O2-sensitive K+ channels in neuroepithelial body-derived small cell carcinoma cells of the human lung

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O'KELLY, I., C. PEERS, AND P. J. KEMP. O2-sensitive K+ channels in neuroepithelial body-derived small cell carcinoma cells of the human lung. Am. J. Physiol. 275 (Lung Cell. Mol. Physiol. 19): L709–L716, 1998.—Neuroepithelial bodies act as airway O2 sensors, but studies of their activity at the cellular level have been severely limited because they are present at such a low density in lung tissue. Small cell lung carcinoma (SCLC) cells are believed to be derived from neuroepithelial body cells and may represent a model system for investigating the mechanisms of airway chemoreception. Here we have used the whole cell patch-clamp technique to investigate the effects of acute hypoxia on voltage-gated ionic currents and membrane potential in H-146 SCLC cells. Step depolarizations evoked transient inward currents due to activation of Na1 and Ca2+ channels, followed by outward K+ currents. K+ currents were partially inhibited by 200 µM Cd2+ (indicative of the presence of a Ca2+-dependent component of the K+ current) and were inhibited by tetraethylammonium (TEA) in a concentration-dependent manner, although even at 100 mM TEA, a residual K+ current could be detected. Hypoxia (Po2 15–20 mmHg) caused a reversible inhibition of outward K+ currents without affecting inward currents. Inhibition by hypoxia was also observed in the presence of Cd2+. Hypoxia and TEA caused membrane depolarization in H-146 cells, and their effects appeared additive. These findings indicate that H-146 cells possess O2-sensitive, Ca2+-independent K+ channels that can influence cell membrane potential. SCLC cells may, therefore, represent a good model for investigating the mechanisms underlying O2 sensing by airway chemoreceptor cells.

hypoxia; ion channels; oxygen sensing; airway; chemoreceptor

ADAPTIVE RESPIRATORY and cardiovascular reflexes evoked in response to acute hypoxic episodes are reliant on sensory information supplied by chemoreceptors. O2 sensing in the airway is believed to occur at neuroepithelial bodies (NEBs) (14, 27). NEBs are discrete clusters of cells situated throughout the airway and, because they localize at bronchial bifurcations, are particularly well positioned to detect Po2 levels in inspired gases. Evidence for their role and importance in O2 sensing is incomplete. However, their prominence in neonatal lungs (6) and the association of pathological respiratory conditions such as apnea of prematurity and sudden infant death syndrome with NEB cell hyperplasia (9) strongly suggest that NEBs are involved in both the initiation of breathing at birth and respiratory control in the adult.

Although very little is known about chemotransduction in NEBs, cellular studies over the past decade have provided many insights into the mechanisms by which hypoxia excites the primary arterial chemoreceptor, the carotid body where hypoxia stimulates transmitter release from sensory glomus cells (11, 22). This transmitter release correlates well with increased afferent chemosensory carotid sinus nerve discharge (10, 11) and arises because hypoxia inhibits specific K+ channels in glomus cells. In normoxia, these channels contribute to the maintenance of resting membrane potential (22). In hypoxia, K+ channel activity is suppressed, glomus cells depolarize, and voltage-gated Ca2+ channels are activated. The concomitant rise in intercellular Ca2+ concentration triggers transmitter release (18).

NEB cells, like carotid body glomus cells, contain an array of transmitters, including amines and peptides, and are commonly identified by positive staining for serotonin [5-hydroxytryptamine (5-HT)] (27). In the lung, 5-HT may be both a neurotransmitter (activating NEB afferent fibers) and, when released into the pulmonary circulation, a regulator of local vasomotor tone (15) and, therefore, of ventilation-perfusion matching. There is evidence to indicate that NEB cells release 5-HT in hypoxia (14, 24), and this has prompted investigations into the cellular mechanisms underlying hypoxia-evoked secretion from NEB cells. Using NEB cells isolated from fetal rabbit lungs, Youngson et al. (27) identified voltage-gated Na+, Ca2+, and K+ channels and demonstrated a selective, reversible inhibition of K+ currents by acute hypoxia. These findings provided preliminary evidence that NEB cells might respond to acute hypoxia in a manner comparable to that seen in carotid body glomus cells. However, there has been a paucity of further information due to the extreme difficulty in preparing isolated NEB cells for such studies: in the fetal lung, NEBs represent 0.2% of lung tissue mass, and this decreases to 0.04% in adult tissue (12). This has prompted us to investigate an alternative model system for studying airway chemoreception.

