Effect of chronic hypoxaemia from birth upon chemosensitivity in the adult rat carotid body *in vitro*

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1. The effect of chronic hypoxaemia upon *in vitro* carotid body chemosensitivity was observed in eight rats > 5 weeks of age born and reared in 12% oxygen. Comparisons were made with eight age-matched normoxic rats.

2. Single exponential functions with offset were fitted to the normalized (percentage of maximum) discharge responses to ramp decreases in P_{O_2} at three steady levels of P_{CO_2}. CO_2 sensitivity was derived from these functions.

3. Increasing hypercapnia increased the horizontal asymptote of the exponential functions in the normoxic ($0{\cdot}15 \pm 0{\cdot}03$% discharge per mmHg P_{CO_2}; $P < 0{\cdot}001$) and chronically hypoxic ($0{\cdot}13 \pm 0{\cdot}04$% discharge per mmHg P_{CO_2}; $P < 0{\cdot}005$) animals but was without effect upon the rate constants in both groups ($-0{\cdot}04 \pm 0{\cdot}18$ mmHg P_{O_2} per mmHg P_{CO_2}, $P > 0{\cdot}50$ and $0{\cdot}63 \pm 0{\cdot}48$ mmHg P_{O_2} per mmHg P_{CO_2}, $P > 0{\cdot}20$, respectively). Rate constants were greater in the chronically hypoxic animals ($P < 0{\cdot}05$) compared with the normoxic animals.

4. CO_2 chemosensitivity increased with decreasing P_{O_2} in normoxic ($P < 0{\cdot}05$) but not in chronically hypoxic ($P > 0{\cdot}50$) rats.

5. Our results show that chronic hypoxaemia from birth attenuates the maturation of CO_2–O_2 interaction at the carotid body.

The carotid body chemoreceptors detect and translate arterial blood gas tensions and pH into a level of afferent chemosensory discharge appropriate for cardio-respiratory homeostasis. Their sensitivity to hypoxia increases or 'resets' during the first few days postnatally in positive correlation with the increase in arterial P_{O_2} (P_{a,O_2}) occurring during this period (Koch & Wendel, 1968). Resetting is measured directly, as an upward and rightward shift in the position of the response curve of these receptors to hypoxia (Blanco, Dawes, Hanson & McCooke, 1984) or, indirectly, as an increased reflex ventilatory response to hypoxia in both animals (Eden & Hanson, 1987*a*) and man (Williams, Smyth, Boon, Hanson, Kumar & Blanco, 1991). Although the mechanisms which underlie this maturation remain unknown, a link between it and postnatal arterial oxygenation has been suggested by experiments in which resetting could be hastened by *in utero* elevations of fetal P_{a,O_2} (Blanco, Hanson & McCooke, 1988). The converse, i.e. a retardation of resetting by the establishment of postnatal chronic hypoxaemia from birth, either naturally (Lahiri, Brody, Motoyama & Velasquez, 1978), experimentally (Eden & Hanson, 1987*b*; Hanson, Kumar & Williams, 1989) or pathologically (Sorensen & Severinghaus, 1968) has also been observed in studies of the ventilatory responses to hypoxia. However, these effects may be confounded by other actions of the chronic hypoxaemia such as changes in a suprapontine drive to breathing (Williams & Hanson, 1989) or changes in haematocrit or whole body oxygen consumption (Mortola, Morgan & Virgona, 1986). The few, direct studies of the influence of chronic hypoxaemia upon peripheral chemoreceptor discharge responses to acute hypoxia have been performed primarily in adult animals and the results are equivocal; both augmented (Barnard, Andronikou, Pokorski, Smatresk, Mokashi & Lahiri, 1987) and attenuated (Tatsumi, Pickett & Weil, 1991) responses have been reported.

The influence of chronic hypoxaemia from birth upon peripheral CO_2 chemosensitivity has been little studied. Interestingly, in one such study, the reflex respiratory response to hypercapnia was not affected (Elnazir, Pepper & Kumar, 1993), suggesting that chronic hypoxaemia may preferentially inhibit the O_2 chemotransduction process. However, recently there have been studies demonstrating a postnatal increase in the degree of multiplicative interaction between hypoxia and hypercapnia (Marchal *et al.* 1992; Carroll, Bamford & Fitzgerald, 1993) and we have

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suggested that this maturation may underlie the resetting of hypoxic chemosensitivity (Pepper, Landauer & Kumar, 1994). The aim of the present work was therefore to use a recently developed *in vitro* rat carotid body preparation to test the hypothesis that chronic hypoxaemia from birth prevents the postnatal increase in the degree of CO_2–O_2 interaction at the level of the peripheral chemoreceptors.

