J. Membrane Biol. 124, 159–167 (1991)
002226319100141R

The Journal of
Membrane Biology

Cyclic GMP Regulation of a Voltage-Activated K Channel in Dissociated Enterocytes

Scott M. O'Grady†, Kim E. Cooper, and James L. Rae
†Department of Physiology and Animal Science, University of Minnesota, St. Paul, Minnesota 55108, and Department of Physiology, Mayo Foundation, Rochester, Minnesota 55905

Summary. Enterocytes from the intestinal epithelium of the winter flounder were isolated by collagenase digestion and incubated in flounder Ringer solution. Conventional whole-cell and amphotericin-perforated whole-cell recording techniques were used to characterize the properties of a voltage-activated K current present in dissociated cells. Resting membrane potentials and series resistances were significantly lower (from −23 to −39 mV and 29 to 13 MΩ, respectively) when amphotericin was used to achieve the whole-cell configuration. When cells were placed in flounder Ringer solution, held at −80 mV and subsequently stepped to a series of depolarizing voltages (from −70 to 0 mV), an outward current was observed that exhibited inactivation at voltages above −20 mV. This current was sensitive to holding potential and was not activated when the cells were held at −40 mV or above. When cells were bathed in symmetric K Ringer solution and the same voltage protocol was applied to the cell, inward currents were observed in response to the negative intracellular potentials. Reversal potentials at two different extracellular K concentrations were consistent with K as the current-carrying ion. $BaCl_2$ (2 mM) and CsCl (0.5 mM) both produced voltage-dependent blockade of the current when added to the bathing solution. Charybdotoxin (300 nM extracellular concentration) completely blocked the current. The IC_{50} for charybdotoxin was 50 nM. Cyclic GMP inhibited the voltage-activated current in flounder Ringer and in symmetric K Ringer solution. The cyclic GMP analog, 8-Br cGMP, lowered the threshold for voltage activation and potentiated inactivation of the current at voltages above −40 mV. Previous studies with intact flounder epithelium showed that K recycling and net K secretion were inhibited by Ba^{2+} and by cGMP. We suggest that the channel responsible for the whole-cell current described in this study may be important in K recycling and secretion.

Key Words cGMP · charybdotoxin · K recycling · K secretion · NaCl absorption

Introduction

The intestinal epithelium of the winter flounder (*Pseudopleuronectes americanus*) absorbs Na and Cl by a mechanism that is similar to the thick ascending limb (TAL) of Henle's loop in mammalian kidney [13]. The uptake of Na, K and Cl from the lumen into the cell is mediated by an electroneutral Na-K-2Cl cotransport system which is blocked by diuretic compounds such as bumetanide and furosemide [15]. The Na that enters the cell across the apical membrane is pumped out across the basolateral membrane by the Na-K ATPase. Chloride, which is present in the cell at a concentration above electrochemical equilibrium, exits across the basolateral membrane through Cl channels and presumably a KCl cotransport pathway [3, 6]. An important component of NaCl absorption in this epithelium is the recycling of K across the apical membrane. Potassium recycling is important in replenishing luminal K so that the Na-K-2Cl cotransport mechanism is not limited by the availability of K [20]. Electrogenic K recycling also maintains the electrical driving force for Cl efflux through Cl channels in the basolateral membrane [2]. Data from electrophysiological experiments indicate that K efflux across the apical membrane is mediated by an electrogenic transport pathway, most likely K channels [6]. These channels are inhibited by mucosal application of $BaCl_2$ (2 mM) [6, 13, 17].

Recent studies of K transport in rabbit TAL suggest that K recycling is mediated by an inwardly rectifying, 22-pS K channel that has a high open probability (0.9) over a wide range of membrane potentials [20]. The channel is blocked by Ba, unaffected by Ca and is sensitive to the cytoplasmic ATP-ADP concentration ratio. A similar ATP-sensitive K channel has also been reported in rat TAL [20]. In *Amphiuma* kidney, ATP alters the Ca sensitivity of a large conductance K channel in cells from the early distal tubule [7]. The mechanism by which ATP blocks the K channel in TAL has not been elucidated but it appears that ATP does not obstruct the channel pore or promote phosphorylation. Regulation of these K channels by cyclic nucleotides has not been described.

In this study, the whole-cell patch-clamp technique was used to investigate electrogenic K transport in primary dissociated enterocytes from the winter flounder. Our objective was to identify and characterize K currents which possess blocker pharmacology and cGMP regulation consistent with previous studies of K recycling and secretion in the intact epithelium. In this paper we describe the properties of a voltage-activated, Ba-sensitive K current that is inhibited by 8-Br cGMP. We also discuss the possible role of this K channel in the process of NaCl absorption across the epithelium.

Materials and Methods

Winter flounder were purchased from the Woods Hole Oceanographic Institution. Fish were held in a 500 gal seawater aquarium at 10°C. Food was withheld from the fish for at least three days prior to experiments.

Cell Dissociation

The intestinal epithelium was stripped of serosal muscle, minced into 2-mm sections and incubated in Ca-free Ringer solution (150 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 3 mM Na_2HPO_4, 5 mM EPPS buffer, 10 mM glucose, 2 mM EGTA, pH = 7.8) containing 0.5 mg/ml of collagenase (15 min at room temperature). The mixture was then filtered through sterile gauze to remove undigested material and centrifuged at low speed to pellet the cells. The cells were washed twice with flounder Ringer solution (150 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 3 mM Na_2HPO_4, 5 mM EPPS buffer, 10 mM glucose, pH = 7.8) to remove cell debris and mucus. The final pellet of cells was resuspended in flounder Ringer solution.

