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Kinetics of Voltage- and Ca^{2+} Activation and Ba^{2+} Blockade of a Large-Conductance K^+ Channel from *Necturus* Enterocytes

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Summary. Potassium channels in membranes of isolated *Necturus* enterocytes were studied using the patch-clamp technique. The most frequent channel observed had a conductance of 170 pS and reversal potential of 0 mV in symmetrical potassium-rich solutions. Channels were highly K^+ selective. Channel activity was modulated by membrane potential and cytosolic Ca^{2+} concentration. Channel openings occurred in characteristic bursts separated by long closures. During bursts openings were interrupted by brief closures. Two gating modes controlled channel opening. The primary gate's sensitivity to intracellular Ca^{2+} concentration and membrane potential crucially determined long duration closures and bursting. In comparison, the second gate determining brief closures was largely insensitive to voltage and intracellular Ca^{2+} concentration. The channel was reversibly blocked by cytosolic barium exposure in a voltage-sensitive manner. Blockade reduced open-state probability without altering single-channel conductance and could be described, at relatively high Ca^{2+} concentration, by a three-state model where Ba^{2+} interacted with the open channel with a dissociation constant of about 10^{-4} M at 0 mV.

Key Words Ca^{2+}-activated K^+ channel · Ba^{2+} blockade · channel kinetics · patch clamp · *Necturus* enterocyte

Introduction

Enterocytes engaged in sodium-coupled amino acid or sugar absorption are subjected to considerable osmotic stress resulting from the intracellular accumulation of substrate. Entry of sodium along with these substrates stimulates the basolaterally located sodium and potassium pump causing ouabain-sensitive K^+ influx to increase (Brown & Sepúlveda, 1985). Potassium accumulation under these conditions is prevented by a regulatory increase in K^+ permeability (Brown, Burton & Sepúlveda, 1983; Grasset, Gunter-Smith & Schultz, 1983). This change in enterocyte K^+ permeability appears to result from the opening of Ca^{2+}-activated K^+ channels sensitive to Ba^{2+} and the bee-venom neurotoxin apamin (Lau, Hudson & Schultz, 1984; Brown & Sepúlveda, 1985). Thus, modulation of enterocyte K^+ conductance during nutrient transport is a potential homeostatic mechanism whereby gross changes in cell volume and membrane potential can be averted.

Patch-clamp studies have provided evidence for the presence of Ca^{2+}-activated K^+ channels in many cell types including enterocytes (Sepúlveda & Mason, 1985; Morris, Gallacher & Lee, 1986). Our knowledge of the electrophysiological consequences of Na^+-coupled nutrient transport comes mainly from studies in *Necturus* enterocytes (Gunter-Smith, Grasset & Schultz, 1982; Grasset et al., 1983; Lapointe, Hudson & Schultz, 1986; Giraldez & Sepúlveda, 1987). We have, therefore, investigated the presence in *Necturus* enterocytes of Ca^{2+}-activated K^+ channels. The following report presents data characterizing a high-conductance K^+ channel which can be activated by voltage and intracellular Ca^{2+}. The possible involvement of this channel in the homeostatic mechanism mentioned above is also discussed.

Preliminary reports of this work have been presented at The Physiological Society (Giraldez, Sepúlveda & Sheppard, 1988) and at the 8th European Intestinal Transport Group meeting (Sheppard, Giraldez & Sepúlveda, 1987).

Materials and Methods

Experiments were performed on enterocytes isolated from the small intestine of *Necturus maculosus* by a modification of a method described for murine enterocytes (Bjerknes & Cheng, 1981). Animals were purchased from Kons Scientific Co., Germantown, Wisconsin, and kept in aquaria containing tap water at room temperature prior to use.

Necturi were anesthetized by immersion in tap water containing 0.1% tricaine methyl sulphonate (Sigma) before being pithed. The intestinal lumen was flushed through with 25 ml of a

calcium- and magnesium-free Ringer (CMF) containing 1 mM DTT (Sigma) at 22°C prior to perfusing the intestinal vasculature with a solution containing 1 mM EDTA in CMF at 22°C for 7 min. The rate of perfusion was approximately 7 ml/min. The proximal one-third of small intestine was then removed and everted onto a small diameter glass rod attached to a Vibromixer®. Brief bursts of shaking released enterocytes into ice-cold CMF before collection by centrifugation. 2-ml aliquots of enterocytes were plated onto small Petri dishes coated with 0.06% polyethylenimine (Sigma) to which they adhered loosely. The majority of attached enterocytes were rounded up with brush border being visible only in some of them, probably owing to their orientation. Clumps of cells varying in size were also observed. Trypan blue-excluding cells constituted about 60% of the cell population. Trypan blue stained cells were generally elongated and, contrary to viable cells, did not show any birefrigency. Nuclei were visible in all viable cells, while many of the stained cells had lost theirs.

Enterocytes were bathed in a normal Ringer solution of the following composition (mM): NaCl 105, KCl 2.5, HEPES 10, $MgCl_2$ 0.5, $CaCl_2$ 1.3 and mannitol 20 buffered to pH 7.2 with Tris. Pipettes were filled with a high potassium solution of the following composition (mM): KCl 100, HEPES 10, $MgCl_2$ 0.5, $CaCl_2$ 1.3 buffered to pH 7.2 with Tris. Membrane patches were obtained by gentle suction from smooth surfaces of attached cells, avoiding apparent brush border regions. We thus assume all our patches to correspond to the basolateral membrane. As discussed previously, however, redistribution of membrane proteins could take place under these conditions (Sepúlveda & Mason, 1985). Solutions used to bathe the intracellular aspect of excised inside/out membrane patches isolated from enterocytes contained (mM): KCl 100, HEPES 10, $MgCl_2$ 1.13 and free calcium concentrations ranging from $<10^{-8}$ to 10^{-6} M Ca^{2+}. For Ca^{2+} concentrations of 10^{-6} M or less, Ca^{2+} was buffered with EGTA (Findlay, Dunne & Petersen, 1985). The Ca^{2+} and EGTA concentrations required were (mM): 1.92 EGTA, 0.97 Ca^{2+} for 1.5×10^{-7} M, 1.73 EGTA and 1.54 Ca^{2+} for 1×10^{-6} M Ca^{2+}; and 5 EGTA and no added Ca^{2+} for $<10^{-8}$ M Ca^{2+}. Barium blockade of single-channel conductance was investigated by adding 5 mM Ba^{2+} to a high potassium bathing solution containing 10^{-4} M Ca^{2+}. All solutions were filtered through 0.2 μm Millipore filters prior to use and experiments performed at room temperature.

