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Characteristics of Chloride Transport in Nasal Mucosa from Patients with Primary Ciliary Dyskinesia

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Abstract

Objectives—Primary ciliary dyskinesia (PCD) is an inherited disorder that produces lifelong difficulties with chronic airway inflammation. Little is known about the role of chronic airway inflammation on chloride ion transport properties in PCD. This study is to assess the cAMP-regulated chloride (Cl) ion transport properties of freshly excised nasal mucosa from PCD compared with normal and chronic rhinosinusitis (CRS).

Study Design—Electrophysiology study utilizing Ussing type hemi-chamber technique with three different types of nasal tissue (normal, CRS, PCD) obtained from patients during endoscopic surgery at tertiary referral center.

Methods—Nasal tissues were examined under short-circuit conditions and gradient-driven Cl currents were continuously recorded. The cAMP elevating agonist (forskolin) was added to stimulate CFTR-mediated Cl secretion. To prevent misinterpretation of flux measurement, Cl transport inhibitors were used at the end of all experiments. Basal Cl currents (I_{Cl}) and changes in I_{Cl} to forskolin (ΔI_{Cl}) were compared between normal, CRS and PCD nasal tissues.

Results—Forskolin stimulated Cl currents across all different types of nasal epithelia. The Cl secretory response was effectively blocked by the Cl ion transport inhibitors. I_{Cl} were significantly higher in normals ($155.0 \pm 9.3 \mu A/cm^2$) compared to CRS ($79.1 \pm 15.0 \mu A/cm^2$) and PCD ($70.9 \pm 20.4 \mu A/cm^2$) ($p = 0.005$). ΔI_{Cl} in CRS ($14.8 \pm 2.3 \mu A/cm^2$) and PCD ($12.2 \pm 2.4 \mu A/cm^2$) were markedly diminished compared to normals ($28.3 \pm 4.7 \mu A/cm^2$) ($p = 0.024$).

Conclusions—PCD tissues were characterized by impaired I_{Cl} and ΔI_{Cl} . Both parameters were reduced by 54.3 % and 56.9 % in PCD when compared to normals.

Keywords

Ussing chamber; electrophysiology; chloride ion transport; primary ciliary dyskinesia; nasal mucosa

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The study protocol has been approved by Stanford University and CHORI Institutional Review Board.

INTRODUCTION

Primary ciliary dyskinesia (PCD) is a genetic oto-sino-pulmonary disease associated with defective ciliary structure and function and has an estimated prevalence of 1:15,000 to 30,000 live births, although this is likely to be an underestimate as under-diagnosis is common.^{1,2} Diagnosis of PCD is made using a combination of careful clinical evaluation and electron microscopic analysis of ciliary ultrastructure and may be difficult in some subjects.³ Although the cystic fibrosis transmembrane conductance regulator (CFTR) gene is not mutated in PCD patients, they resemble patients with cystic fibrosis (CF) in many ways and it has been suggested that treatment of CF can be extrapolated, in part, to PCD and other ciliary syndromes.^{4,5} However, little is known about the ion transport properties in PCD. There is emerging evidence that chronic airway infection and inflammation may lead to an acquired CFTR defect that will affect normal hydration of the airway surface lining fluid⁶⁻¹⁰. For example, the presence of interferon- γ , IL-4, TGF β 1, reactive oxygen and nitrogen species, and *Pseudomonas aeruginosa* toxin pyocyanin have been recently shown to decrease CFTR expression and cAMP-mediated Cl⁻ secretion in human airway epithelial cultures. The purpose of this report was to assess the cAMP-regulated chloride (Cl) ion transport properties of freshly excised nasal mucosa from PCD compared with normal and chronic rhinosinusitis (CRS).

