Molecular diversity and function of $K^+$ channels in airway and alveolar epithelial cells

Olivier Bardou,1,2 Nguyen Thu Nguyen Trinh,1,2 and Emmanuelle Brochiero1,2

1Centre de Recherche, Centre Hospitalier de l’Université de Montréal (CRCHUM), Hôtel-Dieu and 2Département de médecine, Université de Montréal, Montréal, Québec, Canada

Bardou O, Trinh NT, Brochiero E. Molecular diversity and function of $K^+$ channels in airway and alveolar epithelial cells. Am J Physiol Lung Cell Mol Physiol 296: L145–L155, 2009. First published December 5, 2008; doi:10.1152/ajplung.90525.2008.—Multiple $K^+$ channels are expressed in the respiratory epithelium lining airways and alveoli. Of the three main classes [$I$] voltage-dependent or $Ca^{2+}$-activated, 6-transmembrane domains (TMD), $II$ 2-pores 4-TMD, and $III$ inward-rectified 2-TMD $K^+$ channels, almost 40 different transcripts have already been detected in the lung. The physiological and functional significance of this high molecular diversity of lung epithelial $K^+$ channels is intriguing. As detailed in the present review, $K^+$ channels are located at both the apical and basolateral membranes in the respiratory epithelium, where they mediate $K^+$ currents of diverse electrophysiological and regulatory properties. The main recognized function of $K^+$ channels is to control membrane potential and to maintain the driving force for transepithelial ion and liquid transport. In this manner, $K_{V_{1}}$, $K_{C}$, $K_{A_TP}$, and, for example, contribute to the control of airway and alveolar surface liquid composition and volume. Thus, $K^+$ channel activation has been identified as a potential therapeutic strategy for the resolution of pathologies characterized by ion transport dysfunction. $K^+$ channels are also involved in other key functions in lung physiology, such as oxygen-sensing, inflammatory responses, and respiratory epithelia repair after injury. The purpose of this review is to summarize and discuss what is presently known about the molecular identity of lung $K^+$ channels with emphasis on their role in lung epithelial physiology.

$K_{V}$ channels; $K_{C}$ channels; $K_{IR}$ channels; $K_{2P}$ channels; lung; ion transport; epithelial repair; oxygen sensing; inflammation

EPITHELIAL CELLS LINING THE alveoli and airways from the nasal cavities to the bronchioles are involved in many functions essential to pulmonary physiology. Air conduction and gas exchange are the main activities of the respiratory system. However, the respiratory epithelium, frequently exposed to external insults, has also developed various defenses to protect itself against infections and inhaled particles. These defense mechanisms depend, among others, on mucociliary clearance via airway ciliated cells (10), on the capacity to regenerate an intact respiratory epithelium after injury (22, 69), and its ability to participate in inflammatory responses by secreting pro- and anti-inflammatory cytokines (83). Alveolar type II (ATII) cells synthesize and secrete surfactants, which are crucial in lowering surface tension in alveoli (1). Finally, one of the main functions of epithelial cells is to control the ionomic composition and volume of fluids at the surface of the airways and alveoli. In fact, transepithelial transport of ions and liquid is probably the most extensively studied function of epithelial cells. In normal airways (Fig. 1A), the balance between $Na^+$ absorption and $Cl^-$ secretion is necessary to maintain an adequate periciliary liquid volume for proper ciliary function (7). This balance is disturbed in cystic fibrosis patients (CF; Fig. 1A), resulting in reduced periciliary volume and mucus accumulation. In alveoli (Fig. 1B), $Na^+$ and $Cl^-$ absorption is essential for alveolar fluid clearance at birth as well as for the resolution of lung edema in adults (4).

Because of the crucial role of $Na^+$ and $Cl^-$ transport in lung physiology and pathophysiology, $Cl^-$ and $Na^+$ channels, particularly cystic fibrosis transmembrane receptor (CFTR) and epithelial $Na^+$ channel (ENaC), have been most investigated extensively. In contrast, fewer studies have focused on the role of $K^+$ channels in pulmonary epithelial physiology. To date, almost 40 different types of $K^+$ channels have been detected in the airways and alveolar epithelial cells, but the function of many of them is still unknown. These channels belong to the three main $K^+$ channel classes and are characterized by various electrophysiological and regulatory properties. Some of them have been shown to participate in crucial processes of respiratory physiology. One of the main $K^+$ channel functions in epithelia is to control membrane potential and, thus, to maintain an electrochemical gradient for ion and fluid transport. Such a role of $K^+$ channels, recognized for many years in the renal epithelium, is arousing increasing interest in the physiology of the pulmonary epithelium. $K^+$ channels could also participate in other crucial lung epithelial cell functions, including adaptability to oxygen levels or mucosal defense.

