Effects of hypoxia on membrane potential and intracellular calcium in rat neonatal carotid body type I cells

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1. We have studied the effects of hypoxia on membrane potential and $[Ca^{2+}]_i$ in enzymically isolated type I cells of the neonatal rat carotid body (the principal respiratory O_2 chemosensor). Isolated cells were maintained in short term culture (3–36 h) before use. $[Ca^{2+}]_i$ was measured using the Ca^{2+}-sensitive fluoroprobe indo-1. Indo-1 was loaded into cells using the esterified form indo-1 AM. Membrane potential was measured (and clamped) in single isolated type I cells using the perforated-patch (amphotericin B) whole-cell recording technique.

2. Graded reductions in P_{O_2} from 160 Torr to 38, 19, 8, 5 and 0 Torr induced a graded rise of $[Ca^{2+}]_i$ in both single and clumps of type I cells.

3. The rise of $[Ca^{2+}]_i$ in response to anoxia was 98 % inhibited by removal of external Ca^{2+} (+ 1 mM EGTA), indicating the probable involvement of Ca^{2+} influx from the external medium in mediating the anoxic $[Ca^{2+}]_i$ response.

4. The L-type Ca^{2+} channel antagonist nicardipine (10 μM) inhibited the anoxic $[Ca^{2+}]_i$ response by 67 %, and the non-selective Ca^{2+} channel antagonist Ni^{2+} (2 mM) inhibited the response by 77 %.

5. Under voltage recording conditions, anoxia induced a reversible membrane depolarization (or receptor potential) accompanied, in many cases, by trains of action potentials. These electrical events were coincident with a rapid rise of $[Ca^{2+}]_i$. When cells were voltage clamped close to their resting potential (−40 to −60 mV), the $[Ca^{2+}]_i$ response to anoxia was greatly reduced and its onset was much slower. Under voltage clamp conditions, anoxia also induced a small inward shift in holding current.

6. We conclude that anoxia promotes a rise of $[Ca^{2+}]_i$ in type I cells, principally through voltage-gated Ca^{2+} entry which occurs in response to the receptor potential and/or concomitant electrical activity. We propose that this mechanism forms the basis for hypoxic chemotransduction in the carotid body.

In mammals, the ventilatory response to hypoxia relies upon the O_2-sensing properties of arterial chemoreceptors, especially the carotid bodies. The primary sensory element within the carotid body is believed to be the type I cell which responds to a fall of P_{O_2} with a Ca^{2+}-dependent secretion of neurotransmitter substances (Gonzalez, Almaraz, Obeso & Rigual, 1992). The cellular mechanism underlying O_2-sensing by these cells is, however, controversial. Several electrophysiological studies have found that type I cells express K^+ channels which are inhibited by hypoxia (although there is variability in the nature of the O_2-sensitive K^+ channels in different preparations: see Lopez-Lopez, Gonzalez, Urena & Lopez-Barneo, 1989; Delpiano & Hescheler, 1989; Peers, 1990; Stea & Nurse, 1991; Ganfornina & Lopez-Barneo, 1992). This has led to the suggestion that hypoxia depolarizes the type I cell leading to voltage-gated Ca^{2+} entry and neurosecretion. It has been reported that hypoxia both depolarizes neonatal rabbit type I cells (Delpiano & Hescheler, 1989) and increases $[Ca^{2+}]_i$ in adult rabbit type I cells (Biscoe & Duchen, 1990). Surprisingly, however, the data of Biscoe & Duchen (1990) indicated that the rise of $[Ca^{2+}]_i$ in hypoxia resulted predominantly from Ca^{2+} release from internal stores, and it was suggested that the mitochondrion might serve as a source of this Ca^{2+} (Biscoe & Duchen, 1990).

The proposal that chemotransduction relies mainly upon Ca^{2+} release from internal stores is, however, inconsistent with the observation that the increase in catecholamine secretion from the carotid body and the increase in afferent neural discharge in response to hypoxia are both dependent upon extracellular Ca^{2+} and are both inhibited by Ca^{2+} antagonists (Shirahata & Fitzgerald, 1991; Gonzalez *et al.* 1992). To complicate the issue further, Donnelly & Kholwadwala (1992) have recently reported that hypoxia

decreases $[Ca^{2+}]_i$ in isolated rat type I cells and have suggested that there may be species differences in the $[Ca^{2+}]_i$ response to hypoxia.

We have therefore reinvestigated the effects of hypoxia upon both $[Ca^{2+}]_i$ and membrane potential in isolated neonatal rat type I cells. We find compelling evidence that hypoxia raises type I cell $[Ca^{2+}]_i$ and that this occurs principally through membrane depolarization leading to voltage-gated Ca^{2+} entry.

