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Outward-Rectifying Chloride Channels in Cultured Adult and Fetal Human Nasal Epithelial Cells

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Summary. The patch-clamp technique was used to characterize ion channels in the apical membranes of cultured human nasal epithelial cells, dissociated from fetal nasal mucosa and from adult nasal polyps. Outward-rectifying chloride channels were found in 4.3% of the cell-attached patches from fetal cells (n = 258) and in 3.1% of the patches from adult cells (n = 320). After excision the number of patches containing active chloride channels increased threefold to 13% of the patches from the fetal cells and 10% from adult cells. The single-channel conductance at 0 mV in symmetrical 150 mM NaCl solutions was 24.3 ± 0.9 pS (n = 28) and 26.0 ± 1.2 pS (n = 30), respectively, in adult and fetal cells and showed outward rectification in the potential range from −80 to +80 mV. In fetal cells as well as in adult cells the channels were anion selective, and were almost impermeable for larger anions and monovalent cations. In cell-free patches the channels were Ca^{2+} independent. In most of the channels the open probability was voltage independent and high (±0.86); in 20% of the channels, however, the open probability increased with depolarization. In conclusion, fetal nasal epithelial cells contain chloride channels in their apical membranes with single-channel properties and regulatory mechanisms similar to those found in cells from adults.

Key Words chloride channels · nasal epithelium · cell culture · human fetus · adult

Introduction

Ion transport by airway epithelial cells, and more specifically chloride secretion, is important in the regulation of the quantity and the composition of the respiratory tract fluid and thus in effective mucociliary clearance. Outward-rectifying chloride channels in the apical membranes of human (Frizzell, Rechkemmer & Shoemaker, 1986*b*; Welsh, 1986*a*; Gruenert et al., 1988) and canine (Shoemaker et al., 1986; Welsh, 1986*b*) tracheal and human nasal epithelial cells (Li et al., 1988) have been shown to be responsible for the chloride efflux at the apical membrane (Frizzell et al., 1986*a*; Welsh, 1986*a*). Most of the channel properties seem to be common, but the results on Ca^{2+} independence are not unanimous and the single-channel conductance is either around 25 pS (Welsh, 1986*a,b*; Gruenert et al., 1988; Li et al., 1988) or 45 pS (Frizzell et al., 1986*a,b*; Shoemaker et al., 1986). This, however, may be due to different culture and experimental conditions. Therefore, these channels can be regarded as the same type of chloride channel, responsible for the apical chloride permeability.

Chloride channel activity is regulated and stimulated by intracellular cAMP (Al Bazzaz, 1981; Smith et al., 1982). At least one element of this regulatory pathway is considered to be defective in cystic fibrosis since activation of the chloride channel is impaired in both cell-attached patches (Frizzell et al., 1986*b*; Welsh & Liedtke, 1986) and inside-out patches (Schoumacher et al., 1987*b*; Li et al., 1988). Nevertheless the development of the respiratory tract in utero appears not to be affected in cystic fibrosis, since respiratory problems arise mainly after birth.

Outward-rectifying chloride channels were also found in a number of secretory epithelial cells, including human colonic carcinoma cells T84 (Frizzell et al., 1986*a*; Halm, Schoumacher & Frizzell, 1987) and HT29 (Hayslett et al., 1987; Dreinhofer, Gögelein & Greger, 1988), rat colonic cells (Reinhardt et al., 1987), sweat gland secretory cells (Krouse et al., 1987; Schoumacher, Shoemaker & Frizzell, 1987*a*; Welsh, McCann & Dearborn, 1987) and rabbit renal cortical thick ascending limb of Henle's loop (Gögelein, 1988). More recently similar channels were described in human T- and B-lymphocytes (Chen, Schulman & Gardner, 1989) and cultured fibroblasts (Bear, 1988).

For the present study we used nasal polyps rather than tracheal tissue to investigate and characterize chloride channels, because this tissue is more readily available during polypectomy without additional harm or discomfort to the patient and since only a partial description of chloride channels in nasal epithelial cells has been published (Li et al., 1988). The resemblance and similarities between nasal and tracheal epithelium are of course striking and evident: pseudostratified, ciliated and mucus producing, but as there are differences in function and morphology, a study of nasal epithelial cells is justified.

