M. (B) Data points are the means of the K conductance of the 33 axons in A. The solid line is the slope conductance at the reversal potential as a function of the K\(^+\) concentration predicted by the three-barrier, two-site model profile for K illustrated in Fig. 13. The repulsion factor, \(R\), is 2.6.
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500 mM to 2.0 M, external Na\(^+\) was increased from 0 mM to 1.5 M, while external K\(^+\) was maintained at 500 mM. As with the nonelectrolytes, conductance declined as the concentration of Na\(^+\) rose and the corrected conductance vs. [K\(^+\)] relationship still demonstrated a rise and fall. No qualitative difference was seen for conductance change as a function of K\(^+\) concentration when assessed with prepulse voltages to either +100 mV (filled circles) or to the reversal potential (filled triangles). The actual magnitude of the change in conductance was, however, dependent on the prepulse voltage (Fig. 6).

**FIGURE 5.** Normalized slope conductance as a function of symmetrical K\(^+\) or symmetrical nonelectrolyte concentration. The conductance was determined from instantaneous I-V data as either K\(^+\) (filled circles) or the nonelectrolytes glycine or urea (open circles) were increased. All solutions contained at least 500 mM K\(^+\). Experimental and control measurements were obtained from the same axons. The dashed line indicates the estimated conductance after correcting for effects perhaps common to K\(^+\) and nonelectrolytes. The correction was performed by adding the decline in conductance seen in control (nonelectrolyte) conditions back to the change in conductance with K\(^+\) concentration. The values in parentheses are numbers of axons contributing to each point (mean ± SD).

**Current-Voltage Relationships in K\(^+\), NH\(_4^+\), Rb\(^+\), and Tl\(^+\)**

From 100//100 to 300//300, the instantaneous I-V relationships in symmetrical K\(^+\) were linear between −160 and +160 mV. At higher concentrations, although the I-V shape was unchanged over most of the voltage range, the relationship at voltages more negative than −80 mV became supralinear (Fig. 7A). The concentration-dependent shape differences were pronounced when the axon was exposed to asymmetrical K\(^+\) concentrations (Fig. 7B).

In contrast to the findings for symmetrical K\(^+\), the current-voltage curves in symmetrical NH\(_4^+\) or Rb\(^+\) were markedly nonlinear at all voltages and concentrations (Fig. 8). Although the driving forces in symmetrical solutions are equivalent...
for inward and outward currents, inward NH$_4$ or Rb currents were smaller than outward currents. This effect was particularly prominent at low ion concentrations. There was also an enhanced curvature near the reversal potential for the currents at low concentrations. As the permeant ion concentration was raised, the conductances at positive and negative potentials approached one another, making the curvature around the reversal potential less marked.
The I-V relationships found when Tl⁺ was a charge carrier were complex and characterized by two features: an anomalous decrease in outward Iₖ for increases in external Tl⁺ and an apparent voltage-dependent "self-block." When Tl⁺ was substituted for external K⁺, outward K currents were reduced (Fig. 9, A and B), as recently reported by Matteson and Swenson (1986). This reduction is modestly relieved with increasing depolarization, as indicated by the supralinear I-V relation. External substitution of Tl⁺ also resulted in sublinear I-V relations for inward Tl currents at potentials more negative than -100 mV (Fig. 9A).
Similarly, internal Tl\+ led to sublinear I-V relationships for very positive potentials (Fig. 9, B and C). The extreme example of negative slope conductance in Fig. 9C is reminiscent of K channel block by other internal cations (e.g., Cs\+ and Na\+) and suggests "self-block" of Tl current by permeating Tl ions.

Figure 8. Instantaneous I-V relations in symmetrical NH\textsubscript{4}\+ or Rb\+. (A) Instantaneous I-V data from an axon (85-AUG08A) obtained in symmetrical 100 (open triangles), 300 (open circles), or 500 (filled circles) mM NH\textsubscript{4}\+. No correction has been made for junction potential offsets. The solid lines represent fits of the three-barrier, two-site profile for NH\textsubscript{4}\+ with energy levels and spacing as in Fig. 14, with the exception that G34 was moved from 0.59 to 0.66 to improve the fit to the 100//100 data. R = 2.0. The vertical scaling of the computed curves was identical for the three concentrations. (B) Instantaneous I-V data from axon 84-AUG15A obtained in symmetrical 300 (open circles) or 500 (filled circles) mM Rb\+. The solid lines are fits of the three-barrier, two-site profile for Rb\+ using the energy levels and spacing of Fig. 14. R = 2.0.

Permeability Ratios Determined on Both Sides of the K Channel

Perhaps the most common permeability property investigated in membrane channels is the ionic selectivity sequence. It is generally assumed that ion selectivity is independent of the direction of current flow through the channel. However, this is apparently not the case in the squid axon K channel.
Using Eq. 1 in the Methods (Hille, 1971) to determine selectivity in terms of permeability ratios for Rb⁺ or NH₂⁺ to K⁺, differences were detected in these ratios for internal vs. external substitutions of the test ion for K⁺. Table I shows that the permeability ratio is significantly higher when Rb⁺ or NH₂⁺ carries the current out of the cell. This difference in permeability ratio cannot be explained by ion accumulation in the periaxonal space, which would lead to an apparent increase in permeability for inward NH₄ or Rb current.

