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EFFECTS OF MEMORY FROM VAGAL FEEDBACK ON SHORT-TERM POTENTIATION OF VENTILATION IN CONSCIOUS DOGS

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SUMMARY

1. We assessed short-term potentiation of ventilation in response to brief systemic normocapnic hypoxaemia in conscious dogs. Four recumbent dogs were exposed to P_{a,O_2} 35–55 mmHg with P_{a,CO_2} maintained normocapnic for forty to fifty seconds and then abruptly returned to normoxia. Minute ventilation ($\dot{V}_I$) increased 4- to 5-fold during hypoxia due to both increased tidal volume (V_T) and frequency (f). Several trials of hypoxic exposure with normoxic restoration were conducted with animals intact and following bilateral cold blockade of the cervical vagus nerves sufficient to block completely the Hering–Breuer reflex.

2. In the *vagally intact* dog, when normoxia was restored immediately following normocapnic hypoxia ($P_{ET,O_2} = 40$ Torr), expiratory time (T_E) was prolonged to 190 ± 68% of control (mean ± S.E.M., range 53–350%) on the second or third breath and then returned slowly to control values on subsequent breaths. The prolongation of T_E following removal of the hypoxic stimulus was positively correlated with the magnitude of the peak V_T reached during hypoxic exposure. However, V_T and $\dot{V}_I$ remained significantly greater than control over a twenty second or four-breath period following hypoxia.

3. In the *vagally blocked* dog, no prolongation of T_E was observed following isocapnic hypoxia; nor was T_E following hypoxia correlated with the magnitude of the V_T during hypoxia. The time constants of decay of $\dot{V}_I$, V_T and f back to control, following hypoxia averaged 16, 19 and 9 s, respectively.

4. We conclude that short-term potentiation of ventilatory output following peripheral chemoreceptor hypoxic stimulation does exist in the awake dog, but the stimulatory after-effect is masked and T_E is prolonged by a 'memory' of inhibitory vagal feedback. The magnitude of this inhibitory after-effect on T_E prolongation increases in proportion to the increase in tidal volume achieved during the hypoxaemia.

5. This inhibitory effect of vagal memory may contribute to instability of breathing pattern and apnoea following transient disturbances in ventilatory output.

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INTRODUCTION

This study was concerned with the regulation of respiratory motor output and stability of breathing pattern in the period immediately following withdrawal of an hyperpnoeic stimulus. Short-term potentiation of phrenic motor output has been shown repeatedly to follow withdrawal of electrical stimulation of the carotid sinus nerve in anaesthetized dogs (Gesell & Hamilton, 1941) and anaesthetized or decerebrate cats (Eldridge, 1973, 1976; Eldridge & Gill-Kumar, 1978). Similarly, ventilatory output remains augmented following brief stimulation with isocapnic hypoxia in the awake and sleeping human (Georgopoulos, Bshouty, Younes & Anthonisen, 1990; Badr, Skatrud & Dempsey, 1992). Nevertheless, ventilatory output and breathing pattern immediately following hypoxic stimulation may also show either no potentiation or even be frankly inhibited, with reduced tidal volume and/or prolonged expiratory time – even apnoea in humans (Holtby, Berezanski & Anthonisen, 1988; Georgopoulos *et al.* 1990; Badr *et al.* 1992). In the awake or sleeping human, goat or dog, this inhibition of ventilatory output following withdrawal of hypoxic stimulation occurred when P_{a,CO_2} was permitted to fall sufficiently during a hyperpnoeic response (Mitchell, Bainton & Edelist, 1966; Georgopoulos *et al.* 1990; Engwall, Daristotle, Niu, Dempsey & Bisgard, 1991; Badr *et al.* 1992) or when normocapnic hypoxia was sustained for a few minutes (Georgopoulos *et al.* 1990; Badr *et al.* 1992). Thus, ventilatory output and breathing pattern in the post-stimulus phase appear to represent the *net* effect of excitatory *vs.* inhibitory influences on the central respiratory pattern generator.

Our study was aimed at determining the role of slowly adapting stretch receptor activity from the lung as a determinant of ventilatory output and breath timing following removal of a physiological stimulus to hyperpnoea. The concept of a mechanoreceptor-induced inhibitory 'memory' effect is suggested by the continued inhibition of phrenic nerve activity observed following withdrawal of superior laryngeal nerve stimulation and to a lesser, but significant extent, following withdrawal of electrical stimulation of pulmonary vagal afferents (Gesell & Hamilton, 1941; Lawson, 1981; Eldridge & Millhorn, 1986). We sought to determine whether this inhibitory influence also prevailed following the more complex physiological condition of hypoxic-induced hyperpnoea in the awake intact dog. To this end, we induced short-term isocapnic hypoxia in the awake animal; with and without vagal blockade; and observed the effects on ventilatory output and breathing pattern in the period immediately following withdrawal of the chemoreceptor stimulus. The presence of vagal feedback from the lung *during* the hyperpnoeic period had a short-lived but highly significant effect on breathing pattern and therefore on ventilatory stability *following* the removal of the brief hypoxic stimulus.

METHODS

General

Four adult female dogs (20–25 kg) were studied. The dogs were trained to lie quietly on a bed located in an air-conditioned (19–22 °C), sound-proofed chamber. The dogs were unrestrained during the experiments; they chose their body position, prone or lateral. The animal's behaviour in the chamber was monitored by closed circuit television throughout the experiment.

