An Inwardly Rectifying Potassium Channel in Apical Membrane of Calu-3 Cells

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Received for publication, June 1, 2004, and in revised form, August 20, 2004
Published, JBC Papers in Press, August 24, 2004, DOI 10.1074/jbc.M406058200

Patch clamp methods and reverse transcription-polymerase chain reaction (RT-PCR) were used to characterize an apical K⁺ channel in Calu-3 cells, a widely used model of human airway gland serous cells. In cell-attached and excised apical membrane patches, we found an inwardly rectifying K⁺ channel (Kir). The permeability ratio was \( P_{Na}/P_{K} = 0.058 \). In 30 patches with both cystic fibrosis transmembrane conductance regulator and Kir present, we observed 79 cystic fibrosis transmembrane conductance regulator and 58 Kir channels. The average chord conductance was 24.4 ± 0.5 pS \((n = 11)\), between 0 and −200 mV, and was 9.6 ± 0.7 pS \((n = 8)\), between 0 and 50 mV; these magnitudes and their ratio of −2.5 are most similar to values for rectifying K⁺ channels of the Kir4.x subfamilies. We attempted to amplify transcripts for Kir4.1, Kir4.2, and Kir5.1; of these only Kir4.2 was present in Calu-3 lysates. The channel was only weakly activated by ATP and was relatively insensitive to internal pH. Externally Cs⁺ and Ba²⁺ blocked the channel with \( K_{0} \) values in the millimolar range. Quantitative modeling of Cl⁻-secreting epithelia suggests that secretion rates will be highest and luminal K⁺ will rise to 16–28 mM if 11–25% of the total cellular K⁺ conductance is placed in the apical membrane (Cook, D. I., and Young, J. A. (1989) J. Membr. Biol. 110, 139–146). Thus, we hypothesize that the K⁺ channel described here optimizes the rate of secretion and is involved in K⁺ recycling for the recently proposed apical H⁺-K⁺-ATPase in Calu-3 cells.

The Calu-3 cell line (1) is widely used as a model for human airway gland serous cells (2–6). When grown to confluence, Calu-3 cells form polarized monolayers and express CFTR¹ apically at high levels (1). In addition to expressing many typical serous cell markers (7), Calu-3 cells are of special interest because they produce two kinds of anion secretion depending upon the mode of stimulation. When activated via agents that elevate [cAMP], Calu-3 cells secrete a HCO₃⁻-rich fluid, whereas if activated by agents that elevate [Ca²⁺], they secrete a Cl⁻-rich fluid (5), although it still contains a large component of HCO₃⁻ (4, 8). In either case the final step appears to be electrodiffusion of the anions through CFTR (4, 5, 9). It has now been shown directly that anion secretion from Calu-3 cells drives the robust secretion of fluid at rates up to 10 μl/cm²/h (10).

Recently, it was shown that apically secreted HCO₃⁻ is partially neutralized by proton secretion under some conditions (8). The source of the protons appears to be an H⁺-K⁺-ATPase because it is ouabain-sensitive and requires apical K⁺ (8). This raises the issue of the source of the apical K⁺. An apical K⁺ channel in Calu-3 cells is suggested by results of Cowley and Linsdell (6), who showed that 16% of the %i under basal conditions could be blocked with apical Ba²⁺, but nothing else is known about the properties of the putative apical K⁺ channels. In the present experiments, patch clamp methods and RT-PCR were used to characterize apical K⁺ channels in the apical membranes of polarized Calu-3 cells. In excised apical membrane patches, we found abundant copies of a single type of inwardly rectifying K⁺ channel (Kir). The Kir family of channels (reviewed in Ref. 11) are homo- or heterotetramers. Each subunit has two transmembrane domains, a pore loop, and cytoplasmic N and C termini. At least 16 genes (KCNN1–16) have been identified, giving rise to channels grouped into 7 subfamilies that differ in their channel signatures, distribution, and mode of activation. Kir channels differ from the previously identified basolateral K⁺ channels of Calu-3 cells, which are 6 transmembrane domain channels activated by elevations of [Ca²⁺] (11), and [cAMP], respectively. Transcripts for Kir4.2 were amplified in Calu-3 lysates, but the signature of the Calu-3 apical K⁺ channel did not exactly match previous descriptions of Kir4.2 channels, leaving its identity undefined.

