Effects of sleep on the tonic drive to respiratory muscle and the threshold for rhythm generation in the dog

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1. The present study was designed to determine the effect of sleep on the tonic output to respiratory muscle and on the level of chemical respiratory stimulation required to produce rhythmic respiratory output.

2. Chronically implanted electrodes recorded expiratory (triangularis sterni) and inspiratory (diaphragm and parasternal intercostal) electromyographic (EMG) activities in three trained dogs during wakefulness and sleep. The dogs were mechanically hyperventilated via an endotracheal tube inserted into a permanent tracheostomy. During the studies, a cold block of the cervical vagus nerves was maintained to avoid the complicating effects of vagal inputs on respiratory drive and rhythm.

3. During wakefulness, steady-state hypocapnia (partial pressure of CO_2, $P_{CO_2} = 30$ mmHg) abolished inspiratory EMG activity, resulting in apnoea, but the expiratory muscle became tonically active. Compared to wakefulness, the level of the tonic expiratory EMG activity was decreased in non-REM (non-rapid eye movement) sleep (median decrease = 34 %, $P = 0{\cdot}005$) and was further decreased in REM sleep (median decrease = 78 %, $P < 0{\cdot}0001$). During REM sleep, the tonic expiratory EMG activity was highly variable (mean coefficient of variation = 39 % compared to 7 % awake, $P < 0{\cdot}0001$) and in some periods of REM, bursts of inspiratory EMG activity and active breathing movements were observed despite the presence of hypocapnia.

4. During constant mechanical hyperventilation, progressive increases in arterial P_{CO_2} (in hyperoxia) were produced by rebreathing. Measurement of the CO_2 threshold for the onset of spontaneous breathing showed that this threshold was not different between wakefulness and non-REM sleep (mean difference = 0·1 mmHg from paired observations, 95 % confidence interval for the difference = −1·0 to +1·1 mmHg, $P = 0{\cdot}898$).

5. The results show that sleep reduces the tonic output to respiratory muscles but does not increase the CO_2 threshold for the generation of rhythmic respiratory output. These observations suggest that changes in the tonic drives to the respiratory motoneurones may be a principal mechanism by which changes in sleep–wake states produce changes in respiratory output.

Changes in the magnitude of alveolar ventilation and in the pattern of breathing have been observed to accompany changes in sleep–wake state in animals and man (Phillipson & Bowes, 1986). Several mechanisms have been proposed to account for these state-related changes in respiratory activity (Phillipson & Bowes, 1986; Orem, 1988; Henke, Badr, Skatrud & Dempsey, 1992). However, determination of the mechanism(s) underlying the changes in respiratory activity is complicated by the associated changes in other variables that can affect the measurement (e.g. blood gases, upper airway resistance and vagal inputs). Furthermore, changes in alveolar ventilation and breathing pattern in the different sleep–wake states could reflect an effect of sleep on either the phasic or tonic drives to the respiratory motoneurones, in which case the mechanisms involved could be different (Sears, 1977; Berger, 1979; Feldman & Smith, 1989).

The aim of the present study was to determine the mechanism(s) by which changes in sleep–wake state cause changes in respiratory activity whilst minimizing the problems of interpretation noted above. To this end, recordings of inspiratory and expiratory muscle activities were made during periods of wakefulness and sleep in dogs made apnoeic by mechanical hyperventilation; under these conditions, expiratory muscle activity converts from rhythmic to tonic discharge when inspiratory muscle activity and spontaneous breathing are abolished (Horner, Kozar & Phillipson, 1992*a*). To avoid the complicating effects of changes in upper airway resistance during the experiments, the dogs were mechanically ventilated via an endotracheal tube inserted through a chronic tracheostomy. In addition, the level of mechanical hyperventilation was held constant during the studies in order to avoid the

complicating effects of spontaneous changes in blood gases and chemoreceptor inputs on respiratory muscle activity. Furthermore, to avoid the complicating effects of afferent vagal inputs on respiratory drive and rhythm, all studies were performed in the presence of bilateral cold block of the cervical vagus nerves (Phillipson, Hickey, Bainton & Nadel, 1970).

The effects of changes in sleep–wake state on respiratory activity were investigated in two separate studies. The first study was designed to determine the effect of sleep on the level of the tonic expiratory muscle activity that was present during mechanical hyperventilation (Horner *et al.* 1992*a*); this study was designed to test the hypothesis that sleep can reduce the magnitude of the tonic output to respiratory muscle independently of an effect on the magnitude of the phasic respiratory output (Orem, 1988). The second study was designed to determine the effect of sleep on the CO_2 threshold for the generation of phasic respiratory activity; this study was designed to test the hypothesis that sleep can increase the level of chemical respiratory stimulation required to produce rhythmic respiratory activity (Skatrud & Dempsey, 1983; Datta, Shea, Horner & Guz, 1991; Ingrassia, Nelson, Harris & Hubmayr, 1991). The results support the hypothesis of the first study but do not support the hypothesis of the second study, i.e. the results show that sleep reduces the tonic output to respiratory muscle but does not affect the threshold for the generation of rhythmic respiratory output. The implication of these results is that changes in the tonic drives to the respiratory motoneurones may be a principal mechanism by which changes in sleep–wake state affect the magnitude of respiratory output. Some of these results have been reported in preliminary form (Horner, Kozar, Kimoff & Phillipson, 1992*b*).