METHODS

This study was approved by the institutional review boards at Stanford University and Children's Hospital Oakland Research Institute. Normal and diseased nasal mucosa (nasal septum or turbinates) were obtained from patients undergoing endoscopic sinus surgery (ESS) for benign tumor or medically refractory chronic rhinosinusitis (CRS). CRS samples were from CRS without polyps and all CRS patients fulfilled diagnostic criteria endorsed by the American Academy of Otolaryngology.¹¹ PCD was diagnosed when patients were young by electron microscopy at Stanford University Medical Center. Tissue specimens were immediately stored in cold LHC-9 medium (Invitrogen, Carlsbad, CA). A thin layer of epithelial tissue was dissected from the specimen, placed on a slider that holds human biopsies with an open area of 0.031 - 0.1 cm², and mounted between Easy Mount Ussing chambers (Physiologic Instruments Inc., San Diego, CA) and transepithelial short-circuit measurements were performed as described previously in sinus epithelia.¹² Short-circuit current (I_{sc}) was continuously recorded and at 60-second intervals transepithelial voltage was clamped for 1 s from 0 to 1 mV to monitor transepithelial resistance (R_{te}) using Ohm's law. At the beginning of the experiment, tissue specimens were perfused with warmed (37°C) Krebs bicarbonate solution containing (in mM) 120NaCl, 25NaHCO₃, 5KCl, 1.375 NaH₂PO₄, 5.6glucose, 2.0 CaCl₂, and 1.2MgCl₂. After blocking Na⁺ absorption with amiloride (10 μ M), half of the Cl salts were exchanged for gluconate salts in the mucosal reservoir and specimens were equilibrated at least for 30 minutes. Then the cAMP elevating agonist (20 μ M forskolin, bilateral) was added to maximally stimulate CFTR-mediated Cl secretion. To determine the magnitude of transcellular Cl ion movement, CFTR blocker (1 mM glibenclamide, mucosal) and NaKCC inhibitor (500 μ M bumetanide, serosal) were used at the end of each experiment to block Cl exit across apical CFTR Cl channels and cellular uptake of Cl at the basolateral membrane via Na-K-2Cl cotransporter. The stabilized gradient driven (basal) Cl currents (I_{Cl} , μ A/cm²) and the change in forskolin-stimulated Cl currents (ΔI_{Cl} , μ A/cm²) were compared between three different groups of nasal epithelia. Low-resistance tissues ($R_{te} < 20 \Omega \cdot \text{cm}^2$) were excluded from the analysis. Statistical analyses were performed using SPSS software (version 15.0) and $p < 0.05$ was considered significant. The data were subjected to initial testing for normal distribution using Kolmogorov-Smirnov. After normality test, one way ANOVA, followed by the Dunn multiple comparison tests was applied.

RESULTS

Transepithelial Cl currents (I_{Cl}) across freshly excised nasal mucosa were measured in the presence of the sodium channel blocker amiloride and a serosal-to-mucosal directed Cl gradient ($-Cl_{muc}$). Gradient driven Cl currents (I_{Cl}) stabilized after ~30 minutes and were significantly higher in normals ($I_{Cl} = 155.0 \pm 9.3 \mu A/cm^2$) compared to CRS ($I_{Cl} = 79.1 \pm 15.0 \mu A/cm^2$) and PCD ($I_{Cl} = 70.9 \pm 20.4 \mu A/cm^2$) ($p = 0.005$), which was on average 49.0% less in CRS and 54.3% less in PCD than the currents observed in normal nasal epithelia (Figure 1). The corresponding mean resistances (R_{te}) were $31.3 \pm 4.1 \Omega \cdot cm^2$ (Normal), $43.1 \pm 4.9 \Omega \cdot cm^2$ (CRS), and $33.6 \pm 10.8 \Omega \cdot cm^2$ (PCD), respectively and there was no statistical significance between the three groups ($p = 0.254$).

Exposure of the apical surface to cAMP agonist forskolin (20 μM) gradually stimulated I_{Cl} in a sustained fashion over the course of 15–30 minutes. Subsequent additions of the Cl transport inhibitors glibenclamide and bumetanide significantly reduced forskolin-stimulated I_{Cl} to baseline levels indicating that cAMP agonist stimulated Cl transport across the epithelium (Figure 2). Although stimulated by forskolin, the Cl secretory responses in CRS ($\Delta I_{Cl} = 14.8 \pm 2.3 \mu A/cm^2$) and PCD ($\Delta I_{Cl} = 12.2 \pm 2.4 \mu A/cm^2$) were markedly smaller compared to normals ($\Delta I_{Cl} = 28.3 \pm 4.7 \mu A/cm^2$) ($p = 0.024$) (Figure 3). The forskolin-induced increase in Cl secretion was on average 48% less in CRS and 56.9% less in PCD than the increase observed in nasal epithelia from normal subjects. Exposure of CFTR blocker (1mM glibenclamide, mucosal) and NaKCC inhibitor (500 μM bumetanide, serosal) significantly reduced Cl secretion across all nasal tissues (Figure 4). The magnitude of reductions in Cl currents (ΔI_{Cl}) averaged $-11.8 \pm 3.5 \mu A/cm^2$ in CRS, $-5.9 \pm 1.2 \mu A/cm^2$ in PCD, and $-14.7 \pm 3.9 \mu A/cm^2$ in normals, which did not reach statistical significance ($p = 0.154$).

