A large conductance, voltage- and calcium-activated K^+ channel in the basolateral membrane of rat enterocytes

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The patch-clamp technique was employed to record single channel currents in patches of basolateral membrane from enterocytes isolated from rat small intestine. We demonstrate the presence of a large conductance (250 pS), voltage- and calcium-activated, the maxi, K^+ channel in this membrane. Currents in this K^+ channel were blocked by the application of barium (5 mM) to the extracellular membrane face.

K^+ channel *Enterocyte* *Patch-clamp* *Voltage activation* *Ca^{2+} activation* *Ba^{2+}*

1. INTRODUCTION

The mucosa of the mammalian small intestine is capable of both the absorption and secretion of electrolytes. There are common features shared in recent models of both absorptive and secretory cells [1,2]. Both it is suggested have a resting K^+ permeability pathway which is stimulated in parallel with transport processes [2–5]. In both instances this increase in K^+ permeability is reported to be susceptible to blockade by barium [5,6]. There is also evidence that the K^+ permeability in both the absorptive and secretory cells is regulated by changes in cytosolic calcium concentration, i.e. calcium-activated K^+ conductance [4,7,8].

Here, the patch-clamp technique is employed to record single channel currents in both excised and cell-attached patches of basolateral membrane from enterocytes isolated from rat small intestine.

2. MATERIALS AND METHODS

Male Wistar rats (180–200 g), fed ad libitum, were killed by cervical dislocation. The upper two-thirds of the small intestine were removed and the intestine rinsed in cold saline and inverted. The exposed mucosal surface was distended by filling the ligated serosal sac with saline. The intestinal segment was incubated in an Na citrate, phosphate-buffered solution (mM: Na citrate, 27; NaCl, 96; KCl, 1.5; KH_2PO_4, 1.8; Na_2HPO_4, 5.6; pH 7.4) for 10 min at 37°C in a shaking water bath [9]. This incubation medium was discarded and replaced with fresh Na-Hepes buffered solution (mM: NaCl, 120; KCl, 5; Hepes, 20; $MgCl_2$, 1; glucose, 10; pyruvate, 10; ascorbate, 10) containing 1.5 mM EDTA, 0.5 mM dithiothreitol (DTT) and 0.1% BSA [10]. After 10–15 min at 37°C epithelial cells were shaken free and the cell suspension passed through a coarse nylon mesh. The filtrate was washed (3×) by centrifugation in Na-Hepes solution containing 3% BSA and 1.2 mM calcium. A final incubation with hyaluronidase (1 mg/ml) in Na-Hepes for 5 min improved the rate of formation of giga seals. All patch-clamp experiments were carried out between 22 and 24°C.

Single channel currents were recorded in excised or cell-attached patches of basolateral membrane from isolated, single, rat enterocytes.

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An LM-EPC 7 (List Electronics, Darmstadt) patch-clamp amplifier was employed and the signal displayed on a storage oscilloscope and simultaneously recorded for later analysis on magnetic tape (Racal 4DS, Southampton). Fabrication and characteristics of patch-clamp pipettes are as described in [11–14].

The standard high-Na^+ solution contained (mM): 140 NaCl, 4.5 KCl, 1.13 MgCl, 10 glucose, 10 Hepes. The high-K^+ solution contained 145 mM KCl substituted for NaCl. Solutions were titrated to pH 7.2 with KOH. The concentration of free Ca^{2+} in the media was determined by addition of $CaCl_2$ and EGTA buffer mixtures [13–15].

