brief communication

Noise analysis and single-channel observations of 4 pS chloride channels in human airway epithelia

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ABSTRACT Apical membranes of human airway epithelial cells have significant chloride permeability, which is reduced in cystic fibrosis (CF), causing abnormal electrochemistry and impaired mucociliary clearance. At least four types of chloride channels have been identified in these cells, but their relative roles in total permeability and CF are unclear. Noise analysis was used to measure the conductance of chloride channels in human nasal epithelial cells. The data indicate that channels with a mean conductance of 4.5 pS carry most of the chloride current, and that the mean number of such channels per cell is ~4,000. Chloride channels in this conductance range were also seen in single-channel recordings.

INTRODUCTION

Chloride permeability is a crucial component of the normal electrochemistry of airway epithelial cells, regulating chloride and sodium fluxes through the epithelium and thereby influencing the viscoelastic properties of the mucus. In CF, chloride permeability is reduced, leading to a thicker mucus and pathological complications which are major causes of morbidity and mortality (McPherson and Dormer, 1991; Boucher et al., 1988; Welsh, 1987). Expression of the normal form of the cystic fibrosis transmembrane conductance regulator increases chloride currents in cystic fibrosis airway epithelial cells (Rich et al., 1990) and stimulates the appearance of an 8.4 pS anion channel in insect cells (Kartner et al. 1991), but the link between the gene product and the anion conductance is not yet understood.

Single-channel patch clamp experiments have identified four groups of chloride ion channels in airway epithelia, and some of these could contain subgroups (Duszyk et al., 1989; Gögelein, 1988; Vaughan and French, 1989). At least two types of chloride channels are abnormal in CF (Duszyk et al., 1989; Li et al., 1988; Schoumacher et al., 1987) but the relative contributions of different channels to total chloride conductance are unknown. In earlier experiments, we showed that there are significant numbers of chloride channels below 10 pS conductance (Duszyk et al., 1989), and recent findings suggest that a substantial number of chloride channels are of 5 pS or lower conductance in airway cells (Grygorczyk, 1991). In order to estimate the conductance of the majority channels, we have now used a form of noise analysis on whole-cell currents in human nasal airway epithelial cells.

Cells were obtained from human turbinates, and digested overnight with mild agitation in 1 mg/ml type 14 protease in MEM with antibiotics at 4°C before being raised to 37°C in a 5% CO_2 incubator. Cells were separated from the stroma by mild agitation for 1 min, followed by protease neutralization and washing in serum, then plated on Primaria® plates at low density in 5% FBS-DME/F12 and stored at 37°C in 5% CO_2. The medium was replaced every 2 d. Cells were used 1–4 d after plating. Medium was replaced with Ringer solution (three washes) containing (mM) 140 NaCl, 5.4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, pH 7.3. Normal pipet solution contained (mM) 140 KCl, 0.5 $CaCl_2$, 2 $MgCl_2$, 5 EGTA, 10 HEPES, pH 7.3. Sodium replacement experiments used choline chloride instead of NaCl.

Whole-cell experiments were performed on nonconfluent, single cells using pipets made from thin walled borosilicate glass with a two-stage horizontal puller. Pipet tips were fire polished to ~0.5 μm (<5 MΩ) immediately before use. Recording and stimulation was performed by a List EPC-7 amplifier. Voltage protocols and data gathering were performed by a digital computer using 12-bit digital-to-analogue and analogue-to-digital interfaces. The computer also performed dynamic compensation for series resistance. All experiments were conducted at 37°C.

Noise analysis has been used before to estimate single channel conductance in a variety of cells, including intact epithelia (Lindemann and Van Driessche, 1977; Neher and Stevens, 1977; Wills and Zweifach, 1987). The technique uses the fact that ion channels randomly open and close, creating microscopic variations in current around a mean value. The sum of all channel fluctuations creates a noise in the mean current whose variance, σ^2, depends upon the number of channels, N,

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and the single channel conductance, γ:

$$\sigma^2 = I(V - E)\gamma - I^2/N, \quad (1)$$

where I is the total current, V is the membrane potential, and E is the reversal potential (Ehrenstein et al., 1970; Sigworth, 1980). The variance, σ^2, can be estimated from the power spectrum of the noise, where each relaxation state of the channel creates a Lorentzian function:

$$S(f) = S_0/[1 + (f/f_c)^2]. \quad (2)$$

S_0 denotes the plateau of the Lorentzian, f is frequency, and f_0 is the corner frequency of the Lorentzian, which is inversely proportional to the mean channel open time (Lindemann and Van Driessche, 1977). The variance of the channel noise is obtained by integrating all the Lorentzian components.

