

Ion Conductance and Selectivity of Single Calcium-activated Potassium Channels in Cultured Rat Muscle

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ABSTRACT The conductance and selectivity of the Ca-activated K channel in cultured rat muscle was studied. Shifts in the reversal potential of single channel currents when various cations were substituted for K^+ were used with the Goldman-Hodgkin-Katz equation to calculate relative permeabilities. The selectivity was $Tl^+ > K^+ > Rb^+ > NH_4^+$, with permeability ratios of 1.2, 1.0, 0.67, and 0.11. Na^+ , Li^+ , and Cs^+ were not measurably permeant, with permeabilities <0.05 that of K^+ . Currents with the various ions were typically less than expected on the basis of the permeability ratios, which suggests that the movement of an ion through the channel was not independent of the other ions present. For a fixed activity of K^+ (77 mM), plots of single channel conductance vs. activity of K^+ were described by a two-barrier model with a single saturable site. This observation, plus the finding that the permeability ratios of Rb^+ and NH_4^+ to K^+ did not change with ion concentration, is consistent with a channel that can contain a maximum of one ion at any time. The empirically determined dissociation constant for the single saturable site was 100 mM, and the maximum calculated conductance for symmetrical solutions of K^+ was 640 pS. TEA^+ (tetraethylammonium ion) reduced single channel current amplitude in a voltage-dependent manner. This effect was accounted for by assuming voltage-dependent block by TEA^+ (apparent dissociation constant of 60 mM at 0 mV) at a site located 26% of the distance across the membrane potential, starting at the inner side. TEA^+ was much more effective in reducing single channel currents, with an apparent dissociation constant of ~ 0.3 mM.

INTRODUCTION

Calcium-activated potassium currents through cell membranes occur in many different cell types (reviewed by Meech, 1978; Schwarz and Passow, 1983). Single Ca-activated K channels with large conductance have been observed in the surface membranes of chromaffin cells (Marty, 1981), cultured rat muscle (Pallotta et al., 1981; Barrett et al., 1982; Methfessel and Boheim, 1982),

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pituitary cells (Wong et al., 1982), sympathetic neurons (Adams et al., 1982), and acinar cells (Maruyama et al., 1983), and have been isolated from rabbit muscle transverse tubules (t-tubules) (Latorre et al., 1982). These channels, referred to as maxi K channels by Latorre and Miller (1983) and BK channels by Marty (1983b), have many similar properties. They are selective for K^+ over Na^+ and have single channel conductances typically >200 pS in symmetrical 140 mM KCl. These channels are activated by Ca^{2+} at the intracellular membrane surface and are modulated by membrane potential, with depolarization increasing channel activity.

The purpose of this paper is to determine the selectivity sequence and examine some of the conductance properties of the Ca-activated K channel of cultured rat skeletal muscle so that these properties can be compared with those of other K^+ channels. We find that the selectivity to various monovalent cations is identical to that of a Ca-activated K channel in *Aplysia* neurons (Gorman et al., 1982) and similar to the delayed rectifier K^+ channels in frog and squid (Hille, 1973; Conti et al., 1975; Gay and Stanfield, 1978). The selectivity sequence differs from that for delayed rectifier K^+ channels in snail neurons (Reuter and Stevens, 1980) and the relative permeabilities differ from inward rectifier channels in starfish eggs (Hagiwara and Takahashi, 1974). We also find that the flow of ions through the Ca-activated K channel, like other K^+ channels, does not obey the "independence principle" (Hodgkin and Huxley, 1952; reviewed in Hille and Schwarz, 1978; Latorre and Miller, 1983). The relationship between single channel conductance and activity of K^+ was shown to be consistent with a two-barrier model with a single saturable site. This observation, plus the finding that the permeability ratios of Rb^+ and NH_4^+ to K^+ did not change with ion concentration, suggests that the channel can contain a maximum of one ion at any time, which is similar to K^+ channels from the sarcoplasmic reticulum and t-tubules (Coronado et al., 1980; Vergara and Latorre, 1983; Latorre and Miller, 1983).

Tetraethylammonium ion (TEA^+) typically blocks current through K^+ channels (Armstrong, 1975; Latorre and Miller, 1983). We find that TEA^+ applied to either side of the membrane reduces single channel current amplitudes of the Ca-activated K channel from cultured rat muscle, with external application being ~ 200 times more effective than internal application. This finding is consistent with observations on Ca-activated K channels in *Aplysia* neurons (Gorman et al., 1982), sympathetic ganglia neurons (Adams et al., 1982), t-tubules (Vergara and Latorre, 1983), and pituitary cells (Wong et al., 1982).

METHODS

Currents flowing through Ca-activated K channels in the surface membrane of primary cultures of rat skeletal muscle were obtained with the single channel recording technique (Neher and Sakmann, 1976; Hamill et al., 1981). Unless otherwise indicated, experiments were performed with excised patches of membrane using inside-out patches where the normal intracellular side of the membrane was exposed to the bathing solution and the normal extracellular side was exposed to the solution in the pipette. In a few indicated experiments, outside-out patches were used in which the normal extracellular side of the membrane was exposed to the bathing solution and the normal intracellular side was

exposed to the solution in the pipette. Even though the patches of membrane are excised, the terms intracellular and extracellular will be used to refer to the sides of membrane as they are in an intact cell.

Myoblasts were isolated from hind- and forelimbs of 19–20-d-old Sprague-Dawley rat embryos by mechanical and enzymatic treatments and plated on collagen-coated coverslips in a medium containing 15% fetal bovine serum in Dulbecco's Modified Eagle's Medium. The cultures were maintained at 35°C in an atmosphere containing 15% CO₂. Fusion into myotubes usually occurred after ~3 d, and the cells were satisfactory for experimentation for up to ~3 wk in culture.

The solution at the extracellular side of the membrane contained (in mM): 140 KCl, 1 EGTA, 5 TES [*N*-tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid], plus sufficient *N*-methyl-D-glucamine (~4.4 mM) to bring the pH to 7.0.

For experiments investigating the relative permeabilities of various cations, the solution at the intracellular side of the membrane was the same as the extracellular solution (described above), except that 140 mM KCl was replaced by 140 mM of the test salt as chloride, and sufficient Ca²⁺ was added (~0.68–0.96 mM) to give a calculated free Ca²⁺ of 0.4–4 μM. This range of free Ca²⁺ gives appropriate activity of the Ca-activated K channel over the range of membrane potentials examined (Barrett et al., 1982). In experiments in which 140 mM Tl⁺ replaced the 140 mM K⁺, all Cl⁻ on both sides of the membrane was replaced by NO₃⁻ because of the low solubility of TlCl. Only the major cations and anions are referred to in the solutions in text, even though the other ions described above are also present.

Data were corrected for junction potentials as described previously (Hille, 1971; Hagiwara and Ohmori, 1982). These corrections were <0.5 mV for the permeant ions Rb⁺ and NH₄⁺. The junction potential of solutions with Tl⁺ was not measured, but it was assumed to be negligible since the limiting equivalent conductivities of Tl⁺ and K⁺ are similar (Robinson and Stokes, 1959). Activity coefficients of the permeant ions Tl⁺, K⁺, Rb⁺, and NH₄⁺ were assumed to be in the ratios 0.94:1.00:0.99:1.00 (Robinson and Stokes, 1959). No correction for the activity coefficient of TEA was made.

