Interaction between the ventilatory and cerebrovascular responses to hypo- and hypercapnia at rest and during exercise

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Cerebrovascular reactivity to changes in the partial pressure of arterial carbon dioxide (P_{a,CO_2}) via limiting changes in brain [H^+] modulates ventilatory control. It remains unclear, however, how exercise-induced alterations in respiratory chemoreflex might influence cerebral blood flow (CBF), in particular the cerebrovascular reactivity to CO_2. The respiratory chemoreflex system controlling ventilation consists of two subsystems: the central controller (controlling element), and peripheral plant (controlled element). In order to examine the effect of exercise-induced alterations in ventilatory chemoreflex on cerebrovascular CO_2 reactivity, these two subsystems of the respiratory chemoreflex system and cerebral CO_2 reactivity were evaluated ($n = 7$) by the administration of CO_2 as well as by voluntary hypo- and hyperventilation at rest and during steady-state exercise. During exercise, in the central controller, the regression line for the P_{a,CO_2}–minute ventilation ($\dot{V}_E$) relation shifted to higher $\dot{V}_E$ and P_{a,CO_2} with no change in gain ($P = 0.84$). The functional curve of the peripheral plant also reset rightward and upward during exercise. However, from rest to exercise, gain of the peripheral plant decreased, especially during the hypercapnic condition (-4.1 ± 0.8 to -2.0 ± 0.2 mmHg l^{-1} min^{-1}, $P = 0.01$). Therefore, under hypercapnia, total respiratory loop gain was markedly reduced during exercise (-8.0 ± 2.3 to -3.5 ± 1.0 U, $P = 0.02$). In contrast, cerebrovascular CO_2 reactivity at each condition, especially to hypercapnia, was increased during exercise (2.4 ± 0.2 to 2.8 ± 0.2% mmHg^{-1}, $P = 0.03$). These findings indicate that, despite an attenuated chemoreflex system controlling ventilation, elevations in cerebrovascular reactivity might help maintain CO_2 homeostasis in the brain during exercise.

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Numerous enzymes and ion channels which influence neural activity are modified by changes in pH (Chesler, 2003); therefore, the regulation of pH is a vital homeostatic function. The respiratory chemoreflex is an important feedback control system which keeps the partial pressure of arterial carbon dioxide (P_{a,CO_2}) remarkably constant via ventilatory regulation. For example, the periodic nature of inspiration and expiration is carefully controlled by changes in P_{a,CO_2} via central and peripheral chemoreflexes so as to maintain pH nearly constant. The resulting hyper- or hypo-ventilation reduces or increases the CO_2 in the blood, respectively, and therefore in the cerebrospinal fluid.

P_{a,CO_2} serves as an important controlled variable or mediator, especially in the brain. The blood–brain barrier is relatively impermeable to H^+ and HCO_3^- ions; however, molecular CO_2 diffuses across it readily, with the result that the CO_2 in the cerebrospinal fluid parallels the arterial CO_2. Therefore, CO_2 diffuses freely to the cerebrospinal fluid and influences pH which drives ventilation via the central chemoreceptors (Severinghaus *et al.* 1963; Severinghaus & Carcelen, 1964). Moreover, the middle cerebral artery mean blood velocity (MCA V_{mean}), as an index of cerebral blood flow (CBF), is highly sensitive to direct changes in P_{a,CO_2} (Markwalder *et al.* 1984; Rasmussen *et al.* 2006). For example, hypocapnia causes

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cerebral vasoconstriction which reduces MCA V_{mean} and therefore, because of a reduced 'washout', attenuates the fall of brain tissue P_{CO_2}. In contrast, hypercapnia increases MCA V_{mean} by cerebral vasodilatation, which limits elevations in brain tissue P_{CO_2}.

Cerebrovascular reactivity and ventilatory response to CO_2 seems to be tightly linked (Chapman *et al.* 1979; Dempsey, 2005; Xie *et al.* 2005, 2006; Ainslie *et al.* 2007; Peebles *et al.* 2007). Changes in CBF might have an important role in stabilizing the breathing pattern during fluctuating levels of chemical stimuli, especially to P_{a,CO_2} (Xie *et al.* 2006). In fact, an increase in CBF increases diffusion of CO_2 from the cerebrospinal fluid and the brain extracellular fluid to the cerebral vessels. Therefore, $[H^+]$ decreases at the level of the central chemoreceptors when CBF increases. Early work by Severinghaus *et al.* (1963) investigated the regulation of cerebrospinal fluid pH during acclimatization from sea level to high altitude. They proposed three mechanisms for regulating cerebrospinal fluid pH. In addition to active transport across the blood–brain barrier and chemoreflexes, they suggested that cerebral arterioles, which dilate with high P_{CO_2} and constrict with low P_{CO_2}, also reduce the pH variations of cerebrospinal fluid and may be regarded as a third homeostatic means to regulate cerebrospinal fluid pH and therefore central ventilatory control. In goats, Chapman *et al.* (1979) reported that severe brain ischaemia blunted ventilatory responses to CO_2. In addition, reports indicate that cerebrovascular responsiveness to CO_2 is an important determinant of eupnoeic and hypercapnic ventilatory responsiveness in otherwise healthy humans (Xie *et al.* 2006) and those with congestive heart failure and central sleep apnoea (Xie *et al.* 2005), primarily via its effects at the level of the central chemoreceptors. Such reductions in cerebrovascular CO_2 reactivity affect the stability of the breathing pattern by causing ventilatory overshooting during hypercapnia and undershooting during hypocapnia (Xie *et al.* 2005). Therefore, changes in cerebrovascular CO_2 reactivity play a critical role in the ventilatory control of P_{a,CO_2}.

High altitude-induced hyperventilation via peripheral chemoreflex activation reduces P_{a,CO_2} and modifies cerebrospinal fluid pH and central chemoreceptor drive (Severinghaus *et al.* 1963). Therefore, it is possible that exercise-induced hyperpnoea also modifies the respiratory chemoreflex. In fact, exercise increases ventilation via the respiratory chemoreflex, and also modifies the ventilatory response to CO_2 (Asmussen & Nielsen, 1957; Bhattacharyya *et al.* 1968; Poon & Greene, 1985); however, it remains unclear how exercise-induced alterations in the respiratory chemoreflex might influence CBF regulation, in particular cerebrovascular CO_2 reactivity. The potential interactions between cerebrovascular reactivity and ventilatory responsiveness to CO_2 during exercise have not been examined. In order to examine the effect of exercise-induced alterations in ventilatory chemoreflex on cerebrovascular CO_2 reactivity, we evaluated two subsystems of the respiratory chemoreflex system using a new equilibrium diagram model (Miyamoto *et al.* 2004) and cerebral CO_2 reactivity by the administration of CO_2 as well as by voluntary hypo- and hyperventilation at rest and during steady-state exercise. Under a closed-loop condition of the respiratory chemoreflex system, ventilatory output is determined by chemical and metabolic drives (I: central controller), but this ventilatory loading alters these drives in the lung system which feeds back to ventilatory output (II: peripheral plant). We hypothesized that, during exercise, an increase in cerebrovascular CO_2 reactivity will compensate for reductions in the peripheral plant of the respiratory chemoreflex system.

Methods

Seven healthy non-athletic men aged 20 ± 2 years, height 173 ± 8 cm, weight 64 ± 10 kg (mean $\pm$ S.D.) were recruited to participate in the study as approved by the Human Subjects Committee of Morinomiya University of Medical Sciences (No. 001). In addition, they were free of any known cardiovascular and pulmonary disorders and were not using prescribed or over the counter medications. Before the experiment, each subject gave informed written consent and visited the laboratory for familiarization with the techniques and procedures. All procedures conformed to the standards set by the *Declaration of Helsinki*. Subjects were requested to abstain from caffeinated beverages for 12 h and strenuous physical activity and alcohol for at least 24 h before the day of the experiment.

