Ca^{2+}-activated K^+ channels in isolated type I cells of the neonatal rat carotid body

C. N. Wyatt and C. Peers*

Department of Pharmacology, Leeds University, Leeds LS2 9JT, UK

1. Ca^{2+}-activated K^+ (K^+_{Ca}) channels in neonatal rat type I carotid body cells were studied using single channel patch clamp techniques. In outside-out patches, using symmetrical 120 mM [K^+] solutions, channels were observed with a slope conductance of 190 pS and a reversal potential of 0 mV. Reducing $[K^+]_o$ to 5 mM shifted the reversal potential as expected for a K^+-selective channel.

2. With 100 nM Ca^{2+} bathing the cytosolic aspect of patches, channel activity (number of active channels in a patch × open probability, NP_o) increased with depolarization. NP_o also increased with increasing 'cytosolic' [Ca^{2+}] at a fixed membrane potential (0 mV). Using outside-out patches, bath application of 20 or 100 nM charybdotoxin reduced NP_o by >85%. These data indicate the presence of K^+_{Ca} channels in type I cells.

3. At 0 mV, using solutions of identical composition (1 μM Ca^{2+} bathing the cytosolic aspect of the channels), NP_o was higher in outside-out patches than in inside-out patches. NP_o was greatest in recordings using the perforated-vesicle technique.

4. Hypoxia and anoxia were without effect on K^+_{Ca} channels in outside-out patches, but caused significant, reversible reductions of NP_o in channels recorded in perforated vesicles.

5. The whole-cell perforated-patch technique was used to record membrane potential at 35–37 °C. Hypoxia, anoxia and charybdotoxin all depolarized type I cells.

6. Our results suggest an important role for K^+_{Ca} channels in type I carotid body cells, and their activity in relation to a model for chemotransduction is discussed.

Transduction of hypoxic, hypercapnic or acidic stimuli into increased afferent chemosensory fibre discharge by the carotid body involves secretion of neurotransmitters (predominantly dopamine) from type I cells (Fidone & Gonzalez, 1986; Gonzalez, Almarez, Obeso & Rigual, 1992). These cells are in synaptic contact with afferent sensory fibre endings, and evoked neurosecretion is Ca^{2+} dependent (Fishman, Greene & Platika, 1985; Obeso, Rocher, Fidone & Gonzalez, 1992), but differing mechanisms for elevating $[Ca^{2+}]_i$ in response to hypoxic and acidic stimuli have been proposed. Using adult rabbit type I cells, Biscoe & Duchen (1990) have provided evidence that $[Ca^{2+}]_i$ rises in response to hypoxia as a result of release from mitochondrial stores. However, Gonzalez and colleagues (Lopez-Lopez, Gonzalez, Urena & Lopez-Barneo, 1989; Gonzalez *et al.* 1992) have demonstrated that hypoxia suppresses a K^+ current in type I cells of the same species which leads to increases in the frequency of spontaneous action potentials and hence increased Ca^{2+} influx through voltage-gated Ca^{2+} channels. This mechanism is supported by the observation that hypoxia-evoked dopamine release can be inhibited by dihydropyridine blockers of Ca^{2+} channels (Obeso *et al.* 1992).

In contrast to adult rabbit cells, there are no reports in rat type I cells (adult or neonatal) of spontaneous electrical activity, and Na^+ channels are either absent (Peers & Green, 1991; Fieber & McClesky, 1993) or only present at low density (Stea & Nurse, 1991). Nevertheless, these cells possess voltage-gated Ca^{2+} channels (Peers & Green, 1991; Fieber & McCleskey, 1993; Buckler & Vaughan-Jones, 1994) and also K^+ currents, the latter being reversibly inhibited by hypoxia, acidity or other pharmacological stimuli (Peers, 1990*a*, *b*; Peers & Green, 1991; Stea & Nurse, 1991). Whilst the O_2-sensitive K^+ channels in adult rabbit type I cells are voltage gated, Ca^{2+}_i insensitive and of 20 pS conductance (Ganfornina & Lopez-Barneo, 1991), pharmacological evidence using whole-cell recordings has indicated that the K^+ currents sensitive to chemostimuli in the neonatal rat are Ca^{2+} activated and blocked by charybdotoxin (Peers, 1990*a*, *b*). The mechanism of K^+ current inhibition, and its consequences to type I cell

* To whom correspondence should be sent at the following address: Institute for Cardiovascular Research, Leeds University, Leeds LS2 9JT, UK.

functioning in the neonatal rat, have not yet been determined. In this study, we report single channel recordings of Ca^{2+}-activated K^+ channels from rat type I cells, and demonstrate that these channels can be inhibited by lowered P_{O_2}, but this inhibition requires cytosolic factors. Closure of this channel leads to cell depolarization which is sufficient to activate voltage-gated Ca^{2+} channels, and hence would lead to increased transmitter release.