Assuming that the reversal potential, membrane potential, and total current can be measured, Eq. 1 contains two unknowns, the number of channels and their conductance. In order to separate the two unknowns, it is usually necessary to use stimulating or blocking factors to increase or decrease the open probability of the channels and to create more than one independent equation (Lindemann and Van Driessche, 1977). However, if the current–voltage relationship is nonlinear (caused by voltage-dependent open probability and/or channel conductance), it is also possible to obtain multiple independent estimates of σ^2 by changing the membrane potential. In these experiments we found that channel conductance was almost independent of voltage, but open probability increased significantly with voltage, allowing estimation of σ^2 at several different voltages.

Whole-cell currents in isolated cells from normal human nasal epithelia are shown in Fig. 1 *A* and *B*. Membrane potentials were −24.9 ± 12.6 mV (mean ± SD, n = 12) on forming the whole cell configuration but dropped to zero within 5 min of perfusion by the 145 mM chloride solution in the pipet. Currents were unchanged when choline chloride was substituted for sodium chloride in either the bath or the pipet. These findings indicate that chloride is the major ion carrying membrane current, as reported before for airway epithelia (McCann et al., 1989; Schoppa et al. 1989). The maximum current at 80 mV in Fig. 1 *A* is 416 pA. The maximum current at this potential varied between 356 and 2,465 pA for the 12 different cells, reflecting the range of cell diameter.

In more than 80% of cells the current–voltage relationships did not show any significant evidence of time-dependent behavior. Chloride currents, which inactivate with time, have been described previously in human airway epithelial cells (McCann et al., 1989) but other reports show data similar to Fig. 1 *A* (Anderson et al., 1991; Rich et al., 1990). We observed time-varying currents in a small proportion of cells, suggesting that more than one type of epithelial cell may be present in the cultures. We did not observe current increasing with time, as has been reported before for time-dependent currents (McCann et al., 1989). Data reported here are from cells without time-varying currents.

For noise analysis, whole-cell currents of 20 s duration were low pass filtered with a 9-pole, 250 Hz, active filter. Data were sampled at 1-ms intervals and processed by the fast Fourier transform in segments of 1,024 data points. Noise in the whole-cell current records was always well fitted by Eq. 2 (Fig. 2 *A*). An additional term corresponding to $1/f$ noise was included in the fitted equation (Neumcke, 1978). The relative contribution of the Lorentzian component to the total variance in Fig. 2 *A* (62%) was quite typical for the measurements. No clear dependence of $1/f$ noise on voltage was

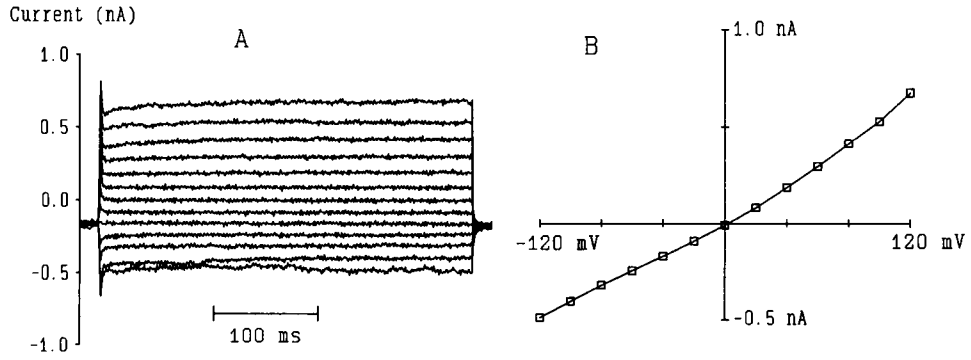

FIGURE 1 Whole cell currents in a human airway epithelial cell. (*A*) Responses to steps from a holding potential of −40 mV to levels of −120 to 120 mV. Note the increased low frequency noise at large negative potentials. (*B*) Current–voltage plot of the same data. The fitted line is a fourth order polynomial in voltage.

