Carbon dioxide sensitivity during hypoglycaemia-induced, elevated metabolism in the anaesthetized rat

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We have utilized an anaesthetized rat model of insulin-induced hypoglycaemia to test the hypothesis that peripheral chemoreceptor gain is augmented during hypermetabolism. Insulin infusion at 0.4 U kg^{-1}min^{-1} decreased blood glucose concentration significantly to 3.37 ± 0.12 mmol l^{-1}. Whole-body metabolism and basal ventilation were elevated without increase in P_{a,CO_2} (altered non-significantly from the control level, to 37.3 ± 2.6 mmHg). Chemoreceptor gain, measured either as spontaneous ventilatory airflow sensitivity to P_{a,CO_2} during rebreathing, or by phrenic minute activity responses to altered P_{a,CO_2} induced by varying the level of artificial ventilation, was doubled during the period of hypermetabolism. This stimulatory effect was primarily upon the mean inspiratory flow rate, or phrenic ramp component of breathing and was reduced by 75% following bilateral carotid sinus nerve section. *In vitro* recordings of single carotid body chemoafferents showed that reducing superfusate glucose concentration from 10 mM to 2 mM reduced CO_2 chemosensitivity significantly from 0.007 ± 0.002 Hz mmHg^{-1} to 0.001 ± 0.002 Hz mmHg^{-1}. Taken together, these data suggest that the hyperpnoea observed during hypermetabolism might be mediated by an increase in the CO_2 sensitivity of the carotid body, and this effect is not due to the insulin-induced fall in blood glucose concentration.

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Exercise hyperpnoea, the proportional increase in alveolar ventilation with increasing metabolic rate (Casaburi *et al.* 1978), acts to maintain alveolar, and hence arterial P_{CO_2} (P_{a,CO_2} and P_{a,CO_2}, respectively) essentially constant during mild to moderate, dynamic exercise below the anaerobic threshold in humans (Haldane & Priestley, 1905; Wasserman *et al.* 1967; Dempsey *et al.* 1995). This homeostatic mechanism, in combination with the plasma bicarbonate concentration ($[HCO_3^-]$), is vital for the maintenance of brain extracellular pH. A number of animal models have confirmed that exercise hyperpnoea also occurs in non-human species, including goats (Bisgard *et al.* 1982) and rats (Fregosi & Dempsey, 1984), albeit usually associated with a relative degree of hypocapnia that is maintained during the exercise period and that is increased with increasing workload. Together with the absence of hypoxia and the maintenance, or elevation, of arterial pH, this negates a major feedback role for peripheral and/or central chemoreceptors in mediating steady-state exercise hyperpnoea. A number of studies have therefore given rise to a variety of feedforward hypotheses that include key roles for limb muscle and joint afferents (e.g. Kao, 1963; Mitchell *et al.* 1977), non-blood gas humoral signals (e.g. Band & Linton, 1986; Burger *et al.* 1988) and so-called CNS central command (e.g. Eldridge *et al.* 1985). There remains, however, much controversy and the mechanism by which ventilation is coupled so precisely to metabolism remains unclear. One possible mechanism might be an increase in chemoreceptor gain (Briggs, 1920; Asmussen & Nielsen, 1946; Hickam *et al.* 1951) during elevated metabolism. Indeed, the slope of the relation between ventilation and P_{a,CO_2} was almost doubled in exercising humans as a consequence primarily of an increase in peripheral chemoreceptor sensitivity (Weil *et al.* 1972). Such studies of respiratory gain during exercise in humans could be complemented by a suitable anaesthetized, animal model in which ventilation might be increased isocapnically by increasing metabolic rate in the absence of potential neural stimuli arising from moving limbs, or from descending central pathways. We showed recently (Bin-Jaliah *et al.* 2004), that insulin-induced hypoglycaemia could increase ventilation in an anaesthetized rat model as a consequence of the associated doubling of $\dot{V}_{O_2}$, rather than through a fall in blood glucose concentration or through any non-specific effect of insulin. This effect was mediated

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through the carotid bodies, as animals in which the carotid sinus nerves were sectioned bilaterally exhibited no change in ventilation and hence showed an elevated P_{a,CO_2} during the period of increased metabolism while, in intact animals, the increased ventilation occurred isocapnically.

The present series of experiments was designed to measure peripheral chemoreceptor gain *in vivo* and *in vitro*, and our results show that an increased metabolism can elevate carotid body-mediated CO_2 sensitivity. Some of this work has been published previously in abstract form (Bin-Jaliah *et al.* 2003).

Methods

In vivo and *in vitro* studies were utilized to investigate the effect of insulin-induced hypermetabolism upon CO_2 chemosensitivity. Experiments were performed in accordance with the UK Animals (Scientific Procedures) Act 1986.

In vivo studies

Animals and surgical preparation. Anaesthesia of adult male Wistar rats (328 ± 6 g, $n = 20$) was induced with halothane (3–4% in oxygen). The right femoral vein was cannulated and anaesthesia maintained with 650 mg kg^{-1} of 25% w/v ethyl carbamate (urethane, Sigma). The femoral artery was cannulated for continuous recording of arterial blood pressure (NL108T2 Neurolog system, Digitimer Research Instrumentation, Hertfordshire, UK). The left femoral vein was cannulated for I.V. insulin infusion. The trachea was exposed and cannulated.

The carotid artery bifurcations were located bilaterally and carotid sinus nerves (CSNs) were identified and either sectioned bilaterally (CSNX) at their junction with the glossopharyngeal (IXth cranial) nerve or left intact (sham). Denervation was confirmed by the absence of hypoxia-induced hyperventilation prior to experiments. Experimental protocols started after a surgical recovery period of 60 min, at which time baseline cardiovascular and respiratory variables were stable. Rectal temperature was monitored throughout the study and kept at 36.5–37.5°C, using a small homoeothermic blanket system (Harvard Apparatus Ltd, Kent, UK). At the end of the experiment, animals were killed by urethane overdose and decapitation.