METHODS

Type I cells were isolated enzymatically from 9- to 12-day-old rats as previously described (Wyatt & Peers, 1993). In brief, carotid bodies were removed from neonatal rats (anaesthetized by breathing 4% halothane, 96% oxygen through a face mask) and placed in ice-cold phosphate-buffered saline containing 50 μM Ca^{2+}, collagenase (0·05% w/v) and trypsin (0·025% w/v). Donor animals were then killed by decapitation. When carotid bodies had been collected from three or four rats, they were placed in an incubator (37 °C, 5% CO_2) for 20 min then teased apart with fine forceps and incubated for a further 7 min. The digested tissue was then triturated, centrifuged (200 *g*, 5 min) and resuspended in Ham's F-12 culture medium containing 84 u l^{-1} insulin, 100 i.u. ml^{-1} penicillin, 100 μg ml^{-1} streptomycin and 10% fetal calf serum. Following further trituration the dispersed cells were plated onto polylysine-coated coverslips and kept in the incubator for up to 48 h before being used for electrophysiological studies.

On each experimental day, pieces of fragmented coverslip were transferred to a continuously perfused recording chamber (volume, 80 μl; flow rate, 0·5 to 1·0 ml min^{-1}) mounted on the stage of an inverted microscope. The standard 'extracellular' perfusate was composed of (mM): NaCl, 135; KCl, 5; $MgSO_4$, 1·2; $CaCl_2$, 2·5; Hepes, 5; glucose, 10 (pH 7·4, 21–24 °C). In some experiments, the concentration of K^+ was raised to 120 mM by isotonic substitution of NaCl with KCl. Patch clamp techniques (Hamill, Marty, Neher, Sakmann & Sigworth, 1981) were used in these studies, and electrodes were of 6–12 MΩ resistance. The 'internal' solution (bathing the intracellular aspect of channels) was typically composed of (mM): KCl, 120; $CaCl_2$, 1; $MgSO_4$, 2; NaCl, 10; EGTA, 11; Hepes, 11; ATP, 2 (pH 7·2). Changes in $[Ca^{2+}]$ were obtained by adjusting the levels of added Ca^{2+}, calculated using CAMG software (W. H. Martin, Yale University). For some experiments, we used the perforated-vesicle technique (Levitan & Kramer, 1990; Rae, Cooper, Gates & Watsky, 1991) and in these studies ATP was omitted from the pipette. The pipette solution had a pCa value of 6 and contained 240 μg ml^{-1} amphotericin (predissolved in DMSO at 60 mg ml^{-1}) and 5 μM ionomycin, to elevate $[Ca^{2+}]$ in the vesicle to 1 μM, for comparison with conventional excised patches. Using this technique, channel activity increased over the first minute after patch excision (presumably as the level of Ca^{2+} in the vesicle increased) before stabilizing. Experiments only commenced after stabilization of channel activity. Recordings of membrane potential were also made, at 35–37 °C, using whole-cell amphotericin-perforated patch recordings to prevent cell dialysis during the experiment. In these latter experiments no ionomycin was present, and the pCa of the pipette solution was 7.

Bath hypoxia was acheived by either (a) bubbling the reservoir which fed the perfusion chamber with 100% N_2 and increasing the rate of perfusion to *ca* 10 ml min^{-1}, or (b) bubbling the reservoir with 100% N_2 and blowing a stream of N_2 over the surface of the perfusion chamber (flow rate maintained at 0·5–1·0 ml min^{-1}. The P_{O_2} levels were measured in the perfusion chamber using a commercial needle O_2 electrode (Strathkelvin Instruments, Glasgow, UK). Using method (a), the final P_{O_2} was 25–30 mmHg, and using method (b), the P_{O_2} was 12–20 mmHg (previous studies have shown that P_{O_2} levels of *ca* 25 mmHg can significantly inhibit whole-cell K^+ currents in these cells; Peers, 1990*b*). The time course of the fall of P_{O_2} was variable, but was always complete within 1 min of solution exchange. For this reason, analysis of channel activity was only made 1 min after solution change-over. Anoxia (0 mmHg) was achieved by equilibrating the reservoir with 100% N_2 and addition of 0·5–1 mM sodium dithionite. Addition of dithionite also resulted in a fall in pH from 7·4 to 6·9, and so this stimulus is referred to as acidic anoxia, unless the pH was subsequently adjusted to 7·4 (see Results).