Animal preparation

Under general anaesthesia, induced with thiamylal sodium (20–25 mg/kg I.V.) and maintained with 1% halothane in O_2, a midline tracheostomy was created in all dogs about 2 cm below the larynx. We also exteriorized one carotid artery and both cervical vago-sympathetic trunks and

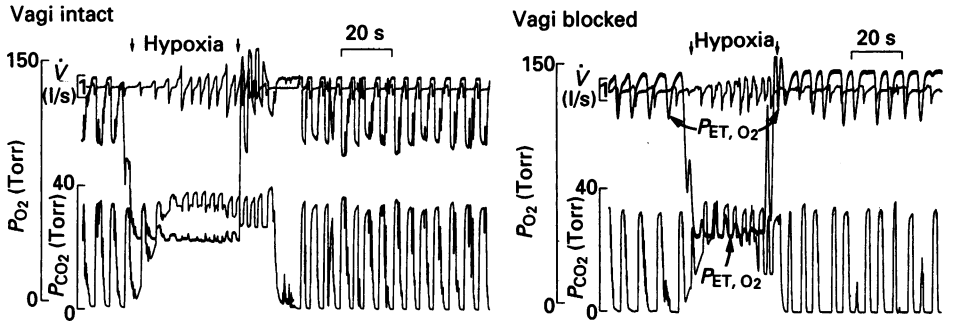

Fig. 1. Two examples of polygraphic recording vagi intact *versus* vagi blocked. Hypoxia was initiated at first arrow and terminated at second arrow. Note the prolongation of T_E on the third breath following normoxic restoration. The end-tidal P_{CO_2} was held slightly > control during hypoxia so that *arterial* P_{CO_2} would be maintained normocapnic (see Methods).

enclosed them in 5 cm long skin flaps. Appropriate analgesics and antibiotics were administered during the post-operative period. We allowed the dogs a recovery period of at least two weeks prior to the studies. The surgical and experimental protocols of this study were approved by the Animal Care and Use Committee of the University of Wisconsin.

Ventilation

The dog was intubated with a cuffed endotracheal tube via the chronic tracheostomy. The endotracheal tube was connected to a thermostatic (37 °C) pneumotachograph system (Hans Rudolph 3700, Kansas City, MO; Validyne MP-45-14-871, Northridge, CA) which in turn was connected to a flow-through gas supply system (20–25 l/min) by means of a T-piece. This system was calibrated daily with five known flow rates. Airway P_{O_2} and P_{CO_2} were sampled and analysed continuously from the endotracheal tube by means of an oxygen analyser (S-3A, Ametek, Pittsburgh, PA) and a CO_2 analyser (SensorMedics LB-2, Anaheim, CA) which were calibrated daily. A parallel 3-way valve system was employed to provide rapid switching between a flow of background gas (21% O_2:79% N_2) and a test gas of variable N_2:O_2 concentrations. CO_2 was added to this flow in appropriate amounts to maintain the desired end-tidal P_{CO_2}.

Flow rate and airway P_{O_2} and P_{CO_2} signals were recorded on a 12-channel polygraphic recorder (Gould ES 2000, Rolling Meadows, IL, USA). The flow signal also passed through an A–D convertor and was stored on the hard disk of a microcomputer for subsequent analysis. Using software developed in our laboratory, the flow signal was analysed breath-by-breath for volume and timing components. P_{ET,O_2} and P_{ET,CO_2} were measured manually from the polygraph record.

Cold block of vagi

We used a method similar to that of Fishman, Phillipson & Nadel (1973) to block nerve traffic reversibly. A metal heat exchanger was secured firmly to each vagal loop and a variable flow of a −10 °C anti-freeze solution was used to regulate heat exchanger outflow temperature. During the

cooling period, a temperature from −2 to −5 °C in the outflow from the heat exchanger was sufficient to obtain a complete vagal blockade within 5–15 min. Criteria used to judge the completeness of the block in the awake dog have been previously documented (Ainsworth, Smith, Johnson, Eicker, Henderson & Dempsey, 1992); they were; (1) complete disappearance of the Hering–Breuer inspiratory inhibitory reflex, i.e. passive lung inflations (volume = 0·75 l) did not prolong T_E; (2) presence of bilateral Horner's syndrome and (3) tachycardia (> 150 beats/min). The total time of the vagal cooling for any experimental day was limited to one hour to avoid skin and nerve damage. The cold block was reversed within 30–60 s following rewarming of the radiators. No subsequent effects of the cold block were observed.

Arterial blood gases and blood pressure

We obtained sets of serial arterial blood samples throughout each of three hypoxia trials for each dog (see Protocol). Prior to the session, a 20 gauge catheter was inserted aseptically, under local anaesthesia, into one carotid artery loop. To minimize dead space, we used a short catheter system only 30 cm long from carotid artery to the sampling point. For each trial, 1 ml blood samples were collected continuously at 10 s intervals during normoxic eupnoea, throughout the subsequent hypoxic period, and during the initial 40 s period of the return to normoxia. P_{O_2}, P_{CO_2}, and pH were analysed with an automated blood gas analyser (ABL-2, Radiometer, Copenhagen, Denmark), calibrated daily with 3-point tonometry and two precision buffers. All values were corrected to the dogs' rectal temperatures (range 37·5–38·5 °C).

Intra-arterial blood pressure was monitored using a pressure transducer connected to the carotid arterial catheter (Viggo–Spectromed, Model P23XL, Oxnard, CA, USA). The effects of vagal blockade on blood pressure were determined in three dogs (Dogs O, P, R). We also determined the effects of normocapnic hypoxia and restoration of normoxia on blood pressure (see Protocol below) during several trials on one dog (Dog O).

Experimental protocol

Our protocol was aimed at determining the immediate 'after-effects' of brief hypoxic stimulation of ventilation, on the breathing pattern in the awake dog and the role of vagally mediated influences on this 'after-effect'. A single trial in one dog with vagi intact and following vagal blockade, is shown in Fig. 1. We used brief isocapnic hypoxia (40–50 s) induced by the inhalation of hypoxic–hypercapnic mixtures sufficient to stimulate the peripheral chemoreceptors (P_{ET,O_2} = 40–60 mmHg) and to maintain arterial P_{CO_2} at normocapnia. Then end-tidal P_{O_2} was quickly restored to normoxic levels and normocapnia maintained by suddenly replacing the hypoxic inspirate with 0·23 fraction of oxygen in inspirate (F_{I,O_2}), balance N_2 and 2–3 % CO_2. For the first few breaths of the transition from hypoxia to normoxia, this increase in F_{I,O_2} together with the animal's hyperpnoea was sufficient to raise $P_{ET,O_2} > 90$ mmHg in the first breath, to 100–120 mmHg by the second breath and to maintain P_{ET,CO_2} near normocapnic levels. For the remainder of the recovery period the dog breathed room air.

