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Reconstitution of a Calcium-Activated Potassium Channel in Basolateral Membranes of Rabbit Colonocytes into Planar Lipid Bilayers

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Summary. A highly enriched preparation of basolateral membrane vesicles was isolated from rabbit distal colon surface epithelial cells employing the method described by Wiener, Turnheim and van Os (Weiner, H., Turnheim, K., van Os, C.H. (1989) *J. Membrane Biol.* **110**:147–162) and incorporated into planar lipid bilayers. With very few exceptions, the channel activity observed was that of a high conductance, Ca^{2+}-activated K^+ channel. This channel is highly selective for K^+ over Na^+ and Cl^-, displays voltage-gating similar to "maxi" K(Ca) channels found in other cell membranes, and kinetic analyses are consistent with the notion that K^+ diffusion through the channel involves either the binding of a single K^+ ion to a site within the channel or "single-filing" ("multi-ion occupancy"). Channel activity is inhibited by the venom from the scorpion *Leiurus quinquestriatus*, Ba^{2+}, quinine, and trifluoperazine. The possible role of this channel in the function of these cells is discussed.

Key Words Ca^{2+}-activated K^+ channels · rabbit colon · basolateral membranes · Ba^{2+} · quinine · scorpion venom · trifluoperazine · lipid bilayers · reconstitution

Introduction

Potassium channels comprise the principal conductive pathways across the basolateral membranes of all sodium-absorbing epithelial cells studied to date (Schultz, 1986). In recent years it has become clear that the properties of these "leak" pathways are not fixed. Instead, there is compelling evidence, derived from studies on a number of Na^+-absorbing epithelia, that the K^+ conductance of the basolateral membranes parallels the rate of Na^+ entry across the apical membranes and, in turn, the rate of Na^+-K^+ pump activity at the basolateral membrane. The physiological utility of this "pump-leak parallelism" has been discussed in a number of publications (Schultz, 1981, 1986, 1989). The intracellular signal(s) responsible for these "homocellular" regulatory processes is(are), however, unresolved.

Two methods have been developed that permit the investigation of the properties and regulation of single ion channels in biological membranes: namely, the patch-clamp technique and techniques for reconstituting ion channels into planar phospholipid bilayers. The application of the patch-clamp technique to the study of ion channels in the basolateral membranes of many Na^+-absorbing epithelial cells is precluded, however, by the presence of a basement membrane and layers of subepithelial connective tissue and smooth muscle that prevent the formation of giga-ohm seals with that barrier. One way to circumvent this problem is by isolating these cells, and several groups have recently successfully patch-clamped the basolateral membranes of isolated small intestinal (Sepulveda & Mason, 1985; Morris, Gallacher & Lee, 1986; Sheppard, Giraldez & Sepúlveda, 1988*a*) and colonic (Richards & Dawson, 1986) epithelial cells; the results of those studies are considered below.

We have previously described an alternate approach: namely, the reconstitution of a highly enriched preparation of basolateral membranes into planar phospholipid bilayers (Costantin et al., 1989). In this study we employed this approach to examine the properties of a K^+ channel present in basolateral membrane vesicles isolated from surface absorptive cells of rabbit distal colon.

Materials and Methods

Segments of distal colon were obtained from white rabbits (2–4 kg) that were euthanized by intravenous injection of T-61 Euthanasia Solution® (Hoechst). The method employed to isolate, from mucosal scrapings, a highly enriched fraction of basolateral membranes (BLMV) that are derived from *surface* absorptive cells followed that described by Wiener, Turnheim and van Os (1989). These investigators reported that this preparation is enriched 34-fold in the (Na^+ + K^+)-ATPase, a generally accepted enzyme marker for basolateral membranes, and that it is minimally contaminated by enzyme markers for the apical membrane

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(e.g., alkaline phosphatase, ouabain-insensitive K^+-phosphatase) or intracellular organelles (e.g., acid phosphatase, succinic dehydrogenase, NADH reductase). The isolated BLMV were divided into small aliquots, stored in liquid nitrogen and thawed immediately prior to use.

