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LONG-TERM MODULATION OF THE EXERCISE VENTILATORY RESPONSE IN GOATS

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SUMMARY

1. To test the hypothesis that repeated associations of exercise and increased respiratory dead space elicit mechanisms that augment future ventilatory responses to exercise alone, experiments were conducted on normal adult goats familiarized with experimental procedures.

2. Measurements of ventilation, arterial blood gases and CO_2 production were made at rest, during mild steady-state exercise (4 km h^{-1}; 5 % grade) and with increased dead space at rest in seven goats before and after training. In Series I experiments, training consisted of fourteen to twenty exercise trials explicitly paired with increased dead space (0·8 l) over 2 days. Increased dead space predominantly represents a CO_2 chemoreceptor stimulus with only mild hypoxic stimulation. Post-training measurements were made 1–6 h and 1 week after training was completed.

3. The same goats repeated a slightly modified protocol several months later (Series II; 6 trials per day for 4 days) to determine if responses were both repeatable and reversible, and to investigate training effects on dynamic ventilatory responses at the onset of exercise.

4. In Series I experiments, resting minute ventilation and breathing frequency were elevated 1–6 h post-training compared to baseline (44 and 74 % respectively), whereas resting tidal volume decreased (14 %). One week post-training, resting values had returned to baseline. Series II training had no significant effects on resting measurements.

5. Relative to baseline, arterial partial pressure of CO_2 (P_{a,CO_2}) values decreased significantly more from rest to exercise 1–6 h post-training in both Series I ($2·7 \pm 0·2$ *vs.* $1·8 \pm 0·9$ mmHg) and Series II ($3·4 \pm 0·6$ *vs.* $2·0 \pm 0·6$ mmHg). The exercise ventilatory response increased 25–28 % 1–6 h post-training (both series), largely due to a greater exercise frequency response, but returned to baseline 1 week post-training. Training had no effect on ventilatory responses to CO_2 at rest, suggesting that decreases in CO_2 chemoreceptor responsiveness did not cause the greater exercise ventilatory response. Model estimates indicate that the net feedforward exercise ventilatory stimulus was increased 40–50 % by training.

6. Training had no discernable effects on ventilatory dynamics at the onset of

exercise. However, post-training differences in P_{a,CO_2} regulation and ventilation were established early in exercise, prior to steady state.

7. Collectively, these experiments suggest a previously unsuspected degree of repeatable and reversible plasticity in the control system subserving the exercise ventilatory response. Such plasticity may contribute to the development of normal exercise hyperpnoea and to adaptive responses of the ventilatory control system in adult animals.

INTRODUCTION

The hypocapnic ventilatory response during mild or moderate exercise in most non-human mammalian species can be explained by (i) a primary ventilatory stimulus in excess of that necessary to maintain arterial isocapnia, and (ii) ventilatory inhibition due to reduced CO_2 chemoreceptor stimulation (Pan, Forster, Bisgard, Kaminski, Dorsey & Busch, 1983; Dempsey, Vidruk & Mitchell, 1985; Mitchell, 1990). The primary exercise stimulus operates in a feedforward manner with respect to arterial blood gas regulation (Houk, 1988), and has traditionally been regarded as an inflexible property of the respiratory control system (Dejours, 1964; Miyamoto, 1990; Eldridge & Waldrop, 1991). However, recent evidence indicates a degree of modulation or plasticity in the exercise ventilatory response. These mechanisms of plasticity operate in both short (i.e. within trial, 'short-term modulation'; Bach, Lutcavage & Mitchell, 1993) and long time domains (i.e. alterations in future trials, 'long-term modulation'; Mitchell, Douse & Foley, 1990).

'Short-term modulation' of the exercise ventilatory response is revealed when resting ventilation and blood gases are altered experimentally. For example, small increases in respiratory dead space increase the exercise ventilatory response, maintaining P_{a,CO_2} regulation with respect to its resting level (Mitchell, 1990; Poon, 1992). Modulation of the exercise ventilatory response with increased dead space cannot be accounted for by changes in chemoreceptor feedback from rest to exercise (Mitchell, 1990), and is similar to the modulation observed with most other experimental treatments that increase resting ventilation including: acid–base changes, hormonal alterations and manipulation of certain neurotransmitter systems (cf. Dempsey, Mitchell & Smith, 1984; Mitchell, Smith & Dempsey, 1984; Schaefer & Mitchell, 1989). Because a wide range of experimental treatments elicit similar modulation, we postulated that a common mechanism links the exercise ventilatory response to resting ventilatory drive by a mechanism independent of chemoreception *per se* (Mitchell *et al.* 1984; Schaefer & Mitchell, 1989). We refer to this mechanism as 'short-term modulation' because the exercise ventilatory response is reversibly altered within a single exercise trial.

Experiments on goats with thoracic sensory denervation via thoracic dorsal rhizotomy (TDR) provided evidence for a different, longer lasting form of modulation, ('long-term modulation') that alters ventilatory responses in future exercise trials (Mitchell, Foley, McGuirk, Selby, Schaefer & Lange, 1988; Mitchell *et al.* 1990). Following TDR, goats exhibited severe ventilatory failure and progressive hypercapnia during exercise with a respiratory mask. However, over successive exercise trials, the goats exhibited remarkable functional recovery,

eventually compensating for resistance and/or dead space associated with the mask despite a persistent lack of feedback from thoracic sensory receptors. Functional recovery following TDR may relate to recovery from neural injury or disuse, but is more likely to result from adaptive control mechanisms that actively change components of the respiratory control system. It was proposed that repeated associations of exercise and chemoreceptor feedback elicit mechanisms of 'long-term modulation' analogous to associative motor learning (Mitchell *et al.* 1990), thus altering future exercise ventilatory responses and compensating for the sensory deficit following TDR by (1) restoring respiratory motoneurone excitability, or (2) overriding continued motoneurone hypoexcitability by increasing descending central respiratory drive.

The primary objective of the present study was to determine if 'long-term modulation' occurs in normal adult goats by addressing the following question: Do repeated, paired presentations of exercise and increased dead space (hypercapnia) augment future responses to exercise alone? Training experiments, designed to simulate aspects of ventilatory failure after TDR, consisted of repeated associations of exercise and large increases in respiratory dead space (Series I). A modified training protocol (Series II) was completed by the same goats several months later to determine if the outcome of Series I experiments could be repeated, and to investigate more thoroughly the effects of training on ventilatory dynamics at the onset of exercise. The results of both training protocols suggest a degree of repeatable and reversible plasticity, established in a remarkably short period (hours to days). Preliminary results of these experiments have been published as an abstract (Martin, Mitchell, Brown & Kaarakka, 1990).

