A large conductance, Ca^{2+}-activated K^+ channel in a human lung epithelial cell line (A549)

F.P. Gillian Ridge [a], Marek Duszyk [b], Andrew S. French [a,*]

[a] *Department of Physiology, Dalhousie University, Halifax, Nova Scotia, Canada*
[b] *Department of Physiology, University of Alberta, Edmonton, Alberta, Canada*

Abstract

A large conductance, Ca^{2+}-activated K^+ channel in a human lung epithelial cell line (A549) was identified using the single channel patch clamp technique. Channel conductance was 242 ± 33 pS ($n = 67$) in symmetrical KCl (140 mM). The channel was activated by membrane depolarization and increased cytosolic Ca^{2+}. High selectivity was observed for K^+ over $Rb^+ (0.49) > Cs^+ (0.14) > Na^+ (0.09)$. Open probability was significantly decreased by Ba^{2+} (5 mM) and quinidine (5 mM) to either surface, but TEA (5 mM) was only effective when added to the external surface. All effects were reversible. Increasing cytosolic Ca^{2+} concentration from 10^{-7} to 10^{-6} M caused an increase in open probability from near zero to fully activated. ATP decreased open probability at ~ 2 mM, but the effect was variable. The channel was almost always observed together with a smaller conductance channel, although they could both be seen individually. We conclude that A549 cells contain large conductance Ca^{2+}-activated K^+ channels which could explain a major fraction of the K^+ conductance in human alveolar epithelial membranes. © 1997 Elsevier Science B.V.

Keywords: Epithelium; Alveolar epithelium; Potassium conductance; A549 cell; Ion transport

1. Introduction

Efficient gas exchange in the lung requires that alveolar sacs be kept almost dry. One function of Type II cells in the lung is to reduce alveolar fluid by transepithelial transport of sodium from the apical to the basolateral surface. However, sodium is not the only ion moving within these cells, because several different transport processes work together to maintain alveolar homeostasis. Type II cells also synthe-size and secrete surfactant to allow lung expansion against the surface tension of the alveolar fluid, and differentiate into Type I cells, which cover most of the alveolar surface.

Na^+ moves into Type II alveolar cells from the apical surface, followed by Cl^- and water, as in some other airway epithelia [1]. Na^+ is absorbed at the apical membrane and extruded actively from the cell through the basolateral membrane via the Na^+-K^+ pump [2]. In rat Type II cells, the apical absorption has been shown to occur via conductive pathways such as Na^+ channels, non-selective cation channels, Na^+-glucose co-transport, Na^+-H^+ exchange and Na^+-amino acid co-transport [2,3]. The activity of the Na^+-K^+ pump, while creating a

* Corresponding author. Department of Physiology and Biophysics, Dalhousie University, Halifax, Nova Scotia, Canada B 3H 4H 7. Fax: +1 (902) 494-2050; E-mail: andrew.french@dal.ca

favourable electrochemical gradient for Na^+ absorption also leads to the accumulation of K^+ ions within the cell. K^+ leaks back through the basolateral membrane where it can be recycled by the pump. Previous work has indicated that alveolar Type II cells possess at least two types of cation conductance that could be responsible for this movement of K^+ through the basolateral membrane [4,5]. However, these studies did not characterize any conductances at the single-channel level, and no evidence for Ca^{2+}-sensitivity of either conductance was presented.

Large Ca^{2+}-activated potassium channels have been found in epithelia from tissues outside the airway that have similar transport mechanisms to airway cells. However, although many single-channel studies have been carried out in epithelial cells from several regions of the airway, only one has reported the presence of a large conductance Ca^2-activated K^+ channel. This was in nasal cells, where a basolateral Ca^{2+}-activated K^+ channel of 302 pS conductance was observed [6].

Human lung alveolar tissue is not readily available, and the use of isolated cells from lungs of other species has inherent problems due to species variation. Other problems with acutely dissociated cells are possible contamination by micro-organisms and damage to ion channel proteins by the digestive processes that must be used for dissociation. A cell line that does not have these disadvantages could be used as a model for human lung alveolar cells. The A549 cell line, which originated from a human alveolar cell carcinoma, possesses many characteristics of Type II alveolar cells [7], including multilamellar cytoplasmic inclusion bodies, and the ability to synthesize phospholipids, consistent with the synthesis of pulmonary surfactant, a well described function of Type II cells [2]. Importantly for the present work, it has recently been shown that chloride transport in A549 cells follows a similar mechanism to other epithelial tissues [8].