Mueller-Rudin planar bilayers containing phosphatidylethanolamine (10 mg/ml) and phosphatidylserine (10 mg/ml) dissolved in decane were painted over a 0.33-mm aperture in a Delrin cup that was inserted into a cut-away PVC block as described elsewhere (Alvarez, 1986). The cup contained the *cis* solution to which the BLMV were added; the *trans* compartment was formed by the PVC block. In all experiments the *cis* solution initially contained 450 mM KCl and the *trans* compartment contained 50 mM KCl; in addition, both compartments contained Ca^{2+} (*see below*) and were buffered at pH 7.0 with 10 mM K^+-HEPES. As discussed elsewhere (Cohen, 1986), the large initial osmolarity difference across the bilayer was employed to promote fusion. After the addition of BLMV (1–10 μg protein per ml), the *cis* compartment was stirred using a magnetic stirring bar; stirring was terminated when channel activity was detected. In many experiments, the KCl in the *cis* compartment was subsequently diluted; in the remainder of this paper we employ the notation (K_c/K_t) to designate the KCl *concentrations* in the *cis* and *trans* compartment, respectively; e.g., the initial condition is designated (450/50). The notations (K), (Na) and (Ca) are used to designate the ionic *activities* of K^+, Na^+ and Ca^{2+}, respectively; the subscripts *c* or *t* following the parentheses refer to the *cis* or *trans* compartments, respectively.

All experiments were carried out at room temperature (22°C).

Channel activity was monitored by a List EPC-7 amplifier, visualized using a digital oscilloscope and recorded in digital form on videotape employing an analog-digital converter (Medical Systems, New York, PCM-2). For analyses, the analog signal derived from the digital record was passed through an 8-pole Bessel filter with the corner frequency (−3 dB) set at 500 Hz and then digitized (Kiethley, System 570) with a sampling frequency of 3000-4000 Hz. Data were analyzed using a program written by Dr. Hubert Affolter and kindly provided by Dr. Roberto Coronado. Open events are detected through the use of two discriminators; the first (the *closed* discriminator) was set at 1 SD above the mean baseline current, and the second (the *open* discriminator) was set at 1 SD below the mean single channel current. Open events are defined as transitions that cross both discriminators and remain above the open discriminator for at least two sampled points (i.e., > 0.66 msec). The "holding voltage" V_m is defined as the electrical potential of the *cis* compartment with reference to that of the *trans* (ground) compartment and, as per the accepted convention, a positive current represents the flow of cations from the *cis* to the *trans* compartment or the flow of anions in the opposite direction.

At the conclusion of every experiment, the contents of the two compartments were removed for analyses of K^+, and where relevant, Na^+, concentrations by flame photometry; activities of these ions were estimated using the activity coefficients published by Robinson and Stokes (1959). Different Ca^{2+} activities were obtained using the Ca^{2+}-EGTA buffering technique described by others (Fabiato & Fabiato, 1979; Findlay, Dunne & Petersen, 1985*a*) and verified using an Ca^{2+}-selective electrode (Orion, Model 93-20) corrected for ambient KCl concentrations. In the absence of added Ca^{2+}, the Ca^{2+} concentrations in the solutions resulting from contamination of the salts or distilled water was negligible.

Leiurus quinquestriatus venom (LQV), trifluoperazine (TFP) and quinine were obtained from Sigma Chemical (St. Louis, MO); all other chemicals were "reagent quality."

Results are expressed as the mean ± SEM.

Results

The BLMV fused with the bilayers readily, and in all but a few instances these membranes possessed one, two and sometimes three identical cation-selective channels. As demonstrated below, these channels proved to be Ca^{2+}-activated, K^+ "maxi" channels.

Ca^{2+} Activation and Voltage Dependence

Figure 1 illustrates the behavior of a single cationic channel in the presence of (K_c/K_t) of (200/50). The tracing shown in Fig. 1*a* was observed when the Ca^{2+} activity in the *cis* compartment, $(Ca)_c$, was ≈ 10 μM. Following the addition of K^+-EGTA to the *cis* compartment sufficient to reduce $(Ca)_c$ to ≈ 0.2 μM, channel activity was abolished (Fig. 1*b*). The subsequent addition of Ca^{2+}-EGTA to the *cis* compartment sufficient to increase $(Ca)_c$ to ≈ 1 μM restored channel activity (Fig. 1*c*). Finally, the addition of *Leiurus quinquestriatus* venom (LQV) to the *cis* solution alone to concentrations as high as 290 μg/ml had no effect on channel activity whereas the addition of LQV to the *trans* solution (80 μg/ml blocked channel activity (Fig. 1*d*). LQV contains a number of polypeptides that are effective blockers of some Na^+ and K^+ channels (Moczydlowski et al. 1988); one of these, charybdotoxin, is an effective blocker of many Ca^{2+}-activated K^+ channels (Latorre, 1986; Anderson et al., 1988; Moczydlowski, Lucchesi & Ravindran, 1988). Inasmuch as LQV is only effective from the extracellular solution, these findings unequivocally establish the "sidedness" of the reconstituted channel; namely, the *cis* compartment corresponds to the cell interior.