Membrane potentials are reported as the voltage at the normal intracellular side of the membrane minus the voltage at the normal external side. Inward currents are recorded as downward or negative deflections. Currents were recorded on a frequency-modulated tape recorder (frequency response of 0–2.5 kHz) and analyzed either by hand from a chart record obtained at reduced playback speed of the tape recorder or by a Digital Equipment Corp. (Marlboro, MA) PDP 11/10 computer. Single channel current amplitudes could be obtained with the computer by visually fitting cursor lines to closed and open single channel current levels in displayed current records, or by plotting histograms of number of observations vs. current and measuring the distance between the peaks, which represented closed and open current levels.

The Ca-activated K channel was identified by its Ca and voltage sensitivity, as described in Barrett et al. (1982), and this was the channel most commonly observed under the conditions of these experiments. Contributions from other membrane channels would be unlikely either because appropriate permeant ions were not included in the bathing solution or because holding potentials were such that other channels, such as delayed rectifier and Na channels, would be inactivated. Other channels were, however, occasionally observed, particularly in solutions with high [KCl]_i. Experiments were rejected if the records were contaminated by these other channels.

The shift in reversal potential (Hille, 1971, 1973) when a test ion X⁺ was substituted for K⁺ was used to determine the relative permeability of X⁺ to K⁺ under conditions of zero net current. The relationship between the permeability ratio and the shift in reversal

potential is obtained from the Goldman-Hodgkin-Katz equation (Goldman, 1943; Hodgkin and Katz, 1949). For the condition of X_i^+ being replaced by K^+ , the shift in reversal potential is

$$V_x - V_k = \frac{RT}{F} \cdot \ln \frac{P_k K_o}{P_x X_i} - \frac{RT}{F} \cdot \ln \frac{K_o}{K_i}, \quad (1)$$

where V_x and V_k are the reversal potentials with X^+ and K^+ at the inner membrane surface, respectively; P_x and P_k are the permeabilities of the channel to X^+ and K^+ , respectively; K_o , K_i , and X_i are the activities of the indicated ions; and $F/RT = 0.0394 \text{ mV}^{-1}$ at 22°C . The permeability ratio of X^+ to K^+ is then obtained by rearrangement:

$$\frac{P_x}{P_k} = \frac{K_i}{X_i} \cdot \exp[(V_k - V_x)F/RT]. \quad (2)$$

For the selectivity experiments presented in this paper, $[K^+]_o$ and $[K^+]_i$ were both 140 mM, so that $V_k = 0$.

Data for determining reversal potentials were obtained by first measuring single channel amplitudes in symmetrical K^+ at a series of potentials positive and negative to the reversal potential. This procedure was then repeated with test ion X^+ replacing K_i^+ . Reversal potential measurements in the test solutions were always bracketed by control measurements in the reference solution. If the reversal potential in the reference solution obtained before and after the test ion differed by $>2 \text{ mV}$, the experiment was rejected. Few experiments were rejected for this reason, as the experimental preparation and recording electrodes were typically stable within the 2-mV range (most experiments were stable to within 0.5 mV) for 30 min to $>2 \text{ h}$.

Plots of single channel current amplitude vs. membrane potential were nonlinear for most of the ions studied. This nonlinearity precluded using simple linear regression lines through the entire current-voltage plot to determine the reversal potential. To minimize possible errors arising from nonlinearities, we fit a regression line through the points closest to the apparent reversal potential and took the voltage intercept on the zero-current axis as the reversal potential. That is, all reported reversal potentials are interpolated values between currents that were observed to reverse. In experiments, such as with Na^+ , where reversal of the currents was not observed, "reversal potentials" are reported as being greater than an extrapolated value, but these data should not be interpreted to mean that the currents would actually reverse if the potential were made large enough.

The single channel chord conductance, g , was determined as

$$g = i/(V - V_R), \quad (3)$$

where i is the single channel current amplitude at membrane potential V , and V_R is the observed reversal potential for the single channel currents.

Experiments were performed at room temperature ($21\text{--}25^\circ\text{C}$).

RESULTS

Permeability of Tl^+ Is Greater Than That of K^+

A typical experiment to determine relative permeability from reversal potential measurements (Hille, 1971, 1973) is shown in Fig. 1. Single channel currents recorded over a range of membrane potentials are shown in Fig. 1A. There were two Ca-activated K channels in this membrane patch and the characteristic

flickering of the channel current toward the closed state current level is evident (Barrett et al., 1982). For these records, the normal extracellular membrane surface was bathed in 140 mM KNO_3 and the normal intracellular membrane surface was bathed first in 140 KNO_3 (left panel) and then in 140 TlNO_3 (right panel). With either K_i^+ or Tl_i^+ , the currents reversed direction, from inward at negative potentials to outward at positive potentials. Plotting the single channel current amplitude against membrane potential in Fig. 1B shows that the currents reverse direction at ~ 0 mV for each ion. Thus, Tl^+ moves through the Ca-activated channel about as well as K^+ at 0 mV with these solutions. When the activity coefficients of K^+ and Tl^+ are taken into account, together with the 85% ionization of TlNO_3 (Sillen and Martell, 1964), Tl^+ is found to be more permeant than K^+ , with a permeability ratio $P_{\text{Tl}}/P_{\text{K}}$ of 1.2 (calculated with Eq. 2 in Methods).

An interesting effect of Tl^+ was the decrease in the apparent single channel conductance at potentials >10 mV, as shown in Fig. 1B. Such a voltage-dependent blocking effect of Tl^+ could result if Tl^+ binds to a blocking site within the electrical field of the membrane (see, for example, Armstrong, 1975). The effects of Tl^+ were completely and rapidly reversed by replacing Tl^+ with K^+ , as shown in Fig. 1B.

Rb⁺ and NH₄⁺ Are Less Permeant Than K⁺

Single channel currents recorded with 140 KCl_o and 140 mM KCl , RbCl , or NH_4Cl at the intracellular membrane surface are shown in Fig. 2A. It is apparent that channel gating kinetics are affected by the permeant ion, but this effect was not examined, as the estimates of selectivity obtained in this study depend only on the shifts in reversal potential. Plots of the single channel current amplitude vs. membrane potential are presented in Fig. 2B. Replacing K_i^+ with Rb^+ shifted the reversal potential from 0 to 11 mV. Since the activities of K^+ and Rb^+ are similar, this shift in reversal potential indicates that Rb^+ is not as permeant as K^+ ; an additional outward force on the ions imposed by an 11-mV membrane potential was required for the outward flux of Rb^+ to equal the inward flux of K^+ . Calculations with Eq. 2 give a Rb^+/K^+ permeability ratio of 0.65.

NH_4^+ was even less permeant than Rb^+ . Replacing K_i^+ with NH_4^+ shifted the reversal potential to 60 mV for the experiment in Fig. 2, giving a calculated NH_4^+/K^+ permeability ratio of 0.09.

