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A new device for *in vivo* measurement of nasal transepithelial potential difference in cystic fibrosis patients and normal subjects

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A new device for in vivo *measurement of nasal transepithelial potential difference in cystic fibrosis patients and normal subjects. O. Duperrex, P-Y. Berclaz, D. Bertrand, J.S. Lacroix, N. Pochon, D. Belli, T. Rochat. ©ERS Journals Ltd 1997.*

ABSTRACT: Measurement of transepithelial potential difference (PD) on the nasal mucosa has been proposed to test for defective ion transport in cystic fibrosis (CF), and its possible correction after gene therapy or other treatments. The "classical" method records nasal PD under the inferior turbinate, with the disadvantage that the tip of the electrode is not seen by the operator. We have developed a purpose-designed perfusion electrode for PD recording on the visible, medial/posterior aspect of the turbinate. We wanted to determine whether such PD recordings adequately discriminate between CF patients and normal subjects.

Measurements of baseline PD and response to a standardized perfusion protocol were performed in 20 normal subjects and 12 CF patients. Solutions of amiloride, with or without low chloride buffer were applied for 3 min.

Increased baseline PD and depolarization after amiloride discriminated CF patients from normal subjects. Only one CF patient overlapped with the normal range. Superfusion of low chloride buffer with amiloride and terbutaline caused repolarization in 18 out of 20 normal subjects (90%), consistent with physiological Cl^- secretion process, but in none of the CF patients.

We conclude that measurements of potential difference on the medial/posterior aspect of the turbinate can discriminate between cystic fibrosis patients and normal subjects. At this site, visual control of the measurement is possible. and the mucosa is easily accessible for subsequent cytological sampling or biopsy.
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Measurements of transepithelial potential difference (PD) on the nasal mucosa of normal subjects and patients with cystic fibrosis (CF) were first described by KNOWLES and co-workers [1, 2] in 1981. The purpose of these measurements was to provide a method, in human subjects, for noninvasive studies on ion transport through the respiratory epithelium, which is characteristically altered in CF. In 1990, ALTON *et al*. [3] proposed the use of measurements of nasal PD as a clinical tool for the diagnosis of CF when results of the sweat test are equivocal.

Recently, the development of trials of gene therapy in CF patients, with the aim of transferring the cystic fibrosis transmembrane conductance regulator (CFTR) complementary deoxyribonucleic acid (cDNA) into the epithelial cells of the respiratory mucosa, has opened new perspectives for the application of nasal PD measurements [4]. Because of the numerous unsolved problems in this area, the nasal mucosa has acted as an interesting surrogate for the bronchial mucosa in clinical experimentation. As compared with the bronchial mucosa, it is much easier to access, and safety problems are substantially decreased. Despite the fact that the nasal epithelium is not equivalent to the bronchial epithelium, they have many similarities, in particular the same pattern of transepithelial ion transport and the same defect in CF patients [2]. Thus, nasal PD measurements represent, for the moment, one of the best available tests to monitor the epithelial cell function in the setting of CFTR gene transfer experiments, a test that can be performed repeatedly and noninvasively.

Two recent articles have described protocols of nasal PD measurements that allow assessment of Na^+ absorption, as well as basal and cyclic adenosine monophosphate (cAMP)-mediated Cl^- secretion by successive "superfusion" of different buffers and drugs on the nasal mucosa [5, 6]. These protocols allow better discrimination between normal and CF-modified ion transport patterns than simple measurement of baseline PD. They have also been used to detect partial correction of the ion transport defect in CF patients after CFTR gene transfer by adenoviral vectors and by liposomes [7–9]. The authors of these protocols recommend that nasal PD measurements be performed on the floor of the nasal cavity, under the inferior turbinate, because the original work by KNOWLES and co-workers [1, 2] has shown that the epithelium of this area includes a majority (67–78%) of ciliated epithelial cells, while the medial surface of the inferior turbinate is often the site of cuboidal metaplasia, with only 40–42% of ciliated epithelial cells

[1]. It is also easier to stabilize the position of the perfusion catheter and exploring electrode under the turbinate than in the middle of the nasal cavity.

Measuring the nasal PD under the inferior turbinate, however, presents a drawback, in that the tip of the exploring electrode cannot be visualized during the procedure. It is, therefore, difficult to be sure that measurement is made on the precise area of the mucosa where the gene vector has been applied, that the mucosa has remained intact in this area, and that subsequent sampling by cytological brush or biopsy will be performed at the same place.