The development of the respiratory tract is a complex process. The various cell types differentiate at different times, e.g., ciliated cells appear before goblet cells (Poulsen & Tos, 1975). In contrast with the many studies on morphology and its development, little is known about the electrophysiological properties of fetal respiratory tract epithelium and their development, neither in vivo, nor in vitro. For these reasons, we also studied fetal nasal epithelial cells at 16 weeks gestation when ciliary differentiation is complete, but when the first goblet cells only just appear.

In another report (Jorissen et al., 1990*b*) we described nonselective cation channels in the apical membranes of cultured fetal nasal epithelial cells, with identical properties as in adult cells. Here, we report on outward-rectifying chloride channels in cultured fetal nasal epithelial cells and show the similarity of properties such as current-voltage relation, single-channel conductance, ion selectivity, open probability, channel distribution, influence of Ca^{2+} and finally channel activity in cell-attached patches, to those found in adult cells.

Materials and Methods

Cell Isolation and Cell Culture

Human nasal epithelial cells were obtained from 11 adults, treated surgically for polyposis nasi, and from 4 aborted fetuses, 16 to 19 weeks gestational age. The tissue fragments were digested with 0.1% Pronase (Sigma) in DME (Dulbecco's Modified Eagle's) (Gibco) containing penicillin (50 IU/ml) and streptomycin (50 μg/ml) during 16 to 24 hr at 4°C. After three washes in culture medium, the dissociated epithelial cells were plated on rat tail collagen gels (0.1 ml/cm^2) in 2-cm^2 wells (Costar) at a density of $\pm$2,000 cells/cm^2. The culture medium consisted of Ham's F12-DME 1/1 (Gibco) supplemented with penicillin (50 IU/ml), streptomycin (50 μg/ml), choleratoxin (10 ng/ml) (Sigma) and 10% NU-serum (Flow laboratories). The medium (1 ml) was changed after 1 day and further three times a week. The cells were cultured at 37°C up to 28 days before use. For the patch-clamp experiments, the collagen gel with the epithelial cells was placed in a 0.7-ml bath at room temperature and continuously perfused with standard bath solution.

Solutions

All solutions were buffered with 10 mM HEPES and adjusted to pH 7.4 with 1 M KOH. The pipette solution contained (in mM): 150 NaCl and 2 $CaCl_2$. Standard bath solution contained (in mM): 140 NaCl, 1 $CaCl_2$, 2 $MgCl_2$ and 5 KCl. The standard bath solution for inside-out patches contained (in mM): 150 NaCl and 1 $CaCl_2$. The solutions for determination of the selectivity contained 2 mM $CaCl_2$ and a variable component: 150 mM KCl, CsCl or CholineCl. Also 300 mM and 50 mM NaCl were used, as well as replacement of 100 mM Cl^- by 100 mM gluconate$^-$, 100 mM isethionate$^-$ or 50 mM sulphate^{2-}. These deviations of the composition of the bath solution are explicitly mentioned in Results. Ca^{2+}-free solutions contained (in mM): 150 NaCl and 1 EGTA.

Experimental Setup

Seals were made in confluent areas of cells according to the technique described by Hamill et al. (1981). Patch electrodes of 4 to 10 MΩ, pulled from borosilicate glass, were fire polished. Backfilling of the pipettes was used after the tip was filled by capillarity. A 1 M KCl 1% agar bridge, connecting the Ag-AgCl reference electrode to the bath solution, reduced contact potentials in asymmetrical chloride concentrations to less than 1 mV. Cells and pipettes were visualized with an invert microscope (IM35 Zeiss) and monitored on closed television circuit. After contact with the cell membrane was made, cell-attached patches were obtained by controlled suction. The seal resistance varied between 3 to 100 GΩ. Currents were recorded from cell-attached, inside-out and occasionally outside-out patches. Currents were amplified by a List patch clamp EPC5, and were recorded and stored on FM-tape (Racal Store 4DS) at 19 cm/sec. After 10 min of spontaneous cell-attached observation, the cell was stimulated with adrenalin (1 μg/ml) for 10 min. If ion channels opened during the cell-attached recording, currents were recorded at different potentials to identify the channel and the patch was excised. Inside-out patches were formed by abruptly lifting the pipette. Sometimes excision led to vesicle formation. These were ruptured by brief exposure to air.