The arterial blood gas data obtained on selected trials (see above) showed that the end-tidal to arterial P_{CO_2} difference averaged 1–3 mmHg higher during the brief isocapnic hypoxia stimulation periods than in control; thus, we added sufficient F_{I,CO_2} to hold P_{ET,CO_2} 2–3 mmHg > control during the hypoxic stimulation (see Fig. 1). This blood gas analysis also confirmed that the arterial P_{O_2} was returned to 110–120 mmHg and the arterial P_{CO_2} was maintained within ±1 mmHg of normocapnia in the initial 10–15 s transition period when normoxia was restored following hypoxic stimulation.

Data analysis and statistics

We used an ensemble-average method to obtain group mean values on a breath-by-breath basis over the time course of the ventilatory response to hypoxia for multiple trials of each ventilatory parameter in each dog. Significant differences among mean values obtained in prehypoxic control and at each of the first ten breaths of normoxic restoration (following hypoxia) were determined by one-way ANOVA across all mean values, combined with a post hoc Tukey's test between the group mean control values and the mean of each of the recovery breaths.

We calculated the time constants of the ventilatory decay following hypoxia for each trial. We chose the starting point for our calculations as the value (P_s) of the first breath following 10 s

posthypoxia, which was assumed to be the *maximum* circulatory time delay from the lungs to the carotid bodies. The time constant for each variable of ventilatory output was calculated as the time when the variable reached a level (P_{tc}) which was equal to 63% of the total decay from P_s to the preceding normoxic control value (C), i.e. $P_{tc} = P_s - 0{\cdot}63\ (P_s - C)$.

RESULTS

Effects of vagal blockade on eupnoea (see Table 1)

Bilateral cold blockade on the vagi eliminated the Hering–Breuer reflex and significantly altered breathing pattern in all dogs. The most consistent effect was an increase in tidal volume (V_T) to 1·5–4·8 times control. Breathing frequency was reduced significantly in three of the four dogs due primarily to an increase in expiratory time (T_E) and in two dogs to a prolongation of both T_E and inspiratory time (T_I). We also found a substantial variability among the four dogs in the effect of vagal blockade on breathing pattern (range of T_E with blockade = +26–240% and V_T = +68–380%). Arterial blood gases were unchanged by vagal blockade in three of the dogs with a slightly increased P_{a,CO_2} in the fourth dog.

Ventilatory response during hypoxia

The time course of the mean ventilatory response of all dogs to normocapnic hypoxia (P_{ET,O_2} = 40 mmHg) is shown in Fig. 2. By the end of the 50 s period of isocapnic hypoxia: (a) $\dot{V}_I$ increased almost 4-fold in the intact trials and about 4·2-fold in the vagally blocked trials, (b) V_T increased 3-fold in both intact and vagally blocked trials, thereby remaining 0·5 l larger in the vagally blocked trials, (c) mean T_E decreased 40–50% in both conditions, thus T_E remained 35% longer in the vagally blocked trials, (d) mean T_I fell 10% in the vagally intact trials, but was unchanged after 50 s of normocapnic hypoxia in the vagally blocked trials.

After-effects of hypoxia

The average breath-by-breath ventilatory response and breath timing following removal of the hypoxic stimulus is shown in Fig. 2. On the first breath at a restored normoxic P_{ET,O_2} in both the intact and vagally blocked trials, V_T and $\dot{V}_I$ remained elevated relative to control and T_E was shorter than control. This initial breath occurred during the first 4–6 s of normoxic restoration and probably represented the response to continued hypoxaemia acting at the carotid bodies due to the circulatory delay from alveoli to carotid bodies.

On the second breath of normoxia in the *vagally intact animal*, T_E was significantly prolonged to an average of 190±68% of control ($P < 0{\cdot}05$). The maximum prolongation of T_E was usually reached on the second but on some occasions on the third breath of normoxic return. On the subsequent breaths, T_E was shortened but still remained significantly greater than control ($P < 0{\cdot}05$) until the seventh normoxic breath, at which time group mean T_E had returned to normoxic control values. In contrast to the transient prolongation of T_E following hypoxia, breath-by-breath mean V_T and therefore $\dot{V}_I$, remained significantly greater than control for the first four normoxic breaths. V_T and $\dot{V}_I$ gradually fell to control values ($P > 0{\cdot}05$) by the fifth normoxic breath (i.e. after 20 s of normoxic restoration).

TABLE 1. Ventilatory parameters and blood gases

Dog	V_T (l)		f (br/min)		$\dot{V}_I$ (l/min)		T_I (s)		T_E (s)		P_{a,O_2} (Torr)		P_{a,CO_2} (Torr)	
	V+	V−	V+	V−	V+	V−	V+	V−	V+	V−	V+	V−	V+	V−
O	0·22 ±0·01	0·37* ±0·02	11·4 ±0·3	10·5 ±0·6	2·5 ±0·1	3·8* ±0·3	1·8 ±0·0	2·1 ±0·1	3·8 ±0·2	4·7* ±0·4	98·6 ±1·5	90·3* ±2·7	36·7 ±0·3	38·0* ±0·5
P	0·17 ±0·01	0·83* ±0·03	18·2 ±1·1	5·1* ±0·3	3·4 ±0·3	4·1 ±0·1	1·5 ±0·1	3·8* ±0·1	2·6 ±0·3	8·7* ±0·6	88·8 ±1·8	86·4 ±1·7	38·2 ±0·4	39·2 ±0·6
R	0·22 ±0·01	0·46* ±0·03	12·5 ±0·4	7·2* ±0·5	2·6 ±0·1	2·9 ±0·1	1·9 ±0·1	2·6* ±0·1	3·3 ±0·1	6·8* ±0·4	89·6 ±1·7	89·1 ±1·0	36·0 ±0·6	34·5 ±0·5
T	0·18 ±0·00	0·28* ±0·01	14·7 ±0·3	12·8* ±0·4	2·7 ±0·1	3·5* ±0·2	1·5 ±0·0	1·8 ±0·0	2·8 ±0·1	3·2* ±0·1	99·5 ±1·4	97·7 ±0·9	35·3 ±0·5	34·9 ±0·5