Whole-Cell Voltage-Clamp Experiments

Dissociated cells were placed in an acrylic plastic chamber and mounted on a custom-built stage which allowed viewing of the cells with a Nikon Diaphot inverted microscope. Whole-cell currents were measured with a model 1-B patch clamp from Axon Instruments. Data was digitized using a TL-1-40 12-bit A/D converter (Axon Instruments) with a maximum digitization rate of 40 kHz. Whole-cell current data was analyzed with an IBM-AT microcomputer using pCLAMP software. Electrodes (2–7 MΩ resistance) were made from Corning 7052 or KG-12 glass and filled with intracellular solution containing (in mM): 15 NaCl, 130 K-methane sulfonate, 5 HEPES, 3 EGTA, 1 $CaCl_2$, 1.5 $MgCl_2$, pH = 7.1. The whole-cell configuration was achieved by either rupturing the cell membrane with suction (conventional whole-cell recording) or by including amphotericin (240 μg/ml) in the electrode solution [16]. The voltage required to set the membrane current to zero was defined as the resting voltage of the cell. In most experiments, the pulse duration was 150 msec unless otherwise indicated. Holding potentials for each experiment are given in each figure legend. In experiments where symmetric K solutions were used, NaCl was replaced with KCl or K-methane sulfonate. The Donnan equilibrium resulting from the presence of amphotericin in the patch pipette produced a voltage offset that was less than 10 mV. A correction for this offset was incorporated in the data presented. Leak subtraction was not used in any of the experiments performed in this study. All experiments were carried out at room temperature (23°C).

Table. Comparison of the electrical properties of dissociated enterocytes from the winter flounder intestine under conventional whole-cell and amphotericin-permeablized whole-cell conditions

Parameter	Conventional configuration	Amphotericin configuration
Diameter (μm)	12 ± 0.3	12 ± 0.2
Resting voltage (mV)	−23 ± 6.0	−39 ± 3.0[a]
Capacitance (pf)	4.8 ± 0.6	5.8 ± 0.5
Series resistance (MΩ)	29.3 ± 3.4	13.2 ± 1.3[a]
Number of cells (n)	16	25

[a] Significantly different from the conventional configuration at $P < 0.05$ using an unpaired "t" test.

Results

Properties of Whole-Cell Currents Using Conventional and the Amphotericin-Perforated Patch Technique

The Table compares the mean values of diameter, resting membrane potential, capacitance and series resistance for cells used in conventional and amphotericin-perforated whole-cell voltage-clamp experiments. Cell diameter and capacitance were not significantly different. The mean values for whole-cell capacitance under both conditions were in good agreement with the calculated capacitance of a spherical cell (4.5 pF) with a diameter of 12 μm (assuming that $C = 1\ \mu F/cm^2$). Estimates of resting membrane potential were significantly more negative in amphotericin-treated cells compared to conventional whole-cell conditions. In addition, significantly lower series resistances were obtained with the amphotericin technique compared to conventional whole-cell recording. The difference in resting voltage was presumably due to a higher seal resistance obtained with the use of amphotericin.

Morphologic Characteristics of Dissociated Enterocytes

Previous ultrastructural studies have revealed that the epithelium was very homogeneous with respect to cell type and that the crypt-villous type organiza-

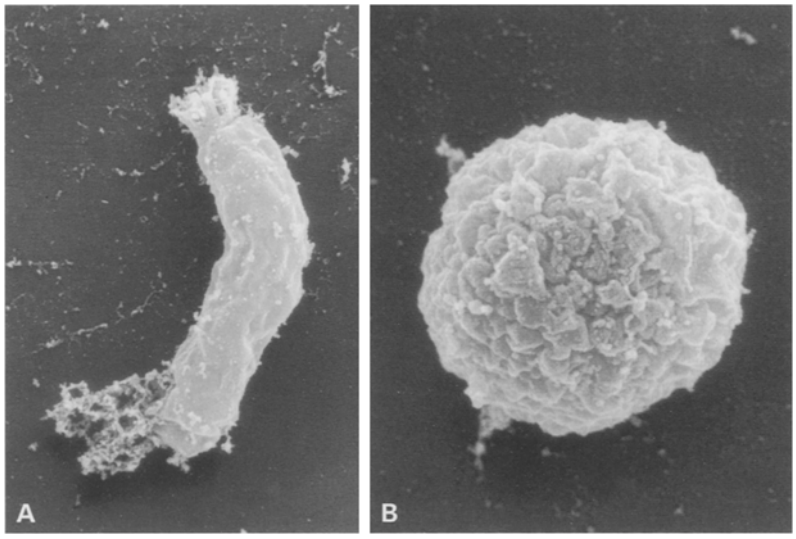

Fig. 1. (*A*) Scanning electron micrograph of a dissociated enterocyte prior to assuming a spherical shape. The apical membrane at the top of the cell can be clearly identified by the presence of microvilli. (*B*) Scanning electron micrograph of an epithelial cell several minutes after dissociation. The diameter of the cell was estimated to be 9 μm

tion present in mammalian intestine does not exist in the flounder intestine [4]. The epithelial cells were elongated (60 μm) and narrow (3.5 μm) with their nuclei located in the basal region of the cell. Figure 1 shows two isolated enterocytes from the flounder intestinal mucosa. Immediately following dissociation, these cells have an elongated appearance with a clearly visible brush-border membrane (Fig. 1*A*). Within minutes, the cells assume a spherical geometry (Fig. 1*B*). Cell diameters ranged from 12–16 μm in freshly dissociated cells. Estimates of cell diameter in cells prepared for scanning electron microscopy were approximately 20% lower than live cells, presumably due to dehydration during sample preparation.

Voltage-Activation Experiments in Flounder Ringer Solution

Figure 2*A* and *B* shows the results of an experiment where the holding potential of the cell was set at −80 mV and the effects of depolarizing voltage steps on whole-cell current were determined using conventional whole-cell (Fig. 2*A*) and amphotericin-perforated (Fig. 2*B*) whole-cell recording techniques. Both techniques showed that an outward current was activated as the membrane was depolarized. Analysis of the tail currents (Fig. 2*C*) indicated that the threshold for activation was approximately −50 mV. In the experiment shown in Fig. 1*A* (conventional whole-cell configuration) complete activation occurred at +10 mV and no inactivation was observed. However, in the amphotericin configuration, maximum activation occurred at −30 mV and a consistent inactivation was observed at higher depolarizing voltages. All subsequent experiments were performed under conditions where amphotericin was used to achieve the whole-cell configuration.

