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IDENTIFICATION OF A BASOLATERAL MEMBRANE POTASSIUM CHANNEL FROM TELEOST INTESTINAL EPITHELIAL CELLS

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Summary

Using patch-clamp techniques, a Ca^{2+}-dependent, voltage-gated K^+ channel [K(Ca) channel] was isolated from the basolateral membrane of NaCl-absorbing intestinal epithelial cells of the goby *Gillichthys mirabilis*. This K(Ca) channel had a high conductance (approximately 150 pS) in the physiological range of membrane potential. Conclusive identification as a K^+ channel is supported by dependence of the reversal potential for single-channel current on the K^+ concentration gradient and the ability of Ba^{2+}, Cs^+ and other pharmacological agents to block the channel. The channel was highly selective for K^+ over Na^+ (P_{Na}/P_K=0.04). Channel activity, expressed as open probability (P_o), was dependent on membrane potential with depolarization increasing P_o over the physiological range in the presence of Ca^{2+}. Channel activity was also dependent on cytoplasmic-side Ca^{2+}. P_o was reduced to near-zero levels following EGTA chelation of Ca^{2+} in the solution bathing the cytoplasmic face of excised membrane patches; channel activity was most sensitive to changes in Ca^{2+} concentration between 10 nmol l^{-1} and 10 μmol l^{-1}. This K(Ca) channel may be one of several avenues for K^+ exit across the basolateral cell membrane and, as such, may play roles in both transepithelial salt transport and maintenance of intracellular ionic composition.

Introduction

The intestinal epithelium in teleost fishes plays an important role in osmoregulation through the active absorption of Na^+ and Cl^- from the gut lumen. The general mechanisms of ion transport have been described for several species of teleost fish and are in most respects very similar (Field *et al.* 1978; Duffey *et al.* 1979; Frizzell *et al.* 1979; Ramos and Ellory, 1981; Musch *et al.* 1982; Loretz, 1983). Na^+ and Cl^- transport proceed continuously in the net absorptive direction despite changes in drinking rate and gut fluid composition, which vary as functions of the salinity of adaptation (Loretz and Bern, 1982). The absorption of Na^+ and Cl^- begins with the coupled transport of these ions across the apical (luminal)

Key words: K^+ channel, fish intestinal epithelium, patch-clamp, *Gillichthys mirabilis*.

membrane *via* a furosemide-sensitive Na^+/Cl^- or $Na^+/K^+/2Cl^-$ cotransporter driven by the Na^+ electrochemical gradient. Once admitted into the cell, Na^+ is actively extruded across the basolateral cell membrane by the Na^+/K^+-ATPase while Cl^-, accumulated above electrochemical equilibrium, exits down its gradient into the serosal solution *via* basolateral membrane anion (Cl^-) channels (Loretz and Fourtner, 1988), Cl^-/HCO_3^- exchange (Dixon and Loretz, 1986) or K^+/Cl^- cotransport (Stewart *et al.* 1980). K^+ is accumulated in the cell above electrochemical equilibrium by the Na^+/K^+-ATPase and the cotransporter, and it can leave the cell across either the apical or the basolateral cell membranes according to their relative permeabilities to this ion, producing either net secretion or absorption, respectively. In addition to this role in K^+ absorption and/or secretion, K^+ conductance may be regulated in order to maintain constancy of the intracellular K^+ concentration in the face of altered NaCl absorption (Dawson and Richards, 1990).

Evidence for K^+ exit across the cell membranes of teleost intestinal epithelial cells comes predominantly from electrophysiological studies using conventional intracellular glass microelectrodes and also from radiotracer flux studies. Previous work on the goby and flounder intestines suggests that apical membrane K^+ exit is largely conductive, whereas basolateral membrane transport occurs as nonconductive K^+/Cl^- cotransport (Stewart *et al.* 1980; Halm *et al.* 1985*a*,*b*; Loretz *et al.* 1985). Control of apical and basolateral K^+ transport, *via* intracellular regulators such as Ca^{2+}, would permit control over net K^+ transport. Such possibilities are supported empirically by observations that mucosal Ba^{2+} addition, which blocks apical membrane K^+ conductance, will reverse net transepithelial K^+ secretion into absorption (Stewart *et al.* 1980; Halm *et al.* 1985*a*,*b*); mucosal Ba^{2+} treatment has no effect on net Cl^- absorption in the goby (Loretz *et al.* 1985), however, suggesting an avenue for basolateral K^+ exit in addition to K^+/Cl^- symport.

This report presents the results of patch-clamp studies designed to identify and characterize K^+ channels from the basolateral membrane of intestinal epithelial cells of the gobiid teleost *Gillichthys mirabilis*. We describe a high-conductance, voltage-gated, Ca^{2+}-dependent K^+ channel which probably functions in basolateral membrane K^+ transport.

Some of this material has been reported previously in abstract form (Loretz and Fourtner, 1989, 1990).

Materials and methods

Dissociated intestinal cell preparation

All experiments were performed on dissociated cells from the stripped mucosal layer of the intestine of the euryhaline goby *Gillichthys mirabilis* Cooper. Gobies weighing 20–50 g were obtained from commercial suppliers in California and fully adapted in the laboratory to Instant Ocean artificial sea water (Aquarium Systems, Mentor, OH) at a salinity of 34 ‰. Fish were maintained under a constant 12 h:12 h L:D photoperiod at 12°C. Experiments were conducted at room

Table 1. *Composition (mmol l^{-1}) of standard and experimental solutions*

Constituent	K-ES	GBR	G35MES
Na^+		161.4	35
K^+	140	2.5	35
Ca^{2+}	1	2.5	
Mg^{2+}		1	
Cl^-	142	144.5	35
Isethionate$^-$		20	
Mes$^-$			35
HCO_3^-		5	
HPO_4^{2-}		0.7	
Hepes	10		10
EGTA			0.1
Sucrose	60		170
Glucose		5	

GBR was bubbled with 99 % O_2:1 % CO_2; others were bubbled with 100 % O_2.
Final pH of all solutions was 7.6.

temperature (18–20°C). A full description of the cell isolation procedure appears elsewhere (Loretz and Fourtner, 1988). Briefly, following decapitation and pithing of the fish in accordance with accepted laboratory animal care guidelines, the mucosal layer of intestinal tissue was stripped from isolated segments of posterior intestine using the edge of a glass microscope slide and then dissociated with collagenase (0.1–0.3 mg ml^{-1}) in chilled *Gillichthys* bicarbonate-buffered Ringer's solution (GBR, composition in Table 1). Dissociated cells were collected by gentle centrifugation (500 *g*, 5 min); the pellet, composed predominantly of epithelial cells, was resuspended in fresh GBR, filtered through Nitex cloth, and plated onto 35 mm polystyrene culture dishes. Individual cells, many exhibiting a clearly visible brush border membrane, were selected for study. Identity as epithelial cells and viability of this preparation have been assessed previously (Loretz and Fourtner, 1988). Dishes with cells were kept cool and used within 4–6 h of preparation.

Solutions

Table 1 shows the basic composition of the several solutions used in these experiments. In the text, solutions are referred to by the abbreviations in the table. To facilitate observation and recording of K^+ channel currents, a pipette solution with a high K^+ concentration [K^+ electrode solution (K-ES), 140 mmol l^{-1} K^+] was used rather than the GBR (2.5 mmol l^{-1} K^+) as in previous studies (Loretz and Fourtner, 1988). The formulation of the G35MES bath solution was such as to yield unique calculated reversal potentials for the major ionic species, specifically: Na^+, a large negative (undefined) membrane potential; K^+, +35 mV; Cl^-, −35 mV. Free Ca^{2+} concentrations in the G35MES bath solution (buffered to pH 7.6 with 10 mmol l^{-1} Hepes) were set by adjustment of the EGTA and Ca^{2+}

concentrations to values derived from the tables and methods of Bartfai (1979), Fabiato and Fabiato (1979) and Durham (1983); for these experimental conditions, $K^{Ca\text{-}EGTA}$ at pH 7.6 was calculated to be $3.82\times10^7\,l\,mol^{-1}$. The stated free Ca^{2+} concentrations were achieved with the following combinations of Ca^{2+} and EGTA: $0.5\,mmol\,l^{-1}$ free Ca^{2+}, $0.1\,mmol\,l^{-1}$ EGTA + $0.6\,mmol\,l^{-1}$ Ca^{2+}; $10^{-4}\,mol\,l^{-1}$ free Ca^{2+}, $0.1\,mmol\,l^{-1}$ EGTA + $0.2\,mmol\,l^{-1}$ Ca^{2+}; $10^{-5}\,mol\,l^{-1}$ free Ca^{2+}, $1.0\,mmol\,l^{-1}$ EGTA + $1.0\,mmol\,l^{-1}$ Ca^{2+}; $10^{-8}\,mol\,l^{-1}$ free Ca^{2+}, $0.7\,mmol\,l^{-1}$ EGTA + $0.2\,mmol\,l^{-1}$ Ca^{2+}. Osmotic pressures of all solutions were routinely monitored and never differed from $320\,mosmol\,l^{-1}$ by more than $5\,mosmol\,l^{-1}$; where necessary, osmotic pressures were adjusted by mannitol or sucrose addition. Ba^{2+} or Cs^+ treatment of excised patches was achieved by addition of a small amount of concentrated $BaCl_2$ or CsCl stock solution to the culture dish followed by gentle mixing.