Measurements

All studies were performed at a constant room temperature between 23 and 24°C with external stimuli minimized. Heart rate (HR) was monitored using a lead II electrocardiogram (ECG). A catheter (0.47 mm i.d., 24 gauge) was placed in the brachial artery of the non-dominant arm for arterial blood samples and measurement of the arterial blood pressure (ABP) with a pressure transducer (DX-200, Nihon-Koden, Tokyo, Japan) positioned at the level of the right atrium in the mid-axillary line, fastened to the subject and connected to a pressure-monitoring system (RM-6000, Nihon-Koden). Arterial blood samples were obtained at rest and after reaching steady state in each experimental condition. Samples were immediately analysed for pH, P_{a,CO_2} and the partial pressure of arterial oxygen (P_{a,O_2}) using a blood gas analyser (IL 1620, Instrumentation Laboratory, USA). The middle cerebral artery blood velocity (MCA V) was obtained by transcranial Doppler ultrasonography (WAKI, Atys Medical, St

Genislaval, France). A 2 MHz Doppler probe was placed over the temporal window and fixed with an adjustable headband and adhesive ultrasonic gel (Tensive, Parker Laboratories, Orange, NJ, USA). The MCA V waveform was isonated at the same depth (5 cm from the skin surface of the temple window) in all subjects. Ventilatory responses were measured using an open-circuit apparatus. The subjects breathed through a face mask attached to a low-resistance one-way valve with a built-in hot-wire flow meter. The valve mechanism allowed subjects to inspire room air or a selected gas mixture from a 200 l Douglas bag containing 0.0, 3.5 or 5.0% CO_2 in 40% O_2 with nitrogen (N_2) balance. These concentrations of CO_2 administration were determined by previous studies (Ellingsen *et al.* 1987*a*,*b*). The respiratory CO_2 sensitivity is close to constant within the range 0–5% CO_2 in the inspired gas. We used three progressive CO_2 stimulus points within the range 0–5% CO_2 to identify respiratory chemoreflex in the model. The total instrumental dead space was 200 ml. Respiratory and metabolic data during the experiments were recorded by an automatic breath-by-breath respiratory gas-analysing system consisting of a differential pressure transducer, sampling tube, filter, suction pump and mass spectrometer (ARCO2000-MET, Arcosystem, Chiba, Japan). We digitized expired flow, CO_2 and O_2 concentrations, and derived tidal volume (V_T), minute ventilation ($\dot{V}_E$), end-tidal O_2 (P_{ET,O_2}) and end-tidal CO_2 (P_{ET,CO_2}). Flow signals were computed to single breath data, and matched to gas concentrations identified as single breaths using the peak P_{ET,CO_2}, after accounting for the time delay in gas concentration measurements. The corresponding O_2 uptake and CO_2 output values for each breath were calculated from inspired–expired gas concentration differences, and by expired ventilation, with inspired ventilation being calculated by N_2 correction. During each protocol, HR, ABP, $\dot{V}_E$, P_{ET,O_2}, P_{ET,CO_2} and MCA V were recorded continuously at 200 Hz.

Experimental protocol

On the first day, each subject performed maximal cycle exercise for the measurement of maximal oxygen uptake ($\dot{V}_{O_2,max}$). In addition, with the exception of arterial blood gas sampling, each subject underwent the same experiment procedures as those used during the main experimental day to ensure familiarization with the experimental protocols.

On the experimental day, subjects arrived at the laboratory at least 2 h after a light meal. Following instrumentation, the subjects rested in a comfortable chair. Five minutes of baseline data were recorded whilst the subjects breathed room air, wearing the face mask. To characterize the central controller and peripheral plant, subjects underwent two experimental procedures, which consisted of the $\dot{V}_E$ response to hypercapnia and the P_{a,CO_2} response to hypo- and hyperventilation, at rest and during exercise ($\dot{V}_{O_2}$, 1.0 l min^{-1}).

Exercise capacity. The $\dot{V}_{O_2,max}$ was assessed with an incremental protocol on a cycle ergometer (Corival1000SS, Lode, Groningen, the Netherlands). The workload was set at 20 W and was increased by 20 W every minute until the subject could no longer maintain the pedalling frequency at 60 r.p.m. despite strong verbal encouragement. The subjects breathed through a facemask attached to a volume transducer while gases were continuously sampled for analysis of fractional concentrations of O_2, CO_2 and N_2. The respiratory gas analysis system was calibrated before each test using known standard gases.

$\dot{V}_E$ response to hypercapnia (CO_2 administration). The $\dot{V}_E$ response to hypercapnia consisted of three trials (fraction of inspired CO_2 (F_{I,CO_2}) 0.00, 0.035, 0.05), which was induced by rapidly changing the F_{I,CO_2}. Each F_{I,CO_2} trial ran for 12 min at approximately 10–15 min intervals. This duration is long enough to permit CO_2 to reach its new steady-state value at the central chemoreceptors (Honda *et al.* 1983; Poon & Greene, 1985; Pianosi *et al.* 1994; Teppema *et al.* 2000). During the interval periods, the subjects inspired room air. Each subject performed these three trials at rest and during exercise. The order of the trials was randomized for each subject. We performed all trials under the hyperoxic condition to abolish the O_2-sensitive chemoreflex (Ohyabu *et al.* 1982; Robbins, 1988; Mohan & Duffin, 1997).

P_{a,CO_2} response to hypo- and hyperventilation (voluntary changes in respiration). The P_{a,CO_2} response to ventilation consisted of three trials: two periods of hyperventilation and one period of hypoventilation. To avoid the possible effects of different breathing patterns on the $\dot{V}_E - P_{a,CO_2}$ relationship, in the hyperventilation trials, both V_T and breathing frequency were altered deliberately by matching the breathing pattern to that recorded during hypercapnia trials, whilst inhaling 0% CO_2 in 40% O_2 with N_2 balance. In the hypoventilation trial, $\dot{V}_E$ was set to 80% of $\dot{V}_E$ during the 0.00 F_{I,CO_2} trial (i.e. during spontaneous breathing). The breathing pattern was estimated from the relationships between $\dot{V}_E$ and V_T in each subject. Each trial ran for 12 min with an interval of 10–15 min. Each subject performed these three trials at rest and during exercise, and the order of the trials was randomized.

During hypo- and hyperventilation trials, the inspired and expired volume curves were continuously displayed on a screen monitor. Visual and audio signals were constructed from the breathing pattern of the subjects

during the hypercapnia trials. The target V_T level was simultaneously displayed on the same screen monitor in each trial. The subjects were instructed to match their volume curve with the target V_T level and to breathe according to the sound of the metronome. As a result, both the V_T and breathing frequency, and thus $\dot{V}_E$, were precisely controlled by the visual feedback.

Since our preliminary measurements indicated that P_{a,CO_2} responses to $\dot{V}_E$, and the $\dot{V}_E$ response to P_{a,CO_2} reached steady states within 8–12 min, we represented each response by averaging it in the last 2 min. The arterial blood sample (2.5 ml) was collected at minute 11.5 of each trial period. The measured values of operating points (OPs) in the subjects were defined to be the steady-state values for $\dot{V}_E$ and P_{a,CO_2} that were obtained during the 0.00 F_{I,CO_2} trial without visual feedback (i.e. during spontaneous breathing).

Data analysis

The cerebrovascular and ventilatory equilibrium diagram model is depicted in Fig. 1. The respiratory chemoreflex system consists of two subsystems, the central controller (I) and peripheral plant (II). These subsystems act as a feedback control system, which regulates the systemic CO_2 level. In the brain, CBF (III) is influenced by systemic CO_2 and regulates CO_2 at the brain level, which then feeds back into the central controller.

Central controller (I). Change in P_{a,CO_2} (input) stimulates chemoreceptor activity and alters ventilation (output) via the central controller. To characterize the central controller, we used a protocol of CO_2 administration (three levels), a conventional linear equation, $\dot{V}_E = S(P_{a,CO_2} - B)$, and determined the slope S and inter-

