Hypoxia-induced inhibition of whole cell membrane currents and ion transport of A549 cells

Christoph Karle,1 Tobias Gehrig,2 Ralf Wodopia, Sabine Höschle,2 Volker A. W. Kreye,3 Hugo A. Katus,1 Peter Bärtsch,2 and Heimo Mairbäurl2

1Section III-Cardiology, 2Section VII-Sports Medicine, Department of Internal Medicine, Medical Clinic and Policlinic, and 3Department of Physiology and Pathophysiology, University of Heidelberg, 69115 Heidelberg, Germany

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Karle, Christoph, Tobias Gehrig, Ralf Wodopia, Sabine Höschle, Volker A. W. Kreye, Hugo A. Katus, Peter Bärtsch, and Heimo Mairbäurl. Hypoxia-induced inhibition of whole cell membrane currents and ion transport of A549 cells. Am J Physiol Lung Cell Mol Physiol 286: L1154–L1160, 2004. First published January 16, 2004; 10.1152/ajplung.00403.2002.—In excitable cells, hypoxia inhibits K channels, causes membrane depolarization, and initiates complex adaptive mechanisms. It is unclear whether K channels of alveolar epithelial cells reveal a similar response to hypoxia. A549 cells were exposed to hypoxia during whole cell patch-clamp measurements. Hypoxia reversibly inhibited a voltage-dependent outward current, consistent with a K current, because tetraethylammonium (TEA; 10 mM) abolished this effect; however, iberiotoxin (0.1 μM) does not. In normoxia, TEA and iberiotoxin inhibited whole cell current (~35%), whereas the K-channel inhibitors glibenclamide (1 μM), barium (1 mM), chromanol B293 (10 μM), and 4-aminopyridine (1 mM) were ineffective. 86Rb uptake was measured to see whether K-channel modulation also affected transport activity. TEA, iberiotoxin, and 4-h hypoxia (1.5% O2) inhibited total 86Rb uptake by 40, 20, and 35%, respectively. Increased extracellular K also inhibited 86Rb uptake in a dose-dependent way. The K-channel opener 1-ethyl-2-benzimidazolinone (1 mM) increased 86Rb uptake by 120% in normoxic and hypoxic cells by activation of Na-K pumps (+60%) and Na-K-2Cl cotransport (+170%). However, hypoxic transport inhibition was also seen in the presence of 1-ethyl-2-benzimidazolinone, TEA, and iberiotoxin. These results indicate that hypoxia, membrane depolarization, and K-channel inhibition decrease whole cell membrane currents and transport activity. It appears, therefore, that a hypoxia-induced change in membrane conductance and membrane potential might be a link between hypoxia and alveolar ion transport inhibition.

whole cell patch clamp; calcium-activated potassium channels; sodium-potassium pump; sodium-potassium-chloride cotransport; membrane potential

Hypoxia inhibits alveolar epithelial Na transport, which generates the osmotic gradient required for the reabsorption of water from the alveolar space (19, 20, 29). A reduced trans-epithelial transport capacity has been associated with an impaired clearance of pulmonary edema (6). Long-term exposure to hypoxia decreases expression and copy number of Na transporters in alveolar epithelial cells (29, 36). However, decreased expression of Na transporters cannot account for the rapid inhibition of Na transport by hypoxia (20).

In excitatory cells, such as the peripheral chemoreceptor and small pulmonary artery smooth muscle cells, K-channel inhibition by hypoxia initiates the rapid adjustment of ventilation (17) and pulmonary vasoconstriction (35). In epithelial cells, K channels are required for the recycling of K taken up by the Na-K pump in exchange for Na. Therefore, these channels contribute to the negative membrane potential required for the uptake of Na and control transepithelial transport (3, 7). It is unclear whether these K channels respond to changes in oxygen tension similar to what is found in excitory cells. We studied, therefore, whether oxygen-dependent K-channel modulation might cause inhibition of Na transport of alveolar epithelial cells by hypoxia. Patch-clamp measurements indicate that hypoxia reversibly inhibits tetraethylammonium (TEA)-sensitive channels. Tracer flux measurements indicate that K-channel inhibitors also inhibit transport activity similar to what was found in hypoxia, whereas K-channel openers blunt the hypoxia-induced inhibition of ion transport.