Data Acquisition and Analysis

The analog currents were filtered at 500 Hz, sampled at 1 kHz using a 12-bit AD converter and stored in records of 2,048 points. Baseline current was subtracted. Single-channel current amplitudes and open probabilities were determined from amplitude histograms. Current-voltage curves were drawn by eye and the slope conductances were measured at different potentials. Records were displayed on a Hewlett Packard *X-Y* plotter (model 7470A). The sign of the clamp voltage refers to the bath with respect to the patch pipette for the cell-attached and inside-out patches and the opposite for the outside-out patches. Positive (outward) currents implicate flow of positive charges from the cytoplasmic side to the extracellular side. Data from series are given as mean $\pm$ SEM.

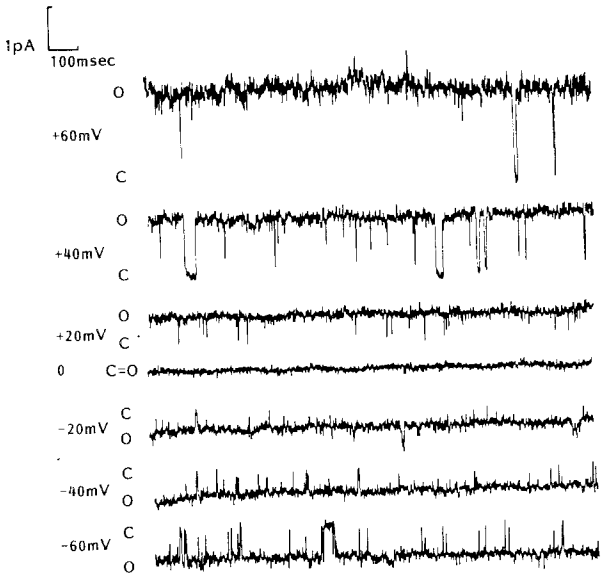

Fig. 1. Single-channel currents from an inside-out patch of cultured fetal nasal epithelial cells in symmetrical conditions at different potentials. Pipette and bath solutions contained 150 mM NaCl, 10 mM HEPES and 1 and 2 mM $CaCl_2$, respectively. The filter frequency was 500 Hz. *C* indicates the baseline current and *O* the current level when the chloride channel is open. The single-channel current amplitudes are larger at the positive than at the corresponding negative potentials and most of the time the current remains at the open level

