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Influence of External Barium and Potassium on Potassium Efflux in Depolarized Frog Sartorius Muscles

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Summary. Efflux of $^{42}K^+$ was measured in frog sartorius muscles equilibrated in depolarizing solutions with external K^+ concentrations ($[K^+]_o$) between 75 and 300 mM and NaCl concentrations of 60, 120, or 240 mM. For several combinations of KCl and NaCl, steady-state internal potentials (V_i) were the same for different $[K^+]_o$. For the range of V_i examined, K^+ efflux occurs principally through the K^+ inward rectifier channels. When external K^+ is removed V_i remains constant for 2 to 3 hr because of the high membrane conductance to Cl^-, but K^+ efflux drops by about one order of magnitude.

External Ba^{2+} in the presence or absence of external K^+ produces an inhibition of K^+ efflux described by a relation of the form $u = (u_1/(1 + C[Ba^{2+}]_o)) + u_2$, where u is the uninhibited fraction of K^+ efflux; u_1, u_2 and C are constants; and $u_1 + u_2 = 1$. C depends both on $[K^+]_o$ and V_i. When $[K^+]_o \geq 75$ mM, increasing $[K^+]_o$ at constant V_i reduces Ba^{2+} sensitivity. For constant $V_i \geq -30$ mV, Ba^{2+} sensitivity is less when $[K^+]_o = 0$ than when $[K^+]_o \geq 75$ mM. When $[K^+]_o = 0$, Ba^{2+} sensitivity decreases as V_i is made more positive. The dependence of the Ba^{2+} sensitivity on V_i at constant $[K^+]_o$ is greater when $[K^+]_o = 0$ than when $[K^+]_o \geq 75$ mM.

Both the activation of K^+ efflux by external K^+ and the Ba^{2+} inhibition of K^+ efflux can be explained on the basis of two membrane control sites associated with each channel. When both sites are occupied by K^+, the channels are in a high flux state. When one or both sites are empty, the channels are in a low, nonzero flux state. When Ba^{2+} occupies either site, K^+ efflux is further reduced. The reduction of Ba^{2+}-sensitivity by increasing $[K^+]_o$ at high $[K^+]_o$ is attributable to the displacement of Ba^{2+} from the control sites by K^+. The increased Ba^{2+} sensitivity produced by going from $[K^+]_o = 0$ to $[K^+]_o \geq 75$ mM when $V_i \geq -30$ mV is attributable to states in which Ba^{2+} occupies one site and K^+ the other when $[K^+]_o \neq 0$. The smaller V_i dependence of the Ba^{2+} sensitivity when $[K^+]_o \geq 75$ mM compared to $[K^+]_o = 0$ is attributable to the necessity that Ba^{2+} displace K^+ at the control sites when $[K^+]_o$ is high but not when $[K^+]_o = 0$.

Key Words anomalous rectifier · inward rectifier · potassium efflux · barium · muscle membrane

Introduction

In frog skeletal muscle fibers immersed in isotonic K_2SO_4 the membrane conductance is relatively high for inward current but low for outward current (Katz, 1949). Hodgkin and Horowicz (1959) observed that the K^+ permeability was high when $(V_i - V_K)$ was negative and low when $(V_i - V_K)$ was positive, where $V_K = (RT/F)\ln([K^+]_o/[K^+]_i)$, both at physiological V_i and $[K^+]_o$ and at depolarized potentials produced by initial equilibration in high $[K^+]_o$. More recent studies by Leech and Stanfield (1981) have demonstrated that the conductance properties of this "inward rectifier" system depend on V_i and $[K^+]_o$ but not on $[K^+]_i$.

Unidirectional tracer K^+ efflux decreases as $[K^+]_o$ is decreased at a constant depolarized V_i; when $[K^+]_o = 0$, K^+ efflux is at least one order of magnitude less than its maximum in high $[K^+]_o$ (Adrian, 1962; Horowicz, Gage & Eisenberg, 1968; Spalding, Senyk, Swift & Horowicz, 1981). In addition, starting in K^+-free solutions at constant V_i, addition of external Rb^+ or external K^+ increases K^+ efflux (Adrian, 1962; Spalding, Swift, Senyk, and Horowicz, 1982). For low concentrations of K^+ or Rb^+ the increment of K^+ efflux is proportional to the second power of either $[K^+]_o$ or $[Rb^+]_o$ and the proportionality constant depends on V_i (Spalding et al., 1981; 1982). The conclusion from these studies is that the K^+ inward rectifier channel is activated from a state of low flux to a state of high flux when at least two sites within the membrane associated with the channel are occupied by K^+ coming from the external solution.

External Ba^{2+} ions block the inwardly rectifying K^+ channels in frog skeletal muscle (Sperelakis, Schneider & Harris, 1967; Standen & Stanfield, 1978), in starfish egg (Hagiwara, Miyazaki, Moody & Patlak, 1978), and in cardiac Purkinje fibers (DiFrancesco, Ferroni & Visentin, 1984). In such preparations, the more negative the internal potential, the more effective external Ba^{2+} is in reducing inward current. Standen and Stanfield (1978) also showed that when $[K^+]_o$ was doubled, $[Ba^{2+}]_o$ had

to be increased by about a factor of four to obtain the same fractional reduction of inward currents.

The experiments described in this report were undertaken to examine two issues. The first was to determine whether the inhibition produced by external Ba^{2+} and the protection by external K^+ against Ba^{2+} inhibition can be accommodated by a model in which the sites that Ba^{2+} occupies to inhibit K^+ efflux are the same as those that K^+ occupies to activate K^+ efflux. The second was to explore whether the inhibition by Ba^{2+} of the small K^+ efflux through the inward rectifier channels in K^+-free solutions can be ascribed to Ba^{2+} interacting with the same sites with which it interacts when external K^+ is present; in other words, whether the Ba^{2+} sensitivities of K^+ efflux in the presence and absence of external K^+ can be attributed to a common mechanism.

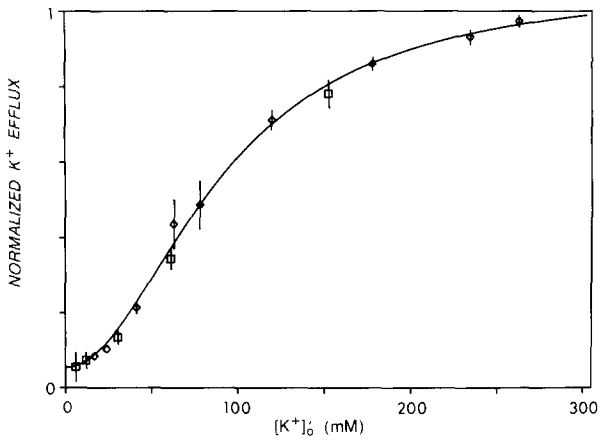

Fig. 1. Dependence of activation of K^+ efflux on $[K^+]'_o$. The K^+ efflux rate coefficient, normalized to that in 305-K^+ solution, is plotted against $[K^+]'_o$. Rate coefficients from semitendinosus bundles have been reduced by 0.051, the difference in the normalized rate coefficients in K^+-free solution measured in bundles and whole sartorius. Data from Spalding et al., 1981, (□) and 1982 (◇)

Materials and Methods

The efflux of $^{42}K^+$ from sartorius muscles from the frog *Rana pipiens* was measured as described in Spalding et al. (1982) except for details of K^+ loading of the muscles. Muscles were loaded with K^+ by a 1-hr soak in one of the high-K^+ solutions described below, preceded by a 30-min soak in an isotonic K_2SO_4 solution. After the transient volume changes were over, muscles were placed in high-K^+ solution prepared from ^{42}KCl (New England Nuclear) for another hour. Except where otherwise noted, experiments were performed at room temperature (23°C).

K^+ efflux was calculated as the fraction of counts lost from the muscle and expressed as an apparent efflux rate coefficient, k. Throughout this paper, this efflux rate coefficient is referred to simply as "K^+ efflux" and has the units of min^{-1}.

The solutions used in this study are identified by the concentration (in mM) of K^+ and Na^+ (as the chloride salt) used in their preparation, for example "150-K^+, 120-Na^+ solution." In addition, solutions contained 5 mM $MgCl_2$, 1 mM $CaCl_2$ and Tris, HEPES or PIPES buffer. Solutions containing barium are prepared by using $BaCl_2$ to replace an equimolar concentration of $MgCl_2$ up to 5 mM $BaCl_2$, or to replace NaCl at the rate of 3 mM NaCl per 2 mM divalent salt beyond 5 mM.

Results

Stimulation of K^+ Efflux by External Potassium Ions

As discussed in the Introduction, lowering the external potassium ion concentration at constant internal potential reduces isotopic K^+ efflux through the inward K^+ rectifier (Adrian, 1962; Horowicz et al., 1968; Spalding et al., 1981). Since K^+ efflux varies as the square of $[K^+]_o$ at low $[K^+]_o$ (Spalding et al., 1981, 1982), this suggests than at least two sites associated with each channel need to be occupied by K^+ from the external solution for conversion from a low to a high flux state. Since we will examine the possibility that inhibition by external Ba^{2+} results from interaction with either of the sites involved in the conversion, we first fit a simple two-site model to data on stimulation of K^+ efflux by external K^+. The parameters so obtained will then be used in fitting the inhibition by Ba^{2+} at different $[K^+]_o$.

The relation between K^+ efflux and $[K^+]_o$ is shown in Fig. 1 along with a curve from fitting the model of Appendix B to the points (data from Table 2 of Spalding et al., 1981, and Table 2 of Spalding et al., 1982). The results include measurements from whole sartorius muscles and from small bundles of fibers dissected from semitendinosus muscle, initially equilibrated in solutions at pH 7.2 in which the major constituents were 305 mM K^+, 4 mM Ca^{2+}, 2 mM Mg^{2+}, 120 mM Cl^-, and 96 mM SO_4^{2-}. When equilibrated in this solution, muscle fiber volume is about the same as in normal Ringer's fluid and the internal potential is within 2 mV of 0 mV. Low $[K^+]_o$ solutions were made by replacing K^+ with Na^+ on a mole-for-mole basis. The data from sartorius muscles have been corrected for the slow repo-

larizations which occur when $[K^+]_o$ is lowered during the relatively longer periods required for K^+ efflux measurements in sartorius muscles compared to small bundles from semitendinosus muscles (Spalding et al., 1982). Thus the data plotted in Fig. 1 are applicable to a transmembrane potential of 0 mV. Potassium efflux is plotted as the measured efflux rate coefficient in a given solution normalized to that in the equilibrating (305 mM K^+) solution.