Rebreathing technique. Ventilatory CO_2 chemosensitivity was determined using a modified Read rebreathing technique (Read, 1967) in hyperoxia ($P_{a,O_2} > 300$ mmHg). The rebreathing circuit consisted of a 40 ml volume bag connected, during the run, to the tracheal cannula and to the input and exhaust ports of a fast response CO_2 analyser (Type FM1, ADC Gas Analysis Limited, Herts, UK). Movements of the rebreathing bag were measured as air displacement through a respiratory flow head (F10L, gm instruments Ltd, Kilwinning, UK), which was connected to a pneumotachometer (electrospirometer CS8, Mercury Electronics Ltd, Glasgow, UK) which integrated the signal to derive tidal volume. Both percentage CO_2 and the tidal volume signals were then recorded using a computer. At the start of each run, the rebreathing bag was filled with ~5–6% CO_2, in accordance with the pre-run P_{a,CO_2}, in 94–95% O_2 and the run was stopped when rebreathing had increased the CO_2 to ~8–10%. Partial pressures of O_2 and CO_2, pH, bicarbonate and glucose concentration of the arterial blood were sampled using 150 μl glass capillary tubes (Stat Profile pHOx Plus L Analyser; Nova Biomedical, Deeside, Flintshire, UK), just before the start of a rebreathing run at 40, 30, 20 and 10 min prior to insulin (from bovine pancreas, 28.6 USP units mg^{-1}, Sigma, St Louis, MO, USA) infusion at 0.4 U kg^{-1}min^{-1} and at 20, 40, 60, 90 and 120 min after the start of insulin infusion.

Phrenic nerve recordings. Integrated phrenic nerve responses were measured as previously described (Bin-Jaliah *et al.* 2004) in urethane anaesthetized (650 mg kg^{-1}; I.V.), vagotomized and paralysed (pancuronium bromide; David Bull Laboratories, Warwick, UK; 3 mg kg^{-1}, I.V.) adult rats that were artificially ventilated (Harvard rodent ventilator, model 683; Harvard Apparatus, Holliston, MA, USA) with 30% O_2 in N_2. Adequacy of anaesthesia was confirmed throughout the procedure by continuous measurement of arterial blood pressure and lack of cardiovascular response to a strong paw pinch. Supplementary anaesthesia, urethane (50 mg kg^{-1}; I.V.) was given as required.

Measurement of adjustable artificial ventilation. The level of artificial pulmonary ventilation ($\dot{V}_E$), utilized in anaesthetized, vagotomized and paralysed animals, was measured from integrated tracheal airflow by placing the respiratory flow head (F10L, gm instruments Ltd) between the tracheal cannula and the ventilator. The respiratory flow head was in turn connected to the pneumotachometer (electrospirometer CS8, Mercury Electronics Ltd) which integrated the signal to derive tidal volume. The tidal volume signal was then recorded using a computer, together with the phrenic signal. The P_{a,CO_2} could be varied by altering the degree of artificial ventilation and thus both the $\dot{V}_E$–P_{a,CO_2} relation and CO_2 chemosensitivity (respiratory controller equation) could be assessed simultaneously.

Data analysis. All data were recorded and analysed via a MICRO 1401 connected to a computer running

Spike 2 software (Cambridge Electronics Design, Cambridge, UK). Mean arterial blood pressure (MABP) and heart rate (HR) were calculated from the blood pressure trace. A CED Spike2 script was used to determine tidal volume (V_T), inspiratory time (T_i) and expiratory time (T_e) breath by breath from the integrated airflow, and these variables were used to calculate $\dot{V}_E$. Inspired percentage CO_2 was measured by the script for each breath and $\dot{V}_E$ was increased linearly by increasing CO_2. Linear regression analysis was used to derive the CO_2 sensitivity. Another CED Spike2 script was applied on the phrenic trace to determine burst frequency (f), peak amplitude, timing and phrenic ramp of integrated phrenic activity ($\int Phr$). The minute phrenic activity then was calculated and corrected for body weight.

In vitro studies

Adult rats (115 ± 17 g, $n = 5$) were anaesthetized with halothane (3–4% in oxygen for induction, and maintained at 2%). Left and right carotid bifurcations were removed as previously described (Pepper *et al.* 1995). Animals were killed by halothane overdose and decapitation.

Electrophysiological recordings of single or few-fibre chemoafferents of the carotid sinus nerve were made with glass suction electrodes (GC150T-10, Harvard Apparatus) and analysed as previously described (Bin-Jaliah *et al.* 2004). Chemoreceptor discharge was discriminated as activity exceeding a level which was at least 25% of the amplitude of the baseline noise above the noise itself and which responded to a decrease in superfusate P_{O_2} with a brisk, reversible increase in discharge frequency. Single fibres were discriminated on the basis of their amplitude and shape. CO_2 chemosensitivity, in Hz mmHg^{-1}, was calculated from the difference in the steady-state chemoafferent discharge at 40 and 80 mmHg P_{CO_2} during either 10 or 2 mM glucose perfusion.

Data from both *in vivo* and *in vitro* studies are expressed as means ± S.E.M. and significance ($P < 0.05$) tested with a paired t test, unless indicated otherwise, using Statview (SAS Institute Inc., North Carolina, USA) software.

Results

Effect of insulin infusion upon arterial blood parameters

Venous infusion of insulin at a rate of 0.4 U kg^{-1}min^{-1} decreased blood glucose (BG) significantly from 8.96 ± 0.34 mmol l^{-1} to 3.37 ± 0.12 mmol l^{-1} ($P < 0.0001$, $n = 8$). This hypoglycaemia was associated with a slight but significant reduction in arterial blood potassium levels from a basal control level of 4.24 ± 0.08 mmol l^{-1} to 3.98 ± 0.08 mmol l^{-1} ($P < 0.007$, $n = 8$). Blood lactate levels during hypoglycaemia were not significantly altered from basal levels (from 1.78 ± 0.13 mmol l^{-1} to 1.83 ± 0.22 mmol l^{-1}; $P > 0.64$, $n = 8$). Bicarbonate also did not vary during hypoglycaemia (from 25.09 ± 0.39 mmol l^{-1} to 24.47 ± 0.41 mmol l^{-1}; $P > 0.15$, $n = 8$).

Effect of insulin-induced hypoglycaemia upon cardiovascular variables

Mean arterial blood pressure (MABP) did not change significantly from its basal levels during the hypoglycaemic testing period from 85.3 ± 7.0 mmHg to 86.24 ± 4.4 mmHg; $P > 0.86$, $n = 8$. In addition, insulin-induced hypoglycaemia did not alter the mean HR (from 456 ± 19 beats min^{-1} (bpm) to 457 ± 14 bpm; $P > 0.94$, $n = 8$).

