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Chronic hypoxia remodels voltage-gated Ca^{2+} entry in a human airway chemoreceptor cell line

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Abstract

Arterial and airway chemoreceptors respond to acute hypoxia by depolarizing, thereby activating voltage-gated Ca^{2+} channels and so permitting Ca^{2+} entry to trigger transmitter release. Following periods of prolonged hypoxia, these cells undergo a form of remodelling which involves altered expression of ion channels. Here, we use microspectrofluorimetric recordings of voltage-gated Ca^{2+} entry (activated by exposure of cells to 50 mM K^+) to show that chronic hypoxia suppresses such Ca^{2+} entry in model airway chemoreceptor (H146) cells. Furthermore, Ca^{2+} entry via L-type channels is suppressed, whilst entry via N-type channels is greatly enhanced. The suppressed response, together with dramatic remodelling of routes available for voltage-gated Ca^{2+} entry, is likely to alter significantly the acute O_2 sensing properties of these cells.

Keywords: Chemoreceptor; Neuroepithelial body; H146 cell; Chronic hypoxia; Voltage-gated Ca^{2+} channel; Microfluorimetry; $[Ca^{2+}]_i$

Acute hypoxia is now recognized as an important modulator of ion channel activity, and hypoxic inhibition of various classes of ion channels contributes to important physiological processes [6,8,14]. These include hypoxic vasodilation in the systemic circulation, vasoconstriction in the pulmonary circulation [8,19] and, in neurones and neuronally-derived cells, modulation of transmitter release [2,4]. In addition, rapid modulation of ion channels in arterial and airway chemoreceptors underlies the physiological responses of these cells to hypoxia, initiating important reflexes which ensure optimal ventilation–perfusion matching [15].

In contrast to these effects of acute hypoxia to alter the activity of existing ion channels, prolonged (chronic) hypoxia regulates ion channel expression. For example, in the model neuroendocrine pheochromocytoma PC12, chronic hypoxia selectively up-regulates L-type Ca^{2+} channels, whilst other voltage-gated Ca^{2+} channels are unaffected [5]. In arterial chemoreceptor (carotid body) glomus cells, such remodelling of ion channel expression underlies the adaptive response of this organ to prolonged hypoxia (an important component of high altitude acclimatization) and, conversely, suppresses postnatal maturation of chemosensitivity [16,20]. Neuroepithelial bodies (NEBs), which act as airway chemoreceptors, exhibit many commonalities with arterial chemoreceptors in their ability to sense acute changes in O_2 levels [2,15]. However, due to difficulties in isolating and studying NEBs, progress in understanding their responses to acute hypoxia is limited, and their adaptive responses to chronic hypoxia are completely unexplored. To address such issues, we have developed a model airway chemoreceptor, the human small cell lung carcinoma, H146 [7,9–12]. We have shown that these cells, like NEBs, express specific K^+ channels which are inhibited by acute hypoxia. This leads to cell membrane depolarization of a magnitude which is sufficient to cause Ca^{2+} influx which in turn permits exocytosis. In the present study, we have investigated the adaptive responses of these cells to chronic hypoxia, and have specifically focussed on voltage-gated Ca^{2+} entry, since this is such a fundamental step in hypoxic signal transduction. Our results, based on the non-invasive use of microspectrofluorimetric recordings, suggest that chronic hypoxia dramatically alters the functional profile of voltage-gated Ca^{2+} channels in these cells.

H146 cells were purchased from American Tissue Type Cell Collection (Rockville, MA) and were of unknown passage number. Upon delivery, cells were thawed rapidly at 37°C, diluted 1:12 with RPMI 1640 culture medium

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(containing L-glutamine) supplemented with 10% fetal calf serum, 2% sodium pyruvate and 2% penicillin/streptomycin (all from Gibco, Paisley, Strathclyde, UK) and incubated at 37°C for 18 h in a humidified atmosphere of 5% CO_2/95% air (~21% O_2). Following this period, cells in suspension culture were removed from the flask, centrifuged at 150 × *g* for 5 min, resuspended in fresh medium and re-seeded 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 passage 1 and 10. To examine the effects of chronic hypoxia, cells were maintained exactly as described above, but for the 24 h period prior to experimentation, they were transferred to a humidified incubator equilibrated with 10% O_2, 5% CO_2 and 85% N_2. On the day of recording, cells were plated onto glass poly-L-lysine coated coverslips, replaced into the appropriate environments (normoxic or hypoxic) and allowed to adhere for at least 1 h before use.