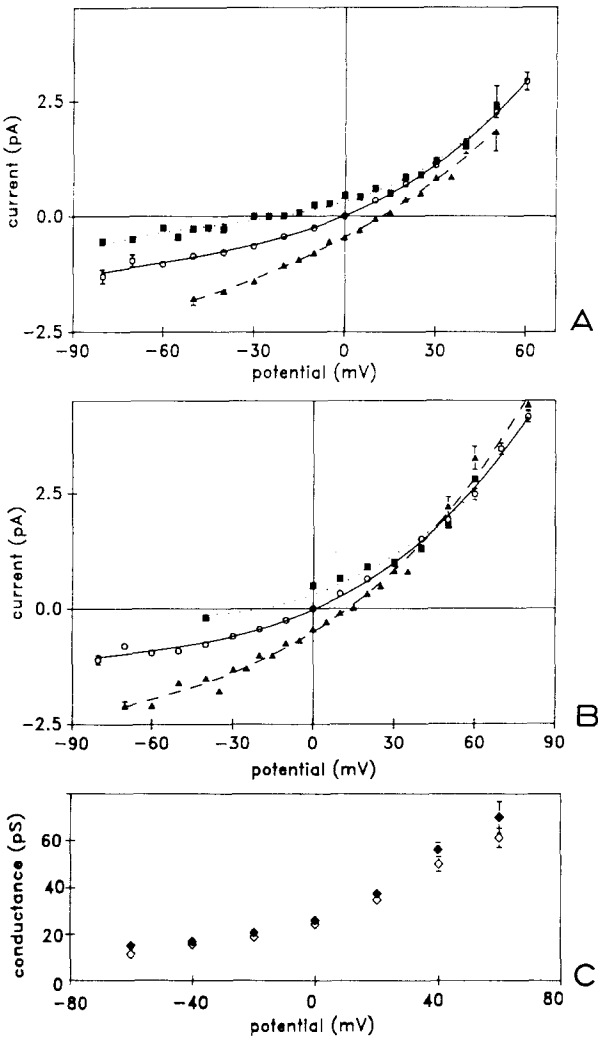

Fig. 2. Single-channel current-voltage relations of outward-rectifying chloride channels from cell-free patches of fetal (*A*) and adult (*B*) cells in different NaCl bath solutions. Pipette solution contained 150 mM NaCl, 2 mM $CaCl_2$ and 10 mM HEPES. Bath solution contained 1 mM $CaCl_2$ in the symmetrical 150 mM NaCl solution and 2 mM $CaCl_2$ in the asymmetrical conditions. Data for 150 mM NaCl (circles) were obtained from 33 patches from fetal cells and 29 patches from adult cells. For 300 mM NaCl (triangles) 19 fetal and 22 adult experiments were used, and for 50 mM NaCl the results from 5 fetal and 2 adult patches were used (squares). The data are plotted as means for 50 mM NaCl in adult cells and as means ± SEM for the other conditions and all are fitted by a third-order polynomial regression. The current-voltage relations are outward rectifying, demonstrate the chloride selectivity and are not different between the fetal and the adult experiments. (*C*) The slope conductances of channels from fetal (filled diamonds) and adult cells (open diamonds) at different potentials in symmetrical 150 mM NaCl are plotted as means ± SEM

Results

General Description of Single-Channel Currents

Figure 1 shows typical current recordings of an inside-out patch from the apical membrane of a cultured fetal nasal epithelial cell in symmetrical 150 mM NaCl solutions. At all potentials, except at 0 mV, two discrete current levels were present with rapid transitions between the two levels. The probability of finding the current at the closed level was far less than at the open level. The amplitude of the single-channel currents was larger at depolarizing, positive potentials than at the corresponding negative potentials indicating outward rectification. The recordings of this and other patches were obtained immediately after excision of the patch in a bath solution containing 1 mM $CaCl_2$ or in a Ca^{2+}-free bath solution. Similar activity was present in 32 out of 320 patches from adult cells (10%) and in 33 out of 258 patches from fetal cells (13%), independent from the time in culture (*data not shown*).

Open-Channel Properties

The resulting average current-voltage relation in symmetrical 150 mM NaCl solutions for the patches from fetal and adult cells are shown in Fig. 2*A* and *B* (open circles). The reversal potential was always 0 mV, since at that potential current events were never observed. A marked outward rectification was observed in the potential range from −80 and +80 mV. The conductance at 0 mV was 26.0 ± 1.2 pS

(n = 30) for fetal and 24.3 ± 0.9 pS (n = 28) for adult cell-free patches. The slope conductances are plotted as a function of the potential for the fetal (filled diamonds) as well as adult cells (open diamonds) in Fig. 2*C*. At −60 mV the slope conductance was only 15.0 ± 1.3 pS (n = 23) for the fetal cells and 11.4 ± 1.0 pS (n = 14) for the adult cells, while at +60 mV the values were respectively 70.0 ± 6.7 pS (n = 16) and 61.2 ± 4.1 pS (n = 15). There was no difference in the current-voltage relation between channels for fetal cells and from adult cells.

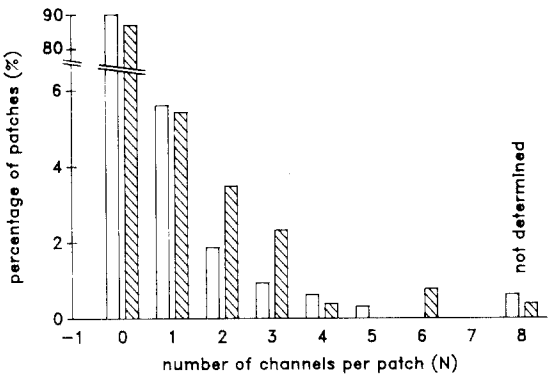

Fig. 3. The number of outward-rectifying chloride channels per patch. The number of channels was determined by the maximal number of simultaneously open channels at moderate depolarizing potentials. The first column contains the blank patches (respectively 90 and 87% for the patches from adult and fetal cells). The last column contains the three patches where the exact number of channels could not be determined