Relative Tl⁺ permeability was difficult to assess on both sides in the same axon owing to variable junction potentials and the short lifetime of Tl⁺-perfused axons. It is clear, however, that Tl⁺ is more permeant than K⁺ when substituted...
on either side of the membrane (mean ± SD: \( P_{Tl}/P_K = 1.60 ± 0.52 \) for internal \( Tl^+ [n = 5] \); \( P_{Tl}/P_K = 1.14 ± 0.05 \) for external \( Tl^+ [n = 3] \)).

**Conductance and Permeability Ratios vs. Activity**

In multi-ion channels, selectivity as well as absolute conductance is predicted to depend on activity. Selectivity may be evaluated either by permeability ratios (as described above) or by conductance ratios. The conductance ratio is determined by simply comparing single channel or macroscopic conductances when the current is carried by different ions in the same cell.

To determine the effect of activity on conductance ratios, instantaneous \( I-V \) relationships were compared for symmetrical \( K^+ \) and \( Rb^+ \) or \( K^+ \) and \( NH_4^+ \) at several activity levels. Conductance ratios were then calculated by comparing slope conductances (see Methods) for each ion species. In the same axons, permeability ratios for external \( Rb^+ \) or \( NH_4^+ \) substitutions for \( K^+ \) were calculated from reversal potentials. Both conductance and permeability ratios are plotted as a function of concentration in Fig. 10. As the concentrations of \( K^+ \) and \( NH_4^+ \) are raised from 200 to 500 mM, the conductance ratio rises, while the permeability ratio falls. The \( Rb^+ /K^+ \) permeability ratio declines over a wide concentration range, while the conductance ratio shows a more complex behavior. Over the concentration ranges examined, the conductance ratio exceeds the permeability ratio for \( NH_4^+ \), while the opposite is observed for \( Rb^+ \).

**Anomalous Mole Fraction Behavior**

In single-occupancy channels, the total current in mixtures of ions \( A \) and \( B \) must be a monotonic function of the mole fraction \([A]/[A] + [B]\). If the current goes through a minimum or a maximum as the mole fraction of \( A \) or \( B \) is increased, then the channel is said to demonstrate an anomalous mole fraction effect (e.g., Hess and Tsien, 1984). In a multi-ion channel, both the conductance and reversal potential may show this anomaly, depending on the energy profiles for each ion species (Hille and Schwarz, 1978).

To determine the existence of an anomalous mole fraction effect, instantaneous current measurements were made to define the reversal potentials and the slope conductances for mole fractions of \( K^+ \) and \( NH_4^+ \), \( K^+ \) and \( Rb^+ \), or \( K^+ \) and \( Tl^+ \). With the internal \( K^+ \) concentration maintained constant at 300 mM, the external \( K^+ \) concentration was increased from 0 to 300 mM while the external \( NH_4^+ \), \( Rb^+ \), or \( Tl^+ \) was decreased from 300 to 0 mM, always maintaining

<table>
<thead>
<tr>
<th>Cation</th>
<th>( n )</th>
<th>( P_{X}/P_K ) (X inside)*</th>
<th>( P_{X}/P_K ) (X outside)</th>
</tr>
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<tbody>
<tr>
<td>( Rb^+ )</td>
<td>8</td>
<td>( 0.76±0.07 )</td>
<td>( 0.54±0.09 )</td>
</tr>
<tr>
<td>( NH_4^+ )</td>
<td>5</td>
<td>( 0.23±0.03 )</td>
<td>( 0.14±0.04 )</td>
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* Mean ± SD.
† \( p < 0.01 \).
a total external monovalent cation concentration of 300 mM. Although no reversal potential minimums or maximums were detected, minimums in conductance for different mole fractions of the three test ions were observed and are depicted by the examples in Fig. 11A. This was a consistent finding, as indicated by the data for six representative axons in NH₄⁺ and Tl⁺ in Fig. 11, B and C. The effect was more subtle for mole fractions of Rb⁺ to K⁺; some axons demonstrated no obvious minimum with this ion pair.

**Figure 10.** Conductance ratios (from calculations of instantaneous I-V slopes near the reversal potential) and permeability ratios (from measurements of the reversal potential) as a function of the concentration of K⁺, NH₄⁺, and Rb⁺. (A) NH₄/K ratios. (B) Rb/K ratios. In each axon, conductance was measured for symmetrical solutions of both K⁺ and the test cation at two or more concentrations. Permeability ratios were obtained for external substitution of the test ion at two or more concentrations. Both conductance and permeability ratios were obtained in 10 of the 14 axons studied.

**Voltage-dependent Block of K Channels by Internal Cs⁺**

In channels allowing only one monovalent ion within their electrical field at any instant in time, blocking ions that penetrate partway through the field exhibit an effective valence (product of the blocking ion's valence and the electrical distance to the blocking site) never greater than 1.0. However, for Cs⁺ block of the voltage-dependent K channels in squid (Adelman and French, 1978; French and Shoukimas, 1985) and lobster axon membranes (Coronado et al., 1984) or
of the inward rectifier in starfish egg cell membranes (Hagiwara et al., 1976),
the effective valence is between 1.0 and 2.0. In the squid K channel, this voltage-
dependent block can apparently be relieved by elevating K⁺ on the side of the
membrane opposite to the blocking ions (Bezanilla and Armstrong, 1972; Adel-
man and French, 1978; Armstrong and Taylor, 1980). Multi-ion rate theory
models predict both high-valence voltage-dependent block and block relief by
permeant ions (Begenisich and Smith, 1984; French and Shoukimas, 1985).