METHODS

Studies were performed on three adult dogs (weight range 20–25 kg) trained to lie quietly in place and to sleep in the laboratory. Each dog laid on one side and this same body position was adopted in all studies. Experimental procedures were approved by the Animal Care Committee of the University of Toronto. Laboratory temperature was maintained between 20 and 23 °C.

Surgical preparation

All surgical procedures were performed under general anaesthesia with aseptic conditions. Surgical procedures were approved by the Animal Care Committee of the University of Toronto. Prior to surgery, each dog was premedicated with atropine (0·02–0·05 mg kg^{-1} I.M.) and a long-acting penicillin (15000–20000 units kg^{-1} I.M.). Induction of anaesthesia was achieved with a short-acting barbiturate (thiamyal sodium, 10–20 mg kg^{-1} I.V.). Depending on the surgical procedure being performed, maintenance of general anaesthesia was achieved with either inhaled halothane (titrated to effect, typically 0·5–2 %) or with a long-acting barbiturate (pentobarbitone, I.V., titrated to effect, typically 30 mg kg^{-1}). Halothane was used for the surgery involving the creation of the exteriorized cervical vagal skin loops and implantation of the EMG electrodes; pentobarbitone was used for the surgery involving the creation of the chronic tracheostomy (see below).

Each dog was prepared with a permanent side-hole tracheostomy and with exteriorized cervical vagal skin loops (Phillipson *et al.* 1970) for purposes of monitoring ventilation and blocking the cervical vagus nerves. These surgical procedures were performed in separate operations months before the beginning of the study. For purposes of monitoring inspiratory and expiratory muscle activities, bipolar recording electrodes were implanted into the parasternal intercostal (PI, interspace 3 or 4), the costal diaphragm (DI, interspace 9 or 10) and the triangularis sterni (TS, interspace 3 or 4); the muscles were located using an anatomical text (Miller, 1964). Pairs of electrodes were sewn into each muscle with the tips separated by 5–10 mm. The electrodes consisted of insulated stainless steel wire with a 0·5 mm outside diameter (3–0 Flexon, 2592-43, Davis and Geck, Cyanid Canada Inc., Montreal, Canada). All leads were tunnelled subcutaneously to a common exit site on the back; when not in use they were housed in a bag, which was protected by a dog jacket. The studies began at least 10 days after surgery when swelling was minimal/absent and the electromyographic (EMG) recordings were stable and consistent.

Vagal blockade

All experiments were performed in the presence of reversible bilateral blockade of the cervical vagus nerves. Vagal blockade was achieved by cooling the vagal skin loops with copper radiators through which cold alcohol (−5 to −3 °C) was circulated. The technique of vagal block and the tests for the effectiveness of this procedure have been described previously (Phillipson *et al.* 1970; Fishman, Phillipson & Nadel, 1973). In summary, effective vagal blockade was indicated by the absence of the Hering–Breuer inflation reflex, a high heart rate (usually > 150 beats min^{-1}), a slow and deep breathing pattern and bilateral Horner's syndrome (Phillipson *et al.* 1970; Fishman *et al.* 1973; Smith, Ainsworth, Henderson & Dempsey, 1990). For each dog, experiments were separated by at least 2 days and were limited to a duration of 1 h in order to prevent cold-induced nerve damage.

Electromyography and electroencephalography

The EMG activity of each muscle was amplified (P5 Series AC preamplifiers, model No. P511K, Grass Instrument Co., Quincy, MA, USA) and filtered (bandpass 50–1000 Hz). The moving-time average of the rectified EMG activities was obtained using a leaky integrator with a 100 ms time constant (Coulbourn S76-01, Lehigh Valley, PA, USA).