METHODS

Cell isolation

The methods for cell isolation have been described previously (Buckler & Vaughan-Jones, 1993*a*). Briefly, 8- to 14-day-old Sprague–Dawley rat pups were anaesthetized with 4% halothane and their carotid bodies excised and stored in cold phosphate-buffered saline (PBS, Sigma). The rats were then killed by decapitation. Type I cells were isolated by a mixture of enzymic treatment (collagenase–trypsin) and mechanical dispersion, plated onto poly-D-lysine-coated coverslips and kept in Ham's F-12 culture medium (Sigma) until use (3–36 h).

$[Ca^{2+}]_i$ measurement

$[Ca^{2+}]_i$ was measured using indo-1, as previously described (Buckler & Vaughan-Jones, 1993*a*). Cells were loaded with indo-1 by incubation in a 2·5 μM solution of indo-1 AM (Molecular Probes, Inc.) in Ham's F-12 culture medium for 1 h at room temperature. Indo-1 fluorescence was excited at 340 nm and measured at 405 and 495 nm.

Perforated-patch voltage recordings and voltage clamp

Voltage and current clamp recordings were performed using the perforated-patch, whole-cell recording technique (Rae, Cooper, Gates & Watsky, 1991). Electrodes were fabricated from thick-walled borosilicate glass tubing and, in most cases, were Sylgard-coated and fire-polished. The internal filling solution contained (mM): 140 potassium gluconate, 10 Hepes, 1 EGTA, 5 $MgCl_2$, adjusted to pH 7·2 at 37 °C with NaOH. To this solution was added 240 μg ml^{-1} amphotericin B (prepared as a stock solution of 60 mg ml^{-1} dimethyl sulphoxide). Membrane potential and current were measured using an Axopatch-1D; signals were filtered at 1 kHz, digitized and then stored on a microcomputer for analysis.

Solutions

The standard HCO_3^--buffered Tyrode solution contained (mM): 117 NaCl, 4·5 KCl, 23 $NaHCO_3$, 1·0 $MgCl_2$, 2·5 $CaCl_2$, 11 glucose, and was equilibrated with 5% CO_2–95% air, pH at 37 °C was 7·4–7·45. Hypoxic solutions were equilibrated with 5% CO_2 and 0, 1, 2·5 or 5% O_2, the remainder being N_2. Anoxia was produced by equilibrating solutions with 5% CO_2–95% N_2 plus the addition of 100–500 μM $Na_2S_2O_4$. Addition of $Na_2S_2O_4$ had no significant effect upon the pH of the anoxic solutions. All experiments were conducted at 35–37 °C.

Quantification of results

Unless otherwise stated all averaged data have been expressed as means ± standard error of the mean (S.E.M.). The effects of interventions on the hypoxic-induced rise of $[Ca^{2+}]_i$ were quantified as follows. The rise of $[Ca^{2+}]_i$ was calculated as the difference in the mean (averaged over a 1–2 min period) $[Ca^{2+}]_i$ during hypoxia minus the mean basal $[Ca^{2+}]_i$ (determined before the hypoxic challenge), both under control and test conditions. Results are expressed as percentage inhibition (i.e. {(control − test)/control} × 100). Statistical significance of the results was assessed by paired *t* test.