MATERIALS AND METHODS

Cells and Cell Culture—Experiments were conducted using Calu-3 cells grown as previously described by Shen et al. (1). Briefly, the Calu-3 cell line was obtained from the American Type Culture Collection (Rockville, MD). After thawing, cells were grown at 37 °C in T_{w} culture fluid (Costar, Pleasanton, CA) containing a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12, plus 15% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine in an atmosphere of 5% CO₂, 95% O₂. Cells were passaged at 1:8 dilution and plated at 5 × 10⁵/cm² onto a 35-mm Petri dish coated with human placental collagen. Cells were grown to partial confluency and used after 1–7 days in culture. To ensure that recordings were made from apical membranes, we only include recordings from cells that were surrounded by other cells within the islands; cells located at edges of the islands were ignored.

Patch Clamp Recording and Solutions—Cell attached and inside-out single channel patch clamp recordings were made at ~23 °C. Pipettes were pulled from Prism LA165SA16 Glass (Dagan Corp.) on a P-87 Brown-Flaming puller. Pipettes with smaller tips were pulled from thick wall glass (SA16 inner diameter = 0.75, outer diameter = 1.65 μm) and those with larger tips from thin wall glass (LA16 inner diameter = 1.10, outer diameter = 1.65 μm). The different tip sizes were used to record either single channels for kinetic analyses or multiple channels when averaged data was required. Currents were amplified and filtered at 500 Hz or 2 kHz with an Axopatch 1C amplifier. Data acquisition was controlled by pClamp 8.0 (Axon Instruments). Data were sampled and digitized at a rate of 5 kHz and stored on disk. Liquid junction potentials between patch

¹ This work was supported by National Institutes of Health Grants DK-51817 and the Cystic Fibrosis Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The abbreviations used are: CFTR, cystic fibrosis transmembrane conductance regulator; RT, reverse transcription.
pipette and bath solution were electrically nulled by adjusting the amplifier prior to making seals. Membrane voltage was held at −60 mV unless otherwise indicated.

Pipettes were filled with a solution (in mM) of 150 KCl, 2.5 CaCl₂, and 10 HEPES, with pH adjusted to 7.3 with KOH, and osmolality adjusted to 320 mosmol. The standard bath and cytosolic control solution was (in mM) 130 K-gluamate, 20 KCl, 0.5 EGTA, 2.5 MgCl₂, 1 CaCl₂, and 10 HEPES titrated to pH 7.3 with KOH. Osmolarity was adjusted to 320–340 mosmol. The free calcium level was 500 μM as computed by MaxChelator (www.stanford.edu/~quatton/maxc.html). Buffers were titrated with KOH to pH values of 7.3, 6.9, 6.7, or 8.0.

**Solution Change**—The valve-controlled solution changer consisted of a manifold with 4 inlet ports, each connected to a different solution reservoir, and one outlet port connected to a perfusion pipette. The manifold was located −2 mm from the tip of the perfusion pipette. Solutions were switched manually in less than 2 s. In this setup the patch and perfusion pipette have fixed positions, which assures identical access to each solution.

**Ussing Chamber Experiments**—A small number of short-circuit (Iₑ) experiments were carried out with monolayers of Calu-3 cells grown for ~4 weeks on Snapwell filters coated with human placental collagen. Cells were fed from the basolateral side and grown at the air interface. Inserts were mounted in an Ussing chamber (4 ml volume each chamber), the voltage was short circuited with a Physiologic Instrument. Solutions were switched manually in less than 2 s. In this setup the patch and perfusion pipette have fixed positions, which assures identical access to each solution.

**Apical K⁺ Channel in Calu-3 Cells**

| Table I | Kir primers | Size | Tₛ |
|---------|-------------|------|----|
| Kir.1 sense | 5'-ATCCACTGAGGCCTCTCCTTCAGC-3' | 596 | 74 |
| Kir.1 antisense | 5'-TGTGGAACCTCTGAGCCCAAAG-3' | 68 |
| Kir.2 sense | 5'-TTCAAATCTACCCCGCTGCATC-3' | 598 | 66 |
| Kir.2 antisense | 5'-ATAAGATGTGCGCTGCTGAGC-3' | 66 |
| Kir.1 sense | 5'-CAGGACGTATCATATATATCATGGC-3' | 1223 | 70 |
| Kir.1 antisense | 5'-AGAGATCTGTTTAAAGTCAGAG-3' | 70 |

KCNJ10, KCNJ15, and KCNJ16, respectively) yielded amplified products of the expected size.

**Statistics**—All data are expressed as mean ± S.E. unless otherwise indicated. Statistical difference was determined by Student’s t test. A value of p < 0.05 was considered statistically significant.