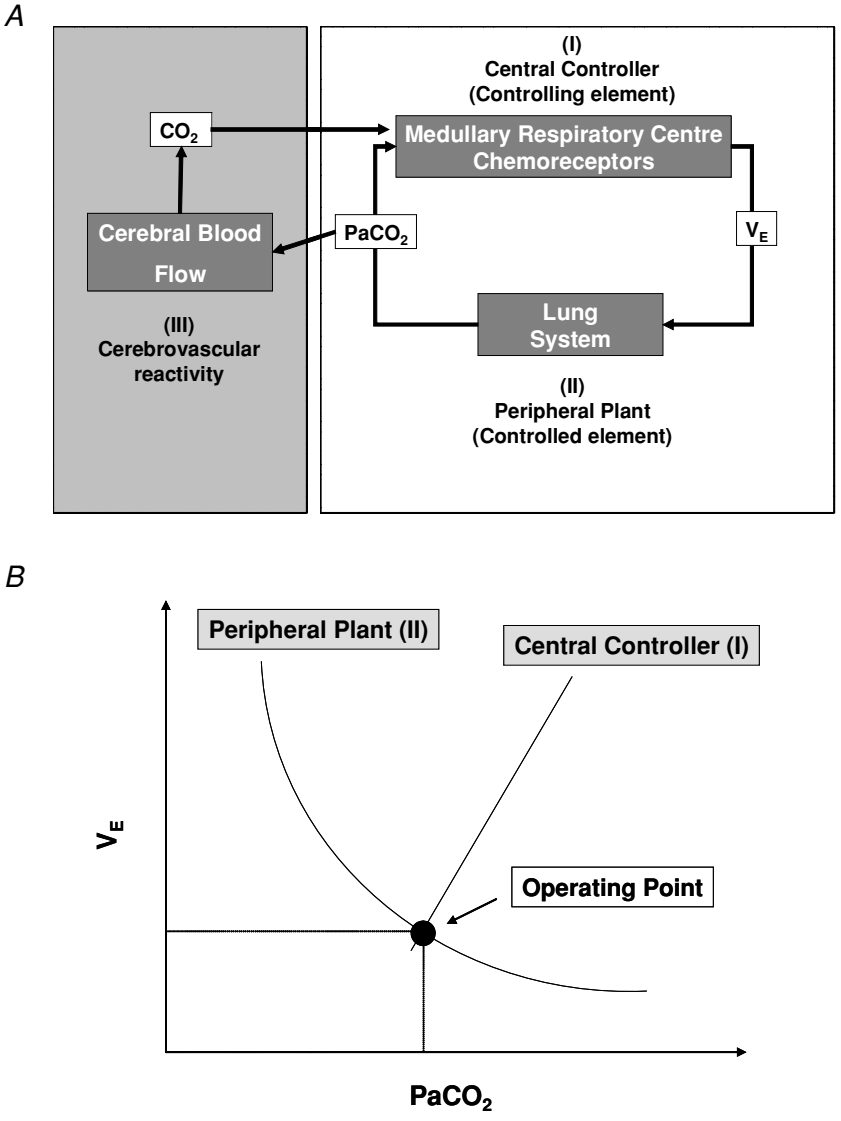

Figure 1. Equilibrium diagram model
A, systemically, partial pressure of arterial carbon dioxide (P_{a,CO_2}) is controlled by the respiratory chemoreflex system which consists of two subsystems: the central controller (controlling element; I) and peripheral plant (controlled element; II). In the brain, cerebral CO_2 is strongly regulated by change in cerebral blood flow (CBF; III). In addition, brain tissue CO_2 influences the central chemoreflex, and can be characterized by observing CBF response to changes in CO_2. *B*, in the central controller the input parameter is P_{a,CO_2}, the output parameter is minute ventilation ($\dot{V}_E$). To characterize the central controller, fraction of inspired CO_2 (0, 3.5, 5% CO_2 in 40% O_2 with N_2 balance) and the $P_{a,CO_2} - \dot{V}_E$ relationship were measured. The central controller can be characterized by observing changes in $\dot{V}_E$ in response to changes in P_{a,CO_2}. In the peripheral plant, input is $\dot{V}_E$, and output is P_{a,CO_2}. To characterize the peripheral plant, $\dot{V}_E$ was altered by hyper- and hypoventilation using a visual feedback method, which made it possible to control both tidal volume and breathing frequency; the $\dot{V}_E - P_{a,CO_2}$ relationship was then quantified. Since both relationships share common variables, the resultant operating point of ventilatory response under the closed-loop condition is determined by intersection of the two relationships.

cept B using a least-squares regression method. The slope (S) also identifies the gain of the central controller (G_C); $G_C = \Delta \dot{V}_E / \Delta P_{a,CO_2} = S$.

Peripheral plant (II). The central controller-induced changes in ventilation (input) also alters P_{a,CO_2} (output). To characterize the peripheral plant, we used a protocol of voluntary changes in respiration (four levels), the modified metabolic hyperbola as $P_{a,CO_2} = A/\dot{V}_E + C$, and determined the values of A and C by the least-squares regression method. During hyper- and hypocapnia, the gain of the peripheral plant (G_P) to operating point (OP) was calculated from the following equations under hyper- ($\dot{V}_E$: $-2\,l\,min^{-1}$ from OP) and hypocapnia ($\dot{V}_E$: $+2\,l\,min^{-1}$ from OP) conditions, respectively.

$$G_P = \Delta P_{a,CO_2} / \Delta \dot{V}_E = -A/\dot{V}_E^2$$

$$(G_P \text{ of OP}) = -A/(\dot{V}_E \text{ at OP})^2$$

$$(G_P \text{ of hypercapnia}) = -A/(\dot{V}_E \text{ at } -2\,l\,min^{-1} \text{ from OP})^2$$

$$(G_P \text{ of hypocapnia}) = -A/(\dot{V}_E \text{ at } +2\,l\,min^{-1} \text{ from OP})^2$$

Total respiratory loop gain (I + II). Total respiratory loop gain (G_{TR}) to OP, hyper- and hypocapnia was calculated by the following equations.

$$G_{TR} = G_C G_P$$

$$(G_{TR} \text{ of OP}) = G_C (G_P \text{ of OP})$$

$$(G_{TR} \text{ of hypercapnia}) = G_C (G_P \text{ of hypercapnia})$$

$$(G_{TR} \text{ of hypocapnia}) = G_C (G_P \text{ of hypocapnia})$$

Cerebral CO_2 reactivity (III). P_{a,CO_2} (input) alters CBF (output) via cerebral CO_2 reactivity. To characterize cerebrovascular reactivity to CO_2, we used protocols of CO_2 administration and voluntary changes in respiration (six levels), an exponential function, %MCA $V_{mean} = K \exp(RP_{a,CO_2})$, and determined the values of K and R. The cerebral CO_2 reactivity (G_B) to OP, hyper- and hypocapnia was calculated from the following equations at the OP, and under hyper- (P_{a,CO_2}: +5 mmHg from OP) and hypocapnic (P_{a,CO_2}: −5 mmHg from OP) conditions, respectively.

$$G_B = \Delta \%\text{MCA } V_{mean} / \Delta P_{a,CO_2} = KR\exp(RP_{a,CO_2})$$

$$(G_B \text{ of OP}) = KR\exp(RP_{a,CO_2} \text{ at OP})$$

$$(G_B \text{ of hypercapnia}) = KR\exp(RP_{a,CO_2} \text{ at } +5 \text{ mmHg from OP})$$

$$(G_B \text{ of hypocapnia}) = KR\exp(RP_{a,CO_2} \text{ at } -5 \text{ mmHg from OP})$$

Table 1. Ventilatory and haemodynamic variables at rest and during exercise

	Rest (R)	Exercise (E)	R *versus* E
P_{ET,CO_2} (mmHg)	38.2 ± 1.2	44.1 ± 1.7	$P = 0.004$
P_{a,CO_2} (mmHg)	43.2 ± 1.7	44.6 ± 01.2	$P = 0.294$
P_{a,O_2} (mmHg)	225 ± 17	244 ± 2	$P = 0.578$
pH	7.40 ± 0.01	7.39 ± 0.01	$P = 0.668$
$\dot{V}_E$ (l min^{-1})	11.6 ± 0.5	26.0 ± 1.2	$P < 0.001$
V_T (ml)	1059 ± 330	1431 ± 163	$P = 0.156$
$\dot{V}_{O_2}$ (ml min^{-1})	330 ± 63	942 ± 40	$P < 0.001$
$\dot{V}_{CO_2}$ (ml min^{-1})	338 ± 24	849 ± 32	$P < 0.001$
MAP (mmHg)	92 ± 1	97 ± 2	$P = 0.169$
HR (beats min^{-1})	69 ± 5	98 ± 8	$P < 0.001$
MCA V_{mean} (cm s^{-1})	51.4 ± 4.5	53.3 ± 4.2	$P = 0.085$

Values are means ± S.E.M. P_{ET,CO_2}, end-tidal carbon dioxide (CO_2) tension; P_{a,CO_2}, partial pressure of arterial CO_2; P_{a,O_2}, partial pressure of arterial O_2; $\dot{V}_E$, minute ventilation; V_T, tidal volume; $\dot{V}_{O_2}$, oxygen uptake; $\dot{V}_{CO_2}$, CO_2 uptake; MAP, mean arterial pressure; HR, heart rate; MCA V_{mean}, middle cerebral artery mean blood velocity.