The electroencephalogram (EEG) was monitored via platinum coated subdermal needle electrodes (Type E2, Grass Instrument Co.) placed in the scalp on either side of the mid-line. A similar electrode placed in the neck was used as a common ground for the EEG and EMG signals. The presence of relaxed wakefulness, rapid-eye-movement (REM) sleep and non-REM sleep was documented according to electroencephalographic (EEG) and behavioural criteria described previously (Phillipson, Murphy & Kozar, 1976; Sullivan, Murphy, Kozar & Phillipson, 1979). In summary, during wakefulness the dogs lay quietly and the EEG displayed low-voltage, high-frequency activity. During non-REM sleep, the EEG was dominated by high-voltage, low-frequency activity and the dogs were unresponsive to moderately loud noises. During REM sleep, the EEG was dominated by low-voltage, high-frequency activity and frequent rapid eye

movements were observed visually and were recorded on the EEG signal as large voltage deflections. Two broad categories of REM sleep were identified (Sullivan *et al.* 1979): (i) those periods without phasic twitches of the nose, ears and limbs (REM sleep); and (ii) those periods with phasic twitches of the nose, ears and limbs (P-REM sleep).

Breathing circuit and mechanical hyperventilation

A schematic diagram of the apparatus is shown in Fig. 1. During the experiments, the dogs breathed via a cuffed endotracheal tube (10 mm internal diameter) inserted through the chronic tracheostomy. The dogs were attached to the breathing circuit via this endotracheal tube. Airflow was measured via a heated pneumotachograph (Fleisch No. 2, P. K. Morgan Ltd, Rainham, Kent, UK) and this signal was integrated to obtain a volume signal. Airway CO_2 (LB-2, Beckman Instruments Inc., Fullerton, CA, USA) and airway pressure (Statham P23Db transducer, Gould Inc., Oxnard, CA, USA) were also measured (Fig. 1).

During the experiments, the dogs were mechanically hyperventilated with 100 % O_2 using a volume-cycled ventilator (model No. 613 ventilator, Harvard Apparatus Inc., South Natick, MA, USA) to reduce the end-tidal partial pressure of CO_2 (P_{ET,CO_2}) and to abolish spontaneous breathing movements. Under these conditions, two separate studies were performed. In each study, multiple observations and interventions were performed in the different sleep–wake states in each dog; the number of observations and interventions that were performed was dependent upon how readily each dog was able to fall asleep on any given day.

Study 1. The effect of sleep on tonic respiratory muscle activity during apnoea

The dogs were mechanically hyperventilated to a steady-state hypocapnic P_{CO_2} of 30 mmHg; this degree of hypocapnia was sufficient to abolish the inspiratory (DI and PI) EMG activity and spontaneous breathing movements, whereas the expiratory (TS) EMG activity converted from rhythmic to tonic discharge during apnoea (see Results). Once the steady-state CO_2 level had been achieved (i.e. > 5 min), the level of mechanical ventilation was held constant for the remainder of the experiment. The level of tonic TS EMG activity was quantified as the mean minimum of the moving-time averaged signal above the level recorded with the amplifier inputs grounded. The level of tonic TS EMG activity during apnoea was quantified during 15–30 s periods of relaxed wakefulness and compared to subsequent periods of non-REM and REM sleep that occurred closest in time to the analysed periods of wakefulness.

Study 2. The effect of sleep on the CO_2 threshold for the onset of spontaneous breathing

The dogs were mechanically ventilated to a steady-state hypocapnic P_{CO_2} that was approximately 5 mmHg below spontaneous breathing levels. Once the steady-state CO_2 level had been achieved (i.e. > 5 min), the level of mechanical ventilation was held constant for the remainder of the experiment. Following the establishment of steady-state hypocapnia, increases in arterial P_{CO_2} were then produced by switching the circuit such that the dogs were now mechanically ventilated with gas drawn from a rebreathing bag (Fig. 1). The rebreathing bag was initially filled with a mixture of CO_2 (5·5–6·1 %) in O_2; in each dog, the concentration of CO_2 and the volume of gas in the rebreathing bag were such that an identifiable mixed venous plateau was produced on the CO_2 record in less than three breaths. After the mixed venous plateau had been established, the CO_2 concentration increased linearly during the course of the rebreathing run. Rebreathing was continued until spontaneous breathing occurred, identified by the onset of phasic inspiratory EMG activity and the production of inspiratory volumes and changes in airway pressure that were out of synchrony with the ventilator. The rebreathing runs were discontinued after at least five consecutive spontaneous breaths had been observed. To allow the P_{CO_2} to return to control (steady-state) levels, at least 5 min were allowed to elapse before the end of one rebreathing run and the beginning of another.

Rebreathing runs were performed during periods of relaxed wakefulness and during periods of non-REM sleep; in these states, the onset of spontaneous breathing could be clearly identified from the chart records (see Results). Rebreathing runs were not performed during REM sleep because it was not possible to determine the CO_2 threshold for the onset of spontaneous breathing with any confidence; in periods of REM sleep, spontaneous inspiratory events occurred both before and during rebreathing runs and the occurrence of these events appeared to be unrelated to the CO_2 stimulus (see Results).

