

An Ion's View of the Potassium Channel

The Structure of the Permeation Pathway as Sensed by a Variety of Blocking Ions

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ABSTRACT We have studied the block of potassium channels in voltage-clamped squid giant axons by nine organic and alkali cations, in order to learn how the channel selects among entering ions. When added to the internal solution, all of the ions blocked the channels, with inside-positive voltages enhancing the block. Cesium blocked the channels from the outside as well, with inside-negative voltages favoring block. We compared the depths to which different ions entered the channel by estimating the "apparent electrical distance" to the blocking site. Simulations with a three-barrier, double-occupancy model showed that the "apparent electrical distance," expressed as a fraction of the total transmembrane voltage, appears to be less than the actual value if the blocking ion can pass completely through the channel. These calculations strengthen our conclusion that sodium and cesium block at sites further into the channel than those occupied by lithium and the organic blockers. Our results, considered together with earlier work, demonstrate that the depth to which an ion can readily penetrate into the potassium channel depends both on its size and on the specific chemical groups on its molecular surface. The addition of hydroxyl groups to alkyl chains on a quaternary ammonium ion can both decrease the strength of binding and allow deeper penetration into the channel. For alkali cations, the degree of hydration is probably crucial in determining how far an ion penetrates. Lithium, the most strongly hydrated, appeared not to penetrate as far as sodium and cesium. Our data suggest that there are, minimally, four ion binding sites in the permeation pathway of the potassium channel, with simultaneous occupancy of at least two.

INTRODUCTION

The potassium channel of nerve membranes is highly selective for ions that pass through its pore, but it appears to be almost completely nonselective for entry

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of ions into its inner mouth. Only potassium, rubidium, ammonium, and thallium readily carry current through the channel (Binstock and Lecar, 1969; Hille, 1973), but an enormous variety of ions impede current flow when they are present on the axoplasmic side. These blocking ions include alkali cations (Chandler and Meves, 1965; Adelman and Senft, 1966; Bergman, 1970; Bezanilla and Armstrong, 1972), barium (Eaton and Brodwick, 1980; Armstrong and Taylor, 1980; Armstrong et al., 1982; Woll, 1982), and a large array of quaternary ammonium ions (Armstrong and Binstock, 1965; Armstrong, 1969, 1971; Armstrong and Hille, 1972; French and Shoukimas, 1981; Swenson, 1981). These observations suggest that the delayed rectifier channel of nerve possesses a relatively large, nonselective mouth at its axoplasmic end ($\sim 100 \text{ \AA}^2$ in cross-sectional area) and a much narrower, highly selective tunnel ($\sim 10 \text{ \AA}^2$) extending toward the extracellular surface of the membrane (for reviews, see Armstrong, 1975; Latorre and Miller, 1983). These structural conclusions have been based almost exclusively on studies of permeation and of block by large quaternary ammonium ions.

The larger quaternary ammonium ions probe a highly restricted portion of the channel. From the dependence of the degree of steady state block on voltage, they appear to pass through 14–20% of the total transmembrane voltage (Hille, 1975; French and Shoukimas, 1981; Swensen, 1981). The action of numerous other blocking ions is much more steeply modulated by voltage. Of these, two ion species, cesium (Adelman and Senft, 1968; Bezanilla and Armstrong, 1972; Dubois and Bergman, 1975, 1977; Adelman and French, 1978; Clay and Shlesinger, 1983) and barium (Armstrong and Taylor, 1980; Armstrong et al., 1982), block the channel from either the external or the internal side. Sodium does not block the channel significantly from the outside (Adelman and French, 1978, Fig. 1), but internal sodium causes a strongly voltage-dependent block that is partially relieved at very high voltages by sodium passing completely through the channel (French and Wells, 1977). The use of these and other steeply voltage-dependent blockers offers a means of probing the deeper reaches of the channel.

Blocking ions may be empirically classified according to their strength of binding and their kinetics of action. The blocking alkali cations (Bezanilla and Armstrong, 1972) and some small organic ions are effective at concentrations in the 10–100-mM range, and the block equilibrates essentially instantaneously following a voltage step (see also French and Wells, 1977; Adelman and French, 1978; French and Shoukimas, 1981). Larger quaternary ammonium ions bind sufficiently tightly that they are effective in the submillimolar range and show relaxation time constants on the order of milliseconds. For alkyl-triethylammonium and tetraalkylammonium ions, variation of these relaxation times is dominated by the dissociation rate constants, which decrease as the alkyl chain length is increased (Armstrong, 1969; French and Shoukimas, 1981). Thus, binding strength increases with increasing alkyl chain length, and the presence of hydrophobic groups in the vicinity of the channel mouth can be inferred. In this way, studies of these blocking interactions have yielded chemical as well as geometric information about the channel structure.

The fact that the blocking ions mentioned are either impermeant or very

weakly permeant, even though they enter the channel easily, indicates that the energy barrier to movement forward from the blocking site effectively approaches infinite height. The kinetics and voltage dependence of the blocking reaction thus reflect the process of penetration up to that point. Consequently, these blocking reactions allow us to experimentally dissect out and study one part of the energy barrier profile in the permeation pathway. Here we have adopted this approach in an attempt to determine how the energetic profiles that the channel presents to different ions differ from one another.

We conclude that depth of penetration into the channel is inversely related to the size of the entering ion. However, chemical structure, as well as size, plays a role in determining the fraction of the channel to which a blocking ion has access. Ions with hydroxyl groups on their surfaces appear to penetrate further than those with only unsubstituted alkyl chains. Functional size, which determines the depth of penetration, also appears to depend on the degree of hydration. Preliminary reports of some of this work have appeared (French et al., 1979, 1981; French and Shoukimas, 1979).

EXPERIMENTAL METHODS

Voltage Clamp and Perfusion

Experiments were performed on medial giant axons from the squid, *Loligo pealei*, using standard axial wire voltage-clamp and internal-perfusion techniques (for details, see French and Shoukimas, 1981; French and Wells, 1977). Between pulses, the membrane potential was held at -60 or -80 mV (inside minus outside). Except for the data in Figs. 3 and 5 (previously unpublished data of R. J. French and J. B. Wells), the linear leakage and capacitive components were removed by summing the digitized record from the command pulse sequence with records from two pulse sequences of half the amplitude and of opposite polarity. We routinely compensated for $2-3 \Omega \cdot \text{cm}^2$ series resistance. The chamber temperature was held at $8 \pm 0.2^\circ\text{C}$ by a Peltier device, unless otherwise indicated.

Prevention of Periaxonal K Accumulation

To compare the action of blocking ions that may compete with K for intrachannel sites, it was necessary to control the K concentration at both the inner and outer surfaces of the axolemma. Thus, the external K concentration was raised to 300 mM and a prepulse to the nominal reversal potential (0 mV) was used to activate a large fraction of the K conductance without passing any appreciable current through the channels (Figs. 6 and 8). Thus, no periaxonal accumulation (Frankenhaeuser and Hodgkin, 1956; Adelman et al., 1973) occurred and the reversal potential at the end of a 30-ms prepulse remained very close to 0 mV (Figs. 7A and 9A). Prepulses to voltages that fully activate the K conductance produce significant shifts in the reversal potential even with 300 mM external K. For these experiments, the use of a prepulse that activates a fixed fraction of the conductance allows a straightforward quantitative comparison of the effects of the different ions, since the block equilibrates essentially instantaneously after a voltage step, before any change in the number of open-gated channels can occur. The analysis does not require that all of the conductance be activated.

A nominal (before junction potential correction) holding potential of -80 mV was used for experiments with 300 mM K outside since we observed that the holding current was minimized ($\sim 10-20 \mu\text{A} \cdot \text{cm}^{-2}$) at this voltage.

Solutions

External solutions contained 0.5–1.0 μM tetrodotoxin (TTX), 10 mM CaCl_2 , and 50 mM MgCl_2 and were buffered with 10 mM Tris-Cl to pH 7.0 at $\sim 20^\circ\text{C}$. In addition to these constituents, 10 mM K TTX artificial seawater (ASW) contained 10 mM KCl and 430 mM NaCl, while 300 mM K TTX-ASW contained 300 mM KCl and 140 mM NaCl.

The standard internal solution in these studies contained (in mM): 300 K^+ , 50 F^- , 300 glutamate $^-$, 25 HPO_4^- , and 505 sucrose. Free glutamic acid was added to give a pH of 7.1 ± 0.1 . To make the solutions designated "100 X, 300 K," 200 mM sucrose was replaced by 100 mM of the desired salt (cf. French and Wells, 1977). Fluoride salts (Alpha Products, Danvers, MA) were used for Na and Cs, and the chlorides of tetramethylammonium (TMA) (Eastman Kodak Co., Rochester, NY), D-glucose-ammonium (GA), and tris(hydroxymethyl)aminomethane (Tris) (Sigma Chemical Co., St. Louis, MO) were used.

Since lithium fluoride and phosphates are not highly soluble, studies on lithium were done using a control solution containing (mM): 200 KCl, 100 KF, 5 Tris-Cl, and 470 sucrose. 100 mM LiCl (made from the hydroxide; Alpha Products) was substituted for sucrose to make the 100 mM Li, 300 mM K solution. The inclusion of 5 mM Tris in these solutions caused a slight degree of channel block, as noted later in the paper. Similar solutions, with methylammonium instead of lithium, were used in one experiment.

