J. Membrane Biol. 86, 89–97 (1985)

The Journal of
Membrane Biology

Barium Modifies the Concentration Dependence of Active Potassium Transport by Insect Midgut

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Summary. The rate of active K^+ transport by the isolated lepidopteran midgut shows a rectangular hyperbolic relation to $[K^+]$ over the range 20 to 70 mM K^+ in the absence of any divalent cation. Addition of Ba^{++} to the hemolymph (K^+ uptake) side introduces a linear component to the concentration dependence, such that active K^+ transport is decreased at $[K^+]$ of 55 mM or less, but increased transiently at higher $[K^+]$. As $[Ba^{++}]$ is increased over the range 2 to 8 mM the linear component increases and the saturating component decreases; in 8 mM Ba^{++} the concentration dependence is dominated by the linear component. The effect of Ba^{++} cannot easily be accounted for by simple competition with K^+ for basal membrane uptake sites. Similar effects might be exercised by other alkali earth cations, since the concentration dependence of active K^+ transport possesses a substantial linear component in solutions containing 5 mM Ca^{++} and 5 mM Mg^{++} (the alkali earth metal concentrations of standard lepidopteran saline).

Key Words Ba^{++} · active K^+ transport · lepidopteran insect midgut · short-circuit current · transport kinetics

Introduction

The alkali earth metal barium (Ba^{++}) has been advanced as a specific blocker of K^+ permeability in a variety of epithelial and nonepithelial cells, including vertebrate cardiac muscle (Hermsmeyer & Sperelakis, 1970), skeletal muscle (Sperelakis et al., 1967), frog skin (Nagel, 1979), gastric mucosa (Pacifico et al., 1969), tracheal mucosa (Welsh, 1983) and squid giant axon (Eaton & Brodwick, 1980). This effect of Ba^{++} is of considerable potential in exploration of the molecular architecture of K^+-translocating sites and the role of K^+ permeability in supporting transepithelial K^+ transport and other activities of epithelia. The midgut of larval lepidoptera is potentially an exceptionally useful tissue for investigation of the mode of action of Ba^{++} because of the following characteristics:

The midgut actively transports K^+ from hemolymph to lumen. An electrogenic K^+ pump is located at the apical (luminal) surface of the single layer of epithelial cells. There are two major cell types, columnar and goblet, and the electrogenic K^+ pump is probably confined to goblet cells (Dow et al., 1984). Entry of K^+ to the transport system via the basal (hemolymphal) border appears to be thermodynamically passive (Moffett et al., 1982). Effects of Ba^{++} on K^+ transport described in this paper are most easily ascribed to an effect on some aspect of the K^+ entry step.

The isolated midgut actively transports K^+ at rates as high as 1 to 3 μeq/cm^2 min and can maintain K^+ transport for several hours even in simple media containing only KCl, sucrose and buffer. The $[K^+]$ of lepidopteran hemolymph is 20 to 38 mM (Jungreis et al., 1973). The isolated midgut can transport K^+ quite well from $[K^+]$ as low as 10 mM and as high as 90 mM, a range that is unphysiological for most vertebrate tissues.

We show here that in this K^+-transporting system effects of Ba^{++} are not easily explained by simple competition with K^+; our results also suggest that effects of Ba^{++} are not species specific but can be exercised by other divalent cations.

Preliminary reports of some aspects of these studies have been presented (Moffett & Koch, 1982, 1983).

Materials and Methods

The morphologically distinct posterior midgut of fifth instar larvae of the tobacco hornworm *Manduca sexta* was excised, mounted and short-circuited as in previous studies (Moffett, 1979). The aperture of the chamber was 0.22 cm^2; I_{sc} and isotopic K^+ flux values shown in figures of single representative experiments are for this area.

Bathing solutions contained 166 mM sucrose, 5 mM Tris (pH 8.0) and KCl to give desired K^+ concentrations. In the present paper solutions are named according to their $[K^+]$ (for example, "70K" indicates a solution that contains 70 mM KCl, buffer and

