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SELECTIVE BLOCK OF SPECIFIC K^+-CONDUCTING CHANNELS BY DIPHENYLAMINE-2-CARBOXYLATE IN TURTLE COLON EPITHELIAL CELLS

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SUMMARY

1. The conduction and gating properties of K^+-conducting channels were studied in isolated turtle colon cells in an attempt to identify the single channels responsible for specific components of the macroscopic conductance of the basolateral membrane. Three types of Ca^{2+}-activated channel were identified, two of which were selective for K^+ over Na^+ and a third which was selective for monovalent cations over anions, but did not discriminate between K^+ and Na^+.

2. One of the K^+-selective channels was a large-conductance 'maxi' K^+ channel. A second was characterized by a lower conductance and pronounced inward rectification.

3. The inward-rectifying K^+ channel was selectively blocked by diphenylamine-2-carboxylate (DPC). Neither the maxi K^+ channel nor a previously identified K^+-selective channel thought to be activated by cell swelling was affected by this compound. DPC also blocked the non-selective cation channel.

4. An inward-rectifying, DPC-sensitive current was prominent in whole cell-recordings, and DPC blocked basolateral K^+ currents in colonic cell layers apically permeabilized with amphotericin-B. In addition, the compound blocked active Na^+ absorption.

5. The selective block of a class of epithelial K^+ channels by DPC may be a useful tool for determining the contribution of this specific subpopulation to macroscopic conductance and transepithelial salt transport.

INTRODUCTION

Channel-specific blockers are important tools for evaluating the contribution of specific ion channel populations to the macroscopic transport properties of an epithelium. Unfortunately, the channels which inhabit the membranes of epithelial cells are not the targets of highly specific toxins such as those which selectively block a variety of voltage-dependent channels in excitable membranes. Diphenylamine-2-carboxylate (DPC) first gained attention as a Cl^- channel blocker in shark rectal gland and rabbit thick ascending limb (Di Stefano, Withner, Schlatter, Lang, Englert & Greger, 1985), but DPC blocks other types of ion channels as well. In pancreatic acinar cells (Gögelein & Pfannmüller, 1989) and rabbit proximal tubule

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cells (Gögelein & Greger, 1986), DPC blocks a class of non-selective cation channels. In the turtle colon, a model NaCl-absorbing epithelium, DPC blocks a basolateral K^+ conductance as well as a basolateral Cl^- conductance in epithelial sheets apically permeabilized with digitonin (Chang & Dawson, 1988).

We investigated the specificity of K^+ channel block by DPC in isolated turtle colon epithelial cells. The membranes of these cells contain three classes of K^+-selective channels, and DPC selectively blocked one of these as well as a major part of the whole-cell K^+ conductance. In addition, DPC selectively blocked a specific component of the basolateral K^+ conductance in sheets of turtle colon apically permeabilized with amphotericin-B and also blocked active Na^+ transport in the intact epithelium. The selectivity of DPC block may be useful in evaluating the contribution of specific DPC-sensitive, K^+-conducting channels to the overall process of transepithelial salt transport.

METHODS

Cell isolation

Colons were removed from doubly pithed freshwater turtles (*Pseudemys scripta*) and the outer muscle layers dissected away. Colons were then everted, distended and incubated in a Ca^{2+}-free solution containing 56 mM NaCl, 56 mM KCl, 2·5 mM $KHCO_3$, 10 mM glucose, 10 mM Hepes and 10 mM ethylenediamine tetraacetate (EDTA); pH 7·4. After 10 min, epithelial cells were dislodged by rapid shaking. Cells in suspension were pelleted by centrifugation and washed in isolation medium containing 1 mM $CaCl_2$ without EDTA. The final suspension included 1 mg/ml DNase (Sigma) to reduce clumping of cells. All solutions were used at room temperature.

Patch-clamp procedure

Details of methods for single-channel recording have been described elsewhere (Richards & Dawson, 1986) and apply here with a few exceptions. Briefly, isolated cells were placed in a 1–2 ml chamber on the stage of a Nikon Diaphot inverted microscope equipped with Nomarski optics and observed at 600×. High resistance seals were made to cell membranes according to Hamill, Marty, Neher, Sakmann & Sigworth (1981) using pipettes fabricated from Corning No. 8161 or Kimble No. R-6 glass. The output of an Axopatch 1-A or 1-B patch clamp amplifier (Axon Instruments, Burlingame, CA, USA) was digitized and stored on videocassette tape for later analysis (Bezanilla, 1985). Mean current and fractional open time were calculated as previously described (Richards & Dawson, 1986). All procedures were performed at room temperature.

Solutions

Solutions used included NaCl-Ringer solution (112 mM NaCl, 2·5 mM $KHCO_3$, 10 mM Hepes, 10 mM glucose, 1 mM $CaCl_2$; pH 7·4) and KCl-Ringer solution, in which KCl replaced NaCl. In some experiments, Ca^{2+}-buffered solutions were used which contained 112 mM KCl, 2·5 mM EGTA, 10–20 mM Pipes (piperazine-*N*-*N*'-bis(2-ethanesulphonic acid)); pH 7·0. Sufficient $CaCl_2$ was added to yield calculated free Ca^{2+} concentrations of 0·1–100 μM as described by Chang, Hsieh & Dawson (1988). A similar solution containing potassium aspartate was used in the pipette for whole-cell recordings. Diphenylamine-2-carboxylate (DPC; also known as *N*-phenylanthranilic acid) was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). A DPC stock solution was prepared by dissolving DPC in dimethyl sulphoxide (DMSO). The final DMSO content in Ringer solution did not exceed 0·4% (v/v).

RESULTS

Channel types in colonic cells

A 17 pS, K^+-selective channel thought to be activated by cell swelling was previously identified in isolated turtle colon epithelial cells (Richards & Dawson, 1986). We report here the identification of three additional classes of single K^+-

conducting channels. Two of these channels were selective for K^+ over Na^+. One was characterized by a very short open time and displayed pronounced inward rectification. The other possessed characteristics which identified it as a large-conductance, 'maxi' K^+ channel (Latorre & Miller, 1983). The third channel was