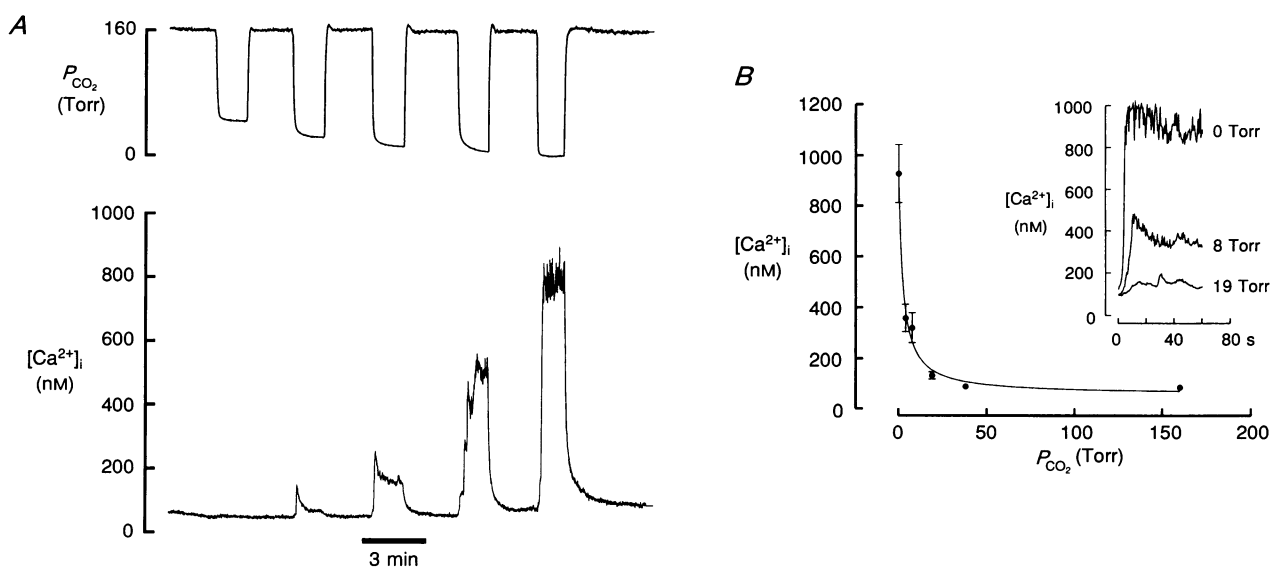

Figure 1. Effects of hypoxia on $[Ca^{2+}]_i$ in rat type I cells

A shows a single recording from a cluster of type I cells. Changes in P_{O_2} were monitored using an O_2 electrode placed in the superfusing medium downstream of the cells. Final challenge is anoxia achieved by addition of 500 μM $Na_2S_2O_4$. *B* is a plot of mean $[Ca^{2+}]_i$ against P_{O_2} for 15 recordings; error bars are ± S.E.M. (for each level of P_{O_2} in each recording $[Ca^{2+}]_i$ was averaged over a 1 min period). Continuous line is the best fit to a hyperbolic function of form $[Ca^{2+}]_i = (a + c) - \{(aP_{O_2})/(b + P_{O_2})\}$; where $a = 820$ nM, $b = 2{\cdot}47$ Torr, and $c = 54{\cdot}8$ nM. Inset shows averaged ($n = 15$) $[Ca^{2+}]_i$ responses to 3 levels of hypoxia.

RESULTS

Effects of lowered P_{O_2} on $[Ca^{2+}]_i$ in type I cells

Both single rat type I cells and type I cell clusters were found to respond to hypoxia with a *rise* of $[Ca^{2+}]_i$ (e.g. Fig. 1*A*). This $[Ca^{2+}]_i$ response was graded in both single and clumps of cells, with the rise of $[Ca^{2+}]_i$ increasing with increasingly severe hypoxia (Fig. 1*A* and *B*). The 'threshold' P_{O_2} at which a $[Ca^{2+}]_i$ response was detectable varied, with 47 % of recordings showing a rise at 38 Torr, and 93 % at 19 Torr (total $n = 15$). In none of the experiments was there any evidence of the response saturating (i.e. anoxia always produced a larger rise in $[Ca^{2+}]_i$ than did severe hypoxia ($P_{O_2} \approx 4$–5 Torr). Figure 1*B* shows the average level of $[Ca^{2+}]_i$ attained over a range of P_{O_2} values. The relationship between $[Ca^{2+}]_i$ and P_{O_2} could be approximated by a hyperbolic function (continuous line). The inset to Fig. 1*B* shows the averaged time course of the $[Ca^{2+}]_i$ response following a reduction of P_{O_2} from 160 Torr to 19, 8 and 0 Torr. Note that, at low P_{O_2}, $[Ca^{2+}]_i$ rises rapidly. Following the initial rapid rise in $[Ca^{2+}]_i$ a secondary decline was often evident at $P_{O_2} = 0$ Torr (see Figs 1 and 2) and also at $P_{O_2} = 8$ Torr (Fig. 1), but at $P_{O_2} = 19$ Torr there was, on average, little subsequent change in $[Ca^{2+}]_i$ (Fig. 1).

Effects of Ca^{2+} removal and Ca^{2+} channel antagonists on the $[Ca^{2+}]_i$ response to anoxia

In previous studies Biscoe & Duchen (1990) found that the removal of extracellular Ca^{2+} only partially inhibited the $[Ca^{2+}]_i$ response to hypoxia. Figure 2*A* shows that, in the rat type I cell, Ca_o^{2+} removal (Ca^{2+} excluded from the bathing medium and 1 mM EGTA added) almost completely inhibited the $[Ca^{2+}]_i$ response to anoxia (mean inhibition 98 ± 1·5 %, $n = 7$). This effect was reversible. The suppression of the $[Ca^{2+}]_i$ response to anoxia in Ca^{2+}-free medium was observed within 1 min of removal of Ca_o^{2+} ($n = 4$). The rise in $[Ca^{2+}]_i$ in response to anoxia is therefore critically dependent upon extracellular calcium which suggests a role for Ca^{2+} influx.