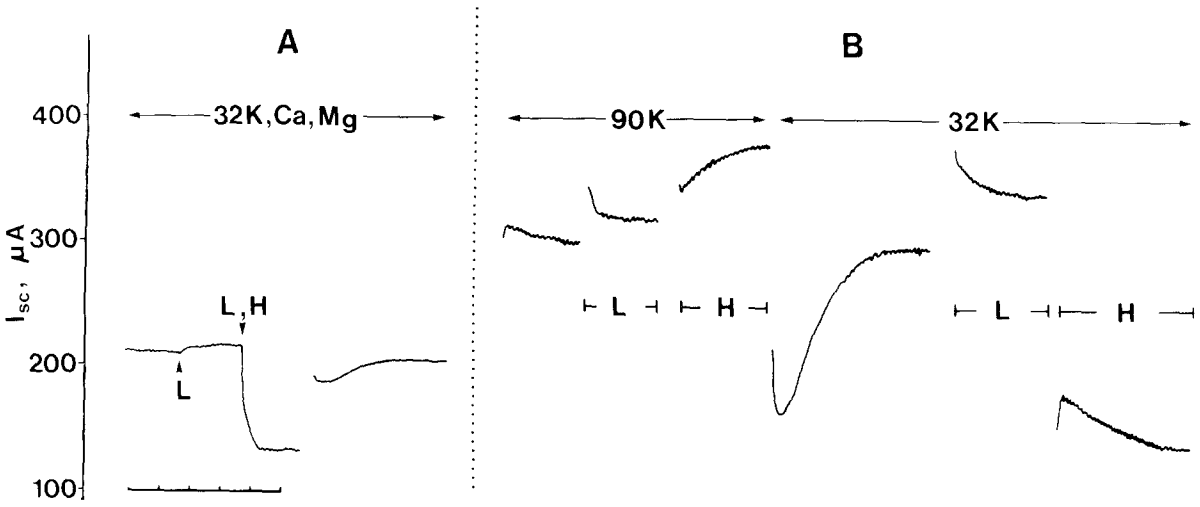

Fig. 1. Responses of I_{sc} to unilateral Ba^{++} addition. The vertical scale is in μA. The horizontal time bar represents 5 min; each tick is 1 min. Sections *A* and *B* were recorded from different preparations. In *A*, $[K^+]$ was 32 mM; $[Ca^{++}]$ and $[Mg^{++}]$ were both 5 mM. At *L*, Ba^{++} was added to the luminal side. The small increase in I_{sc} can be calculated to be entirely attributable to the electrical conductivity of the added $BaCl_2$. At *L*, *H*, Ba^{++} was added to the hemolymph side as well. The break in the record represents a return to Ba^{++}-free solution. The experiment shown in *B* starts in 90K. Ba^{++} was added first to the luminal side only (*L*), then to the hemolymph side only (*H*), followed by a change to 32K and repetition of the unilateral additions

sucrose). Where indicated, the solutions also contained 5 mM $CaCl_2$ and 5 mM $MgCl_2$ (as in "32KS," Zerahn, 1977). $BaCl_2$ was added to half-chambers as a 1 M solution to give final concentrations indicated. Addition of $BaCl_2$ solution resulted in a change in $[K^+]$ of less than 0.4%. Except as noted, Ba^{++} was added in equal concentrations simultaneously to both half-chambers.

Because of the relatively high electrical resistance of bathing solutions, maintenance of the midgut under short-circuit conditions requires a compensation for the voltage drop between voltage-measuring bridges. Solution resistance ranged between 250 and 900 ohms in these experiments. Tissue resistance was of the range 90 to 250 ohms. The effect of solution resistance was fully compensated for by a feedback system (Cornell, 1982) that adjusted the clamp circuit to maintain a voltage difference between the measuring bridges such that the voltage across the tissue itself was zero.

To determine the side-specificity of Ba^{++} in solutions containing Ca^{++} and Mg^{++}, Ba^{++} was added to one or the other half-chamber without compensating for its effect on bathing solution resistance; the latter effect is trivial under these conditions. In the absence of Ca^{++} and Mg^{++} it was necessary to balance the effect on resistance by simultaneous addition of choline chloride to the opposite half-chamber. Addition of choline chloride to a final concentration of 20 mM was determined to confer a change on bathing solution resistance equal to that caused by 8 mM $BaCl_2$. When 8 mM $BaCl_2$ is added to one half-chamber and 20 mM choline chloride to the other, the bathing solution resistance can be correctly compensated for, but there are electrochemical gradients of Ba^{++}, choline and (to a smaller extent) Cl^- across the tissue. The small error apparent in determination of short-circuit current (I_{sc}) under these conditions (*see* Fig. 1*B*) was preferable to the large error that would result if $BaCl_2$ were added to one half-chamber without a balancing electrolyte and without a compensation for its effect on solution resistance.

Hemolymph-to-lumen fluxes of ^{42}K were measured under short-circuit conditions with the preparation bathed in either 32K or 90K on both sides. The fluid bathing the lumen side was pumped through a flow-through scintillation detector (Nuclear-Chicago model 6770) and then recirculated. The counts were passed to a ratemeter (Nuclear-Chicago model 8731) and the ratemeter output was recorded on one channel of a two-channel servorecorder. Isotopic K was added to the hemolymph side after 45 min of equilibration. Flux measurements started 15 min later. The continuous trace of isotope activity on the lumen side was smoothed by eye and readings were taken at 5-min intervals. All readings were then corrected for isotopic decay. The average flux over an interval was computed as the difference between successive 5-min readings of lumen side activity divided by the specific activity of the hemolymph side solution. The activity of the luminal solution was always less than 1% of that on the hemolymph side. A continuous trace of compensated I_{sc} was recorded on the other channel of the servorecorder. Average values were estimated by eye from the midpoint of each flux period.

There are distortions between the rate at which the isotope crosses the epithelium and the rate at which the recorded count rate rises. The lumen side chamber had a volume of 28 ml and this introduces a phase lag in the rate of change of the activity. The mixed sample from this chamber was pumped through a small tube to the detector, so both a time delay and distortions due to streamline flow were introduced. Subsequent to counting, the fluid was returned to the lumen side chamber where its activity, then approximately 0.5 min obsolete, was mixed with the current contents of the chamber. Precise correction of the distortion of the data due to these processes is difficult. A time shift of 1.25 min was used as an approximate correction. I_{sc} at 1.25 min into the flux period was computed by linear interpolation and was used as the average current during each flux period. The correction is adequate when neither flux nor current change greatly during a flux period, but may fail for periods that include very rapid changes. Accordingly, the first 5-min flux period after addition of Ba^{++} was not analyzed.