Na⁺, Cs⁺, and Li⁺ Are Not Measurably Permeant

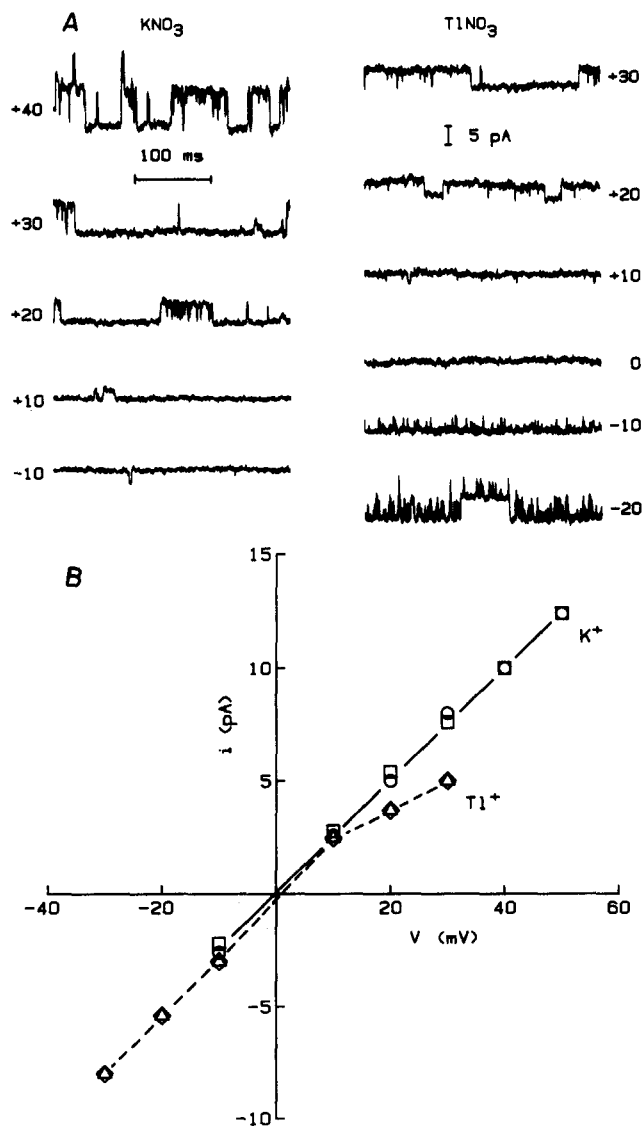
When K_i^+ was replaced by Na^+ , no outward single channel currents were detected for potentials up to the maximum examined potential of 100 mV. Typical single channel current records are presented in Fig. 2A and a plot of single channel current amplitude vs. membrane potential is presented in Fig. 3. These figures show that the inward currents became progressively smaller, appearing to approach the zero-current axis asymptotically as the membrane potential was made more positive. Since the single channel currents were not observed to reverse, it was not possible to calculate a permeability ratio, but it was possible to set an upper limit for the permeability of Na^+ . In experiments of this type, the projected

reversal potential was >110 mV, giving an upper limit for the Na^+/K^+ permeability ratio of 0.01.

Results similar to those shown in Fig. 3 were also observed for replacement of K_i^+ by Cs^+ or Li^+ . Single channel currents were not observed to reverse with these ions for examined membrane potentials up to 80 mV. The projected reversal potentials were >80 mV for Cs^+ and >100 mV for Li^+ , giving upper limits for the Cs^+/K^+ and Li^+/K^+ permeability ratios of 0.05 and 0.02, respectively.

Selectivity Sequence at the Reversal Potential

Table I summarizes reversal potential measurements and calculated permeability



ratios for a series of experiments like those presented in Figs. 1–3. The relative permeabilities of the ions tested were: $P_{\text{Tl}} > P_{\text{K}} > P_{\text{Rb}} > P_{\text{NH}_4}$. Cs^+ , Li^+ , and Na^+ were not measurably permeant.

Deviation of Single Channel Currents from Independence

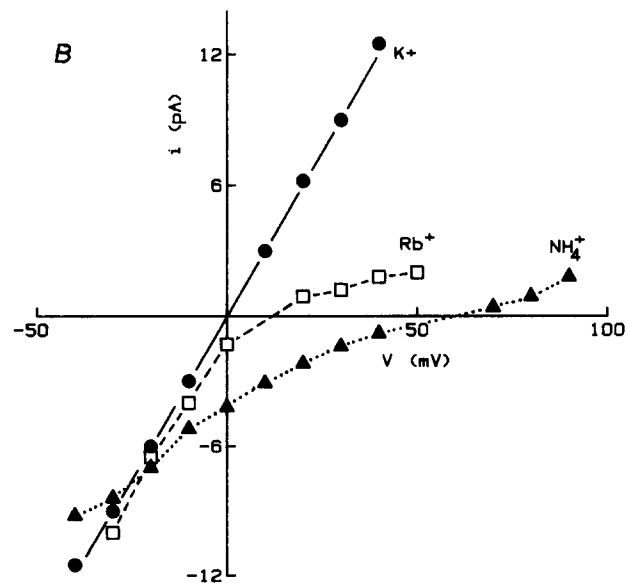
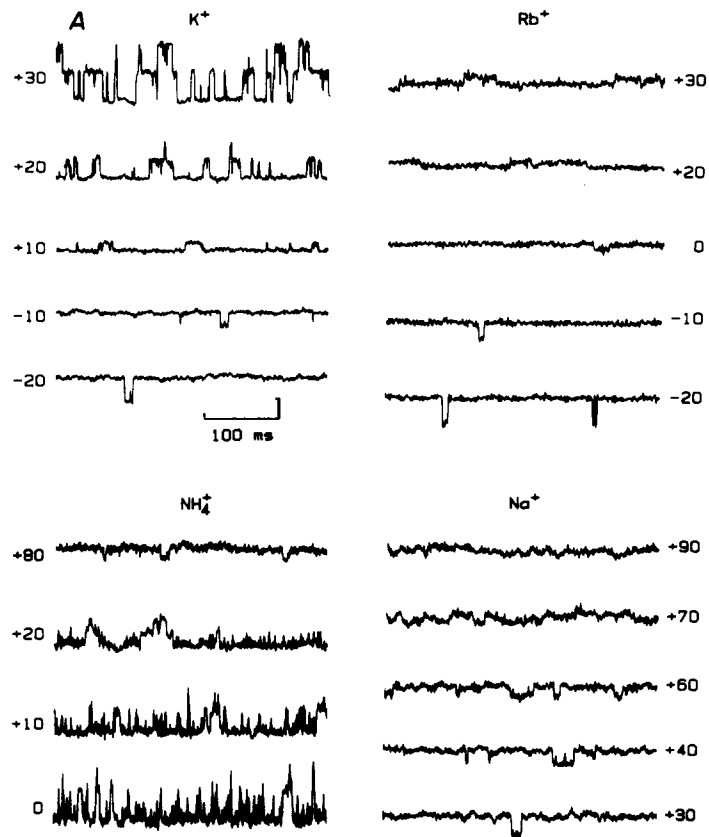
If the probability that an ion will pass through a channel is independent of all other ions present, including those of the same type (independence principle; see Hodgkin and Huxley, 1952; Hille, 1975*a, b*), then the single channel current amplitude with ion X^+ replacing K_i^+ would be related to the permeability ratio P_X/P_K by

$$\frac{i_X}{i_K} = \frac{K_o' - \frac{P_X}{P_K} \cdot X_i \cdot \exp(VF/RT)}{K_o - K_i \cdot \exp(VF/RT)}, \quad (4)$$

where i_X is the single channel current amplitude recorded with activities of K_o' and ion X_i inside; i_K is the single channel current amplitude recorded with activities K_o and K_i ; V is the membrane potential; and $F/RT = 0.0394 \text{ mV}^{-1}$ (see Hodgkin and Huxley, 1952; Hille, 1975*a*). Eq. 4 is derived from the Goldman-Hodgkin-Katz flux equation (Goldman, 1943; Hodgkin and Katz, 1949) with the assumption that if independence holds, then $X_i P_X/P_K$ can be substituted for K_i' .