Tetrakis(1-hydroxyethyl)ammonium bromide (tetrakis), obtained from Eastman Kodak Co., was recrystallized from water by addition of ethanol, washed with acetone, and dried under vacuum before use. Tetrakis is a significantly more potent blocker than the ions mentioned above; thus, for the studies reported here, 10 mM tetrakis-Br, rather than 100 mM, was used to produce a convenient degree of block.

N-methylstrychnine (NMS) iodide was prepared by methylation of strychnine free base with an excess of methyl iodide (both reagents from Aldrich Chemical Co., Milwaukee, WI) as described by Shapiro (1977). The white precipitate of NMS was recrystallized from water, washed with cold methanol, and dried under vacuum before use. Since NMS blocks K channels at submillimolar concentrations, it was dissolved directly in the standard 300 mM K control solution, and then diluted with more 300 mM K solution to give the desired NMS concentrations in the range of 0.1–1.0 mM.

Junction potentials were measured as previously described (French and Wells, 1977). Corrections for measured junction potentials have been made in all of the current-voltage and conductance-voltage relations. Nominal voltages, before junction potential corrections, are indicated on the original records.

Quantitative Analysis of Conductance-Voltage Relations

Chord conductances were derived from the instantaneous current-voltage relations using the expression

$$g(E) = I(E)/(E - E_K), \quad (1)$$

where $g(E)$ is the conductance and $I(E)$ is the current at voltage, E . The conductance obtained in the presence of a blocking ion, g_{x+k} , was fit to the following relation:

$$g_{x+k}(E) = rg_K(E)/\{1 + \exp[szF(E - E_{0.5})/RT]\}, \quad (2)$$

where g_K is the conductance obtained with the control solutions; $r = \lim_{E \rightarrow -\infty} g_{x+k}(E)/g_K(E)$ as $E \rightarrow -\infty$ (or $+\infty$ for Cs acting externally); $z = 1$, the valence of all blocking ions used in this study; and F , R , and T are the Faraday constant, the gas constant, and the temperature (in degrees Kelvin), respectively. $E_{0.5}$ is the voltage at which half the channels are blocked, and s is the apparent electrical distance from the membrane-solution interface to the blocking site, expressed as a fraction of the total transmembrane voltage per unit charge

on the blocking ion. The product, sz , is often termed the "apparent valence" of the blocking ion.

Treating the blocking as if it were a one ion per channel reaction, the apparent dissociation constant at $E = 0$ is

$$K_x = [X]\exp(-szFE_{0.5}/RT), \quad (3)$$

where $[X]$ is the concentration of the blocking ion. The analysis is equivalent to those of Woodhull (1973) and Strichartz (1973), who studied block of Na channels in frog node.

Simulation of Instantaneous I-E Relations

Rate theory calculations of open channel currents were carried out for a three-barrier, double-occupancy channel with two ion species that were able to enter the channel. The general approach is similar to that of Hille and Schwarz (1978), but the system of nine equations for the different occupancy states of the channel was solved by direct matrix inversion (cf. Begenisich and Cahalan, 1980a). For the calculations shown, each of the energy barriers was symmetric and spanned one-third of the total voltage drop across the membrane. Although some calculations, including ionic repulsion effects, were done, and these are important in simulating "cross-over effects" (Hille and Schwarz, 1978), none are presented here. The major quantitative deficiency of our simple simulations shown is that the control I - E relations bend upward too sharply at large depolarizations.

The goals of the calculations that we present are limited to (a) demonstrating the capability of this class of model to generate I - E curves of the forms that we observed, and (b) showing the effect, on the empirical parameters used to describe the data, of making the blocking ion sufficiently permeant to allow appreciable relief of block at high voltages.

The "barriers" are purely operational and imply no particular physical mechanism, which leaves open the possibility that the limiting barrier may represent such phenomena as either pore distortion, under stress from an ion in the field, or the stripping of a crucial water molecule to dehydrate an ion sufficiently for it to pass on through the channel. In either case, the energy to overcome the limiting barrier could originate from the action of the electric field on an ion moving along the channel (cf. Begenisich and Cahalan, 1980b).

A completely different theoretical approach was used by Clay and Shlesinger (1983, 1984) to describe voltage-dependent block. Their model, based on a strict "knock-on" mechanism, adequately describes the blocking phenomena, but, as they explain, does not account for the voltage-dependent flux ratio exponents observed by Begenisich and De Weer (1980). A strict knock mechanism is consistent with, but not proven by, the data of J. R. Clay (manuscript submitted for publication).

RESULTS

"Instantaneous" Block by Internal Ions, with Physiological K Concentrations

Figs. 1 and 2, illustrating the effect of internal Li, show the general features of the instantaneous, voltage-dependent block of K channels analyzed in this paper. In Fig. 1B, it is clear that the block reaches a new steady state level within 60 μ s of voltage step. The block is favored by membrane depolarization to an extent sufficient to produce a region of negative slope in the instantaneous I - E relation (Fig. 2A). The dominant feature in the conductance-voltage relation (Fig. 2B) is the steep decrease in conductance at positive voltages, but two more subtle points ought to be noted. First, in the control, a slight decrease in conductance at

positive voltages is due to the blocking action of Tris (French and Wells, 1977; and the following section of this paper). Tris was used to buffer these solutions only because of the low solubility of lithium phosphate, phosphate being the internal solution buffer for the other experiments. Control I - E curves rose linearly at high voltage when phosphate was the internal buffer (e.g., Figs. 3 and

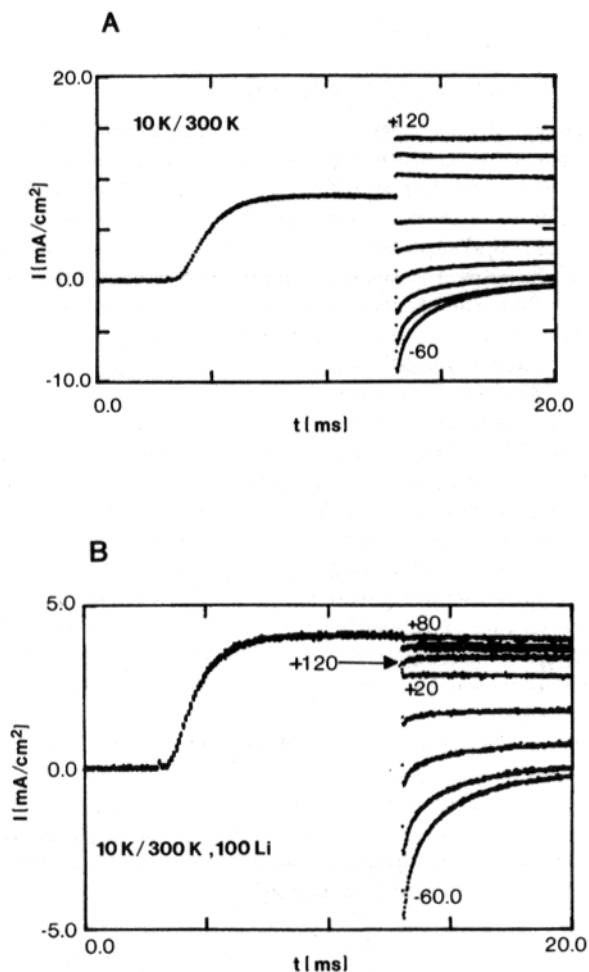


FIGURE 1. K channel block by internal Li, with a low-K external solution (10 mM K TTX-ASW). Sample intervals: prepulse, 50 μ s/pt; test pulse, 10 μ s/pt. (A) Control voltage-clamp current records for steps to $E = -60$ to +120 mV (except $E = 60$) following a 10 ms prepulse to $E = 60$ mV. The cations in the internal solution were 300 mM K and 5 mM Tris. Experiment F78.05; temperature, 8°C. Nominal holding potential, -80 mV. (B) A family of records, as in A, except that there was 100 mM Li in the internal solution. The current at the beginning of the test pulse decreases with increasing voltage above $E = 80$ mV, which indicates an instantaneous, voltage-dependent block of the current by the Li ions.