We examined Cs⁺ block and relief by perfusing axons with 300 mM K⁺
solutions containing 50 mM Cs⁺ and exposing them to various extracellular K⁺
concentrations. In the presence of 50 mM external K⁺, voltage-dependent block
by internal Cs⁺ resulted in a region of negative slope conductance at positive
membrane potentials (e.g., Fig. 12, open triangles). Elevation of the external K⁺
concentration to 300 mM reduced the block (Fig. 12, open circles). A comparison
of the I-V relationships in 300 mM symmetrical K⁺ with and without internal Cs⁺
revealed no effect of Cs⁺ on inward currents. An equilibrium analysis of internal
Cs⁺ block was performed following the general approach used by others (see,
e.g., Woodhull, 1973; Coronado and Miller, 1979; French and Shoukimas, 1985), assuming a linear \( I-V \) relationship for outward currents in the absence of \( \text{Cs}^+ \), and no block in the linear portions of the curves in the presence of \( \text{Cs}^+ \) for the 50 mM external \( \text{K}^+ \) case. The \( I-V \) data were analyzed according to

\[
I_k(\text{Cs}) = \frac{I_k(\text{control})}{1 + [\text{Cs}^+] \exp(-DFV/RT)/K_0},
\]

where \( D \) equals the effective valence and \( K_0 \) is the \( \text{Cs}^+ \) concentration required to

FIGURE 12. Voltage-dependent block of outward \( \text{K} \) currents by internal \( \text{Cs}^+ \). Instantaneous \( I-V \) data were obtained in an axon (82-JUL14A) perfused with 300 mM \( \text{K}^+ \) plus 50 mM \( \text{Cs}^+ \) with external \( \text{K}^+ \) concentrations of 50 (open triangles) and 300 (open circles) mM. Data in 50 K+/300 K, 50 Cs have been vertically scaled by 1.26 to account for the decreased number of channels opened during the 8-ms prepulse to +60 mV relative to records in the absence of \( \text{Cs}^+ \). Control 300 K+/300 K data are also plotted (filled circles). The solid lines represent three-barrier, two-site model fits to the data using the \( \text{K}^+ \) (Fig. 13) and \( \text{Cs}^+ \) (Fig. 14) profiles described in the text. The spacings for the \( \text{K} \) profiles were (G12 to G34) 0.1, 0.5, 0.53, 0.56, and 0.89 for 50 mM external \( \text{K} \) and 0.32, 0.5, 0.53, 0.56, and 0.76 for 300 mM external \( \text{K} \). The Cs profile spacing is given in the legend of Fig. 14. \( R = 2.5 \). (Inset) Semilog plot of the data according to Eq. 3 to obtain the effective valence of the \( \text{Cs}^+ \) blocking reaction. Data points for 50 (open triangles) and 300 (open circles) mM \( \text{K}^+ \) are fitted with straight lines to yield \( D \) values of 1.03 and 0.51, respectively.

block 50% of the channels at 0 mV. Eq. 2 can be linearized (Eq. 3) and fitted to the data as plotted in the inset of Fig. 12 to obtain \( D \) and \( K_0 \):

\[
\ln [I_k(\text{control})/I_k(\text{Cs}) - 1] = \ln [\text{Cs}^+]/K_0 + DFV/RT.
\]

The apparent electrical distance determined in this axon decreased with increasing external \( \text{K}^+ \) concentrations, yielding values of 1.03, 0.92, and 0.51 for 50, 135 (data not shown), and 300 mM \( \text{K}^+ \), respectively. The apparent dissociation constant for \( \text{Cs}^+ \) block at +100 mV was estimated according to

\[
K_D(100) = [\text{Cs}^+](r/1 - r).
\]
where \( r \) is the fraction of unblocked conductance at +100 mV. Values of 50 and 119 mM were calculated for 50 and 300 mM K\(^+\) conditions, respectively.

In contrast to the effects of external K\(^+\) concentration on Cs\(^+\) block, elevation of the external Cs\(^+\) or NH\(_4^+\) concentrations failed to relieve the voltage-dependent block by 50 mM internal Cs\(^+\) (data not shown), despite the documented ability of these ions to interact with the K channel permeation pathway from the external side (Adelman and French, 1978; this study).

**Barrier Models of the Ion Permeation Pathway**

In view of the evidence presented for multi-occupancy and nonindependent ion movement, the general approach of Hille and Schwarz (1978) was followed in modeling our data. We chose the three-barrier, two-site model to explore whether the minimal multi-occupancy scheme (two sites) could account for our experimental observations. Flux ratio measurements (e.g., Begenisich and De Weer, 1980) have suggested more than two binding sites in the profile, but the determination of the exact number is limited by experimental uncertainties of such measurements (Begenisich and Smith, 1984). Although the three-barrier, two-site model is limited, by combining our data for symmetrical solutions with that for the biionic cases of K\(^+\) vs NH\(_4^+\), Rb\(^+\), Tl\(^+\), and Cs\(^+\), general characteristics and limits of the profiles emerged.