METHODS

Experiments were conducted on seven female adult goats (33–54 kg; 2–4 years of age), familiarized with laboratory procedures such as wearing a tight-fitting respiratory mask and exercising on a motor-driven treadmill (Quinton, Model Q65, USA). The goats were prepared with a translocated carotid artery, thus allowing repeated placement of arterial catheters. The goats were premedicated with atropine sulphate (0·044 mg kg^{-1} I.V.), intubated after administration of thiamylal sodium (10–15 mg kg^{-1} I.V.), and maintained under halothane anaesthesia during the carotid translocation. Penicillin (20000 IU kg^{-1} I.M.) was administered following surgery (3 days) and after each experiment (1 day). A minimum of 2 weeks passed between surgery and the first experiment. Prior to experiments, the goats were fasted overnight with water *ad libitum*.

Measurements

Heparinized arterial blood samples (0·8 ml) were collected in triplicate (Series I, see below) or quadruplicate (Series II) at rest or during steady-state exercise, or at 10 s intervals following the onset of exercise (Series II only). The samples were capped and stored on ice until analysed for pH, P_{CO_2} and P_{O_2} with an automated blood gas analyser (ABL 330, Radiometer, Denmark); values were corrected with respect to measured rectal temperature. Blood gas values were also corrected with reference to tonometered blood, equilibrated with three gas mixtures of known composition on each experimental day.

A respiratory mask, fitted with a non-rebreathing valve (Hans Rudolf, series 2600, USA) was used to allow measurements of ventilation and mixed expired gas composition. A pneumotachograph (Fleisch, No. 2, Switzerland) was attached to the inspiratory port of the non-rebreathing valve, and pressure differences across the pneumotachograph were measured with a variable reluctance differential pressure transducer (Validyne, MP-45, USA) and a carrier

demodulator (Validyne, CD15, USA). This pressure signal (proportional to flow) was integrated (Gould, USA) to obtain the tidal volume. The mixed expired CO_2 fraction was measured in a mixing chamber with an infrared CO_2 analyser (Sensor Medics, LB–2, USA) for calculation of CO_2 production ($\dot{V}_{CO_2}$; see Mitchell, 1990 for details). Ventilatory and metabolic data were collected with an on-line computer acquisition system designed in our laboratory.

Experimental protocol

Steady-state measurements were made after 5 min in each resting or exercise condition while the goats were breathing air. Carbon dioxide chemoreceptor responsiveness at rest (S) was estimated by the slope of the ventilatory response to changes in arterial partial pressure of CO_2 (P_{a,CO_2}) elicited by an increased dead space of 0·8 l ($S = \Delta\dot{V}_I/\Delta P_{a,CO_2}$, where $\dot{V}_I$ is the inspiratory ventilation). Dead space tubes were made of the same spiral tubing (3·5 cm diameter; W. E. Collins, USA) as the much longer inspiratory and expiratory hoses. Short additions of such tubing provide essentially a hypercapnic stimulus, without appreciable effects on the overall system resistance (Mitchell, 1990). Since ambient air was inspired, measurements of S were associated with slight hypoxaemia ($P_{O_2} = 80$–90 mmHg). Twenty minutes of rest were allowed between trials of exercise or hypercapnia at rest.

To ensure that the goats did not receive visual cues indicating the addition of dead space: (1) the treadmill was fitted with side panels to minimize visibility of experimental manipulations; and (2) the dead space tube was disguised so that the goats could not distinguish it from the normal inspiratory hose. Furthermore, to impose a consistent temporal association between exercise and increased dead space, either the inspiratory hose or the dead space (as appropriate) was connected to the non-rebreathing valve within 5 s after the onset of exercise.

Series I: training with exercise and dead space (steady-state responses)

Seven goats were used in Series I experiments to determine if repeated hypercapnic exercise elicits 'long-term modulation'. Baseline measurements were made on 2 days, approximately 1 week prior to training. Baseline measurements were made at rest and during exercise (4 trials per day; 4 km h^{-1}, 5 % grade) with two measurements of CO_2 responsiveness at rest per day. No less than 48 h passed between baseline experiments.

Exercise (4 km h^{-1}, 5 % grade; 5–7 min) and increased dead space (0·8 l) were repeatedly paired for fourteen to twenty training trials on two consecutive days (10–16 trials on day 1, 4 trials on day 2). Twenty minutes of rest were allowed between training trials. Between training days, the goats were housed in restricted quarters to limit spontaneous activity unpaired with increased dead space. During training, the goats were never exercised without increased dead space, nor were they presented with increased dead space at rest.

Following the last of four training trials on day 2, the goats were allowed 1 h to recover while standing quietly on the treadmill. Post-training measurements were made 1–6 h and 1 week following training and consisted of rest, followed by exercise (4 trials; 4 km h^{-1}, 5 % grade; 5–7 min), with two measurements of CO_2 responsiveness at rest. During post-training experiments, exercise and dead space were never presented simultaneously and, thus, were once again unpaired.

Series II: dynamic responses

Training with paired exercise and dead space was repeated several months later in six of the seven goats used in Series I. These experiments were conducted to determine if steady-state Series I results were repeatable, but were modified to: (1) determine if a greater number of training days would enhance 'long-term modulation' of the exercise ventilatory response; and (2) allow an assessment of changes in dynamic ventilatory responses at the onset of exercise resulting from training. Baseline ventilatory and arterial blood gas measurements were made on one day at rest and during exercise (3 trials; 4 km h^{-1}, 5 % grade), with two measurements of CO_2 responsiveness at rest. During the transition from rest to exercise, arterial blood samples were collected at 10 s intervals from the onset through 3 min of exercise.

Training began approximately 1 week after baseline measurements. Exercise and dead space were paired for twenty-one to twenty-five trials over four training days. Measurements of ventilation and blood gases were made during the first three training trials (day 1) and the last three training trials (day 4). Goats were housed in restricted quarters between training days.

After the final training trial on day 4, the goats stood quietly for 1 h. Post-training experiments were performed 1–6 h and 1 week post-training.