Patch-clamp apparatus

The patch-clamp system used in our laboratory has been fully described (Loretz and Fourtner, 1988) and was used in these studies with only slight modification. Briefly, the experimental apparatus is based around a List-Medical model LPC-7 patch-clamp amplifier (List-Medical, Darmstadt, West Germany) with associated electronics for voltage-clamp command, as well as data storage, filtering, display and analysis. The major specific modification to our published system was the replacement of FM tape recorder storage with digital video cassette tape recorder (VCR) storage; the frequency response of the VCR system (16 kHz, −3 dB) exceeded that of the filtered single-channel data output from the patch-clamp amplifier (10 kHz, −3 dB, four-pole low-pass Bessel filter).

The K^+ channel appeared as a large inward membrane current in either on-cell or excised patch recordings in any of the bath solutions when the recording pipette solution was K-ES and the membrane potential (V_m) was near physiological values. Inward current refers to the flow of positive charge from the pipette solution to the cytoplasmic side of the membrane patch; inward currents appear as downward deflections in our current traces and as negative currents in figures. In the on-cell configuration, V_m equals the magnitude of the resting cell membrane potential (approximately −60 mV) plus an offset equal to the potential applied from the patch-clamp amplifier (V_p), with V_m and V_p reported relative to the patch pipette solution; in excised, inside-out membrane patches, $V_m = V_p$. Data collection generally began with the pipette in the on-cell configuration where single-channel current fluctuations were often observed. Excision of the patch from the cell (excised, inside-out configuration) allowed for change of the solution bathing the cytoplasmic face of the patch. Apparent vesicle formation by the membrane patch in the pipette tip was seen as a cessation in channel activity; brief exposure of the pipette tip to air generally restored observable channel activity.

For analysis, recorded data were filtered to yield an effective bandwidth (f_c) of either 2.5 or 1.5 kHz (−3 dB, eight-pole Bessel filter) as specified, digitized at 20 kHz and stored on computer hard disk. Single-channel data were analyzed

using the IPROC2 program (principles and application described in Sachs *et al.* 1982; Colquhoun and Hawkes, 1983; Colquhoun and Sigworth, 1983; Sachs, 1983; Auerbach and Sachs, 1984); representative data recordings 20–60 s or more in duration for each membrane potential and experimental treatment were subjected to analysis. IPROC2 analysis yielded single-channel characteristics including: single-channel current (I_c) and lifetimes (half-times) for the exponentially distributed open and closed states. Single-channel conductance (g_c) was calculated as the slope of plots relating I_c to V_m; regression analysis was applied to the linear portions of the graph that included the physiological range of membrane potential. The plots of I_c *versus* V_m were typically linear over a broad physiological range (V_m=0 to −100 mV) but exhibited reductions in slope when the membrane patch was strongly depolarized (V_m>0 mV). The open probability of a single channel (P_o) was calculated from the total current amplitude histogram as the proportion of time spent in the open state. For membrane patches containing more than one channel, P_o was calculated from the relative peak areas in total current amplitude histograms using binomial distribution and with the assumption of independence.

Statistics

Data are presented as the mean±1 S.E.M. Specific statistical tests were applied as specified in the text with a critical level for significance of $P<0.05$. Statistical tests were performed using the Crunch Statistical Software Package (Version 3.1; Crunch Software Corporation, 1987).

Results

As reported previously (Loretz and Fourtner, 1988), patch-clamp data collected from isolated intestinal epithelial cells probably reflect single-channel activity in membrane patches sampled from the basolateral membrane of the enterocyte, since the relative surface area of the basolateral membrane greatly exceeds that of the apical membrane and also since the complex geometry of the brush border is an impediment to gigaohm seal formation on that surface. In practice, the patch recording pipette could easily be manoeuvred into position on the smooth basolateral surface of the cell. Although a potential complication regarding the assignment of observed single-channel activity to the basolateral membrane is the apparent ability of membrane channel proteins to migrate between apical and basolateral membrane domains, the presence of Ca^{2+} in the bathing solutions during cell dissociation and patch formation and the maintenance of cells at temperatures no greater than room temperature may retard such redistribution (Ziomek *et al.* 1980; Sepulveda and Mason, 1985). Seal resistance of basolateral membrane patches generally exceeded 10 GΩ.

In prior studies on the characterization of an anion channel from this same tissue (Loretz and Fourtner, 1988), single-channel records from an apparent K^+ channel were obtained but the single-channel currents (<2 pA) and conductance (20–40 pS) were small owing to the low K^+ concentrations in the pipette and bath

solutions. Reliable and repeatable observation of K^+ channels required the use of a KCl-based patch pipette solution in place of the normal NaCl-based saline used in our isolation of anion channels. Yellen (1984*a*,*b*) reported that internal Na^+ will block K(Ca) channels *via* a fast or flickery block mechanism and that external K^+ will relieve that blockade. Perhaps in our earlier studies using a NaCl-based pipette solution, excision of the patch into normal (NaCl-rich) GBR produced such a Na^+ blockade which could not be relieved with the K^+-poor pipette solution. Of more than 25 membrane patches containing single ion channels, 15 patch recordings were obtained for which complete analysis for several V_m values and bath solutions were performed.

Fig. 1 illustrates the typical appearance of the single-channel current record of this K^+ channel. In our recording configuration, channel openings appeared as downward deflections to a stable I_c characterized by increased noise and brief flicker closings to the zero current level. Openings of single K^+ channels were often clustered together as bursts which, arbitrarily, for the purpose of analysis were defined as a series of single-channel openings separated by closed periods of less than 2 ms.

The K^+ channels observed in these experiments showed uniformly large conductances and exhibited reductions in conductance with strong depolarization. There was no significant difference between the conductance of channels bathed in GBR compared with those bathed in G35MES in the physiological range (143±6 pS in GBR and 168±15 pS in G35MES over the range V_m=0 to −100 mV; P=0.11, two-tailed t-test, N=9). However, in the range of strong membrane depolarization (V_m>0 mV), channels bathed in GBR showed a lower conductance compared with those bathed in G35MES (38±17 pS in GBR and 74±6 pS in G35MES; P<0.05, two-tailed t-test, N=5). The observed reductions in conductance were precisely those predicted by the Goldman–Hodgkin–Katz (GHK) current equation (Hille, 1984) for a K^+ conductance in a membrane bathed in the asymmetrical solutions used here.

Fig. 2 presents I_c–V_m relationships for a single K^+ channel in an excised inside-out membrane patch bathed first in GBR and then in G35MES. As predicted from the calculated K^+ reversal potential of +101 mV in GBR, I_c fell towards zero at large positive values of V_m. Increasing the K^+ concentration in the solution bathing the cytoplasmic face of the channel from 2.5 to 35 mmol l^{-1} by replacement of GBR with G35MES produced the reduction in reversal potential of the channel to a less positive value of V_m that would be expected for a K^+ channel and increased g_c at V_m>0 mV in accordance with the GHK current equation (Hille, 1984). The average reversal potential for six patches bathed in G35MES with added Ca^{2+} (+33.7±1.9 mV) was not significantly different from the predicted value of V_m=+35 mV for a K^+-selective channel.

As illustrated in Figs 1, 6 and 10, the channel is voltage-gated over a wide range of V_m, encompassing the physiological range. P_o decreases from about 0.9 to near zero with hyperpolarization from V_m=0 to −100 mV.

