Published in final edited form as:

Am J Respir Cell Mol Biol. 2003 March ; 28(3): 363–372.

Large Conductance Ca^{2+} -Activated K^+ Channels Sense Acute Changes in Oxygen Tension in Alveolar Epithelial Cells

Sofija Jovanović, Russell M. Crawford, Harri J. Ranki, and Aleksandar Jovanović

Tayside Institute of Child Health, Ninewells Hospital & Medical School, University of Dundee, Scotland, United Kingdom

Abstract

The rise in alveolar oxygen tension (PO_2) that occurs as the newborn infant takes its first breaths induces removal of liquid from the lung lumen due to ion transport across the alveolar epithelium and the activity of alveolar Na^+ channel (ENaC). In the present study, we have aimed to identify an ion conductance in alveolar epithelial A549 cells that responds to acute changes in PO_2 . Variation in PO_2 did not affect single-channel ENaC activity. However, in these cells we have detected single-channel conductance having properties similar to those of large conductance Ca^{2+} -activated K^+ (BK_{Ca}) channels. Reverse transcriptase–polymerase chain reaction and Western blotting demonstrated presence of $\alpha\text{-BKCa}$ channel subunit and iberiotoxin, a blocker of BK_{Ca} channels, inhibited whole cell K^+ current. Chronic changes in PO_2 did not affect expression, recruitment, or function of BK_{Ca} channels in A549 cells. In contrast, acute changes of PO_2 regulated the BK_{Ca} channel activity by controlling the channel mean open time. This effect of PO_2 was insensitive to inhibitor of flavoproteins, diphenylene iodonium. In addition, decrease in PO_2 and iberiotoxin induced membrane depolarization and Ca^{2+} oscillations in A549 cells. We conclude that BK_{Ca} channels serve as oxygen sensors in human alveolar A549 epithelial cells.

The rise in alveolar PO_2 that occurs as the newborn infant takes its first breaths induces removal of liquid from the lung lumen, which is vital for alveolar gas exchange and newborn survival during the first few minutes after birth (1). It is generally accepted that removal of liquid from the lungs is driven by ion transport across the alveolar epithelium and that the activity of alveolar Na^+ channel (ENaC) controls the lung liquid clearance (2,3). It has been demonstrated that gene expression of ENaC and some other channels and transports in alveolar epithelium is regulated by chronic changes in oxygen tension (4). However, no evidence has been provided so far to suggest that the activity of ENaC may be changed promptly in response to changes in environmental O_2 .

Up to now, no ion conductance was identified in alveolar epithelium that serves as sensor for acute changes in oxygen tension. On the other hand, it has been shown that acute modulation of ion channel activity by oxygen is crucial to the mechanisms underlying chemosensing in carotid body, excitability of nervous system, neuroepithelial body, and vascular smooth muscle (5). Crucial to these adaptive physiologic and cellular responses is the acute inhibition of K^+ channels by hypoxia (6). Although oxygen-sensitive tissue express different channel subtypes, oxygen-mediated regulation of BK_{Ca} is suggested to be central to the cellular mechanism of oxygen sensing in many tissues (7).

In the present study, we have aimed to identify ion channels in alveolar epithelium that respond to acute changes in oxygen tension. We report that ENaC is not regulated by fast changes in oxygen tension, whereas BK_{Ca} channel, an ion conductance we found to be present and not

regulated by chronic changes in environmental oxygen in human A549 alveolar epithelial cells, promptly responds to changes in oxygen tension by changes in probability of the channel opening. The regulatory effect of oxygen seems to be direct and in a channel-specific ligand-dependent manner. This is the first report describing the channel in alveolar epithelium capable of sensing fast changes in oxygen which, by virtue of this ability, may transduce changes in environmental oxygen tension into changes in fluid clearance in lungs.

Materials and Methods

A549 cells

A549 cells (American Type Culture Collection, Teddington, UK) were cultured in a tissue flask (at 5% CO₂ and 21% O₂ or at 5% CO₂ and 3% O₂ where appropriate) containing Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 2 mM glutamine.

Patch Clamp Electrophysiology

To monitor on-line behavior of single channel molecules, the gigohm seal patch-clamp technique was applied in cell-attached (for ENaC; 8) or the inside-out configuration (for BKCa channels; 7). For cell-attached recordings cells were superfused with (in mM) 140 NaCl, 4.5 KCl, 2.5 CaCl₂, 1 MgCl₂, 10 HEPES, and 5 D-glucose (pH 7.35); the pipette solution contained (in mM) 140 LiCl, 3 MgCl₂, and 10 HEPES (pH 7.35) (9). The conductance of single channel extracted using these solutions was ~ 10 pS and was sensitive to 30 μM of amiloride, confirming that the observed conductance was ENaC (8,9). For inside-out recordings, cells were superfused with the following solution (in mM): NaCl 10, KCl 117, MgCl₂ 2, and HEPES 11 (pH 7.2), with Ca²⁺ buffered 1 μM using EGTA and CaCl₂ in appropriate ratios. Fire-polished pipettes, coated with Sylgard (resistance 7–15 MΩ), were filled with (in mM): NaCl 135, KCl 5, MgCl₂ 1.2, HEPES 5, CaCl₂ 2.5, and D-glucose 10 (pH = 7.4). In some experiments, 112 mM KCl was replaced with 112 mM NaCl in bathing solution, whereas in some other experiments free Ca²⁺ concentration in the bathing solution was decreased from 1 μM to 3 nM. Single-channel activity was monitored on-line and stored on a PC. Data were reproduced, low-pass filtered at 1 KHz (–3 dB), sampled at 100 μs rate, and further analyzed using the pClamp8 software (Axon Instruments, Inc., Foster City, CA). Channel activity, assayed by digitizing segments of current recordings and forming histograms of baseline and open level data points, were expressed as NP_o (N, number of channels in the patch; P_o, probability of each channel to be open). Amplitude, open-, and closed-dwell time histogram was constructed and fitted by the sum of 1–2 exponents (10).