The effects of V_m and $(Ca)_c$ on the kinetics of this channel are illustrated in Fig. 2. Clearly, the open-time probability (P_o) of the channel is affected by both $(Ca)_c$ and V_m. At a fixed $(Ca)_c$, increasing V_m results in an increase in P_o, and this voltage dependence is much more marked at the lower values of $(Ca)_c$ than at the higher values. Changes in $(Ca)_c$ did not affect the single-channel conductance (*data not shown*). Given the orientation of the channel, the voltage gating is such that at fixed intracellular Ca^{2+} activity, P_o decreases as the cell interior

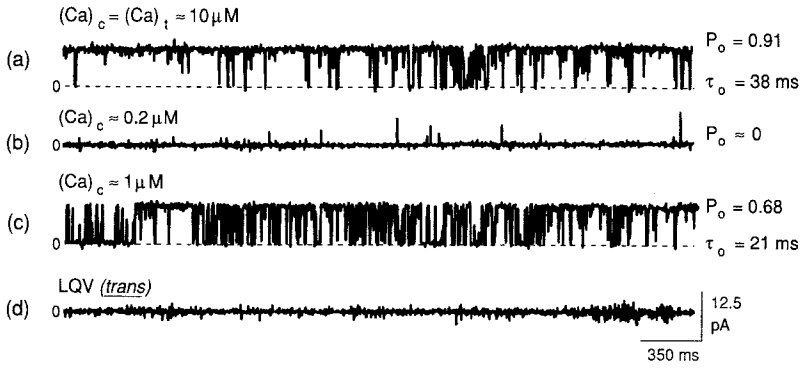

Fig. 1. Effects of $(Ca)_c$ and LQV on channel activity (V_m = 0 mV). Also given are the values of the open-time probabilities (P_o) and the mean open-times (τ_o) under these conditions

becomes electrically more negative with respect to the serosal solution.[1]

Finally, the sensitivity of these channels to activation by Ca^{2+} displayed considerable variability in the range 1 μM < $(Ca)_c$ < 10 μM (Fig. 2). But, in general when V_m = 0 mV, $P_o > 0.9$ when $(Ca)_c \approx 10$ μM and $P_o < 0.1$ when $(Ca)_c$ < 500 nM.

Current-Voltage Relations, Ion Selectivity and Conductance

An example of the current-voltage ($I_c - V_m$) relation of the channel when first exposed to asymmetric KCl solutions and then after the addition of NaCl to the *trans* compartment is shown in Fig. 3. In both instances the $I_c - V_m$ relations are linear. In the absence of Na^+, the conductance of the channel, g_c, was 320 pS and the reversal potential (E_r) did not differ significantly from the Nernst equilibrium potential for K^+ (E_K), indicating that the permeability of the channel to K^+ (P_K) is much greater than that to Cl^- (P_{Cl}). After the addition of NaCl to the *trans* solution there was a small increase in E_r consistent with a small conductance of the channel to Na^+. In this experiment (P_K/P_{Cl}), calculated from the equation for a biionic diffusion potential (Schultz, 1980) was 20 and (P_K/P_{Na}), calculated using the Goldman-Hodgkin-Katz "constant field" equation, was 14. In similar studies, (P_K/P_{Cl}) averaged 49 ± 10 (n =