For these reasons, we considered that a thin perfusion electrode that could be placed on the medial part of the inferior turbinate, and maintained with constant visualization of the contact point with the mucosa, would be an advantage in studies of CFTR gene transfer. Cuboidal metaplasia in this area would represent a problem only if it altered the epithelial ion transport to the point that nasal PD would no longer be able to discriminate between normal and CF pattern. In addition, it is important to note that the ultimate target of treatment, namely the CF bronchial epithelium, is frequently affected by the same type of cuboidal metaplasia [10].

To address this important technical question, we devised a very thin, original, perfusion electrode, and used it to measure baseline PD in normal subjects and CF patients, on various areas of the nasal mucosa where the contact point can be easily and permanently seen by telescopic rhinoscopy. PD measurements were then performed on the medial part of the inferior turbinate, with superfusion of successive buffers and drugs, according to the protocols of KNOWLES *et al*. [6], and MIDDLETON *et al*. [5]. The objective was to determine whether such measurements would discriminate between normal and CF epithelial physiology, and eventually be suitable for trials of CFTR gene transfer, or other forms of treatment of the ion transport defect of CF.

Materials and methods

Subjects

Measurements of baseline PD values were obtained in 32 normal subjects (12 females and 20 males; mean age 34 yrs, range 22–61 yrs) and 13 CF patients (7 females and 6 males; mean age 26 yrs, range 18–49 yrs). Superfusion studies were performed in 20 normal subjects and in 12 CF patients. The CF patients all had pancreatic insufficiency, and CF diagnosis was confirmed both by clinical history and an abnormal sweat test. All participants gave informed consent and the protocol was approved by our institutional Ethics Committee.

Methods

The principle of the measurement of transepithelial nasal PD has been described in detail by others [3, 6]. It consists in connecting an exploring electrode in contact with the nasal mucosa, and a reference electrode in contact with the subcutaneous tissue, to a high impedance voltmeter (World Precision Instruments (WPI), Berlin, Germany). A silver/silver chloride electrode (electro-cardiograph (ECG) type), taped on the volar side of the forearm after a light abrasion of the skin epithelium, was used as a reference electrode.

Our exploring electrode consisted of a Teflon-coated silver wire, 0.125 mm in diameter (WPI), that had been uncoated and chlorinated over the last 10 mm from the tip. The wire was passed into a thin intravenous catheter (outer diameter (OD) 0.63 mm, length 30 cm; Portex, UK) with the tip near the end of the lumen. The thin catheter passed through a rubber joint and then through a three-way connection. At the outlet of the three-way connection, the thin catheter entered a larger catheter from the same manufacturer (OD 1.34 mm), which was connected by a Luer lock system. The larger, outer catheter was used for perfusion with solutions coming from the side port of the three-way connection. The length of the larger catheter was adjusted such that the thin catheter protruded a few millimetres at the extremity. The protruding tip of the thin catheter, with the silver wire inside, was slightly curved and bell-shaped by gentle heating. It was then filled with hot 4% agar that had been diluted with basal perfusion buffer. It was found that inclusion of the silver wire in an agar bridge considerably reduced the junction potential that occurred when low chloride buffer was perfused through the outer catheter. Under these conditions, the junction potential was found to be always ≤ 2 mV.

This perfusion electrode was a concentric, double lumen system, with an outside diameter of 1.34 mm and a slightly curved extremity, which facilitated positioning and observation of the contact point with the mucosa. Figure 1 gives a scheme of the device, and figure 2 shows the electrode in contact with the nasal mucosa, on the medial part of the inferior turbinate, as observed *via* a telescopic rhinoscope during measurement.

The zero offset of the electrodes was determined before and after each recording, making it possible to recognize a shift due to a defective electrode. Measurements of baseline PD were made under visual control, at four different sites: nasal septum, anterior tip, medial part and posterior part of the inferior turbinate. The values were recorded after at least 15 s of stable PD. Values obtained from left and right nostrils were averaged.