The following approach was used. The amplitudes and positions of barriers and wells in the three-barrier, two-site model were adjusted by trial and error to determine the best fit for the I-V relationships. The fits were positioned along the voltage axis to adjust for offsets owing to solution junction potentials and to match as closely as possible the measured reversal potentials. The best fit of the simulated curves that most closely matched the data points was determined by eye. Fits to the data were attempted for many experiments as the exact shapes varied among axons. After the best average fit was determined for the symmetrical case, fits were generated for asymmetrical K\(^+\) concentrations. Next, reversal potentials simulated by the model for the biionic cases of NH\(_4^+\), Rb\(^+\), or Tl\(^+\) substituted for K\(^+\) on either side of the membrane were determined. At this point, the model reversal potentials were not equivalent to those determined experimentally. Therefore, adjustments were made in the profiles to optimize the fit to the average reversal potentials for both inside and outside cation substitutions without compromising the fits to the I-V shapes. After much trial and error, we arrived at a satisfactory fit to both the I-V shapes and the reversal potentials.

Next, the profile for K\(^+\) movement through the K channel was tested for its ability to accurately match the measured conductance changes as a function of ion activity. The profile selected to match the I-V relationships and the reversal potentials qualitatively reflected the conductance-activity relationship. Minor adjustments were made in the model to improve the fit to these data and to allow for fits to the NH\(_4^+\)/K\(^+\), Rb\(^+\)/K\(^+\), and Tl\(^+\)/K\(^+\) conductance ratio data. A return to the I-V shape and reversal potential data revealed no significant loss of accuracy as a result of these last adjustments in the model. Finally, the K profile was used to generate a profile for Cs\(^+\) block and relief of block by K\(^+\).
Energy Profiles for Cation Movement through the K Channel

The three-barrier, two-site profile that best fits our data for K⁺ movement through the K channel is depicted in Fig. 13. The profile is quite symmetric in well and barrier magnitude and electrical positions. This symmetry was essential for fitting the potassium I-V relationships. The solid lines through the data points in Fig. 7A demonstrate the excellent fit of the K profile to the data at 100 and 500 mM symmetrical K⁺. The increasing supralinearity in the negative limb of the I-V with elevations in concentration was accounted for in the model fits by allowing the external barriers to shift with concentration. For example, as K⁺ was raised to 500 mM, the external barriers were moved closer to the electric field midpoint. Similarly, the difference in shape between the asymmetric and symmetric cases in Fig. 7B was fitted by moving only the external barrier (G12)

![Figure 13. Three-barrier, two-site energy profiles for K ion permeation through the K channel under conditions of relatively low (solid lines) or relatively high (dashed lines) ion concentration. G1 and G4 represent the external and internal surfaces of the channel, respectively. Energy values (RT units) for the barriers are G12 = 9.9, G23 = 7.8, and G34 = 10.0, and for the wells are G2 = G3 = -3.5 for both high- and low-K profiles. The fractional electrical distances between G1 (0.0) and G4 (1.0) serve to define the positions of barriers and wells (D values). Nominal positions (G12, G2, G23, G3, G34) for the low-K case are 0.1, 0.5, 0.53, 0.56, and 0.95, while those for the high-K case are 0.32, 0.5, 0.53, 0.56, and 0.77.

into the field as the external K⁺ concentration was elevated. The consequences of simulating low-K data with the high-K profile or vice versa are illustrated by the poor fits (dashed lines) in Fig. 7B.

Unlike the importance of the lateral barrier positions to I-V shape, the magnitude of the simulated slope conductance near the reversal potential was largely independent of barrier positions. Either K profile in Fig. 13 predicted a rise in conductance as the K⁺ concentration was increased from 0.1 to 1.0 M and a subsequent fall in conductance with further elevations in concentrations (Fig. 4B). Preliminary attempts to fit the biphasic K conductance-activity data using a four-barrier profile (Begenisich and Smith, 1984) yielded a monotonic saturating function.

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Fig. 14 depicts the average channel energy profiles for NH₄⁺, Rb⁺, Cs⁺, and TI⁺. These profiles are quite dissimilar, as might be expected given the differences in I-V shapes, permeabilities, and conductances for these ions. To produce the nonlinear I-V relations in NH₄⁺, unequal well depths or binding sites were introduced into the profile, with the deeper well positioned near the external membrane surface. To improve the fit to the I-V relationship for NH₄⁺, it was sometimes necessary to move G34 further into the electric field by a tiny fraction as the concentration of symmetrical NH₄ was increased from 100 to 500 mM. In Fig. 8A, the solid curves represent fits to the data from one experiment using the NH₄ profile of Fig. 14. The fit is excellent for the majority of the data set.

![Energy Profiles](https://example.com/energy_profiles.png)

**FIGURE 14.** Three-barrier, two-site energy profiles for NH₄⁺, Rb⁺, Cs⁺, and TI⁺ permeation of the K channel in squid. Energy levels (RT units) for barriers and wells are indicated for each profile. The nominal positions for barriers and wells (G12, G2, G3, G34) are: NH₄: 0.05, 0.10, 0.14, 0.17, and 0.59; Rb⁺: 0.19, 0.30, 0.40, 0.46, and 0.70; Cs⁺: 0.20, 0.30, 0.35, 0.56, and 0.76; and TI⁺: 0.20, 0.25, 0.60, 0.90, and 0.95.