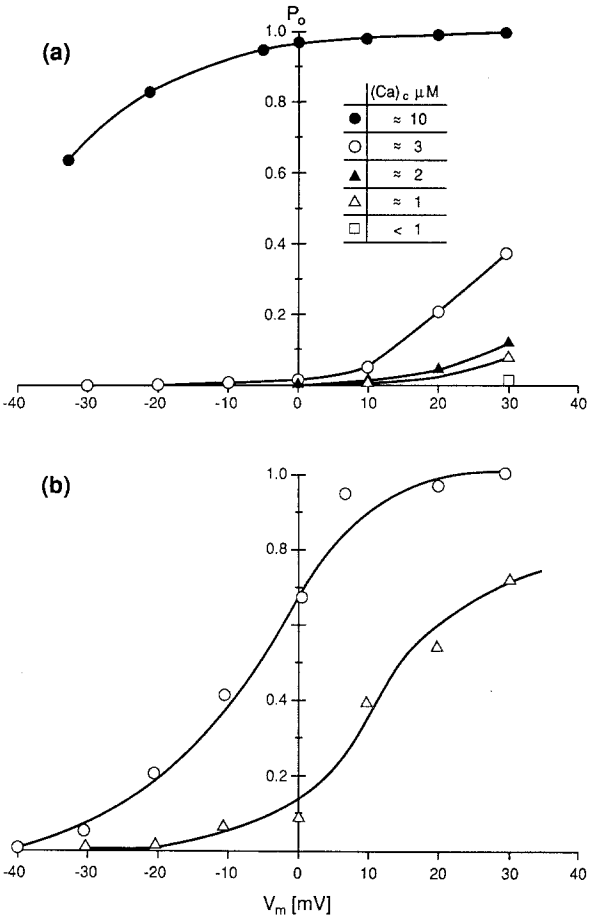

Fig. 2. Two examples of the effects of $(Ca)_c$ and V_m on P_o that illustrate the variability in the sensitivity of the channel to $(Ca)_c$. The relation shown in *a* when $(Ca)_c \approx 10$ μM is typical of many experiments. What is notable is the marked differences in the sensitivities to $(Ca)_c$ over the range between 1–3 μM

[1] The BLMV prepared according to the method of Wiener et al. (1989) have a mixed orientation: approximately 50% are in the right-side-out configuration, 25% are in the inside-out configuration, and 25% are leaky. In a number of our experiments the reconstituted channels exhibited an orientation opposite to that displayed by the channels whose activities are illustrated in Figs. 1 and 2. This was readily detected from the effect of V_m on channel activity displayed on the oscilloscope; thus, in these instances, channel activity *decreased* with increasing V_m. But, in every such instance, LQV blocked channel activity only when added to the *cis* compartment and Ca^{2+} was an activator only when present in the *trans* compartment. Thus, in every experiment Ca^{2+} activated and LQV blocked the channels from opposite sides. Throughout this manuscript we employ the convention that the *cis* compartment corresponds to the cell interior.

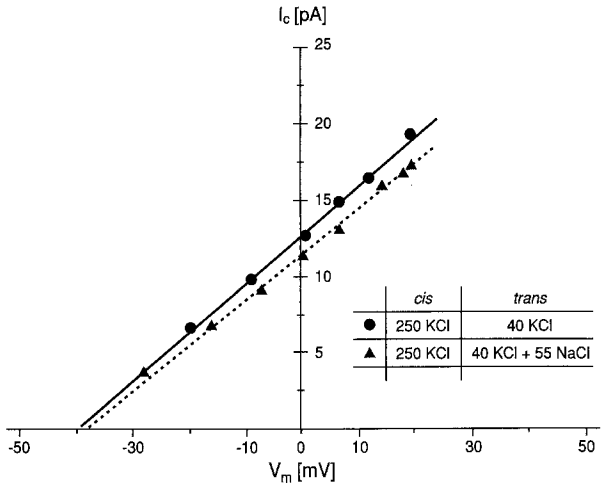

Fig. 3. Relations between I_c and V_m in the presence of asymmetric KCl solutions (●) and after addition of NaCl to the *trans* solution (▲). Activities are given in the inset

