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EFFECTS OF SPECIFIC CAROTID BODY AND BRAIN HYPOXIA ON RESPIRATORY MUSCLE CONTROL IN THE AWAKE GOAT

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SUMMARY

1. We assessed the effects of specific brain hypoxia on the control of inspiratory and expiratory muscle electromyographic (EMG) activities in response to specific carotid body hypoxia in seven awake goats. We used an isolated carotid body perfusion technique that permitted specific, physiological, steady-state stimulation of the carotid bodies or maintenance of normoxia and normocapnia at the carotid bodies while varying the level of systemic, and therefore, brain oxygenation.

2. Isolated brain normocapnic hypoxia of up to 1·5 h duration increased inspired minute ventilation ($\dot{V}_I$) by means of increases in both tidal volume (V_T) and respiratory frequency (f_R). Electromyographic activities of both inspiratory and expiratory muscles were augmented as well. These responses were similar to those produced by low levels of whole-body normoxic hypercapnia. We conclude that moderate levels of brain hypoxia ($P_{a,O_2} \approx 40$ mmHg) in awake goats caused a net stimulation of ventilatory motor output.

3. Hypoxic stimulation of the carotid bodies alone caused comparable increases in V_T and f_R, and EMG augmentation of both inspiratory and expiratory muscles whether the brain was hypoxic or normoxic. These responses were quite similar to those obtained over a wide range of whole-body normoxic hypercapnia. We conclude that the integration of carotid body afferent information is not affected by moderate brain hypoxia in awake goats.

4. We found no evidence for an asymmetrical recruitment pattern of inspiratory *vs.* expiratory muscles in response to carotid body hypoxia or in response to brain hypoxia alone.

5. Our data support the concept that moderate brain hypoxia results in a net stimulation of respiratory motor output. These findings question the significance of 'central hypoxic depression' to the regulation of breathing under physiological levels of hypoxaemia in the awake animal.

INTRODUCTION

Whole-body hypoxia is believed to have both stimulatory and inhibitory effects on respiratory motor output. Hyperventilation prevails presumably because of the

dominance of carotid body chemoreceptor stimulation, but it is commonly supposed that this stimulation is opposed by a direct depression of ventilatory output by brain hypoxia (Dumke, Schmidt & Chiodi, 1941; Neubauer, Melton & Edelman, 1990). The 'roll-off' of the ventilatory response following acute exposure to hypoxia (Huang *et al.* 1984) and the reduction or elimination of the short-term potentiation of ventilation following a few minutes of hypoxia (Georgopoulos, Bshouty, Younes & Anthonisen, 1990) have been suggested as manifestations of hypoxic depression of the brain. This depressant effect of brain hypoxia has been demonstrated in anaesthetized animals in the absence of peripheral chemoreceptor stimulation, accomplished either by means of carotid body denervation in conjunction with hypoxic hypoxia or CO inhalation in intact animals (Dumke *et al.* 1941; Melton, Neubauer & Edelman, 1988; Yu, Neubauer, Melton, Wasicko, Li, Krawciw & Edelman, 1988). However, in the awake animal brain hypoxaemia either does not decrease, or only transiently decreases, ventilatory output; to the contrary, minute ventilation is usually increased in the steady state. Tachypnoea is always a feature of this hyperventilation but tidal volume is frequently increased as well (Moyer & Beecher, 1942; Watt, Dumke & Comroe, 1943; Davenport, Brewer, Chambers & Goldschmidt, 1947; Tenney & Brooks, 1966; Santiago & Edelman, 1976; Bouverot, Candas & Libert, 1973; Miller & Tenney, 1975; Gautier & Bonora, 1983; Daristotle, Engwall, Niu & Bisgard, 1991).

It is also commonly accepted that when ventilation is stimulated by hypoxia expiratory muscles are either frankly inhibited, or are augmented substantially less than they would be with a hypercapnic stimulus, for a given level of ventilation. This selective reduction of respiratory muscle activity has been attributed to a preferential recruitment of inspiratory motor neurons via carotid body stimulation or to a selective depressant effect of brain hypoxia (St John, 1981; Sears, Berger & Phillipson, 1982).

Our study was designed to determine the effects of specific brain hypoxia on the control of inspiratory and expiratory muscle electromyographic (EMG) activities in response to specific carotid chemoreceptor stimulation in the awake animal. Our approach was to utilize the technique of isolated carotid body perfusion in the awake goat (Busch, Bisgard & Forster, 1985). This technique allowed us to stimulate the carotid bodies of unanaesthetized goats with a physiological stimulus (normocapnic hypoxia) in the steady state, or maintain the carotid bodies in a normoxic and normocapnic state, while varying the level of brain oxygenation.

METHODS

Seven unanaesthetized goats were studied, three females and four castrated males (mean weight $46{\cdot}1 \pm 1{\cdot}3$ kg). At least 2 weeks prior to study each goat was equipped with bipolar EMG electrodes in three inspiratory muscles: crural diaphragm (CR), costal diaphragm (CO), parasternal intercostal (PS); and two expiratory muscles: triangularis sterni (TS) and transversus abdominis (TA). This technique has been described in detail previously (Smith, Ainsworth, Henderson & Dempsey, 1989). At the same time the initial surgery to prepare the goat for carotid body perfusion was performed. This technique has been described in detail previously (Busch *et al.* 1985). The arterial supply to the brain of the goat allows surgical rearrangement for carrying out unilateral carotid body perfusion with blood of independent gas tensions and pH from that perfusing the systemic arterial system, including the brain. The initial phase was to ligate the internal maxillary and lingual arteries on one side (carotid body perfusion side) and, contralaterally, excise the carotid

body and ligate the occipital artery (brain perfusion side). After a minimum of 1 week of recovery and at least 1 week before study the second phase was completed on the carotid body intact side. This procedure included insertion of a polyvinyl catheter into the proximal common carotid artery for subsequent blood sampling and systemic blood pressure measurement. Silastic cannulae were placed in the distal common carotid artery for carotid body perfusion and right atrium via the external jugular vein for drawing blood into the extracorporeal circuit. An arteriovenous shunt was formed by connecting these two cannulae, so that during the non-study period blood flowed from the ipsilateral occipital artery, through the upper segment of the carotid artery and returned via the externalized shunt to the right atrium. Sodium heparin (40000 U subcutaneously) was administered daily following this procedure. During experiments the extracorporeal perfusion circuit drew blood from the venous cannula into a reservoir (Medtronic, USA) by means of one head of a perfusion pump (Travenol, USA). Blood was pumped from the reservoir by a second pump head through an oxygenator (Medtronic, USA) and blood filter (Medtronic, USA) and into the carotid artery perfusion cannula. Blood was sampled from the perfusion cannula and the gas tensions were controlled by changing the relative concentration of O_2, CO_2, and N_2 flowing through the oxygenator. Perfusion pressure was measured using a pressure transducer connected to a T-piece located just proximal to the carotid artery cannula. Perfusion pressure was maintained 15–20 mmHg above systemic arterial pressure. The aortic bodies were left intact as it has been shown (Weizhen, Engwall, Daristotle, Pizarro & Bisgard, 1992) that maximal stimulation of these receptors in goats did not elicit a significant ventilatory response. All surgery was performed under general anaesthesia (approximately 1% halothane, 30% nitrous oxide, balance oxygen) using sterile technique. Paralysing agents were never used. Appropriate analgesics and antibiotics were administered post-operatively.