The primary purpose of this review is to summarize current knowledge of the molecular identity and electrophysiological as well as regulatory properties of $K^+$ channels expressed along airway and alveolar epithelia. Its secondary aim is to focus
Fig. 1. Schematic model of ion transport across alveolar and airway epithelia. A: equilibrium between Na⁺ absorption and Cl⁻ secretion across the airway epithelium is necessary to maintain adequate periciliary liquid volume and mucociliary clearance. Cl⁻ secretion involves Cl⁻ entry through a basolateral Na⁺ -K⁺ -2Cl⁻ or K⁺ -Cl⁻ cotransporters, followed by its exit via apical Cl⁻ channels, such as the cystic fibrosis transmembrane conductance regulator (CFTR). Cystic fibrosis (CF) mutations resulting in dysfunctional CFTR channel cause reduced Cl⁻ and fluid secretion. PCL, periciliary liquid. B: Na⁺ absorption across the alveolar epithelium (alveolar type I and II cells: ATI and ATII, respectively) involves passive Na⁺ entry via apical Na⁺ channels [mainly epithelial Na⁺ channel (ENaC)] with subsequent extrusion through basolateral Na⁺ -K⁺ -2Cl⁻ pump, Na⁺ -K⁺ -2Cl⁻ and K⁺ -Cl⁻ cotransporters, aquaporine (AQP).

on the proposed functions of K⁺ channels in lung physiology and their potential role in the resolution of certain lung pathologies.

Molecular Identity of K⁺ Channels Expressed in Airway and Alveolar Epithelial Cells

Since the cloning of the first K⁺ channel, Shaker from Drosophila (58) in 1987, more than 100 other K⁺ channels have been identified at the molecular level. These channels have been classified in three main groups, according to their predictive number of transmembrane domains (TMD). The first class with six TMD is divided in two subgroups: the voltage-dependent K⁺ channels (Kv) and Ca²⁺-activated K⁺ channels (KCa). K⁺ channels from the second class are characterized by four TMD and two pores (K₂P). The third class is composed of two TMD, inward-rectified K⁺ channels (Kir).

Despite this structural heterogeneity, all K⁺ channels possess a highly conserved signature within the pore region, the GYG (Gly-Tyr-Gly) sequence. In some types of K⁺ channels, including human ether-a-go-go-related gene (hERG) or ATP-sensitive K⁺ (KATP), the GYG sequence is, however, replaced by GFF (Gly-Phe-Gly). This GY/FG sequence forms the selectivity filter of K⁺ channels. Another common property of K⁺ channels is their high selectivity for K⁺ over Na⁺ (>100 to 1), an essential feature for their function (19, 54).

More than 30 different K⁺ channels from these three main classes have been detected in the respiratory epithelium (Tables 1 and 2). A phylogenetic tree of K⁺ channels is presented in Fig. 2.

Six-TMD, Kv channels. The human KvLQT1 gene (KCNQ1, Kv7.1), responsible for human cardiac arrhythmia, was identified by positional cloning in 1996 (89). KvLQT1 expression was subsequently reported in nasal (50), tracheal (16, 23, 95), bronchial (Calu-3, 16HBE14o−, NuLi, CuFi, ciliated cells from terminal bronchioles) (2, 12, 16, 50, 82), and ATII (44) epithelial cells (Table 1). It was then established that KvLQT1 coassemble with MinK to form the channel responsible for slow delayed rectifier K⁺ current (I₅/Kᵣ) cardiac currents (95). In the lungs, KvLQT1 could be associated with MinK-related protein (MiRP), possibly with MiRP1 (KCN2, Calu-3), MiRP2 (KCN3, trachea, Calu-3), or MiRP3 (KCN4, lung), which are expressed in the airways and lungs (12, 23, 79) (Table 1). This very-small-conductance K⁺ channel (<3 pS) is activated by cAMP and inhibited by chromanol 293B (91). Among all Kv channels from the respiratory epithelium, KvLQT1 is probably the most studied, and evidence of their functional expression and physiological role has been documented. A great deal of evidence originated from Ussing chamber experiments showing chromanol 293B- or clofilium-sensitive short-
circuit currents across the airway epithelia in normal and CF nasal cells (50), tracheal cells (23), Calu-3 cells (12, 53), normal NuLi, and CF CuFi bronchial cell lines (82) as well as in alveolar cell monolayers (81). It should be noted, however, that we observed lower KvLQT1 expression and currents in CF CuFi compared with normal NuLi bronchial epithelial cells. Our Ussing chamber experiments, performed with apically permeabilized monolayers, demonstrated KvLQT1 currents through the basolateral membrane of NuLi and CuFi bronchial cells (82). Nevertheless, Moser et al. (53) detected KvLQT1 channels and chromanol 293B-sensitive currents also at the apical membrane of Calu-3 monolayers by immunocytochemistry and in electrophysiological experiments, respectively.