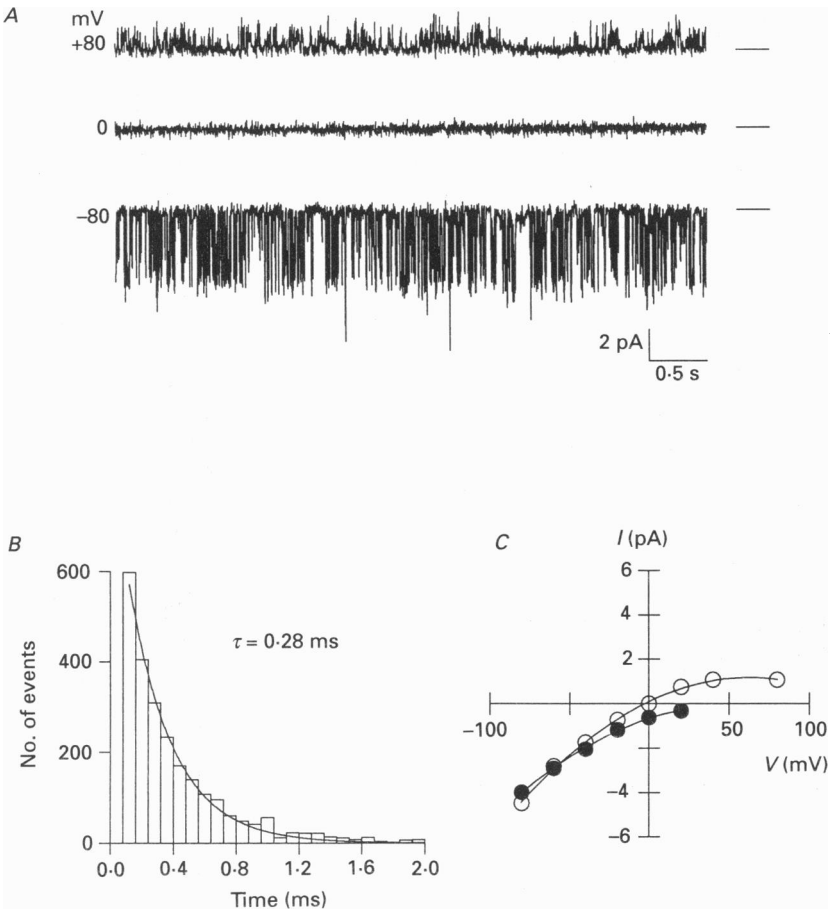

Fig. 1. *A*, inward-rectifying K^+ channel currents in turtle colon cell membranes. Traces are from an inside-out patch bathed on both sides with KCl-Ringer solution. The closed current level is indicated to the right of each trace. Holding potential is expressed as the potential of the cell interior so that −80 mV, for example, drives inward current. *B*, open duration histogram constructed using an inside-out patch with a KCl–EGTA–1 μM Ca^{2+} solution in the pipette and KCl-Ringer solution in the bath at a holding potential of −60 mV. The current trace was digitized at 25 kHz and filtered at 5 kHz for this analysis. The histogram was adequately described by a single exponential with a mean open time of 0·28 ms. *C*, current–voltage relations for the channel shown in *A*. ○, the current–voltage relation when the patch was bathed with symmetric KCl-Ringer solution. Imposing an inwardly directed K^+ gradient by changing the bath to NaCl-Ringer solution shifted the zero current potential to a more positive value (●) and reduced the slope conductance near 0 mV from 35 to 15 pS.

selective for monovalent cations over anions but did not discriminate between Na^+ and K^+.

Inward-rectifying K^+ channel

Single-channel currents recorded in an inside-out patch bathed with symmetric KCl-Ringer solutions are shown in Fig. 1*A*, which illustrates two prominent

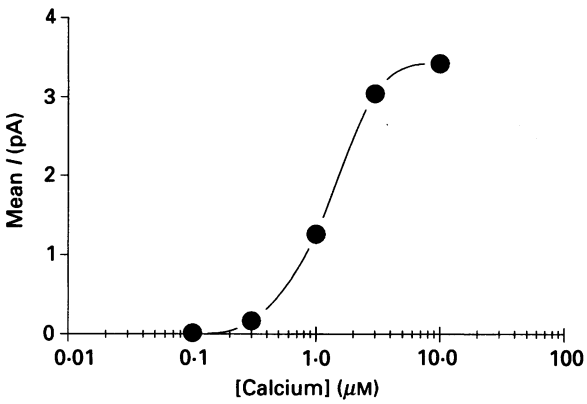

Fig. 2. Calcium activation of the inward-rectifying K^+ channel. Mean current was calculated using an inside-out patch containing several inward-rectifying K^+ channels. The pipette contained a KCl–EGTA–1 μM Ca^{2+} solution and the bath was a similar solution with varying free Ca^{2+} concentrations. The holding potential was −60 mV. Increasing the free Ca^{2+} concentration in the range of 0·3–10 μM at the cytoplasmic surface of the membrane caused a progressive increase in the mean current across the patch.

characteristics displayed by this channel type. First, the open durations were quite short (less than 1 ms; Fig. 1*B*) imparting a characteristic ragged appearance to the current record due to the incomplete resolution of channel openings. Second, the channel displayed pronounced inward rectification, i.e. the amplitudes of inward currents were greater than outward current amplitudes under conditions of equal driving force. In the presence of symmetric high-KCl solutions, currents reversed at 0 mV (Fig. 1*C*). The slope conductance in the vicinity of −60 mV was approximately 70 pS while the conductance near 0 mV was 35 pS. Imposing an inwardly directed K^+ gradient by changing the bath solution to NaCl-Ringer solution shifted the reversal potential in the positive direction, indicating that the channel is selective for K^+ over Na^+. Although an accurate determination of the reversal potential could not be made due to the noisy character and small amplitudes of the single-channel currents, the reversal potential was greater than 20 mV in this case and the conductance near 0 mV was approximately 15 pS. This channel type was observed in approximately 25% of patches. The typical patch contained between one and three channels.

The inward-rectifying K^+ channel was activated by Ca^{2+} at the cytoplasmic face of the membrane. Figure 2 shows a Ca^{2+}-activation curve obtained from an inside-out patch which contained several inward-rectifying K^+ channels. The mean current across the patch was essentially zero when the inside membrane was bathed with a solution containing 0·1 μM Ca^{2+} but increased progressively as the Ca^{2+} was

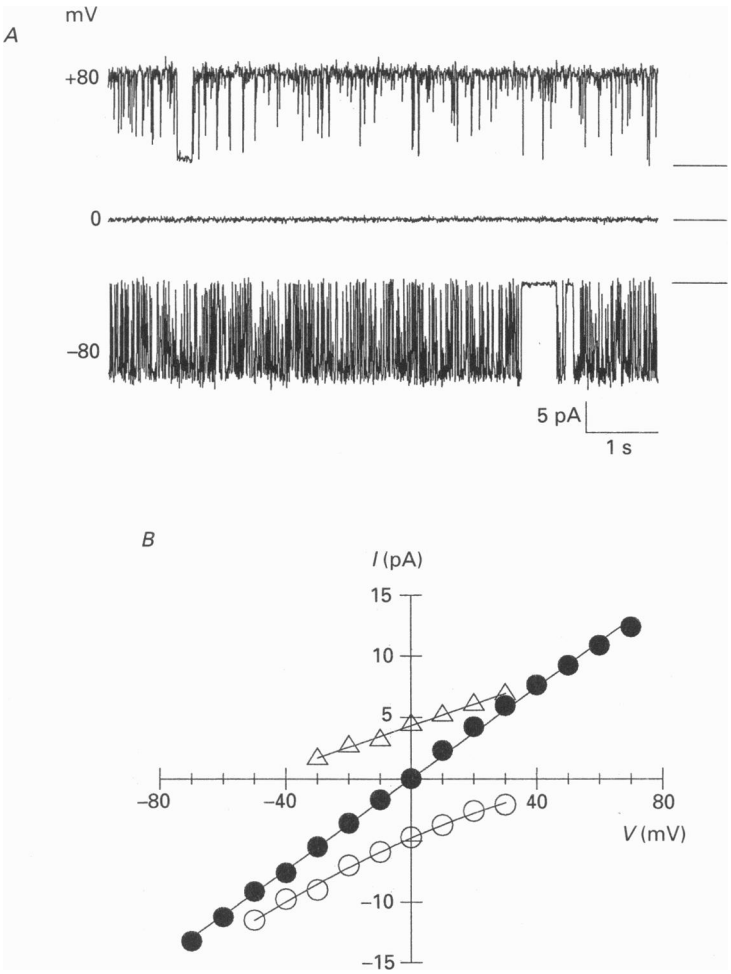

Fig. 3. *A*, large-conductance K^+ channel currents recorded in turtle colon epithelial cell membranes. Traces are from an inside-out patch with KCl-Ringer solution in both the pipette and bath. The closed current level is indicated to the right of each trace. The holding potential is expressed as the potential of the cell interior. *B*, the figure shows representative current–voltage relations of the large-conductance K^+ channel. In inside-out patches bathed with symmetric KCl-Ringer solutions (●), currents reversed near 0 mV and had a slope conductance of 188 ± 5 pS ($n = 3$). In inside-out patches with NaCl-Ringer solution in the pipette and KCl-Ringer solution in the bath (△), the extrapolated reversal potential for outward current was approximately −50 mV and slope conductance was 85 ± 5 pS ($n = 3$). In outside-out patches with identical solutions (○), inward currents had a slope conductance of 94 ± 13 pS ($n = 3$) and the extrapolated reversal potential was between +40 and +50 mV.

increased in the range 0·3–10 μM. A similar activation by Ca^{2+} was observed in three other patches.

Large-conductance K^+ channel

Figure 3*A* shows single-channel currents recorded from a detached, inside-out patch bathed by symmetric KCl-Ringer solutions and containing a single, large-conductance K^+ channel. Currents exhibited a characteristic gating, with large-amplitude openings interrupted by numerous brief flickery closures and by less frequent closures of longer duration. Flickery behaviour was typically more prominent when the channel conducted inward current (i.e. movement of K^+ from pipette to bath) than when current was outward.