Small cell lung carcinoma (SCLC) is a common form of lung cancer, and SCLC cells are likely to originate from the same population of committed pulmonary neuroendocrine precursor cells that give rise to NEB cells (8). SCLC and NEB cells share numerous features including the presence of 5-HT, peptide transmitters, and voltage-gated ion channels (20). Such similarities suggest that SCLC cells might represent an immortal cell model for airway chemoreceptors, just as pheochromocytoma (PC-12) cells have been suggested to be a representative model for the glomus cell (5). In the present study, therefore, we have characterized the
electrophysiological properties of one SCLC cell line, NCI-H-146, and investigated whether these cells might act as O$_2$ sensors in a manner comparable to that of acutely isolated NEB cells. The H-146 cell line was selected in preference to other SCLC lines because it shares similar K$^+$-current characteristics with NEB cells and both possess mRNA encoding $K_V3.3$ (26).

Part of this work has been presented in abstract form (19).

**METHODS**

**Cell Culture**

H-146 cells were purchased from American Type Culture Collection (Manassas, VA) and were of unknown passage number. On delivery, the cells were thawed rapidly at 37°C, diluted 1:12 with RPMI 1640 culture medium (containing L-glutamine) supplemented with 10% fetal calf serum, 2% sodium pyruvate, and 2% penicillin-streptomycin (all from Gibco, Paisley, UK), and incubated at 37°C for 18 h in a humidified atmosphere of 5% CO$_2$-95% air. After this period, cells in suspension culture were removed from the flask, centrifuged at 150 g for 5 min, resuspended in fresh medium, and reseeded in flasks at low density. This point was designated passage 1. Subsequently, the medium was changed every 2 days, and cells were passaged every 6–7 days by splitting in the ratio 1:5. Cells were used between passages 1 and 10.

**Electrophysiology**

Solutions and chemicals. Unless stated otherwise, all chemicals were of the highest grade available and were purchased from Sigma (Poole, UK). The standard pipette solution was K$^+$ rich and contained (in mM) 10 NaCl, 117 KCl, 2 MgSO$_4$, 10 HEPES, 1 CaCl$_2$, and 2 Na$_2$ATP, pH 7.2 with KOH; Ca$^{2+}$ concentration = 27 nM. The standard bath solution was Na$^+$ rich and contained (in mM) 135 NaCl, 5 KCl, 1.2 MgCl$_2$, 5 HEPES, 2.5 CaCl$_2$, and 10 D-glucose, pH 7.4 with NaOH. Tetraethylammonium (TEA) and CdCl$_2$ were added to the bath solution where indicated. When TEA was employed at concentrations $\approx$ 10 mM, osmolarity was maintained by the isosmotic substitution of NaCl.

All tubing was gas impermeant (Tygon tubing, BDH, Atherstone, UK) and kept as short as possible. Normoxic solutions were equilibrated with room air. Solutions were made hypoxic, where appropriate, by bubbling with N$_2$ for at least 30 min before perfusion of the cells. This procedure produced no shift in pH. The solution flow rate was $\approx$ 5 ml/min. In some experiments, P O$_2$ was measured (at the cell) with a calibrated carbon-fiber electrode (17); P O$_2$ under hypoxic conditions ranged from 15 to 20 mmHg for the experiments reported.

Whole cell recording. A 0.5-ml aliquot of cells was removed from a culture flask on the morning of the experiment. After titration (10 passes through a 1-ml automatic pipette tip), the cells were allowed to adhere to poly-L-lysine-coated glass coverslips at 37°C for at least 1 h before being placed in a temperature-controlled perfusion chamber (Brook Industries, Lake Villa, IL) mounted on the stage of a Nikon TMS inverted microscope. All experiments were carried out at 21 ± 1°C. Patch-clamp pipettes were manufactured from standard-walled borosilicate glass capillary tubing on a two-stage Narishige PP-83 pipette puller (Narishige Scientific Instrument Laboratory, Tokyo, Japan), were routinely heat polished on a Narishige microforge, and had measured tip resistances of 3–8 MΩ (when filled with a K$^+$-rich pipette solution).

Resistive-feedback voltage clamp was achieved with an Axopatch 200A amplifier (Axon Instruments, Foster City, CA). Voltage protocols were generated and currents were recorded with pClamp 6.0.3 software with the use of a Digidata 1200 analog-to-digital converter (Axon Instruments). Data were filtered (4-pole Bessel) at 2 kHz and digitized at 5 kHz. After successful transition to the whole cell recording mode (13), capacitance transients were compensated for and measured. To evoke ionic currents in H-146 cells, three voltage protocols were used: 1) current-voltage (I-V) protocol: holding potential = −70 mV, step increments = 10 mV from −100 to +60 mV, step duration = 50 ms, and frequency = 0.1 Hz; 2) ramp protocol: holding potential = −70 mV, −100 to +60 mV, ramp duration = 1 s, and frequency = 0.1 Hz; and 3) time series: holding potential = −70 mV, single increment to 0 mV, step duration = 50 ms, and frequency = 0.1 Hz.