The perfusion electrode was then placed on the medial or slightly posterior part of the inferior turbinate. It was slowly moved until the largest PD value was obtained, together with stable recording, and constant observation of the contact point with the mucosa. The electrode was maintained in this position by attachment to an arm affixed to a helmet on the patient's head. The perfusion solutions, preheated to 37°C in a water bath, were administered with a peristaltic pump at a flow of 5 mL·min^{-1}. When switching from one perfusion to another,

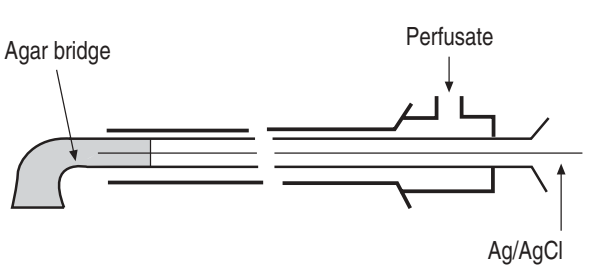

Fig. 1. – Scheme of the original perfusion electrode that was devised for the present study. Outside diameter is 1.34 mm. See text for full description.

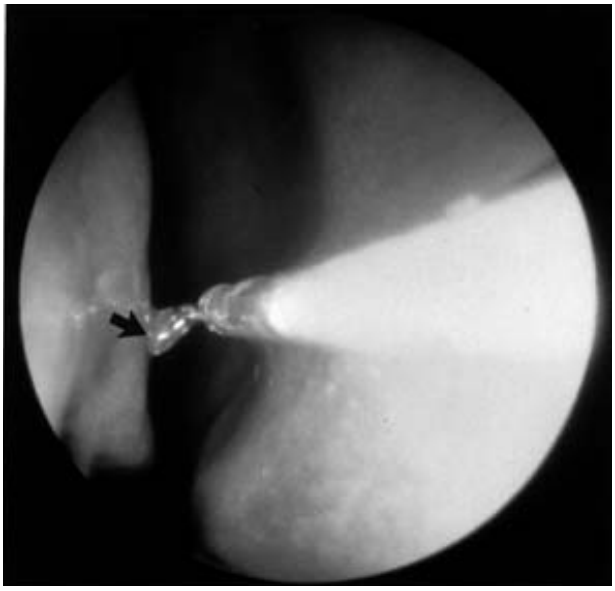

Fig. 2. – The perfusion electrode is seen by telescopic rhinoscopy. The tip, which is slightly curved to the right, is in contact with the mucosa of the medial aspect of the inferior turbinate (arrow).

the recording was interrupted for about 20 s whilst the tubing was purged with the new solution. In each of four CF patients, baseline nasal PD on the medial part of the inferior turbinate was obtained 5–6 times on different days. One investigator performed the measurements on three of these patients, and another investigator on the fourth. The two investigators positioned the tip of the electrode precisely on the same site of the mucosa for all measurements.

The basal buffer contained (mM): Na^+ 140, K^+ 6, Mg^{2+} 1, Ca^{2+} 2, Cl^- 152, hydroxyethylpiperazine ethanesulphonic acid (HEPES) 10. A low chloride buffer was prepared by substituting NaCl and KCl with equimolar gluconate, resulting in a final Cl^- concentration of 6 mM. The pH of all buffers was adjusted to 7.40. Solutions were filtered and packed under sterile conditions by the pharmacist at our institution. Osmolarity and electrolyte content were checked by direct determination. Fresh stock solution of terbutaline in 4% ascorbic acid, and amiloride in water, were prepared less than 4 h before the procedure.

Perfusion protocol

After stabilization of the baseline PD under superfusion of basal buffer for at least 1 min (Phase 1), the solutions were applied in the following order: amiloride 100 µM in basal buffer until stabilization of the new baseline (Phase 2); amiloride 100 µM in low chloride buffer for at least 3 min (Phase 3); amiloride 100 µM + terbutaline 10 µM in low chloride buffer for another 3 min (Phase 4).

Statistical analysis

Results are indicated as mean±SEM. Unpaired t-test was used for comparison between normal subjects and CF patients and differences were considered significant at p-values less than 0.05.

