Contribution of the IsK (MinK) Potassium Channel Subunit to Regulatory Volume Decrease in Murine Tracheal Epithelial Cells*

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The cell volume regulatory response following hypotonic shocks is often achieved by the coordinated activation of K\(^+\) and Cl\(^-\) channels. In this study, we investigate the identity of the K\(^+\) and Cl\(^-\) channels that mediate the regulatory volume decrease (RVD) in ciliated epithelial cells from murine trachea. RVD was inhibited by tamoxifen and 1,9-dideoxyforskolin, two agents that block swelling-activated Cl\(^-\) channels. These data suggest that swelling-activated Cl\(^-\) channels play an important role in cell volume regulation in murine tracheal epithelial cells. Ba\(^{2+}\) and amphotericin B, inhibitors of K\(^+\) channels, were without effect on RVD, while tetraethylammonium had little effect on RVD. In contrast, clofilium, an inhibitor of the KvLQT/IsK potassium channel complex potently inhibited RVD, suggesting a role for the KvLQT/IsK channel complex in cell volume regulation by tracheal epithelial cells. To investigate further the role of KvLQT/IsK channels in RVD, we used IsK knock-out mice. When exposed to hypotonic solutions, tracheal cells from IsK\(^{+/+}\) mice underwent RVD, whereas cells from IsK\(^{−/−}\) failed to recover their normal size. These data suggest that the IsK potassium subunit plays an important role in RVD in murine tracheal epithelial cells.

When exposed to hypotonic solutions most cells swell rapidly before recovering their original volume, a response known as regulatory volume decrease (RVD).\(^1\) RVD involves the activation of ionic pathways, mainly ion channels, which permit the passive loss of electrolytes and osmotically obliged water (1). Although the ability of maintaining a constant volume in the face of osmotic stress is important for all cells in the body, the process assumes particular significance in epithelial cells. In the case of the airways, the luminal face of the epithelial cells is covered by a liquid (airway surface liquid, ASL) that modifies its osmolality under different situations, becoming hyperosmolar (e.g., during cold or dry ventilation) or hypoosmolar (e.g., breathing fog) (2–4). These changes in the osmolality of the ASL affect other airway functions, including protein and hydroelectrolytic secretion, and release of inflammatory mediators (5, 6). Despite the significance of ASL to airway physiology and pathophysiology the molecular basis for the ion pathways involved in cell volume regulation is largely unknown. Although the molecular identity of the swelling-activated Cl\(^-\) channels has been the subject of several studies in recent years (7–9), little is known about the identity of the swelling-activated K\(^+\) channels involved in RVD. In this study we have characterized the ionic conductances underlying the RVD response in murine tracheal cells. We have found that the potassium channel complex KvLQT/IsK (10) plays an important role in cell volume regulation by murine tracheal cells.

EXPERIMENTAL PROCEDURES

Animals—For this study, we used mice from two different outbred genetic backgrounds. One group yielded control animals, while the other gave wild-type isk\(^{+/+}\) and null isk\(^{−/−}\) mice (11). Mice (4–20 weeks old) were killed by asphyxiation with carbon dioxide prior to tissue removal. Animals were maintained and experiments performed according to the guidelines issued by the Animal Care Committees at the respective institutions involved.