Effects of Holding Potential on Whole-Cell Currents

Figure 3 shows the effects of holding potential on voltage-activated whole-cell current. When the cell was held at −40 mV and stepped through a series of depolarizing voltage steps, very little time-dependent outward current was observed. Outward current appeared when the cell was held at −50 mV and increased in magnitude at each depolarizing voltage step when the holding potential was lowered to −60 and −80 mV. A reversal of tail current polarity between −60 and −80 mV was also observed in these experiments (*data not shown*), suggesting that the reversal potential for the current in flounder Ringer solution lies within this voltage range.

Current-Voltage Relationships in Flounder Ringer and Symmetric K Ringer Solutions

The effect of changing the bathing solution from flounder Ringer to symmetric K Ringer solution (KCl) on whole-cell current is presented in Fig. 4*A*. In this experiment the cell was held at −80 mV and stepped through a series of depolarizing voltages ranging from −60 to +60 mV. In symmetric K solution, inward current was observed in response to negative voltage steps. Figure 4*B* shows a tail cur-

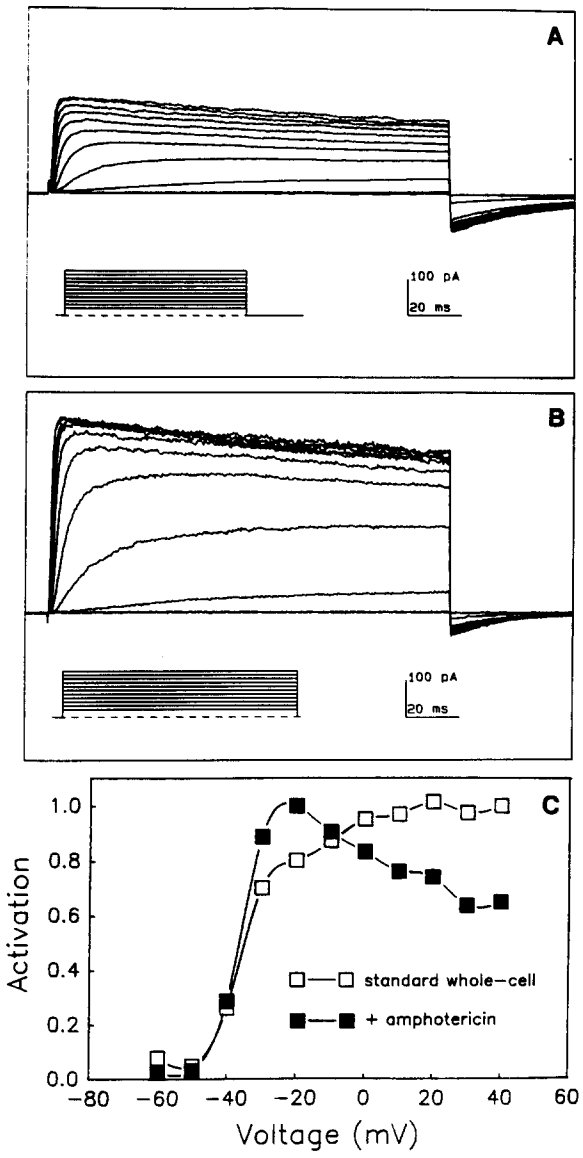

Fig. 2. (*A*) Conventional whole-cell recording of outward current from an enterocyte held at −80 mV and stepped through a series of depolarizing voltages ranging from −60 to +40 mV (pulse interval = 150 msec). (*B*) Whole-cell recording of outward current from an enterocyte where amphotericin was used to achieve the whole-cell configuration. The holding potential, voltage step protocol and pulse duration were identical to that in *A*. (*C*) Analysis of tail currents from data in *A* and *B* at each voltage step. The ratio of peak tail current at each voltage step to the maximum peak current recorded in the experiment is plotted as a function of voltage

rent experiment where the cell was held at −80 mV, depolarized to +20 mV to activate the channels and then stepped through a series of voltages ranging from −70 to +30 mV (5-mV steps). The instantaneous current-voltage relationship determined from these voltage steps was plotted in Fig. 4*C*. It is clear from this experiment that the instantaneous current

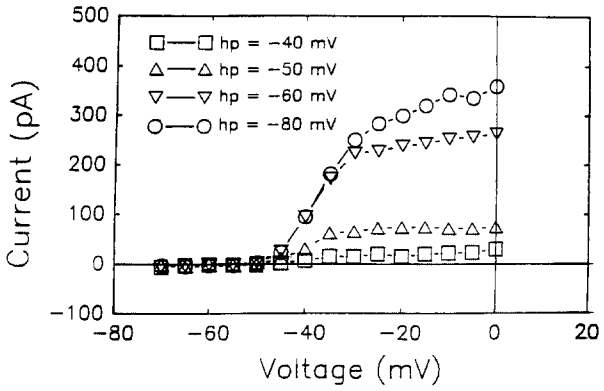

Fig. 3. Effects of holding potential on the magnitude of voltage-activated outward current from an amphotericin-treated cell bathed in flounder Ringer solution. In this experiment the cell was held at various potentials (−40, −50, −60 and −80 mV) and then stepped through a series of voltages ranging from −70 to 0 mV. The steady-state current is plotted as a function of voltage at each holding potential

is inwardly rectifying with a reversal potential of 0 mV.

Similar tail current experiments were performed on cells bathed in flounder Ringer solution over a range of voltages from −120 to +40 mV. The instantaneous current-voltage relationship is also presented in Fig. 4*C*. The estimated reversal potential was −79 mV. The reversal potential was very close to the equilibrium portential for K at 23°C (−83 mV), suggesting that the current was carried by K ions moving out of the cell and that the conductive pathway was highly selective for K relative to Na.