3. RESULTS

The isolated, single, enterocytes were viewed at $\times 400$ magnification. In 10–15% of these cells a brush border membrane was clearly visible at one pole of the cell. Giga seals were formed at the opposite pole on the brush border-free membrane. The membrane patches were excised from the cells to form predominantly inside-out patches of basolateral membrane. Fig.1 shows single channel currents in such a patch exposed to symmetrical 145 mM KCl solutions on either side of the patch membrane. When the solution bathing the intracellular membrane face contains no added calcium and 1 mM EGTA ($[Ca^{2+} \leqslant 10^{-9}$ M) the channel is clearly voltage-sensitive. At negative membrane potentials inward currents are seen but the frequency and duration of channel opening are low. Membrane depolarizations are associated with an increased frequency and duration of channel opening. The currents in this situation reversed at 0 mV and outward currents are seen at positive transmembrane potentials. Fig.1 shows a plot of current amplitude as a function of membrane potential. The current-voltage relationship in this

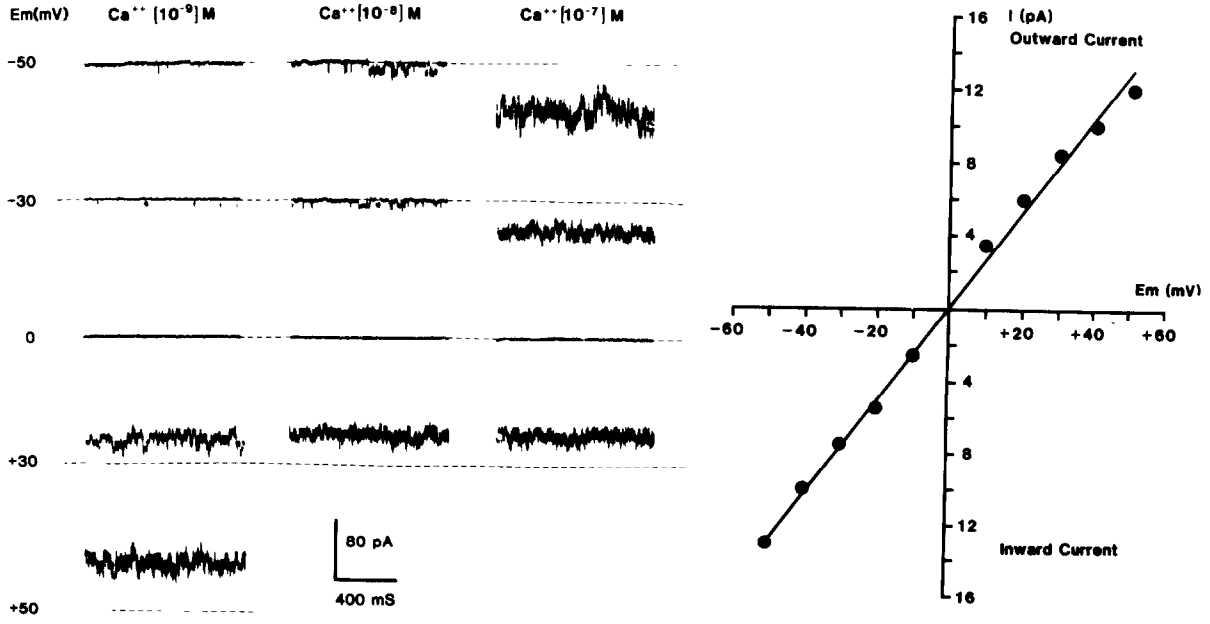

Fig.1. Sections of a continuous recording from a single excised, inside-out, patch of basolateral membrane from rat enterocyte. The patch pipette was filled with the 145 mM KCl solution, 1 mM EGTA but no Ca^{2+} was added. The bathing solution was an identical 145 mM KCl solution but the free ionized Ca^{2+} concentration was buffered to between 10^{-9} and 10^{-7} M. The figure shows the effect of both voltage (top to bottom) and increasing ionized calcium concentration (left to right) on the frequency and duration of single channel currents. E_m indicates transmembrane potential. The dotted lines indicate all channels closed. Inward currents are shown as downward deflections, outward currents, upwards. The plot to the right shows single channel current amplitude as a function of transmembrane potential.

and each of five other experiments was linear with a mean slope conductance of 256 ± 5.7 pS. Fig.1 also shows, in the same patch, the effect of increasing $[Ca^{2+}]$ in the solution bathing the intracellular membrane face. The frequency and duration of channel opening are increased with increasing $[Ca^{2+}]$ concentration till at 10^{-7} M all channels are predominantly open at all voltages. This pattern was confirmed in two other patches. The 250 pS channel is then voltage- and calcium-sensitive.