METHODS

Animals and hypoxic chamber

Two groups of Wistar rats were studied: normoxic adult controls (49 ± 7 days old, 175 ± 17 g body weight) and adult rats which had been made chronically hypoxic from birth (46 ± 1 day old, 113 ± 2 g body weight). The two groups were not different in postnatal age ($P > 0.50$) but were different in body weight ($P < 0.01$). The chronically hypoxic group had been born into and subsequently reared in a normobaric, hypoxic chamber (volume *ca* 1·0 m^3), operating under a 12 h light–dark cycle, from time-mated females which had been placed into the chamber 2–3 days prior to giving birth. The chamber air was circulated at *ca* 15 l min^{-1} (i.e. *ca* one complete change per hour) and the O_2 concentration measured continuously by an oxygen analyser (P. K. Morgan, Chatham, UK) and set to 12% (range 11·75–12·25%) by a system whereby deviations from the desired concentration were met by addition of nitrogen or air, through feedback-controlled solenoid valves. Ambient CO_2 in the chamber was measured (NovaStat 3, Nova Biomedical, Waltham, MA, USA) and maintained at normal levels (*ca* 0·03%) by circulating the gas through soda lime. Ambient temperature was measured and maintained at 22–24 °C, and humidity measured and maintained between 40 and 50% by circulating the chamber gas through a freezer unit and silica gel. Ammonia in the chamber was removed by circulation through a molecular sieve (Type 3A, Fisons).

Carotid body preparation

Anaesthesia was induced and maintained with 1·5–2·5% halothane in 100% O_2 or 12% O_2 (balance N_2) for the normoxic and chronically hypoxic groups, respectively. The right carotid bifurcation, including the carotid body, sinus nerve and glossopharyngeal nerve, was isolated, the common carotid artery ligated and the bifurcation excised within 15 s of ligation of the blood supply. Donor animals were killed by decapitation whilst under halothane anaesthesia. The resulting bifurcation and its associated tissues were then immersed in chilled, gassed (95% O_2 and 5% CO_2) bicarbonate-buffered saline (125 mM NaCl, 3 mM KCl, 1·25 mM NaH_2PO_4, 5 mM Na_2SO_4, 1·3 mM $MgSO_4$, 24 mM $NaHCO_3$, 2·4 mM $CaCl_2$ and 10 mM glucose at pH 7·38). The bifurcation was pinned on Sylgard (Dow Corning) in a small volume (*ca* 0·2 ml) tissue bath and superfused at 3 ml min^{-1} with warmed (36·7–37 °C) saline solution whilst the excess connective tissue around the bifurcation was removed. The overlying occipital artery was retracted and removed to expose the carotid body, and the sinus nerve was sectioned at its intersection with the glossopharyngeal nerve. The remaining tissue was partially digested in a gassed enzyme solution (0·06% collagenase (Type II, Sigma), 0·02% protease (Type IX, Sigma)) for 25 min at 37 °C to facilitate the recording of neuronal activity.

Electrophysiological recording

Extracellular recordings of afferent single- or few-fibre activity were recorded from the cut end of the carotid sinus nerve using glass suction electrodes (tip diameter *ca* 15 μm), and amplified and filtered (bandwidth 30 Hz to 1·5 kHz) with standard Neurolog modules. A reference silver chloride-coated silver wire was placed nearby in the bath. The P_{O_2} and temperature of the superfusate were continually monitored in the superfusion line immediately before the bath by a membrane oxygen electrode and meter (WPI ISO2, WPI, Aston, Stevenage, UK; 90% response in 10 s) and thermocouple. Superfusate P_{CO_2} was determined from samples collected and measured by a blood gas analyser (NovaStat 3). Afferent spike activity and superfusate P_{O_2} were monitored on an oscilloscope (Gould 1602) and recorded throughout each experiment on a standard VHS video recorder via a DC modified PCM digital unit (Sony 701ES). In addition, spike activity was converted to transistor–transistor logic pulses via a window discriminator and integrated to give spike frequency. This integrated signal was monitored throughout the experiment on a thermal pen recorder to determine the point of chemoreceptor failure and possible spike degradation with time.