The P_{CO_2} at the onset of spontaneous breathing was taken as the P_{ET,CO_2} of the ventilator cycle that just preceded the onset of

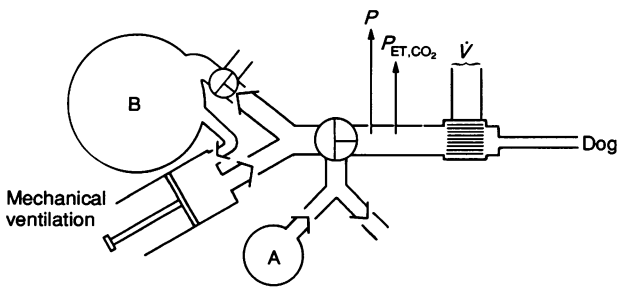

Figure 1. Schematic diagram of the apparatus and breathing circuit
The dogs were attached to the breathing circuit via an endotracheal tube inserted through a tracheostomy. Airflow ($\dot{V}$), airway pressure (P) and end-tidal P_{CO_2} (P_{ET,CO_2}) were measured. During spontaneous breathing, the dogs inspired from bag A. During mechanical hyperventilation, the dogs were ventilated with gas from bag B. See text for further details. The symbol, >, shows the direction of airflow through one-way valves. Bag A is a 13 l rubber breathing bag; bag B is a 100 l Douglas bag.

the first spontaneous breath. Measurements were made only if the rebreathing runs were not interrupted by movement and/or changes in behavioural state for at least 30 s before the onset of spontaneous breathing.

Recording of data

Signals were recorded on chart paper (at 5 mm s^{-1}) and on magnetic tape (Hewlett Packard 3968A, 0·2–625 Hz bandwidth). The raw EMG signals were monitored with an oscilloscope and loudspeaker to confirm the adequacy of each signal and to observe the activities of the individual muscles. The activity of an individual muscle was said to have been abolished if observation of the raw signal on the oscilloscope trace showed an absence of EMG activity. On these occasions, the moving-time averaged signal approached the zero level of the integrator and was only influenced by cardiac artifact (if present).

Statistical analyses

The analyses performed for each statistical test are included in the text where appropriate. For all tests, differences were considered significant if the null hypothesis was rejected at a level of $P < 0{\cdot}05$ using a two-tailed test. The decision to use either a parametric or a non-parametric statistical test was made after an analysis of the data distribution. For each paired analysis, the paired observations obtained from each individual dog were pooled for a single paired analysis (i.e. where 'n' is the total number of paired observations). This was performed after preliminary qualitative (visual) and quantitative (statistical) analyses showed that the results obtained within a dog were similar between dogs and that the process of pooling did not alter the overall data interpretation. In this way, the power of the statistical comparisons was increased and overall data interpretation was unaffected. Confidence intervals (CI) for the mean (Gardner & Altman, 1986) or median (Brown & Hollander, 1977) were calculated where appropriate.

RESULTS

Electromyography

An example of the raw EMG activities recorded from the chronically implanted electrodes during spontaneous breathing is shown in Fig. 2. In each dog during spontaneous breathing, inspiratory activity was recorded from the DI and PI muscles and expiratory activity was recorded from the TS muscle. However, the activity recorded from the TS was susceptible to contamination by inspiratory activity (e.g. see Fig. 3, first panel). Observation of the raw EMG signals suggested that the source of this contamination was inspiratory activity in the PI muscle, which is anatomically adjacent to the TS (Miller, 1964).

With the vagi blocked, the pattern of breathing consistently became slower and deeper compared with when the vagi were intact (Fig. 2). In addition, TS EMG activity was present throughout the long expiration associated with this pattern of breathing; inhibition of this tonic expiratory activity preceded the onset of inspiration (Fig. 2).

Mechanical hyperventilation

A reduction in arterial P_{CO_2}, by mechanical hyperventilation, abolished inspiratory EMG activity and spontaneous breathing. However, the TS muscle remained tonically active during apnoea (see Fig. 3, second panel). In the presence of constant mechanical hyperventilation, the dogs spontaneously fell asleep and experienced several changes in sleep–wake states during the experiments. The different sleep–wake states usually lasted for several minutes, thereby allowing enough time for the desired observations and interventions to be performed.

