Long-term facilitation of ventilation following repeated hypoxic episodes in awake goats

Duncan L. Turner* and Gordon S. Mitchell

*Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706-1102, USA and *Department of Physiology, The Medical School, University of Leeds, Leeds LS2 9NQ, UK*

1. This study tested two hypotheses: (1) that episodic hypoxia elicits long-term facilitation (LTF) in respiratory neurons that is manifest as an increase in ventilation in awake goats; and (2) that LTF causes complex changes in respiratory pattern which are responsible for the increase in ventilation.

2. Each goat participated in two protocols. In the first, inspired gas mixtures were alternated between isocapnic normoxia and hypoxia (arterial partial pressure of oxygen, $P_{a,O_2} = 47$ mmHg) for ten cycles. Each hypoxic episode lasted 3 min and normoxic intervals were 5 min. Ventilatory variables were measured during the last minute of each episode and periodically for up to 1 h following the last hypoxic episode. The second, sham protocol was undertaken at least 2 weeks later and was identical to the first, except that isocapnic hypoxia was replaced with normoxia.

3. Inspired ventilation ($\dot{V}_I$) increased during the first isocapnic hypoxic episode and reached progressively higher levels in subsequent hypoxic episodes. $\dot{V}_I$ also increased progressively among normoxic intervals, such that by the tenth normoxic interval, it had increased 68% relative to the comparable sham value ($P < 0{\cdot}05$). Respiratory frequency (F_R), tidal volume and mean inspiratory flow all contributed to the augmented $\dot{V}_I$ during both isocapnic normoxia and hypoxia. The increase in $\dot{V}_I$ lasted up to 40 min after the final hypoxic episode, with an increased F_R making the greatest contribution. The persistent increase in $\dot{V}_I$ strongly suggests that episodic hypoxia elicits LTF in respiratory neurons in the awake goat. Complex changes in respiratory pattern underpin the ventilatory manifestation of LTF.

Episodic, isocapnic hypoxia increases phrenic nerve discharge in anaesthetized, vagotomized rats (Erickson & Millhorn, 1991; Hayashi, Coles, Bach, Mitchell & McCrimmon, 1993; Bach & Mitchell, 1996*a*). There are several recognizable time-dependent responses, including a progressive increase or decrease in ventilatory activity that lasts seconds to minutes during and immediately following each hypoxic exposure (i.e. short-term potentiation; see Eldridge & Millhorn, 1986), and a prolonged augmentation of ventilatory activity that persists for minutes to hours after the final hypoxic episode (Eldridge & Millhorn, 1986; Hayashi *et al.* 1993; Bach & Mitchell, 1996*a*). This last phase has been termed long-term facilitation (LTF) and appears to be underpinned by a serotonergic neural mechanism (Millhorn, Eldridge & Waldrop, 1980; Bach & Mitchell, 1996*a*).

Hypoxia-induced LTF leads to a persistent increase in ventilatory activity in awake, vagally intact dogs (Cao, Zwillich, Berthon-Jones & Sullivan, 1992). However, neither arterial blood gases nor body temperature were reported in this study. Furthermore, there was significant time-dependent hyperventilation in sham control studies (Cao *et al.* 1992). Imprecise regulation of arterial carbon dioxide partial pressure (P_{a,CO_2}) and/or thermally induced hyperventilation would alter the ventilatory manifestation of LTF. These uncontrolled influences may also explain differences in the magnitude and duration of LTF in anaesthetized, vagotomized rats (Hayashi *et al.* 1993; Bach & Mitchell, 1996*a*) compared with awake dogs (Cao *et al.* 1992). Alternatively, differences in species, arousal state (anaesthetized *vs.* awake) and presence or absence of vagal reflex mechanisms may account for the altered manifestation of LTF.

The predominant component of increased ventilatory activity in anaesthetized rats during hypoxia-induced LTF is phrenic burst amplitude (Bach & Mitchell, 1996*a*). Similarly, the predominant effect is on tidal volume (V_T) in awake dogs (Cao *et al.* 1992). In anaesthetized rats, breathing frequency (F_R) increases during hypoxic episodes (Hayashi *et al.* 1993), but quickly decreases to below baseline values after the hypoxia ends (post-hypoxic depression; Bach & Mitchell, 1996*b*). F_R then increases progressively to levels above baseline, 15–60 min later (i.e. during LTF; Bach & Mitchell, 1996*a*). This time-dependent F_R response

Table 1. Ventilatory, metabolic and blood gas variables in goats before and after the manifestation of LTF due to episodic hypoxia (Hypox), or during control (Sham) studies