Changes in $[Ca^{2+}]_i$ were measured in H146 cells attached to glass coverslip fragments using methods described previously [17,18]. In brief, cells were incubated with bath solution (see below for composition) containing 4 μM Fura2-AM (Molecular Probes, Cambridge, UK) at room temperature for 1 h (in a normoxic or hypoxic environment, as appropriate). The cell layer was then rinsed with bath solution and changes in $[Ca^{2+}]_i$ were measured with a Joyce Loeble PhoCal apparatus (Applied Imaging, Newcastle, UK). The $[Ca^{2+}]_i$ was monitored in groups of approximately 8–20 cells for each recording, and was measured from the fluorescence emitted at 510 nm due to alternating excitation at 340 and 380 nm using a rotating filter wheel. Due to inherent calibration inaccuracies associated with this methodology [3], data are expressed as changes in ratio units (r.u.), rather than being converted to absolute $[Ca^{2+}]_i$ values.

During all recordings, cells were continually perfused with a solution of composition (in mM): 135 NaCl, 5 KCl, 1.2 $MgCl_2$, 5 HEPES, 2.5 $CaCl_2$, 10 D-glucose; pH 7.4 with NaOH. To depolarize cells, $[K^+]_o$ was raised to 50 mM and the $[Na^+]_o$ was reduced accordingly to maintain osmolarity. In some experiments, extracellular Ca^{2+} was replaced with 1 mM EGTA (Ca^{2+}-free solution). All other drugs were applied in the perfusate, except peptide toxins, which were applied for 1 h in a static bath prior to recordings. Results are presented as mean ratio changes with SEM values, and statistical analysis was carried out using unpaired *t*-tests.

Basal Ca^{2+} levels, recorded whilst cells were perfused with solution containing 5 mM K^+, were not significantly different ($P > 0.5$) in the two cell groups, being 0.48 ± 0.01 r.u. in controls ($n = 29$ recordings) and 0.47 ± 0.01 r.u. in chronically hypoxic cells ($n = 14$). Exposure of H146 cells to 50 mM K^+ caused rapid and reversible rises of $[Ca^{2+}]_i$ (e.g. Fig. 1A, top left) and these rises were due to Ca^{2+} influx, since they were abolished in Ca^{2+}-free solutions (e.g. Fig. 1B, top left). Furthermore, the Ca^{2+} influx occurred via voltage-gated Ca^{2+} channels, since no rises

