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RESPIRATORY MUSCLE RECRUITMENT DURING SELECTIVE CENTRAL AND PERIPHERAL CHEMORECEPTOR STIMULATION IN AWAKE DOGS

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SUMMARY

1. In four awake dogs we measured EMG activity of three inspiratory and four expiratory muscles during sustained central chemoreceptor stimulation (CO_2 inhalation), and peripheral chemoreceptor stimulation (intravenous infusion of almitrine bismesylate (almitrine)). By using this selective pharmacological stimulation of the peripheral chemoreceptors and reversibly cold-blocking pulmonary stretch receptors, we were able to determine the effects of each type of stimulation on respiratory muscle recruitment in the absence of such complicating influences as pulmonary stretch receptor feedback, cerebral hypoxia or hypocapnia, and differences in breathing pattern.

2. During 10 min of steady-state hyperpnoea (minute ventilation $\dot{V}_I$, approximately twice eupnoea) caused by either hypercapnia or isocapnic stimulation of the carotid bodies with almitrine, all three inspiratory and all four expiratory muscles demonstrated significant and sustained elevations in EMG activity.

3. With both types of chemoreceptor stimulation, as tidal volume, V_T, increased, so did the mean electrical activities of the crural diaphragm ($r = 0{\cdot}88$), costal diaphragm ($r = 0{\cdot}93$), parasternals ($r = 0{\cdot}82$), triangularis sterni ($r = 0{\cdot}74$), transversus abdominis ($r = 0{\cdot}77$), external obliques ($r = 0{\cdot}68$) and internal intercostals ($r = 0{\cdot}75$).

4. In each dog, the response of ventilation and of the diaphragmatic EMG to a given level of central or peripheral chemoreceptor stimulation is highly reproducible from one test day to the next. On the other hand, accessory inspiratory and expiratory abdominal and rib cage muscles in two of the four dogs showed highly significant changes from day to day in the amount of their EMG activity at any given V_T.

5. During steady-state ventilatory stimulation, 2 min intervals were chosen during which the two types of chemoreceptor stimulation had caused hyperpnoeas with similar values for V_T, total time per breath (T_{TOT}) and inspiratory time divided by the total time (T_I/T_{TOT}). Comparison of EMG activities during these matched hyperpnoeas revealed that there were no differences in the activities of any of the

muscles between the two forms of stimulation. We conclude that peripheral chemoreceptor stimulation causes significant and sustained recruitment of expiratory muscles even in the absence of pulmonary feedback and that both expiratory and inspiratory muscles are recruited to the same extent during peripheral chemoreceptor stimulation as they are during an identical hyperpnoea caused by central chemoreceptor stimulation.

INTRODUCTION

The pattern of inspiratory and expiratory muscle recruitment during a hyperpnoea is thought to be strongly influenced by the nature of the hyperpnoeic stimulus, that is, whether it acts on central or peripheral chemoreceptors. Specifically, it has been demonstrated that a hypercapnia-induced hyperpnoea (predominantly central chemoreceptor stimulation) results in a greater recruitment of abdominal and rib cage expiratory muscles than does a hyperpnoea caused by hypoxia (Sears, Berger & Phillipson, 1982; Fregosi, Knuth, Ward & Bartlett, 1987; Smith, Ainsworth, Henderson & Dempsey, 1989). These observations, that the non-specific carotid body stimulant hypoxia causes either a 'relative' or 'absolute' inhibition of expiratory muscles, have led some investigators to conclude that carotid body stimulation causes a pattern of muscle recruitment that is qualitatively different from that observed during a hypercapnia-induced hyperpnoea. This position fits well with neuroanatomical data which indicates that stimulation of peripheral chemoreceptors results in activation of only a subgroup of the medullary respiratory neurons which are activated by central chemoreceptor stimulation (St John, 1981).

Previous studies which have attempted to compare the effects of peripheral *vs.* central chemoreceptor stimulation on respiratory motor output have included complicating factors which make their interpretation difficult. The present study attempts to eliminate or to control several of these complicating factors. First, we have avoided the use of hypoxia for stimulation of peripheral chemoreceptors since hypoxia may have secondary effects on respiratory motor output via central nervous system excitation (Miller & Tenney, 1975) or depression (Neubauer, Melton & Edelman, 1990) and/or its ability to cause increases in cerebral blood flow. Second, the often unpredictable effects of anaesthesia and its depth, type and duration on the selective 'responsiveness' of the various respiratory muscles (Gilmartin, Ninane & De Troyer, 1987) makes it essential that a study such as the present one, which seeks to compare muscle responsiveness during different types of stimulation, be performed in unanaesthetized animals. Third, eliminating vagally mediated feedback from the lungs is critical when attempting to determine as much as possible the isolated effect of either carotid body or central chemoreceptor stimulation on respiratory muscle recruitment, since stimulation of pulmonary stretch receptors has been shown to alter the pattern of respiratory muscle recruitment during a hyperpnoea in unanaesthetized dogs (Ainsworth, Smith, Johnson, Eicker, Henderson & Dempsey, 1990). In fact some investigators believe that stimulation of pulmonary stretch receptors is *mandatory* for recruitment of abdominal expiratory muscles – at least in anaesthetized animals (Russell & Bishop, 1976). Finally, recent studies have noted that respiratory muscles often respond in a heterogeneous way to a given resistive load, hyperpnoeic stimulus, or postural change (De Troyer & Ninane, 1987;

Ainsworth, Smith, Eicker, Henderson & Dempsey, 1989; Leevers & Road, 1989). These observations highlight the necessity of measuring the activity of as many different muscles as possible, including both rib-cage and abdominal muscles, and of controlling posture.

The purpose of this study was to compare, in awake dogs and in the absence of vagally mediated feedback from the lungs, the pattern of respiratory muscle recruitment observed during systemic hypercapnia to that observed during the isocapnic stimulation of the carotid bodies via infusion of the drug almitrine, which has been shown to selectively stimulate the carotid bodies (Bisgard, 1981). Special attention was paid to comparing the patterns of respiratory muscle recruitment during equal levels of ventilatory output and similar breathing patterns.

METHODS

Animal instrumentation. Four female dogs weighing between 20 and 35 kg were chronically instrumented in a series of three or four surgical sessions conducted under general anaesthesia (0·5–1·5% halothane in O_2) using aseptic procedures. During the first of these sessions both vagosympathetic trunks and one carotid artery were relocated into separate exteriorized skin folds. After healing was complete, a chronic tracheostomy was constructed in each dog. During the final one or two sessions, stainless-steel bipolar electrodes for recording electromyograms (EMGs) were placed in three inspiratory muscles, namely the crural and costal portions of the diaphragm and the parasternal intercostals, a chest-wall muscle. EMG electrodes were also placed in two chest-wall expiratory muscles (internal intercostal, triangularis sterni) and two abdominal expiratory muscles (transversus abdominis, external oblique) (Ainsworth *et al.* 1989; Smith *et al.* 1989). After recovery from surgery it was observed that some electrodes did not function adequately. Because of this, data from the parasternal muscles was only recorded in three of the four dogs and data from the costal diaphragm, triangularis sterni and external oblique muscles were only measured in two of the four dogs. Surgical procedures were performed at least 1 month prior to data collection sessions. All procedures were approved by the Animal Care Committee, University of Wisconsin, Madison.

Protocol. On the day of an experiment, the dog to be studied was intubated with a cuffed endotracheal tube through which it breathed for the duration of the experiment. A pneumotachograph, which had been calibrated using five known flow rates, was then attached to the endotracheal tube. A T-piece was attached to the pneumotachograph so that the dog would draw its inspired air from a 'bias flow' of 30–40 l/min. End-tidal CO_2 concentration was measured with an LB-2 CO_2 analyser. The dog was then placed in a stanchion where it either stood or sat for the duration of the data collection session.