Baseline ventilation and ventilatory CO_2 chemosensitivity, measured by rebreathing, increased during hypoglycaemia in sham but not in CSNX animals

Basal ventilation and ventilatory CO_2 chemosensitivity in sham and CSNX animals ($n = 6$ for both) was determined from the relation between $\dot{V}_E$ and the percentage of inspired CO_2 during rebreathing (Fig. 1). Significance between sham and CSNX before and during hypoglycaemia was determined with two-way ANOVA (with denervation and hypoglycaemia as factors) with *post hoc* Bonferroni/Dunn test. Basal ventilation, at 40 mmHg P_{a,CO_2}, increased significantly during hypoglycaemia in sham operated rats ($P < 0.002$, Fig. 2), but not in CSNX animals ($P > 0.11$, Fig. 2). In sham animals, no significant changes in P_{a,CO_2} or pH from baseline occurred during hypoglycaemia (43.1 ± 0.8 mmHg to 42.2 ± 1.0 mmHg, $P > 0.56$, and 7.402 ± 0.007 to 7.389 ± 0.005, $P > 0.13$). In contrast, CSNX rats exhibited a respiratory acidosis with P_{a,CO_2} increasing significantly during insulin-induced hypoglycaemia by ~6 mmHg (41.3 ± 0.7 mmHg to 47.3 ± 0.5 mmHg; $P < 0.0001$), which was correlated with a significant fall in pH from 7.402 ± 0.024 to 7.320 ± 0.006 ($P < 0.0002$). The basal P_{a,O_2} was lower in CSNX than in sham animals (67.3 ± 1.6 mmHg *versus* 73.4 ± 2.1 mmHg, $P < 0.02$) and these levels did not change significantly during hypoglycaemia in both groups (CSNX; 65.7 ± 2.1 mmHg; $P > 0.59$ and sham; 75.9 ± 1.6 mmHg; $P > 0.91$).

Basal, pre-insulin levels of CO_2 chemosensitivity were not significantly different between sham and CSNX animals ($P > 0.69$, Fig. 2). In contrast, insulin-induced hypoglycaemia, in the sham operated rats, was associated with a considerable, ~50% increase in ventilatory CO_2 chemosensitivity from basal levels ($P < 0.004$, Fig. 2),

while CO_2 chemosensitivity in CSNX animals remained unchanged ($P > 0.21$, Fig. 2). The difference, in sham animals, between CO_2 chemosensitivity in euglycaemia and hypoglycaemia represents the total (peripheral plus CNS) augmentation of chemoreceptor drive during hypoglycaemia ($19.2 - 12.6 = 6.6$ ml min^{-1} kg^{-1} mmHg^{-1}). The same difference, when measured in CSNX animals, represents only the CNS component of the augmentation ($13.8 - 12.1 = 1.7$ ml min^{-1} kg^{-1} mmHg^{-1}). Therefore, the peripheral-only component of the augmentation can be found as $6.6 - 1.7 = 4.9$ ml min^{-1} kg^{-1} mmHg^{-1} which represents ~75% of the total augmentation. The increased ventilatory CO_2 chemosensitivity in sham animals during hypoglycaemia was due to a significant rise of the mean inspiratory flow rate ($MIF = V_T/T_i$; $P < 0.02$, Fig. 2), rather than the timing (T_i/T_{tot}; $P > 0.09$, Fig. 2) component of each breath. Mid-rebreathing-run arterial blood samples were taken for P_{a,O_2} which was found to be 325 ± 20 mmHg (sham, $n = 3$) and 311 ± 22 mmHg (CSNX, $n = 3$).

Increased CO_2 chemosensitivity in anaesthetized rats during elevated metabolism

Given that, at any point of time, an animal has a singular $\dot{V}_E$ and a singular P_{a,CO_2} that is governed by the rate of metabolism, the effect of $\Delta\dot{V}_E$ upon P_{a,CO_2} and the effect of $\Delta P_{a,CO_2}$ upon phrenic nerve activity can be computed simultaneously with the intersection of the $\dot{V}_E$–P_{a,CO_2} curve and respiratory controller equations indicating a unique, steady-state level of P_{a,CO_2} for any particular metabolic rate. Steady-state phrenic nerve activity was measured (Fig. 3) prior to and during insulin infusion in anaesthetized, vagotomized, paralysed and artificially

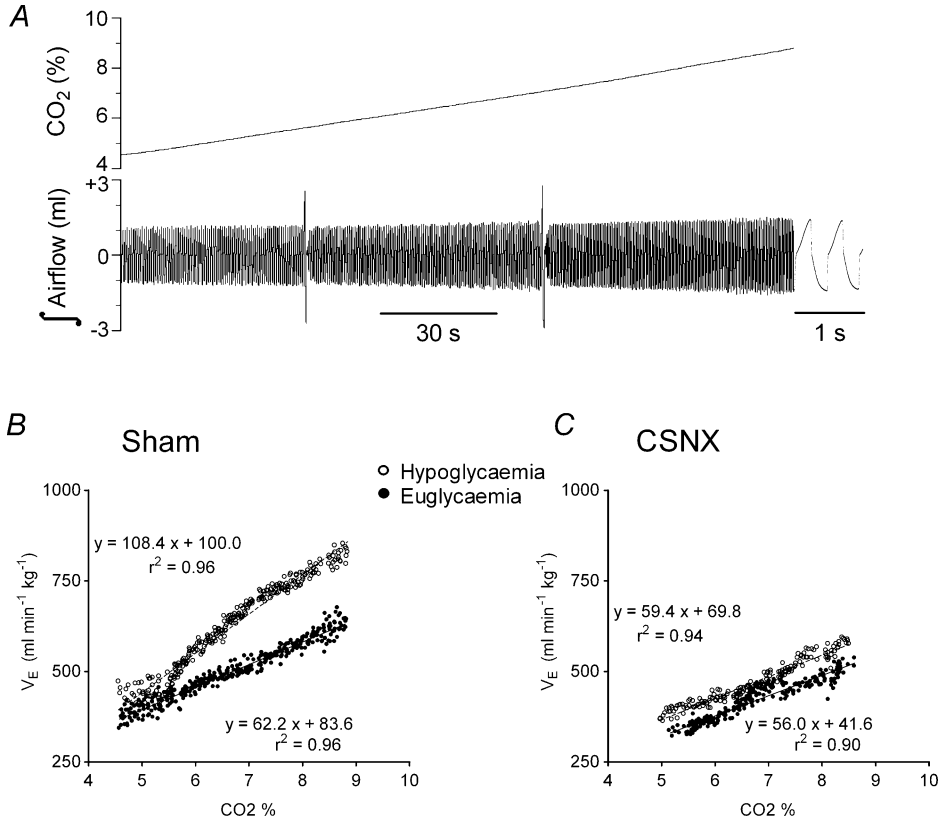

Figure 1. Carotid body-mediated augmentation of ventilatory CO_2 chemosensitivity measured during rebreathing