The main features of the model are as follows. First, it is assumed that there are at least two membrane sites associated with each rectifier channel related to 'conversion' or 'activation.' When K^+ occupies both sites, the channel is in a high flux or high conductance state. When both of the sites are empty K^+ efflux is about one order of magnitude less. Further, it is assumed that the major source for K^+ at these sites is the external solution and the sites are at equilibrium with external K^+ (although there is a net efflux of K^+ for external K^+ concentrations less than 305 mM). An additional simplyfying approximation is that the fraction of channels with one activation site occupied by K^+ and the other empty is negligible for all the conditions explored.

For such a model, the normalized rate coefficient for K^+ efflux, y, follows an equation of the form

$$y = \frac{y_o + SA([K^+]'_o)^2}{1 + A([K^+]'_o)^2} \quad (1)$$

where y_o is the value of y when $[K^+]_o = 0$, A is a function of the equilibrium constants of the reaction network describing the model and S is a dimensionless scale factor introduced to allow for the fact that the K^+ efflux at $[K^+]_o = 305$ mM (used for normalization) may be less than the maximum possible (*see* derivation of Eq. (B16) in Appendix B). Use of the effective external K^+ concentration, $[K^+]'_o = [K^+]_o \exp(-V_iF/RT)$, is based on the observation that the activation sites respond quantitatively to changes of internal potential at constant $[K^+]_o$ as if they were sensitive to the entire transmembrane potential difference (Spalding et al., 1981, 1982). After the initial equilibration, the internal K^+ concentration does not change under the conditions of these experiments.

The curve drawn in Fig. 1 is the result of a nonlinear least squares procedure (Scarborough, 1966) used to fit Eq. (1) to the data points from the small bundles and sartorius muscles. The two-site model for activation of channels fits the K^+ efflux *vs.* $[K^+]'_o$ data reasonably well with $S = 1.074$ and $A = 1.210 \times 10^{-4}$ mM^{-2}.

Internal Potential and Potassium Concentration in Various KCl plus NaCl Solutions

For studies on the effects of varying V_i and $[K^+]_o$ on barium inhibition, solutions of various [KCl] and [NaCl] were devised after Boyle and Conway (1941). Six KCl levels, ranging from 75 to 300 mM, were chosen, and NaCl concentrations of 60, 120, and 240 mM were employed for each. The internal potentials, measured with microelectrodes, of sartorius muscles equilibrated in each of the solutions are given in Table 1*A*. Very few muscles survive in 300-K^+, 240-Na^+ solution, and consequently the results from this solution are not included. It is apparent that at any given $[K^+]_o$ the internal potential becomes more negative as external NaCl increases. The increment in potential varies from 14 mV in 75 mM KCl to 8 mV in 250 mM KCl for increasing NaCl from 60 to 240 mM.

For frog skeletal muscle equilibrated in high potassium $V_i = V_K$. Assuming that the potassium ion activity coefficients inside and outside the cell are equal over the range of ionic strengths used, the internal potassium ion concentration can be calculated from the relation $[K^+]_i = [K^+]_o \exp(-V_iF/RT)$. The results of this calculation are given in Table 1*B* using the measured internal potentials from Table 1*A*.

The calculated internal potassium ion concentrations are plotted as a function of the external potassium ion concentration in Fig. 2. For each concentration of NaCl the line obtained by the method of least squares has a slope of 1.0. In other words, at any given level of NaCl the equilibrated $[K^+]_i$ increases with $[K^+]_o$ on a one-for-one basis. Furthermore, at any given $[K^+]_o$, $[K^+]_i$ increases as the concentration of NaCl increases. These findings are in accord with those of Boyle and Conway (1941). Finally, for muscles equilibrated in 120 mM NaCl, $[K^+]_i$ approaches 140.5 mM as $[K^+]_o$ approaches 0. This value corresponds reasonably well with the measured $[K^+]_i$ in normal Ringer's fluid (*see* review of Venosa, 1979).

Table 1*C* summarizes the mean K^+ efflux rate coefficient for the equilibrating solutions employed. At any constant internal potential, k increases with $[K^+]_i$, as expected since in high K^+ unidirectional K^+ efflux is proportional to $[K^+]_i^2$ (Horowicz et al.,

Table 1. Properties of muscles soaked in various equilibrating solutions

A. Membrane potentials, V_i

$[Na^+]_o$	60		120		240	
$[K^+]_o$	V_i	n	V_i	n	V_i	n
75	−21.6 ± 0.5	20	−27.4 ± 0.2	29	−35.4 ± 0.4	21
100	−18.1 ± 0.3	40	−21.9 ± 0.4	30	−29.0 ± 0.5	20
150	−11.8 ± 0.2	57	−16.2 ± 0.3	35	−22.0 ± 0.3	75
200	−10.7 ± 0.4	20	−14.1 ± 0.4	19	−19.8 ± 0.7	10
250	− 8.1 ± 0.3	23	−11.6 ± 0.3	17	−16.0 ± 0.6	22
300	− 7.7 ± 0.2	79	− 9.6 ± 0.3	10		

B. Internal potassium concentrations

$[Na^+]_o$ / $[K^+]_o$	60	120	240
75	175	220	302
100	204	237	313
150	239	284	356
200	305	348	436
250	344	394	469
300	406	438	

C. K^+ efflux rate coefficients ($\times 10^{-3}$ min^{-1})

$[Na^+]_o$	60		120		240	
$[K^+]_o$	k	n	k	n	k	n
75	7.08 ± 0.21	68	7.50 ± 0.68	16	16.19 ± 0.57	16
100	7.38 ± 0.21	48	13.79 ± 0.52	8	16.43 ± 0.43	8
150	10.20 ± 0.28	120	16.14 ± 0.31	76	22.69 ± 0.87	104
200	9.32 ± 0.28	24	15.60 ± 0.49	24	31.59 ± 1.97	40
250	10.14 ± 0.66	8	18.82 ± 0.53	24	27.96 ± 2.64	40
300	13.74 ± 0.99	8	22.33 ± 1.30	16		

Muscles were equilibrated in solutions prepared with the indicated Na^+ and K^+ concentrations (in mM) as described in Materials and Methods. The tabulated membrane potentials (V_i) are presented as mean ± SE from n measurements with microelectrodes and are given in mV. The internal potassium concentrations (in mM) are calculated from the equation $[K^+]_i = [K^+]_o \exp(-V_i F/RT)$. Efflux rate coefficients are presented as mean ± SE from measurements on n muscles.

1968) and the rate coefficient is the efflux divided by $[K^+]_i$. A detailed analysis of the results along this line of reasoning does not yield any further information since there is a substantial seasonal variation in the K^+ efflux rate coefficient that confounds the results.

Effect of Varying $[K^+]_o$ or V_i on Ba^{2+} Inhibition of K^+ Efflux

Figure 3 shows Ba^{2+} inhibition of K^+ efflux from a pair of muscles equilibrated in 75-K^+, 60-Na^+ solution (panel *A*) and from a pair equilibrated in 150-K^+, 240-Na^+ solution (panel *B*). The internal potentials of muscle fibers equilibrated in these two solutions were the same within the error of measurement (*see* Table 1*A*). In each panel, one muscle was not exposed to Ba^{2+} and served as a control for the slight drift in the K^+ efflux rate coefficient. Increases in $[Ba^{2+}]_o$ promptly reduced K^+ efflux to a new steady value. When external Ba^{2+} was removed, K^+ efflux recovered to values close to those for the untreated muscles. The fraction of K^+ efflux inhibited by Ba^{2+} was greater in the solutions with the lower $[K^+]_o$, particularly for the lower concentrations of Ba^{2+}.

Figure 4 shows average values from several experiments of the fraction of the K^+ efflux, u, remaining after the addition of Ba^{2+} plotted as a func-

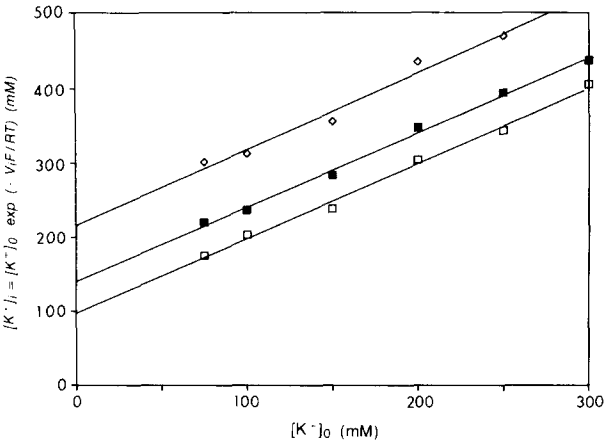

Fig. 2. Internal K^+ concentrations, $[K^+]_i = [K^+]_o \exp(-V_iF/RT)$, calculated from measured resting potentials, and plotted as a function of K^+ concentration in the equilibrating solution. The lines are drawn with slopes of 1.01, 1.00 and 1.03 and intercepts of 97.6, 140.5, and 216 mM for external Na^+ concentrations of 60 mM (□), 120 mM (■) and 240 mM (◇), respectively

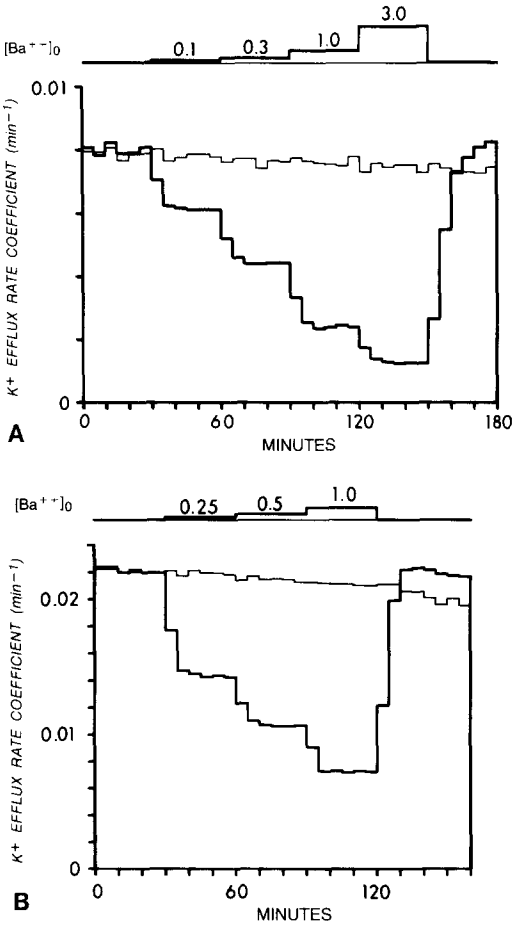

Fig. 3. Comparison of the reduction in K^+ efflux by Ba^{2+} in muscles bathed in 75-K^+, 60-Na^+ solution (*A*) or 150-K^+, 240-Na^+ solution (*B*). K^+ efflux rate coefficients during each collection period in the presence (thick line) or absence (thin line) of the $[Ba^+]_o$ indicated are plotted