Statistical analysis

A paired t test was used to assess the differences in the steady-state haemodynamic variables between rest and exercise conditions. Two-way analysis (CO_2 and exercise) of variance with repeated measures was used to assess the differences in the G_P, G_{TR} and G_B between all conditions. A Student–Newman–Keul's test was employed *post hoc* when main effects were significant, i.e. $P < 0.05$. Data are expressed as mean ± S.E.M. and analyses were conducted using SigmaStat (Jandel Scientific Software, SPSS Inc., Chicago, IL, USA).

Results

Averaged O_2 uptake during the cycling exercise was 32 ± 2% $\dot{V}_{O_2,max}$. This mild exercise increased HR and caused slight elevations in MAP (92 to 97 mmHg; $P = 0.169$) and MCA V_{mean} (51.4 to 53.3 cm s^{-1}; $P = 0.085$, Table 1). During exercise, both $\dot{V}_E$ ($P < 0.001$) and P_{ET,CO_2} ($P = 0.004$) were increased whilst pH and P_{a,CO_2} were unchanged.

In the central controller, the regression line of the $P_{a,CO_2} - \dot{V}_E$ relation was reset to higher $\dot{V}_E$ and P_{a,CO_2} during exercise (Fig. 2) without a change in gain (G_C) (1.7 ± 0.3 to $1.8 \pm 0.5\,l\,min^{-1}\,mmHg^{-1}$, $P = 0.837$; Fig. 3). The functional curve of the peripheral plant also reset to higher $\dot{V}_E$ and P_{a,CO_2} during exercise (Fig. 2); however, the change in gain (G_P) was different from that of G_C, i.e. the G_P at the OP, during both hypercapnia and hypocapnia, was decreased from rest to exercise; the change in G_P was especially marked during hypercapnia (-4.1 ± 0.8 to $-2.0 \pm 0.2\,mmHg\,l^{-1}$ min, $P = 0.009$; Fig. 3). Therefore, total respiratory loop gain

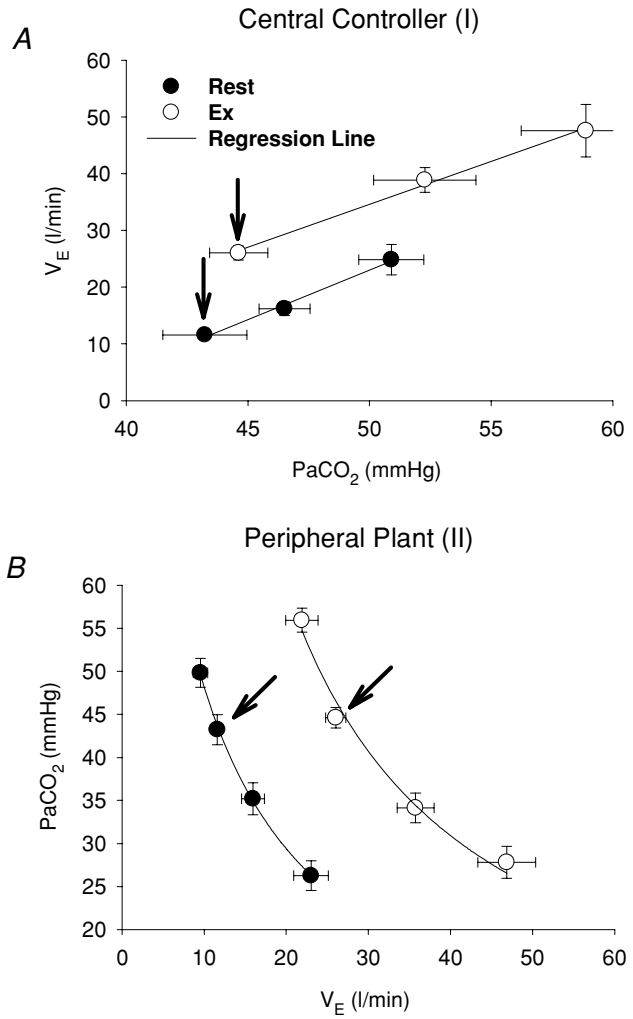

Figure 2. Characteristics of central controller (I; *A*) and peripheral plant (II; *B*) at rest and during exercise
A (central controller), $\dot{V}_E$ linearly increased with P_{a,CO_2} at rest and during exercise. The averaged regression lines were $\dot{V}_E = 1.74(P_{a,CO_2} - 29.2)$ and $\dot{V}_E = 1.81(P_{a,CO_2} - 21.3)$ at rest and during exercise, respectively. *B* (peripheral plant), the peripheral plant was characterized by a modified metabolic hyperbola. The averaged fitted hyperbolae were $P_{a,CO_2} = 344/\dot{V}_E + 13.2$ and $P_{a,CO_2} = 1124/\dot{V}_E + 3.1$ at rest and during exercise, respectively. Arrows denote operating points.

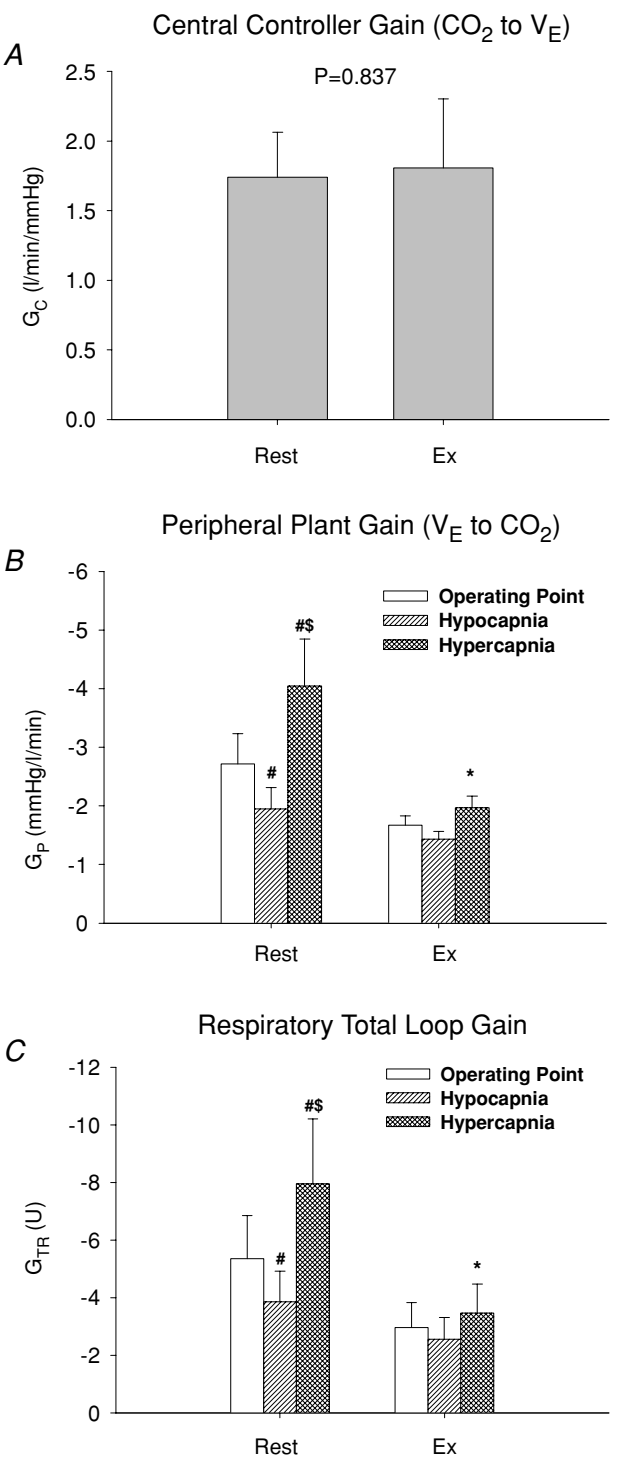

Figure 3. Group-averaged central controller gain (G_C, *A*), peripheral plant gain (G_P, *B*) and respiratory total loop gain (G_{TR}, *C*) at rest and during exercise
The central controller was characterized by a conventional linear equation, thus the gain under hypocapnic conditions was the same as that at the operating point (OP) and hypercapnia. The peripheral plant was characterized by a hyperbola therefore gains at OP, hypo- and hypercapnia were analysed. Values are means ± S.E.M. $^*P < 0.05$, different from rest; $\#P < 0.05$, different from operating point; $\$P < 0.05$, different from hypocapnia.

