MEASUREMENT OF HUMAN NASAL POTENTIAL DIFFERENCE TO TEACH THE THEORY OF TRANSEPITHELIAL FLUID TRANSPORT

Ulrich Kersting,[1] Albrecht Schwab,[2] and Alexandra Hebestreit[3]

[1]*Institute of Clinical Biochemistry and Pathobiochemistry, Medical University Clinic,* [2]*Department of Physiology, and* [3]*University Children's Hospital, University of Würzburg, 97078 Würzburg, Germany*

We describe a novel student course in membrane physiology in which students record their own nasal potential difference, i.e., the transepithelial potential difference of the respiratory mucosa in the nose. The nasal potential difference monitors directly, and in vivo, changes in the apical cell membrane potential of the respiratory mucosa induced by activators and inhibitors of ion channel activities. Basic principles of transepithelial fluid transport are taught by applying an appropriate perfusion protocol to the respiratory epithelium to either depolarize or hyperpolarize the membrane potential of the luminal cell side, thereby increasing or decreasing the nasal potential difference. This course was given at the Department of Physiology at the University of Würzburg in 1997, and responses of the students as reported on questionnaires were mainly positive.

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Transport of fluid is one of the main functions of many tissues, e.g., kidney, intestine, exocrine glands, and lung. Despite its clinical importance, students often have difficulties understanding the basic principles of transepithelial fluid transport. We therefore developed a new course of membrane physiology in which students record the transepithelial electric potential difference of the respiratory mucosa in their own noses, i.e., the nasal potential difference. It is generally understood that changes in the nasal potential difference can be caused by activation or inhibition of Na^+ and Cl^- channels in the apical cell membrane of the respiratory epithelium and correlated to transepithelial ion transport (2–4, 6, 7). Fluid secretion depends on Cl^- efflux at the apical cell membrane; Na^+ follows Cl^- electrostatically, and water follows salt transport osmotically (5, 7, 9). Students are first introduced to the membrane concepts of ion gradients, diffusion potentials, and membrane conductances by measuring the cell membrane potential of living *Xenopus laevis* oocytes (8). Subsequently, the basic principles of transepithelial fluid transport are taught by recording the nasal potential difference. The clinical relevance of transepithelial fluid transport is exemplified by cystic fibrosis (CF), which is characterized by blockade of cAMP-activated apical Cl^- channels (1, 7). Measurements of nasal potential differences can be easily performed by nonelectrophysiologists and do not require extensive electrophysiological training.

METHODS

Experimental setup. The exploring electrode consists of a flexible umbilical vessel catheter with an outer diameter of ~1.2 mm (Sherwood Medical, Schwalbach, Germany), which is continuously perfused with isotonic NaCl solution. The umbilical vessel catheter is connected to the measuring elec-

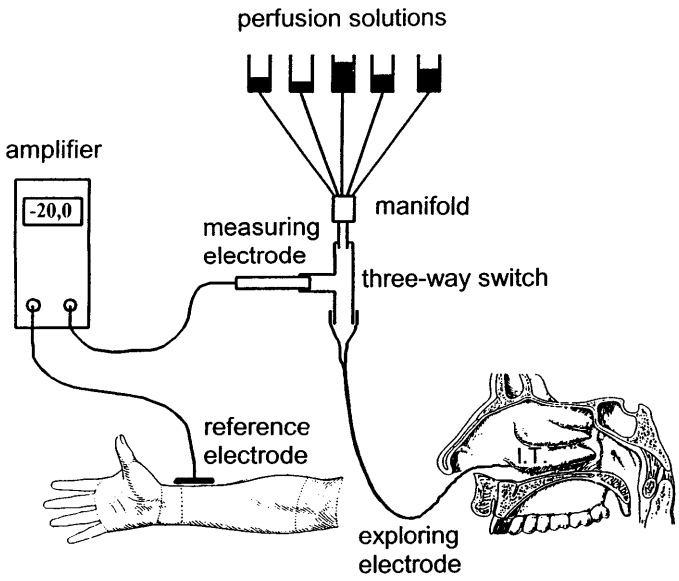

FIG. 1.
Schematic drawing of experimental setup to record nasal potential difference. Inferior turbinate of nose is marked in anatomic drawing. I.T., inferior turbinate.

trode (KCl outflow electrode; World Precision Instruments, Sarasota, FL) by a conventional three-way switch (Connecta TH Luer-Lock; Ohmeda, Helsingborg, Sweden). The tip of the umbilical vessel catheter is placed onto the respiratory mucosa between the inferior turbinate and the floor of the nose. The reference electrode is a silver/silver chloride electrode that is covered by gel (3M RedDot; Medica, Borken, Germany) and taped to the forearm. The KCl outflow electrode and the reference electrode are connected to the input of a battery-operated high-impedance voltmeter (World Precision Instruments). Figure 1 shows schematically the experimental setup.