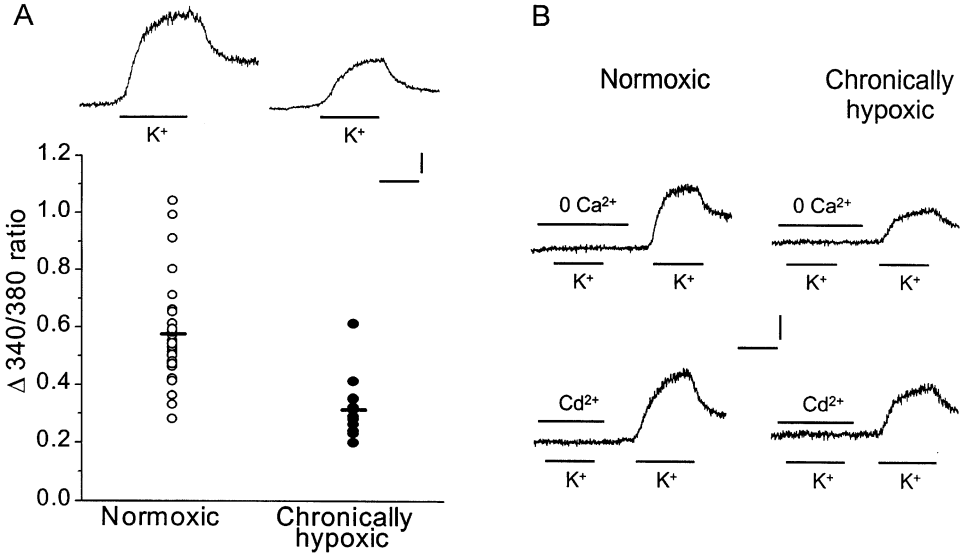

Fig. 1. (A) Example recordings of $[Ca^{2+}]_i$ rises in response to cell depolarization, evoked by application of 50 mM K^+ (K^+, applied for the periods indicated by the horizontal bars). Recordings were made from normoxic (top left) and chronically hypoxic (top right) cells. Also plotted (lower panel) are peak rises of $[Ca^{2+}]_i$ evoked by 50 mM K^+ in all normoxic cells (open symbols) and all chronically hypoxic cells (filled symbols). Each point represents the individual responses of 29 control recordings and 14 recordings from chronically hypoxic cells. Horizontal bars represent mean response for each group. (B) Typical effects of Ca^{2+}-free perfusate on K^+-evoked rises of $[Ca^{2+}]_i$ in normoxic (top left) and chronically hypoxic (top right) cells. Also shown are typical effects of 200 μM Cd^{2+} on K^+-evoked rises of $[Ca^{2+}]_i$ in normoxic (lower left) and chronically hypoxic (lower right) cells. For all examples, the first application of 50 mM K^+ was during perfusion with Ca^{2+}-free or Cd^{2+}-containing perfusate and the second application was following reperfusion with normal bath solution (as indicated). Scale bars: vertical, 0.2 r.u.; horizontal, 30 s. These apply to all traces shown in (A,B).

were evoked by 50 mM K^+ in the presence of 200 μM Cd^{2+}, a non-selective blocker of voltage-gated Ca^{2+} channels (e.g. Fig. 1B, lower left). Following 24 h exposure to 10% O_2, 50 mM K^+ was still capable of evoking rises of $[Ca^{2+}]_i$ (e.g. Fig. 1A, top right), and these rises remained attributable to Ca^{2+} influx through voltage-gated Ca^{2+} channels, since they were abolished in Ca^{2+}-free perfusate (e.g. Fig. 1B, top right) and in the presence of 200 μM Cd^{2+} (e.g. Fig. 1B, lower right). However, the mean magnitude of the rises evoked by 50 mM K^+ was significantly reduced ($P < 0.0001$) from 0.57 ± 0.04 r.u. in control cells ($n = 29$) to 0.31 ± 0.03 r.u. ($n = 14$) in chronically hypoxic cells (Fig. 1A).

The above-described suppression of K^+-evoked rises of $[Ca^{2+}]_i$ observed in chronically hypoxic cells is likely due to down-regulation of voltage-gated Ca^{2+} channels in H146 cells. These and related cell lines have been shown to express multiple types of voltage-gated Ca^{2+} channels [1,13], some or all of which would contribute to depolarization-evoked rises of $[Ca^{2+}]_i$. To investigate the contribution of different classes of Ca^{2+} channels to K^+-evoked rises of $[Ca^{2+}]_i$, we examined the ability of various selective Ca^{2+} channel blockers to suppress evoked rises of $[Ca^{2+}]_i$ in both control and chronically hypoxic cells. Results are summarized in Fig. 2. For normoxic cells, the L-type channel blocker, nifedipine (2 μM), suppressed K^+-evoked rises of $[Ca^{2+}]_i$ by ca. 33% ($P < 0.001$, Fig. 2A), whereas in chronically hypoxic cells the same concentration of nifedipine was without significant effect (Fig. 2B). In contrast, pre-exposure of cells to 1 μM ω-conotoxin GVIA (ω-CgTx), a selective inhibitor of N-type channels, caused no significant reduction of K^+-evoked rises of $[Ca^{2+}]_i$ in normoxic cells, whereas in chronically hypoxic cells K^+-evoked rises of $[Ca^{2+}]_i$ were suppressed by 80% following ω-CgTx treatment ($P < 0.0001$). Exposure of cells to 200 nM ω-agatoxin GIVA (ω-Aga; a selective inhibitor of P/Q type channels) for 1 h prior to recordings caused similar reductions of K^+-evoked rises of $[Ca^{2+}]_i$ in the two cell groups (40% reduction in controls, $P < 0.01$, Fig. 2A; 48% reduction in chronically hypoxic cells, $P < 0.002$, Fig. 2B).