Kinetic data were derived from nine membrane patches each containing a single

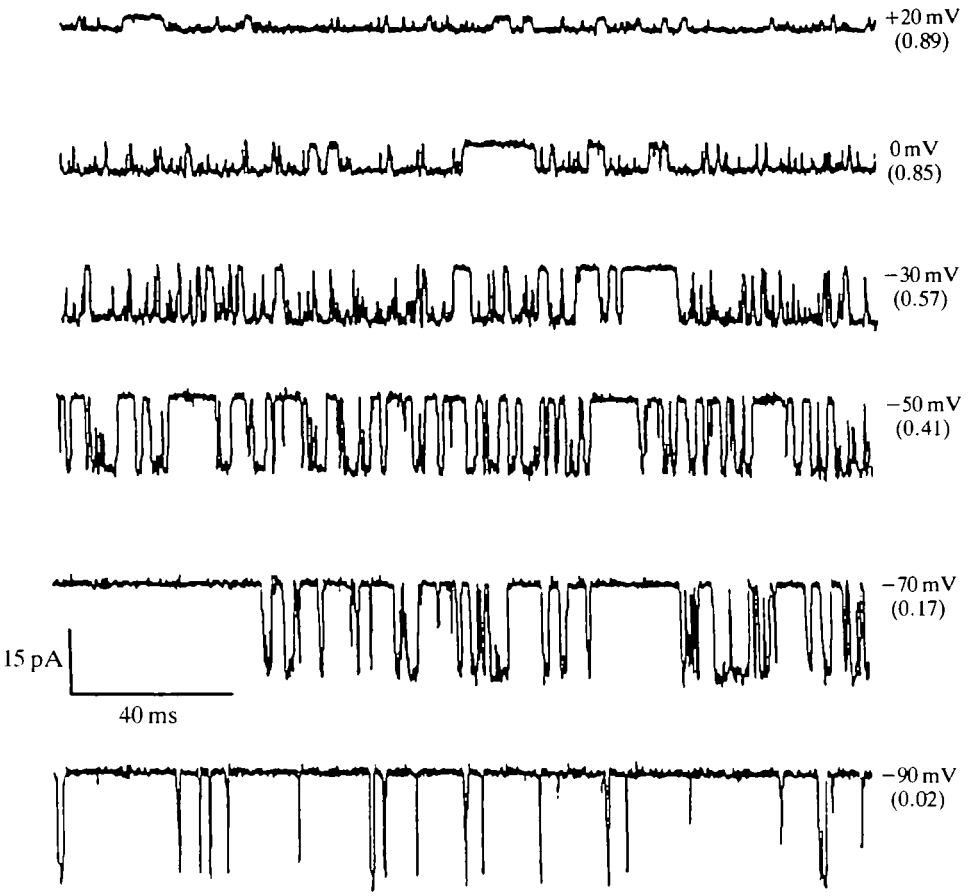

Fig. 1. Typical single-channel current records from a basolateral membrane K^+ channel. For this excised, inside-out patch recording, the pipette contained K-ES (140 mmol l^{-1} K^+) and the bath contained G35MES+0.5 mmol l^{-1} Ca^{2+} (35 mmol l^{-1} K^+). In the physiological range of V_m (approximately −60 mV), channel openings appear as downward deflections in the current record relative to the current level of the closed state. Channel activity is generally steady at any V_m with the open state characterized by greater noise and rapid flickering. Occasionally, as shown here, channels show short periods of inactivity. V_m is the electrical potential across the membrane patch with the patch pipette solution designated as ground. The probability of the channel being found in the open state (P_o, displayed in parentheses) decreased with membrane hyperpolarization (f_c=1.5 kHz, g_c=179 pS).

ion channel for which data were collected over a wide range of V_m (Fig. 3A–D). Burst length and the number of open events per burst decreased with hyperpolarization, as did single-channel open time. Closed time increased with hyperpolarization. Deviations from the basic trend of these data in the strongly depolarized region (V_m=+50 to +100 mV) reflect, in part, skewing due to representation by only about half of the membrane patches in this voltage range.

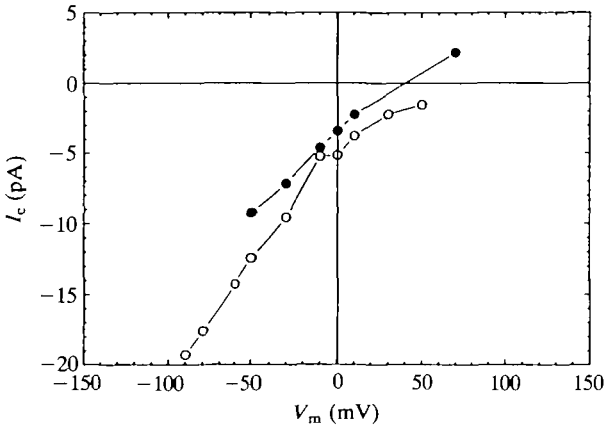

Fig. 2. I_c–V_m plot for a K^+ channel in an excised, inside-out membrane patch bathed in 2.5 mmol l^{-1} K^+ (GBR, ○) and in 35 mmol l^{-1} K^+ (G35MES+0.1 mmol l^{-1} Ca^{2+}, ●). The reversal potential for the patch bathed in G35MES+0.1 mmol l^{-1} Ca^{2+} was +30 mV (extrapolated from data points from V_m = −50 mV to +10 mV), whereas that for the patch bathed in GBR was extrapolated to be about +100 mV.

K^+ selectivity of this channel is further supported by the ability of both Ba^{2+} and Cs^+ to block the channel in excised, inside-out membrane patches. Addition of 2 mmol l^{-1} Ba^{2+}, a concentration known to produce a complete block of apical membrane K^+ conductance in this tissue (Loretz *et al.* 1985), slightly diminished I_c and g_c (see Fig. 5); Ba^{2+} also acts in this tissue by reducing P_o (Fig. 4; see Fig. 6). Interestingly, the Ba^{2+} blockade is voltage-dependent, with the effect being most striking on channels in depolarized membrane patches ($V_m > -50$ mV); Ba^{2+} has virtually no effect on P_o in normally polarized and hyperpolarized patches ($V_m < -50$ mV) when added to the cytoplasmic face. The data presented in Figs 4–6 are typical of experiments on at least four membrane patches. In contrast to the slow channel block produced by Ba^{2+}, addition of Cs^+ at the high concentration of 10 mmol l^{-1} to the solution bathing the cytoplasmic face of the membrane patch produced rapid flicker in the current record typical of a fast channel block (Fig. 7). In the several membrane patches tested, Cs^+ blockade showed only slight, if any, voltage dependence and reduced I_c and g_c (data not shown). Although less marked in effect, Cs^+ treatment at lower concentrations (50–100 μmol l^{-1}) also blocked the channel (data not shown).

Channel activity is also dependent on Ca^{2+}. Removal of Ca^{2+} from the bathing solution by substitution of EGTA-containing G35MES for GBR dramatically reduced P_o and in some cases abolished channel activity with little effect on I_c or g_c (Figs 8–10). Following substitution with low-Ca^{2+} media, voltage sensitivity is retained, although it is weaker (e.g. 10^{-5} mol l^{-1} Ca^{2+} in Fig. 10). The site of Ca^{2+} action is at the cytoplasmic face of the channel since the K-ES bathing the extracellular face contains 1 mmol l^{-1} Ca^{2+}. Addition of Ca^{2+} to the Ca^{2+}-free G35MES bathing the cytoplasmic face of membrane patches increased single-