Experimental protocol

Baseline chemoreceptor discharge was obtained by bubbling the superfusate with 95% O_2 and 5% CO_2 which resulted in a dissolved P_{O_2} of *ca* 450 mmHg and P_{CO_2} of 35 mmHg. Superfusate P_{O_2} was decreased three times between *ca* 450 and 75 mmHg over 3–4 min at each of three fixed levels of P_{CO_2} (35, 46 and 61 mmHg) by precision mixing of the gases which were bubbling through the superfusate reservoir (volume *ca* 70 ml). The superfusate was returned to 95% O_2 and 5% CO_2 levels for 4 min between every hypoxic challenge and then maintained at each level of P_{CO_2} for 3 min before each hypoxic challenge. At the end of each experiment, a more prolonged (*ca* 5 min) hypoxic or asphyxial stimulus was applied to the superfusate to establish the viability of the preparation and to determine the maximum level of chemoreceptor discharge. The entire protocol lasted *ca* 40 min.

Data and statistical analysis

Few-fibre chemoreceptor discharge was determined as the activity exceeding a level which was *ca* 50% of the amplitude of the baseline noise above the noise itself. Nerve activity and superfusate P_{O_2} were sampled (10 kHz per channel) on- or off-line by a computer (Macintosh IIci with National Instruments NB-MIO-16 DA and NB-MIO-8G DMA cards) running customized LabVIEW 2 (National Instrument Co., Austin, TX, USA) software. Action potentials were counted and binned into 10 s periods, expressed as the percentage of the maximum binned frequency found during the experiment and correlated against the mean P_{O_2} during the 10 s period. A single exponential with offset (equivalent to the horizontal asymptote, i.e. the theoretical discharge at infinite P_{O_2}) was fitted to each response to hypoxia using an iterative routine with robust weighting (Ultrafit, Biosoft, Cambridge, UK), for every animal at each level of CO_2 according to the following equation:

$$\text{Discharge} = \text{offset} + \text{discharge at 70 mmHg } P_{O_2} \times \exp\left(\frac{-(P_{O_2} - 70)}{\text{rate constant}}\right),$$

where the rate constant is the increase in mmHg P_{O_2} required to decrease discharge to 1/e of its initial value. The software also calculated a goodness-of-fit index from the incomplete gamma function using the χ^2 test and the number of degrees of freedom, each halved, as its arguments, and expressing the index as a probability, where values near to 0 indicated a poor fit and a value of 1 indicated a perfect fit. Data points which corresponded to a

consistent fall in discharge as P_{O_2} was decreased (failure) were excluded from the analysis.

All data are expressed as means ± S.E.M. and tested for significant differences by t test, ANOVA or regression analysis, as indicated.

RESULTS

Hypoxic response curves

Successful recording through an entire protocol was obtained in eight normoxic and eight chronically hypoxic animals. In all cases, decreasing superfusate P_{O_2} caused an increase in chemoreceptor discharge frequency that was reversible upon return to hyperoxia. Figure 1 shows an example of this in one chronically hypoxic animal at a superfusate P_{CO_2} of 35 mmHg. A failure to maintain an increased discharge during the hypoxic challenge was observed in only two normoxic and one chronically hypoxic preparation (s) during the highest steady level of P_{CO_2} and it was necessary, for the remaining experiments, to increase the degree of hypoxic and hypercapnic stimulation at the end of the protocol to establish a maximum discharge frequency from which discharge could be normalized. During hypoxic challenges that did not exhibit failure, the peak in chemoreceptor discharge occurred one bin (10 s) later than the lowest bin value of P_{O_2}. Each response discharge (expressed as a percentage of maximum) was therefore correlated with the previous binned P_{O_2} level and exponential functions fitted. These functions gave good fits to the data points at all levels of hypercapnia (Fig. 2), although the response curves of the normoxic group were significantly better fitted with exponential functions than the chronically hypoxic group (0·72 ± 0·08 and 0·40 ± 0·09, respectively; $P < 0·02$).