(G_{TR}) during hypercapnia decreased during exercise (-8.0 ± 2.3 to -3.5 ± 1.0 U, $P = 0.019$) despite no change in G_{TR} at OP and under hypocapnia. If the change in P_{a,CO_2} is 2 mmHg, $\dot{V}_E$ changes are 3.5 ± 0.7 and 3.6 ± 1.0 l min^{-1} at rest and during exercise, respectively. However, these similar $\dot{V}_E$ changes cause a different correction in P_{a,CO_2} between the hypo- and hypercapnia conditions. Under conditions of hypocapnia, $\dot{V}_E$ change similarly alters P_{a,CO_2} at rest (8 ± 2 mmHg) and during exercise (5 ± 2 mmHg); however, under conditions of hypercapnia, large differences in alterations in P_{a,CO_2} are observed between those at rest (16 ± 5 mmHg) and those during exercise (7 ± 2 mmHg).

Hypercapnia resulted in an exponential elevation in MCA V_{mean} during exercise as well as at rest (Fig. 4). However, the functional curve of cerebral CO_2 reactivity was not reset during exercise because of small changes in P_{a,CO_2} and MCA V_{mean}. In contrast to the ventilatory chemoreflex, all cerebrovascular reactivities (G_B) to OP, hyper- and hypocapnia were increased during exercise despite unremarkable changes in both K ($P = 0.662$) and R ($P = 0.286$) of these curves (Table 2 and Fig. 5). The increases in G_B were more marked in the hypercapnic condition (2.4 ± 0.2 to 2.8 ± 0.2 % mmHg^{-1}, $P = 0.025$) compared to other conditions (OP, $P = 0.049$; hypocapnia, $P = 0.086$).

Discussion

The main finding of the present investigation was that, under conditions of hypercapnia and exercise, the total respiratory loop gain was markedly reduced. These changes in total loop gain occurred independently of the change in central controller gain because of a marked decrease in peripheral plant gain. Furthermore, cerebrovascular CO_2 reactivity during each condition, especially during hypercapnia, was increased during exercise. These findings indicate that, despite an attenuated chemoreflex system controlling ventilation, elevations in cerebrovascular reactivity might help maintain CO_2 homeostasis in the brain during exercise.

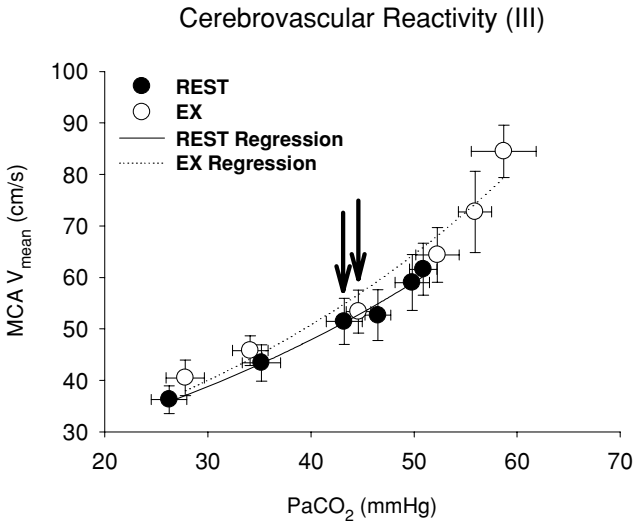

Figure 4. Characteristics of cerebrovascular CO_2 reactivity (III) at rest and during exercise
The cerebrovascular CO_2 reactivity was characterized by an exponential function. The averaged fitted exponential equations were MCA $V_{mean} = 20.5 \exp(0.0216 P_{a,CO_2})$ and MCA $V_{mean} = 20.4 \exp(0.0232 P_{a,CO_2})$ at rest and during exercise, respectively. Arrows denote operating points.

The respiratory chemoreflex

The respiratory chemoreflex is a powerful feedback control system which acts to maintain P_{a,CO_2} or pH remarkably constant; the tight regulation of pH is critical to maintain homeostatic function for all tissues (Chesler, 2003), especially neural activity. Exercise, which activates muscle metabolism and produces CO_2, causes hyperpnoea via the ventilatory chemoreflex. The exercise-induced hyperpnoea was reflected in elevations in $\dot{V}_E$ from 12 to 26 l min^{-1} ($P < 0.001$). The mechanism(s) subserving ventilatory control during exercise remain controversial (Ward, 2007); however, traditionally these mechanisms are proposed to include elements of proportional feedback, central and carotid chemosensory, and feedforward systems, central command and muscle reflex (Dempsey *et al.* 2006; Waldrop & Iwamoto, 2006; Ward, 2007). Acute hypoxia causes hyperventilation and alkalosis at the medullary chemoreceptors, which reduce their drive (Crawford & Severinghaus, 1978). In addition, the change of this cerebrospinal fluid alkalosis modifies the respiratory control (Severinghaus *et al.* 1963). Although the mechanism of exercise-induced hyperpnoea is different from that associated with hyperventilation at high altitude, it seems that exercise-induced hyperpnoea alters central chemoreflex. The role of the ventilatory chemoreflex in the regulation of exercise hyperpnoea has been extensively investigated (Cunningham, 1987). The ventilatory sensitivity to hypoxia is increased from rest during exercise (Bhattacharyya *et al.* 1968); however, the effect of exercise on the respiratory chemoreflex remains controversial. For example, Asmussen & Nielsen (1957) demonstrated that the ventilatory–P_{CO_2} relationship line shifted to the left without a change in its sensitivity. In contrast, Poon & Greene (1985) showed that the slope of the ventilatory–P_{CO_2} relationship was increased by exercise. In addition, the 'chemoreflex response' is not dictated by the level of chemical drive. Such an integrative response involves a dynamic interaction between the respiratory controller and the chemical drive, and is influenced by respiratory mechanical constraints (Poon *et al.*, 2007). Under a closed-loop condition (Fig. 1), ventilatory output is determined by chemical and

Table 2. Characteristics of central controller, peripheral plant and cerebrovascular reactivity at rest and during exercise

	Rest (R)	Exercise (E)	R *versus* E
Central controller (I)			
S	1.74 ± 0.32	1.81 ± 0.49	$P = 0.837$
B	29.2 ± 8.51	21.28 ± 6.62	$P = 0.073$
Peripheral plant (II)			
A	344 ± 54	1124 ± 118	$P < 0.001$
C	13.2 ± 3.0	3.1 ± 2.8	$P = 0.004$
Cerebrovascular CO_2 reactivity (III)			
K	40.0 ± 2.5	38.9 ± 3.1	$P = 0.662$
R	0.0216 ± 0.0013	0.0232 ± 0.0016	$P = 0.286$

Values are means ± S.E.M. Central controller (I), $\dot{V}_E = S(P_{a,CO_2} - B)$; peripheral plant (II), $P_{a,CO_2} = A/\dot{V}_E + C$; cerebrovascular reactivity to CO_2 (III), MCA $V_{mean} = K\exp(RP_{a,CO_2})$.

metabolic drives, although this ventilatory loading alters these drives in the lung system which feeds back to ventilatory output. However, previous studies have failed to consider the importance of metabolic changes due to the work of breathing (Miyamoto *et al.* 2004). We have used a new equilibrium diagram model (Miyamoto *et al.* 2004) to resolve this question. The previous studies by Severinghaus *et al.* (Severinghaus *et al.* 1963; Severinghaus & Carcelen, 1964; Crawford & Severinghaus, 1978) demonstrated the regulation of cerebrospinal fluid pH during the hyperventilation associated with high altitude by using a similar model for ventilatory control. The respiratory model of the present study has a limitation in identifying the regulation of cerebrospinal fluid pH or the interaction between central and peripheral chemoreflexes. In the present study, the equilibrium diagram model demonstrates the effect of CO_2 change on respiratory control or the effect of respiratory change on cerebral CO_2 haemodynamics during exercise. Compared with the model used in previous work (Severinghaus *et al.* 1963; Severinghaus & Carcelen, 1964; Crawford & Severinghaus, 1978), our model gave similar information about respiratory control during conditions of exercise rather than at high altitude.