The magnitudes of the barriers in the K and NH₄ profiles are set by the measured permeability ratios. To produce the "sidedness" in permeability ratios (PNH₄/PK), the barrier heights for NH₄⁺ must be unequal, with G34-NH₄ greater than G12-NH₄. The depths and positions of the wells in both NH₄ and K profiles, as well as the barrier heights, affect the permeability ratios. For example, decreasing the magnitude of the K wells leads to a shift in the biionic reversal potentials toward zero membrane potential. Although satisfactory permeability ratios can be obtained with a wide variety of well positions, in order to fit simultaneously the I-V shapes, the conductance vs. concentration data, and the permeability ratios, the NH₄ wells must be placed to one side of the electric field and the wells for either K⁺ or NH₄⁺ must be positioned close to one another. The
model predicts a $P_{\text{NH}_4}/P_K$ ratio for internal substitution of NH$_4^+$ of 0.19 and for external substitution of 0.11. This compares favorably with the measured ratios of $0.23 \pm 0.03$ and $0.14 \pm 0.04$ (see Table I). The depths of the wells are further constrained by the experimental conductance ratios. The simulated $g_{\text{NH}_4}/g_K$ of 0.44 compares well with the experimental ratio of 0.41.

The profile for Rb$^+$ permeation has features in common with both K and NH$_4$ profiles. The barrier amplitudes for Rb$^+$ are similar to those for K$^+$, but the positions of barriers and wells are more like those of NH$_4^+$. The profile provides an excellent fit to the data for 300 and 500 mM symmetrical Rb$^+$ (Fig. 8B). It also simulates (a) increases in conductance for increases in Rb$^+$ concentration, (b) accurate Rb$^+/K^+$ conductance ratios (0.36 at 300 mM as compared with 0.33 found experimentally), and (c) higher permeability ratios for internal as compared with external substitution of Rb$^+$ for K$^+$ (0.6 vs. 0.5).

The fits to the I-V shapes for Tl$^+$ are not as good as for the other ions. The anomalous decrease in outward current during external Tl$^+$ substitution can be described by the profile (Fig. 9A) owing to the deep external well (G2). The profile produces a slight supralinearity for outward current, which agrees with the apparent voltage-dependent relief of conductance seen experimentally. Despite adjustments in many parameters, we were unable to quantitatively account for the apparent "self-block" without compromising the general shape of the I-V relations, and the conductance and permeability ratios. The profile does, however, demonstrate sublinearity for inward current, in qualitative agreement with this phenomenon. It is conceivable that the apparent self-block reflects the presence of small amounts of Tl ions in these solutions. Simulating this possibility would require the addition of a third energy profile combined with those for K$^+$ and Tl$^+$. Although the profile falls short of a quantitative description of thallium I-V shapes, it does predict a lower Tl$^+$ conductance and a higher Tl$^+$ permeability (for either external or internal Tl$^+$ substitution), in agreement with experimental results.

The high-energy barriers in the Cs profile demonstrate graphically one possible explanation for voltage-dependent block by this ion. The fits of I-V data using this profile with 50 mM Cs$^+$ inside the cell and 50 or 300 mM K$^+$ outside are shown in Fig. 12. The model simulates voltage-dependent internal Cs$^+$ block of outward currents and relief of block with external K$^+$ elevation. Voltage-dependent block can be obtained with either G12, G23, or G34 being rate-limiting, if the barriers and wells are carefully placed within the electric field. However, in order to account for voltage-dependent block by both internal and external Cs$^+$ (French and Shoukimas, 1985) and relief of block by elevations in K$^+$ on either side of the membrane, the rate-limiting Cs barrier must be the central one in a three-barrier, two-site model. For internal Cs$^+$ block, the depth and position of the inner well, G3, largely determines the magnitude of the block as a function of voltage, although the magnitudes of all barriers and wells, as well as their positions, influence the block. The fit to the data is improved by moving the outer K barriers as a function of K$^+$ concentration (Fig. 12), consistent with the concentration-dependent changes required to fit symmetrical (Fig. 7A) or asymmetrical (Fig. 7B) potassium I-V relations.
We have demonstrated that squid axon K channels exhibit the following behaviors: (a) saturation and decline in conductance with increasing symmetrical K⁺ activity, (b) ion-specific and activity-dependent I-V shapes, (c) permeability ratios that depend on the side on which ion substitution takes places, (d) activity-dependent conductance and permeability ratios, (e) a minimum in conductance as a function of the mole fraction of Rb⁺, Tl⁺, or NH₄⁺ to K⁺, and (f) voltage-dependent block by internal Cs⁺ with relief of block by external K⁺, but not by external Cs⁺ or NH₄⁺. In addition, we have described energy barrier profiles for K⁺, NH₄⁺, Rb⁺, and Tl⁺ permeation and Cs⁺ block, which provide adequate fits to a large portion of our dataset.