	NI_1		HE_1		N60+	
	Sham	Hypox	Sham	Hypox	Sham	Hypox
$\dot{V}_I$ (l_{BTPS} min^{-1})	8·4 ± 0·7	9·2 ± 0·7	9·4 ± 0·8	19·9 ± 1·4*	7·8 ± 0·7	9·4 ± 1·0
F_R (breaths min^{-1})	17 ± 1	18 ± 1	18 ± 1	28 ± 2*	17 ± 2	22 ± 3
V_T (l_{BTPS})	0·45 ± 0·03	0·47 ± 0·03	0·49 ± 0·04	0·67 ± 0·05*	0·45 ± 0·05	0·40 ± 0·03
V_T/T_I (l_{BTPS} s^{-1})	0·42 ± 0·04	0·47 ± 0·04	0·46 ± 0·05	0·81 ± 0·07*	0·39 ± 0·04	0·47 ± 0·05
T_I/T_{TOT}	0·31 ± 0·01	0·30 ± 0·01	0·32 ± 0·02	0·37 ± 0·01*	0·31 ± 0·01	0·30 ± 0·01
T_I (s)	1·1 ± 0·1	1·1 ± 0·1	1·1 ± 0·1	0·8 ± 0·1*	1·1 ± 0·1	0·9 ± 0·1
T_E (s)	2·6 ± 0·2	2·5 ± 0·2	2·5 ± 0·2	1·5 ± 0·1*	2·6 ± 0·2	2·2 ± 0·3
V_D/V_T	0·54 ± 0·02	0·51 ± 0·04	0·53 ± 0·03	n.m.	0·58 ± 0·03	0·55 ± 0·02
$\dot{V}_{CO_2}$ (l_{STPD} min^{-1})	0·20 ± 0·02	0·22 ± 0·03	0·22 ± 0·02	n.m.	0·17 ± 0·02	0·21 ± 0·02
F_H (beats min^{-1})	80 ± 7	86 ± 9	83 ± 5	107 ± 4*	82 ± 12	75 ± 7
MABP (mmHg)	106 ± 2	96 ± 3*	108 ± 2	104 ± 3	107 ± 4	98 ± 4
T_B (°C)	39·0 ± 0·1	39·1 ± 0·2	39·1 ± 0·2	39·2 ± 0·2	39·7 ± 0·1	39·9 ± 0·2
P_{a,O_2} (mmHg)	98 ± 3	102 ± 2	96 ± 2	48 ± 3*	98 ± 2	105 ± 3
$\Delta P_{a,CO_2}$ (mmHg)	0	0	1 ± 1	−1 ± 1	2 ± 1	0 ± 1
pH	7·42 ± 0·01	7·41 ± 0·01	7·42 ± 0·01	7·41 ± 0·01	7·41 ± 0·01	7·40 ± 0·01
$[HCO_3^-]$ (mequiv l^{-1})	27 ± 1	25 ± 1	27 ± 1	24 ± 1*	27 ± 1	24 ± 1*

NI_1, normoxia interval no. 1; HE_1, hypoxia episode no. 1; and N60+, 60 min after episodic hypoxia. Values are means ± S.E.M., $n = 6$. * Significantly different from sham at same time point ($P < 0·05$). $\dot{V}_I$, inspired minute volume; F_R, breathing frequency; V_T, tidal volume; V_T/T_I, mean inspiratory flow; T_I/T_{TOT}, inspiratory duty cycle; T_I, inspiratory time; T_E, expiratory time; V_D/V_T, physiological dead space; $\dot{V}_{CO_2}$, CO_2 production; F_H, heart rate; MABP, mean arterial blood pressure; T_B, rectal temperature; P_{a,O_2}, O_2 partial pressure; $\Delta P_{a,CO_2}$, change in CO_2 partial pressure; and $[HCO_3^-]$, bicarbonate concentration. BTPS, body temperature and pressure, saturated; STPD, standard temperature and pressure, dry. P_{a,CO_2} during NI_1 was 44 ± 1 mmHg (Hypox) and 43 ± 1 mmHg (Sham), $P > 0·05$. n.m., not measured.

implies that several mechanisms may modify the manifestation of LTF, and that the balance between prevailing mechanisms may change in a time-dependent manner. Although Cao *et al.* (1992) reported that F_R was not significantly different from sham studies in vagally intact, awake dogs, this finding must be viewed with caution since F_R increased markedly during the sham control studies. Thus, it is unclear whether or not LTF will lead to an increase in F_R in awake, vagally intact animals. The distinction between increases in V_T *vs.* F_R has important implications concerning the underlying neural mechanism responsible for LTF.

The objective of this study was to test two hypotheses: (1) that episodic hypoxia elicits LTF which is manifest as an increase in ventilation; and (2) that LTF causes complex changes in respiratory pattern leading to the increased ventilation in awake, vagally intact animals. Preliminary results have been presented as an abstract (Turner & Mitchell, 1995).

METHODS

Six female goats (33–45 kg; 1–3 years old) were familiarized with laboratory procedures and with wearing a tight-fitting respiratory mask whilst standing quietly on a motor-driven treadmill (model Q65; Quinton, Seattle, WA, USA). All measures were taken to ensure the animals did not suffer distress at any time and the study was approved by a local animal care committee. In each goat, a translocated carotid artery allowed repeated transcutaneous placement of a catheter to sample arterial blood. Artery translocation was performed at least 4 weeks before experiments under halothane anaesthesia (1–3%). Goats were fasted overnight before a study.

Measurements

Arterial blood samples (0·8 ml) were drawn into heparinized 1 ml tuberculin syringes. The samples were capped and stored on ice (< 30 min) until they were analysed for arterial oxygen and carbon dioxide partial pressures (P_{a,O_2} and P_{a,CO_2}) and pH by an automated blood gas analyser (ABL 330; Radiometer, Copenhagen, Denmark). All values were corrected to the rectal temperature of the animal (T_B; YSI temperature probe, model 401, Yellow Springs, OH, USA) and with reference to the goat's own blood, tonometered with three gas mixtures of known composition on each experimental day.

