Silver/silver chloride electrodes for measurement of potential difference in human bronchi

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Abstract

***Background*—An easy and reliable method to measure potential difference (PD) in the lower airways would be of interest in the field of cystic fibrosis. We have developed silver/silver chloride (Ag/AgCl) electrodes to measure PD in the lower airways.**

***Methods*—To validate this technique the nasal PD measured with Ag/AgCl electrodes and with conventional perfused electrodes was compared in 16 patients. The range of PD measured with Ag/AgCl electrodes in the lower airways during fibreoptic bronchoscopy was determined in 14 adult patients and in nine the reproducibility of this technique was examined.**

***Results*—Nasal PD values measured with Ag/AgCl and perfused electrodes were highly correlated ($r = 0.985$, $p<0.0001$) and the limits of agreement (mean ±2SD of the difference) between the two methods were –1.91 mV and 1.53 mV. In the lower airways a progressive and slight decrease in PD values with decreasing airway diameter was observed in most patients. The mean (2SD) of the differences between the two tracheal measurements was 0.21 (1.73) mV.**

***Conclusions*—The use of Ag/AgCl electrodes gives a reliable and reproducible measurement of PD in the lower airways in humans.**

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Keywords: silver/silver chloride electrodes; potential difference; airways

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A transepithelial electric potential difference (PD) across mammalian airway epithelium is generated by electrogenic ion transport.[1] Nasal PD can be measured in vivo in humans and is higher in patients with cystic fibrosis than in normal control subjects.[2 3] Although it would be of interest in the field of cystic fibrosis and especially in gene therapy studies to be able to measure PD easily in the lower airways, few studies published a decade ago have attempted to measure PD in the bronchi.[4–6] In these studies PD was recorded during fibreoptic bronchoscopy with the same type of Ringer's perfused electrodes as are used in the nose. With these electrodes we have attempted to measure transepithelial PD in the lower airways of eight subjects during fibreoptic bronchoscopy. However, in this preliminary study we found it difficult to obtain stable and reproducible PD values in the lower airways, mostly because of air bubbles that formed at the tip of the very thin electrode and because of an early flooding of the airway lumen by the perfused fluid. In the present study we have therefore prepared silver/silver chloride (Ag/AgCl) nonpolarisable electrodes to measure more easily transepithelial PD in the lower airways.

Methods

EXPERIMENTAL DESIGN AND SUBJECTS

The agreement between Ag/AgCl electrodes and perfused electrodes was determined by measurement of nasal PD with both methods in 16 adult patients (six men) of mean (SD) age 51 (19) years. The range of PD in the lower airways was determined with Ag/AgCl electrodes in 14 adult patients (six men) of mean (SD) age 60 (19) years during fibreoptic bronchoscopy required because of lung cancer (n = 8) or interstitial lung disease (n = 6). In nine of these 14 patients the reproducibility of PD measurements with Ag/AgCl electrodes was determined by two measurements at five minute intervals at the same location of the distal trachea.

The investigation conformed to the regulations of the Institutional Ethics Committee.

PREPARATION OF AG/AGCL WIRE ELECTRODES

Silver wires were used to prepare the Ag/AgCl electrodes (World Precision Instruments, Hertfordshire, UK). The silver wire was inserted into a Teflon catheter (Bioblock Scientific, Illkirch, France; fig 1). The end of the silver wire was heated to form a small atraumatic bead which was covered by a layer of silver chloride by anodising in HCl solution using a potentiostat/galvanostat. Two new Ag/AgCl electrodes were prepared for each patient and sterilised by autoclaving. One served as a reference bridge and the silver bead was taped on to a scarification on the skin at the anterior face of the forearm. The other electrode served as the exploring bridge and the silver bead allowed the contact with the airway wall. Both branches of the bridge were connected to a high impedance millivoltmeter.

MEASUREMENT OF TRANSEPITHELIAL PD IN THE NOSE AND THE LOWER AIRWAYS

Before each measurement bridge conductivity was verified. For measurement of nasal PD each patient underwent two measurements in random order, one with Ag/AgCl electrodes and the other with conventional electrodes perfused with Ringer's lactate and prepared as previously described.[7] The exploring electrode was positioned 3–4 cm from the anterior tip of