Single channel analysis was performed off-line using PAT or VCAN software (J. Dempster, Strathclyde University). Channel activity is expressed as NP_o (number of active channels in a patch × open probability), rather than P_o, since we were not always confident of determining the absolute number of channels in a given patch. NP_o was determined at fixed membrane potentials using 1 or 2 min periods of recordings either by (i) measuring mean current during a recording and dividing this value by the unitary channel amplitude, or (ii) summating the time spent at each unitary current level (as determined by all-point histograms generated by PAT) and expressing this as a fraction of the total recording duration. All results are presented as means ± S.E.M. and statistical comparisons were made using Student's paired or unpaired *t* tests, as indicated in the results.

RESULTS

Ca^{2+}-activated K^+ channels in isolated type I carotid body cells

Using symmetrical 120 mM K^+ solutions, channels were identified in outside-out patches with a slope conductance of 187 ± 4 pS ($n = 9$ patches), and a reversal potential of 0 mV (open circles, Fig. 1*A*). When external $[K^+]$ was reduced to 5 mM, channel openings 5·50 ± 0·18 pA in amplitude became apparent at 0 mV, and the current–voltage relationship became outwardly rectifying, following a line fitted with the Goldmann–Hodgkin–Katz equation (filled circles, Fig. 1*A*). The extrapolated reversal potential (dashed continuation of line, Fig. 1*A*) was between −80 and −90 mV, indicative of a K^+-selective channel. Channel activity was steeply voltage dependent at any fixed $[Ca^{2+}]_i$ (Fig. 1*B*, where pipette $[Ca^{2+}]$ was 100 nM. Note logarithmic *Y*-axis). NP_o values in outside-out patches were dependent on pipette ('intracellular') Ca^{2+} levels (Fig. 1*C*, open bars), as determined at 0 mV. In five experiments, changing pCa from 7 to 6 caused a significant increase in channel activity in inside-out patches ($P < 0·002$, paired *t* test, data not shown). However, Fig. 1*C* also indicates that the level of channel activity at 0 mV (with pCa 6) depended on the recording configuration used; activity was lowest in inside-out patches (filled bar, Fig. 1*C*) and highest in perforated-

vesicle patches (hatched bar, Fig. 1*C*), suggesting that cytosolic factors are required for physiological channel activity to be observed.

The results described in Fig. 1 suggested that we had identified the channels underlying the Ca^{2+}-activated component of the whole-cell K^+ current (see Introduction). To confirm this, we investigated the effects of charybdotoxin (ChTX, 20 or 100 nM) using either outside-out or perforated-vesicle recordings (pCa 6, 0 mV). At either concentration, ChTX caused substantial reductions of NP_o, ranging from 62 to 99% (mean inhibition 87·4 ± 4·3%, $n = 7$ recordings). An example of the inhibitory effect of 20 nM ChTX is shown in Fig. 2*A*. It is noteworthy that in the presence of ChTX, a much smaller channel was revealed of approximately 1·5 pA in amplitude (seen more clearly in the expanded trace of Fig. 2*B*). Since recordings were usually dominated by high conductance K^+_{Ca} channels, we have not studied these small channels in detail, but their presence during ChTX application suggests that ChTX inhibits K^+_{Ca} channels selectively. The inhibitory effect of ChTX further substantiates our suggestion that the high conductance K^+_{Ca} channels identified here underlie the chemosensitive Ca^{2+}-activated component of the whole-cell K^+ current in these cells (Peers, 1990*a*, *b*).