<table>
<thead>
<tr>
<th>Name</th>
<th>Gene</th>
<th>Locus</th>
<th>Cell Type</th>
<th>Evidence</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kv1.1</td>
<td>KCNA1</td>
<td>12p13.32</td>
<td>ATII (ap)</td>
<td>R, P&lt;sub&gt;a&lt;/sub&gt;, G&lt;sub&gt;b&lt;/sub&gt;</td>
<td>55</td>
</tr>
<tr>
<td>Kv1.3</td>
<td>KCNA3</td>
<td>1p13.3</td>
<td>ATII (ap)</td>
<td>R, P&lt;sub&gt;a&lt;/sub&gt;, G&lt;sub&gt;b&lt;/sub&gt;</td>
<td>55</td>
</tr>
<tr>
<td>Kv1.4</td>
<td>KCNA4</td>
<td>11p14</td>
<td>ATII (ap)</td>
<td>R</td>
<td>11</td>
</tr>
<tr>
<td>Kv1.5</td>
<td>KCNA5</td>
<td>12p13</td>
<td>Lung</td>
<td>R</td>
<td>11</td>
</tr>
<tr>
<td>Kv1.7</td>
<td>KCNAB2</td>
<td>8q</td>
<td>ATII</td>
<td>R&lt;sub&gt;a&lt;/sub&gt;</td>
<td>55</td>
</tr>
<tr>
<td>Kv2.2</td>
<td>KCNAB1</td>
<td>Xp11.23</td>
<td>ATII</td>
<td>R&lt;sub&gt;a&lt;/sub&gt;</td>
<td>55</td>
</tr>
<tr>
<td>Kv4.1</td>
<td>KCND1</td>
<td>7q31</td>
<td>ATII (ap)</td>
<td>R&lt;sub&gt;a&lt;/sub&gt;, P&lt;sub&gt;a&lt;/sub&gt;, G&lt;sub&gt;b&lt;/sub&gt;</td>
<td>55</td>
</tr>
<tr>
<td>Kv4.2</td>
<td>KCND2</td>
<td>1p13.3</td>
<td>ATII (ap)</td>
<td>R&lt;sub&gt;a&lt;/sub&gt;, P&lt;sub&gt;a&lt;/sub&gt;, G&lt;sub&gt;b&lt;/sub&gt;</td>
<td>55</td>
</tr>
<tr>
<td>Kv6.1</td>
<td>KCNQ1</td>
<td>20q13</td>
<td>Lung</td>
<td>R</td>
<td>11</td>
</tr>
<tr>
<td>KvLQT1 (Kv7.1)</td>
<td>KCNQ1</td>
<td>11p15.5</td>
<td>Nasal (wt/CF)</td>
<td>R&lt;sub&gt;a&lt;/sub&gt;, P&lt;sub&gt;a&lt;/sub&gt;, G&lt;sub&gt;b&lt;/sub&gt;</td>
<td>49, 50</td>
</tr>
</tbody>
</table>

Molecular identity of voltage-dependent and calcium-activated 6-transmembrane domain (TMD) K<sup>+</sup> channels expressed in respiratory epithelia. The names of the channels and genes as well as their chromosomal localization (locus) and cell types expressing the channels are indicated. The experimental approaches to mRNA (R), protein (P) and/or functional (F) evidence are also reported: PCR (a), in situ hybridization (b), Northern blotting (c), immunoblotting (d), immunohistochemistry (e), immunofluorescence (f), patch-clamp (g), Ussing (h). mb, Membrane; ap, apical membrane; bas, basolateral membrane; lat, lateral membranes; ciliated term bronchioles, ciliated cells from the terminal bronchioles; resp. bronchioles, respiratory bronchioles; wt and CF, normal and cystic fibrosis cells, respectively; ATII, alveolar type II cells; Kv, voltage-gated K<sup>+</sup> channels; BK Ca, large-conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels; MiRP, MinK-related protein; HBE, human bronchial epithelial.
Kv channels constitute a large family, and several homologs have been reported in lung epithelial cells (Table 1). In addition to the KvLQT1-Kv7.1 channel, KCNQ3 (Kv7.3) and KCNQ5 (Kv7.5) proteins have been detected at the apical membrane of Calu-3 cells (53). Kv7.2 and Kv7.5 have been identified in human bronchial 16HBE14o cells (53). Transcripts of other Kv channel subtypes, i.e., Kv1.1, -1.3, -1.4, -2.2, -4.2, -4.3, and -9.3, have been found in ATII cells (42). Among them, Kv1.1, -1.3, -1.4, -4.2, and -4.3 have been detected at the protein level and localized at the apical membrane (42, 55, 56). Finally, other types (Kv1.5, -1.7, and -6.1) have been measured at the apical membrane of 16HBE cells (20). Transcripts of other 2-TMD, inward-rectified K channels, such as Kir2.1, Kir2.2, Kir3.1, and Kir3.2, have been detected in the respiratory epithelia (15, 61). The most frequently observed was the low-threshold (n-type) channel resembling delayed-rectifier K channels. This 12-pS K+ channel, sensitive to 4-aminopyridine, is activated at −40 to −20 mV membrane potential. The second type, the l-type or high-threshold type, is activated at more positive potentials (−20 or −10 mV). It had to be noted that these currents represent a combination of the Kv channel activities associated to α-subunits mentioned above.