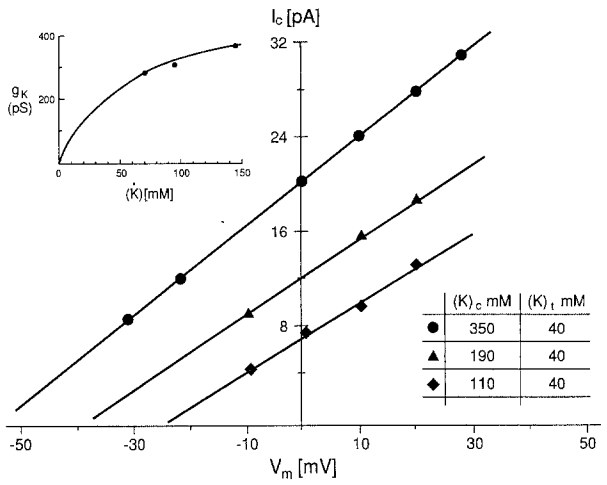

Fig. 4. Example of the relation between I_c (or I_K) and V_m when $(K)_c$ is decreased from 350 to 110 mM in the presence of a constant $(K)_t$. The inset illustrates the relation between the observed values of g_K and (K̇); the curve corresponds to the relation $g_K = 526\ (\dot{K})/(60 + (\dot{K}))$

11) and (P_K/P_{Na}) averaged 57 ± 7 (n = 7).[2] Thus, the channel is highly selective for K^+ over both Cl^- and Na^+.

Further insight into the kinetic properties of this channel was derived from studies in which $I_c - V_m$ relations were determined when $(K)_c$ was varied in the presence of constant $(K)_t$. A typical result is illustrated in Fig. 4. In each instance, the $I_c - V_m$ relation is linear but g_c (under these conditions, g_K) is only minimally affected by large decreases in $(K)_c$. The inset shows the relation between g_K and the logarithmic mean K^+ activity across the membrane, (K̇), given by $\{[(K)_c - (K)_t]/\ln[(K)_c/(K)_t]\}$ (Schultz, 1980). In 11 representative experiments, g_K averaged 340 ± 11 pS when (K̇) averaged 178 mM and 326 ± 17 pS when (K̇) averaged 96 mM. If K^+ passage through the channel conformed to the "independence principle" one would expect a linear relation between g_K and (K̇) that passes through the origin (Schultz, 1980; Hille, 1984). The present findings indicate that this is not the case, but that instead K^+ passage involves either the binding and debinding of single K^+ ions to a site within the channel or multi-ion occupancy of the channel (i.e., "single-filing") (Hille, 1984). Single-file diffusion has been reported for Ca^{2+}-activated K^+ channels in human erythrocytes (Vestergaard-Bogind, Stampe & Christophersen, 1985) and striated muscle membranes (Latorre, 1986; Eisenman, Latorre & Miller, 1986).[3]

Finally, the high conductance of this channel clearly places it in the category of Ca^{2+}-activated K^+ "maxi" channels (Latorre & Miller, 1983); in the remainder of this paper we refer to these channels as BK(Ca)[4].

Channel Inhibition

Barium is an effective blocker of a wide variety of K^+ channels (Latorre & Miller, 1983; Hille, 1984). The results of a typical experiment (n = 6) illustrating the effect of adding Ba^{2+} to the *trans* compartment on P_o as functions of V_m and $(K)_c$ are shown in Fig. 5. Initially, in the presence of (450/50) and with $(Ca)_c \approx 300\ \mu M$, P_o was close to unity at all values of V_m. Following the addition of 7 mM Ba^{2+} to the *trans* compartment, channel activity was inhibited in a voltage-dependent manner such that the blocking effectiveness of Ba^{2+} increased (i.e., P_o decreased) with decreasing V_m; Ba^{2+} (*trans*) did not affect the conductance of these channels (Fig. 6*a*).

[2] In a number of experiments (P_K/P_{Cl}) and (P_K/P_{Na}) did not differ significantly from ∞; for purposes of statistics these were assigned values of 100.

[3] Laver, Fairley and Walker (1989) have suggested that these deviations from the predictions of the "independence principle" could be the result of diffusion limitation to the entrance of the permeating ion into the channel.

[4] Two abbreviations for Ca^{2+}-activated K^+ channels have emerged in the literature: namely, K(Ca) (*cf.* Hille, 1984) and CaK (*cf.* Latorre, 1986). We feel that the former is a better descriptor of a K^+-selective channel that is activated by Ca^{2+} whereas the latter is somewhat ambiguous with respect to the ionic selectivity of the channel. Further, the abbreviations BK and SK have been employed to distinguish between high (i.e., "big") and low (i.e., "small") conductance Ca^{2+}-activated K^+ channels (*cf.* Moczydlowski et al., 1988); these abbreviations do not, however, indicate the dependence on Ca^{2+}. Thus, we adopt the notation K(Ca) to designate Ca^{2+}-activated channels and BK(Ca) to designate those that fall into the category of high-conductance ("maxi") channels.