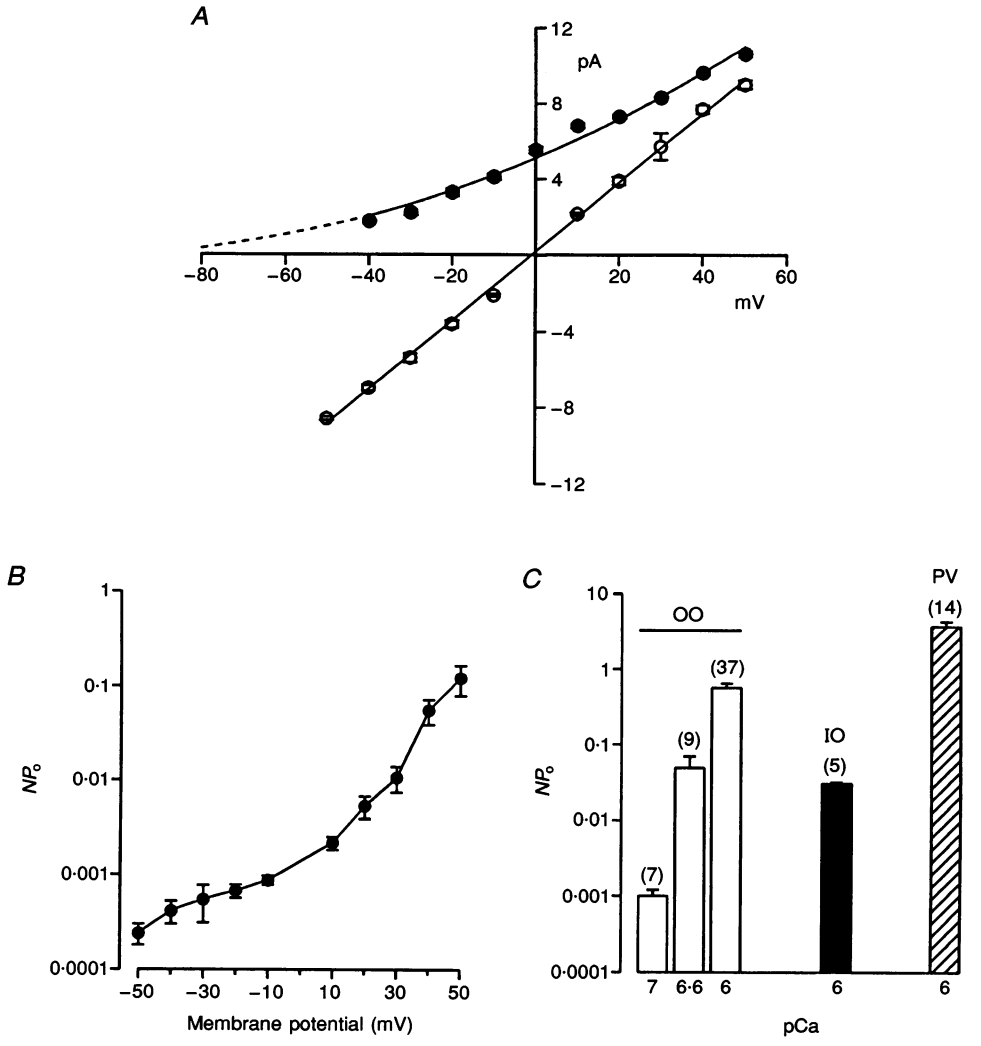

Figure 1. Properties of single K^+_{Ca} channels in type I carotid body cells

A, unitary current–voltage relationships from outside-out patches (pipette $[Ca^{2+}]$, 100 nM) using symmetrical (120 mM) $[K^+]$ solutions (○) or a 120 : 5 mM $[K^+]$ gradient (●). The latter data have been fitted with a curve derived from the Goldmann–Hodgkin–Katz current equation, as described by Hille (1992). *B*, plot of NP_o *versus* membrane potential, determined from outside-out patches; pipette $[Ca^{2+}]$, 100 nM. Values have been pooled from 5–9 patches for each point. *C*, channel activity NP_o (taken from number of patches indicated above each bar) at different pCa values, all determined at 0 mV (120 : 5 $[K^+]$ gradient) using outside-out patches (OO, □), inside-out patches (IO, ■) or perforated vesicles (PV, ▨).

Table 1. Effects of hypoxia and anoxia on Ca^{2+}-activated K^+ channel activity

Recording configuration	Stimulus	Inhibition of NP_o (%)	Number of recordings
Perforated vesicle	Hypoxia (12–20)	35·4 ± 5·5*	6
Perforated vesicle	Anoxia (0)	55·3 ± 8·7**	8
OO patch	Hypoxia (25–30)	10·2 ± 9·9	4
OO patch	Anoxia (0)	7·4 ± 12·2	6

Percentage inhibition values shown are means ± S.E.M. Channel activity was determined at 0 mV with pCa 6 in pipette solutions and standard external solution (see Methods). For perforated-vesicle recordings, ionomycin was present in the pipette solution. No ionomycin was present in outside-out (OO) recordings. Anoxic solutions had a pH value of 6·9. Significant reductions caused by hypoxia or anoxia: $*P < 0.002$, $**P < 0.005$ (paired t test). Numbers in parentheses are the P_{O_2} values (mmHg).