Current–voltage relations for the channel are shown in Fig. 3*B*. Currents recorded from inside-out patches bathed with symmetric KCl-Ringer solutions reversed near 0 mV and had a slope conductance of 188 pS. No obvious rectification was observed over the voltage range tested. Imposing a K^+ gradient across the patch shifted the reversal potential as expected for a K^+-selective channel.

The large-conductance K^+ channel was not frequently observed in isolated turtle colon epithelial cell membranes. On the rare occasions in which this channel was found, it tended to occur in clusters (i.e. more than one channel per patch). The channel was activated by Ca^{2+} at the cytoplasmic face of the membrane and by depolarizing the membrane potential (not shown), characteristics which identify it as a member of the class of 'maxi' K^+ channels (Latorre & Miller, 1983).

Non-selective cation channel

Figure 4*A* shows typical current records obtained from an inside-out patch which contained several cation channels. Currents reversed near 0 mV in the presence of opposing Na^+ and K^+ gradients (NaCl-Ringer solution in the pipette, KCl-Ringer solution in the bath). The current–voltage relation was linear under these conditions and the slope conductance was approximately 30 pS (Fig. 4*B*). Isosmotic replacement of KCl in the bath with choline chloride abolished outward current and shifted the zero current potential to a more positive value, consistent with cation flow. A similar result obtained if bath KCl was replaced isosmotically with sucrose; however, replacement of Cl^- with aspartate or gluconate did not significantly alter outward current or reversal potential. The channel therefore appeared to be selective for monovalent cations over anions but did not discriminate between Na^+ and K^+.

The majority of detached patches contained one or more of these channels, though the channels tended to inactivate within minutes after patch excision, particularly in inside-out patches. It was not uncommon for a single patch to contain so many cation channels that the current record appeared as noise. The gating of the cation channels was slow compared to the inward-rectifying K^+ channels. Openings were clearly resolved and exhibited little or no open channel noise. The gating of the channels was voltage dependent such that the open durations tended to be much longer at depolarized potentials, often lasting up to several seconds before closing.

Non-selective cation channels were not typically observed in cell-attached patches. The sudden appearance of these channels in inside-out patches immediately after patch excision into a Ca^{2+}-containing medium was the first indication that the

opening of non-selective cation channels depended on the concentration of Ca^{2+} at the cytoplasmic membrane surface. Further studies revealed that Ca^{2+} was indeed required for channel opening; however, the Ca^{2+} dependence of the channel was not straightforward, particularly in inside-out patches. Channel openings were readily

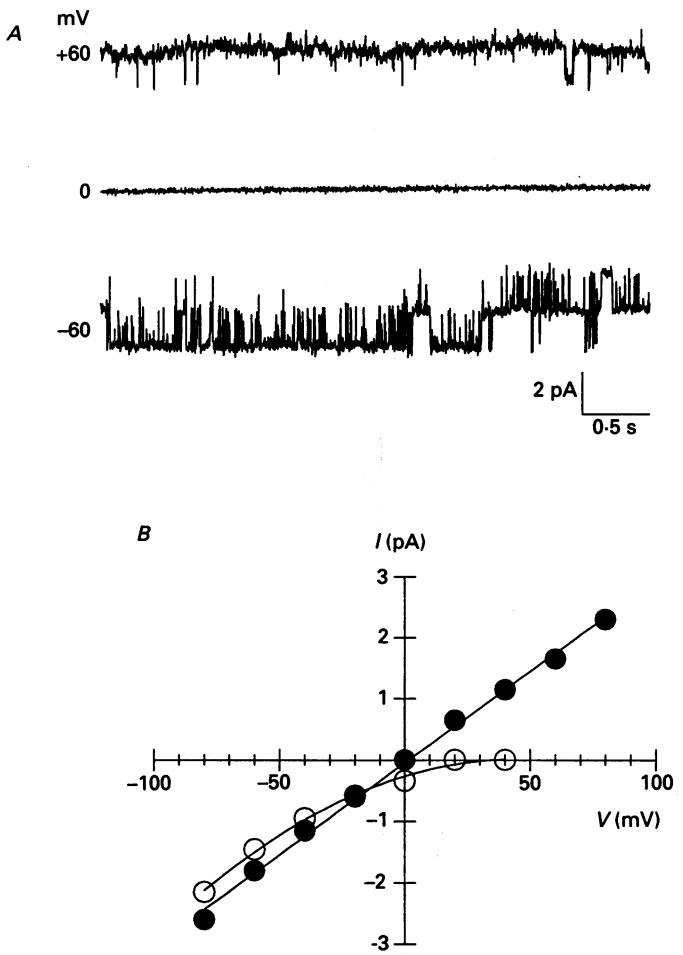

Fig. 4. *A*, non-selective cation channel currents in turtle colon cell membranes. Traces were recorded from an inside-out patch with NaCl-Ringer solution in the pipette and KCl-Ringer solution in the bath. The holding potential is expressed as the potential at the cell interior. This patch contained multiple channels so a clear closed current level cannot be defined. *B*, current–voltage relation of single-channel currents in *A* (●). Slope conductance was approximately 30 pS and currents reversed near 0 mV. Replacement of KCl in the bath with choline chloride shifted the reversal potential to a more positive value (○).

observed in inside-out patches when the bath was a Ringer solution containing 1 mM $CaCl_2$. Subsequent replacement of the bath with Ca^{2+}-free solution (with or without EGTA) always caused the channel to immediately close, and this effect was readily reversible by replacing the bath with standard Ringer solution. However,

activation of the channels by precisely defined EGTA-buffered solutions containing free Ca^{2+} concentrations up to 100 μM could not be consistently demonstrated. Cation channels could be consistently recorded, however, in outside-out patches if the pipette contained an EGTA-buffered solution with 1 μM free Ca^{2+}. The reason(s) for this discrepancy are unclear.

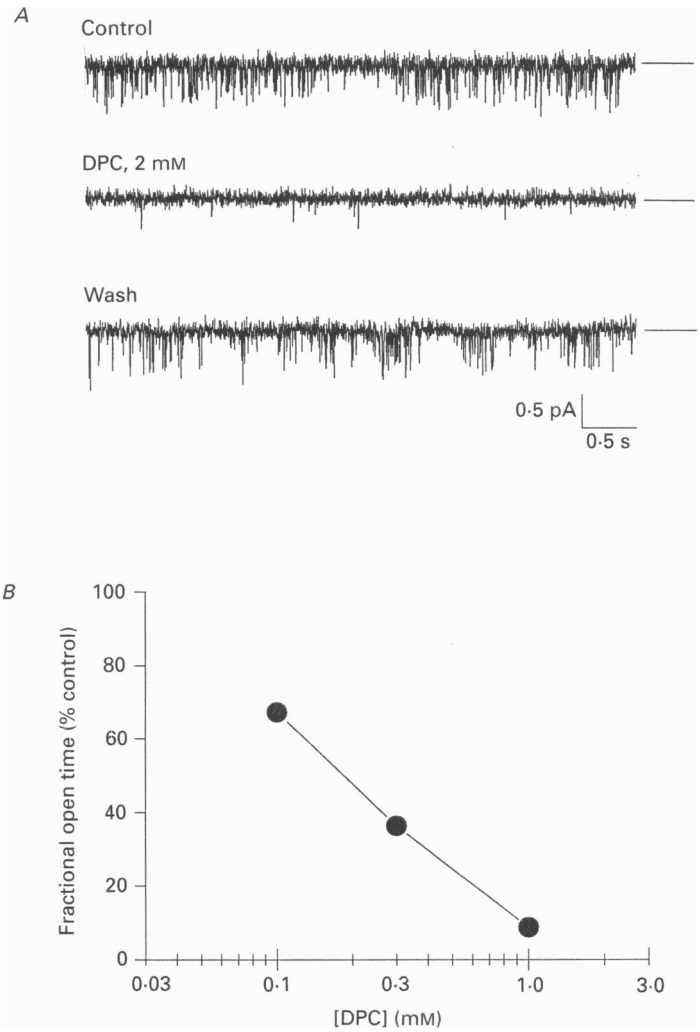

Fig. 5. *A*, effect of DPC on the inward-rectifying K^+ channel. Traces show inward currents recorded from an inside-out patch with KCl-Ringer solution in the pipette and NaCl-Ringer solution in the bath at a holding potential of 0 mV. The closed current level is indicated to the right of each trace. The top trace shows inward currents in the absence of drug. The second trace shows the virtual abolition of channel openings by the addition of 2 mM DPC to the bath solution. The final trace shows the reversibility of the DPC effect. *B*, dose–response curve for DPC inhibition of inward rectifying K^+ channel. Fractional open time was calculated using an inside-out patch with KCl–EGTA–1 μM Ca^{2+} in the pipette and KCl-Ringer solution in the bath at a holding potential of −60 mV. Current records were digitized at 25 kHz and filtered at 5 kHz for this analysis.