Here, we used the single-channel patch clamp technique to identify K^+ permeable channels in the A549 cell line. We discovered that a frequently observed channel in this cell was a large Ca^{2+}-activated K^+ channel, with similarities to channels found in other epithelia, including nasal cells [6]. This channel could explain a large fraction of the K^+ conductance of human alveolar epithelial membranes.

2. Materials and methods

2.1. Cell culture

Studies were performed on A549 cells, obtained from the American Type Culture Collection. This cell line was derived from an adult human lung carcinoma and is described as 'Type II cell like' [7]. The cells were grown in Ham's F-12 K media (Sigma) and maintained in a humidified 5% CO_2-95% air incubator at 37°C. The growth media was supplemented with gentamycin (50 μg/ml), penicillin-G (60 μg/ml), streptomycin (100 μg/ml) and 5% fetal calf serum and changed every 2 days. Confluent monolayers were subcultured by treatment with 50 μg/ml trypsin. Cells were used within 3 days of plating. Experiments were performed on cells over 20 consecutive passages.

2.2. Patch-clamp recording

Single-channel current recordings were obtained using the standard patch clamp technique for excised, inside-out and outside-out patch configurations [9]. Pipets were made from thick-walled borosilicate glass using a two stage vertical puller (Narishige, Tokyo, Japan). Pipet tips were subsequently coated with sylgard (Dow Corning) to reduce capacitance and fire polished, resulting in a final resistance of ~ 10 MΩ. Recordings commenced immediately after an excised patch had been formed and exposed to the air/liquid interface. All experiments were performed at room temperature (~ 20°C).

The standard pipet solution was (mM): 140 KCl, 2 $MgCl_2$, 1 $CaCl_2$, and 10 HEPES (pH 7.4). The standard bath solution was (mM): 140 KCl, 2 $MgCl_2$, 1 $CaCl_2$, and 10 HEPES (pH 7.4). Modified bath solutions were made by replacing the KCl with 140 NaCl, CsCl, RbCl, 70 KCl +140 mannitol or 70 K gluconate +70 KCl, as described below. For channel blocking experiments the blocking agents were added to the standard bath solution prior to solution exchange. Free Ca^{2+} concentration was varied between 10^{-9} and 10^{-3}M. To achieve these free Ca^{2+} concentrations, EGTA and Ca^{2+} concentration ratios were calculated according to Stockbridge [10]. All chemicals and drugs were obtained from Sigma (USA).

Once an excised patch was obtained, it was moved to a smaller chamber where solution changes could be performed using a pathway exchange system [11] with a small dead space for rapid solution changes.

Recordings were performed by a List EPC-7 amplifier (List Medical, Germany) and the channel current signals were stored on video tape, via a digital VCR adaptor (Medical Systems, PCM-1). Pipet offset potentials were compensated prior to forming a seal. Data analysis was based on procedures described by Colquhoun and Sigworth [12]. Following Gaussian digital filtering, the half amplitude criteria was used to distinguish between the open and closed states. The probability of the channels being open was calculated as a percentage of time spent in the open stated relative to the total time at a given potential, using a minimum total time of 8.25 s. Voltages are reported in reference to zero in the extracellular solution and positive currents are outward currents throughout. Data are presented as original recordings (filtered at 1 kHz) or as means ± s.d.

3. Results

Inside-out patches from the A549 human lung cell line often contained a spontaneously active, large conductance K^+ channel. From a total of 740 patches, 38% had at least one such channel. Some patches had more than one channel, up to a maximum of 4 channels. The mean channel density for all patches was approximately 0.75 channels/patch. Fig. 1A shows a single-channel recording from an inside-out patch in symmetrical KCl (140 mM). Under these conditions the mean slope conductance at zero membrane potential was 242 ± 33 pS ($n = 67$), reversing close to 0 mV and there was evidence of saturation at large driving potentials as shown in Fig. 1B. Channel opening varied with membrane potential, depolarization of the cell membrane leading to increased open probability (Fig. 1C). The large conductance channel was rarely observed completely alone ($< 1\%$ of patches). It was usually accompanied by a smaller, inwardly rectifying channel of approximately 27 pS. In addition, 22 pS linear and combinations of multiple large and small conductance channels were seen in 60% of patches.
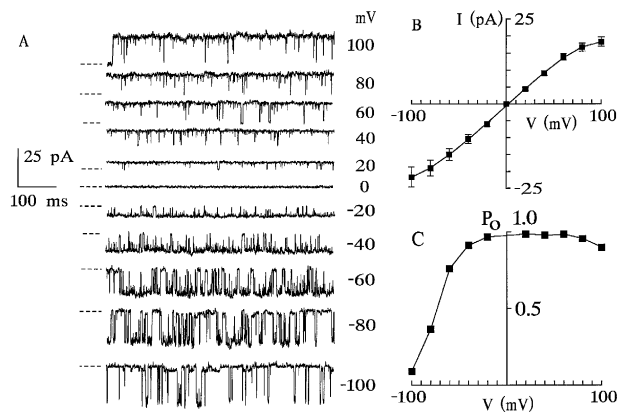