The effectiveness of vascular isolation with this technique has been described in detail by Busch *et al.* (1985) who used microspheres, polymer arterial casts, and cerebral angiography as well as physiological criteria. On the day of an experiment in the present study the presence of a functional carotid body on the carotid body perfusion side was confirmed by close arterial injection of NaCN (100 μg) on that side. Removal of the carotid body on the brain perfusion side, and functional isolation of the two sites, was confirmed by a lack of a ventilatory response to intravenous injection of NaCN (50 μg/kg) during perfusion of the carotid body side by means of the extracorporeal circuit. Engwall, Daristotle, Niu & Bisgard (1991) used normoxic and normocapnic carotid body perfusion to show that carotid body perfusion *per se* and/or the sudden switching between systemic carotid body perfusion and extracorporeal carotid body perfusion and extracorporeal carotid body perfusion had no effects on ventilation and only small effects (< 5 mmHg) on systemic blood pressure.

During an experiment raw EMGs were amplified (bandpass 50–500 Hz) and recorded on analog tape for subsequent analysis. The signals on the analog tape were played back through amplifiers, rectified, and moving time averaged (100 ms time constant). The moving time averaged signals passed through an A–D convertor and were acquired and stored on an AT class personal computer using custom-written software. EMGs were calculated breath-by-breath as mean electrical activities (area of the moving time averaged signal/duration of electrical activity; Ledlie, Pack & Fishman, 1983) and were normalized to the response achieved in each goat to breathing an inspired fraction of CO_2 (F_{I,CO_2}) of 0·065 ('percentage of reference'). The goats were always studied in the standing position.

The goats wore tight-fitting muzzle masks equipped with one-way valves. Inspired air flow was measured with a pneumotachograph calibrated daily with a chain-compensated spirometer. This signal was also recorded on analog tape for subsequent computer analysis.

Systemic arterial and extracorporeal circuit blood gases and pH were measured at appropriate points throughout the experiment (see Protocol). The blood gas machine was validated repeatedly throughout the experiment with blood tonometered with a known gas mixture at a known temperature. The blood gas values were corrected to the goat's rectal temperature.

Systemic arterial and extracorporeal circuit pressures were monitored continuously throughout the experiment as were end-tidal P_{CO_2} and P_{O_2}.

Our experimental and surgical protocols were approved by the University of Wisconsin-Madison institutional animal care and use committee.

Linear regression was performed with the least-squares method. Differences between slopes were determined by means of an F test. Differences between means were determined with a t test. Differences were considered significant if $P < 0·05$.

Protocol

Brain normoxia

In this condition the systemic circulation (and hence brain) was maintained normoxic and normocapnic throughout. In this report, for clarity, we have termed this condition 'brain normoxia' but it should be understood that the normoxia was systemic except for the carotid body circulation when it was perfused with hypoxic blood. Control periods consisted of 5 min intervals in which the carotid bodies were perfused by systemic arterial blood, i.e., the extracorporeal circuit was not supplying the carotid bodies. Carotid body stimulation was superimposed on this normoxic background by abruptly perfusing the carotid bodies from the extracorporeal circuit. These stimulation periods were either 5 or 10 min in duration. Following the stimulation period the carotid bodies were switched back to perfusion by the systemic circulation thus beginning another control period. Blood gases were obtained in the fourth minute of the control period and in the second, fourth, and eighth (if applicable) minute of each carotid body stimulation period. Steady-state ventilatory and EMG measurements were obtained over the last 80 s of the control period and the last 80 s of the carotid body stimulation period. All data in each 80 s period were analysed. One to five such trials (typically four) were performed in each goat. The steady-state control values were averaged for all trials. There were no significant differences between the steady-state values of the 5 and 10 min carotid body hypoxic stimulation trials so these results were averaged together. The total duration of the successive control and stimulated periods during brain normoxia ranged between 1 and 1·5 h.

Brain hypoxia

The protocol for this portion of the experiment was essentially the same as that for brain normoxia except that the systemic arterial circulation (and, therefore, brain) was maintained hypoxic and normocapnic throughout the 1–1·5 h duration of these trials. In this report, for clarity, we have termed this condition 'brain hypoxia' but it should be understood that the hypoxia was systemic except for the carotid body circulation when it was perfused with normoxic blood. The goats were exposed to at least 15 min of brain hypoxia before any data were collected. Then a control period ensued consisting of 5 min of systemic (brain) hypoxia while the carotid body was perfused by normoxic and normocapnic blood from the extracorporeal circuit. To achieve carotid body hypoxia the carotid bodies were abruptly switched back to the systemic circulation and were thus presented with the same hypoxic and normocapnic blood supplying the brain and the rest of the body. Care was taken to maintain arterial isocapnia as ventilation increased. This cycle was then repeated one or more times.

CO_2 responses

With the systemic circulation perfusing the carotid body conventional normoxic CO_2 responses were obtained with two levels of elevated F_{I,CO_2}, 0·02 and 0·065 (arterial P_{CO_2} (P_{a,CO_2}) = 36·8 ± 1·4 and 44·1 ± 1·6 mmHg). Each level was maintained for 10 min and steady-state values were obtained from the entire last 80 s of each level.

All trials of brain normoxia, brain hypoxia, and CO_2 responses for a given goat were accomplished on the same experimental day.