For whole-cell electrophysiology (11), cells were superfused with Tyrode solution (in mM: 136.5 NaCl; 5.4 KCl; 1.8 CaCl₂; 0.53 MgCl₂; 5.5 glucose; 5.5 HEPES-NaOH; pH 7.4). Pipettes (resistance 3–5 MΩ), were filled with (in mM): KCl 140, EGTA 5, CaCl₂ 1.67, MgCl₂ 1, HEPES-KOH 10 (pCa 7.0 at pH 7.4), and ATP 2 (pH 7.4). To measure whole cell membrane currents, the membrane potential was normally held at –40 mV and the currents evoked by a series of 400 ms depolarizing and hyperpolarizing current steps (–100 mV to +80 mV in 20-mV steps) recorded directly to hard disk using an Axopatch-200B amplifier, Digidata-1321 interface, and pClamp8 software (Axon Instruments, Inc., Foster City, CA). The capacitance compensation was adjusted to null the additional whole-cell capacitive current. The slow capacitance component measured by this procedure was used as an approximation of the cell surface area, and allowed normalization of current amplitude (i.e., current density). Currents were low-pass filtered and digitized at 5 kHz. For measurement of the membrane potential, whole cell patch clamp method was used in current clamp mode (12).

Measurement of RNA Levels

To determine whether transcription of BK_{Ca} channels is affected by chronic changes in oxygen tension, we have measured mRNA of BK_{Ca} channel α -pore-forming subunit using reverse transcriptase–polymerase chain reaction (RT-PCR) (13,14). Total RNA was isolated using a commercial kit (RNeasy, Mini Kit; Qiagen, Crawley, UK) according to the manufacturer's instructions. First-strand cDNA was synthesized with random hexanucleotides from 1 mg of total RNA using Reverse Transcription System kit (Promega, Southampton, UK). PCR reactions were done using ReadyMix Red Tag (Sigma, Dorset, UK) in a thermal cycler Model Phoenix (Helena Biosciences, Sunderland, UK) under the following conditions: denaturation at 94°C for 0.5 min, annealing 0.5 min at 55°C, 0.5 min primer extension at 72°C for 28 cycles. For the 266 base-long product for human BK_{Ca} α -subunit the primers had the following sequences: sense, 5'-CAGTATCACAACAAGGCCCATCTG-3'; antisense, 5'-AAGGACAGACCCACGAAGGCA-3'. The loading of RNA was checked by human GAPDH-primers: sense, 5'-CATCACCATCTTCCAGGAGCGA-3'; antisense, 5'-GTCTTCTGGGTGGCAGTGATGG-3', the size of GAPDH-product was 341 bp; PCR conditions were as above just 22 cycles instead of 28 were used. The nature of PCR product was confirmed by DNA sequencing. The PCR product band intensities were analyzed using the Quantiscan software (15).

Western Blotting Analysis

A549 cells were snap-frozen and ground to a powder under liquid nitrogen (16). The powder was resuspended in 10 ml of buffer (20 mM Hepes, 150 mM NaCl, Triton-X 100 [1%], pH 7.5) and homogenized. Protein concentration was determined using the method of Bradford. A quantity of 10 μ g of the anti- α -BK_{Ca} antibody (Alomone, Munich, Germany) was pre-bound to Protein-G Sepharose beads and used to immunoprecipitate from 50 μ g of protein extract. The pellets of this precipitation were run on SDS polyacrylamide gels for Western analysis. Western blot probing was performed using 1/200 and 1/300 dilutions of antibody, and detection was achieved using Protein-G horseradish peroxidase and enhanced chemiluminescence reagents. The band intensities were analyzed using the Quantiscan software (17).

Ca²⁺ Imaging

A549 cells were loaded with the Ca²⁺-sensitive fluorescent probe Fluo-3AM (Molecular Probes, Eugene, OR), and Ca²⁺ levels were imaged with a Zeiss LSM-510 (Zeiss, Gottingen, Germany) laser-scanning confocal microscope using the Ar/Kr or Ar/UV laser to “excite” dye at 488 nm, as we have previously described (18). Emission light by photomultiplying tubes was detected at 520 nm (19).

Control of Partial Oxygen Tension in Environment Surrounding A549 Cells

A549 cells were normally cultured in incubator at partial oxygen pressure (PO₂) 144 mm Hg. When the effect of chronic changes in oxygen tension was elucidated, the cells were cultured for 24 h in PO₂ = 23 mm Hg. Under control conditions, solutions used for patch-clamp recordings always had PO₂ of 140 mm Hg (due to equilibrium with atmospheric O₂). PO₂ was decreased in environment surrounding A549 cells by continuous bubbling bathing solutions with 100% argon with simultaneous prevention of exchange of O₂ between solution in the chamber and air by nitrogen jet. The PO₂ under these conditions was 20 mmHg (*see Refs.* 11,14,15).

