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A K^+-selective channel in the colonic carcinoma cell line: CaCo-2 is activated with membrane stretch

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CaCo-2 is a human colonic carcinoma cell line which becomes differentiated in culture to form a polarized epithelium exhibiting several of the functional characteristics of native colonic tissue. In the present study, CaCo-2 cells have been used for a patch-clamp study of colonic ion conductance pathways. A large, 120 pS K^+-selective channel was found in cells forming subconfluent monolayers in culture. Unlike Maxi-K^+ channels found in other epithelial cells, this channel was not activated with elevations in cytosolic Ca^{2+}. Channel activity was stimulated with membrane depolarization and most markedly with membrane stretch. The application of negative pressure (20 mm-Hg) to both cell-attached and excised, inside-out membrane patches caused a burst of channel activity which disappeared within seconds of suction removal. Single-channel conductance of the pressure-activated channel was decreased when quinine (100 μM) was present in the patch pipette.

Introduction

The CaCo-2 cell line is a colonic cell line which undergoes spontaneous differentiation in culture. These cells exhibit epithelial characteristics, ie. brush-border microvilli, tight junctions and dome formation [1–3]. The bioelectric properties of the cells have been investigated in micropuncture and Ussing chamber studies. While these cells fail to exhibit amiloride-sensitive Na^+ conductance, an important feature of colonic epithelium [4,5], cyclic AMP activated Cl^- transport has been detected [3] suggesting that some normal colonic function is retained and that this cell line can be used as a convenient model for study of ion conductance in human colonic epithelium.

In general, the role of K^+ channels in epithelial secretion remains uncertain. According to the model first proposed by Silva et. al. [6] for the shark rectal gland and developed by Petersen [7] for mammalian exocrine systems, electrolyte secretion is driven primarily by Cl^- ion conductance across the apical membrane through anion selective channels with K^+ conductance across the basolateral membrane serving a permissive role. However, in the colonic cell line, T_{84}, carbachol-mediated secretion may be driven by calcium-activated K^+ conductance causing hyperpolarization which in turn stimulates cellular Cl^- efflux [8].

The participation of K^+ channels in epithelial cell volume regulation has been quite well studied. An increase in basolateral K^+ conductance following cell swelling has been reported in a number of cell types [9–13]. However, the signal which triggers enhanced K^+ conductance appears to vary from one cell type to another. Cell swelling caused by exposure to hypotonic shock or organic solute uptake is associated with a transient elevation in calcium in some epithelial cell types and therefore it has been suggested that cytosolic calcium may signal the changes in K^+ permeability necessary for volume regulation [9,11–13]. Swelling results in activation of Ca^{2+}-activated K^+ channels in choroid plexus epithelium [11]. On the other hand, membrane stretch, resulting from cell swelling provides the primary trigger for increased basolateral K^+ permeability through pressure-activated K^+ channels in *Necturus* kidney proximal tubule [14].

In the present study, a large, 120 pS K^+-selective channel has been described in CaCo-2 cells using the patch-clamp technique. Although its role in colonic water and electrolyte secretion is uncertain, this channel may function in cell volume regulation as it is activated with membrane stretch.

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Methods

Cell culture procedures. The CaCo-2 cell line was obtained from American Type Culture Collection, ATCC and grown in alpha medium (Princess Margaret Hospital, Toronto, Canada) supplemented with 10% fetal calf serum and antibiotics on 35 mm plastic cell culture dishes under a 5% CO_2 atmosphere. Prior to patch clamp studies, the cells were rinsed at least three times with Na^+-Hepes buffer (described below). For cell volume measurements, the CaCo-2 cells were first dissociated by trypsinization , washed twice in the above culture medium and then kept in suspension at room temperature in bicarbonate-free RPMI medium buffered with 20 mM Hepes, (pH 7.4) until experimentation.