RESULTS

Time course of response

The isolated, perfused carotid body preparation permitted a rapid transition (1–2 s) from the control condition to carotid body stimulation and this stimulation could be maintained as long as required to achieve a new ventilatory steady state. Typical examples of the time course of response are presented in Fig. 1. Figure 2 plots breath-by-breath responses for all trials of carotid body stimulation with and without systemic hypoxia in the same goat as shown in Fig. 1. The within-goat controls and responses to carotid body stimulation were quite reproducible. Figures

1 and 2 also show the typical overall time course of carotid body stimulation against a background of brain normoxia and systemic hypoxia. Note that for both conditions the onsets of the ventilatory and EMG responses were quite rapid. A new steady state was achieved in about 90 s and there was no tendency for ventilation or respiratory

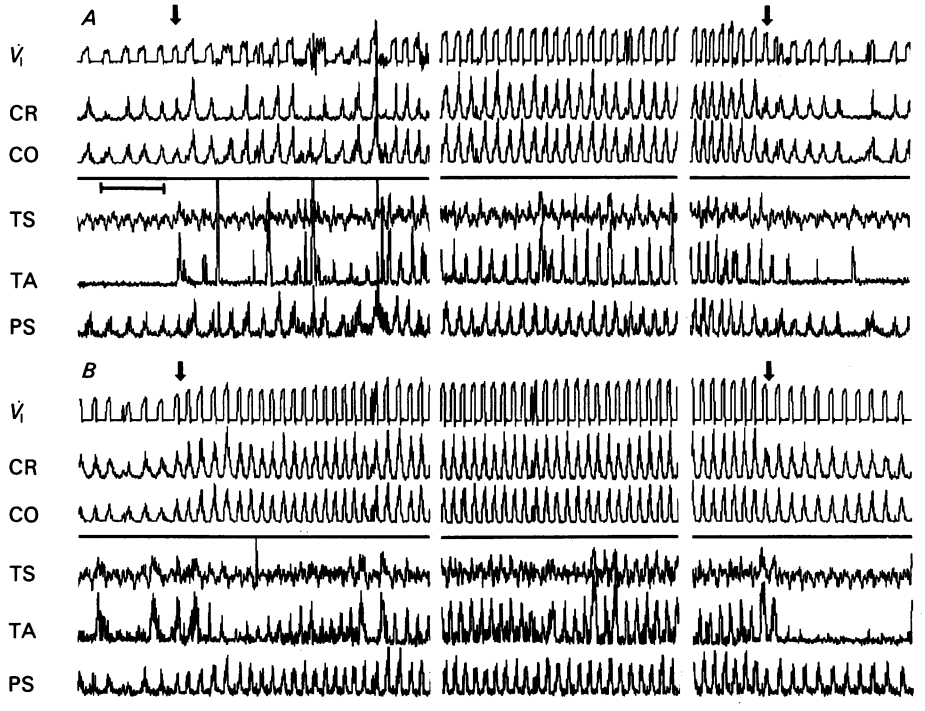

Fig. 1. Computer-generated record of representative carotid body stimulation trials during brain normoxia (panel *A*) and brain hypoxia (panel *B*) in the same goat. The carotid bodies were made hypoxic at the first arrows and returned to normal 10 min later at the second arrows. The time bar represents 10 s. Gains on all channels were the same throughout both trials. The record is not continuous; the centre segment begins after approximately 5 min of elapsed time from the beginning of stimulation. The right-hand segment begins a few seconds before the carotid bodies are returned to normoxia after 10 min of stimulation. Abbreviations: $\dot{V}_I$, inspired air flow; moving time averages of the EMGs of CR (crural diaphragm), CO (costal diaphragm), TS (triangularis sterni), TA (transversus abdominis), PS (parasternal intercostal). *A*: mean V_T during the steady state of stimulation was 1 l; f_R, 36 breaths/min; P_{CB,O_2}, 42 mmHg; P_{a,O_2}, 116 mmHg. *B*: mean V_T during the steady state of stimulation was 1·1 l; f_R, 36 breaths/min; P_{CB,O_2}, 44 mmHg; P_{a,O_2}, 45 mmHg.

muscle activity to decrease during the period of stimulation. The off-transient, while rapid, did show a post-stimulus potentiation.

Blood gases

Mean blood gas values for all seven goats are summarized in Table 1. Both systemic and carotid body P_{CO_2} were maintained isocapnic throughout. Carotid body P_{O_2} was higher during systemic hypoxia than during brain normoxia (106 *vs.*

79 mmHg, control; 48 *vs.* 39 mmHg, stimulated). During systemic hypoxia carotid body stimulation caused an increase in P_{a,O_2} of 5 mmHg.

Ventilatory output

Systemic hypoxia *per se* stimulated ventilation significantly in the control (no carotid body stimulation) condition. Moderate normocapnic hypoxia (P_{a,O_2} =

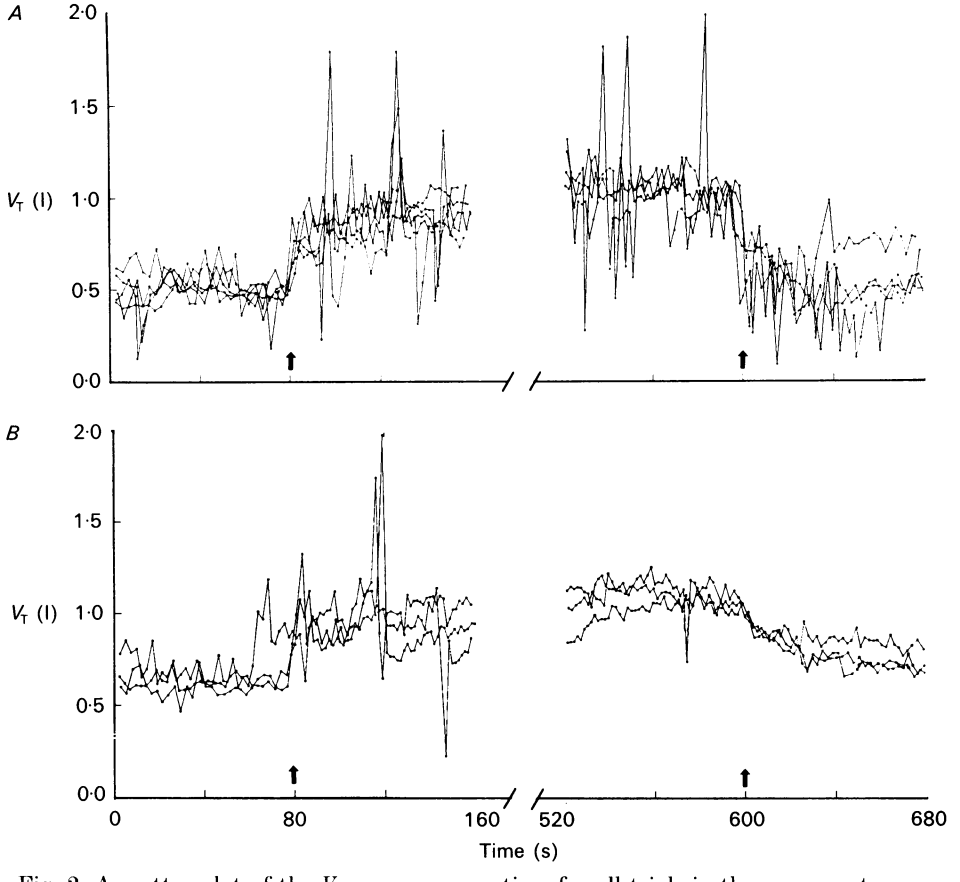

Fig. 2. A scatter plot of the V_T response over time for all trials in the same goat as was represented in Fig. 1; brain normoxia (*A*), brain hypoxia (*B*). Same abbreviations and conditions as listed in Fig. 1. Record is not continuous; note break between 160 and 520 s.