Prior to each testing session and before vagal cooling, EMGs were recorded during a hyperpnoea induced by exposing the dog to 6·5% inspired CO_2 in air for 5–10 min. All subsequent EMG values were divided by the value measured during this hyperpnoea and expressed as a percentage of this reference EMG activity.

To determine the strength of the Hering–Breuer reflex in each dog prior to vagal blockade, three passive hyperinflations of 1 l each were administered at end-expiration. These were performed by simultaneously occluding an expiratory valve and injecting 1 l of air through an inspired valve via a large syringe. The resulting apnoeas averaged 15 s or 3·5 times control expiratory time (T_E).

To block neural transmission in the vagi, cold propylene glycol (−3 to −8 °C) was circulated through radiators surrounding each vagosympathetic trunk (Phillipson, Hickey, Bainton & Nadel, 1970). In order to conclude that a 'cold' vagal block had been established three criteria had to be met: (1) a 1 l hyperinflation at end expiration produced no prolongation of T_E, (2) tachycardia (vagal block increased heart rate from an average of 109 to 185 beats/min), and (3) the presence of bilateral Horner's syndrome. The dogs then remained vagally blocked for the duration of the experiment.

Once vagal block had been established (5–15 min after the start of vagal cooling) and the dog demonstrated a steady, relaxed breathing pattern, collection of ventilatory and EMG data was begun. After collection of 5–10 min of eupnoeic control data, inspired CO_2 was elevated to a fixed

concentration between 2·5 and 4% for 10 min. This was followed by a 1–2% further increase in inspired CO_2 concentration for an addition 5–10 min. During some experiments a third level of elevated inspired CO_2 was administered for at least 5 min. Following a second air breathing control period of 5 min almitrine bismesylate was administered into a leg vein via an acutely placed

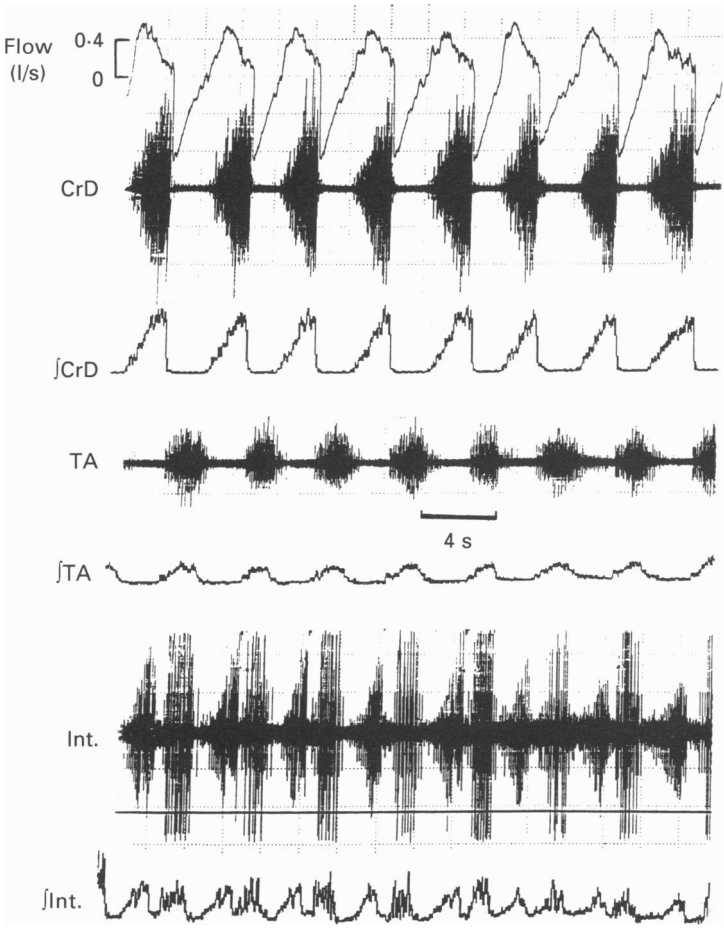

Fig. 1. Example of breath-by-breath flow rate (l/s BTPS (body temperature and pressure when saturated with water vapour), inspiration up) and raw respiratory muscle EMGs with corresponding moving time averages for selected muscles during 3·5% CO_2 inhalation. Note the 'contamination' of internal intercostal EMG with an inspiratory muscle EMG. CrD, crural diaphragm; TA, transversus abdominis; Int., internal intercostal.

catheter. Pilot data had established that in order to create a steady hyperpnoea with intravenous almitrine, a bolus of 14 ml (1 mg/ml) administered over 25 s followed by a continuous infusion at a rate of 0·4 ml/min would give the best results. This bolus–infusion protocol resulted in a hyperventilation which lowered end-tidal CO_2 partial pressure (P_{ET,CO_2}) within 1 min. In order to re-establish isocapnia, 1–2% CO_2 was added to the inspired air. This isocapnic hyperpnoea was maintained for 10 min. During several sessions, a second bolus of almitrine (3 ml) followed by an infusion, was administered at the end of the 10 min of isocapnic hyperpnoea. This resulted in an additional increase in minute ventilation ($\dot{V}_I$) which was maintained isocapnic for 6–10 min more. Infusion of the carrier solution alone during two pilot experiments resulted in no change in $\dot{V}_I$.

Data analysis. EMG signals were amplified, rectified and moving-time-averaged with a time constant of 100 ms. The moving-time-averaged signals passed through an A–D board onto a microcomputer for storage. Custom software allowed real-time viewing of both the moving-time-averaged EMGs and flow signal on a microcomputer during the experiment (Fig. 1).

Analysis of the moving-time-averaged EMGs and flow signal were performed using software which had previously been validated by comparison with hand-digitized moving-time-averaged EMG signals. This software first determined the location (timing) of inspiration and expiration by examining the flow signal for zero crossings. The accuracy of the computer in detecting breath locations was then checked by hand and reset where necessary. The program could then be instructed to examine a moving-time-averaged EMG signal and quantify phasic bursts of activity occurring during either inspiration or expiration depending on the muscle in question. EMGs are reported as the mean electrical activity (MEA) for a breath. MEA is calculated as the area of the moving-time-averaged EMG for a breath divided by the duration of its electrical activity (Ledlie, Pack & Fishman, 1983). MEA for each breath (for each muscle) was then expressed as a percentage of the MEA measuring during 6·5 % CO_2 breathing before vagal cooling.

During each testing session five to twelve 2 min sections of data were analysed. Sections chosen for analysis included the 2 min prior to the start of CO_2 breathing, minutes 1–3, 5–7 and 8–10 after the start of each level of CO_2 breathing, the 2 min prior to the bolus–infusion of almitrine and 1–3, 5–7 and 8–10 min after the start of the isocapnic almitrine-induced hyperpnoea. During each of these 2 min sections, MEA EMGs, flow rate and P_{ET,CO_2} were measured for each breath. During several testing sessions, movement of the dog during one of these 2 min periods necessitated that the data be excluded from further analysis.

At least two successful sessions were completed on each of four dogs. In three of the four dogs, arterial blood gases were sampled from a catheter placed in the carotid artery as described by Smith *et al.* (1989) to determine how well arterial isocapnia was being maintained during the almitrine infusion.