A, representative traces showing increasing percentage inspired CO_2 (upper trace) and corresponding spontaneous, integrated tracheal airflow (lower trace) during a single rebreathing experiment in one animal. Inspiratory and expiratory flows were separately integrated and depicted in a single trace with + indicating inspiratory volume and − indicating expiratory volume. Timescale expanded at right side. *B*, breath by breath $\dot{V}_E$ against percentage CO_2 in a single sham animal, during euglycaemia (●; from the example data shown in *A*) and hypoglycaemia (○). $\dot{V}_E$ increased linearly with increasing CO_2 ($P < 0.0001$; linear regression), and the ventilatory CO_2 sensitivity was taken as the slope of the regression. During insulin-induced hypoglycaemia there was an increase of ventilatory CO_2 sensitivity. *C*, breath by breath $\dot{V}_E$ against percentage CO_2 in a single CSNX different animal, during euglycaemia (●), and hypoglycaemia (○). $\dot{V}_E$ increased linearly with increasing CO_2 ($P < 0.0001$; linear regression) but the ventilatory CO_2 sensitivity did not change during hypoglycaemia.

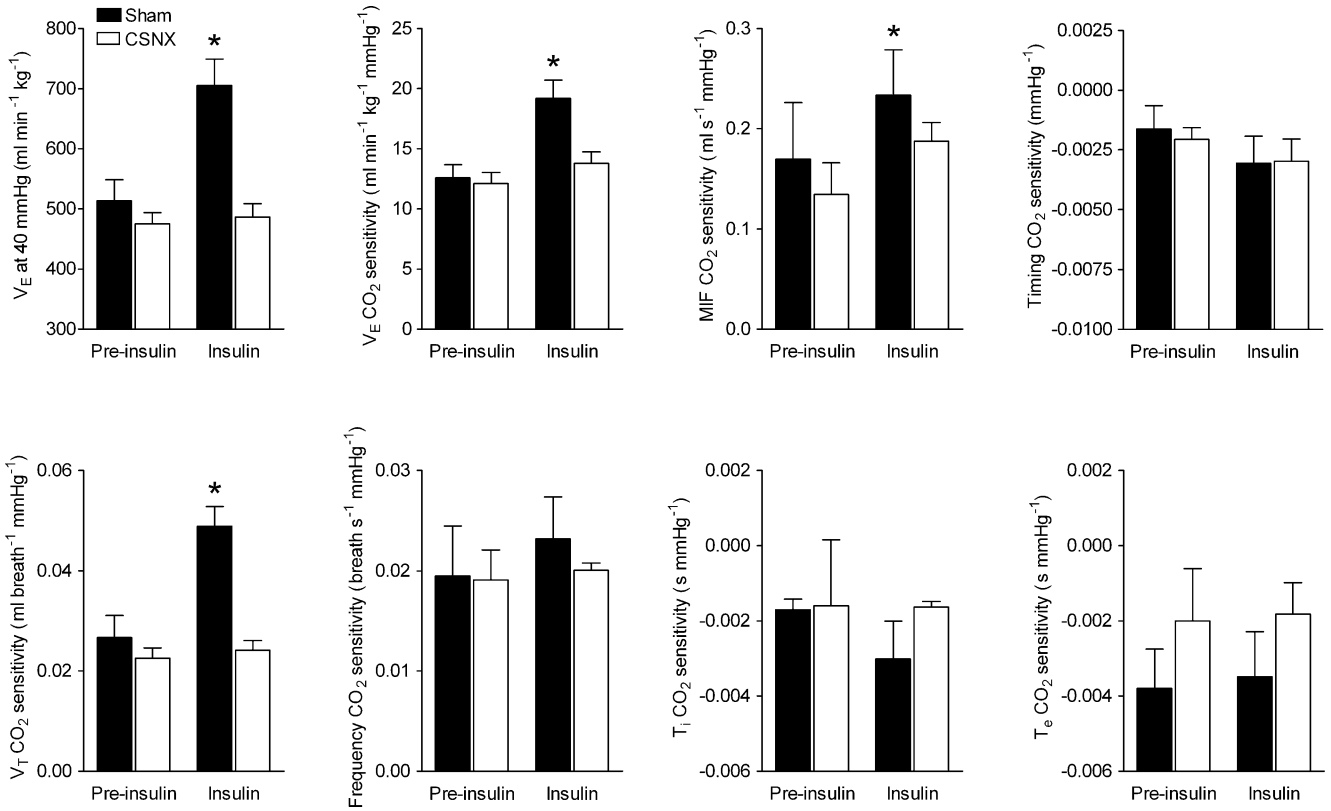

Figure 2. Carotid body-mediated augmentation of basal spontaneous ventilation and ventilatory CO_2 chemosensitivity measured during rebreathing

Means ± S.E.M. of $\dot{V}_E$ at 40 mmHg, $\dot{V}_E$ CO_2 sensitivity, mean inspiratory flow rate (MIF = V_T/T_i) CO_2 sensitivity, timing (T_i/T_{tot}) CO_2 sensitivity, tidal volume (V_T) CO_2 sensitivity, frequency CO_2 sensitivity, inspiratory time (T_i) CO_2 sensitivity and expiratory time (T_e) CO_2 sensitivity in sham ($n = 6$) and CSNX ($n = 6$) animals, pre-insulin (40–10 min prior) and during (20–120 min post-) insulin infusion at 0.4 u min^{-1} kg^{-1}. *Significant difference ($P < 0.05$) from pre-insulin levels.