Effects of lowered P_{O_2} on Ca^{2+}-activated K^+ channels

Since previous whole-cell recordings have shown the Ca^{2+}-activated K^+ current to be inhibited by lowering of P_{O_2} (Peers, 1990*b*), we investigated whether this phenomenon could be observed at the single channel level. Figure 2*A* exemplifies the observation that in perforated-vesicle recordings, hypoxia (P_{O_2}, 12–20 mmHg) caused reversible inhibition of ChTX-sensitive K^+_{Ca} channel activity without affecting unitary current amplitude. The mean effect of hypoxia is shown in Table 1, which also indicates that acidic anoxia (pH 6·9) inhibits NP_o further, but still incompletely. In contrast to these findings using perforated

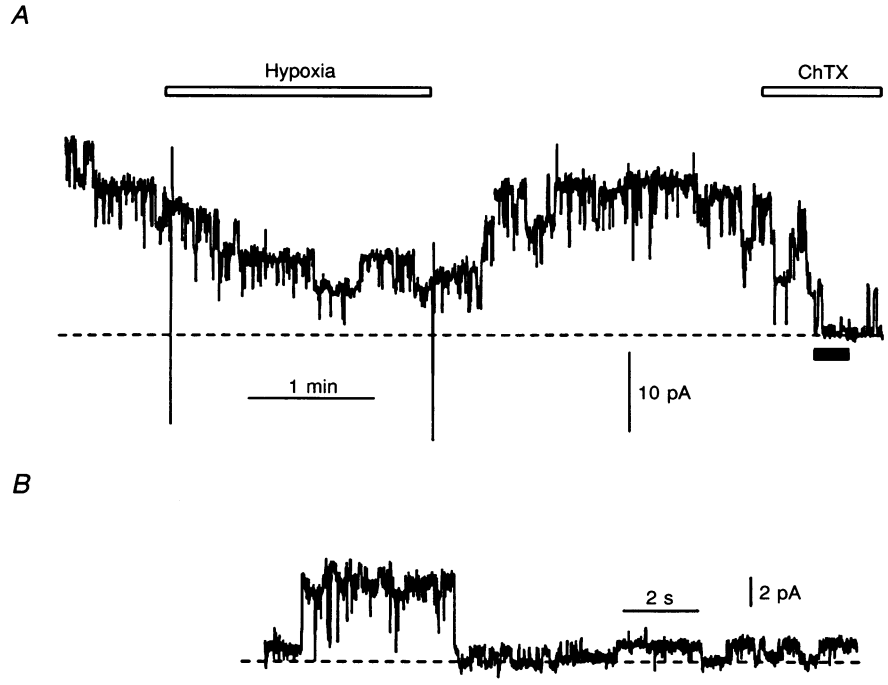

Figure 2. Effects of hypoxia and charybdotoxin on K^+_{Ca} channels

A, perforated-vesicle recording (see Methods for experimental details) at 0 mV with pipette pCa 6 and containing ionomycin. Open horizontal bars show the periods of time during which the perfusing solution was exchanged for a hypoxic one (final P_{O_2}, 12–20 mmHg) or for one containing 20 nM charybdotoxin (ChTX), as indicated. Dashed line represents baseline (no channels open). *B*, expanded section of trace *A*, corresponding to the section indicated by the filled bar in *A*, which shows the activity of a much smaller conductance channel alongside an opening of a K^+_{Ca} channel.

vesicles, we found that neither hypoxia nor acidic anoxia significantly affected NP_o in conventional outside-out patches (Table 1). These results suggest that hypoxic inhibition of K^+_{Ca} channels is not determined by a closely coupled, membrane-limited mechanism. Instead, it appears that cytosolic component(s) are required not only for maintaining channel activity (Fig. 1*C*), but are also required for hypoxia to cause channel inhibition.

Effects of charybdotoxin and hypoxia on membrane potential

As outlined in the Introduction, there is compelling evidence to suggest that chemostimuli trigger neurosecretion in type I cells by causing cell depolarization and hence activation of voltage-gated Ca^{2+} channels (Gonzalez *et al.* 1992; Buckler & Vaughan-Jones, 1994). A possible mechanism underlying this effect in rat type I cells might be closure of K^+_{Ca} channels, but this would depend on these channels being active at the resting membrane potential. Since we found channel activity varied according to the recording configuration used (Fig. 1), we have addressed this question by recording membrane potential using whole-cell perforated-patch recordings at 37 °C (neither ATP nor ionomycin were included in the pipette solution). In fifty-three cells studied, we found the mean resting potential to be −42·7 ± 1·5 mV (range −64 to −29 mV). In ten cells, bath application of 100 nM ChTX caused a reversible depolarization from