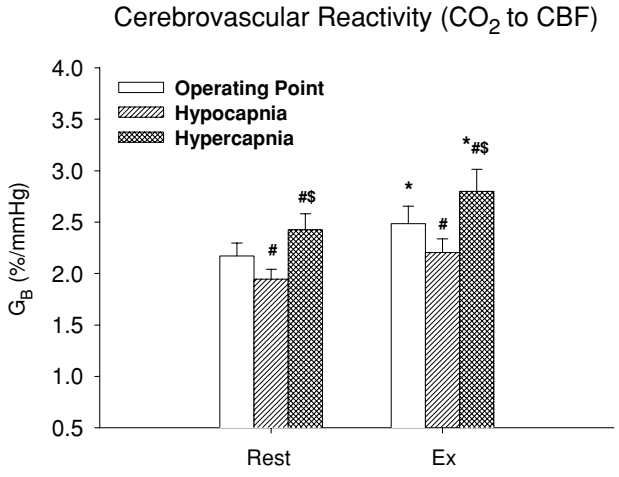

Figure 5. Group-averaged cerebrovascular CO_2 reactivity (G_B) at rest and during exercise
The cerebrovascular CO_2 reactivity was characterized by an exponential function therefore G_B values at the operating point and under hypo- and hypercapnia were analysed. Values are means ± S.E.M. *$P < 0.05$, different from rest; #$P < 0.05$, different from operating point; \$$P < 0.05$, different from hypocapnia.

The regression line of the central controller was shifted rightward and upward around the higher operating point of $\dot{V}_E$ and P_{a,CO_2} during exercise (Fig. 2). Both neural and humoral mechanisms may be involved in the ventilatory chemoreflex responses of the central controller associated with exercise. During exercise, increases in peripheral chemoreflex hypoxic sensitivity can be related to lactic acidosis (Asmussen & Nielsen, 1958; Wasserman *et al.* 1975), circulating catecholamines (Cunningham *et al.* 1963) and potassium (Linton *et al.* 1984; Qayyum *et al.* 1994). However, the sensitivity of the central controller (G_C) was unchanged during exercise ($P = 0.837$, Fig. 3). This finding may be related to the small changes in lactate, catecholamines and potassium concentrations during such a light exercise workload (32% $\dot{V}_{O_2,max}$), or a differential influence of CO_2 on peripheral chemoreflex activity as opposed to hypoxia. Moreover, because oscillations in pH increase during exercise (Band *et al.* 1980), these changes might also modify the results of respiratory control during exercise as identified in the present study.

The lung system (peripheral plant) is an important subsystem of respiratory chemoreflex, because it is an effector to change CO_2 systemically via an alteration in ventilation (Miyamoto *et al.* 2004). The sensitivity of peripheral plant is non-linear and is changed by ventilation (Fig. 2). At rest, G_P was increased due to a decrease in ventilation, suggesting that the peripheral plant is more effective in controlling CO_2 at low $\dot{V}_E$ levels. This effect of ventilatory loading is particularly acute during

hyperventilation as the respiratory apparatus is subject to increasing mechanical limitations, i.e. dead space (Poon *et al.* 2007). Therefore, considering the multiple effects of these subsystems, the central controller is generally more pronounced at low $\dot{V}_E$ than high $\dot{V}_E$ levels (Clark *et al.* 1980; Poon, 1989*a*,*b*). The respiratory total loop gain (G_{TR}) was much higher under the hypercapnic condition caused by hypoventilation compared with normal ($P < 0.001$) and hypocapnic ($P < 0.001$) conditions at rest (Fig. 3).

During exercise the functional curve of the peripheral plant also reset rightward and upward around the higher $\dot{V}_E$ and P_{a,CO_2} (Fig. 2). However, the change in G_P was different from that of G_C. From rest to exercise, during hypercapnia and hypocapnia, there was a decrease in G_P at the operating point (OP). Importantly, the change in G_P at hypercapnia was larger (−51%) compared with that at other conditions (OP, −39% and hypocapnia −27%; Fig. 3). The sensitivity of the peripheral plant is non-linear and was decreased exponentially during elevations in ventilation. Therefore, these exercise-induced G_P reductions were related such that the OP moved rightward on the functional curve of the peripheral plant during exercise compared with rest (Fig. 2). The rightward shift of the exercise OP was determined by the resetting of central controller (rightward and upward shift), indicating that the mechanism of the change in G_P during exercise depends on the interaction with alteration in the central controller. As a consequence, total respiratory loop gain (G_{TR}) at hypercapnia decreased during exercise despite no changes in G_{TR} at the OP and under the hypocapnic condition. These findings suggest that the respiratory chemoreflex was attenuated during exercise under the hypercapnic condition despite no change in the sensitivity of the central controller. The interaction between the central controller and the plant was non-linear. Moreover, these results were not consistent with the traditional chemoreflex feedback model, which ignores the mechanical plant. The ventilatory response to chemical or exercise inputs is also potentiated by increases in physiological dead space or shunt (Poon *et al.* 2007). In addition, congestive heart failure patients with increased physiological dead space are reported to have an augmented $\dot{V}_E - \dot{V}_{CO_2}$ sensitivity (Wasserman *et al.* 1997). Therefore, an interaction with the attenuation in peripheral plant gain may be another mechanism underlining the lack of change in the controller gain during exercise.

Cerebrovascular CO_2 reactivity

At rest, hypercapnic cerebral CO_2 reactivity was greater than the hypocapnic reactivity (Fig. 5) because of the increase in CO_2 exponentially elevated MCA V_{mean} when a wider range of CO_2 challenge was applied (Rasmussen *et al.* 2006). Animal studies indicate that the mechanisms underlying the normal greater reactivity to hypercapnia compared with hypocapnia may be related to a greater influence of vasodilator mediators on intracranial vascular tone compared with vasoconstrictive mediators (Toda & Okamura, 1998). During exercise, cerebral CO_2 reactivity (G_B) to the OP, in both the hyper- and hypocapnia conditions, was increased. Enhanced cerebral CO_2 reactivity at OP with exercise has been reported (Rasmussen *et al.* 2006). Our new finding is that the increase in G_B during the hypercapnic condition was much larger compared with other conditions at rest and during exercise. Moreover, cerebral CO_2 reactivity (G_B) to OP ($P = 0.049$) and hypercapnia ($P = 0.025$) was increased during exercise while G_B to hypocapnia was unchanged ($P = 0.086$, Fig. 5). This enhanced cerebral CO_2 reactivity during exercise may relate to interactions with the central controller; however, the mechanism(s) underpinning such changes remain unclear.

The role of autonomic neural control of the cerebral circulation is controversial and, despite rich sympathetic nerve innervation of the cerebral arteries (Nielsen & Owman, 1967; Nelson & Rennels, 1970; Edvinsson, 1975), the traditional thinking is that changes in sympathetic tone appear to have a limited effect on CBF. In contrast, Meadows *et al.* (2003) found that sleep decreased cerebral CO_2 reactivity, suggesting that the level of cerebral activation influences the cerebrovascular reactivity to CO_2. In addition, sympathetic nervous activation attenuates the CO_2-induced increase in CBF at rest (Jordan *et al.* 2000). Therefore, exercise-induced physiological changes (e.g. autonomic neural control) may also modify the cerebral CO_2 reactivity. However, these findings contrast with a study which reported that sympatho-excitation induced with lower body negative pressure did not alter the cerebral CO_2 reactivity (LeMarbre *et al.* 2003). Collectively, the mechanisms underlying heightened sympathetic nerve activity during exercise on the regulation of CBF remain unclear.

Cerebral autoregulation is well maintained during mild and moderate dynamic exercise (Brys *et al.* 2003; Ogoh *et al.* 2005*a*,*b*, 2007), suggesting that CBF regulation is not influenced by ABP during exercise. However, at rest in the supine position, Aaslid *et al.* (1989) have reported that cerebral autoregulation is also affected by the basal vascular tone and it is attenuated by hypercapnia. Via sympathoexcitation, arterial blood pressure increases with CO_2 administration (Ainslie *et al.* 2005). Thus, because of an attenuation in normal cerebral autoregulation under hypercapnic conditions, CBF may be influenced by an increased ABP with CO_2 administration and this phenomenon may be further altered by exercise. During exercise the additional CO_2-induced elevations in blood pressure and a lowered cerebral autoregulation might explain the exponential change in cerebral CO_2 reactivity during hypercapnia.