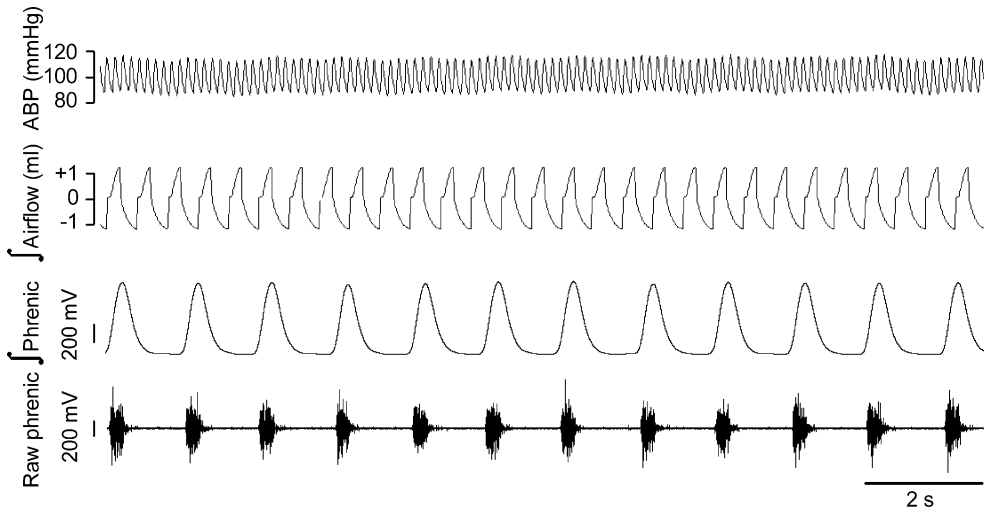

Figure 3. Phrenic nerve activity during adjustable artificial ventilation

Steady-state measurement of phrenic nerve discharge, artificial ventilation and blood gases were recorded in anaesthetized, vagotomized, paralysed and artificially ventilated rats. By modifying the level of artificial ventilation, different levels of P_{aCO_2} could be achieved. Illustrative traces from a single experiment are shown (from top down): arterial blood pressure, integrated tracheal airflow, integrated phrenic activity (100 ms time constant) and raw phrenic nerve discharge. For each steady state, ventilation could be measured as integrated airflow and as phrenic minute activity. By determining the effect of varying airflow upon P_{a,CO_2} and the effect of P_{a,CO_2} upon phrenic minute activity, both $\dot{V}_E$–P_{a,CO_2} curves and CO_2 respiratory controller equations could be derived simultaneously.

ventilated rats ($n = 6$). The level of artificial pulmonary ventilation was increased and decreased by adjusting the ventilator tidal volume and frequency settings. At any given metabolic rate, P_{a,CO_2} varies inversely with ventilation as predicted by the conservation of matter. The values of artificial ventilation and P_{a,CO_2} were used to create $\dot{V}_E$–P_{a,CO_2} relation curves during euglycaemic (control) and hypoglycaemic conditions, according to the hyperbolic equation:

$$y = D(1 + (A/(x - C)))$$

where $y = \dot{V}_E$, $x = P_{a,CO_2}$, A is the shape parameter, C is the vertical asymptote and D is the horizontal asymptote. A representative hyperbolic fit from a single experiment is shown in Fig. 4*A* (NB axes reversed). In euglycaemia, the mean hyperbolic function was:

$$\dot{V}_E = 13.70 \pm 6.36(1+(6583 \pm 4392/(P_{a,CO_2} - 10.03 \pm 3.32)))$$

which, during hypoglycaemia was:

$$\dot{V}_E = 27.89 \pm 12.98(1+(6855 \pm 5015/(P_{a,CO_2} - 14.88 \pm 4.72)))$$

On average, in all rats, the P_{a,CO_2} was significantly elevated by 4.6 ± 1.2 mmHg ($P < 0.01$) at a fixed (euglycaemic, eucapnic; P_{a,CO_2} 40 mmHg) level of $\dot{V}_E$, indicating an increase in metabolic rate.

Insulin-induced hypoglycaemia significantly increased the CO_2 chemosensitivity of minute phrenic activity as measured by linear regression, by more than two-fold (from 1.33 ± 0.13 V min^{-1} kg^{-1} mmHg^{-1} to 3.31 ± 0.28 V min^{-1} kg^{-1} mmHg^{-1}; $P < 0.0001$). Representative data, taken from the same rat utilized for Fig. 4*A*, are shown in Fig. 4*B*. This elevation was mediated via an increase in the phrenic ramp component of each breath (from 0.030 ± 0.009 V s^{-1} mmHg^{-1} to 0.073 ± 0.021 V s^{-1} mmHg^{-1}; $P < 0.02$) with no change in the timing component (from -0.0011 ± 0.0005 mmHg^{-1} to -0.0019 ± 0.0006 mmHg^{-1}; $P > 0.15$).

A conversion and normalization procedure was used to compare the units of ventilation obtained from airflow (ml min^{-1} kg^{-1}) and the phrenic minute activity (V min^{-1} kg^{-1}). For each rat, we computed a single euglycaemic, eucapnic (P_{a,CO_2} 40 mmHg) value for each of these measurements of ventilation. A conversion factor was calculated from these and this was applied to all phrenic minute activity levels for this particular animal. Finally, the ventilation level at which both curves crossed during euglycaemia (i.e. at 40 mmHg) was then normalized to 1. An overlay of the normalized mean $\dot{V}_E$–P_{a,CO_2} curves and linear CO_2 sensitivities, from all rats, during euglycaemia and hypoglycaemia is shown in Fig. 5 which shows that the increase of CO_2 chemosensitivity when coupled to hypermetabolism acts to maintain a relative eucapnia or slight hypocapnia (the P_{a,CO_2} was tuned to 37.3 ± 2.6 mmHg from the basal setting of 40 mmHg; $P > 0.21$).

Low glucose decreased CO_2 chemosensitivity in the *in vitro* carotid body preparation

Baseline, chemoreceptor discharge was obtained at 400 mmHg P_{O_2}, 40 mmHg P_{CO_2} and 10 mM glucose.