**Conductance Declines at High Ion Activity: Interpretation and Cautions**

In Ca channels, Ca-activated K channels, and the K channel in sarcoplasmic reticulum, conductance has been shown to rise and saturate with elevations in permeant cation activity (Coronado et al., 1980; Hagiwara and Byerly, 1981; Blatz and Magleby, 1984). Much of this information has been fitted by single-occupancy models, although anomalous mole fraction effects suggest that some Ca channels may be multi-ion (Almers and McClesky, 1984; Hess and Tsien, 1984). In preliminary experiments with the squid axon K channel, Clay (1985) saw no evidence for saturation with increases in the internal K⁺ concentration from 200 to 500 mM. Also, in preliminary work by C. M. Armstrong reported by Begenisich and Smith (1984), the outward K current of the squid axon increased steeply as the internal K⁺ concentration was elevated from 50 to 100 mM and then approached a nearly linear phase with subsequent increases in K⁺ concentration to 550 mM. It appears that the range of activities employed in these two studies was too narrow for conductance saturation to be observed.

In the squid K channel, saturation of conductance apparently occurs at a higher concentration than in other channels. This behavior has been suggested to reflect a high lateral to central barrier ratio and/or significant ion repulsion within the channel (Hille and Schwarz, 1978; Begenisich and Smith, 1984). The three-barrier, two-site energy profile that best fits our K data has a lateral/central barrier ratio of only 1.3 and a repulsion factor of 2.6. Although the fits to the shapes of the potassium I-V relationships at large voltages are improved by allowing the lateral barrier positions to shift as concentration is changed over a wide range, these shifts are not necessary to simulate the conductance-activity relationship.

When ion activity is increased to effect conductance changes, osmolality as well as viscosity and ionic strength are coincidently increased. Although control experiments to test for the effects of increases in osmolality and ionic strength were performed, the contribution of these factors to the total K conductance-activity relationship is not entirely clear. The major constraint in all controls of this sort is the necessity for inert substitutes for K⁺. An inert substitute is one that neither permeates the channel nor alters channel permeability or gating properties. Attempts to control for independent effects of ionic strength were hampered by the lack of such an inert cation to substitute for K⁺.
least at biological concentrations, external Na\(^+\) is thought not to enter the K channel, internal Na\(^+\) enters and causes a voltage-dependent block (French and Wells, 1977). The same appears true to some degree for all monovalent cations. When the concentration of nonelectrolytes was increased in experiments to control for changes in osmolality, obvious changes in solution viscosity, not seen in solutions of high electrolyte concentration, were noted. Addition of nonelectrolytes changes a variety of properties of the aqueous environment surrounding membrane channels, including water activity, ion mobility, viscosity, and density (French and Horn, 1983). Nonelectrolytes are not inert with regard to channel gating kinetics and have been shown to increase the time to peak current and decrease current magnitude apparently because of changes in the microscopic viscosity sensed by ions in the solution (Shoukimas et al., 1981). In contrast, bulk solution viscosity or osmolality are believed to have comparatively less effect on ionic currents (Kukita and Yamagishi, 1979). Additionally, it should be noted that compared with experiments in which ion concentrations were elevated, elevations in nonelectrolytes led to greater increases in leak currents and a general instability of ionic currents.

Elevations in permeant cation concentrations to the levels used here could produce alterations in the gating properties of K channels. Some alterations could conceivably lead to decreases in the number of open channels during the activating prepulses of instantaneous current measurements. We examined these possibilities in terms of channel activation kinetics and steady state voltage dependence. Although there was little difference in K current kinetics at 0.5 and 1.0 M concentrations, elevations in the K\(^+\) concentration (symmetrically or in internal K\(^+\) concentration alone) above 1.0 M led to a slowing of the “turn-on” or activation of the currents. Determination of activation half-times at a number of voltages revealed that activation was slower at all potentials, not merely shifted along the voltage axis. In addition, steady state conductance-voltage relations were shifted in the positive direction along the voltage axis by several millivolts for axons exposed to K\(^+\) concentrations >500 mM. Both the kinetic changes and a positive voltage shift in conductance would result in lower numbers of channels being activated for short prepulses to moderate depolarizations during instantaneous current measurements. Such a shift in activation could explain the greater decrease in macroscopic conductance at high electrolyte concentration for protocols using prepulse voltages near the reversal potential as compared to protocols with prepulses that lie within the saturating regions of the activation time or conductance vs. voltage curves. As the concentration is increased and activation is therefore shifted toward more positive voltages, prepulses near the reversal potential would tend to activate fewer channels, complicating analysis of an apparent change in conductance of a population of channels. However, as noted in the Methods, we used prepulse voltages that saturated the I-V relation for all concentrations and scaled the conductance measured for brief prepulses to the total number of open channels measured for a long pulse to that voltage. Thus, we feel confident that the gating changes noted above do not account for the concentration-dependent changes in K\(^+\) conductance. The decline in conductance at high concentrations could also be due to “death” of channels in these
solutions. However, since the conductance again increased during recovery to the 500 mM symmetrical case (e.g., Fig. 2), loss of channels cannot explain the conductance changes observed. For these reasons, we believe the conductance-activity relationships reported here represent changes in channel conductance rather than changes in channel numbers. However, without single channel studies and the availability of an inert cation, changes in channel numbers cannot be entirely ruled out.

In the gramicidin channel, where wide activity ranges have also been tested, saturation and subsequent declines in conductance have been demonstrated. Hladky and Haydon (1972) saw a decline in Cs⁺ conductance for gramicidin in lipid bilayer membranes when they elevated the Cs⁺ concentration to 5 M. The single channel conductance of gramicidin channels has also been found to decrease at high K⁺ activities (Eisenman et al., 1978). Thus, there is experimental precedent at the single channel level for our observation of conductance decline. The energy barrier profile that best fitted the gramicidin data was an extension of a three-barrier model to include binding sites external to the lateral barriers at the channel mouths (three-barrier, four-site model; Eisenman and Sandblom, 1983).