6-TMD, KCa channels. KCa channels are composed of two subfamilies, Slo and SK channels. SK4, also known as the Gardos channel in red blood cells, IKca or, more recently, KCa3.1, was cloned in 1997 (33). In the lungs (Table 1), KCa3.1 has been detected by immunohistochemistry in the trachea (at the basolateral membrane), in bronchi, and bronchioles, but not in alveoli (80). Nevertheless, we found KCa3.1 mRNA and protein in primary cultures of ATII cells (44). We also demonstrated the presence of KCa3.1 in non-CF (NuLi) and CF (CuFi) bronchial cells, although in the latter one, KCa3.1 protein expression and current were reduced compared with NuLi (82). KCa3.1 was also detected in the bronchoalveolar cell line A549 (78), in non-CF and CF nasal polyps (49), 16HBE14o- (2) and Calu-3 cells (12). KCa3.1, encoding for intermediate-conductance (~16 pS) KCa channels, sensitive to clotrimazole, charybdotoxin, and TRAM34, is activated by 1-ethyl-2-benzimidazolinone (1-EBIO) and its derivatives. Functional evidence of KCa3.1 channels comes from short-circuit current experiments in Ussing chamber with CF and non-CF nasal cells (49), 16HBE14o- (2), Calu-3 (12), NHBE (20), NuLi, and CuFi cells (82) and whole cell patch-clamp experiments (78). Commonly located at the basolateral membrane of pulmonary epithelia, KCa3.1 currents have also been measured at the apical membrane of bronchial 16HBE14o- (2) and NuLi cells (37). Transcripts of another SK family member, SK1, have been detected in 16HBE14o- (2) and NuLi cells (37).

Table 2. Identification of 2- and 4-TMD K+ channels in airways and alveolar epithelium

<table>
<thead>
<tr>
<th>Name</th>
<th>Gene</th>
<th>Locus</th>
<th>Cell Type</th>
<th>Evidence</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kir2.1</td>
<td>KCNJ2</td>
<td>17q23-1q24.2</td>
<td>Fetal ATII</td>
<td>R, F</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RERFLC-MA</td>
<td>R</td>
<td>70</td>
</tr>
<tr>
<td>Kir3.1</td>
<td>GIRQ1</td>
<td>2q24.1</td>
<td>WBA, H69, H146, A549, H727, H2170, H226, H520</td>
<td>R, P</td>
<td>64</td>
</tr>
<tr>
<td>Kir3.2</td>
<td>GIRQ2</td>
<td>2q22.13-2q22.2</td>
<td>WBA, H69, H209, H526, A549, H727, H530</td>
<td>R</td>
<td>64</td>
</tr>
<tr>
<td>Kir3.3</td>
<td>GIRQ3</td>
<td>1q21-2q23</td>
<td>SAEC, WBA, H69, H146, H187, H209, H526, A549, H727, H2170, H226, H520</td>
<td>R</td>
<td>64</td>
</tr>
<tr>
<td>Kir3.4</td>
<td>GIRQ4</td>
<td>11q24</td>
<td>SAEC, WBA, H69, H146, H187, H209, H526, A549, H727, H2170, H226, H520</td>
<td>R</td>
<td>64</td>
</tr>
<tr>
<td>Kir4.2</td>
<td>GIRQ5</td>
<td>21q22.2</td>
<td>NuLi</td>
<td>R</td>
<td>*</td>
</tr>
<tr>
<td>Kir6.1</td>
<td>KCNJ8</td>
<td>12p11.23</td>
<td>Lung</td>
<td>F</td>
<td>71</td>
</tr>
<tr>
<td>Kir7.1</td>
<td>KCNJ13</td>
<td>2q37</td>
<td>Lung</td>
<td>R</td>
<td>18</td>
</tr>
</tbody>
</table>

**Four-TMD, 2 pore K+ channels**

| Task 1  | KCNK1    | 1q42.4-q43  | H441      | R, F      | 25       |
|         |          |             | Calu3     | R         | 14       |
| Task 2  | KCNK6    | 19q13.1     | H441      | R, F      | 25       |
|         |          |             | Calu3 (ap. mb) | R         | 14       |
| Task 3  | KCNK9    | 8q24.3      | H441      | R, F      | 25       |
| Task 4  | KCNK17   | 6p21.2-p21.1 | H441     | R, F      | 25       |
|         |          |             | Calu3 (vesicles) | R         | 14       |
| Thik 1  | KCNK13   | 14q31-2q32  | H441      | R, F      | 25       |
|         |          |             | H441      | R, F      | 25       |

Molecular identity of 2-pore, 4-TMD and inward-rectified, 2-TMD channels. *Unpublished results.*
investigated in Calu-3 cells: Twik-2 and Trek-1 have been detected at the plasma membrane level, mostly at the apical membrane, whereas Task-2 seems to be localized in intracellular vesicles beneath the apical membrane (14). Agents such as bupivicaine, lidocaine, and quinidine have highlighted the presence of functional K2P currents through the apical membrane of Calu-3 monolayers.