**RESULTS**

**Functional K⁺ Channels Expressed in the Apical Membrane**—When cell-attached patches were formed on polarized Calu-3 cells using pipettes filled with 150 mM KCl, the most abundant channel activity observed was CFTR, which tended to mask other channel activity. However, immediately upon excision of inside-out patches into an ATP-free, 150 mM K-gluamate solution, K⁺ channel activity became apparent in ~45% of patches made with smaller (thick wall) pipettes and ~100% of patches made with larger tipped (thin wall) pipettes. K⁺ channel activity was observed even when Ca²⁺ and Mg²⁺ levels in the bath were <100 mM. The relative numbers of cell-attached CFTR and excised Kir channels observed in two samples of patches made with thick wall pipettes is shown in Table II.

Each of these samples was made in conditions designed to optimize recording of Kir or CFTR, respectively. To establish more definitively that these two types of channel occur in the same apical membrane patch and to estimate their relative abundance, recordings were made under conditions in which each type of channel could be observed, and ramp voltages were used to isolate the two kinds of channel currents (Fig. 1, top trace). In 28 excised patches that had both channel types, we observed a total of 79 CFTR and 58 Kir channels. The mean ± S.D. number of channels was 2.8 ± 0.40 CFTR channels and 2.1 ± 0.25 Kir channels per patch, with a mean CFTR:Kir ratio of 1.8 ± 0.33.

**Unitary Conductance and Inward Rectification**—In solutions containing symmetrical K⁺, unitary current traces at different membrane potentials revealed asymmetrical amplitudes characteristic of inward rectification (Figs. 1, lower trace, and 2A). The rectification was quantified in I–V curves taken from 8 to 11 patches (Fig. 2B). The average inward chord conductance taken between 0 and −200 mV was 24.4 ± 0.5 pS (n = 11) and the outward chord conductance taken between 0 and 50 mV was 9.6 ± 0.7 pS (n = 8). The magnitude of the unitary conductances and their rectification ratio of −2.5 are most similar to K⁺ channels of the Kir4.x family.

**Selective K⁺ Permeation**—To determine permeation-selectivity between K⁺ and Na⁺, we recorded the single channel currents in the inside-out configuration with pipettes filled with different concentrations of K⁺ (substituted with Na⁺) to determine reversal potentials. Unitary I–V curves at three different [K⁺]o are shown in Fig. 2B. Each symbol represents the average from 4 to 11 patches ± S.E. At negative membrane potentials ≤−100 mV, the I–V curves for lower [K⁺]o are no longer linear, suggesting that [Na⁺] blocks permeation. Low [K⁺]o data were least squares fit with a 3rd order polynomial to extract reversal potentials from the linear portions. The relative reversal potentials (Eᵢ) with respect to that in symmetrical
The Kir channels remained active in multichannel patches for more than 30 min after excision, but the open probability \( P_o \) fluctuated markedly. This complicated gating behavior is illustrated in Fig. 4A, a 35-min recording of a single Kir channel in 150 mM [KCl]/[K-glutamate]. \( P_o(t) \) was computed from the normalized average of 5000 data points for every 10 s, and the resulting time-dependent \( P_o \) was plotted (Fig. 4B). For this channel the mean overall was \( P_o = 0.55 \pm 0.27 \) (S.D.).

An expanded portion of the single channel current trace (Fig. 4C) shows that the gating consisted of at least three modes that were captured sequentially in this sample, which shows a long burst, followed by short bursts and then the start of a long closure, which could last for several or tens of seconds. Fig. 4D further expands the long burst portion to show interruptions by very fast closures, and Fig. 4E details the short burst portion that includes multiple opening and closing events lasting a few tenths of a second.

To further quantify multi-modal gating, we determined the \( P_o \) for each gating mode. An intuitive and convenient way to illustrate this analysis is depicted in Fig. 4F. First, the single channel current trace was reduced by averaging every 2-s interval of the original data. Then the reduced data were binned to make an open probability \( (P_o) \) distribution histogram with a bin width of 0.05. Three major peaks appeared in the histogram, and these were fitted with Gaussian distributions using least squares fits. The fitted peak with the lowest \( P_o \) corresponded to the long closures and had an average \( P_o = 0.10 \) and an area (representing the percent time in this mode) of 5.1. The middle peak corresponded to the short bursts and had an intermediate \( P_o = 0.42 \) and an area of 31.4. The third peak, corresponding to the long bursts, had a \( P_o = 0.80 \) and an area of 16.6. The middle peak was several folds wider (0.58) than the left (0.08) or right (0.16) peaks, suggesting that it might represent more than one gating mode.

