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A synthetic, chloride-selective channel that alters chloride transport in epithelial cells

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Abstract

An Ussing chamber was used to demonstrate that synthetic amphiphilic anion transporters function as chloride transporters in mammalian airway epithelial cells.

Proteins are generally required for the transport of ionic and molecular species through bilayer membranes. Because these proteins are both critical and complex, bioorganic chemists have devised a variety of functional model systems. Two decades ago, synthetic ion channels were virtually unknown, but several worthy examples have emerged recently.¹ In our laboratory, we have designed and characterized structurally different families of synthetic cation-² and anion-selective³ channels. We refer to the former as hydraphiles;⁴ this family of cation channels is now well characterized. The cation channels incorporate macrocyclic “crown” polyethers and are thought to function as monomers within the bilayer. In contrast, the anion channels developed in our laboratory are amphiphilic peptides^{3,5,6,7} that function, at least in some cases, as dimers.⁸ The active molecules have the general structure (R¹)₂NCOCH₂OCH₂CO-(Gly)₃Pro(Gly)₃-OR². Extensive structural variation⁵⁻⁷ and selectivity studies⁹ have been conducted in liposomes.

Vectorial ion transport across lung epithelial cell layers is essential for homeostasis in multicellular organisms and disruption of this process is pathological.^{10,11} Epithelial cell layers control mass transport of water and solutes in alimentary, pulmonary, renal, and a variety of secretory organs. Anion regulation is critical in the function of epithelial layers. We now report that a synthetic anion channel, based on the general structure shown above, mediates anion regulation in airway epithelial cells.

The preparation of (H₃₇C₁₈)₂NCOCH₂OCH₂CO-(Gly)₃-Pro(Gly)₃-OCH₂Ph, **1**, has been reported.⁵ The *ca.* six-fold less effective variant, (H₃₇C₁₈)₂NCOCH₂OCH₂CO-(Gly)₃Leu(Gly)₃-OCH₂Ph, **2**,³ was chosen as a control. When **1** was added to DOPC-DOPA (7 : 3) liposomes containing chloride ion, an increase in Cl⁻ permeability was observed. Compound

1 produced a membrane potential in liposomes when the membrane impermeant SO_4^{2-} replaced the permeant NO_3^- anion in the external buffer. When the known potassium-selective ionophore valinomycin was added, rapid K^+ transport dissipated the membrane potential, accelerating the release of intravesicular Cl^- . This experiment verified the substantial chloride selectivity that was previously observed in planar lipid bilayers.³

Epithelial layers consist of multiple cell layers in which individual cells are joined by tight junctions, and form a water and electrolyte barrier between the outside (apical) and inside (basolateral) compartments.¹² In airway epithelia, the cells regulate ion concentration in the water-mucus layer that clears particles and bacteria from the lung airways. The specific localization of transport proteins on each side of the epithelium affords directional ion transport of water and electrolytes across it. Epithelial cell layers, therefore, constitute a stringent challenge to the activity of a synthetic ion channel.

The apical membranes of vital epithelial cells contain amiloride-sensitive Na^+ channels, referred to as ENaC,^{13,14} and cAMP-activated Cl^- channels (CFTR).¹⁵ Together these two channels mediate the transcellular movement of NaCl. Under baseline conditions, epithelial layers absorb Na^+ . Sodium ions enter the cell at the apical (exterior) surface through ENaC channels and leave the cytoplasm on the basolateral side. The basolateral-side transport process involves a potassium coupled mechanism (Na^+ - K^+ -adenosine triphosphatase, ATPase) that provides energy for ion movement in the epithelium. Chloride ions enter the cytoplasm across the basolateral membrane *via* an electrically neutral co-transport process, coupled to Na^+ and K^+ (Na^+ - K^+ -2 Cl^- -co-transporter). Potassium ions that accumulate within the cell leave passively through basolateral membrane K^+ channels.

amiloride

forskolin

A schematic of the device Ussing developed in 1949 (“Ussing chamber”) to study the electrical properties of epithelial tissue¹⁶ is shown in Fig. 1. We used primary epithelial layers differentiated from murine (mouse) tracheal epithelia on permeable supports in culture as previously described.¹⁷ The support is the plastic “insert” of the Ussing chamber (EC8225 Warner Instruments, Inc.) and is shown in panel a of Fig. 1. The cell layer separates two chambers containing different aqueous solutions. One chamber bathes the apical surface while another bathes the basolateral surface of the cells. The solutions are held at 37 °C and are ventilated with 5% CO₂ in O₂. Electrodes inserted in the solutions are used to monitor electrical changes (Fig. 1b) following additions of various substances. Fig. 1c shows the change in

electrical response of the system when forskolin is introduced into the bathing solutions. Hyperpolarization occurs during ~200 seconds.

In this experiment, the magnitude of the transepithelial potential, V_{te} , is equal to the difference between the apical and basolateral membrane potentials, and is directly proportional to the epithelial layer resistance. In one approach, a voltage (potential) is applied and the resulting current change is measured (voltage clamping). Transepithelial ion transport under these conditions, however, may not be accurately reflected since the applied voltage also forces electrolytes through the cell. An alternative method, called an “open circuit”, does not expose the epithelium to external voltage. In this case, the voltage difference between the basolateral and apical sides is recorded.