Current recordings. Single-channel currents were recorded according to Hamill et al. [15] using a List EPC-7 patch-clamp amplifier (Medical Systems, Great Neck, NY, U.S.A.) Pipettes were fabricated from borosillicate glass type 7052 (Garner Glass Co.) using a two-stage Narishige pipette puller. When filled with Na^+-Hepes solution, pipette resistances were approx. 2–5 MΩ. The bath electrode was a Ag-AgCl wire connected to the bathing solution via an agar bridge. Current output was monitored on a Tektronix oscilloscope and recorded on video tape after A/D conversion by a video adaptor (PCM 2, Medical Systems). In some experiments suction was applied to the pipette interior via a side port on the pipette holder. Suction (20 mmHg) was generated using a vacuum pump equipped with a regulator, (Vacu-Trol, Lab. Vac., Spectrum, Med. Ind., Inc., Los Angeles, CA, U.S.A.).

Solutions. The standard bath and pipette solutions contained (mM): 140 NaCl or 140 KCl, 1 $MgCl_2$, 10 glucose and 10 Hepes. The pH was adjusted to 7.2 using NaOH or KOH. In excised patch studies the bath Ca^{2+} concentration was buffered using EGTA (ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid). Experiments were done at 22–25 °C.

Single-channel data analysis. Current records stored on video cassette tape were transferred to the hard disk of an IBM-AT compatible computer using the FETCHEX program of pCLAMP (Version V) software (Axon Inst.). FETCHAN and pSTAT programs were then employed for analysis of single channel data.

Volume measurements. A Coulter counter with a Channelanalyzer (Model ZM 256) was used for cell volume measurements. Changes in volume were estimated by the shift in median size based on appropriate calibrations with particles of known size suspended in various media. The Coulter counter has been used for volume determinations for several different types of cell including toad bladder epithelial cells [9]. As with toad bladder there was considerable variability observed between different CaCo-2 cell suspensions in terms of size distribution and volume changes accounting for large standard deviations within each experimental group. Due to this variation each experiment was performed with a paired control and volume responses reported relative to the initial measurement obtained prior to volume manipulations, ie. exposure to hypotonic solutions. For these studies cell aliquots were separated from the culture medium RPMI, by centrifugation and the pellet resuspended in the Na^+-Hepes solution. A sample of this cell suspension was used to determine the initial mean cell volume. The remaining cell suspension was exposed to hypotonic shock by dilution with an equal volume of distilled water so that the final osmolarity of the medium was 67% of control and samples were taken at various times for cell volume determinations. These experiments were performed at 37 °C.

Statistical analysis. The mean and standard deviation of data groups have been calculated. The differences between paired data were assessed for statistical significance using the paired '*t*'-test.

Results

Stretch activated channels in cell-attached membrane patches

In most cases, channel openings were rarely observed at pipette potentials ranging from −80 to +80 mV in cell-attached patches. The application of negative pressure (20 mmHg) through the patch pipette of cell-attached patches resulted in a burst of channel openings in 24 of 35 trials, regardless of the applied pipette potential. Fig. 1 shows an example of marked activation of a single channel with pressure application. Mean pressure-evoked channel open probability was 0.12 ± 0.11 at $V_p = -22$ mV. As indicated by the large standard deviation, there was much variability in the single-channel response to pressure application. Generation of the current-voltage relationship for this pressure-evoked channel required the continuous application of suction (20 mmHg) to the pipette (Fig. 2). The unitary channel conductance was nonlinear with a maximum conductance in the voltage range $V_p = -40$ to

Fig. 1. Stretch-activated channel in cell-attached patches. The application (↑) of negative pressure (20 mmHg) through the pipette interior of a cell-attached patch ($V_p = -50$ mV) resulted in a burst of outward current steps lasting until the suction was removed (↓). For this record the bath and pipette solution contained a Na^+-rich Hepes buffer.