RESULTS

Time course of $\dot{V}_I$ and EMG responses to chemoreceptor stimulation

The time-dependent effects of CO_2 inhalation and isocapnic almitrine infusion on ventilatory output and respiratory muscle recruitment are shown on a breath-by-breath basis for one experiment in Fig. 2*A* and *B*. After the onset of 4 % CO_2 inhalation at time = 240 s (arrow) in Fig. 2*A*, $\dot{V}_I$, crural diaphragm EMG activity and parasternal EMG activity reached their steady-state values in 3–4 min and remained nearly constant for the remaining 6–7 min. Expiratory muscle EMGs also increased to their steady-state values in approximately 4 min, with abdominal muscles in this example demonstrating an additional brief augmentation of activity at time = 700–720 s. In Fig. 2*B* the bolus–infusion of almitrine was started at 290 s (arrow) and an increase in $\dot{V}_I$ (with a transient decrease in P_{ET,CO_2}) was observed at 330 s. CO_2 was gradually added to the inspired air with isocapnia achieved at 500 s. After establishing isocapnia, $\dot{V}_I$, crural diaphragm and parasternals remained constant for the remainder of the experiment. Expiratory muscle EMGs increased and remained elevated, with the abdominal muscles again demonstrating a brief additional augmentation of activity at time = 1200 s.

Time courses for changes in ventilation and EMG activities are shown in Fig. 3 as averages of all ten trials in the four dogs during one level of CO_2 inhalation and one infusion dose of isocapnic almitrine. During the hypercapnic stimulation P_{ET,CO_2} rose to its steady-state level (10 mmHg > control) within 1 min of the onset of CO_2 inhalation. The ventilatory response to this hypercapnia consisted entirely of an increase in tidal volume (V_T) with no change in breathing frequency. V_T and $\dot{V}_I$ reached their steady-state values approximately 3 min after the start of CO_2

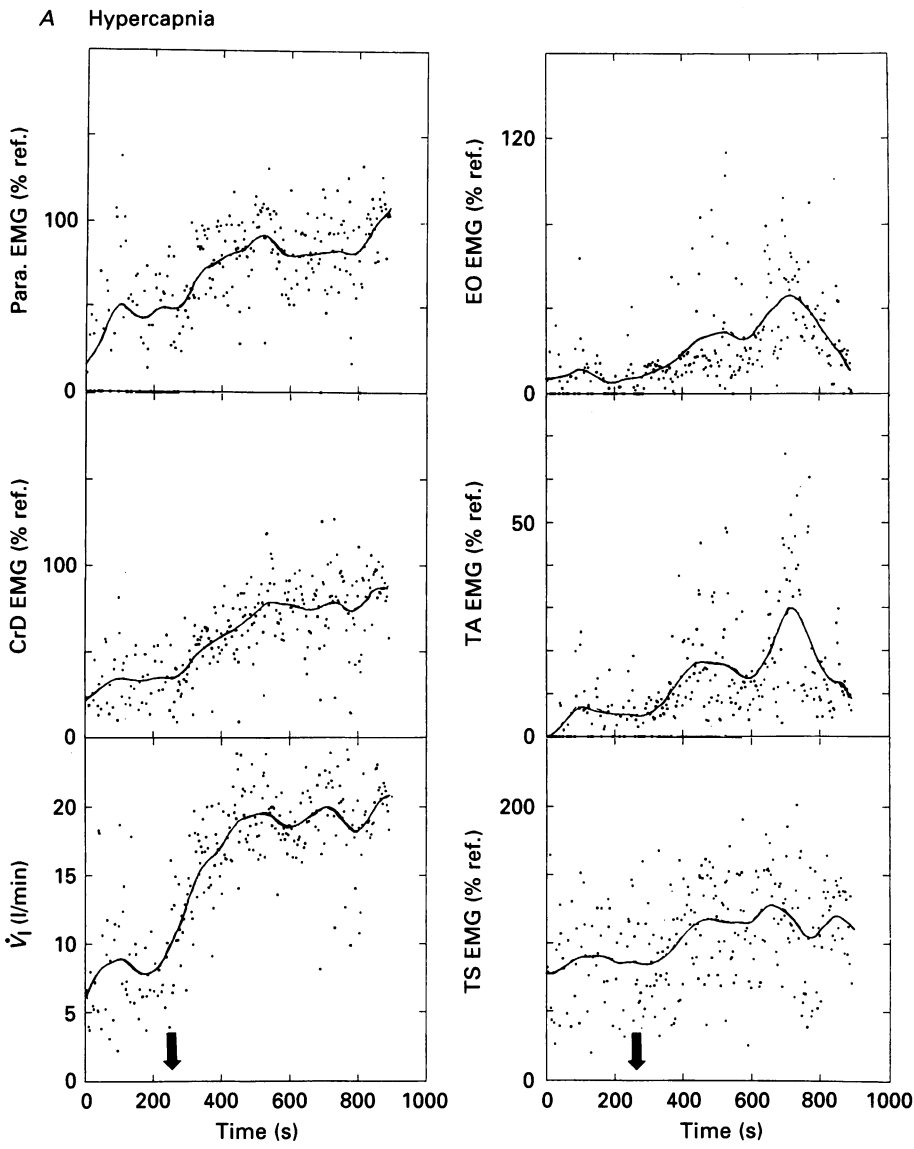

Fig. 2. For legend see facing page.

inhalation. EMG activities increased with a time course very similar to that of V_T, generally reaching their steady-state values after 3–4 min of CO_2 inhalation. The one exception to this was the external oblique muscle which continued to increase in activity for 5 min. All muscles demonstrated a sustained elevation of activity throughout the 10 min of elevated inspired CO_2.

Almitrine administration resulted in an increase in $\dot{V}_I$ and a decrease in P_{ET,CO_2} within 30 s of the end of the bolus. Once this hyperventilation had begun, enough CO_2 was quickly added to the inspired air so that P_{ET,CO_2} was subsequently maintained within 1 mmHg of the pre-stimulation control values. During the three