Analysis

Measurements at rest and responses to steady-state exercise were averaged to yield mean responses per goat for baseline, 1–6 h post-training and 1 week post-training. Population means were calculated from per goat means for each experimental series and reported ± one standard error of the mean. Statistical significance was determined via paired *t* tests with the correction for multiple comparisons suggested by Bonferroni (Wallenstein, Zucker & Fleiss, 1980). Three comparisons were made (baseline *vs.* 1–6 h post-training, baseline *vs.* 1 week post-training, and 1–6 h post-training *vs.* 1 week post-training). Differences were considered significant if overall probability of an error was $P < 0{\cdot}05$.

For Series I, the statistical significance of differences between conditions (baseline, 1–6 h and 1 week post-training) was also tested for the change in P_{a,CO_2} from rest to exercise ($\Delta P_{a,CO_2}$) and $\Delta \dot{V}_I / \Delta \dot{V}_{CO_2}$ by analysis of variance with repeated measures (ANOVA; Systat Inc., Evanston, IL, USA). Using this approach, a paired comparison between conditions was not possible, but the analysis retained variance from trial to trial within condition and goat.

For the dynamic analysis of Series II experiments, breath-by-breath ventilatory measurements were assigned in temporal sequence to ten time bins (20 s) and per bin means were calculated. Values in each bin were averaged in comparable conditions to create per goat mean responses per time bin. Blood gases were assigned to the corresponding time bins and averaged to yield means per time bin per goat. The per bin data for each goat were then averaged across all goats in an experimental series to yield mean responses per time bin for baseline, training day 1, training day 4, 1–6 h post-training and 1 week post-training. The change from rest to exercise in $\dot{V}_I$ ($\Delta \dot{V}_I$) was also normalized as a fraction of the steady-state change. This normalization allows direct comparisons of the time course in different experimental conditions, despite differences in the magnitude of steady-state responses (Mitchell & Osborne, 1978). Statistical significance of the dynamic data was tested using two-way ANOVA.

A previously described mathematical model was used to estimate the net feedforward stimulus, characterized by an exercise gain (G_{EX}; Mitchell, 1990). Based on model assumptions, G_{EX} reflects net ventilatory stimulation resulting from all stimulatory or inhibitory inputs that are correlated with metabolic CO_2 production during exercise, but unrelated to arterial CO_2 chemoreceptor feedback. Although simplistic, the model is useful in making inferences concerning relative changes in feedforward *versus* additive CO_2 chemoreceptor feedback due to experimental manipulations (Mitchell, 1990). The overall system ventilatory response to exercise is characterized by the slope of the relationship between $\dot{V}_I$ and $\dot{V}_{CO_2}$ or the system gain: $G_{SYS} = \Delta \dot{V}_I / \Delta \dot{V}_{CO_2}$, and results from the balance of feedforward and CO_2 feedback mechanisms. Thus, G_{EX} was calculated from measured values of G_{SYS}, ventilatory responsiveness to P_{a,CO_2} at rest (S), and the slope of the relationship between P_{a,CO_2} and $\dot{V}_{CO_2}$ during exercise:

$$G_{EX} = G_{SYS} - S\ (\Delta P_{a,CO_2} / \Delta \dot{V}_{CO_2}).$$

In this analysis, any effect that is not attributable to additive P_{a,CO_2} effects, but is related to an interaction between P_{a,CO_2} and exercise, will be assigned to G_{EX}. The reasons for this assumption (and the use of resting S in model calculations) have been argued previously (Mitchell, 1990).

RESULTS

Chemoreceptor stimuli during training trials

During exercise trials with increased dead space, P_{a,CO_2} increased $10{\cdot}0 \pm 1{\cdot}7$ mmHg while P_{a,O_2} decreased 22 ± 4 mmHg from rest to exercise on the second training day of Series I (Table 1). Exercise with increased dead space increased P_{a,CO_2} approximately 10–12 mmHg while decreasing P_{a,O_2} 27–31 mmHg in Series II experiments (Table 1). Arterial blood gas regulation from rest to exercise on training day 4 *vs.* training day 1 was not significantly different.

TABLE 1. Measured values of P_{a,CO_2}, P_{a,O_2}, and $\dot{V}_I$ during training trials

	Series I ($n = 4$)	Series II ($n = 5$)	
	Day 2	Day 1	Day 4
Resting values			
P_{a,CO_2} (mmHg)	41·6 ± 1·1	41·9 ± 0·7	41·8 ± 0·8
P_{a,O_2} (mmHg)	108 ± 2	112 ± 3	115 ± 4
$\dot{V}_I$ (l_{BTPS} min^{-1})	9·7 ± 1·0	9·7 ± 1·0	11·1 ± 1·4
Exercise values			
P_{a,CO_2} (mmHg)	51·6 ± 1·1	53·7 ± 1·8	51·6 ± 2
P_{a,O_2} (mmHg)	85 ± 2	81 ± 4	88 ± 4
$\dot{V}_I$ (l_{BTPS} min^{-1})	59·5 ± 5·9	78·3 ± 4·1	82·1 ± 6·4

Values are means ± 1 S.E.M. Series I and Series II training trials consisted of normoxic rest (5 min) followed by hypercapnic exercise (4 km h^{-1}, 5 % grade; increased dead space = 0·8 l; 5–7 min).

TABLE 2. Measured values at rest

	Baseline	1–6 h post-training	1 week post-training
Series I ($n = 7$)			
P_{a,CO_2} (mmHg)	39·9 ± 0·7	41·9 ± 1·2	41·2 ± 0·8
$\dot{V}_I$ (l_{BTPS} min^{-1})	8·2 ± 0·3	11·3 ± 1·3*†	7·2 ± 0·5
V_T (l_{BTPS})	0·42 ± 0·02	0·37 ± 0·02*	0·40 ± 0·03
f (breaths min^{-1})	19·1 ± 1·2	31·9 ± 5·0*†	17·7 ± 1·7
$\dot{V}_{CO_2}$ (l_{STPD} min^{-1})	0·18 ± 0·01	0·20 ± 0·01	0·17 ± 0·02
V_T/t_I (l_{BTPS} s^{-1})	0·43 ± 0·02	0·62 ± 0·06*†	0·39 ± 0·03
t_I/t_{TOT}	0·28 ± 0·01	0·28 ± 0·01	0·28 ± 0·01
T_B (°C)	39·6 ± 0·2	39·8 ± 0·1†	39·4 ± 0·1
Series II ($n = 6$)			
P_{a,CO_2} (mmHg)	40·1 ± 0·4	42·0 ± 0·9	41·2 ± 1·3
$\dot{V}_I$ (l_{BTPS} min^{-1})	10·0 ± 0·8	11·5 ± 1·2	9·8 ± 0·9
V_T (l_{BTPS})	0·50 ± 0·04	0·42 ± 0·02	0·48 ± 0·04
f (breaths min^{-1})	19·6 ± 1·6	26·1 ± 2·6	20·4 ± 1·9
$\dot{V}_{CO_2}$ (l_{STPD} min^{-1})	0·23 ± 0·02	0·22 ± 0·01	0·22 ± 0·02
V_T/t_I (l_{BTPS} s^{-1})	0·54 ± 0·06	0·62 ± 0·05	0·51 ± 0·05
t_I/t_{TOT}	0·29 ± 0·01	0·28 ± 0·01	0·30 ± 0·01
T_B (°C)	39·3 ± 0·1	39·3 ± 0·1	39·2 ± 0·2