Effects of K-Channel Blockers

The effect of Ba^{2+} on whole-cell current in symmetric K Ringer solution is shown in Fig. 5*A*. The open symbols show the control condition while the filled symbols show the remaining current following $BaCl_2$ (2 mM) addition to the bath. Ba^{2+} at this concentration produces a complete block of inward current at −45 mV. At greater depolarizing voltages, inward current can be observed in the presence of Ba^{2+}. Figure 5*B* shows similar effects of Cs^+ on inward current in symmetric K Ringer solution. Again, open symbols show the whole-cell current under control conditions and filled symbols show the remaining current following addition of 0.5 mM CsCl to the bathing solution. These results indicate that the effect of Ba^{2+} and Cs^+ on inward current is voltage dependent. Figure 6*A* shows the effect of charybdotoxin on whole-cell current in symmetric K Ringer solution. Charybdotoxin is a peptide toxin isolated from scorpion venom (*Leirus quinquestriatus*) that blocks K channels in a variety of cell types [1, 12].

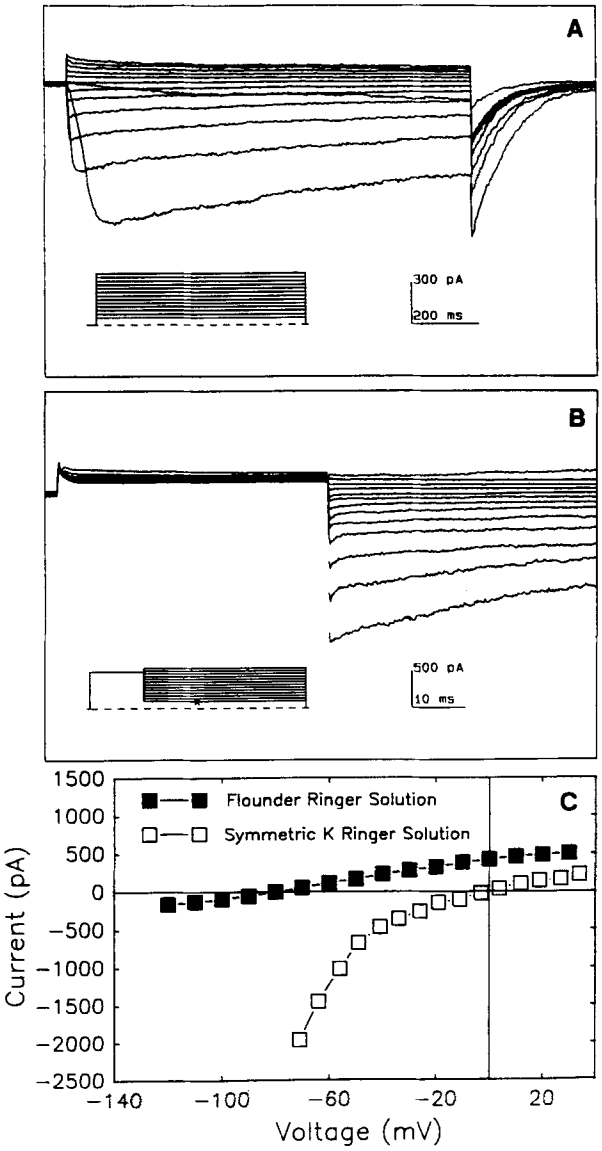

Fig. 4. (*A*) Whole-cell current from an amphotericin-perforated cell bathed in symmetric KCl Ringer solution. The cell was held at −80 mV and stepped through a series of voltages from −60 to +60 mV (10-mV steps) with a pulse duration of 1.2 sec. (*B*) Tail current experiment in symmetric KCl Ringer solution. The cell was held at −80 mV, depolarized to +20 mV for 50 msec to activate the channels and then stepped through a series of hyperpolarizing and depolarizing voltages to produce the tail currents. (*C*) Instantaneous current-voltage relationships obtained from the peak tail current response at each voltage step. Open symbols show inward rectification of current when cells were bathed in symmetric KCl Ringer solution. Filled symbols show the nearly linear instantaneous current-voltage relationship when the cell is bathed in flounder Ringer solution at a holding potential of −80 mV. Note that the reversal potential in flounder Ringer is −79 mV and that E_K = −83 mV

As shown in Fig. 6*A*, charybdotoxin produces complete inhibition of the voltage-activated inward current at 300 nM (filled symbols). In Fig. 6*B*, the concentration-response relationship indicated that the IC_{50} for charybdotoxin was 50 nM.

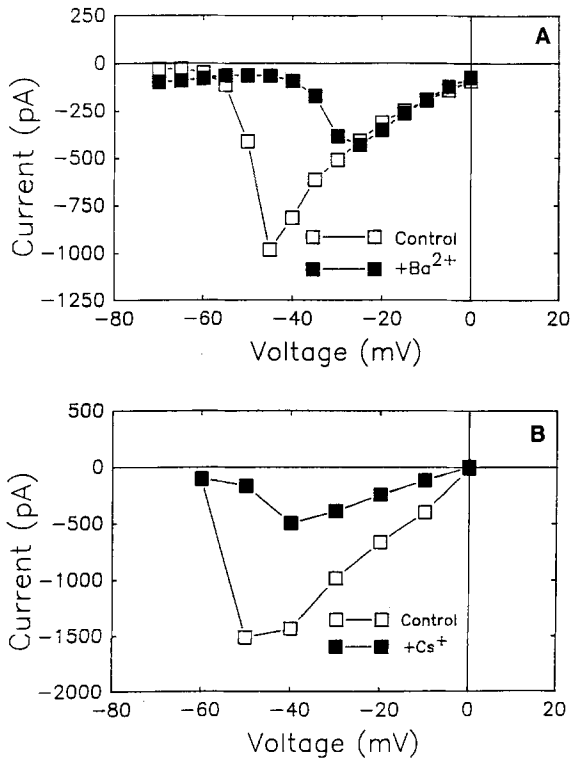

Fig. 5. (*A*) Voltage-dependent effects of 2 mM $BaCl_2$ (in the bathing solution) on the steady-state current of a cell bathed in symmetric KCl Ringer solution. The cell was held at −80 mV, and the current was activated by depolarization from −70 to 0 mV (5-mV steps). (*B*) Voltage-dependent effects of 0.5 mM CsCl on the steady-state current of a cell in symmetric KCl Ringer solution. This cell was held at −80 mV, and the current was activated by depolarization from −60 to 0 mV (10-mV steps)