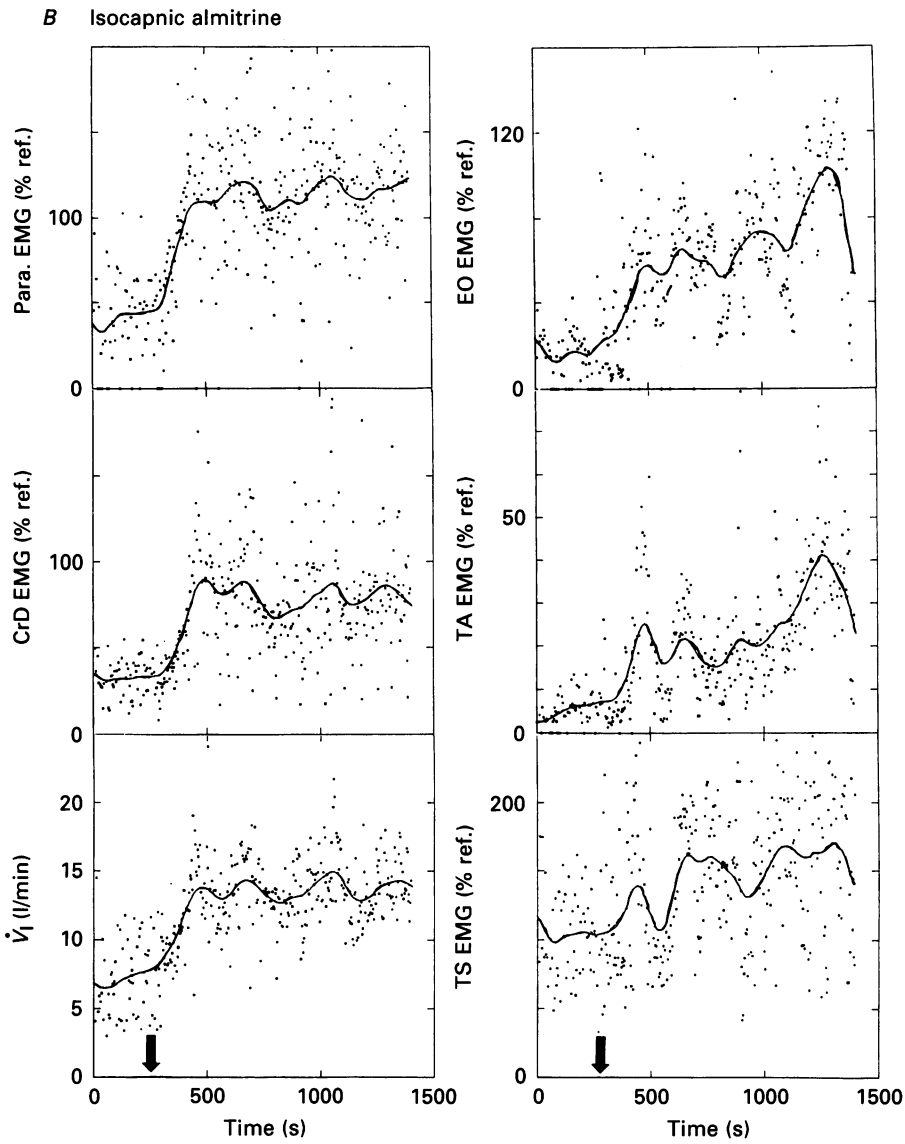

Fig. 2. Breath-by-breath ventilatory and mean electrical activity EMG data from dog B during one trial of hypercapnia (inspiratory CO_2 fraction, $F_{I,CO_2} = 0{\cdot}04$) (*A*), isocapnic almitrine (*B*). Minute ventilation and two inspiratory muscles are in the three left panels of each figure. Expiratory muscles are in the three right panels of each figure. EMGs are expressed as percentage of the value measured during reference hyperpnoea ($F_{I,CO_2} = 0{\cdot}065$, pre-vagal block). Lines through the data are least-squares regression lines fitted by computer. Arrows indicate start of CO_2 inhalation and start of almitrine bolus–infusion (see text). Para, parasternal intercostal; EO, external oblique; TS, triangularis sterni.

experiments where arterial blood gases were measured during steady-state almitrine infusion, arterial CO_2 pressure, P_{a,CO_2}, was within +1 to −2 mmHg of control P_{a,CO_2}, with the average being 0·2 mmHg below control (Table 1). As was the case with

hypercapnic stimulation, the increase in $\dot{V}_I$ was the result of an increase in V_T with no increase in breathing frequency. V_T and $\dot{V}_I$ reached their steady-state values approximately 2 min after isocapnia had been established and remained constant for the remaining 10 min. Muscle recruitment followed a time course very similar to that

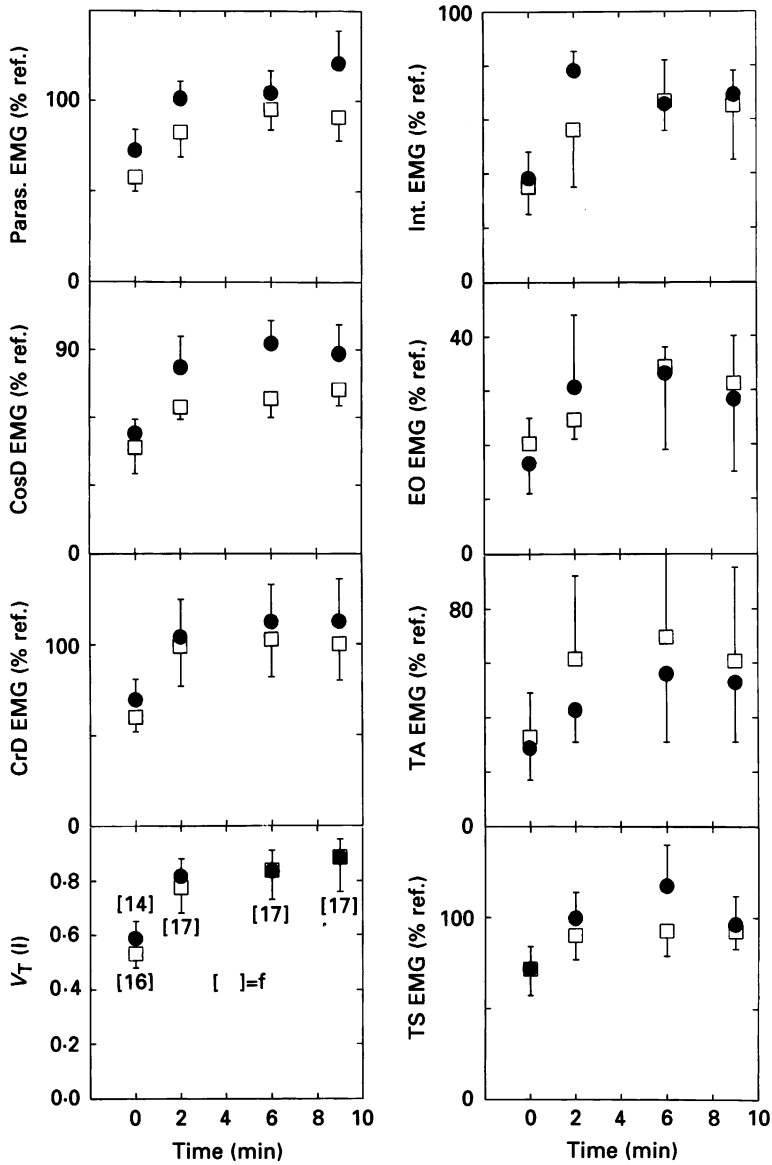

Fig. 3. Average time courses of the ten trials (performed in four dogs) for increases in ventilation and EMGs during both hypercapnia and isocapnic almitrine induced hyperpnoeas. ●, Almitrine; □, hypercapnia. Cos D, costal diaphragm.

of V_T with all inspiratory and expiratory muscles reaching their steady-state levels of activity within 2–3 min of the start of isocapnia. The activity of all of the muscles remained elevated for the duration of the ventilatory stimulation.

Relationships between V_T and EMG activities during both types of ventilatory stimulation

Fig. 4*A–D* summarizes all of the experiments performed on each dog. Dogs A, C and D were always sitting during an experiment while dog B remained standing throughout the experiments. Each point represents a 2 min average (25–40 breaths)

TABLE 1. End-tidal P_{CO_2}, arterial blood gases and pH during control breathing and steady-state hyperpnoeas caused by hypercapnia or isocapnic almitrine (mean ± S.E.M.)

	P_{ET,CO_2} (mmHg)	P_{a,CO_2} (mmHg)	P_{a,O_2} (mmHg)	pH
Control ($n = 4$)	33·8 ± 2·1	32·9 ± 2·6	101·1 ± 2·8	7·393 ± 0·017
Isocapnic almitrine ($n = 5$)	34·1 ± 1·4	32·7 ± 1·1	121·5 ± 2·5	7·398 ± 0·008
Hypercapnia ($F_{I,CO_2} = 0·03$–$0·05$; $n = 5$)	44·3 ± 2·8	41·9 ± 2·6	114·0 ± 7·1	7·320 ± 0·018

P_{a,O_2}, arterial oxygen pressure; F_{I,CO_2}, inspiratory carbon dioxide fraction.

taken either during control or minutes 1–3, 5–7 or 8–10 of each level of almitrine infusion or hypercapnia. A strong linear relationship was found between V_T and $\dot{V}_I$ (average $r = 0·90$) indicating that breathing frequency was fairly constant throughout all of the experimental sessions. We found no difference between hypercapnia and almitrine administration in the relationship of increasing V_T to EMG activity; thus data from both types of stimulation were pooled to determine the correlation coefficients for each muscle's response in each dog. During each of the testing sessions V_T correlated strongly with inspiratory muscle EMG activity, with r values averaging 0·88, 0·93 and 0·82 respectively for crural diaphragm, costal diaphragm and parasternals. Expiratory muscle activities also showed significant positive correlations with V_T. These correlations were slightly lower than those between V_T and inspiratory muscle EMG averaging 0·74 ($n = 5$ trials), 0·77 ($n = 8$ trials), 0·68 ($n = 6$ trials) and 0·75 ($n = 6$ trials) for triangularis sterni, transversus abdominis, external oblique and internal intercostals respectively.