Data are mean resting values ± 1 S.E.M. for Series I and Series II experiments. * denotes significant differences from baseline, $P < 0·05$. † denotes significant differences from 1 week post-training, $P < 0·05$. V_T, tidal volume; f, breathing frequency; t_I, inspiratory time; t_{TOT}, total respiratory time; T_B, mean body temperature.

Series I: training with exercise and dead space

Several resting variables were significantly changed 1–6 h post-training in Series I experiments (Table 2). Mean $\dot{V}_I$ increased 44 ± 16 % ($P < 0{\cdot}05$) 1–6 h post-training relative to baseline, and 61 ± 21 % ($P < 0{\cdot}05$) relative to 1 week post-training. This increase was caused by a 74 ± 20 % increase in resting frequency *versus* baseline ($P < 0{\cdot}01$) and 79 ± 26 % *versus* 1 week post-training ($P < 0{\cdot}01$). Resting tidal volume (V_T) decreased 14 ± 3 % 1–6 h post-training *versus* baseline ($P < 0{\cdot}01$), but was not significantly different from 1 week post-training. Mean body temperature (T_B) was within a range of 0·5 °C (Table 2); T_B was significantly elevated (0·4 °C; $P < 0{\cdot}01$) 1–6 h *versus* 1 week post-training, but no other comparisons were significant. V_T/t_I was increased 1–6 h post-training relative to baseline (48 ± 16 %; $P < 0{\cdot}05$) and 1 week post-training (65 ± 22 %; $P < 0{\cdot}01$). The ratio of inspiratory time to total respiratory time (t_I/t_{TOT}) was unchanged by training. Neither resting P_{a,CO_2} (Table 2) nor S (Fig. 1*A*) were significantly affected by training.

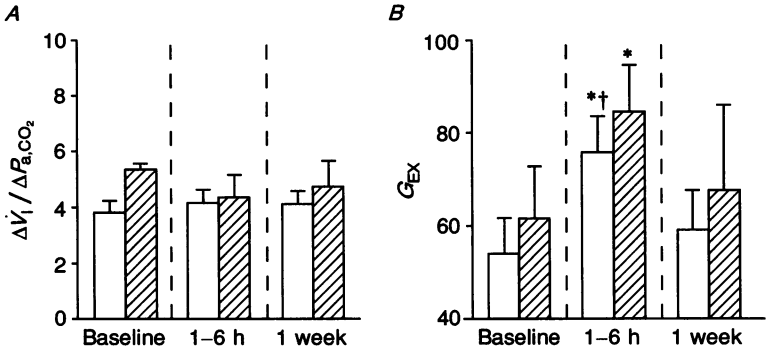

Fig. 1. Effects of Series I (open bars) and Series II (hatched bars) training on feedforward and feedback components of the exercise ventilatory response. In *A*, CO_2 responsiveness at rest ($S = \Delta\dot{V}_I/\Delta P_{a,CO_2}$; $l_{BTPS}\,min^{-1}\,mmHg^{-1}$) is illustrated. Following training, S is unchanged, suggesting that changes in CO_2 responsiveness cannot account for 'long-term modulation'. In *B*, estimated exercise gain (G_{EX}) is significantly elevated 1–6 h post-training in both experimental series, suggesting that an augmented feedforward mechanism accounts for 'long-term modulation'. G_{EX} returns to baseline levels at 1 week. Values are means ± 1 S.E.M. * denotes significant difference from baseline, $P < 0{\cdot}05$. † denotes significant difference from 1 week post-training, $P < 0{\cdot}05$.

Changes in P_{a,CO_2} from rest to exercise ($\Delta P_{a,CO_2}$) and the exercise ventilatory response are illustrated in Fig. 2. P_{a,CO_2} decreased nearly 2 mmHg more from rest to exercise 1–6 h post-training *vs.* baseline ($P < 0{\cdot}01$). The P_{a,CO_2} decrease at 1 week was also greater than baseline ($P < 0{\cdot}05$), but less than that at 1–6 h post-training ($P < 0{\cdot}01$), indicating at least a partial return to normal. G_{SYS} was increased 25 ± 8 % from baseline 1–6 h post-training ($P < 0{\cdot}05$), but had returned to baseline 1 week post-training. Increased G_{SYS} was due to a greater exercise frequency response (81 ± 31 %; $P < 0{\cdot}05$; Fig. 3*B*), with a diminished tidal volume response (37 ± 11 %; $P < 0{\cdot}05$; Fig. 3*A*). The change in $\dot{V}_{CO_2}$ from rest to exercise was

unaffected by training. The greater post-training exercise P_{a,CO_2} decrease, without change in S, suggests an increased feedforward exercise stimulus. In accordance, estimates of G_{EX} increased $47 \pm 12\%$ 1–6 h post-training relative to baseline ($P < 0{\cdot}01$), but had returned to baseline at 1 week (Fig. 1*B*).