To determine the kinetics of K^+ transport with respect to extracellular K^+, the mounted midguts were equilibrated under short-circuit conditions for one hour in the highest K^+ concentration to be used, with several changes of bathing solution. Then test solutions were applied in descending order of K^+ concentration; Ba^{++} was added to each test solution so that values of I_{sc} under control conditions and corresponding values in the presence of Ba^{++} were determined closely in time. Some experiments were also conducted in which the order of presentation of solutions was randomized. Such experiments involved large differences in $[K^+]$ between successive solutions. The general pattern of results was similar to that of the sequential experiments, but the variability was much greater. The I_{sc} of mounted midguts declines rapidly during the first hour after mounting and more slowly during the subsequent hour (Moffett, 1979). In the present studies we minimized the effect of this "intrinsic" decay on determinations of the transport kinetics by performing the experiments as rapidly as possible. Because the midgut typically reaches a new quasistable I_{sc} within 3 min after a change of extracellular $[K^+]$ or addition of Ba^{++}, I_{sc} values for up to 5 $[K^+]$ values could be determined within 30 min. A typical experiment is shown in Fig. 3.

Standard statistical analyses were used, except as noted. Where appropriate, one-way or multi-way ANOVA was used to test homogeneity of the data. Means, standard deviations, and standard errors were normally conventional. Estimates of V_{max} and K_m were taken from linear regression of the Eadie-Hofstee plot. The values of $\bar{\delta}$ in the Table were weighted by their corresponding variances. This set of data was then "jackknifed" so that the grand mean given is the average, and the standard error is the standard error of the pseudovalues. The jackknife technique gives more accurate estimates of mean and confidence limits of nonnormal distributions than do classical methods (Miller, 1974; Mosteller & Tukey, 1977).

Table. Difference between I_{sc} and hemolymph to lumen ^{42}K flux (F)[a]

	N	Δ	$\bar{\delta}$	σ_δ
32KS				
1	11	0.150	0.209	0.254
2	10	−0.042	0.413	0.145
3	11	−0.040	0.126	0.098
4	11	0.154	0.068	0.101
5	12	0.264	0.043	0.150
Mean		0.094	0.137	0.150
SEM		0.060	0.057	
90K				
1	14	−0.208	0.298	0.125
2	11	0.182	0.328	0.149
3	10	−0.064	0.590	0.139
4	11	−0.065	0.452	0.114
5	11	−0.111	0.309	0.134
6	11	−0.191	0.359	0.173
Mean		−0.077	0.393	0.140
SEM		0.058	0.048	

[a] N is the number of experimental periods. Δ is the difference between the average of δ for the pre-Ba^{++} periods and that for the post-Ba^{++} periods. $\bar{\delta}$ is the overall average difference between the isotopic flux and I_{sc} including both pre-Ba^{++} and post-Ba^{++} periods; this is our best estimate of the backflux. The means and SEM's given below each data set were obtained by the jackknife procedure. The mean of δ is not significant in 32K or in 90K. σ_δ is the standard deviation of the values for each experiment and is a direct measure of the reliability of I_{sc} as an estimate of F.

Results

Rapidity, Reversibility and Side-Specificity of Ba^{++} Effect

Initial studies in bathing solution containing 5 mM Ca^{++} and 5 mM Mg^{++} showed that Ba^{++} concentrations greater than 0.1 mM partially inhibit I_{sc}. The inhibitory effect of Ba^{++} in the presence of Ca^{++} and Mg^{++} is specific for the hemolymph side of the midgut (Fig. 1*A*). Dose-effect experiments showed that in the presence of 5 mM Ca^{++} and 5 mM Mg^{++} nearly the maximal effect is obtained with 2 mM Ba^{++}. In Ca^{++}, Mg^{++}-free bathing solution 8 mM Ba^{++} is required for comparable effect (Fig. 1*B*). As we show (Fig. 5, Fig. 6), in the absence of Ca^{++} and Mg^{++}, Ba^{++} inhibits I_{sc} at low K^+ concentrations and stimulates it at high K^+ concentrations. Side-specificity was therefore determined in 32K and in 90K (*see* Materials and Methods). Figure 1*B* shows that addition of 8 mM Ba^{++} only to the hemolymph side stimulates I_{sc} in 90K and inhibits it in 32K, a result compatible with those of experiments in which Ba^{++} was added symmetrically (*see below*). When Ba^{++} is added only to the lumen side there is a small increase in I_{sc} both in 90 and in 32K. This result is not compatible with those obtained from simultaneous bilateral addition of Ba^{++}, but would be expected if the tissue permeability to choline were greater than that to Ba^{++}.

Figure 1 shows that both the stimulatory and the inhibitory effects of Ba^{++} are rapid and easily reversed if the period of Ba^{++} application is 5 min or less. Figure 2*B* shows that the stimulatory effect does not lead to a sustained high I_{sc}, but instead the I_{sc} begins to decline after about 10 min of continuous exposure to Ba^{++}. These results and others not shown indicate that continuous exposure to Ba^{++} for periods of 10 min or longer leads to secondary deleterious effects that are not rapidly reversed upon return to control conditions. Irreversible effects were not elicited by our protocol of brief, repeated Ba^{++} intervals.

I_{sc} and Hemolymph-to-Lumen $^{42}K^+$ Flux

Much previous work (reviewed by Harvey & Zerahn, 1972, and reported most recently by Cioffi

& Harvey, 1981) has shown that I_{sc} arises from and is a close measure of net transepithelial K^+ flux; this is especially true in the absence of Ca^{++} and Mg^{++}. The experiments described in this section test whether I_{sc} is a good measure of the effect of Ba^{++} on net K^+ transport. Hemolymph-to-lumen fluxes of $^{42}K^+$ (F) were measured in 11 experiments. In five experiments the bathing solution was 32K, a concentration of K^+ in which 8 mM Ba^{++} reliably inhibits I_{sc}. In six experiments the bathing solution was 90K, a concentration in which 8 mM Ba^{++} typically causes a transient stimulation of I_{sc}. Figure 2 shows the time course of F and I_{sc} for two typical experiments, one in 32K and one in 90K.