The effect of CO_2 upon the baseline discharge and the shape of the exponential, hypoxic response curves was assessed by linear regression analysis of the values found for the horizontal asymptote and the rate constant. This showed a significant increase in baseline discharge, with increasing P_{CO_2}, in both normoxic (0·15 ± 0·03% discharge per mmHg P_{CO_2}; $P < 0·001$) and chronically hypoxic (0·13 ± 0·04% discharge per mmHg P_{CO_2}; $P < 0·005$) animals (Fig. 3*A*). At each level of P_{CO_2}, the horizontal asymptotes were not different between the two groups ($P > 0·05$, unpaired t test). The rate constant was unaffected by P_{CO_2} in the normoxic group (−0·04 ± 0·18 mmHg P_{O_2} per mmHg P_{CO_2}; $P > 0·500$). Although increasing P_{CO_2} affected the rate constant to a greater degree in the chronically hypoxic group, this too was not significant (0·63 ± 0·48 mmHg P_{O_2} per mmHg P_{CO_2}; $P > 0·200$) (Fig. 3*B*). However, the absolute levels of the rate constant were significantly different between the two groups at each level of P_{CO_2} ($P < 0·05$, unpaired t test).

Effect of P_{O_2} upon CO_2 sensitivity

From the fitted exponential functions it was possible to derive values for chemoreceptor discharge at three levels of

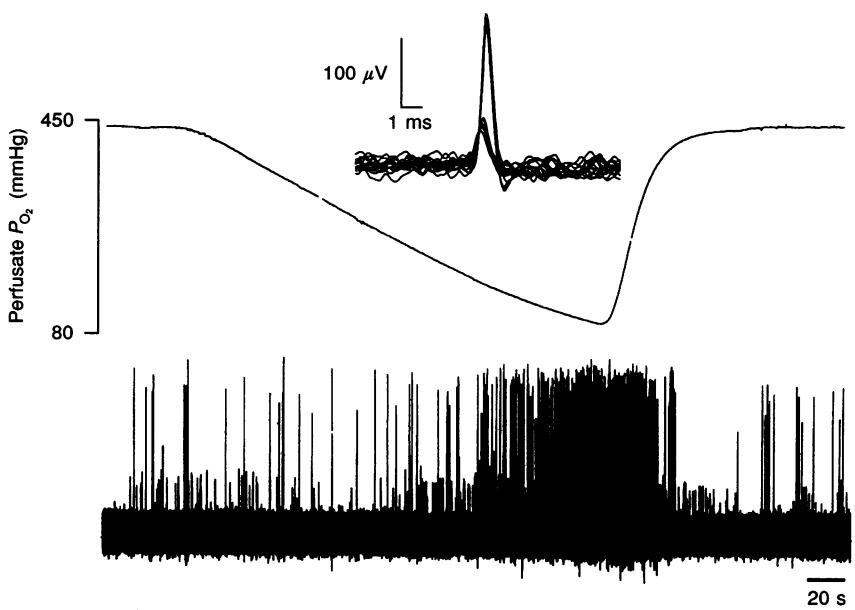

Figure 1. Decreasing P_{O_2} increases carotid body chemoreceptor discharge *in vitro*

The bottom trace shows an example of the increase in the frequency of action potentials, recorded from the carotid sinus nerve of a 40-day-old rat which had been made chronically hypoxic from birth, as superfusate P_{O_2} was reduced from *ca* 450 mmHg to *ca* 80 mmHg (middle trace) and the subsequent return to baseline discharge as P_{O_2} was increased. The inset (at the top) shows, on a different time scale, a composite of sixteen of the action potentials, triggered by amplitude, to demonstrate the uniformity of shape and height of two fibres in the preparation. Superfusate P_{CO_2} was 35 mmHg throughout.