A respiratory mask, fitted with a non-rebreathing valve (Series 2600; Hans Rudolph, Kansas City, MO, USA) was used to measure ventilatory variables. A pneumotachograph (Fleisch no. 2, Zurich, Switzerland) was attached to the inspiratory side of the non-rebreathing valve and pressure differences across the pneumotachograph were measured with a variable reluctance differential pressure transducer (MP-45; Validyne, Northridge, CA, USA) excited by a carrier demodulator (CD15; Validyne). The pressure signal (proportional to flow) was integrated to obtain the tidal volume (V_T; Gould Instruments, Cleveland, OH, USA). Inspired minute volume ($\dot{V}_I$) was calculated by multiplying V_T and breathing

frequency (F_R) for each breath. Heart rate (F_H) and mean arterial blood pressure (MABP) were calculated from the arterial pulse pressure measured with a blood pressure transducer (Statham P-23id, Gould Instruments, Oxnard, CA, USA) and displayed on a pen recorder (Gould). End-tidal, inspired and mixed expired carbon dioxide fractions (F_{ET,CO_2}, F_{I,CO_2} and F_{E,CO_2}, respectively) and the inspired oxygen fraction (F_{I,O_2}) were monitored by rapid gas analysers (Sensormedics LB-2, Anaheim, CA, USA; Amatek S-3A, Sunnyvale, CA, USA). Carbon dioxide production ($\dot{V}_{CO_2}$) was calculated as the product of F_{E,CO_2} and $\dot{V}_I$ (see Mitchell, 1990). Other variables such as the inspiratory (T_I) and expiratory (T_E) times, physiological dead space (V_D/V_T from the Bohr equation using P_{a,CO_2} values), mean inspiratory flow (V_T/T_I) and the inspiratory duty cycle (T_I/T_{TOT}, where T_{TOT} is the total breath time) were also calculated with an on-line computer acquisition system designed in our laboratory. $\dot{V}_{CO_2}$ and V_D/V_T were not calculated when F_{I,CO_2} was greater than 0·03.

Experimental protocol

Each goat participated in two protocols. In the first, goats were subjected to episodic, isocapnic hypoxia (Hypox). Initially, goats stood quietly, breathing ambient air for 10–15 min; at the end of this time, baseline measurements of ventilatory, blood gas and metabolic variables were made (normoxia interval no. 1; NI_1). F_{I,CO_2} was adjusted continuously throughout the following protocol to maintain isocapnia with respect to the baseline P_{a,CO_2} and F_{ET,CO_2}. The inspired gas was switched to a hypoxic gas mixture ($F_{I,O_2} = 0·09–0·10$, balance nitrogen) for 3 min. Ventilatory measurements were made and a blood sample was taken during the final minute (hypoxia episode no. 1; HE_1). At the end of HE_1, the inspired gas was switched back to ambient air for 5 min and ventilatory measurements and blood sampling were performed during the last minute of this normoxic interval (NI_2). This cycle of isocapnic normoxia and hypoxia was repeated ten times (i.e. until NI_{10}/HE_{10}). Following HE_{10}, it was necessary to maintain F_{I,CO_2} above zero for 20 min to achieve isocapnia and thereafter, F_{I,CO_2} was returned to zero. Ventilatory and blood gas measurements were made at 5, 20, 40 and 60 min after episodic hypoxia had ended (N5+, N20+, N40+ and N60+, respectively). At least 2 weeks later, each goat performed a similar protocol, in which episodic hypoxia was replaced by room air breathing (sham). The sham protocol served as a control for time-dependent factors not associated with episodic hypoxia.

Statistics

Data are presented as means ± 1 S.E.M. Student's paired t tests were used to identify significant differences between Hypox and sham means at different time points. Bonferroni corrections were made to account for multiple comparisons, and significance was set at an overall $P < 0·05$ level.

RESULTS

There were no significant differences between variables during NI_1 in Hypox *vs.* sham protocols, with the exception of MABP (Table 1). During HE_1, hypoxia elicited a number of cardiorespiratory responses compared with sham, most prominently in $\dot{V}_I$, F_R, V_T, V_T/T_I and F_H, all of which increased. By N60+, all Hypox variables were once again