Comparing data from different days demonstrates not only that the $\dot{V}_I$:V_T relationship remained constant within a testing session, but that it did not change between testing sessions. Similarly the V_T:crural EMG activity and V_T:costal diaphragm EMG activity relationships from different days were always nearly identical for a given dog, as can be seen in Fig. 4*A–D*. Of the three dogs with parasternal EMG electrodes, dogs B and D showed little between-test variability while in dog A parasternals were recruited to a much greater extent on day 1 than on day 2. Similarly, in dogs A and B, where expiratory muscle EMGs and V_T were highly correlated *within* a session, there were systematic *day-to-day* changes in the magnitude of the recruitment of these expiratory muscles. This can be seen in the triangularis sterni and transversus abdominis of dog A, and the transversus abdominis and external oblique of dog B. In dogs C and D on the other hand, the amount of expiratory muscle EMG activity at a given V_T remained very similar from day-to-day. In all cases where day-to-day changes were observed in V_T:EMG relationships, the differences held whether the relationships were described using EMG activity in relative terms (% reference, as plotted) or in absolute (arbitrary) units.

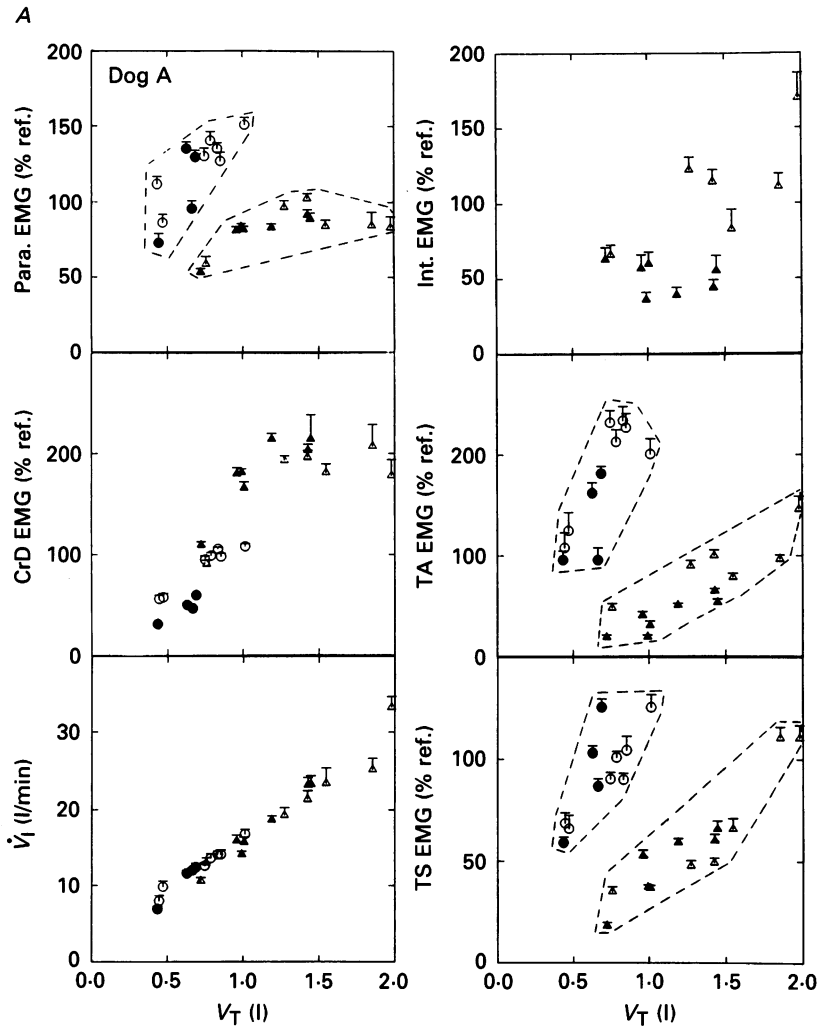

Fig. 4*A*. For legend see page 625.

It should be pointed out that whether or not day-to-day changes were observed in the V_T *vs.* EMG relationship for a given muscle at a given V_T on a given day, the amount of inspiratory or expiratory muscle EMG is essentially the same during both the isocapnic almitrine infusion and CO_2 inhalation conditions. One exception to this occurred with the internal intercostal muscle in dog A which did not increase EMG activity during isocapnic almitrine stimulation, but which did recruit during CO_2 inhalation.

Matched hyperpnoeas

As previously mentioned, the major aim of this study was to compare the amount of respiratory muscle activity during hyperpnoeas caused by two different forms of ventilatory stimulation. This type of comparison can best be made when the two hyperpnoeas are (1) very similar in their magnitude and breath cycle timing and (2)

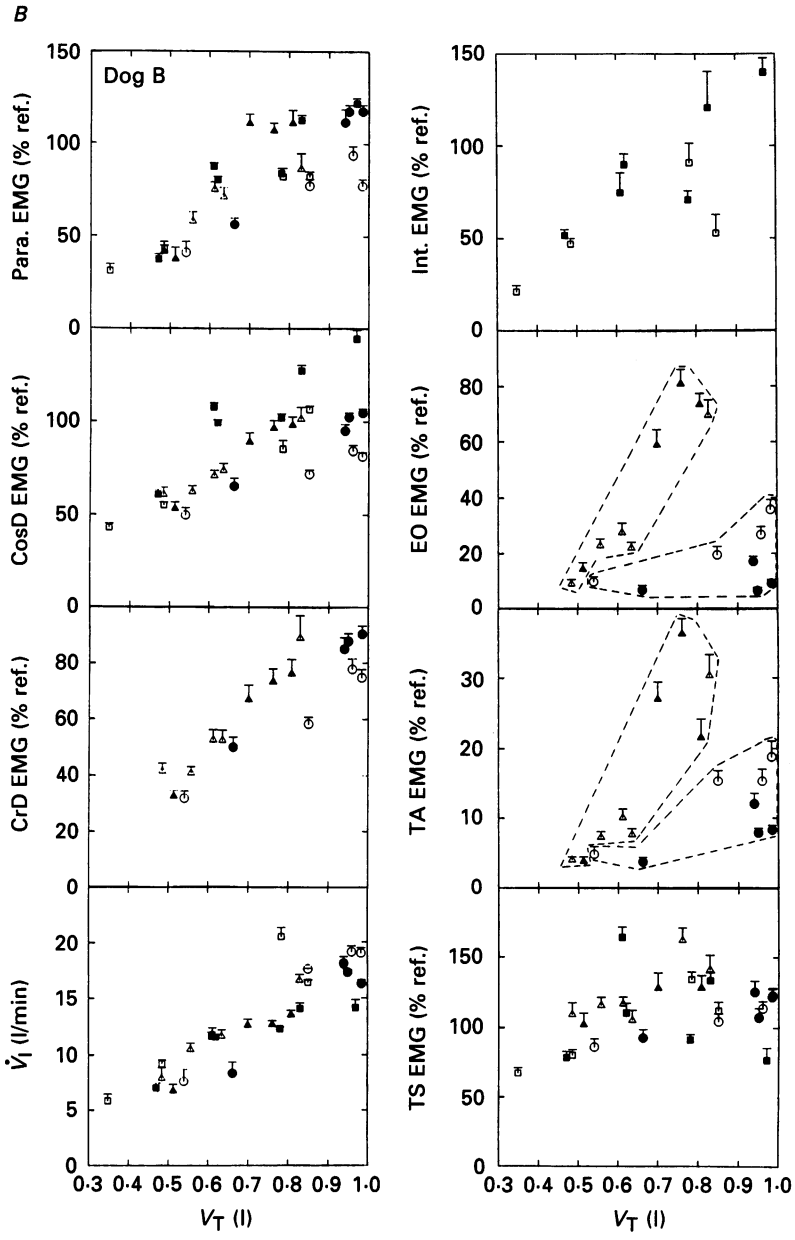

Fig. 4*B*. For legend see page 625.

recorded during the same testing session. In fact during most trials, hyperpnoeas caused by CO_2 inhalation and isocapnic almitrine infusion were similar enough so that this type of comparison could be made (Fig. 5).