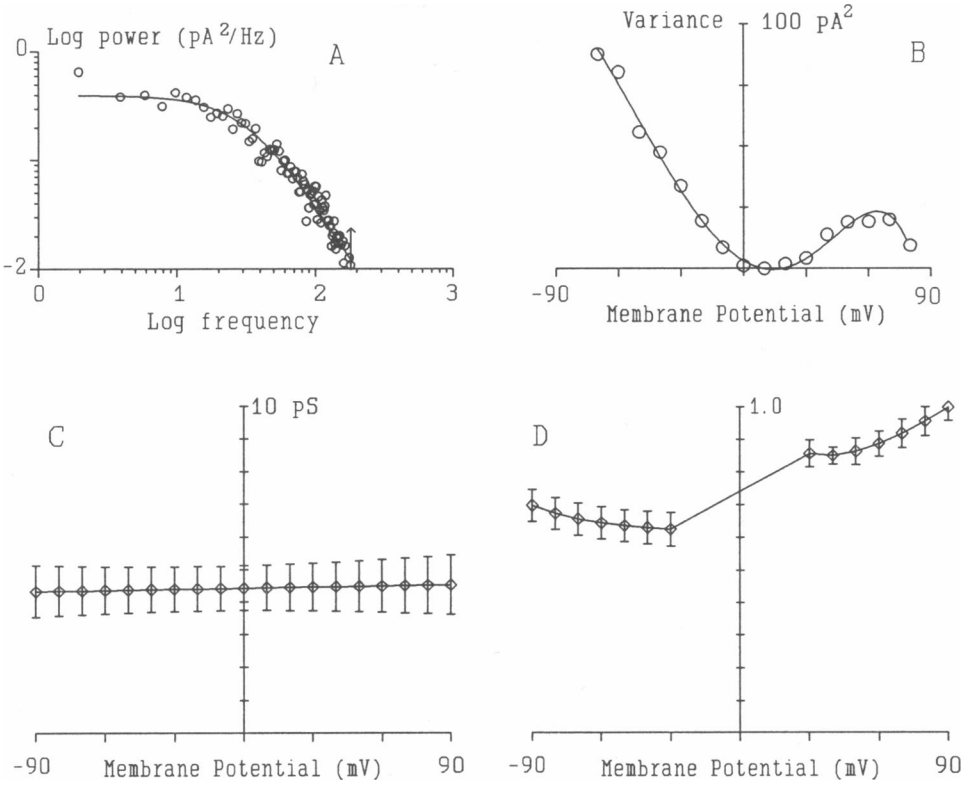

FIGURE 2 Noise analysis of whole-cell records. (*A*) Power spectrum of the noise at 70 mV in 20 s of current data from the experiment shown in Fig. 1. Noise having a $1/f$ spectrum has been removed from this data. The solid line is the best fit from Eq. 2, using the minimum χ^2 method of Levenberg-Marquardt (Press et al., 1990). In this example, the Lorentzian component contributed 62% of total power at all frequencies. Data were fitted in the range 0–180 Hz, where the filter output was 99–100% of input. (*B*) Total Lorentzian variance as a function of membrane potential for the same cell. The solid line is the best fit of Eq. 1 using the same nonlinear fitting algorithm, with fitted parameters: $\alpha = 4.87$ pS, $\beta = 0.009$ pS/V, $N = 3{,}151$. (*C*) Single channel conductance, γ (mean ± SD), as a function of membrane potential for 12 whole-cell recordings. (*D*) Mean open probability, P_o, for the 12 whole-cell recordings calculated from Eq. 4. P_o was not calculated for ±20 mV around the reversal potential because the small values of I and V–E made it unreliable.

observed. There was no evidence for a second Lorentzian component in the data, indicating that a single group of channels dominates the chloride permeability. The mean value of the corner frequency, f_c, was 13.7 ± 7.9 Hz (n = 132 Lorentzian fits on 12 cells).