Statistical Analysis

Data are presented as mean $\pm$ SEM, with *n* representing the number of samples (RT-PCR and Western blotting), patched cells or excised membrane patches. Mean values obtained were compared by the paired or unpaired Student's *t* test or by Rank test where appropriate using

SigmaStat program (Jandel Scientific, Chicago, IL). $P < 0.05$ was considered statistically significant.

Results

ENaC Does Not Respond to Acute Changes in Oxygen Tension in Alveolar Epithelial A549 Cells

Under cell-attach configuration of patch-clamp technique, current flowing through single ENaC channels was extracted (Figure 1A) (19, 20). Fast changes in PO_2 (from 140 to 20 mm Hg) did not affect the channel activity (Figures 1A and 1B). The average N_{po} was 0.92 ± 0.05 in cells exposed to $PO_2 = 140$ mm Hg, and 0.87 ± 0.04 in cells exposed to $PO_2 = 20$ mm Hg (Figure 1C; $n = 6$, $P = 0.427$).

BK_{Ca} Channels Are Present in Alveolar Epithelial A549 Cells

Because ENaC did not respond to changes in PO_2 , we have hypothesized that some other ion conductance in alveolar epithelial cells may serve as oxygen sensor and regulator of alveolar fluid clearance. Therefore, we have examined, using inside-out configuration of patch-clamp technique under “physiologic” ionic conditions existence of other ion conductance that may be responsive to O_2 . Upon excision of a membrane patch from A549 cell, vigorous current suggestive of ion channel opening was observed (Figure 2A). Under 0 mV holding potential the observed single channel current was ~ 8 pA (Figure 2AB). The activity of this channel was dependent on the presence of K^+ and Ca^{2+} ions in internal solution (Figures 2B and 2C). The single-channel amplitude, as well as probability of the channels opening, increased with more positive membrane potential (Figure 2D, D2). The calculated single-channel slope conductance at asymmetrical K^+ conditions was 112 pS. Because the single-channel properties were suggestive of those for BK_{Ca} channels (*see* Refs. 7, 20) we have further tested this possibility. Iberitoxin (100 nM), a selective antagonist of BK_{Ca} channels (21), significantly inhibited whole-cell K^+ membrane current (Figure 3A; at 80 mV current density was 25.0 ± 2.8 pA/pF in the absence and 15.7 ± 2.8 pA/pF in the presence of iberitoxin, $n = 6$, $P < 0.01$). Because all these findings at both single-channel and whole-cell levels were typical for BK_{Ca}, we have probed A549 cells for α -BK_{Ca} channel subunit mRNA and protein. RT-PCR with α -BK_{Ca} primers, as well as Western blotting with anti- α -BK_{Ca} antibody, revealed single signals migrating at the size expected for the α -BK_{Ca} channel subunit (Figure 3B).

Expression, Recruitment, and Function of BK_{Ca} Channels Are Not Affected by Chronic Changes in PO_2

All proteins involved in epithelial electrolyte transport are apparently regulated by changes in oxygen tension (4). We have examined whether 24-h-long incubation (incubation time sufficient for O_2 -mediated up- or downregulation of proteins involved in electrolyte transport in lungs [4]) of A549 cells to $PO_2 = 23$ mm Hg would change levels of BK_{Ca} channels in comparison with cells cultured at $PO_2 = 144$ mm Hg. Semiquantitative RT-PCR has demonstrated that levels of α -BK_{Ca} channel subunit mRNA are not significantly changed by changes in PO_2 (PCR product band intensity was 24 ± 3 and 26 ± 3 arbitrary units [AU] for $PO_2 = 140$ mm Hg and $PO_2 = 20$ mm Hg, respectively; $n = 3$ of each, $P = 0.69$; Figure 4A). Western blotting also did not show significant difference between cells cultured at different PO_2 (band intensity was 12.1 ± 1.9 and 13.0 ± 1.8 AU for $PO_2 = 140$ mm Hg and $PO_2 = 20$ mm Hg, respectively; $n = 3$ of each, $P = 0.38$; Figure 4B). In addition, iberitoxin-sensitive components of whole cell membrane current were not significantly different between cells cultured in different oxygen tensions (current density of iberitoxin-sensitive component at 80 mV was 9.3 ± 1.3 pA/pF and 7.3 ± 1.8 pA/pF for $PO_2 = 140$ mm Hg and $PO_2 = 20$ mm Hg, respectively; $n = 5-6$, $P = 0.36$; Figure 4C, C2).

