

Single channel recordings obtained from basolateral membranes of isolated rabbit enterocytes

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Epithelial cells isolated by hyaluronidase incubation from rabbit small intestine were used to explore the presence of ionic channels by the patch-clamp method. Recordings were made from cell-attached or excised patches of basolateral membrane. Evidence was obtained for the presence of at least two kinds of channels conducting potassium currents. One of these can be shown to be activated by Ca^{2+} .

Epithelial intestinal cell Potassium channel Ca^{2+} sensitivity Patch clamp Single channel recording

1. INTRODUCTION

Recent radioisotope flux studies have provided evidence for the presence in rabbit intestinal cells of a barium- and apamin-sensitive, calcium-activated potassium permeability pathway. This permeability can be activated in the presence of the calcium ionophore A23187 and during the transport of sugars and amino acids [1–3]. Microelectrode studies have also provided evidence for the presence of a potassium-conducting pathway in basolateral membranes of *Necturus* and goldfish intestinal cells [4,5]. Activation of this permeability pathway is also observed during transport of sugars and amino acids in *Necturus* small intestine [6]. However, a direct demonstration of active ionic channels in small intestinal cells by either noise analysis or the patch clamp technique has not been forthcoming. Here, we demonstrate that it is possible to record single channel activity in membranes of isolated intestinal epithelial cells by the patch clamp method [7]. Recordings of currents in patches made from the basolateral membranes of rabbit enterocytes reveal the presence of at least 2 classes of potassium channels differentiated on the basis of unit conductance.

2. METHODS

Cells were isolated from rabbit jejunum by incubation in the presence of hyaluronidase as described in [8]. After isolation cells were suspended in a modified Hanks buffer [8] and then plated on plastic petri dishes which had previously been coated with polylysine (1 mg/ml).

Voltage-clamp recordings of single ion channel activity were made by established methods [7]. Sometimes after formation of a giga seal ($>5 \times 10^9 \Omega$) the pipette was withdrawn from the cell in order to excise a patch of membrane. The pipette usually contained a high potassium concentration solution of the following composition (in mM): 140 KCl, 10 NaCl, 1.8 CaCl_2 , 1 MgCl_2 , 10 Hepes, pH 7.3 (KOH). The solution bathing the inside face of the patch was usually changed for one of the same composition but containing no added calcium. The free calcium concentration in this buffer, measured with a calcium-selective electrode [9], was around 1 μM (pCa ranged from 5.8 to 6.0). All experiments were conducted at room temperature.

3. RESULTS

Fig.1 shows a phase contrast image under our experimental conditions of a single enterocyte following enzyme dispersion. The basolateral plasma membrane is in contact with a patch microelectrode. The brush border membrane can be clearly seen at the opposing pole of the cell. In most cases it appeared that cells adhered rather loosely to the plastic by their brush border membrane. In all instances the micropipette appeared to make contact with the smooth, non-brush border part of the cell. Clumps of cells were also observed but no attempt was made to record from these. After successful giga seal formation, the electrode was sometimes withdrawn and an inside-out excised patch of membrane was obtained.

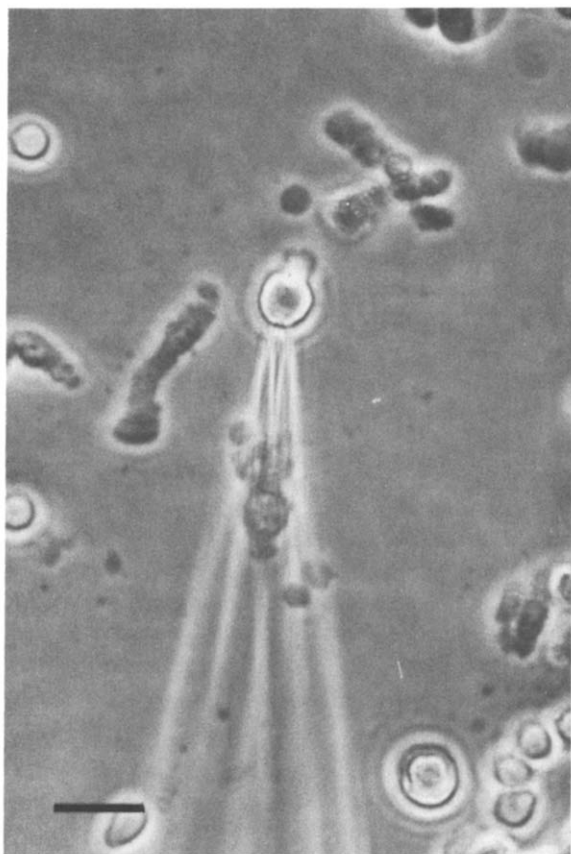


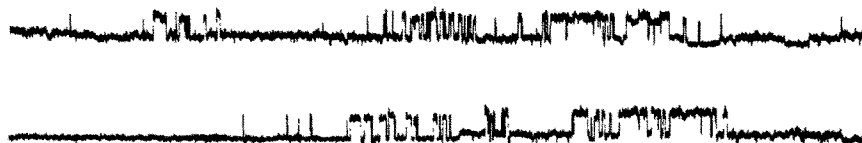
Fig.1. Phase contrast image of an isolated enterocyte with a patch pipette touching its basolateral membrane. Calibration bar = 20 μm .