Control values (mean ± S.E.M.) of ventilatory parameters and blood gases during vagally intact (V+) and blocked (V−) on all four dogs. The number of observations averaged was 22–155. *Means significant difference from the vagally intact value ($P < 0·05$).

The major effect of *vagal blockade* on posthypoxic ventilation was on T_E. In the vagally blocked trials, T_E was never prolonged following hypoxia; rather, T_E returned quickly to control values by the second breath (8–10 s) of normoxic restoration. As in the intact animal, V_T and $\dot{V}_I$ were significantly greater than

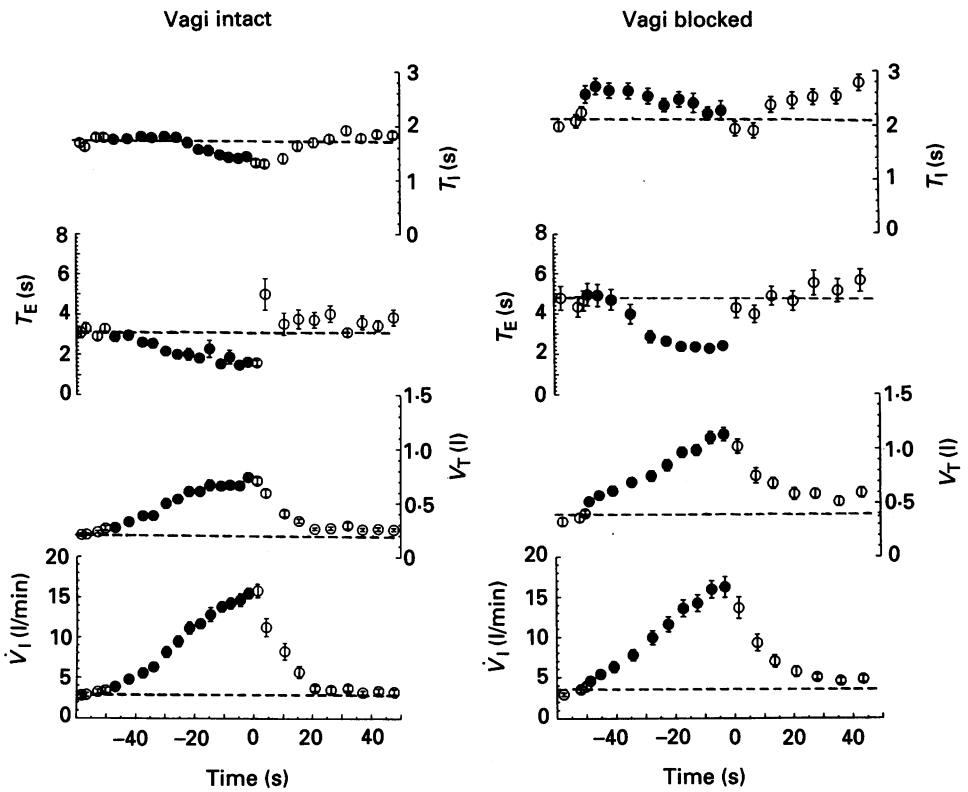

Fig. 2. Time course of ventilatory response to brief hypoxia ($F_{I,O_2} = 0{\cdot}06$, $P_{a,O_2} = 40$ Torr) and normoxic restoration. The data are the breath-by-breath ensemble-averaged values (mean ± S.E.M.) of the trials from all dogs when vagi were intact (left panel, $n = 48$) or blocked (right panel, $n = 40$). The filled symbols represent hypoxic breaths and the open symbols represent normoxic breaths. The dashed lines show the average control value for each ventilatory parameter.

control on the second normoxic breath and thereafter gradually fell but remained significantly elevated, relative to control ($P < 0{\cdot}05$) until the fifth recovery breath in normoxia, at which time the V_T and $\dot{V}_I$ returned to control levels ($P > 0{\cdot}05$).

We also described the recovery from hypoxia by determining the time constants for the different parameters of ventilatory output beginning at the first breath following 10 s of normoxic restoration. In the vagally blocked trials, we found time constants for $\dot{V}_I$ (16·4 ± 2·3 s, mean ± S.E.M.), V_T (19·0 ± 6·7 l) and f (9·4 ± 3·0 breaths/min). In the vagally intact trials, also starting after the initial 10 s of normoxic restoration, the time constants were shorter, amounting to 8·9 ± 3·0 s for $\dot{V}_I$, 14·0 ± 2·0 l for V_T and 5·2 ± 1·8 breaths/min for f.