Currents resulting from the I-V and time-series protocols were recorded at two analog channels; one unsubtracted and one on-line subtracted with a P/N protocol, where P is pulse amplitude and N is number of prepulses, each 1/N of P.
Where necessary, series resistance compensation was used at 100%.

Fast current clamp was achieved with the same amplifier, and the solutions were the same as those used in the voltage-clamp experiments. Cells were clamped at $I = 0$ pA, and the recorded voltage was filtered at 2 kHz and digitized at 2 kHz.

Data handling and calculations. The magnitude of the steady-state outward currents (in both the I-V and time-series protocols) was measured as the mean current between 46 and 49 ms of the voltage pulse. Transient inward currents were measured at their peak. Where indicated, currents were normalized to a nominal cell area by dividing by measured cell capacitance (to yield current density). In the time-series plots, the currents from each separate experiment were normalized by dividing the current at each point by the mean of the first five currents in that series; in Figs. 1–7, values are means ± SE; $n$ refers to the number of cells. Statistical comparisons were made with paired Student’s t-test, with $P < 0.05$ being considered significant.

RESULTS

Characterization of Whole Cell Currents in H-146 Cells

Figure 1A shows a representative family of whole cell currents evoked in H-146 cells by the I-V protocol. The currents had two main characteristics: in response to step depolarizations, rapidly activating, transient inward currents were observed, which were followed (at potentials positive to $-30$ mV) by sustained outward currents. Mean cell capacitance was $5.98 \pm 0.47$ pF (range = 2.9–11.2 pF; $n = 38$). The mean current density versus voltage relationships ($n = 27$) for these two currents are shown in Fig. 1B. The inward currents were transient and were maximal in amplitude at 0 mV. The mean current density of the inward currents, measured at 0 mV, was $-61.5 \pm 7.1$ pA/pF (range = $-7.0$ to $-131.7$ pA/pF). Bath application of 1 µM TTX significantly reduced the inward currents by $82.1 \pm 8.2\%$ ($P < 0.003; n = 5$; Fig. 2, A and B), indicating that the largest component of inward current was attributable to voltage-gated Na$^+$ currents. When the nonselective blocker of voltage-gated Ca$^{2+}$ channels (Cd$^{2+}$, 200 µM) was applied (Fig. 2, C and D), inward currents were reduced in amplitude by $13.1 \pm 5.0\%$ ($P < 0.03; n = 9$). Thus inward currents in H-146 cells arose due to activation of voltage-gated Na$^+$ and Ca$^{2+}$ channels.

Outward currents (mean density at 0 mV = $36.1 \pm 3.8$ pA/pF, range = 10.9 to 79.6 pA/pF; $n = 27$) were...
activated at voltage steps positive to −30 mV and were blocked in a concentration-dependent manner by a bath application of TEA (Fig. 3). The calculated IC₅₀ value for externally applied TEA was 1.24 ± 0.27 mM (n = 20). In the majority of cells, there was a small TEA-resistant component of the current even when the blocker was present at 100 mM (Fig. 3B). As well as reducing the amplitude of inward currents (Fig. 2, C and D), the bath application of 200 µM Cd²⁺ significantly depressed the outward currents to 75.6 ± 5.2% of control values (P < 0.005; n = 9; Fig. 4), suggesting that a significant proportion of the outward current was attributable to current flow through Ca²⁺-activated K⁺ channels.

Effect of Hypoxia on Ionic Currents in H-146 Cells

Reduction of perfusate PO₂ from that of atmospheric air to 15–20 mmHg resulted in a sustained and reversible inhibition of the outward currents at all activating test potentials studied (Fig. 5). Hypoxic inhibition of K⁺ currents was observed with both the voltage-step (Fig. 5A) and ramp (Fig. 5B) protocols. With the step protocol, hypoxia induced a significant inhibition of 15.4 ± 1.6% (P > 0.05; n = 30). Interestingly, the effect of hypoxia (determined employing the ramp protocol and measuring current amplitudes at 0 mV) was larger (29.7 ± 3.1%; P < 0.0001; n = 8) and is shown in Fig. 5C. Maximal inhibition under these experimental conditions was achieved within 3 min, and the effect reversed on removal of hypoxia within 2 min. With the time-series protocol, inward currents were seen to be unaffected by hypoxia (Fig. 5A).