Effects of 8-Br cGMP on Whole-Cell Current

Figure 7*A* and *B* demonstrates the effect of 8-Br cGMP on whole-cell current in flounder Ringer solution. Figure 7*A* shows the control condition and Fig. 7*B* shows the resulting current 5 min after addition of 300 μM 8-Br cGMP to the bathing solution. 8-Br cGMP produced a dramatic inhibition of outward current at voltages above −30 mV when cells were bathed in flounder Ringer solution. Maximum inhibition of the current always occurred within 5 min following application of 8-Br cGMP to the bathing solution. This effect was observed in all cells (six enterocytes from three animals) that were tested. In Figure 8*A* and *B*, the effects of 8-Br cGMP on cells bathed in symmetric K Ringer solution are presented. Figure 8*A* shows the control condition where Na and Cl were replaced with K and methane sulfonate. An inward current with properties similar to results with symmetric KCl Ringer solution (shown in Fig. 4*A*) was observed. 8-Br cGMP produced a decrease in inward current and nearly abolished the

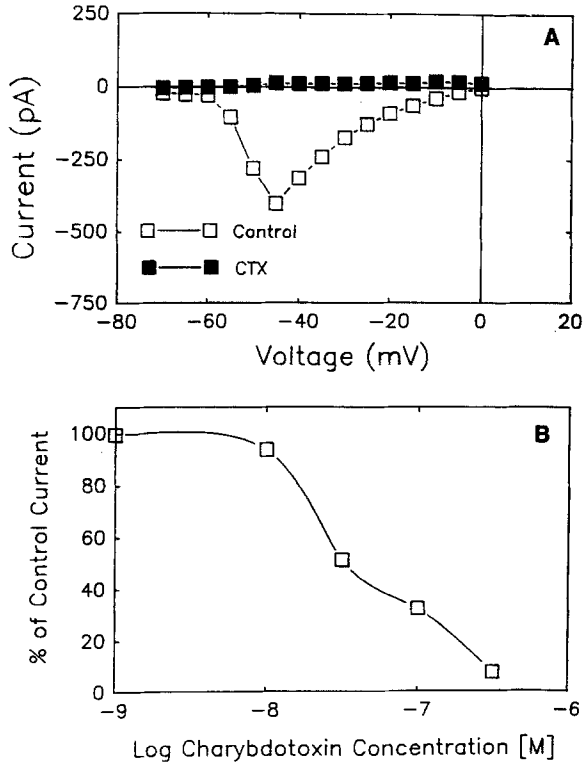

Fig. 6. (*A*) Effects of 0.3 μM charybdotoxin on the steady-state current of a cell bathed in symmetric KCl Ringer solution. The cell was held at −80 mV and step depolarized from −70 to 0 mV. Charybdotoxin was added to the bathing solution and produced complete inhibition of the current at all voltages. (*B*) Concentration-response curve showing the % of the control current (at −45 mV) remaining after treatment with various concentrations of charybdotoxin. The IC_{50} was estimated to be 50 nM using linear-regression analysis of the data points between 10 and 300 nM

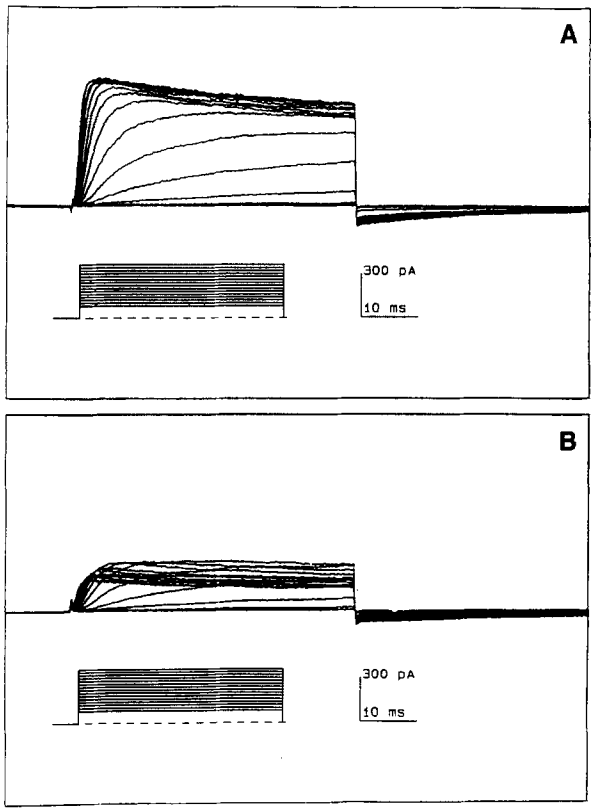

Fig. 7. (*A*) Whole-cell current from an enterocyte bathed in flounder Ringer solution and held at −80 mV (control condition). Outward current was activated by depolarizing the cell from −70 to 0 mV (5-mV steps, pulse duration = 50 msec). (*B*) Effects of 300 μM 8-Br cGMP (5-min exposure) on the whole-cell current following the voltage step protocol described in *A*