Relationship of peak V_T during hypoxia to T_E prolongation following hypoxia

In order to determine whether the prolongation of T_E during the posthypoxic period is dependent on the tidal volume reached during hypoxic stimulation, we carried out correlational analyses between these two variables. For this analysis we

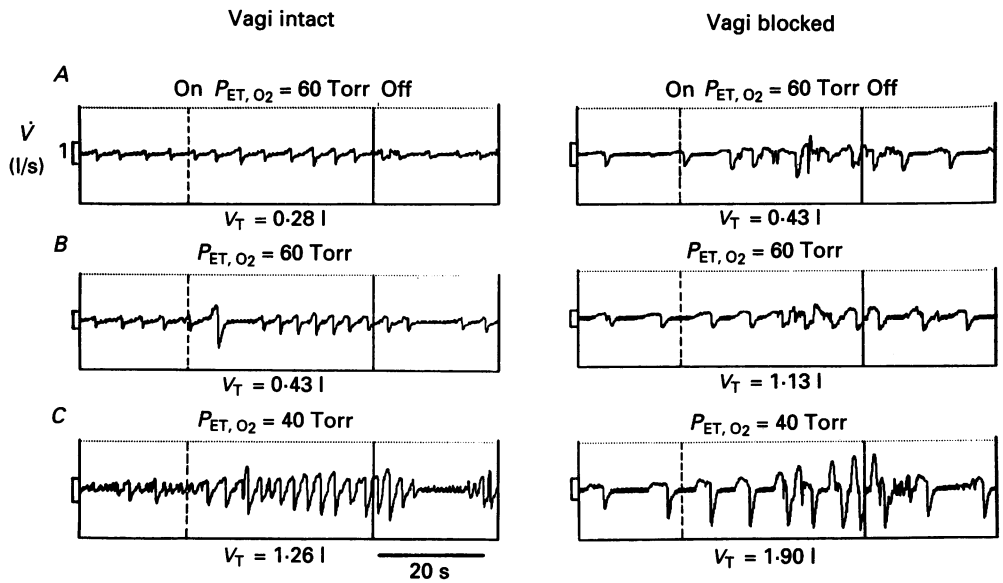

Fig. 3. Three hypoxic exposure trials showing the effects of different levels of hypoxia on the V_T response (low in *A*, medium in *B*, high in *C*) and on breathing pattern following restoration of normoxia. Left panel shows that in trials with vagi intact the prolongation of T_E increased when V_T was greater, but this effect of increasing V_T was abolished after vagal blockade (right panel). Note the spontaneous sigh at the onset of hypoxia (left panel, *B*), which are frequently seen in vagally intact dogs but not in the vagally blocked dogs.

included all trials conducted at $P_{ET,O_2} = 40$ mmHg and at $P_{ET,O_2} = 60$ mmHg in order to obtain a wide range of tidal volume responses.

Typical examples of the effect of varying V_T during the two levels of hypoxic stimulation on the time course of recovery of T_E and V_T are shown in one dog (P) in Figs 3 and 4. In both the polygraph traces of Fig. 3 and the multiple trials showing breath-by-breath T_E plotted in Fig. 4 we note that, in general, the more severe the normocapnic hypoxia the greater the increase in V_T and reduction in T_E, although considerable variability in response occurred among trials even at the same P_{ET,O_2}. Furthermore, note that in the trials with *vagi intact*, the larger V_T response was generally accompanied by a greater prolongation of T_E following hypoxia, with little discernible effect on the continued elevation in V_T during recovery. On the other hand, in the trials with vagal blockade following hypoxia, T_E was either not prolonged or only minimally so; furthermore, T_E during recovery did not differ as V_T was increased between the two levels of hypoxia.

In Fig. 5 we plotted the longest T_E obtained in the second or third posthypoxic

breath ($T_{E,max}$ following hypoxia) against the V_T reached just at the termination of hypoxic stimulation (V_T at end-hypoxia) in each trial in all four dogs. In vagally intact trials a significant *positive* relationship between $T_{E,max}$ in recovery and V_T in hypoxia was found. On the other hand, in the vagally blocked trials, T_E following