Study 1. The effect of sleep on tonic respiratory muscle activity during apnoea

Figure 3 demonstrates the effects of sleep on the level of tonic TS EMG activity during apnoea. This example shows that during constant mechanical ventilation, the mean level of tonic TS EMG activity was decreased in non-REM sleep, compared to wakefulness, and was further decreased in REM sleep. The example shown in Fig. 3 also shows that the TS EMG activity was sometimes abolished for brief periods in REM sleep. In other examples the TS EMG activity was observed to be abolished for long periods in (and sometimes throughout) REM sleep and only a cardiac artifact (if

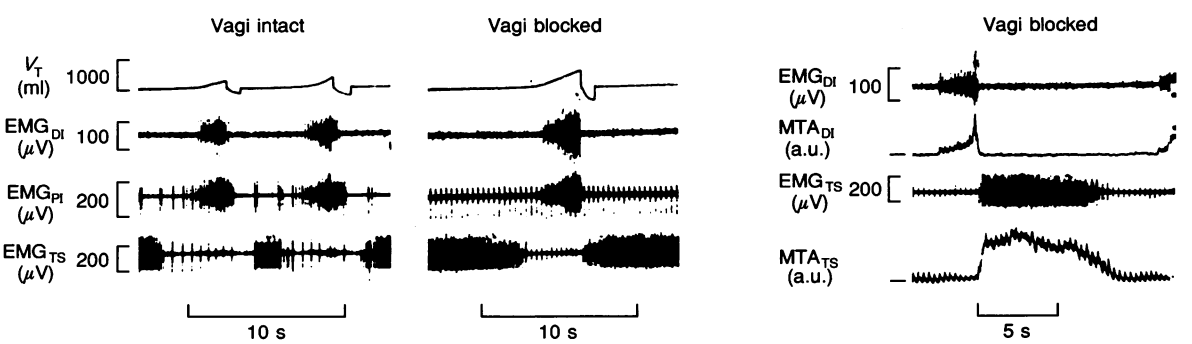

Figure 2.
Tidal volume (V_T) and the electromyographic (EMG) activities recorded from the diaphragm (DI), parasternal intercostal (PI) and triangularis sterni (TS) muscles during spontaneous breathing. In the panel on the right, the EMG_{DI} and EMG_{TS} are shown together with their moving-time averages (MTA) displayed in arbitrary units (a.u.). The MTA is displayed on subsequent figures. Note the high heart rate during vagal block (see cardiac artifact on the EMG_{PI}).

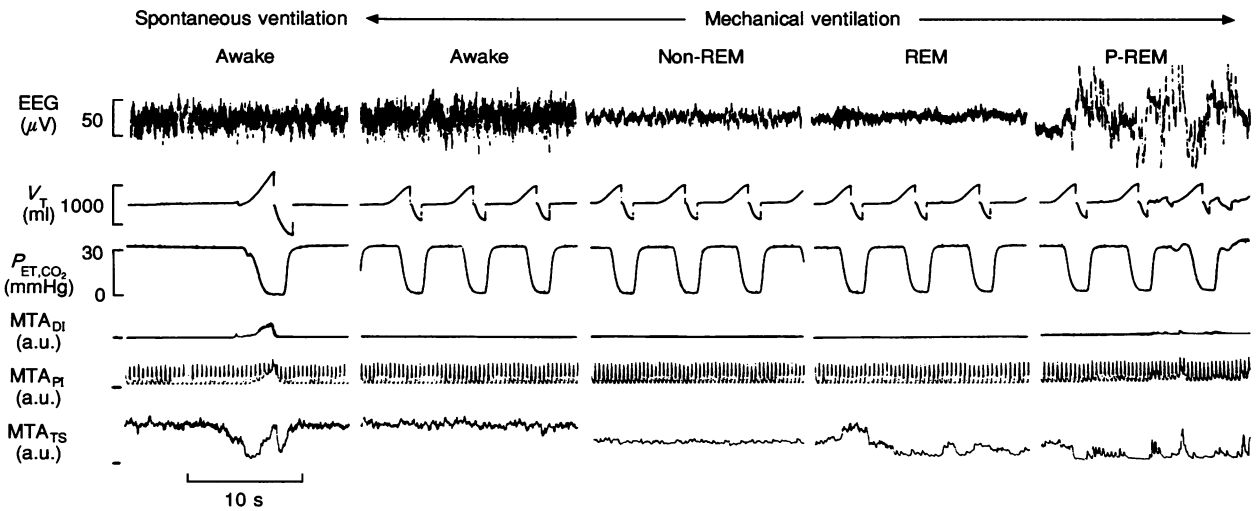

Figure 3. The effects of sleep on the level of tonic expiratory muscle activity during apnoea
During spontaneous ventilation, rhythmic inspiratory (DI and PI) and expiratory (TS) muscle activities were observed. Mechanical ventilation abolished inspiratory EMG activity, but the EMG_{TS} converted from rhythmic to tonic discharge. In the presence of apnoea produced by constant mechanical ventilation, the level and pattern of tonic EMG_{TS} activity was different in the different sleep–wake states. See text for specific points of interest. All EMGs are displayed as the moving-time averages (a.u.). The baseline of the integrator (i.e. electrical zero) is shown for each EMG. Note the high heart rate during vagal block (see cardiac artifact on the EMG_{PI} signal). All abbreviations are consistent with those used in the text.