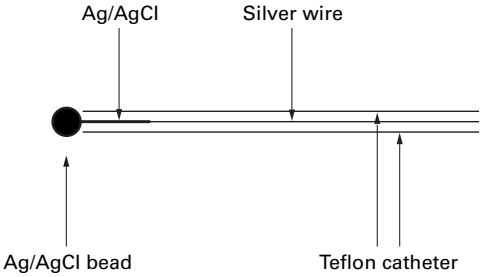

Figure 1 Scheme of the Ag/AgCl electrode that was devised for the present study. Outside diameter 1.93 mm.

the turbinate until a maximal and stable PD for over 10 s was obtained. Measurements of PD in the lower airways were made with Ag/AgCl electrodes under general anaesthesia (propofol and lidocaïne 2%). No local anesthesia was used. PDs were measured with the exploring electrode inserted through the suction channel of the fibreoptic bronchoscope and positioned on the airway surface of the different bronchi.

STATISTICAL ANALYSIS

PDs were negative and were expressed as absolute values. Nasal PD was expressed as the arithmetic mean of the values of the two nostrils. Statistical analysis was performed according to Bland and Altman.[8]

Results

Measurement of PDs with Ag/AgCl electrodes in the nose and the lower airways proved to be safe, easy, and gave stable values.

AGREEMENT BETWEEN AG/AGCL ELECTRODES AND PERFUSED ELECTRODES FOR NASAL PD MEASUREMENTS

The range of nasal PDs was 10.4–33.1 mV (median 14.0) with perfused electrodes and 9.6–32.4 mV (median 13.4) with Ag/AgCl electrodes. Nasal PDs measured with Ag/AgCl and perfused electrodes were highly correlated (r = 0.985, p<0.0001) (fig 2A). The mean (SD) bias of the differences was –0.19 (0.86) mV and the limits of agreement (mean ± 2SD of the difference) between the two methods were –1.91 mV and 1.53 mV. The range of the differences was –2 to 1 mV (fig 2B). The 95% confidence interval for the lower limits of agreement was –2.70 to –1.12 mV and for the upper limits was 0.74 to 2.32 mV. Thus, the mean difference between the two methods was close to zero and the limits of agreement were small enough to demonstrate the validity of Ag/AgCl electrodes for measurement of the transepithelial PD in the airways.

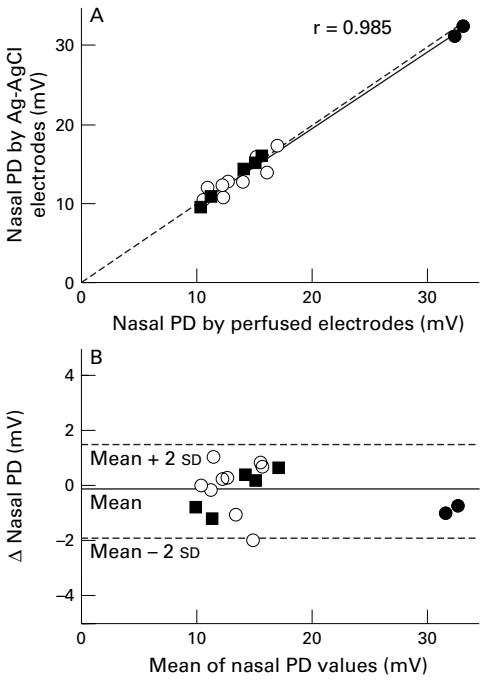

Figure 2 Agreement between Ag/AgCl electrodes and conventional perfused electrodes for nasal potential difference (PD) measurement in 16 subjects. (A) Correlation between nasal PD measured with Ag/AgCl electrodes and with perfused electrodes (— = regression line, --- = line of identity). (B) Difference between nasal PDs measured with perfused electrodes and with Ag/AgCl electrodes (Δ nasal PD) plotted against mean of nasal PDs measured with both methods. ● = patients with cystic fibrosis, ○ = patients with bronchiectasis, and ■ = healthy subjects.

DETERMINATION OF THE NORMAL RANGE OF TRANSEPITHELIAL PD IN THE LOWER AIRWAYS WITH AG/AGCL ELECTRODES

A precise location of the bead was easily secured. A progressive but slight decrease in PDs with decreasing airway diameter was observed in most patients and for the group as a whole: mean (SE) in distal trachea, 13.4 (1.7) mV; in main stem bronchi, 12.3 (1.8) mV; in lobar bronchi, 12.5 (5.2) mV; in segmental and subsegmental bronchi, 10.6 (3.1) mV.