Fluctuations like those shown in Fig. 4 were sometimes superimposed upon a gradual decrease in \( P_o \) that occurred overall during recordings of 10–60 min. This slow rundown was inconsistent from patch to patch, but when it happened it was characterized by a decrease in the long burst open time and an increase in the long closed time. The presence of 2 mM ATP in the bath did not prevent rundown.

Characteristics of Cs⁺- and Ba²⁺-Block—Cs⁺ block from the extracellular surface was examined by recording channel activity in the inside-out configuration with pipettes containing various levels of Cs⁺. In the presence of [Cs⁺]o, current traces showed apparent reductions in unitary current amplitude as a result of fast block (Fig. 5A). I–V curves (Fig. 5B) revealed that the inward currents were blocked at hyperpolarized potentials, whereas outward currents were virtually unchanged.

Normalizing the Cs⁺-blocked I–V curves to the control I–V curves gave the remaining fractions in the presence of [Cs⁺]o shown in Fig. 5A. Error bars represent transformed S.E. Data were least square fitted with the Boltzmann function. The half-maximum potentials are −255, −158, and −84 mV for [Cs⁺]o levels of 1, 5, and 20 mM, respectively. Fig. 6A depicts the Cs⁺-blocked currents normalized to that of the control versus [Cs⁺]o. The solid lines represent the least squares fit to the Hill equation. The fitted \( K_d(V) = 2.9, 3.6, 3.9, 5.4, 6.9, 11.6, 20.6, \) and 39.0 mM corresponding the membrane potentials from −200 to −60 mV with a step of 20, respectively. The voltage dependence of these Cs⁺ dissociation constants is plotted in the inset of Fig. 6B and fitted by a Woodhull-type exponential relationship that results in an electrical distance of \( \delta = 0.69 \).

Ba²⁺ block of Kir was examined in the same way as Cs⁺ block, by filling the pipette with 0.5 mM Ba²⁺. In contrast to the Cs⁺ block, which reduced the unitary current, Ba²⁺ induced a voltage-dependent, discrete block (Fig. 7). It appears that at hyperpolarized potentials, Ba²⁺ caused an increase in the closed time. These results with blockers provide an additional signature for this channel.

The basolateral membranes of Calu-3 cells contain K⁺ channels that are strongly and directly activated by 1-EBIO and are

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**Table II**

| Channel count statistics in patch clamp |
|----------------------------------------|
| Kir  | CFTR |
| Patches (n)  | 169 | 158 |
| Patches with channels (n)  | 76 (45%) | 77 (49%) |
| Channels (n)  | 145 | 221 |
| Channels per active patch (n)  | 1.9 | 2.9 |
inhibited by clotrimazole. To determine whether the apical K⁺ channel shared these properties, the apical K⁺ conductance was tested in Ussing chambers using 5 mM Ba²⁺ added to the apical chamber. The magnitude of the apical Ba²⁺ block (under conditions mentioned under “Materials and Methods”) was compared before and after 1-EBIO (500 μM, n = 8), and before and after clotrimazole (50 μM, n = 8). Neither compound caused a significant difference in the magnitude of the Ba²⁺-blockable K⁺ conductance.

ATP Weakly Activated Kir—ATP was not required for channel opening, as shown by our routine observation of Kir activity in ATP-free conditions. Furthermore, when partial rundown was observed it was not prevented by the presence of 2 mM ATP. To look for more subtle effects of ATP on channel gating, we used larger tipped pipettes to obtain patches with multiple K⁺ channels, so that fluctuation-induced error would be reduced relative to the signal. Then, the multiple (n > 2) channel patches were rapidly and repeatedly switched among several ATP levels by moving them into different streams of a multibarrel perfusion outlet.

Under these conditions, responses to ATP could be observed even in the raw traces (Fig. 8A). For analysis, control currents (0 ATP) from before and after a given ATP concentration were averaged (mean at -60 mV = -4.23 ± 0.29 pA, n = 29) and the percentage increases relative to the average were computed. This method minimized the remaining fluctuations and possible rundown. Data were compiled in this way for 6 patches, with 8–12 trials each (Fig. 8B). The data show a 35% increase in Kir currents for ATP levels of 2 or 10 mM versus 0 or 0.5 mM (p < 0.05 and p < 0.001, t test). Note that if a Pₜ of ~0.5 in 0 ATP (see Fig. 4) is considered typical, the maximal increase possible is an approximate doubling of current unless new channels are recruited.