**I-V Relationships Reveal Profile Shapes**

Eisenman and Sandblom (1983) have reported distinctive differences in I-V shapes in the gramicidin A channel when current is carried by either Cs⁺, Rb⁺, K⁺, or Na⁺. These observations were only apparent when ionic activity was kept quite low, where the shapes of the I-V relations reflected the voltage dependence of the ion's entry step. More recently, species-dependent I-V shapes have been described for single large-conductance Ca-activated K channels in planar lipid bilayers (Eisenman et al., 1986). We have observed ion-specific and concentration-dependent I-V shapes when squid axon K channels are exposed to symmetrical concentrations of K⁺, NH₄⁺, or Rb⁺.

The fairly linear I-V relationships for symmetrical K solutions are usually thought to indicate that the energy barrier profile for K⁺ is fairly symmetrical or has many barriers and wells. However, by carefully choosing the positions and energies of the wells and highest barrier, Cukierman et al. (1985) modeled linear I-V relationships equally well with a four-barrier asymmetric or a three-barrier symmetric profile for the K channel of sarcoplasmic reticulum. Although I-V relationships can be made more or less linear by changing barrier heights or well depths (Woodbury, 1971; Hille, 1975b), for both static and fluctuating single-site models, I-V shapes can be relatively insensitive to barrier or well position (Eisenman and Dani, 1986). However, we have found that with the three-barrier, two-site model, I-V shapes are extremely sensitive to external barrier positions. For example, even though fairly linear relationships could be generated with a number of different energy profiles, the changes in the shapes of the current-voltage relationships as a function of K⁺ concentration were most consistently fitted by systematic shifts in barrier positions. This modification provided fits for both the symmetric and asymmetric case at all concentrations as well as giving the best fit to relief of internal Cs⁺ block with elevations in the external K⁺
concentration. No other change in parameters provided adequate fits to all these data.

The nonlinear ammonium and rubidium I-V relationships suggest an asymmetric profile for these less conductive ions with a deeper well on one side of the membrane. Consistent with this, the profiles chosen to best represent our experimental results include asymmetric well depths with the deeper well placed closest to the outer electrical surface. In a multi-ion asymmetric channel, the currents are predicted to be largest when the ions traverse the highest barrier first (Hille and Schwarz, 1978). This prediction is consistent with the Rb and NH$_4$ data sets and the fits in Fig. 8. Here outward currents are larger than inward currents and correspondingly G34 is larger than G12. The supralinearity in the I-V relations for these two ions is similar to that seen recently in single Ca-activated K channels (Eisenman et al., 1986).

**Permeability Ratios Reflect More than Relative Barrier Energies**

As with the nonlinear I-V shapes for NH$_4^+$ and Rb$^+$, permeability ratios, which depend on the direction of current flow across the channel, suggest an asymmetric energy profile. Both experimental and modeled permeability ratios are greater when either NH$_4^+$ or Rb$^+$ flows from inside the cell to the external bath.

Recently, Matteson and Swenson (1986) have described three-barrier, two-site profiles for the squid K channel based upon instantaneous I-V relationships obtained with external ion substitution. Although the energy levels used to fit the present data are in general similar to theirs, our profiles have shallower wells for K$^+$, Rb$^+$, and NH$_4^+$ and lower barriers to Rb$^+$ and NH$_4^+$. Assuming fairly symmetrical placement of wells and barriers, the NH$_4^+$ profile described by these authors produces low permeability ratios with higher ratios for external substitution of NH$_4^+$ as well as an NH$_4^+$/K$^+$ conductance ratio $>1.0$ in symmetrical solutions. Their profile for Rb$^+$ simulates permeability ratios that are higher than those found experimentally with similar ratios for external or internal substitution.

The K channel profiles that fit our data do not meet the offset energy peak condition (Hille, 1975b); i.e., the barrier peaks for different permeant ions differ by more than an additive constant. Since this condition (for which there is no experimental evidence) is not met, and since well depths may play a role in determining selectivity for this channel, the flux equations for this system cannot be easily simplified to derive the Goldman-Hodgkin-Katz voltage and current equations. Even though these equations may not hold strictly (Hille, 1975b), almost identical permeability ratios were determined with Eq. 1 using either experimentally determined reversal potentials or those generated by the proposed energy profiles.

**Permeability and Conductance Ratios Depend on Activity**

In the K$^+$-selective sarcoplasmic reticulum channel, not only does conductance saturate as a function of activity, the $g_K/g_Na$ also increases and finally saturates. However, the $P_K/P_Na$ ratio remains constant with activity changes (Coronado et al., 1980). This behavior is fitted by a single-site model. Some experimental
evidence suggests that the voltage-dependent Na channel is also a single-site pore. However, for the squid axon Na channel, the \( P_{Na}/P_K \) ratio is apparently a function of the internal K\(^+\) concentration, an observation that is inconsistent with single occupancy (Cahalan and Begenisich, 1976; Ebert and Goldman, 1976). The gramicidin channel, now widely considered a multi-ion channel, has been found to exhibit permeability and conductance ratios that vary in opposite directions as activity is increased (Myers and Haydon, 1972). We have determined that, for the squid axon K channel, not only do permeability and conductance ratios change with activity, but also the pattern of change is unique for each ion. The very fact that the ratio changes are different for each ion makes a common phenomenon such as an osmolality change less likely to be responsible for our findings.