If Ca^{2+} influx were to occur via voltage-gated channels, then Ca^{2+} channel antagonists would be expected to inhibit the $[Ca^{2+}]_i$ response to hypoxia. We therefore tested the effects of the L-type Ca^{2+} channel antagonist nicardipine (10 μM) upon the $[Ca^{2+}]_i$ response to anoxia. Whilst nicardipine substantially reduced the $[Ca^{2+}]_i$ response it did not completely inhibit it (inhibition was 67 ± 6·2 %, $n = 8$). This suggests a major role for voltage-activated L-type Ca^{2+} channels in mediating the anoxia-induced rise of $[Ca^{2+}]_i$. However, because nicardipine failed to inhibit completely the $[Ca^{2+}]_i$ response, we also tested the effects of a non-specific Ca^{2+} channel antagonist, Ni^{2+}. At a concentration of 2 mM, Ni^{2+} inhibited the $[Ca^{2+}]_i$ response to anoxia by 77 ± 8·2 % ($n = 5$). It was notable, however, that even 2 mM Ni^{2+} was not as effective at blocking the anoxic $[Ca^{2+}]_i$ response as was Ca_o^{2+} removal.

Effects of hypoxia on membrane potential

The foregoing experiments suggest that the anoxic $[Ca^{2+}]_i$ response is mainly dependent upon Ca^{2+} influx from the external medium and that this influx occurs, at least in part, through L-type Ca^{2+} channels. The simplest explanation for these data is that anoxia causes cell membrane depolarization leading to voltage-gated Ca^{2+} entry (as has recently been described for acidic stimuli; Buckler & Vaughan-Jones, 1994). We therefore studied the effects of anoxia on both membrane potential and $[Ca^{2+}]_i$ in

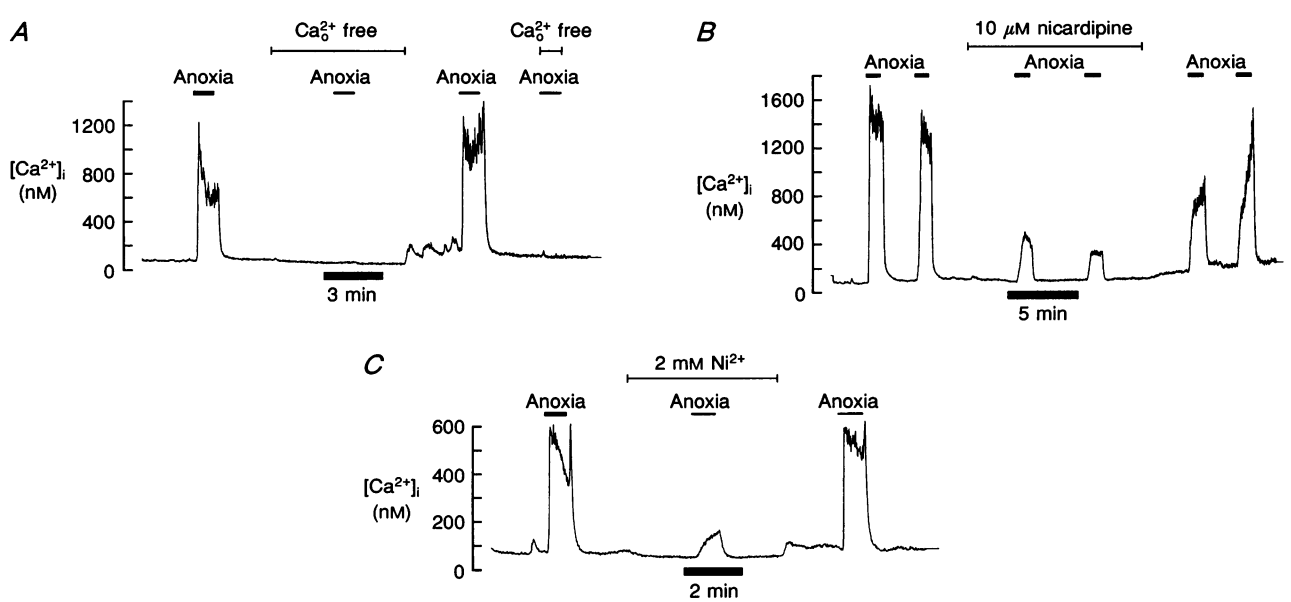

Figure 2.
Effects of Ca_o^{2+} removal (Ca^{2+}-free solutions contained no added $CaCl_2$ and 1 mM EGTA; *A*), 10 μM nicardipine (*B*) and 2 mM $NiCl_2$ (*C*) upon the $[Ca^{2+}]_i$ response to anoxia.