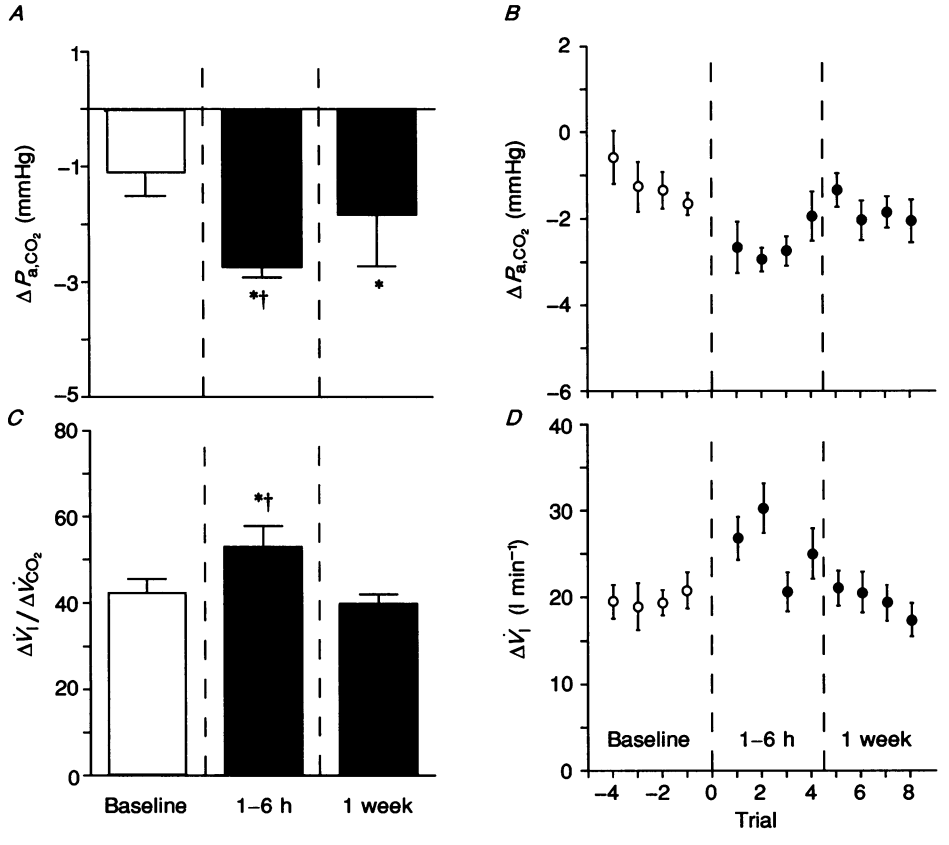

Fig. 2. Series I results demonstrating 'long-term modulation' following repeated trials of hypercapnic exercise. In *A*, the P_{a,CO_2} decrease from rest to exercise ($\Delta P_{a,CO_2}$) is significantly greater 1–6 h post-training and remains elevated from baseline at 1 week post-training. In *B*, when $\Delta P_{a,CO_2}$ is plotted as a function of exercise trial, the greatest changes from rest to exercise occur during the first three post-training trials. By trial 4, $\Delta P_{a,CO_2}$ approaches baseline levels and is restored to near baseline levels at 1 week. In *C*, the slope of the ventilation *versus* CO_2 production relationship or system gain ($G_{SYS} = \Delta \dot{V}_I / \Delta \dot{V}_{CO_2}$) is increased 25% from baseline 1–6 h post-training and returns to baseline levels at 1 week. In *D*, $\Delta \dot{V}_I$ is plotted as a function of exercise trial, revealing a time course similar to $\Delta P_{a,CO_2}$ (*B*). Values are means ± 1 S.E.M. * denotes significant difference from baseline, $P < 0{\cdot}05$. † denotes significant difference from 1 week post-training, $P < 0{\cdot}05$.

Per trial changes in P_{a,CO_2} and $\dot{V}_I$ from rest to exercise are illustrated in Fig. 2. The greater P_{a,CO_2} decrease 1–6 h post-training is associated with an increased $\dot{V}_I$ response. Both $\Delta P_{a,CO_2}$ and $\Delta \dot{V}_I$ show the greatest changes in post-training trials 1–2, and return towards baseline by trial 4, less than 6 h post-training. During

trial 3, one goat experienced difficulties during exercise manifested by extremely unco-operative behaviour in a normally tolerant animal; P_{a,CO_2} increased 4 mmHg during this exercise trial. Inspection of the respiratory mask revealed that the goat's breathing may have been partially obstructed during this trial. Rejection of

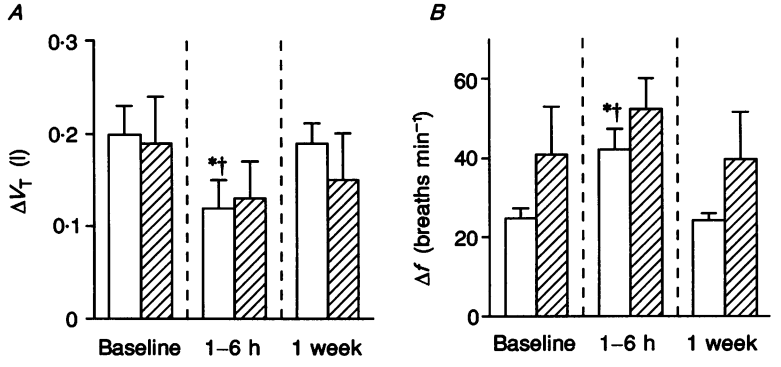

Fig. 3. Changes in ventilatory pattern before and after training in Series I (open bars) and Series II (hatched bars) experiments. *A*, changes in tidal volume (V_T; l_{BTPS}) from rest to exercise. *B*, changes in breathing frequency (f; breaths min^{-1}) from rest to exercise. The decreased V_T response 1–6 h post-training is offset by a greater proportionate increase in the f response. Values are means ± 1 S.E.M. * denotes significant difference from baseline, $P < 0{\cdot}05$. † denotes significant difference from 1 week post-training, $P < 0{\cdot}05$.

this goat from the data set did not change statistical significance of the results. Therefore, responses of trials 2 (e.g. $\Delta P_{a,CO_2} = -3{\cdot}7$ mmHg; $\Delta \dot{V}_I = +32{\cdot}5$) and 4 ($\Delta P_{a,CO_2} = -3{\cdot}8$ mmHg; $\Delta \dot{V}_I = +30{\cdot}8$) for this goat were averaged and used in place of the measured trial 3 responses to calculate the mean values used in Fig. 2.

ANOVA with repeated measures confirmed the Bonferroni corrected, paired t tests and indicated a significant effect of condition (baseline, 1–6 h and 1 week post-training) between animals for $\Delta P_{a,CO_2}$ and $\Delta \dot{V}_I / \Delta \dot{V}_{CO_2}$ ($P < 0{\cdot}05$). Including within animal variability indicated no significant effect of trial number or an interaction between trial and condition.