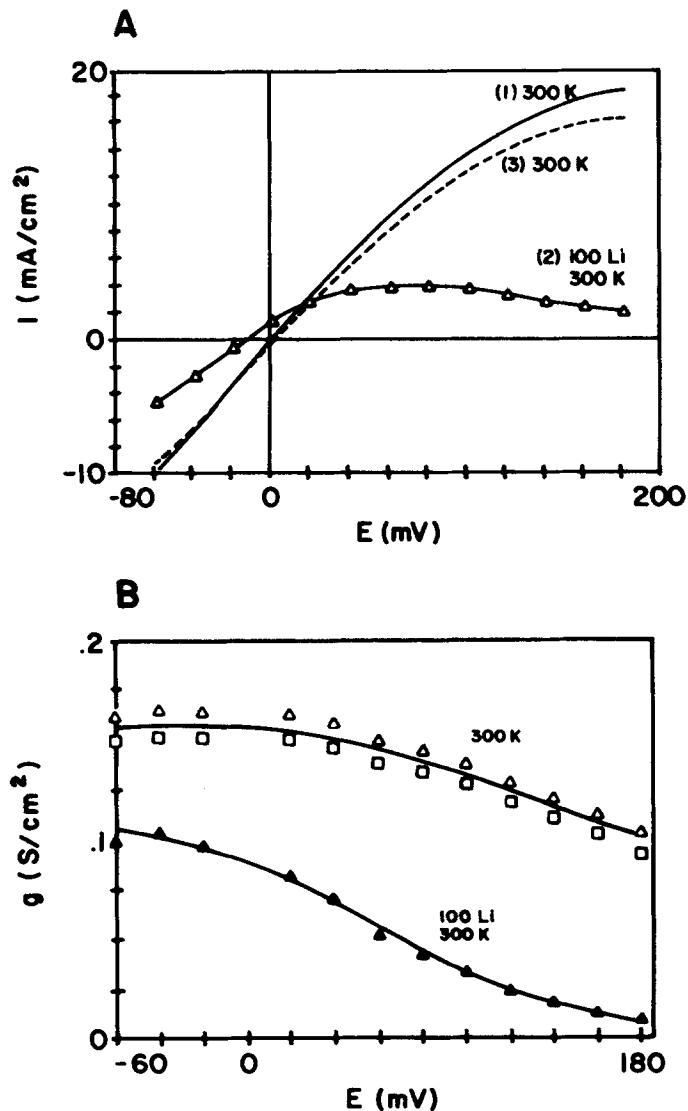


FIGURE 2. Voltage-dependent block of K channel currents by internal Li ions. (A) Instantaneous current-voltage relations from the experiment shown in Fig. 1, obtained by plotting the current at $60 \mu\text{s}$ after the end of the prepulse against the test voltage. Note the decrease in current as the test potential is increased above $E = 80 \text{ mV}$, producing a region of negative slope in the I - E relation. Numbers in parentheses indicate the order in which the sets of I - E data were obtained. (B) Chord conductances determined from the I - E relationships in part A. The slight decrease in conductance at positive voltages in the control case is due to the use of Tris (5 mM) as a buffer. Addition of 100 mM Li causes a strong voltage-dependent reduction in the conductance. The smooth curve through the Li data is derived from the control data using Eq. 2 in the text.

5). Second, the reduced conductance at the most negative voltages is due largely, if not entirely, to block of outward currents at the prepulse voltage (+60 mV) rather than at negative test pulse levels. Reduced prepulse currents lessen the accumulation of K in the periaxonal space during the prepulse, as suggested by the more negative reversal potential in the presence of Li (cf. Bezanilla and Armstrong, 1972). The lower periaxonal K concentration is responsible for the reduction of the limiting value of the conductance at negative potentials when the internal blocking ion is present. Because of the difficulty of controlling periaxonal K accumulation with low-K (10 mM) external solutions, quantitative

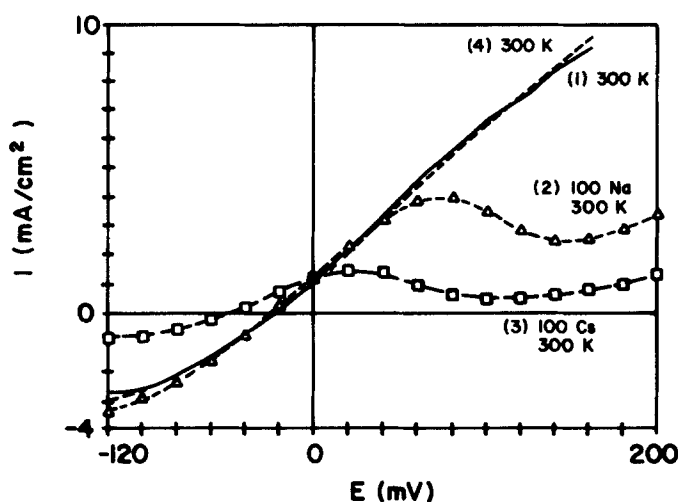


FIGURE 3. Relief of block by Na and Cs ions is seen at highly positive voltages, in contrast to the data in the preceding figures. Instantaneous I - E relations are plotted. Note that current increases with increasing voltage above $E = 100$ – 140 mV. In this case, current was measured at $100 \mu\text{s}$ after the voltage step, without summation of records to remove slow components of the capacitive transient, as in French and Wells (1977). Axon bathed in 10 mM K TTX-ASW. Experiment 76.49; nominal holding potential, -60 mV; temperature, 9.0 – 9.4°C .

comparison of the block by different ions rests primarily on experiments in which the K concentration was fixed at 300 mM both inside and outside the axon. Nonetheless, it is useful to make a brief, qualitative comparison of the action of different blocking ions using external solutions containing physiological levels of K.

Figs. 3 and 4 illustrate the block of K channel currents by Na, Cs, GA, Tris, and TMA. All of these ions, present at 100 mM inside the axon, produce a region of negative slope in the instantaneous I - E relation. A notable qualitative difference between the data for Na and Cs (Fig. 3) and those for the other ions is that at ~ 100 (for Cs) and 140 mV (for Na), the I - E relation turns upward and current again increases with increasing voltage. A detailed series of experiments by French and Wells (1977) led to the conclusion that Na is forced through the

channel at high voltages, relieving the block. The same interpretation also appears to apply to Cs, since 40 mM TEA in the internal solution blocks essentially all of the outward current seen with 100 mM Cs, 300 mM K inside, even at voltages up to +240 mV (French, R. J., and J. B. Wells, unpublished data).

The three organic ions, whose actions are compared in Fig. 4, block the currents in a manner very similar to Li (Figs. 1 and 2). There is no upward bend of the I - E curves up to $E = 180$ mV, and thus we have no indication of relief of block caused by sluggish permeation by the blocking ion for any of these ionic species. This is not surprising for the organic ions since all three have diameters

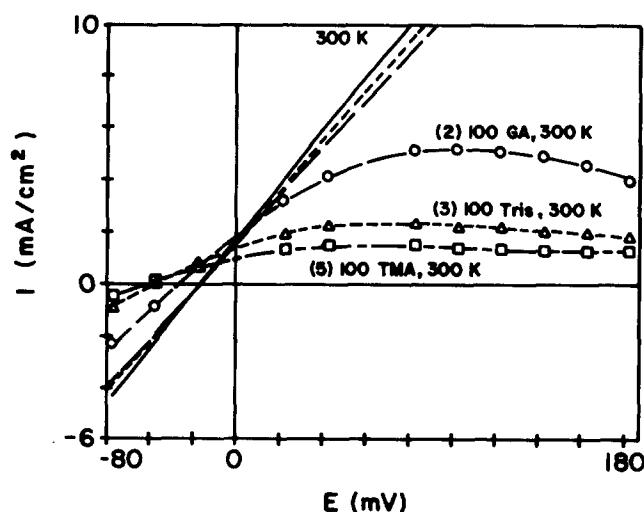


FIGURE 4. GA, Tris, and TMA each blocks K channels in a manner similar to the action of Li ions. Instantaneous K channel current-voltage relations determined as in Fig. 2, with 100 mM GA, Tris, or TMA added to the internal solution. The replicate control runs obtained throughout the experiment indicate the reversibility of the block produced by these ions. Numbers in parentheses indicate the order in which the I - E curves were determined. Experiment 78.18; external solution, 10 mM K TTX-ASW; nominal holding potential, -80 mV.

greater than the 3-Å upper limit, noted by Hille (1973), for ions that can pass through the K channels in frog node of Ranvier.

The precise form of instantaneous I - E relations also depends on the concentration of the blocking ion, as illustrated for Cs in Fig. 5. The striking N-shaped I - E relation is seen for 25, 50, and 100 mM Cs, but for 5 and 10 mM, the degree of block is sufficiently slight that the curves merely show inflections that hint at the block-enhancing effects of moderate depolarizations. For the six ions compared in detail in the first sections of this study, a concentration of 100 mM provides a compromise that allows the ions' actions to be compared at the same ionic strength, and allows a wide modulation of the degree of block over experimentally attainable voltages. The latter feature is important for a comparison of the apparent site of action of these ions.

K Channel Block in the Presence of Symmetric K Concentrations

K channel block by alkali cations can be affected by changing the concentration of K on the opposite side of the membrane, both for internal (Bezanilla and Armstrong, 1972) and external (Adelman and French, 1978) blocking ions. Hence, we devised the following protocol, which allowed comparison of different

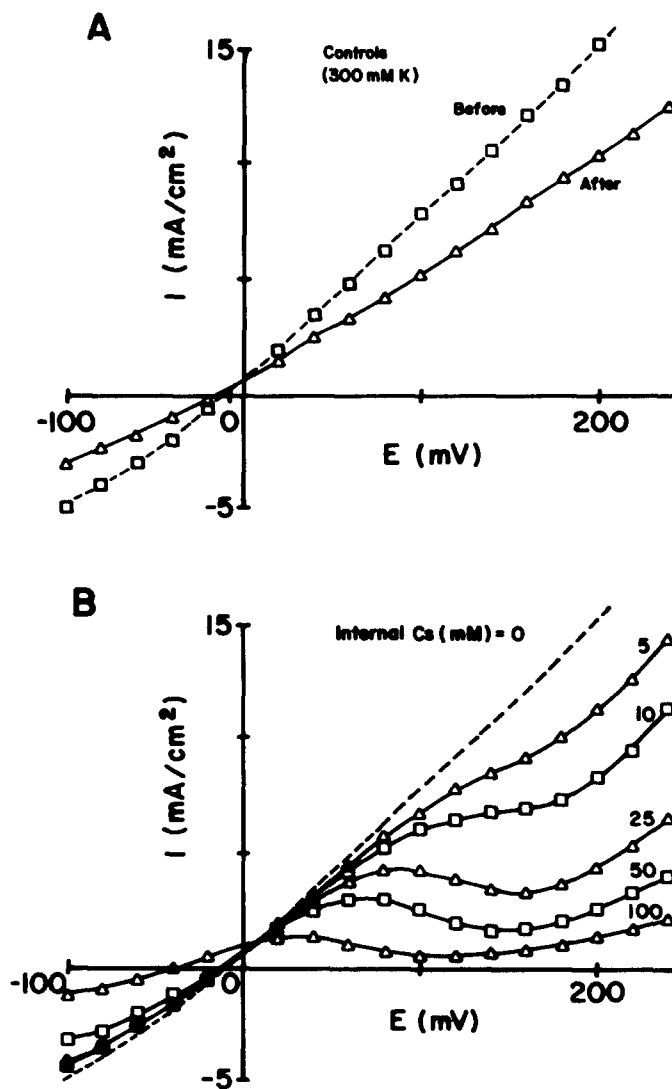


FIGURE 5. A family of instantaneous current-voltage relations, showing K channel block by internal Cs concentrations from 5 to 100 mM. External solution, 10 mM K TTX-ASW. Experiment 76.59; nominal holding potential, -60 mV; temperature, 8.8 – 9.4 °C. In this experiment, current was measured 100 μ s after the step to the test potential without any correction for the slow component of the capacitive transient. Data from an experiment by R. J. French and J. B. Wells.