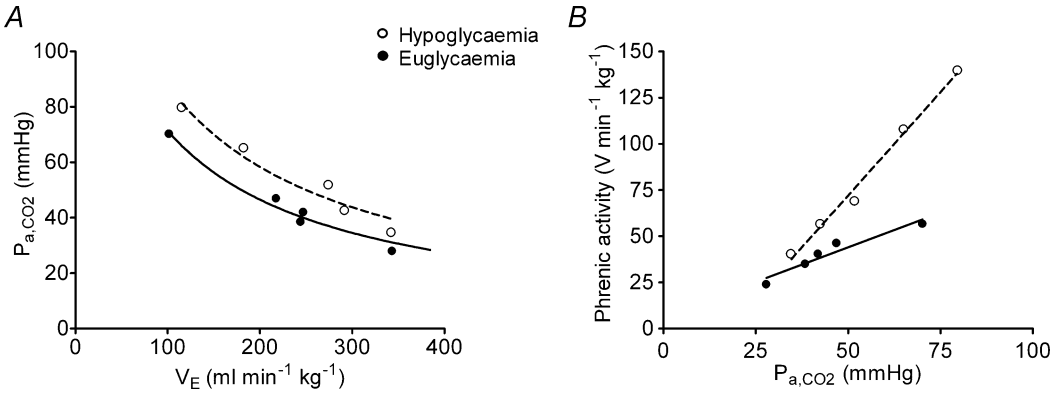

Figure 4. The effect of $\Delta\dot{V}_E$ upon P_{a,CO_2} and the effect of ΔP_{aCO_2} upon phrenic nerve activity; increased metabolism and CO_2 chemosensitivity during hypoglycaemia
A, representative data taken from a single experiment. Steady-state P_{a,CO_2} is sampled at various levels of artificial $\dot{V}_E$ measured as integrated tracheal airflow during euglycaemia (●) and hypoglycaemia (○). Data are shown fitted by hyperbolic functions: $P_{a,CO_2} = 0.4(1 + (3.3 \times 10^4/(\dot{V}_E + 85.3)))$ and $P_{a,CO_2} = 0.5(1 + (3.7 \times 10^4/(\dot{V}_E + 97.3)))$ during euglycaemia ($r^2 = 0.98$) and hypoglycaemia ($r^2 = 0.95$), respectively. During insulin-induced hypoglycaemia, there was an upward shift in the position of the $\dot{V}_E$–P_{a,CO_2} curves. *B*, representative raw data from the same animal. Phrenic minute activity is measured at the same steady-state levels of P_{a,CO_2} shown in *A*. Data are shown fitted by linear regression: phrenic minute activity $= 0.75P_{a,CO_2} + 6.6$ and phrenic minute activity $= 2.24P_{a,CO_2} - 39.9$ during euglycaemia ($r^2 = 0.92$) and hypoglycaemia ($r^2 = 0.99$), respectively. Linear regression showed an increase of phrenic CO_2 sensitivity during insulin-induced hypoglycaemia.

All preparations responded in a graded fashion to a ramp decrease in P_{O_2}. CO_2 control chemosensitivity was determined from the difference between the baseline discharge and that obtained at 80 mmHg P_{CO_2}, with the P_{O_2} and glucose unchanged. Raising the superfusate P_{CO_2} from 40 mmHg to 80 mmHg, significantly increased the chemoreceptor single-fibre discharge frequency from 0.40 ± 0.20 Hz to 0.70 ± 0.25 Hz, respectively; $P < 0.04$, $n = 7$, and CO_2 chemosensitivity was calculated as 0.007 ± 0.002 Hz mmHg^{-1}. Lowering the superfusate glucose from 10 mM to 2 mM by substitution with sucrose blunted the effect of hypercapnia upon chemoafferent discharge (0.27 ± 0.15 Hz at 40 mmHg P_{CO_2} and 0.31 ± 0.14 Hz at 80 mmHg P_{CO_2}; $P > 0.58$, $n = 7$) and CO_2 chemosensitivity was significantly reduced to 0.001 ± 0.002 Hz mmHg^{-1} from the basal level in 10 mM glucose ($P < 0.03$; Fig. 6). In addition, in a separate series of experiments, lowering superfusate glucose to 0 mM by complete substitution with sucrose in most cases led to slowly reversible decreases or even abolition of discharge frequency with prolonged exposure of up to 20 min. Baseline discharge and CO_2 chemosensitivity were therefore greatly reduced.

Discussion

We have shown that an increase in the gain of the peripheral chemoreceptor C-mediated, reflex response to CO_2 occurs during a period of increased metabolism, induced by insulin-dependent, hypoglycaemia. In the present study, and in our previous study (Bin-Jaliah *et al.* 2004), we have controlled for and excluded insulin and hypoglycaemia as determinants of the ventilatory response in this model. The most likely remaining stimulus is that of increased metabolism, or at least some factor related to it. The model we have used resembles exercise only in terms of increased metabolic rate and isocapnic hyperpnoea. Given the absence of moving limbs, and the presence of anaesthesia, it is therefore not as complete an animal model as, for example, conscious, exercising ponies (Forster *et al.* 1983) or goats (Bisgard *et al.* 1982), but does offer a simple method for the rapid, reproducible and concurrent generation of both $\dot{V}_E$–P_{a,CO_2} relation curves and respiratory controller equations. This has not previously been described and the use of phrenic recordings in artificially ventilated animals might provide an efficient means by which to assess fully and test specific hypotheses regarding the hyperpnoea of exercise when used in conjunction with the hypermetabolism associated with insulin infusion. Insulin-induced hypoglycaemia is, however, a non-physiological situation that might induce non-stimulus-specific responses from a variety of receptors. For example, the hypoglycaemia induced by the insulin infusion may cause an increase in muscle sympathetic nerve activity (MSNA) as seen in man (Fagius *et al.* 1986), which itself may be liable for part of the observed hypermetabolism.

In the present study we have employed neuromuscular blockade with pancuronium. Neuromuscular blocking agents are known to reduce hypoxia chemosensitivity in man by around 28% but appear to be without effect upon CO_2 chemosensitivity (Eriksson, 1996). In all our experiments on paralysed animals, we only measured CO_2 chemosensitivity and this in the absence of hypoxia. Any non-specific effect of pancuronium might therefore be expected to be minimal. In the spontaneously breathing animals, although blood P_{a,O_2} fell to mildly hypoxic levels between rebreathing runs, which might have raised CO_2 chemosensitivity slightly and decreased metabolic rate (Mortola, 1999), all CO_2 rebreathing runs were measured on a background of hyperoxia to remove or minimize these confounding effects.

We have observed a significant lateral shift in the position of the $\dot{V}_E$–P_{a,CO_2} curves, as expected during hypermetabolism which was of the right magnitude to account for the elevation in P_{a,CO_2} we observed previously during insulin-induced hypoglycaemia when ventilation was kept constant (Bin-Jaliah *et al.* 2004). The relation between $\dot{V}_E$ and P_{a,CO_2} is only strictly correct if alveolar ventilation, $\dot{V}_A$, and not total ventilation, $\dot{V}_E$, is utilized. We have measured physiological dead space in adult rats (unpublished data) using the Bohr method by simultaneous measurement of expiratory volume