Effects of DPC and other blockers on single channels

Inward-rectifying K^+ channel

Figure 5*A* shows current traces taken from an inside-out patch which contained a single, inward-rectifying K^+ channel. The upper trace shows inward current at

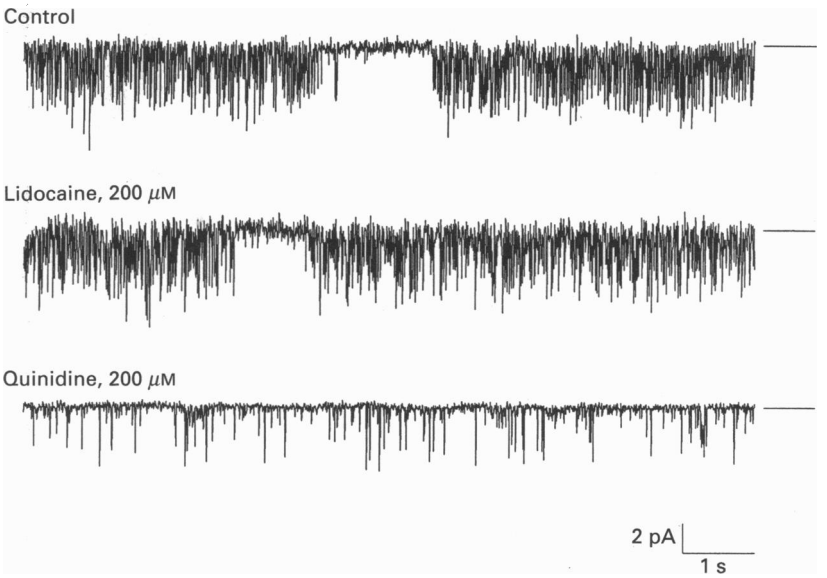

Fig. 6. Effect of lidocaine and quinidine on the inward-rectifying K^+ channel. Traces show inward currents recorded from an outside-out patch with KCl in the bath and a potassium aspartate–EGTA–1 μM Ca^{2+} solution in the pipette at a holding potential of −80 mV. The closed current level is indicated to the right of each trace. Adding 200 μM lidocaine to the bath had no obvious effect on the chamber but the subsequent addition of 200 μM quinidine caused a profound block, reducing fractional open time in this case from 0·33 to 0·04.

0 mV in the presence of an inwardly directed K^+ gradient. Addition of 2 mM DPC to the solution bathing the cytoplasmic surface of the channel resulted in an almost complete cessation of channel openings, and the block was readily reversed by washing DPC out of the bath. Figure 5*B* shows a dose–response curve obtained from a second patch, from which an IC_{50} of approximately 200 μM was estimated.

Quinidine and lidocaine were also tested on the inward-rectifying K^+ channel as these agents selectively block one type of macroscopic K^+ conductance in the turtle colon (Germann, Ernst & Dawson, 1986*a*; Germann, Lowy, Ernst & Dawson, 1986*b*) and also block the single channels thought to be responsible for that conductance (Richards & Dawson, 1986). As shown in Fig. 6, lidocaine had no obvious effect while quinidine caused a profound block of the channel. Quinidine block of the inward-rectifying K^+ channel appeared to be of the 'slow' variety, in that the drug induced clearly distinguishable blocked intervals in the current record without appreciably reducing current amplitude.

The channel was also blocked by 5 mM Ba^{2+} at the extracellular face of the channel (not shown).

Maxi K^+ channel

Figure 7 shows inward current recorded in an inside-out patch containing a single, large-conductance K^+ channel before and after adding 1 mM DPC to the solution

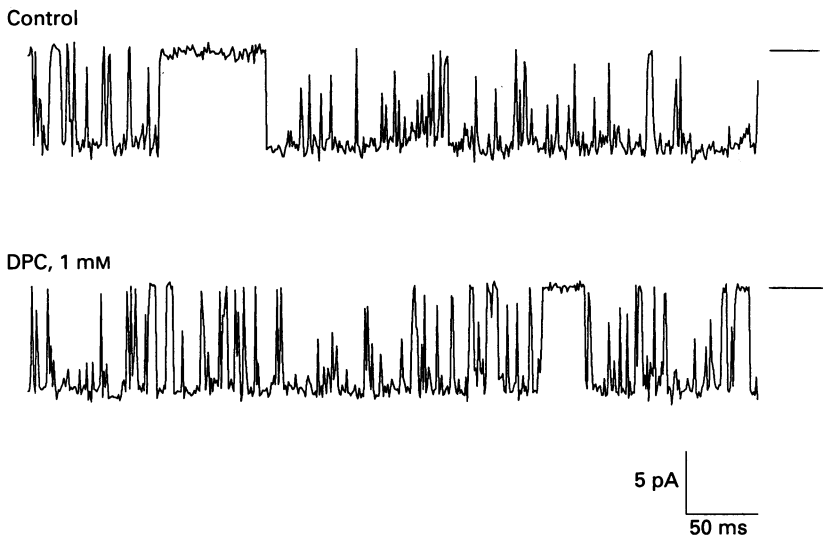

Fig. 7. Effect of DPC on the large-conductance K^+ channel. The top trace shows normal inward currents recorded in an inside-out patch bathed with symmetric KCl-Ringer solutions at a holding potential of −60 mV. The addition of 1 mM DPC to the bath solution had no effect on the channel. The closed current level is indicated to the right of each trace.

bathing the cytoplasmic surface of the patch. DPC had no effect on the gating or conductance of the channel.

Lidocaine did not affect the large-conductance K^+ channel (Fig. 8), but quinidine produced a 'fast', amplitude-reducing block. The channel was also completely blocked by 2 mM TEA at the extracellular face of the membrane (not shown).

Non-selective cation channel

The non-selective cation channel was reversibly blocked by DPC. Figure 9 shows the effect of adding 200 μM DPC to the solution bathing the cytoplasmic membrane of an inside-out patch. DPC did not produce a 'fast' block of the cation channels, i.e. it did not induce a noticeable flicker in the single-channel record. The total time that the channels spent in the open state was obviously greatly reduced, but when the channels did open, apparently normal gating kinetics were observed.