43 ± 3 mmHg; $P_{a,CO_2} = 36 \pm 1$ mmHg) increased mean inspired minute ventilation ($\dot{V}_I$) by 9 l/min ($P < 0{\cdot}05$) with respect to brain normoxia due to a 40% increase in tidal volume (V_T) (+0·2 l; $P < 0{\cdot}05$) and a 24% increase in respiratory frequency (f_R) (+6 breaths/min; n.s.) (Table 1, Fig. 3).

When the brain was normoxic, carotid body stimulation increased mean $\dot{V}_I$ by 16 l/min due to an increase in f_R (+10 breaths/min) and an increase in V_T (+0·3 l). The increase in f_R was due almost entirely to a decrease in expiratory time (T_E) of

TABLE 1. Mean steady-state blood gas and ventilatory values for the brain normoxia and brain hypoxia portions of the protocol

	Systemic		Carotid body									
	P_{O_2} (mmHg)	P_{CO_2} (mmHg)	P_{O_2} (mmHg)	P_{CO_2} (mmHg)	$\dot{V}_I$ (l/min)	f_R (breaths/min)	V_T (l)	Crural (% ref)	Costal (% ref)	Parasternal (% ref)	Triangularis (% ref)	Transversus (% ref)
Brain normoxia, control	85±4	36±4	79±3	36±1	13±1	25±2	0·5±0·03	51±5	51±10	54±9	54±14	20±8
Brain normoxia, CB hypoxia	106±6	35±1	39±1	35±1	29±2	35±3	0·8±0·06	79±7	83±17	80±8	85±6	74±8
Brain hypoxia, control	43±3*	36±3	106±3*	36±1	22±3*	31±3	0·7±0·04*	60±4	55±12	60±12	75±9	37±8*
Brain hypoxia, CB hypoxia	48±3	36±1	48±4	35±1	35±5	38±4	0·9±0·07	95±8	98±15	93±13	104±15	96±16

$n = 7$, mean ± S.E.M., *significant difference ($P < 0·05$) between brain normoxia control and brain hypoxia control. % ref, percentage of reference ventilation (see Methods).

0·6 s (Fig. 3). Inspiratory time (T_I) was essentially unchanged by carotid body stimulation and therefore V_T/T_I was increased.

When carotid body stimulation was superimposed on systemic hypoxia response slopes for all components of ventilation *vs.* carotid body P_{O_2} (P_{CB,O_2}) were not

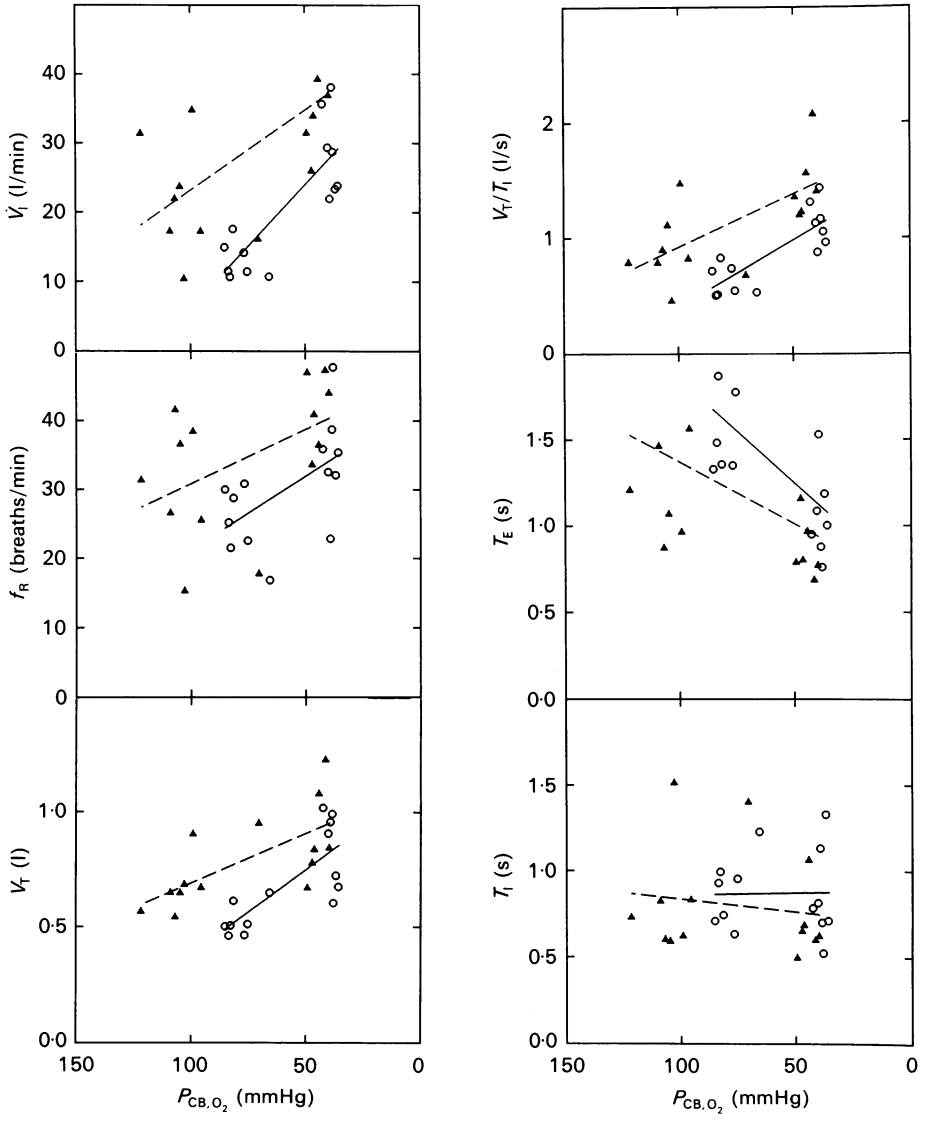

Fig. 3. Mean response slopes of the components of ventilation for all goats during brain normoxia (continuous line, ○) and brain hypoxia (dashed line, ▲) as a function of P_{CB,O_2}.