In every experiment, the mean F was greater than the mean I_{sc}. If changes in I_{sc} can be used to measure changes in net K^+ flux, the difference (δ) between F and I_{sc} should be constant within an experiment. This statement includes both the requirement that δ should not change consequent to the addition of Ba^{++} and that any other spontaneous or time-dependent changes in F should be reflected by similar changes in I_{sc}. The first requirement was tested by comparing, within each experiment, the average values of δ obtained from the control periods to those obtained after Ba^{++} had been added. As shown in the Table, Ba^{++} itself led to no consistent change in δ, either in 32K (where both F and I_{sc} fell) or in 90K (where both rose). The Table gives F as averaged over the pre-Ba^{++} periods, the mean δ and the standard deviation of δ for all experiments. The grand means of δ were obtained by jackknife of the weighted averages from each experiment. The variance within each experiment was the weighting factor. The mean of the standard deviations of δ for both pre- and post Ba^{++} intervals was only about 10% of the measured F. This means that an individual measurement of I_{sc} estimates the active flux to within 10% about two-thirds of the time. These results allow us to conclude that changes in I_{sc} induced by Ba^{++} are attributable specifically to an effect on F.

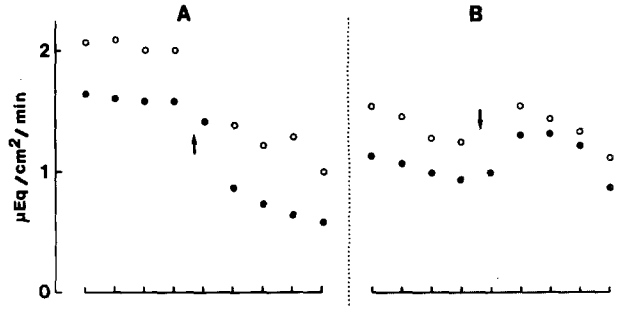

Fig. 2. Comparison of F (open circles) and I_{sc} (filled circles). The horizontal axis represents time with each tick representing 5 min. The arrows show the addition of 8 mM Ba^{++}. Section *A* shows an experiment in 32K; section *B* shows a separate experiment in 90K

Relationships between I_{sc}, $[K^+]$ and $[Ba^{++}]$

The experiments described in this section determine the relationship between I_{sc} and $[K^+]$ under control conditions (Fig. 4) and with varying $[Ba^{++}]$ (Fig. 5). A typical experiment is shown in Fig. 3. Each experiment can be viewed in two ways: as a set of direct measurements of the effect of Ba^{++} on I_{sc} each at a different $[K^+]$, or as a simultaneous

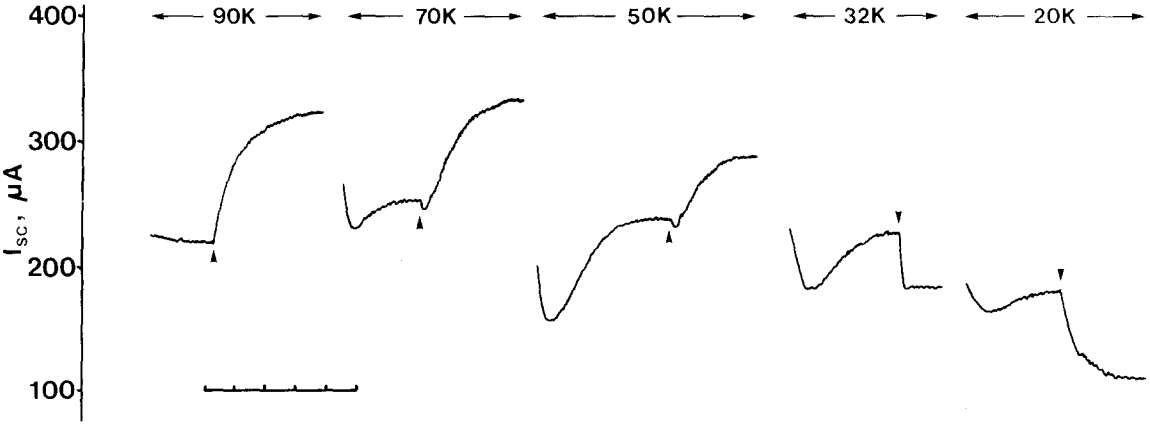

Fig. 3. Response of I_{sc} to addition of Ba^{++} (8 mM) in varied $[K^+]$. The horizontal time bar represents 5 min with each tick representing 1 min. All traces were recorded sequentially from the same preparation. This is a representative experiment from the series for which data are given in Fig. 5*C*. For each $[K^+]$, the first part of the trace is the control reading. At the arrow, Ba^{++} was added to both half-chambers and the response is displayed. Each break in the record indicates a change of bathing solution. Changes of bathing solution required about 2 min; the record was condensed for figure presentation by not showing some of this time

tracing of two functional relations between I_{sc} and $[K^+]$, one in the presence and one in the absence of Ba^{++}. Each measurement of I_{sc} is expressed in terms of the I_{sc} obtained in 32K. This normalization eliminated intrinsic variation between preparations and allowed the results of different experiments to be pooled. The mean value of I_{sc} at the normalization point was 1165 ± 67 $\mu A/cm^2$ for all experiments shown in Figs. 4 and 5 (n = 30). The points shown are the average experimental values, with their standard errors. The number above or below each point is the number of values. The pooled values for the control experiments shown in the body of Fig. 4 were plotted on an Eadie-Hofstee plot (inset to Fig. 4). For values of $[K^+]$ from 20 to 70 mM, points fit Michaelis-Menten kinetics very well. The points for 10 and for 90K are distinctly off the straight line. Grounds for excluding these points are presented in the Discussion. However, since transport kinetics are probably not determined by a single-site mechanism, we are terming this pattern of concentration dependence "Michaelis-Menten-like" or "saturating" without implying that the pattern is determined by a single reactive site. Linear regression of the points from 20 through 70K gives estimates of V_{max} equal to 1.46 times the mean value of I_{sc} in 32K, or 1770 $\mu A/cm^2$, and K_m of 15.1 mM. The hyperbola with these parameters is represented by the solid curve in Figs. 4, 5 and 7.