It was noted that DPC blocked the channels almost immediately after being added to the bath in outside-out patches, while block of cation channels in inside-out

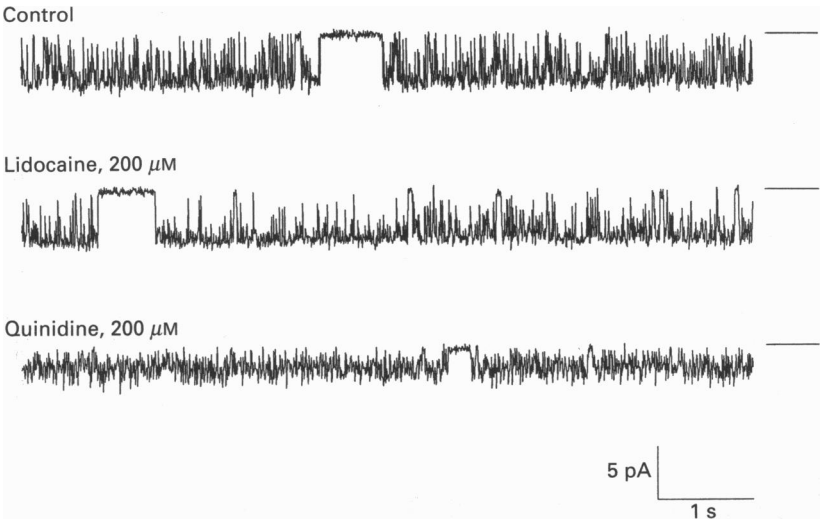

Fig. 8. Effect of lidocaine and quinidine on the large-conductance K^+ channel. Traces show inward currents recorded from an outside-out patch with KCl-Ringer solution in the bath and NaCl-Ringer solution in the pipette at a holding potential of 0 mV. Lidocaine (200 μM) had no effect but quinidine (200 μM) caused a fast, amplitude-reducing block of the channel. The closed current level is indicated to the right of each trace.

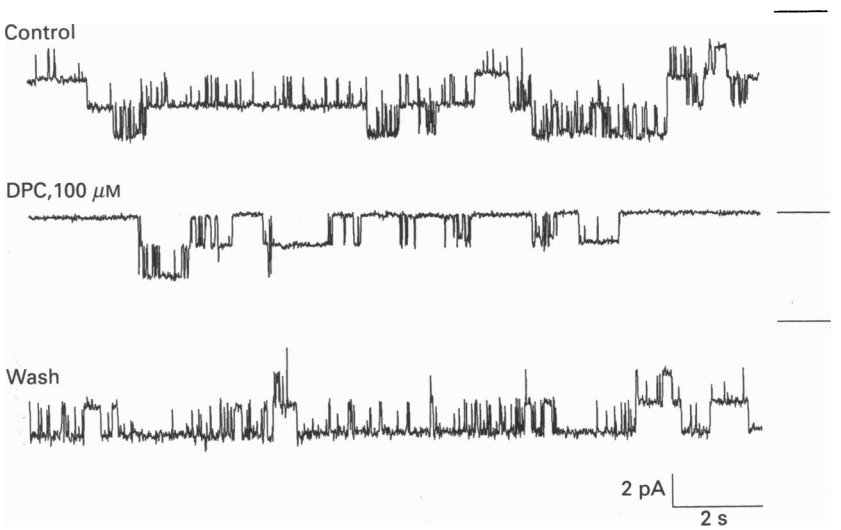

Fig. 9. Block of the non-selective cation channel by DPC. Traces show inward currents recorded from an inside-out patch with KCl-Ringer solution in the pipette and NaCl-Ringer in the bath at a holding potential of −60 mV. This patch contained at least four channels (top trace). Addition of 100 μM DPC to the bath solution inhibited channel activity (middle trace) and this effect was readily reversible (lower trace). The closed current level is indicated to the right of each trace.

patches was more gradual and sometimes less complete. Such a difference in the rate of block may indicate that the blocking site is on the extracellular face of the channel. The hydrophobic character of DPC suggests that it would be able to cross cell

membranes rather easily, and this may explain the slower but still significant block in inside-out patches. In both inside-out and outside-out patches, 1–2 mM DPC was required for complete block.

The cation channel was also completely blocked by Mn^{2+} or Ni^{2+} (1–10 mM) but only at the extracellular face of the membrane (not shown). Similar concentrations

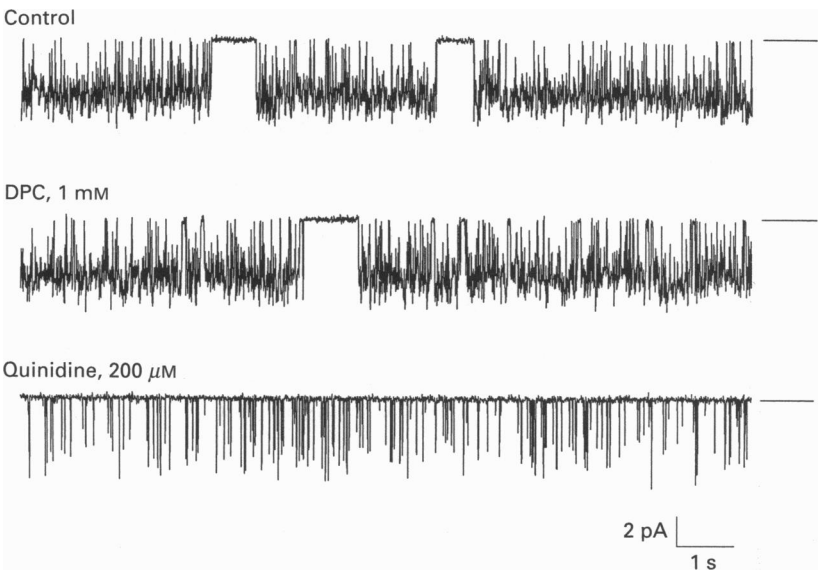

Fig. 10. Effect on DPC on the swelling-activated K^+ channel. The top trace shows normal inward currents recorded from a cell-attached patch bathed with symmetric KCl-Ringer solutions at a holding potential of −60 mV. The middle trace shows the lack of effect after adding 1 mM DPC to the bath solution. However, the swelling-activated channel was still characteristically blocked by 200 μM quinidine (lower trace). The closed current level is indicated to the right of each trace.

of these cations at the inside membrane did not block and in fact tended to further activate channels. Extracellular Ba^{2+} was ineffective.

Swelling-activated K^+ channel

Another K^+-selective channel, which is blocked by lidocaine and quinidine and thought to be the channel underlying a macroscopically defined, swelling-activated basolateral K^+ conductance in the turtle colon, was identified previously in the membranes of isolated turtle colon epithelial cells (Richards & Dawson, 1986). The channel, which has a blocker specificity and ion selectivity identical to the macroscopic conductance, is spontaneously active in isolated turtle colon epithelial cells bathed in a high-K^+ solution, possibly because the isolated cells are swollen due to the dissociation process and/or the recording conditions. The effect of DPC on this channel was also tested (Fig. 10). DPC had little if any effect on the K^+ channel, while quinidine produced its characteristic 'slow' block (Richards & Dawson, 1986).

Effects of DPC on macroscopic currents

Whole-cell K^+ currents

Whole-cell currents recorded with a potassium aspartate solution (1 μM Ca^{2+}) in the pipette and KCl-Ringer solution in the bath are shown in Fig. 11. Whole-cell