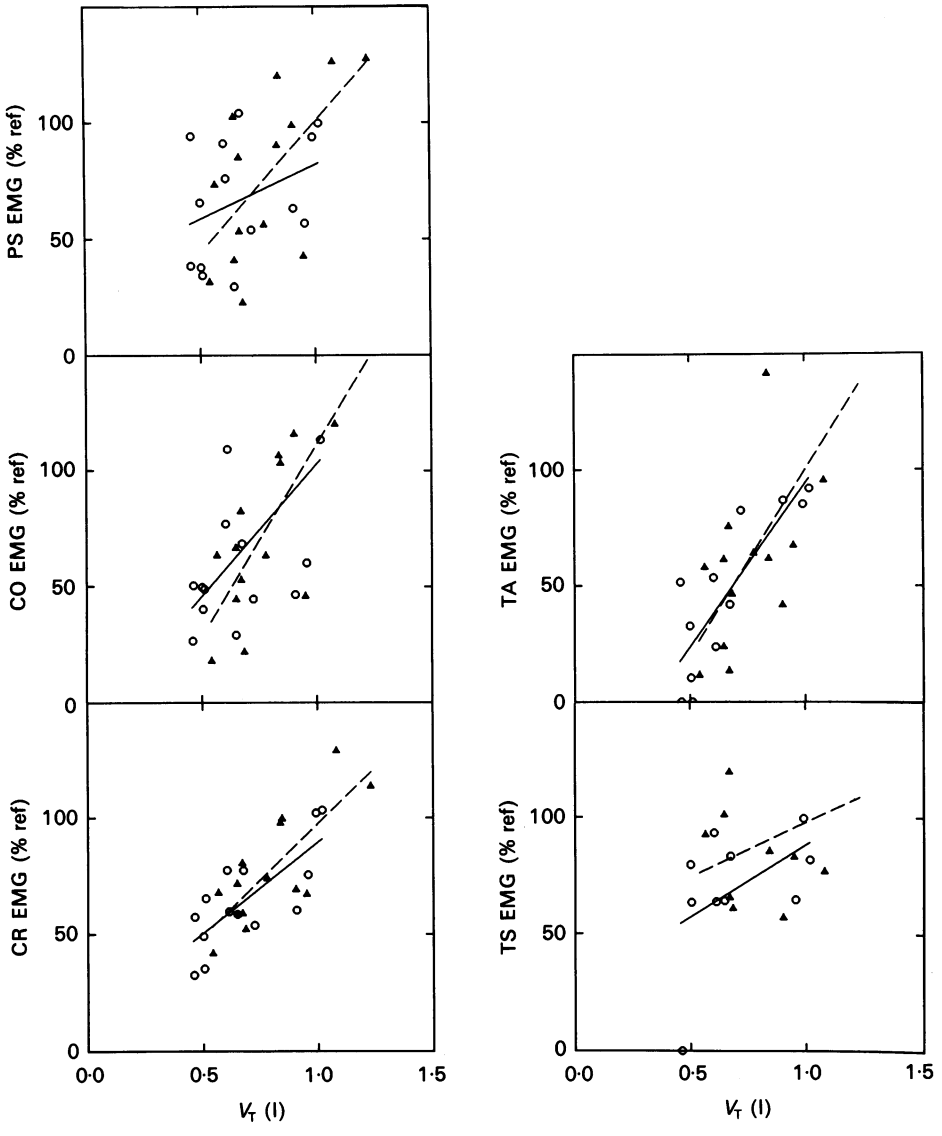

Fig. 4. Mean response slopes of the mean electrical activities (see text) of respiratory muscle EMGs as a function of V_T in response to carotid body stimulation during brain normoxia (continuous line, ○) and brain hypoxia (dashed line, ▲). There were no significant differences in slopes between the two conditions for CR or CO diaphragm or for TA. There were no clear differences between brain normoxia and brain hypoxia for PS and TS even though the slopes of their regression lines were not different from zero for the PS during brain normoxia or during either condition for the TS. See Table 2 for regression data.

significantly different from those obtained with brain normoxia (Fig. 3). The control values were offset as described above.

Respiratory muscles

With a normoxic brain and in the absence of carotid body stimulation (eupnoea) inspiratory muscles were always phasically active and expiratory muscles were

TABLE 2. Regression data for EMG mean activities of all goats in response to carotid body stimulation as a function of tidal volume

	CB hypoxia + brain normocapnic normoxia		CB hypoxia + brain normocapnic hypoxia		Whole-body normoxic hypercapnia	
	Slope	r^2	Slope	r^2	Slope	r^2
CR	78·3*	0·58	96·6*	0·61	65·5*	0·59
CO	114·0*	0·34	165·4*	0·64	76·6*	0·59
PS	46·4	0·13	112·9*	0·39	66·7*	0·42
TS	62·0	0·23	47·0	0·12	41·4	0·11
TA	141·0*	0·71	159·9*	0·52	87·7*	0·59

* Indicates a slope significantly different from zero.

usually phasically active. In two goats the transversus abdominis and in one goat the triangularis sterni were not phasically active during eupnoea (Fig. 4).

Systemic hypoxia *per se* increased the mean electrical activity of both inspiratory and especially expiratory muscles (Table 1) coincident with the increase in ventilation described above.

With a normoxic brain, carotid body stimulation augmented the EMG activities of both inspiratory (crural and costal diaphragm, parasternal intercostals) and expiratory muscles (triangularis sterni, transversus abdominis). The slopes of the regression lines of EMG mean activities as a function of increasing V_T (V_T, Fig. 4; or $\dot{V}_I$, not shown) were always positive and always significantly different from zero for all muscles except for the parasternal and triangularis sterni (Table 2).

When carotid body stimulation was superimposed during systemic hypoxia the response slopes were always positive and always significantly different from zero for all muscles except the triangularis sterni and the parasternal intercostals during brain normoxia (Fig. 4).

Comparison with whole-body CO_2 response

When plotted as a function of $\dot{V}_I$, it is clear that the response to systemic hypoxia alone (data below a $\dot{V}_I$ of about 22 l/min) is essentially no different than the response to low-level F_{I,CO_2} over the same range of $\dot{V}_I$. Similarly, the response slopes of the components of ventilation for both brain normoxia and systemic hypoxia were very similar to those obtained during whole-body normoxic CO_2 responses (Fig. 5). The increased $\dot{V}_I$ was due to both an increase in f_R and V_T. The increase in f_R was due largely to a decreased T_E.

The EMG response slopes to carotid body stimulation are summarized in Fig. 6 and contrasted with the responses to whole-body CO_2 over a similar range of

increasing V_T. The EMG response slopes during carotid body stimulation in both brain normoxia and systemic hypoxia were always slightly greater than (especially costal diaphragm and transversus abdominis) or equal to those observed during whole-body CO_2 responses.