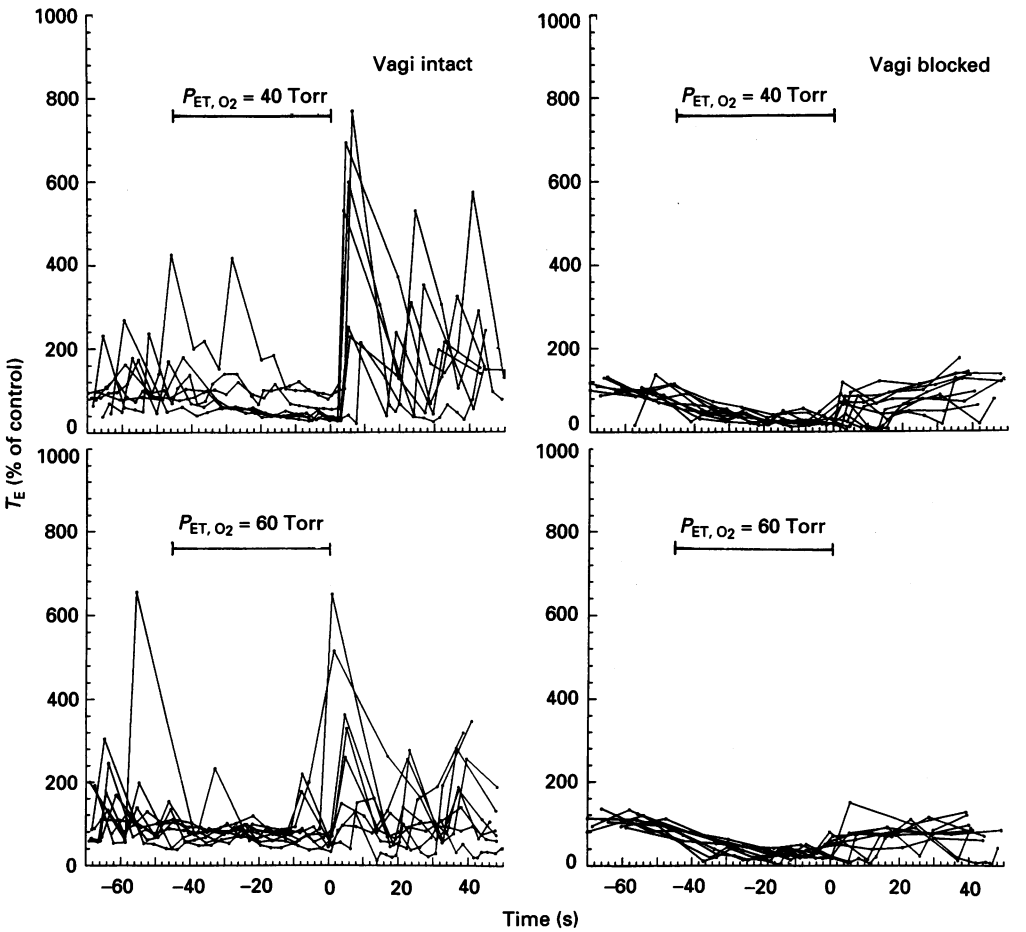

Fig. 4. Breath-by-breath changes in T_E are shown during and following moderate normocapnic hypoxia (P_{ET,O_2} = 60 Torr) and more severe normocapnic hypoxia (P_{ET,O_2} = 40 Torr) in vagally intact and vagally blocked trials (also see Fig. 3). Average V_T in the *intact trials* was 0·17 ± 0·01 l in normoxic control and 0·46 ± 0·12 l at P_{ET,O_2} = 60 Torr and 0·73 ± 0·06 l at P_{ET,O_2} = 40 Torr. Average V_T in the *vagi blocked trials* was 0·83 ± 0·03 l in normoxic control, 0·76 ± 0·10 l at P_{ET,O_2} = 60 Torr and 1·54 ± 0·04 l at P_{ET,O_2} = 40 Torr.

hypoxia remained unchanged and therefore was not significantly related to an increasing V_T over a wide range of V_T.

Effects on blood pressure

With the vagi intact, during the steady state of normoxic control conditions, carotid arterial systolic/diastolic blood pressure averaged 125/72 mmHg in the three dogs studied. With brief normocapnic hypoxia, blood pressure increased an average

of about 5 mmHg to 130/75 over the final 10 s of hypoxia; with normoxic restoration, blood pressure returned to control levels in the first few seconds, fell slightly below control levels during the T_E prolongation and then returned quickly again to control. In the steady state of vagal blockade during normoxic control, blood

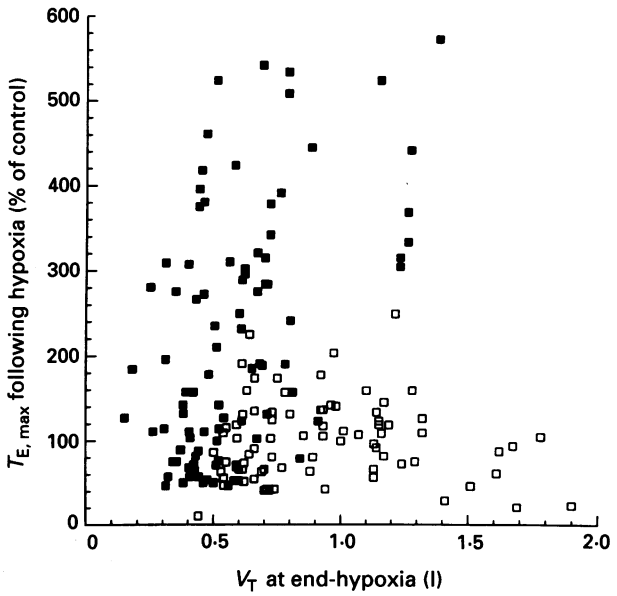

Fig. 5. Plot of the $T_{E,max}$ obtained immediately *following* the removal of hypoxia against the V_T obtained *during* the end of the brief hypoxic stimulation period. Data shown from all trials on the four dogs. Filled symbols represent the trials during vagally intact; open symbols represent the trials during vagally blocked. The $T_{E,max}$ is plotted as the percentage of its pre-hypoxia mean control value for each dog. During pre-stimulus control conditions (not shown here), the group mean T_E and V_T for vagal intact trials was 3·1 s and 0·20 l and for vagal blocked trials was 5·9 s and 0·49 l. The correlation of the V_T at end-hypoxia to $T_{E,max}$ following hypoxia for vagally intact trials was significant ($r = 0{\cdot}50$, $n = 92$, $P < 0{\cdot}01$, $T_{E,max}$ following hypoxia (% of control T_E) = $48{\cdot}4 + 287{\cdot}7$ V_T at end-hypoxia), whereas the correlation for vagally blocked trials was not significant ($r = 0{\cdot}07$, $n = 79$, $P > 0{\cdot}05$).

pressure increased to 143/112 mmHg. Hypoxia caused systolic and diastolic blood pressure to increase 40–50 mmHg (to an average of 185/127 mmHg); immediately upon restoration of normoxia, blood pressure fell slightly, but required 40–60 s before gradually returning to control levels.

DISCUSSION

Our major finding is that expiratory time is significantly prolonged immediately following a brief period of hypoxic-induced, normocapnic hyperpnoea in the awake, intact dog. The magnitude of the T_E prolongation following hypoxia is positively correlated with the V_T achieved during hypoxia. Furthermore, the prolongation of T_E following hypoxia and the correlation of V_T to T_E prolongation are virtually eliminated

by vagal blockade. The time constant of the decay in ventilatory output following hypoxic stimulation is also prolonged by the absence of vagal afferents. We interpret these findings as evidence for an inhibitory 'memory' effect of vagally mediated pulmonary stretch receptor activity which persists *following* the removal of the primary (hypoxic) stimulus and causes prolongation of T_E and opposes the facilitory, 'short-term potentiation' or after-discharge effect from chemoreceptor stimulation.