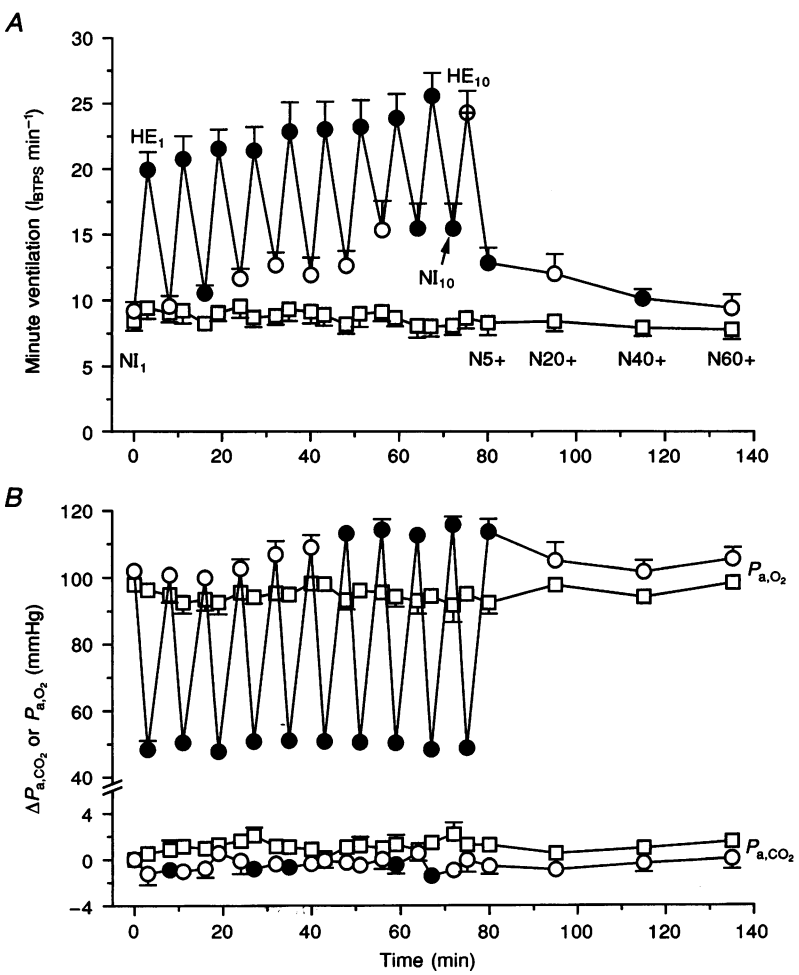

Figure 1. The effect of episodic hypoxia on ventilation and blood gases

Minute ventilation (measured at body temperature and pressure, saturated; BTPS) (*A*), and arterial oxygen tension (P_{a,O_2}) and the change in arterial carbon dioxide tension ($\Delta P_{a,CO_2}$) (*B*) from the first normoxic episode (NI_1) during and after episodic hypoxia (○) or continuous normoxia (sham, □). N5+, N20+, N40+ and N60+ represent normoxic time points after the tenth hypoxic episode (HE_{10}). Filled symbols indicate values significantly different from sham at the same time point ($P < 0·05$); ⊕, significantly different from first hypoxic episode (HE_1; $P < 0·05$). Values are means ± S.E.M.; missing error bars are within the symbol.

similar to sham values, with the exception of bicarbonate concentration ($[HCO_3^-]$), which was slightly lower during the Hypox protocol.

Repeated, episodic isocapnic hypoxia led to a progressive increase in $\dot{V}_I$ among the normoxic intervals which lasted for up to 40 min after the last hypoxic episode (Fig. 1*A*), thus providing evidence for LTF. The precise end-point of LTF is difficult to establish from the present results. Nevertheless, $\dot{V}_I$ was significantly elevated above sham values at N40+, but not at N60+, suggesting that the duration of LTF was between 40 and 60 min. The progressive increase in $\dot{V}_I$ among the isocapnic, normoxic intervals between hypoxic episodes (i.e. NI_1–NI_{10}) attained statistical significance by NI_9. This progressive increase in normoxic $\dot{V}_I$ presumably reflects the development of LTF. The development of LTF was accompanied by an increase in P_{a,O_2} among normoxic intervals. There was a tendency (although not significant) for $\dot{V}_I$ among normoxic intervals to begin to decrease after ten hypoxic episodes (e.g. NI_{10} *vs.* N5+; Fig. 1*A*). This suggests that the present protocol may have 'captured' the full expression of LTF in awake goats (i.e. approximately 70% increase in $\dot{V}_I$; NI_{10} *vs.* NI_1).

Although the change in ventilation in response to the same level of isocapnic hypoxia ($P_{a,O_2} = 47$ mmHg) was similar when compared as HE_1–NI_1 *vs.* HE_{10}–NI_{10}, $\dot{V}_I$ was significantly greater during HE_{10} *vs.* HE_1 (Fig. 1*A*). The greater $\dot{V}_I$ during hypoxia therefore appears to be the consequence of an augmented initial $\dot{V}_I$ (i.e. LTF).

The maintenance of isocapnia throughout the protocol is documented in Fig. 1*B*. P_{a,CO_2} was significantly different from its value during NI_1 at only five out of twenty-four time points throughout the whole protocol. When there was an error in P_{a,CO_2} regulation it was small and towards relative hypocapnia.

The progressive and persistent increase of $\dot{V}_I$ was brought about by significant increases in F_R, V_T/T_I and V_T at NI_{10}, whereas a significant increase in only F_R was predominantly responsible for the elevated $\dot{V}_I$ between N5+ and N40+ (Fig. 2).

There were no significant changes (compared with NI_1) in any ventilatory variable during the sham protocol. Furthermore, there were no significant differences between Hypox and sham protocols in F_H, MABP, T_B, pH, $\dot{V}_{CO_2}$ and V_D/V_T