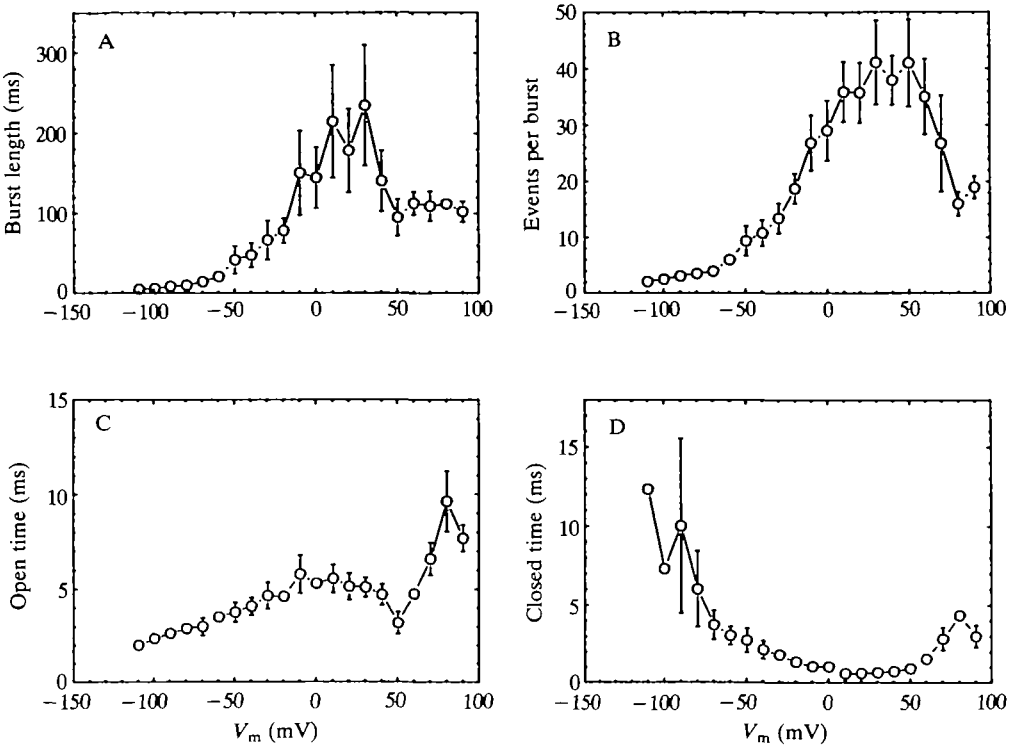

Fig. 3. Relationship of single-channel kinetics to V_m for nine excised, inside-out membrane patches bathed in either GBR or G35MES with added Ca^{2+}. (A) Burst length, (B) events per burst, (C) open time, (D) closed time. Data are expressed as the mean±1 S.E.M. See text for details.

channel activity (expressed as P_o; Fig. 10). The data illustrated in Figs 8–10 are typical of experiments on four membrane patches. In each case, channel activity was most sensitive to changes in Ca^{2+} concentration between 10 nmol l^{-1} and 10 μmol l^{-1}. Addition of Ca^{2+} at concentrations of 10^{-4} mol l^{-1} or greater to the G35MES solution bathing membrane patches generally restored channel activity to a level near that seen in the GBR control.

Discussion

Application of the patch-clamp technique to goby intestinal epithelia has permitted direct observation of ion-transporting membrane channels previously investigated by electrophysiological and radiotracer flux measurements (Loretz, 1983; Loretz *et al.* 1985; Dixon and Loretz, 1986). In our previous studies on goby intestine, we described a basolateral membrane anion channel which probably functions *in vivo* as one of several avenues for basolateral membrane Cl^- exit (cf. Dixon and Loretz, 1986; Loretz and Fourtner, 1988). In this report, we describe a high-conductance, voltage-gated, Ca^{2+}-dependent K^+ channel [K(Ca) channel,

using the terminology of Hille, 1984] from the basolateral membrane of goby intestinal epithelium.

Patch-clamp identification of a basolateral membrane K(Ca) channel

In our previous report (Loretz and Fourtner, 1988), we argued that membrane patches with anion channels were captured from the basolateral membrane

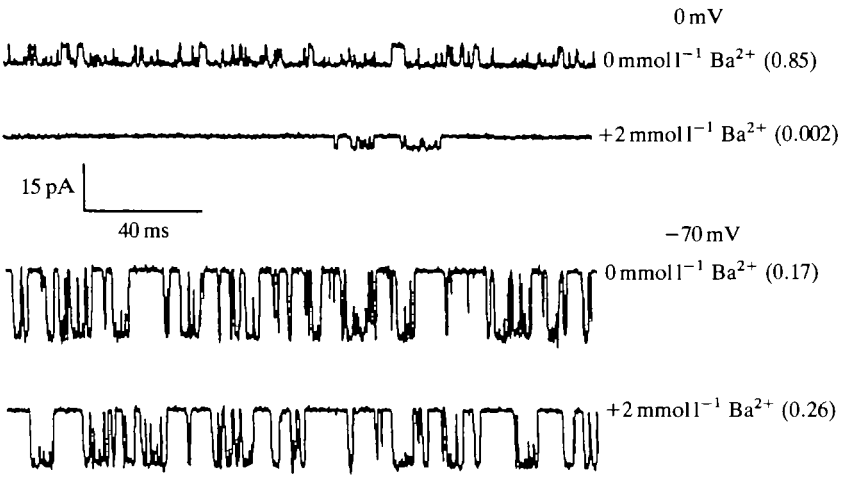

Fig. 4. Single-channel current records from a basolateral membrane K^+ channel in an excised, inside-out membrane patch bathed in 35 mmol l^{-1} K^+ (G35MES+0.5 mmol l^{-1} Ca^{2+}) at V_m=0 and −70 mV in the absence and presence of 2 mmol l^{-1} Ba^{2+}. Addition of 2 mmol l^{-1} Ba^{2+} to the bathing solution reduced channel activity (expressed as P_o, displayed in parentheses) in depolarized (0 mV) membrane patches but had little effect in hyperpolarized (−70 mV) membrane patches (f_c=1.5 kHz)

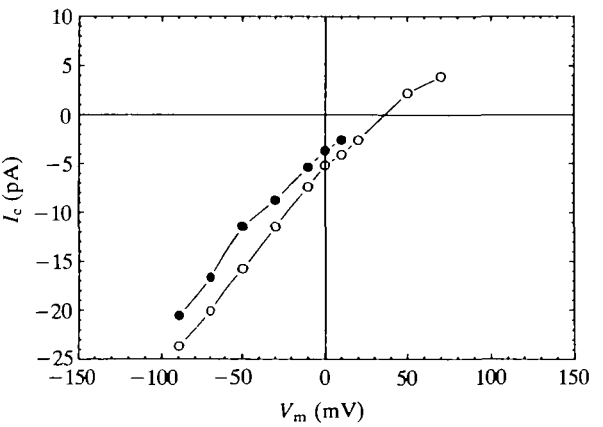

Fig. 5. I_c–V_m plots for a basolateral membrane K^+ channel in an excised, inside-out membrane patch bathed in G35MES+0.5 mmol l^{-1} Ca^{2+}. Comparison of the plots for solutions without (○, g_c=179 pS) and with (●, g_c=181 pS) 2 mmol l^{-1} Ba^{2+} demonstrates that Ba^{2+} has no effect on single-channel conductance and only slightly reduces single-channel current.

because of its relatively large surface area and simple topography (compared with the relatively small but morphologically complex apical membrane). For these same reasons, the K(Ca) channel we have identified is probably located in the basolateral membrane. In our earlier experiments, K^+ channels were seldom observed using GBR (2.5 mmol l^{-1} K^+) as the patch pipette solution and, when observed, they were typified by low single-channel currents and conductances

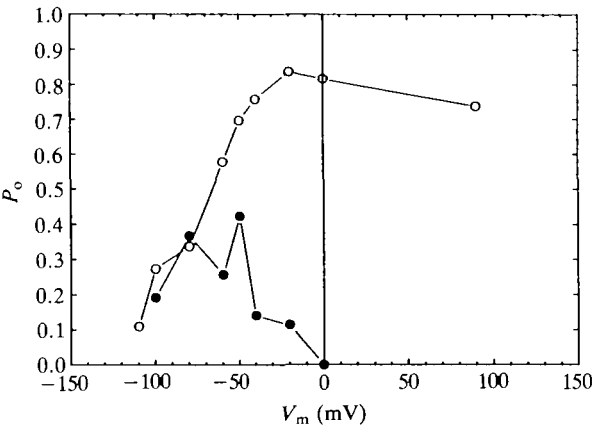

Fig. 6. Relationship of P_o to V_m for a basolateral membrane K^+ channel in an excised, inside-out membrane patch bathed in G35MES+10^{-4} mol l^{-1} Ca^{2+} without (○) and with (●) 2 mmol l^{-1} Ba^{2+}. Ba^{2+} blockade of K^+ channel activity (as P_o) is voltage-dependent, being more effective with membrane depolarization.