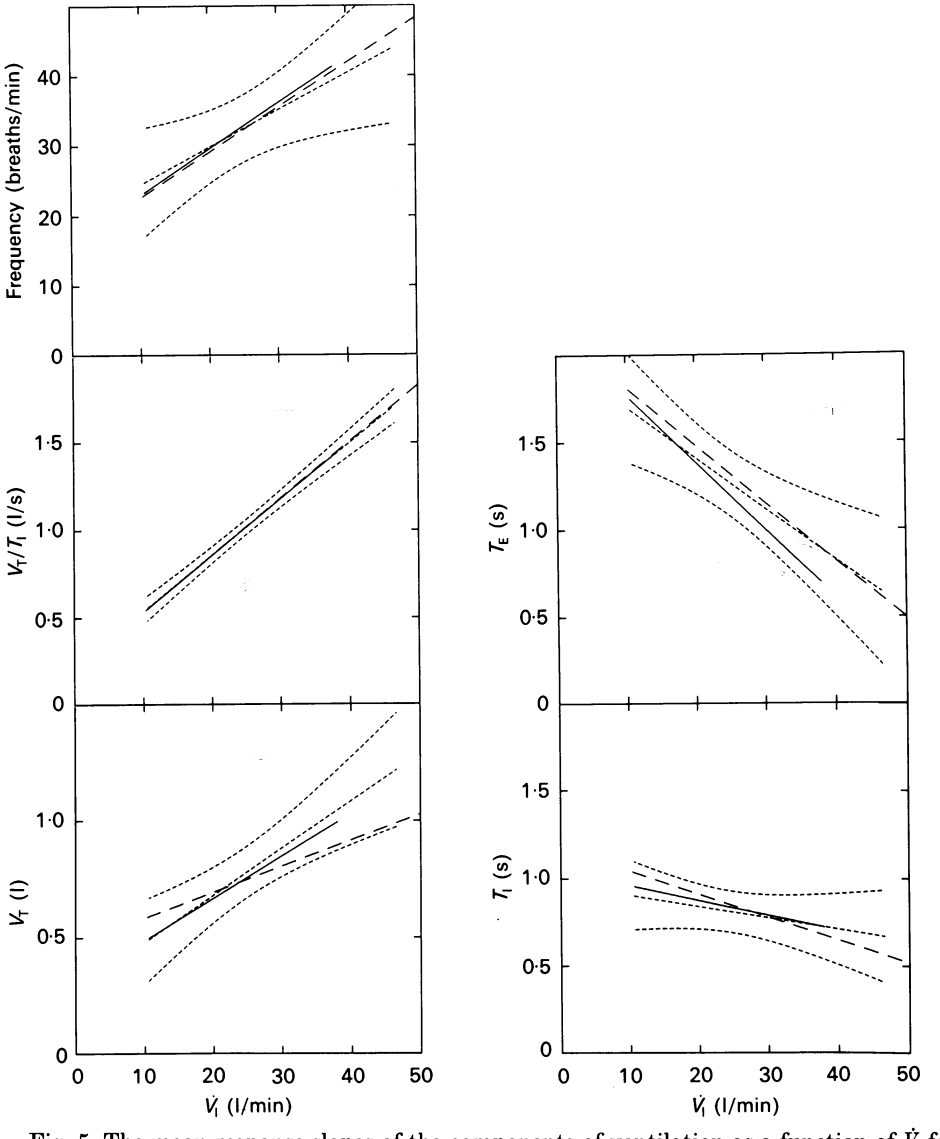

Fig. 5. The mean response slopes of the components of ventilation as a function of $\dot{V}_I$ for carotid body hypoxia with and without brain hypoxia (derived from Fig. 3) superimposed on the mean slope of the normoxic whole-body CO_2 response. Continuous line, brain normoxia; dashed line, brain hypoxia. Note that all hypoxic response slopes are within the 95% confidence limits for the whole-body CO_2 responses (dotted lines).

Blood pressure and heart rate

During brain normoxia carotid body stimulation caused a mean decrease of 14 mmHg in mean blood pressure and a 9 beats/min fall in heart rate. Systemic hypoxia resulted in a 23 mmHg decrease in blood pressure relative to brain normoxia