Fig. 1. A single K^+ channel in an excised inside-out patch from an A549 cell in symmetric KCl (140 mM). (A) Typical single channel recording over a range of holding potentials. In this and the following recordings, the dashed line indicates the closed state and a downward deflection represents inward current i.e. movement of K^+ from pipet to bath solution. In Fig. 1Fig. 2Fig. 3, error bars represent the standard deviations of individual current amplitudes for a single channel at each potential. (B) Current-voltage relationship for the channel recording of (A), fitted by a 5th order polynomial. The conductance of this individual channel, calculated from the slope of the curve at $V = 0$ mV was 253 pS. (C) Open probability (P_o) of the channel from A, showing voltage dependence.

3.1. Selectivity

To measure the selectivity of the large channel for K^+ over Cl^- two approaches were used. First, the reversal potential of the channel was observed when the patch was exposed to asymmetrical K^+ solutions (Fig. 2A). Here, the pipet solution contained 70 mM KCl (mannitol was used to maintain the osmolarity) and the bath solution was 140 mM KCl. This caused a shift of reversal potential (Er) to -16.4 ± 1.7 mV ($n = 5$), indicating a high selectivity for K^+ over anions. The liquid junction potential of the solution was calculated at the reversal potential and adjusted accordingly [11], which accounts for the shift in values along the voltage axis in Fig. 2A. Using the Nernst equation, the predicted Er for a K^+ channel under these conditions would be -17.5 mV, a value close to that obtained here.

Secondly, the Cl^- concentration in the bathing solution was reduced to 70 mM by replacement with gluconate, a large anion. This reduction of permeant anions in the bath solution had no effect on the
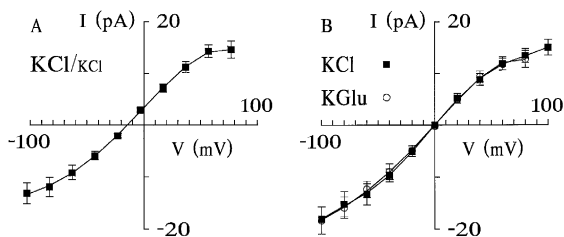
Fig. 2. Cation selectivity of the K^+ channel. (A) Current-voltage relationships for a representative channel in asymmetric KCl (70 mM pipet and 140 mM bath). The shift in reversal potential corresponds to that expected for a K^+ selective channel. (B) Current-voltage relationship for a single representative channel where the bath Cl^- concentration (KCl 140 mM) (■) was reduced to half the normal value (70 mM potassium gluconate and 70 mM KCl) (○) with no effect on channel activity, consistent with a cation permeable channel.

channel current amplitude (Fig. 2B). The observed conductances were $G = 243 \pm 35$ pS in reduced Cl^- and G $= 236 \pm 21$ pS in control (symmetric 140 mM KCl) solutions ($n = 9$). Single $-$ channel open probability was also apparently unaffected. These data are consistent with a channel that is highly selective for cations over anions.

3.2. Relative cation permeability

The relative permeability of the large K^+ channel to other cations was assessed by completely replacing KCl with equal concentrations of RbCl, CsCl or NaCl (Fig. 3A, Cs^+ data not shown). Relative permeabilities were calculated from the reversal potentials. The channel was highly selective for K^+ over the other cations, as shown by the strong rectification. Neither Cs^+ nor Na^+ carried significant currents at positive potentials (Fig. 3B). The resulting permeability sequence relative to K^+ was: K^+ (1.0) $\gg$ Rb^+ (0.49 $\pm$ 0.05) > Cs^+ (0.14 $\pm$ 0.05) > Na^+ (0.09 $\pm$ 0.03), which corresponds to the Eisenman permeability sequence IV [13]. Open probability was not strongly affected by cation replacement, and the voltage dependence of channel activity was maintained with all cations (Fig. 3C).

3.3. Effects of K^+ channel blockers

Addition of Ba^{2+} to the internal surface of the cell membrane had no effect on channel current ampli-

tude (Fig. 4A) with a conductance $G = 230 \pm 43$ pS relative to $G = 248 \pm 47$ pS in the absence of Ba^{2+} (Fig. 4C, $n = 8$) but caused a dramatic reduction in the open probability (P_o) of the K^+ channel (Fig. 4D). It can be seen that the blockade was slightly voltage dependent at negative potentials. At a holding potential of $+60$ mV P_o was reduced from 0.89 ± 0.071 to 0.011 ± 0.019 in the presence of Ba^{2+}. This effect was completely reversible and the block was independent of the side of the membrane to which the Ba^{2+} was added.