Figure 6 shows the fractional change of I_{sc} resulting from 2, 4 and 8 mM Ba^{++}. Lower concentrations of Ba^{++} produced qualitatively similar effects. Barium invariably decreased I_{sc} when $[K^+]$ was 32 mM or less, while at $[K^+]$ of 55 mM or greater the

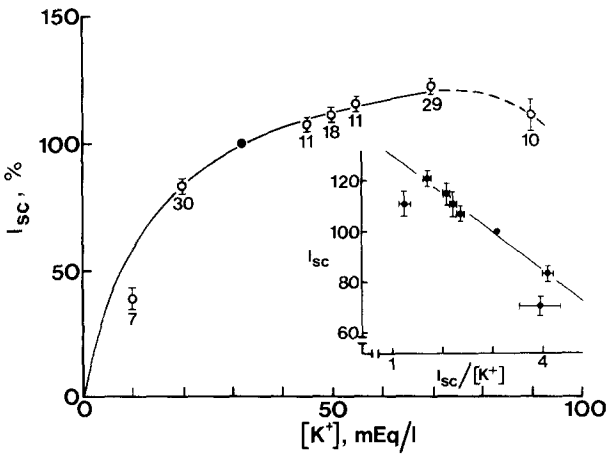

Fig. 4. Dependence of I_{sc} on $[K^+]$. This figure shows grouped control values for three series of experiments for which data are shown in Fig. 5. The three series comprise 30 individual experiments. For each experiment, values were normalized to the value of I_{sc} recorded in 32K (filled circle). Brackets show ± 1 SEM. The solid line is the rectangular hyperbola with V_{max} = 1.46 times the value in 32K, and K_m = 15.1 mM. These values are taken from the Eadie-Hofstee fit of the central six points (*see* inset). The two points below the straight line in the inset are those for 90K (left) and 20K (right). The broken line from 70 to 90 meq/liter is drawn by eye. In this figure and in Figs. 5 and 7 the open circles are average values ± 1 SEM. The number next to each point gives the number of values. The hyperbola fitted to the control points as described above is shown in Figs. 5 and 7 for comparison with the experimental points

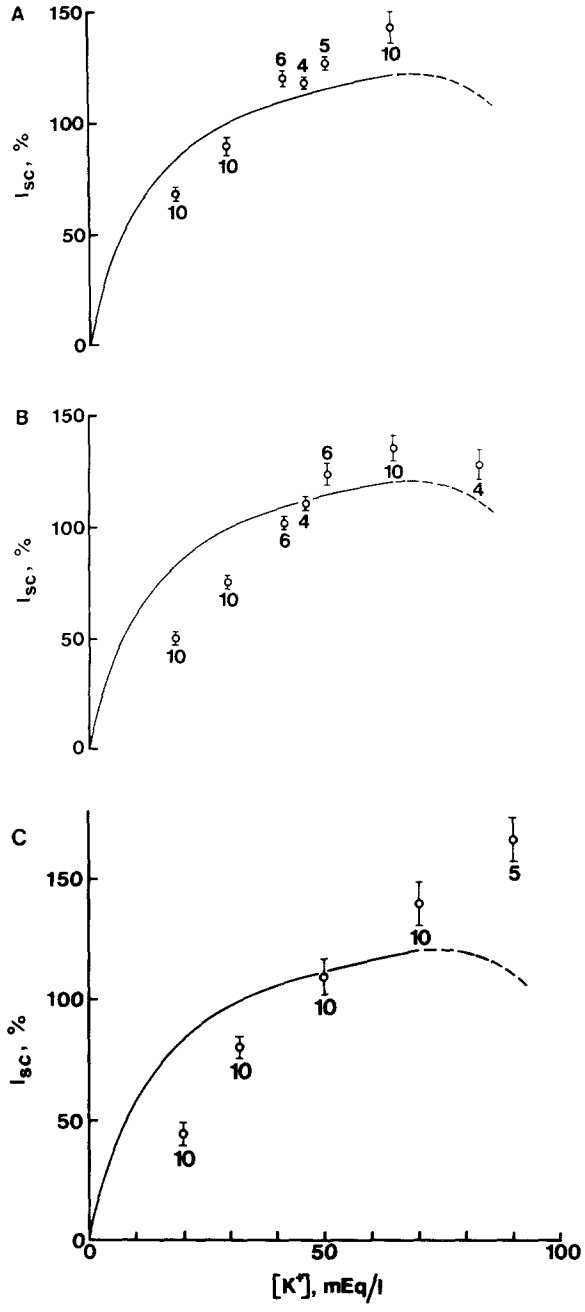

Fig. 5. Dependence of I_{sc} on $[K^+]$ in the presence of 2 mM Ba^{++} (*A*), 4 mM Ba^{++} (*B*) and 8 mM Ba^{++} (*C*). Values are normalized to the control value in 32K