Fig. 2 *B* shows noise variance versus membrane potential from a typical set of current recordings, fitted by Eq. 1. In order to allow for variable channel conductance with voltage, as reported for some epithelial chloride channels (Welsh, 1987), the variable γ in Eq. 1 was initially replaced by a second-order polynomial series in voltage. However, only slight voltage dependence was observed in any experiments, so a first order approximation was used:

$$\gamma = \alpha + \beta V. \tag{3}$$

Eqs. 1 and 3 were fitted to data from 12 experiments using a nonlinear fitting method. In each case the algorithm converged rapidly after a small number of iterations. Convergence was insensitive to starting conditions in a wide range, and the results were all obtained from fitting with initial values of $\alpha = 50$ pS, $\beta = 0$ pS/V, $E = 0$ V, $N = 1{,}000$. The probability of the channels being open was calculated at each voltage from:

$$P_o = I/[N(V - E)\gamma]. \tag{4}$$

Figs. 2 *C* and *D* show mean ± SD values of γ and P_o as functions of voltage. Single channel conductance was almost independent of voltage with a mean value of 4.5 ± 1.7 pS. The number of channels per cell was 4,000 ± 2,000. P_o increased with depolarization, reaching values close to unity by 90 mV. This reduced the noise variance at depolarized voltages because channels that are mainly open create no noise.

Single channel measurements were made by the excised inside-out patch clamp technique on similar

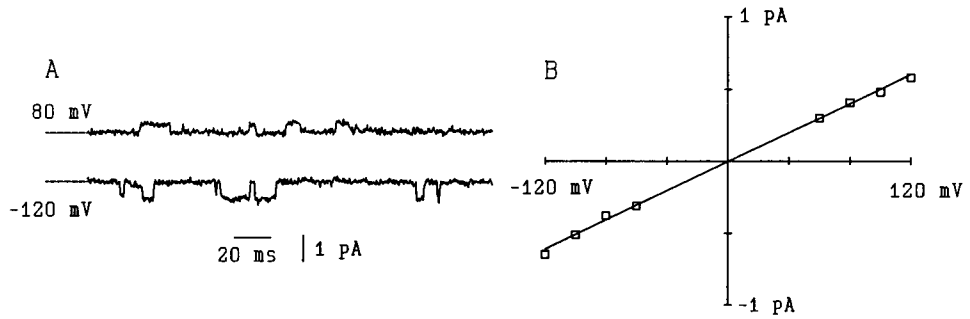

FIGURE 3 Single channel observations of a small chloride channel. (*A*) Single channel current records. Dashed lines indicate the closed condition in each case. (*B*) Current–voltage plot for the channel. The solid line is a linear regression fit of 4.97 pS. Solutions were (mM), bath: 140 NaCl, 2 $CaCl_2$, 2 $MgCl_2$, 5 HEPES, pH 7.3; pipet: 140 choline chloride, 2 $CaCl_2$, 2 $MgCl_2$, 5 HEPES, pH 7.3. Pipets were fabricated from thick walled borosilicate glass using a two-stage horizontal puller and fire-polished to a final resistance of ~15 MΩ. Procedures for stimulating, recording, and data analysis have been described in detail previously (Duszyk et al., 1990).

groups of nasal epithelial cells using procedures described previously (Duszyk et al., 1989, 1990). Fig. 3 *A* shows examples of recordings from one channel, and Fig. 3 *B* the current-voltage relationship. The single channel conductance in this case was 4.97 pS. It was not possible to estimate the proportion of channels in this conductance range because of the relative difficulty of identifying channels below ~5 pS in our recordings. Current–voltage relationships were always linear, in agreement with the noise results.

The open probabilities of single 4 pS channels were typically below 0.3, which is significantly lower than obtained from the noise analysis. The difference could be due to reduced channel activation in the excised patches, which would be expected if the channels are regulated by a process such as cAMP-dependent phosphorylation.

The noise experiments reliably indicated that channels of ~4.5 pS carry most of the chloride current in these cells. Noise analysis can underestimate single channel conductance if Lorentzian components with high corner frequencies are not detected. However, single channels of this size were seen in excised patches, and single chloride channels with linear conductance in this range have recently been reported in another study of human airway epithelia (Grygorczyk, 1991). Linear conductance anion channels with conductances of 6–7 pS have also been reported in human pancreatic epithelia (Gray et al., 1990). Although single-channel work has so far emphasized the role of larger channels (> 5 pS), it seems likely that improved single-channel measurements will discover more channels below this value in the future. Our data indicate that most chloride conductance is due to such small channels. Therefore, severe abnormalities of chloride conductance, as seen in CF, would be expected to involve small channels, as suggested by the CF gene expression work (Kartner et al., 1990).

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Note added in proof: Dalemans et al. have found that normal and ΔF508 CFTR gene expression produce linear chloride channels of 4.9 and 4.3 pS, respectively. The open probability of the normal channels was ~0.3, as reported here for excised patches.

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