When the internal surface of a patch was exposed to TEA (Fig. 4A) it had no effect on channel current amplitude ($G = 250 \pm 24$ pS compared to control value $G = 258 \pm 23$ pS, $n = 6$) (Fig. 4C) or open probability (Fig. 4D). At a holding potential of $+60$ mV, $P_o = 0.84 \pm 0.14$ in the presence, and $P_o = 0.88 \pm 0.07$ in the absence of TEA. In contrast, when TEA was added to the external surface of an outside-out patch, it caused a total but reversible block of K^+ channel activity (Fig. 4B). Quinidine had identical effects when added to either side of a channel. Fig. 4B shows quinidine added to the external surface,
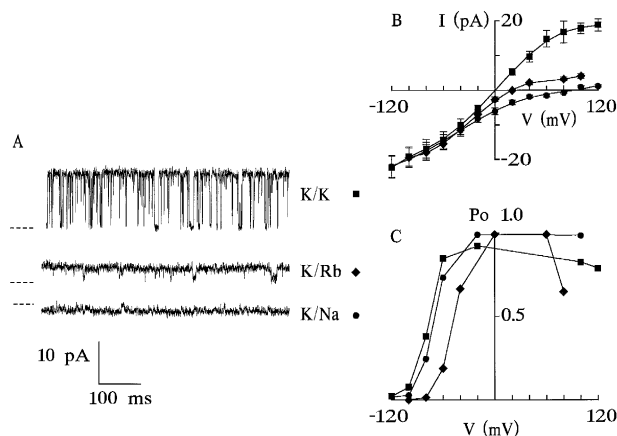

Fig. 3. Effect of complete bath cation replacement on K^+ channel activity. (A) Typical single channel recording showing reduced current amplitude when KCl (140 mM) in the bath solution was replaced by RbCl (140 mM) or NaCl (140 mM). The pipet contained 140 mM KCl throughout and the patch was held at $+60$ mV. (B) Current-voltage relationship for the channel of A over a range of holding potentials when the bath solution KCl (140 mM) (■) was replaced with RbCl (140 mM) (♦) or NaCl (140 mM) (●). (C) Open probability of the channel shown in A was unaffected by cation replacement.

producing a rapid flickery block of the channel. Because of the rapid block, this data was filtered at 3 kHz rather than 1 kHz to ensure that the filtering did not obscure the channel activity. Again, channel conductance was unaffected by quinidine, with $G = 267 \pm 31$ pS in the presence, and 244 ± 23 pS in the

absence of 5 mM quinidine (Fig. 4C, $n = 8$, data was combined from inside-out and outside-out patch recordings), but P_o was reduced from 0.92 ± 0.05 to 0.073 ± 0.064 in the presence of quinidine (Fig. 4D).

3.4. Ca^{2+} dependence

The Ca^{2+} dependence of the large K^+ channel was examined by varying the free Ca^{2+} concentration in the bath (i.e. the cytosolic side of inside-out patches) between 10^{-3} M and 10^{-9} M, as illustrated in Fig. 5A. When these data were plotted as the relationship between open probability and free $[Ca^{2+}]$ as depicted in Fig. 5B it was clearly seen that free Ca^{2+} concentrations below 10^{-6} M caused a large decline in channel opening.