Tissue Isolation—Tracheal epithelial cells were isolated using sterile conditions. The trachea proximal to the bronchial bifurcation was excised from the mice and adherent adipose tissue removed. The tracheal tissue was opened longitudinally, cut into three pieces, and incubated in Ca\(^{2+}\)- and Mg\(^{2+}\)-free Eagle’s minimal essential medium containing 0.1% (v/v) protease XIV, 0.1 mg/ml deoxyribonuclease I, 2 mM EDTA, and 50 µg/ml gentamicin for 16 h at +4°C with occasional gentle agitation. Following tissue digestion, 10% fetal bovine serum was added to the medium to deactivate enzymes, undigested fragments of tissue were removed and tracheal epithelial cells harvested by low speed centrifugation (5 min at 500 rpm). The collected cells were resuspended in Ham’s F-12 nutrient medium supplemented with 1 µg/ml insulin, 5% fetal bovine serum, 2 mM L-glutamine, and 1 µg/ml gentamicin at +4°C. Tracheal cells remained viable for up to 24 h at +4°C. Tracheal cell volume experiments were performed at 37°C to obtain a larger number of cells with beating cilia (a prerequisite for cell selection). Tracheal epithelial cells were attached to glass coverslips coated with 75 mg/ml 1-ethyl-3(3-dimethylaminopropyl) carbodiimide and 15 mg ml\(^{-1}\) concanavalin A (12), placed in the bottom of a recording chamber, bathed in isotonic Hanks’ solution, and observed under phase contrast optics using an inverted microscope (Leica DMLB). Single cells with well defined membranes and beating cilia were selected and digital images acquired at timed intervals using a Photons camera and Quanticell 500 software (Applied Imaging). Photomicrographs of tracheal epithelial cells were analyzed using NIH Image software (version 1.58) to determine the area of cells. The radius \((R)\) of individual cells was calculated using the following equation.

\[
R = \sqrt{\frac{\text{area}}{\pi}} \tag{Eq. 1}
\]

Assuming that tracheal epithelial cells are spherical in shape, cell volume \((V)\) was calculated using the equation

\[
V = \frac{4}{3} \pi \cdot r^3 \tag{Eq. 2}
\]

and normalized to that measured at time \(t = 0\).

Solutions—The isotonic Hanks’ solution contained (mM): 140 NaCl, 2.5 KCl, 1.2 CaCl\(_2\), 0.5 MgCl\(_2\), 5 glucose, and 10 Hepes, pH 7.25 (osmolality, 302 ± 6 mOsm; \(n = 12\)). The hypotonic Hanks’ solution (osmolality, 205 ± 10 mOsm; \(n = 16\)) was prepared by removing 50 mM NaCl from the isotonic solution and diluting it with the hypotonic solution to achieve the desired osmolality.
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The apical membrane of airway epithelial cells is covered by a thin layer of ASL (22) that provides the right microenvironment for cilia motility and, hence, mucus clearance. The tonicity and/or salt composition of ASL may vary under both physiological (2–4) or pathological conditions such as cystic fibrosis (22, 23), although the changes in tonicity and/or salt composition in cystic fibrosis airways has been highly disputed (24). In the present study we have addressed, for the first time, the homeostatic volume adjustments triggered in the ciliated airway epithelial cells by changes in the tonicity of the medium.

As in many other epithelia (14, 25, 26), ciliated cells of the murine trachea show a biphasic response to hypotonic solutions. They rapidly swell due to the entry of water, and subsequently they initiate a recovery phase, named RVD, toward the initial cell volume. The RVD response in murine tracheal cells appears to depend on the activity of both Cl– and K+ channels as inhibition of either type of channels prevents the full RVD. The RVD is inhibited by DDFSK and tamoxifen, suggesting the participation of the DDFSK- and tamoxifen-sensitive outwardly rectifying swelling activated Cl– channel (9, 13, 14). Whole cell currents obtained by the activation of this channel

Fig. 2. Inhibition of RVD by blockers of swelling-activated Cl– channels. a and b show the effect of 100 μM DDFSK and 5 μM tamoxifen on the RVD response of tracheal epithelial cells from control mice. Cl– channel blockers were added 3 min prior to the addition (at 0 min) of the hypotonic bathing solution, which also contained the appropriate blocker. Data are mean ± S.E. (DDFSK = 6; tamoxifen = 3).

Involvement of IsK in Cell Volume Regulation

Pharmacological Identification of the Potassium Channel Involved in RVD—To investigate the identity of the K+ channels that mediate RVD in murine tracheal epithelial cells, we tested the effect of Ba2+ and tetraethylammonium (TEA) (19, 20) two agents that inhibit a wide range of K+ channels. Apamin, a blocker of small conductance Ca2+-activated K+ channels (21), inhibited RVD in mouse small intestinal cells, therefore, we also tested its effect on murine tracheal epithelial cells.