Results

Figure 3 shows the results of baseline PD obtained at four different sites in normal subjects and in patients with CF. On the medial and posterior part of the inferior turbinate, baseline PD clearly differentiated normal subjects from CF patients, with very little overlap (medial part -10.3±1.2 *versus* -31.0±2.7 mV in normals and CF patients, respectively (p=0.0001); posterior part

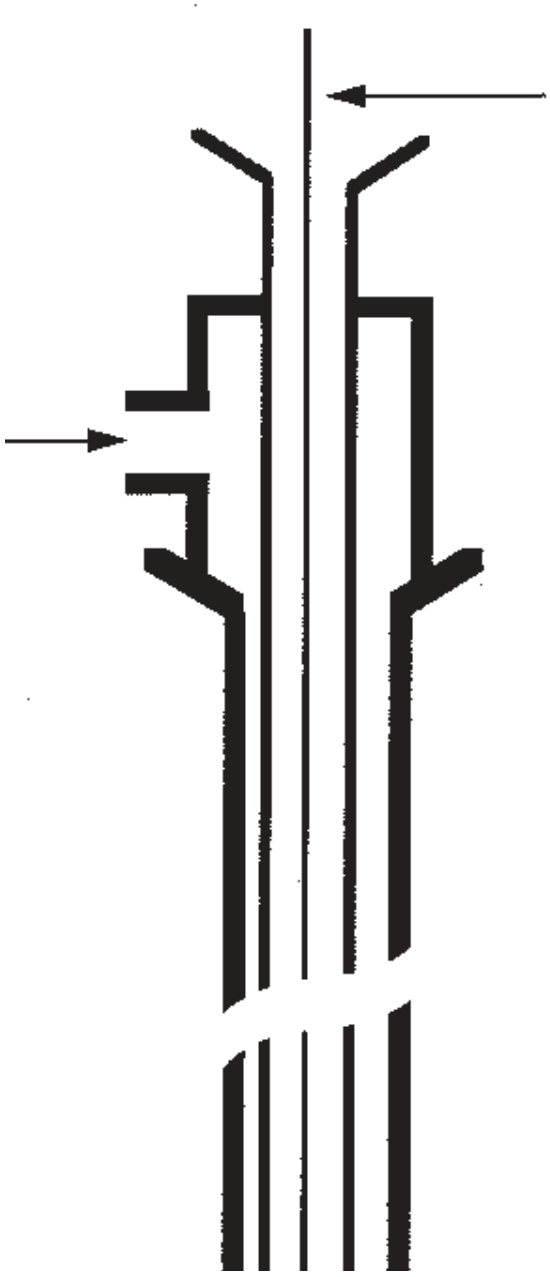

Fig. 3. – Baseline values for nasal transepithelial potential difference (PD) at various sites of the nasal mucosa where direct visualization of the contact point of the electrode is possible. a) nasal septum; b) anterior part of the inferior turbinate; c) medial part of the inferior turbinate; d) posterior part of the inferior turbinate. ● : normal subjects; ❍ : cystic fibrosis (CF) patients. PD values are significantly different between groups for the medial and for the posterior part of the inferior turbinate (p=0.0001). There were 32 normal subjects, except for the posterior part of the inferior turbinate where only 16 normal subjects were tested *versus* 13 CF patients.

-3.2±0.9 *versus* -38.9±1.6 mV, respectively; p=0.0001). By contrast, there was no difference between the two groups for measurements on the nasal septum and on the anterior tip of the inferior turbinate. Left and right sides gave similar values within the same individual, with a mean difference of 3.7±0.4 mV. The coefficient of variation (SD/mean) for baseline nasal PD on the medial part of the inferior turbinate obtained in each of the four CF patients measured 5–6 times was 0.26, 0.27, 0.27 and 0.25, respectively. Thus the variability appeared to be very constant from one subject to the other.

At the start of the perfusion protocol, the exploring electrode was placed at the site of the most negative baseline PD which, depending on the case, was on the medial or slightly posterior part of the inferior turbinate. Baseline values averaged -15.1±1.8 mV for normal subjects, and -42.9±4.7 mV for CF patients. The 95% confidence interval (95% CI) for normal subjects (mean± 2 SD) ranged from +1 to -32 mV. Only one CF patient repeatedly had baseline values in this range (-20 mV).

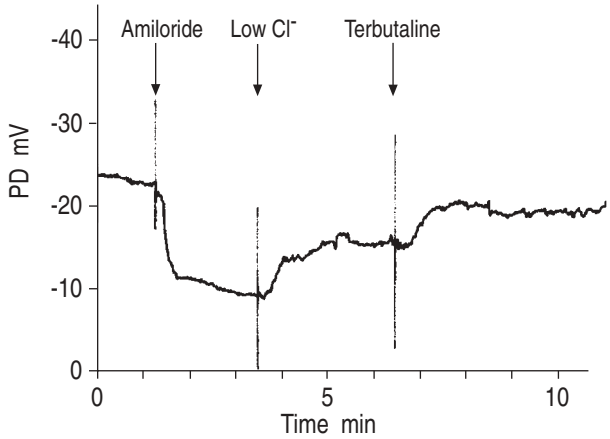

Fig. 4. – Chart recording of nasal transepithelial potential difference (PD) against time in a normal subject during the perfusion protocol. After depolarization by amiloride (100 µM), two successive phases of repolarization occurred after superfusion of low chloride buffer (6 mM) followed by terbutaline (10 µM).