The circles in Fig. 4 plot experimentally observed single channel current amplitudes vs. membrane potential when 140 mM K_i^+ was replaced by 140 mM Tl^+ , Rb^+ , NH_4^+ , or Na^+ . The lines indicate predicted single channel current amplitudes calculated on the basis of the independence principle with Eq. 4. The permeability ratios used in the calculations are listed in Table I. Clearly, none of these ions obeys the independence principle. Tl^+ , Rb^+ , and, to a lesser extent, NH_4^+ block outward currents, with the block increasing with membrane potential. NH_4^+ and Na^+ also block inward currents. Thus, like other ion channels (Hodgkin

FIGURE 1. (*opposite*) Effect of Tl^+ on currents through single Ca-activated K channels. (A) Records of single channel currents. Currents in the left panel were recorded with 140 mM KNO_3 on each side of the membrane patch and show characteristic conductance (250 pS) and voltage sensitivity (increased activity with depolarization) of Ca-activated K channel. Currents in the right panel were recorded after the internal solution was changed to 140 mM TlNO_3 . Holding membrane potentials (in millivolts) are indicated by each current trace. Upward current deflections indicate outward membrane current. The increased current noise when the channel is open is apparent and is useful for distinguishing between open and closed states. In practice, a distinction between open and closed states is easily made during the experiment from the dynamic records and by passing the membrane potential systematically through the reversal potential, which was near ~ 0 mV in both solutions in this experiment; channel opening is associated with upward-going currents at positive potentials and downward-going currents for negative membrane potentials. (B) Single channel current magnitude, i , plotted against membrane potential, V , for currents with K_i^+ (circles, squares) and Tl_i^+ (triangles, diamonds). Order of data collection: circles, diamonds, squares, triangles. Note the reversibility of the effect.



and Keynes, 1955; Armstrong, 1969; Hagiwara and Takahashi, 1974; Hille, 1975*b*; Lewis and Stevens, 1979; Adams et al., 1981; Latorre and Miller, 1983), the Ca-activated K channel does not obey independence.

Concentration Dependence of Channel Conductance

Binding of ions to sites within the channel could lead to deviations of currents from the independence principle. If a permeant ion binds to such a site, then it

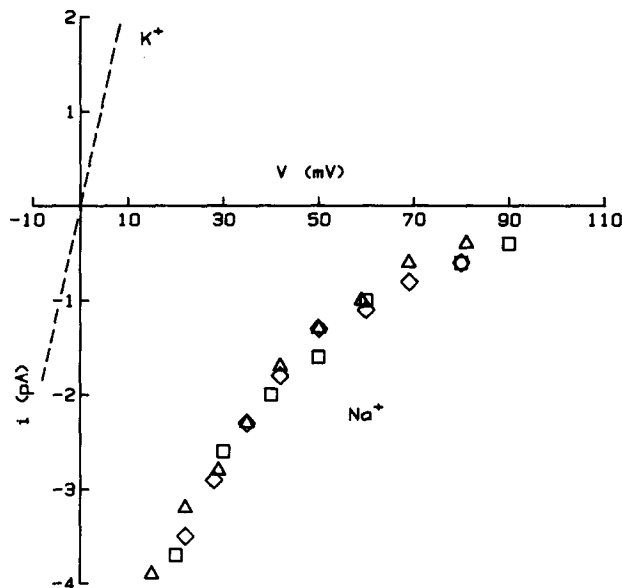


FIGURE 3. Plot of single channel current amplitude vs. membrane potential for data recorded with 140 mM NaCl, and 140 mM KCl. Examples of currents are shown in Fig. 2. Each symbol type represents data obtained from a different patch of membrane. The apparent star is a superimposed square and diamond. Note that extrapolation of data obtained at less positive potentials would lead to an underestimation of the reversal potential and hence an overestimation of Na⁺ permeability. The dashed line indicates a typical *I-V* relationship with symmetrical 140 mM KCl.

might be expected that the flux of ions through the channel would reach a maximum as the concentration of the ion increased (Laüger, 1973; Hille and Schwarz, 1978; Horn and Patlak, 1980). To examine this possibility, the single channel conductance was determined at increasing [K⁺]_i. The single channel

FIGURE 2. (*opposite*) Shift in reversal potential resulting from replacing 140 mM KCl_i with 140 mM RbCl, NH₄Cl, or NaCl. (A) Records of single channel currents. Inward and outward current magnitudes are symmetrical about 0 mV for K⁺, are nonlinear, and reverse at positive potentials with NH₄⁺ or Rb⁺. No outward currents were observed with Na⁺. Vertical bar: 5 pA for K⁺ and Rb⁺; 2.5 pA for NH₄⁺ and Na⁺. (B) Plots of single channel current amplitudes vs. voltage for the three indicated permeant cations.

current amplitude vs. membrane potential is plotted in Fig. 5A for $[K^+]_i$ increasing from 10 mM to 1 M while $[K^+]_o$ was held at 100 mM. The single channel conductance for each $[K^+]_i$ was determined with Eq. 3 from the slope of least-squares fits through the data points and plotted in Fig. 5B as filled circles. As

TABLE I
Reversal Potential Shifts ($V_x - V_K$) and Permeability Ratios (P_x/P_K) Determined for Substitution of 140 mM X_i^+ for 140 mM K_i^+

Ion _i	$V_x - V_K$ mV	n^*	P_x/P_K^\ddagger
Tl ⁺	1.2±0.4	2	1.2±0.15
K ⁺	0±0.02	20	1.0
Rb ⁺	10.4±1.3	4	0.67±0.03
NH ₄ ⁺	57.1±3.1	6	0.11±0.01
Cs ⁺	>80	5	<0.05
Li ⁺	>100	5	<0.02
Na ⁺	>110	5	<0.01

* Number of membrane patches. Three or more separate determinations of reversal potential were typically made in each patch for both K_i and X_i .

† Calculated with Eq. 2. $K_o = 140$ mM. Mean ± SE.

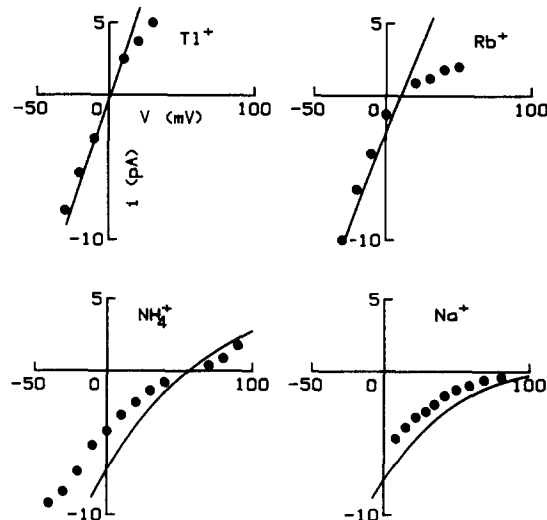


FIGURE 4. The Ca-activated K channel does not obey independence. Plots of single channel current amplitudes vs. membrane potential for data recorded with 140 mM K_o^+ and either 140 mM Tl⁺, NH₄⁺, Rb⁺, or Na⁺ at the inner membrane surface. Filled circles plot experimental observations. Continuous lines are predictions of the independence principle and were calculated with Eq. 4.

$[K^+]_i$ increased, channel conductance increased, first rapidly, and then more slowly to an apparent maximum, which was suggestive of a saturating site involved in movement of K^+ through the channel.