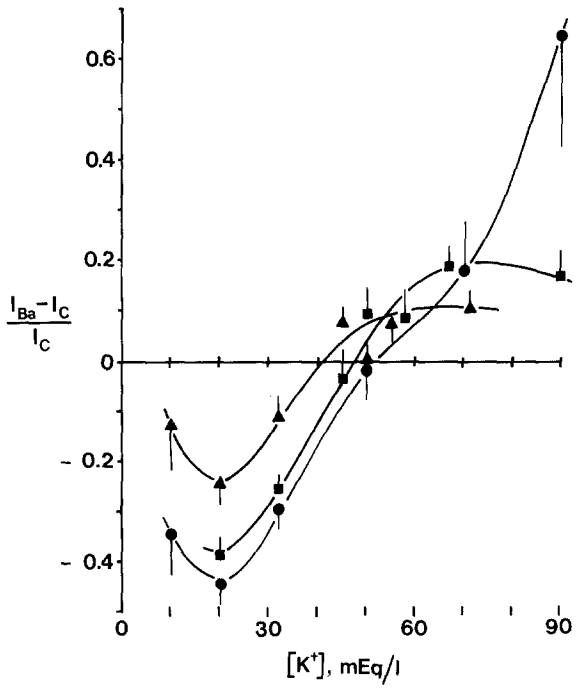

Fig. 6. Fractional change in I_{sc} caused by Ba^{++}, plotted against $[K^+]$. Smooth curves drawn by eye connect three sets of points (2 mM Ba^{++}: triangles; 4 mM Ba^{++}: squares; 8 mM Ba^{++}: circles). This figure summarizes the results presented in Figs. 4 and 5, showing that both the inhibitory and stimulatory effects of Ba^{++} are dose-related over this range of $[Ba^{++}]$

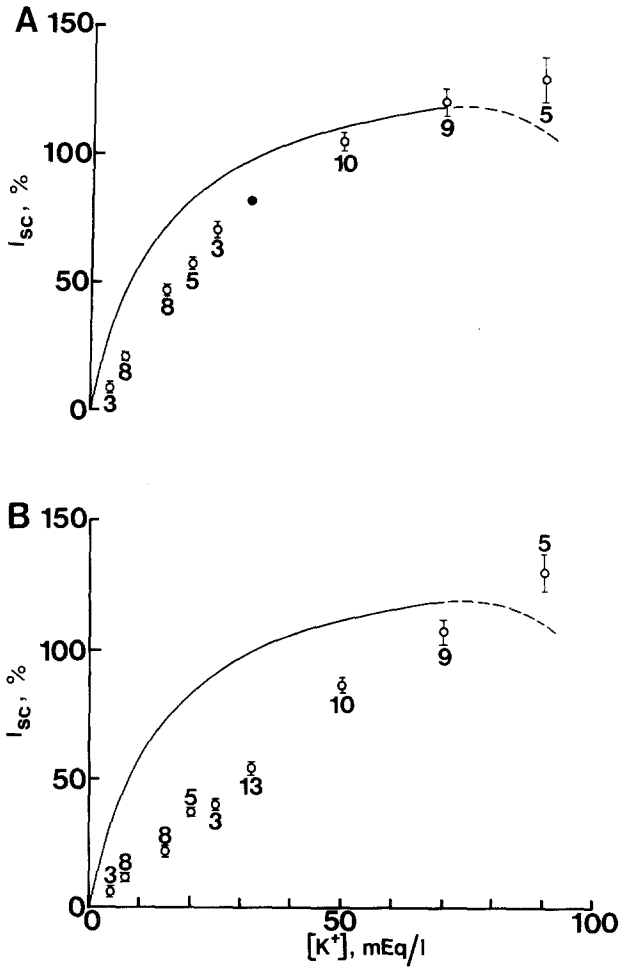

Fig. 7. *A*: Dependence of I_{sc} on $[K^+]$ in the presence of 5 mM Ca^{++} and 5 mM Mg^{++}. All values were normalized to the value in 32K solution containing Ca^{++} and Mg^{++}. The average value of I_{sc} in the latter solution was 0.82 of the average value obtained in experiments with divalent-free 32K (*see* Fig. 4). The values plotted are scaled in this proportion for comparison to the hyperbola obtained in divalent-free solutions (solid curve). The brackets are ± 1 SEM, including both variation around the mean of the values in 32K for the experiments shown and the variation between these values and the values in divalent-free 32K. *B*: Dependence of I_{sc} on K^+ in the presence of 5 mM Ca^{++}, 5 mM Mg^{++} and 2 mM Ba^{++}. Values were normalized to the value in 32K with 5 mM Ca^{++} and 5 mM Mg^{++}, and scaled in the same manner as the control values shown in *A*

average I_{sc} increased in every instance. At any concentration of K^+, Ba^{++} had a monotonic effect with higher concentrations of Ba^{++} leading to a greater effect. The results suggest that Ba^{++} changes the form of the functional relation between $[K^+]$ and I_{sc}.

Figure 7*A* shows the relation under control conditions in the presence of 5 mM Ca^{++} and 5 mM Mg^{++}. Figure 7*B* shows the simultaneously determined relation in the presence of 2 mM Ba^{++}. The presence of Ca^{++} and Mg^{++} alone produced a curve similar to that resulting from 4 mM Ba^{++} (Fig. 5*B*). The addition of 2 mM Ba^{++} to that solution led to a relation intermediate between those shown in Fig. 5*B* (4 mM Ba^{++}, no Ca^{++} or Mg^{++}) and Fig. 5*C* (8 mM Ba^{++}, no Ca^{++} or Mg^{++}).