When cell-attached recordings were made, we nearly always observed ion currents which were net outward currents at normal resting potential. Fig.2 shows a recording from an excised patch in symmetrical solutions containing a high potassium concentration. Voltages which depolarised the patch by 40–120 mV in the presence of 1 μM free calcium (high Ca) elicited frequent openings of a channel conducting outward current. Smaller conductance outward current events are also apparent in this recording. Addition of EGTA to give a final concentration of 50 nM Ca (low Ca) greatly diminished the activity of both channels. The lower part of fig.2 shows the effect of different depolarising potentials on channel activity measured at 1 μM Ca concentration. The slope conductance of the large event observed in fig.2 was 36 pS under the particular ionic composition used. From fig.2 it can be seen that the channel openings are characterised by intermittent bursts of opening and silent periods, with larger openings punctuated with brief flickerings or closings. The experiments at different holding potentials suggested a sensitivity to voltage, with open time probability being low at 56 mV and increasing with increasing depolarising voltage.

Activity of channels with high conductance was also observed in some experiments. One example of these is shown in fig.3, where an inside-out patch of membrane shows outward current events apparently mediated by several independently opening channels. The unit conductance of these was around 90 pS in symmetrical high potassium solutions and the number of open channels increased with increasing depolarisation. Voltage manipulation of the patch in fig.3 revealed the presence of at least 5 channels.

Recording in the cell-attached configuration was also possible as shown in fig.4, which shows a rapidly flickering outward current channel with a unit conductance of around 85 pS. The lower panels in fig.4 show distribution histograms for the open and closed times. Under these experimental conditions, therefore, this channel appeared to be described by a single open-close kinetic scheme, with the channel having a single conducting state and a single non-conducting state. Thus the forward rate constant for this channel was about 558 s^{-1} .

CELL SEP 1-7

 $V_{\text{hold}} = 100\text{mV}$ HIGH Ca^{2+} LOW Ca^{2+} 

10pA

1 sec

 $V_{\text{hold}} =$

160mV



150



145



120



110



85



57



5pA

1 sec

Fig.2. Excised patch recording of channel activity. Upper panel measurements at $1\text{ }\mu\text{M}$ (high Ca) or 50 nM (low Ca) calcium concentration in the bathing medium. Lower panel shows the same patch at $1\text{ }\mu\text{M}$ Ca and different holding voltages.

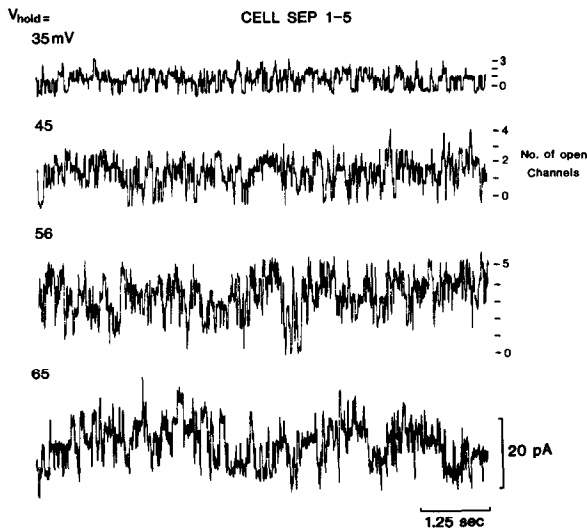


Fig.3. Excised patch recording of channel activity in symmetrical high potassium.

4. DISCUSSION

The cells used in this study were isolated from rabbit jejunum by a method preserving their normal permeability to ions [8]. Phase contrast observation of these cells after plating in polylysine-coated dishes suggests that they attach to the surface through their brush border membranes and that, consequently, membrane patches obtained in these experiments must be of basolateral membrane. Enterocyte isolation by other methods, usually calcium chelation which disrupts tight