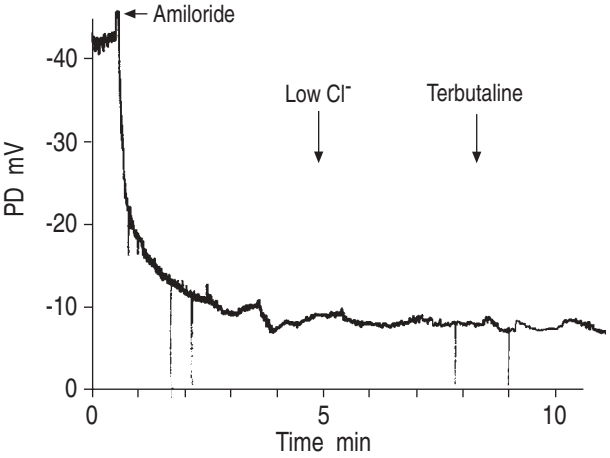

Fig. 5. – Chart recording of nasal transepithelial potential difference (PD) against time in a patient with cystic fibrosis (CF) during the perfusion protocol. After a profound depolarization caused by amiloride, superfusion of low chloride buffer and terbutaline did not cause repolarization (concentrations identical to figure 4). This indicates that no significant chloride secretion occurred in response to increased electrochemical gradient, nor to stimulation by cyclic adenosine monophosphate (cAMP).

Responses of nasal PD to the perfusion protocol are illustrated by two representative cases in figures 4 and 5. Amiloride induced a marked depolarization in both cases. Whilst low chloride buffer and terbutaline induced two successive repolarizations in the normal subject (fig. 4), these manoeuvres did not cause any persistent change of nasal PD in the CF patient (fig. 5).

The individual responses obtained in the group of normal subjects and in the group of CF patients are compared in figure 6. Amiloride caused more pronounced depolarization among CF patients than among normal