The present study reports a remarkable shift in the relative contributions of various classes of voltage-gated Ca^{2+} channels to Ca^{2+} influx stimulated by cell depolarization in H146 cells following a 24 h period of chronic hypoxia. In normoxic cells, Ca^{2+} influx is mediated by L-type and P/Q-type channels, with only a small contribution from N-type, as determined pharmacologically. Following a 24 h period of chronic hypoxia, the most striking effects were the decrease in the influence of L-type channels mediating Ca^{2+} influx, and the dramatic increase in Ca^{2+} influx via N-type channels (Fig. 2B). The possibility that such effects are due to the fact that hypoxic cells were more depolarized under resting conditions (e.g. due to altered K^+ channel expression) is unlikely, since N-type channels are more sensitive to inactivation than L-type under such conditions [13]. This suggests that prolonged hypoxia exerts opposing effects on the functional expression of L-type and N-type Ca^{2+} channels, an effect which is reflected in both absolute and relative contributions to Ca^{2+} influx. Whether these effects are due to altered numbers of expressed channels, or to altered activity of existing channels requires further study. However, our interpretation is clouded somewhat by the apparent partial loss of selectivity of the toxins

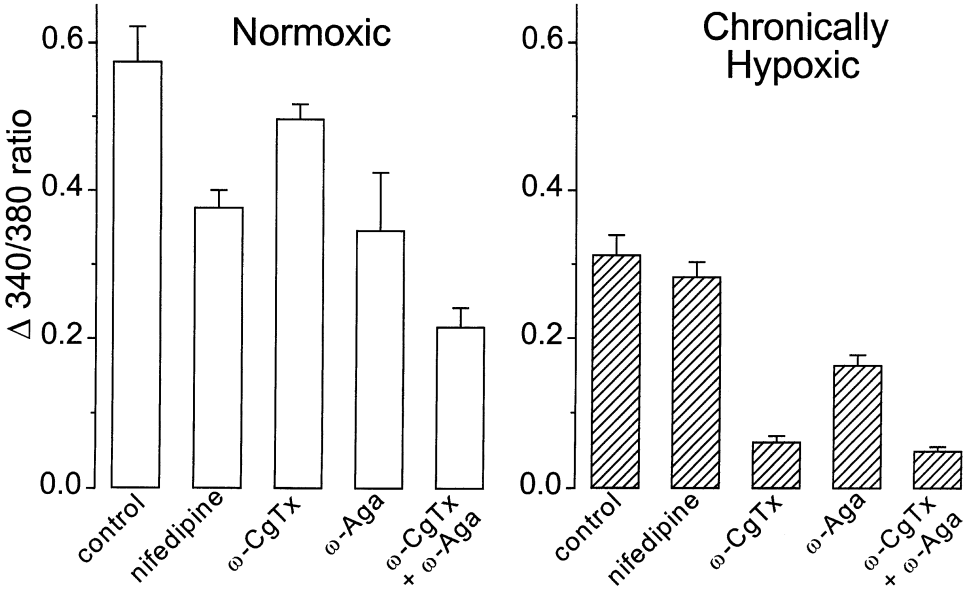

Fig. 2. Mean rises of $[Ca^{2+}]_i$ recorded in normoxic cells (open bars, A) and chronically hypoxic cells (hatched bars, B) in response to application of perfusate containing 50 mM K^+. Recordings were made in the absence of Ca^{2+} channel blockers (control), or in the presence of 2 μM nifedipine, or after pre-incubation with 1 μM ω-conotoxin GVIA or 200 nM ω-agatoxin GIVA, or both toxins, as indicated. Each bar represents the mean response (with vertical SEM bar) taken from the number of recordings shown above each bar.

employed in chronically hypoxic cells. Thus, whilst ω-CgTx reduced responses by 80%, ω-Aga was also able to block 48% of the rises in $[Ca^{2+}]_i$, and when both toxins were applied together, the reduction of the response was not significantly greater than that of ω-CgTx alone (Fig. 2B). This is in marked contrast to the additive effects of the two toxins in control cells (Fig. 2A), and represents an effect we cannot account for at present.

In summary, our results provide the first information concerning remodelling of airway chemoreceptor function in response to chronic hypoxia. The general suppression of voltage-gated Ca^{2+} influx, together with the dramatically altered functional expression profile of specific sub-types of Ca^{2+} channels (both relative and absolute), will doubtless have profound effects on stimulus-evoked transmitter release from these cells and, as such, is likely to represent an important adaptive response to the cells following periods of prolonged hypoxia.

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