BK_{Ca} Channels Sense Acute Changes in PO₂ in Alveolar Epithelial A549 Cells

Exposure of A549 cells from PO₂ = 140 mm Hg to PO₂ = 20 mm Hg induced fast (after ~ 30 s) and significant decrease in whole cell membrane current in A549 cells (whole cell current density at 80 mV was 23.0 ± 2.9 pA/pF in PO₂ = 140 mm Hg and 16.8 ± 2.4 pA/pF in PO₂ = 20 mm Hg, $n = 6$, $P < 0.01$; Figure 5AA1). Under PO₂ = 20 mm Hg, the iberiotoxin-sensitive current component was almost abolished (Figure 5AA2; current density of iberiotoxin-sensitive component at 80 mV was 1.8 ± 1.1 pA/pF, $n = 6$, $P < 0.001$ when compared with those at PO₂ = 140 mm Hg). In membrane patches excised from A549 cells, changes in PO₂ induced immediate changes in BK_{Ca} channel activity (Figure 6A, A1; Npo was 0.80 ± 0.04 in PO₂ = 140 mm Hg and 0.49 ± 0.06 in PO₂ = 20 mm Hg, $n = 6$, $P < 0.01$). The changes in PO₂ did not significantly changed the single-channel amplitude (single amplitude was 8.10 ± 0.10 in PO₂ = 140 mm Hg and 8.17 ± 0.05 in PO₂ = 20 mm Hg, $n = 6$, $P = 0.61$; data not shown). Dwell times of opening and closing events for BK_{Ca} fitted well with a combination of two exponential components (Figure 6A, A2). Changes in PO₂ did not affect significantly closed BK_{Ca} times (short closed time was 0.43 ± 0.07 ms in PO₂ = 140 mm Hg and 0.42 ± 0.02 ms in PO₂ = 20 mm Hg, $P = 0.85$, $n = 6$, whereas long closed time was 4.56 ± 0.47 ms in PO₂ = 140 mm Hg and 4.37 ± 1.48 ms in PO₂ = 20 mm Hg, $P = 0.91$; data not shown) as well as short open time (short open time was 1.18 ± 0.21 ms in PO₂ = 140 mm Hg and 1.38 ± 0.19 ms in PO₂ = 20 mm Hg, Figure 6A, A2; $P = 0.51$, $n = 6$). However, long open time was significantly shortened by decrease in PO₂ (Figure 6A, A2; long open time was 18.6 ± 1.99 ms in PO₂ = 140 mm Hg and 10.29 ± 2.97 ms in PO₂ = 20 mm Hg, $P = 0.04$, $n = 6$).

The observed inhibitory effect of acute hypoxia on BK_{Ca} channels activity was not affected by the presence of wide range flavoproteins inhibitor, diphenylene iodonium (DPI; 10 μ M) (Figure 6B)

PO₂-Mediated Changes of BK_{Ca} Activity Is Associated with Regulation of Membrane Potential and Ca²⁺ Homeostasis

The membrane potential of A549 cells at rest was estimated to be -57.7 ± 6.8 mV (Figure 7A, A1, $n = 5$). Exposure of A549 cells from PO₂ = 140 mm Hg to PO₂ = 20 mm Hg, as well as to iberiotoxin (100 nM), induced significant membrane depolarization (to -48.8 ± 6.4 mV in PO₂ = 20 mm Hg and to -35.8 ± 5.9 mV in iberiotoxin, $n = 5$, $P < 0.01$, Figure 7A, A1). In addition, both decrease in PO₂ from 140 mm Hg to 20 mm Hg and iberiotoxin (100 nM) induced oscillation of Ca²⁺ in A549 cells (Figure 7B).

Discussion

In the present study, we report that BK_{Ca} channels serve as sensor for acute changes in oxygen tension in environment surrounding alveolar epithelial cells. This is the first report of an ion conductance capable of rapid response to changes in PO₂ in alveolar epithelium.

Alveolar gas exchange might be limited by thickness of diffusion barrier, which consists of alveolar lining fluid and alveolar epithelial and capillary endothelial cells as well as their basal membrane. The thickness of the film of lining fluid is determined by the balance between fluid filtration into the alveolar space and fluid reabsorption across the alveolar epithelium (*see* Ref. 22). It is generally accepted that changes in oxygen tension may be associated with changes in electrolyte transepithelial transport. In this respect, it is believed that Na⁺ enters alveolar epithelial cells via cation channels, in particular the ENaC (2,3). Several-hours-long exposure of epithelial cells affect the magnitude of whole-cell membrane current conducted by ENaC, which seems to be due to changes in levels of intracellular ENaC protein (4). However, no evidence so far has been provided to suggest that ENaC could respond to acute changes in oxygen tension. Our results, at the single-channel level, suggest that ENaC activity is not

affected by acute changes in oxygen tension, which would be in accord with recent reports showing that hypoxia regulates ENaC-mediated ion current only after ~ 4-h-long incubation period (23). Therefore, it seems that ENaC is not the channel responsible for fast alveolar response to changes in environmental oxygen tension.

O₂-regulated ion channels initially studied in carotid body glomus cells are found in a broad variety of cells, including several neurosecretory cells, smooth and heart muscle, and central neurons. The nature of majority of O₂-sensitive channels have been identified, and most of them are K⁺-selective (6). Therefore, to identify an ion conductance that may be involved in oxygen-sensing in A549 cells, we have examined single-channel currents occurring under “physiologic” ionic conditions. This strategy revealed K⁺ conductance that was regulated by levels of intracellular Ca²⁺, with voltage-dependant probability of channel opening and the channel amplitude, and with single-channel conductance similar to those previously reported for BK_{Ca} channels (20). Moreover, whole-cell membrane current was sensitive to iberiotoxin, a known inhibitor of BK_{Ca} channels (21), and both mRNA and protein of α -BK_{Ca}, a pore-forming subunit (24), have been found in alveolar epithelial A549 cells. Although it has been previously suggested that these cells may express BK_{Ca} channels (25), this is the first report to provide what seems to be definitive evidence that alveolar A549 cells express BK_{Ca} channels.

