C55

Short-term regulation of cellular and paracellular ion transport pathways in natural airway epithelium

A.N. Poulsen[1], T.L. Klausen[3], N.J. Willumsen[2], P.S. Pedersen[3] and O. Frederiksen[3]

[1]Department of Animal- and Veterinary Basic Sciences, The Royal Veterinary and Agricultural University, DK-1870 Frederiksberg C, Denmark, [2]Institute of Molecular Biology and Physiology, University of Copenhagen, DK-2100 Copenhagen OE, Denmark and [3]Department of Medical Physiology, The Panum Institute, University of Copenhagen. Blegdamsvej 3, DK-2200 Copenhagen N, Denmark

Fast regulation of the depth of the surface liquid layer (ASL) covering ciliated airway epithelium is critical for the function of the muco-ciliary clearance mechanism. Nucleotides (ATP and UTP) released to the surface layer are most likely involved in regulation of NaCl transport through interaction with apical purinergic receptors.

The natural airway epithelium absorbs Na^+ along a cellular pathway through apical ENaC and allows passive Cl^- absorption through the anion selective paracellular pathway. At the same time, a minor cellular Cl^- secretion takes place involving CFTR and calcium activated Cl^- channels.

In order to evaluate the involvement of the different pathways in the regulation of NaCl transport, the effects of nucleotides on cellular Na+ absorption, cellular Cl^- secretion and paracellular diffusion were investigated.

Under short-circuited conditions, tracer fluxes of Na^+, Cl^- and mannitol were measured, in addition to electrophysiological transport parameters short circuit current (I_{sc}) and epithelial conductance (G_t), in rabbit nasal airway epithelia, from humanely killed animals, mounted in Ussing-chambers.

Mucosal nucleotides (ATP or UTP, 200μM) inhibited amiloride-sensitive Na^+ absorption (from 91.1±10.2 to 55.8±9.5 nmol min^{-1} cm^{-2}; n=8; $p<0.01$) but only slightly increased Cl^- secretion (from 35.7±8.8 to 50.2±12.0 nmol min^{-1} cm^{-2}; n=7; NS). From changes in G_t (UTP: -23.4±1.1%; n=101; $p<0.001$) it was calculated that nucleotides also caused a large decrease in paracellular conductance (G_s). Flux measurements showed that nucleotides decreased passive paracellular Cl^- fluxes (from 135.7±8.7 to 114.6±12.5 nmol min^{-1} cm^{-2}; n=14; $p<0.02$) while passive Na^+ fluxes and mannitol permeability were unaffected (Na^+: from 35.1±4.5 to 37.5±4.2; n=8; NS. Mannitol: from 3.29±0.45 to 3.13±0.43 10^{-6} cm s^{-1}; n=16; NS). The effects of nucleotides were mimicked by ionomycin (1μM), which also prevented the effects of nucleotides on I_{sc} and G_t. Stimulation of cAMP production with a purinergic P1 receptor agonist adenosine (200μM) or by forskolin (8μM) treatment had little effect on I_{sc} but increased G_t to an extent that indicated a substantial increase in G_s. In agreement it was found that forskolin increased passive paracellular Cl^- fluxes (from 126.4±19.6 to 173.6±18.2 nmol min^{-1} cm^{-2}; n=6; $p<0.002$).

The results suggest that nucleotides released to the ASL exert an autocrine regulatory function on airway epithelial ion transport, primarily by inhibiting net NaCl absorption, while stimulation of Cl^- secretion is of minor importance. An increase in $[Ca^{2+}]_i$ evoked by purinergic receptors probably mediates inhibition of ENaC channels and down-regulation of paracellular anion (Cl^-) permeability, while the opposite paracellular effect of cAMP suggests that this pathway is under dual control.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C56

Conversion of the human red cell Cl^-/HCO_3^- exchanger into an anion selective conductance