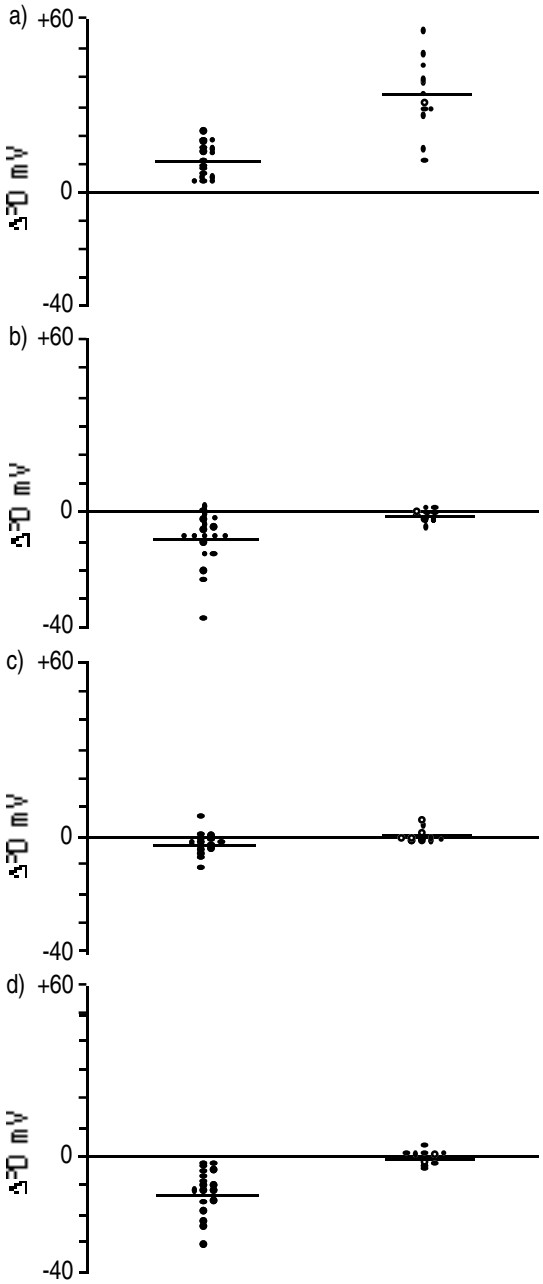

Fig. 6. – Changes in nasal transepithelial potential difference (ΔPD) after superfusion of: a) amiloride (100 µM); b) low chloride buffer (6 mM); c) terbutaline (10 µM); and d) the combined effect of low chloride + terbutaline. ● : normal subjects; ○ : cystic fibrosis (CF) patients. Changes in PD are significantly different between groups for amiloride (p=0.0001), low chloride (p=0.002), terbutaline (p=0.002), and the combined effect of low chloride + terbutaline (p=0.0001). There were 20 normal subjects *versus* 12 CF patients.

subjects (ΔPD after amiloride = 10.1±1.2 *versus* 32.4±3.7 mV for normal subjects and CF patients, respectively; p=0.0001). However, when expressed as percentage of baseline, the changes were not statistically different between the two groups (66±6% *versus* 76±5%, respectively; NS).

Perfusion of low chloride buffer with amiloride caused definite repolarization (ΔPD ≥5 mV) in 15 out of 20 normal subjects, while CF patients displayed no or only minimal repolarization (fig. 6). The difference between the two groups was significant (ΔPD after low chloride = -10.0±2.0 *versus* -1.3±0.5 mV for normal subjects and CF patients, respectively; p=0.002). The presence of terbutaline caused additional repolarization in only 7 out of 20 normal subjects, whilst CF patients did not demonstrate repolarization (fig. 6). The difference between the two groups was again significant (ΔPD after terbutaline =3.2±0.8 *versus* +0.7±0.5 mV, respectively; p=0.002). Finally, the combined effect of low chloride buffer and terbutaline induced definite repolarization in 18 out of 20 normal subjects (90%), but in none of the CF patients (fig. 6).The difference between the two groups was highly significant (ΔPD after low chloride + terbutaline =-13.2±1.8 *versus* -0.7±0.6 for normal subjects and CF patients, respectively; p=0.0001).

Discussion

The aim of this work was to set up a fast, and noninvasive method to assess abnormal ion transport of the respiratory epithelium in CF patients. For this purpose, we developed a new recording electrode allowing stable investigation of nasal mucosa transepithelial potential on the medial aspect of the inferior turbinate. This site of the mucosa is easily accessible for inspection and tissue sampling, if necessary. We found that normal subjects and CF patients differed markedly in their baseline PD, as well as in their response to superfusion of amiloride, low chloride, and terbutaline. These results are in accordance with those obtained on the floor of the nasal cavity, under the turbinate, by MIDDLETON *et al*. [5], and by KNOWLES *et al*. [6], who used a similar perfusion protocol. However, their measurements were made on a site that is not accessible, for the most part, to visual control, and which necessitates local anaesthesia for biopsy or cytological brushing.

The superfusion protocol that we applied has been used with minor differences by various authors [5, 6, 8, 9]. It was designed for detection of two characteristic abnormalities of epithelial ion transport in CF patients. Firstly, the excessive Na^+ absorption through the epithelium, from the lumen to the submucosa; and secondly, the failure to secrete chloride in the opposite direction, following stimulation by cAMP, under an appropriate electrochemical gradient.

Under basal conditions, transepithelial PD is due mainly to continuous absorption of Na^+ from the lumen [11]. Superfusion of amiloride causes the blockade of Na^+ channels at the apical membrane of epithelial cells, and therefore causes depolarization of the transepithelial potential. In CF, as a consequence of the defective CFTR molecule, which contributes to the regulation of Na^+ channels [12], there is increased basal absorption of Na^+. This is the reason for increased nasal PD under basal conditions, and for a larger decrease than normal under superfusion of amiloride.

The mean baseline PD that we observed at the medial or posterior part of the inferior turbinate, among normal subjects (-13.9 mV) and CF patients (-41.5 mV), was in the same range as that reported by ALTON *et al*. [3], who performed their measurements along the floor of the nasal cavity (-19.0 mV for normal subjects and -46.1 mV for CF patients). We found very little overlap between the two groups, with only one CF value within the 95% CI of normal subjects. Finally, the mean difference that we observed between both nasal cavities within the same individuals (3.5 mV) was similar to that reported by ALTON *et al*. [3] (3.6 mV).