blocking ions at the same ionic strength and fixed internal and external (periaxonal) K concentrations. Both internal and external solutions contained 300 mM K. A prepulse to the reversal potential then activated the conductance without passing appreciable current across the membrane (Fig. 6). Since no current flowed during the prepulse, the reversal potential for the instantaneous I - E

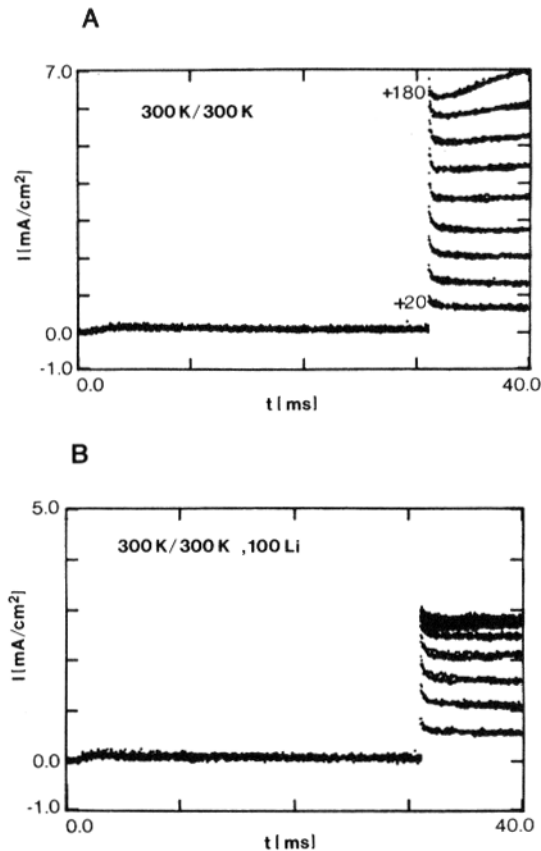


FIGURE 6. K channels may be activated by a prepulse to the reversal potential (0 mV) with internal and external K concentrations equal to 300 mM. Experiment F78.14; nominal holding potential, -80 mV. (A) Family of control current records for test voltage levels of $+20$ to $+180$ mV in 20-mV increments, following a 30-ms prepulse to 0 mV. Note that essentially no current flows during the prepulse, which eliminates the possibility of accumulation changing the periaxonal potassium concentration during this time. (B) Family of records collected with 100 mM Li, in addition to the 300 mM K, internally. The instantaneous current at the beginning of the test pulse decreases at the highest voltages (see Fig. 7A).

relations (Fig. 7A) matched the K equilibrium potential (0 mV) calculated from the solution concentrations (observed: 0.35 ± 0.44 mV, mean \pm SEM, from 46 I - E relations). The data of Fig. 7 allow two further points, important for quantitative analysis, to be made. At the most negative voltages, the conductances, with and without the blocker present, superpose. This suggests that (a)

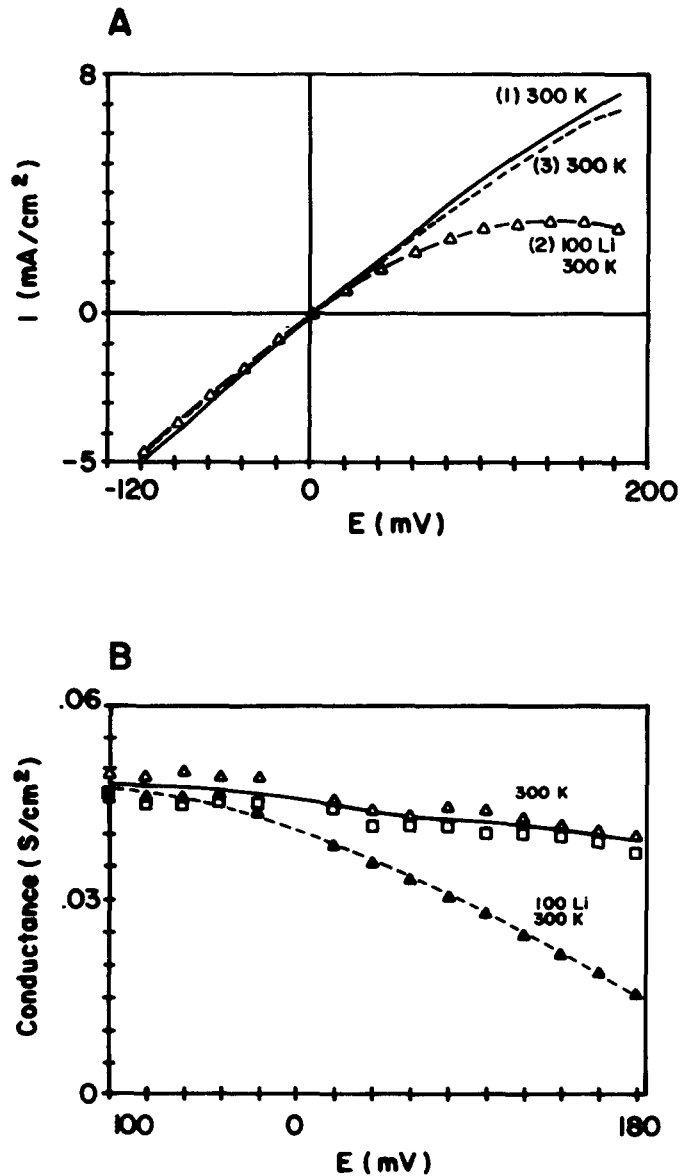


FIGURE 7. K channel block by internal Li, studied with 300 mM K both inside and outside. Experiment F78.14. Also see Fig. 6. (A) Instantaneous I - E relations. Note that there is no significant effect of Li on the current at negative voltages, and that the reversal potentials are approximately zero for all three curves. The lines connect the experimentally measured data points. (B) Chord conductance plotted against transmembrane voltage illustrating the voltage-dependent block by internal Li at positive voltages. The line through the control data is a smoothed curve through the average of the values before and after the exposure to Li. The line through the Li data points is the best fit to Eq. 2 in the text.

constant number of channels is opened during the prepulse, regardless of the presence or absence of the blocker, and (b) the blocking effect at these voltages is negligible.

The fact that the reversal potential remained close to zero when the blocking ions were added suggests that the blockers are effectively impermeant at this voltage. On the average, we observed a slight positive shift (controls: -0.54 ± 0.54 mV [$n = 25$]; 100 mM blocker: 1.4 ± 0.74 mV [$n = 21$]). This is a shift in the direction opposite from that expected if the blockers were significantly permeant and could be explained by a small (~6%) decrease in the activity coefficient for K ions when the ionic strength is raised by including the blocker in the internal solution. This is in the range of decreases in activity coefficients that are observed for a change in molality from 0.3 to 0.4 for a variety of K salts (Robinson and Stokes, 1959, Appendix 8.10, Table 11).

Block of the K channels by Li in the presence of symmetric 300 mM K is qualitatively similar to that seen with low-K external solutions in Figs. 1 and 2. In both cases, the slight droop in the control conductance at positive voltages can be attributed to the blocking action of Tris (5 mM), included as a buffer in the internal solutions. In studies on the other blocking ions, internal solutions were buffered with phosphate, and control conductances remained even more nearly constant throughout the range of voltages studied.

Current-voltage relations of similar form were obtained with Li, Na, Cs, Tris, TMA, and GA as the internal blocker. Using the 300 mM K TTX-ASW, no upturn in the I - E relations was seen, using Cs or Na as the blocker, over the range of voltages studied ($E \leq 180$ mV). Nonetheless, it is likely that the degree of block by these two ions represents a balance between their rate of entry and exit via the axoplasmic end of the channel and their occasional exit by passage onward through the channel. The effects of this phenomenon on a quantitative interpretation of the data are studied with the aid of a numerical simulation given later in the paper.

Thus, for the conditions described here, block is monotonically enhanced by positive voltages, which tend to drive the internally supplied blocking ions deeper into the channel. A rare, but complete, passage through the channel by Cs or Na implies that these ions have access to deeper regions of the channel than are apparently reached by Li, Tris, TMA, and GA. The quantitative analysis that follows suggests that the effective site of binding by Na and Cs is, in fact, deeper in the channel than that for the other four ions.