Cells were viewed using a Leitz Diavert microscope. Single-channel currents were recorded from excised inside/out membrane patches of enterocytes using a LM EPC-7 patch-clamp amplifier (Hamill et al., 1981). The signal was simultaneously viewed on a storage oscilloscope and recorded on videotape following modulation using a modified digital audio processor (Lamb, 1985). Potentials are given as bath (intracellular) relative to patch pipette (extracellular) potential, thus corresponding to conventional membrane potential. Ionic currents produced by positive charge flowing from the bath into the patch pipette are shown as positive currents and correspond to upward deflections of the illustrated single-channel recordings.

Taped current records were analyzed using a personal computer (IBM AT) and a patch-clamp analysis program (J. Dempster, 1987, Department of Physiology and Pharmacology, University of Strathclyde, Scotland). Data was digitized using a Cambridge Electronic Design 1401 interface and collected at 1000–2500 samples per sec. Transitions between the open and closed states were identified using 50% of the open-state current as the detection threshold and average open-state probability (P_o) calculated from current records of 30–45 sec duration. The signal was low-pass filtered at 0.5 kHz, and only patches containing a single channel were used for kinetic analyses. Exponential probability density functions were fitted to the bin amplitudes of histograms of channel residence times using a Levenberg-Marquardt, nonlinear least-squares method. In instances where patches contained multiple channel openings, open-state probability was calculated as described before (Reuter et al., 1982). Mean open, and closed times represent arithmetic means of all observed open or shut intervals.

Results

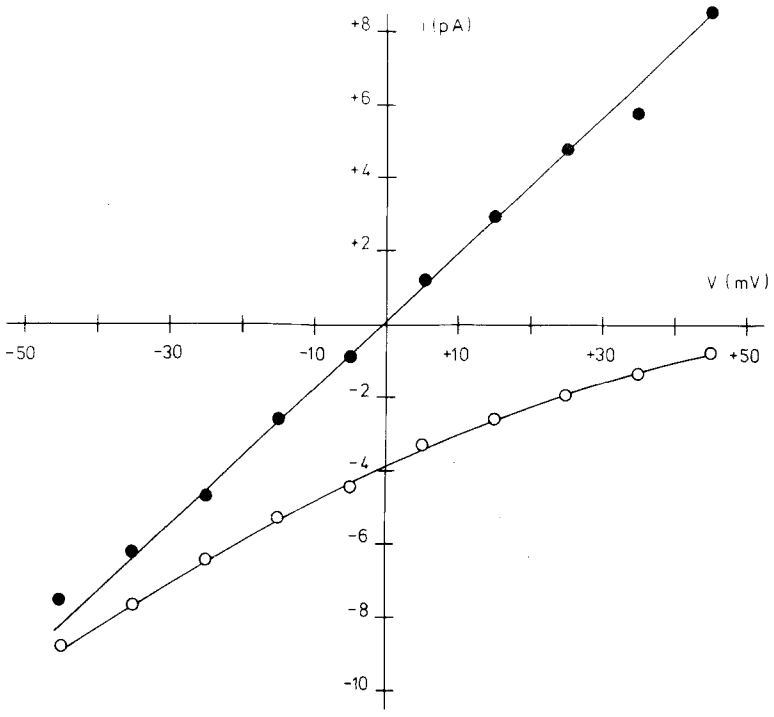
Unitary currents with a range of conductances were observed in inside/out membrane patches of *Necturus* enterocytes. The most frequently observed channel was a high-conductance K^+-selective channel the description of which forms the basis of this report.

Characteristic single-channel currents are shown in Fig. 1 (lower panel) for three membrane potentials. As the membrane was depolarized both the frequency of channel opening and the duration of time spent in the open state increased. The current pattern also demonstrated that channel openings occurred in characteristic bursts, that is channel openings separated by well-resolved closures during which only brief closures were apparent (*see below*).

Figure 1 (upper panel) illustrates the relationship between single-channel current and membrane potential. In symmetrical KCl solutions (filled circles) the *I-V* relationship was linear over the range of potentials studied, and channel conductance was 167 pS with a reversal potential of 0 mV. However, when replacing the cytoplasmic solution by NaCl only inward currents were observed at all potentials. This demonstrates that the channel was highly selective for K^+ over Na^+ and also excludes possible Cl^- selectivity.

Figure 2 (upper panel) demonstrates the effect of varying the Ca^{2+} concentration bathing the intracellular aspect of an excised patch, at a membrane potential of +10 mV. With increasing $[Ca]_i$ there was an increase in the channel open time. The duration of well-resolved closures progressively decreased until at high calcium concentrations the channel was predominantly open. At this high Ca^{2+} concentration most of the closures observed were brief.

Varying membrane potential at a given $[Ca]_i$ also influenced P_o (probability of the open state). As shown in Fig. 2 (lower panel), at $[Ca]_i = 10^{-6}$ M P_o was a sigmoidal function of membrane potential with P_o around 0 at −30 mV and 1 at +40 mV. Increasing $[Ca]_i$ shifted P_o negatively until at $[Ca]_i = 1.3 \times 10^{-3}$ M the curve was essentially flat, since the channel remained open a high proportion of the



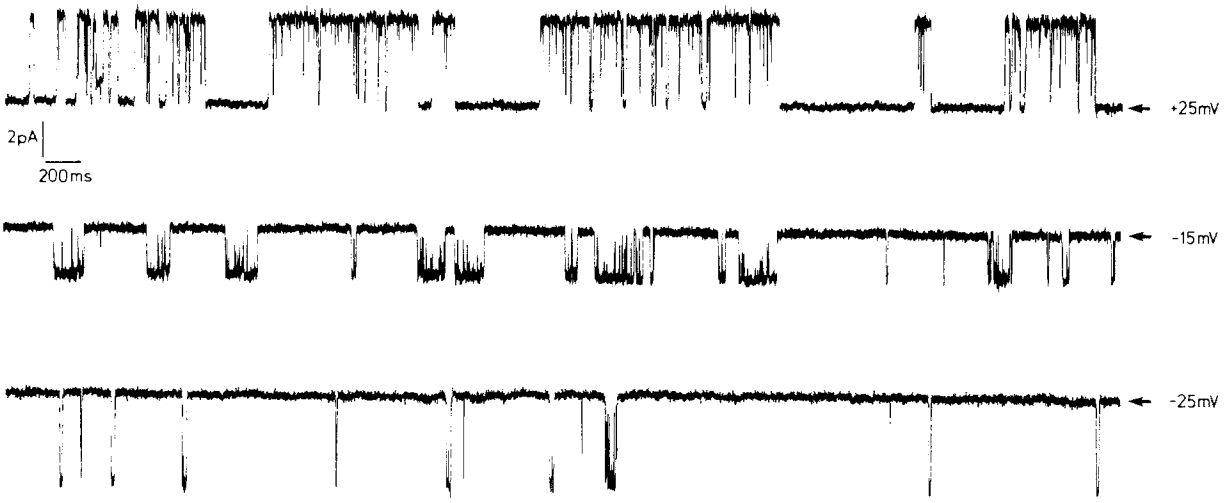


Fig. 1. *Upper panel:* Single-channel current-voltage relationships recorded from an inside/out patch from the basolateral membrane of an isolated *Necturus* enterocyte under the conditions: symmetrical 100 mM KCl solutions (●) and outside NaCl, inside KCl (○). *Lower panel:* Single-channel current traces at indicated potentials recorded from an excised inside/out patch bathed by symmetrical 100 mM KCl solutions and free Ca^{2+} concentration of 10^{-6} M in the bath. Arrows indicate the position of the closed state. Upward deflections represent outward current