large tail currents observed under control conditions. The steady-state current-voltage relationships for the results shown in Fig. 7*A* and *B* are presented in Fig. 9*A*. In the control experiment, the activation-voltage threshold was approximately −55 mV. At −35 mV, nearly complete activation of outward current was observed. In the presence of 8-Br cGMP, the activation threshold was −60 mV and the outward current showed a biphasic response to depolarizing voltage steps with a maximum current at −45 mV. These results indicated that 8-Br cGMP lowers the threshold for activation of the whole-cell current by approximately 5 mV and potentiates the voltage-dependent inactivation of the current at all voltages greater than −45 mV. To determine whether the results reported above were specific for cGMP, we examined the effects of 8-Br cAMP on whole-cell current in flounder Ringer solution. The effects of 8-Br cAMP (600 μM) on whole-cell current are shown in Fig. 9*B*. In contrast to the effects of 8-Br cGMP, cAMP produced no effect on outward current after 10 min at a twofold higher concentration than that used for 8-Br cGMP. In Fig. 10*A* and *B*, a tail current experiment is presented that examines the effects of 8-Br cGMP in symmetric K (K-methane sulfonate) Ringer solution on the open-channel current-voltage relationship. In this experiment cells were held at −80 mV and step depolarized to 0 mV in order to activate all of the channels. After the current stabilized, the cell was stepped through a series of voltages ranging from −120 to +60 mV. In the absence of 8-Br cGMP the tail current results were similar to those in symmetric K (KCl) Ringer solution reported in Fig. 4*B*. The instantaneous current-voltage relationship indicated strong inward rectification (Fig. 10*C*). When 8-Br cGMP was added to the bathing solution a large decrease in inward current was observed. The current-voltage plot shown in Fig. 10*C* shows that 8-Br cGMP produced a proportional decrease in current at each voltage step. The simplest explanation for this effect is that 8-Br cGMP inactivated a large fraction of single channels, resulting in a decrease in the magnitude of whole-cell current at each voltage step compared to the control condition.

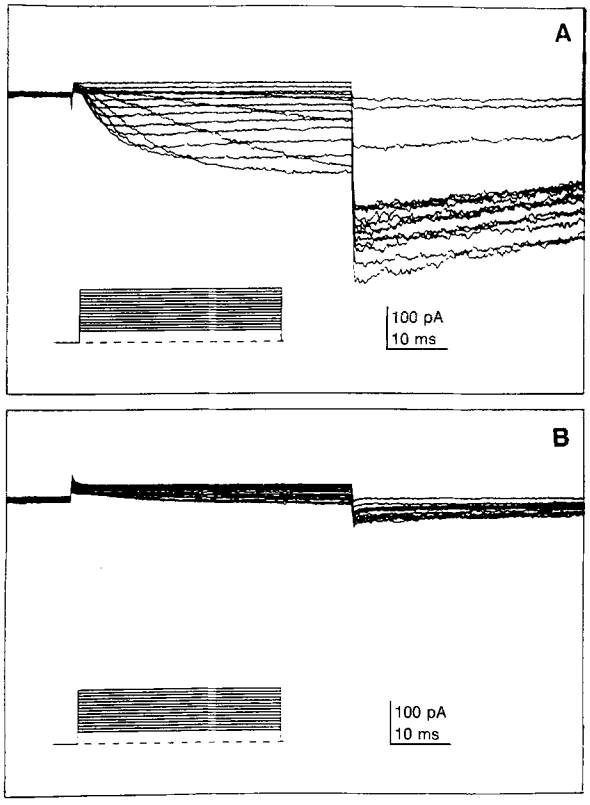

Fig. 8. (*A*) Whole-cell current from a cell bathed in symmetric K-methane sulfonate Ringer solution. Voltage-dependent activation of inward current was achieved with the protocol described in Fig. 7*A*. (*B*) Effects of 300 μM 8-Br cGMP (5-min exposure) on the inward current shown in *A*

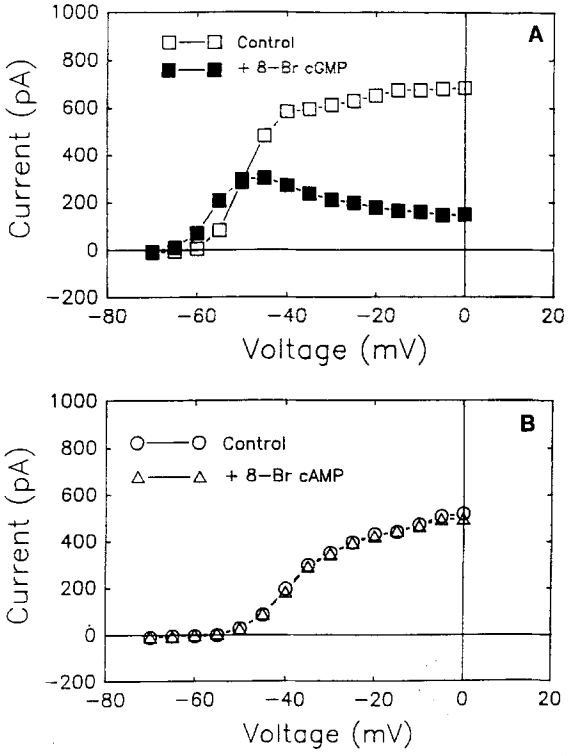

Fig. 9. (*A*) Comparison of the steady-state current-voltage relationship before and after treatment with 300 μM 8-Br cGMP. The cell was held at −80 mV and depolarized from −70 to 0 mV. Cyclic GMP reduced the voltage-activation threshold and potentiated the voltage-dependent inactivation of the current at voltages above −45 mV. (*B*) Comparison of the steady-state current-voltage relationship before and after treatment with 600 μM 8-Br cAMP. The holding potential and voltage step protocol were identical to the conditions reported in *A*. No effect of 8-Br cAMP on whole-cell current was observed

Discussion

In this study we used a variation of the whole-cell recording technique to investigate the properties and regulation of K channels in dissociated flounder enterocytes [16]. When amphotericin was used to achieve the whole-cell configuration, significantly lower series resistances were obtained and significantly more negative resting voltages were observed than with the conventional whole-cell recording technique. In addition, the inactivation observed with increasing depolarization was very reproducible when amphotericin was used, whereas in some cases (*see* Fig. 2), inactivation was not observed under conventional whole-cell conditions. This difference may result from the loss or dilution of an intracellular factor involved in the inactivation process when the membrane patch was ruptured.