Block by External Cs Ions in the Presence of Symmetric K Concentrations

External Cs blocks inward current through the K channel, with enhanced block at negative voltages (Adelman and Senft, 1968; Bezanilla and Armstrong, 1972; Adelman and French, 1978). For the quantitative comparison here, we investigated block by 100 mM external Cs in perfused axons with K concentrations held at 300 mM inside and out as described above. The records are shown in Fig. 8, and the derived instantaneous I - E and g - E relations appear in Fig. 9. These data allowed us to verify, under better-controlled conditions, the analysis

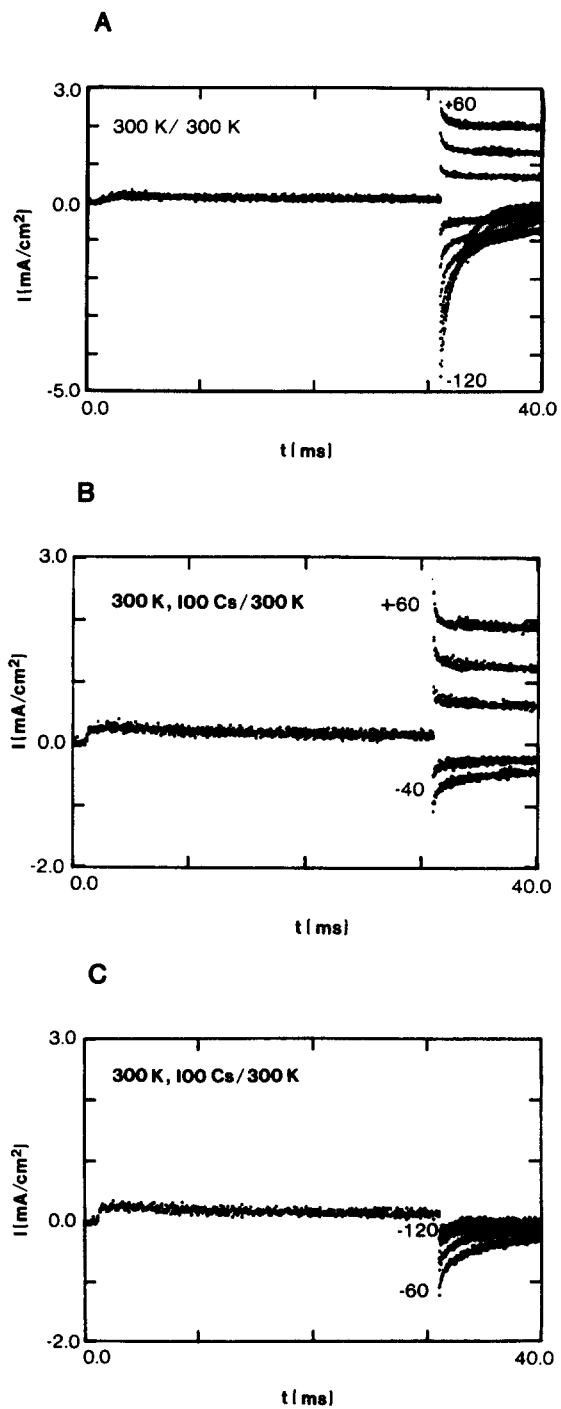


FIGURE 8.

of Adelman and French (1978). More important, the data yield quantitative information about the external mouth of the K channel, which complements that provided by the internal blockers. Cs is the only alkali cation (Adelman and Senft, 1968) that causes strongly voltage-dependent block of the squid K channel from the outside and thus it offers unique input for modeling the permeation pathway. Ba is the only other inorganic ionic blocker that has been shown to enter and block squid axon K channels from the outside (Armstrong and Taylor, 1980; Armstrong et al., 1982).

Quantitative Analysis: K Channel Block by Cs, Na, Li, Tris, TMA, and GA

We fitted all conductance-voltage relations determined for the six ions, including Cs added separately to internal and external solutions, using Eq. 2. This was done not so much to test the validity of a particular model as to provide an empirical, quantitative measure of the potency of each ion species as a blocker and of the efficacy of voltage in modulating the degree of block by each ion. Operationally, $E_{0.5}$, the voltage at 50% block, gives a measure of potency and is related to the apparent dissociation constant and the parameter s by Eq. 3. The apparent electrical distance, s , to the blocking site from the point of entry into the channel is determined by the steepness of the dependence of the fractional block on voltage (see Adelman and French, 1978). The third parameter, r , is the limiting fractional conductance at voltages that favor the unblocked state. Mean values of all these parameters for data obtained with 10 mM K TTX-ASW, as well as with 300 mM K TTX-ASW, are presented in Table I.

An examination of the values of r , the limiting fractional conductance, provides a reason for basing the quantitative comparison on measurements obtained with symmetric K concentrations. For 10 mM K TTX-ASW, r varied from 0.5 to 1.1, which indicates a significant reduction in conductance at negative voltages. For 300 mM K TTX-ASW, the r values clustered around unity (1.05 ± 0.03 , mean \pm SEM, from 21 fits). Thus, for these cases, block is completely relieved at negative voltages. Having the unblocked state well defined within each set (control, blocker, control) of I - E curves enables a more reliable comparison among the different blocking ions, and means that fewer assumptions are required to place a physical interpretation on values of s obtained from the fits.

Values of s for the 10 mM K TTX-ASW data range from 0.3 to 0.92, and are difficult to interpret because the periaxonal concentration of K at the instant of

FIGURE 8. (*opposite*) K channel block by external Cs ions, with 300 mM K in both internal and external solutions. Voltage-clamp current records resulting from an activating pulse to the reversal potential (0 mV) with subsequent steps to the nominal values of +60, +40, +20, -20, -40, . . . -120 mV. Experiment 78.14. (A) Control records, with K as the only cation present internally and externally. (B) Record taken with 100 mM Cs added to the external solution. The currents during the test pulse decrease monotonically as the voltage is made more negative in the range +60 to -40 mV. (C) With 100 mM external Cs, as the test pulse voltage is further decreased, currents become more positive (smaller in amplitude), which indicates that negative voltages enhance channel block by external Cs.

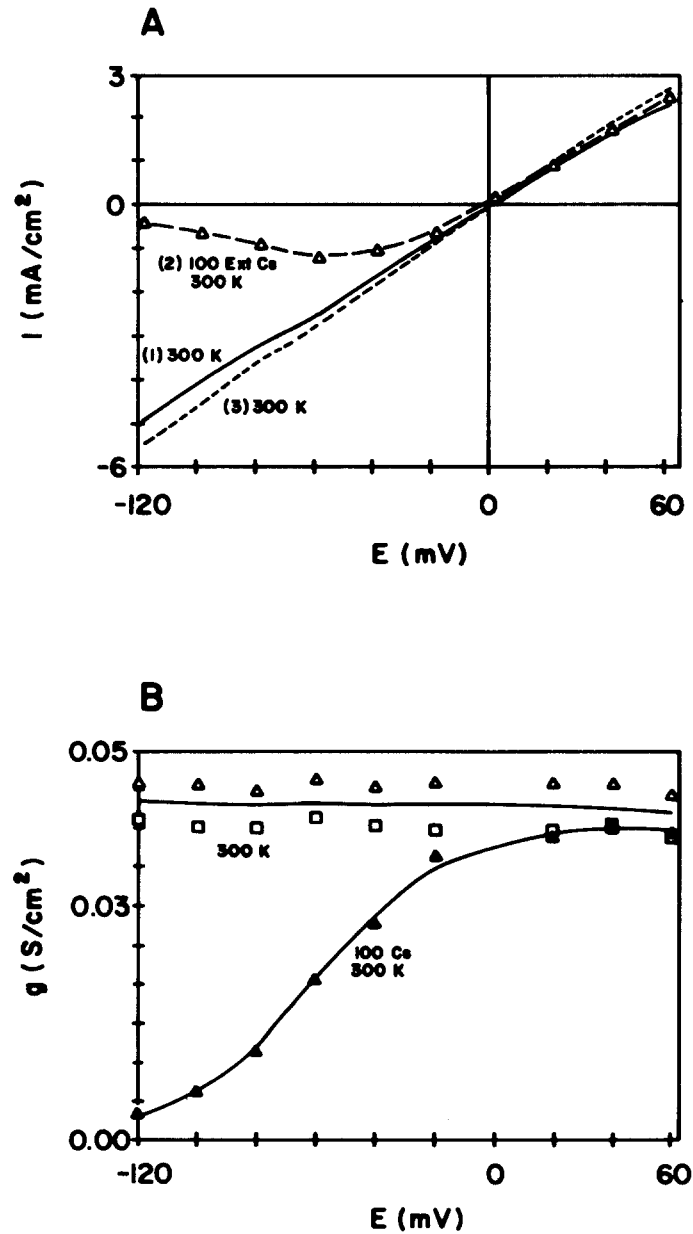


FIGURE 9. K channel block by external Cs. Note that the data qualitatively mirror those for block by internal Li ions (Fig. 7). For external Cs, however, the voltage dependence of block is much steeper (see text). Experiment 78.14. (A) Instantaneous current-voltage relations determined before exposure (1), during exposure (2), and after exposure (3), to external Cs. Lines connect the data points. (B) Chord conductances determined from the instantaneous $I-E$ relations in A. The curve through the Cs data was derived by a fit to Eq. 2 in the text, as in Figs. 2 and 7.

measurement, estimated from the reversal potentials, varied from 130 to 300 mM in the controls and from 50 to 150 mM with a blocking ion present. The best-fit value for s would be influenced by any factor that modifies the shape of the I - E curves, which here include direct changes caused by variation in the concentration of the conducting ion, K, and any possible interactions between the K ions and the blocker. Ideally, one would like to study the variation in the fraction of blocked channels with voltage as the only independent variable. This ideal appears to be closely approached in the experiments using symmetric 300 mM K solutions. In these experiments, the six blockers considered here fall into two functional groups (see Table II): Cs and Na, with $s = 0.48 \pm 0.03$ (mean \pm

TABLE I
Mean Parameter Values from Fit of Data to $g(E)^*$

Blocker		Li	Na	Cs	External Cs	GA	Tris	TMA
300	s	0.37	0.47	0.48	-1.09	0.34	0.32	0.37
	$E_{0.5}$	141	156	101	-44	155	78	37
	r	1.00	0.93	0.94	1.01	1.13	1.13	1.20
10	s	0.45	0.52	0.92	—	0.43	0.37	0.30
	$E_{0.5}$	68	104	55	—	114	52	-11
	r	0.81	1.10	0.75	—	0.76	0.54	0.54

$$* g(E) = \frac{g_s(E)r}{1 + \exp[sF(E - E_{0.5})/RT]}$$

All values are means from three axons except for GA, Tris, and TMA with $[K]_{ext} = 10$ mM. These three sets of values came from a single experiment. The negative sign of s for external Cs reflects that, for this case, s is a measure of "apparent electrical distance" from the outside toward the inside. All other values are positive and reflect the opposite orientation—inside to outside.