Läuger (1973) has shown for symmetrical solutions of permeant ion that the

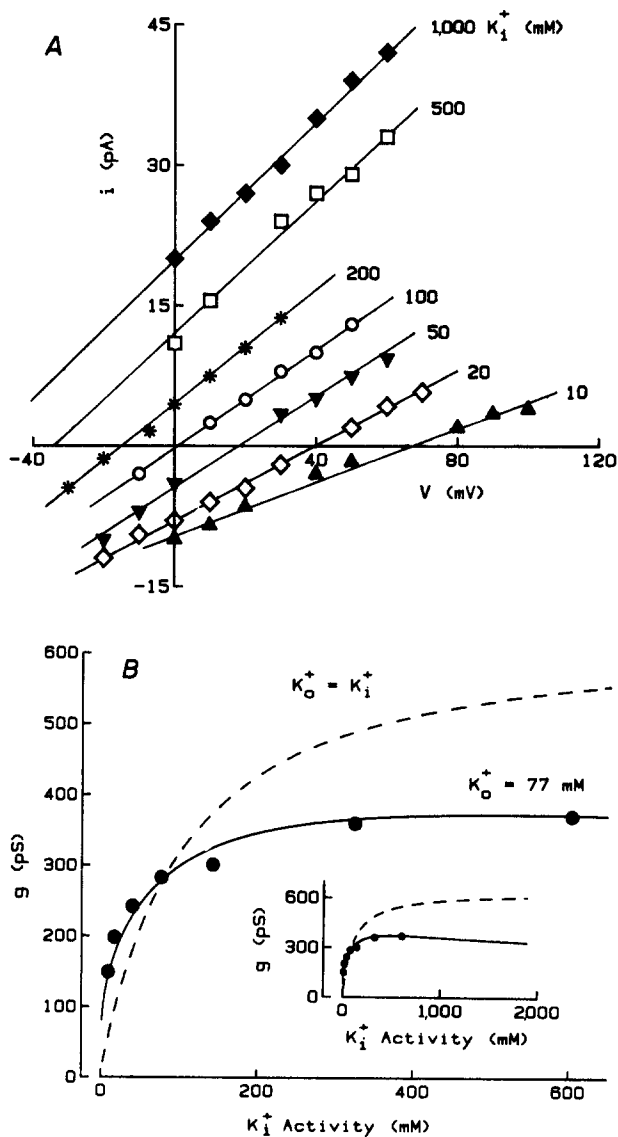


FIGURE 5. Saturation of the Ca-activated K channel. (A) Plots of single channel current amplitudes vs. membrane potential for the concentrations of K_o^+ fixed at 100 mM (activity = 77 mM) and a series of concentrations of K_i^+ , as indicated. (B) Single channel conductance g (Eq. 3) vs. activity of K_i^+ for the data in A plotted as filled circles. The continuous lines were calculated (Eq. 6) for a symmetrical two-barrier model with a single saturable site with a K_D of 100 mM (activity) and a maximum single channel current of 32 pA at 0 mV. The dashed lines indicate the calculated activity-conductance relationship for the same model and parameters assuming symmetrical solutions. For symmetrical solutions the half-maximal conductance (320 pS) occurs at an activity equal to the empirically determined K_D , which is not the case for asymmetrical solutions.

conductance, g , of a channel that can contain, at most, a single ion at a time (single ion channel) should increase as a simple saturation function with increasing activity of the permeant ion:

$$g = \frac{g_{\max} A / K_D}{1 + A / K_D}, \quad (5)$$

where g_{\max} is the maximum single channel conductance; A is the activity of the permeant ion on each side of the membrane; and K_D is the dissociation constant (the activity at half-maximal conductance) for the site in the channel that binds the permeant ion.

Vergara and Latorre (published in Latorre and Miller, 1983) have found that Eq. 5 describes the activity-conductance relationship for the Ca-activated K channel from muscle t-tubule using a K_D of 140 mM. It would be of interest to determine whether the Ca-activated K channel from cultured rat muscle has similar properties. It is not possible, however, to apply Eq. 5 directly to our data in Fig. 5B, as we used asymmetrical solutions. It can be shown (Eq. A11 in Appendix) that the activity-conductance relationship at 0 mV for a single ion channel with asymmetrical solutions, but two symmetrical barriers and a single binding site for the permeant ion, is:

$$g = \frac{\left[\frac{i_{\max} \cdot (A_i - A_o) / K_D}{2 + (A_i + A_o) / K_D} \right]}{\frac{RT}{F} \cdot \ln \frac{A_i}{A_o}}, \quad (6)$$

where A_i and A_o are the activities of K^+ at the inner and outer membrane surface; i_{\max} is the maximum single channel current at 0 mV; $RT/F = 25.4$ mV at 22°C; and K_D is the dissociation constant of the permeant ion from the binding site. Note that in asymmetrical solutions, K_D is not given by the half-maximal conductance, but is determined empirically by fitting Eq. 6 to the data.

The continuous lines in Fig. 5B and its inset were calculated with Eq. 6 assuming a K_D of 100 mM and a maximum single channel current of 32 pA. The description of the data by these lines indicates that a two-barrier model with a single binding site can account for the activity-conductance relationship in asymmetrical solutions.

The theoretical activity-conductance relationship at 0 mV with symmetrical solutions was determined with Eq. 6 by setting A_o equal to $A_i - d$, where d is $\ll A_i$ and is plotted as dashed lines in Fig. 5B and its inset. Eq. 5, with a g_{\max} of 640 pS and a K_D of 100 mM, gave a relationship identical to the dashed lines. These values are similar to the g_{\max} of 500 pS and K_D of 140 mM observed by Vergara and Latorre (data in Latorre and Miller, 1983) for the Ca-activated K channel from t-tubule bathed in symmetrical solutions.

Notice in Fig. 5B that the calculated shape of the conductance-activity relationship for the two-barrier single ion channel (Eq. 6) is markedly different for symmetrical (dashed lines) and asymmetrical solutions (continuous lines). In contrast to the simple saturation curve observed in symmetrical solutions, the

conductance in asymmetrical solutions increases more rapidly to a peak and then declines with increasing activity of K_i . It follows, then, that a simple saturation relationship of the type predicted by Eq. 5 would not necessarily be expected for a single ion channel with asymmetrical solutions.

In 22 experiments, the single channel conductance of the Ca-activated K channel in symmetrical 140 mM KCl was 307 ± 36 pS (mean \pm SD). This value is higher than the mean value of 218 pS reported previously (Barrett et al., 1982). The solutions for the previous experiments contained Na^+ , which can reduce single channel currents (Pallotta et al., 1981; Marty, 1983a), and the temperature in the previous experiments was typically lower, which reduces single channel currents (Barrett et al., 1982).

The Permeability Ratios of Rb^+ and NH_4^+ to K^+ Are Independent of Concentration

The activity-conductance relationship for the Ca-activated K channel (Fig. 5) is consistent with a single ion channel. A further test of this possibility was made by investigating the concentration dependence of the permeability ratios $P_{\text{Rb}}/P_{\text{K}}$ and $P_{\text{NH}_4}/P_{\text{K}}$. Laüger (1973) has shown that for biionic conditions the permeability ratios should be independent of ion concentrations.

Permeability ratios were determined from the shifts in reversal potential when K_i^+ was replaced with an equimolar concentration of Rb^+ or NH_4^+ . For final concentrations of both K_o^+ and Rb_i^+ of 50, 100, and 500 mM, the observed permeability ratios of $P_{\text{Rb}}/P_{\text{K}}$ were 0.65, 0.62, and 0.64, respectively. With 140 mM K_o and 1 M Rb_i , the permeability ratio was 0.67. These values are similar to each other and the value of 0.67 in Table I for 140-mM solutions.