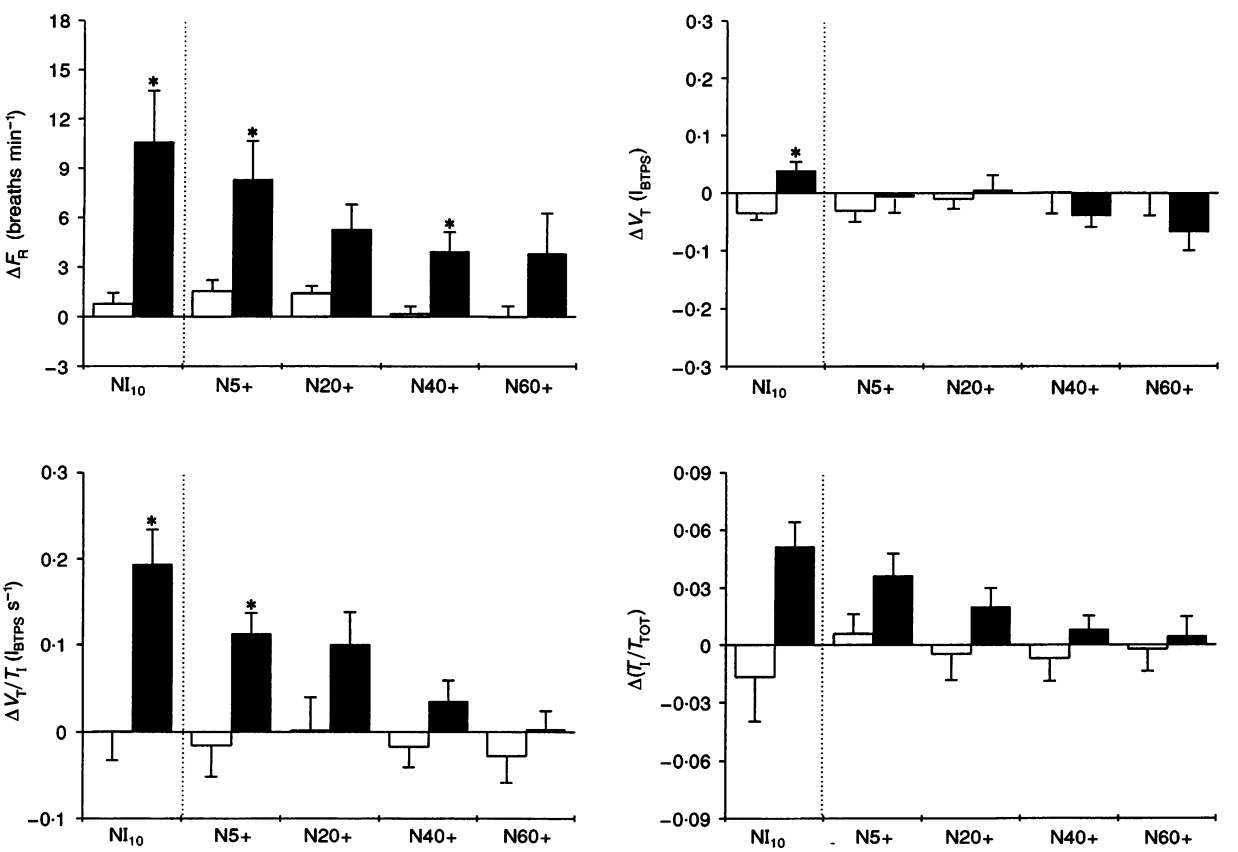

Figure 2. The effect of episodic hypoxia on breathing pattern

Changes (Δ) from the NI_1 value in breathing frequency (F_R), tidal volume (V_T), mean inspiratory flow (V_T/T_I) and inspiratory duty cycle (T_I/T_{TOT}), during (NI_{10}) and after (N5+, N20+, N40+ and N60+) episodic hypoxia (■) or continuous normoxia (sham, □). * Significantly different from sham value at same time point ($P < 0.05$).

following HE_{10} (Fig. 3 and Table 1). There was no evidence of a persistent change in cardiovascular variables during or following hypoxia (i.e. analogous to LTF).

DISCUSSION

Episodic hypoxia led to a progressive and persistent increase of ventilation during normoxia, thus demonstrating long-term facilitation (LTF) in awake, vagally intact goats (see McCrimmon, Mitchell & Dekin, 1995). The increase in $\dot{V}_I$ caused by LTF was modest and was mainly the result of increases in F_R, with lesser increases in V_T and V_T/T_I.

LTF of $\dot{V}_I$ in awake, vagally intact animals

This study and the study of Cao *et al.* (1992) on tracheostomized, recumbent dogs are the only two reports currently available on awake non-human mammals. The manifestation of LTF in goats is predominantly due to increased F_R with only minor increases in V_T or V_T/T_I. In contrast, dogs exhibit primarily an augmented V_T with no statistical change in F_R from sham values (Cao *et al.* 1992). Recently, LTF was shown to occur (Stewart, Berthon-Jones, McNamara, Grunstein & Sullivan, 1994), but also not to occur in humans (McAvoy, Popovic, Saunders & White, 1996). These observations suggest species differences or different degrees of precision in controlling blood gases and body temperature.

In the present study, P_{a,CO_2} was measured and controlled throughout the experimental protocol. We intentionally avoided (slight) hypercapnia since it would have rendered a distinction between LTF-mediated hyperpnoea and hypercapnic hyperpnoea difficult. Based on CO_2–response curves measured in preliminary studies in these goats ($\Delta\dot{V}_I/\Delta P_{a,CO_2}$ was $4{\cdot}7 \pm 1{\cdot}1$ $l_{BTPS}\,min^{-1}\,mmHg^{-1}$ in normoxia and $6{\cdot}9 \pm 0{\cdot}9$ $l_{BTPS}\,min^{-1}\,mmHg^{-1}$ in hypoxia, $n = 6$), even a 1 mmHg relative hypercapnia could have accounted for most, if not all of the observed increase in $\dot{V}_I$. In fact, any error in maintaining isocapnia was a non-significant tendency towards hypocapnia in this study. In other time-dependent processes, such as short-term potentiation (STP), hypocapnia can attenuate and shorten the apparent duration of augmented ventilation following hypoxia in the goat (Engwall, Daristotle, Niu, Dempsey & Bisgard, 1991). Thus, LTF may have been slightly underestimated in the present study, although it was clearly larger and lasted significantly longer than STP as reported in goats (LTF > 40 min *vs.* STP < 2 min; Engwall *et al.* 1991).