SEM, $n = 6$), and Li, GA, TMA, and Tris, with $s = 0.36 \pm 0.02$ (mean \pm SEM, $n = 12$). This difference is statistically significant ($0.01 < P < 0.001$, derived from a two-tailed, unpaired t test), and the simplest interpretation is that Cs and Na reside deeper in the channel when acting as blockers than do the other ions (see also Discussion and Fig. 12).

External Cs block varies even more steeply with voltage ($s = 1.09 \pm 0.07$, $n = 3$) than internal Cs block. These quantitative results, taken together with those for internal block by Cs, place important restrictions on the number and position of energy barriers required to model ion permeation through the K channel (Hille and Schwarz, 1978) and further strengthen the argument by Adelman and French (1978) that Cs block of the K channel must be a multi-ion phenomenon (see Discussion).

For Na and Cs, s values tend to increase when the concentration of the blocker is increased (see Table III). Adelman and French (1978) reported a change in s from 0.6 to 1.3 as external Cs was changed from 5 to 200 mM. We analyzed two further experiments (unpublished data obtained by R. J. French and J. B. Wells)

TABLE II
*Apparent Electrical Distance of Penetration into Squid
 Axon K Channels by Blocking Ions*

Ion(s)*	Apparent electrical distance (s)*		Source of data
	From inside	From outside	
Ba (external)		0.75	Armstrong et al. (1982)
Ba	1.0		Armstrong et al. (1982)
	0.5		Eaton and Brodwick (1980)
Cs (external)		0.6–1.3	Adelman and French (1978)
		1.09	This study
Na, Cs	0.48		This study
Li, TMA, Tris, GA	0.36		This study
Tetrakis	0.28		This study
NMS	0.18		This study
TAA ions, N(C _n H _{2n-1}) ₄ [‡] , n = 2–5	0.15		French and Shoukimas (1981)

* Blocking ions present in the internal solution unless noted.

[‡] s represents the fraction of the transmembrane voltage traversed by the ion to reach its blocking site.

in which axons were bathed in 10 mM K TTX-ASW and the concentration of an internal blocking ion was varied. For internal Cs varying between 5 and 100 mM, s ranged from 0.45 to 0.91. For internal Na varying from 10 to 200 mM, s changed from 0.25 to 0.55. These trends are unlikely to be related to changes in periaxonal K concentration since, for the Na experiment, reversal potentials for all I-E relations, including controls, fell between -12.5 and -14.3 mV. The increasing s values may reflect the increasing probability of double occupancy of channels by the blocker, with an associated increase in the net movement of charge into the transchannel electric field.

TABLE III
*Apparent Electrical Distance (s) to Blocking Site with Various
 Concentrations of "Deep" Blocking Ions*

Concentration of blocker	Na (internal)*	Cs (internal)*	Cs (external) [‡]
<i>mM</i>			
5	—	0.47	0.65
10	0.25	0.45	0.77
20	—	—	0.99
25	0.39	0.66	—
50	0.44	0.77	1.22
100	0.53	0.91	1.26
200	0.55	—	1.33

* Previously unpublished data from the experiments of R. J. French and J. B. Wells. Axons were perfused with 300 mM K and bathed with 10 K TTX-ASW.

[‡] From the experiments of Adelman and French (1978). Nonperfused axons, bathed in 240 K TTX-ASW, were used.

Other "Instantaneous" K Channel Blockers: Tetrakis and Methylammonium

To further investigate the structural features that control ion entry into the K channel, experiments on the effect of tetrakis were initiated. The kinetics of tetrakis block are rapid, but can be resolved at low concentrations and in a restricted range of voltage (French et al., 1981; and manuscript in preparation). However, with an internal concentration of 10 mM tetrakis and 300 mM K inside and out, the block is essentially instantaneous and the data can be analyzed as for the six ions compared above. The only reservation that need be made about a direct comparison of the tetrakis data with the other results is that the ionic strength of the internal solution containing the blocking ion is slightly lower in the case of tetrakis. For tetrakis, we obtained values of $s = 0.28 \pm 0.04$ ($n = 3$), and an apparent dissociation constant, K_{tetrakis} , at $E = 0$ mV, of 27 ± 9 mM ($n = 3$). Two points are of interest. First, it is not essential for a symmetric quaternary ammonium ion to have its side chains terminated in hydrophobic alkyl groups to be an effective K channel blocker. Tetrakis falls between TMA and TEA in blocking potency (cf. French and Shoukimas, 1981). Second, taking s at face value, tetrakis appears somewhat paradoxically to penetrate more deeply than the parent TEA, a smaller ion that does not possess any hydroxyl groups that might form hydrogen bonds with the channel molecule.

One experiment examining block by 100 mM internal methylammonium (MA), in an axon bathed by 10 mM K TTX-ASW, was performed. The effect of MA qualitatively resembled that of the group Li, GA, TMA, and Tris, but the block showed a less steep dependence on voltage ($s = 0.1$). Further experiments, with symmetric 300 mM K, will be necessary for a quantitative comparison. Taken together with the NMS results described below, these data point to a significant, quantitative difference between K channels in frog node and those in squid giant axon. Hille (1975) observed much more steeply voltage-dependent block than we describe here of K channel currents by MA ($s = 0.70$ for block of K currents, and 1.6 for block of NH_4 currents).

NMS Block of K Channels

NMS, the quaternary derivative of strychnine, blocks K channels in a manner similar to the action of the larger tetraalkylammonium ions (French and Shoukimas, 1981) and other quaternary ammonium ions (Armstrong, 1969, 1971; Swenson, 1981). Records from one experiment are shown in Fig. 10. The block caused by 0.1 mM NMS equilibrates over ~ 20 ms following the onset of a depolarizing pulse. A slight dependence of the block on voltage results in the steady state current being almost constant in the range $E = 20$ – 100 mV. With 1 mM strychnine, applied externally but presumably acting from the inside as the protonated form, Shapiro (1977) saw a very clear negative slope in the steady state I - E relation above $E = 40$ mV. The accentuated effect of voltage is immediately obvious in his records (Shapiro, 1977, Fig. 1), where those at the highest voltages cross sharply downward over those for more moderate depolarizations. The frog node data yielded a value of $s = 0.6$ ($s \equiv \delta$ in Shapiro's paper). Our data were analyzed using the approach of French and Shoukimas (1981) to eliminate the contribution of the gating transitions to the apparent voltage

dependence of the steady state block. For the blocking reaction, we arrive at $s = 0.18 \pm 0.01$ and $K_{0.5} = 144 \pm 31 \mu\text{M}$ (mean \pm SEM, eight determinations in three axons). Thus, in squid axon, NMS probably binds to the same site as tetraalkylammonium ions and other TEA derivatives.

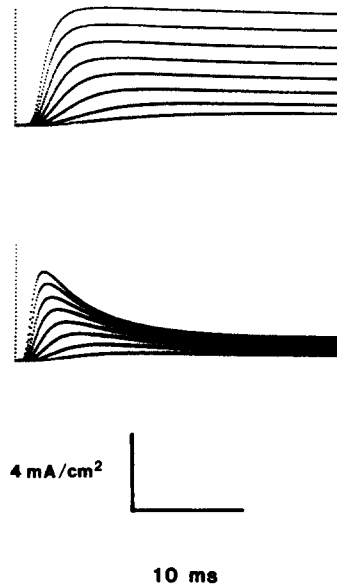


FIGURE 10. Block of K channel currents by NMS. Families of voltage-clamp current records from an axon bathed in 10 mM K TTX-ASW, and perfused with 300 K internally (upper family). When NMS (0.1 mM) is added to the internal perfusate (lower family), a time-dependent block takes effect in a few milliseconds after channel opening. This qualitatively resembles that seen by Shapiro (1977) in frog node, but the squid records here indicate a much less dramatic dependence of the block on voltage. Experiment 28JN83A; nominal holding potential, -80 mV. The total record length is 30 ms. The dotted vertical lines indicate 6 mA/cm^2 .

DISCUSSION

Our discussion is predicated on the assumption that the blocking ions physically enter the permeation pathway. This assertion is based on observations that (a) Na and Cs can pass sluggishly through the channel, particularly at high voltages (French and Wells, 1977; and this paper); (b) block can be antagonized by K added on the side of membrane opposite the blocker (Bezanilla and Armstrong, 1972; Adelman and French, 1978; Eaton and Brodwick, 1980; Armstrong and Taylor, 1980); and (c) several blockers for which there is no direct evidence of measurable permeation act in a manner otherwise qualitatively similar to Na and Cs.