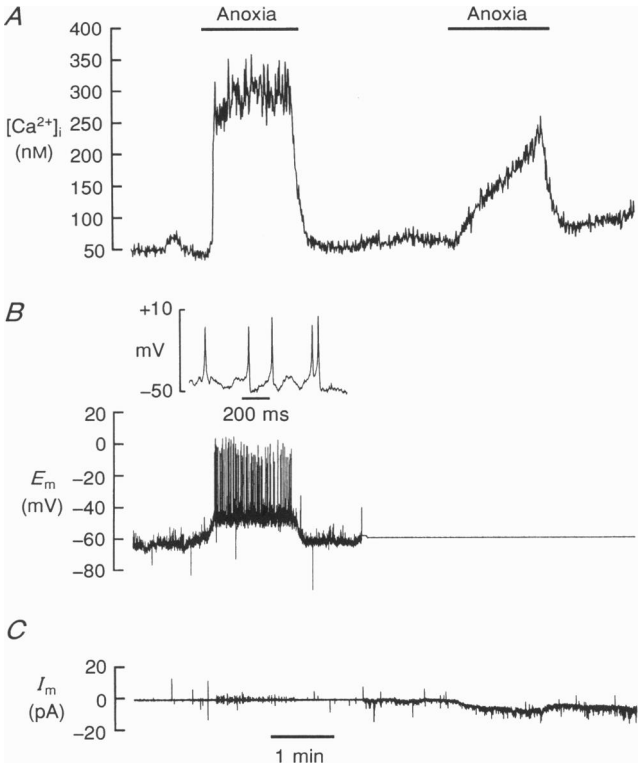

Figure 3. Simultaneous recording of membrane potential and current (using the perforated-patch recording technique) and $[Ca^{2+}]_i$ in a single isolated type I cell
A shows $[Ca^{2+}]_i$, *B* membrane potential (E_m) and *C* current (I_m; current is averaged over 10 ms intervals). The experiment begins in voltage recording mode (i.e. current clamp with $I = 0$; noise in current trace during voltage recording is artifactual). Inset shows action potentials recorded during anoxia on a faster time base. After the first exposure to anoxia the cell is voltage clamped at approximately −60 mV (see change in voltage trace). A second exposure to anoxia is then given.

single isolated type I cells. These voltage recordings were performed using the perforated-patch technique in order to prevent the possible wash-out of any second messengers which might be involved in the O_2 chemotransduction pathway.

Figure 3 shows the results of one such recording. Under voltage recording conditions (current clamp, $I = 0$) anoxia elicited a membrane depolarization of 23 ± 6 mV ($n = 6$). In five of these cells, anoxia also induced action potentials which were typically of small amplitude (e.g. 30–40 mV; see inset to Fig. 3). These electrophysiological changes were associated with a rapid rise in $[Ca^{2+}]_i$. Both the anoxic membrane depolarization and the rise of $[Ca^{2+}]_i$ were reversible. In order to determine the importance of the membrane depolarization (and resultant action potentials) to the $[Ca^{2+}]_i$ response, the cells were then voltage clamped close to their resting potential (between −40 and −60 mV) and the anoxic stimulus reapplied. Under voltage clamp, $[Ca^{2+}]_i$ again rose but this time the rise was very much slower ($d[Ca^{2+}]_i/dt = 254 \pm 45$ nM s^{-1} for control and $3 \pm 0{\cdot}6$ nM s^{-1} for voltage-clamped cells; $n = 6$) and, over the test period (approximately 1–2 min), reached a much lower maximum level (peak $[Ca^{2+}]_i$ in anoxia was 868 ± 265 nM for control and 390 ± 82 nM in voltage-clamped cells; $n = 6$) than under control, non-voltage-clamp conditions. Under voltage-clamp conditions, anoxia also induced a small inward shift in the holding current (maximum $\Delta I_m = -7{\cdot}8 \pm 2{\cdot}0$ pA; $n = 6$) consistent with the observed membrane depolarization seen before voltage clamp.

DISCUSSION

Effects of hypoxia on type I cell $[Ca^{2+}]_i$

Our observation that hypoxia causes a rise in $[Ca^{2+}]_i$ in isolated type I cells of the neonatal rat confirms work in rabbit type I cells (Biscoe & Duchen, 1990; Sato, Ikeda, Yoshizaki & Koyano, 1991; Lopez-Barneo, Benot & Urena, 1993) but is contrary to a recent report that hypoxia *decreases* $[Ca^{2+}]_i$ in rat type I cells (Donnelly & Kholwadwala, 1992). In this latter study, however, hypoxia only lowered type I cell $[Ca^{2+}]_i$ immediately following enzymatic dispersion; after a rest period of a few hours hypoxia raised type I cell $[Ca^{2+}]_i$ (Donnelly & Kholwadwala, 1992).