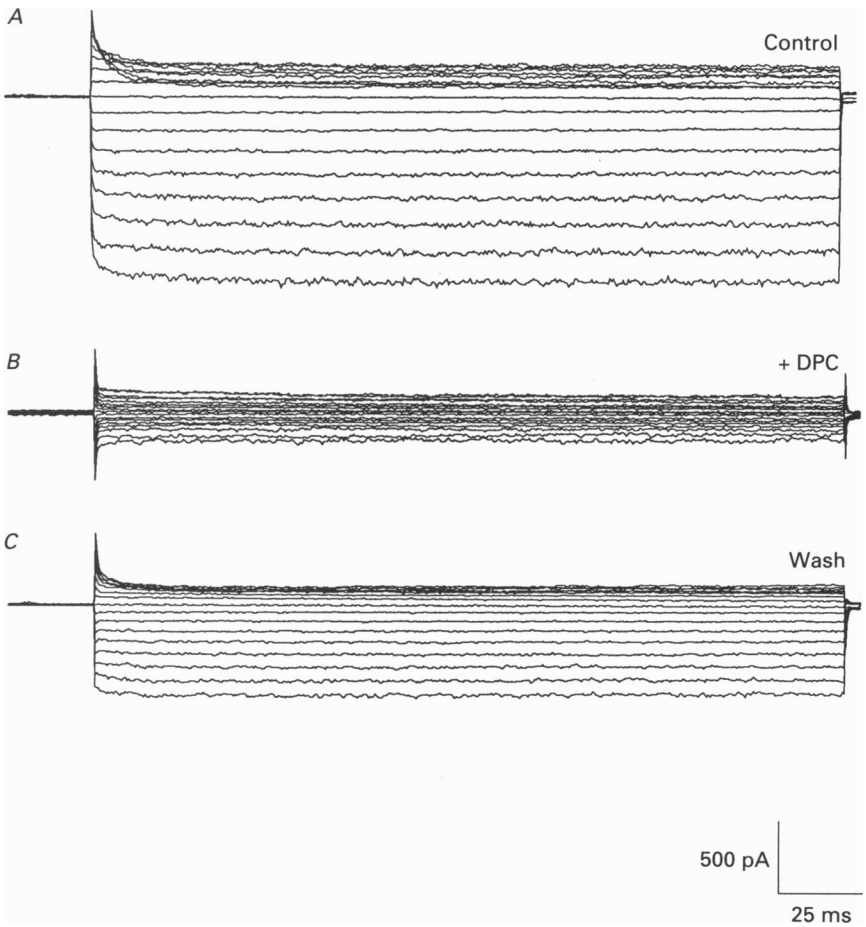

Fig. 11. Whole-cell currents recorded in isolated turtle colon cells. The pipette solution was 112 mM potassium aspartate buffered to 1 μM-free Ca^{2+} with EGTA. The bath solution was KCl-Ringer solution. The holding potential was 0 mV and voltage steps ranging from +80 to −80 mV were applied in 10 mV increments. Currents are shown before addition of DPC (*A*), after block by 1 mM DPC (*B*), and after washing the DPC from the bath (*C*).

currents under these conditions typically displayed two prominent characteristics. First, pronounced inward rectification was observed even though an outwardly directed K^+ gradient was present (152 mM *vs.* 112 mM). Second, in response to a voltage step, outward currents were transiently activated, then relaxed back to a plateau current value which decreased with increasing voltage.

The inward whole-cell currents were largely abolished by the addition of 1 mM DPC to the bath, an effect which was at least partially reversible. Quinidine (200 μM) also blocked inward whole-cell currents but lidocaine (200 μM) was ineffective (not shown).

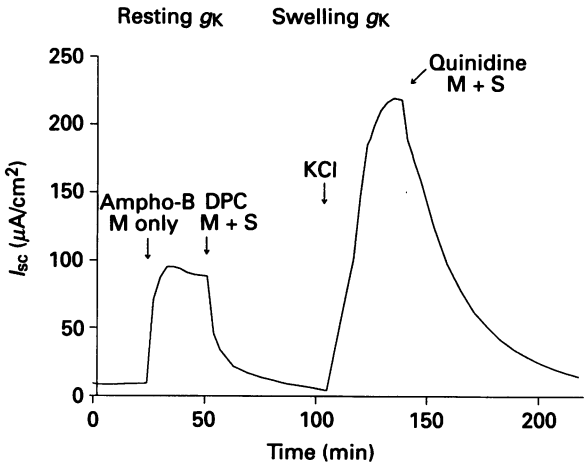

Fig. 12. Short-circuit current across a sheet of turtle colon epithelium bathed on the mucosal side (M) with potassium gluconate-Ringer solution and on the serosal side (S) with NaCl-Ringer solution. The addition of amphotericin (10 μM) to the apical surface results in the appearance of the 'resting' basolateral K^+ conductance (g_K), which is abolished by DPC (2 mM). The continued presence of DPC did not prevent the subsequent activation of the quinidine-sensitive swelling-activated K^+ conductance (induced by changing the apical solution to KCl).

Basolateral K^+ conductance

Germann *et al.* (1986*a*, *b*) identified two distinct K^+ conductance pathways in the basolateral membranes of amphotericin-permeabilized turtle colon epithelium. One, termed the resting K^+ conductance, was present under non-swelling conditions and a second was activated by cell swelling. We tested the effect of DPC on both conductances. Short-circuit current (I_{sc}) across a sheet of turtle colon epithelium was measured as indicated in Fig. 12. At time 0, the serosal bath was NaCl-Ringer solution and the mucosal bath was potassium gluconate-Ringer solution. I_{sc} is near 0 mV under these conditions due to the low K^+ conductance of the apical membranes. The subsequent addition of amphotericin-B to the mucosal solution permeabilizes the apical membranes to monovalent cations and results in a new I_{sc} representing K^+ flow across the basolateral membranes. These conditions do not result in cell swelling and the I_{sc} is defined as being due to the resting K^+ conductance (Germann *et al.* 1986*a*, *b*). The addition of 2 mM DPC to both sides of the cell layer rapidly abolished the resting K^+ conductance. Replacing potassium gluconate in the mucosal solution with KCl results in swelling of the epithelial cells, presumably due to the finite anion conductance of the amphotericin pores. As shown in the figure, this manoeuvre resulted in the appearance of another I_{sc} due to the swelling-activated K^+ conductance despite the continued presence of DPC. Therefore, DPC appears to be

a relatively selective blocker of the resting K^+ conductance. This result was consistently observed in seven tissues from four different animals.

Inhibition of the swelling-activated conductance by quinidine (200 μM), a characteristic blocker of this conductance (Germann *et al.* 1986*a*, *b*) is also shown in

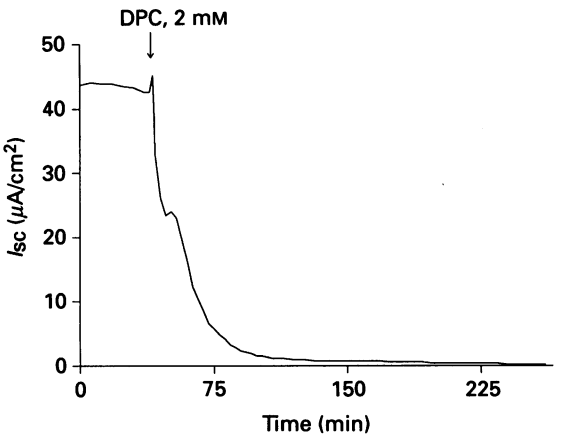

Fig. 13. Short-circuit current across a sheet of turtle colon bathed on both sides with NaCl-Ringer solution. The addition of 2 mM DPC to both sides of the cell layer resulted in a prompt and complete inhibition of short-circuit current, an indicator of active Na^+ transport under these conditions.

the figure. It is noted that the single channels thought to be responsible for that conductance are also blocked by quinidine but not by DPC (Fig. 10 and Richards & Dawson, 1986).

Active Na^+ transport

The resting K^+ conductance is believed to be intimately involved in the regulation of Na^+ transport in the turtle colon. It is this conductance which is thought to carry the outward K^+ current required to balance inward Na^+ current so as to maintain mass and charge balance. The effect of DPC on active Na^+ transport was therefore tested. Figure 13 shows the I_{sc} as a measure of active Na^+ transport across a sheet of turtle colon bathed on both sides with NaCl Ringer solution. Addition of 2 mM DPC to both sides of the cell layer resulted in a prompt and complete inhibition of I_{sc}. A similar block by DPC was observed in nine tissues from five different animals.

DISCUSSION

The available evidence strongly suggests that the basolateral membranes of epithelial cells are inhabited by multiple types of K^+ channels, and the differential expression of these channels under different experimental conditions suggests that each of the K^+ channel types plays a specific role in the life of the cell (Dawson, 1987; Dawson & Richards, 1990). Significant problems exist, however, in the process of identifying a particular type of K^+ channel with a specific transport modality. In

many cases, particularly in gastrointestinal epithelia, single channels cannot be studied in the intact polarized cell layer but must instead by assayed in isolated cells which may not retain their full complement of differentiated properties. In this situation it is essential to develop specific blockers or agonists which can be used to implicate particular channels in an integrated setting.

TABLE 1. Blocker profiles of single channels in turtle colon epithelial cells

	Inward rectifier	Maxi K^+	Swelling activated	Cation
DPC	Yes	No	No	Yes
Quinidine	Yes	Yes	Yes	Yes
Lidocaine	No	No	Yes	No
Ba^{2+}	Yes	Yes	Yes	No

Although the goal of developing a useful pharmacology of epithelial K^+ channels is simple in principle, in practice significant difficulties have been encountered in identifying compounds which recognize specific channel types. It may be unrealistic to expect that we will discover probes which will be generally useful across species of animals. A more limited, but nevertheless useful, goal would be to develop a K^+ channel pharmacology for a single epithelial cell type.