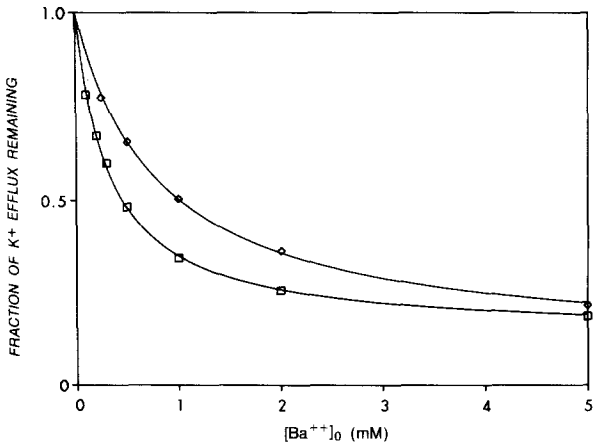

Fig. 4. Comparison of Ba^{2+} inhibition of K^+ efflux for 14 muscle pairs bathed in 75-K^+, 60-Na^+ solution (□) and four pairs bathed in 150-K^+, 240-Na^+ solution (◇). The points are means of the ratio of the K^+ efflux rate coefficient in the $[Ba^{2+}]_o$ indicated to that in the control muscle in Ba^{2+}-free solution at the same time, normalized to this ratio before exposure to Ba^{2+}. The curves are drawn according to Eq. (2) with $C = 3.05$ mM^{-1} and $u_2 = 0.14$ for 75-K^+, 60-Na^+ solution and $C = 1.22$ mM^{-1} and $u_2 = 0.09$ for 150-K^+, 240-Na^+ solution

tion of $[Ba^{2+}]_o$. The curves are plots of an equation of the form

$$u = \frac{u_1}{1 + C[Ba^{2+}]_o} + u_2 \qquad (2)$$

where u_1, u_2, and C are constants. Since $u = 1$ when $[Ba^{2+}]_o = 0$, $u_1 + u_2 = 1$. The constant C is a measure of the Ba^{2+} sensitivity of K^+ efflux; larger values of C represent greater inhibition. In this equation, u_2 represents the fraction of K^+ efflux remaining uninhibited for high values of $[Ba^{2+}]_o$; i.e., when $C[Ba^{2+}]_o \gg 1$. A discussion of the significance of equations having this form is given in Appendix A. A nonlinear least squares procedure (Scarborough, 1966) used to fit Eq. (2) to the data points gives estimates of $C = 3.05$ mM^{-1} and $u_2 = 0.136$ for muscles in 75-K^+, 60-Na^+ solution, and $C = 1.22$ mM^{-1} and $u_2 = 0.094$ for muscles in 150-K^+, 240-Na^+ solution. Thus, Eq. (2) with $[Ba^{2+}]_o$ raised to the first power adequately describes the results. Further, at constant V_i, C decreases as $[K^+]_o$ increases.

This conclusion suggests the complementary question: how does C depend on V_i when $[K^+]_o$ is kept constant? This question was approached by varying the NaCl concentration of the equilibrating solutions. The results at four external K^+ concentrations are displayed in Fig. 5. In each panel, the Ba^{2+}-sensitive K^+ efflux, i.e. $(u - u_2)/(1 - u_2)$, is plotted as a function of $[Ba^{2+}]_o$ using the value of u_2

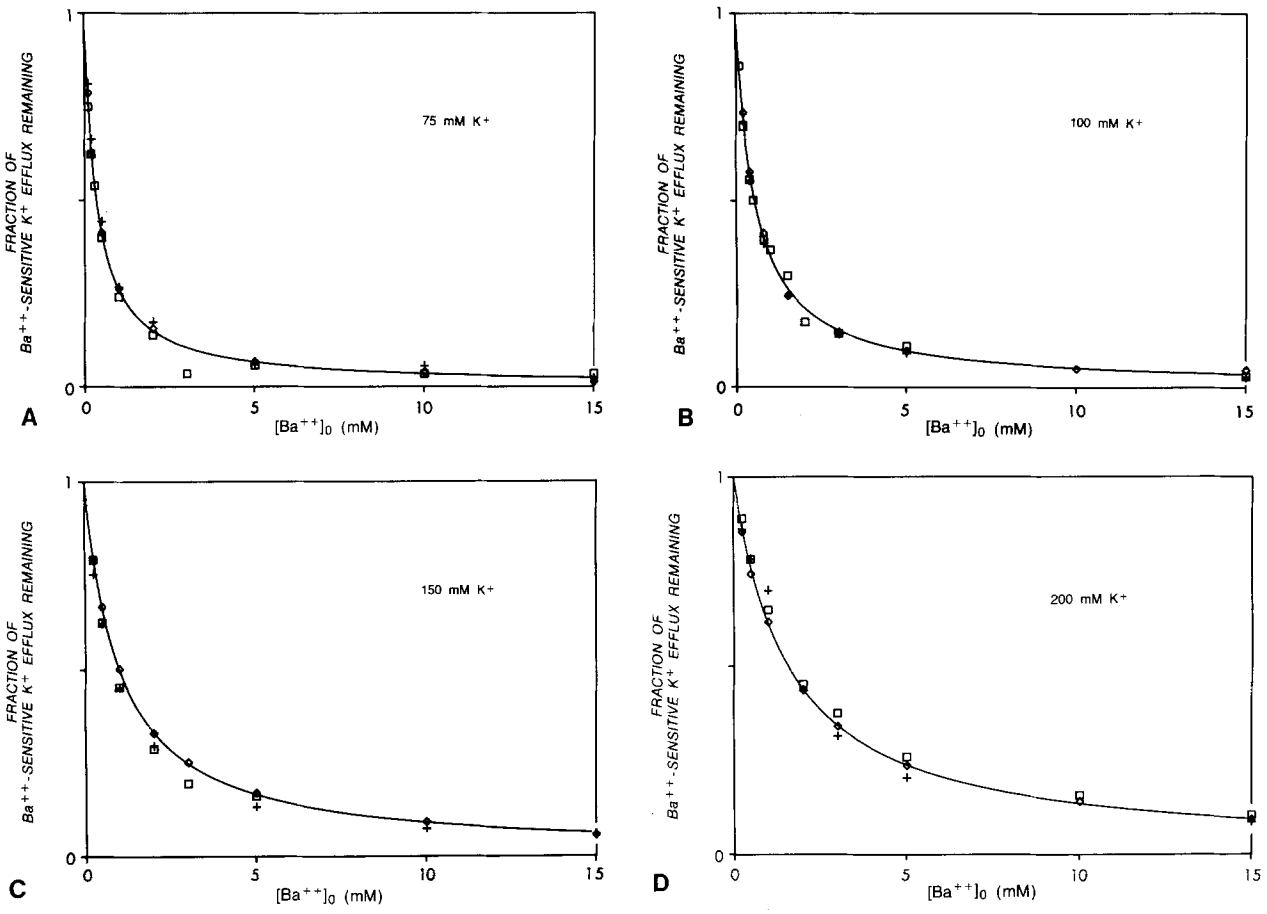

Fig. 5. Dependence of Ba^{2+}-sensitive efflux ($(u - u_2)/(1 - u_2)$ from Eq. (2)) on $[Ba^{2+}]_o$, for muscles bathed in solutions containing 75-K^+ (*A*), 100-K^+ (*B*), 150-K^+ (*C*) and 200-K^+ (*D*) and Na^+ concentrations of 60 mM (□), 120 mM (◇) and 240 mM (+). For each K^+ concentration, the curve is drawn according to $(u - u_2)/(1 - u_2) = 1/(1 + C[Ba^{2+}]_o)$ with the *C* value obtained from the results in the 120-Na^+ solutions and tabulated in Table 2 (2.83, 1.86, 1.00 and 0.64 mM^{-1}, respectively)

obtained for each solution by a nonlinear least squares procedure as in Fig. 4. The values of *C* and u_2 for each solution are given in Table 2*A*. For any given $[K^+]_o$, the Ba^{2+} sensitivity is relatively insensitive to internal potential when V_i is changed by 8 to 14 mV by varying the NaCl concentration in the equilibrating solutions for the range of V_i (−35 to −8 mV) and $[K^+]_o$ (75 to 300 mM) examined.

Ba^{2+} Inhibition of K^+ Efflux at Low Temperature

To determine the effect of lowering temperature on Ba^{2+} inhibition, similar experiments were performed at a temperature of 12°C. The values of the parameters obtained at this temperature are given in Table 2*B*. On the average, the values of *C* at a temperature of 12° are 0.71 of their values at room temperature (~23°C). This gives an apparent reaction heat of Ba^{2+} inhibition in high K^+ of about 5275 cal/mole or a $Q_{10} = 1.41$.

Ba^{2+} Inhibition of K^+ Efflux in Solutions Free of External K^+

After equilibrating muscle fibers in high concentrations of KCl, external K^+ can be removed with little change in V_i for 2 to 3 hr (Hodgkin & Horowicz, 1959). This is due to a fall in the conductance of the K^+ inward rectifier in response to the removal of external K^+ and is made evident by a drop in K^+ efflux (Adrian, 1962) so that the chloride conductance dominates and the internal potential is determined by the chloride equilibrium potential.