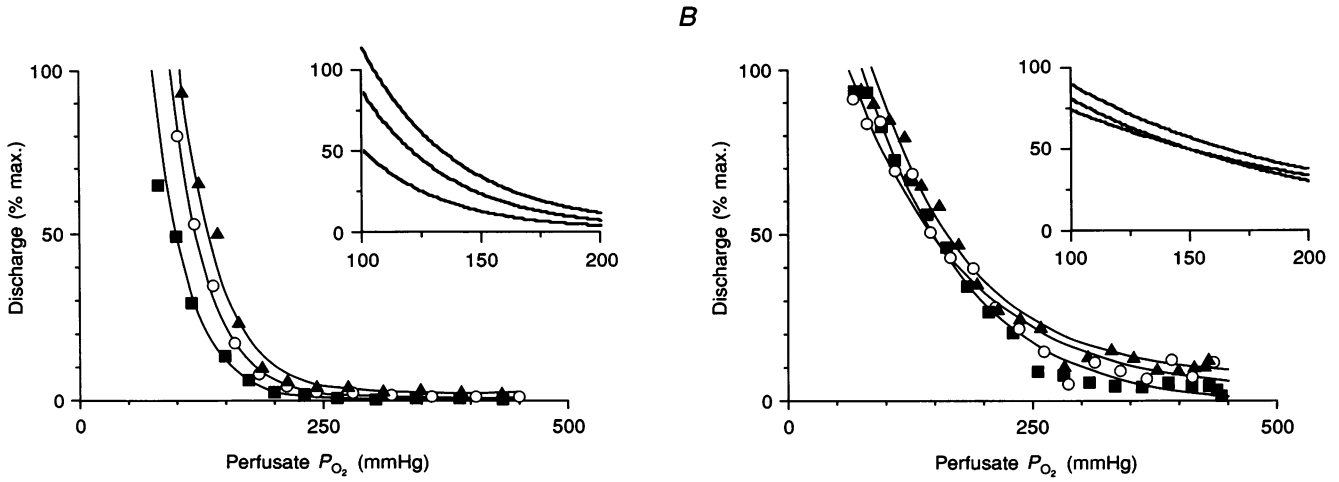

Figure 2. Effect of hypoxia and hypercapnia upon chemoreceptor discharge in normoxic and chronically hypoxic rats

Examples of the three P_{O_2}–response curves obtained during a single experiment in a normoxic (*A*) and a chronically hypoxic (*B*) rat. Symbols represent chemoreceptor discharge during each 10 s of the hypoxic challenge and at different steady levels of P_{CO_2} (■, 35; ○, 46 and ▲, 61 mmHg). Discharge has been normalized as a percentage of the maximum discharge obtained for each preparation. Continuous lines are the fitted single exponentials with offsets. The insets in each graph show an expansion of the axis to illustrate the effect of increasing P_{CO_2} upon the position of the P_{O_2}–response curves. An increasing interaction with decreasing P_{O_2} is apparent in *A* but absent in *B*.

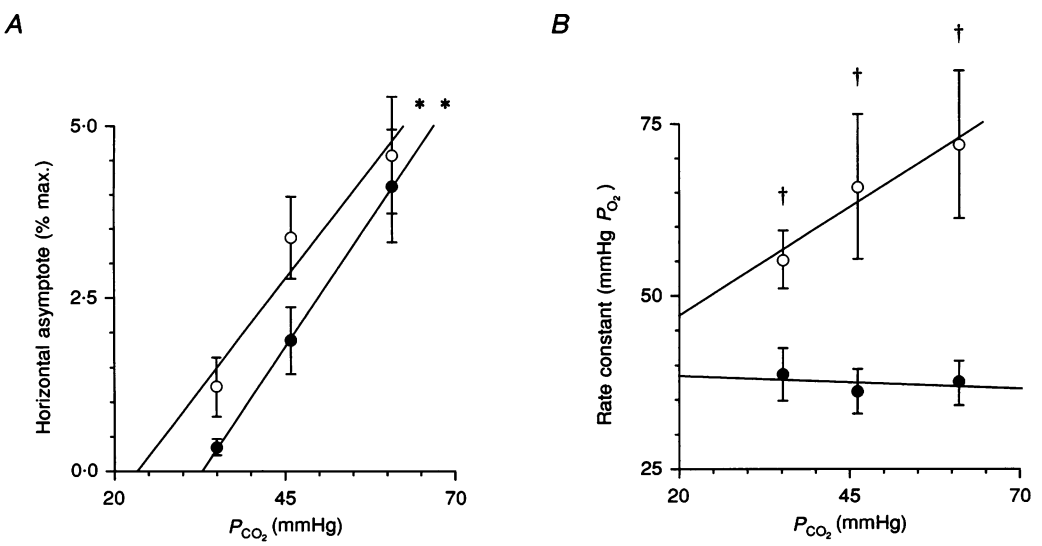

Figure 3. Effect of hypercapnia upon the shape and position of P_{O_2}–response curves in normoxic and chronically hypoxic rats

The mean ± S.E.M. of values obtained for the horizontal asymptote (*A*) and the rate constant (*B*) for all normoxic (●; $n = 8$) and chronically hypoxic (○; $n = 8$) rats at each of three levels of steady P_{CO_2}. Linear regressions (continuous lines) through each set of data points are shown. * at the end of any line indicates a significant effect ($P < 0{\cdot}005$) of P_{CO_2} by linear regression analysis. † indicates a significant difference ($P < 0{\cdot}05$) between normoxic and chronically hypoxic rats at any level of P_{CO_2} by unpaired *t* test.