time at all potentials. In contrast, decreasing $[Ca]_i$ shifted P_o positively, at $[Ca]_i = 1.5 \times 10^{-7}$ M the channel only opened at very depolarizing membrane potentials and at $[Ca]_i < 10^{-8}$ M hardly at all. The dependence of open-state probability on intracellular pCa at a given membrane potential was sigmoidal. At +10 mV four separate experiments gave P_o values of 0.82 ± 0.08, 0.52 ± 0.07 and 0.03 ± 0.02 at 1.3×10^{-3}, 10^{-6} and 1.5×10^{-7} M Ca^{2+}, respectively (mean ± SE). For a holding potential of +10 mV, P_o was 50% of maximum at a pCa of 6.5. Hill plots of this sort of relationship ($P_o/(1 - P_o)$ against pCa) gave a slope of around 1.7 (*not shown*).

A better understanding of the kinetics of gating can be obtained from statistical analysis of channel residence times in the open and closed states. Figure 3*A* shows that the histogram of channel-open times was well fitted by a single exponential probability distribution (time constant, τ_o = 21 msec), compatible with the presence of a single open-state.

Multiple closed states are, however, indicated from the single-channel records of Fig. 1, and this is confirmed by the frequency histograms of channel-closed times (Fig. 3*B* and *C*). In Fig. 3*B* the closed time distribution was fitted to a single exponential probability distribution with time constant $\tau_{c(\text{fast})}$ of

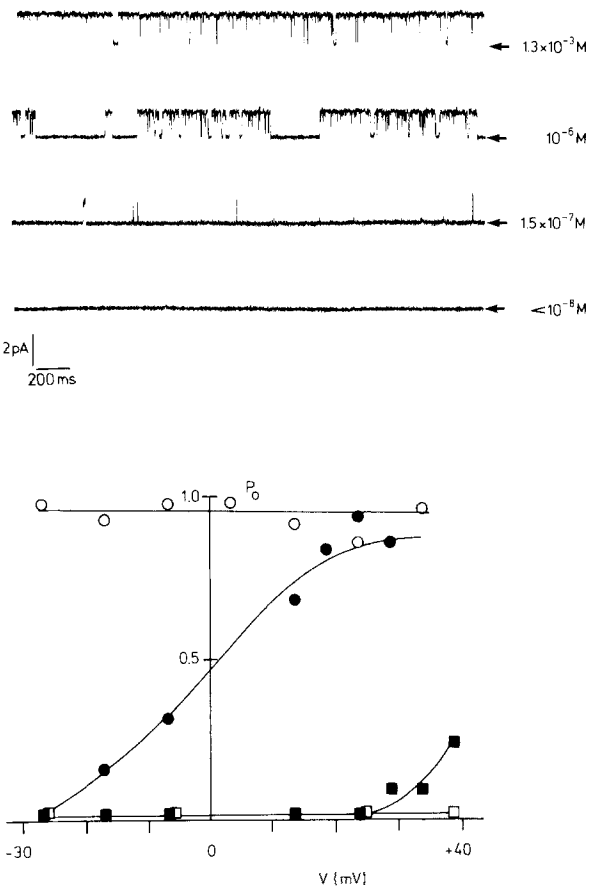


Fig. 2. *Upper panel:* Effect of intracellular Ca^{2+} concentration upon channel activity in an excised inside/out membrane patch. Single-channel current records at different $[Ca]_i$ are presented. The patch was bathed by symmetrical KCl solutions, and the membrane potential was +10 mV. Arrows indicate the closed channel state. *Lower panel:* Channel open probability plotted as a function of membrane potential for the following $[Ca]_i$: 1.3×10^{-3} M (○); 10^{-6} M (●); 1.5×10^{-7} M (■) and $<10^{-8}$ M (□)

1.4 msec using a bin size = 1 msec and a range = 100 msec. However, this fit did not accommodate a significant number of intervals greater than 20 msec, which become apparent when the bin size and time scale are expanded to 40 msec and 2 sec, respectively (Fig. 3*C*). With this bin size the long sojourns in the closed state are more evident, and the distribution can be fitted with a double exponential. This contained the fast closures, shown mainly in the first bin, but gave a second exponential with $\tau_{c(slow)}$ of 454 msec. Careful examination of the current records revealed the presence of a third group of closures interrupting the bursts of channel openings. These very brief closures (< 1 msec) were poorly resolved even under the most favorable recording conditions and for this reason were excluded from the frequency versus channel residence times histograms in order to simplify kinetic analyses. Including the poorly resolved flickering closures there were, therefore, three types of closed times, suggesting the presence of three different closed states.

The main effect of both Ca^{2+} and voltage was to reduce the proportion of long closures and consequently to increase the length of apparent bursts (*see* traces in Figs. 1 and 2). These were examined by the approach of Sakmann and Trube (1984). Openings separated by gaps lasting <30 msec were, by definition, considered to be part of a burst. This was approximately one-tenth of $\tau_{c(slow)}$ in Fig. 3*C*. The lower panel of Fig. 3 illustrates that, like open state probability, burst duration was strongly voltage dependent. Burst duration is seen to decrease about tenfold as the excised membrane patch is hyperpolarized from +35 to −35 mV. However, in each instance the distribution of burst duration was well fitted by a single exponential. The modulation of channel gating by membrane potential and the influence of cytosolic Ca^{2+} upon this process is further examined in Fig. 4. The upper panel demonstrates that at moderate intracellular Ca^{2+} concentrations it is the closed ($\bar{t}_c$) and burst ($\bar{t}_{burst}$) mean times which were critically voltage-sensitive. Upon depolarization significant decreases and increases occurred in $\bar{t}_c$ and $\bar{t}_{burst}$ values, respectively, the most pronounced changes lying between −50 and 0 mV for both parameters. In contrast, mean open time ($\bar{t}_o$) was little changed upon depolarization. In four separate experiments $\bar{t}_{burst}$ was 38 ± 15 and 537 ± 235 msec, $\bar{t}_o$ was 12 ± 6 and 20 ± 4 msec an $\bar{t}_c$ was 121 ± 40 and 39 ± 15 msec at −40 and +40 mV, respectively (mean ± SE). Increasing $[Ca]_i$ drastically altered the voltage dependence of the time constants as illustrated by the voltage insensitivity at 1.3×10^{-3} M Ca^{2+} (Fig. 4, lower panel). At this Ca^{2+} concentration $\bar{t}_c$, $\bar{t}_o$ and $\bar{t}_{burst}$ remained constant at around the same level as that reached at maximum depolarization at the lower $[Ca]_i$. In four separate experiments $\bar{t}_o$ was 15 ± 6 or 17 ± 4 msec, $\bar{t}_c$ was 32 ± 15 or 31 ± 16 msec and $\bar{t}_{burst}$ was 409 ± 70 or 371 ± 80 msec at −40 or +40 mV, respectively (mean ± SE).