The results of experiments presented in Fig. 2 clearly demonstrated that a voltage-activated current was present in dissociated flounder enterocytes. Activation of this current occurred at a threshold which was close to the mean resting membrane potential of these cells (−39 mV). This current was sensitive to holding potential and could not be activated if the cell was held at −40 mV or above. When cells were placed in symmetric K Ringer solution and held at −80 mV, activation of the current by step depolarizations above −50 mV produced inward currents consistent with movement of K into the cell in response to the negative intracellular potential. Analysis of the instantaneous tail currents obtained at various hyperpolarizing and depolarizing voltages indicated that the instantaneous current was inwardly rectifying in symmetric K Ringer solution with a reversal potential consistent with K as the current-carrying ion. Analysis of the tail currents from cells bathed in flounder Ringer solution indicated that the reversal potential lies very close to E_K and suggested that the channel was more selective for K than Na.

Experiments with known blockers of K channels (Ba^{2+}, Cs^+ and charybdotoxin) also supported

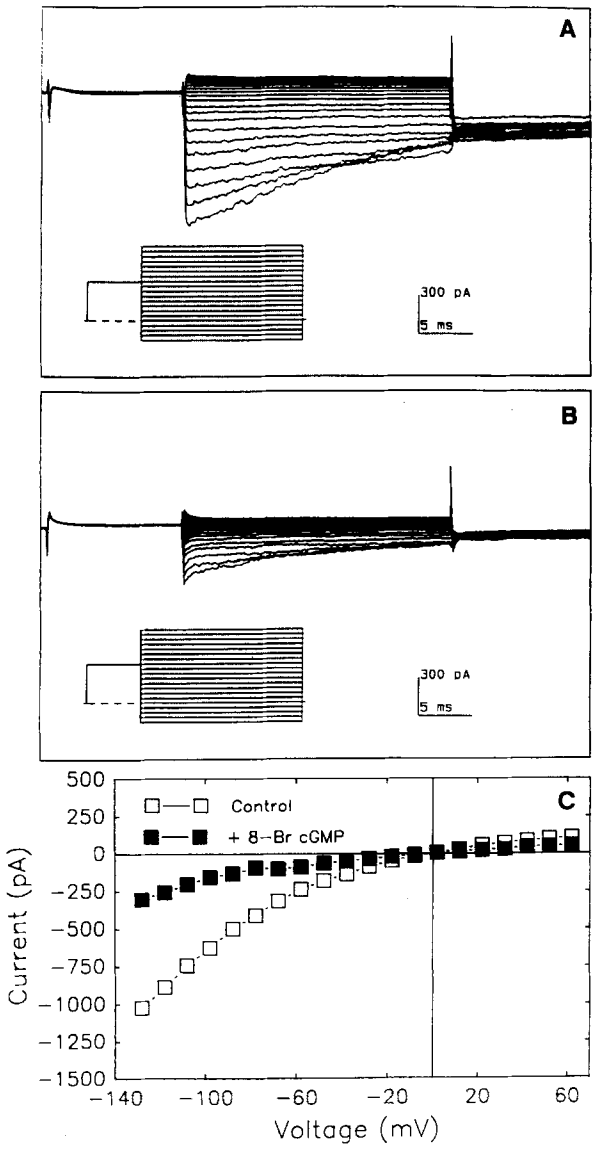

Fig. 10. Tail current experiment in symmetric K-methane sulfonate Ringer solution showing the effects of 8-Br cGMP on inward current. (*A*) Control condition where the cell was held at −80 mV, depolarized to 0 mV for 15 msec and then stepped through a series of voltages from −130 to +60 mV. (*B*) Remaining tail currents after treatment with 300 μM 8-Br cGMP. (*C*) Instantaneous (peak) current-voltage relationship in the presence and absence of 8-Br cGMP

the hypothesis that a K channel was responsible for the voltage-activated current observed in this study. Both Ba^{2+} and Cs^{+} have been previously shown to block voltage-activated channels in a variety of cell types [5, 8, 11, 18]. Charybdotoxin is a basic peptide that inhibits large conductance Ca^{2+}-activated K channels presumably by physical occlusion of the channel opening. All three blockers have been previously shown to inhibit an intermediate conductance (18–60 pS) Ca^{2+}-activated K channel in molluscan neurons and erythrocytes [1, 18].

The results presented in Figs. 7–10 clearly demonstrated that cGMP is an effective inhibitor of the whole-cell current. These effects were selective for cGMP since 8-Br cAMP did not produce a similar response. Previous studies of ion transport in intact epithelial preparations of flounder intestine in vitro indicated that K recycling was blocked by addition of 8-Br cGMP to the bathing solution [6, 17]. This effect could be reversed if cGMP-dependent protein phosphorylation was blocked by subsequent addition of the isoquinoline sulfonamide derivative H-8 [14]. If the K channel described in this study was involved in K recycling and secretion, it would be reasonable to suggest that cGMP-dependent protein phosphorylation is involved in its regulation. It is worth noting that cGMP has been shown to inhibit a nonselective, amiloride-sensitive cation channel in intermedullary collecting duct cells [10]. Inhibition of this channel could be produced by cGMP directly and by activation of a purified cGMP-dependent protein kinase added to excised membrane patches. The phosphorylation-dependent effects of cGMP were reversed by treatment with H-8 [9].

In a recent study by Sullivan et al. [19], poly A containing mRNA isolated from winter flounder enterocytes was injected into *Xenopus* oocytes. These oocytes expressed an inwardly rectifying K channel with greater selectivity for K over Na or Cl. This channel was tonically active and displayed voltage-independent kinetics. Barium, quinine and tetraethylammonium blocked the channel, whereas apamin, charybdotoxin and 4-aminopyridine had no effect. The K channel was rapidly and completely inhibited by 4β-phorbol 12-myristate 13-acetate, suggesting a role for protein kinase C in the regulation of this channel. Thus, it would appear that the channel expressed in *Xenopus* oocytes is distinct from the K channel described in this study. However, it is important to consider the possibility that differences in voltage sensitivity and blocker pharmacology may be due to incomplete expression and assembly of subunit proteins that make up this channel or cytoplasmic factors that may alter the properties of the channel.