In previous studies, at least three types of K^+ conductance have been identified in the basolateral membranes of turtle colon by using permeabilizing techniques which permit the conductive properties of the basolateral membrane to be assayed while the cell layer retains its structural integrity. In the amphotericin-permeabilized colon, one K^+ conductance was activated by cell swelling, was blocked by quinidine or lidocaine and was highly selective for K^+ over Rb^+ (Germann *et al.* 1986*a*, *b*). Channels exhibiting these properties were also identified in isolated cells (Richards & Dawson, 1986). In the absence of cell swelling, the basolateral K^+ conductance was virtually insensitive to quinidine or lidocaine, did not discriminate appreciably between K^+ and Rb^+ and was inactivated by cholinergic agonists (Germann *et al.* 1986*a*, *b*; Venglarik & Dawson, 1986). Because active Na^+ absorption is also unaffected by the substitution of K^+ with Rb^+, this K^+ conductance was referred to as the 'resting' conductance of the cell.

The initial response to carbachol or the calcium ionophore A23187 is a transient activation of the basolateral K^+ conductance. This observation led Venglarik & Dawson (1986) to speculate that the transient activation could be due to a population of Ca^{2+}-activated channels. Direct evidence for a population of Ca^{2+}-activated K^+ channels was obtained by Chang & Dawson (1988), using cell layers apically permeabilized with digitonin to permit clamping of cytosolic pH and free Ca^{2+}. This Ca^{2+}-activated basolateral K^+ conductance was blocked by quinidine and also by the anthranilic acid derivative, DPC. In the present study we identified three types of Ca^{2+}-activated, K^+-conducting channels. Two of the channels were K^+-selective, one of which was selectively blocked by DPC. Table 1 summarizes the pharmacological profiles of the three channels and includes for comparison the profile of a previously identified K^+ channel thought to be activated by cell swelling (Richards & Dawson, 1986). Taken together, the evidence suggests that the DPC-sensitive, inward-rectifying K^+ channel rather than the maxi K^+ channel may play

an important role in the life of the cell. Furthermore, the results suggest that DPC sensitivity may be a useful characteristic with which to identify two subsets (i.e. inward-rectifying and non-selective cation) of K^+-conductive channels, at least in turtle colon.

A physiological role for the DPC-sensitive inward rectifier?

The somewhat surprising finding in isolated cells of a relatively selective block of the inward rectifier by DPC suggests that it should be possible to use this compound to assay for the expression of these channels in various functional states of the basolateral membrane. Chang & Dawson (1988) first identified DPC as a blocker of a specific Ca^{2+}-activated K^+ conductance in digitonin-permeabilized cell layers. It was thus particularly interesting to find that the DPC-sensitive channel was activated by increasing Ca^{2+} activity at the cytoplasmic face of detached patches, although there is a considerable disparity in the sensitivity of Ca^{2+} activation (see below). It is also noteworthy that a DPC-sensitive, inward-rectifying K^+ conductance was the dominant conductive pathway in isolated cells under whole-cell patch-clamp conditions, which should approximate those in the digitonin-permeabilized cell layer. Such results suggest that under the appropriate conditions, the DPC-sensitive channel can make a significant contribution to the basolateral membrane conductance.

In cell layers permeabilized with amphotericin-B, DPC blocked the resting K^+ conductance but not that activated by cell swelling. Although it is tempting to speculate that the resting conductance is due to the DPC-sensitive channel, there are a number of observations which are not consistent with this hypothesis. First, the resting conductance is relatively insensitive to quinidine whereas the inward-rectifier is blocked by this compound. Second, in amphotericin-B treated cell layers, substitution of mucosal Rb^+ for K^+ leads to resting currents of a similar magnitude which are blocked by barium and by DPC. In several experiments we have been unable to measure Rb^+ currents through single, inward-rectifying channels.

It seems most likely at this juncture that there are as many as two DPC-sensitive K^+ channels in the basolateral membrane. One of these is a Ca^{2+}-activated channel which is preserved in digitonin-treated cell layers and which may be responsible for the transient activation of K^+ current following the addition of carbachol. The second is the true 'resting' K^+ channel which has a high Rb^+ conductance and which is subject to inhibitory regulation by muscarinic agonists. It is also possible, however, that in the amphotericin-treated or normally transporting epithelium, DPC, a highly hydrophobic compound, exerts some non-specific effect on the cell which leads indirectly to the inactivation of the resting K^+ conductance.

The maxi K^+ channel: basolateral or apical?

Maxi K^+ channels have been found in a wide variety of cell types, including many epithelia (Petersen, 1986; Hunter, Kawahara & Giebisch, 1988; Dawson & Richards, 1990). The flickery block of the maxi K^+ channel by quinidine in turtle colon cells is similar to that reported for block by quinidine or its stereoisomer quinine of maxi K^+ channels in other cell types (Iwatsuki & Petersen, 1985; Guggino, Guggino, Green & Sacktor, 1987; Glavinovic & Trifaro, 1988; Merot, Bidet, LeMaout, Tanc & Poujcol,

1989; Richards, Lowy, Ernst & Dawson, 1989; Turnheim, Constantin, Chan & Schultz, 1989; Wong, 1989; Bokvist, Rorsman & Smith, 1990; Mancilla & Rojas, 1990; Rae, Dewey, Rae & Cooper, 1990; Segal & Reuss, 1990; Fatherazi & Cook, 1991). However, the signature of the maxi K^+ channel identified in isolated colonic cells does not correspond to any of the macroscopic *basolateral* currents measured in permeabilized cell layers. DPC, a potent blocker of K^+ currents in isolated cells and cell layers had virtually no effect on the maxi K^+ channel. In addition, external TEA does not block basolateral K^+ currents under any of these conditions but is a potent blocker of the maxi K^+ channel. It may be that these channels are only expressed under physiological conditions not attained in the preparations thus far studied. Another possibility, however, is that these channels are *apical* in origin and mediate K^+ secretion. Recent studies show, in fact, that apical K^+ channel noise is abolished by 10 mM TEA applied to the apical surface (Wilkinson & Dawson, 1991).

Non-selective cation channel

Ca^{2+}-activated ion channels which do not discriminate among a variety of monovalent cations have been identified in several epithelial cell types (Maruyama & Petersen, 1982; Maruyama, Gallacher & Petersen, 1983; Marty, Tam & Trautman, 1984; Gögelein & Greger, 1986; Teulon, Paulais & Bouthier, 1987; Partridge & Swandulla, 1988; Kunzelmann, Pavenstädt, Beck, Ünal, Arnt & Greger, 1989; Cook, Poronnick & Young, 1990; Gögelein & Capek, 1990; Champigny, Verrier & Lazdunski, 1991) and it has been reported that DPC or analogues of this compound can block the channels (Gögelein & Greger, 1986; Gögelein & Pfanmüller, 1989; Cook *et al.* 1990; Gögelein & Capek, 1990; Champigny *et al.* 1991). This particular channel type presents us with an interesting paradox. On one hand, this channel is the most frequently recorded in detached patches, but on the other hand there is no evidence for any non-selective cation conductance in isolated cells, permeabilized cell layers or intact cell layers. Manganese, a blocker of the channel, is not an effective blocker of the macroscopic currents under any of these conditions. These channels thus appear to be reasonably abundant in the cells but are inactive under physiological conditions.

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REFERENCES

BEZANILLA, F. (1985). A high capacity data recording device based on a digital audio processor and a videocassette recorder. *Biophysical Journal* **47**, 437–441.

BOKVIST, K., RORSMAN, P. & SMITH, P. A. (1990). Block of ATP-regulated and Ca^{2+}-activated K^+ channels in mouse pancreatic β-cells by external tetraethylammonium and quinine. *Journal of Physiology* **423**, 327–342.