The effects of barium upon K^+ channel activity were also investigated. Figure 5 (top panel) demonstrates that applying 5 mM Ba^{2+} to the intracellular aspect of an excised membrane patch drastically reduced open-state probability. The patch contained five channels which were almost continuously active under control conditions ($[Ca]_i = 10^{-4}$ M). Barium blockade was most pronounced at depolarizing potentials, bursts of channel openings being reduced to brief openings separated by long periods of closure. At hyperpolarizing potentials blockade was reduced, bursts of openings separated

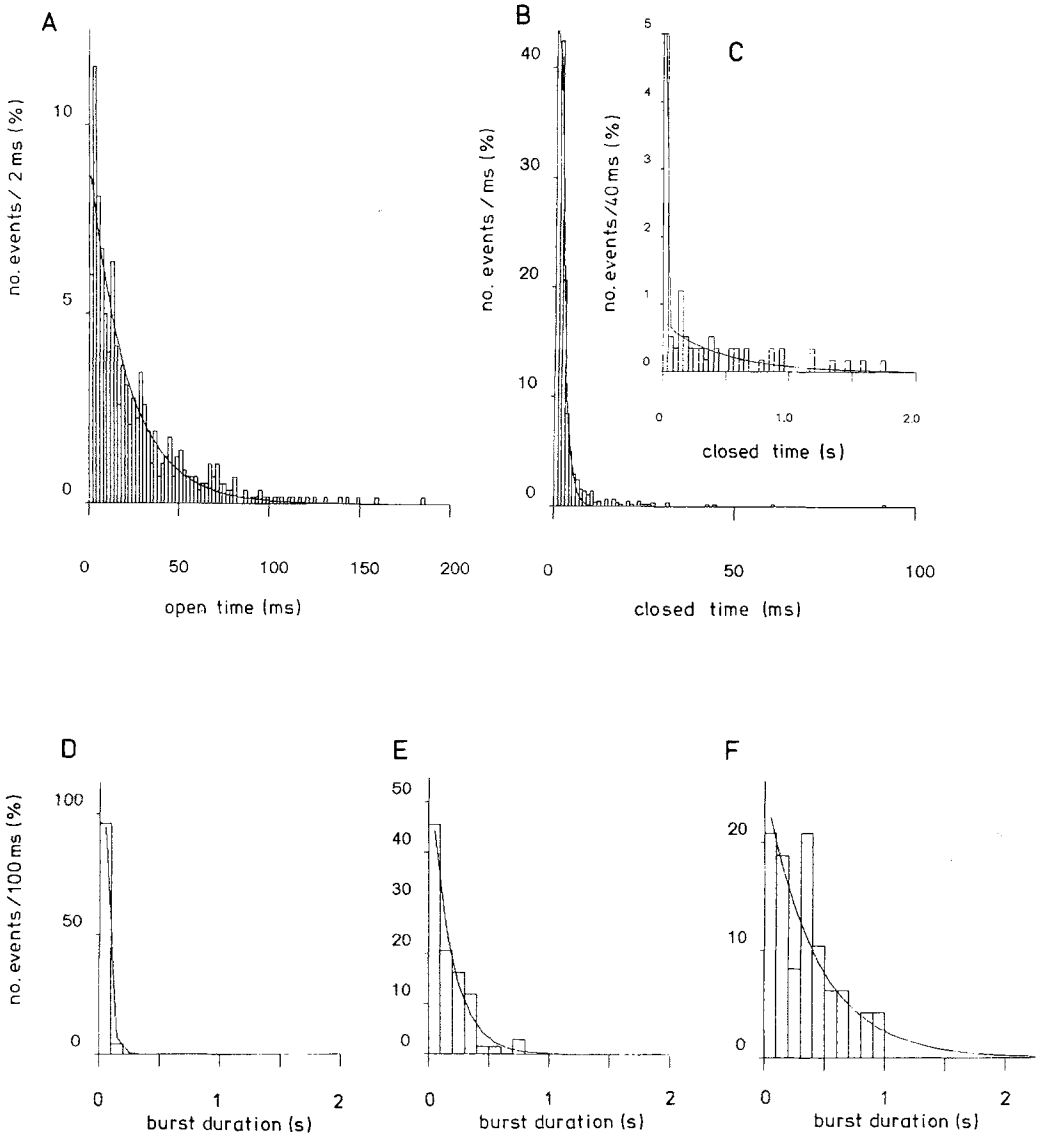


Fig. 3. *Upper panel:* Analysis of single-channel kinetics. Frequency versus time histograms are illustrated for an excised inside/out patch containing a single K^+ channel bathed by symmetrical KCl solutions and free Ca^{2+} concentration of 10^{-6} M in the bath. Membrane potential was +35 mV. The histogram of channel-open times (*A*) was fitted by a single exponential with time constant (τ_o) of 21 msec. The histograms showing closed time frequency distributions were fitted by two exponentials with time constants $\tau_{c(fast)}$ = 1.4 msec (*B*) and $\tau_{c(slow)}$ = 454 msec (*C*). Note the different time scales and bin widths used to calculate closed time constants. Maximum duration of unresolved shut interval was 0.5 msec. *Lower panel:* Voltage dependence of the burst duration. In this analysis channel openings separated by gaps of duration <30 msec are considered as part of a burst. Same time scale and bin width for all histograms. Holding voltages were −35 (*D*), −15 (*E*) and +35 (*F*) mV. The respective time constants for the monoexponential fitting were 39, 171 and 432 msec. Data from same experiment as upper panel

by shorter closures were more frequent and multiple channel openings were now observed. Single-channel current was largely unaffected by Ba^{2+} exposure (*not shown*).