present) was observed on the TS EMG signal. In addition to the decreased mean level of tonic TS EMG activity observed in REM sleep, the TS EMG activity was also highly variable whenever such activity was present in this state. Furthermore, during periods of REM sleep that were accompanied by frequent twitching movements of the nose, ears and limbs (i.e. P-REM sleep), there were occasional episodes of spontaneous inspiratory EMG activity and active breathing movements (see Fig. 3, final panel). Since the activity recorded from the TS muscle was susceptible to contamination by inspiratory activity (e.g. see Fig. 3, first panel) the level of tonic TS EMG activity in this P-REM state was restricted to a qualitative (rather than quantitative) analysis. However, the level of tonic TS EMG activity was quantified for periods of relaxed wakefulness, non-REM sleep and REM sleep (without body twitches), because in these states inspiratory activity was absent/rare (Fig. 3).

The level of tonic TS EMG activity during apnoea

Figure 4 shows the change in the mean level of tonic TS EMG activity that occurred following each change in sleep–wake state in the presence of hypocapnic apnoea. From analysis

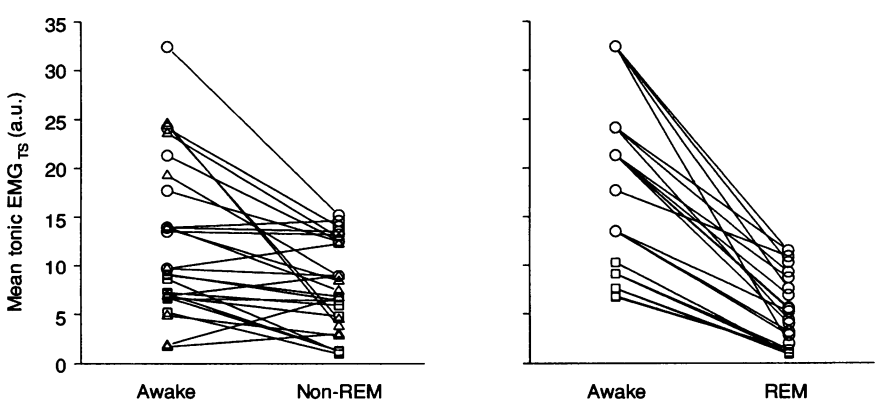

Figure 4. The change in the mean level of tonic TS EMG activity for each change in sleep–wake state in the presence of hypocapnic apnoea
Each dog is represented by a different symbol. One of the dogs (No. 2 in Table 1) spent only very short periods of time in REM sleep, such that insufficient data were obtained for analysis, in this state, in this dog.

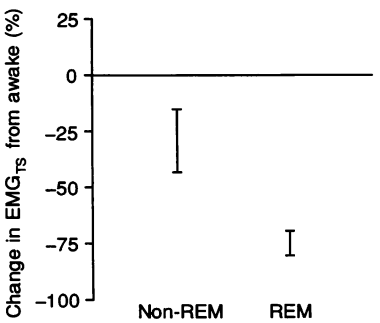

Figure 5.
The 95 % confidence interval for the percentage change in tonic TS EMG activity from the levels observed in wakefulness (from analysis of the pooled data).

of the pairs of raw data shown in Fig. 4, each dog showed a decrease in tonic EMG activity in non-REM sleep (range of $P = 0{\cdot}05$–$0{\cdot}002$) and REM sleep (each $P < 0{\cdot}0001$, paired t tests). To facilitate comparisons of the effects of non-REM and REM sleep on tonic TS EMG activity, the percentage changes in EMG activity from wakefulness to non-REM and REM sleep were calculated in order to normalize the data for the amount of awake EMG activity prior to the onset of sleep. The percentage changes in EMG activity from wakefulness to non-REM and REM sleep are shown in Fig. 5. Analysis of these data showed that the level of tonic TS EMG activity decreased significantly in both non-REM and REM sleep compared to the levels observed during wakefulness. The median decrease in the level of tonic TS EMG activity in non-REM sleep, compared to wakefulness, was 34·4 % (95 % CI for the decrease = 15·8–43·4 %, $P = 0{\cdot}005$, Wilcoxon test) whereas the median decrease in tonic TS EMG activity in REM sleep was 77·8 % (95 % CI for the decrease = 70·2–80·4 %, $P < 0{\cdot}0001$, Wilcoxon test). The decrease in the level of tonic TS EMG activity in REM sleep was significantly more than that observed in non-REM sleep ($P < 0{\cdot}0001$, Mann–Whitney test). The differences in the level of tonic TS EMG activity between non-REM and REM sleep were not due to the time at which the measurements were made; the time interval between the end of an analysed period of wakefulness to the start of an analysed period of non-REM or REM sleep was similar for both states (mean interval for non-REM sleep = 233 s, range 24–765 s; mean interval for REM sleep = 268 s, range 20–843 s).