Series II: dynamic responses

Following Series II training, there were no significant differences in any resting value (Table 2). Changes in steady-state exercise responses following Series II training (Fig. 4) were similar to Series I; the longer training period (4 *vs.* 2 days) did not enhance the magnitude of training effects observed 1–6 h post-training. The greater P_{a,CO_2} decrease from rest to exercise ($3{\cdot}4 \pm 0{\cdot}6$ mmHg, 1–6 h post-training *vs.* $2{\cdot}0 \pm 0{\cdot}6$ mmHg, baseline; Fig. 4*A*) and the G_{SYS} increase (28 ± 9 %; Fig. 4*C*) were both significant 1–6 h post-training *versus* baseline ($P < 0{\cdot}05$), but not *versus* 1 week post-training. There were no significant differences between baseline and 1 week post-training for either value. A 40 ± 14 % increase in G_{EX} was observed 1–6 h *versus* baseline ($P < 0{\cdot}05$), but G_{EX} had returned to baseline by 1 week post-training (Fig. 1*B*). Per trial values of $\Delta P_{a,CO_2}$ and $\Delta \dot{V}_I$ showed patterns similar to Series I experiments, including an apparent return towards normal P_{a,CO_2}

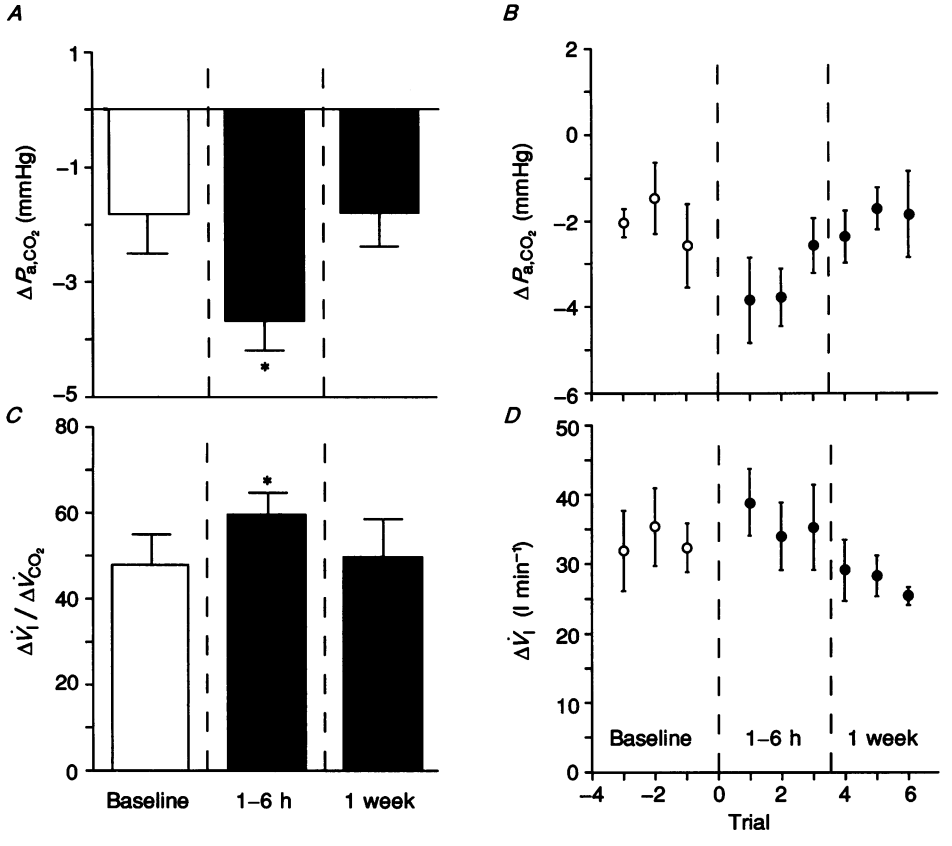

Fig. 4. Series II results demonstrating that 'long-term modulation' is repeatable in the same animals with a modified experimental protocol (see text). For further description of figure, see legend for Fig. 2.

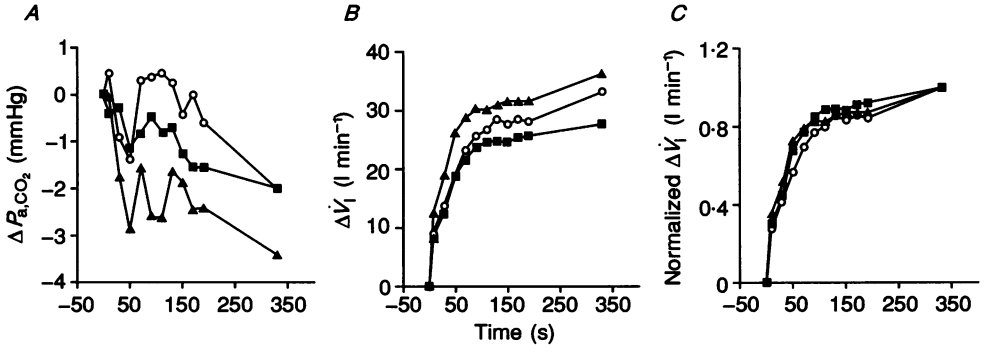

Fig. 5. Dynamic ventilatory responses from rest to steady-state exercise (Series II). *A*, changes in P_{a,CO_2} from rest to exercise ($\Delta P_{a,CO_2}$). *B*, change in minute ventilation from rest to exercise. *C*, normalized minute ventilation ($\Delta \dot{V}_{I_t} / \Delta \dot{V}_{I_\infty}$). ○, baseline responses; ▲, 1–6 h post-training responses; ■, 1 week post-training responses.

regulation within 6 h post-training (Fig. 4*B* and *D*). Collectively, Series II experiments confirm that the augmented steady-state post-training exercise response is both repeatable and reversible, despite continued trials of unpaired exercise or increased dead space.

The dynamics of the approach to steady-state ventilation and blood gases are illustrated in Fig. 5. Two-way ANOVA indicated a significant effect of condition (baseline, 1–6 h post-training and 1 week post-training; $P < 0{\cdot}05$) on $\Delta P_{a,CO_2}$; however, neither time (excluding resting values) nor an interaction between condition and time had significant effects. $\Delta \dot{V}_I$ was significantly affected by condition ($P < 0{\cdot}05$) and time ($P < 0{\cdot}05$), but not an interaction between them. Normalized $\dot{V}_I$ (Fig. 5*C*) shows no differences in the time course of change in ventilation from rest to exercise 1–6 h post-training.