The effect of barium exposure on the voltage dependence of open-state probability is shown in Fig. 5 (lower panel, left). At the high $[Ca]_i$ used in the control condition, open-state probability was voltage insensitive. However, in the presence of Ba^{2+} open state probability exhibited marked voltage-sensitivity, decreasing significantly upon patch depolarization.

Barium blockade was kinetically analyzed in membrane patches containing only one K^+ channel (Fig. 6). Channel residence times in the open state were well fitted by a single exponential probability distribution both in the presence and absence of barium. Barium exposure, however, reduced chan-

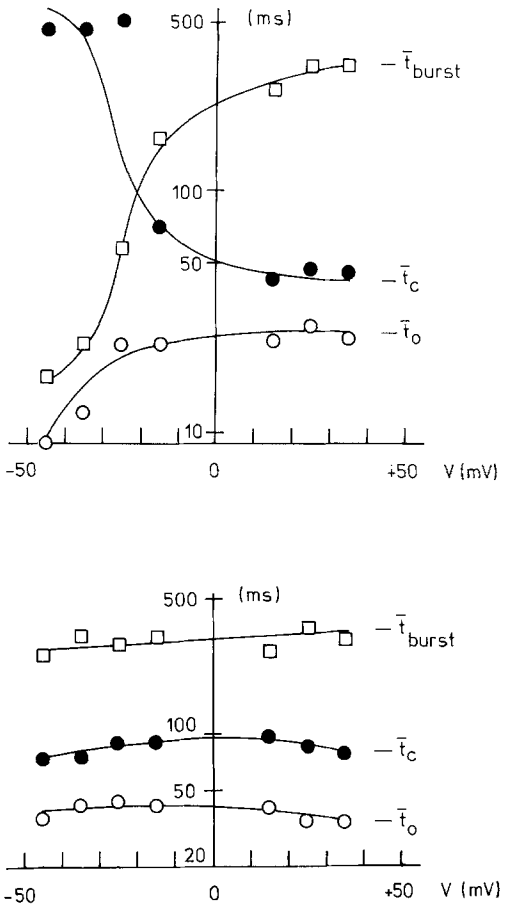


Fig. 4. Influence of intracellular calcium concentration on the voltage dependence of mean open (○), closed (●) and burst (□) times. Data from an excised inside/out membrane patch containing a single K^+ channel bathed by symmetrical KCl solutions. *Upper panel:* $[Ca]_i = 10^{-6}$ M; *lower panel:* $[Ca]_i = 1.3 \times 10^{-3}$ M

nel open times as evidenced by the fall in τ_o from 3.2 to 1.6 msec.

At the relatively high intracellular Ca^{2+} concentrations used channel-closed time distributions were no longer fitted by two exponentials since the influence of $\tau_{c(slow)}$ had been eliminated. Instead, they were described by a single exponential of time constant $\tau_{c(fast)} = 9$ msec. Barium exposure significantly increased channel residence in the closed state. This was evident from the channel-closed time histogram where a two-exponential probability distribution was now required to fit the data. The short duration closures were described by $\tau_{c(fast)}$ of similar magnitude to the control situation but now there was a significant contribution of long duration closed periods, the result of channel blockade by barium.

Figure 7 demonstrates the effect of Ba^{2+} exposure upon the voltage sensitivity of $\bar{t}_o$ and $\tau_{c(slow)}$. Notice that this new $\tau_{c(slow)}$ is different from that used for long shut periods in low Ca^{2+} concentration. In the absence of barium, at the high intracellular Ca^{2+} concentration used for the control condition $\bar{t}_o$ was only slightly potential sensitive. Upon exposure to barium, $\bar{t}_o$ decreased significantly upon depolarization. $\tau_{c(slow)}$ was only strongly voltage sensitive at negative potentials, increasing significantly upon depolarization. $\tau_{c(fast)}$ was poorly voltage sensitive both in the presence and absence of barium (*not shown*).

Discussion

A K^+ channel present in the plasma membrane of isolated *Necturus* enterocytes has been characterized using the excised inside/out membrane patch configuration of the patch-clamp technique. This channel has a large conductance and is highly selective to potassium. Channel open probability was influenced by both membrane potential and cytoplasmic Ca^{2+} concentration and the channel was sensitive to barium blockade. These are properties characteristic of the group of K^+ channels described as maxi K^+ channels (Latorre & Miller, 1983), which have already been observed in a variety of cells and tissues (Marty, 1981; Pallotta, Magleby & Barrett, 1981; Wong, Lecar & Alder, 1982; Maruyama et al., 1983; Tabares, López-Barneo & De Miguel, 1985; Morris et al., 1986; Christensen & Zeuthen, 1987; Gitter et al., 1987).

Calcium- and Voltage Activation

Channel open state probability was shown, in the present work, to be modulated by membrane potential and cytosolic Ca^{2+}. Depolarizing the membrane potential by 20 mV caused a fivefold increase in P_o at a $[Ca]_i = 10^{-6}$ M, while changing pCa from 8 to 6 produced a sixfold increase in P_o at a membrane potential of +10 mV. Although these results suggest that Ca^{2+} and membrane potential have related effects upon P_o, it is the channel's Ca^{2+} sensitivity which is crucial to its function. Without Ca^{2+} the channel did not conduct in the physiological range of membrane potentials. Saturating the channel with Ca^{2+} rendered it completely open, eliminating long closures and abolishing voltage sensitivity.

A wide range of Ca^{2+} sensitivities has been observed for maxi K^+ channels originating from different tissues. Channels originating from muscle membranes (Barrett, Magleby & Pallotta, 1982; Moczydlowski & Latorre, 1983) and choroid plexus (Christensen & Zeuthen, 1987) exhibit low Ca^{2+} sensitivity, while those in pancreatic acinar cells (Maruyama et al., 1983) and clonal anterior pituitary cells (Wong et al., 1982) are exquisitely sensi-