The experiments to be described next compare the Ba^{2+} sensitivity of K^+ efflux in the presence and absence of external K^+ at the same internal potential and examine how the Ba^{2+} sensitivity in the

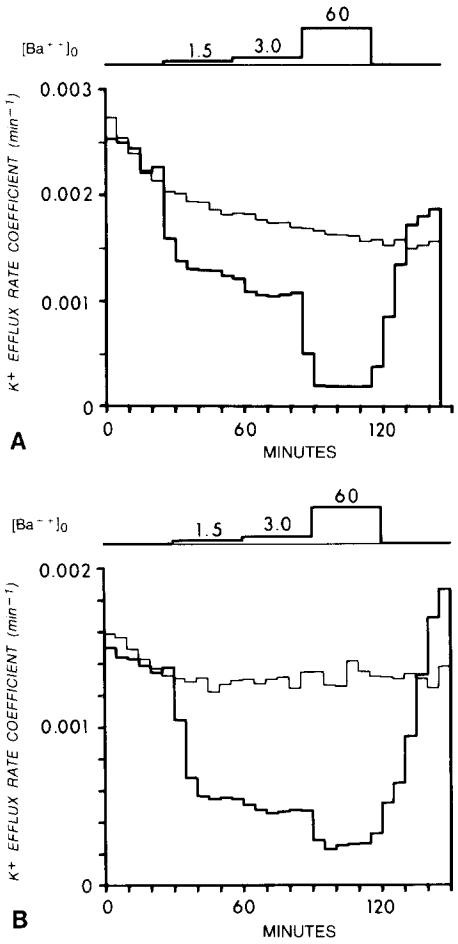

Fig. 6. Comparison of the reduction in K^+ efflux by Ba^{2+} in muscles equilibrated in 150-K^+, 60-Na^+ solution (*A*) or 150-K^+, 240-Na^+ solution (*B*), after switching to a K^+-free bathing solution (K^+ replaced by Na^+). K^+ efflux rate coefficients during each collection period in the presence (thick line) or absence (thin line) of the $[Ba^{2+}]_o$ indicated are plotted

absence of external K^+ varies with internal potential. Muscles were initially equilibrated in high K^+ solutions, and K^+ efflux was determined in the equilibrating solution, after external K^+ was removed, and then after application of Ba^{2+} in K^+-free solutions.

Figure 6 shows the K^+ efflux in K^+-free solutions from muscles equilibrated in two different solutions. Ba^{2+} reduced K^+ efflux to a new level, which depended upon $[Ba^{2+}]_o$. The fraction of K^+ efflux remaining after addition of Ba^{2+}, averaged from several experiments in each K^+-free solution, is plotted as a function of $[Ba^{2+}]_o$ in Fig. 7. The curves were obtained by a nonlinear least squares fit to the data of the equation

$$u_0 = \frac{u_{01}}{1 + C_0[Ba^{2+}]_o} + u_{02} \qquad (3)$$

Table 2. Barium inhibition parameters in various equilibrating solutions

$[Na^+]_o$	60		120		240	
$[K^+]_o$	C	u_2	C	u_2	C	u_2
A. For T = 23°C						
75	3.05	0.136	2.83	0.030	2.53	0.062
100	1.95	0.104	1.86	0.049	2.02	0.038
150	1.19	0.122	1.00	0.037	1.22	0.094
200	0.560	0.068	0.637	0.032	0.634	0.234
250	0.450	0.064	0.508	0.107	0.366	0.210
300	0.326	0.028	0.364	0.034		
B. For T = 12°C						
75	1.99	0.138	2.13	0.065	2.19	0.137
100	1.24	0.108	1.25	0.048	0.763	0.047
150	0.782	0.062	0.560	0.0	0.706	0.057
200	0.668	0.172	0.484	0.052	0.415	0.162
250	0.282	0.102	0.368	0.026	0.295	0.147
300	0.228	0.019	0.297	0.038		

The inhibition parameters C (in mM^{-1}) and u_2 were determined by a two-parameter nonlinear least squares fit of Eq. (2) to data pooled from several experiments (4 to 14 muscle pairs) of the type shown in Figs. 3 and 4. On the average, the values of C at 12° are 0.71 times those at room temperature.

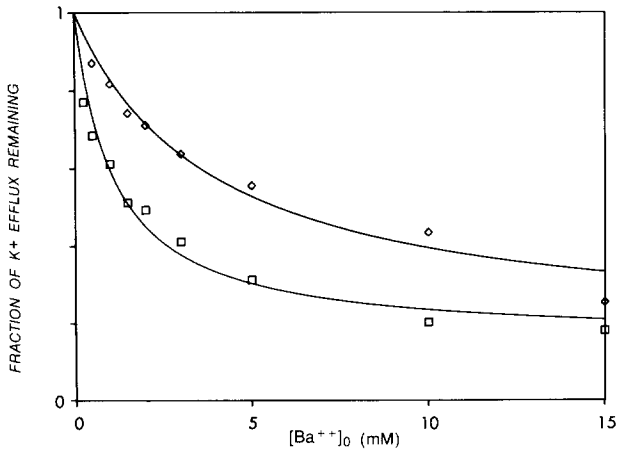

Fig. 7. Comparison of Ba^{2+} inhibition of K^+ efflux in two different K^+-free solutions. Muscle pairs were equilibrated in 150-K^+, 60-Na^+ solution (19 pairs, ◇) or 150-K^+, 240-Na^+ solution (20 pairs, □) and Ba^{2+} inhibition measured in the corresponding K^+-free solutions as in Fig. 6. The points are means of the ratio of the K^+ efflux rate coefficient in the $[Ba^{2+}]_o$ indicated to that in the control muscle in Ba^{2+}-free solution at the same time, normalized to this ratio before exposure to Ba^{2+}. The curves are drawn according to Eq. (5) with C_0 = 0.256 mM^{-1} and u_{02} = 0.16 for the muscles equilibrated in 150-K^+, 60-Na^+ solution and C_0 = 0.924 mM^{-1} and u_{02} = 0.15 for the muscles equilibrated in 150-K^+, 240-Na^+ solution

where u_{01}, u_{02}, and C_0 are constants and $u_{01} + u_{02} = 1$. Equation (3) is analogous in form to Eq. (2) and adequately fits the data. The values of C_0 obtained

Table 3. Barium inhibition parameters in K^+-free solutions

Equilibrating solution	V_i (mV)	C_0 (mM^{-1})	C (mM^{-1})
150-K^+, 60-Na^+	−11.8	0.256	1.19
150-K^+, 120-Na^+	−16.2	0.629	1.00
150-K^+, 240-Na^+	−22.0	0.924	1.22
75-K^+, 60-Na^+	−21.6	1.09	3.05
75-K^+, 120-Na^+	−27.4	0.974	2.83
75-K^+, 240-Na^+	−35.4	2.99	2.53

The inhibition parameter C_0 (in mM^{-1}) was determined by a two-parameter nonlinear least squares fit of Eq. (2) to data pooled from several experiments of the type shown in Figs. 6 and 7, in a manner analogous to that used for C in Table 2. The values of V_i and C are taken from Tables 1*A* and 2, respectively.

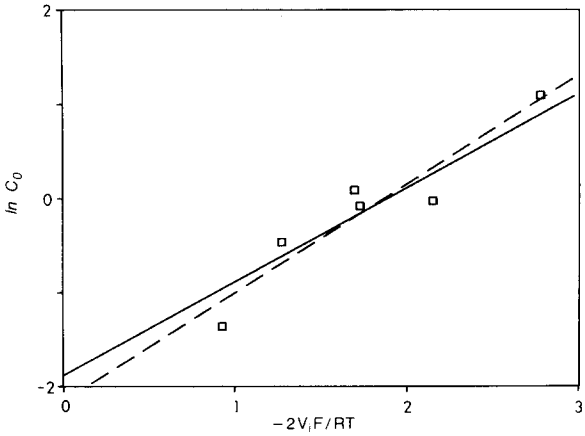

Fig. 8. Dependence of C_0 on internal potential. The value of $\ln(C_0)$ for each solution from Table 3 is plotted against $2V_iF/RT$ as in Eq. (6). The dashed line is drawn with slope $\eta = 1.16$ and intercept $\ln C_0' = -2.15$; the solid line is drawn with slope $\eta = 1$ and intercept $\ln C_0' = -1.88$.

using other equilibrating solutions are given in Table 3.

The K^+ efflux in K^+-free solutions evidently is not due to damaged fibers or nonspecific leaks since most of it is inhibitable by Ba^{2+}. Further, it is apparent from Table 3 that, at constant V_i, K^+ efflux is more sensitive to Ba^{2+} in high external K^+ than in K^+-free solutions. For example, for muscles equilibrated in 150-K^+, 60-Na^+ solution, $C = 1.189\ mM^{-1}$ when $[K^+]_o = 150$ mM but $C_0 = 0.256\ mM^{-1}$ when $[K^+]_o = 0$. This result is interesting, since in high K^+ solutions external K^+ protects against Ba^{2+} inhibition. Ba^{2+} sensitivity is less in K^+-free solutions except for muscles equilibrated in 75-K^+, 240-Na^+ solution.

This exception appears to arise because C_0 depends strongly on internal potential, as shown in Fig. 8. The internal potential used in Fig. 8 was that measured in the equilibrating solutions as indicated in Table 3. (Internal potential measurements of sartorius muscles in K^+-free solutions after equilibration in high K^+ solutions indicated repolarizations of no more than 2 mV over times comparable to those in the K^+ efflux experiments.) In general, the more negative the internal potential the greater the Ba^{2+} sensitivity in K^+-free solutions. The dashed line in the figure is the result of a linear least squares fit to the data of an equation describing C_0 as a voltage-dependent constant of the form

$$\ln C_0 = \ln C_0' + \eta(-2V_i \cdot F/RT) \quad (4)$$

where $C_0' = 0.116 \pm 0.036\ mM^{-1}$ (SE), and $\eta = 1.16 \pm 0.20$. Since η is not significantly different from 1.0, the data were then fitted by the equation

$$\ln C_0 = \ln C_0' + (-2V_iF/RT) \quad (5)$$

the logarithmic form of Eq. (B29b) of Appendix B, shown by the solid line in Fig. 9 with $C_0' = 0.153\ mM^{-1}$.