In numerous reports so far it has been suggested that long-lasting exposure to changed oxygen tension regulate expression of different proteins involved in epithelial electrolyte transport, including ENaC, Na-K-ATPase, and Na/K/2Cl cotransporter (reviewed in Ref. 26; *see also* Ref. 4). In the present study, exposure of A549 cells did not change levels of BK_{Ca} channel mRNA, protein, or BK_{Ca} channel contribution to whole-cell K⁺ current. Accordingly, this would suggest that BK_{Ca} channel transcription, translation, recruitment, or function is not regulated by chronic changes in oxygen tension. In a view of previous reports, it is apparent that BK_{Ca} channel is the first protein involved in electrolyte transport in alveolar epithelial cells reported to be insensitive to chronic changes in environmental oxygen tension.

Numerous tissues express BK_{Ca} channels and, at least in some of them, act as oxygen sensors. Hypoxic inhibition of BK_{Ca} channels has been reported in carotid body, pulmonary smooth muscle, chromaffin cells, and central neurons (reviewed in Ref. 6). In the present study, we have shown that changes in PO₂ in environment surrounding A549 cells regulated the activity of BK_{Ca} channels, which supports the general idea that BK_{Ca} may play a role of fast-responsive oxygen sensors (7). It has been previously suggested that BK_{Ca} channels in central neurons are inhibited by hypoxia via a mechanism which requires cytosolic factors (27), whereas it has been reported that recombinant human BK_{Ca} channel is inhibited by hypoxia, by a mechanism which is membrane-delimited (7). The fact that the inhibition of BK_{Ca} channels in A549 cells was immediate and observed in excised membrane patches would suggest that oxygen regulates the activity of BK_{Ca} by its direct action on channel subunits or closely associated protein in alveolar epithelium cells. DPI is an inhibitor of a wide range of flavoproteins, including nitric oxide synthase, NADPH oxidase, and complex I within the mitochondrial electron transport chain, known to inhibit a variety of responses to hypoxia, such as vasoconstriction and carotid body nerve firing (28,29). Our finding that this agent did not affect the effect of hypoxia on BK_{Ca} channels would further support the idea of direct action of oxygen on the channel proteins itself. Changing in oxygen tension did not affect the channel unitary conductance, but selectively regulated the long open time, suggesting that oxygen does not interfere with BK_{Ca} channel pore function but rather acts in a manner typical for channel ligands targeting regulatory channel subunits or accessory protein (7).

It is well established that decrease in oxygen tension can cause alveolar flooding or that the rise of O₂ initiates fluid reabsorption in the newborn infant (1,30). Traditionally, the central

role for these processes have been ascribed to ENaC (*see* Refs. 31,32), but the findings from this study protein (7).

response of alveolar epithelium to changes in oxygen tension. In contrast, BK_{Ca} channels sense fast changes in PO₂ leading to changes in membrane potential and intracellular Ca²⁺ homeostasis (this study). The observed membrane depolarization induced by inhibition of BK_{Ca} channels would be in accord with the suggestion that BK_{Ca} channels regulate membrane potential in alveolar epithelial cells (25). We have previously demonstrated that ischemia, a challenge similar to hypoxia, induces intracellular Ca²⁺ loading in A549 cells (11). In the present study, we have shown that acute hypoxia induces oscillations of intracellular levels of Ca²⁺, suggesting that closure of BK_{Ca} channels may trigger intracellular signaling cascade(s). It should be mentioned that, in contrast to our study, it has been reported previously that acute exposure of A549 cells to hypoxia does not affect intracellular concentration of Ca²⁺ (33). This difference between our study and that of Papen and coworkers (33) could be explained by using different methods (with different resolutions) to measure intracellular Ca²⁺. Here, we applied laser confocal microscopy (LSM) and measured dynamics of intracellular Ca²⁺ with high spatial resolution, which revealed oscillations of Ca²⁺ in certain intracellular regions, rather than increase of Ca²⁺ throughout the cell. In this regard, we appreciate the fact that Papen and colleagues (33) may not be able to detect localized subcellular changes in Ca²⁺ in A549 cells exposed to hypoxia without using LSM. The opening and closing of K⁺ conductance in alveolar epithelium increases and decreases alveolar fluid clearance, respectively (34). In the present study, we have demonstrated that rise of oxygen tension (as occurs during birth) increases the activity of BK_{Ca}, which would lead to increase in the alveolar fluid clearance and fluid reabsorption (as occurs during birth). In contrast, decrease in oxygen tension inhibits the activity of BK_{Ca}, leading to decrease in fluid clearance and fluid retention (as occurs during rapid ascent to high altitude). Bearing all these in mind, the responsiveness of BK_{Ca} to acute changes in PO₂ may contribute to fast changes in alveolar clearance under certain physiologic and pathophysiologic conditions, including the first breath fluid reabsorption and hypoxia-induced pulmonary edema.