DISCUSSION

PCD is diagnosed using a combination of a careful clinical history together with an examination of ciliary structure. The upper respiratory tract is frequently involved in PCD. Inadequate innate mucus clearance commonly manifests as CRS and some patients develop nasal polyposis.³ Emerging developments in measuring ciliary beat frequency, ciliary beat patterns and nasal NO show promise as diagnostic or screening tools. Primary therapeutic goals in PCD are to diminish mucus viscosity and to enhance mucociliary clearance, definitely similar to the treatment of CF. At this time, there are no therapies adequately studied to definitively prove their efficacy in the treatment of PCD and to correct ciliary dysfunction.³

Epithelial ion transport controls the hydration of mucosal surfaces in the airway and promotes effective mucociliary clearance. The cystic fibrosis transmembrane conductance regulator (CFTR) functions as a chloride (Cl) and bicarbonate (HCO_3) channel and is critical for the normal height of the airway surface liquid (ASL).¹³ Skrzypczak et al. mentioned that one of the possible candidates for the PCD modifier genes is *CFTR* and the heterozygous F508del mutation was found in PCD patients.¹⁴ The observed frequency of $\Delta F508$ carriers in the Polish PCD patients was slightly higher than expected, however the difference was not significant. A higher frequency of mutations in atypical cases of chronic sinusitis (55% of patients) was observed compared with the general population suggested that a heterozygote CFTR gene mutation could be a sinusitis-causing status.¹⁵ Furthermore carriers of a single CF mutation have a higher prevalence of CRS than the general population. In this present study nasal tissues from PCD and CRS patients were not genotyped for possible CF mutations and this aspect should be considered in future clinical studies.

We used freshly excised human tissue to assess its functional chloride ion transport characteristics. Freshly excised human sinonasal epithelia are easily accessible in the clinic or during ESS, yielding satisfactory surface area for ion transport analysis.¹² Good tissue viability is critical for quantitative assessment of bioelectric responses and is best maintained by storing tissue specimens on ice in an appropriate medium and by conducting functional measurements immediately.¹⁶ During the mounting process, it is also important to strip the mucosal layer from underlying cartilage or connective tissue layer without damaging or touching the epithelial layer. We demonstrated that freshly excised human nasal epithelial tissue can be analyzed successfully in Ussing chambers and therefore, believe that analysis offers a novel and effective platform for electrophysiologic studies of the upper respiratory epithelium.

To our knowledge there are no other published Ussing chamber studies using freshly excised sinonasal tissues and there is no reference standard we could use to make a comparison about the variability and distribution of the basal gradient-driven Cl current data in freshly excised human nasal tissues. We may expect that the absolute value of the basal I_{Cl} can be quite variable due to edge damage and paracellular permeability. Interestingly, the gradient-driven Cl currents (I_{Cl}) were two times higher in normal nasal tissues compared to I_{Cl} measured in CRS and PCD suggesting that transepithelial Cl^- movement is reduced in chronic airway inflammation. In our preliminary experiments with CF tissues from homozygous delta F508 mutation patients, basal Cl currents were significantly reduced by 70~80% compared to normals (unpublished data). However, primary cultures derived from CRS specimens exhibited larger sodium absorption and a greater capacity for Cl-secretion when compared to cultures derived from normal subjects.¹⁷ Although the effects of chronic inflammation found in cystic fibrosis airways in vivo persist for at least several days in primary cultures, it is possible that inflammatory mediators or other factors that lead to reduced Cl secretory responses in CRS and PCD seem to be lost on extended culture or in passaged cells.

Forskolin stimulated Cl currents across nasal mucosa from normals, CRS, and PCD. However, forskolin stimulated Cl currents were significantly reduced to almost the same degree in nasal epithelia from diseased mucosa (CRS by 48% and PCD by 57%), suggesting a common underlying factor(s) for compromising CFTR function during chronic airway infection and inflammation. For example interferon- γ , IL-4, TGF β 1, and *Pseudomonas aeruginosa* toxin pyocyanin as well as reactive oxygen and nitrogen species were all reported to decrease CFTR expression and cAMP-mediated Cl-secretion in airway epithelia which may lead to an acquired CFTR defect⁶⁻¹⁰. By using chloride ion transport blockers at the end of each experiment, we examined whether smaller stimulation response might be caused by prior CFTR activation in these tissues. Statistical significance was lacking in the magnitude of the blocker responses among three groups, however CRS tissues showed a trend for a reduced Cl transport blocker response which is another indicator for reduced CFTR function in these tissues. A decrease of the CFTR-mediated Cl secretion into the mucosal surface of the airways may contribute to the development of CRS by depletion of the ASL. Tarran et al showed that active Na^+ absorption and Cl secretion are required to regulate normal ASL volume, whereas respiratory cilia are not required for ASL volume regulation.¹⁸ Without functioning cilia, PCD patients are primarily dependent on mechanical means of clearing respiratory secretions (e.g., nose blowing, coughing).¹⁹ Mucus clearance in PCD may thus be improved by enhancement of chloride secretion, which may increase the ASL volume and thus thin out viscous secretions.