P_{O_2} (400, 200 and 100 mmHg) and three levels of P_{CO_2} (35, 46, 61 mmHg) from which linear P_{CO_2}–response curves could be constructed. Multiple regression analysis was performed on these data sets to give the following equation for all normoxic rats:

$$\text{Discharge} = -93{\cdot}34 + (3{\cdot}79 \pm 1{\cdot}24 \times P_{CO_2}) + (0{\cdot}27 \pm 0{\cdot}23 \times P_{O_2}) + (-0{\cdot}01 \pm 0{\cdot}005 \times P_{CO_2} \times P_{O_2}),$$

where discharge is expressed as a percentage of the maximum and partial pressures in mmHg.

The degree of interaction between P_{CO_2} and P_{O_2} was significant ($P < 0{\cdot}05$), indicating a greater than additive effect of hypoxia upon CO_2 sensitivity. A similar analysis, performed with the chronically hypoxic data yielded the following multiple regression equation:

$$\text{Discharge} = 216{\cdot}91 + (1{\cdot}21 \pm 2{\cdot}42 \times P_{CO_2}) + (-1{\cdot}07 \pm 0{\cdot}76 \times P_{O_2}) + (-0{\cdot}003 \pm 0{\cdot}016 \times P_{CO_2} \times P_{O_2}).$$

The level of interaction in the chronically hypoxic group was not significant ($P > 0{\cdot}50$).

The CO_2 sensitivity at three further levels of hypoxia (300, 150 and 75 mmHg) was derived (Fig. 4) and the effect of hypoxia upon CO_2 chemosensitivity assessed by one factor ANOVA. This showed that, in the normoxic preparations, increasing hypoxia increased CO_2 sensitivity significantly ($P < 0{\cdot}001$). *Post hoc* testing (Scheffe's F test) determined the statistically significant differences ($\alpha = 0{\cdot}05$) between successive levels of P_{O_2} to lie below 200 mmHg P_{O_2}. In contrast, increasing hypoxia in the chronically hypoxic group was without effect upon CO_2 sensitivity ($P > 0{\cdot}999$). In both cases exponential functions with offset could be fitted to the data as shown in Fig. 4. Goodness-of-fit indices were $> 0{\cdot}99$ for both groups. The rate constant for the chronically hypoxic group ($2{\cdot}08 \times 10^5$ mmHg P_{O_2}) was considerably greater than that of the normoxic group (24·21 mmHg P_{O_2}). The viability of the preparation was always confirmed by the reproducibility of the response to hypoxia at the lowest level of P_{CO_2}. In all cases the final hypoxic challenge produced response curves that were slightly shifted to the left compared with the first hypoxic challenge.

DISCUSSION

Our results are the first to demonstrate an impairment of peripheral chemoreceptor maturation by chronic hypoxaemia from birth in an *in vitro* rat carotid body preparation. Our findings suggest that carotid body mechanisms may be entirely responsible for this impairment. Although the actual level of tissue stimulation in an *in vitro* preparation such as ours is not known to the degree that it is *in vivo* (Lahiri, Rumsey, Wilson & Iturriaga, 1993) we have felt it preferable to generate hypoxic response curves over a wide range of superfusate P_{O_2} values, rather than determine chemoreceptor frequency simply in hyperoxia and anoxia (Kholwadwala & Donnelly, 1992), as this latter approach, generating just two data points, would not reveal shape changes in the response curves and could suffer from the adaptation or failure known to occur in this receptor system with prolonged stimulation (Marchal *et al.* 1992; Carroll *et al.* 1993). The level of stimulus at the site of transduction, in an *in vitro* preparation, will clearly be greater than that measured in the superfusate and greater, at any given P_{O_2}, in larger organs due to the increased mass of metabolizing tissue through which gases would need to diffuse. Unfortunately, we do not have data regarding the masses or volumes of the carotid bodies used in this study, nor of their metabolic rates. Previous studies, however, have shown that chronic hypoxaemia induces hypertrophy and hyperplasia of carotid body tissue in man (Arias-Stella, 1969) and animals (Edwards, Heath, Harris, Castillo,