Syringes (50 ml) serve as reservoirs for perfusion solutions. Every solution is filter sterilized before use. Infusion tubes connect the reservoirs via a manifold (Warner Instrument, Hamden, CT) and a three-way switch to the exploring electrode. The manifold exhibits a very small dead space of ~100 µl, allowing a rapid change of perfusion solutions. Flow of perfusion solutions is driven by gravity, and flow rate in each tube is adjusted by a valve to 0.5 ml/min. The control perfusion solution is an isotonic NaCl solution (308 mosmol/l). A low-Cl^- solution is prepared by substituting NaCl with equimolar gluconate, giving a final Cl^- concentration of 15 mmol/l. Amiloride (100 µmol/l) and isoproterenol (10 µmol/l) are dissolved in perfusion solutions shortly before class begins. Perfusion solutions are stored frozen as 50-ml aliquots.

Experimental procedure. The setup for measurements of nasal potential difference is renewed daily during the course with sterile disposable infusion syringes, infusion tubes, valves, and three-way switches. A fresh sterile umbilical vessel catheter is used for each measurement. The measuring KCl outflow electrode is prepared shortly before the recording and connected to the three-way switch.

During the experiment, the student sits comfortably in a dental chair. The student's head is tilted slightly back to facilitate the placement of the exploring electrode below the inferior turbinate. To create a suitable site of zero potential for the reference electrode, the epidermal surface of the skin of the forearm is treated with a special polishing gel and cleaned with 70% ethanol. Before measurements are taken, the exploring electrode, which is already perfused with NaCl solution, is placed in close contact to the reference electrode and adjustments are made to compensate for the recorded offset values. The explor-

ing electrode is then passed along the floor of the nose until a stable nasal potential difference in the range of −10 to −20 mV is obtained. Nasal potential difference reaches a maximum when the exploring electrode is placed between the inferior turbinate and the floor of the nose, ~0.5 cm distal from the anterior tip of the inferior turbinate. At this position the recording of the nasal potential difference is maximal and very stable. Maximal nasal potential difference can be lowered reproducibly by moving the tip of the exploring electrode to slightly sites more proximal or distal from the inferior turbinate. The correct placement of the exploring electrode may also be controlled by viewing through an otoscope. Perfusion solutions drop from the floor of the nose into the throat without discomfort, according to the test persons. Each perfusion solution is applied with a constant flow rate of 0.5 ml/min for a time period of ~5 min. Tightly controlling the perfusion rate assures that the amounts of amiloride and isoproterenol applied during the course stay well below pharmacologically relevant doses.

Because amiloride is contraindicated during pregnancy and lactation period, pregnant or nursing students were excluded as test persons. Nasal potential differences were recorded in groups of about five students. One student from each group was expected to volunteer as a test person, and at least one student was expected to place the exploring catheter. All students were involved in preparing the experimental setup and data analysis. The students placed the exploring catheters after the lab instructors in each group demonstrated how to do so. This was, in principle, not difficult, but correct positioning of the tip of the exploring catheter below the inferior turbinate required some experience and was closely supervised by the lab instructors.

RESULTS

Figure 2 shows changes in transepithelial potential differences, apical cell membrane potentials, and ion transport of the Cl^--secreting respiratory epithelium in the nose that were induced by the respective experimental maneuvers. The perfusion protocol was optimized to measure apical Na^+ and cAMP depending Cl^--channel activities in Cl^--secreting airway epithelium (6). The numerical values of transepithelial potential differences in Fig. 2 correspond to data shown in Fig. 3.

The inferior turbinate of the nose was perfused with isotonic NaCl solution until a stable nasal potential value, e.g., −12 mV, was recorded.