Limitations

Our aim was to determine the role of vagal feedback on the regulation of ventilation in the period immediately following ventilatory stimulation. On the one hand, we believe our use of the awake animal and physiological (hypoxic) chemoreceptor stimuli does permit us to draw realistic inferences about the sensitivity and relative importance of mechanisms which may be operative in physiological states involving the sudden appearance and withdrawal of transient ventilatory stimuli. Furthermore, the use of reversible vagal blockade permitted us to use each animal as its own control and to compare vagally intact *vs.* vagally blocked conditions in experiments conducted within the same testing session. Both of these factors enhanced our ability to discriminate and quantify the effects of vagal feedback. On the other hand, there were drawbacks to our approach because we could not *immediately* stop carotid chemoreceptor stimulation and restore normoxic conditions, as is possible with the use of electrical stimulation of the carotid sinus nerve in the anaesthetized animal (Gesell & Hamilton, 1941; Eldridge, 1973, 1976) or isolated perfusion of the carotid bodies in the awake goat (Engwall *et al.* 1991). This limitation jeopardized the accuracy of our time constant calculations in two ways. First, since we could not be sure of the precise time at which cessation of the primary carotid body stimulus had occurred, we took a conservative estimate of 10 s following the breath in which P_{ET,O_2} had risen > 90 mmHg. We began calculating our time constant for the short-term potentiation from this point. Judging from the fact (in vagally blocked animals) that the significant reduction in V_T following hypoxia occurred prior to this 10 s period we were underestimating the true time constant and therefore the magnitude of the short-term potentiation. An additional, and more serious problem with the time constant calculation occurs in the vagally intact dog which showed a significant prolongation of T_E *prior to* the initiation of our time constant calculation. Thus, the subsequent change in ventilatory output following this T_E prolongation reflected the after effects of the apnoea *in addition* to any effect of short-term potentiation, *per se*. Accordingly, we interpreted the time constants in the two conditions to be only a means of determining whether: (1) the dog showed any significant manifestations of short-term potentiation following brief hypoxic stimulation, which did occur, as evidenced by the significant time constants for V_T and $\dot{V}_I$ in the vagally blocked condition; and (2) the presence of vagal feedback affected the duration of return of ventilatory output to control conditions, which also occurred, as shown by the shorter time constants for recovery of V_T and $\dot{V}_I$ during vagal blockade.

Other potentially confounding factors in our study were the dynamic changes in blood pressure during and immediately following hypoxia as well as the higher blood pressure during normoxia and greater increase in pressure during and following

hypoxia in the vagally blocked trials. It has been previously established that baroreceptor stimulation will reflexly affect ventilatory output and breath timing (Brunner, Sussman, Greene, Kallman & Shoukas, 1982). Accordingly, we would expect some modulation of ventilatory output secondary to the systemic blood pressure changes we observed during and following hypoxia. However, we would not predict that the accentuated rise in systemic blood pressure we observed in the vagally blocked trials could explain the failure of T_E to lengthen following the hypoxia, as it did in the intact trials; to the contrary, the increase in arterial blood pressure, by itself, would be expected to slow breathing frequency and to lengthen T_E (Brunner *et al.* 1982).

Excitatory vs. inhibitory effects following hypoxic stimulation

What factors are known to determine the post-stimulus or post-hyperpnoea respiratory motor output? Two approaches have been made to this question in quite different experimental preparations. First, in the anaesthetized, vagotomized, paralysed, servo-ventilated animal using electrical stimulation of the carotid sinus nerve to provide the sensory input, peak phrenic nerve activity was shown to persist, decaying in an exponential manner following removal of the sensory stimulus (Gesell & Hamilton 1941; Eldridge 1973, 1976; Eldridge & Gill-Kumar, 1978). This after-effect has been termed 'short-term potentiation' and attributed to an intrinsic response of neurones in the respiratory control system to their own increased activity. Second, in sleeping normal humans (Badr *et al.* 1992) and awake goats with separately perfused carotid chemoreceptors (Engwall *et al.* 1991), brief ventilatory stimulation with isocapnic hypoxia is also followed by continued elevation in respiratory output.

This short-term potentiation of ventilatory output may be significantly affected and even overridden by inhibitory chemosensory influences. For example, when hypocapnia was permitted during brief hypoxic exposures in the sleeping human (Badr *et al.* 1992) as in the awake goat (Engwall *et al.* 1991) much of the short-term potentiation was eliminated and both T_E prolongation and reduced V_T were observed immediately following removal of the hypoxia. This inhibitory effect of hypocapnia was not as obvious in the awake human at rest (Georgopoulos *et al.* 1990) or during exercise (Fregosi, 1991). Furthermore, if hypoxic stimulation was prolonged for as little as 5–20 min, even if normocapnia was maintained, apnoea commonly occurred immediately following withdrawal of the hypoxic stimulation (Holtby *et al.* 1988; Georgopoulos *et al.* 1990; Badr *et al.* 1992). We propose that the persistent effect of vagal feedback from the lungs represents yet another inhibitory effect on ventilatory output following stimulus withdrawal.

When will this vagal inhibitory 'memory' effect be manifested under physiological conditions? The answer seems to be simply that both *inhibitory* influences such as hypocapnia and vagal memory, and *excitatory* influences, secondary to short-term potentiation, are operative following removal of the primary stimulus; their summation dictates the ventilatory pattern and amplitude during this recovery period. For example, we note that intact humans and goats do manifest a continued hyperpnoea without prolongation of T_E following brief normocapnic–hypoxic stimulation, and that this differs from what we observed in our intact dogs, even though the conditions of hypoxic stimulation and response were very similar. This

difference may be attributed to the relative strengths of the pulmonary stretch receptor (PSR) inhibitory reflex among species and the magnitude of the V_T (or PSR stimulus) reached *during* hypoxic-induced hyperpnoeas. This difference in findings does not mean that an inhibitory effect attributed to vagal 'memory' does not exist in the human; rather the fact that ventilatory output stayed high and T_E shortened in the human immediately following normocapnic hypoxic stimulation may mean only that the stimulatory effects of short-term potentiation dominated the coincident persistent inhibitory effect of PSR stimulation during the hyperpnoea. Just as we observed in the dog, in the human or other species with a significant Hering–Breuer reflex, we would predict that removal of vagal inhibition would enhance the manifestation of short-term potentiation following withdrawal of the primary stimulus.