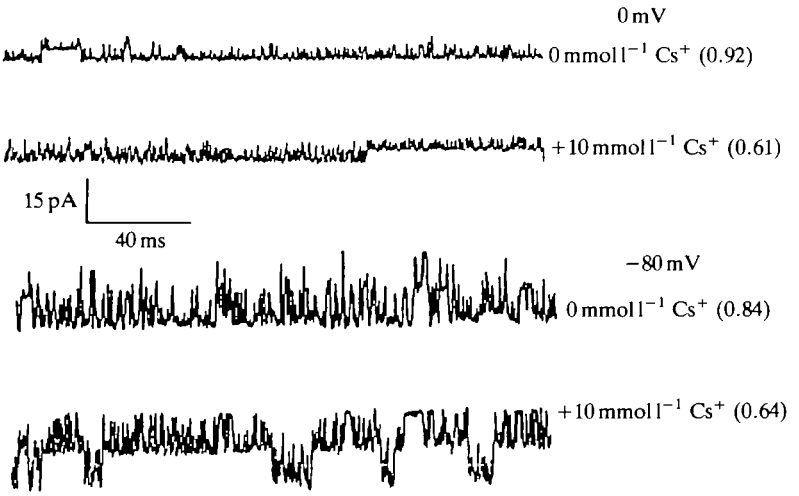

Fig. 7. Single-channel current records from a basolateral membrane K^+ channel in an excised, inside-out membrane patch bathed in GBR (2.5 mmol l^{-1} K^+) at V_m=0 and −80 mV in the absence and presence of 10 mmol l^{-1} Cs^+. Addition of Cs^+ to the bathing solution produced a fast flicker block, reducing P_o (displayed in parentheses) and slightly reducing I_c (V_m=0 mV: −Cs^+ 3.5 pA, +Cs^+ 3.3 pA; V_p=−80 mV: −Cs^+ 11.3 pA, +Cs^+ 10.8 pA) and g_c (−Cs^+ 110 pS, +Cs^+ 98 pS) (f_c=2.5 kHz).

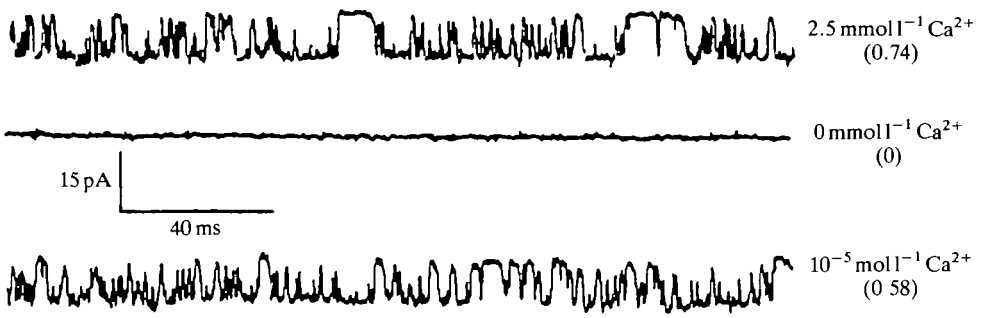

Fig. 8. Single-channel current records from a basolateral membrane K^+ channel in an excised, inside-out membrane patch at $V_m = -60$ mV in solutions of varying calcium concentration. The channel was active (expressed as P_o, displayed in parentheses) in the presence of 2.5 mmol l^{-1} Ca^{2+} (GBR). Channel activity was abolished in the absence of Ca^{2+} (G35MES+0 mmol l^{-1} Ca^{2+}) and restored once again following the addition of Ca^{2+} to the bathing medium (G35MES+10^{-5} mol l^{-1} Ca^{2+}) (f_c=1.5 kHz).

(C. A. Loretz and C. R. Fourtner, unpublished observations, see above). Use of K-ES as the patch-pipette solution substantially improved the success in observing K^+ channels. With K-ES as the pipette solution, single-channel K(Ca) currents were consistently large (up to 20–25 pA) and single-channel conductance was observed to be roughly 150 pS in the physiological range of V_m. Membrane patches containing the voltage-gated goby intestinal anion channel were also observed with the K-ES pipette solution.

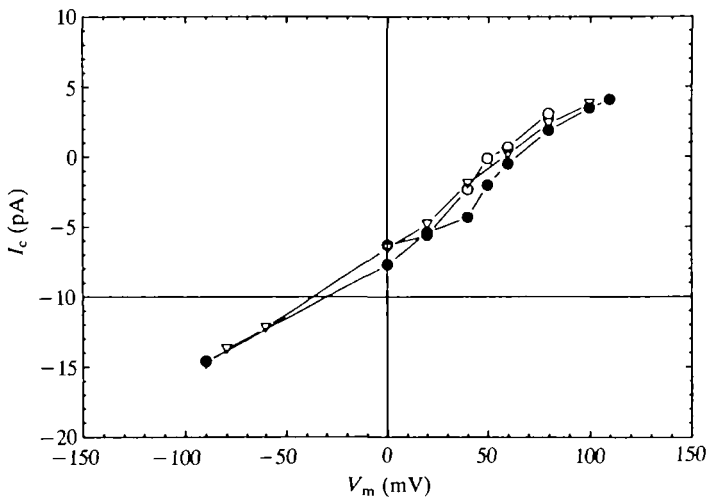

Fig. 9. I_c–V_m plots for a basolateral membrane K^+ channel in an excised, inside-out membrane patch bathed in G35MES solutions with varying Ca^{2+} concentrations. Coincidence of the plots for solutions containing 10^{-8} mol l^{-1} Ca^{2+} (○, g_c=127 pS), 10^{-5} mol l^{-1} Ca^{2+} (●, g_c=98 pS) and 10^{-4} mol l^{-1} Ca^{2+} (△, g_c=100 pS) demonstrates that Ca^{2+} has no effect on single-channel conductance.

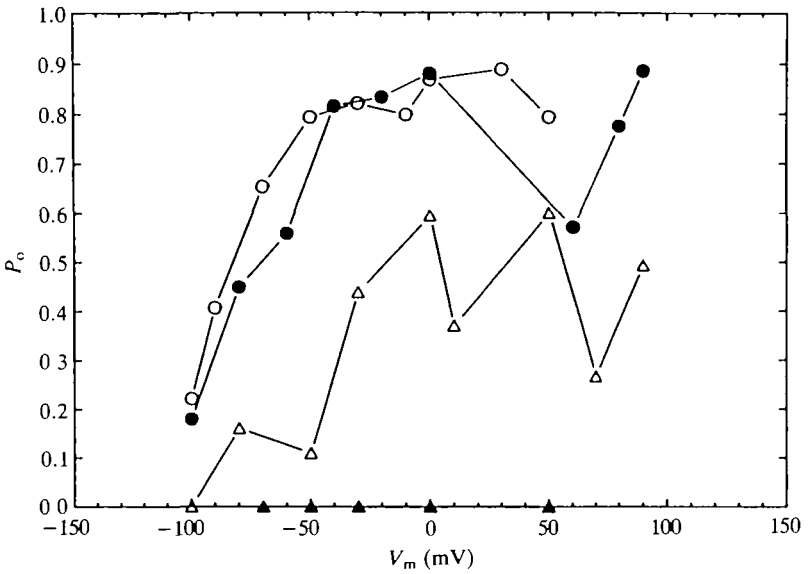

Fig. 10. Relationship of P_o to V_m for a basolateral membrane K^+ channel in an excised, inside-out membrane patch bathed in solutions with varying Ca^{2+} concentrations. Lowered Ca^{2+} concentrations in the bathing solution brought about reductions in open probability. GBR (2.5 mmol l^{-1} Ca^{2+}), ○; G35MES+10^{-4} mol l^{-1} Ca^{2+}, ●; G35MES+10^{-5} mol l^{-1} Ca^{2+}, △; G35MES+0 mmol l^{-1} Ca^{2+}, ▲.

Identification of this channel as a K^+ channel is supported by two lines of evidence. First, I_c–V_m plots demonstrated the dependence of I_c on the K^+ concentration gradient with the observed reversal potential for the single-channel current very nearly that predicted for K^+ by the Nernst equation. Second, the single-channel current was blocked by Ba^{2+} and Cs^+ (this report) and also by tetraethylammonium (TEA^+), quinidine and lidocaine (Loretz and Fourtner, 1990; C. A. Loretz and C. R. Fourtner, in preparation), consistent with the known sensitivity to these agents of K^+ channels in many tissues, including fish intestinal epithelia (Armstrong *et al.* 1982; Hille, 1984; Halm *et al.* 1985*a*,*b*; Loretz *et al.* 1985; Armstrong and Matteson, 1986; Sheppard *et al.* 1988; Castle *et al.* 1989; Turnheim *et al.* 1989). A full report on the dose-dependence of pharmacological blockade in this channel is in preparation. In addition to these rigorous criteria for identifying this K^+ channel, it can readily and practically be distinguished from the anion channel by the large conductance and more frequent openings with greater flicker activity (see Loretz and Fourtner, 1988).