Ion Selectivity

The selectivity of the channels was measured in inside-out patches by varying the NaCl concentration of the bath solution (Fig. 2) and by replacements of Na^+ or Cl^- in the bath solution by different cations or anions, respectively. The bath solution always contained 2 mM $CaCl_2$. In a first step, P_{Cl}/P_{Na} was determined by increasing NaCl in the bath solution to 300 mM (Fig. 2, filled triangles): the reversal potential shifted in fetal cells to +12.6 ± 0.3 mV (n = 17) and to +13.5 ± 0.4 mV (n = 22) in patches from adult cells. Using the Goldman-Hodgkin-Katz equation for each patch, the P_{Cl}/P_{Na} was 11.5 ± 3.0 for the channels in fetal cells and 16.6 ± 3.0 in adult cells. Lowering the NaCl concentration in the bath solution to 50 mM (filled squares) shifted the reversal potential to −25.3 ± 1.5 mV (n = 5) in fetal cells and to −24.0 mV in two patches from adult cells, resulting in a P_{Cl}/P_{Na} of 79.2 ± 31.7 and 23.9, respectively. In outside-out patches from adult cells (n = 2), the reversal potentials were +13 mV in 300 mM NaCl and −23 mV in 50 mM NaCl. In outside-out patches from fetal cells (n = 3), it shifted to +15 mV in 300 mM NaCl. Therefore, in all conditions studied the channels were at least 10 times more permeable for Cl^- than for Na^+ in fetal cells as well as in adult cells.

The permeability for larger anions was determined in inside-out patches from adult cells by replacing 100 mM NaCl in the bath solution by equimolar amounts of sodium isethionate, sodium glucuronate or by 50 mM sodium sulphate. Since the reversal potentials were, respectively, −23.3 ± 1.1 mV (n = 3), −23.3 ± 2.0 mV (n = 3) and −23.3 ± 1.4 mV (n = 4), the effect of chloride replacement was identical to that of chloride reduction and none of these larger anions permeated well (P_{Cl}/P larger anion > 15).

When, for inside-out patches, Na^+ was replaced by K^+ in the bath solution (seven fetal and seven adult experiments), by Cs^+ (four fetal and three adult experiments) or by choline$^+$ (three fetal and four adult experiments), neither the reversal potential, nor the single-channel currents changed (*data not shown*). So, the channel did not discriminate between these different cations.

Number of Channels and Open Probability

The number of channels per patch was determined as the maximal number of simultaneously open chloride channels at depolarizing potentials, because the open probability was high. The distribution of the number of channels per patch is illustrated in Fig. 3. Of the 320 patches from adult cells and 258 patches from fetal cells, only 32 and 33, respectively, appeared to contain functional outward-rectifying chloride channels. In 3 of these 65 patches the number of chloride channels could not be measured accurately. The mean number of channels per patch for the patches with chloride channels was 2.6 ± 0.4 (n = 32) for the fetal cells. This was not significantly different from the adult cells: 1.7 ± 0.2 (n = 30).

The open probability was calculated from amplitude histograms of inside-out patches with chloride channels from 22 fetal cells and from 26 adult cells. For each amplitude histogram a minimal period of 10 sec (most were longer than 30 sec) with representative activity was analyzed. Since the open probability did not vary extensively between different test periods, a 10-sec period was sufficient and representative (*data not shown*). For the majority (n = 39) of these 48 patches, the open probability was high and voltage independent, *see* Fig. 4. There was no significant difference between fetal (n = 17) (filled circles) and adult cells (n = 22) (open circles):