The interaction between total respiratory chemoreflex and cerebrovascular reactivity

An increase in cerebrovascular CO_2 reactivity compensated an attenuated respiratory chemoreflex system during steady-state exercise, especially under the hypercapnic condition. Although the interaction between systemic and cerebral CO_2 controlling mechanisms during exercise remains unknown, previous investigations (Chapman *et al.* 1979; Dempsey, 2005; Xie *et al.* 2005, 2006; Ainslie *et al.* 2007; Peebles *et al.* 2007) indicate that cerebral CO_2 reactivity is linked with the ventilatory response to CO_2. Changes in cerebrovascular CO_2 reactivity affect the stability of the ventilatory responsiveness to CO_2 via alterations in the degree of washout in central chemoreceptor hydrogen $[H^+]$; these changes have been documented in a range of physiological (Xie *et al.* 2006; Ainslie *et al.* 2007) and pathophysiological disorders (Xie *et al.* 2005). Peebles *et al.* (2007) reported that hypercapnic cerebral CO_2 reactivity was inversely related to the increase in ventilatory change and suggested that a reduced cerebral CO_2 reactivity resulted in less central CO_2 washout and greater ventilatory stimulus. However, our findings indicate that the relationship between the two systems during exercise cannot be explained only by these mechanisms, because the central controller gain was unchanged during exercise despite an enhanced cerebral CO_2 reactivity. This dissociation may depend on a peripheral chemoreflex distribution to the central controller or CBF distributions to central chemoreflex that are different between rest and exercise. These findings highlight the interdependence of total respiratory chemoreflex to many other variables through a complex and probably non-linear relationship.

Technological considerations

P_{ET,CO_2} measurement has been used as an estimate of P_{a,CO_2}. The difference between P_{a,CO_2} and P_{ET,CO_2} is influenced by metabolic CO_2 production and tidal volume and the relationship between these two variables is not altered by breathing frequency and exercise (Jones *et al.* 1979). In addition, the estimated different cerebral CO_2 reactivity between rest and exercise from P_{a,CO_2} was the same as that from P_{ET,CO_2} (Rasmussen *et al.* 2006). However, P_{ET,CO_2} is higher than P_{a,CO_2} when metabolic CO_2 production and $\dot{V}_E$ are increased (Jones *et al.* 1979). Peebles *et al.* (2007) demonstrated that cerebrovascular CO_2 reactivity is underestimated by P_{ET,CO_2} when compared with P_{a,CO_2}; therefore, we have made the calculations of central controller, peripheral plant and cerebral CO_2 reactivity using P_{a,CO_2}. Another important consideration is that P_{CO_2} from the internal jugular vein is likely to be a closer index of brain tissue P_{CO_2} than P_{a,CO_2} (Xie *et al.* 2006) and the medullary central chemoreceptors are not stimulated directly by P_{a,CO_2}; rather, they are stimulated by $[H^+]$ via alterations in brain tissue CO_2 tension (Peebles *et al.* 2007). Whilst studies have 'corrected' ventilatory reactivity against brain tissue P_{CO_2} (Xie *et al.* 2006), these experiments were conducted *at* rest (Fencl, 1986; Peebles *et al.* 2007). Because exercise would modify the relationship between P_{a,CO_2} and internal jugular vein P_{CO_2} as an index of brain tissue P_{CO_2}, we decided not to 'correct' ventilatory reactivity against brain tissue P_{CO_2}. Another potential limitation of estimating MCA V using transcranial Doppler ultrasonography is that changes in the diameter of the isonated vessels could modulate MCA V independently of flow. However, the MCA diameter appears to remain relatively constant in humans under several conditions (Giller *et al.* 1993; Schreiber *et al.* 2000; Serrador *et al.* 2000). In addition, the changes in MCA V_{mean} during submaximal dynamic exercise appear to be similar to the changes in CBF determined by other techniques, i.e. internal carotid artery blood flow (Hellström *et al.* 1996) and the ^{133}Xe clearance technique (Jørgensen *et al.* 1992*a*,*b*). It should be noted, however, that the functional anatomy of the arteries supplying the brain varies between individuals. In addition, the proportion of total flow in any single vessel may not be constant, and flow redistribution between major cerebral vessels may occur. Therefore, accurate measurement of global CBF cannot be assured unless simultaneous flow in all major vessels is measured. Third, the change in MCA V_{mean} in relation to a 'central controller' *versus* a 'peripheral plant' could not be evaluated in the model of the present study. Each mechanism was identified separately at rest and during exercise. Thus, its relationship remains unclear at rest and during exercise and further studies incorporating linear and non-linear models are needed to provide further insight.

References

Aaslid R, Lindegaard KF, Sorteberg W & Nornes H (1989). Cerebral autoregulation dynamics in humans. *Stroke* **20**, 45–52.

Ainslie PN, Ashmead JC, Ide K, Morgan BJ & Poulin MJ (2005). Differential responses to CO_2 and sympathetic stimulation in the cerebral and femoral circulations in humans. *J Physiol* **566**, 613–624.

Ainslie PN, Murrell C, Peebles K, Swart M, Skinner MA, Williams MJ & Taylor RD (2007). Early morning impairment in cerebral autoregulation and cerebrovascular CO_2 reactivity in healthy humans: relation to endothelial function. *Exp Physiol* **92**, 769–777.

Asmussen E & Nielsen M (1957). Ventilatory response to CO_2 during work at normal and at low oxygen tensions. *Acta Physiol Scand* **39**, 27–35.

Asmussen E & Nielsen M (1958). Pulmonary ventilation and effect of oxygen breathing in heavy exercise. *Acta Physiol Scand* **43**, 365–378.

Band DM, Wolff CB, Ward J, Cochrane GM & Prior J (1980). Respiratory oscillations in arterial carbon dioxide tension as a control signal in exercise. *Nature* **283**, 84–85.

Bhattacharyya NK, Cunningham DJ, Goode RC, Howson MG & Lloyd BB (1968). The effects of hypoxia and light exercise on the respiratory response to CO_2 in man. *J Physiol* **194**, 14*P*–16*P*.

Brys M, Brown CM, Marthol H, Franta R & Hilz MJ (2003). Dynamic cerebral autoregulation remains stable during physical challenge in healthy persons. *Am J Physiol Heart Circ Physiol* **285**, H1048–H1054.

Chapman RW, Santiago TV & Edelman NH (1979). Effects of graded reduction of brain blood flow on chemical control of breathing. *J Appl Physiol* **47**, 1289–1294.

Chesler M (2003). Regulation and modulation of pH in the brain. *Physiol Rev* **83**, 1183–1221.

Clark JM, Sinclair RD & Lenox JB (1980). Chemical and nonchemical components of ventilation during hypercapnic exercise in man. *J Appl Physiol* **48**, 1065–1076.

Crawford RD & Severinghaus JW (1978). CSF pH and ventilatory acclimatization to altitude. *J Appl Physiol* **45**, 275–283.

Cunningham DJ (1987). Studies on arterial chemoreceptors in man. *J Physiol* **384**, 1–26.

Cunningham DJ, Hey EN, Patrick JM & Lloyd BB (1963). The effect of noradrenaline infusion on the relation between pulmonary ventilation and the alveolar PO_2 and PCO_2 in man. *Ann N Y Acad Sci* **109**, 756–771.

Dempsey JA (2005). Crossing the apnoeic threshold: causes and consequences. *Exp Physiol* **90**, 13–24.

Dempsey JA, Romer L, Rodman J, Miller J & Smith C (2006). Consequences of exercise-induced respiratory muscle work. *Respir Physiol Neurobiol* **151**, 242–250.

Edvinsson L (1975). Neurogenic mechanisms in the cerebrovascular bed. Autonomic nerves, amine receptors and their effects on cerebral blood flow. *Acta Physiol Scand Suppl* **427**, 1–35.

Ellingsen I, Liestol K, Sydnes G, Hauge A & Nicolaysen G (1987*a*). Arterial PCO_2 and lung ventilation in man exposed to 1–5% CO_2 in the inspired gas. *Acta Physiol Scand* **129**, 269–276.

Ellingsen I, Sydnes G, Hauge A, Zwart JA, Liestol K & Nicolaysen G (1987*b*). CO_2 sensitivity in humans breathing 1 or 2% CO_2 in air. *Acta Physiol Scand* **129**, 195–202.

Fencl V (1986). Acid-base balance in cerebral fluids. In *Handbook of Physiology,* section 3, *The Respiratory System,* vol. 2, *Control of Breathing,* ed. Fishman, AP, Cherniack NS, Widdicombe JG & Geiger SR, pp. 115–140. American Physiology Society, Bethesda, MD, USA.

Giller CA, Bowman G, Dyer H, Mootz L & Krippner W (1993). Cerebral arterial diameters during changes in blood pressure and carbon dioxide during craniotomy. *Neurosurgery* **32**, 737–741; discussion 741–742.