Fig. 3, a and b, demonstrates that Ba2+ (5 mM) and apamin (2.5 μM) failed to prevent RVD in ciliated cells from control mouse trachea, while Fig. 3c shows that TEA (5 mM) only partially inhibited RVD. The half-maximal inhibitory concentration of TEA was 4.3 mM, but the maximal level of inhibition was only about 50% (data not shown). In contrast, Fig. 3d shows that clofilium (100 μM), an inhibitor of the KvLQT/IsK potassium channel complex, almost completely inhibited RVD in ciliated tracheal cells.

To explore further the role of the KvLQT/IsK potassium channel complex in RVD, we used a mouse model that has a complete loss of the isk gene product (11). Like ciliated tracheal cells from outbred control mice (Fig. 1b), wild-type mice, isk(+/+) showed around 75% RVD response, measured 30 min after switching to a hypotonic solution (Fig. 4). In contrast, isk(−/−) litter mates presented a reduced RVD response (−25%) over the same 30-min exposure to hypotonic shock. These data suggest that the IsK potassium channel subunit plays a key role in RVD by epithelial cells from mouse trachea.

FIG. 1. Regulatory volume decrease in murine tracheal cells. a, digital micrographs of an isolated murine tracheal cell taken in isotonic solution (left), 2 min (middle), and 30 min (right) after replacement of the isotonic solution with a 25% hypotonic solution. b, relative changes in cell volume measured before and after replacement at time t = 0 of isotonic solution with a 25% hypotonic solution in tracheal cells from control mice (n = 10). Scale bar: 7 μm.

NaCl from the isotonic Hanks’ solution and adjusting it with D-mannitol when necessary. All chemicals were purchased from the Sigma-Aldrich Co. Ltd. (Poole, UK).

Statistics—Results are expressed as means ± S.E. of n observations. To compare sets of data, we used Student’s t test. Differences were considered statistically significant when p < 0.05.

RESULTS

Regulatory Volume Decrease in Murine Tracheal Cells—Fig. 1 shows phase contrast images of a single ciliated tracheal cell. Images were taken under isotonic conditions (Fig. 1a, left) and after 2 min (Fig. 1a, middle) and 30 min (Fig. 1a, right) in a 25% hypotonic solution. Superfusion of the cell with the hypotonic solution resulted in a clear increase in cell size. After the initial swelling, the cell returned close to its preswelling size by undergoing a RVD response. Fig. 1b quantifies the RVD response of control tracheal cells, which achieved 80 ± 6% volume recovery at 30 min.

Pharmacological Identification of the Chloride Channel Involved in RVD—Swelling-activated chloride channels, Cl(swell), have been described in many cell types (7–9). Among the different Cl(swell) channels, the outwardly rectifying, ATP-dependent channel, also known as volume-regulated anion channel or volume-sensitive organic osmolyte/anion channel, has been implicated in the RVD response of several cell types (13, 14). This channel is blocked by 1,9-dideoxyforskolin (DDFSK) (13, 15, 16) and the antioestrogen tamoxifen (13, 14, 17, 18). Both drugs have also been shown to inhibit the RVD response in T84 epithelial cells (14). Based on this data, we examined the effect of DDFSK and tamoxifen on the RVD response of control murine ciliated tracheal cells (Fig. 2). DDFSK at 100 μM and tamoxifen at 5 μM were added to the bathing solution 3 min before exposure to a 30% hypotonic shock. Fig. 2a demonstrates that both compounds prevented RVD. These data suggest that swelling-activated Cl– channels play an important role in RVD in murine tracheal epithelial cells.

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DISCUSSION

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2 M. A. Valverde, unpublished observation.
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Figure 3. Inhibition of RVD by K+ channel blockers. The indicated K+ channel blocker was added 3 min prior to the addition (at 0 min) of the hypotonic bathing solution, which also contained the appropriate blocker. a, 5 mM Ba2+ (n = 4); b, 2.5 μM amamin (n = 6); c, 5 mM TEA (n = 4); d, 100 μM clofilium (n = 3).

