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Measurement of ion fluxes across epithelia

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ABSTRACT

Epithelial tissues line all wet surfaces of vertebrate bodies. Their major function is directional transport of ions and water. Cells forming an epithelial layer are bound together by a tight junction that forms a barrier to ion flux. Ions and water are transported via specialized molecules. The presence of a defect in a single ion channel molecule leads to cystic fibrosis – the most common, fatal, human genetic disease. The paper describes ion transport data obtained by means of different experimental techniques. Special attention is given to radiochemical tracers, transepithelial resistance determination, open circuit potential and short circuit current measurements, the nasal potential difference in healthy and cystic fibrosis patients, the use of ion selective electrodes, and electrochemical mapping of the cell membrane surface. The effect of different activators and blockers of ion transport molecules on measured parameters are also discussed.

Contents

1. Introduction

Epithelium is a common tissue present in all organisms. It lines all wet surfaces of the body, i.e., lungs, intestines, the reproductive tract, and secretory organs. The major function of epithelium is the directional transport of solutes and water. In epithelium there are also mucin secreting goblet cells and protein secreting acinar cells undifferentiated basal cells ciliated cells in different proportions and local distribution (Hollenhorst et al., 2011). Epithelial tissues are made of a single cell layer bound together by tight junctions forming a barrier to diffusion. Water across the epithelium is transported passively following the electro-neutral transport of ions. The osmotic water transport mechanism is very effective, since the transport of a single cation and a single anion is accompanied by 370 water molecules. Epithelial cells are polarized—they have different transport molecules on apical and basolateral surfaces. There are different ion channels, transporters, and pump molecules present in epithelial cell membranes (e.g., Jentsch et al., 2004; Palmer, 2007; Bachmann et al., 2011; Hollenhorst et al., 2011; Novak, 2011; Toczylowska-Maminska and Dolowy, 2012; Hobbs et al., 2013; Reichhart and Strauss, 2014; Ohana, 2015). For the

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purpose of this paper, only a few ion transport molecules, including their blockers and activators, will be discussed. All of them are presented in Fig. 1.

Defective ion and water transport across epithelial tissue is the cause of cystic fibrosis (CF), which is the most common, fatal, genetic disorder among people of Northern and Central European descent, affecting 1:3000 newborns. CF is caused by a defect in a single gene that encodes an anion channel present in the apical face of the epithelial cell layer called the CFTR (Cystic Fibrosis Transport Regulator). The defect in the anion channel protein influences the electro-neutral transport of salt and passive osmotic water transport across the epithelium. The defective water transport leads to dense mucus formation, making sufferers of CF prone to opportunistic bacterial infections. There are many hypotheses of how the defect in the CFTR molecule influences the water flux in CF epithelial cell monolayers. One suggestion is that in CF, there is insufficient water secretion, while others claim that there is enhanced water absorption (Kunzelmann and Schreiber, 2012). Yet another theory is that there is defective bicarbonate secretion (Quinton, 2010; Chen et al., 2010).

The mechanism of water and ion transport across epithelium is a very complex one. There are four ions: sodium, potassium, chloride, and bicarbonate, which simultaneously flow across the epithelium in both directions. Additionally, calcium and hydrogen ions affect the transport. Calcium (Windhager and Taylor, 1983) and cAMP (Anderson et al., 1991) act as secondary messengers activating transport proteins, while the change in pH or cytoplasm volume activates processes of homeostasis involving ion transport (see e.g., Dolowy, 2015). There are numerous studies showing positive or negative correlation between CFTR protein and other channels and transporters (see for review e.g. Kunzelmann et al., 2005). It is not clear whether channels interact directly (Klein et al., 2016), via biochemical or electrochemical equilibria.