REPRODUCIBILITY OF PD MEASUREMENTS IN THE DISTAL TRACHEA USING AG/AGCL ELECTRODES

The mean (SD) difference was 0.21 (0.86) mV and the coefficient of reproducibility[9] was 1.73 mV. These results demonstrate a good reproducibility of the method using Ag/AgCl electrodes for transepithelial PD measurements in the trachea.

Discussion

Commercially available Ag/AgCl electrodes are currently used in cardiography to measure monophasic action potentials. Their low polarisation properties ensure reliable potential measurements and they have already been used for measurements of nasal PD.[10] As our Ag/AgCl electrodes were devised to measure PDs in the lower airways, we did not find any advantage over the perfusion technique of these electrodes in the nose. However, in the lower airways the Ag/AgCl electrodes allowed reliable and reproducible PD measurements. It is noteworthy that the reproducibility we have observed in the lower airways with Ag/AgCl electrodes was similar to the one described with the usual perfusion technique in the nose.[2 10]

One drawback of Ag/AgCl electrodes is that the silver chloride layer which gives the electrode its distinctive black coloration and its low polarisation properties disappears with rubbing. This is why new electrodes had to be used for each patient and they could not be just washed and sterilised after each use. This drawback was offset by their non-perfused property, their easy use in the lower airways, and the stable and reproducible PD measurements they allowed. The silver bead was easily seen and placed precisely against the airway wall.

Although our study was performed on a small number of subjects, the use of Ag/AgCl electrodes appear to provide a reliable, reproducible and easy method for measurement of transepithelial PD in the lower airways. As this method will be applied almost uniquely in

cystic fibrosis, it needs to be validated in patients with cystic fibrosis and should be adapted to measure PDs during pharmacodynamic tests. However, it may prove a useful technique for the study of ion transport mechanisms in the lower airways and for the assessment of the functional efficacy of in vivo gene transfer in cystic fibrosis.

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Endogenous nitric oxide in patients with stable COPD: correlates with severity of disease

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Abstract

***Background*—Increased levels of exhaled nitric oxide (eNO) have been reported in asthmatic subjects but little information is available on eNO in patients with advanced chronic obstructive pulmonary disease (COPD). A study was undertaken to evaluate the levels of eNO in patients with stable COPD of different degrees of severity.**

***Methods*—Peak and plateau values of eNO (PNO and PLNO, respectively) were evaluated in 53 patients with COPD and analysed according to the level of forced expiratory volume in one second (FEV_1) and the presence of cor pulmonale (CP) (group 1, FEV_1 <35% predicted with CP, n = 15; group 2, FEV_1 <35% predicted without CP, n = 15; group 3, FEV_1 >35% predicted, n = 23). Seventeen normal subjects served as controls.**

***Results*—All the patients with COPD had reduced levels of PLNO compared with the controls (mean (SD) 6.3 (3.0) and 9.4 (2.8) ppb, respectively). In groups 1 and 2 PLNO levels were significantly lower than in subjects in group 3 (5.5 (2.9), 5.7 (3.5), and 7.1 (2.7) ppb, respectively; p<0.01 ANOVA). In all subjects % predicted FEV_1 correlated slightly with PLNO but not with PNO.**

***Conclusion*—Patients with severe stable COPD have reduced levels of eNO compared with normal subjects. eNO levels are slightly related to the severity of airflow obstruction.**

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Keywords: exhaled nitric oxide; chronic obstructive pulmonary disease

Increased levels of exhaled nitric oxide (eNO), an index of NO synthesis in the respiratory system,[1] have been detected in patients with asthma whereas smokers exhibit reduced levels of eNO.[2 3] Similar levels of eNO were found in patients with chronic obstructive pulmonary disease (COPD) and in healthy subjects.[4] More recently Maziak *et al*[5] have shown a negative correlation between forced expiratory volume in one second (FEV_1) and eNO in patients with stable and exacerbated COPD. We wondered whether the levels of eNO might be influenced by the degree of severity of COPD as assessed by airway obstruction and by the presence or absence of cor pulmonale (CP). The aim of this study was therefore to measure the concentration of eNO in patients with stable COPD with different levels of airway obstruction, with and without CP.

Methods

SUBJECTS

Fifty three patients with COPD diagnosed according to the American Thoracic Society (ATS) criteria[6] were enrolled, with a mean increase in FEV_1 following inhaled bronchodilator (200 mg salbutamol) of 6 (2)% of the baseline. All patients were ex-smokers (mean pack years 24 (8)) without a history of atopy. At the start of the study they were all in a stable condition and had been free from exacerbations in the preceding four weeks. Patients with other organ failure, cancer, and inability to cooperate