Materials and Methods

Reagents. Reagents used were of analytic grade, and media were prepared from deionized water (18 MΩ). Oatbain, bumetanide, TEA, 4-aminopyridine (4-AP), BaCl2, glibenclamide, amiloride, ATP, nifedipine, DMSO, and trypsin were from Sigma Chemical (Deisenhofen, Germany); chromanol 293B was from Hoechst (Frankfurt, Germany); iberiotoxin and 1-ethyl-2-benzimidazolinone (1-EBIO) were from Tocris (Ballwin, MO); and phosphate-buffered saline, Ham's F-12, Hank's balanced salt solution (HBSS), penicillin/streptomycin, FCS, and HEPES were from Life Technologies (Karlsruhe, Germany). Stock solutions of chromanol 293B (10 mM), iberiotoxin (1 mM), and amiloride (100 mM) were prepared in DMSO. At its highest concentration of 1%, DMSO had no effect on the currents measured in patch-clamp experiments.

Cells. Experiments were performed on A549 cells (ATCC, Manassas, VA) grown to confluence in Ham's F-12 culture medium substituted with 7% FCS, penicillin-streptomycin (1:100), 10 mM HEPES, and 14 mM sodium bicarbonate. Experiments were carried out on confluent cells 5–10 days after seeding.

For exposure to different atmospheres, cells were cultured in a CO2-controlled incubator (normoxia, room air with 5% CO2; Heraeus, Hanau, Germany) or, for exposure to hypoxia, in an O2/CO2-controlled incubator (NUNC, Wiesbaden, Germany) adjusted to 1.5% O2, 5% CO2, and balance N2 at 37°C. To initiate hypoxia, the culture medium of cells grown in normoxia was replaced with fresh culture medium equilibrated with the respective gas. Any agent or the respective solvent was added at this time.

Whole cell patch-clamp measurements. Confluent A549 cells grown in normoxia were scratched off the surface and allowed to adhere to untreated glass slides (4–8 h). The slides were mounted in
a chamber on an inverted microscope and perfused (2 ml/min) with bathing media equilibrated to room air or N\textsubscript{2}. Perfusion with N\textsubscript{2}-equilibrated bathing medium resulted in a Po2 of ~12 Torr in the perfusion chamber.

The bathing medium contained (in mM) 135 NaCl, 5 KCl, 1 MgCl\textsubscript{2}, 1.5 CaCl\textsubscript{2}, 10 HEPES, and 5 glucose, pH = 7.4. Amiloride (100 \mu M) and nifedipine (10 \mu M) were added to block epithelial Na channel-type Na channels, nonselective cation channels, and L-type Ca channels, respectively. To characterize K currents, screening concentrations resulting in inhibition of K channels in different tissues of glibenclamide (1 \mu M), BaCl\textsubscript{2} (1 mM (30)), chromanol B293 [10 \mu M (32)], 4-AP [1 mM (1)], TEA [10 mM (10)], andiberiotoxin [0.1 \mu M (16)] were added. The pipette solution contained (in mM) 130 KCl, 2 MgCl\textsubscript{2}, 5 NaCl, 1 CaCl\textsubscript{2}, 11 EGTA, 10 HEPES, and 2 ATP, pH = 7.2. The calculated K equilibrium potential was –86 mV.

Membrane currents were measured with an EPC-7 amplifier (List, Darmstadt, Germany) at a sampling rate of 1 kHz and a filter frequency of 250 Hz, recorded online, and analyzed with pCLAMP 6 software (Axon Instruments, Foster City, CA). Experiments were performed at room temperature in the whole cell configuration of the patch-clamp technique. The holding potential was ~80 mV. For measurement of outward currents, the potential was stepwise increased to +70 mV; inward currents were analyzed by decreasing the potential from –40 mV to –150 mV. In both settings, voltage steps were 10 mV, and test pulses lasted for 400 ms. No subtraction of leak currents was performed. The stability of patches was tested by evaluating series resistance (30–50 M\textOmega) at the beginning and end of each experiment. Current measurements were made 50 ms after the onset of the voltage pulse (early plateau phase).