During seven trials the average tidal volume for both stimuli was 0·86 l, the values for the total time per breath (T_{TOT}) were 3·65 and 3·73 s and the ratios for inspiratory time divided by total time (T_I/T_{TOT}) were 0·53 and 0·51 respectively. This V_T represents an increase of approximately 56% over control conditions. Since not all

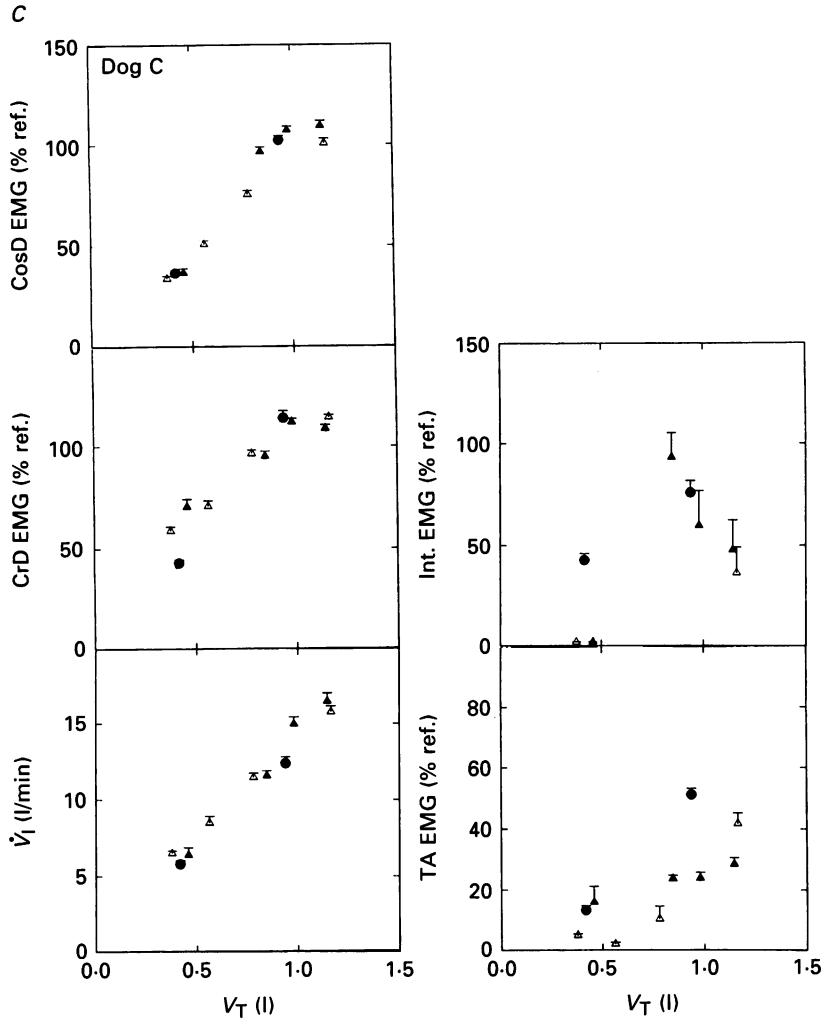

Fig. 4*C*. For legend see facing page.

of the dogs had functioning electrodes in all seven of the muscles, the number of trials with each muscle is listed in the legend. There were no significant ($P < 0{\cdot}05$) differences in the amounts of EMG activity during the two forms of ventilatory stimulation. All three inspiratory muscles did, however, show a slightly elevated level of activity during isocapnic almitrine stimulation as compared to hypercapnia.

For eight trials a second set of matched hyperpnoeas was established at a slightly higher V_T and slightly longer T_{TOT}. During these hyperpnoeas V_T averaged 1·01 l for both conditions, T_{TOT} averaged 4·19 and 4·07 s, and T_I/T_{TOT} were 0·52 and 0·49 for almitrine- and hypercapnia-induced hyperpnoeas, respectively. EMG values during these matched hyperpnoeas are shown in Fig. 5. Again, there were no significant differences between the amounts of EMG activity during the two types of ventilatory stimulation for any of the muscles, but all three inspiratory muscles were slightly more active during isocapnic almitrine stimulation than during hypercapnia.

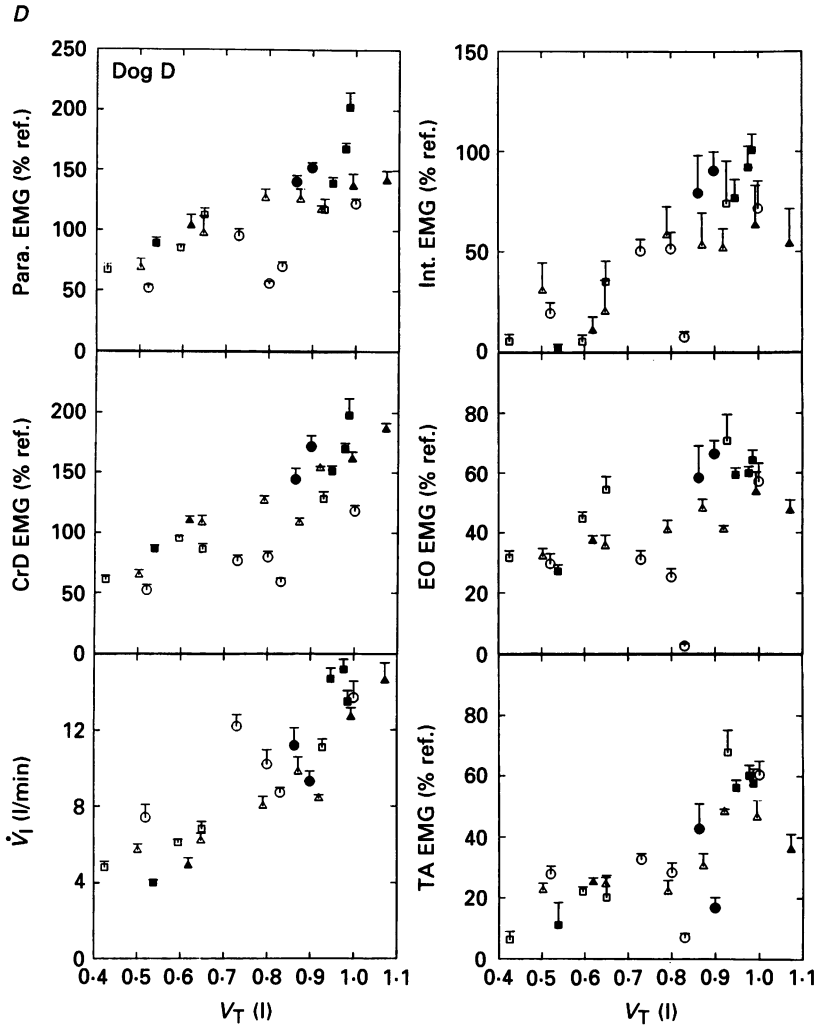

Fig. 4. Data from all testing sessions on four dogs A–D. Each point is the average for all (25–40) breaths within a 2 min period ± S.E.M. Dashed lines enclose data points from each testing session where muscles demonstrated obvious day-to-day changes in their recruitment patterns. Note that within a testing session, and in some cases when all testing sessions are combined, both the breaths stimulated by hypercapnia and isocapnic almitrine infusion appear to fall on the same line. Eupnoeic control data are the two or three points with the smallest V_T for each day. Circles indicate data from day 1, triangles data from day 2 and squares data from day 3. Open symbols, hypercapnia; filled symbols, isocapnic almitrine infusion.