A typical experiment was conducted as follows. The epithelial layer was fitted into the Ussing chamber and the appropriate solutions were added to both sides of the membrane. The transepithelial potential, V_{te} , was allowed to stabilize (10-30 min) before addition of the agents under study.¹⁸

Fig. 2a shows the change in transepithelial voltage upon application of amiloride (50 μ M, first arrow) to the apical surface. The amiloride-sensitive channels are blocked and V_{te} falls from -12.9 mV to -5.0 mV within 100 s. The second arrow indicates addition of the adenylylase activator, forskolin (5 μ M), which results in recovery of the transmembrane potential to -23.2 mV. Forskolin is a cAMP agonist, which causes CFTR Cl^- channels in the apical membrane to open and hyperpolarize the epithelium (as apparent in Fig. 2a), by activation of apical chloride transport.

Fig. 2b shows the result of sequential addition of amiloride (50 μ M), **1** (40 μ M), and forskolin (5 μ M). Application of amiloride to the apical surface (first arrow) reduced the transepithelial voltage from -29.2 to -2.0 mV ($\Delta V = 27.2$ mV, ~90% change). The average depolarization caused by amiloride was 17 ± 8 mV (5 replicates (*i.e.*, $n = 5$)). After the voltage stabilized, a solution of **1** in 2-PrOH¹⁹ was added so that the final concentration of **1** was 40 μ M. Addition of **1** to the apical bath caused hyperpolarization of the epithelium from -2.0 to -5.6 mV within 250 s. Subsequent addition of 5 μ M forskolin further hyperpolarized the epithelium, reaching -19.9 mV in 5 minutes. The effect of **1** ($\Delta V_{te} = -2.1 \pm 1.4$ mV, $n = 3$) was ~20% of the transepithelial voltage change observed after apical stimulation with forskolin ($\Delta V_{te} = -12.5 \pm 4.8$ mV, 5 replicates). The transepithelial resistance, R_{te} , was measured during each phase of the experiment to assess the integrity of the epithelium. Membranes treated with **1** showed no change in transepithelial resistance as compared to the controls treated only with amiloride and forskolin. This experiment demonstrates that **1** influences ion transport across the epithelial layer.

The results shown in panels b and c of Fig. 2 confirm that chloride transport is critical for the effect of **1** observed in Fig. 2b. This was demonstrated by performing an identical experiment in which Cl^- was not present in the buffer solution. If no chloride anion is present, endogenous chloride channels are not expected to affect the experiment. Thus, chloride-free Ringers solution,²⁰ normally 140 mM in Cl^- , was prepared by replacing chloride with gluconate and then applying to the epithelia. As above, amiloride (50 μ M) caused depolarization of the epithelium ($\Delta V_{te} = 23.2 \pm 14.5$ mV, $n = 3$). However, addition of **1** did not alter the observed voltage (*i.e.*, $\Delta V_{te} = 0$ mV, $n = 3$). Application of forskolin solution caused initial, rapid hyperpolarization of the membrane which stabilized in 5 minutes ($\Delta V_{te} = -11.0 \pm 5.9$ mV, $n = 3$). We note that the response of these cells to added forskolin confirms their vitality.

The forskolin-stimulated voltage changes observed in this experiment are not mediated solely by activation of endogenous chloride channels. Apical to basolateral chloride transport simply

cannot occur when no chloride ions are present in the media. We speculate that the observed voltage change results from stimulation of cAMP-activated sodium channels. These results indicate that the hyperpolarization observed upon addition of forskolin in previous experiments may have more components than simple activation of apical CFTR. We note that cAMP stimulated sodium absorption in Cl⁻-free gluconate solutions has been previously reported.²¹ We postulate that **1** establishes a new pathway for chloride transport at the apical side of the epithelium and fosters vectorial Cl⁻ transport in conjunction with the known basolateral Na⁺-K⁺-2Cl-co-transporter.

Despite the results obtained above, it seemed possible that compound **1** stimulated epithelial potential changes by activation of endogenous chloride channels. A control study with **2** was undertaken. Compounds **1** and **2** are identical except for the Pro→Leu replacement (see above). As in the experiment described above, the membrane was bathed with Ringers solution, the apical side was treated with amiloride solution, and epithelial depolarization was observed ($\Delta V_{te} = 8.9$ mV, Fig. 2d). Subsequent addition of 40 μ M **2** ((H₃₇C₁₈)₂NCOCH₂OCH₂CO-(Gly)₃Leu(Gly)₃-OCH₂Ph) to the apical bath resulted in no change in V_{te} ($\Delta V_{te} = 0.0$ mV, $n = 2$). Our attempts to use a higher concentration of **2** were unsuccessful owing to limited solubility. Final treatment with 5 μ M forskolin hyperpolarized the membrane ($\Delta V_{te} = -6.0$ mV), again confirming cellular vitality.

We report here what is, to our knowledge, the first example of Cl⁻ transport in mouse trachea epithelial cells that is stimulated by a completely synthetic amphiphilic peptide ionophore. The voltage change was achieved without compromising membrane integrity, and our heptapeptide-based chloride transporter altered Cl⁻ transport throughout the epithelium. This effect was not observed for a structurally similar heptapeptide with leucine at its center. We conclude that **1** establishes a new pathway for chloride transport in the apical membrane that is independent of native chloride channels present in the mouse trachea epithelial cells.

Acknowledgments

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Notes and references

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Fig. 1.
(a) Schematic representation of the plastic insert used in an Ussing chamber. An epithelial cell layer was grown on the permeable support. (b) Electrical circuitry of the Ussing chamber. (c) Transepithelial voltage increase caused by addition of forskolin solution to the apical membrane.

Fig. 2.

(a) Response of airway epithelia to amiloride and forskolin. (b) Hyperpolarization caused by addition of **1**. (c) Experimental conditions identical to (b) except chloride free buffer was used. (d) Experimental conditions identical to (b) except **2** was added rather than **1**.