Expression of K channels. Total RNA of A549 cells was extracted with TriStar reagent, according to the manufacturer’s instructions (Hybaid-AGS, Heidelberg, Germany). RNA was reversely transcribed with Superscript II (Life Technologies, Karlsruhe, Germany) by using random hexamucleotide primers. For the detection of K-channel expression, 30 cycles of PCR reactions were performed by using the primers listed in Table 1, 1 unit Platinum Taq (Life Technologies), a Mg concentration of 1.6 mM, and an annealing temperature of 60°C. Based on pharmacological evidence from our patch-clamp studies and the published O\textsubscript{2} sensitivity of K channels (4, 5), the expression of the human large-conductance Ca- and voltage-sensitive K channel \alpha-subunit (MaxiK; U11058), the human voltage-gated K channel KCN1A (K\textsubscript{, 1.2}; NM_000217), the human voltage-gated K channel KCN5 (K\textsubscript{, 1.5}; XM_006988), and the human delayed-rectifier K channel DRK1 (K\textsubscript{, 2.1}; AF026005) was studied. Primers were designed from the published base sequences by using the Primer 3 software (Whitehead Institute/MIT Center for Genome Research). In all cases, a single fragment of the expected size was amplified (see Fig. 4).

\textsuperscript{86}Rb uptake. The activity of ion transport was determined by measuring the unidirectional uptake of \textsuperscript{86}Rb (Amersham-Pharmacia, Freiburg, Germany). After exposure to normoxia or hypoxia, A549 cells grown in 24-well plates (Costar) were washed twice and incubated with 250 \mu M HBSS buffered with 10 mM HEPES (pH 7.4 at RT) for 15 min at RT. The HBSS was equilibrated with the respective normoxic or hypoxic CO\textsubscript{2}-free gas. The cells were allowed to equilibrate at room temperature for 20 min in a box flushed with the respective CO\textsubscript{2}-free gas (room air or a gas mixture composed of 1.5% O\textsubscript{2} and 98.5% N\textsubscript{2}) to allow adjustment to the serum- and CO\textsubscript{2}-free condition. Fluxes were started by adding 25 \mu M of HBSS containing \textsuperscript{86}Rb (final activity: 2 \mu Ci/ml) as well as ouabain and/or bumetanide (final concentration: 0.1 and 0.05 mM, respectively) to quantify the activity of the Na-K pump and of Na-K-2Cl cotransport (NKCC), respectively. Control reactions contained respective volumes of DMSO. Fluxes were stopped after 8 min by removing supernatant medium and five washes with ice cold washing medium composed of 150 mM NaCl and 2 mM HEPES (pH = 7.4 at 4°C). Cells were lysed by adding 1 ml of 0.1 M NaOH. Radioactivity was counted in aliquots of cell lysates (model TR 2100; Canberra Packard, Dreieich, Germany). For quantification, the radioactivity was normalized to the protein content of the cell lysates measured with a colorimetric assay (BioRad Laboratories, Hercules, CA).

Statistical evaluation. Experiments were repeated on several monolayers obtained from at least two different cell preparations. Patch-clamp data are presented as mean values ± SE of the indicated number of experiments. Results from flux measurements are mean values ± SD of the indicated number of measurements. Results from more than two experimental groups were analyzed by ANOVA by using Tukey’s post hoc tests. Student’s (paired) t-tests were used to determine the significance between two group means. In all cases, the level of significance was P < 0.05.

RESULTS

Patch-clamp measurements. Patch-clamp measurements were performed to see whether total whole cell current of A549 cells changes on exposure to hypoxia. Figure 1A shows representative whole cell current tracings of A549 cells in normoxia, after 20 min of hypoxia, and after reoxygenation. Figure 1B summarizes the time course of changes of whole cell currents (n = 8) at a holding potential of +70 mV during hypoxia and reoxygenation. Exposure to hypoxia caused a decrease in membrane currents that amounted to ~50% and reached a plateau after ~14 min. Rereoxygenation caused a rapid increase in membrane currents to almost twice the hypoxic values. The percent decrease in whole cell current of A549 cells on exposure to hypoxia was very similar in individual experiments. In contrast, the degree of increase in currents on reoxygenation varied considerably. Membrane currents of control cells were stable when kept at normoxia for the same length of time.

Various inhibitors of K channels were applied to specify, on a pharmacological basis, the kinds of K channels present in the membrane of A549 cells that might be affected by hypoxia.