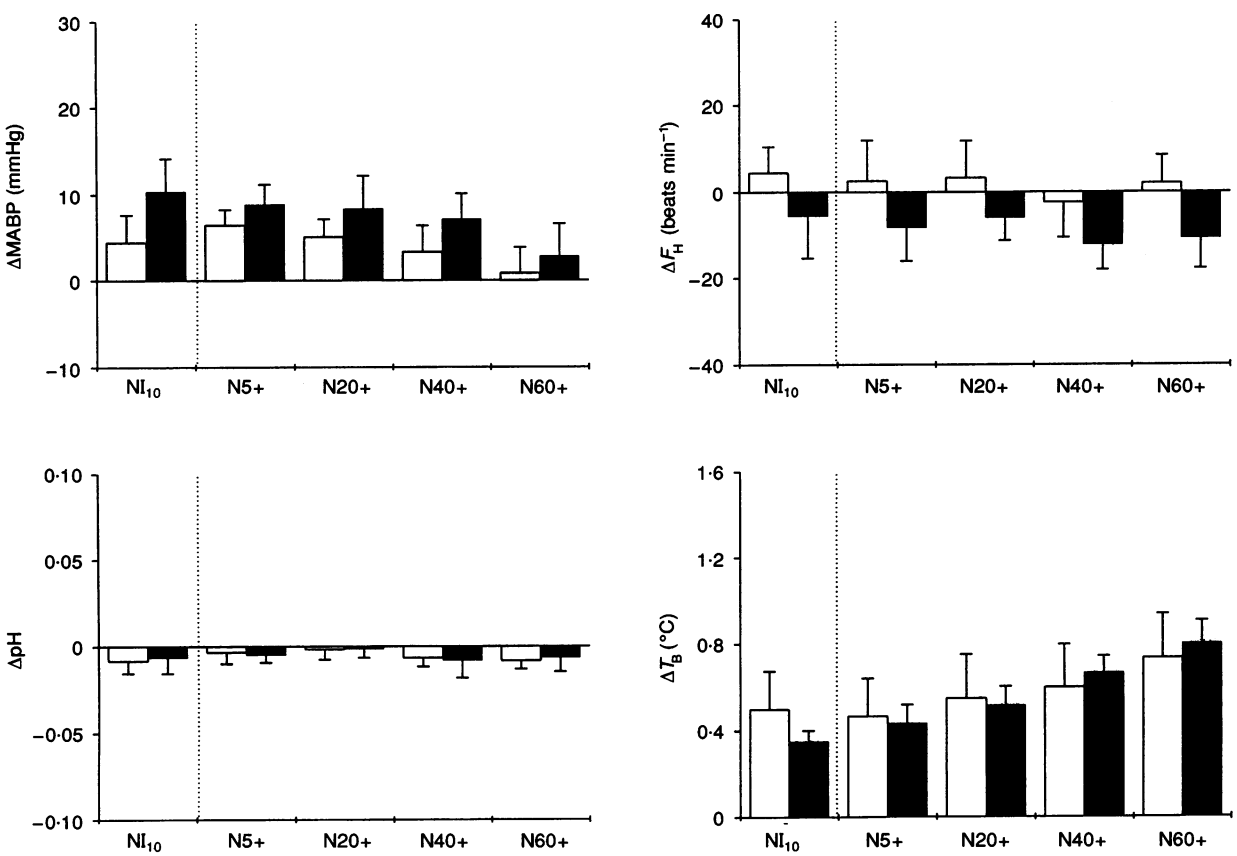

Figure 3. The effect of episodic hypoxia on cardiovascular and other variables

Changes (Δ) from NI_1 value in heart rate (F_H), mean arterial blood pressure (MABP), pH and body temperature (T_B), during (NI_{10}) and after (N5+, N20+, N40+ and N60+) episodic hypoxia (■) or control normoxia (sham, □). There were no significant differences in any variable (hypoxia *vs.* sham).

Magnitude of LTF

LTF seems to be less robust (in terms of duration) in awake, vagally intact goats (40 min) than in anaesthetized, vagotomized rats, where LTF has been demonstrated to last at least 1 h after episodic hypoxia (e.g. Bach & Mitchell, 1996*a*). The reasons for this difference in the duration of LTF are unknown, but procedural factors, such as hypo/hypercapnia or baseline raphe neural activity, may be responsible (see below). Alternatively, there may be other, counteracting mechanisms that attenuate respiratory drive and ventilatory manifestation of LTF in goats. Firstly, LTF is significantly attenuated in anaesthetized cats which have intact vagi compared with anaesthetized, vagotomized cats (Mateika & Fregosi, 1996). Secondly, the tendency for $\dot{V}_I$ to peak at NI_{10} and, if anything, begin to decrease at N5+ in awake goats may indicate that additional hypoxic exposures would induce inhibitory mechanisms that counteract the facilitatory effects of episodic hypoxia on $\dot{V}_I$. Intracarotid infusions of both noradrenaline (NA) and substance P (SP) inhibit $\dot{V}_I$ during continuous hypoxia in awake goats (see Ryan, Hedrick, Pizarro & Bisgard, 1993; Pizarro, Ryan, Hedrick, Xue, Keith & Bisgard, 1995). Thus, NA and SP release may reduce the ventilatory manifestation of LTF after a critical duration of hypoxia.

The critical number of hypoxic episodes necessary to elicit LTF in awake or anaesthetized animals is not known at present. However, the critical number appears to be lower in the anaesthetized rat (three 5 min episodes; Hayashi *et al.* 1993; Bach & Mitchell, 1996*a*) than in the awake goat (at least eight 3 min episodes in this study). It is also not presently known what decrease in P_{a,O_2} is necessary to elicit LTF in awake or anaesthetized animals. This may be a critical issue since a lower level of P_{a,O_2} may not only enhance LTF, but also other inhibitory mechanisms which may modify respiratory muscle activity and $\dot{V}_I$. For example, in humans, phasic genioglossus electrical activity was markedly depressed, whereas diaphragmatic electrical activity and $\dot{V}_I$ showed two distinct phases of augmentation following an episodic hypoxia protocol similar to that used in this study (ten 2 min hypoxic episodes; oxygen saturation during hypoxia, 80–84%; McAvoy *et al.* 1996).

Comparison of the effects of continuous *vs.* episodic hypoxia

The neural mechanism for LTF appears to be different from that occurring during ventilatory acclimatization to continuous hypoxia (VAH). While serotonin depletion with para-chlorophenylalanine attenuates stimulation-induced LTF in anaesthetized cats (Millhorn *et al.* 1980), VAH is neither attenuated nor delayed by similar treatment in awake rats (Olson, 1987). Furthermore, VAH is not accompanied by a post-hypoxic ventilatory augmentation in isocapnic, awake goats (Engwall & Bisgard, 1990; Ryan, Hedrick & Bisgard, 1995). Recently, 30 min of continuous, isocapnic hypoxia was shown not to induce LTF in goats (Dwinell, Janssen & Bisgard, 1996), in contrast to episodic hypoxia (i.e. ten 3 min hypoxic episodes in this study). The reasons for these differences in the expression of LTF and VAH may lie in the nature of peripheral chemoreceptor afferent fibre coding of episodic *vs.* continuous hypoxia and/or the possibility of differences in central neural integration (McCrimmon *et al.* 1995).