Figure 4. Role of IsK in RVD by murine tracheal epithelial cells. Comparison of the percent RVD of 10 ciliated tracheal cells obtained from three wild-type mice (IsK+/+) and the percent RVD of 16 ciliated tracheal cells obtained from four knock-out mice (IsK−/−). Relative volume changes were normalized to those obtained at the peak volume increase calculated at time t = 2 min to account for different cell swelling in response to the same osmotic stimulus. Percent RVD values obtained for IsK−/− cells were significantly different from IsK+/+ cells; p < 0.05.

have been characterized in canine (27, 28) and human (29, 30) tracheal cells.

Clofilium, a blocker of RVD in murine airway epithelial cells, is also an inhibitor of the IK current (10) that result from the activity of a channel complex formed by the association of a pore forming α subunit, KCNQ1 (KvLQT1) (31), and a β subunit, IsK (MinK or KCNE) (10, 32). However, the effect of clofilium may not be specific for KvLQT/IsK channels as it blocks other types of voltage-dependent potassium channels (33, 34). Clofilium has also been shown to block volume-sensitive potassium currents and RVD in Ehrlich cells (35), although in this case the authors, on the basis of the lack of voltage-dependence of the K+ current, concluded that KvLQT1/IsK channels could not underlie the volume-sensitive macroscopic K+ currents.

A role for KvLQT1/IsK channels in the RVD of murine tracheal cells was suggested by our pharmacological studies. This hypothesis was further examined by the use of IsK knock-out mice. The tracheal cells obtained from this animal model showed marked reduction of the RVD response (25% RVD versus 80% RVD in the wild-type mice). The molecular mechanism by which the presence of IsK improves the RVD response in tracheal cells is unknown at present. However, our data suggest two possible mechanisms. First, IsK might act as the volume sensor within the channel complex. In response to cell swelling, it may undergo a conformational change that opens the KvLQT channel pore. Second, IsK might shift the activation of the channels to potentials close to the resting membrane potential of tracheal cells and hence accelerate the potassium efflux under hypotonic conditions. Consistent with this idea, co-assembly of KCN3 (IsK) with KCNQ1 potassium channels in intestine modulates the voltage dependence of channel activation (36). The first hypothesis would involve a principal role for the activation of potassium channels to activate the RVD response, while the second would confer them a more passive role, with the activation of swelling-activated chloride channels being the starting signal. Although we cannot discard the first hypothesis, we favor the second possibility as basolateral IK currents have been identified in tracheal cells under isosmotic conditions (37). In that respect, IK current has been associated to the maintenance of transepithelial chloride secretion in the airways (37, 38) and colonic cells (36) and potassium secretion in the stria vasculäris (39).

The association of IsK with cell volume regulatory mechanisms has been previously proposed in vestibular dark cells (40), although a more recent study disagrees with this view (11). In this respect, it is worth mentioning that both studies measured the peak increase in cell height, which reflects the osmometric behavior of the cells, rather than the RVD, which represents the cell regulatory adjustments following a hypotonic shock. A more direct approach to study the modulation of IK by cell swelling, using electrophysiological techniques, has shown the increase in IK current following hypotonic shock in oocytes expressing IsK (41).

In conclusion, it appears that the KvLQT1/Isk potassium channel complex plays important roles in the physiology of murine secretory epithelia, i.e., maintenance of Cl− (36–38) and K+ (39) secretion and cell volume regulation (this study). Although we still do not know which of the 5 identified KCNQ subunits (36, 42) or the 2 KCNE subunits (42) are expressed in the murine trachea, it will be interesting to know whether the IsK role in cell volume control also applies to other epithelia and/or other species.

Acknowledgments—We thank D. Vetter and S. Heinemann (The Salk Institute, San Diego, CA) for providing the wild-type and Isk knock-out mice and A. Grace (University of Cambridge, United Kingdom) for his help with the handling of the animals and D. Sheppard for his comments on the manuscript.

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