For final concentrations of both K_o^+ and $\text{NH}_4_i^+$ of 20, 50, and 500 mM, the observed permeability ratios of $P_{\text{NH}_4}/P_{\text{K}}$ were 0.13, 0.12, and 0.11, respectively. With 140 mM K_o^+ and 1 M $\text{NH}_4_i^+$, the permeability ratio was 0.10. These values are similar to each other and the value of 0.11 in Table I for 140-mM solutions.

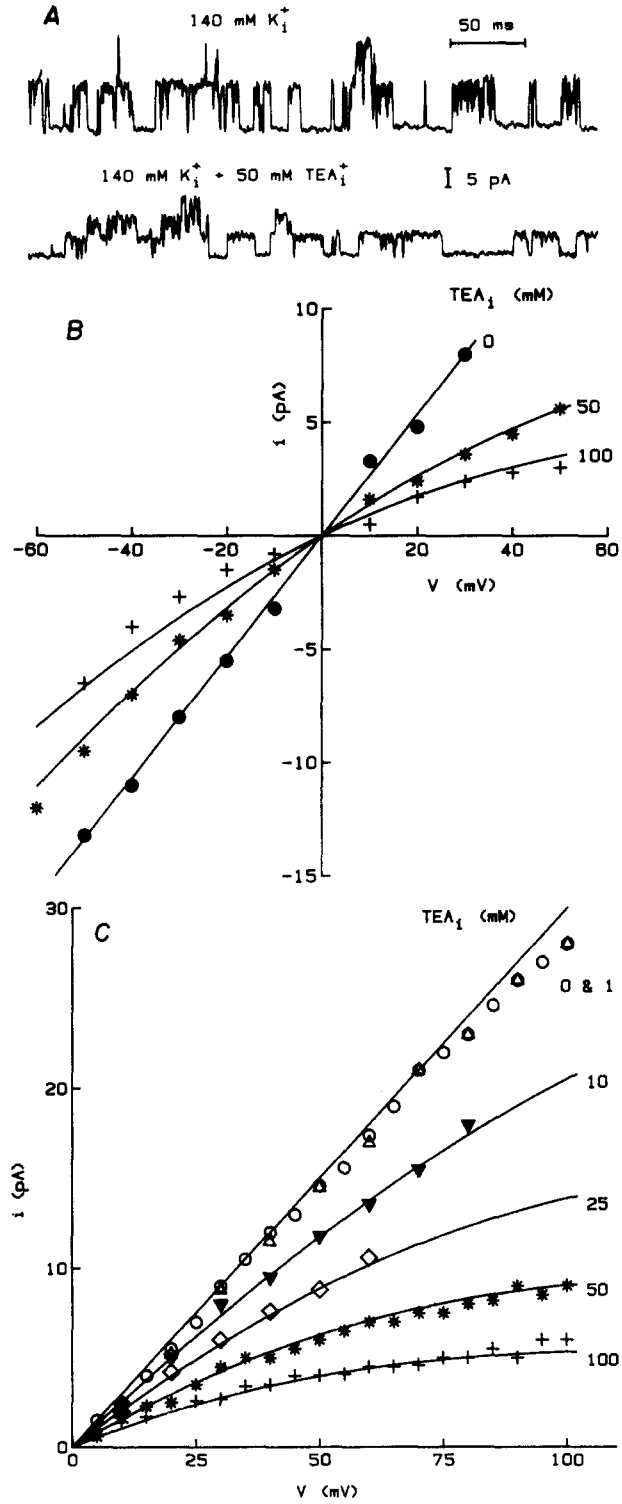
The observation that the permeability ratios of Rb^+ and NH_4^+ to K^+ are independent of ion concentration would be expected if the Ca-activated K channel is a single ion channel.

Cl^- Is Not Permeant

The shifts in reversal potential with decreasing $[\text{KCl}]_i$ in experiments of the type shown in Fig. 5 were in agreement with those predicted by the Nernst equation for a channel permeable to K^+ and impermeable to Cl^- . For example, the calculated reversal potential, assuming a perfect K^+ electrode with 100 mM K_o^+ and 20 mM K_i^+ (activities of 77 and 17.2 mM, respectively), was 38.1 mV compared with an observed reversal potential of 38.8 mV.

Voltage-dependent Block by TEA_i^+

TEA^+ at the intracellular side of the membrane reversibly reduced single channel current amplitudes at all membrane potentials. The experimental records in Fig. 6A show the reduction in single channel currents with 50 mM TEA-Cl added to the 140 mM KCl at the intracellular side of the membrane. Fig. 6, B and C, plots single channel current amplitudes against membrane potential for two experi-



ments, each of which examines a different range of membrane potentials. For these experiments, TEA_i^+ was increased from 0 to 100 mM while KCl_i was maintained at 140 mM. Two major effects can be seen. At all potentials the single channel current amplitudes are depressed by TEA_i^+ , and the blocking action is voltage dependent, becoming greater at more positive membrane potentials. For example, 100 mM TEA_i^+ reduced single channel current amplitudes 50% at -50 mV and 80% at 100 mV.

One explanation for a voltage-dependent reduction of currents is that the blocking ion binds to a blocking site within the electric field of the membrane (Armstrong, 1969, 1975; Armstrong and Hille, 1972; Woodhull, 1973; Hermann and Gorman, 1981; Coronado and Miller, 1982). Positive membrane potentials would then drive the positively charged TEA^+ toward a blocking site within the membrane. The binding of TEA^+ to the blocking site would then slow or prevent the passage of K^+ through the channel. If the time constants for blocking and unblocking are rapid compared with the time constant of the current-recording system, then partial or complete block of the channel by TEA^+ could appear as a reduction in single channel current amplitude, as shown in Fig. 6A.

Using the approach detailed by Woodhull (1973), it is possible to determine both the dissociation constant for the proposed blocking site and the location of this site within the membrane field. The ratio of the single channel conductance in the absence and presence of blocking ion at the intracellular side of the channel, g_o/g_B , would be given by

$$\frac{g_o}{g_B} = 1 + \frac{B_i}{K_D(0)} \cdot \exp(zdVF/RT), \quad (7)$$

where z , B_i , $K_D(0)$ are the blocker valence, concentration, and zero-voltage dissociation constant, respectively, and d is the fraction of voltage drop at the blocking site measured from the intracellular side of the membrane (Coronado and Miller, 1982). When Eq. 7 is linearized by taking the natural log of each side, the slope of a plot of $\ln[(g_o/g_B) - 1]$ vs. membrane potential equals zdF/RT ; thus, $d = \text{slope}(RT/Fz)$, and $K_D(0) = B_i/\exp(\text{y-intercept})$.

Such a plot is shown in the inset in Fig. 7 for 50 mM TEA_i^+ added to 140 mM K_i^+ . For this experiment, $d = 0.26$, which indicates a blocking site for TEA^+ 26% across the membrane potential from the inside, and $K_D(0) = 60$ mM, which indicates a 50% reduction in the single channel current amplitude at 60 mM TEA_i^+ .

FIGURE 6. (*opposite*) TEA_i^+ produces a voltage-dependent reduction of single channel current amplitude. (A) Single channel currents from a membrane patch internally bathed in symmetrical 140 mM KCl containing either no TEA^+ or 50 mM TEA_i^+ . The overall activity level remains about the same, but the single channel current amplitudes are markedly reduced with TEA_i^+ . $V = +40$ mV. (B and C) Single channel current amplitudes vs. membrane potential for data recorded with the indicated $[\text{TEA}^+]_i$. Lines were calculated with Eq. 7 (substituting the current ratio for the conductance ratio) with $d = 0.26$ and $K_D(0) = 60$ mM.