A simple explanation for the results in K^+-free solutions can be given in terms of the model. In the absence of external K^+, the activation sites are empty and the channel is in the low flux state. Ba^{2+} from the external solution can occupy either one of these sites and further lower K^+ efflux. The Ba^{2+} sensitivity is determined by the equilibrium constants for binding and the fact that the activity of the ions being bound responds to the entire transmembrane potential difference. These features of the model lead to Eqs. (3) and (5) (*see* Appendix B for development of Eqs. (B29a) and (B29b)). When external K^+ is present, the sensitivity to Ba^{2+} is increased, at constant V_i in the range of 0 to −30 mV, because channels assume new states (K^+,Ba^{2+}) and (Ba^{2+},K^+) to a significant extent (*see* Fig. 11 for state diagram and also Appendix B). The K^+ exit probabilities for these mixed states are lower than for state (K^+,K^+).

Analysis of Ba^{2+} Inhibition of K^+ Efflux when Muscles are Equilibrated in High KCl Solutions

The purpose of the analysis which follows is to determine whether these results can be accommodated by a model in which Ba^{2+} inhibits by occupying the same sites that K^+ occupies when activating K^+ efflux through the inward rectifier channels. In the model, when Ba^{2+} occupies either site, K^+ efflux is lower than when Ba^{2+} is absent. In such a model external K^+ has two opposing ways of alter-

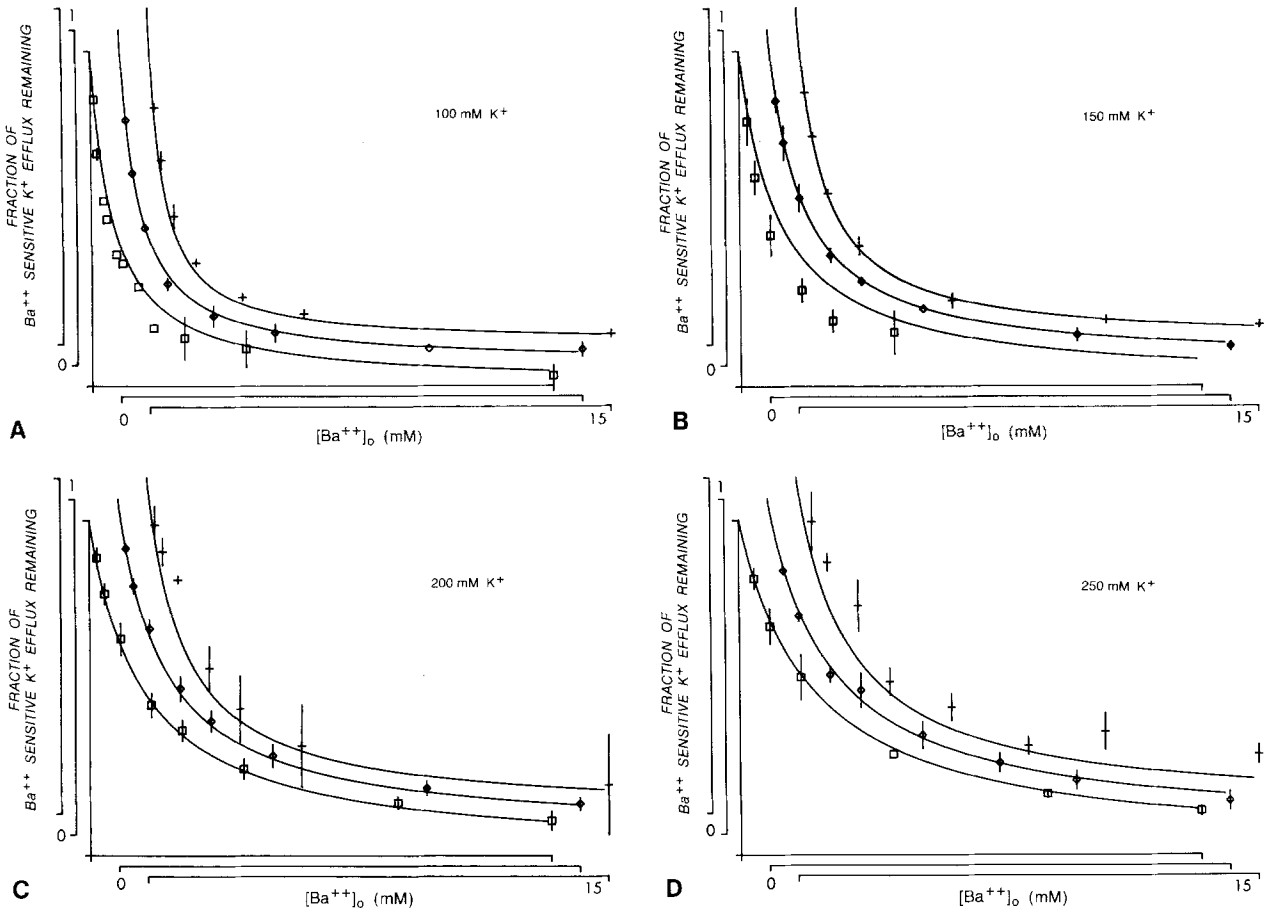

Fig. 9. Fit of the model to Ba^{2+} inhibition data. Ba^{2+}-sensitive K^+ efflux, $(u - u_2)/(1 - u_2)$, with standard deviation bars, is plotted as a function of $[Ba^{2+}]_o$ for muscles bathed in solutions containing 100-K^+ (*A*), 150-K^+ (*B*), 200-K^+ (*C*) and 250-K^+ (*D*) and Na^+ concentrations of 60 mM (□), 120 mM (◇) and 240 mM (+) as in Fig. 5. The curves are drawn according to $(u - u_2)/(1 - u_2) = 1/(1 + C[Ba^{2+}]_o)$ with the *C* values tabulated in Table 4, calculated according to the model presented in the text. For clarity, the graphs in each Na^+ concentration have been offset slightly

ing the effects of Ba^{2+}: (i) protection against inhibition by promoting state (K^+,K^+) and (ii) enhancement of inhibition by promoting the inhibitory states (K^+,Ba^{2+}) and (Ba^{2+},K^+). Which effect predominates depends on the values of the equilibrium constants of the reactions that determine the distribution of states.

Since the two sites controlling activation respond to the entire transmembrane potential difference (Spalding et al., 1981, 1982), the constant *C* should be related to $[K^+]_o$ and V_i by the relation

$$C = \frac{\{C_0' + B[K^+]_o \cdot \exp(-V_iF/RT)\} \exp(-2V_iF/RT)}{1 + A([K^+]_o \cdot \exp(-V_iF/RT))^2} \qquad (6)$$

where *A*, *B*, and C_0' are constants, and *R*, *T*, and *F* have their conventional significance (*see* Eq. (B44) in Appendix B). According to the model, *A* appears also in the analysis of external K^+ activation of K^+ efflux (in the absence of Ba^{2+}) at 0 mV (*see* Eq. (1)), and C_0' in the analysis of Ba^{2+} inhibition of K^+ efflux in K^+-free solutions (*see* Fig. 8). This leaves only *B* to be determined from the Ba^{2+} inhibition data in high K^+. To obtain *B*, Eq. (6) can be rewritten as follows:

$$B = \frac{C[1 + A([K^+]_o \exp(-V_iF/RT))^2] - C_0' \exp(-2V_iF/RT)}{[K^+]_o \exp(-3V_iF/RT)} \qquad (7)$$

For each equilibrating solution *B* can be calculated from *C* and V_i (both measured), $[K^+]_o$, and the values of *A* and C_0' obtained earlier. Using the results in Table 2*A* and averaging the values of *B*, one obtains $B = 9.69 \pm 0.62 \times 10^{-3}$ mM^{-2}. Using this value of *B* with the values of *A* and C_0' determined above, the Ba^{2+}-sensitivity predicted by Eq. (6) agrees satisfactorily with the experimental data.

Table 4 and Fig. 9 show this comparison. In

Table 4. Barium sensitivity calculated from model

$[Na^+]_o$	60		120		240	
$[K^+]_o$	C(calc.)	% dev.	C(calc.)	% dev.	C(calc.)	% dev.
75	2.14	30	2.86	1	4.12	63
100	1.46	25	1.75	6	2.41	19
150	0.789	34	0.963	4	1.24	2
200	0.588	5	0.680	7	0.863	36
250	0.430	4	0.498	2	0.597	63
300	0.357	10	0.386	6		

Values of C (in mM^{-1}) were calculated according to Eq. (3) using parameters obtained as described in the text; % dev. is the percent deviation of the calculated value of C from the experimental value tabulated in Table 2.

Fig. 9 the calculated curves are within the standard deviation bars of the data points in most cases. In 15 out of the 17 solutions, the value of C predicted by the model is within 37% of the value of C estimated from the data. Overall, the model predicts only small changes in C when internal potential is changed by 8 to 14 mV at constant $[K^+]_o$. It should be noted that in the model B is a measure of the degree to which external K^+ enhances Ba^{2+} inhibition by favoring the inhibitory states (K^+,Ba^{2+}) and (Ba^{2+},K^+).

The conclusion, therefore, is that in high external K^+, Ba^{2+} inhibits by occupying one or the other of the same sites which produce activation of K^+ efflux when both sites are occupied by K^+. In other words, the action of Ba^{2+} is consistent with the notion that it prevents activation of the inward rectifier channels by K^+. Furthermore, the lower Ba^{2+} sensitivity in K^+-free solutions is due to the absence of inhibitory states in which one control site is occupied by Ba^{2+} and the other by K^+.

Discussion

It appears that associated with each inward rectifier channel there are at least two membrane sites, whose occupancy by external K^+ or Rb^+ alters the rate of K^+ efflux (Spalding et al., 1982). The results of this report show that Ba^{2+} inhibition of K^+ efflux can be explained by interactions of Ba^{2+} with these same sites.

Others have also presented models for inward rectification involving binding of ions by multiple sites. Hille and Schwarz (1978) considered a single-file pore with three sites which may bind K^+ or a blocking ion, while in the model of Ciani, Krasne, Miyazaki and Hagiwara (1978) the availability of the permeability pathway depends on the binding of three K^+ ions. The model we consider here is not tied to any specific molecular mechanism, but demonstrates that our K^+ and Ba^{2+} results can be described by a formalism which arises from consideration of binding by two sites.

There are additional sites associated with inward rectifier channels which influence K^+ efflux. For example, a third site is required to explain the decrease in K^+ efflux as $[Rb^+]_o$ is increased above 30 mM (Spalding et al., 1982). This site does not interact appreciably with external K^+, since increasing $[K^+]_o$ does not change Rb^+ sensitivity in high external K^+. Consequently, it is unlikely that external Ba^{2+} interacts with this site since increasing $[K^+]_o$ does reduce Ba^{2+} sensitivity. In the following paper, evidence will be presented to show that at least two additional sites are needed to account for Zn^{2+} inhibition, but these do not interact with Ba^{2+}. Thus, only the activation sites appear to be involved in Ba^{2+} inhibition.