The selectivity of the channel for K^+ was demonstrated in two excised, inside-out, patches exposed to asymmetrical ionic gradients (fig.2). The recording pipette contains the 145 mM KCl solution and the bathing medium is the control saline (140 mM NaCl, 145 mM KCl). Inward currents are seen at all potentials tested including 0 mV. The current-voltage relationship is no longer linear but shows rectification, in this situation, at positive membrane potentials. The zero current potential is not demonstrated but by extrapolation it can be estimated to be between 60 and 80 mV. This current-voltage plot is as would be predicted in this situation for a channel which is highly selective for K^+ [13].

Cell-attached (in situ) recordings were made from a number of enterocytes. Fig.3 is an example of such a recording, typical of five. At the normal resting membrane potential (ΔE_m = 0 mV) inward currents are observed. The frequency and duration of opening of the in situ K^+ channels are low. As the patch membrane is progressively depolarized

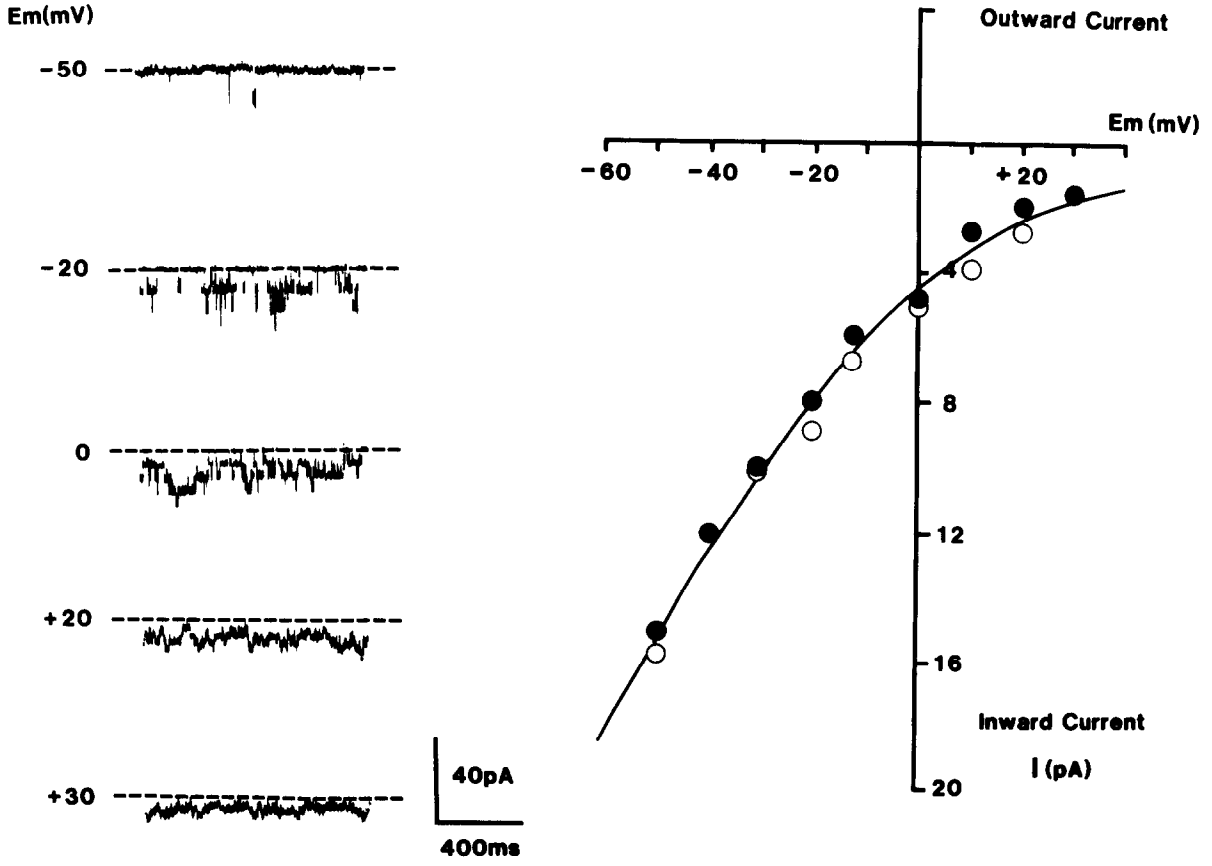

Fig.2. Sections of a recording from an excised, inside-out patch, exposed to asymmetrical ionic gradients. The pipette contained the 145 mM KCl solution with no Ca^{2+} and 1 mM EGTA added. The bathing solution was the control (140 mM NaCl, 4.5 mM KCl) saline, with no Ca^{2+} and 1 mM EGTA added. The records to the left show single channel currents at different potentials. The current-voltage plot is shown on the right.