The relative selectivity of this K(Ca) channel for Na^+ with reference to K^+ (P_{Na}/P_K) is estimated to be 0.04 from application of the GHK equation to six membrane patches bathed in G35MES-based solutions where current reversal was observed and reliable estimates of reversal potential could be derived graphically and by linear regression analysis. For several of these six membrane patches, complete I_c–V_m plots were obtained for several bathing solutions of G35MES with

various Ca^{2+} concentrations; for these patches, the observed reversal potential and g_c were independent of Ca^{2+} concentration, suggesting that Ca^{2+}-dependence operates as a simple gating mechanism without affecting the distribution and/or geometry of charged sites in the channel, which may determine selectivity or conductance.

The K(Ca) channel we describe here is not unlike the large-unitary-conductance Ca^{2+}-activated K^+ channels [maxi-K channels, BK(Ca) channels] from a number of other tissues. The conductance of the goby basolateral membrane K(Ca) channel (approximately 150 pS) is within the range of single-channel conductances (90–307 pS) reported by Latorre (1986) and Castle *et al.* (1989) for a number of maxi-K channels, including those from renal tubule epithelium (Hunter *et al.* 1984; Guggino, 1986) and intestinal epithelium (Sheppard *et al.* 1988; Turnheim *et al.* 1989). Also, like other maxi-K channels, the goby K(Ca) channel is highly selective for K^+ over Na^+ and is voltage-dependent (Latorre, 1986; Sheppard *et al.* 1988; Turnheim *et al.* 1989).

The K(Ca) channel is voltage-dependent

The K(Ca) channel from goby intestine is voltage-dependent over the physiological range of membrane potential, with depolarization bringing about a several-fold increase in P_o. The K(Ca) channel lacks the long and frequent interburst intervals typical of the voltage-gated anion channel from goby intestinal epithelium (Loretz and Fourtner, 1988). Hyperpolarization of the K(Ca) channel brings about a general and uniform decrease in activity. Over the physiological range of membrane potential ($V_m = -100$ to -30 mV), average channel current, $\bar{I}$ ($=I_c \times P_o$), increases with membrane patch depolarization, suggesting that total basolateral membrane Ca^{2+}-dependent K^+ current *in vivo* also increases with depolarization. Measurement of cell membrane potential in intact epithelial sheets using conventional glass intracellular microelectrodes indicates that in healthy cells the membrane potential is in the range from -30 to -90 mV (Loretz *et al.* 1985; Loretz, 1987, 1990). This corresponds nicely to the range of V_m over which channel activity is voltage-gated and suggests that only during the strongest transport stimulation, when depolarization is observed, or during inhibition, when hyperpolarization results, would P_o approach values of unity or zero, respectively.

K(Ca) channel kinetics

The goby K(Ca) channel exhibits rapid open–close kinetics at physiological membrane potentials, with openings of single channels grouped as bursts. Bursts are generally long in duration; this is especially apparent in depolarized membrane patches, where the open probability is high. With hyperpolarization, the flicker closings within the bursts increase in duration and divide long bursts into shorter bursts composed of fewer individual channel openings. This trend continues with increasing V_m until, at strongly hyperpolarized potentials, the current record contains almost exclusively uniformly spaced, single, short-duration ion channel openings. The voltage-dependence of both open and closed times suggests that the

transition rate from closed to open states decreases with hyperpolarization, whereas that from open to closed states increases. At all holding potentials, bursts of single-channel activity are very occasionally separated by long closed periods lasting up to several hundred milliseconds. These longer closed periods constitute another type of closed state and suggest another mode of regulation (e.g. phosphorylation–dephosphorylation).

Ba^{2+} blocks the K(Ca) channel in a voltage-dependent manner

Ba^{2+} blocks the K(Ca) channel when added to the bathing solution of excised, inside-out membrane patches. The efficacy of the Ba^{2+} block is voltage-dependent, with the effect on P_o progressively increasing with membrane depolarization; complete blockade is achieved as V_m approaches zero. Clearly, Ba^{2+} is more effective when the electrical gradient favours Ba^{2+} entry into the channel, a finding also reported previously for a basolateral membrane K^+ channel from turtle colon epithelial cells (Richards and Dawson, 1986). Ba^{2+} blockade of K^+ channels in the squid giant axon is voltage-dependent in the same manner; the effect is symmetrical, with Ba^{2+} application either internally or externally being fully effective in the appropriately directed electrical field (Armstrong and Taylor, 1980; Armstrong *et al.* 1982). The Ba^{2+} blockade of the K(Ca) channel is not permanent since return of a depolarized, blocked channel to normal physiological values of V_m will reversibly restore activity. The voltage-dependence of the Ba^{2+} blockade also supports the notion of others (Armstrong and Taylor, 1980; Hille, 1984) that Ba^{2+} does not bind irreversibly to block the channel but, instead, may be permeable yet pass only very slowly through the channel.

Based on the strong voltage-dependence of the Ba^{2+} blockade, a Ba^{2+} binding site within the electrical field of the membrane, and probably within the pore itself, can be proposed. If this is the case, then the steepness of the voltage-dependence for Ba^{2+} blockade is a function of the electrical distance of the Ba^{2+} site through the membrane field. Using a modification of the method of Armstrong and Taylor (1980) for the calculation of the equilibrium dissociation constant (K_D) for the blockade of K^+ channels by Ba^{2+}, and applying it to single channels:

$$K_D = \frac{[Ba^{2+}](1 - \%B)}{\%B},$$

where %B is the percentage blockade by Ba^{2+}, we determined the dependence of K_D on V_m to be e-fold per 14.4 mV for single K(Ca) channels in three membrane patches. This magnitude of voltage-dependence suggests that the Ba^{2+} site experiences about 90 % of the electrical field and, therefore, that the Ba^{2+} site may be located almost all of the way through the channel from the cytoplasmic mouth of the pore. This estimate of the electrical distance of the Ba^{2+} binding site compares favourably with the values for K(Ca) channels from guinea pig arterial and rabbit intestinal smooth muscle cell membranes, where the Ba^{2+} binding site is located about 95 % of the way through the channel from the cytoplasmic side

(Benham *et al.* 1985), and in basolateral membrane K^+ channels from turtle colon epithelial cells, where the Ba^{2+} binding site is about 90 % of the way through the channel (calculated from the published data of Richards and Dawson, 1986). In some other K^+ channels, the Ba^{2+} binding site experiences less of the electrical field. For example, an electrical distance of half to three-quarters was reported previously for the delayed rectifier K^+ channel of squid giant axon (Armstrong and Taylor, 1980; Eaton and Brodwick, 1980) and a Ba^{2+} site approximately 80 % of the way through the channel from the *cis* (internal), or Ca^{2+}-sensitive, side was found for the large-unitary-conductance Ca^{2+}-activated K^+ channel of T tubule from rabbit skeletal muscle (Vergara and Latorre, 1983; Latorre, 1986; Yellen, 1987).

The K(Ca) channel is Ca^{2+}-activated

The activity of the K(Ca) channel, expressed as P_o, is dependent on the presence of Ca^{2+}. In the absence of Ca^{2+}, the channel is inactive, regardless of the membrane potential. Addition of Ca^{2+} to the solution bathing the cytoplasmic face of the membrane patch increases P_o in a roughly concentration-dependent manner and exposes voltage-dependent activity over the physiological range of V_m. Channel activity is dependent only on cytoplasmic-side Ca^{2+}, apparently, since complete removal of Ca^{2+} from the solutions bathing excised patches abolished activity, despite the presence of 1 mmol l^{-1} Ca^{2+} in the pipette solution.