CHAMPIGNY, G., VERRIER, B. & LAZDUNSKI, M. (1991). A voltage, calcium, and ATP sensitive non selective cation channel in human colonic tumor cells. *Biochemical and Biophysical Research Communications* **176**, 1196–1203.

CHANG, D. & DAWSON, D. C. (1988). Digitonin-permeabilized colonic cell layers: Demonstration of calcium-activated basolateral K^+ and Cl^- conductances. *Journal of General Physiology* **92**, 281–306.

CHANG, D., HSIEH, P. S. & DAWSON, D. C. (1988). CALCIUM: A program in BASIC for calculating the composition of solutions with specified free concentrations of calcium, magnesium and other divalent cations. *Computers in Biology and Medicine* **18**, 351–366.

COOK, D. I., PORONNIK, P. & YOUNG, J. A. (1990). Characterization of a 25-pS nonselective cation channel in a cultured secretory epithelial cell line. *Journal of Membrane Biology* **114**, 37–52.

DAWSON, D. C. (1987). Properties of epithelial K channels. *Current Topics in Membranes and Transport* **28**, 41–71.

DAWSON, D. C. & RICHARDS, N. W. (1990). Basolateral K conductance: Role in regulation of NaCl absorption and secretion. *American Journal of Physiology* **259**, C181–195.

DI STEFANO, A., WITTNER, M., SCHLATTER, E., LANG, H. J., ENGLERT, H. & GREGER, R. (1985). Diphenylamine-2-carboxylate, a blocker of the Cl^--conductive pathway in Cl^--transporting epithelia. *Pflügers Archiv* **405**, suppl. 1, S95–100.

FATHERAZI, S. & COOK, D. L. (1991). Specificity of tetraethylammonium and quinine for three K channels in insulin-secreting cells. *Journal of Membrane Biology* **120**, 105–114.

GERMANN, W. J., ERNST, S. A. & DAWSON, D. S. (1986*a*). Resting and osmotically induced basolateral K conductances in turtle colon. *Journal of General Physiology* **88**, 253–274.

GERMANN, W. J., LOWY, M. E. & ERNST, S. A. & DAWSON, D. C. (1986*b*). Differentiation of two distinct K conductances in the basolateral membrane of turtle colon. *Journal of General Physiology* **88**, 237–251.

GLAVINOVIC, M. I. & TRIFARO, J. M. (1988). Quinine blockade of currents through Ca^{2+}-activated K^+ channels in bovine chromaffin cells. *Journal of Physiology* **399**, 139–152.

GÖGELEIN, H. & CAPEK, K. (1990). Quinine inhibits chloride and nonselective cation channels in isolated rat distal colon cells. *Biochimica et Biophysica Acta* **1027**, 191–198.

GÖGELEIN, H. & GREGER, R. (1986). A voltage-dependent ionic channel in the basolateral membrane of late proximal tubules of the rabbit kidney. *Pflügers Archiv* **407**, S142–148.

GÖGELEIN, H. & PFANNMÜLLER, B. (1989). The nonselective cation channel in the basolateral membrane of rat exocrine pancreas. Inhibition by 3′,5-dichlorodiphenylamine-2-carboxylic acid (DCDPC) and activation by stilbene disulfonates. *Pflügers Archiv* **413**, 287–298.

GUGGINO, S. E., GUGGINO, W. B., GREEN, N. & SACKTOR, B. (1987). Blocking agents of Ca^{2+}-activated K^+ channels in cultured medullary thick ascending limb cells. *American Journal of Physiology* **252**, C128–137.

HAMILL, O. P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F. J. (1981). Improved patch clamp techniques for high-resolution current recording from cells in cell-free membrane patches. *Pflügers Archiv* **391**, 85–100.

HUNTER, M., KAWAHARA, K. & GIEBISCH, G. (1988). Calcium-activated epithelial potassium channels. *Mineral and Electrolyte Metabolism* **14**, 48–57.

IWATSUKI, N. & PETERSEN, O. H. (1985). Inhibition of Ca^{2+}-activated K^+ channels in pig pancreatic acinar cells by Ba^{2+}, Ca^{2+}, quinine and quinidine. *Biochimica et Biophysica Acta* **819**, 249–257.

KUNZELMANN, K., PAVENSTÄDT, H., BECK, C., ÜNAL, Ö., EMMRICH, P., ARNDT, H. J. & GREGER, R. (1989) Characterization of potassium channels in respiratory cells. *I. General properties. Pflügers Archiv* **414**, 291–296.

LATORRE, R. & MILLER, C. (1983). Conduction and selectivity in potassium channels. *Journal of Membrane Biology* **71**, 11–30.

MANCILLA, E. & ROJAS, E. (1990). Quinine blocks the high conductance, calcium-activated potassium channel in rat pancreatic beta-cells. *FEBS Letters* **260**, 105–108.

MARTY, A., TAM, Y. P. & TRAUTMANN, A. (1984). Three types of calcium-dependent channels in rat lacrimal glands. *Journal of Physiology* **357**, 293–325.

MARUYAMA, Y., GALLACHER, D. V. & PETERSEN, O. H. (1983). Voltage- and Ca^{2+}-activated K^+ channel in basolateral acinar cell membranes of mammalian salivary glands. *Nature* **302**, 827–829.

MARUYAMA, Y. & PETERSEN, O. H. (1982). Single-channel currents in isolated patches of plasma membrane from basal surface of pancreatic acini. *Nature* **299**, 159–161.

MEROT, J., BIDET, M., LEMAOUT, S., TAUC, M. & POUJEOL, P. (1989). Two types of K^+ channels in the apical membrane of rabbit proximal tubule in primary culture. *Biochimica et Biophysica Acta* **978**, 134–144.

PARTRIDGE, L. D. & SWANDULLA, D. (1988). Calcium-activated non-specific cation channels. *Trends in Neuroscience* **11**, 69–72.

PETERSEN, O. H. (1986). Calcium-activated potassium channels and fluid secretion by exocrine glands. *American Journal of Physiology* **251**, G1–13.

RAE, J. L., DEWEY, J., RAE, J. S. & COOPER, K. (1990). A maxi calcium-activated potassium channel from chick lens epithelium. *Current Eye Research* **9**, 847–861.

RICHARDS, N. W. & DAWSON, D. C. (1986). Single potassium channels blocked by lidocaine and quinidine in isolated turtle colon epithelial cells. *American Journal of Physiology* **251**, C85–89.

RICHARDS, N. W., LOWY, R. J., ERNST, S. A. & DAWSON, D. C. (1989). Two K^+ channel types, muscarinic agonist-activated and inwardly rectifying, in a Cl-secretory epithelium: the avian salt gland. *Journal of General Physiology* **93**, 1171–1194.

SEGAL, Y. & REUSS, L. (1990). Ba^{2+}, TEA^+, and quinine effects on apical membrane K^+ conductance and maxi K^+ channels in gallbladder epithelium. *American Journal of Physiology* **259**, C56–68.

TEULON, J., PAULAIS, M. & BOUTHIER, M. (1987). A Ca^{2+}-activated cation-selective channel in the basolateral membrane of the cortical thick ascending limb of Henle's loop of the mouse. *Biochimica et Biophysica Acta* **905**, 125–132.

TURNHEIM, K., CONSTANTIN, J., CHAN, S. & SCHULTZ, S. G. (1989). Reconstitution of a calcium-activated potassium channel in basolateral membranes of rabbit colonocytes into planar lipid bilayers. *Journal of Membrane Biology* **112**, 247–254.

VENGLARIK, C. J. & DAWSON, D. C. (1986). Cholinergic regulation of Na absorption by turtle colon: Role of basolateral K conductance. *American Journal of Physiology* **251**, C563–570.

WILKINSON, D. J. & DAWSON, D. C. (1991). Apical K^+ channels mediating K^+ secretion by turtle colon are specifically identified by TEA^+ sensitivity. *FASEB Journal* **5**, A688.

WONG, B. S. (1989). Quinidine blockade of calcium-activated potassium channels in dissociated gastric smooth muscle cells. *Pflügers Archiv* **414**, 416–422.