Table 1. Characteristics of RT-PCR primers

<table>
<thead>
<tr>
<th>K Channel</th>
<th>Primer</th>
<th>Sequence of Primer</th>
<th>Location in Published Sequence (Predicted Size)</th>
<th>Genbank No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MaxiK</td>
<td>Left</td>
<td>ttcttaggaagcgccacagtct</td>
<td>4545–4745 (201)</td>
<td>U11058</td>
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<tr>
<td></td>
<td>Right</td>
<td>ccgctcatccctgcttaaaa</td>
<td>563–814 (252)</td>
<td>NM_000217</td>
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<tr>
<td>K\textsubscript{, 1.2}</td>
<td>Left</td>
<td>tccgggatggaatgagacacatagt</td>
<td>1534–1783 (250)</td>
<td>XM_006988</td>
</tr>
<tr>
<td>K\textsubscript{, 1.5}</td>
<td>Left</td>
<td>tccagggtgtagagagactact</td>
<td>766–986 (221)</td>
<td>AF026005</td>
</tr>
<tr>
<td>K\textsubscript{, 2.1}</td>
<td>Left</td>
<td>aagttcttccaaaggccacact</td>
<td>679–986 (221)</td>
<td>AF026005</td>
</tr>
</tbody>
</table>

maxiK, large-conductance Ca- and voltage-sensitive K channel \alpha-subunit; K\textsubscript{, 1.2}, voltage-gated K channel KCN1A; K\textsubscript{, 1.5}, voltage-gated K channel KCN5; K\textsubscript{, 2.1}, delayed rectifier K channel DRK1.
None of the inhibitors significantly affected whole cell currents except for iberiotoxin and TEA, which decreased currents by ~35% and 60%, respectively (Figs. 2 and 3). Figure 3 also summarizes the combined effects of hypoxia, TEA, and iberiotoxin. It shows that hypoxia caused no further inhibition of whole cell currents in the presence of TEA. Iberiotoxin caused a 35% inhibition of whole cell current. However, in iberiotoxin-treated A549 cells, hypoxia still caused a decrease in whole cell membrane current, reaching the same low level as with TEA or hypoxia alone.

Figure 4 summarizes the current-voltage (I-V) curves of A549 cells exposed to hypoxia, TEA, and iberiotoxin. Figure 4A shows the I-V curves obtained from the tracing indicated in Fig. 1A. In this example, the reversal potential was ~30 mV in

Fig. 1. Time course of hypoxic inhibition of whole cell currents of A549 cells. A549 cells grown in normoxia were allowed to adhere to glass coverslips and mounted into the perfusion chamber. Patch-clamp measurements were performed as described in MATERIALS AND METHODS. After initial recordings in normoxia, the bathing medium was switched to one equilibrated with N₂ (P O₂ = 12 Torr) and, after 20 min, back to the normoxic one. Control cells were perfused with normoxic medium for the entire period. A: typical recordings of currents after step changes of voltages (holding potential −80 mV, 10-mV steps, 400 ms) in normoxia (control), hypoxia, and after reoxygenation. B: mean values ± SE of whole cell currents at a test pulse potential of +70 mV of 6 (normoxia) and 9 (hypoxia) experiments, including the 4 experiments in which reoxygenation was studied. *P < 0.05 compared with normoxia current voltage curves of this experiment.

Fig. 2. Summary of effects of different K-channel blockers on whole cell currents of A549 cells. To test for inwardly rectifying currents (solid bars), test pulses ranging from −150 to −70 mV (400 ms, 10-mV steps) were applied. For the recordings of outward currents (shaded bars), test pulses ranged from −80 to +70 mV. The holding potential was −80 mV. Inward currents were analyzed at a test potential of −150 mV (400 ms, 10-mV steps); outward currents were analyzed at +70 mV. Values are means ± SD relative to control cells. Inhibitors used were glibenclamide (glibencl.; 1 μM; n = 4), BaCl₂ (Ba²⁺; 1 mM; n = 6), chromanol 293B (croma.; 10 μM; n = 5), and 4-aminopyridine (4-AP; 1 mM; n = 5). *P < 0.05 compared with control cells.