CONCLUSION

Freshly excised nasal mucosa from PCD patients were characterized by impaired basal Cl secretion and Cl secretory responses to forskolin, which were reduced by 54.3% and 56.9%, respectively when compared to nasal mucosa from normal subjects. Therefore, up-regulating CFTR-mediated Cl secretion may provide a potential target for the improvement of mucociliary clearance treatment of thickened secretions in PCD.

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Figure 1.

Basal Cl currents (I_{Cl} , $\mu A/cm^2$) in nasal tissue surgical specimen from normal subjects and patients with chronic rhinosinusitis (CRS), and PCD. I_{Cl} was measured in presence of a serosal-to-mucosal gradient for chloride after currents had stabilized (see Methods). I_{Cl} are plotted from individual experiments and average values are shown as horizontal bars. Basal I_{Cl} were significantly higher in nasal tissues from normal specimen ($I_{Cl} = 155.0 \pm 9.3 \mu A/cm^2$) compared to specimen obtained from CRS ($I_{Cl} = 79.1 \pm 15.0 \mu A/cm^2$) and PCD patients ($I_{Cl} = 70.9 \pm 20.4 \mu A/cm^2$). N refers to the number of single measurements from different individuals. * $P < .05$ ($p = 0.005$) compared with I_{Cl} among three groups, one way ANOVA followed by the Dunn multiple comparison test. Error bars represent SEM (standard error of the mean). ● : Normal nasal tissue, ○ : CRS, ○ : PCD.

Figure 2.

Stimulation of transepithelial Cl secretion by forskolin in freshly excised nasal tissue from PCD patient. Transepithelial Cl currents (I_{Cl}) were measured in the presence of the sodium channel blocker amiloride (10 μ M, mucosal) and a serosal to mucosal directed Cl gradient ($-Cl_{muc}$) (120 mM: 60 mM). Gradient driven Cl currents stabilized after ~30 minutes and transepithelial resistance was 44.4 $\Omega \cdot cm^2$ in this experiment. Addition of the cAMP agonist forskolin (20 μ M, bilateral) stimulated I_{Cl} and the forskolin-stimulated I_{Cl} was sequentially blocked by addition of the CFTR blocker glibenclamide (1 mM) and the NaKCC inhibitor bumetanide (200 μ M).

Figure 3.

Changes in basal Cl currents after stimulation with forskolin (ΔI_{Cl}) in freshly excised nasal tissues from normal subjects (Normal), and patients with CRS and PCD. Forskolin stimulated Cl currents are plotted from individual experiments and average values are shown as horizontal bars. The largest Cl secretory response to forskolin was noted in nasal epithelia from normal subjects. Both nasal epithelia from CRS and PCD patients showed lower Cl secretory responses to forskolin compared to their normal counterparts. N refers to the number of single measurements from different individuals. * $P < 0.05$ ($p = 0.024$) compared with ΔI_{Cl} among three groups, one way ANOVA followed by the Dunn multiple comparison test. Error bars represent SEM. ● : Normal nasal tissue, ○ : CRS, ○ : PCD.

Figure 4.

Mean changes (ΔI_{Cl}) in Cl currents after stimulation with forskolin (ΔI_{Cl}) and inhibition of Cl secretion by using CFTR blocker (1mM glibenclamide, mucosal) and NaKCC inhibitor (500 μ M bumetanide, serosal). The magnitude of reductions in Cl currents (ΔI_{Cl}) from blockers are $-11.8 \pm 3.5 \mu A/cm^2$ (Normal), $-5.9 \pm 1.2 \mu A/cm^2$ (CRS), and $-14.7 \pm 3.9 \mu A/cm^2$ (PCD). There was no statistical significance in the magnitude of Cl blocking among three groups ($p = 0.154$). N refers to the number of single measurements from different individuals. $P > 0.05$, one way ANOVA followed by the Dunn multiple comparison test. Error bars represent SEM. ■: Normal nasal tissue, □: CRS, Hatched square: PCD