M.D. Parker[1], M.T. Young[2], M.J. Tanner[3], W.F. Boron[1] and R.W. Meech[4]

[1]Department of Cellular and Molecular Physiology, Yale School of Medicine, New Haven, CT, USA, [2]Institute of Molecular Physiology, University of Sheffield, Sheffield, UK, [3]Department of Biochemistry, University of Bristol, Bristol, UK and [4]Department of Physiology, University of Bristol, Bristol, UK

The anion transporter AE1 (band 3) mediates electroneutral exchange of Cl^- for HCO_3^- across red blood cell membranes. Human AE1 is a 911-amino acid protein thought to traverse the plasma membrane as many as 14 times (Tanner, 1997). Previous work (Groves et al. 1998) showed that when human AE1 is expressed as two separate non-complementary fragments representing the entire protein but lacking the putative transmembrane (TM) helices numbers 6 and 7, the assembly can mediate ^{36}Cl influx into *Xenopus* oocytes. We have now created a single construct (band3(1:5-8:14)) composed of the N-terminal fragment of AE1, consisting of TM helices 1-5, covalently linked to the C-terminal fragment consisting of TM helices 8-14. Using a combination of pH-sensitive microelectrodes and a two-electrode voltage clamp, we have demonstrated that, while still capable of low level Cl^--HCO_3^- exchange, the protein also mediates the conductive passage of anions.

Xenopus oocytes expressing the 'linked' AE1 construct (band 3(1:5-8:14)), had a markedly higher membrane conductance (4.50 μS) than control AE1-expressing oocytes (0.2 μS). Substituting Cl^- in the bathing medium for gluconate, reduced the oocyte membrane conductance and shifted the zero current potential as expected for an anion selective pathway. Its permeability sequence was NO_3^->NO_2^->I^->Cl^-~Br^->formate$^-$>HCO_3^->gluconate$^-$.

Analysis of oocyte membrane patches expressing band 3(1:5-8:14) revealed single-channel events with a unitary conductance of ~35 pS, and an open probability of 2-3%. Such events were never observed in control oocytes. Each channel-bearing patch had a multiple of two functional channels, as if each AE1 dimer contained two channel pores.

As proposed by Jennings (1989) the structure of AE1 includes an aqueous channel that penetrates deep into the membrane allowing anions access to the translocation site at the permeability barrier. Our finding, that the removal of TM helices 6-7 from AE1 is sufficient to give it channel-like properties, reinforces the idea that ion transporters and ion channels are not distinct membrane mechanisms, but are part of a continuum of ion-carrying proteins. Thus the anion selectivity of the access channel of AE1 may contribute to the high degree of selectivity exhibited by the native transporter.

Groves JD et al. (1998). Biochem J 332, 161-171.

Jennings ML (1989). Annual Review of Biophysics and Biophysical Chemistry 18, 397-430.

Tanner MJA (1997). Mol Membr Biol 14, 155-165.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C57

Aquaporins in the rat uterine epithelium and its potential involvement in fluid formation