3.5. Effect of ATP

ATP also affected the probability of channel opening in a dose-dependent manner without affecting conductance. The application of 1 mM ATP to the internal surface of an inside-out patch had no noticeable effect on the K^+ channel current amplitude or
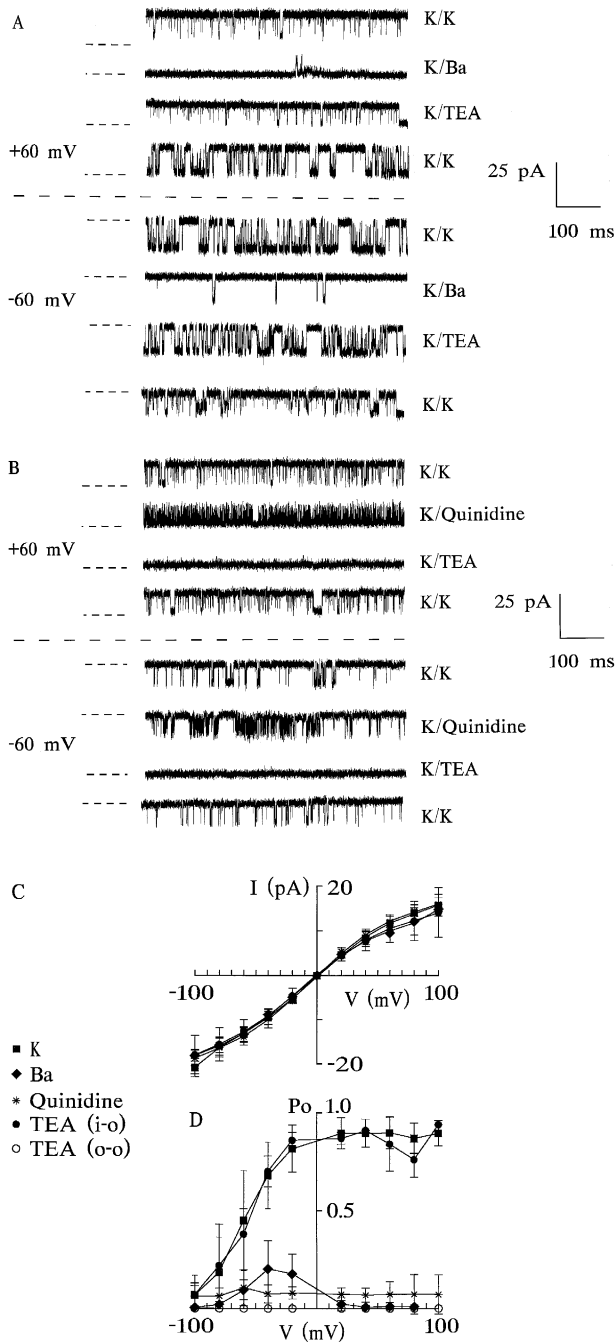

Fig. 4. Effect of blockers on K^+ channel activity. (A) Representative recordings showing the reversible ability of Ba^{2+} to reduce the open probability and the lack of effect of TEA on a single inside-out patch at a holding potential of ± 60 mV (filtered at 3 kHz). Pipet and bath solutions were symmetric 140 mM KCl with either 5 mM Ba^{2+} or 5 mM TEA added to the bath solution (internal surface). (B) Representative recordings of a single channel from an outside-out patch at a holding potential of ± 60 m (filtered at 3 kHz). Note that quinidine produced a flickery block and TEA completely blocked channel activity. Pipet and bath solutions were symmetric 140 mM KCl with either 5 mM quinidine or 5 mM TEA added to the bath solution (external surface). (C) Current-voltage relationship over a range of holding potentials for inside-out patches showing that the current amplitude was not affected by the addition of 5 mM Ba^{2+} (♦), 5 mM quinidine (∗) or 5 mM TEA (●) to the bathing solution containing 140 mM KCl with 140 mM KCl in the pipet. Data are means $\pm$ s.d. for 5-8 experiments. (D) Open probability of K^+ channels over a range of holding potentials for inside-out (closed symbols) i.e. internal surface and outside-out (open symbols) i.e. external surface, patches in the presence of 5 mM Ba^{2+} (♦), 5 mM quinidine (∗) or 5 mM TEA (● and ○) to the bath solution containing 140 mM KCl with 140 mM KCl in the pipet. Data are means $\pm$ s.d. for 5-8 experiments.
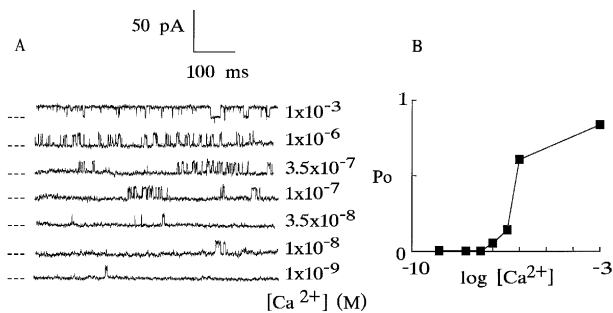
Fig. 5. Effect of internal free $[Ca^{2+}]$ on K^+ channel activity. (A) Recording of a single inside-out patch at a holding potential of $+60$ mV. Pipet and bath solutions were symmetric KCl (140 mM) with 1 mM Ca^{2+} in the pipet. The $[Ca^{2+}]$ in the bath solution (cytosolic) was buffered with EGTA to give the required free $[Ca^{2+}]$. (B) Open probability data from A vs. free cytosolic $[Ca^{2+}]$ at a holding potential of $+60$ mV showing that K^+ channel activity was strongly calcium-dependent.