Two-TMD, Kir. Transcripts of many two-TMD Kir have been found in the lungs (Table 2): Kir2.1 [in fetal ATII cells (52) and in the small cell lung cancer cell line RERF-LC-MA (70)], Kir3.1, -3.2, -3.3, and -3.4 [also called GIRK1, -2, -3, and -4, expressed in cancer cell lines, including A549 cells (64)], Kir4.2 (in NuLi cells, unpublished data), Kir6.1 [in ATII (43, 81), NuLi, CuFi cells (82)], and Kir7.1 [in total lung extracts (18)]. Functional evidence of two of them, Kir6.1 and Kir2.1, has been documented. An IRK1 (Kir2.1), inward-rectifying current, with 31-pS unitary conductance, has been observed in fetal alveolar cells (52). In adult alveolar cells, we identified a KATP channel, formed from the inwardly rectifying, pore-forming subunit Kir6.1 and sulfonylurea receptor SUR2B subunits (43). This channel, sensitive to ATP, is activated by pinacidil and inhibited by glibenclamide. Proof of functional KATP channels at the basolateral membrane of bronchial (NuLi, CuFi) (82) and alveolar cells (43) has been generated in Ussing chamber short-circuit current experiments. However, we noted that KATP currents and Kir6.1 protein expression are reduced in CuFi compared with NuLi cells (82).

Thus >30 different pore-forming α-subunits, plus several regulatory β-subunits, have been detected in the respiratory epithelium. This huge molecular diversity is intriguing. Unfortunately, the large number of K+ channels, expressed in the same cell, and often at the same membrane of polarized epithelium, complicates the task of precisely establishing the cellular function of each K+ channel type. In following sections, we will analyze what is currently known about their function in lung physiology and physiopathology.

**K+ Channel Function in Lung Epithelial Physiology**

Gas exchange and alveolar stability. Lung ability to rapidly adapt to environmental changes in Po2 is crucial for survival. Neuroepithelial bodies (NEB) localized in the airway epithelium, arterial carotid bodies, and pulmonary smooth muscle cells are the three main Po2-sensing systems (35, 36). These cells express several ion channels, particularly K+ channels that are sensitive to O2 levels. Indeed, the two-pore channel Task-3 and the voltage-dependent channel Kv3.3, expressed in native NEB and the immortalized model of NEB (the H146 cell...
line), have been shown to be inhibited after acute hypoxia (35, 36, 87). Other types of Kv channels, e.g., Kv1.5 and Kv2.1, could be responsible for O$_2$-sensitive currents in vascular smooth muscle cells (VSMC) (for review, see Ref. 46). Kv1.1, Kv4.3, and Kv9.3 channels, which are expressed in alveolar cells (55), are also regulated by O$_2$ (63). Moreover, it has been shown that Kv1.1, Kv4.3, and Kv9.3 mRNA expression is decreased after chronic hypoxia in VSMC (63). Inhibition of K$^+$ channels seems to be a key element of O$_2$ detection and a crucial determinant of the cellular response to hypoxia. Indeed, membrane depolarization, due to hypoxic K$^+$ channel inhibition, is thought to induce Ca$^{2+}$ influx through voltage-gated Ca$^{2+}$ channels with subsequent transmitter release by NEB cells (Fig. 3) (for review, see Refs. 35 and 46).

A third K$^+$ channel class, i.e., Ca$_{2+}$-activated channels, could also act as an O$_2$ sensor. Indeed, the open time of the BK$_{Ca}$ channel is severely reduced after Po$_2$ decreases in alveolar AS49 cells (34), whereas chronic hypoxia has no effect on channel expression. Another study reported two types of K$^+$ currents activated by reoxygenation after hypoxia in this cell line; the first is a delayed-rectifier type, and the second, a slow-inactivated type (39).

The nature of the O$_2$ sensor and the mechanisms whereby changes in O$_2$ regulate K$^+$ channel activity remain uncertain. Because BK$_{Ca}$ channels expressed in HEK293 are still inhibited by hypoxia after patch-excision, it could be postulated that a soluble intracellular component was not required (45). Although intrinsic O$_2$ sensitivity of the pore-forming subunit cannot be excluded, the mechanism of K$^+$ channel inhibition is most probably dependent on O$_2$-sensitive accessory proteins. It has been consistently shown that the Kv$\beta$1.2 subunit confers oxygen sensitivity to the Kv4.2 channel expressed in HEK293 cells (62). Several studies have also proposed the existence of a redox model of O$_2$ sensing that involves reactive oxygen species produced by NADPH oxidase or mitochondria (Fig. 3) (for review, see Refs. 35 and 46).

Thus the K$^+$ channels of O$_2$-sensing systems, e.g., NEB and pulmonary smooth muscle cells, are key elements of the adaptive function of the lungs in response to environmental changes in O$_2$ levels. It has been also proposed that rapid detection of O$_2$ variation by alveolar K$^+$ channels and subsequent modulation in K$^+$ channel activity could result in changes in ion transport and fluid clearance through alveolar epithelia (34, 55, 56). Finally, it has been postulated that alteration of O$_2$ sensing by K$^+$ channels could participate in pathological conditions, including pulmonary hypertension (46, 63).