Fig. 3. Combined effects of hypoxia, tetraethylamonium (TEA), and iberiotoxin (iberioTX) on whole cell currents of A549 cells. Test pulses ranged from −80 to +70 mV (400 ms, 10-mV steps); holding potential was −80 mV. Values are means ± SD of outward currents analyzed of normoxic and hypoxic A549 cells at +70 mV in absence and presence of TEA (10 mM; n = 6) and iberioTX (0.1 μM; n = 4) relative to normoxic control cells (n = 6). *P < 0.05 compared with normoxic controls. *P < 0.05 compared with hypoxic control cells. **Significant effect of hypoxia in iberioTX-treated cells, P < 0.05.
normoxic cells. Hypoxia shifts the I-V curve to the right, resulting in a reversal potential of zero in this example. The average of six such experiments on normoxic A549 cells was $-18 \pm 7$ mV. In hypoxia, the reversal potential was increased by $22.6 \pm 7.3$ mV. The hypoxia-sensitive whole cell current shows little rectification with an almost linear relation and a reversal potential of about $-70$ mV, which is close to the expected K equilibrium potential (Fig. 4A). In contrast, the TEA-sensitive whole cell current (Fig. 4B) shows the voltage-dependence of activation expected of a delayed rectifier or large-conductance Ca- and voltage-activated K (BKCa)-type ion channel with increasing currents at more polarized potentials. The iberiotoxin-sensitive current (Fig. 4C) exhibits a basically similar pattern with an exponential increase of current at positive potentials, a plateau between +20 mV and $-50$ mV, when it bends, showing a reversal potential of about $-65$ mV. This I-V curve is typical for BKCa.

Expression of K channels. RT-PCR was used to detect whether A549 cells actually express Ca- and voltage-dependent K channels that are reported to be sensitive to inhibition by hypoxia. Figure 5 shows that the mRNAs of BKCa (MaxiK channel), Kv 1.2, Kv 1.5, and Kv 2.1 are present in A549 cells.

Tracer flux experiments. Measurements of the activity of the Na-K pump and of NKCC were performed on normoxic and hypoxic A549 cells to see whether modulation of the membrane potential and of K channels also affected transport activity in a way that might explain hypoxic transport inhibition. Figure 6A shows that membrane depolarization by increasing extracellular K (5–20 mM) resulted in a dose-dependent inhibition of total $^{86}$Rb uptake as well as inhibition of the Na-K pump and of NKCC. The ouabain- plus bumetanide-insensitive portion of $^{86}$Rb uptake was increased with increasing extracellular K, indicating that this flux represented a K leak. The same pattern of changes in transport activity was seen when extracellular K was increased by the addition of KH$_2$PO$_4$ (Fig. 6B), which indicates that the change in activity was not due to the addition of chloride. To account for an increase in osmolarity by high-KCl concentrations, fluxes were also measured after the addition of NaCl. In this case, NKCC was slightly activated ($20 \pm 3\%$ at 20 mM NaCl), which was probably due to activation by decreasing the cell volume (12), but no effect on Na-K pump-mediated $^{86}$Rb uptake was seen (Fig. 6C).

Figure 7 summarizes the results of flux measurements in the presence of the K-channel inhibitors TEA and iberiotoxin, as well as of the K-channel opener 1-EBIO. Exposure of A549 cells to TEA (Fig. 7A) for 4 h (normoxia) caused an inhibition of total $^{86}$Rb uptake by $\sim 30\%$ of the activity of the Na-K pump ($\sim 18\%$) and of NKCC (55%). The degree of inhibition was about the same in normoxic and hypoxic cells. Iberiotoxin (Fig. 7B) caused an inhibition of total $^{86}$Rb uptake of the Na-K pump and of NKCC of 15–30%, which is less than transport inhibition by hypoxia. In iberiotoxin-treated cells, exposure to hypoxia caused a slight but statistically significant decrease in total $^{86}$Rb uptake, but inhibition was less pronounced than in normoxic cells. In iberiotoxin-treated cells, hypoxia had no effect on the activity of the Na-K pump, whereas inhibition of NKCC was still seen but less pronounced than in normoxic cells. In iberiotoxin-treated cells, hypoxia had no effect on the activity of the Na-K pump, whereas inhibition of NKCC was still seen but less pronounced than in normoxic cells. In contrast, addition of the K-channel opener 1-EBIO doubled total $^{86}$Rb uptake both in normoxic and hypoxic A549-cells (Fig. 8), and NKCC was stimulated threefold. Stimulation of the Na-K pump and of the ouabain- and bumetanide-insensitive portion of $^{86}$Rb uptake was seen in normoxic cells only.