In the formulation of the model used to analyze Ba^{2+} inhibition, the states (K^+,Ba^{2+}) and (Ba^{2+},K^+) are assumed to be inhibitory. This assumption is supported by the observation that for V_i between 0 and −30 mV, Ba^{2+} sensitivity is lower in K^+-free solutions than in K^+-containing solutions. Nevertheless, at high external K^+, increasing K^+ reduces Ba^{2+} sensitivity, due to the overriding tendency to produce the (K^+,K^+) state.

External K^+ enhances inhibition of K^+ movements by certain other ions. For example, increasing external K^+ promotes inhibition by external Cs^+ of K^+ currents through the inward rectifier in starfish egg membranes (Hagiwara, Miyazaki & Rosenthal, 1976) and frog skeletal muscle (Senyk, 1984*a*,*b*). Increasing external K^+ concentration at constant internal potential also increases the inhibition of K^+ efflux by external tetraethylammonium ions (TEA^+). Figure 10 shows TEA^+ experiments done on pairs of sartorius muscles initially equilibrated in 75-K^+, 60-Na^+ solution or 150-K^+, 240-

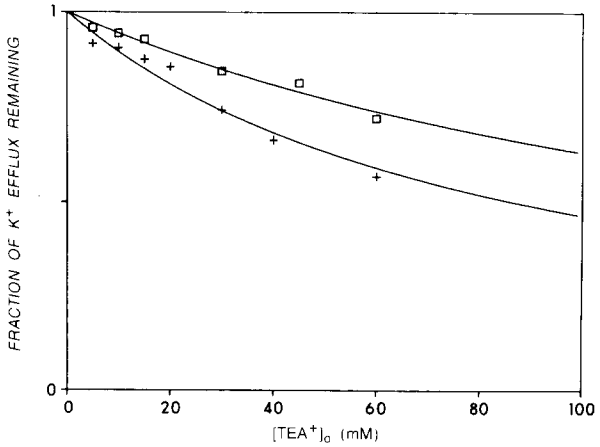

Fig. 10. Comparison of inhibition of K^+ efflux by TEA^+ for six muscle pairs bathed in 75-K^+, 60-Na^+ solution (□) and 11 pairs bathed in 150-K^+, 240-Na^+ solution (+). The points are means of the ratio of the K^+ efflux rate coefficient in the $[TEA^+]_o$ indicated to that in the control muscle in TEA^+-free solution, normalized to this ratio before exposure to TEA^+. The curves are drawn according to $u = 1/(1 + C[TEA^+]_o)$ with $C = 0.0058$ mM^{-1} for 75-K^+, 60-Na^+ solution and $C = 0.0115$ mM^{-1} for 150-K^+, 240-Na^+ solution

Na^+ solution (as in the Ba^{2+} experiments in Fig. 4). In both solutions V_i was about −22 mV. In these experiments TEA^+ replaced equimolar amounts of Na^+. It is clear that TEA^+ is about twice as potent in 150 mM K^+ (calculated half-inhibition concentration of 87 mM) as in 75 mM K^+ (half-inhibition concentration of 172 mM). Thus, enhancement of Ba^{2+} inhibition by external K^+ is not without homologous cases. A minimum of two sites is required to produce this stabilization of inhibitory configurations.

The experiments on Ba^{2+} inhibition in the presence of external K^+ were done with K^+ at electrochemical equilibrium throughout the system. This implies that all membrane sites are occupied by K^+ to the extent determined by the equilibrium constants independent of the source of the K^+ ions. If Ba^{2+} cannot penetrate into the fibers, then sites accessible to external Ba^{2+} also reach equilibrium occupancy with respect to Ba^{2+}. Under these conditions the inhibition of K^+ fluxes is determined by equilibrium relations independent of the unidirectional rate constants.

These relations no longer apply when there is a net current. For example, at a constant $[K^+]_o$ and a given V_i no net K^+ movement occurs if the internal K^+ concentration is such that $V_i = V_K$. If $[K^+]_i$ is less than this equilibrium level, then the K^+ occupancy of the sites in the transport sequence is less and net inward movement of K^+ occurs. Further, if the site with which Ba^{2+} interacts is either a site in the transport sequence, or a site not in the transport sequence but whose occupancy is influenced by the occupancy of the transport sites, then external Ba^{2+} will be more effective in inhibiting K^+ fluxes when there is net inward current, because the occupancy of the sites by K^+ is less even though both $[K^+]_o$ and V_i are the same. A similar argument can be made for the case where V_i is made more negative while internal and external concentrations remain the same: making $V_i < V_K$ reduces the K^+ occupancy of the sites, allowing net inward movement of K^+ and making external Ba^{2+} more effective as an inhibitor.

This effect likely explains the smaller voltage dependence of Ba^{2+} inhibition compared to the results of Standen and Stanfield (1978), where the internal potential dependence was determined under conditions of net inward K^+ current. Standen and Stanfield found that when $[K^+]_o = 230$ mM the Ba^{2+} sensitivity changes e-fold for a 19.5 mV change of internal potential. Using Eq. (6) we calculate an e-fold change in Ba^{2+}-sensitivity for a 25-mV change of internal potential when $[K^+]_o = 230$ mM. That the internal potential dependence of Ba^{2+} sensitivity is a function of the current is evidence that Ba^{2+} inhibits by interacting with sites that are part of or influenced by the sequence of transport sites in the channel.

In K^+-free solutions Ba^{2+} sensitivity changes e-fold for a 12.5 mV change of internal potential (*see* Fig. 8). The dependence of Ba^{2+} sensitivity on internal potential when $[K^+]_o$ is high (e-fold for a 25-mV change) is weaker because Ba^{2+} must then displace K^+ from the sites. As expected, the internal potential dependence of Ba^{2+} sensitivity for inward currents (Standen & Stanfield, 1978) is between the value when the system is in equilibrium with high external K^+ and the value in K^+-free solutions.

Ba^{2+} inhibition in both K^+-free and K^+-containing solutions is well described by a model in which K^+ efflux depends upon the occupancy of two sites, and in which it is assumed that the K^+ efflux in K^+-free solutions is a measure of the K^+ exit probability when the control sites are free of K^+. This assumption is supported to the extent that the model adequately describes Ba^{2+} inhibition in both K^+-free and K^+-containing solutions. On the other hand, the presence of a substantial K^+ conductance in parallel with the inward rectifier has been assumed by most investigators in this field. This notion was introduced by Adrian and Freygang (1962) based on current-voltage relations measured with microelectrodes in high K^+ solutions containing impermeant anions. In such solutions there is a component of outward current which depends linearly on internal potential at very positive potentials. External Rb^+ reduces the large inward currents in these solutions

without altering the linear outward currents (Adrian, 1964). In part, this component has been ascribed to leaks introduced by the microelectrodes, but this is not the entire story since there is evidence that muscle fibers equilibrated in solutions with high $[K^+]_o$ $[Cl^-]_o$ products lose considerable amounts of internal K^+ and Cl^- when V_i is made positive by reducing external Cl^- (Hodgkin & Horowicz, 1959). Thus there is no clear evidence that outward currents use a pathway other than the inward rectifier channels. A detailed discussion supporting the view that the K^+ efflux from depolarized muscles in K^+-free solutions occurs through the inward rectifier channels in a low flux state is given by Spalding et al. (1981).

Nevertheless, there is evidence that K^+ channels other than the delayed and inward rectifiers are present in muscle membranes. Myotubes and adult skeletal muscle contain K^+ channels opened by internal Ca^{2+} (Barrett, Magleby & Pallotta, 1982; Latorre, Vergara & Hidalgo, 1982; Fink, Hase, Lüttgau & Wettwer, 1983), and frog muscle contains K^+ channels that open at low ATP concentrations (Spruce, Standen & Stanfield, 1985). However, there is no evidence to indicate that the ionized Ca^{2+} concentrations in depolarized, relaxed muscle are sufficient to open such Ca^{2+}-activated K^+ channels as might be present in frog skeletal muscle, and since frog muscle depolarized by high external K^+ does not have significantly reduced levels of ATP (Edwards & Carlson, 1964) the ATP-sensitive channels also are likely not involved in the studies reported here.

In summary, we conclude that external Ba^{2+} inhibits K^+ efflux through the inward rectifier by interacting with the same sites that external K^+ ions occupy when activating K^+ efflux. In addition, we conclude that the major portion of the K^+ efflux in K^+-free solutions utilizes the inward rectifier channels in a low conductance state and exhibits relatively low Ba^{2+} sensitivity because external K^+ is not available to stabilize the inhibitory complexes formed between Ba^{2+} and the control sites.

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Appendix A

Significance of the Two-Parameter Inhibition Equation

The purpose of this section is to illustrate that Eq. (2), describing the dependence of the uninhibited K^+ efflux, u, on the external concentration of inhibitor, $[I]$, can arise in two ways.

Two classes of K^+ channels are taken to be present in the cell membranes: the channels of one class are assumed sensitive to the inhibitor, while channels of the other class are insensitive. Each channel in the sensitive class has an inhibitor binding site, denoted by ( ). At equilibrium, the number of channels with bound ligand, $[(I)]$, is

$$[(I)] = C[(\mathrm{O})][I] \tag{A1}$$

where $[(\mathrm{O})]$ is the number of channels with an empty site and C is the equilibrium constant. Assuming the number of sensitive channels, T, remains constant, the fraction of channels with bound inhibitor, f_1, is

$$f_1 = [(I)]/T \tag{A2a}$$

and the fraction with empty sites, f_0, is

$$f_0 = [(\mathrm{O})]/T. \tag{A2b}$$

Since $f_1 + f_0 = 1$, solving for f_0, gives

$$f_0 = 1/(1 + C[I]). \tag{A3}$$

There are no analogous equations for the insensitive channels. It is assumed that their total number, T', remains constant.