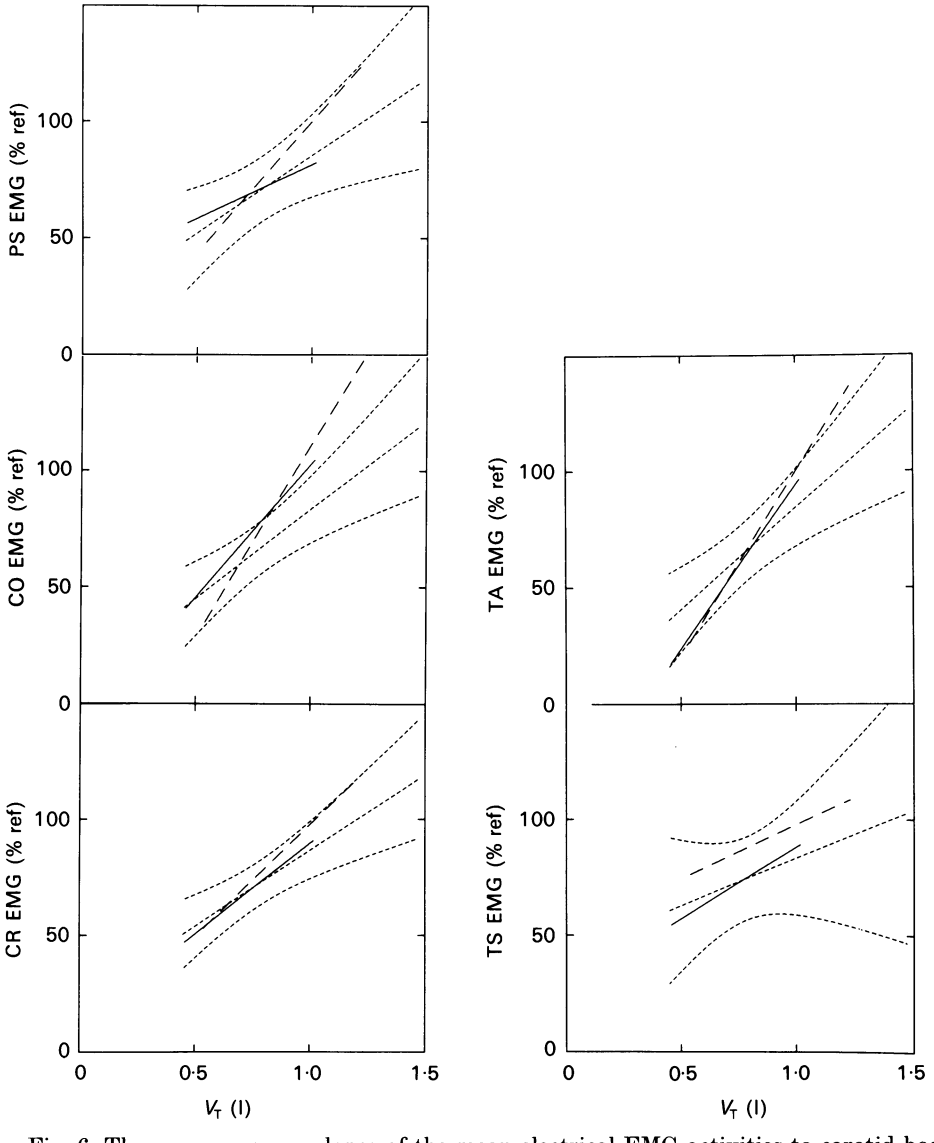

Fig. 6. The mean response slopes of the mean electrical EMG activities to carotid body hypoxia with and without brain hypoxia (from Fig. 4) superimposed on the mean slope of the normoxic whole-body CO_2 responses (dotted lines) and their corresponding 95% confidence interval for the slope. Continuous line, brain normoxia, dashed line, brain hypoxia. Note that with only one exception (PS, brain normoxia) all hypoxic response slopes are equal to or greater than that of the CO_2 response. See Table 2 for regression data.

and a 15 beats/min increase in heart rate. When carotid body stimulation was superimposed on systemic hypoxia blood pressure increased 11 mmHg and heart rate was essentially unchanged (Table 3). None of these differences was statistically significant.

TABLE 3. Steady-state (5th minute) mean blood pressure and heart rate for all conditions

Condition	Mean blood pressure (mmHg)	Heart rate (beats/min)
Brain normoxia, control	125±9	130±16
Brain normoxia, CB hypoxia	111±6	121±15
Brain hypoxia, control	102±7	145±4
Brain hypoxia, CB hypoxia	113±8	143±9

Mean±S.E.M., $n = 5$ (blood pressure data unavailable in two goats).

DISCUSSION

Our major findings were as follows. (1) Isolated systemic hypoxia by itself increased $\dot{V}_I$ by means of increases in both V_T and f_R; the EMG activities of inspiratory and expiratory muscles were augmented as well. (2) Stimulation of the carotid bodies alone, with or without systemic hypoxia, caused a pattern of EMG augmentation of inspiratory and expiratory muscles which was quite similar to that obtained with whole-body hypercapnia. (3) We found no evidence for an asymmetrical recruitment pattern (inspiratory shift) of respiratory muscles in response to specific carotid body hypoxia.

Ventilatory stimulation via systemic hypoxia

Systemic hypoxia by itself increased $\dot{V}_I$, f_R, V_T and inspiratory and expiratory muscle EMG activities in our unanaesthetized goats. These changes were not distinguishable from increases in ventilation or EMG activities due to elevated F_{I,CO_2} or increased carotid body stimulation. To us the most straightforward interpretation of these data is that moderate systemic hypoxia alone acts as a stimulant to breathing in unanaesthetized goats. Furthermore, in terms of either breathing pattern or respiratory muscle recruitment over a wide range of hyperpnoea, the responses to carotid body stimulation in the presence of systemic hypoxia were comparable to whole-body, normoxic CO_2 breathing or to carotid body stimulation when the brain was normoxic (Figs 5 and 6). Our interpretation of these findings is that systemic hypoxia had no apparent effect on the integrative capability of the brainstem respiratory controller(s).

How do our observations fit with the literature? In general our results are consistent with similar studies in unanaesthetized animals but our interpretation of our findings is not consistent with the notion of hypoxic depression of the brain in the unanaesthetized animal, at least at the moderate levels of hypoxic hypoxia used in this study.

Hypoxia-induced hyperpnoeas in the absence of carotid body stimulation (carotid body denervation, CO breathing, or isolated carotid body perfusion) are well

documented in the unanaesthetized state in the goat (Tenney & Brooks, 1966; Santiago & Edelman, 1976; Daristotle *et al.* 1991; present study) and in other species (Davenport *et al.* 1947; Bouverot *et al.* 1973; Miller & Tenney, 1975; Gautier & Bonora, 1983) although the degree and duration of hypoxia required to produce it varied. The tachypnoeic nature of this hyperpnoea in response to brain hypoxia has received considerable emphasis in the literature. This tachypnoea has been attributed to disinhibition of diencephalic rate-facilitatory respiratory centres secondary to hypoxic depression of cerebral cortical neurons (Neubauer *et al.* 1990) or to direct stimulation of diencephalic facilitatory centres (Miller & Tenney, 1975). Our data and, we think, data from most studies in awake animals favour a stimulatory or (see below) 'net' stimulatory effect of brain hypoxia. We emphasize that in goats with no carotid bodies, or perfused normoxic carotid bodies, or exposed to CO, the hyperpnoeic response to brain hypoxaemia was always achieved by an increased V_T of 10–40% (i.e. +22% at arterial oxygen saturation (S_{a,O_2}) = 39%, Santiago & Edelman, 1976; +10% at P_{a,O_2} = 30 mmHg, Daristotle *et al.* 1991; +25% at P_{a,O_2} = 40 mmHg, Weizhen *et al.* 1992; and +40% at P_{a,O_2} = 43 mmHg, present study) as well as by an increased f_R. In cats and dogs with denervated carotid bodies the hyperpnoea is accounted for solely by tachypnoea, as V_T usually falls slightly. The sources of this variability have not been studied systematically but may be significant. Such factors as the severity and duration of the hypoxaemia as well as the mode of induction of hypoxaemia, the species under study, and whether the peripheral chemoreceptors are intact or denervated may all play a role in this variability. We reiterate that in the goat the aortic bodies elicit no significant response to severe stimulation (Weizhen *et al.* 1992).

In the present study of unanaesthetized, neurally intact goats at a moderately severe level of hypoxic hypoxia it is noteworthy that the response of both inspiratory and expiratory muscles to brain hypoxia as well as that of ventilation was near-identical to that achieved with low levels of normoxic hypercapnia. We also emphasize that the hyperpnoea caused by the increased V_T and f_R was a sustained response that showed no systematic variation for up to 1·5 h of systmeic hypoxia. Taken together, we believe this evidence is the most comprehensive offered to date in support of a stimulatory effect of brain hypoxia on respiratory motor output.