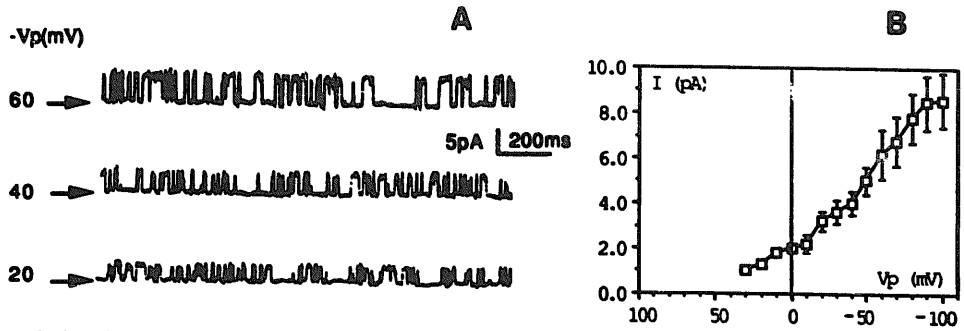

Fig. 2. Current-voltage relation for stretch-activated channel. (A) Current records have been shown for cell-attached patch, (pipette = NaCl, bath = NaCl) at varying pipette potentials. To obtain these traces the cell-attached patch was exposed to the continuous application of negative pressure (20 mmHg) through the pipette (B) The mean current-voltage relationship for 21 cell-attached patches has been shown. S.D. values have been included.

-100 mV of 83 ± 10 (S.E.) pS. The reversal potential of the current-voltage relationship of this channel was estimated at $V_R = 60$ mV, a pipette potential corresponding to a negative membrane potential. This relatively large channel was only observed in subconfluent monolayers of CaCo-2 cells suggesting that this channel may be localized to the basolateral membrane surface in the differentiated state.

Application of the calcium ionophore: A23187 (μM) failed to activate the channel in six patches containing the stretch-activated channel.

Excised, inside-out membrane patches

This channel was also studied in excised inside-out membrane patches. In several studies it was possible to stretch-activate the channel in both the cell-attached and excised patch configuration. A typical experiment is shown in Fig. 3. In 14 cells, examined under depolarizing conditions with a high KCl-bath, channel steps of the same current level, were activated prior to and following patch excision. This observation indicates that activation in the cell-attached mode is not related to the action of a cytosolic modulator but is most likely due to a direct mechanical effect. In some cases, patch excision of cell-attached patches to form inside-out membrane patches alone resulted in transient channel activation which subsided within varying time intervals between one and three minutes. In 11 cases, the large channel appeared upon patch excision when it was not previously observed in the cell-attached configuration.

The *I-V* relationships for the channel when exposed to quasi-physiological (NaCl pipette, KCl bath) and Symmetrical (KCl pipette and bath) ion gradients in the excised patch configuration have been shown in Fig. 4. In the presence of quasi-physiological ion gradients only outward currents were observed and the *I-V* relationship was nonlinear with a maximum single-channel conductance of 111 ± 7 pS ($n = 8$) in the voltage range of 0 to 60 mV . Extrapolation of the *I-V* relationship to zero current yielded an estimated reversal potential of $V_R = -60$ mV. Potassium is likely to be the current carrying ion as K^+ is the only ion with a negative equilibrium potential under standard ionic gradients. Furthermore, in the presence of symmetrical KCl solutions the reversal potential of the *I-V* relationship became 0 mV , as expected for K^+-selective current. With symmetrical KCl solutions the calculated single-channel conductance was 120 ± 14 pS ($n = 6$).

Membrane depolarization caused a slight increase in spontaneous channel open probability. Open probability was highly variable from one membrane patch to another as observed in Fig. 3c. Nevertheless, the in-

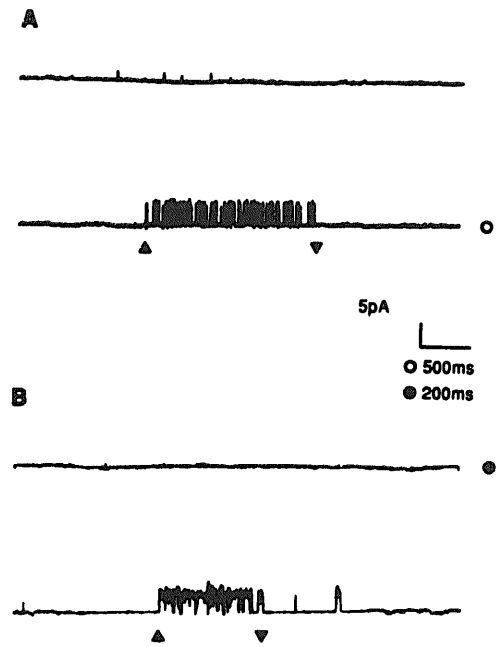

Fig. 3. Stretch activation in cell attached and excised membrane patches. (A) The application and cessation of suction through the patch pipette (20 mmHg) to a cell-attached membrane patch have been indicated with (▲) and (▼), respectively. The cell was bathed in a high KCl (140 mM)-containing Hepes buffer, the pipette contained a high NaCl (140 mM)-containing Hepes buffer and the pipette potential was $V_p = -22$ mV. (B) Activation of a channel with a similar current step amplitude at -22 mV was observed in an excised, inside-out membrane patch held at $V_p = -22$ mV following the application of negative pressure (20 mmHg). The bath and pipette solutions were the same as above.