open probability. Increasing the exposure time to 1 mM ATP also failed to produce any changes. A higher ATP concentration (2 mM) caused some reduction of channel open probability after immediate addition and a dramatic decrease was observed after 5 min, although this effect varied with time and between individual patches. There was a significant decline in open probability as a result of 2 mM ATP addition which was clearly voltage dependent (Fig. 6).
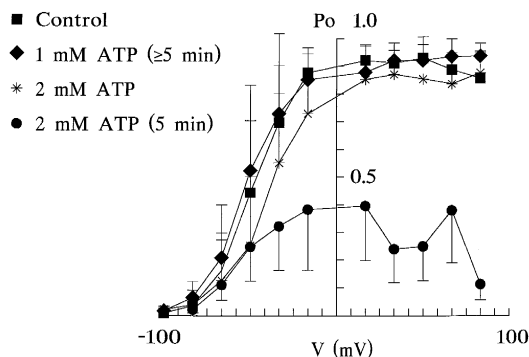
Fig. 6. Effect of ATP on K^+ channel activity. The open probability of a K^+ channel in an inside-out patch at a range of holding potentials when ATP was added to the bath. Channel activity was not affected by the presence of 1 mM ATP (◆, $n = 6$), and this did not change with time during experiments of at least 5 minutes duration. Some inhibition was observed immediately after adding 2 mM ATP (∗, $n = 8$), and there was a significant decrease in open probability after 5 min (●, $n = 4$). Data points are means $\pm$ s.d.

4. Discussion

The appearance of the A549 cells and the biophysical properties of the ion channels remained constant throughout the course of this work, which involved approximately 20 cell passages. The use of this cell line to study transport in human alveolar cells has obvious advantages compared to obtaining fresh human tissues. The cell line was relatively easy to maintain, and readily formed confluent monolayers.

One of the most commonly observed channels was a highly selective, large conductance (~ 242 pS) K^+ channel, activated by Ca^{2+} and membrane depolarization. Channels with similar characteristics have been found in a variety of other epithelia [14] including distal colon, 275 pS [15], vas deferens, 250 pS [16] and the only single-channel report from the airway, a 302 pS channel in nasal epithelia [6]. Therefore, this work presents the first characterization of a Ca^{2+}-activated K^+ channel in lung epithelial cells.

4.1. K^+ channels in alveolar cells

Information about K^+ channels in airway epithelia has been obtained using a variety of techniques, including Ussing chambers which measure the short circuit current (I_{sc}) and ion fluxes of intact or cultured epithelia under voltage clamp conditions [1,2,17] or patch clamp studies using various patch configurations [6]. Less is known about K^+ channels in alveoli than in upper airways but some data are available from whole-cell patches from rat alveolar cells either freshly isolated or in primary culture [4,5], radioisotope flux studies [18] and isolated lung [19]. No previous descriptions of large conductance alveolar K^+ channels are available at the single-channel level.

Previous studies identified two types of K^+ channels in rat alveolar type II cells either in primary culture [4] or freshly isolated cells [5] and A549 cells [20]. The most commonly observed K^+ channels, called *n*-type or low threshold (LT) were found in 75% of cells with an estimated conductance of 12 pS, and activated at membrane potentials of -40 mV to -20 mV. These channels were highly selective for K^+ over Na^+ and were blocked by TEA and quinine. Less common *l*-type or high threshold (HT) K^+

channels were seen in 25% of cells, and were distinguished by activation at more positive potentials, greater sensitivity to TEA blockade and voltage-dependent block by quinine. The two channel types were never observed in the same cell.

The *n*-type and *l*-type channels were said to resemble delayed rectifier and Ca^{2+}-activated K^+ channels respectively [5,18]. However, these studies did not test for Ca^{2+} sensitivity. Later, it was argued strongly that neither of the two populations were Ca^{2+}-dependent [4].

4.2. Membrane specialization

Epithelial cells are polarized, and transport proteins are usually localized to a particular membrane. Here, we used cells that had not yet reached confluence, which gave access to a wide range of cellular ion channels, but prevented definite identification of the membranes in which they would normally be located. Numerous earlier studies in epithelia [15,21], including upper airway epithelia [6], suggest that the large conductance Ca^{2+}-activated K^+ channel is located in the basolateral membrane of intact epithelia. However, this type of channel has also been described in the apical membranes of some epithelia, with a possible role in transepithelial K^+ secretion [16]. At least one study [19] has reported the presence of an apical K^+ channel in alveolar cells, although its other characteristics are unknown.