In rat type I cells the rise of $[Ca^{2+}]_i$ in response to hypoxia is both rapid in onset and is graded with the severity of hypoxia, as is the response of the intact organ (Fig. 1; see also Biscoe & Duchen, 1990). Furthermore, the general shape of the P_{O_2}–$[Ca^{2+}]_i$ curve is similar to arterial P_{O_2}–afferent discharge curves reported for intact carotid bodies from other species (i.e. it is approximately hyperbolic: Hornbein, 1968; Biscoe, Purves & Samson, 1970). It is, however, notable that the P_{O_2} *sensitivity* of $[Ca^{2+}]_i$ is somewhat lower than previous reports of the P_{O_2} sensitivity of neural discharge from the carotid body. There are a number of possible reasons for this apparent discrepancy. Perhaps most importantly, it has been observed that in perfused carotid bodies, tissue (microvascular) P_{O_2} is significantly lower than that of the perfusate (Rumsey, Iturriaga, Spergel, Lahiri & Wilson,

1991). Thus the relationship between neural discharge and *arterial* P_{O_2} may overestimate the real O_2 sensitivity of type I cells. Another contributory factor might be a difference in species or age; it is notable, however, that the P_{O_2} sensitivity of the $[Ca^{2+}]_i$ response in our 2-week-old rat type I cells is similar to that previously reported for adult rabbit type I cells (Biscoe & Duchen, 1990). Finally, the P_{O_2} sensitivity of type I cells $[Ca^{2+}]_i$ *in vivo* may be modulated by extrinsic factors (e.g. neurotransmitters). Overall the observed hypoxic rise of type I cell $[Ca^{2+}]_i$ is consistent with the proposed role for $[Ca^{2+}]_i$ as a second messenger in hypoxic chemotransduction.

What causes the $[Ca^{2+}]_i$ rise in hypoxia?

We have observed that the hypoxic $[Ca^{2+}]_i$ response is almost completely, and very rapidly, abolished by Ca_o^{2+} removal, indicating a fundamental role for Ca^{2+} influx. A similar observation has recently been reported in the rabbit type I cell (Lopez-Barneo *et al.* 1993), in which the removal of extracellular Ca^{2+} also abolished a hypoxia-induced rise of $[Ca^{2+}]_i$. These results differ markedly from those of Biscoe & Duchen (1990), who found that Ca_o^{2+} removal only partially inhibited the rise of $[Ca^{2+}]_i$ under hypoxic conditions. One possible reason for the difference between our results and those of Biscoe & Duchen (1990) is that we routinely included a Ca^{2+} chelator (EGTA) in our Ca^{2+}-free media. In the work of Biscoe & Duchen (1990) the absence of a Ca^{2+} chelator may have allowed a sufficiently high level of $[Ca^{2+}]_o$ to support some Ca^{2+} influx.

The proposal that the hypoxic $[Ca^{2+}]_i$ response results primarily from Ca^{2+} influx is further supported by the observation that it was considerably attenuated by 2 mM Ni^{2+} and by 10 μM nicardipine. More specifically, the data with nicardipine imply a role for L-type voltage-sensitive Ca^{2+} channels, which supports the hypothesis that membrane depolarization triggers the rise of $[Ca^{2+}]_i$.

Simultaneous measurement of membrane potential and $[Ca^{2+}]_i$ revealed that the hypoxia-induced $[Ca^{2+}]_i$ rise was coincident with both depolarization of resting potential and the occurrence of spontaneous action potentials. When these electrophysiological responses were prevented by voltage clamping the membrane potential, the $[Ca^{2+}]_i$ response was greatly slowed and attenuated. This shows that in the unclamped cell, the rapid rise of $[Ca^{2+}]_i$ in anoxia is attributable primarily to membrane depolarization, indicating that Ca^{2+} entry is through a voltage-dependent pathway. Since depolarization promotes Ca^{2+} influx through voltage-gated channels in these cells (e.g. as opposed to an electrogenic Na^+–Ca^{2+} exchanger; see Buckler & Vaughan-Jones, 1994, for further details), we must conclude that the anoxia-induced Ca^{2+} influx also occurs primarily through voltage-gated Ca^{2+} channels. Whilst a major route for this voltage-gated Ca^{2+} entry would appear to be through dihydropyridine-sensitive L-type Ca^{2+} channels, other voltage-gated Ca^{2+} channels may also play a role, since dihydropyridines do not completely block the $[Ca^{2+}]_i$ rise in response to high K_o^+ (Buckler & Vaughan-Jones, 1994) and only partially block the high threshold Ca^{2+} current (Fieber & McCleskey, 1993).