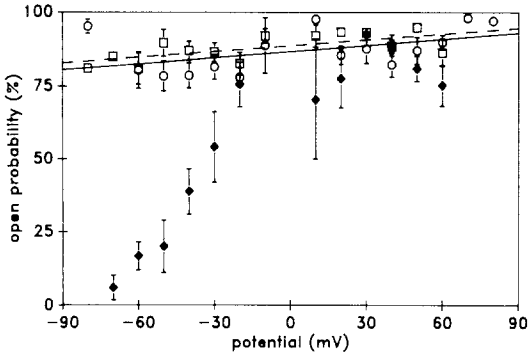

Fig. 4. Open probability *versus* potential. The open probabilities of the majority of the chloride channels from fetal cells (open squares) and adult cells (open circles) and of a minor (different) group from both fetal and adult cells (filled diamonds) are plotted at different potentials as means ± SEM. The bath solution for these inside-out patches contained 150 mM NaCl and 1 mM $CaCl_2$. For 80% of the channels (n = 17/22 from fetal and 22/26 from adult cells) the open probability is high (86 ± 1%) and voltage independent. The dashed line (fetal cells) and the solid line (adult cells) were obtained by linear regression. There is no siginficant difference between the fetal and the adult cells. A minority (20%) of the chloride channels from fetal (n = 5/22) and adult cells (n = 4/26) has a voltage-dependent open probability (filled diamonds), less than 50% at potentials ranging from −80 to −40 mV and more than 80% at +30 to +50 mV.

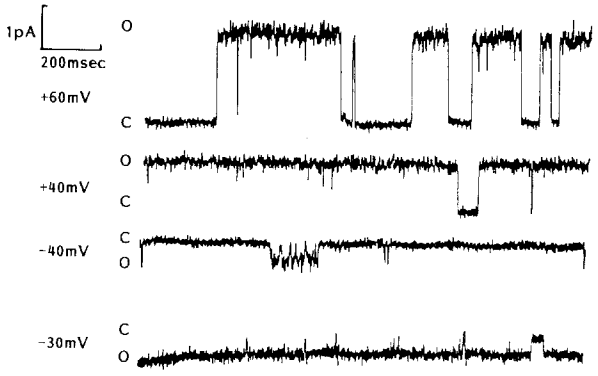

Fig. 5. Single-channel currents at different potentials obtained from an inside-out patch of cultured fetal nasal epithelium, which shows a different (voltage-dependent) open probability. The pipette and bath solution contained 150 mM NaCl and 2 and 1 mM $CaCl_2$, respectively. The filter frequency was 500 Hz. The upper traces were recorded immediately after excision. At moderate depolarizing potentials the current remains most of the time at the open level, but at hyperpolarizing and strongly depolarizing potentials the channel is closed most of the time. The lowest trace was recorded 10 min later, when the open probability at hyperpolarizing potentials had increased. The single-channel current did not change

88.5 ± 1.0% (122 measurements) *versus* 83.9 ± 1.3% (138 measurements).

In a minority (n = 9) of the patches, five from fetal cells and four from adult cells, channels with identical single-channel currents but with different kinetics were present (Fig. 5). At hyperpolarizing potentials and for the illustrated channel also at depolarizing potentials of more than +40 mV, the open probability was much lower than at potentials around +20 mV. At hyperpolarizing potentials, very long closed times were interrupted by bursts of openings (minimal one open and two closed times). At more positive potentials the channel was most of the time in the open state with much shorter closures (minimal one open and one closed time). At increasing depolarization, long closed times appeared again and were interrupted by periods of channel activity that resembled that of around +30 mV and that were clearly different from the bursting pattern that was observed at hyperpolarization (minimal one open and two closed times). The mean ± SEM for these nine patches are plotted as diamonds in Fig. 4. In four of these nine patches the open probability at hyperpolarizing potentials increased to a value comparable to that of the majority after 10 to 15 min inside-out recording, as is illustrated at −30 mV in the lowest trace of Fig. 5. In two other patches the voltage dependence of the open probability was still present after 1 hr recording under different conditions. The chloride channels in these nine patches were not significantly different from the ones with voltage-independent open probability for cell-attached activity (n = 2) and for excision in Ca^{2+}-free bath solution (n = 3).

The single-channel currents were not influenced at any potential by varying Ca^{2+} concentration in the bath solution from 2 mM to less than 10^{-8} M in inside-out patches from fetal cells (n = 15) as well as adult cells (n = 13). The open probability was not altered. These outward-rectifying chloride channels were thus not dependent on the presence of Ca^{2+} at the cytoplasmic side. In one outside-out patch from an adult cell, the channel was also found to be Ca^{2+} independent at the extracellular side. There was no evidence for loss of Ca^{2+} dependence after excision, since a number of patches that were excised in a Ca^{+}-free bath solution, contained active chloride channels.