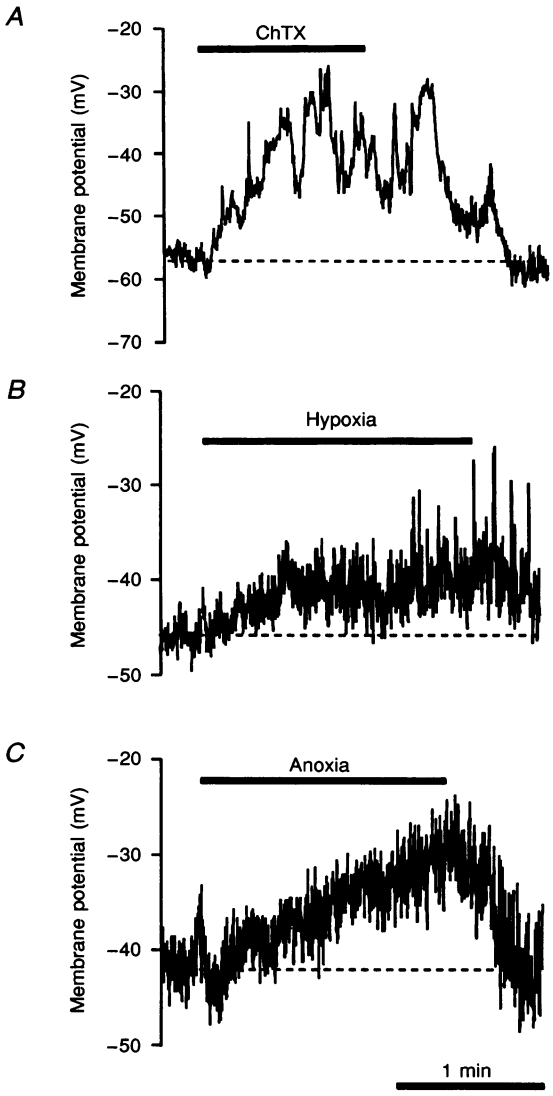

Figure 3. Charybdotoxin, hypoxia and anoxia depolarize type I carotid body cells

A, example of membrane potential recording from a type I cell at 35–37 °C (using the perforated-patch technique), and the effects of 100 nM charybdotoxin (ChTX; period of toxin application indicated by the horizontal bar). *B*, membrane potential recording from another type I cell, and the effect of hypoxia (final P_{O_2}, 12–20 mmHg) which was applied for the period indicated by the horizontal bar. *C*, effects of anoxia (applied for the period indicated by the horizontal bar, pH_o 7·4) on membrane potential. Time scale applies to all traces.

$-43{\cdot}1 \pm 3{\cdot}1$ to $-32{\cdot}3 \pm 3{\cdot}2$ mV ($P < 0{\cdot}0005$, e.g. Fig. 3*A*), and in a further ten cells 20 nM ChTX depolarized cells from $-43{\cdot}4 \pm 2{\cdot}2$ to $-36{\cdot}6 \pm 2{\cdot}2$ mV ($P < 0{\cdot}0001$). Hypoxia (P_{O_2} 12–20 mmHg) reversibly depolarized type I cells, from $-43{\cdot}7 \pm 0{\cdot}8$ to $-35{\cdot}1 \pm 1{\cdot}9$ mV ($P < 0{\cdot}0001$, $n = 8$ cells, e.g. Fig. 3*B*), and a similar effect was observed when cells were exposed to acidic anoxia: in fifteen cells tested, anoxia caused reversible depolarizations from $-42{\cdot}7 \pm 1{\cdot}1$ to $-32{\cdot}0 \pm 1{\cdot}3$ mV ($P < 0{\cdot}0001$, e.g. Fig. 3*B*). This effect is not likely to be due to the fall of pH_o, since, in a further ten cells, anoxic solutions in which the pH was adjusted to 7·4 after addition of dithionite, membrane potentials were again reversibly depolarized, from $-40{\cdot}8 \pm 1{\cdot}0$ to $-31{\cdot}3 \pm 1{\cdot}3$ mV ($P < 0{\cdot}0002$, e.g. Fig. 3*C*).