To investigate further the nature of the hypoxia-sensitive K⁺ current in H-146 cells, we examined the effects of hypoxia during blockade of Ca²⁺-activated K⁺ currents with 200 µM Cd²⁺ (Fig. 6). When the step protocol was used, hypoxia, in the presence of Cd²⁺, produced a significant (P < 0.001) inhibition of outward K⁺ currents that was similar in magnitude (19.5 ± 3.0% of the control currents, i.e., before the addition of Cd²⁺; n = 10) to that seen in the absence of Cd²⁺ (Fig. 5). This finding supports the hypothesis that hypoxia selectively inhibits a Ca²⁺-insensitive K⁺ current in H-146 cells.
Effects of Hypoxia and TEA on Membrane Potential in H-146 Cells

With the use of the same bath and pipette solutions as those employed in voltage-clamp recordings, the resting membrane potential of H-146 cells was found to be $-40.8 \pm 2.1$ mV ($n = 27$) under normoxic conditions. When the bath perfusate $P_{\text{O}_2}$ was reduced to 15–20 mmHg, all H-146 cells tested ($n = 26$) reversibly depolarized (Fig. 7A shows a typical recording). Membrane potentials obtained in H-146 cells before, during, and after exposure to hypoxia are plotted in Fig. 7B. Depolarization of the cells was also consistently observed during bath application of a concentration of TEA (30 mM) that maximally inhibited $K^+$ currents (see Fig. 3), and, wherever tested ($n = 29$), both hypoxia and TEA separately caused membrane depolarization (Fig. 7B). Furthermore, the depolarizing effects of hypoxia and TEA appeared additive (Fig. 7).

DISCUSSION

In agreement with a previous study (20) using this and other SCLC cell lines, the present study has identified the presence of voltage-gated $Na^+$, $Ca^{2+}$, and $K^+$ channels in H-146 cells. Inward currents were attributable to activation of both $Na^+$ and $Ca^{2+}$ channels. $Na^+$ channels accounted for ~80% of the inward current, and $Ca^{2+}$ channels accounted for the remaining 20% on the basis of the degree of inhibition caused by TTX and $Cd^{2+}$, respectively (Fig. 2). Outward $K^+$ currents were also present in H-146 cells, indicating that this SCLC cell line has an array of voltage-gated ion channels expected of cells of neuroendocrine origin. Although $Ca^{2+}$-channel subtypes have been investigated in detail in SCLC cells (4, 23, 25), little is known about the properties of the outward $K^+$ currents in these cells. Through bath application of 200 $\mu$M $Cd^{2+}$, we have identified $Ca^{2+}$-dependent and $Ca^{2+}$-independent components of the whole cell $K^+$ current (Fig. 4). As described for carotid body glomus cells (21), $Cd^{2+}$ would be expected to prevent $Ca^{2+}$ influx through voltage-gated $Ca^{2+}$ channels and so, indirectly, would prevent activation of $Ca^{2+}$-dependent $K^+$ channels.

$K^+$ currents in H-146 cells were found to be inhibited in a concentration-dependent manner by TEA, a $K^+$-channel blocker that is only poorly selective between subtypes of the $K^+$ channel. However, even at TEA concentrations as high as 100 mM, a significant residual outward current remained during step depolar-
fetal rabbit lungs (27), which show selective inhibition of K⁺ current by hypoxia, whereas inward currents were unaffected. A further study (28) suggested that the contribution of Ca²⁺-activated K⁺ currents in the hypoxic response is small. Youngson et al. (27, 28) did not investigate further the nature of the O₂-sensitive K⁺ current in NEB cells, so no further information is available in this tissue. In the carotid body, different classes of K⁺ channel have been shown to be O₂ sensitive: in rabbit glomus cells, a Ca²⁺-insensitive, voltage-gated K⁺ current that can be blocked by 4-aminopyridine has been identified (7, 16), whereas in glomus cells of the rat carotid body, both a high-conductance, Ca²⁺-dependent K⁺ current (21) and a voltage-insensitive, TEA- and 4-aminopyridine-resistant, low-conductance K⁺ current (1) have been shown to be inhibited by hypoxia. In PC-12 cells, which have been proposed as a model for a glomus cell, the hypoxia-sensitive K⁺ current was shown to be the TEA-sensitive Kv1.2 (5). Clearly, there are numerous K⁺-channel types capable of being inhibited by hypoxia, even within the same tissue type, and molecular identification of these subclasses of K⁺ channel will be required before a more complete understanding of the mechanisms underlying channel inhibition by hypoxia can be achieved.