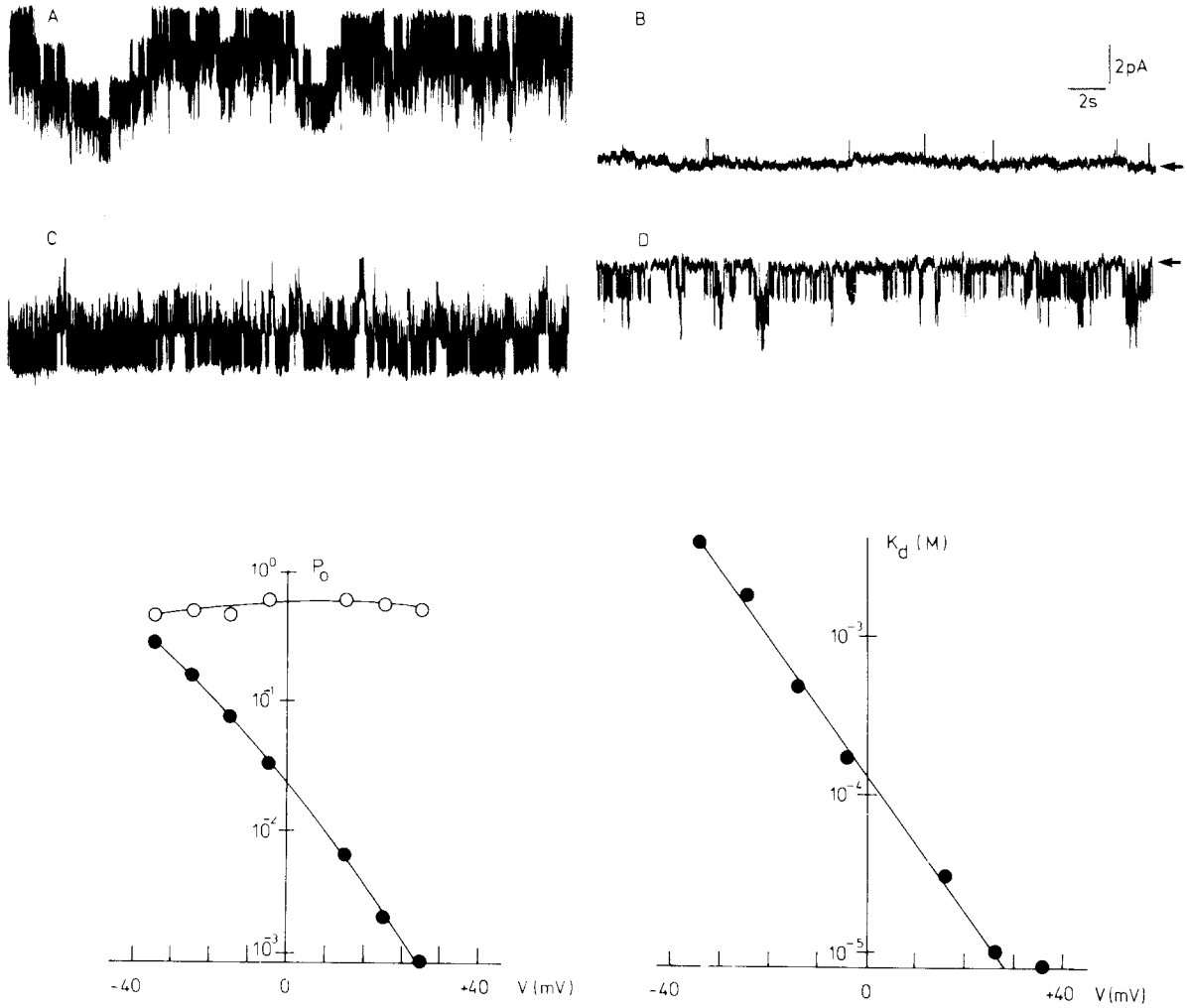


Fig. 5. *Upper panel:* K^+-channel activity in an excised inside/out membrane patch in the absence (*A*,*C*) and presence (*B*,*D*) of 5 mM Ba^{2+} bathing the intracellular membrane face. Membrane potential was +30 mV (*A*,*B*) or −30 mV (*C*,*D*). The patch contained five channels, and arrows indicate the current level at which no channels appeared to be open. The membrane patch was bathed by symmetrical 100 mM KCl solutions and the cytoplasmic face exposed to 10^{-4} M Ca^{2+}. *Lower panel:* Voltage dependence of open state probability (P_o) and the voltage-dependent dissociation constant (K_d). Lower panel, left: probability of channel being in open state in control (○) and in the presence of 5 mM Ba^{2+} (●). Lower panel, right: K_d of barium calculated as described in the Discussion. Data for graphs derived from same patch used for illustrative purposes in upper panel

tive to Ca^{2+}. The sensitivity of the channel reported here lies intermediate between the two groups. However, it is significantly less sensitive than the maxi K^+ channel isolated from basolateral membranes of rat enterocytes which at 10^{-7} M Ca^{2+} is predominantly open at all potentials tested (Morris et al., 1986). This heterogeneity in Ca^{2+} sensitivity is probably a reflection of the evolutionary adaptation of maxi K^+ channels to a diverse range of biological functions and/or their location in different membrane environments. Another possible reason for this heterogeneity in Ca^{2+} sensitivity might be the unavoidable loss of unknown cytoplasmic regulators and/or loosely attached membrane components that could occur upon excision of a membrane patch.

The kinetic description of modulation of maxi K^+ channel activity by cytosolic Ca^{2+} and membrane potential is complex. Comprehensive investigations of maxi K^+ channels from skeletal muscle have revealed the presence of multiple open and closed states differing in residence times and Ca^{2+} sensitivity (Methfessel & Boheim, 1982; Moczydlowski & Latorre, 1983; Magleby & Pallotta, 1983). The results shown here demonstrate the presence of at least two closed states on the basis of the distribution of closed times. Very rapid events (<1 msec) were not resolved; therefore, an additional open state or a third closed state characterizing the 'flickering,' although probably present, was not studied. Channel substates were also observed during experiments (*results not shown*) but these