Vagally mediated inhibitory memory effects

It has been emphasized, based on findings in the vagally denervated animal, that short-term potentiation will increase with increasing strength of the chemoreceptor stimulus (Eldridge & Gill-Kumar, 1978). On the contrary, in the vagally intact, awake dog, we have observed a paradoxical effect. That is, the prolongation of T_E during recovery was *greater* and the time constant for minute ventilation was *shortened*, the more severe the preceding hypoxic stimulus and the *greater* the magnitude of the tidal volume response during hypoxic stimulation. In other words, in the intact, physiological state, there appear to be simultaneous excitatory and inhibitory effects occurring during the hyperpnoea which have counteracting influences on respiratory motor output and timing in the subsequent post-stimulus period.

Thus, our data show that an inhibitory 'vagal memory' is accumulated during the hyperpnoeic period presumably when the slowly adapting stretch receptors in the lungs are stimulated intermittently and approximately in proportion to the increased tidal volume accompanying hypoxic stimulation. The effect of this 'accumulation' of inhibitory influences is to oppose the short-term potentiation of ventilatory output following removal of the hypoxic stimulus resulting in a significant but short-lived prolongation of T_E, often to the point of frank apnoea. As might be expected from the effects of increased PSR activity, the inhibitory memory effects were confined strictly to T_E prolongation with no persistent effects on the magnitude of tidal volume, which remained elevated following all pauses in breathing pattern. Our data do not address exactly how or whether the vagal inhibitory memory effect and the short-term potentiation facilitory effect influence each other at the level of the inspiratory–expiratory cycling neuronal network in the brain stem (Eldridge & Millhorn, 1986).

Our interpretation of a stretch-receptor-induced inhibitory memory effect is supported by studies of the after-effects of electrical stimulation of the vagi in anaesthetized dogs, cats and piglets. In their classic description of the respiratory-neural 'after-discharge' mechanism in the dog, Gesell & Hamilton (1941) carried out simultaneous intermittent stimulation of vagi and constant stimulation of a cutaneous sensory (saphenous) nerve. Stimulating the saphenous nerve alone usually produced an excitatory after-discharge of ventilation; whereas, immediately following stimulation of both nerves a significant inhibition of spontaneous breathing

pattern occurred, consisting of an apnoea of about 10 s duration and a reduction in V_T over an additional 20 s. Similarly, Lawson (1981) in piglets showed brief 10 s periods of inhibition of phrenic nerve activity following vagal nerve electrical stimulation. On the other hand, Eldridge & Gill-Kumar (1978) used brief electrical stimulation of the vagi in the anaesthetized cat and observed post-stimulus inhibition in 'some cases' but not in the majority of trials. Of further interest in this regard is the additional data of Lawson (1981) which showed a substantial 'inhibitory memory' or after-effect on reduced phrenic nerve activity following laryngeal nerve electrical stimulation; and this inhibitory effect lasted much longer than that following vagal stimulation. Perhaps, then, in the intact awake animal undergoing hyperpnoea via the upper airway, the inhibitory after-effects on respiratory motor output may be even more prolonged than we have found in the tracheotomized animal.

The effects of vagal denervation on short-term potentiation following carotid sinus nerve stimulation have been tested in the anaesthetized cat (Eldridge, 1973, 1976) and some but not all findings are in apparent agreement with our data obtained in the awake dog following brief hypoxic stimulation. Our findings that vagal blockade results in longer time constants for elevation of the ventilatory output, frequency and tidal volume following hypoxic exposure are consistent with the longer time constants for elevated phrenic nerve activity following carotid sinus nerve electrical stimulation in the vagotomized cat (compared to the vagally intact cat) (Eldridge, 1976). However, other studies in the spontaneously breathing anaesthetized cat (Eldridge, 1973) show no clear effect of vagotomy on the timing of phrenic motor nerve activity following carotid sinus nerve stimulation. There are, of course, many very significant differences between studies including species and the relative strength of the Hering–Breuer reflex, wakefulness *vs.* anaesthesia, and the contrasting types of sensory stimuli, i.e. hypoxemia *vs.* carotid sinus nerve electrical stimulation. Of special importance may be the relatively small magnitude of the increase in tidal volume in the intact cats which averaged about 170% of control during carotid sinus nerve stimulation. According to the V_T:$T_{E,max}$ correlation found in our study (see Fig. 5) these increases in V_T would not be expected to elicit major prolongations in T_E following withdrawal of chemoreceptor stimulation.

Implications for breathing instability

What is the importance of our findings to breathing stability and periodic breathing? During sleep in normoxia and especially in hypoxia, the levels of ventilatory stimuli commonly undergo brief but frequent changes. These perturbations might include changes in one or more of P_{a,O_2} or P_{a,CO_2}, sleep state, upper airway resistance or body posture. The facilitory, short-term potentiation mechanism may be viewed as a stabilizer of rhythmic ventilatory output under these conditions, because breathing pattern will remain normal and tidal volume will remain high for the period immediately following stimulus removal. On the other hand, the inhibitory effects of vagal memory would be expected to exert a destabilizing influence on ventilatory output following removal of the primary stimulus. This short-lived mechanoreceptor-induced inhibitory effect might contribute, along with transient hypocapnia, to the T_E prolongation, apnoeas and periodicities in breathing pattern often observed in non steady-state periods, especially during sleep. We would

also predict that the relative contribution from the 'vagal memory' inhibitory mechanism would vary directly with the magnitude and perhaps duration of the stretch receptor activation and the strength of the Hering–Breuer inflation reflex.

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