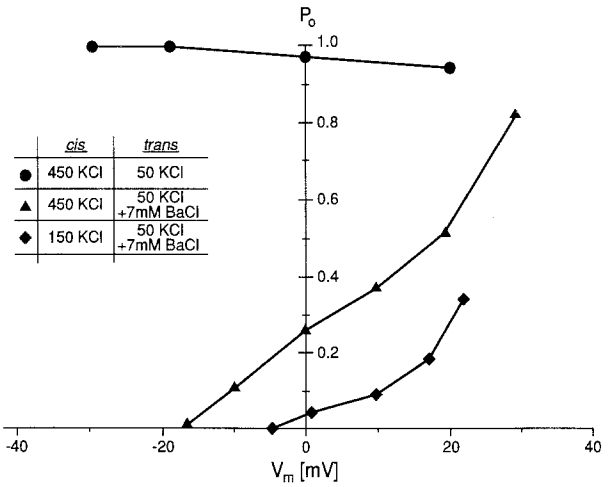

Fig. 5. Effect of Ba^{2+} (*trans*) on relation between P_o and V_m in the presence of 0.3 mM Ca^{2+}. The nominal concentrations of KCl in the *cis* and *trans* compartments are given in the inset. Note that lowering $(K)_c$ markedly increased the blocking action of Ba^{2+}

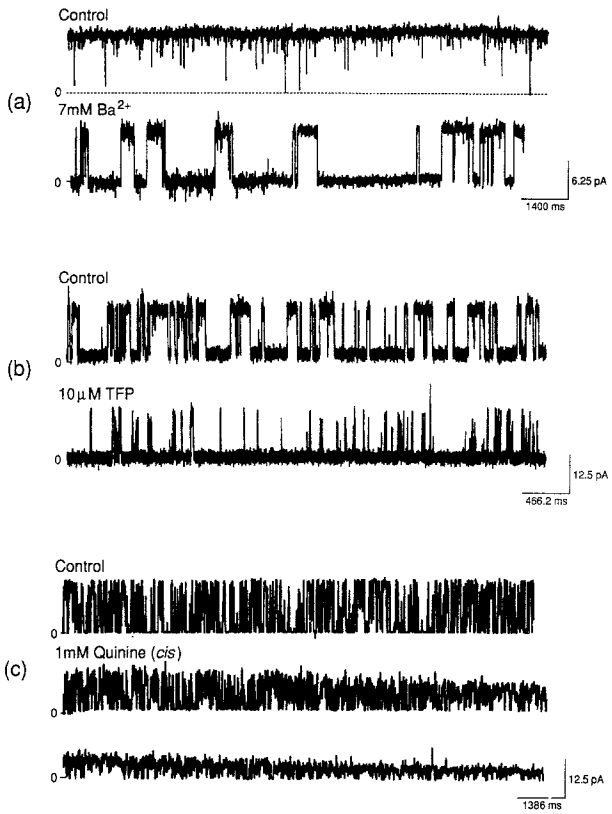

Fig. 6. Effects of (*a*) Ba^{2+} (*trans*) and (*b*) TFP (*cis*) on channel activity when $V_m = 0$; note that inhibition is the result of a decrease in P_o with no effect on I_K. (*c*) Effect of quinine on channel activity when $V_m = 0$; note that this agent brings about a rapid and marked decrease in I_K

Further, the blocking effectiveness of Ba^{2+} was markedly increased by decreasing $(K)_c$. These characteristics of Ba^+ inhibition have been reported for a number of K^+ channels and are consistent with the notion that Ba^{2+} is a slowly reversible blocker that acts by occluding the pathway for K^+ diffusion through the channel (Vergara & Latorre, 1983; Miller, Latorre & Reisin, 1987).

As illustrated in Fig. 6, channel activity was also inhibited by the addition of trifluoperazine (TFP) (10 μM) to the *cis* compartment ($n = 9$) and by the addition of quinine (0.5–1 mM) to either the *cis* ($n = 2$) or *trans* ($n = 2$) compartments. In the case of TFP, inhibition was the result of a decrease in P_o with no effect on I_K, whereas quinine resulted in a marked decrease in I_K. These findings are consistent with the notion that, unlike Ba^{2+} and TFP, the effect of quinine mimics that of a rapidly reversible "inhibitor" of the channel (Hille, 1984).