Lung surfactant, synthesized and secreted by ATII cells, reduces surface tension in alveoli, which prevents alveolar collapse during expiration, and plays a protective role against infection. Several signaling pathways are involved in the control of surfactant secretion via exocytosis of lamellar bodies by ATII cells (for review, see Ref. 1). Many studies have highlighted the crucial role of Ca$^{2+}$ signals in that process (1, 24). Furthermore, it has been shown that Ca$^{2+}$ uptake in lamellar bodies is dependent on the following two mechanisms: pH gradient and K$^+$ transport. Indeed, Ca$^{2+}$ uptake in lamellar bodies is greater in the presence of K$^+$, and this K$^+$-dependent Ca$^{2+}$ uptake is prevented by the K$^+$ channel inhibitors TEA and 4-aminopyridine (86). K$^+$ transport could thus participate in the control of surfactant secretion, through Ca$^{2+}$ signal modulation. Considering the lack of extensive studies on the topic, this hypothesis needs to be verified.

Epithelial repair after injury. The respiratory epithelium is continuously exposed to dust and pathogenic agents. The first line of defense against these aggressions is provided by the mucociliary clearance of particles and pathogens trapped in mucus, combined with the action of antimicrobial/immune molecules. In spite of these efficient defenses, bacterial infections and inflammatory responses can develop, leading to epithelial injury (Fig. 4A). Indeed, chronic inflammatory diseases, such as CF or asthma, for example, are characterized by tissue damage and remodeling. After injury, epithelial repair is crucial to restore respiratory functions and barrier integrity against bacterial infections. Several processes are sequentially engaged in pulmonary epithelial regeneration (Fig. 4A): 1) dedifferentiation, spreading, and migration of healthy cells, 2) cell proliferation, and 3) redifferentiation (3, 22, 69). As seen in Fig. 4B, similar processes are observed in our mechanical wound-healing model of alveolar cell monolay-

AJP-Lung Cell Mol Physiol • VOL 296 • FEBRUARY 2009 • www.ajplung.org

![Diagram](http://example.com/diagram.png)

**Fig. 3.** Schematic model of O$_2$ sensing and cellular responses to hypoxia in lung neuroepithelial body (NEB) cells. Postulated cellular responses to hypoxia (1) in NEB of the lungs are presented. Various O$_2$ sensors (2) have been proposed, including NADPH-dependent and -independent mechanisms, coupled with K$^+$ channel activity (3). Hypoxic inhibition of voltage-gated K$^+$ (Kv), TASK, or Ca$_{2+}$-activated K$^+$ (BK$^{Ca}$) channels (3) then leads to, sequentially, membrane depolarization (4), activation of voltage-gated Ca$_{2+}$ channels (5), Ca$^{2+}$ influx (6), and neurotransmitter release (7).
ers (81). Several external mediators and intracellular signals control repair processes of the respiratory epithelium. Growth factors, such as epidermal growth factor (EGF), hepatocyte growth factor, transforming growth factor-β, and keratinocyte growth factor, through their respective receptors, induce mitogenic, motogenic, and morphogenic responses that are crucial for epithelial repair. Because K⁺ channel activity has been shown to modulate proliferation and migration of various cell types (9, 13, 29, 38, 48, 59, 60, 67, 88, 94), we recently hypothesized that K⁺ channels could also be involved in respiratory epithelia repair. We then demonstrated that alveolar and bronchial repair processes are highly dependent on K⁺ channel activity (81, 82) (Fig. 4C). More precisely, K<sub>ATP</sub> or KvLQT1 inhibition reduces alveolar EGF-stimulated wound healing, cell migration, and proliferation. Conversely, alveolar wound healing is stimulated by pinacidil, a K<sub>ATP</sub> channel activator, which also increases cell migration (81). In NuLi bronchial epithelia, K<sub>ATP</sub>, KvLQT1, and KCa3.1 inhibitors elicit a significant and additive inhibitory effect on wound healing, cell migration, and proliferation, under EGF-stimulated conditions. Furthermore, our results suggest that the delay in wound healing observed in CF bronchial cell monolayers (CuFi) could be the result of defective EGF receptor signaling coupled with reduced K⁺ channel function and expression (82).

The mechanisms that link K⁺ channel activity to cell migration and proliferation processes are not clearly defined. However, the role of KCa3.1 channels in kidney cell migration has been studied extensively, and it has been established that a rise in intracellular Ca<sup>2+</sup> and massive K⁺ efflux through KCa3.1 channels, causing cell shrinkage, induces retraction of the rear part of migrating cells (72, 73). In addition, changes in K⁺ channel activity have been postulated to control cell proliferation by altering membrane potential, intracellular Ca<sup>2+</sup>, and cell volume as well as growth factor-mediated mitogenic signals (90). Indeed, coupling between growth factor signaling and K⁺ channel function has been reported in multiple cell types. The open probability of 4-aminopyridine-sensitive Kv channels, for example, is increased by EGF through protein