Respiratory pattern during LTF

The complexity of ventilatory responses to episodic hypoxia implies that neural mechanisms regulating both rhythmogenesis (i.e. timing or F_R) and burst pattern formation (i.e. V_T and V_T/T_I) are operative during LTF in the awake, vagally intact animal. It could be predicted that awake animals with intact vagi would express LTF with a shift towards an increase in F_R as a consequence of feedback sensing an increased V_T or V_T/T_I (thereby shortening T_I). However, in this study, T_I was not shortened significantly (Hypox *vs.* sham at NI_{10}, $0{\cdot}8 \pm 0{\cdot}1$ *vs.* $1{\cdot}0 \pm 0{\cdot}1$ s; $P > 0{\cdot}05$), but T_E was (Hypox *vs.* sham at NI_{10}, $1{\cdot}6 \pm 0{\cdot}3$ *vs.* $2{\cdot}5 \pm 0{\cdot}2$ s; $P < 0{\cdot}05$).

Anatomical and physiological evidence suggests that serotonergic raphe projections innervate both brainstem and spinal neurons (see below), possibly accounting for effects on both rhythm generation and pattern formation during LTF. Indeed, increases in both frequency and phrenic amplitude during LTF in the anaesthetized rat are attenuated by methysergide, a serotonergic receptor antagonist (Bach & Mitchell, 1996*a*).

Neural mechanisms responsible for LTF: awake *vs.* anaesthetized

Physiological and anatomical evidence suggests that serotonergic raphe neurons play an important role in the neural mechanism of LTF (see McCrimmon *et al.* 1995). Episodic hypoxia-induced activity in, or electrostimulation of, carotid body afferent nerve fibres, results in c-*fos* expression in raphe nuclei of both anaesthetized and awake rats (Erickson & Millhorn, 1991). Furthermore, a persistent (1000 s) increase in raphe neuronal activity has been observed in cats during and following repeated carotid body activation (Morris, Arata, Shannon & Lindsey, 1996). Stimulation of raphe serotonergic neurons elicits LTF leading to an increase in phrenic nerve activity (Millhorn, 1986) and release of serotonin in the cervical ventral horn (Brodin *et al.* 1990). Both raphe-spinal projections to respiratory motoneurons, and serotonin-containing nerve terminals near phrenic motoneurons have been demonstrated in the cat (Holtman, Vascik & Maley, 1990; Pilowsky, de Castro, Llewellyn-Smith, Lipski & Voss, 1990). In addition, there are neural connections between raphe nuclei and brainstem respiratory groups (Voss, de Castro, Lipski, Pilowsky & Jiang, 1990). Finally, hypoxia-induced LTF leading to increased phrenic and hypoglossal nerve activity is abolished by pretreatment with systemic methysergide in the anaesthetized rat and cat (Millhorn *et al.* 1980; Bach &

Mitchell, 1996*a*). Collectively, this evidence supports an important role for raphe serotonergic neurons in the mechanism of LTF in anaesthetized animals.

It is likely that LTF in awake animals will also be dependent on serotonergic pathways. However, differences in the basal serotonergic neuronal activity may explain why the peak magnitude and duration of changes in ventilation in the awake goat (+70%, 40 min) and dog (+40%; Cao *et al.* 1992) are somewhat lower than that recorded in anaesthetized rats (+70 to +100%, > 60 min; Hayashi *et al.* 1993; Bach & Mitchell, 1996*a*). Anaesthesia lowers the activity of serotonergic neurons in brainstem raphe nuclei (Grahn & Heller, 1989) in a similar fashion to sleep. For example, the average firing rate of raphe neurons in cats decreases from $5{\cdot}5 \pm 0{\cdot}5$ spikes s^{-1} during alert waking to $0{\cdot}6 \pm 0{\cdot}1$ spikes s^{-1} during rapid eye movement sleep (Veasey, Fornal, Metzler & Jacobs, 1995). The peak firing rate of raphe neurons in the awake cat is approximately 12 spikes s^{-1} in response to hypercapnia and locomotion, a 2-fold increase that is correlated with increases in $\dot{V}_I$ (Veasey *et al.* 1995). Consequently, if the raphe nuclei are stimulated by episodic hypoxia during anaesthesia when their basal firing rate is low, the potential range of augmentation of raphe activity and therefore $\dot{V}_I$ (i.e. LTF) would be greater than in the awake state.

Bach, K. B. & Mitchell, G. S. (1996*a*). Hypoxia induced long term facilitation of respiratory activity is serotonin dependent. *Respiration Physiology* **104**, 251–260.

Bach, K. B. & Mitchell, G. S. (1996*b*). Post-hypoxic depression of phrenic burst frequency in rats requires alpha-2 adrenergic receptors. *The Physiologist* **39**, A185.

Brodin, E., Linderoth, B., Goiny, M., Yamamoto, Y., Gazelius, B., Millhorn, D. E., Hokfelt, T. & Ungerstedt, U. (1990). *In vivo* release of serotonin in cat dorsal vagal complex and cervical ventral horn induced by electrical stimulation of the medullary raphe nuclei. *Brain Research* **535**, 227–236.

Cao, K.-Y., Zwillich, C. W., Berthon-Jones, M. & Sullivan, C. E. (1992). Increased normoxic ventilation induced by repetitive hypoxia in conscious dogs. *Journal of Applied Physiology* **73**, 2083–2088.

Dwinell, M. R., Janssen, P. L. & Bisgard, G. E. (1996). Do awake goats exhibit long term facilitation and roll-off with continuous isocapnic hypoxia? *The Physiologist* **39**, A186.