DISCUSSION

The purposes of this study were (1) to determine if selective, sustained stimulation of the carotid bodies would cause recruitment or inhibition of expiratory muscles, and (2) to contrast quantitatively respiratory muscle recruitment during carotid body stimulation to that observed during an equal level of central chemoreceptor

stimulation. Our data demonstrate that when ventilation is increased in the awake dog by sustained, selective peripheral chemoreceptor stimulation, expiratory muscles are not only recruited, but are recruited to the same extent as during an identical hyperpnoea caused by central chemoreceptor stimulation. This sustained increase in

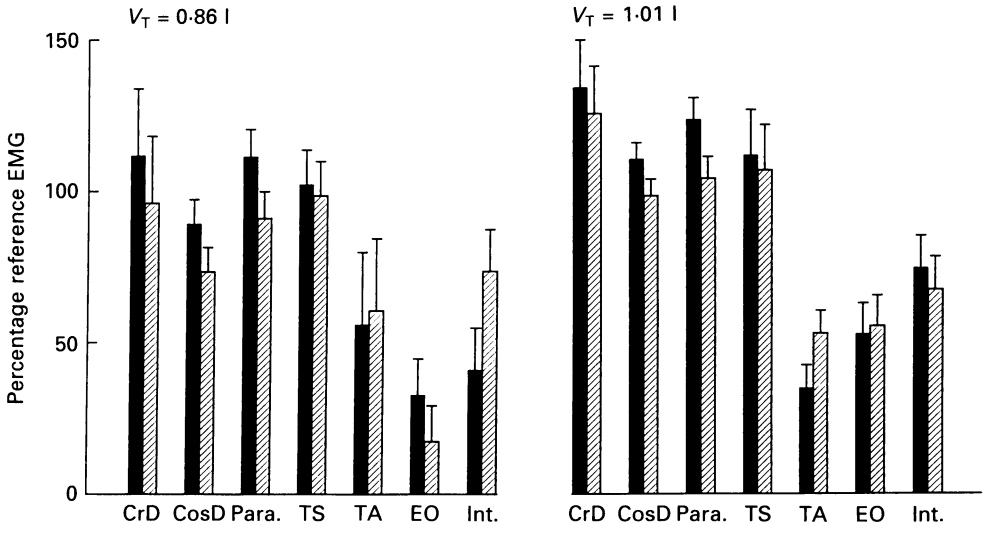

Fig. 5. Comparison of EMG activities (mean ± S.E.M.) during matched steady-state hyperpnoeas caused by hypercapnia ▨ and isocapnic almitrine ■ infusion. For match 1, V_T = 0·86 l, T_{TOT} = 3·73 and 3·65 s, T_I/T_{TOT} = 0·51 and 0·53 for CO_2 and almitrine stimulation respectively. Of the seven trials included in match 1, crural diaphragm, parasternals and transversus abdominis were recorded in six, costal diaphragm and triangularis sterni were recorded in five, and external oblique and internal intercostals were recorded in three. For match 2, V_T = 1·01, T_{TOT} = 4·07 and 4·19 s, T_I/T_{TOT} = 0·49 and 0·52 for CO_2 and almitrine-induced stimulation respectively. Of the eight trials included in match 2 crural diaphragm, parasternals and transversus abdominis were recorded in seven, internal intercostals were recorded in six, external oblique was recorded in five, and costal diaphragm and triangular sterni were recorded in four.

EMG activity was apparent in all four of the expiratory muscles we studied and was independent of vagal feedback. It was also observed that over the range of tidal volumes studied, diaphragmatic EMGs increased linearly with V_T and demonstrated little day-to-day variability. On the other hand expiratory muscle and accessory inspiratory muscle EMG activities, while usually correlated with V_T, often showed marked day-to-day changes in their level of activity at a given V_T.

Advantages and limitations of our experimental model

Our chronically instrumented, vagally blocked dog model provides us with several advantages when attempting to compare respiratory muscle recruitment patterns during central *vs.* peripheral chemoreceptor stimulation. First, our use of awake animals ensured that the responsiveness of respiratory muscles to chemical stimuli was not compromised by anaesthetics. This was critical since it has been noted that anaesthetics selectively inhibit some respiratory muscles to a greater extent than

others (Fregosi *et al.* 1987), and that this selectivity of inhibition may or may not be the same during central *vs.* peripheral chemoreceptor stimulation. Using awake animals also permitted us to study respiratory muscle recruitment patterns during a wide range of hyperpnoeas up to four times greater than control ventilation in some instances. Second, in our vagally blocked animals the ventilatory response to both types of chemoreceptor stimuli was almost entirely one of an increase in tidal volume, thus we were able to compare respiratory muscle recruitment during the two types of chemoreceptor stimuli at equal breathing frequencies, tidal volumes, and inspiratory and expiratory times. This fortuitous occurrence overcame the serious problem encountered in previous studies when peripheral chemoreceptor stimuli usually caused a more tachypnoeic response than did CO_2 stimulation, making it difficult to compare EMG responses to the different stimuli.

Third, we were able to control factors which may mask the effects of carotid body stimulation *per se* on muscle recruitment. To this end, we avoided the effects of CNS hypoxia and secondary medullary hypocapnia by using a pharmacological stimulus to the carotid body. Additionally, by using vagal blockade we avoided secondary effects on inspiratory and expiratory muscle recruitment due to activation of pulmonary stretch receptors (Russell & Bishop, 1976). Finally, the morphological diversity of respiratory muscles with regard to important characteristics such as muscle spindle density (Duron & Marlot, 1980) and fibre type, makes it increasingly clear that each muscle is unique and that findings made in one inspiratory or expiratory muscle cannot necessarily be extrapolated to others. Examples of stimuli which cause *selective* recruitment (or derecruitment) of respiratory muscles include chemical stimuli, posture, vagal blockade and anaesthetics (De Troyer & Ninane, 1987; Fregosi *et al.* 1987; Smith *et al.* 1989; Ainsworth *et al.* 1990). The inability to generalize about respiratory muscle control was addressed in the present study by recording from three inspiratory and four expiratory muscles.

Our approach also has limitations. First, we used almitrine to stimulate carotid bodies in order to avoid the secondary complications of cerebral hypoxia. However, we cannot be sure that almitrine precisely mimicked the effects of hypoxia on the carotid body. None the less, we feel that the effects of almitrine on the carotid bodies must at least approximate those of hypoxia because (1) both hypoxia and almitrine elicit similar patterns of discharge in few-fibre recordings of the carotid sinus nerve (Bisgard, 1981), (2) the hyperpnoea caused by almitrine infusion is abolished by carotid body denervation (Bisgard, 1981; De Backer, Vermeire, Bogaert, Janssens & Van Maele, 1985).

Second, it might be questioned whether our experimental model using awake animals was advisable since it is well established that changes in posture and/or chest-wall configuration, such as those that may occur in awake animals, will exert tonic or phasic influences on expiratory muscle activity of the trunk (Duron & Marlot, 1980; Farkas, Baer, Estenne & De Troyer, 1988) (see below). While this added to the variability in our measurements, we believe it was not a serious problem, since within a testing session in a given dog, the increase in inspiratory and expiratory muscle EMG per increase in V_T remained constant from the beginning to the end of a test, a phenomenon one would not expect if there were large time-dependent postural effects on muscle activity occurring during the testing session.