A failure to see both Cl^- and K^+ channels in the same patches from nasal airway cells was used to argue that membranes are already separated in some non-confluent cells [6]. Our similar failure to observe such pairing does not necessarily indicate polarity, because of a possible lack of cAMP- and Ca^{2+}-activated Cl^- channels in alveolar cells Type II cells [22], although a high conductance Cl^- channel has been described in apical membranes of cultured alveolar type II cells [23].

Basset et al [19] proposed a 'head-to-tail' model for adult rat alveolar transport. They suggested that two different cell types were present, one performing Na^+ absorption, as previously described [20], while a second type absorbed K^+ at the apical membrane via a Na^+-K^+ ATPase. Both types would probably contain apical and basolateral K^+ channels. This model has yet to be supported by further evidence.

4.3. Conductance and voltage dependence

The A549 cell K^+ channel had a conductance of approximately 240 pS. Its large conductance, voltage dependence and high K^+ selectivity clearly place it in the class of maxi-K channels, which have conductances in the range 200–300 pS [24]. Maxi-K channels have been described in a wide range of epithelial and non-epithelial cells [14,24]. The most closely related tissue in which similar channels have been reported is nasal polyp epithelium, which like the alveolus is a net Na^+ absorbing epithelium [1]. The channel described here possesses some similarities to these basolateral nasal K^+ channels, which had a conductance of 302 pS in symmetrical KCl solutions [6].

The voltage dependence of large conductance K^+ channels has been widely observed in epithelia [14,24,25]. In nasal polyp cells, the channel was described as weakly voltage-dependent, but this was probably due to a limited experimental range of -40 to $+50$ mV. We found that open probability declined significantly at potentials below -40 to -60 mV (Fig. 1C). Whole-cell work on alveolar cells [5] found that the small conductance, low-threshold (LT) or *n*-type K^+ channels activated at potentials of ~ -40 mV, similarly to the A549 maxi-K channel, while the high-threshold (HT) or *l*-type channels required potentials closer to -20 mV for activation.

4.4. Selectivity and permeability

In spite of its large conductance (Fig. 1), the channel was highly selective for K^+ over anions or other cations ($P_{Na}/P_K = 0.09 \pm 0.03$, Fig. 3). The permeability sequence corresponds to Sequence IV of Eisenman [13] suggesting that the channel binding site has relatively weak field strength compared to the dehydration energy of the permeating cations. The high selectivity of Ca^{2+}-activated K^+ channels for K^+ over Na^+ has been demonstrated in several other epithelia [24]. In nasal polyps the Ca^{2+}-activated channel was also highly selective for K^+ over Na^+, but had similar permeabilities to Rb^+ and K^+. In contrast, the A549 K^+ channel discriminated strongly between K^+ and Rb^+ ($P_{Rb}/P_K = 0.49 \pm 0.05$, Fig. 3), which agrees with other reports for maxi-K channels [24].

Transepithelial studies on intact tracheal tissues suggested that there were 3 populations of K^+ channels in basolateral membranes, based on ionic selectivities and sensitivity to blocking chemicals [17]. Of these, the most similar to the A549 channel had $P_{Rb}/P_K = 0.555$, was blocked by Ba^{2+}, quinidine and lidocaine, and was not stimulated by epinephrine. The second type was activated by epinephrine, blocked only by Ba^{2+} (not quinidine or lidocaine) and did not discriminate between K^+ and Rb^+. The third channel was more similar to the first but even more selective ($P_{Rb}/P_K = 0.1$) and was activated under swelling conditions.

4.5. Channel blockade

Ba^{2+}, which blocks a variety of K^+ channels, also blocked the large conductance Ca^{2+}-activated K^+ channel of A549 cells by reducing the channel open probability in a slightly voltage-dependent manner. The channel closed for longer periods, with bursts of activity, as seen in nasal epithelial K^+ channels [6] and other epithelia [24].

Quinidine, which fairly selectively blocks many Ca^{2+}-activated K^+ channels, produced a distinct flickery voltage-independent block of the channel. Blockade by quinidine or its isomer quinine has been used to discriminate between the different sub-populations of basolateral K^+ channels in trachea [17] and alveolar cells [5]. Quinidine blocked the highly K^+-selective tracheal basolateral channel, as it did here, but not the unselective, epinephrine-activated K^+ channel [5]. In alveolar cells, the LT and HT channels were both blocked by quinine but with different voltage-dependence. The voltage-independent blockade by quinine (Fig. 4C) is also closer to the findings for LT, rather than the voltage dependent block in HT channels [5].