DISCUSSION

In this study we have characterized single K^+_{Ca} channels which underlie the Ca^{2+}-activated component of whole-cell K^+ currents in rat type I cells. This current is of interest since it has previously been shown to be inhibited by hypoxia and acidic stimuli which excite the intact carotid body (Peers, 1990*a*, *b*; Peers & Green, 1991). It is noteworthy that hypoxia inhibits K^+ currents similarly in both whole-cell and perforated-patch recordings (Stea & Nurse, 1991), which might suggest that channels retain normal activity regardless of cell dialysis. However, in these studies there were no reported attempts to gauge the rate or degree of cell dialysis. We would suggest that normal physiological channel activity and O_2 sensitivity depends on cytosolic factors which may be loosely coupled to the channel, since we saw markedly less activity in inside-out patches (where the intracellular aspect of the channel is exposed to a continuously perfused bath) than in outside-out patches (where the intracellular aspect is protected from flowing solutions). In perforated vesicles, where the intracellular aspect of the channel remains in contact with a small volume of cell cytosol (Levitan & Kramer, 1990), channel activity was at the highest level, and channels were always inhibited by hypoxia and anoxia. Thus dialysis of whole cells with pipette solutions may be sufficiently slow or gentle for physiological channel activity to be preserved. The nature of regulatory cytosolic factors remains to be determined, but others have indicated or speculated that cyclic AMP may regulate O_2-sensitive K^+ channels (reviewed by Gonzalez *et al.* 1992) or that the channel redox state may be influenced by an O_2-sensitive, haem-linked NADPH oxidase (Cross, Henderson, Jones, Delpiano, Hentschel & Acker, 1990; Acker, Bolling, Delpiano, Dufau, Gorlach & Holtermann, 1991).

The present studies contrast with recent reports in which hypoxia reversibly inhibits a Ca^{2+}-insensitive, small-conductance K^+ channel in excised patches from rabbit type I cells (Ganfornina & Lopez-Barneo, 1991). However, these authors did not examine the effects of hypoxia on channels in perforated vesicles, and the relationship between P_{O_2} and channel activity revealed that inhibition was seen over a P_{O_2} range which does not excite the intact organ (Lopez-Lopez *et al.* 1989; Ganfornina & Lopez-Barneo, 1991). This suggests that cytosolic factors may also be required to see the true physiological response of these channels to hypoxia. It should be noted that apart from the nature of the O_2-sensitive K^+ channel in rat and rabbit type I cells being different, other electrophysiological properties differ strikingly between the two species. For example, spontaneous firing of action potentials in rabbit cells (Lopez-Lopez *et al.* 1989) is not apparent in rat cells, and Na^+ currents, which are present in rabbit cells are either absent or only present at low density in rat cells (Peers & Green, 1991; Stea & Nurse, 1991). Since a recent study has also reported a lack of Na^+ channels in adult rat cells (Fieber & McCleskey, 1993), there seem to be genuine species-related (rather than age-related) differences in type I cell functioning.

Our finding that charybdotoxin can depolarize type I cells (Fig. 3*A*) provides evidence that K^+_{Ca} channels are active at rest in unstimulated cells, and thus provide a hyperpolarizing influence on the membrane potential. The depolarizations caused by charybdotoxin, hypoxia and anoxia are far more modest than previously seen in fetal rabbit type I cells (Delpiano & Hescheler, 1989) but compare well with receptor potentials seen in rat type I cells in response to acidic stimuli (Buckler & Vaughan-Jones, 1994). The question then arises, is this depolarization sufficient to cause opening of voltage-gated Ca^{2+} channels? Fieber & McCleskey (1993) reported that Ca^{2+} currents only activate at potentials positive to *ca* -20 mV, but these workers recorded currents using 110 mM Ba^{2+} solutions and such a high concentration of divalent cations would be expected to give rise to a surface charge screening effect (Hille, 1992). Indeed, Ca^{2+} channel currents recorded using a lower Ba^{2+} concentration of 10 mM have shown activation beginning at *ca* -40 mV (Peers & Green, 1991). Perhaps most convincing, however, are the recent studies of Buckler & Vaughan Jones (1994) who measured $[Ca^{2+}]_i$ as a function of membrane potential under physiological conditions and demonstrated that voltage-gated Ca^{2+} influx increases steeply between -40 and -20 mV.

In situ, type I cells are likely to be under the tonic influence of the various neurotransmitters they release (Eyzaguirre, Monti-Bloch & Woodbury, 1990), and also on mediators released from afferent nerve endings (e.g. Wang, Stensaas, Bredt, Dinger & Fidone, 1994). Any of these factors might influence type I cell membrane potential or excitability by acting on different ionic channels (Benot & Lopez-Barneo, 1990; Wyatt & Peers, 1993). It would therefore seem highly unlikely that K^+_{Ca} channels alone determine resting membrane potential in rat type I cells. Nevertheless, the findings reported here that charybdotoxin, hypoxia and anoxia (and also hyper-

capnic stimuli in the studies of Buckler & Vaughan-Jones, 1994) similarly depolarize type I cells sufficiently to cause Ca^{2+} influx via voltage-gated Ca^{2+} channels point to the regulation of K^+_{Ca} channels as being a potentially important mechanism in carotid body chemotransduction in this species.