Basolateral membrane ion channels have a role in transport

Establishment of a complete cellular model defining physiological roles for ion channels, and their regulation, in epithelial transport across goby intestinal cells is difficult, since single ion channels from the luminal membrane have not been characterized. Nonetheless, a partial scheme can be proposed. In our earlier report, we suggested that the voltage-dependence of the basolateral membrane anion channel provided a mechanism for coupling cellular Cl^- exit into the serosal medium to cellular ion accumulation across the luminal membrane *via* the $Na^+/K^+/2Cl^-$ cotransporter in response to factors such as the transport-stimulating neuropeptide urotensin II (UII), which causes cellular depolarization (Loretz *et al.* 1985; Loretz and Fourtner, 1988; Loretz, 1990). In goby intestine, cytoplasmic Ca^{2+} acts *via* a calmodulin-dependent mechanism to inhibit NaCl absorption (Loretz, 1987); UII stimulates NaCl absorption across the goby intestine *via* a decrease in cytoplasmic Ca^{2+} concentration (Loretz and Assad, 1986). In our proposed scheme, the UII-induced reduction in cytoplasmic Ca^{2+} concentration, through a consequent reduction in basolateral membrane K^+ conductance, may bring about the observed cellular depolarization and, thereby, the increased activity of the basolateral membrane anion channel promoting Cl^- exit during transport stimulation. Supporting the notion that intracellular Ca^{2+} may, in fact, be a physiological regulator of K(Ca) channel activity are electrophysiological studies on goby intestine where treatment with the Ca^{2+} ionophore A23187 produced cellular hyperpolarization (Loretz, 1987).

The cellular depolarization that follows K(Ca) channel inactivation (i.e. decrease in P_o) will tend to self-limit the decrease in P_o and stabilize channel activity. Not unlike the stability proposed by Dawson and Richards (1990) to result from a closed-loop feedback system, with sensitivities to both Ca^{2+} and voltage in the physiological ranges, the K(Ca) channels will be regulated and will tend to restrict fluctuations in membrane potential to some particular range. Given the strong dependence on Ca^{2+} in the physiological range of V_m, as suggested by the data of Fig. 10, reduction in cytoplasmic Ca^{2+} will probably produce some degree of depolarization, despite any modulation through voltage-dependence. Greater dependence on Ca^{2+} than on V_m is also supported by data from basolateral membrane K(Ca) channels of *Necturus* enterocytes and rabbit colonocytes (Sheppard *et al.* 1988; Turnheim *et al.* 1989). Precise assessment of Ca^{2+} sensitivity in the physiological range will require accurate chelation of cytoplasmic Ca^{2+} in the submicromolar range characteristic of these cells (Loretz and Assad, 1986).

Regulation of the apical membrane cotransporter by a Ca^{2+}–calmodulin-dependent mechanism would permit coordinated regulation by UII *via* Ca^{2+} of apical and basolateral membrane transport processes. Ca^{2+}–calmodulin dependence of apical membrane transport processes is suggested by the studies of Donowitz *et al.* (1984) demonstrating Ca^{2+}–calmodulin-dependent changes in phosphorylation state of intestinal brush-border membrane proteins in rabbit, although the identity of these proteins as transport-related is uncertain. There are also reports on reconstituted membrane systems proposing that Ca^{2+}–calmodulin inhibition of K(Ca) channel function is directly on the channel protein and independent of phosphorylation (Klaerke *et al.* 1987; Turnheim *et al.* 1989, 1990).

The fractional resistance of the apical membrane (relative to the basolateral membrane) of goby intestinal epithelial cells increases with UII stimulation of NaCl absorption (Loretz *et al.* 1985). One interpretation of this finding is that the basolateral membrane K^+ conductance decreases less than the Cl^- conductance increases in response to UII; alternatively, a decrease in another Ca^{2+}-dependent conductance (another K^+ channel?) in the apical membrane may contribute to the increase in fractional resistance. Future characterization of single ion channels from the apical membrane will shed light on this question.

Recently, Dawson and Richards (1990) have outlined the importance of K^+ conductance in the maintenance of cellular mass and charge balance in ion-transporting epithelial tissues. In the fish intestine, for example, stoichiometric balance of apical and basolateral membrane ion fluxes may involve a multiplicity of ion transport pathways for K^+ and Cl^-. Consider the case for stoichiometries of (for our purposes here) Na^+:K^+:2Cl^- for the apical membrane cotransporter and 3Na^+:2K^+ for the basolateral membrane Na^+/K^+-ATPase. Extrusion of 3Na^+ by one cycle of the Na^+/K^+-ATPase would be matched by three cycles of the cotransporter which, taken together, would result in the cellular accumulation of 5K^+ and 6Cl^-. Since there are multiple avenues for Cl^- exit (anion channel, Cl^-/HCO_3^- exchange, K^+/Cl^- symport), which may variably be active and only one of which involves K^+, the basolateral membrane K^+ conductance provides an

exit path to balance inward movement. There is no *a priori* requirement that the entire K^+ imbalance be dissipated across the basolateral cell membrane. There is a substantial K^+ conductance in the apical membrane suggestive of K^+ secretion in both goby and flounder intestine (Halm *et al.* 1985*a*,*b*; Loretz *et al.* 1985). K^+ secretion can be reversed to become absorption by Ba^{2+} blockade of apical membrane K^+ conductance (Stewart *et al.* 1980; Halm *et al.* 1985*a*,*b*). Moreover, since the apical membrane cotransporter may apparently operate in different modes ($Na^+/K^+/Cl^-$ or Na^+/Cl^-; Halm *et al.* 1985*a*,*b*), schemes for stoichiometric mass and charge balance may be more complex, and especially so when the regulatory effects of intracellular mediators are included, allowing for both K^+ absorption and secretion.

Through patch-clamp studies of single voltage-dependent K(Ca) channels, we have demonstrated in this report that Ca^{2+} may serve as an intracellular regulator of conductive basolateral K^+ exit in goby intestine, where hormonal regulators of NaCl absorption such as UII bring about alterations in free cytoplasmic Ca^{2+} concentrations (Loretz *et al.* 1985; Loretz and Assad, 1986; Loretz, 1987). Further, involvement of Ca^{2+} suggests the potential for coordinated regulation of both NaCl absorption and K^+ transport through an intercellular messenger system.

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References

ARMSTRONG, C. M. AND MATTESON, D. R. (1986). The role of calcium ions in the closing of K channels. *J. gen. Physiol.* **87**, 817–832.

ARMSTRONG, C. M., SWENSON, R. P., JR AND TAYLOR, S. R. (1982). Block of squid axon K channels by internally and externally applied barium ions. *J. gen. Physiol.* **80**, 663–682.

ARMSTRONG, C. M. AND TAYLOR, S. R. (1980). Interaction of barium ions with potassium channels in squid giant axons. *Biophys. J.* **30**, 473–488.

AUERBACH, A. AND SACHS, F. (1984). High-resolution patch-clamp techniques. In *Voltage and Patch Clamping with Microelectrodes* (ed. T. Smith, H. Lecar, S. Redman and P. Gage), pp. 121–149. Baltimore: Waverly Press.

BARTFAI, T. (1979). Preparation of metal–chelate complexes and the design of steady-state kinetic experiments involving metal nucleotide complexes. In *Advances in Cyclic Nucleotide Research*, vol. 10 (ed. G. Brooker, P. Greengard and G. A. Robison), pp. 219–242. New York: Raven Press.

BENHAM, C. D., BOLTON, T. B., LANG, R. J. AND TAKEWAKI, T. (1985). The mechanism of action of Ba^{2+} and TEA on single Ca^{2+}-activated K^+-channels in arterial and intestinal smooth muscle cell membranes. *Pflügers Arch. ges. Physiol.* **403**, 120–127.

CASTLE, N. A., HAYLETT, D. G. AND JENKINSON, D. H. (1989). Toxins in the characterization of potassium channels. *Trends Neurosci.* **12**, 59–65.

COLQUHOUN, D. AND HAWKES, A. G. (1983). The principles of the stochastic interpretation of ion-channel mechanisms. In *Single-Channel Recording* (ed. B. Sakmann and E. Neher), pp. 191–263. New York: Plenum Press.

COLQUHOUN, D. AND SIGWORTH, F. J. (1983). Fitting and statistical analysis of single-channel records. In *Single-Channel Recording* (ed. B. Sakmann and E. Neher), pp. 135–175. New York: Plenum Press.

CRUNCH SOFTWARE CORPORATION (1987). *Crunch Statistical Package*. Oakland, CA.

DAWSON, D. C. AND RICHARDS, N. W. (1990). Basolateral K conductance: role in regulation of NaCl absorption and secretion. *Am. J. Physiol.* **259**, C181–C195.