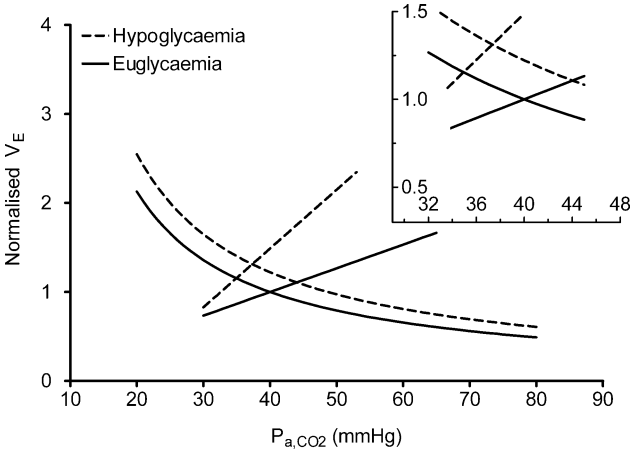

Figure 5. The normalized mean $\dot{V}_E$–P_{a,CO_2} curves and linear CO_2 sensitivities during euglycaemia and hypoglycaemia
The mean $\dot{V}_E$–P_{a,CO_2} curves and the mean CO_2 sensitivities for all animals ($n = 6$) determined by averaging the equation parameters determined from each experiment. The curves are shown overlaid after correction for different units of $\dot{V}_E$ and normalization (see text for details). The inset is an expanded portion of the graph. The intercept during euglycaemia (solid line) and the intercept during hypoglycaemia (broken line) represent the unique steady state $\dot{V}_E$ and P_{a,CO_2} in each condition and indicate that P_{a,CO_2} is not elevated during the hypermetabolism of hypoglycaemia due to the increase in the CO_2 sensitivity. Standard errors have not been shown in order to aid clarity.

and P_{a,CO_2}, and have shown that it is not significantly altered by varying the degree of total ventilation over the range utilized in this study. We have therefore made the assumption that dead space ventilation remains a constant fraction of total ventilation during hyperpnoea – an assumption that seems borne out from previous studies (Wasserman *et al.* 1967).

The potential for chemoreceptor gain to be altered during acute changes in metabolism is of physiological and, potentially, pathological significance. Augmented peripheral chemoreceptor gain during exercise as a potential mechanism mediating hyperpnoea was discussed by Cunningham (1963, 1973) and subsequently tested. Weil *et al.* (1972) described, in humans, that hypercapnic and hypoxic ventilatory sensitivity were both greater during mild, muscular, dynamic exercise of sufficient intensity to double metabolic rate as measured by $\dot{V}_{O_2}$. In our model, insulin infusion at 0.4 U kg^{-1}min^{-1} also caused an approximate doubling of $\dot{V}_{O_2}$ (Bin-Jaliah *et al.* 2004) and the concomitant, approximate doubling of CO_2 sensitivity in our present study is of a similar magnitude to that observed by Weil *et al.* (1972) at this relative rate of metabolism. Our mean data demonstrate that such an increase in chemoreceptor gain during augmented metabolism could reduce P_{a,CO_2} by up to 2 mmHg, while a failure to increase gain would lead to a calculated elevation of P_{a,CO_2} of ∼4 mmHg, a value very similar to our previously measured increase of ∼5 mmHg (Bin-Jaliah *et al.* 2004). Use of arterial blood samples for determination of blood gas tensions excludes potential problems of validating either alveolar or end-tidal gas tensions that, during exercise or increased metabolism, may not precisely reflect arterial values (Whipp & Wasserman, 1969). In further support of an increase in peripheral chemoreceptor gain, direct, *in vivo*, recordings of carotid chemoreceptor afferents showed an increase in discharge frequency during passive hindlimb movements in the cat (Biscoe & Purves, 1967) that arose as a consequence of an increase in efferent sympathetic discharge to the carotid body. Subsequently, however, blockade of the sympathetic innervation of the carotid body in human subjects by lignocaine (lidocaine) anaesthesia of the stellate ganglia failed to alter the ventilatory response to moderately heavy exercise (Eisele *et al.* 1967), but while this would seem to rule out a neurogenic origin for the increased chemoreceptor gain, it does not alter the possibility of a humoral mediator. In our *in vivo* experiments, although limb movement could not be seen, the elevated metabolic rate and presumed increase in MSNA might indicate some muscle fibre contraction and hence a degree of muscle neurogenic afferent activity. In our *in vitro* experiments, the decrease in superfusate glucose concentration to 2 mM does not

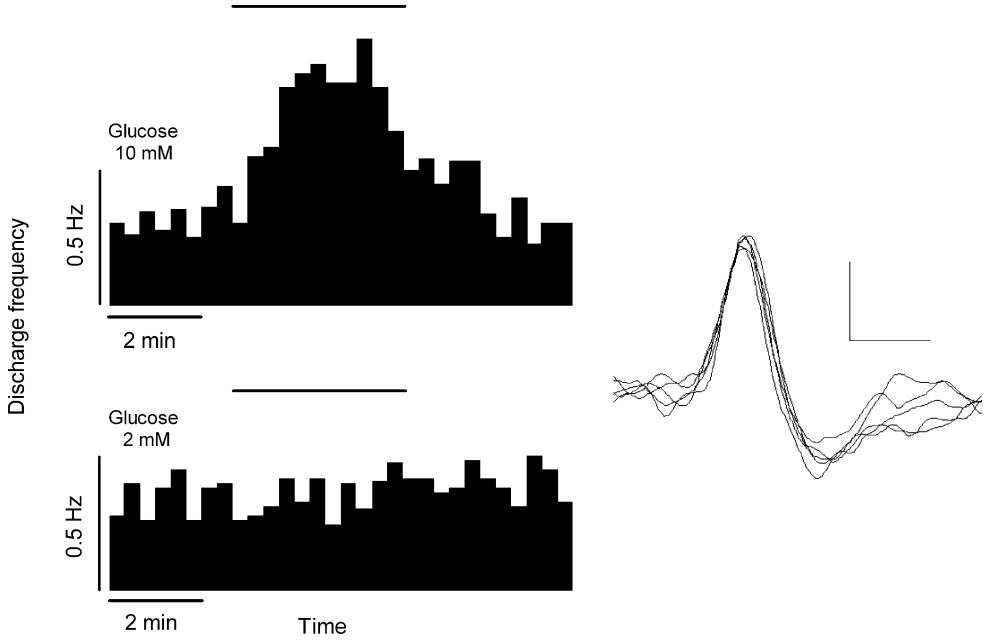

Figure 6. *In vitro* carotid sinus nerve CO_2 chemosensitivity is not increased by decreasing [glucose]
Single-fibre chemoafferent discharge recorded from one carotid body during control (10 mM glucose; above) and low-glucose superfusion (2 mM glucose; below). Discharge was binned into 20-s periods and frequency calculated as impulses s^{-1} (Hz). Hypercapnia, raising the superfusate P_{CO_2} from 40 mmHg to 80 mmHg, indicated by the horizontal bars, increased the chemoreceptor discharge frequency during control glucose perfusate. This effect was absent during low glucose superfusion. On the right are shown four superimposed afferent action potentials from this recording. The vertical scale bar is 100 μV, horizontal scale bar 0.4 ms.

account directly for any elevation in carotid chemoreceptor CO_2 sensitivity. This contrasts with the findings of Pardal & Lopez-Barneo (2002) who found that lowering superfusate glucose concentration could induce catecholamine secretion in a novel, thin slice preparation of the carotid body. We cannot presently account for this discrepancy except to note that, in the presence of hyperoxia, the catecholaminergic response of the thin slice carotid body to reduced glucose was relatively low, especially at the less severe levels of lowered glucose concentration.