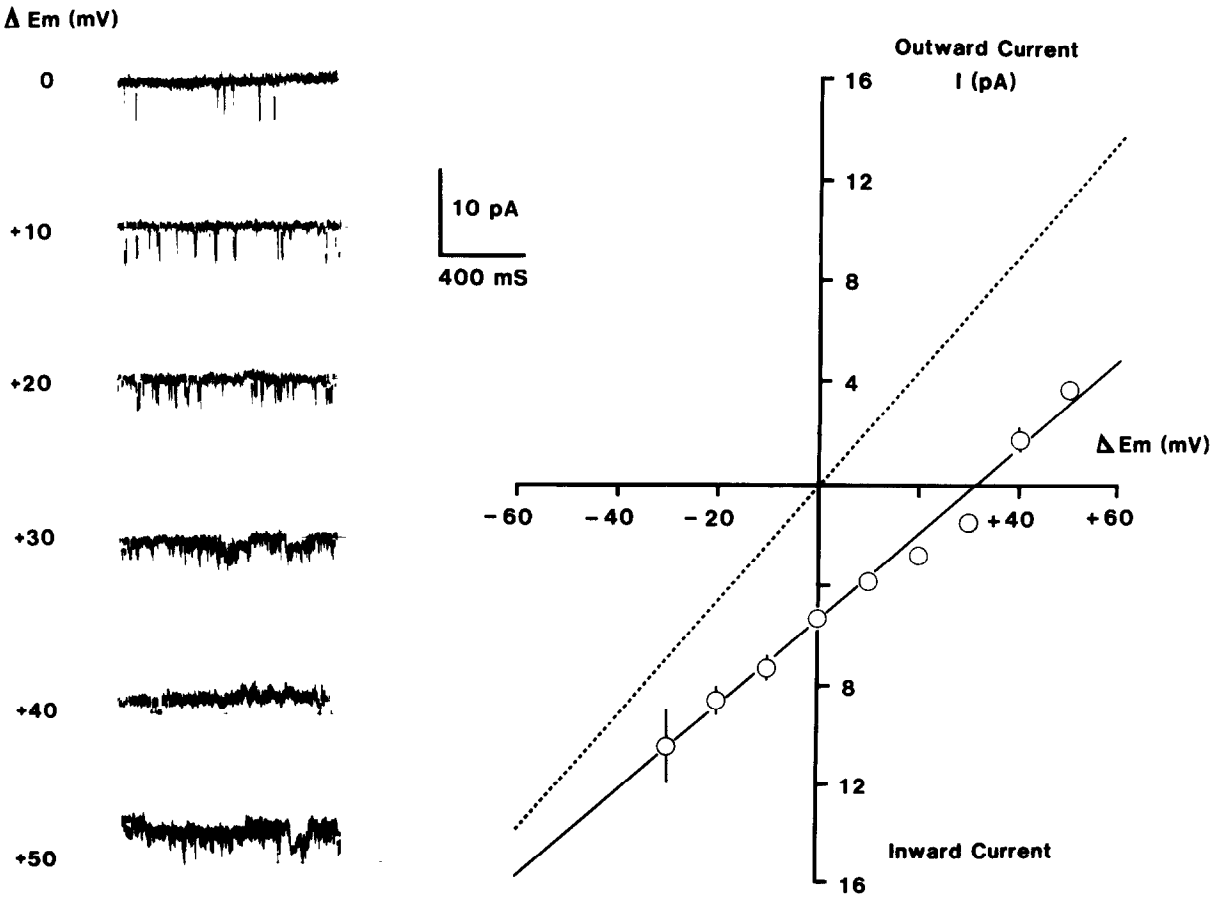

Fig.3. Records to the left are from a single cell-attached patch. The recording pipette contains 145 mM KCl, with no Ca^{2+} and 1 mM EGTA added. The transmembrane potential across the patch is varied from the normal resulting potential (ΔE_m = 0 mV) by voltage-clamping at depolarizing voltages (ΔE_m = positive). The current-voltage plot, mean of five experiments, is shown to the right. The dotted line corresponds to the current, voltage plot obtained in excised patches exposed to symmetrical 145 mM KCl solutions.

(i.e. ΔE_m = positive) the open probability of the channels increases. Current reversal occurs when the membrane is depolarized by some 30–40 mV. This figure also shows the mean current amplitude as a function of changing voltage. The mean single channel conductance was 155 ± 10 pS. In two of these experiments the cell-attached patches were subsequently excised as inside-out patches and exposed to symmetrical 145 mM KCl solutions. In this situation the single channel conductance was close to 250 pS.

The effects of barium blockade was tested on two excised, outside-out membrane patches. Fig.4 shows one of these recordings. The patches were exposed to symmetrical 145 mM KCl solutions. The K^+ channels in the patch were activated by voltage-clamping the patch at depolarizing voltages, +50 mV.

In this situation in the example in fig.4B currents are seen in between 4 and 6 channels. The patch membrane was then transferred to a bathing solution containing 5 mM barium (i.e. extracellular membrane face exposed to barium). As shown (fig.4B) the currents in the large conductance K^+ channel were rapidly abolished upon exposure to barium. The inset (fig.4C) demonstrates that