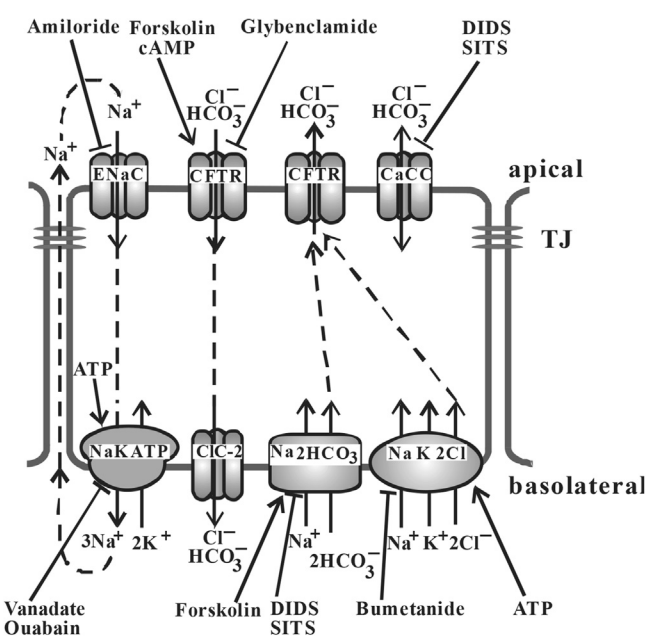

Fig. 1. Major molecules involved in ion transport across human bronchial epithelium, their activators denoted by ↓ and inhibitors denoted by ⊥. TJ – tight junction.
ENaC – Epithelial sodium channel, CFTR – Cystic Fibrosis Transport Regulator, CaCC – Calcium activated Chloride Channel, NaKATP – sodium potassium pump, ClC-2–Chloride Channel, Na2HCO3–transporter, NaK2Cl – transporter.
Dashed lines denote the likely routes of ion movements. Only sodium can move via the paracellular mode.

Concentrations of all major ions differ in all compartments (apical, cytoplasm and basolateral), and each ion flowing across the epithelium has different equilibrium potential at which it does not flow. The change in the transmembrane potential influences fluxes of all ions in the system. Thus, when studying total electrical properties of epithelium, i.e. resistance, open circuit potential difference, or short circuit current, one cannot recognize between cations flowing "out" from anions flowing "in," not to mention the differentiation between cation or anion species. With the help of specific ion channels blockers or activators, one can ascribe the change in electric properties of the epithelium to a particular ion transport molecule. However, the use of ion channel blockers and activators have side effects, which influence the whole tissue physiology (see e.g., Szewczyk et al., 2010). Thus, in spite of almost 70 years of studies of ion transport across the epithelium, we are far from understanding the process and its malfunction in cystic fibrosis. This paper reviews different methods of measuring ion transport across epithelium, their drawbacks, and results obtained.

2. Ion fluxes measured by the radioisotope tracers

The discovery of artificial radioisotopes led three Danish, Nobel Prize-winning scientists, August Krogh, Niels Bohr, George de Hevesy, and their young collaborator Hans Ussing, to apply them to biology (Ussing, 1980; Larsen, 2002). The measurement of epithelial transport started in the 1930s. In the beginning, heavy water (Von Hevesy et al., 1935) and chloride transport across frog skin was measured (Krogh, 1937, 1946). Ussing and Zerahn (Ussing and Zerahn, 1951) first used $^{24}Na^{+}$ ions to study active sodium transport through isolated frog skin. They built an apparatus that is the forefather of present-day measuring devices called the Ussing chamber. The Ussing chamber consists of two rectangular containers with side openings. Each container is equipped with the Ag/AgCl (or other non-polarizable) electrode often connected to a chamber via a salt bridge. Epithelial tissue or a plastic cup with epithelial cells grown on a porous support is placed and sealed between the two containers. The electric potential across epithelial tissue is either measured or short circuited. By adding another working electrode to each container and galvanostat or amperostat, one can introduce a given stable potential difference across the tissue or the cell monolayer and measure current flowing across the epithelium.

Presently, all the radioactive ions necessary to study epithelial ion transport are readily available ($^{22}Na^{+}$,$^{36}Cl^{-}$,$^{42}K^{+}$). It is also possible to use ($^{86}Rb^{+}$ as a substitute for potassium transport and $^{125}I^{-}$ to study chloride anion transport). Usually, two twin Ussing chambers are used in a single experiment. Identical tissues or cell monolayers are positioned in each chamber, and the radiotracer is added. In the first Ussing chamber, the radiotracer is added to the apical fluid and in the second chamber to the basolateral one. Samples of the medium from the "cold" chambers are then taken out for measurement and replaced by the same amount of bathing solution to maintain the fluid volume. The isotope amount in the chamber is measured, and the difference between the "apical to basolateral" and "basolateral to apical" transport is determined. If the net radiotracer flux difference is zero, then there is no active transport. Otherwise, the transport of ions is active at least in one direction. To determine the specific transport pathways, activators or blockers can be introduced into the Ussing chambers. The major limitations of the radiotracer method are the requirements of high safety standards and relatively long time intervals of a few minutes between the experimental points. Usually, only one ion species is measured in a single experiment. The fluxes of sodium and chloride ions across different epithelial tissues measured by radioisotopes are shown in Table 1 and for cell monolayers grown on a porous