Whilst voltage-gated Ca^{2+} entry is mainly responsible for the rapid $[Ca^{2+}]_i$ rise in response to anoxia, there is also a second, smaller component. This second component is revealed under voltage clamp conditions as a slow rise in $[Ca^{2+}]_i$. Since the anoxic rise in $[Ca^{2+}]_i$ is totally abolished in Ca^{2+}-free media, it is likely that this second component is also dependent upon Ca^{2+} entry, even though it does not require membrane depolarization. There are at least two possible explanations for this slow rise of $[Ca^{2+}]_i$ seen with anoxia under voltage-clamp conditions: (1) it may represent an increase in Ca^{2+} influx, either through a channel or a transporter, or (2) it may result from a decrease in Ca^{2+} efflux which normally balances an inward Ca^{2+} leak. The former possibility is attractive because the activation of a Ca^{2+}-permeable channel could contribute to the receptor potential (i.e. the hypoxic depolarization) and may therefore be an important part of the O_2 chemotransduction pathway. In contrast, the latter possibility might simply result from a metabolic compromising of Ca^{2+}-ATPases, which may only occur with prolonged and severe hypoxia. Therefore, whilst this component contributes little, directly, to the immediate rise of $[Ca^{2+}]_i$, its importance to hypoxic chemotransduction cannot be evaluated until its cause is known.

Effects of hypoxia on membrane potential

Whilst one of the most popular theories of O_2 chemotransduction is that it involves membrane depolarization, there has until now been little evidence to support this proposal (although an increase in action potential frequency has been reported in current-clamped rabbit type I cells; Lopez-Lopez *et al.* 1989). The data presented here demonstrate that hypoxia can cause both a depolarization of resting type I cell membrane potential, which we refer to as the receptor potential, and can initiate electrical activity. We have recently reported that acidosis has similar effects on membrane potential in type I cells. Thus the mechanisms for chemotransduction of both hypoxia and acidosis appear to converge upon a common final pathway, i.e. the generation of a receptor potential and electrical activity.

What is the cause of the receptor potential? Numerous groups have reported that hypoxia inhibits various classes of voltage-activated K^+ channel in type I cells (Lopez-Lopez *et al.* 1989; Delpiano & Hescheler, 1989; Peers, 1990; Stea & Nurse, 1991; Ganfornina & Lopez-Barneo, 1991). In the adult rabbit the channel concerned is of medium conductance (40 pS in symmetrical K^+) and is Ca^{2+} insensitive (Ganfornina & Lopez-Barneo, 1992), whereas in the rat the channel that is P_{O_2} sensitive seems to be the large-conductance Ca^{2+}-activated K^+ channel (Peers, 1990).

The role of this latter channel in mediating the electrophysiological response to hypoxia in rat type I cells is as yet unclear. Whilst the inhibition of maxi K^+ channels could (a) help to maintain the receptor potential as $[Ca^{2+}]_i$ rises and (b) facilitate action potential generation, it is less certain whether these channels contribute to the resting membrane potential such that their inhibition could then initiate the receptor potential. What is clear from our voltage clamp experiments is that anoxia induces an inward shift of holding current at potentials close to the resting potential (i.e. -50 to -60 mV). At these potentials, an inward shift of holding current could result from either the suppression of an outward K^+ current or, for example, the activation of inward Na^+ or Ca^{2+} currents. The precise cause of the receptor potential now needs to be investigated.

Site of O_2 chemoreception

Our data argue persuasively for a hypoxic chemotransduction mechanism which operates through changes in the type I cell's electrical properties, and not through the release of Ca^{2+} from internal stores. This is not consistent with the mitochondrial Ca^{2+} release hypothesis for hypoxic chemotransduction (Biscoe & Duchen, 1990). We cannot, however, exclude a role for the mitochondrion in O_2-sensing. Indeed we have observed that mitochondrial uncouplers (which are potent chemostimulants) have very similar effects upon type I cell $[Ca^{2+}]_i$ and membrane potential to those reported here for anoxia (Buckler & Vaughan-Jones, 1993*b*). Mitochondrial metabolism may therefore be linked to the control of membrane potential and excitability in type I cells. If so, and given the exceptionally high P_{O_2} sensitivity of mitochondrial metabolism in type I cells (Duchen & Biscoe, 1992), the mitochondrion could still play an important role in hypoxic chemotransduction.