Fig. 5. Expression of K channels in A549 cells. A549 cells were cultured in normoxia. Total RNA was extracted and transcribed by using random primers. cDNAs (1 µg) were enhanced by PCR by using the primers listed in Table 1 under the conditions described in MATERIALS AND METHODS. Result are shown from 1 of 2 preparations with similar results. maxiK, large-conductance Ca- and voltage-sensitive K channel $\alpha$-subunit; Kv 1.2, voltage-gated K channel KCNA1; Kv 1.5, voltage-gated K channel KCNA5; Kv 2.1, delayed rectifier K channel DRK1.
DISCUSSION

This study was performed to test whether hypoxia affects the membrane conductance and K channels of alveolar epithelial cells and, if so, whether these changes relate to the hypoxia-induced inhibition of ion transport. The results show that hypoxia markedly decreased the whole cell current of A549 cells. The hypoxia effect is abolished by TEA and, in part, by iberiotoxin, both of which are potent blockers of MaxiK channels, which are expressed in A549 cells. Inhibitors of K

Fig. 7. Combined effects of hypoxia, TEA, and iberiotoxin on unidirectional 86Rb uptake of A549 cells. Cells were exposed to normoxia and hypoxia (1.5% O2) in absence and presence of 10 mM TEA (A) and iberiotoxin (0.1 μM; B) for 4 h. Values are means ± SD of 6 experiments on 3 different batches of cells. *Statistically significant difference to normoxic control cells, P < 0.05. # Statistically significant difference between normoxic and hypoxic TEA- or iberiotoxin-treated cells, P < 0.05.

Fig. 6. Effects of increased KCl, KH2PO4, and NaCl on unidirectional 86Rb uptake of A549 cells. The activity of the Na-K pump was the portion of 86Rb uptake inhibited by 100 μM ouabain; Na-K-2Cl cotransport was the bumetanide-sensitive (50 μM) portion. Residual indicates ouabain- and bumetanide-insensitive 86Rb uptake. A: membrane depolarization was achieved by increasing the extracellular K concentration (Ko) by addition of KCl to the flux medium. B: to control for Cl effects, KH2PO4 was added to the flux medium; C: NaCl was increased to control for effects induced by the elevated K and osmolarity. *Statistically significant difference to transport activity at an Ko of 5 mM, P < 0.05 (A). Values are means ± SD of 6 experiments on 3 different batches of cells (B and C). Legends indicate final concentrations of KCl (A), KH2PO4 (B), and NaCl (C). Mean values are from triplicate flux measurements on a single batch of cells. Prot., protein.

Fig. 8. Effects of 1-ethyl-2-benzimidazolinone (1-EBIO) and hypoxia on 86Rb uptake of A549 cells. Cells were exposed to normoxia and hypoxia (1.5% O2) in absence and presence of 1 mM 1-EBIO (9) for 4 h. Values are means ± SD of 6 experiments on 3 different batches of cells. *Statistically significant difference to normoxic controls, P < 0.05. # Statistically significant difference between normoxic and hypoxic 1-EBIO-treated cells, P < 0.05.
channels and membrane depolarization by increased extracellular K inhibited $^{86}$Rb uptake via the Na-K pump and NKCC, whereas K-channel stimulation activated $^{86}$Rb uptake. However, K-channel modulators could not prevent hypoxia effects on transport activity.

K channels control the resting membrane potential and are responsible for recycling of K taken up via the Na-K pump in exchange for Na and by other transporters, such as NKCC (11, 22). Thus, in epithelial cells, K recycling is an essential step in the control of transepithelial transport (7). Any inhibition of this conductance would therefore, prevent K recycling, depolarize the membrane potential, and inhibit ion transport (7). This has also been observed in rat alveolar epithelial cells where Ba$^{2+}$ but not TEA inhibited the short-circuit current (25). In contrast, our own results from tracer flux measurements on normoxic A549 cells show that application of TEA and iberiotoxin, as well as membrane depolarization by increasing extracellular K, causes inhibition of total $^{86}$Rb uptake, of the Na-K pump, and of NKCC. We did not study the effects of Ba$^{2+}$. In these experiments, transport inhibition by increased extracellular K was not caused by the elevated concentration of chloride, as indicated by replacement with phosphate and not by the elevated osmolarity, which is known to stimulate NKCC on cell shrinkage in hypertonic media (12), which indicates an essential role of K in transport inhibition, probably an inhibition of K recycling and/or depolarization. These results are in accordance with the interpretation proposed by Venglarik and Dawson (33) that the inhibition of a basolateral K conductance might be the primary event in the inhibition of Na reabsorption. On the other hand, activation of K channels by application of 1-EBIO, an opener of epithelial Ca-dependent K channels (9), increased $^{86}$Rb uptake by stimulation of the Na-K pump and NKCC in normoxia, again pointing out the significance of K channels in the control of transport activity.