Relative Insensitivity to pH—Kir4.2 is reported to be regulated by intracellular pH, as are other Kir channels including Kir1.1 and Kir4.1. Given the sensitivity of the above protocol, we used it again to test the sensitivity of the Calu-3 apical Kir to pH. As shown in Fig. 9, changing the pH to 8.0 and 6.7 did not affect activity. At the extreme value of pH 6.0, half of the patches tested (n = 15) were again unresponsive, but in 8 patches the Pₜ was reduced from 0.5 ± 0.03 to 0.20 ± 0.04 (n = 8). This large inhibitory effect was slow to develop, requiring 62 ± 15 s on average to reach the plateau level of inhibition (n = 6).

**DISCUSSION**

The research reported here had a simple purpose: to ascertain if the apical membranes of Calu-3 cells contain K⁺ channels, and if so to characterize those channels. We found abundant copies (about half as abundant as CFTR) of a single type of inwardly rectifying K⁺ channel whose properties are most similar to previous descriptions of Kir4.2, but that nevertheless differed from those descriptions in important respects. Thus, although Calu-3 cells express transcripts for Kir4.2, we cannot yet conclude that they are components of the apical Kir.

**Comparison of the Apical K⁺ Channel Properties with Prior Descriptions of Kir Channels**

**Unitary Conductance**—The unitary conductance of 24.4 pS in the inward direction (γᵢᵣ) differs significantly from unitary conductances for all Kir channels except Kir2.1 (γᵢᵣ = 24 or 26.9 pS) (16, 17), Kir4.1 (γᵢᵣ = 22.8–27.2 pS) (18–21), and Kir4.2 (γᵢᵣ = 25.2 pS) (22).

The **Unitary Conductance Ratio** (γᵢᵣ/γₒ) —This value must be measured at comparable levels of intracellular Mg²⁺. When measured in the presence of 2.5 mM Mg²⁺, the unitary conductance ratio of the apical Kir channel was 2.5. This weak rectification differs markedly from Kir2.1, which strongly rectifies with a ratio >20 (23, 24). Kir4.1 and Kir4.2 have been reported...
to have unitary conductance ratios between 2 and 6, consistent with what we observed.

Expression of Kir4.2 Transcripts in Calu-3 Lysates—RT-PCR was performed to amplify transcripts of Kir4.1 and 4.2; in addition we looked for Kir5.1, which is capable of forming heteromultimers when co-expressed with Kir4.1 (21, 25) or Kir4.2 (22, 26). Evidence was obtained for expression of Kir4.2, but not for Kir4.1 or Kir5.1.

Tissue Distribution—The evidence that Kir4.2 but not Kir4.1 is expressed in Calu-3 cells is consistent with prior descriptions of tissue distributions. Kir4.1 was primarily detected in brain >> kidney; whereas Kir4.2 was most readily detected in kidney >> pancreas >> lung (14). In embryonic mice, Kir4.2 was found in heart, thymus, thyroid gland, perichondrium, kidney, bladder, stomach, and lung (27). Taken together, our results suggest that the apical K+ channel in Calu-3 cells may include Kir4.2 subunits, but further study will be required to establish the molecular identity of this channel.

Probing Kir with Blockers and Activators

We tested the effects of Cs+ and Ba2+ to extend the signature properties of Kir. Block by external Cs+ was highly voltage sensitive, and required >20 mM at −80 mV. No other studies have been reported for human Kir4.2, but in mouse Kir4.2, Cs+ blocks in the 100 μM (~120 mV) range (13). Kir channels display a broad range of sensitivities to Cs+ block, with Kd values ranging over a 100-fold range near resting membrane potentials. Kir channels with micromolar sensitivity to external Cs+ include hKir2.1 at −92 mV (28), hamKir2.1 at −80 mV (29), mKir3.2 at −90 mV (30), rat Kir4.1 at −75 mV (31), and mKir4.2 at −120 mV (13). Kir channels with millimolar sensitivity to Cs+ block include Kir1.1 at −80 mV (32),...
Kir2.1 at -110 mV (17), Kir2.4 at -80 mV (33), and hKir7.1 at -60 mV (34). Values have not been reported for human Kir4.1 or Kir4.2.