The variability of tonic TS EMG activity during apnoea

Figure 6 shows the effect of sleep on the coefficient of variation (c.v.) of tonic TS EMG activity during apnoea. When TS EMG activity was still present in REM sleep (15 of the 24 analysed epochs), this activity was highly variable compared to the activity observed in wakefulness; the mean c.v. of tonic TS EMG activity in REM sleep was 39 % compared with 7 % observed during wakefulness. This difference in the c.v. of the tonic TS EMG activity between wakefulness and REM sleep was significant (95 % CI for the difference = 22–41 %, $P < 0{\cdot}0001$, paired t test). However, when tonic TS EMG activity was still present in non-REM sleep (23 of the 28 analysed epochs) the variability of this activity was not different from that observed in wakefulness (95 % CI for the difference in c.v. between wakefulness and non-REM sleep = −5 to +5 %, $P = 0{\cdot}997$, paired t test).

The spontaneous occurrence of inspiratory activity during hypocapnia

Table 1 shows, for each dog, the time spent in steady-state hypocapnia in each sleep–wake state and also shows the frequency with which inspiratory events (i.e. some inspiratory EMG activity and active breathing movements) occurred in each state. This table shows that inspiratory events were rarely observed in hypocapnia during periods of relaxed wakefulness, non-REM sleep and REM sleep, but shows that such events were observed more frequently in P-REM sleep. Analysis of these data showed that the frequency of occurrence of inspiratory events in P-REM sleep was significantly increased compared with the other sleep–wake states (Table 1). On most occasions (77, 67 and 88 % in each of the three dogs, respectively) the bursts of inspiratory EMG activity that gave rise to the inspiratory events in P-REM sleep took the form of clusters of spikes that were observed on the raw and processed EMG signals (e.g. see Fig. 3, final panel). However, on the remaining occasions (23, 33 and 12 % in each of the three dogs, respectively), the bursts of inspiratory EMG activity in P-REM sleep appeared to have an augmenting pattern of discharge similar to that observed during spontaneous breathing.

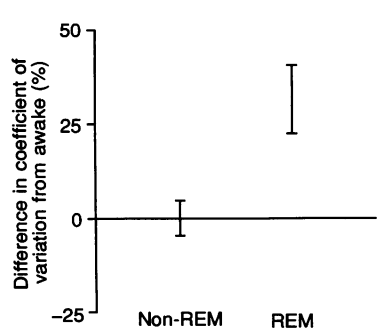

Figure 6.
The 95 % confidence interval for the difference in the coefficient of variation of tonic TS EMG activity from the levels observed during wakefulness (from analysis of the pooled data).

Table 1. The time spent in steady-state hypocapnia in each sleep–wake state and the frequency with which inspiratory events (i.e. inspiratory muscle activation and active breathing movements) were observed

Dog		W	Non-REM	REM	P-REM
1	Time	14 min 32 s	12 min 2 s	14 min 59 s	16 min 54 s
	Frequency (min^{-1})	0·41	0	0·40	2·66
2	Time	97 min 53 s	9 min 19 s	2 min 4 s	4 min 17 s
	Frequency (min^{-1})	0·15	0	0	2·10
3	Time	40 min 49 s	14 min 16 s	16 min 11 s	1 min 50 s
	Frequency (min^{-1})	0·15	0	0·31	4·36
Mean	Frequency (min^{-1})	0·24	0	0·24	3·04*
S.D.		0·15	0	0·21	1·18

Abbreviations: W, wakefulness; REM, rapid eye movement sleep; P-REM, phasic REM sleep; S.D., standard deviation. *The calculated Fisher's least significant difference ($P < 0·05$) showed that this value was significantly different from the values observed in the other sleep–wake states. (Fisher's least significant difference was calculated after one-way analysis of variance with repeated measures showed significant differences between the means, $F = 18·10$, $P = 0·002$.)