The probability of internal K^+ exiting through a channel in the sensitive class per unit time will be denoted p_0 for state (O), and p_1 for state (I). For a K^+ channel of the insensitive class, the probability of K^+ exit will be denoted p'. The total probability of K^+ exit, k, is

$$k = p_0 f_0 T + p_1 f_1 T + p'T' \tag{A4}$$

or

$$k = f_0(p_0 - p_1)T + p_1 T + p'T' \tag{A5}$$

since $f_1 = 1 - f_0$.

When $[I] = 0$, then $f_0 = 1$; and denoting k for this condition by $k(0)$, one obtains

$$k(0) = p_0 T + p'T'. \tag{A6}$$

If one wishes to determine how the probability of K^+ exiting depends on $[I]$, it is convenient to define a variable u by the ratio

$$u = k/k(0). \tag{A7}$$

Substituting Eqs. (A5) and (A6) into (A7), one obtains

$$u = f_0(p_0 - p_1)T/(p_0 T + p'T') + (p_1 T + p'T')/(p_0 T + p'T'). \tag{A8}$$

Substituting Eq. (A3) into (A8)

$$u = u_1/(1 + C[I]) + u_2 \tag{A9}$$

where $u_1 = (p_0 - p_1)T/(p_0 T + p'T')$ and $u_2 = (p_1 T + p'T')/(p_0 T + p'T')$, which is the same as Eq. (2). Note that $u = 1$ when $[I] = 0$, $u = u_2$ when $C[I] \gg 1$, and $u_1 + u_2 = 1$.

There are two adjustable parameters in Eq. (A9); we have taken u_2 and C as the adjustable parameters.

Nonzero values of u_2 can arise because $p_1 T > 0$ or $p'T' > 0$ or both; that is, because sensitive channels with bound inhibitor allow K^+ exit ($p_1 > 0$) or because insensitive channels are present ($T' > 0$) that allow K^+ exit ($p' > 0$). For either case C is an adequate measure of the equilibrium constant for binding of inhibitor to the sites associated with K^+ channels of the sensitive class.

We are unaware of any K^+ channel proven to be Ba^{2+} insensitive, but we do not rule out the possibility of such a K^+ channel. Another possibility is that there are Ba^{2+}-insensitive channels which have insufficient selectivity for K^+ to be considered K^+ channels but which permit detectable movements of K^+.

Although nonzero values of u_2 can arise in different ways, the main point of this appendix is to indicate that nonzero K^+ efflux in high inhibitor concentrations is compatible with the existence of channels where inhibitor does not reduce K^+ exit to zero.

Appendix B

Two-Site Model for K^+ Activation and Ba^{2+} Inhibition

This section presents a model for the actions of external K^+ and external Ba^{2+} on K^+ efflux through inward rectifier channels. We assume that two sites associated with each channel are related to activation of K^+ efflux by external K^+, to inhibition of K^+ efflux by Ba^{2+}, and to the alterations of the sensitivity of inhibition by Ba^{2+} produced by varying external K^+.

The reaction network describing the various states possible is given in Fig. 11. An empty site is denoted by zero. The state in which Ba^{2+} occupies both sites is omitted since there is no evidence in the experimental results to indicate that this state is present. The occupancy of the sites by either of the two external ions is affected by the transmembrane potential.

Equilibrium with K^+ and Ba^{2+} in the external solutions is assumed. The Ba^{2+} inhibition studies in high K^+ solutions are in fact done when K^+ is in electrochemical equilibrium by experimental design. The justification for using equations derived with this assumption when fitting the activation of K^+ efflux by external K^+ under conditions of net K^+ loss is that the conductance determined by $[K^+]_o$ and V_i is not measurably affected by changes in internal K^+ concentration (Stanfield, Standen, Leech & Ashcroft, 1981).

Finally, it is assumed that internal K^+ exits with a high probability only when both sites are occupied by K^+, i.e., in state (K^+,K^+). All other states are assumed to reduce K^+ exit. Experimental results indicate that the K^+ exit probability is about 10 or 20 times less for states (O,O), (O,K^+) and (K^+,O) than for state (K^+,K^+). K^+ exit probability is at least a factor of 5 less for states (O,Ba^{2+}) and (Ba^{2+},O) than for state (O,O). For the mixed states (Ba^{2+},K^+) and (K^+,Ba^{2+}) the measurements suggest K^+ exit probabilities intermediate between those of state (O,O) and states (O,Ba^{2+}) and (Ba^{2+},O).

Activation of K^+ Efflux by External K^+

In the absence of external Ba^{2+}, four states are present: (O,O), (O,K^+), (K^+,O), and (K^+,K^+). At equilibrium, the occupancy of each site depends on $[K^+]_o$ and V_i. On the basis of previous experiments (Spalding et al., 1981, 1982) both sites are assumed to be sensitive to the entire transmembrane potential difference. The concentrations of channels in each of the four states are related as follows:

$$[O,O] + [O,K^+] + [K^+,O] + [K^+,K^+] = T \tag{B1}$$

where T is a constant. At equilibrium the following relations hold:

$$[K^+,O] = K_1[K^+]'_o[O,O] \tag{B2}$$

$$[O,K^+] = K_2[K^+]'_o[O,O] \tag{B3}$$

$$[K^+,K^+] = K_3[K^+]'_o[O,K^+] = K_3K_2([K^+]'_o)^2[O,O] \tag{B4}$$

where K_1, K_2, and K_3 are the equilibrium constants for the individual reactions and $[K^+]'_o = [K^+]_o \exp(-V_iF/RT)$. Substituting Eqs. (B2), (B3), and (B4) into (B1), one obtains

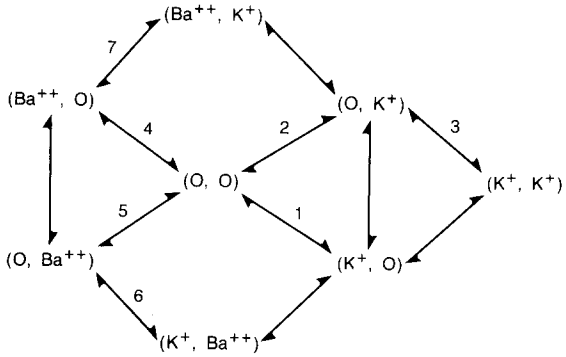

Fig. 11. State diagram for the model described in the text. The symbol for each state indicates the occupancy of the two sites by K^+ or Ba^{2+}. An empty site is denoted O. The numbers refer to the reactions whose equilibrium constants are used in the equations in Appendix B

$$[O,O] \cdot Q = T \tag{B5}$$

where

$$Q = 1 + (K_1 + K_2)[K^+]'_o + K_3 \cdot K_2([K^+]'_o)^2. \tag{B6}$$

The fraction of channels in any of the four states is given by the relations:

$$f_{OO} = [O,O]/T;\ f_{KO} = [K^+,O]/T;\ f_{OK} = [O,K^+]/T \tag{B7}$$

and

$$f_{KK} = [K^+,K^+]/T. \tag{B8}$$

If the probability of K^+ exit in each state is denoted by p_{OO}, p_{KO}, p_{OK}, and p_{KK}, then the total probability of K^+ exit, k, is

$$k = (p_{OO}f_{OO} + p_{KO}f_{KO} + p_{OK}f_{OK} + p_{KK}f_{KK})T. \tag{B9}$$

In analyzing the results on activation of K^+ efflux it is useful to normalize to a reference level at close to maximal K^+ efflux. This procedure compensates for variations in the maximal rate of efflux between muscles from different animals. The normalized K^+ efflux, y, is

$$y = \frac{k}{k'} = \frac{p_{OO}f_{OO} + p_{KO}f_{KO} + p_{OK}f_{OK} + p_{KK}f_{KK}}{p_{OO}f'_{OO} + p_{KO}f'_{KO} + p_{OK}f'_{OK} + p_{KK}f'_{KK}} \tag{B10}$$

where the primed symbols represent the values of the variables at the reference K^+ concentration. If one defines a factor, S', by the relation

$$S' = 1/(p_{OO}f'_{OO} + p_{KO}f'_{KO} + p_{OK}f'_{OK} + p_{KK}f'_{KK}) = 1/k' \tag{B11}$$

then

$$y = S'(p_{OO}f_{OO} + p_{KO}f_{KO} + p_{OK}f_{OK} + p_{KK}f_{KK}). \tag{B12}$$

S' is a constant because the reference K^+ concentration is a constant, and enters into the analysis as a scale factor.

If one assumes that the low K^+ exit probabilities are nearly equal (i.e., $p_{OO} \simeq p_{KO} \simeq p_{OK}$), then one can rewrite Eq. (B12) as

$$y = \frac{S'(p_{OO}(1 + (K_1 + K_2)[K^+]'_o) + p_{KK}K_3K_2([K^+]'_o)^2)}{1 + (K_1 + K_2)[K^+]'_o + K_3K_2([K^+]'_o)^2}. \quad \text{(B13)}$$

Since the reference K^+ concentration used in the activation experiments was high ($[K^+]'_o = 305$ mM), relative to the K^+ concentration producing 50% of maximal K^+ efflux, f'_{KK} in these experiments is about a factor of 10 greater than ($f'_{OO} + f'_{OK} + f'_{KO}$). In addition, experiments indicate that p_{KK} is about a factor of 10 greater than ($p_{OO} + p_{OK} + p_{KO}$). Thus, to a good approximation $S' = 1/p_{KK}f'_{KK}$, and

$$y = \frac{S((p_{OO}/p_{KK})(1 + (K_1 + K_2)[K^+]'_o) + K_3K_2([K^+]'_o)^2)}{1 + (K_1 + K_2)[K^+]'_o + K_3K_2([K^+]'_o)^2} \quad \text{(B14)}$$

where $S = 1/f'_{KK}$. The relative rate of K^+ efflux in K^+-free solutions, y_o, is $y_o = Sp_{OO}/p_{KK}$. Thus,

$$y = \frac{y_o(1 + (K_1 + K_2)[K^+]'_o) + SK_3K_2([K^+]'_o)^2}{1 + (K_1 + K_2)[K^+]'_o + K_3K_2([K^+]'_o)^2}. \quad \text{(B15)}$$

Since the increment in K^+ efflux is proportional to the second power of the concentration of external K^+ at low concentrations (Spalding et al., 1981, 1982), the terms containing the factor $(K_1 + K_2)[K^+]'_o$ are small relative to the other terms in the equation. Hence Eq. (B15) can be further approximated by the relation

$$y \simeq \frac{y_o + SA([K^+]'_o)^2}{1 + A([K^+]'_o)^2} \quad \text{(B16)}$$

where $A = K_3 \cdot K_2$. If one takes the average measured relative rate coefficient for K^+ efflux in K^+-free solutions for y_o, then Eq. (B16) can be fit to the activation data by adjusting two constants, S and A, where S is near 1. Equation (B16) adequately fits the activation data, as shown in Fig. 1, indicating that f_{OK} and f_{KO} do not become significantly large as external K^+ concentration is increased from zero to high values.