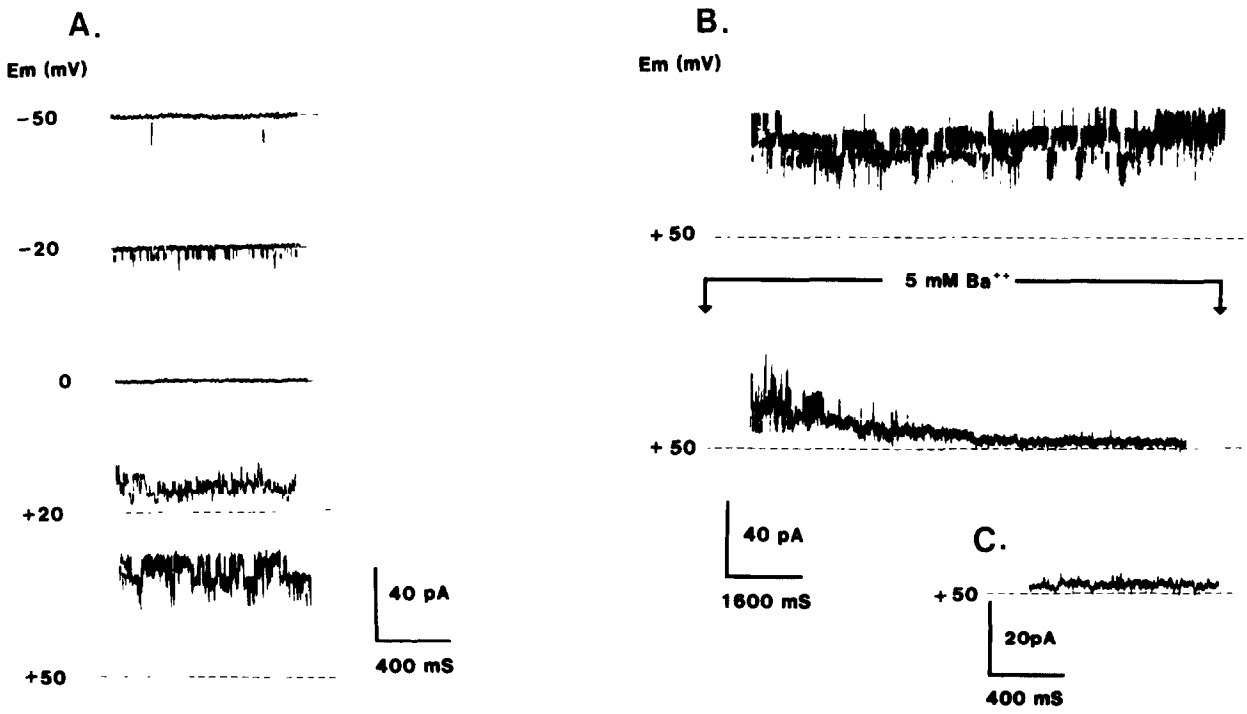

Fig.4. (A) Recording of single channel currents in excised, outside-out, membrane patch. The solutions in the recording pipette and bath are identical 145 mM KCl solutions with no Ca^{2+} and 1 mM EGTA added. (B) The patch membrane is voltage-clamped at 50 mV to activate the 4–6 channels present in this patch (upper record). In the lower record the patch has been transferred to an identical solution but containing 5 mM barium. The currents in the large conductance channel are abolished. (C) The inset shows at greater amplification that there is a residual conductance after barium blockade of the large K^+ channel.

although barium has blocked currents in the large conductance channel a residual conductance still persists, i.e. barium-insensitive.

4. DISCUSSION

The present study clearly demonstrates, for the first time, the presence of the large conductance, voltage- and calcium-activated K^+ channel (the maxi K^+ channel [15]) in excised patches of basolateral membrane from rat enterocytes. In the cell-attached recording mode the voltage-activated K^+ channels had a conductance of only 150 pS compared to the 250 pS in excised patches. We consider however that the currents recorded in situ and in excised patches are due to the same, the maxi K^+ channel. It has been demonstrated that the single channel conductance measured by cell-attached recording techniques can be underestimated, particularly if recording from small, single cells [17]. In this situation the cell membrane potential does not remain constant as the electrode potential is varied. Two observations suggest that this was true for our cell-attached recordings; the reversal potential in the cell-attached mode was often seen to fluctuate and the channels in cell-attached patches upon excision had a conductance of 250 pS. A recent patch-clamp study of rabbit intestinal cells reported a number of small conductance K^+ channels in these enterocytes [18]. No maxi K^+ channel was reported. The channels in the rabbit enterocytes were not fully characterized, however, and it is not yet clear whether these two studies reflect a real species difference or if a more detailed investigation of rabbit enterocytes will reveal a maxi K^+ channel. It is possible that the residual conduc-

tance we report in the presence of barium is due to one of these smaller K^+ channels.

The maxi K^+ channel demonstrated in the rat enterocyte membrane is one which has now been reported in a wide variety of fluid and electrolyte transporting epithelia [19]. The properties of calcium activation and barium blockade are consistent with a role for this channel in either intestinal secretion and/or resorption.

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