Third, it should be remembered that electromyography is only an indirect method of assessing the neural drive to, and mechanical output of, a muscle. None the less, in spontaneously breathing animals a close correspondence has been demonstrated between changes in diaphragmatic EMG activity and phrenic nerve efferent activity

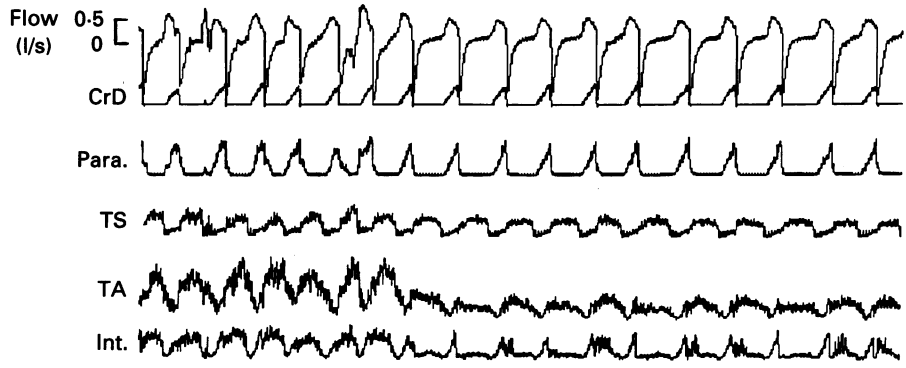

Fig. 6. Eighty seconds of breath-by-breath flow rate (l/s BTPS) and moving-time-averaged EMG data during an almitrine-induced hyperpnea. Note that during this period of very stable breathing transversus abdominis and internal intercostal muscles markedly decrease their EMG activity at breaths 7–8 and parasternals and diaphragm EMG activities remained unchanged with little if any effect on flow rate or V_T. Also note that while the internal intercostal EMG is 'contaminated' with an inspiratory muscle EMG, that only the expiratory portion of this signal decreases at breaths 7–8.

(Lourenco, Cherniack, Malm & Fishman, 1966; Road & Leevers, 1988). Furthermore, in awake dogs, diaphragmatic EMG has been shown to correlate strongly with the amount of diaphragmatic shortening during a breath (Ainsworth *et al.* 1992). These relationships seem to hold even when lung volume, and therefore muscle length, are altered. These findings together with the high level of reproducibility demonstrated in the V_T:diaphragm EMG relationship during our repeated testing in the same animal (see below) support the use of indwelling EMG electrodes as a valid and reliable indicator of respiratory muscle and motor nerve activities.

Finally, while we were able to eliminate pulmonary afferent feedback travelling in the vagi, muscle afferents located in the chest and abdominal walls remained intact. During a hyperpnoea, increased activation of these muscle spindles and other afferents could conceivably enhance the recruitment of expiratory muscle (Russell & Bishop, 1976), thereby masking differences in respiratory muscle activation patterns between the two types of chemoreceptor stimulation.

Non-chemoreceptor effects on EMG activity

Our data demonstrate that expiratory and accessory inspiratory muscle EMG activity is strongly affected by non-chemoreceptor stimuli. This is manifest in the observation that at times when chemical ventilatory drive remained relatively constant as indicated by stable values for P_{ET,CO_2}, V_T and diaphragm EMG, the EMG activity of non-diaphragmatic muscles could vary. Figure 6 presents an example of a steady hyperpnoea (in this case an almitrine-induced hyperpnoea) during which

transversus abdominis and internal intercostal muscle activities varied transiently with no noticeable changes in air flow rate, tidal volume or diaphragmatic EMG. This phenomenon is also shown in Fig. 2*A* and *B* where transversus abdominis and external oblique EMG activities are augmented for 20–30 breaths near the end of the tests. These alterations in muscle activity occurred during both types of ventilatory stimulation and were most probably due to small, unnoticeable changes in posture. Duron & Marlot (1980) observed a similar phenomenon in awake and sleeping cats, where subtle postural changes caused marked and selective changes in the EMG activity of internal intercostal muscles, which these authors attributed to activation of muscle spindles.

In addition to these changes in accessory muscle activity during steady-state hyperpnoeas, our data also show that from day to day different combinations of accessory muscle activities could be used to generate equivalent hyperpnoeas. Figure 4*A* presents a dog whose parasternals, triangularis sterni and transversus abdominis EMG activities within a day are linearly related to V_T, but who demonstrates a significant change in 'gain' for the muscle (Δ EMG/Δ V_T) between days. Interestingly, these changes in gain are only evident in respiratory muscles with a combined postural and respiratory role as there were not observable compensatory changes in diaphragm EMG activity to correspond with these changing levels of activity in the accessory muscles. Thus a given level of V_T and $\dot{V}_I$ was achieved with substantial differences in the sum total of measured EMG activity. Several possible explanations for how these day-to-day changes in accessory muscle recruitment could occur without observable compensatory changes in other muscles or in V_T include: (1) other respiratory muscles which were not studied may have shown compensatory changes in EMG activities, (2) day-to-day changes in EMG activity may have been so small that they meant little with regard to force development by these muscles, (3) mechanical outcomes other than V_T, such as functional residual capacity or diaphragmatic length (Ainsworth *et al.* 1989) may have been influenced, and (4) small shifts in thoraco-abdominal configuration from one day to the next could have altered the length of these muscles so that a change in measured EMG activity produced the same force.

Effects of chemoreceptor stimulation on respiratory muscle activity

Several investigators have observed that hypoxic stimulation of ventilation results in an increase in inspiratory motor activity but either a decrease or no change in expiratory motor activity (Sears *et al.* 1982; Fregosi *et al.* 1987; Smith *et al.* 1989; Brice, Forster, Pan, Lowry & Murphy, 1990). Excitation of carotid body afferents has been implicated as a possible cause of this 'inspiratory shift' by Sears *et al.* (1982) who measured the effects of hypoxia on phrenic and internal intercostal nerve activity during spontaneous breathing and apnoea in anaesthetized cats. Sears also noted that upon transection of the carotid sinus nerve no inspiratory shift was elicited by hypoxia.

Other investigators who have observed this inspiratory shift have attributed it to either medullary hypocapnia or hypoxic depression of the brain stem. To distinguish among these possible explanations, investigators have measured inspiratory and expiratory muscle activities during selective stimulation of either the carotid bodies

(Fregosi *et al.* 1987; Smith *et al.* 1989) or the carotid sinus nerve (Eldridge, 1976; Marek & Prabhakar, 1985). These experiments have demonstrated that *brief* selective carotid body stimulation causes increases in both inspiratory and expiratory muscle activity, but the necessity of keeping this stimulation very transient has led some investigators to speculate that sustained, selective carotid body stimulation would cause a time-dependent decrease in expiratory muscle activity (Fregosi *et al.* 1987). This point of view is apparently supported by data of Smith *et al.* (1989) which demonstrate that hypoxic stimulation causes an initial increase in expiratory muscle activity followed by a decrease. This decrease in expiratory muscle EMG activity occurs at a time when ventilation and diaphragmatic EMG activity remain elevated.

To the contrary, we believe our data demonstrate that in vagally blocked, awake dogs equivalent levels of hyperpnoea caused by selective central and peripheral chemoreceptor stimulation are associated with patterns of respiratory muscle recruitment which are very similar. This was shown to be the case in three inspiratory and four expiratory muscles which were studied over a wide range of hyperpnoeas. From these data we speculate that the lack of expiratory muscle recruitment observed during hypoxic stimulation is a result of either the accompanying hypocapnia or the effect of hypoxia on the central nervous system rather than a direct result of peripheral chemoreceptor stimulation. Recent work (Smith, Engwall, Dempsey & Bisgard, 1991), has shown that in the awake goat central nervous system hypoxia of 40 mmHg had no effect on the pattern of inspiratory or expiratory muscle EMG activity during hyperpnoea induced by selective stimulation of the isolated carotid bodies. This is strong evidence that moderate CNS hypoxia does *not* have a significant, selective inhibitory effect on expiratory muscle recruitment in awake animals. We favour the hypothesis that hypocapnia, most probably acting on the medullary chemoreceptors, provides a powerful and selective inhibition or disfacilitation of expiratory muscle activity during hypoxic hyperventilation.

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