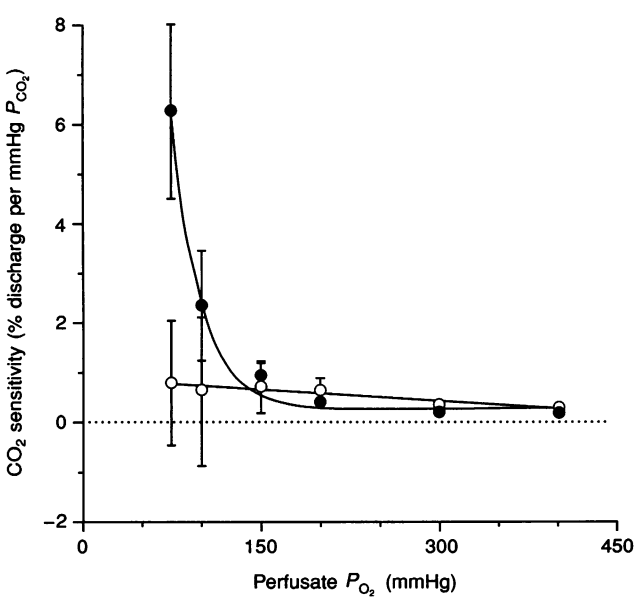

Figure 4. Mean effect of P_{O_2} upon CO_2 chemosensitivity in normoxic and chronically hypoxic rats

The effect of superfusate P_{O_2} upon the mean ± S.E.M. of CO_2 sensitivity (expressed as a percentage change in chemoreceptor discharge per mmHg P_{CO_2}) in all normoxic (●; $n = 8$) and chronically hypoxic (○; $n = 8$) rats. Values have been derived from the slopes of CO_2–response curves, at six levels of P_{O_2}, generated from the exponential fits to the P_{O_2}–response curves. Continuous lines through each set of data are the best-fit single exponentials with offset. A significant effect ($P < 0{\cdot}001$, single factor ANOVA) of P_{O_2} was found in the normoxic group but not ($P > 0{\cdot}999$) in the chronically hypoxic group.

Kruger & Arias-Stella, 1971), a phenomenon that appears due to a local effect within the carotid body (Mills & Nurse, 1993). There is no reason to suppose that a similar increase in growth did not occur with our chronically hypoxic carotid bodies and the possibility arises therefore that, due to their larger size, the chronically hypoxic carotid bodies of our study were subjected to a relatively greater tissue stimulation than were the normoxic controls, at any level of superfusate P_{O_2}, although, of course, this may be counteracted by the larger size of the normoxic rats. Any increase in the metabolic rate of the chronically hypoxic group relative to the normoxic group would further increase any difference in stimulation intensity. This would tend to produce P_{O_2}–response curves that were relatively right-shifted in the chronically hypoxic group. Although our data would seem to suggest that this might not be the case, we have not made a direct comparison of the position of the hypoxic response curves between the two groups, for the reasons outlined above, and have only compared P_{O_2}-dependent results within single animals or within a single group of animals.

However, our approach does not preclude a comparison of the effect of P_{CO_2} upon the P_{O_2}-independent components of each exponential P_{O_2}–response curve. In this respect we have found that P_{CO_2} increased the level of chemoreceptor discharge in hyperoxia (horizontal asymptote) in both groups of animals. Chronic hypoxia does not therefore appear to affect the CO_2 chemotransduction process in any qualitative way. However, although P_{CO_2} was without effect upon the shape (rate constant) of the P_{O_2}–response curve in both groups, the absolute level of the rate constant was significantly greater in the chronically hypoxic group, giving rise to 'flatter' response curves. As this flatness may arise from a relative lack of CO_2–O_2 interaction, we suggest that chronic hypoxaemia does not prevent a chemoreceptor response to hypoxia or to hypercapnia *per se* but instead, leads to a decreased interaction between the effects of hypoxia and hypercapnia given together as an asphyxial stimulus. This would account for the larger rate constants in the chronically hypoxic group. Thus the normal adult multiplicative interaction between these two stimuli (Lahiri & Delaney, 1975) appears to be undeveloped and the results of the chronically hypoxic group appear similar to those obtained in 5- to 7-day-old, normoxic rat pups (Pepper *et al.* 1994). The influence of the increased postnatal arterial oxygenation therefore appears important to the process of resetting at the level of the carotid body, as implied in previous studies (Blanco *et al.* 1988; Eden & Hanson, 1987*b*; Hanson *et al.* 1989), with resetting now defined as a maturational increase in the level of chemoreceptor discharge to either a CO_2 or a hypoxic stimulus, brought about by an increasing interaction with the other stimulus.