TEA blocks Ca^{2+}-activated K^+ channels from the outside membrane surface [24]. But higher concentrations (> 10 mM) can also result in some blockage from the inside [5,14,26]. In agreement with other studies, we saw a complete block of the A549 channel when 5 mM TEA was added externally, although we occasionally ($2/8$ patches) saw channel block when this concentration was presented to the internal surface. In nasal polyp epithelia [6] an identical concentration of TEA (5 mM) blocked the K^+ channel

following addition to the internal surface, although this was claimed to act on the outside surface following penetration of the membrane. These data do not clearly discriminate between the nasal and alveolar channels, although nasal channels may be more sensitive to TEA. In whole-cell recordings of alveolar cells, TEA blocked both populations of K^+ channels, although LT channels were less sensitive (20 mM TEA) than HT channels [4,5] while only one population of channels showed any TEA sensitivity in A549 cells [20].

4.6. Ca^{2+} dependence

Ca^{2+} regulates maxi-K channels in many epithelia [14], including nasal airway [6]. However, Ca^{2+}-sensitivity varies significantly [24]. For example, Ca^{2+}-sensitivity was high in pancreatic acinar cells with maximal activation at 0.2 μM [21], while maxi-K channels in cultured rat muscle required > 10 μM Ca for maximal activation [24]. In A549 cells, open probability increased from near zero to near unity over a relatively small range of Ca^{2+} concentrations (10^{-7}–10^{-6} M) which correlates well with nasal epithelia [6]. In contrast, both sub-populations of whole-cell alveolar K^+ currents were Ca^{2+}-insensitive [4]. Therefore, some or all of the whole-cell currents could be due to small conductance Ca^{2+}-sensitive channels as described in trachea [1]. In rat alveolar type II cells, a small conductance, non-selective, amiloride and Ca^{2+}-sensitive channel was present in the apical membrane. This channel required relatively high Ca^{2+} concentrations (> 10 μM) for complete activation [3].

4.7. Effect of ATP

The effect of ATP on K^+ channel activity in A549 cells was variable, and sensitive to both concentration and time. 1 mM ATP did not affect channel activity, while 2 mM ATP produced a voltage-dependent reduction in open probability. This effect was sometimes seen immediately after application, but required up to 5 min to be maximal in some patches. Sensitivity to ATP varies considerably among different tissue [27], but the inhibitory effect of ATP on several other epithelial Ca^{2+}-activated K^+ channels has been observed [15]. Nasal cells were inhibited over the range

of 0.1–1 mM ATP, but A549 channels were less sensitive. The role and mechanism of ATP blockade of these channels is not clear. Local changes in ATP levels or ATP/ADP ratio may couple basolateral K^+ conductance to the pump rate [6]. In Type II cells, ATP also stimulates surfactant secretion [28].

4.8. Smaller channels

A much smaller conductance channel was often observed in the same patch as the large conductance channel that we have described here, as well as independently in other patches. We have not yet studied this channel in detail, but preliminary data indicate that it has a conductance of ~ 27 pS in symmetrical 140 mM KCl, is inwardly rectifying and voltage insensitive. This could be similar to the 20 pS inwardly rectifying channel of tracheal epithelia [22] which was selective for K^+ over Na^+, and Ca^{2+}- but not voltage-activated. Alternatively, it could be related to the 24 pS non-selective cation channel that has been described as abundant in nasal airway [29] and rat alveoli [3]. A number of other small conductance channels were also observed but not fully characterized.

4.9. Summary

We have described a large, Ca^{2+}-activated K^+ channel in A549 cells that has many similarities to maxi-K channels from a variety of tissues, including nasal airway epithelia. There were some differences from the nasal epithelial channels, in conductance, cation selectivity, and probably in ATP blockade and TEA-sensitivity, but their significance is not yet clear. It is known that ion channels in airway epithelia vary between both regions and species [1]. Some preliminary experiments have also suggested that the A549 K^+ channels are pH-sensitive, unlike nasal channels, but this investigation is incomplete. In contrast, it is hard to relate the A549 channels to any of the conductances seen in whole-cell alveolar epithelial experiments, because there was no clear correlation between the voltage- and chemical-sensitivities of the A549 channels and either of the two currents seen in the whole-cell experiments. In particular, the A549 channels were clearly Ca^{2+}-activated maxi-K chan-

nels, while alveolar K^+ currents have been described as Ca^{2+}-insensitive [4].

The voltage- and calcium-dependence of the A549 channel would be expected to play important functional roles because the activation range is close to the resting potential in these cells [30] and the channel would be expected to repolarize cells following depolarization or calcium entry. In contrast, the small conductance K^+ channels previously described in tracheal basolateral membranes [22] and the small conductance cation-selective channels of nasal epithelia [29] and rat alveolar type II cells [3] were clearly voltage-independent.

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