In carotid body glomus cells, the physiological significance of hypoxic inhibition of K⁺ channels is clear:
channel inhibition results in cell depolarization, activation of voltage-gated Ca\textsuperscript{2+} channels, and a resulting Ca\textsuperscript{2+} influx that triggers neurosecretion. The little available evidence suggests that a similar mechanism may operate in the NEB cell; Youngson et al. (27) reported that, in hypoxia, the firing frequency of current-clamped NEB cells increased due to an increased slope of pacemaker potential resulting from K\textsuperscript{+}-current inhibition. However, this study was conducted in cells that were induced to fire action potentials by brief, supramaximal depolarizing current injections. It is not known whether NEB cells spontaneously fire action potentials in normoxia or whether they are quiescent. Indeed, it is not known whether hypoxia alone can induce membrane depolarization in NEB cells. To investigate this in H-146 cells, we used current-clamp (I = 0 pA) recordings and found most cells to be quiescent, with a mean resting potential of approximately −40 mV. Hypoxia consistently and reversibly depolarized H-146 cells. This effect was considered quite modest, and we cannot at present determine whether such a depolarization is sufficient to activate voltage-gated Ca\textsuperscript{2+} entry (this depends on how close to the resting membrane potential the threshold for significant Ca\textsuperscript{2+}-channel activation lies). However, it is noteworthy that in rat glomus cells, which are also usually quiescent in normoxia, similar modest depolarizations can cause dramatic voltage-gated Ca\textsuperscript{2+} entry (2).

It is possible that the O\textsubscript{2}-sensitive K\textsuperscript{+} current recorded at 0 mV may not be the same O\textsubscript{2}-sensitive K\textsuperscript{+} current that is responsible for contributing to resting membrane potential. However, the observation that hypoxia causes membrane depolarization in H-146 cells suggests that the O\textsubscript{2}-sensitive current recorded in voltage clamp is active at (and so contributing to) the resting membrane potential. We also found that TEA caused membrane depolarization in H-146 cells (Fig. 7), which might suggest that the O\textsubscript{2}-sensitive K\textsuperscript{+} current that is responsible for membrane depolarization is TEA sensitive (as is the case in PC-12 cells (5)). However, we found that the effects of TEA and hypoxia were additive in terms of membrane depolarization (Fig. 7), even when a concentration of TEA (30 mM) that maximally inhibited the outward K\textsuperscript{+} currents was used, suggesting that TEA and hypoxia act at different K\textsuperscript{+} channels to cause membrane depolarization. This conclusion is not obviously consistent with our voltage-clamp data, which indicate that the degree of K\textsuperscript{+} current inhibited by hypoxia (Fig. 5) was greater than the fraction of current remaining in the presence of high concentrations of TEA (Fig. 3). However, these data were obtained at a membrane potential of 0 mV, and it is likely that the relative proportion of K\textsuperscript{+} currents active at this potential are not the same as those active at the cell resting membrane potential.

In summary, our findings have revealed that, like NEB cells, the SCLC line H-146 possesses voltage-gated Na\textsuperscript{+}, Ca\textsuperscript{2+}, and K\textsuperscript{+} channels and that a Ca\textsuperscript{2+}-insensitive component of the K\textsuperscript{+} current is reversibly inhibited by hypoxia. Although we have not investigated the nature of the O\textsubscript{2}-sensitive K\textsuperscript{+} channel in greater detail, we have found that it contributes to the resting membrane potential and that its inhibition gives rise to cell depolarization. These latter findings have also provided evidence to suggest that the O\textsubscript{2}-sensitive K\textsuperscript{+} current is not TEA sensitive. The lack of available data concerning cellular studies on NEB cells precludes detailed comparisons with the H-146 cells as reported herein. However, wherever possible, our findings compare favorably and are also consistent with studies of carotid body glomus cells. Furthermore, a previous study (26) has identified a putative O\textsubscript{2} sensor, NADPH oxidase, in both NEB and SCLC cells. Our findings now show that the major functional feature of NEB cells (an O\textsubscript{2}-sensitive K\textsuperscript{+} channel) is present in an SCLC cell line. Therefore, although H-146 cells may not be the chosen model in which to study neurotransmitter release [due to the documented quantitative differences in, for example, neurosecretory granule content (3)], we suggest that this SCLC cell line represents a useful model system for investigating in detail the cellular and molecular mechanisms underlying O\textsubscript{2} sensing by airway chemoreceptors.

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