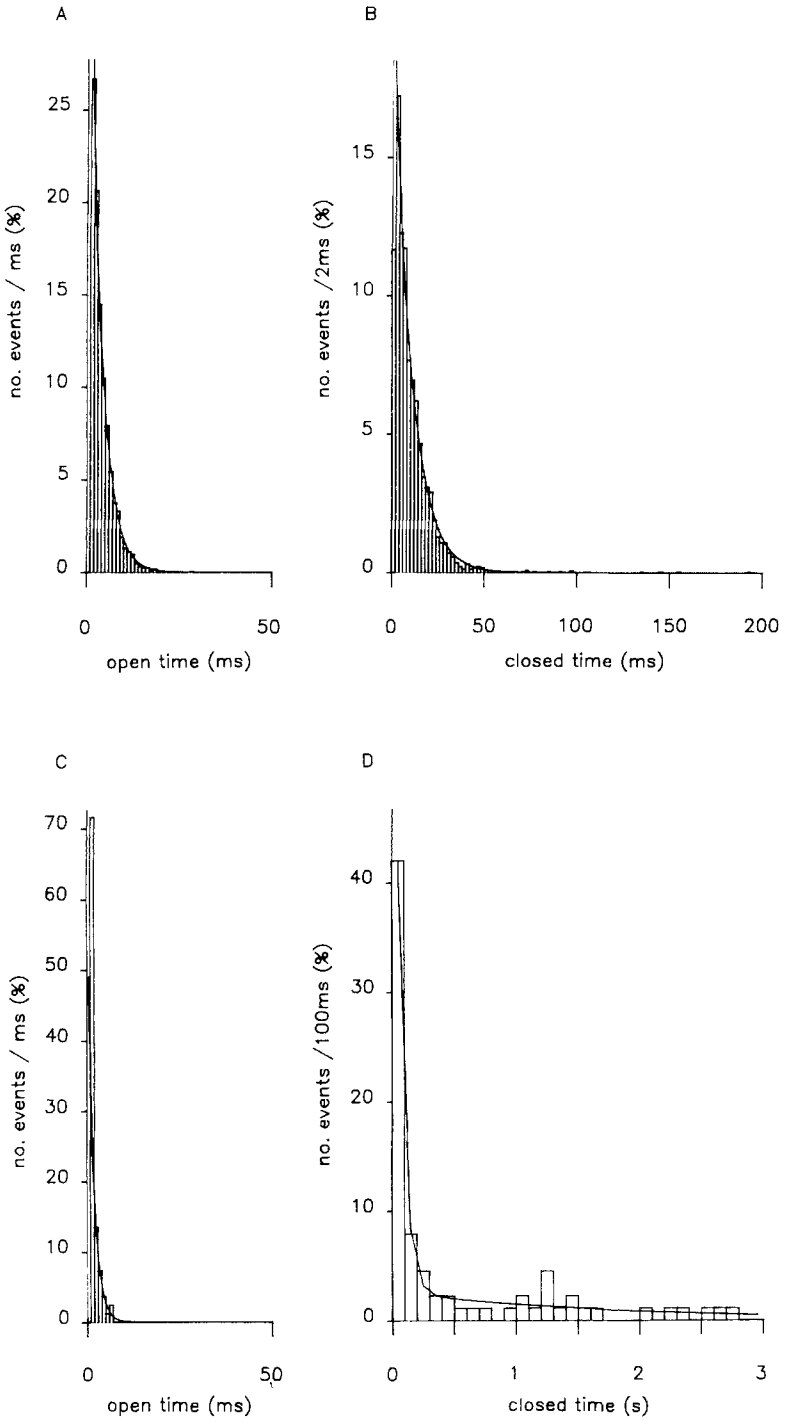


Fig. 6. Effect of barium on single-channel kinetics. Frequency versus time histograms are illustrated for an excised inside/out patch containing a single K^+ channel bathed by symmetrical KCl solutions in the absence (*A,B*) and presence (*C,D*) of 5 mM Ba^{2+}. Bath Ca^{2+} concentration was 10^{-4} M; membrane potential was +35 mV. Histograms of channel-open times (*A,C*) were both fitted by single exponentials with τ_o of 3.2 and 1.6 msec, respectively. Closed time distribution in the absence of Ba^{2+} (*B*) was described by a single exponential ($\tau_{c(\text{fast})}$ = 9 msec), while in the presence of Ba^{2+} closed time frequency distribution was fitted by two exponentials with time constants $\tau_{c(\text{fast})}$ and $\tau_{c(\text{slow})}$ of 7.4 msec and 1.4 sec, respectively. A long time scale and large bin size is used in *D* for illustrative purposes only

were low probability events and were also excluded to simplify the kinetic description. With these provisos, it sufficed to describe channel gating in terms of fluctuations between a single open state and two closed states. A kinetic model adequately accommodating such findings is (Del Castillo & Katz, 1957)

$$C_1 \underset{\alpha_s}{\overset{\beta_s}{\rightleftharpoons}} C_2 \underset{\alpha_f}{\overset{\beta_f}{\rightleftharpoons}} O \qquad (1)$$

where the long duration closed state (C_1) was the principal closed state separating bursts of channel openings and C_2 consists of brief closures interrupting the open state (O) record. β_s and α_s and β_f and α_f

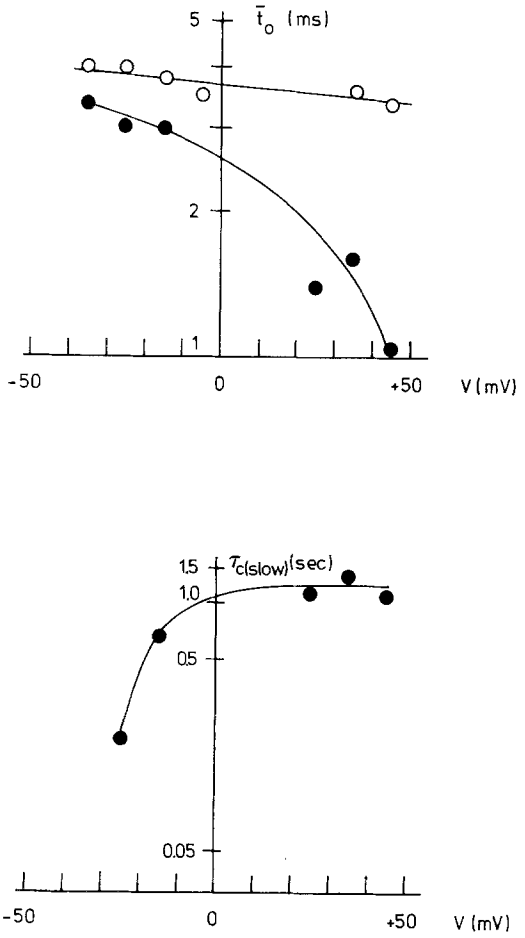


Fig. 7. Effect of Ba^{2+} exposure on the voltage dependence of open and closed times. In the upper panel the voltage dependence of mean open time ($\bar{t}_o$) in the presence (●) or absence (○) of Ba^{2+} is shown. The lower panel shows the voltage dependence of $\tau_{c(slow)}$ in the presence of Ba^{2+}. Data are from analyses illustrated in Fig. 5

were the rate constants describing transitions between the states. Full expressions for the derivation of these rate constants from the time constants of the residence times have been published previously by others (Colquhoun & Hawkes, 1981; Sakmann & Trube, 1984). It must be stressed that for a sequential model as that in Eq. (1), although the distribution of all shut periods will have two exponential terms, it will not have constants with simple physical significance.

The primary gate-modulating channel function lay between the two closed states in the above model. It exhibited marked dependence upon membrane potential and cytosolic Ca^{2+} as shown by the strong voltage and Ca^{2+} sensitivity of $\bar{t}_c$ and $\bar{t}_{burst}$ (Fig. 4). The decrease in $\bar{t}_c$ could be purely attributed to the gradual disappearance of the long closures ($\tau_{c(slow)}$) with increasing Ca^{2+} or depolarization, without affecting $\tau_{c(fast)}$[1]. In contrast, the second gate determining the brief closures varied little with changes in membrane potential and cytosolic Ca^{2+} as witnessed by the relative constancy of $\bar{t}_o$ (Fig. 4).