After a stable baseline value was obtained, amiloride (100 μmol/l; dissolved in isotonic NaCl solution) was applied. Amiloride blocks Na^+ channels in the apical cell membrane of the respiratory epithelium, thereby inhibiting cellular Na^+ uptake. Consequently, the membrane potential of the luminal cell side hyperpolarized, which in turn led to a decrease in the nasal potential difference to −5 mV (Fig. 2*B*).

The perfusion was then continued with a low-Cl^- solution (15 mmol/l Cl^-), with amiloride still present. The increased Cl^- gradient across the luminal cell side increased the efflux of Cl^- through cAMP-activated Cl^- channels. Increased Cl^- efflux at the luminal cell side depolarized the apical cell membrane potential, which in turn led to a rise in the nasal potential difference to −24 mV (Fig. 2*C*).

To further stimulate Cl^- efflux across the apical cell membrane, isoproterenol (10 μmol/l) was added to the low-Cl^- solution, with amiloride still present. Isoproterenol opens cAMP-activated CF transmembrane conductance regulator (CFTR)-Cl^- channels in the apical cell membrane. The stimulated luminal Cl^- efflux further reduced the apical cell membrane potential and led to an increase in the nasal potential difference to −26 mV (Fig. 2*D*).

Finally, control Ringer solution was applied to check whether the basal nasal potential difference was kept constant during the experiment (−12 mV).

During the course, which was held in winter, it became apparent that nasal mucosal infections decrease the nasal potential difference. Therefore, Fig. 3 represents only data from students without any sign of mucosal inflammation or viral infection of the upper respiratory tract. Basal nasal potential difference was -12.4 ± 1.3 mV ($n = 16$). Inhibition of apical

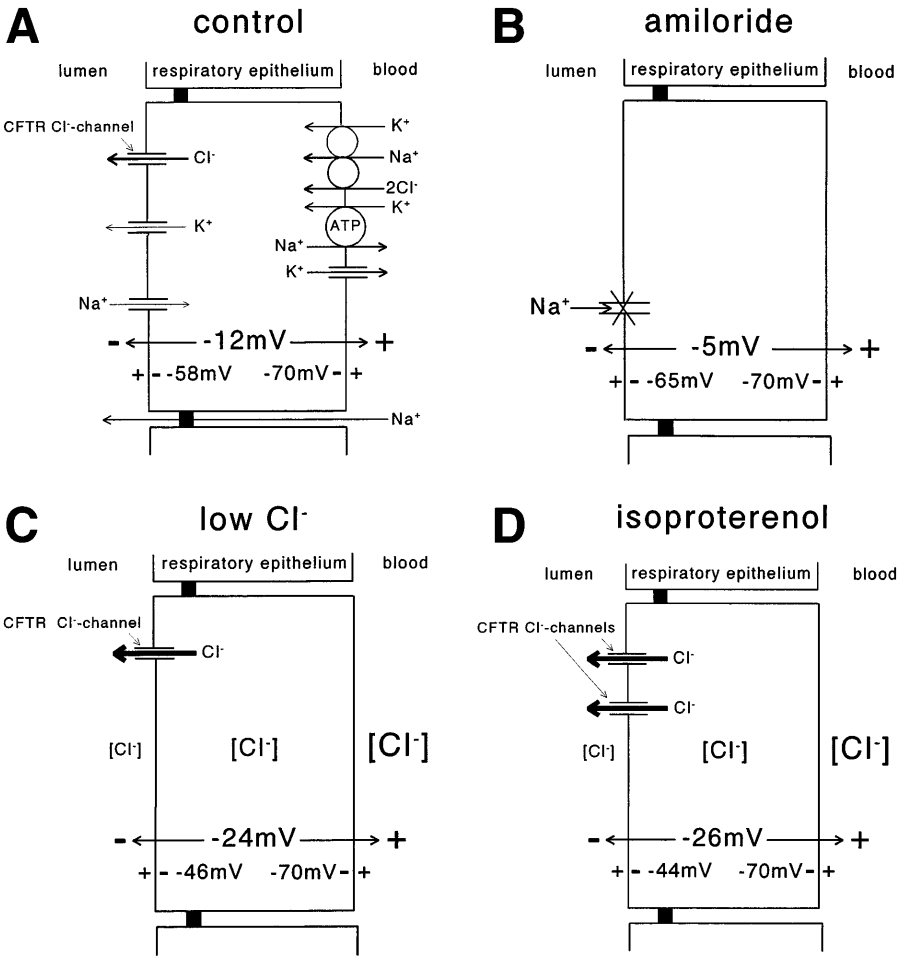

FIG. 2.