Discussion

Side-Specificity Experiments

The rapidity of onset and reversal of the Ba^{++} effect suggest that Ba^{++} acts at an external site. The specificity of Ba^{++} for the hemolymph side suggests that the action is on the K^+ uptake step rather than on the apical pump. The normal route for entry of actively transported Ca^{++} and Mg^{++} is the apical side (Wood et al., 1975; Wood & Harvey, 1976). Results of side-specificity and flux-current experiments suggest that Ba^{++} is not transported by the Ca^{++} or Mg^{++} transport system and does not follow the uptake route for those ions in the course of exerting its effect.

The unexpected stimulation of K^+ transport by

Ba^{++} in solutions lacking Ca^{++} and Mg^{++} and containing high $[K^+]$ led us to perform side-specificity experiments in Ca^{++}, Mg^{++}-free solutions. These experiments (Fig. 1*B*) were the only experiments performed here in which the solutions were not identical on both sides of the preparation. Thus in these, but in no other experiments, there is reason to expect some passive ion movement. At both concentrations of K^+, there was a small increase in I_{sc} when Ba^{++} was on the lumen side and choline was on the hemolymph side. This change was in the opposite direction from that seen after luminal addition of Ba^{++} in 32K. When Ba^{++} was added to the hemolymph side only, the response was qualitatively similar to that seen after symmetric addition. In the experiment shown in Fig. 1*B*, I_{sc} increased by about 35 μA when Ba^{++} was added to the luminal side of the preparation in 90K. In 32K, luminal addition of Ba^{++} led to an increase of 45 μA. Thus in both cases there is an additional hemolymph-to-lumen current of about 40 μA. This could be expected if the preparation were more permeable to choline than to Ba^{++}. If this is true, there should be a passive current of the same magnitude and opposite polarity when choline is added to the lumen side; this passive current would be superimposed on any Ba^{++} effect. For the experiment of Fig. 1*B*, addition of Ba^{++} to the hemolymph side in 90K increased I_{sc} from 300 to 450 μA, a 50% increase. Assuming a 40-μA current due to choline-Ba^{++} asymmetry, the active current really increased to 490 μA. This increase of 63% compares well with the 65% increase we obtained with symmetric solutions (Fig. 6). When Ba^{++} was added to the hemolymph side in 32K, I_{sc} fell from 295 to 150 μA. Correction as before for the choline-Ba^{++} asymmetry gives a net decrease of 35%, close to the 30% decrease seen with symmetric solutions (Fig. 6). The results are most compatible with the following explanation:

a. Ba^{++} acts from the hemolymph side in Ca^{++}, Mg^{++}-free solutions, just as in the presence of Ca^{++} and Mg^{++}.

b. The midgut is more permeable to choline than to Ba^{++} under these conditions.

Flux-Current Comparison

The reason for conducting the flux experiments was to test whether I_{sc} could be used as an accurate estimate of the active flux of K^+ in the main body of the experiments. Transport of the other ions present during the control interval (Cl^-, Tris) can be ruled out on the basis of previous work (Harvey & Zerahn, 1972). Active transport of Ba^{++} could not be ruled out *a priori*, especially since the midgut is capable of active transport of Ca^{++} and Mg^{++} (Wood et al., 1975; Wood & Harvey, 1976). Active transport of Ba^{++} would have changed the relation between F and I_{sc}. Addition of 8 mM $BaCl_2$ to 32K makes a solution in which Ba^{++} is about $\frac{1}{5}$ of the total cation. The finding that δ did not change after addition of Ba^{++} means that under these conditions any net transport of Ba^{++} is too slight to have detectable effect on the relationship between I_{sc} and F. The difference between F and I_{sc} is the difference between isotope exchange and net movement. Every ion of ^{42}K that crossed the preparation reflected crossing of a fixed number of charges according to the relation given by the specific activity of the hemolymph-side solution. Some of the charge movement led to a current, I_{sc}. The remainder of it was δ and when these charges crossed the midgut there was equal and opposite movement (backflux) of nonisotopic K^+ from lumen to hemolymph side. Thus δ is an estimate of the passive backflux of K^+. The Table gives the average values of δ in 32 and in 90K. These values are our best estimates of the backfluxes. The ratio of δ in 90K to δ in 32K was 3.93/1.37 = 2.87. The ratio of K^+ concentrations was 90/32 = 2.81. This agreement suggests that, in this preparation, the passive flux is in simple proportion to $[K^+]$.

Dependence of I_{sc} on K^+

Figure 4 shows the dependence of I_{sc} on external $[K^+]$ in the absence of divalent ions. The curve is drawn for Michaelis-Menten kinetics with V_{max} = 1.46 × I_{sc} in 32K and K_m = 15.1 mM. These estimates were taken from the Eadie-Hofstee plot of the central six points (Fig. 4,, inset). The correlation coefficient of this plot is 0.992. There are some grounds for elimination of the 10K values and the 90K values from the estimation of the transport kinetic parameters.

First, the electrical resistance of 10K is more than 5 times that of the midgut tissue, so that with this solution we are working near the limit of our ability to compensate for solution resistance. While the deviation of the control value of I_{sc} in this solution from the hyperbolic could be taken as evidence of cooperativity, no Hill plot of the curve brings this point into line. Also, in experiments in which Ca^{++} and Mg^{++} were present, the conductance provided by these ions kept solution resistance within an acceptable range for $[K^+]$ as low as 4 mM. As we discuss below, the relation between I_{sc} and $[K^+]$ as measured in divalent-free solutions retains some of the properties of the control curve when Ca^{++} and

Mg^{++} are present. No sigmoid property is present in the latter curve (Fig. 7*A*).