The question arises whether K-channel inhibition initiates hypoxic transport inhibition. This hypothesis seemed feasible because K channel inhibition appears to be the primary event in hypoxic pulmonary vasoconstriction and in the peripheral chemoreceptor response to hypoxia (17, 35). However, although K-channel inhibition by inhibitors of BKCa mimics the effects of hypoxia and BKCa channel opening stimulates transport even in hypoxic cells, in both situations hypoxia still exerts its inhibitory effect on $^{86}$Rb uptake. Therefore, if BKCa were the only target of hypoxia, no further change in $^{86}$Rb uptake on exposure to hypoxia should be observed in the presence of TEA, iberiotoxin, or 1-EBIO. Therefore, these results suggest that the typical targets of these reagents, which are Ca-dependent K channels, might not be the primary target affected by hypoxia to inhibit transport activity.

Because whole cell currents were inhibited by hypoxia in the presence of inhibitors of Na and Ca channels and K-specific effects of membrane depolarization on tracer fluxes, we screened primarily for hypoxia effects on K channels. Various types of K channels have been reported to be present in A549 cells. A large-conductance, Ca-activated K channel and a small-conductance K channel have been identified by using the single-channel patch-clamp technique (31). There, most of the K conductance was blocked by TEA, barium, and quinidine but was only moderately sensitive to ATP (31). In cultured primary rat fetal ATII cells, a quinine- and Ca-sensitive K conductance was activated by insulin (21). This channel might be involved in the insulin-stimulated control of cell volume (21). Our own data confirm the presence of MaxiK in A549 lung alveolar epithelial cells based on the presence of specific mRNA (34) and on the sensitivity of whole cell membrane currents to TEA and iberiotoxin. The oxygen-sensitivity of recombinant human MaxiK channels expressed in HeLa cells has recently been reported (15). Oxygen sensitivity of another K channel present in lung-derived neuroepithelial cells, TASK 1, has been reported in H146 cells and in HEK293 cells (14, 26). In contrast, Koong et al. (13) found an activation of K channels of A549 cells only on reoxygenation of hypoxia-exposed cells but not during exposure to hypoxia. It is not known whether other K channels described in cultured alveolar epithelial cells (8, 23, 27) are also sensitive to changes in oxygen. PCR measurements revealed that other typically oxygen-sensitive channels such as Kv 1.2, Kv 1.5, and Kv 2.1 (28) are expressed in A549 cells, but seem not to be involved in the control of ion transport because 4-AP (24) had no effect on whole cell currents.

In further experiments, we compared I-V relationships of hypoxia-, TEA-, and iberiotoxin-sensitive currents. The results show that the hypoxia-sensitive current shows no rectification but rather exhibits a linear I-V relation, whereas TEA- and iberiotoxin-sensitive currents show the rectification properties and reversal potentials typical for voltage-dependent, Ca-activated K channels. This result does not exclude a contribution of MaxiK channel-mediated currents to the hypoxia-induced decrease in whole cell currents but indicates that other membrane conductances not studied here might be involved.

Membrane depolarization and inhibition of K channels by hypoxia initiates pulmonary vasoconstriction and the hemodynamic changes that subsequently lead to alveolar flooding (18). Hypoxia also inhibits alveolar epithelial Na and water reabsorption (29). Therefore, blunting of hypoxia-induced membrane depolarization with K-channel openers might prevent edema formation, not only by preventing hypoxic pulmonary vasoconstriction (2) but also by preventing hypoxic inhibition of alveolar epithelial Na transport due to a stimulation of the recycling of K across the basolateral plasma membrane.

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