If Woodhull's (1973) channel blocking model can account for the voltage-dependent block by TEA_i^+ , then Eq. 7, with the fixed parameters $d = 0.26$ and $K_D(0) = 60$ mM, should account for the effect of membrane potential on single channel current amplitude and conductance over a range of concentrations of TEA_i^+ . That this is the case is shown by the lines in Figs. 6, A and B, and 7. The calculated conductance was extended over a larger range of membrane potentials in Fig. 7 than the experimental data to show the theoretical shape of the blocking effect on conductance.

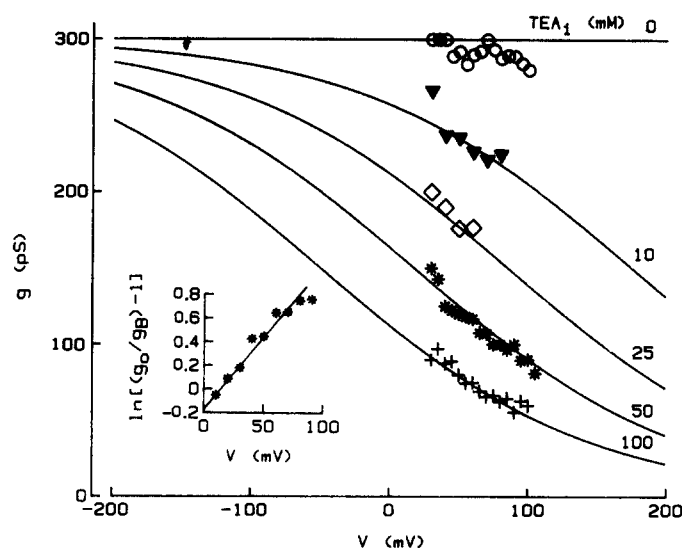


FIGURE 7. Single channel conductance plotted against membrane potential for $[\text{TEA}_i^+]$ ranging from 10 to 100 mM. The lines were calculated with Eq. 7 with the same parameters as in Fig. 6B. The lines were extended beyond the data to show the general shape of the relationship. The inset plots $\ln(g_o/g_B - 1)$ vs. membrane potential, where g_o and g_B are the single channel conductance in the absence and presence of 50 mM TEA_i^+ , respectively. The parameters $K_D(0)$ and d used with Eq. 7 are derived from this plot as explained in the text.

TEA⁺ Blocks More Effectively from the Extracellular Side of the Channel

The blocking effect of TEA^+ was also studied using outside-out membrane patches. The addition of 0.1 mM TEA_o^+ resulted in an ~25% decrease in single channel current amplitudes, and 1 mM reduced the single channel current amplitude below the limit of resolution in these experiments, which suggests a K_D of ~0.3 mM. Thus, the Ca-activated K channel from cultured muscle is asymmetrically sensitive to TEA block, with the extracellular side of the channel exhibiting at least 200 times the sensitivity to block as the intracellular side.

While examining the effect of TEA^+ at the extracellular membrane surface, we occasionally encountered a channel that was not blocked by 1–10 mM TEA_o^+ , but had a conductance similar to the Ca-activated K channel in the

absence of TEA⁺. The kinetic properties of this TEA⁺-resistant channel were relatively voltage independent, and this channel only seemed to be observed in the presence of millimolar external TEA⁺.

DISCUSSION

The results presented in this paper indicate that the selectivity sequence of the Ca-activated K channel in cultured rat muscle, as determined from the reversal potential shifts of single channel currents, is: Tl⁺ > K⁺ > Rb⁺ > NH₄⁺ ≫ Cs⁺, Li⁺, and Na⁺, with permeability ratios relative to K⁺ of: 1.2, 1.0, 0.67, 0.11, <0.05, <0.02, <0.01 (Table I). Cs⁺, Li⁺, and Na⁺ were not measurably permeant, and the permeability ratios given for these ions simply set upper limits. Cl⁻ was also not measurably permeant in our experiments, and Barrett et al. (1982) have shown that single channel conductance is independent of [Ca²⁺] in the millimolar range. Under physiological conditions, then, K⁺ would be the major charge carrier in this channel. The channel did not obey the independence principle (Hodgkin and Huxley, 1952), as both permeant and impermeant ions could have blocking effects (Figs. 4 and 5).

In agreement with our observations, Methfessel and Boheim (1982) found for the Ca-activated K channel in cultured rat muscle that Na⁺ and Cs⁺ are considerably less permeant than K⁺. The permeability ratios they report for Na⁺ (0.05–0.07) and Cs⁺ (0.10–0.25) are greater than our upper limits for these ions, but these differences may result from differences in extrapolating the current-voltage curve to the projected reversal potential, as the curves become highly nonlinear for the apparently impermeant ions as the current approaches the zero-current axis (Fig. 3).

The selectivity sequence we observed for the Ca-activated K channel in cultured rat muscle is almost identical to that found by Gorman et al. (1982) for the Ca-activated K channel in *Aplysia*: Tl⁺ (0.99) ~ K⁺ (1.0) > Rb⁺ (0.69) > NH₄⁺ (0.11) ≫ Cs⁺ (0.03), Li⁺ (<0.011), Na⁺ (<0.009).

Similar to these two Ca-activated K channels, the Ca-activated K channel from rabbit muscle t-tubule is not measurably permeable to Na⁺ and Cs⁺ (Latorre and Miller, 1983). The high permeability ratio for Na⁺/K⁺ initially reported for the channel from t-tubule (Latorre et al., 1982) was probably a result of extrapolating the linear portion of the current-voltage curve to determine the reversal potential. Unlike the Ca-activated K channels in *Aplysia* and cultured rat muscle, the Ca-activated K channel from rabbit muscle t-tubule is apparently impermeable to Rb⁺ (Latorre and Miller, 1983).

The selectivity sequence we observed for the Ca-activated K channel in cultured rat muscle is the same as found for delayed rectifier K currents in squid axon (Conti et al., 1975), node of Ranvier in frog (Hille, 1973), and frog skeletal muscle (Gay and Stanfield, 1978). These similarities suggest similarities in the selectivity filters in these K channels.

The selectivity filter must be somewhat different in inward rectifier K channels, where the relative permeability of NH₄⁺ to K⁺ is considerably reduced (Hagiwara and Takahashi, 1974), and the selectivity filter must also be somewhat different

in snail neuron delayed rectifier K^+ channels, where Cs^+ is more permeant than NH_4^+ (Reuter and Stevens, 1980).

In contrast to other K^+ channels, the K^+ channels from rabbit and frog sarcoplasmic reticulum are less selective, allowing Na^+ , Li^+ , and many organic cations to pass through (Coronado et al., 1980; Labarca and Miller, 1981; Coronado and Miller, 1982).

We found that the conductance-activity relationship of the Ca-activated K channel from cultured rat muscle (Fig. 5) saturates in a manner consistent with a channel that is occupied by, at most, a single ion at a time (Laüger, 1973). Another finding consistent with a single ion channel (Laüger, 1973) was that the permeability ratios of Rb^+ and NH_4^+ to K^+ were independent of concentration. Such single ion behavior is similar to that of the Ca-activated K channel from muscle t-tubule, the K channel from sarcoplasmic reticulum (Latorre and Miller, 1983), and the Cl^- channel from electroplax (White and Miller, 1981). The constraints of high selectivity and high conductance have led Latorre and Miller (1983) to suggest that the Ca-activated K channel has large access mouths that lead to a narrow constriction of short length, with a further constriction at the point of the selectivity filter.

Since the conductance of the Ca-activated K channel is at least 20 times greater than delayed and inward rectifier K channels, although the selectivity sequence is similar, it would appear that the differences in conductance arise from factors other than the selectivity filter.