ATP was not required to maintain Kir activity in Calu-3 cells, but enhancement of channel activity occurred somewhere between 0.5 and 2 mM ATP. These results are in sharp contrast to the ATP dependence of Kir2.1 (35) and Kir4.1 (31). The Kir6.x subfamily of channels is modulated by ATP in a dual fashion, with activity sustained at low cytoplasmic concentrations via a phosphorylation-dependent step (36, 37), but inhibited at higher concentrations via non-hydrolytic binding (36, 38, 39). The molecular basis underlying ATP modulation of Kir channels is under active investigation. ATP has been suggested to activate via interactions with a Walker type-A domain found in both Kir1.1 and Kir4.1 (31, 40, 41), or to inhibit via positive residues in the C terminus of Kir6.2 (42).

Modulation of Apical Kir by Internal pH

In the present study, the response of apical Kir from Calu-3 cells to cytosolic acidification was variable, with only half of the patches showing a delayed inhibition upon changing the cytosolic pH to 6.0. This result differs from the observation that Kir4.2 expressed in oocytes was consistently inhibited by reduced pH with a pKₐ of 6.7 in whole cell and 7.1 in excised, inside-out patches (22), and greatly reduces the probability that the apical Kir channel is a homomeric form of Kir4.2. The difference of -0.4 pH units between pKₐ values we measured in the different patch configurations, and the absence of any pH sensitivity in half of the channels we tested further suggests that Kir pH sensitivity depends upon additional components or states of the channel. When expressed as a homomeric channel, Kir4.2 is ranked as one of the most pH-sensitive of all homomeric Kir channels with a pKₐ sequence of Kir4.2 (pKₐ = 7.1 (22)) > Kir1.1 (pKₐ = 6.73 (19)) > Kir4.1 (pHₐ = 6.0–6.1 (19),...
KCNN4/KCNE2. 1) Kir is not Ca\(^{2+}\) channel from the basolateral channels KCNQ1/KCNE3 and serve to minimize that possibility and distinguish the present possibility that the channel studied here might be a misplaced not fully polarized. Therefore, it was important to consider theations of apical and basolateral membrane channels if they are domains, respectively.

which also code for proteins with 6 and 1 transmembrane domains, increased [cAMP] product of the

Thus, Kir4.2, which has 2 transmembrane domains and is the blocked by clotrimazole; and 5) it is co-located with CFTR.

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reversible inhibition by the low pH buffer in about half of the patches. 

heteromeric Kir4.1–Kir5.1 is drastically shifted in pH sensitiv-

20)). All of the heteromeric Kir4.x–Kir5.1 channels described to date are also inhibited by cytosolic acidification. Among them,

channels expressed from both clofilium and clotrimazole, and are probably made up of channel complexes that include KCNQ1/KCNE3 gene products, which code for proteins with 6 and 1 transmembrane domains, respectively. Ca\(^{2+}\) activated increases in \(I_{\text{sc}}\) were blocked by both clofilium and clotrimazole, and are probably made up of channels expressed from KCNN4/KCNE2 gene products, which also code for proteins with 6 and 1 transmembrane domains, respectively.

Epithelial cells grown in culture can display mixed popula-

Contrasting Properties of the Apical Channel in Comparison with Basolateral K\(^{+}\) Channels in Calu-3 Cells

Basal and stimulated secretion in Calu-3 cells requires basolateral K\(^{+}\) channels, and two types have been identified (6, 43). Most basal \(I_{\text{sc}}\) and essentially all \(I_{\text{sc}}\) stimulated by increased [cAMP], is blocked by the ammonium-derived compound clofilium, but not by clotrimazole. The clotrimazole-insensitive K\(^{+}\) channel is probably made up of channel complexes that include KCNQ1/KCNE3 gene products, which code for proteins with 6 and 1 transmembrane domains, respectively. Ca\(^{2+}\) activated increases in \(I_{\text{sc}}\) were blocked by both clofilium and clotrimazole, and are probably made up of channels expressed from KCNN4/KCNE2 gene products, which also code for proteins with 6 and 1 transmembrane domains, respectively.

Acknowledgment—We thank Dennis Lee for expert technical help.