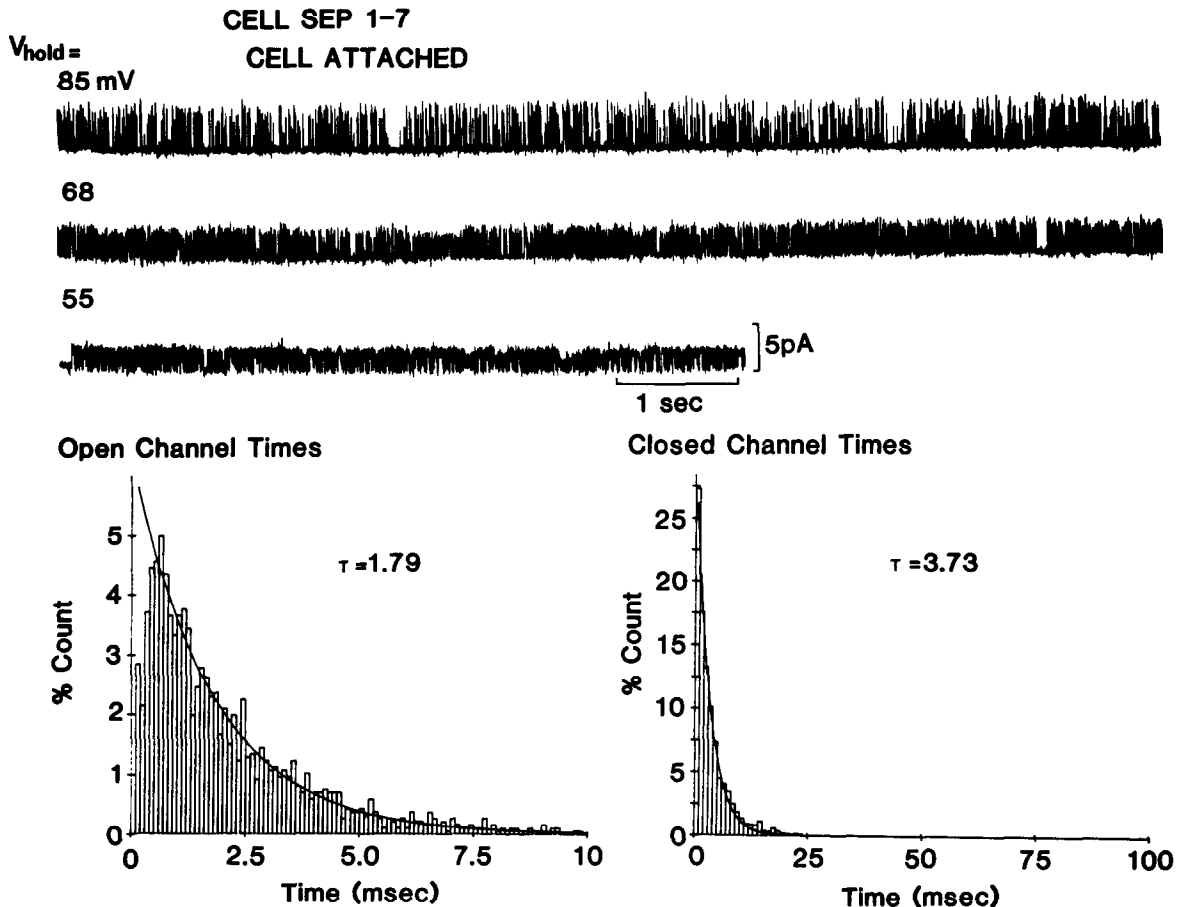


Fig.4. Cell-attached recording of basolateral membrane channel activity. Pipette solution was high potassium containing 1.8 mM Ca. Histograms show the distribution of open and closed times for the recording at 68 mV pipette voltage.

junction structure, leads to mixing of proteins of brush border and basolateral origin [10]. Such a redistribution cannot be discounted in the present experiments but the fact that physiological calcium levels were used throughout the isolation procedure suggests that segregation might have been maintained.

The outward currents recorded across membrane patches in the present experiments, and also in whole cell recordings, are consistent with the presence of potassium channels in the basolateral membranes of rabbit enterocytes. Although the presence of potassium channels in enterocytes has been suggested on the basis of radioisotope flux experiments in isolated cells [1-3] and from intracellular microelectrode studies in intact epithelium [5,6] this is the first direct demonstration that active ion channels, as recorded by the patch clamp technique, might underlie these observations.

In the patches examined to date we have detected so far at least 3 types of channels showing different conductances. Two of these show conductances of about 35 and 90 pS respectively in symmetrical high potassium solutions. Classification of these channels in the categories put forward by Latorre and Miller [11] will have to await detailed pharmacological and ionic selectivity characterisation of the channels, including their calcium and voltage sensitivity. The observation that the activity of these channels is dependent on

the presence of calcium at the cytoplasmic face of the membrane suggests them as possible mediators in the increase in potassium permeability observed in enterocytes transporting sugars or amino acids [1,2]. Studies in the cell-attached mode, which we also show are feasible with this preparation, should yield information about this point.

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