Cell-Attached Mode Activity

From the 56 inside-out patches with chloride channels, chloride channels were already active in the cell-attached configuration in 37.5%. Spontaneous activity was present in nine patches from fetal cells and seven from adult cells. The outward rectification of the current-voltage relation and the conductance

of about 25 pS at 0 mV indicated that they were chloride channels. In addition, the reversal potential was about 0 mV, as expected for chloride channels, since the intracellular Cl^- activity is around 35 mM and the potential difference over the apical membrane is 40 mV (Welsh, 1987). The reversal potentials for K^+ and Na^+ channels would be $> +60$ and -30 mV, respectively, based on the presence of 150 mM NaCl in the pipette solution and intracellular K^+ and Na^+ activities of 83 and 11 mM, respectively (Welsh, 1987). In two cell-attached patches from fetal cells and three from adult cells, chloride channels activated after stimulation with adrenalin. In the remaining 17 patches from fetal cells and 18 patches from adult cells chloride channels were found only after excision.

Discussion

In this report we described outward-rectifying chloride channels in the apical membranes of cultured human nasal epithelial cells from adults and fetuses. The channels found in the fetal cells cannot be distinguished from those found in cells from adults.

In culture, respiratory epithelial cells maintain their polarization, with the apical membrane exposed to the medium and the basolateral membrane in contact with the collagen gel (Jorissen et al., 1989). Since only cells in patches of epithelial cells were tested, these channels must be located in the apical membranes.

The channels found after 2 to 3 weeks in culture are identical to those found immediately after plating. Therefore, the specific electrophysiological properties appear to be well conserved in culture. Respiratory epithelial cells in culture maintain not only their polarization, but also most of the specific electrophysiological properties, like a transepithelial potential difference and regulatory mechanisms of ion transport (Welsh, 1985, 1986*a*; Yankaskas et al., 1985*a*; Cotton et al., 1987) and in cultured cells from cystic fibrosis (Widdicombe, Welsh & Finkbeiner, 1985) the apical membrane chloride conductance is reduced.

The channels described here have the properties of chloride channels. First of all, the reversal potentials in asymmetrical chloride solutions indicate anion selectivity and chloride permeability. The P_{Cl}/P_{Na} of about 13 is similar to the values published (Frizzell et al., 1986*b*; Welsh, 1986*a*). Secondly, in cell-attached patches the reversal potential of around 0 mV agrees well with the equilibrium potential for chloride being near the resting apical membrane potential, and is different from the expected reversals for other ions, like Na^+ and K^+. Thirdly, larger anions are less permeable than chloride.

The current-voltage relation of the chloride channels in fetal and adult cells is outward rectifying with a conductance of 25 pS at 0 mV in symmetrical 150 mM NaCl solutions. No significant differences are found between the slope conductances of the fetal and adult channels. Outward rectifying chloride channels are also found in tracheal epithelial cells from human (Frizzell et al., 1986*b*; Welsh, 1986*a*) and canine origin (Shoemaker et al., 1986; Welsh, 1986*b*) and also in other cell types (*see* references in Introduction). A conductance of about 25 pS for the outward-rectifying chloride channels, as found in this study, is reported for human and canine tracheal epithelial cells (Welsh, 1986*a*,*b*), human nasal epithelial cells (Li et al., 1988) and sweat gland secretory cells (Krouse et al., 1987).