M.C. Branes, P. Gallardo, B. Nunez and M.J. Villalon

Pontificia Universidad Catolica de Chile, Santiago, Chile

The volume of the fluid in the female reproductive tract fluctuates during the oestrus cycle suggesting that water availability is under hormonal control. Water movement across epithelial barriers occurs through water channels formed by aquaporins (AQPs). We have previously demonstrated that rat AQP9 levels are increased during the periovulatory period of the reproductive cycle in an oestrogen- and progesterone-dependent manner (1). Now we have explored the functionality of these channels in fluid formation and the possible AQPs involved in water movement through the rat uterine epithelium. Rats used in all experiments were anaesthetized intraperitoneally with sodium pentobarbital (55 mg/kg weight) before any experimental procedure including humane killing. The instillation of uterine horns from rats in proestrus with 100 µl of 200 µM $HgCl_2$ produced 6 h later a 52.4 ± 18.6% ($p<0.05$) reduction in the fluid volume compared to that of horns instilled with 0.9% NaCl. Using RT-PCR of total RNA from uterine epithelium of rats in proestrus, we identified AQP2, -3 and -9. By immunoblotting of total membrane homogenates we detected a 29 kDa band for AQP2 and a 52 kDa band for AQP9, suggesting that this epithelium expresses a modified form of AQP9. Both AQPs were detected during the whole oestrus cycle. While levels of AQP2 were relatively constant, those of AQP9 increased mainly during oestrus and less prominently during proestrus (0.205 ± 0.020, 0.108 ± 0.022, 0.029 ± 0.004 and 0.017 ± 0.003 correspond to AQP9 relative levels in oestrus, proestrus, diestrus 1 and 2, respectively, n=3). Immunohistochemical studies of AQP9 in uterine sections showed positive reaction only in glandular and luminal epithelial cells. Our results suggest that water channels formed, at least by AQP2 and -9 may mediate water movement across this epithelial barrier and that up regulation of AQP9 may account for an increase in uterine epithelial membrane water permeability. Further experiments are in progress to evaluate the localization of AQP2 and -9 in basolateral or apical membrane as well as to determine if other AQPs are also present in this epithelium.

Branes MC et al. (2005). Am J Phys Cell Phys 288 ajpcell.00420.2003.

Supported by FONDECYT grants 3030066 (MCB) and 1040804 (MJV).

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

SA25

Surface and glandular epithelia and airway surface liquid pH

S.K. Inglis

Maternal and Child Health Sciences, University of Dundee, Dundee, UK

The airways are equipped with a number of innate defence mechanisms that protect them against pathogens and other harmful particles that are inhaled, and many of these mechanisms are sensitive to ambient pH. Given the pH sensitivity of these defensive processes, we may expect that lung diseases will be associated with changes in luminal pH (pH_{Lumen}). Although measurements of the pH of the fluid bathing the airway lumen are difficult to make, there is evidence that it does change during lung disease, particularly that associated with inflammation, for example cystic fibrosis. The role that alterations in pH_{Lumen} may have in contributing to the pathogenesis of these pulmonary diseases is not yet known, but it is possible that the disease process may be ameliorated by restoring pH_{Lumen} to normal. We are a long way from being able to manipulate airway luminal pH, however, since the mechanisms by which it is controlled are not understood, and indeed the normal range of luminal pH is still under debate as a result of the difficulties in measuring this parameter. We have therefore studied some of the mechanisms that may be involved in regulating airway pH_{Lumen}.

We and others have shown that stimulation of submucosal glands in intact, isolated airways that contain both glandular and surface epithelia results in HCO_3^- secretion, and this secretion of HCO_3^- by airway submucosal glands is essential for normal liquid and mucus secretion (3). We may therefore expect that the pH of ASL is likely to be relatively alkaline, particularly during periods of glandular secretion. However, numerous measurements of ASL pH, both in vitro (e.g. 7) and in vivo (6), have shown that ASL is acidic relative to plasma. This led us to hypothesise that the surface epithelium may acidify the HCO_3^- -rich glandular secretions, whilst others have suggested that proximal regions of the glands carry out this acidification (8). We isolated distal bronchi from porcine lungs, perfused them with a lightly buffered solution and demonstrated that they are capable of both acidifying and alkalinising their lumen (5). The alkalinisation was stimulated by the gland secretagogue acetylcholine and was inhibited by removal of bath HCO_3^-, the Na^+/H^+ exchange blocker dimethylamiloride and the anion channel blocker NPPB. It thus matched the properties previously described for ACh-evoked submucosal gland HCO_3^- secretion from these tissues (4). The bronchi also secreted acid equivalents into the lumen, and this was inhibited 65% by the H^+-ATPase blocker, bafilomycin A_1. Immunohistochemistry confirmed the presence of H^+-ATPase in the surface epithelium, and removal of this epithelium reduced luminal acidification suggesting that the surface epithelium can acidify the airway lumen. Other studies of surface epithelia have revealed activities of the non-gastric form of the K^+/H^+ -ATPase (1) and a zinc-sensitive proton conductance (2), both of which may also be involved in luminal acidification. It thus seems possible that the surface epithelium may indeed acidify the airway lumen, and that the glandular and surface epithelia together determine the pH_{Lumen} in glandular airways. However, others have demonstrated that Calu-3 cells, thought to be representative of submucosal gland serous cells, also acidify their luminal surface through activity of a K^+/H^+ -ATPase (9). It is possible, therefore, that both glandular and surface regions of airway epithelia can acidify the luminal surface. The mechanisms by which they regulate pH_{Lumen}, however, are as yet unknown.