Permeation by the Blocker Affects the "Apparent Electrical Distance" to the Blocking Site

The simulations in Figs. 11 and 12A show that a three-barrier, vacancy-diffusion, rate theory model can simulate the general features of block of K channels by internal impermeant and slightly permeant ions. Fig. 11 shows a set of open

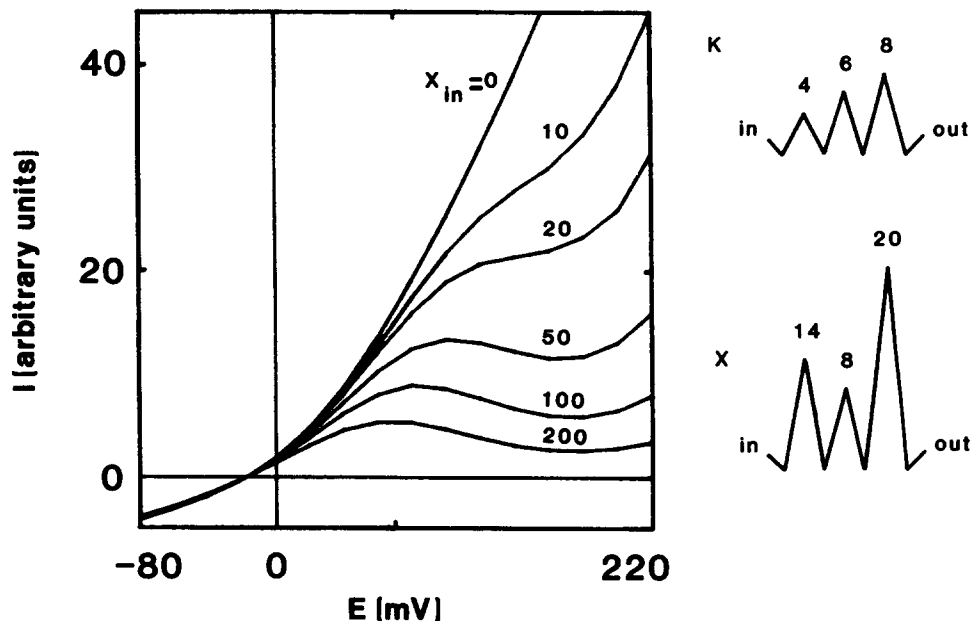


FIGURE 11. A three-barrier, double-occupancy model simulation of a family of open channel I - E relations with 0, 10, 20, 50, 100, or 200 mM of an internal, poorly permeant blocking ion present. This family shows a striking qualitative resemblance to the data in Fig. 5. Further details and some limitations of the simulation are discussed in the text. Concentration, given in millimolar as outside/inside were: 150/300 K; 430/0, 10, 20, 50, 100, 200 blocker, X. Barrier profiles, with heights in kilocalories per mole, are indicated on the figure. Each barrier is symmetric and spans one-third of the transmembrane voltage. Current is in relative units. Further details in the Methods. Between $E = -60$ and $+160$ mV, these data were well fit by Eq. 2 of the text with the parameters given below.

Concentration	s	K_x
<i>mM</i>		<i>M</i>
10	0.541	0.515
50	0.584	0.587
200	0.614	0.533

The increase in s as blocker concentration increases may reflect the combined influence of increased double occupancy, and increased blocker permeation at the largest depolarizations. Blocker permeation would reduce s values estimated for low blocker concentrations. Variation of s as a function of blocker concentration is even more dramatic in the experimental data (see Discussion and Table III).

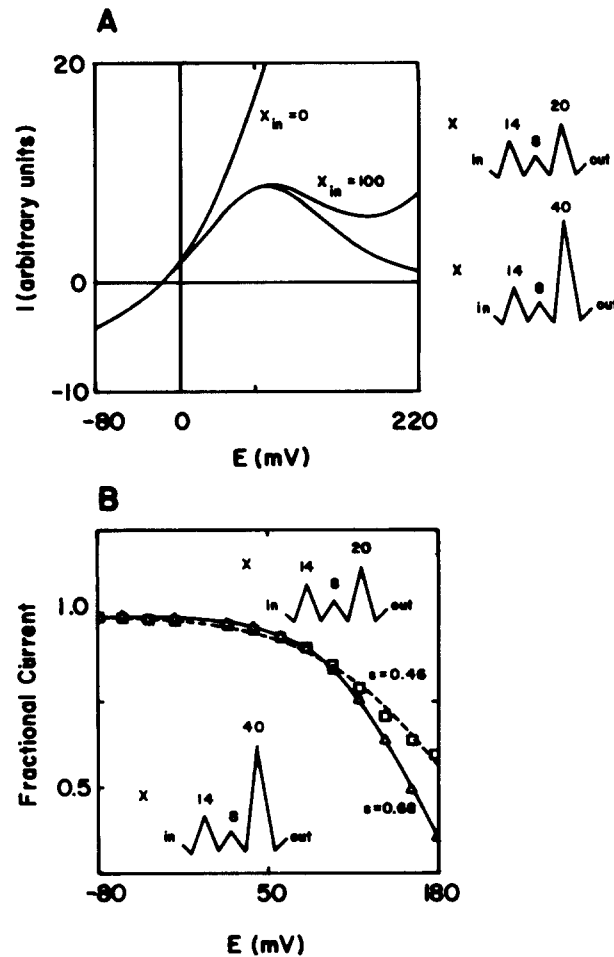


FIGURE 12. Effect of partial permeation of a blocking ion on the shape of the current-voltage relations and on the "effective electrical distance" that the blocking ion penetrates. (A) Open channel current-voltage relations, simulated using a three-barrier, double-occupancy model. I - E relations are shown without internal blocker (upper curve) with a partially permeant blocking ion (middle curve) and with a virtually impermeant blocking ion (lower curve). Concentrations in millimolar, as outside/inside, were 150/300 K; 0/100 X. Other details of the simulation as in Fig. 11. (B) Fractional currents, I_{X+K}/I_K , seen with partially permeant (open squares) and virtually impermeant (filled triangles) blocking ions. Currents were simulated as in A, except that blocker concentrations were 0/10. The lines are best fits, using Eq. 2 to the simulated data points. The "effective electrical distance," s , of penetration is reduced if the blocking ion is significantly permeant, even though the position of the binding sites for the simulation was the same for both cases. This effect on the apparent blocking site position must be borne in mind when placing a physical interpretation on our experimental data (see text). Note that the fitted points do not include those at extreme voltages for which the I - E curve turns up for the permeating blocker (see part A, above).

channel I - E relationships in which there is significant relief of block caused by the blocker passing forward through the channel at high voltages. Results in this family closely resemble observations with internal Cs (Fig. 5) and Na (French and Wells, 1977). The N-shaped I - E curves represent the most complicated form observed. When the blocker is almost completely impermeant, the region of negative slope continues monotonically toward zero at high, positive voltages (Fig. 12A). It is apparent that the region of negative slope is less steep when the blocker passes through more easily. When the simulated data were analyzed over a restricted voltage range, as were the experimental data, the value for s changed from 0.46 to 0.68 as the exit barrier impeding the blocker's passage was raised (Fig. 12B). Thus, the "apparent electrical distance" is smaller for a slightly permeant blocker than for an impermeant one, even though the actual electrical distances to the ion binding sites were unchanged. This supports our assertion that Na and Cs do, in blocking the channel, penetrate further than the other ions used in this study. Given the observations which suggest that Na and Cs can sluggishly pass completely through, this is clearly a possibility. However, these observations do not provide an a priori reason that the location of stable binding must be deeper for Na and Cs. That conclusion rests directly on the quantitative analysis. Na and Cs yielded the largest values of s in this study. The simulations show that if these values are in error, they are likely to underestimate the distances to which Na and Cs penetrate, relative to the other blocking ions.

Physical Interpretation of the "Apparent Electrical Distance" to the Blocking Site

In this discussion, we make the assumption that the voltage dependence of a transition from one steady state condition (e.g., unblocked) to another (blocked) arises from the redistribution of the ions in the electric field within the channel. Different values of s reflect different distributions of ions among the channel binding sites in the blocked state. There is no intent to necessarily associate a particular value of s , even when the value is <1 , with the existence and position of a single, discrete binding site.¹ When the data yield values of $s > 1$, the only physically reasonable interpretation is that more than a single univalent ion has moved in the field (Hille, 1975; Adelman and French, 1978; Hille and Schwarz, 1978). Further, it may generally be true that some of the charge redistributed in going from open to blocked can be ascribed to the permeant ion (see also Armstrong et al., 1982). Despite these reservations, it seems reasonable to us to interpret the different s values, which we observe with symmetric K concentrations, as reflecting different degrees of penetration of the different blocking ions into the channel.²

¹ In the voltage-dependent K channel from the SR, single occupancy and a single discrete site for the binding of monovalent blocking ions appear to be the rule (Coronado and Miller, 1982). Divalent methonium ions, while apparently conforming to the single-occupancy restriction, show voltage dependence that depends on the length of the alkyl chain connecting the two charged methonium groups (Miller, 1982). An imaginative analysis of these data allowed Miller to estimate the physical distance from the channel mouth to the blocking site and thus obtain the relation between physical and electrical distance in this part of the channel.

² In another case of channel block, even this conclusion is subject to question, since a variety of competitively acting toxins, including saxitoxin and tetrodotoxin, which have different net

Additional complications arise if the concentrations of either permeant or blocking ions vary. A general trend was that values of s were lower with high K on the outside (see Table I). This suggests that the internal blocking ion penetrates less deeply when there is a higher likelihood of occupancy of external sites by K, which would electrostatically repel the blocking ion. Values of s increased as the chemical potential gradient driving the blocking ion into the channel increased (see Table III).