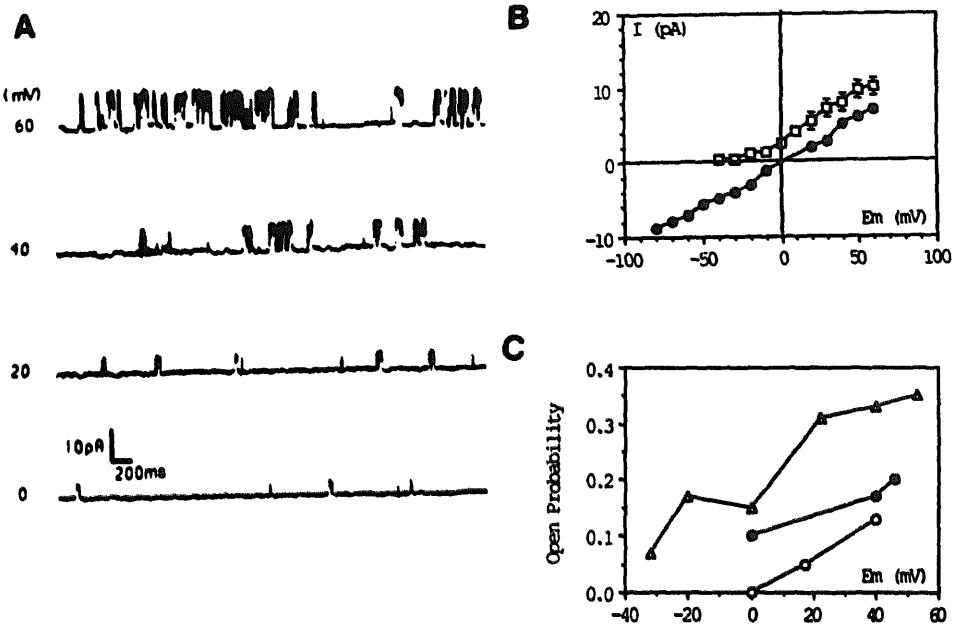

Fig. 4. *I-V* relationship for channel observed in excised. Inside-out membrane patches. (A) Current records have been shown for excised membrane patch exposed to quasi-physiological ion gradients (pipette = NaCl, bath = KCl) at varying membrane potentials. The concentration of free Ca^{2+} in the bath was $< 10^{-9}$ M (no added $CaCl_2$ with 1 mM EGTA). (B) Mean current-voltage relationship for excised, inside-out membrane patches exposed to quasi-physiological (□) ($n = 28$), and symmetrical (pipette = KCl, bath = KCl) (●) ($n = 6$) have been shown. The amplitudes of single-channel currents were measured both by direct determination from the oscilloscope and amplitude distributions generated during record analysis. Amplitudes determined using both of these methods were identical. (C) The relationship between channel open probability and membrane potential for three separate excised, inside-out membrane patches exposed to quasi-physiological ion gradients has been shown. The difference between open probability observed at 0 mV and 40 mV is statistically significant ($P < 0.05$), (paired '*t*'-test).

crease in open probability from 0 to +40 mV was statistically significant ($P < 0.01$) for three excised membane patches. The degree of variability in open probability between patches may relate to variability in tension or stretch experienced by each patch.

Potassium channel activity in three excised, inside-out patches was not enhanced with elevations in ionized Ca^{2+} concentration bathing the cytosolic membrane surface, i.e., from 10 nM to 1 μM (data not shown). These results are consistent with our earlier observations in the cell-attached configuration in which the calcium ionophore A23187 failed to stimulate channel activity.