Schematic drawing of nasal potential differences, apical cell membrane potentials, and ion transporters at respiratory epithelium. In *B-D*, only those ion transporters that are affected by the respective experimental protocols are shown. Water movement is not shown. *A*: perfusion with isotonic NaCl (control). CFTR, cystic fibrosis transmembrane conductance regulator. *B*: application of amiloride (100 μmol/l). *C*: perfusion with a low-Cl^- solution (15 mmol/l Cl^-). *D*: addition of isoproterenol (10 μmol/l) to low-Cl^- solution. Nasal potential difference is transepithelial membrane potential difference of luminal and serosal cell sides. Transepithelial potential differences correspond to data shown in Fig. 3. For detailed explanations of experimental protocol, see RESULTS.

Na^+-channel activity by amiloride decreased the nasal potential difference to -4.65 ± 1.1 mV. Perfusion of the luminal side of the respiratory mucosa with a low-Cl^- solution increased the nasal potential difference to -24.12 ± 0.95 mV. Isoproterenol further increased the nasal potential difference to -25.5 ± 1.6 mV. Nasal potential difference decreased to basal values, -12 ± 6.8 mV, after perfusion with isotonic NaCl for ~10 min.

At the end of the course, the students completed a questionnaire, anonymously rating the quality of the course on a scale from 1 (very good) to 5 (insufficient) (Fig. 4).

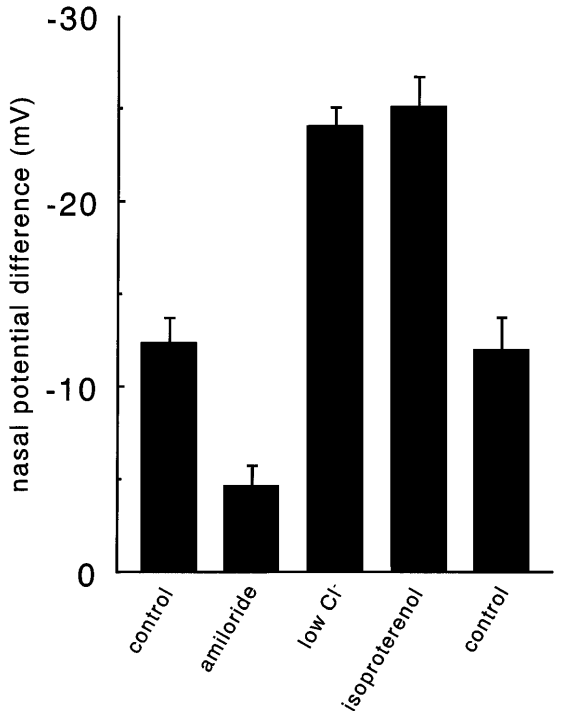

FIG. 3.
Changes of nasal potential differences after application of amiloride, low-Cl^- solution, and isoproterenol in students. Stable values of nasal potential differences were recorded 5 min after perfusion solutions were switched.