DISCUSSION

If the mechanism(s) controlling ventilation during exercise are 'hard wired' and inflexible, post-training exercise ventilatory responses and the precision of P_{a,CO_2} regulation should remain unaltered. If, on the other hand, experiences such as repeated exercise with increased dead space elicit neural mechanisms underlying 'long-term modulation' of the exercise ventilatory response, the effects should persist when the hypercapnic challenge is no longer present, resulting in greater hyperventilation and P_{a,CO_2} decrease from rest to exercise. Since the exercise ventilatory response was augmented 1–6 h post-training in both Series I and Series II experiments, a form of repeatable and reversible 'long-term modulation' has been demonstrated in normal adult animals. Since the exercise ventilatory response was enhanced without detectable effects on ventilatory responsiveness to hypercapnia at rest, it appears that training augmented the exercise ventilatory response by increasing the feedforward exercise stimulus, and not via persistent changes in chemoreceptor responsiveness *per se*. The augmented exercise ventilatory response may result from neural processes analogous to motor learning, or from persistent circulating or neuro-humoral factors. Regardless of the specific mechanism, these experiments suggest a previously unsuspected degree of plasticity in the control system subserving the exercise ventilatory response.

Experimental design

The protocols used in these experiments were designed to simulate aspects of functional recovery after thoracic dorsal rhizotomy (TDR; Mitchell *et al.* 1988, 1990), but in normal animals. It was postulated that the recovery of function after TDR was the result of adaptive control mechanisms analogous to associative motor learning (Mitchell *et al.* 1990). Therefore, the association of exercise and CO_2 chemoreceptor stimulation via increased dead space (0·8 l) was chosen because their combination caused hypercapnia similar to levels observed during ventilatory failure following TDR.

Small increases in dead space (0·2 l) elicit 'short-term modulation' in goats, augmenting the exercise ventilatory response sufficiently to maintain P_{a,CO_2} slightly

below its resting value. Larger dead space (0·6 l) at higher exercise intensities exceeds the operational range of 'short-term modulation' in goats, resulting in hypercapnic hyperpnoea during exercise (Mitchell, 1990). Thus, the large dead space volume used in this study (0·8 l) exceeds the limits of 'short-term modulation' and provides a powerful CO_2 chemoreceptor stimulus of 10–12 mmHg. The use of dead space *versus* inspired CO_2 to elicit hypercapnia has implications concerning the dynamic signals associated with the CO_2 load (Goode, Brown, Howson & Cunningham, 1968; Swanson, 1978). However, CO_2 responsiveness at rest is the same in goats when assessed with inspired CO_2 *versus* increased dead space during hyperoxia (Mitchell, 1990).

The goats wore a respiratory mask, which inherently increases dead space and resistance (Smith, Mitchell, Jameson, Musch & Dempsey, 1983). However, since the mask was worn during all measurements, its effects should have been constant in all conditions. Although the inspiratory, expiratory and dead space tubes had only minimal effects on the resistance of the experimental apparatus (Mitchell, 1990), endogenous resistance within the goat's airways may increase at elevated $\dot{V}_I$ caused by hypercapnia, since airflow is unlikely to be laminar. Thus, it is possible that changes in endogenous resistance or the physical act of hyperpnoea contribute to the training response via effects on proprioceptors. All conclusions attributing 'long-term modulation' to chemoreceptor activation must remain tentative, pending evaluation of the role played by mechanoreceptor feedback or hyperpnoeic stress. This limitation does not threaten the essential goal of this study: to determine if repeated sensory feedback during hypercapnic exercise alters future exercise ventilatory responses.

It is difficult to define an appropriate control study for these experiments since ventilation increased 300 % more during exercise with increased dead space than with the mask alone. We have not devised a means to match the 'stress' of this hyperpnoea without pairing chemoreception and exercise. When goats are subjected to a similar number and time course (cf. Series I) of exercise trials that are not paired with increased dead space, there is no statistically significant augmentation of the exercise ventilatory response (Martin & Mitchell, 1992). Furthermore, both pre-training and post-training studies (Series I and II) represent repeated, unpaired presentations of dead space and exercise, yet the goats had no obvious pre-training changes in P_{a,CO_2} regulation, and actually began to reverse the training effect within 1–6 h post-training (Figs 3 and 5).

A relatively short interval (1 h) was allowed between the end of training and the beginning of post-training trials to maximize the possibility of detecting training related changes in behaviour. This is insufficient time to discriminate between training effects due to neural alterations similar to associative motor learning, or due to unknown and persistent neuro-humoral factors released during repeated hyperpnoea.

Adult goats were used in these investigations of 'long-term modulation' in normal animals because of the extensive literature available concerning their exercise ventilatory responses (cf. Bisgard, Forster, Mesina & Sarazin, 1982; Smith *et al.* 1983; Mitchell *et al.* 1984; Schaefer & Mitchell, 1989; Mitchell, 1990).

However, some of the goats were still growing between Series I and Series II experiments (mean increase, 4 kg). While both Series I and Series II protocols resulted in a consistent chemoreceptor stimulus of +10 to 12 mmHg, $\dot{V}_I$ during training trials in Series I (59 l min^{-1}) was less than in Series II (78–82 l min^{-1}), probably reflecting the larger size of the goats.

Effects on resting ventilation

Alterations in resting ventilatory pattern following Series I training (Table 2) are not easily explained. Increased T_B causes ventilatory pattern shifts towards rapid shallow breathing in goats (Baker, 1989). However, there were no consistent increases in T_B that would account for a thermolytic tachypnoea 1–6 h after Series I training (Table 2). Very high exercise intensities (92–100 % of the maximum rate of O_2 uptake) cause post-exercise tachypnoea in humans (Younes & Burks, 1985), an effect attributed to the development of pulmonary congestion and interstitial oedema. Since training with exercise and increased dead space provided an intense respiratory challenge, it is possible that oedema had developed in the goats, thus accounting for the shift in respiratory pattern. Similar alterations in breathing patterns have been reported in goats following induction of respiratory muscle fatigue by resistive loading (Oliven, Lohda, Adams, Simhai & Kelsen, 1988). Fatigue may be a factor in the post-training changes in ventilatory pattern, but cannot explain the increase in resting ventilation observed after training since respiratory muscle fatigue reportedly decreases ventilation in goats (Oliven *et al.* 1988). There was an insignificant trend towards tachypnoea and shallow breathing during sham training without increased dead space (Martin & Mitchell, 1992), suggesting that some of these effects are due to repeated exercise and are not necessarily specific to paired presentation of ventilatory stimuli.