Stretch-activated K^+ channel inhibited by quinine

The addition of, quinine (100 μM) to the pipette caused a reduction of unitary conductance of the pressure activated channels in excised, inside-out membrane patches (Fig. 5). As previously reported, the maximum single-channel conductance of the pressure-activated channel was 111 ± 7 pS ($V_p = 0$ to $V_p = +60$ mV) in excised patches exposed to quasi-physiological ion gradients. With quinine in the pipette, the maximum unitary conductance of the pressure-activated channel was 53 ± 3 pS.

The addition of gadolinium (100 μM) [16], a blocker of stretch-activated nonselective cation channels, to the pipette contents produced no significant change in

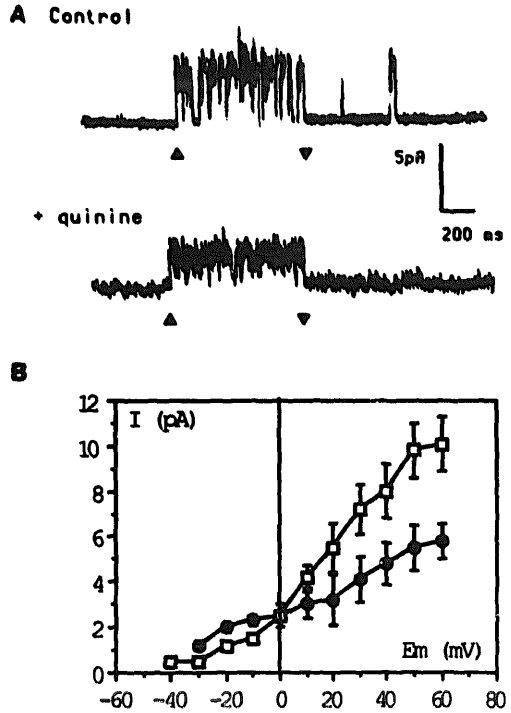

Fig. 5. Quinine Inhibition of K^+ channel. (A) Negative pressure (20 mmHg) was applied to (▲) an excised, inside-out membrane patch held at $V_p = -22$ mV in the upper panel. The release of negative pressure is indicated as (▼). In the lower panel the record shows the channel openings evoked with pressure , on (▲), off (▼) when the patch pipette contained quinine (100 μM). (B) The *I-V* relationships for the pressure activated channel in excised patches exposed to physiological ion gradients with (●) ($n = 6$) or without (□) ($n = 28$) quinine in the pipette have been shown.

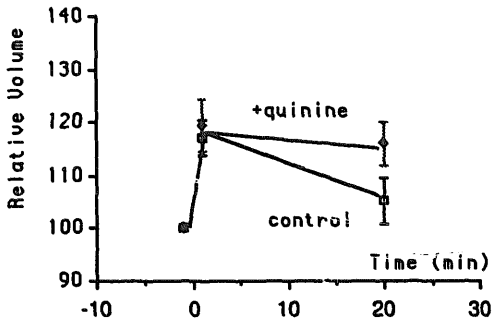

Fig. 6. Quinine inhibits regulatory volume decrease: Mean cell volume was assessed using a Coulter Counter prior to and following exposure of cells to hypotonic shock (bath dilution to 67% isotonicity) at time zero. Mean values and standard deviation have been shown. Twenty minutes after hypotonic shock the difference in mean volume between control and quinine-treated cells was significantly different ($P < 0.05$).

stretch-activation of the K^+-selective channel. Furthermore, gadolinium had no effect on stretch-activation of K^+ channels when applied to the cytosolic membrane surface in three excised, inside-out patches.

Regulatory volume decrease is inhibited by quinine (100 μM)

Like most cells, CaCo-2 cells are capable of regulating their cell size following swelling. Swelling was caused by exposure to hypotonic shock (67% of isotonicity) in the present experiments (Fig. 5). After the initial, immediate swelling phase, CaCo-2 cells began to volume regulate and the mean cell volume at 20 min was not significantly different from that of pre-swollen cells. However, the addition of quinine (100 μM) one minute prior to hypotonic shock resulted in inhibition of the volume regulatory response. These results suggest that the quinine-inhibitable, pressure-activated K^+ channel previously described may be involved in regulatory volume decrease in these cells.