Acker, H., Bolling, B., Delpiano, M. A., Dufau, E., Gorlach, A. & Holtermann, G. (1992). The meaning of H_2O_2 generation in carotid body cells for PO_2 chemoreception. *Journal of the Autonomic System* **41**, 41–52.

Benot, A. R. & Lopez-Barneo, J. (1990). Feedback inhibition of Ca^{2+} currents by dopamine in glomus cells of the carotid body. *European Journal of Neuroscience* **2**, 809–812.

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.

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* **478**, 157–171.

Cross, A. R., Henderson, L., Jones, O. T. G., Delpiano, M. A., Hentschel, J. & Acker, H. (1990). Involvement of an NAD(P)H oxidase as a pO_2 sensor protein in the rat carotid body. *Biochemical Journal* **272**, 743–747.

Delpiano, M. A. & Hescheler, J. (1989). Evidence for a pO_2-sensitive K^+ channel in the type-I cell of the rabbit carotid body. *FEBS Letters* **249**, 195–198.

Eyzaguirre, C., Monti-Bloch, L. & Woodbury, J. W. (1990). Effects of putative neurotransmitters of the carotid body on its own glomus cells. *Pflügers Archiv* **2**, 77–88.

Fidone, S. & Gonzalez, C. (1986). Initiation and control of chemoreceptor activity in the carotid body. In *Handbook of Physiology*, section III, *The Respiratory System*, ed. Cherniack, N. S. & Widdicombe, J. G., pp. 247–312. American Physiological Society, Bethesda, MD, USA.

Fieber, L. A. & McCleskey, E. W. (1993). L-type Ca^{2+} channels in type I cells of the rat carotid body. *Journal of Neurophysiology* **70**, 1378–1384.

Fishman, M. C., Greene, W. L. & Platika, D. (1985). Oxygen chemoreception by carotid body cells in culture. *Proceedings of the National Academy of Sciences of the USA* **82**, 1448–1450.

Ganfornina, M. D. & Lopez-Barneo, J. (1991). Single K^+ channels in membrane patches of arterial chemoreceptor cells are modulated by O_2 tension. *Proceedings of the National Academy of Sciences of the USA* **88**, 2927–2930.

Gonzalez, C., Almarez, L., Obeso, A. & Rigual, R. (1992). Oxygen and acid chemoreception in the carotid body chemoreceptors. *Trends in Neurosciences* **15**, 146–153.

Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. (1981). Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches. *Pflügers Archiv* **391**, 85–100.

Hille, B. (1992). *Ionic Channels of Excitable Membranes*, pp. 337–361. Sinauer Associates, Sunderland, MA, USA.

Levitan, E. S. & Kramer, R. H. (1990). Neuropeptide modulation of single calcium and potassium channels detected with a new patch clamp configuration. *Nature* **348**, 545–547.

Lopez-Lopez, J., Gonzalez, C., Urena, J. & Lopez-Barneo, J. (1989). Low pO_2 selectively inhibits K channel activity in chemoreceptor cells of the mammalian carotid body. *Journal of General Physiology* **93**, 1001–1015.

Obeso, A., Rocher, A., Fidone, S. & Gonzalez, C. (1992). The role of dihydropyridine-sensitive Ca^{2+} channels in stimulus-evoked catecholamine release from chemoreceptor cells of the carotid body. *Neuroscience* **47**, 463–472.

Peers, C. (1990*a*). Selective effect of lowered extracellular pH on Ca^{2+}-dependent K^+ currents in type I cells isolated from the neonatal rat carotid body. *Journal of Physiology* **422**, 381–395.

Peers, C. (1990*b*). 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.

Peers, C. & Green, F. K. (1991). Intracellular acidosis inhibits Ca^{2+}-activated K^+ currents in isolated type I cells of the neonatal rat carotid body. *Journal of Physiology* **437**, 589–602.

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.

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.

Wang, Z.-Z., Stensaas, L. J., Bredt, D. S., Dinger, B. & Fidone, S. J. (1994). Localization and actions of nitric oxide in the cat carotid body. *Neuroscience* **60**, 275–286.

Wyatt, C. N. & Peers, C. (1993). Nicotinic acetylcholine receptors in isolated type I cells of the neonatal rat carotid body. *Neuroscience* **54**, 275–281.

Acknowledgements

We are grateful to The Wellcome Trust for their support of this work. We also thank K. J. Buckler and R. D. Vaughan-Jones (Oxford University) for a preprint of their studies.

Author's present address

C. N. Wyatt: Department of Pharmacology, Royal Free Hospital Medical School, Rowland Hill Street, London NW3 2PF, UK.

Received 17 December 1993; accepted 25 August 1994.