A possible cortical involvement in post-training ventilatory pattern shifts may be suggested by the observation that the same goats exhibiting tachypnoea in Series I experiments produced no statistically significant effects after Series II training. This difference might be due to the previous experience, minimizing the stress or anxiety levels of the animals when a similar training protocol was repeated several months later.

Exercise ventilatory responses

The augmented post-training ventilatory response to steady-state exercise was characterized by an increased frequency response. Since breathing frequency changes are under the control of neural circuits, it can be deduced that the mechanism underlying the augmented ventilatory response during exercise is due to neural control mechanisms rather than changes in pulmonary mechanics or respiratory muscles.

Ventilatory responses and $\Delta P_{a,CO_2}$ were already returning towards normal by the third post-training trial (Figs 2 and 4). This return may be time dependent, or it may reflect an active process analogous to the extinction of a learned response (cf. Thompson, 1986) since dead space and exercise were no longer paired. In either case, it clearly demonstrates that the necessary stimulus for the augmented

exercise ventilatory response is present during hypercapnic exercise only. When the stimuli are no longer paired, the effects are reversed even though hypercapnia and exercise are presented independently.

Paired exercise and CO_2 chemoreceptor stimulation do not cause detectable changes in the ventilatory dynamics of approach to steady state (Fig. 5). However, the dynamic analysis suggests that differences in P_{a,CO_2} regulation and ventilation observed during steady-state exercise post-training begin early in exercise, and continue through the approach to steady state.

Increased contributions from the exercise feedforward stimulus and/or decreased chemoreceptor responsiveness are expected to cause greater hypocapnia during exercise (Bennett & Fordyce, 1988; Mitchell, 1990). Since post-training increases in G_{SYS} were accompanied by constant S at rest in both experimental series, G_{EX} increased (Fig. 1*A*). Changes in the exercise stimulus *per se* cannot be distinguished from changes in a P_{a,CO_2}–exercise interaction using this analysis. However, the analysis does exclude changes in the additive contribution of chemofeedback to the overall exercise ventilatory response (Mitchell, 1990).

Possible mechanisms

The augmented exercise ventilatory response 1–6 h post-training provides new evidence for plasticity in the system(s) controlling ventilation during exercise. Few examples of long-lasting effects on future ventilatory responses are known (cf. Dempsey & Forster, 1982; Eldridge & Millhorn, 1986). However, plasticity in neural systems controlling a variety of motor behaviours is well established (Thompson, 1986; Houk, 1988; Stein & Capaday, 1988; Wolpaw & Carp, 1990). 'Long-term modulation' of the exercise ventilatory response resulting from paired exercise and dead space could be explained by several potential mechanisms, but almost certainly requires supraspinal involvement since the exercise frequency response was increased.

Classical conditioning, using the temporal conjunction of two stimuli, is often used as a means of demonstrating associative learning (Schreurs, 1989). The repeated presentation of exercise and dead space in a specified order and temporal spacing satisfies some of the traditional characteristics which define classical conditioning. While the use of classical conditioning techniques is not commonly employed in the study of ventilatory control during exercise (Tobin, Perez, Guenther, D'Alonzo & Dantzker, 1986), several investigators have demonstrated conditioned reflexes in resting breathing of humans (Gallego & Perruchet, 1991) and invertebrates (Levy & Susswein, 1990). Associative learning could result from repeated, paired stimulation of separate respiratory neural pathways, resulting in long-term synaptic changes at a point of convergence (i.e. creating new synapses or strengthening pre-existing ones). In *Aplysia*, paired presentations of shock and lowered environmental pH increase future respiratory pump responses to decreased pH alone (Levy & Susswein, 1990). In vertebrate models, there is considerable precedent for synaptic plasticity in the cerebellum, motor cortex, brainstem and even the spinal cord (cf. Thompson, 1986; Wolpaw & Carp, 1990). Similar neuronal alterations may underlie associative motor learning in the exercise ventilatory response (Houk, 1988; Somjen, 1992).

Conditioned responses, resulting from paired auditory stimuli with chemoreceptor stimulation, have been demonstrated in human subjects (Gallego & Perruchet, 1991), and anticipatory reactions to exercise are capable of stimulating ventilation at rest (Tobin *et al.* 1986). Furthermore, recent experiments on humans, using repeated exercise trials with increased dead space, demonstrated post-training changes in P_{a,CO_2} regulation for subjects with limited prior experience of the exercise test (Adams, Moosavi & Guz, 1992). Other subjects who underwent repeated trials of exercise alone prior to the paired stimulus presentation had post-training responses that were indistinguishable from controls. Thus, stress, anxiety or volitional influences on breathing may play a role in mediating 'long-term modulation' in humans; prior experience with laboratory procedures may cause a degree of habituation, preventing an augmented exercise ventilatory response. However, since 'long-term modulation' was observed in both Series I and Series II experiments, it does not appear that habituation or accommodation to the training protocols occurs to a similar degree in goats.

'Long-term modulation' of the exercise ventilatory response could also result from non-associative processes, sensitizing or increasing the ventilatory response to exercise, or desensitizing (i.e. habituation) mechanisms with an inhibitory effect on ventilation during exercise. Repeated hyperpnoea (via intrinsic CNS or proprioceptor feedback), muscular work or repeated CO_2 chemoreceptor stimulation could provide non-associative breathing stimuli that elicit mechanisms of plasticity.

Significance

'Short-' and 'long-term modulation' suggest an ability to adapt the exercise ventilatory response so that it remains appropriate in the face of changing conditions, such as changes in acid–base or hormonal status (e.g. pregnancy). These findings, if confirmed, have wide ranging implications for the design and interpretation of many studies on ventilatory control since this is a control system that is commonly assumed to be inflexible or 'hard wired'. Furthermore, these mechanisms may also be significant during normal development and ageing, or during unnatural circumstances when wearing a respiratory apparatus during activity (e.g. firefighters or scuba divers). We also suspect that these processes may be significant in compensating for the onset of disease, helping to maintain an appropriate exercise ventilatory response despite progressively deteriorating mechanics and gas exchange. Pulmonary disease is often characterized by progressively increasing respiratory ('alveolar') dead space and hypoxaemia. Without appropriate modifications in the exercise ventilatory response, even mild activity would exacerbate the hypoxaemia and hypercapnia, thereby limiting activity. Finally, long-term changes in the exercise ventilatory response based on experience may implicate that at least part of the normal exercise ventilatory response is learned.

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