In our experiments cell-attached activity is present in 37.5% of the patches that contain chloride channels after excision. The current-voltage relation of the channels in cell-attached patches is similar to that of inside-out patches with a reversal potential around 0 mV. The jump in baseline current level upon lifting the pipette indicates that spontaneous excision of the patches did not occur and that cell free was regarded as cell attached. The frequency of total cell-attached activity in this study is situated between that found for human trachea (56 and 58%) (Frizzell et al., 1986*b*; Welsh, 1986*a*) and that for canine trachea (28%) (Welsh, 1986*b*). In vivo, apical membrane chloride conductance is low in resting conditions and increases by elevation of intracellular cAMP levels (Al Bazzaz, 1981; Smith et al., 1982). More specifically in tracheal epithelial cells cAMP activates about 30% of the apical chloride channels (Frizzell et al., 1986*b*; Welsh, 1986*a*). In nasal epithelial cells the apical membrane chloride conductance increases also by cAMP elevation (Yankaskas et al., 1985*b*; Cotton et al., 1987). Choleratoxin, added in this study to the culture medium to enhance cell growth and prolong the time span for cultured cells to be used, increases cellular cAMP (Finkelstein, 1973; Gill & King, 1975). The so-called spontaneously active channels in this study may thus already be cAMP-stimulated channels but, in five additional cell-attached patches, chloride channels activated after stimulation by the beta-adrenergic agent, adrenalin, which increases cAMP. This relative low percentage of adrenergic response contrasts with human trachea, where an equal amount of patches showed spontaneous and stimulated activity (Welsh, 1986*a*) and with canine trachea, where even most cell-attached recordings were obtained only after beta-adrenergic stimulation (Welsh, 1986*b*).

In our experiments there is no difference in

channel activity between Ca^{2+}-rich (1 mM) and Ca^{2+}-free ($<10^{-8}$ M) bath solution. Even after excision in Ca^{2+}-free solutions, chloride channels activate without depolarization of the patch. The channel is therefore Ca^{2+} independent. This is also reported for the 25-pS channel in human tracheal epithelial cells (Welsh, 1986*a*). Calcium dependence, however, is reported for the 45-pS chloride channel (Frizzell et al., 1986*b*).

For the open probability of the chloride channels we find two populations equally distributed over the adult and fetal cells. The open probability of most of the channels is high (±86%) and does not show a significant voltage dependence and is not influenced by Ca^{2+}. This high and voltage-independent open probability is present in 80% of our experiments on both adult and fetal cells and contrasts with the high interchannel and interstudy variability, the lower open probabilities and the variable voltage dependence reported. In human tracheal cells the open probability of the channels is reported to be variable, but not clearly voltage dependent (Frizzell et al., 1986*a*; Welsh, 1986*a*; Li et al., 1988). In other cell types, however, the open probability of the outward-rectifying chloride channels is voltage dependent; it increases with depolarization in shark rectal gland (Greger, Schlatter & Gögelein, 1987), in HT29 colonic carcinoma cells (Hayslett et al., 1987) and T84 colonic carcinoma cells (Frizzell et al., 1986*a*; Halm et al., 1987), and in human lymphocytes (Chen et al., 1989), but decreases with depolarization in canine tracheal cells (Shoemaker et al., 1986).

In a minority of the patches (20%) the chloride channels have different kinetics at hyperpolarizing potentials with long closed periods, interrupted by bursts of flickering openings, as is also observed in colonic cells (Frizzell et al., 1986*a*; Hayslett et al., 1987) and lymphocytes (Chen et al., 1989). At depolarizing potentials the open probability and kinetic appearance are not different from those of the majority of the chloride channels. The open probability of these channels is thus voltage dependent with increasing values for increasing potentials, but at strongly depolarizing potentials (> +40 mV), the open probability decreases again. This voltage-dependent open probability is not related to the patch configuration of the first channel activity or to the calcium concentration in the bath solution used during excision and is similar in the patches from fetal and adult cells. In a number of patches this voltage dependence, observed immediately after excision to an inside-out patch, changes within 15 min into the kinetic behavior of the majority. It cannot be excluded that depolarization induces this change.

The data on nasal epithelial cells from adults in this study, agree well with those found in tracheal cells. Based on the similarity with the chloride channels in tracheal epithelial cells, shown to be responsible for the chloride secretion (Frizzell et al., 1986*a*; Welsh, 1986*a*), the striking morphological and functional similarities between nasal and tracheal epithelium and the presence of spontaneous and β-adrenergic-evoked activity in this study, it is likely that the channel described here is involved in chloride secretion in nasal epithelium.

In fetal nasal epithelial cells channels identical to the adult ones are present with similar regulatory mechanisms. This leads us to conclude that at 16 weeks gestational age, when organogenesis is incomplete (Poulsen & Tos, 1975), human fetal nasal epithelial cells not only express the nonselective cation channels (Jorissen et al., 1990), but they also express functional chloride channels in their apical membranes with all the properties of those found in adults.

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