In conclusion, this study provides compelling evidence that hypoxic chemoreception by type I cells is mediated through the modulation of the type I cell's electrical properties (probably K^+ channels) which leads to a depolarizing receptor potential, an increase in electrical activity and voltage-gated Ca^{2+} entry.

REFERENCES

Biscoe, T. J. & Duchen, M. R. (1990). Responses of type I cells dissociated from the rabbit carotid body to hypoxia. *Journal of Physiology* **428**, 39–59.

Biscoe, T. J., Purves, M. J. & Sampson, S. R. (1970). The frequency of nerve impulses in single carotid body chemoreceptor afferent fibres recorded *in vivo* with intact circulation. *Journal of Physiology* **208**, 121–131.

Buckler, K. J. & Vaughan-Jones, R. D. (1993*a*). Effects of acidic stimuli on intracellular calcium in type-I cells of the neonatal rat carotid body. *Pflügers Archiv* **425**, 22–27.

Buckler, K. J. & Vaughan-Jones, R. D. (1993*b*). Effects of the metabolic uncoupler DNP on intracellular pH and Ca^{2+} in enzymically isolated neonatal rat carotid body type-I cells. *Journal of Physiology* **459**, 345*P*.

Buckler, K. J. & Vaughan-Jones, R. D. (1994). Effects of hypercapnia on membrane potential and intracellular calcium in rat carotid body type I cells. *Journal of Physiology* (in the Press).

Delpiano, M. A. & Hescheler, J. (1989). Evidence for a P_{O_2}-sensitive K^+ channel in the type-I cell of the rabbit carotid body. *FEBS Letters* **249**, 195–198.

Donnelly, D. F. & Kholwadwala, D. (1992). Hypoxia decreases intracellular calcium in adult rat carotid body glomus cells. *Journal of Neurophysiology* **67**, 1543–1551.

Duchen, M. R. & Biscoe, T. J. (1992). Relative mitochondrial membrane potential and $[Ca^{2+}]_i$ in type I cells isolated from the rabbit carotid body. *Journal of Physiology* **450**, 33–62.

Fieber, L. A. & McCleskey, E. W. (1993). L-type calcium channels in type-I cells of the rat carotid body. *Journal of Neurophysiology* **70**, 1378–1384.

Ganfornina, M. D. & Lopez-Barneo, J. (1992). Potassium channel types in arterial chemoreceptor cells and their selective modulation by oxygen. *Journal of General Physiology* **100**, 401–426.

Gonzalez, C., Almaraz, L., Obeso, A. & Rigual, R. (1992). Oxygen and acid chemoreception in the carotid body chemoreceptors. *Trends in Neurosciences* **4**, 146–153.

Hornbein, T. F. (1968). The relationship between stimulus to chemoreceptors and their response. In *Arterial Chemoreceptors*, ed. Torrance, R. W., pp. 65–78. Blackwell, Oxford.

Lopez-Barneo, J., Benot, A. R. & Urena, J. (1993). Oxygen sensing and the electrophysiology of arterial chemoreceptor cells. *News in Physiological Sciences* **8**, 191–195.

Lopez-Lopez, J., Gonzalez, C., Urena, J. & Lopez-Barneo, J. (1989). Low P_{O_2} selectively inhibits K channel activity in chemoreceptor cells of the mammalian carotid body. *Journal of General Physiology* **93**, 1001–1015.

Peers, C. (1990). Hypoxic suppression of K^+-currents in type I carotid body cells: Selective effect on the Ca^{2+}-activated K^+-current. *Neuroscience Letters* **119**, 253–256.

Rae, J., Cooper, K., Gates, P. & Watsky, M. (1991). Low access resistance perforated patch recordings using amphotericin B. *Journal of Neuroscience Methods* **37**, 15–26.

Rumsey, W. L., Iturriaga, R., Spergel, D., Lahiri, S. & Wilson, D. F. (1991). Optical measurements of the dependence of chemoreception on oxygen pressure in the cat carotid body. *American Journal of Physiology* **261**, C614–622.

Sato, M., Ikeda, K., Yoshizaki, K. & Koyano, H. (1991). Response of cytosolic calcium and cyanide in cultured glomus cells of newborn rabbit carotid body. *Brain Research* **551**, 327–330.

Shirahata, M. & Fitzgerald, R. S. (1991). Dependency of hypoxic chemotransduction in cat carotid body on voltage-gated calcium channels. *Journal of Applied Physiology* **71**, 1062–1069.

Stea, A. & Nurse, C. A. (1991). Whole-cell and perforated-patch recordings from O_2-sensitive rat carotid body cells grown in short- and long-term culture. *Pflügers Archiv* **418**, 93–101.

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