There is neurophysiological evidence for both direct cortical inhibition and subcortical excitation by hypoxia. Studies using medullary (Haddad & Donnelly, 1990) or hypothalamic (Dillon & Waldrop, 1991) slices have demonstrated that hypoxia can have direct stimulatory effects on some neurons in these regions whereas those recording from cortical neurons observed hypoxia-induced depression of neuronal activity (Fujiwara, Higashi, Shimoji & Yoshimura, 1987; Krnjević & Leblond, 1989). Given the striking similarities we obtained in our goats' responses (both ventilatory output and respiratory muscle activities) to specific systemic hypoxia, carotid body hypoxia, and low levels of whole-body normoxic hypercapnia, we must favour the hypothesis that the net effect of systemic hypoxia on the ventilatory control system is one of stimulation.

Our data also cast doubt on whether moderate brain hypoxia in awake animals causes depression, or even reduced gain, of neurons in the brainstem respiratory centres. It is in these lower centres that the integrative capability of the respiratory

control system resides. Had there been significant depression of these lower centres we should have observed a decrease in the EMG and ventilatory response slopes to carotid body stimulation when we compared brain normoxia with systemic hypoxia. However, as Figs 3–6 illustrate, this was not the case. That is, none of the ventilatory and EMG responses to carotid body stimulation were significantly changed, the only noticeable effect being a slight but not significant reduction in slope of the V_T *vs.* $\dot{V}_I$ response.

Our observations extend those in anaesthetized animals in which (a) phrenic nerve activity had been reduced or abolished via severe CO hypoxaemia yet significant ventilatory responses to carotid body stimulation were retained (Melton *et al.* 1988), or (b) the vascularly isolated brain was made hypoxic and yet normal responses to hypoxia or hypercapnia were retained (Van Beek, Berkenbosch, De Goede & Olievier, 1984). Evidence of another type has been provided by Engwall *et al.* (1991) who also used the isolated carotid body perfusion technique in the awake goat. They demonstrated that systemic hypoxia had no effect on the ventilatory manifestation of the short-term potentiation phenomenon observed when the carotid body hypoxic stimulation was suddenly withdrawn.

In summary, our data are consistent with the absence of any significant depressive effect of moderate systemic hypoxia on the regulation of breathing pattern, respiratory muscle recruitment, or the integration of sensory input in the unanaesthetized goat. If these data in the goat prove to be applicable to humans, then currently held explanations for some ventilatory effects of sustained hypoxia would have to be questioned. For example, hypoxic depression of the brain could not be implicated in the time-dependent reduction in ventilation ('roll-off') with short-term hypoxia (Huang *et al.* 1984) or the apnoea observed upon abrupt imposition of hyperoxia following several minutes of sustained isocapnic hypoxia (Holtby, Berezanski & Anthonisen, 1988; Georgopoulos *et al.* 1990). Nor could it be implicated as an inhibitory influence counteracting the normal peripheral chemoreceptor stimulation during whole-body hypoxia. The situation is quite different in the anaesthetized animal where brain hypoxia causes a clear depression of ventilatory output and phrenic nerve activity (Neubauer *et al.* 1990). This central depressant effect might also apply to the sleeping animal (Santiago, Neubauer & Edelman, 1986). It might also explain at least some of the biphasic ventilatory response to hypoxia in the neonate (Haddad & Donnelly, 1990).

Expiratory muscles and chemical stimuli

Hypoxia has been shown to cause less augmentation of expiratory muscle EMG activity relative to that produced by hypercapnia at similar levels of hypernpnoea. Sears *et al.* (1982) have attributed this to carotid body stimulation causing an activation of inspiratory muscles and a simultaneous inhibition of expiratory muscles ('inspiratory shift'). In contrast to this idea we have suggested (Smith *et al.* 1989; Smith, Ainsworth, Henderson & Dempsey, 1990) that brain hypoxic depression and/or hypocapnia could account for the time-dependent inhibition of expiratory muscle activity observed during several minutes of poikilocapnic hypoxia.

Our current findings speak to both of these possibilities. Firstly, we found that specific carotid body stimulation with hypoxia in the presence of central normoxia

caused an augmentation of inspiratory and expiratory muscle EMG activities that were not distinguishable from those seen in progressive hypercapnia when compared at equal levels of ventilatory output (Fig. 6). These results argue against an 'inspiratory shift'. These data do support the idea that carotid body stimulation either by means of hypoxia or almitrine during isocapnic conditions (Smith *et al.* 1989, 1990; Saupe, Smith, Henderson & Dempsey, 1992) acts to augment both inspiratory and expiratory muscles similarly for a given increment in V_T. At the medullary level Lipski, Trzebski, Chodobska & Kruk (1984) demonstrated that carotid body stimulation caused roughly equal activation of inspiratory- and expiratory-related neurons. Secondly, we found that carotid body hypoxia caused equal levels of inspiratory and expiratory muscle EMG activities at any given V_T whether the brain was hypoxic or normoxic. These data speak against our suggestion that brain hypoxia caused selective inhibition of expiratory neurons (Smith *et al.* 1989) thus leaving a selective effect of central hypocapnia as the most likely cause of expiratory muscle inhibition. This possibility was not addressed in the present study.

Carotid body stimulation *per se* is certainly not the only determinant of the pattern of respiratory muscle recruitment during chemically-induced hyperpnoea in the intact animal. Lung and/or chest wall mechanoreceptor feedback is important to expiratory muscle recruitment during hyperpnoea. In the awake standing dog, vagal afferents do affect the absolute level of abdominal expiratory muscle activation and consequently the functional residual capacity and length of the diaphragm (Ainsworth, Smith, Johnson, Eicker, Henderson & Dempsey, 1992*a*, *b*). However, pulmonary vagal feedback was not required for a near-normal linear augmentation of expiratory muscle activity in response to carotid body stimulation or to whole-body hypercapnia (Ainsworth *et al.* 1992*a*, *b*; Saupe *et al.* 1992). Evidence from anaesthetized, rhizotomized preparations suggest that chest wall afferents might also contribute to expiratory muscle recruitment (Bishop, 1964) as do the clear effects of changes in posture on inspiratory and expiratory muscle EMG activity (Remmers & Bartlett, 1977; Russell, Bishop & Hyatt, 1987; Farkas, Baer, Estenne & De Troyer, 1988).

Taken together, we believe that the available data suggest that carotid body stimulation in the awake goat activates both inspiratory and expiratory neurons equally as well as does CO_2 for a given ventilatory output. At least one set of mechanoreceptor feedback influences (pulmonary, thoracic, or abdominal) may be required for expiratory muscle activation. Moderate brain hypoxia has no significant depressant effect on this partitioning of responses between the inspiratory and expiratory muscles.

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