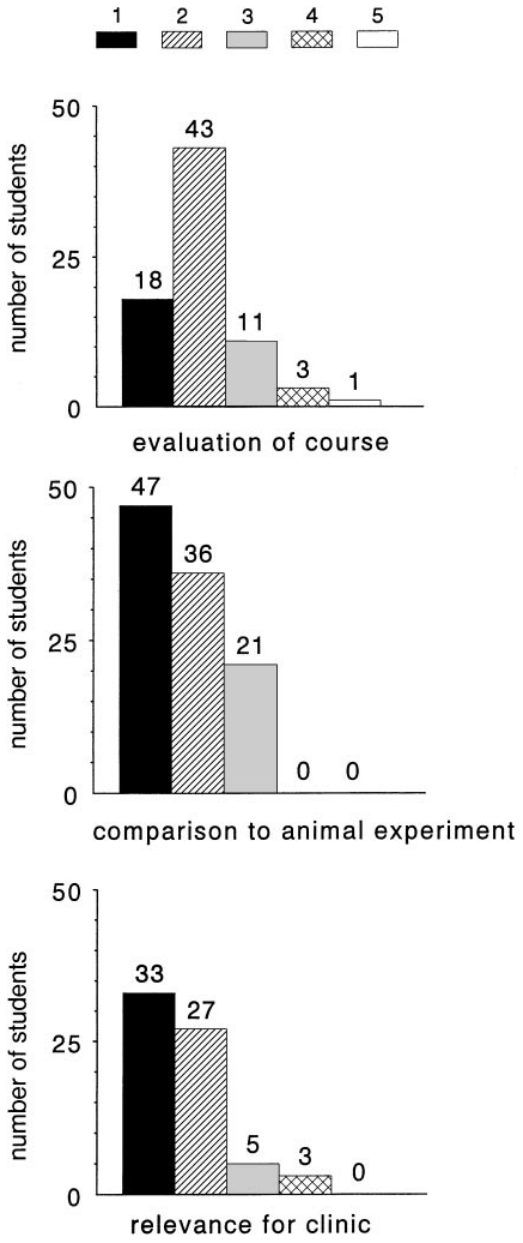

FIG. 4.
Students anonymously ranked course quality on a scale from 1 (very good) to 5 (insufficient).

DISCUSSION

Recording the nasal potential difference is a suitable model for teaching membrane physiology for several reasons. First, it is an easy and reliable technique that can be established in almost every laboratory at low cost. Second, it shows all the advantages and risks of a ''live'' experiment without being an animal experiment. Finally, the students gain hands-on experience regarding the effects of inhibitors and activators of transepithelial ion transport. Placement of the umbilical vessel catheter below the nasal inferior turbinate does not pose any danger to the subject but requires concentration and the ability of the experimenter to take responsibility. These demands greatly increased the motivation of the students in our course on membrane physiology.

After the practical part of the course was completed, the changes in the nasal potential differences were discussed in detail. The observed changes in the nasal potential differences were explained by changes in the apical cell membrane potentials, which were induced by alterations of luminal ion transport by amiloride, low-Cl^- solution, and isoproterenol. On the basis of their personal measurements of the nasal potential difference, the students could realize the

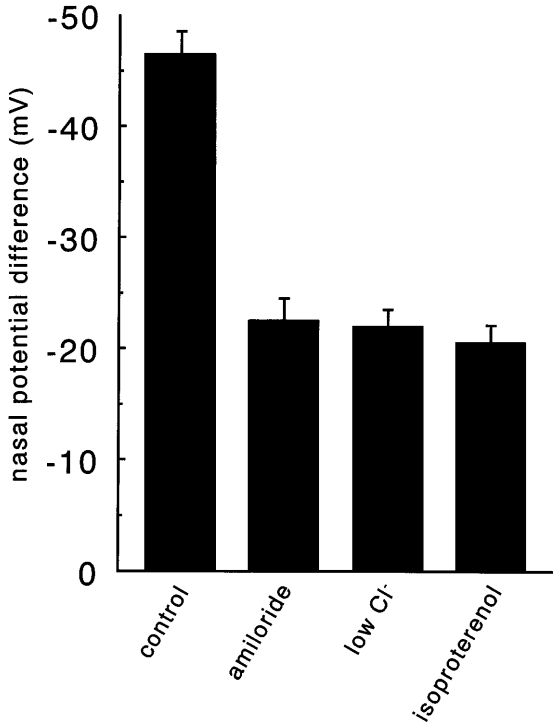

FIG. 5.
Changes in nasal potential differences in cystic fibrosis patients ($n = 6$). Data were taken from Ref. 6.

importance of the transepithelial potential difference as a driving force for transepithelial fluid transport through the paracellular space. So far, the students were showing a lively interest not only during the measurements but also during this theoretical part of the course. As the survey shows, the students rate our new course of membrane physiology very positively.

The clinical significance of disturbed transepithelial transport was stressed by discussing the pathophysiology of CF, the most common inherited lethal disease in Europeans and Caucasian North Americans. For comparison, Fig. 5 shows mean data of nasal potential differences from CF patients. The hyperpolarization in low-Cl^- and isoproterenol-containing solution is missing because of the blockade of luminal Cl^- efflux. Inhibition of fluid secretion in the lung of CF patients induced dehydration of the mucous, thereby causing lung damage. During our course in membrane physiology, the main symptoms of CF were discussed on the pathophysiological basis of disturbed transepithelial ion transport.

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Address for reprint requests: U. Kersting, Inst. of Clinical Biochemistry and Pathobiochemistry, Medical Univ. Clinic, Versbacher Str. 5, 97078 Würzburg, Germany.

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