Hellström G, Fischer-Colbrie W, Wahlgren NG & Jogestrand T (1996). Carotid artery blood flow and middle cerebral artery blood flow velocity during physical exercise. *J Appl Physiol* **81**, 413–418.

Honda Y, Hayashi F, Yoshida A, Ohyabu Y, Nishibayashi Y & Kimura H (1983). Overall 'gain' of the respiratory control system in normoxic humans awake and asleep. *J Appl Physiol* **55**, 1530–1535.

Jones NL, Robertson DG & Kane JW (1979). Difference between end-tidal and arterial PCO_2 in exercise. *J Appl Physiol* **47**, 954–960.

Jordan J, Shannon JR, Diedrich A, Black B, Costa F, Robertson D & Biaggioni I (2000). Interaction of carbon dioxide and sympathetic nervous system activity in the regulation of cerebral perfusion in humans. *Hypertension* **36**, 383–388.

Jørgensen LG, Perko M, Hanel B, Schroeder TV & Secher NH (1992*a*). Middle cerebral artery flow velocity and blood flow during exercise and muscle ischemia in humans. *J Appl Physiol* **72**, 1123–1132.

Jørgensen LG, Perko G & Secher NH (1992*b*). Regional cerebral artery mean flow velocity and blood flow during dynamic exercise in humans. *J Appl Physiol* **73**, 1825–1830.

LeMarbre G, Stauber S, Khayat RN, Puleo DS, Skatrud JB & Morgan BJ (2003). Baroreflex-induced sympathetic activation does not alter cerebrovascular CO_2 responsiveness in humans. *J Physiol* **551**, 609–616.

Linton RA, Lim M, Wolff CB, Wilmshurst P & Band DM (1984). Arterial plasma potassium measured continuously during exercise in man. *Clin Sci (Lond)* **67**, 427–431.

Markwalder TM, Grolimund P, Seiler RW, Roth F & Aaslid R (1984). Dependency of blood flow velocity in the middle cerebral artery on end-tidal carbon dioxide partial pressure – a transcranial ultrasound Doppler study. *J Cereb Blood Flow Metab* **4**, 368–372.

Meadows GE, Dunroy HM, Morrell MJ & Corfield DR (2003). Hypercapnic cerebral vascular reactivity is decreased, in humans, during sleep compared with wakefulness. *J Appl Physiol* **94**, 2197–2202.

Miyamoto T, Inagaki M, Takaki H, Kawada T, Yanagiya Y, Sugimachi M & Sunagawa K (2004). Integrated characterization of the human chemoreflex system controlling ventilation, using an equilibrium diagram. *Eur J Appl Physiol* **93**, 340–346.

Mohan R & Duffin J (1997). The effect of hypoxia on the ventilatory response to carbon dioxide in man. *Respir Physiol* **108**, 101–115.

Nelson E & Rennels M (1970). Innervation of intracranial arteries. *Brain* **93**, 475–490.

Nielsen KC & Owman C (1967). Adrenergic innervation of pial arteries related to the circle of Willis in the cat. *Brain Res* **6**, 773–776.

Ogoh S, Brothers RM, Barnes Q, Eubank WL, Hawkins MN, Purkayastha S, O-Yurvati A & Raven PB (2005*a*). The effect of changes in cardiac output on middle cerebral artery mean blood velocity at rest and during exercise. *J Physiol* **569**, 697–704.

Ogoh S, Dalsgaard MK, Secher NH & Raven PB (2007). Dynamic blood pressure control and middle cerebral artery mean blood velocity variability at rest and during exercise in humans. *Acta Physiol (Oxf)* **191**, 3–14.

Ogoh S, Fadel PJ, Zhang R, Selmer C, Jans O, Secher NH & Raven PB (2005*b*). Middle cerebral artery flow velocity and pulse pressure during dynamic exercise in humans. *Am J Physiol Heart Circ Physiol* **288**, H1526–H1531.

Ohyabu Y, Yoshida A, Hayashi F & Honda Y (1982). Ventilatory response to CO_2 after brief stimulations of the peripheral chemoreceptors in man. *Jpn J Physiol* **32**, 627–636.

Peebles K, Celi L, McGrattan K, Murrell C, Thomas K & Ainslie PN (2007). Human cerebrovascular and ventilatory CO_2 reactivity to end-tidal, arterial and internal jugular vein P_{CO_2}. *J Physiol* **584**, 347–357.

Pianosi P, Grondin D, Desmond K, Coates AL & Aranda JV (1994). Effect of caffeine on the ventilatory response to inhaled carbon dioxide. *Respir Physiol* **95**, 311–320.

Poon CS (1989*a*). Effects of inspiratory elastic load on respiratory control in hypercapnia and exercise. *J Appl Physiol* **66**, 2400–2406.

Poon CS (1989*b*). Effects of inspiratory resistive load on respiratory control in hypercapnia and exercise. *J Appl Physiol* **66**, 2391–2399.

Poon CS & Greene JG (1985). Control of exercise hyperpnea during hypercapnia in humans. *J Appl Physiol* **59**, 792–797.

Poon CS, Tin C & Yu Y (2007). Homeostasis of exercise hyperpnea and optimal sensorimotor integration: the internal model paradigm. *Respir Physiol Neurobiol* **159**, 1–13; discussion 14–20.

Qayyum MS, Barlow CW, O'Connor DF, Paterson DJ & Robbins PA (1994). Effect of raised potassium on ventilation in euoxia, hypoxia and hyperoxia at rest and during light exercise in man. *J Physiol* **476**, 365–372.

Rasmussen P, Stie H, Nielsen B & Nybo L (2006). Enhanced cerebral CO_2 reactivity during strenuous exercise in man. *Eur J Appl Physiol* **96**, 299–304.

Robbins PA (1988). Evidence for interaction between the contributions to ventilation from the central and peripheral chemoreceptors in man. *J Physiol* **401**, 503–518.

Schreiber SJ, Gottschalk S, Weih M, Villringer A & Valdueza JM (2000). Assessment of blood flow velocity and diameter of the middle cerebral artery during the acetazolamide provocation test by use of transcranial Doppler sonography and MR imaging. *AJNR Am J Neuroradiol* **21**, 1207–1211.

Serrador JM, Picot PA, Rutt BK, Shoemaker JK & Bondar RL (2000). MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis. *Stroke* **31**, 1672–1678.

Severinghaus JW & Carcelen A (1964). Cerebrospinal fluid in man native to high altitude. *J Appl Physiol* **19**, 319–321.

Severinghaus JW, Mitchell RA, Richardson BW & Singer MM (1963). Respiratory control at high altitude suggesting active transport regulation of CSF pH. *J Appl Physiol* **18**, 1155–1166.

Teppema L, Sarton E, Dahan A & Olievier CN (2000). The neuronal nitric oxide synthase inhibitor 7-nitroindazole (7-NI) and morphine act independently on the control of breathing. *Br J Anaesth* **84**, 190–196.

Toda N & Okamura T (1998). Cerebral vasodilators. *Jpn J Pharmacol* **76**, 349–367.

Waldrop TG & Iwamoto GA (2006). Point: supraspinal locomotor centers do contribute significantly to the hyperpnea of dynamic exercise. *J Appl Physiol* **100**, 1077–1079.

Ward SA (2007). Muscle-energetic and cardio-pulmonary determinants of exercise tolerance in humans: Muscle-energetic and cardio-pulmonary determinants of exercise tolerance in humans. *Exp Physiol* **92**, 321–322.

Wasserman K, Whipp BJ, Koyal SN & Cleary MG (1975). Effect of carotid body resection on ventilatory and acid-base control during exercise. *J Appl Physiol* **39**, 354–358.

Wasserman K, Zhang YY, Gitt A, Belardinelli R, Koike A, Lubarsky L & Agostoni PG (1997). Lung function and exercise gas exchange in chronic heart failure. *Circulation* **96**, 2221–2227.

Xie A, Skatrud JB, Khayat R, Dempsey JA, Morgan B & Russell D (2005). Cerebrovascular response to carbon dioxide in patients with congestive heart failure. *Am J Respir Crit Care Med* **172**, 371–378.

Xie A, Skatrud JB, Morgan B, Chenuel B, Khayat R, Reichmuth K, Lin J & Dempsey JA (2006). Influence of cerebrovascular function on the hypercapnic ventilatory response in healthy humans. *J Physiol* **577**, 319–329.

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