REFERENCES

1. Shen, B. Q., Finkbeiner, W. E., Wine, J. J., Mrsny, R. J., and Widdicombe, J. H. (1994) Am. J. Physiol. 266, L493–L501
2. Haws, C., Finkbeiner, W. E., Widdicombe, J. H., and Wine, J. J. (1994) Am. J. Physiol. 266, L502–L512
3. Moon, S., Singh, M., Krouse, M. E., and Wine, J. J. (1997) Am. J. Physiol. 273, L1208–L1219
4. Lee, M. C., Penland, C. M., Widdicombe, J. H., and Wine, J. J. (1998) Am. J. Physiol. 274, L450–L453
5. Devor, D. C., Singh, A. K., Lambert, L. C., DeLuca, A., Frizzell, R. A., and Bridges, R. J. (1999) J. Gen. Physiol. 113, 743–760
6. Cowley, E. A., and Linedell, P. (2002) J. Physiol. 538, 747–757
7. Finkbeiner, W. E., Carrier, S. D., and Teresi, C. E. (1993) Am. J. Respir. Cell Mol. Biol. 9, 547–556
8. Krouse, M. E., Talbott, J. F., Lee, M. M., Joo, N. S., and Wine, J. J. (2004) Am. J. Physiol. 287, in press
9. Illek, B., Yankaskas, J. R., and Machen, T. E. (1997) Am. J. Physiol. 272, L752–L761
10. Irakawa, T., Krouse, M. E., Joo, N. S., Wu, J. V., and Wine, J. J. (2004) Am. J. Physiol. Lung Cell Mol. Physiol. 287, L784–L793
11. Bichet, D., Haase, F. A., and Jan, L. Y. (2003) Nature 424, 957–967
12. Dewson, G., Conley, E. C., and Bradding, P. (2002) BMC Genomics 3, 22
13. Pearson, W. L., Dourado, M., Schreiber, M., Salkoff, L., and Nichols, C. G. (1999) J. Physiol. 514, 639–653
14. Shuck, M. E., Piser, T. M., Bock, J. H., Slighston, J. L., Lee, K. S., and Bienkowski, M. J. (1997) J. Biol. Chem. 272, 586–593
15. Kusaka, S., Horio, Y., Fujita, A., Matsushita, K., Inanobe, A., Gotow, T., Uchiyama, T., Tano, Y., and Kurachi, Y. (1999) J. Physiol. 520, 373–381
16. Tare, M., Prestwich, S. A., Gerdienko, D. V., Parvener, S., Carver, J. E., Robinson, C., and Bolton, T. B. (1998) J. Physiol. 506, 303–318
17. Hayashi, M., Komazaki, S., and Ishikawa, T. (2003) J. Physiol. 547, 255–269
18. Yang, Z., and Jiang, C. (1999) J. Physiol. 520, 921–927
19. Xu, H., Yang, Y., Cui, N., Wang, L., Wu, D., Qian, S., and Jiang, C. (2000) J. Physiol. 528, 267–277
20. Tanemoto, M., Kittaka, N., Inanobe, A., and Kurachi, Y. (1997) Am. J. Physiol. 514, 113, 743–760
21. Tanemoto, M., Kittaka, N., Inanobe, A., and Kurachi, Y. (1997) Am. J. Physiol. 514, 113, 743–760
22. Pessia, M., Imbrici, P., D’Adamo, M. C., Salvatore, L., and Tucker, S. J. (2001) J. Biol. Chem. 276, 15, 335–341
23. Finkbeiner, W. E., Carrier, S. D., and Teresi, C. E. (1993) Am. J. Physiol. 272, L784–L793
24. Pearson, W. L., Dourado, M., Schreiber, M., Salkoff, L., and Nichols, C. G. (1999) J. Physiol. 514, 639–653
25. Shuck, M. E., Piser, T. M., Bock, J. H., Slighston, J. L., Lee, K. S., and Bienkowski, M. J. (1997) J. Biol. Chem. 272, 586–593
26. Kusaka, S., Horio, Y., Fujita, A., Matsushita, K., Inanobe, A., Gotow, T., Uchiyama, T., Tano, Y., and Kurachi, Y. (1999) J. Physiol. 520, 373–381
27. Tare, M., Prestwich, S. A., Gerdienko, D. V., Parvener, S., Carver, J. E., Robinson, C., and Bolton, T. B. (1998) J. Physiol. 506, 303–318
28. Hayashi, M., Komazaki, S., and Ishikawa, T. (2003) J. Physiol. 547, 255–269
29. Yang, Z., and Jiang, C. (1999) J. Physiol. 520, 921–927
30. Xu, H., Yang, Y., Cui, N., Wang, L., Wu, D., Qian, S., and Jiang, C. (2000) J. Physiol. 528, 267–277