The data arising regarding changes in the peripheral chemoreceptor gain to CO_2 during exercise in humans is, however, equivocal, with other studies demonstrating no change or perhaps even a fall in CO_2 sensitivity during exercise (Miyamura *et al.* 1976; Duffin *et al.* 1980; Kelley *et al.* 1982). These latter findings, however, may have been affected by respiratory mechanical limitation which is known to reduce ventilatory output (Clark *et al.* 1980; Poon, 1987). It was not our aim to assess mechanical limitation, and our approximate doubling of $\dot{V}_{O_2}$ would not equate to severe exercise, and therefore should not have been mechanically limiting. Many of the studies measuring chemoreceptor gain in humans and animals have, additionally, utilized a similar methodology of raising P_{a,CO_2} by increasing the inspired P_{CO_2}. This is in marked contrast to the normal physiological situation where inspired P_{CO_2} remains unaltered during exercise. Any elevation of inspired P_{CO_2} would act to increase alveolar P_{CO_2} and hence decrease the amplitude of any potential oscillating signal in blood gas tension that may be related to the metabolic rate (Yamamoto & Edwards, 1960; Yamamoto, 1962; Band *et al.* 1980; Kumar *et al.* 1988). In our model of hypermetabolism, any such oscillations would not be dampened as inspired gas tensions are not altered and might therefore provide a proportional signal to the peripheral chemoreceptors that would be independent of steady-state arterial blood gas tensions. Our model therefore is best viewed alongside earlier attempts to elevate $\dot{V}_{CO_2}$ in anaesthetized animals, through exchange transfusion of high-CO_2-equilibrated saline into the inferior vena cava (Grant *et al.* 1981; Band & Linton, 1989), or electrically induced exercise (Cross *et al.* 1982). It is interesting to note that, while the ventilatory response to hypoxia during exercise is much more accepted as showing a graded increase (Asmussen & Nielsen, 1957; Asmussen, 1967; Weil *et al.* 1972), it is clear, given the non-discriminatory nature of the carotid body neural output (Kumar *et al.* 1988), that there might not be any chemoreflex response to hypoxia that is independent of P_{a,CO_2} (Duffin & Mahamed, 2003) even below the ventilatory CO_2 threshold. Thus, if hypoxia sensitivity is altered during exercise, this is due to an alteration in either, or both of the chemoreflex sensitivity or threshold to CO_2.

No single factor, identified to date, appears to account for the full ventilatory response to exercise. Various individual chemical and non-chemical components make proportionally more or less contribution to the overall drive at varying phases of the exercise response and at varying intensities. That the carotid body plays an important part in this tight regulation of blood gas tensions has been long recognized (Wasserman *et al.* 1975; Phillipson *et al.* 1981; Whipp, 1994), but no consensus has been achieved for which products or consequences of exercise are essential. Thus, a number of studies, proposing different mechanisms, each seem to be able to account for the entire hyperpnoea. This may point to a high degree of redundancy in this important control system, whereby multiple factors acting together veil each other. Our data from CSNX animals are quite clear in showing that approximately 75% of the increase in CO_2 chemosensitivity during hypermetabolism arises from the carotid body. We have not determined the source of the remaining contribution, but it may be that the factor(s) augmenting peripheral chemoreceptor gain may also affect, e.g., central chemoreceptor gain, although to date, there is little evidence supporting a role for central chemoreceptors in mediating exercise hyperpnoea (Dempsey *et al.* 1979; Smith *et al.* 1988). That the peripheral chemoreceptors do contribute to CO_2 sensitivity in humans has been demonstrated in subjects with uni- or bilateral carotid body resection (Fatemian *et al.* 2003).

A role for the carotid body in exercise hyperpnoea is not universally accepted (see e.g. Dempsey *et al.* 1995), as subjects with bilateral carotid body resection (Wasserman *et al.* 1975) appear to retain the ability to control P_{a,CO_2} during mild, steady-state exercise. However, the dynamics of the ventilatory response were altered from control subjects although the resected human subjects all had an underlying and serious asthma condition that might, itself, have been causal in the effects noted by altering ventilatory mechanics (Forster *et al.* 1993), making them, perhaps, an unsuitable model to assess the role of the carotid body in exercise. A more commonly used, and non-invasive, method to assess carotid body contribution to ventilation is the so-called Dejours test (Dejours, 1962) which utilizes the breathing of 100% O_2 to abolish peripheral respiratory chemosensitivity. This is the case when chemoreceptor discharge is basal or elevated by hypoxia or hypercapnia. There is, however, no clear evidence that 100% O_2 can abolish the carotid chemoreceptor response to other stimulatory substances and, for example, the ventilatory response to elevated plasma $[K^+]$ is not abolished by hyperoxia (Sneyd *et al.* 1988), and nor is the carotid chemoafferent response to adenosine (Vandier *et al.* 1999). The finding that hyperoxia is without significant effect upon the hyperpnoea of exercise below the anaerobic threshold in humans (Wasserman *et al.* 1979) is therefore not conclusive. Perhaps more confusing is the finding that in the pony (Pan *et al.* 1983), the role of the chemoreceptors appears

to be to reduce the ventilatory response to exercise and thus to limit the resulting hypocapnia, However, this attenuating effect was most apparent only during the initial few seconds of beginning mild exercise, after which time chemodenervated animals demonstrated a relative hypoventilation during steady-state exercise when compared to their intact controls (see Fig. 4 in Dempsey *et al.* 1995) which is more supportive of our findings.

In conclusion, our results suggest that a proportional increase in the sensitivity, or gain, of the carotid body to P_{aCO_2}, coupled to the upward elevation in the position of the $\dot{V}_E$–P_{a,CO_2} curve can act to ensure the constancy of arterial blood gas tensions and pH during increased metabolism. The mechanism(s) underlying the increase in sensitivity is not yet known.

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