Discussion

A 120 pS K^+-selective channel has been detected in the basolateral membrane of CaCo-2 cells. While this channel is similar to large K^+-selective channels observed in the basolateral membrane of comparable epithelial cells with respect to its activation with depolarization [7,11,17], it is distinctive with respect to it's stimulation with membrane stretch.

The list of channels that are activated with membrane stretch is increasing and includes channels which are nonselective pores (failing to discriminate between anions or cations) [17], nonselective cation channels (permeable to K^+, Na^+ and Ca^{2+}) [16,18,19], Cl^--selective channels [20,21] and finally, channels which are highly selective for K^+ [14,22]. The proposed functions for these stretch-activated channels are as varied as the types of tissue in which they are observed and may mediate sound transduction in cochlear cells [23] and volume regulation in epithelial cells [11,14,18]. Comparison with other stretch-activated channels indicates that the K^+ channel described in the present study resembles the stretch-activated K^+ channel found in renal proximal tubule cells of *Necturus* [14] and demonstrates a clear preference for K^+ over other cations.

The role of the stretch-activated, K^+ selective channel reported in this study in cell volume regulation is unclear. Quinine blocked both the 120 pS stretch-activated K^+ channel and regulatory volume decrease in these cells, evidence supporting a role for this channel in cell volume regulation. Indeed, quinine inhibition of cell volume regulation has been observed in several epithelial cell types, including; rat hepatocyte [24] and Madin-Darby canine kidney (MDCK) cells [13].

However, quinine, like so many channel blockers, is relatively nonspecific, blocking K^+ channels of varying unitary conductances and activation properties [25]. Therefore it is possible that other K^+ channels in addition to the 120 pS channel discussed in this paper are involved in CaCo-2 cell volume regulation.

Low open probability of the stretch-activated K^+ channel suggests that it may provide a minor contribution to the total membrane conductance under resting or swollen conditions. An estimate of the whole cell conductance (G) contributed by the 120 pS K^+ channel following activation with membrane stretch can be obtained from the equation:

$$G = g \cdot N \cdot P_0 / A$$

where g is the single-channel conductance, N the number of channels per patch, P_0 the average open probability and A the area of membrane forming a patch. Assuming that $N = 0.66$, $g = 120$, $P_0 = 0.05$ to 0.10 and the patch area is 1.5 μm^{-2}, the stretch-activated channel may contribute 0.04 to 0.1 $\mu S/cm^2$ to the total membrane conductance. However, the resting membrane conductance measured in preliminary whole-cell patch clamp studies is approx. 2 $\mu S/cm^2$. To conclude, it appears likely that other K^+ channels in addition to the stretch-activated channel discussed in this report may be involved in K^+ efflux in resting cells and in cells undergoing volume regulatory decrease.

In *Necturus* choroid plexus epithelium [11] stretch-activated calcium permeable, nonselective cation channels act as signal transducers, permitting Ca^{2+}-influx and the subsequent activation of Ca^{2+}-dependent K^+ channels on the basolateral membrane. In contrast, the K^+-channel in CaCo-2 cells is not stimulated with changes in intracellular Ca^{2+} hence, it's activity is unlikely to be secondary to opening of Ca^{2+}-permeable channels.

Clearly, volume regulatory decrease requires the activation of anion-selective channels by cell swelling to permit anion efflux to accompany K^+ conductance. As yet, such volume activated Cl^- conductance paths have not been characterized in CaCo-2 cells.

Calcium-activated K^+ channels have been detected in the basolateral membrane of the colonic cell line, T_{84} [8] and may be of primary importance in the generation of carbachol-evoked secretion. Cellular hyperpolarization resulting from carbachol evoked K^+ conductance increases the electrochemical driving force for Cl^- exit across the apical membrane. It is difficult to explain why CaCo-2 cells should fail to possess such a mechanism. CaCo-2 cells appear to most closely resemble fetal colonic cells, displaying cAMP-evoked Cl^-conductance but failing to exhibit aldosterone-stimulated Na^+ conductance. Perhaps the expression of Ca^{2+}-activated K^+ channels is dependent in some way on cellular differentiation.

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