In conclusion, BK_{Ca} channels are present in human alveolar A549 epithelial cells, where they act as sensors for acute, but not chronic, changes in PO₂ which may be involved in transduction of changes in environmental oxygen tension into changes in fluid clearance in lungs.

Acknowledgments

This research was supported by grants from the Anonymous Trust, BBSRC, BHF, TENOVUS-Scotland, and the Wellcome Trust.

Abbreviations

BK_{Ca}, large conductance Ca²⁺-activated K⁺; ENaC, alveolar Na⁺ channel; PO₂, alveolar oxygen tension; RT-PCR, reverse transcriptase–polymerase chain reaction.

References

1. Bland RD. Dynamics of pulmonary water before and after birth. *Acta Paediatr. Scand* 1983;305:12–20. [PubMed: 6351531]
2. Olver RE, Ramsden CA, Starng LB, Walters DV. The role of amiloride-blockable sodium transport in adrenaline-induced lung liquid reabsorption in the fetal lamb. *J. Physiol* 1986;376:321–340. [PubMed: 3795077]
3. Hummler E, Baker P, Gatzky J, Berrmann F, Verdumo C, Schmidt A, Boucher R, Rossier RC. Early death due to defective neonatal lung liquid clearance in α -ENaC-deficient mice. *Nat. Genet* 1996;12:325–328. [PubMed: 8589728]

4. Wodopia R, Ko HS, Billian J, Wiesner R, Bartsch P, Mairbaurl H. Hypoxia decreases proteins involved in epithelial electrolyte transport in A549 cells and rat lung. *Am. J. Physiol. Lung Cell. Mol. Physiol* 2000;279:L1110–L1119. [PubMed: 11076801]
5. Vergara C, Latorre R, Marrion NV, Adelman JP. Calcium-activated potassium channels. *Curr. Opin. Neurobiol* 1998;8:321–329. [PubMed: 9687354]
6. Haddad GG, Liu H. Different O₂-sensing mechanisms by different K⁺ channels. *Adv. Exp. Med. Biol* 2000;475:441–452. [PubMed: 10849685]
7. Lewis A, Peers C, Ashford MLJ, Kemp PJ. Hypoxia inhibits human recombinant large conductance Ca²⁺-activated K⁺ (maxi-K) channels by a mechanism which is membrane delimited and Ca²⁺ sensitive. *J. Physiol* 2002;540:771–780. [PubMed: 11986367]
8. Lazrak A, Samanta A, Venetsanou K, Barbry P, Matalon S. Modification of biophysical properties of lung epithelial Na(+) channels by dexamethasone. *Am. J. Physiol. Cell Physiol* 2000;279:C762–C770. [PubMed: 10942727]
9. Itani OA, Auerbach SD, Husted RF, Volk KA, Ageloff S, Knepper MA, Stokes JB, Thomas CP. Glucocorticoid-stimulated lung epithelial Na(+) transport is associated with regulated ENaC and sgk1 expression. *Am. J. Physiol. Lung Cell. Mol. Physiol* 2002;282:L631–L641. [PubMed: 11880287]
10. Jovanović S, Jovanović A. Diadenosine tetraphosphate-gating of cardiac K_{ATP} channels requires intact actin cytoskeleton. *Naunyn-Schmiedeberg's Arch. Pharmacol* 2001;364:276–280. [PubMed: 11521171]
11. Crawford RM, Budas GR, Jovanović S, Ranki HJ, Wilson TJ, Davies AM, Jovanović A. M-LDH serves as a sarcolemmal K_{ATP} channel subunit essential for cell protection against ischemia. *EMBO J* 2002;21:3936–3948. [PubMed: 12145195]
12. Jovanović S, Jovanović A. Delivery of genes encoding K_{ATP} channel subunits in conjunction with pinacidil prevents membrane depolarisation in cells exposed to chemical hypoxia-reoxygenation. *Biochem. Biophys. Res. Commun* 2001;282:1098–1102. [PubMed: 11302727]
13. Ohya S, Yamamura H, Muraki K, Watanabe M, Imaizumi Y. Comparative study of the molecular and functional expression of L-type Ca²⁺ channels and large-conductance, Ca²⁺-activated K⁺ channels in rabbit aorta and vas deferens smooth muscle. *Pflüger's Arch. (Eur. J. Physiol.)* 2001;441:611–620. [PubMed: 11294242]
14. Ranki HJ, Budas GR, Crawford RM, Jovanović A. Gender-specific difference in cardiac ATP-sensitive K⁺ channels. *J. Am. Coll. Cardiol* 2001;38:906–915. [PubMed: 11527652]
15. Ranki HJ, Crawford RM, Budas GR, Jovanović A. Ageing is associated with decrease in number of sarcolemmal ATP-sensitive K⁺ channels in a gender-dependent manner. *Mech. Ageing Dev* 2002;123:695–705. [PubMed: 11850031]
16. Crawford RM, Ranki HJ, Booting CH, Budas GR, Jovanović A. Creatine kinase is physically associated with the cardiac ATP-sensitive K⁺ channel *in vivo*. *FASEB J* 2002;16:102–104. [PubMed: 11729098]
17. Ranki HJ, Budas GR, Crawford RM, Davies AM, Jovanović A. 17 β -estradiol regulates expression of K_{ATP} channels in heart-derived H9c2 cells. *J. Am. Coll. Cardiol* 2002;40:367–374. [PubMed: 12106946]
18. Alekseev AE, Jovanović A, Lopez JR, Terzic A. Adenosine slows the rate of K⁺-induced membrane depolarization in ventricular cardiomyocytes: possible implications in hyperkalemic cardioplegia. *J. Mol. Cell. Cardiol* 1996;28:1193–1202. [PubMed: 8782061]
19. Jovanović S, Jovanović A. Pinacidil prevents membrane depolarisation and intracellular Ca²⁺ loading in single cardiomyocytes exposed to severe metabolic stress. *Int. J. Mol. Med* 2001;7:639–643. [PubMed: 11351278]
20. Mistry DK, Garland CJ. Characteristics of single, large-conductance calcium-dependent potassium channels (BK_{Ca}) from smooth muscle cells isolated from the rabbit mesenteric artery. *J. Membr. Biol* 1998;164:125–138. [PubMed: 9662557]
21. Giangiacomo KM, Garcia ML, McManus OB. Mechanism of iberiotoxin block of the large-conductance calcium-activated potassium channel from bovine aortic smooth muscle. *Biochemistry* 1992;31:6719–6727. [PubMed: 1379069]
22. Matthay MA, Fukuda N, Frank J, Kallet R, Daniel B, Sakuma T. Alveolar epithelial barrier: role in lung fluid balance in clinical lung injury. *Clin. Chest Med* 2000;21:477–490. [PubMed: 11019721]