31. Tanemoto, M., Kittaka, N., Inanobe, A., and Kurachi, Y. (2000) J. Physiol. 525, 587–592
32. Pesina, M., Imbrici, P., D’Adamo, M. C., Salvatore, L., and Tucker, S. J. (2001) J. Physiol. 532, 359–367
33. Fakler, B., Schultz, J. H., Yang, J., Schulte, U., Brandle, U., Zernner, H. P., Jan, L. Y., and Ruppersberg, J. P. (1996) EMBO J. 15, 4093–4099
34. Bradley, K. K., Jagg, J. H., Benev, A. D., Hepper, T. J., Flynn, R. R., Nelson, M. T., and Horowitz, B. (1999) J. Physiol. 515, 639–651
35. Yang, Z., Xu, H., Cui, N., Qiu, A., Chanechuvapal, S., Shen, W., and Jiang, C. (2000) J. Gen. Physiol. 114, 35–45
26. Lourdel, S., Paulais, M., Cluzeaud, F., Bens, M., Tanemoto, M., Kurachi, Y., Vandewalle, A., and Teulon, J. (2002) J. Physiol. 538, 391–404
27. Thiery, E., Gosset, P., Damotte, D., Delezide, A. L., de Saint-Sauveur, N., Vayssettes, C., and Creau, N. (2000) Mech. Dev. 95, 313–316
28. Klein, H., Garneau, L., Coady, M., Lemay, G., Lapointe, J. Y., and Sauve, R. (1999) J. Membr. Biol. 167, 45–52
29. Thompson, G. A., Leyland, M. L., Ashmole, I., Suttle, M. J., and Stanfield, P. R. (2000) J. Physiol. 526, 231–249
30. Lesage, F., Guillaumare, E., Pink, M., Duprat, F., Heurteaux, C., Fosset, M., Romey, G., Barhanin, J., and Lazdunski, M. (1995) J. Biol. Chem. 270, 28660–28667
31. Takumi, T., Ishii, T., Horio, Y., Morishige, K., Takahashi, N., Yamada, M., Yamashita, T., Kiyama, H., Sohmiya, K., Nakanishi, S., and Kurachi, Y. (1995) J. Biol. Chem. 270, 16339–16346
32. Bhandari, S., and Hunter, M. (2001) Kidney Blood Press Res. 24, 142–148
33. Topert, C., Doring, F., Wischmeyer, E., Karschin, C., Broekhaus, J., Ballanyi, K., Derst, C., and Karschin, A. (1998) J. Neurosci. 18, 4096–4105
34. Krapivinsky, G., Medina, I., Eng, L., Krapivinsky, L., Yang, Y., and Clapham, D. E. (1998) Neuron 20, 995–1005
35. Fakler, B., Brandle, U., Bond, C., Glowatzki, E., Konig, C., Adelman, J. P., Zengner, H. P., and Ruppersberg, J. P. (1994) FEBS Lett. 356, 199–203
36. Findlay, I., and Dunne, M. J. (1986) Pflogers Arch. 407, 238–240
37. Wang, W., and Giebisch, G. (1991) J. Gen. Physiol. 98, 35–61
38. Cook, D. L., and Hales, C. N. (1984) Nature 311, 271–273
39. Noma, A. (1983) Nature 305, 147–148
40. Ho, K., Nichols, C. G., Lederer, W. J., Lytton, J., Vassilev, P. M., Kanaizirska, M. V., and Hebert, S. C. (1993) Nature 362, 31–38
41. McNicholas, C. M., Yang, Y., Giebisch, G., and Hebert, S. C. (1996) Am. J. Physiol. 271, F275–F285
42. Trapp, S., Haider, S., Jones, P., Sansom, M. S., and Aschcroft, F. M. (2003) EMBO J. 22, 2903–2912
43. Cuthbert, A. W., and MacVinish, L. J. (2003) Br. J. Pharmacol. 140, 81–90
44. Cook, D. L., and Young, J. A. (1989) J. Membr. Biol. 110, 139–146
45. Silva, P., Stoff, J., Field, M., Fine, L., Forrest, J. N., and Epstein, F. H. (1977) Am. J. Physiol. 233, F298–F306
46. Fujita, A., Horio, Y., Higashi, K., Mouri, T., Hata, F., Takeguchi, N., and Kurachi, Y. (2002) J. Physiol. 540, 85–92
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J. Biol. Chem. 2004, 279:46558-46565.
doi: 10.1074/jbc.M406058200 originally published online August 24, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M406058200

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