Ba^{2+} Inhibition of K^+ Efflux in K^+-Free Solutions

In K^+-free solutions, three states are present: (O,O), (O,Ba^{2+}) and (Ba^{2+},O). The condition of constancy of total numbers of channels gives

$$[O,O] + [Ba^{2+},O] + [O,Ba^{2+}] = T. \quad \text{(B17)}$$

At equilibrium,

$$[Ba^{2+},O] = K_4[Ba^{2+}]'_o[O,O] \quad \text{(B18)}$$

$$[O,Ba^{2+}] = K_5[Ba^{2+}]'_o[O,O] \quad \text{(B19)}$$

where K_4 and K_5 are the equilibrium constants and $[Ba^{2+}]'_o = [Ba^{2+}]_o \exp(-2V_iF/RT)$.
As before,

$$[O,O] \cdot Q' = T \quad \text{(B20)}$$

where

$$Q' = 1 + (K_4 + K_5)[Ba^{2+}]'_o. \quad \text{(B21)}$$

The probability k_o of K^+ exit in K^+-free solutions is

$$k_0 = (p_{BaO}f_{BaO} + p_{OBa}f_{OBa} + p_{OO}f_{OO})T \quad \text{(B22)}$$

where

$$f_{Bao} = \frac{[Ba^{2+},O]}{T} = \frac{K_4[Ba^{2+}]'_o}{Q'}; \; f_{OBa} = \frac{[O,Ba^{2+}]}{T} = \frac{K_5[Ba^{2+}]'_o}{Q'}$$

and

$$f_{OO} = \frac{[O,O]}{T} = \frac{1}{Q'}. \quad \text{(B23)}$$

If we assume that the K^+ exit probabilities for the two Ba^{2+}-occupied states are about equal (i.e., $p_{BaO} \simeq p_{OBa}$) then

$$k_0 = (p_{BaO}(K_4 + K_5)[Ba^{2+}]'_o + p_{OO})T/Q'. \quad \text{(B24)}$$

In analyzing the dependence of Ba^{++} inhibition of K^+ efflux it is useful to introduce a variable, u, to denote the fraction of K^+ efflux that remains in the presence of external Ba^{2+}. For K^+-free solution, it is defined by the ratio

$$u_0 = \frac{k_0([Ba^{2+}]_o \neq 0)}{k_0([Ba^{2+}]_o = 0)}. \quad \text{(B25)}$$

When $[Ba^{2+}]_o = 0$, $f_{BaO} = 0 = f_{OBa}$ and $f_{OO} = 1$, so

$$k_0([Ba^{2+}]_o = 0) = p_{OO}T. \quad \text{(B26)}$$

Substituting Eqs. (B26), (B23) and (B22) into (B25), one obtains

$$u_0 = (1 + (p_{BaO}/p_{OO})(K_4 + K_5)[Ba^{2+}]'_o)/Q' \quad \text{(B27)}$$

or

$$u_0 = \frac{1 + u_{02}C'_0[Ba^{2+}]'_o}{1 + C'_0[Ba^{2+}]'_o} \quad \text{(B28)}$$

where $u_{02} = (p_{BaO}/p_{OO})$ and $C'_0 = (K_4 + K_5)$. This equation can be transformed to

$$u_0 = \frac{u_{01}}{1 + C_0[Ba^{2+}]_o} + u_{02} \quad \text{(B29a)}$$

where

$$C_0 = C'_0 \exp(-2V_iF/RT) \text{ and } u_{01} + u_{02} = 1. \quad \text{(B29b)}$$

Equations (B29a) and (B29b) are used to fit the experimental results obtained in K^+-free solutions.

Ba^{2+} Inhibition of K^+ Efflux in K^+ Containing Solutions

When both external Ba^{2+} and K^+ are present, two additional states become available: (Ba^{2+},K^+) and (K^+,Ba^{2+}). Now,

$$[O,O] + [O,K^+] + [K^+,O] + [K^+,K^+] + [Ba^{2+},O] + [O,Ba^{2+}] + [Ba^{2+},K^+] + [K^+,Ba^{2+}] = T. \quad \text{(B30)}$$

At equilibrium,

$$[K^+,Ba^{2+}] = K_6[K^+]'_o[O,Ba^{2+}] = K_6K_5[K^+]'_o[Ba^{2+}]'_o[O,O] \quad \text{(B31)}$$

$$[Ba^{2+},K^+] = K_7[K^+]'_o[Ba^{2+},O] = K_7K_4[K^+]'_o[Ba^{2+}]'_o[O,O] \quad \text{(B32)}$$

where K_4, K_5, K_6 and K_7 are the equilibrium constants for the individual reactions.
As before,

$$[O,O] \cdot Q'' = T \quad \text{(B33)}$$

where

$$Q'' = 1 + (K_1 + K_2)[K^+]'_o + K_3K_2([K^+]'_o)^2 + (K_4 + K_5)[Ba^{2+}]'_o + (K_6K_5 + K_7K_4)[K^+]'_o[Ba^{2+}]'_o. \quad \text{(B34)}$$

The experimental results indicate that the K^+ exit probabilities for the mixed states (Ba^{2+},K^+) and (K^+,Ba^{2+}) are substantially lower than that of the (K^+,K^+) state and somewhere in the range of K^+ exit probabilities between those of the (O,O) and (Ba,O) states. For convenience, we can group the K^+ exit probabilities according to the following relations: $p_{BaK} \simeq p_{KBa}$; $p_{BaO} \simeq p_{OBa}$; and $p_{OO} \simeq p_{OK} \simeq p_{KO}$. Using these approximations, the total probability of K^+ exit, k, is

$$k = [p_{KK}f_{KK} + p_{OO}(f_{OO} + f_{OK} + f_{KO}) + p_{BaO}(f_{BaO} + f_{OBa}) + p_{BaK}(f_{BaK} + f_{KBa})]T \quad \text{(B35)}$$

where

$$f_{KK} = \frac{K_3K_2([K^+]'_o)^2}{Q''} \quad \text{(B36a)}$$

$$f_{OO} + f_{OK} + f_{KO} = \frac{1 + (K_1 + K_2)[K^+]'_o}{Q''} \quad \text{(B36b)}$$

$$f_{BaO} + f_{OBa} = \frac{(K_4 + K_5)[Ba^{2+}]'_o}{Q''} \quad \text{(B36c)}$$

and

$$f_{BaK} + f_{KBa} = \frac{(K_6K_5 + K_7K_4)[K^+]'_o[Ba^{2+}]'_o}{Q''}. \quad \text{(B36d)}$$

The fraction of K^+ efflux that remains in the presence of external Ba^{2+}

$$u = \frac{k([Ba^{2+}]_o \neq 0)}{k([Ba^{2+}]_o = 0)} \quad \text{(B37)}$$

is

$$u = (1 + D[Ba^{2+}]_o) \cdot \frac{Q''([Ba^{2+}]_o = 0)}{Q''([Ba^{2+}]_o \neq 0)} \quad \text{(B38)}$$

where $Q''([Ba^{2+}]_o = 0)$ is the value of Q'' in the absence of external Ba^{2+}, $Q''([Ba^{2+}]_o \neq 0)$ is the value of Q'' in the presence of external Ba^{2+} at the concentration applied to the muscle fibers, and D is a constant given by

$$D = \frac{(p_{BaO}(K_4 + K_5) + p_{BaK}(K_6K_5 + K_7K_4)[K^+]'_o) \exp(-2V_iF/RT)}{p_{OO}(1 + (K_1 + K_2)[K^+]'_o) + p_{KK}K_3K_2([K^+]'_o)^2}. \quad \text{(B39)}$$

Equations (B38) and (B39) follow from the fact that the K^+ exit probabilities, the total number of channels, and the equilibrium constants are all Ba^{2+} independent, plus the fact that $[K^+]_o$ and V_i are constant by experimental design. Using Eq. (B34) one obtains

$$\frac{Q''([Ba^{2+}]_o = 0)}{Q''([Ba^{2+}]_o \neq 0)} = \frac{1}{1 + C[Ba^{2+}]_o} \quad \text{(B40)}$$

where

$$C = \frac{((K_4 + K_5) + (K_6K_5 + K_7K_4)[K^+]'_o) \exp(-2V_iF/RT)}{1 + (K_1 + K_2)[K^+]'_o) + K_3K_2([K^+]'_o)^2} \quad \text{(B41)}$$

and, hence

$$u = \frac{1 + D[Ba^{2+}]_o}{1 + C[Ba^{2+}]_o}. \quad \text{(B42)}$$

Using the definitions $u_2 = D/C$ and $u_1 + u_2 = 1$, this equation can be rewritten as

$$u = \frac{u_1}{1 + C[Ba^{2+}]_o} + u_2. \quad \text{(B43)}$$

If one recalls that $C'_0 = (K_4 + K_5)$, $A = K_3K_2$ and $(K_1 + K_2)[K^+]'_o \ll 1 + K_3K_2([K^+]'_o)^2$ then Eq. (B41) can be rewritten as

$$C = \frac{(C'_0 + B[K^+]_o \exp(V_iF/RT)) \exp(-2V_iF/RT)}{1 + A([K^+]_o \exp(-V_iF/RT))^2} \quad \text{(B44)}$$

where

$$B = (K_6K_5 + K_7K_4).$$

Equation (B44) shows explicitly how C, the constant which measures the Ba^{2+} sensitivity of the K^+ efflux, depends on $[K^+]_o$ and V_i. The constant A can be obtained from fitting the results of external K^+ activation of K^+ efflux in the absence of external Ba^{2+} with Eq. (B16). The constant C'_0 can be obtained from fitting the results of Ba^{2+} inhibition in the absence of external K^+ using Eqs. (B29a) and (B29b). Finally, the constant B can then be obtained by fitting the measured values of C when muscles are equilibrated in solutions having different external K^+ concentrations and different internal potentials using Eq. (B44) and the values of A and C'_0.