**Cs\(^+\) Block and Relief Can Be Described by the Three-Barrier, Two-Site Model**

The voltage-dependent block by internal Cs\(^+\) is not a new finding. Our value for the apparent electrical distance to the Cs\(^+\) blocking site of 1.03 is an underestimate owing to the assumption that the outward current in the absence of Cs\(^+\) was linear, clearly at odds with asymmetrical potassium \( I-V \) relations (e.g., Fig. 7B). Although it has previously been shown that external K\(^+\) could relieve block by internal Cs\(^+\), it remained unclear whether this was a specific effect of K\(^+\). At the concentrations used in our experiments, the less permeant NH\(_4^+\) and almost impermeant Cs\(^+\) were ineffective both in relieving the Cs\(^+\) block experimentally and in computer simulations.

French and Shoukimas (1985) were able to qualitatively account for block of K channels by internal Cs\(^+\) using a simplified double-occupancy, three-barrier, two-site model where the well depths were fixed at 0 RT. Their Cs profile incorporates a rate-limiting exit barrier (G12) to account for the block. As pointed out by these authors, this raises the paradox that both G12-Cs and G34-Cs must be rate-limiting to account for voltage-dependent block by external and internal Cs\(^+\) in the same channels. In this regard, by making the central barrier of our Cs profile rate-limiting, we have been able to successfully simulate both bidirectional Cs\(^+\) block and relief by K\(^+\) using the same profile.

**Anomalous Mole Fraction Effects Reveal the Limits of This Three-Barrier, Two-Site Model**

The anomalous mole fraction effect has been found in several types of K channels, Ca channels, and the gramicidin channel (Neher, 1975; Hagiwara et al., 1977; Ashcroft and Stanfield, 1983; Almers and McClesky, 1984; Hess and Tsien, 1984; Eisenman et al., 1986), although only recently reported for a delayed rectifier K channel (Plant, 1986). If, as suggested by Hille and Schwarz (1978), such minima require interactions among ions and their channel binding sites and are functions of the relative occupancies of these sites, the three distinct minima suggest either different relative \( X^+ / K^+ \) binding site affinities for each test ion or different electrical distances to the binding sites. The profiles chosen for NH\(_4^+\), Rb\(^+\), and Tl\(^+\) have both different binding site affinities and different positions for binding. We attempted to simulate an anomalous mole fraction effect using
our K and NH₄ profiles. A minimum in slope conductance could not be obtained. However, a minimum in chord conductance, as measured by others, was produced by the model at 10% K⁺. This apparent discrepancy reflects changes in I-V shape at very positive or negative voltages for progressive increases in the K⁺ concentration (linear I-V) at the expense of the NH₄ concentration (nonlinear I-V). Slope conductances measured near the reversal potential are nearly insensitive to I-V shape changes, whereas chord conductances determined using current values distant from the reversal potential are influenced by such changes in shape.

Perhaps a model with more binding sites would have allowed a fit to the anomalous mole fraction slope conductance data. Effects on net current contributed by an additional binding site, perhaps important for both ion types, could be significant. However, it seems equally possible that alterations in one ion's profile due to the presence of another type of ion in the channel might affect net current near the reversal potential and therefore slope conductance. In addition, complex changes in both profiles as a function of changing concentrations of each permeant species could be important.

Conclusions

After manipulating the parameters to achieve those profiles that best fit our squid axon K channel data, certain generalizations can be made. Such generalizations should be helpful to others attempting to use such a model. First, small changes in well depths lead to large changes in dissociation constants and therefore current magnitude. Second, changes in well depths or barrier heights cause changes in the limiting rate of ion exit and therefore affect the magnitude of conductance. Third, the positions of wells and barriers largely determine the I-V shape and these positions are apparently concentration dependent. Fourth, permeability ratios are sensitive to both the differences in barrier heights between two ion types and the position and depth of the wells. Furthermore, the barrier heights for different ions may not be different by a constant additive amount. This asymmetry in barrier peak differences, plus the importance of binding site affinities and positions should not be ignored in investigations of channel selectivity. Finally, parameters that provide the best fit to one experimental observation frequently do not provide the best fit to another, making compromises in the profiles necessary. This final observation could reflect limitations of static three-barrier, two-site models, limits of the data, or the need for a high-resolution, objective fitting algorithm when approaching such a large data set. Future attempts at refining model fits to the data will probably require a more detailed examination of numbers of binding sites, concentration dependence (Eisenman and Sandblom, 1983), or incorporation of time-dependent energy profiles (Lauger, 1984; Eisenman and Dani, 1986).

The squid axon K channel is undoubtedly a multi-ion channel. The historical evidence for this has been supplemented by a test of the theoretical predictions for such a channel. The experimental measurements have provided data to further the development of a model for cation transport through this channel. The profiles provide some surprises in their lack of symmetry, their concentration...
dependence, and the disparate well positions for the different ions. They are surely not the only profiles that could be generated to fit the data. They do, however, suggest directions for more sophisticated attempts at this difficult task.

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