23. Mairbaur H, Mayer K, Kim KJ, Borok Z, Bartsch P, Crandall ED. Hypoxia decreases active Na transport across primary rat alveolar epithelial cell monolayers. *Am. J. Physiol. Lung Cell. Mol. Physiol* 2002;282:L659–L665. [PubMed: 11880290]
24. Shen KZ, Lagrutta A, Davies NW, Standen NB, Adelman JP, North RA. Tetraethylammonium block of Slowpoke calcium-activated potassium channels expressed in *Xenopus* oocytes: evidence for tetrameric channel formation. *Pflüger's Archiv (Eur. J Physiol.)* 1994;426:440–450. [PubMed: 7517033]
25. Ridge FP, Duszyk M, French AS. A large conductance, Ca^{2+} -activated K^{+} channel in human lung cell line (A549). *Biochim. Biophys. Acta* 1997;1327:249–258. [PubMed: 9271267]
26. Clerici C, Matthay MA. Hypoxia regulates gene expression of alveolar epithelial transport proteins. *J. Appl. Physiol* 2000;88:1890–1896. [PubMed: 10797154]
27. Liu H, Moczyldowski E, Haddad GG. O_2 deprivation inhibits Ca^{2+} -activated K^{+} channels via cytosolic factors in mice neocortical neurons. *J. Clin. Invest* 1999;104:577–588. [PubMed: 10487772]
28. Stuehr DJ, Fasehun OA, Kwon NS, Gross SS, Gonzalez JA, Levi R, Nathan CF. Inhibition of macrophage and endothelial cell nitric oxide synthase by diphenyleneiodonium and its analogs. *FASEB J* 1991;5:98–103. [PubMed: 1703974]
29. Marshall C, Mamary AJ, Verhoeven AJ, Marshall BE. Pulmonary artery NADPH-oxidase is activated in hypoxic pulmonary vasoconstriction. *Am. J. Respir. Cell Mol. Biol* 1996;15:633–644. [PubMed: 8918370]
30. Bartsch P. High altitude pulmonary edema. *Respiration* 1997;64:435–443. [PubMed: 9383819]
31. Saumon G, Basset G. Electrolyte and fluid transport across the mature alveolar epithelium. *J. Appl. Physiol* 1993;74:1–15. [PubMed: 8444679]
32. Pitkanen O. Lung epithelial ion transport in neonatal lung disease. *Biol. Neonate* 2001;80:14–17. [PubMed: 11359039]
33. Papen M, Wodopia R, Bartsch P, Mairbaur H. Hypoxia-effects on Ca_i signaling and ion transport activity of lung alveolar epithelial cells. *Cell. Physiol. Biochem* 2001;11:187–196. [PubMed: 11509826]
34. Sakuma T, Takahashi K, Ohya N, Nakada T, Matthay MA. Effects of ATP-sensitive potassium channel opener on potassium transport and alveolar fluid clearance in the resected human lung. *Pharmacol. Toxicol* 1998;83:16–22. [PubMed: 9764421]

Figure 1.

The effect of oxygen tension (PO_2) on ENaC activity. (A) Recording of ENaC activity in cell-attached configuration of patch-clamp technique under $PO_2 = 140$ mm Hg and $PO_2 = 20$ mm Hg. Holding potential: -60 mV. Dotted lines correspond to the zero current levels. (B) Channel activity expressed as NPo under conditions in A. (C) Average NPo under conditions in A obtained on six cells. Vertical bars represent mean $\pm$ SEM ($n = 6$ for each).

Figure 2.