---

**Fig. 4. Repair mechanisms of pulmonary epithelia.**

**A:** After injury (step 1), several mechanisms are sequentially engaged in airway and alveolar epithelial repair: spreading, dedifferentiation (step 3); and migration (step 4) of healthy epithelial cells, followed by proliferation and redifferentiation of stem cells (steps 5 and 6). **B:** In vitro model of mechanical wound injury (81, 82). Images of alveolar monolayers during repair are presented. Cells in the wound are undifferentiated (absence of blue, phosphatase alkaline staining), spread, and lamellipodia stretch out to connect the wound edges. Some cells, with 2 nuclei, are in division. **C:** Schematic model of epidermal growth factor (EGF)- and K⁺ channel-dependent pulmonary epithelial repair based on our in vitro results (81, 82). In this model, EGF, released by injured/repairing alveolar or bronchial epithelial cells (step 1), activates EGF receptor (EGFR; step 2), leading to the stimulation of K<sub>ATP</sub> and KvLQT1 currents (step 3) through the basolateral membranes of bronchial and alveolar monolayers. Chronic treatments with EGF also increase K<sub>ATP</sub> and KvLQT1 expression in alveolar cells (step 4). K⁺ channel inhibition prevents, in large part, EGF-stimulated cell migration (step 5) and proliferation (step 6). K⁺ channel activity, coupled with EGF/EGFR signaling, is then a crucial component of cell migration and proliferation processes favoring epithelial repair. Direct stimulation of cell migration and proliferation by other EGF signaling pathways could also be involved (step 7).
kinase A-dependent phosphorylation in myeloblastic cells (88). EGF also stimulates KCa channels in mucous airway cells (28). We consistently observed a stimulation of alveolar and bronchial K⁺ channels after exposure to EGF (81, 82).

Thus K⁺ channel activity controls cell proliferation, migration, and, subsequently, repair mechanisms of the respiratory epithelia. K⁺ channel activation could then represent a promising strategy to enhance epithelial repair after injury. On the other hand, K⁺ efflux, after K⁺ channel activation, has been identified as one of the first steps of cell apoptosis, especially in tumor cells (90). Activation of the caspases and endonucleases could be a consequence of cell shrinkage and intracellular K⁺ depletion after K⁺ efflux and/or to the secondary activation of proapoptotic molecules (90). Although the role of K⁺ channels in lung epithelial cell apoptosis has not been studied extensively, it could be hypothesized that these channels play a very complex role in the balance between cell migration, proliferation, and apoptosis during lung epithelial regeneration after injury.

**Inflammatory responses.** The inflammatory responses occurring in several lung pathologies could be involved in both injury and repair processes of the pulmonary epithelium. In addition, inflammation could regulate K⁺ channel function and expression. The impact of inflammation on K⁺ channels has been studied in several other tissues. It has been reported, for example, that inflammation stimulates K_ATP channels (30) and decreases BKCa open probability (47) in smooth muscle cells. The proinflammatory cytokine tumor necrosis factor (TNF) also exerts a complex action on K⁺ channels by upregulating some of them (21, 85, 93) and downregulating others (66, 85). In the airways, bronchial KCa3.1 currents have been shown to be stimulated by interleukin (IL)-13 (20). Moreover, our experiments have disclosed that TNF treatment enhances KvLQT1 protein in bronchial cells (unpublished data). In inflammatory pathologies, such as CF, modulation of K⁺ channel expression and function by inflammatory molecules could interfere with ion and fluid transport (see section below) as well as repair processes of the injured epithelium (see section above).

Some studies have also evaluated the impact of K⁺ channel modulation on inflammatory responses. On the one hand, nicorandil, a K_ATP opener used as a vasodilator, reduces TNF release by lymphocytes (92). On the other hand, K_ATP channel inhibition decreases neutrophil infiltration as well as TNF and IL-6 levels in the lungs and intestine in an ischemic-reperfusion model (65). It has also been shown that lipopolysaccharide (LPS) and TNF both enhance Kv1.3 currents and expression in macrophages (85). Moreover, LPS- and TNF-induced macrophage activation as well as macrophage proliferation are reduced after inhibition of Kv1.3 channels (85). In addition, inhibition of K⁺ channels by quinine prevents LPS-induced TNF expression and release by alveolar macrophages (51). Thus K⁺ channels could participate in complex inflammatory responses in lungs by regulating immune cell functions.

**Control of ionic and liquid transepithelial transport.** The ion and fluid transport functions of the pulmonary epithelium are essential to lung physiology in all stages of life. Indeed, Cl⁻ and liquid secretion is crucial to fetal lung development (57), whereas Na⁺ absorption favors alveolar liquid clearance at birth. This phenomenon is also essential for the resolution of pulmonary edema(4). In the airways, equilibrium between Cl⁻ secretion and Na⁺ absorption is necessary to maintain adequate periciliary volume crucial for ciliary function (5, 6). In CF patients, reduced Cl⁻ secretion and Na⁺ hyperabsorption lead to inefficient mucociliary clearance and mucus accumulation (CF; Fig. 1A) that favor chronic infections and inflammation (8). Adequate control of Na⁺ absorption, Cl⁻ secretion, and liquid transport is thus necessary in normal lung function as well as for the resolution of pathologies characterized by ion transport abnormalities. As detailed below, K⁺ channels, by controlling membrane potential and creating an electrochemical gradient for Na⁺ and Cl⁻ transport, are thus key elements in the control of liquid volume and composition.