In summary, the results of this study demonstrated the presence of a voltage-activated K current in dissociated flounder enterocytes. This current was blocked by known inhibitors of K channels (Ba^{2+}, Cs^{+} and charybdotoxin) and inhibited by 8-Br cGMP. Since the whole-cell recording technique and dissociated enterocytes were used in this study, it could not be determined whether this K channel was located in the apical or basolateral membrane of the cell. It is worth noting that previous

electrophysiological studies of flounder enterocytes in the intact epithelium have demonstrated that the K conductance of the basolateral membrane is very low relative to the apical membrane. Moreover, Ba produced significant depolarizations of the apical membrane consistent with inhibition of K channels. Similarly, Ba and 8-Br cGMP also inhibit net K secretion across the intact epithelium. Although the exact function of the K channel described in this study remains uncertain, the results of previous studies and those presented in this paper provide evidence for a possible role in K recycling and secretion across the epithelium.

The authors wish to thank Cheryl Buller and Jon Charlesworth for their help in the preparation of dissociated cells for scanning electron microscopy. This work was supported by National Institutes of Health research grants AM-38197 (to S.M.O.), EY-03282 and EY-06005 to J.L.R.

References

1. Castle N.A., Haylett, D.G., Jenkinson, D.H. 1989. Toxins in the characterization of potassium channels. *Trends Neurosci.* **12:**59–65
2. Dawson, D.C., Richards, N.W. 1990. Basolateral K conductance: Role in regulation of NaCl absorption and secretion. *Am. J. Physiol.* **259:**C181–195
3. Duffey, M.E., Thompson, S.M., Frizzell, R.A., Schultz, S.G. 1979. Intracellular chloride activities and active chloride absorption in the intestinal epithelium of the winter flounder. *J. Membrane Biol.* **50:**331–341
4. Field, M., Karnaky, K.J., Jr., Smith, P.L., Bolton, J.E., Kinter, W.B. 1978. Ion transport across the isolated intestinal mucosa of the winter flounder, *Pseudopleuronectes americanus:* I. Functional and structural properties of cellular and paracellular pathways for Na and Cl. *J. Membrane Biol.* **41:**265–293
5. Hagiwara, S., Miyasaki, S., Rosenthal, N.P. 1976. Potassium current and the effects of cesium on this current during anomalous rectification of the egg cell membrane of the starfish. *J. Gen. Physiol.* **67:**621–638
6. Halm, D.R., Krasny, E.J., Frizzell, R.A. 1985. Electrophysiology of the flounder intestinal mucosa. I. Conductance properties of the cellular and paracellular pathways. *J. Gen. Physiol.* **85:**843–864
7. Hunter, M., Geibisch, G. 1988. Calcium-activated K channel of *Amphiuma* early distal tubule: Inhibition by ATP. *Pflueg-ers Arch.* **412:**331–333
8. Lewis, R.S., Cahalan, M.D. 1990. Ion channels and signal transduction in lymphocytes. *Annu. Rev. Physiol.* **52:**415–430
9. Light, D.B., Corbin, J.D., Stanton, B.A. 1990. Dual ion channel regulation by cGMP and cGMP-dependent protein kinase. *Nature* **344:** 336–339
10. Light, D.B., McCann, F.V., Keller, T.M., Stanton, B.A. 1988. Amiloride sensitive cation channel in apical membrane of inner medullary collecting duct. *Am. J. Physiol.* **255:**F278–F286
11. McCann, J.D., Welsh, M.J. 1990. Regulation of Cl and K channels in airway epithelium. *Annu. Rev. Physiol.* **52:**115–135
12. Miller, C., Moczydlowski, E., Latorre, R., Phillips, M. 1985. Charybdotoxin, a protein inhibitor of single Ca-activated K channels from mammalian skeletal muscle. *Nature* **313:**316–318
13. Musch, M.W., Orellana, S.A., Kimberg, L.S., Field, M., Halm, D.R., Krasny, E.J., Frizzell, R.A. 1982. Na-K-Cl cotransport in the intestine of a marine teleost. *Nature* **300:**351–353
14. O'Grady, S.M., DeJonge, H.R., Vaandrager, A.B., Field, M. 1988. Cyclic nucleotide-dependent protein kinase inhibition by H-8: Effects on ion transport. *Am. J. Physiol.* **254:**C115–C121
15. O'Grady, S.M., Palfrey, C.H., Field, M. 1987. Characteristics and functions of Na-K-Cl cotransport in epithelial tissues. *Am. J. Physiol.* **253:**C177–C192
16. Rae, J., Cooper, K., Gates, P., Watsky, M. 1991. Low access resistance perforated patch recordings using amphotericin B. *J. Neurosci. Methods* **37:**15–26
17. Rao, M.C., Nash, N.T., Field, M. 1984. Differing effects of cGMP and cAMP on ion transport across flounder intestine. *Am. J. Physiol.* **246:**C167–C171
18. Rudy, B. 1988. Diversity and ubiquity of K channels. *Neuroscience* **25:**729–749
19. Sullivan, S.K., Swamy, K., Greenspan, N.R., Field, M. 1990. Epithelial K channel expressed in *Xenopus* oocytes is inactivated by protein kinase C. *Proc. Natl. Acad. Sci. USA* **87:**4553–4556
20. Wang, W., White, S., Geibel, J., Giebisch, ■. 1990. A potassium channel in the apical membrane of the rabbit thick ascending limb of Henle's loop. *Am. J. Physiol.* **258:**F244–F253

Received 18 March 1991; revised 20 June 1991