Superfusion of amiloride caused a larger depolarization in all CF patients than in normal subjects, with the exception of only one patient, who also had baseline PD in the normal range. This large, amiloride-induced depolarization is in agreement with the concept that increased baseline PD in CF patients is essentially due to excessive sodium reabsorption.

As pointed out by KNOWLES and co-workers [1, 6], it is important to realize that inflammation and light trauma may decrease the transepithelial electrical resistance, which in turn will decrease baseline PD and the absolute effect of amiloride, without affecting the process of sodium hyperabsorption. Inflammation and/or epithelial damage is probably the best explanation for the low baseline PD, with low response to amiloride, that we found in one CF patient in the present study. This observation also stresses the fact that changes in baseline PD, and in the response to amiloride, in gene therapy or pharmacological trials aimed at improving ion transport, must be interpreted with caution because of the difficulty of ruling out an effect on transepithelial resistance in each case.

The other objective of the perfusion protocol was the assessment of the chloride secretion process across the epithelium. By blocking Na^+ absorption, amiloride creates an electrical gradient that favours secretion of Cl^- into the lumen. The addition of low chloride buffer on the mucosa adds a chemical gradient. This will translate into chloride secretion, and a repolarization of the transepithelial PD in subjects whose epithelia have normal chloride permeability [5, 6]. The addition of a cAMP stimulator, such as terbutaline or isoproterenol, will open new chloride channels at the apical membrane of epithelial cells, allowing increased passage of Cl^- into the lumen, and causing additional repolarization of transepithelial PD in normal subjects [5, 6]. The CFTR molecule has been shown to be a cAMP-responsive chloride channel, but also a cAMP-dependent regulator of other types of chloride channels that are present at the apical membrane of respiratory epithelial cells [13]. The defective CFTR molecule in CF will be the cause of the reduced ability to secrete Cl^- in response to a favourable electrochemical gradient, and of the lack of response upon cAMP stimulation.

We found that marked repolarization (arbitrarily defined by a change of PD of ≥5 mV) occurred in 18 out of 20 normal subjects (90%) under the combined effect of low chloride buffer (Cl^- 6 mM) and terbutaline (10 μM). By contrast, none of the 12 CF patients responded

to the same stimulation by a repolarization of more than -4 mV, most of them showing no change of PD at all.

We compared our results with those reported by KNOWLES and co-workers [6] who studied the combined effect of amiloride, low chloride and isoproterenol, in 20 studies performed in 17 normal subjects. Repolarization averaged about 30 mV in their subjects, whilst we observed a mean of 13 mV in ours. The difference could be related to a difference in potency of isoproterenol and terbutaline, both being used at 10 µM concentration. We are not aware of studies comparing the two drugs under these precise conditions. Despite this limitation, the perfusion protocol that we applied on this site of the mucosa was able to discriminate between normal subjects and CF patients.

As opposed to the medial aspect of the inferior turbinate, measurements of baseline PD on the anterior tip of the turbinate and on the mucosa of the nasal septum did not discriminate between normal subjects and CF patients. The mucosa at the anterior tip is covered by a stratified squamous epithelium, while the mucosa of the septum contains predominantly goblet cells, with very few ciliated cells [1]. For this reason, we did not apply the perfusion protocol on these sites.

In summary, the present data indicate that measurement of nasal potential difference with a perfusion protocol, on the visible, medial or posterior part of the inferior turbinate, allowed good discrimination between normal subjects and patients with cystic fibrosis. Measurement of nasal potential difference has been shown to be of diagnostic value when patients are suspected to have some clinical form of cystic fibrosis, but have sweat chloride values in the normal range [14]. The method that we are describing here is not intended to replace the "classical" method, with measurements under the inferior turbinate, when the objective is focused on such a diagnostic purpose. Rather, it offers a more suitable approach to evaluate the effect of new forms of treatment that have recently been proposed to correct the functional defect of ion transport in cystic fibrosis. These include gene therapy, with viral and nonviral vectors, but also pharmacological treatments, for example with "chemical chaperones" [15], or compounds such as phenylbutyrate [16], which have been shown *in vitro* to restore the function of mutated cystic fibrosis transmembrane conductance regulator. In clinical trials aimed at evaluating such substances, the assessment of ion transport of the respiratory mucosa would be best performed on a site that is easily visible, and accessible for tissue sampling, with no (or minimal) manipulation. The method that we have presented here could satisfy these criteria.

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