(A, A1) Single-channel conductance occurring in excised membrane patches of A549 cells. Recording of single-channel activity (A and A1 are lower- and higher-time resolution, respectively) in membrane patches excised from A549 cells. Holding potential: 0 mV. (B, C) Recording of single-channel activity in excised membrane patches in asymmetrical and symmetrical K^+ concentrations (B) and in the presence of 1 μ M (high Ca^{2+}) or 3 nM (low Ca^{2+}) free intracellular Ca^{2+} (C). Holding potential: 0 mV. (D) Recording of single-channel activity in excised membrane patches in asymmetrical K^+ under depicted membrane potentials. Dotted lines correspond to the zero current levels. Probability of the channel opening (D1) and single-channel current (D2) under conditions in D.

Figure 3.

Iberitoxin sensitivity of whole cell K⁺ current and presence of α-BK_{Ca} channel subunit in A549 cells. (A) Membrane currents evoked by identical families of 400 ms voltage pulses in cell that was first maintained under control conditions and then exposed to 100 nM iberitoxin for 1 min. (A1) Iberitoxin-sensitive component of current was obtained by digital subtraction of currents in the absence and presence of iberitoxin. I-V relationship under conditions in A. Each point represents a mean ± SEM of six experiments. (A2) I-V relationship of iberitoxin-sensitive component of current under conditions in A. Each point represents a mean ± SEM of six experiments. (B) RT-PCR product obtained with α-BK_{Ca} channel subunit-specific primers from A549 cells and western blot (W) with anti-α-BK_{Ca} channel subunit antibody using A549 cells protein extract.

Figure 4.

The effect of 24 h-long exposure to $\text{PO}_2 = 23$ mm Hg on expression, recruitment and activity of BK_{Ca} channels. (A) RT-PCR product obtained with $\alpha\text{BK}_{\text{Ca}}$ channel subunit-specific primers from A549 cells exposed to $\text{PO}_2 = 144$ mm Hg and $\text{PO}_2 = 23$ mm Hg. (B) Western blotting with anti- $\alpha\text{BK}_{\text{Ca}}$ channel subunit antibody using protein extract of A549 cells exposed either to $\text{PO}_2 = 144$ mm Hg or $\text{PO}_2 = 23$ mm Hg for 24 h. (C) Membrane currents evoked by identical families of 400 ms voltage pulses in cell that was exposed to 24-h-long incubation in $\text{PO}_2 = 23$ mm Hg in the absence (control) and presence of 100 nM iberiotoxin. The iberiotoxin-sensitive component was obtained by digital subtraction of currents in the absence and presence of iberiotoxin. (C1) I-V relationship in cells incubated 24 h in $\text{PO}_2 = 23$

mm Hg in the absence (control) and presence of 100 nM iberiotoxin. Each *point* represents mean $\pm$ SEM of five experiments. (C2) Iberiotoxin-sensitive component of current in cells incubated in $PO_2 = 144$ mm Hg (control) or $PO_2 = 23$ mm Hg (chronic hypoxia). Each *point* represents mean $\pm$ SEM of five to six experiments.

A **Figure 5.**

The effect of PO_2 on the whole-cell K^+ current and iberiotoxin-sensitive component of the current. (A) Membrane currents evoked by identical families of 400 ms voltage pulses in cell exposed first to $PO_2 = 140$ mm Hg and then 30 s to $PO_2 = 20$ mm Hg, in the absence and presence of iberiotoxin (100 nM). Iberiotoxin-sensitive component of current under $PO_2 = 20$ mm Hg is obtained by digital subtraction of currents in the absence and presence of iberiotoxin in cells exposed to $PO_2 = 20$ mm Hg. Measurement was performed 1 min after iberiotoxin was introduced. (A1) I-V relationships under conditions in A as well as I-V relationship of whole-cell K^+ current in the presence of iberiotoxin (100 nM) at $PO_2 = 140$ mm Hg. (A2) Iberiotoxin-

sensitive component in cells exposed to $\text{PO}_2 = 140$ mm Hg and $\text{PO}_2 = 20$ mm Hg. Each *point* in graphs represents mean $\pm$ SEM of six experiments.

Figure 6.

The effect of PO_2 on single BK_{Ca} channel activity. (A) Recording of BK_{Ca} activity in a membrane patch excised from A549 cell under $PO_2 = 140 \text{ mm Hg}$ and $PO_2 = 20 \text{ mm Hg}$. Holding potential: 0 mV. Dotted lines correspond to the zero current level. (A1) Channel activity expressed as NPo under conditions in A. (A2) Open-time histograms obtained from membrane patch presented in A, plotted with a bin width size of 50 μs and fitted by two exponential function. τ_1 and τ_2 stand for first and second mean open times, respectively. (B) Recording of BK_{Ca} activity in a membrane patch excised from A549 cell under $PO_2 = 140 \text{ mm Hg}$ and $PO_2 = 20 \text{ mm Hg}$ when diphenylene iodonium (10 μM) was present throughout the experiment. Holding potential: 0 mV. Dotted lines correspond to the zero current level. Essentially the same result was obtained in three more experiments.

Figure 7.

The effect of PO₂ on membrane potential and intracellular Ca²⁺ in A549 cells. (A) Time-course of membrane potential (A) and average values of membrane potential (A1) in A549 cells under depicted conditions. Bars represent mean ± SEM (*n* = 5), and stars indicate *P* < 0.01 when compared with the control. (B) Time-course of Fluo-3 fluorescence in selected subcellular region in A549 cell under depicted conditions. AU: arbitrary units. Similar results were obtained in three additional experiments.