Eldridge, F. L. & Millhorn, D. E. (1986). Oscillation, gating and memory in the respiratory control system. In *Handbook of Physiology*, section 3, *The Respiratory System*, vol. 2, ed. Cherniack, N. S. & Widdicombe, J. G., pp. 93–114. American Physiological Society, Bethesda, MD, USA.

Engwall, M. J. A. & Bisgard, G. E. (1990). Ventilatory responses to chemoreceptor stimulation after hypoxic acclimatization in awake goats. *Journal of Applied Physiology* **69**, 1236–1243.

Engwall, M. J. A., Daristotle, L., Niu, W. Z., Dempsey, J. A. & Bisgard, G. E. (1991). Ventilatory afterdischarge in the awake goat. *Journal of Applied Physiology* **71**, 1511–1517.

Erickson, J. T. & Millhorn, D. E. (1991). Fos-like protein is induced in neurons of the medulla oblongata after stimulation of the carotid sinus nerve in awake and anesthetized rats. *Brain Research* **567**, 11–24.

Grahn, D. A. & Heller, H. C. (1989). Activity of most rostral ventromedial medulla neurons reflect EEG/EMG pattern changes. *American Journal of Physiology* **257**, R1496–1505.

Hayashi, F., Coles, S. K., Bach, K. B., Mitchell, G. S. & McCrimmon, D. R. (1993). Time-dependent phrenic nerve response to carotid afferent stimulation: intact *vs.* decerebellate rats. *American Journal of Physiology* **265**, R811–819.

Holtman, J. R., Vascik, D. S. & Maley, B. E. (1990). Ultrastructural evidence for serotonin-immunoreactive terminals contacting phrenic motoneurons in the cat. *Experimental Neurology* **109**, 269–272.

McAvoy, R. D., Popovic, R. M., Saunders, N. A. & White, D. P. (1996). Effects of sustained and repetitive isocapnic hypoxia on ventilation and genioglossal and diaphragmatic EMGs. *Journal of Applied Physiology* **81**, 866–875.

McCrimmon, D. R., Mitchell, G. S. & Dekin, M. S. (1995). Glutamate, GABA and serotonin in ventilatory control. In *Regulation of Breathing*, ed. Dempsey, J. A. & Pack, A. I., pp. 151–218. Marcel Dekker Inc., New York, USA.

Mateika, J. H. & Fregosi, R. F. (1996). Long term facilitation of upper respiratory muscle activity in the vagotomized and vagally intact cat. *The Physiologist* **39**, A186.

Millhorn, D. E. (1986). Stimulation of raphe (obscurus) nucleus causes long-term potentiation of phrenic nerve activity in cat. *Journal of Physiology* **381**, 169–179.

Millhorn, D. E., Eldridge, F. L. & Waldrop, T. G. (1980). Prolonged stimulation of respiration by endogenous central serotonin. *Respiration Physiology* **42**, 171–188.

Mitchell, G. S. (1990). Ventilatory control during exercise with increased respiratory dead space in goats. *Journal of Applied Physiology* **69**, 718–727.

Morris, K. F., Arata, A., Shannon, R. & Lindsey, B. G. (1996). Long-term facilitation of phrenic activity in cats: responses and short time scale correlations of medullary neurones. *Journal of Physiology* **490**, 463–480.

Olson, E. B. (1987). Ventilatory adaptation to hypoxia occurs in serotonin-depleted rats. *Respiration Physiology* **69**, 227–235.

Pilowsky, P. M., de Castro, D., Llewellyn-Smith, I., Lipski, J. & Voss, M. D. (1990). Serotonin immunoreactive boutons make synapses with feline phrenic motoneurons. *Journal of Neuroscience* **10**, 1091–1098.

Pizarro, J., Ryan, M. L., Hedrick, M. S., Xue, D. H., Keith, I. M. & Bisgard, G. E. (1995). Intracarotid Substance P infusion inhibits ventilation in the goat. *Respiration Physiology* **101**, 11–22.

Ryan, M. L., Hedrick, M. S. & Bisgard, G. E. (1995). Lack of long term facilitation in ventilation after acclimatization to hypoxia in awake goats. *FASEB Journal* **9**, A566.

Ryan, M. L., Hedrick, M. S., Pizarro, J. & Bisgard, G. E. (1993). Carotid body noradrenergic sensitivity in ventilatory acclimatization to hypoxia. *Respiration Physiology* **92**, 77–90.

Stewart, D., Berthon-Jones, M., McNamara, S., Grunstein, R. & Sullivan, C. (1994). Ventilatory responses to repetitive hypoxia in normal males. *American Journal of Respiratory and Critical Care Medicine* **149**, A260.

Turner, D. L. & Mitchell, G. S. (1995). Long-term facilitation of ventilation following episodic hypoxia in awake goats. *Journal of Physiology* **483.P**, 91*P*.

Veasey, S. C., Fornal, C. A., Metzler, C. W. & Jacobs, B. L. (1995). Response of serotonergic caudal raphe neurons in relation to specific motor activities in freely moving cats. *Journal of Neuroscience* **15**, 5346–5359.

Voss, M. D., de Castro, D., Lipski, J., Pilowsky, P. M. & Jiang, C. (1990). Serotonin immunoreactive boutons form close appositions with respiratory neurons of the dorsal respiratory group in the cat. *Journal of Comparative Neurology* **295**, 208–218.

Acknowledgements

This study was funded by NIH (HL36780). Steve Bartholow and Michele Rosenshield offered valuable technical assistance.

Author's email address

D. L. Turner: d.turner@leeds.ac.uk

Received 30 September 1996; accepted 18 November 1996.