Discussion

These results conclusively demonstrate the presence of a "maxi" K(Ca), or BK(Ca), channel in the membranes of rabbit villus colonocytes. The presumption that this channel originates from the basolateral membrane of these epithelial cells is strongly supported by the fact that the membrane preparation incorporated into the planar lipid bilayers is highly enriched in ($Na^+ + K^+$)-ATPase and is minimally contaminated by enzyme markers for apical or intracellular membranes (Wiener et al., 1989). Further, in view of the consistent properties exhibited by these reconstituted channels it seems highly unlikely that they originate from accidental, minor contaminants of the membrane preparation. Nonetheless, patch-clamp studies of the K^+ channels in the basolateral membranes of isolated rabbit colonocytes will be necessary to definitively substantiate this presumption.

Comparison of the Properties of these Channels with those of other K(Ca) Channels

The properties of the BK(Ca) channel described in this study closely resemble those of "maxi" K(Ca) channels found in other cell membranes with respect to: (i) single channel conductance; (ii) voltage gating; and (iii) block by Ba^{2+} and by LQV (Latorre & Miller, 1983; Latorre, 1986; Moczydlowski et al., 1988). Quinine is also an effective inhibitor of this channel's activity as is the case for other K(Ca) channels (Latorre & Miller, 1983) but the effect of this agent does not appear to be specific for K(Ca) channels (Findlay et al., 1985*b*). Finally, TFP, an established inhibitor of the interaction between Ca-calmodulin complexes and calmodulin acceptor

proteins (Weiss & Levin, 1978; Moore & Dedman, 1982), also markedly inhibits the activity of this channel as is the case for K(Ca) channels in erythrocytes (Yingst & Hoffman, 1984), fibroblasts (Okada et al., 1987) and cardiac sarcolemmal vesicles (Wen, Famulski & Carofoli, 1984). This finding is consistent with the notion that calmodulin [or other calcimedins (Dedman, 1986)] may mediate the interaction between cell Ca^{2+} and the K(Ca) channel as has been definitively established for K(Ca) channels in *Paramecium* (Hinrichesen et al., 1986), fibroblasts (Okada et al., 1987) and human erythrocytes (Pape & Kristensen, 1984). However, inasmuch as TFP appears to be a nonspecific inhibitor of hydrophobic interactions (Moore & Dedman, 1982), this suggestion awaits more definitive evidence.

High conductance ("maxi") K(Ca) channels have been identified, employing the patch-clamp technique, in the basolateral membranes of isolated rat (Morris et al., 1986), rabbit (Sepulveda & Mason, 1985) and *Necturus* (Sheppard et al., 1988*a*) small intestinal cells; and, pancreatic, parotid, lacrimal (Petersen & Maruyama, 1984) and tracheal (Welsh & McCann, 1985) secretory cells. They have also been found in the "apical membranes" of *Necturus* choroid plexus "secretory" cells; but, inasmuch as these "apical" membranes also possess the $(Na^+ + K^+)$-ATPase, they are analogous to the basolateral membranes of Na^+-absorbing epithelial cells (Christensen & Zeuthen, 1987; Brown, Loo & Wright, 1988).

The principal differences among these channels appear to reside in their different sensitivities to activation by intracellular Ca^{2+}. Thus, the K(Ca) channels in the basolateral membranes of pancreatic and parotid acinar, tracheal epithelial and isolated rat small intestinal cells are extremely sensitive to $(Ca)_c$ in the range between 10^{-8}–10^{-7} M. The channel described in the present study and that identified in the basolateral membrane of *Necturus* enterocytes (Sheppard et al., 1988*a*) have much lower sensitivities and are essentially inactive when $(Ca)_c < 1$ μM, particularly when $V_m < 0$ mV. The K(Ca) channel identified in the apical membrane of *Necturus* choroid plexus likewise displays very little activity when $(Ca)_c < 1$ μM (i.e., $P_o = 0.001$ when $V_m = 0$) and is not fully active even when $(Ca)_c$ is as high as 75 μM (Christensen & Zeuthen, 1987; Brown et al., 1988).