An inhibitory process is present in 90K; the average value of I_{sc} is lower in 90K than in 70K. This depression is significant at the 5% level. The hyperbola predicts a higher value of I_{sc} in 90K than in 70K. It is even less likely than 5% that the data we obtained are from a distribution centering around the predicted value for 90K. Because of the very good fit of the central 6 points to a rectangular hyperbola, we are choosing to regard the inhibition as a process that occurs only in the highest $[K^+]$. However, the apparent saturation of the transport system could reflect a substrate-dependent inhibitory process that we only see clearly in 90K.

In summary, the control data can be described in terms of two processes: a Michaelis-Menten-like process and an inhibitory process that becomes evident when $[K^+]$ is greater than 70 mM. we believe, but cannot prove, that accurate measurement of I_{sc} in 10K would give a value about 60% of that in 32K. In earlier studies of the relation between I_{sc} and $[K^+]$ (Moffett, 1979), efforts were made to correct for the intrinsic decay of I_{sc} and to keep solutions isosmotic by substitution of NaCl for KCl. The relation of I_{sc} to $[K^+]$ for control conditions in the presence of Ca^{++} and Mg^{++} obtained in the present study agrees quite well with that of the earlier study. This agreement suggests that the kinetics of the transport system are independent of solution electrical resistance or the osmotic effect of changing extracellular $[K^+]$, at least for the brief time needed for the determinations of the transport kinetics.

Mode of Action of Ba^{++}

The similarity of the crystal ionic radii of Ba^{++} and K^+ has suggested that the apparent affinity of Ba^{++} for K^+ channels stems from an initial step in K^+ permeation in which the permeating ion passes through a selectivity filter (Standen & Stanfield, 1978; Armstrong & Taylor, 1980; Eaton & Brodwick, 1980). If transport of K^+ is closely related to the rate of K^+ permeation, simple competition between Ba^{++} and K^+ for permeation sites would seem to be ruled out in the case of the midgut, since at all concentrations tested Ba^{++} stimulates K^+ transport at K^+ concentrations of 55 mM or greater (Figs. 5 and 6). Our results suggest that the effect of Ba^{++} is really to change the form of the concentration dependence of K^+ transport. It happens that the form of the dependence seen in the presence of Ba^{++} leads to reduced transport when the extracellular $[K^+]$ is low but to increased transport when it is high: the overall effect is to linearize the concentration dependence of K^+ transport. If Ba^{++} does indeed competitively block K^+ permeation in the midgut, the kinetics of the transport system must be responsive to secondary consequences of this effect.

Interaction of Ca^{++}, Mg^{++} and Ba^{++}

In the presence of Ca^{++} and Mg^{++} the dependence of I_{sc} on $[K^+]$ (Fig. 7*A*) differs from that in the absence of any divalent. The curve for Ca^{++}, Mg^{++} medium is similar to those obtained with intermediate concentrations of Ba^{++} (*see* Fig. 5). Analogizing the effect of the presence of Ca^{++} and Mg^{++} to that of Ba^{++} as discussed above, the effect of Ca^{++} and Mg^{++} is to introduce a partial linearization of the transport kinetics. Only 2 mM Ba^{++} is needed to complete this conversion (Fig. 7*B*). This analogy may not be completely satisfactory since the slope of the linearized kinetics differs somewhat between the two sets of experiments (*compare* Fig. 5*C* and Fig. 7*B*). Nevertheless, these results suggest that Ca^{++} + Mg^{++} may exercise an effect on the kinetics similar to that of brief exposure to Ba^{++}. The ability of other alkali earth metals to mimic the action of Ba^{++} varies from tissue to tissue. For example, both Ba^{++} and Sr^{++} can block inward rectification in skeletal muscle (Standen & Stanfield, 1978), while neither Ca^{++}, Sr^{++} nor La^{3+} can exercise effects like those of Ba^{++} on gastric mucosa (McLennan et al., 1980). The results from the midgut suggest that a particularly close match to the crystal ionic radius of K^+ is not important for the effect of Ba^{++}. One possibility consistent with the results is that Ba^{++} and other divalent ions might screen fixed negative charges specifically associated with K^+ permeation sites. Lindemann (1982) suggested such fixed charges could impede ion flow when channel occupancy is high and accelerate it when channel occupancy is low. This mechanism would be consistent with "saturating" kinetics for the transport process in divalent-free medium, and with quantitatively similar and additive effects of Ba^{++} and Ca^{++} + Mg^{++}. However, further studies will be needed to distinguish a possible primary effect of divalents on K^+ permeation across the basal cell membrane from its consequential effects on intracellular $[K^+]$ and cell membrane potentials since the role of such variables in determining apical pump activity and tissue transport kinetics is as yet not worked out.

Finally, it is possible that the susceptibility of the transport system to effects of divalent cations could play an important role in determining the rate of K^+ transport *in situ*. Mature larvae of *Manduca*

sexta have hemolymph concentrations of 38 mM K^+, 50 mM Mg^{++} and 10 mM Ca^{++} (Jungreis et al., 1973). Even if it is assumed that some divalent is complexed by organic molecules in the hemolymph, it is likely that the kinetics of the system *in situ* are more similar to those of Fig. 7*A* than those of Fig. 4.

This work was supported by PHS GM 26287 and NSF PCM 8315739. We thank L.B. Kirschner for a critical reading of the manuscript.

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Received 10 October 1984; revised 5 March 1985