Supporting this suggestion is the observation that K channels of small conductance appear to have quite different architecture. More than one ion may occupy these channels at a time, and at least a portion of the conducting pathway restricts the ions into a single file (reviewed by Hille and Schwarz, 1978), which suggests a long, narrow pore for the channel.

We found that the Ca-activated K channel from cultured rat muscle is sensitive to block by TEA^+ from both the intracellular and extracellular sides. Block from the intracellular side was voltage dependent, with a K_D of 60 mM at 0 mV. The blocking site was located 26% of the distance through the membrane field starting from the inside. Block by TEA^+ from the outside was much more sensitive, with a K_D of ~ 0.3 mM. Our findings are similar to those of Vergara and Latorre (1983) for the Ca-activated K channel from rabbit muscle t-tubules. These investigators report K_D 's for TEA^+ block at 0 mV of 45 mM at the *cis* (intracellular?) side and 0.29 mM at the *trans* side of their lipid bilayers, respectively. Block at the *cis* side was voltage dependent, with the blocking site detecting 35% of the total membrane field.

Ca-activated K channels in sympathetic neurons (Adams et al., 1982) and molluscan neurons (Hermann and Gorman, 1981) are similarly sensitive to block by external TEA^+ , with K_D 's of ~ 1 and 0.4 mM, respectively. Ca-activated K channels in pituitary cells appear more sensitive to block by internal TEA^+ than those in rabbit t-tubule and cultured rat muscle, as 20 mM TEA_i abolishes single channel currents (Wong et al., 1983).

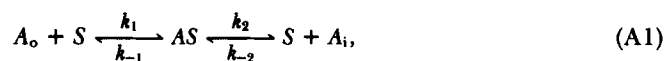
Interestingly, the slow after-hyperpolarization following an action potential in frog motoneurons and cultured rat muscle cells is not blocked by millimolar

amounts of TEA⁺ (Barrett and Barrett, 1976; Barrett et al., 1981). This slow after-hyperpolarization may arise from a different type of channel than the Ca-activated K channel studied in this paper. Marty (1983*b*) has emphasized that there may be more than one type of Ca-activated K channel, and Adams et al. (1982) find that the TEA⁺-sensitive Ca-activated K channels in sympathetic neurons serve a function similar to that of the delayed rectifier K channel.

APPENDIX

Expressions for the steady state current and conductance for a channel with a single saturable binding site are presented for the simplified condition of zero net membrane potential. Our approach follows from more detailed models that have been presented previously and which included the effects of membrane potential (Woodbury, 1971; Heckmann et al., 1973; Luger, 1973; Woodhull, 1973; Hille, 1975; Hille and Schwarz, 1978; Lewis and Stevens, 1979; Horn and Brodwick, 1980).

A kinetic scheme for a channel with two barriers and a single binding site separating permeant ions of activity A_o and A_i outside and inside the cell, respectively, is



where S and AS are the fraction of binding sites free and bound, respectively, such that $S + AS = 1$.

The inward flux is given by the rate of formation of AS from A_o times the probability that AS dissociates into $S + A_i$ instead of $S + A_o$. Thus,

$$\text{flux}_{\text{in}} = A_o S k_1 \cdot \frac{k_2}{k_{-1} + k_2}. \quad (\text{A2})$$

It follows that the outward flux is

$$\text{flux}_{\text{out}} = A_i S k_{-2} \cdot \frac{k_{-1}}{k_{-1} + k_2}. \quad (\text{A3})$$

The net flux is then $\text{flux}_{\text{out}} - \text{flux}_{\text{in}}$:

$$\text{flux}_{\text{net}} = \frac{A_i k_{-2} k_{-1} - A_o k_1 k_2}{k_{-1} + k_2} \cdot S. \quad (\text{A4})$$

S , the fraction of sites free, is given by the percentage of time the site spends in the S state. This can be calculated from the mean lifetime of state S divided by the sum of the mean lifetimes of states S and AS . The mean lifetime of state S is $1/(A_o k_1 + A_i k_{-2})$, and the sum of the mean lifetimes of states S and AS is $1/(A_o k_1 + A_i k_{-2}) + 1/(k_{-1} + k_2)$.

Simplification and substitution for S in Eq. A4 gives

$$\text{flux}_{\text{net}} = \frac{A_i k_{-2} k_{-1} - A_o k_1 k_2}{k_{-1} + k_2} \cdot \frac{k_{-1} + k_2}{k_{-1} + k_2 + A_o k_1 + A_i k_{-2}}. \quad (\text{A5})$$

For symmetrical barriers, $k_1 = k_{-2}$ and $k_{-1} = k_2$ and

$$\text{flux}_{\text{net}} = \frac{(A_i - A_o) k_1}{2 + (A_i + A_o) k_1 / k_{-1}}. \quad (\text{A6})$$

For asymmetrical solutions, the maximum flux for large concentrations of A_i can be calculated from Eq. A6 in the limit $A_i \rightarrow \infty$ to obtain

$$\text{flux}_{\max} = \lim_{A_i \rightarrow \infty} \frac{(A_i - A_o)k_1}{2 + (A_i + A_o)k_1/k_{-1}} = k_{-1}. \quad (\text{A7})$$

Eq. A7 indicates that at very high A_i , site S is instantly saturated to form AS , such that the maximum rate of flux is given by the dissociation rate constant k_{-1} . Thus, as pointed out by Armstrong (1975), the rate of transport is slowed by increasing the binding constant (the depth of the well).

Single channel flux is related to single channel current i by

$$\text{flux} = i/e, \quad (\text{A8})$$

where $e = 1.6 \times 10^{-19}$ C.

For symmetrical barriers, the dissociation constant K_D for site S is

$$K_D = k_{-1}/k_1. \quad (\text{A9})$$

Substitution of Eqs. A7–A9 into Eq. A6 gives the single channel current i for symmetrical barriers at 0 mV membrane potential:

$$i = \frac{i_{\max} \cdot (A_i - A_o)/K_D}{2 + (A_i + A_o)/K_D}. \quad (\text{A10})$$

Single channel conductance at 0 mV for symmetrical barriers can then be calculated by combining Eqs. 3 and A10 and using the Nernst equation to define the effective driving force at 0 mV to obtain

$$g = \frac{\left[\frac{i_{\max} \cdot (A_i - A_o)/K_D}{2 + (A_i + A_o)/K_D} \right]}{\frac{RT}{F} \cdot \ln \frac{A_i}{A_o}}. \quad (\text{A11})$$

The continuous lines in Fig. 5B show that Eq. A11 describes single channel conductance as a function of A_i for the Ca-activated K channel assuming $K_D = 100$ mM and $i_{\max} = 32$ pA at 0 mV. These same parameters in Eq. A10 also described the single channel current amplitude (not shown). For a maximum single channel conductance of 32 pA at 0 mV, $k_{-1} = 2 \times 10^8 \text{ s}^{-1}$ (Eqs. A7 and A8) and $k_1 = 2 \times 10^9 \text{ s}^{-1}\text{M}^{-1}$ (Eq. A9).

The symmetrical two-barrier model described by Eqs. A10 and A11 was the simplest model that described the observed effects of activity on single channel current and conductance. The differential blocking effects of TEA show, however, that the channel is not symmetrical for this blocking ion, and our results do not exclude the possibility that the channel is asymmetrical for permeant ions. More barriers will also most likely be needed to account for other properties of the channel (see, for example, Woodbury, 1971; Hille and Schwarz, 1978).

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