The reason(s) for this wide variability in sensitivity to Ca^{2+} among K(Ca) channels, despite marked similarities in many other respects, is(are) unknown. This variability could be due to intrinsic differences among the channels and/or the membranes into which they are inserted. It is also possible that the sensitivity to Ca^{2+} is influenced by intracellular regulators, loosely attached membrane components, etc., that are lost (perhaps to variable extents) when the membrane patch is excised or, as in the present studies, when basolateral membrane vesicles are isolated (e.g., calmodulin). In this respect, it may be of interest that we observed considerable variability in Ca^{2+} sensitivity over the range of 1–10 μM among different vesicle preparations, within the same vesicle preparation and, sometimes, in a single channel during the course of an experiment. Moczydlowski and Latorre (1983) have reported similar experiences with BK(Ca) channels from rat muscle transverse tubule membranes reconstituted into planar lipid bilayers. The identification of the reasons for this variability could provide important insight into the factors that regulate these channels.

Speculation on the Physiological Function of these Channels

The intracellular Ca^{2+} activity in rabbit distal colonic epithelial cells is estimated to be between 50–100 nM (Potter, Tran & Sellin, 1989) and the electrical potential difference across the basolateral membrane is approximately 40 mV, cell interior negative with respect to the serosal bathing solution (Schultz, Frizzell & Nellans, 1977). Thus, if the properties of the BK(Ca) channel described in this study approximate those in the basolateral membranes of the intact cell, this channel would be inactive under physiological conditions. Sheppard et al. (1988*a*) arrived at the same conclusion for the case of BK(Ca) channels in the basolateral membrane of isolated *Necturus* enterocytes as did Christensen and Zeuthen (1987) for the BK(Ca) channel in the apical membrane of *Necturus* choroid plexus.

What, then, is the physiological significance of these basolateral membrane K(Ca) channels? While the answer to this question is by no means clear, there are several reports that suggest a possible role of these channels in cell volume regulation. Thus, Sheppard, Giraldez and Sepúlveda (1988*b*) have reported that the addition of L-alanine to the solution bathing isolated *Necturus* enterocytes results in the activation of normally quiescent K(Ca) channels in the basolateral membranes of these cells, and a similar finding has been reported by Bear and Petersen (1987) for isolated hepatocytes. Previous studies from this laboratory have demonstrated that sugar or amino acid transport by *Necturus* small intestine is associated with an increase in the K^+ conductance of the basolateral membrane (Gunter-Smith, Grasset & Schultz, 1982; Grasset, Gunter-Smith & Schultz, 1983) and evidence has been presented that

this may be a response to cell swelling resulting from the intracellular accumulation of these solutes in osmotically active forms (Lau, Hudson & Schultz, 1984, 1986). In addition, Na^+-coupled alanine uptake by isolated hepatocytes is accompanied by an increase in membrane K^+ permeability which , likewise, has been attributed to an increase in cell volume (Kristensen, 1986). Dawson and his coworkers (Dawson, 1987; Germann, Ernst & Dawson, 1986; Chang & Dawson, 1988; Dawson, van Driessche & Helman, 1988) have presented evidence for a Ca^{2+}-activated K^+ channel in the basolateral membrane of turtle colon that is inhibited by quinidine; this channel is distinct from the K^+ channel that is "normally" responsible for the "resting" conductance of that membrane but is activated in response to cell swelling.

Wong and Chase (1986) have reported an increase in $(Ca)_c$ in isolated toad urinary bladder cells in response to swelling. Further, Christensen (1987) has identified Ca^{2+} channels in the apical membrane of *Necturus* choroid plexus that are "normally" closed but are activated by membrane stretch; the activation of these channels results in an influx of Ca^{2+} which, in turn, activates nearby BK(Ca) channels that are also "normally" closed. Christensen has suggested that this is the mechanism responsible for volume regulation in response to cell swelling. It should be noted that while the values of $(Ca)_c$ needed to activated the K(Ca) channels described in this study and those in *Necturus* enterocytes (Sheppard et al., 1988*a*) and choroid plexus (Christensen & Zeuthen, 1987; Brown et al., 1988) are high, it is not inconceivable that such concentrations could be achieved in the unstirred layer adjacent to the inner surface of the basolateral membrane in response to stretch-activation of Ca^{2+} channels in that barrier.

Thus, it is quite possible that the BK(Ca) channels in the basolateral membranes of rabbit colonocytes, while not active under normal conditions, are activated in response to cell swelling. Studies on intact cells will be needed to test this notion.

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