Under conditions of saturating levels of Ca^{2+} ions the equilibrium lies far to the right to the effect that the channel operates in the mode

$$C_2 \underset{\alpha_f}{\overset{\beta_f}{\rightleftharpoons}} O \qquad (2)$$

hence, the channel is predominantly open, shows little voltage sensitivity and only brief closures are observed. Very detailed kinetic descriptions of Ca^{2+}-activated K^+ channels have been published before by Methfessel and Boheim (1982), Magleby and Pallotta (1983), and Moczydlowski and Latorre (1983). Although we have not studied the enterocyte channels in such detail, it is clear that their behavior with respect to voltage and Ca^{2+} dependence is very similar to that of those reported before. For instance, if the open probability within bursts is examined it is found that log K (where $K = P_o/(1 - P_o)$ in Methfessel and Boheim's nomenclature) is linearly related to voltage, with a slope approaching $2F/RT$. In addition, plotting K against pCa yields slopes approximating 2. This suggests that two Ca^{2+} ions are required to lead to the open state of the channel. From our observations, therefore, the kinetic behavior of *Necturus* enterocyte membrane maxi K^+ channels, although described here by a simplified model, is comparable to those originating from skeletal muscle membranes (Methfessel & Boheim, 1982, Magleby & Pallotta, 1983; Moczydlowski & Latorre, 1983).

Barium Blockade

Results described above demonstrate that millimolar concentrations of barium in the solution bathing the intracellular patch face block the maxi-K^+ channel. The cause of this block is the reduction of channel open state probability (Fig. 5). Channel conductance was unaltered in the presence of Ba^{2+}, consistent with the idea that Ba^{2+} acts as a 'slow'

[1] Comparison of $\tau_{c(fast)}$ in Figs. 3*B* and 6*B* might suggest that Ca^{2+} prolongs the closed state of the short lifetime. This is not borne out if experiments in one single patch are considered, and we would attribute this discrepancy to variability between different patches. However, another argument that might be entertained is that at 10^{-4} M Ca^{2+} the short closures of duration 1.4 msec may have become impossible to detect above a background of a large number of long shut periods of 9 msec average duration.

blocker only influencing P_o upon binding the channel (Hille, 1984). The efficacy of Ba^{2+} as a maxi K^+ channel blocker is attributed to the selectivity properties of K^+ channels and the similarity in crystal ionic radii of potassium and barium ions (Standen & Stanfield, 1978). Barium ions are proposed to enter the wide channel mouth but not to pass the selectivity filter. On the basis of this evidence Vergara and Latorre (1983) suggest that the selectivity filter represents the location of the Ba^{2+} binding site within the channel.

As mentioned above, at the high Ca^{2+} concentration used, only one closed state normally occurs. The closures appearing in the presence of Ba^{2+} can then be characterized as a new population of long closures arising from Ba^{2+} blockade. Hence the following model (Benham et al., 1985) can be used to describe the effects of Ba^{2+} on K^+ channels:

$$C \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} O + Ba^{2+} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} OBa^{2+}. \tag{3}$$

In the absence of Ba^{2+} the mean open time is related, at a given potential, to the backward rate constant, α_f, by: $\bar{t}_o = \alpha_f^{-1}$. While in the presence of Ba^{2+} the mean open time ($\bar{t}_{oBa}$), is described by the rates of the reactions leading from open to closed and to blocked states: $\bar{t}_{oBa} = (\alpha_f + k_1[Ba])^{-1}$.

The product $k_1[Ba]$ was calculated according to the formula: $k_1[Ba] = (\bar{t}_o - \bar{t}_{oBa})/(\bar{t}_o \cdot \bar{t}_{oBa})$. In the absence of Ba^{2+}, at high intracellular Ca^{2+} concentrations, $\tau_{c(slow)}$ was absent (Fig. 6). Hence $\tau_{c(slow)}$ in the presence of Ba^{2+} was equivalent to $(k_1)^{-1}$. Thus, from values derived for $\bar{t}_o$, $\bar{t}_{oBa}$ and $\tau_{c(slow)}$ it was possible to calculate values for k_1 and k_{-1} from whence a value for K_d (k_{-1}/k_1) could be derived. By this approach a value for K_d of 1.2×10^{-4} M was obtained at 0 mV.

In multichannel patches the kinetics of barium blockade could be analyzed according to the relationship (Benham et al., 1985)

$$\frac{k_{-1}}{k_1} = K_d(V) = \frac{[Ba^{2+}]P_{Ba} \cdot P}{P - P_{Ba}} \tag{4}$$

where K_d is the voltage-dependent dissociation constant and P_{Ba} and P are the open probabilities in the presence and absence of barium, respectively. Using the data in Fig. 5 (lower panel, left) it was possible to derive an estimate for K_d. The value of K_d (0 mV) obtained from Fig. 5 (lower panel, right) by interpolation was 1.4×10^{-4} M. This was very close to that derived above from the kinetic constants, providing good support for the model described.

Barium blockade was voltage dependent, being more effective upon depolarization. This finding is consistent with previous observations (Vergara & Latorre, 1983; Benham et al., 1985). The voltage sensitivity of Ba^{2+} effect relates to the mechanism of blockade. Thus the fact that k_1 was significantly more voltage-sensitive than k_{-1} is explained by suggesting that the blocking barium ion is shielded from the membrane field by interaction with charged groups at the binding site, such that unbinding is little influenced by the potential (Colquhoun & Hawkes, 1981).

Physiological Role

It is difficult to ascribe a physiological role to the Ca^{2+}-activated K^+ channel described here purely on the basis of data obtained from excised patches of membrane. However, the characteristics of activation described here set constraints for their possible involvement in cellular functions. In cell-attached patches the channel was rarely seen to be active, and in these circumstances large depolarizing voltages had to be applied for it to be observed. Similar observations have been reported for the choriod plexus maxi K^+ channel (Christensen & Zeuthen, 1987). Previous observations in rat enterocytes (Morris et al., 1986) show a very high sensitivity of maxi-K^+ channels to Ca^{2+}, which suggests that they are active at the low Ca^{2+} concentrations expected in the cytoplasm of enterocytes. In *Necturus* enterocytes, however, significant openings at physiological Ca^{2+} concentrations were seen only after depolarizing the patch to +30 or +40 mV, equivalent to a depolarization of about 70 mV in the intact cell. It is proposed then that for this channel to participate in the regulatory adjustments observed during Na^+-coupled substrate transport (Brown et al., 1983; Grasset et al., 1983; Lau et al., 1984; Brown & Sepúlveda, 1985) an increase in cytoplasmic Ca^{2+} would have to be postulated.

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