1. Coakley, R. D., B. R. Grubb, A. M. Paradiso, J. T. Gatzy, L. G. Johnson, S. M. Kreda, W. K. O'Neal, and R. C. Boucher. Proceedings of the National Academy of Science of the United States of America 100: 16083-16088, 2003.
2. Fischer, H., J. H. Widdicombe, and B. Illek. American Journal of Physiology 282: C736-C743, 2002
3. Inglis, S. K., M. R. Corboz, and S. T. Ballard. American Journal of Physiology 274: L762-L766, 1998.
4. Inglis, S. K., and S. M. Wilson. Pediatric Pulmonology In press, 2005.
5. Inglis, S. K., S. M. Wilson, and R. E. Olver. American Journal of Physiology 284: L855-L862, 2003.
6. Ireson, N. J., J. S. Tait, G. A. MacGregor, and E. H. Baker. Clinical Science 100: 327-333, 2001.
7. Jayaraman, S., Y. Song, and A. S. Verkman. American Journal of Physiology 281: C1508-C1511, 2001.
8. Joo, N. S., Y. Saenz, M. E. Krouse, and J. J. Wine. Journal of Biological Chemistry 277: 28167-28175, 2002.
9. Krouse, M. E., J. F. Talbott, M. M. Lee, N. S. Joo, and J. J. Wine. American Journal of Physiology 287: L1274-L1283, 2005.

This work was funded by the Wellcome Trust

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

SA26

Airway surface liquid volume homeostasis in health and disease

R. Tarran

Cystic Fibrosis Center, University of North Carolina, Chapel Hill, NC, USA

Human airways normally regulate the volume of a thin liquid layer, the periciliary liquid (PCL), to provide the mucus clearance component of lung defense (REF 1). Studies under standard (static) culture conditions revealed that normal airway epithelia possess an adenosine (ADO)-regulated pathway that blends Na^+ absorption and Cl^- secretion to optimize PCL volume. In CF, the absence of CFTR results in a failure of ADO regulation of PCL volume, which is predicted to initiate mucus stasis and infection. However, under conditions that mimic the phasic motion of the lung *in vivo*, ATP release into PCL was increased, CF ion transport rebalanced, and PCL volume restored to levels adequate for lung defense. This ATP signaling system was vulnerable, however, to insults that trigger CF bacterial infections, such as viral (RSV) infections, which upregulated extracellular ATPase activity and abolished motion-dependent ATP regulation of CF PCL height. These studies demonstrate (i) how the normal coordination of opposing ion transport pathways to maintain PCL volume is disrupted in CF; (ii) the hitherto unknown role of phasic motion in regulating key aspects of normal and CF innate airways defense; and (iii) that maneuvers

directed at increasing motion-induced nucleotide release may be therapeutic in CF patients.

Tarran, R. 2004. Regulation of Airway Surface Liquid Volume and Mucus Transport by Active Ion Transport. Proceedings of the American Thoracic Society. 1: 42-46.

Supported by the North American Cystic Fibrosis Foundation and the NIH

Where applicable, the experiments described here conform with Physiological Society ethical requirements.