DIXON, J. M. AND LORETZ, C. A. (1986). Luminal alkalinization in the intestine of the goby. *J. comp. Physiol.* B **156**, 803–811.

DONOWITZ, M., COHEN, M. E., GUDEWICH, R., TAYLOR, L. AND SHARPE, G. W. G. (1984). Ca^{2+}–calmodulin–cyclic AMP- and cyclic GMP-induced phosphorylation of proteins in purified microvillus membranes of rabbit ileum. *Biochem. J.* **219**, 573–581.

DUFFEY, M. E., THOMPSON, S. M., FRIZZELL, R. A. AND SCHULTZ, S. G. (1979). Intracellular chloride activities and active chloride absorption in the intestinal epithelium of the winter flounder. *J. Membr. Biol.* **50**, 331–341.

DURHAM, A. C. H. (1983). A survey of readily available chelators for buffering calcium ion concentrations in physiological solutions. *Cell Calcium* **4**, 33–46.

EATON, D. C. AND BRODWICK, M. S. (1980). Effects of barium on the potassium conductance of squid axon. *J. gen. Physiol.* **75**, 727–750.

FABIATO, A. AND FABIATO, F. (1979). Calculator programs for computing the composition of the solutions containing multiple metals and ligands used for experiments in skinned muscle cells. *J. Physiol., Paris* **75**, 463–505.

FIELD, M., KARNAKY, K. J., JR, SMITH, P. L., BOLTON, J. E. AND 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. Membr. Biol.* **41**, 265–293.

FRIZZELL, R. A., SMITH, P. L., VOSBURGH, E. AND FIELD, M. (1979). Coupled sodium–chloride influx across brush border of flounder intestine. *J. Membr. Biol.* **46**, 27–39.

GUGGINO, S. (1986). Channels in kidney epithelial cells. In *Ionic Channels in Cells and Model Systems* (ed. R. Latorre), pp. 207–220. New York: Plenum Press.

HALM, D., KRASNY, E. J., JR AND FRIZZELL, R. A. (1985*a*). Electrophysiology of flounder intestinal mucosa. I. Conductance properties of the cellular and paracellular pathways. *J. gen. Physiol.* **85**, 843–864.

HALM, D., KRASNY, E. J., JR AND FRIZZELL, R. A. (1985*b*). Electrophysiology of flounder intestinal mucosa. II. Relation of the electrical potential profile to coupled NaCl absorption. *J. gen. Physiol.* **85**, 865–883.

HILLE, B. (1984). *Ionic Channels of Excitable Membranes*. Sunderland, MA: Sinauer.

HUNTER, M., LOPES, A. G., BOULPAEP, E. L. AND GIEBISCH, G. H. (1984). Single channel recordings of calcium-activated potassium channels in the apical membrane of rabbit cortical collecting tubules. *Proc. natn. Acad. Sci. U.S.A.* **81**, 4237–4239.

KLAERKE, D. A., PETERSEN, J. AND JORGENSEN, P. L. (1987). Purification of Ca^{2+}-activated K^+ channel protein on calmodulin affinity columns after detergent solubilization of luminal membranes from outer renal medulla. *FEBS Lett.* **216**, 211–216.

LATORRE, R. (1986). The large calcium-activated potassium channel. In *Ion Channel Reconstitution* (ed. C. Miller), pp. 431–467. New York: Plenum Press.

LORETZ, C. A. (1983). Ion transport by the intestine of the goby, *Gillichthys mirabilis*. *Comp. Biochem. Physiol.* **75A**, 205–210.

LORETZ, C. A. (1987). Regulation of goby intestinal ion absorption by the calcium messenger system. *J. exp. Zool.* **244**, 67–78.

LORETZ, C. A. (1990). Recognition by goby intestine of a somatostatin analog, SMS 201–995. *J. exp. Zool. Suppl.* **4**, 31–36.

LORETZ, C. A. AND ASSAD, J. A. (1986). Urotensin II lowers cytoplasmic free calcium concentration in goby enterocytes: measurements using quin2. *Gen. comp. Endocr.* **64**, 355–361.

LORETZ, C. A. AND BERN, H. A. (1982). Prolactin and osmoregulation in vertebrates. *Neuroendocrinology* **35**, 292–304.

LORETZ, C. A. AND FOURTNER, C. R. (1988). Functional characterization of a voltage-gated anion channel from teleost fish intestinal epithelium. *J. exp. Biol.* **136**, 383–403.

LORETZ, C. A. AND FOURTNER, C. R. (1989). Patch clamp study of a calcium-dependent, voltage-gated K channel from fish intestinal epithelium. *Am. Zool.* **29**, 154A.

LORETZ, C. A. AND FOURTNER, C. R. (1990). Pharmacological blockade of single K(Ca) channels from fish intestinal epithelium. *Am. Zool.* **30**, 66A.

LORETZ, C. A., HOWARD, M. E. AND SIEGEL, A. J. (1985). Ion transport in goby intestine: cellular mechanism of urotensin II stimulation. *Am. J. Physiol.* **249**, G284–G293.

MUSCH, M. W., ORELLANA, S. A., KIMBERG, L. S., FIELD, M., HALM, D., KRASNY, E. J. AND FRIZZELL, R. A. (1982). Na^+–K^+–Cl^- co-transport in the intestine of the marine teleost. *Nature* **300**, 351–353.

RAMOS, M. M. AND ELLORY, J. C. (1981). Na and Cl transport across the isolated anterior intestine of the plaice *Pleuronectes platessa*. *J. exp. Biol.* **90**, 123–142.

RICHARDS, N. W. AND DAWSON, D. C. (1986). Single potassium channels blocked by lidocaine and quinidine in isolated turtle colon epithelial cells. *Am. J. Physiol.* **251**, C85–C89.

SACHS, F. (1983). Automated analysis of single channel records. In *Single-Channel Recording* (ed. B. Sakmann and E. Neher), pp. 265–285. New York: Plenum Press.

SACHS, F., NEIL, J. AND BARKAKATI, N. (1982). The automated analysis of data from single ionic channels. *Pflügers Arch.* **395**, 331–340.

SEPULVEDA, F. V. AND MASON, W. T. (1985). Single channel recordings obtained from basolateral membranes of isolated rabbit enterocytes. *FEBS Letts* **191**, 87–91.

SHEPPARD, D. N., GIRALDEZ, F. AND SEPULVEDA, F. V. (1988). Kinetics of voltage- and Ca^{2+} activation and Ba^{2+} blockade of a large-conductance K^+ channel from *Necturus* enterocytes. *J. Membr. Biol.* **105**, 65–75.

STEWART, C. P., SMITH, P. L., WELSH, M. J., FRIZZELL, R. A., MUSCH, M. W. AND FIELD, M. (1980). Potassium transport by the intestine of the winter flounder, *Pseudopleuronectes americanus*: evidence for KCl cotransport. *Bull. Mt Desert Isl. biol. Lab.* **20**, 92–96.

TURNHEIM, K., COSTANTIN, J., CHAN, S. AND SCHULTZ, S. G. (1989). Reconstitution of a calcium-activated potassium channel in basolateral membranes of rabbit colonocytes into planar lipid bilayers. *J. Membr. Biol.* **112**, 247–254.

TURNHEIM, K., COSTANTIN, J. AND SCHULTZ, S. G. (1990). Properties of single K^+ channels from the basolateral membrane of rabbit colon epithelium. *Naunyn-Schmiedeberg's Arch. Pharmac.* **341**, R39.

VERGARA, C. AND LATORRE, R. (1983). Kinetics of Ca^{2+}-activated K^+ channels from rabbit muscle incorporated in planar bilayers. *J. gen. Physiol.* **82**, 543–568.

YELLEN, G. (1984*a*). Ionic permeation and blockade in Ca^{2+}-activated K^+ channels of bovine chromaffin cells. *J. gen. Physiol.* **84**, 157–186.

YELLEN, G. (1984*b*). Relief of Na^+ block of Ca^{2+}-activated K^+ channels by external cations. *J. gen. Physiol.* **84**, 187–199.

YELLEN, G. (1987). Permeation in potassium channels: implications for channel structure. *A. Rev. Biophys. biophys. Chem.* **16**, 227–246.

ZIOMEK, C. A., SCHULMAN, S. AND EDEDIN, M. (1980). Redistribution of membrane proteins in isolated mouse intestinal epithelial cells. *J. Cell Biol.* **86**, 849–857.