Our results support the idea that either long-term or intermittent chronic hypoxaemia from birth may lead to a failure to reset chemosensitivity, which could then lead to a compounding of the chronic hypoxaemia and a further delay in chemoreceptor maturation in a positive feedback loop. Thus the immature carotid body may indeed play a role in sudden infant death syndrome (Hunt, McCulloch & Brouillette, 1981; Hunt, 1992). The reversibility of the effects of chronic hypoxia upon chemosensitivity have been less studied than the onset of any such changes. However, in one such study (Okubo & Mortola, 1990), it was found that rats exposed to 10% inspired O_2 for the first six postnatal days exhibited only 50% of the ventilatory response to acute hypoxic challenges at 50 days of age when compared with normoxic age-matched controls, and also rats that had been given the same duration of exposure to chronic hypoxia but only during post-weaning, adult life. These authors make the intriguing suggestion that the blunting of hypoxic chemosensitivity seen in high-altitude natives may result from their exposure to chronic hypoxia at birth rather than to any genetic differences. The implications of this for non-altitude resident infants who experience chronic hypoxaemia in the neonatal period may also be of clinical importance (Calder, Williams, Smyth, Boon, Kumar & Hanson, 1994). Interestingly, in the rats studied by Okubo & Mortola (1990), the ventilatory response to hyperoxia was little affected. If carotid body CO_2–O_2 interaction is attenuated by chronic hypoxia, then one might predict little effect upon the response to acute hyperoxia.

It is now generally accepted that the common final stages of chemotransduction in the carotid body involve an elevation of intracellular $[Ca^{2+}]$ (Buckler & Vaughan-Jones, 1994*a*, *b*) which initiates neurotransmitter release from the presynaptically located type I cell. In this respect the carotid body is similar to many other neuro-secretory systems and its uniqueness therefore presumably lies with its exquisite sensitivity to its natural stimuli. Although the mechanism(s) which underlie this sensitivity in the adult are disputed, it is conceivable that the postnatal maturation of CO_2–O_2 interaction may involve an oxygen-dependent change in their sensitivity. Postulated mechanisms include a hypoxia-sensitive intracellularly located cytochrome (Biscoe & Duchen, 1990; Mulligan, Lahiri & Storey, 1981) and/or a putative membrane-bound haem protein (Lopez-Lopez, Gonzalez, Urena & Lopez-Barneo, 1989) or NADPH-oxidase (Acker, Dufau, Huber & Sylvester, 1989) linked to a K^+ channel (Delpiano & Hescheler, 1989; Lopez-Lopez *et al.* 1989; Peers, 1990). Interestingly, these potential sensors operate over different ranges of P_{O_2} (Lahiri, 1994) and a maturational change in the relative contribution of each to chemoreception cannot be ruled out. The effect of changes in $P_{CO_2}/[H^+]_i$ upon the sensitivity of these chromophores to oxygen is also not known. A significant resting Na^+ conductance in the type I cell of the normoxic carotid body has been described (Buckler & Vaughan-Jones, 1994*b*) and a cAMP-mediated increase in newborn rat carotid body type I cell Na^+ current density with 2 weeks of chronic hypoxia has also been reported (Stea, Jackson & Nurse, 1992). A deter-

mination of the role of any of these potential mechanisms in resetting will require further experimentation. Whether similar or other mechanisms to those mentioned above are involved in the attenuation or 'blunting' of the ventilatory response to an acute hypoxic challenge in residents of high altitude (Severinghaus, Bainton & Carcelen, 1966) is not known.

In conclusion, we have shown that 5 weeks of chronic hypoxia from birth can attenuate the degree of CO_2–O_2 interaction at the level of the carotid body and therefore a postnatal elevation in arterial oxygenation appears to be a prerequisite for the normal maturation of this organ. This impairment will greatly reduce the chemoreceptor discharge frequency at any level of asphyxial stimulation and we speculate that such an impairment may have clinical relevance to infants who experience a pathologically imposed chronic hypoxaemia in early postnatal life.

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