This suggests that, on the average, deeper sites are occupied as the driving force on the blocker increases. Thus, there are probably two sites, easily accessible to internal Na and Cs between the inner mouth of the channel and the rate-limiting barrier to outward movement of these ions. In addition, there appear to be two sites accessible to external Cs between the outer mouth and the rate-limiting barrier to inward Cs movement.

The suggestion of four ion binding sites in the channel is foreshadowed in the work of Hodgkin and Keynes (1955) and Begenisich and De Weer (1980). The latter authors observed flux ratio exponents, $n' > 3$, which suggest three or more K ions moving in single file through the pore. No single feature of our data appears to demand simultaneous occupancy of the channel by more than two ions, although that possibility is certainly not precluded. An alternative possibility is that the same sites are occupied by blocking ions entering the channel from either side. The approximately equal values for the apparent dissociation constant, at $E = 0$, estimated for both external and internal Cs (Table III) seem to support this. However, this would require a large exit barrier for the blocker in either direction and is thus difficult to reconcile with the weak binding of Cs to the channel. To account for both blocking and flux data, four ion binding sites with triple occupancy under some conditions is probably a minimal requirement (see also Begenisich and Smith, 1984).

Characteristics of "Deep" and "Shallow" Blockers

"Deep" blockers are typified by the inorganic ions Na, Cs, and Ba. "Shallow" blockers are typified by the larger quaternary ammonium ions. Between these two extremes falls an intermediate group (see Table II). Deep and shallow blockers can be distinguished on the basis of the following functional characteristics. (a) Apparent electrical distance, s , of penetration: deep blockers can penetrate 50% or more of the transmembrane voltage, whereas shallow blockers penetrate 15–20% (see Tables I–III; also French and Shoukimas, 1981; Swenson, 1981). (b) Penetration as a function of ionic concentrations: for deep blockers, s varies as the concentration of the permeant ion (Table I) or the blocking ion (Table III) is varied. For shallow blockers, a single value of s accounts for data from a wide range of blocker concentrations (French and Shoukimas, 1981). (c) Dependence of the apparent stoichiometry on voltage: for external Cs, a deep

charges, show the same fractional change of the degree of block of Na channels with changes of voltage (see French et al., 1984, and the published comments by O. S. Andersen and E. Moczydlowski that accompany that paper; see also Green et al., 1984; Moczydlowski et al., 1984).

blocker, the apparent stoichiometry changes from 1:1 to 2:1 as the electrical driving force increases (Adelman and French, 1978). For shallow blockers, data can be fit with 1:1 stoichiometry for a wide range of conditions (Armstrong, 1966; French and Shoukimas, 1981; Swenson, 1981). (d) Degree of interaction with permeant ions on the opposite side of the membrane: deep blockers are strongly affected, showing a "cross-over" of *I-E* relations (Bezanilla and Armstrong, 1972; Adelman and French, 1978; Hille and Schwarz, 1978), as well as other evidence of relief of block (Eaton and Brodwick, 1980; Armstrong and Taylor, 1980; Schauf and Bullock, 1982). Interactions may be complex. Block by external Ba can either be enhanced or reduced by other ions that enter the channel from the outside (Armstrong et al., 1982). By contrast, the effect of external K on the action of internal, shallow blockers is weaker. There is little effect on the block of outward currents (Swenson, 1981), although unblocking is enhanced by high external K when current flow is inward (Armstrong, 1971). (e) Relief of block by permeation at extreme voltages: block by deep blockers can be relieved when the blocking ions are forced onward through the channel by very large depolarizations (Fig. 3; also French and Wells, 1977). The shallow blocker TEA blocks current almost completely over the same range (French and Wells, 1977).

Binding Affinities of Various Blockers

The apparent dissociation constant at $E = 0$ was calculated from Eq. 3. This is not the ideal way to obtain a precise estimate of this parameter since in most experiments a large percentage block occurred only at voltages far removed from zero. The numbers obtained are useful in that they give an indication of the relative binding affinities of the different ions. The sequence of binding affinities obtained in this study was $NMS > tetrakis > TMA > Tris > Cs > Li \approx GA > Na$. The values of the apparent dissociation constants are presented, with values from other studies on squid axon, in Table IV. There does not appear to be a systematic relation between binding affinity and the apparent depth of penetration of the blocking ion.

Dependence of Apparent Depth of Penetration on Ionic Size

A broad look at the data obtained from experiments on *Loligo pealei* in this and other studies indicates a general inverse correlation between molecular size and the apparent electrical distance that blocking ions penetrate into the channel from its inner mouth. Data collected from this and other studies are shown in Table II. The largest ions studied, quaternary ammonium ions, including NMS and the tetraalkylammonium ions, $N(C_nH_{2n-1})_4^+$, for $n \geq 2$, are least affected by voltage, sensing ~15% of the transmembrane voltage (French and Shoukimas, 1981). Swensen (1981) derived a slightly larger number ($\geq 20\%$) for a variety of quaternary ammonium ions, but did not account for the addition of a component of voltage dependence arising from the voltage dependence of the gating process.

The most deeply acting ions are Na, Cs, and Ba, all of which can pass completely through the channel, presumably in a largely dehydrated state. A group showing intermediate voltage dependence includes Li and a variety of organic ions. Thus,

the smallest ions (in dehydrated form) act most deeply in the field, and the largest act nearest the surface. The intermediate group is scrutinized more closely below.

Effect of Molecular Structure on Penetration into the Inner Mouth of the K Channel

It is notable that three ions—Tris, GA, and tetrakis—which have diameters as large as, or larger than, TEA, penetrate at least twice the apparent electrical

TABLE IV
*Apparent Dissociation Constants for
Various Blockers of Squid Axon K Channels*

Blocking ion	Apparent disso-	Solution K	Source
	ciation constant at $E = 0$ mV	concentra- tions, inside/ outside	
	mM	mM	
Na	2,000	300/300	This study
GA	890	300/300	This study
Li	870	300/300	This study
Cs (internal)	750	300/300	This study
Cs (external)	720	300/300	This study
Tris	290	300/300	This study
TMA	180	300/300	This study
Tetrakis	20	300/300	This study
NMS	0.14	300/10	This study
Tetraalkylammonium, $N(C_nH_{2n-1})_4^+$, $n = 2$ to 5	0.4–0.03	300/10	French and Shoukimas (1981)
Quaternary Ammonium ions*	0.008–4.4	275/0	Swenson (1981)
Ba (internal) [‡]	7×10^{-5}	100/10	Eaton and Brodwick (1980)
Ba (external) [§]	150	275/0	Armstrong et al. (1982)

* The range of values represents 16 quaternary ammonium ions of various structures. Tabulated values are calculated assuming $s = 0.2$ from apparent K_d 's determined at +100 mV.

‡ Calculated assuming $s = 0.5$, using the data of Fig. 3 in Eaton and Brodwick (1980), determined at $E = +60$ mV. Armstrong and Taylor (1980) and Armstrong et al. (1982) used total internal barium concentrations in the millimolar range to study block, but did not explicitly give dissociation constants because of uncertainty about the level of free ionized Ba^{2+} .

§ Calculated assuming an e-fold change for 18 mV, from the data of Fig. 7 in Armstrong et al. (1982), determined at -70 mV.

distance into the channel. On examining their molecular structure, it is striking that each of the ions has a number of hydroxyl groups exposed on its surface. Hille (1971) noted a parallel anomaly in his study of the permeability of the Na channel to various organic ions. No ions having a methyl group were measurably permeant. On the other hand, several ions larger in cross section than MA showed significant permeation through the channel. Hille suggested that these

ions might fit through the pore by making hydrogen bonds with surrounding oxygen atoms of the pore. A similar rationale may explain the deeper penetration into the K channel mouth by relatively large blocking ions having polarizable surface groups.

The data of Swensen (1981) show a similar tendency. Rate parameters for hydroxylated quaternary ammonium ions depend more steeply on voltage than do those for compounds having simple alkyl chains. In that study, however, voltage dependence of the steady state block was not determined directly.

Differences Between K Channels in Frog Node and Squid Axon

Two blockers, NMS and MA, appear to enter more deeply into the inner mouth of K channels of frog node than of squid axon. On the other hand, in frog node, Ba may bind somewhat closer to the axoplasmic surface than it does in squid (see Woll, 1982). Other differences of detail have been previously noted, both in gating kinetics and in blocking behavior. For example, external TEA blocks K channels of frog node but not those of squid, while internal quaternary ammonium ions act similarly in both preparations (Armstrong and Hille, 1972). However, the differences in detail point to the need for a comprehensive set of electrical and flux data, from a single preparation, as the input for a detailed model of ion permeation through the K channel.

Future experiments on the interaction between blockers and other permeant ions such as ammonium and rubidium may reveal additional features of the permeation pathway by altering the most likely location of binding by the blocking ions. A possible reason for the particularly steep voltage dependence of MA block in frog node (Hille, 1975) when the permeant ion was ammonium ($s = 1.6$) is that the relative probabilities of ammonium occupying different sites in the K channel are different from those for K ($s = 0.7$ for block of K currents). The redistribution of charge when a blocker occupied the channel would thus be quantitatively different for different permeant ions. Intricate electrostatic calculations by Jordan (1984) are beginning to define, more precisely, physical dimensions and properties of channels that would yield the electrical "dimensions" estimated from voltage-clamp data.

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