**K⁺ channels and Cl⁻ secretion in the airways.** In the airways, two main K⁺ channel types, namely, KvLQT1 and KCa3.1 channels, have been associated with the control of Cl⁻ secretion (Fig. 1A). Indeed, pharmacological inhibition of KvLQT1 and KCa3.1 channels strongly reduces Cl⁻ transport in nasal, tracheal, and bronchial cells (2, 12, 17, 23, 49, 50). Conversely, it has been shown that KCa3.1 activation, by compounds such as 1-EBIO (49), DC-EBIO (75), 4-chlorobenzof[1]isoquinoline (CBIQ) (77), and chlorzoxazone (74), for example, stimulates Cl⁻ secretion through CFTR and/or Ca²⁺-activated Cl⁻ channels (CaCC) in non-CF airflow cells. In nasal cells from CF patients with ΔF508 mutation, no Cl⁻ secretory response is detected in the presence of chlorzoxazone (74). Another study revealed that 1-EBIO increases UTP-activated Cl⁻ secretion, whereas it has no effect on cAMP-induced Cl⁻ secretion, indicating that KCa3.1 activation could enhance Cl⁻ transport through alternative CaCC in the absence of CFTR in nasal CF cells (49). In primary cultures of human bronchial cells from ΔF508 patients, 1-EBIO failed to stimulate Cl⁻ secretion at 37°C. However, this compound significantly enhanced Cl⁻ currents in cells cultured at 26°C, which increased ΔF508 CFTR processing at the membrane (17). Since then, KCa3.1 channel activation has been proposed as a promising strategy to favor Cl⁻ transport in CF tissues through residual or partially rescued CFTR as well as alternative Cl⁻ channels (CF; Fig. 1A).

**K⁺ channels and Na⁺ absorption.** As illustrated in our schematic model of alveolar absorption (Fig. 1B), Na⁺ absorption depends on passive Na⁺ entry through apical Na⁺ channels (cyclic nucleotide gated, highly selective channel, and/or nonselective channel) and active exit by basolateral Na⁺-K⁺-ATPase, whereas K⁺ are recycled by basolateral K⁺ channels, in agreement with the Ussing model (84). In ATI cells, K⁺ currents have been detected, but the exact molecular identity of these K⁺ channels is still unknown (ATI; Fig. 1B) (31, 32). In ATII cells, we have identified three types of K⁺ channels, i.e., KvLQT1, K_ATP, and KCa3.1 channels, at the basolateral membrane primary culture. These channels play a prominent role in Cl⁻ and Na⁺ alveolar transport (43). Interestingly, we also observed that long-term modulation of K⁺ channels (24-h treatments with KvLQT1 or K_ATP inhibitors or activators) regulates not only Na⁺ and Cl⁻ transport but also controls ENaC and CFTR expression as well as liquid absorption through ATII monolayers (44). These results are in agreement with a study by Sakuma et al. (71) demonstrating that K_ATP activation increases alveolar clearance in the resected human lung. In Na⁺-absorbing H441 human airway epithelial cells, for example, another class of K⁺ channels located at the
basolateral membrane, i.e., K, P K+ channels, has been proposed to maintain the driving force for Na+ absorption (25).

Emerging role of apical K+ channels in transepithelial transport. Although it has been assumed that K+ channels, such as KVLQ1 and KCa3.1, controlling the electrochemical gradient for Na+ and Cl− transport are located at the basolateral membrane, apical localization of these channels in bronchial cells was reported recently (2, 53). Furthermore, XE-991, an inhibitor of KCNQ channels, inhibited the short-circuit currents through Calu-3 monolayers measured in basal conditions, indicating that these channels participate in the basal Cl− secretion (53). The same group also identified K, P K+ channels at the apical membrane of Calu-3 cells. These channels may participate in K+ exit/recycling across the apical membrane and could help transepithelial anion secretion (14). Finally, many Kv channels have also been detected at the apical membrane in ATII cells (42, 55). It has been proposed that they could be involved in K+ secretion.

Conclusion. In summary, it has been clearly established that K+ channels play a central role in the respiratory epithelium by participating in the control of alveolar and airway surface liquid composition and volume. Other functions, including O2 sensing and epithelial repair, have been highlighted. However, among >30 different types of K+ channels detected in alveolar and airway epithelial cells, the physiological roles of many of them remain unknown and deserve future study.

ACKNOWLEDGMENTS

We acknowledge the editorial assistance of Ovid Da Silva, Research Support Office, Centre de Recherche, Centre Hospitalier de l’Université de Montréal (CRCHUM).

GRANTS

This work was supported by the Cystic fibrosis Foundation, Natural Sciences and Engineering Research Council of Canada, and the CRCHUM foundation.

REFERENCES

Review

K⁺ CHANNEL FUNCTION IN EPITHELIAL CELLS


and KCNN4 potassium channels in Calu-3 human airway epithelial cells. 