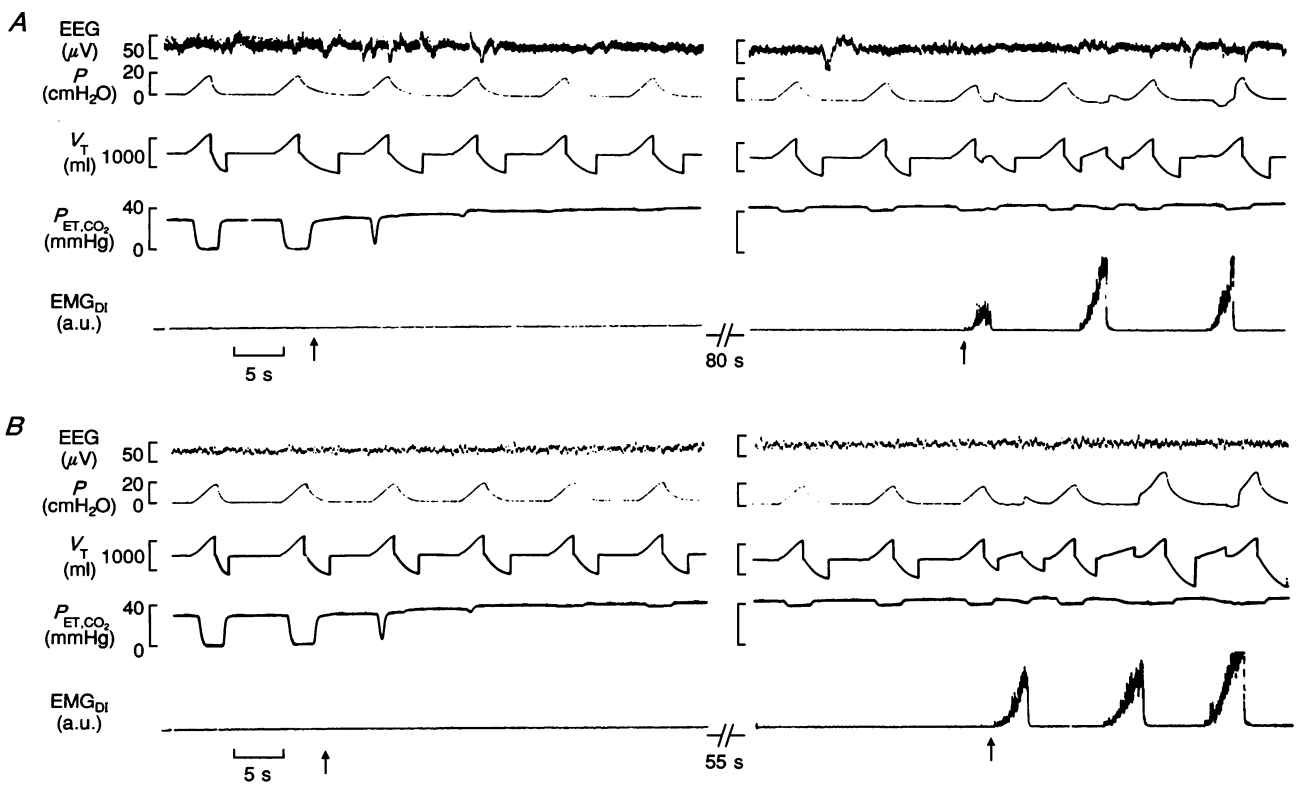

Figure 7. The P_{ET,CO_2} at the onset of spontaneous breathing in wakefulness (*A*) and non-REM sleep (*B*)

During constant mechanical hyperventilation (see swings in airway pressure, *P*), progressive increases in arterial P_{CO_2} were produced by rebreathing (see text for details). In *A* and *B*, the onset of rebreathing is shown by the first arrow and the onset of spontaneous breathing is shown by the second arrow. This figure shows that progressive increases in the level of arterial P_{CO_2} caused the onset of spontaneous breathing in wakefulness and non-REM sleep and that the P_{ET,CO_2} at which this occurred was not different between these two states. All abbreviations are consistent with those used in the text. The baseline of the integrator is shown for the EMG_{DI}.

Study 2. The effect of sleep on the CO_2 threshold for the onset of spontaneous breathing

Measurements of the CO_2 threshold for the onset of spontaneous breathing were made during periods of wakefulness and non-REM sleep. Measurements were not made during REM sleep, because it was not possible to determine the CO_2 threshold for the onset of spontaneous breathing in this state with any confidence; the occurrence of periods of P-REM sleep during the rebreathing runs, with the associated occurrence of spontaneous inspiratory activity, made such measurements in REM sleep unreliable.

Figure 7 shows an example of the onset of spontaneous breathing in response to a progressive increase in the level of arterial P_{CO_2} during rebreathing. This figure shows that an increasing level of P_{CO_2} caused the onset of spontaneous breathing at the same P_{ET,CO_2} in wakefulness and non-REM sleep.

Multiple determinations of the level of CO_2 at the onset of spontaneous breathing were made during periods of relaxed wakefulness and non-REM sleep in each dog. The number of measurements that were made in each state was dependent upon how readily each dog fell asleep on any given day. For this reason, in each dog the number of measurements made during wakefulness was larger than the number of measurements made during non-REM sleep. Because of this unequal number of measurements in wakefulness and non-REM sleep, the data were subjected to two separate analyses.

Unpaired analysis

Figure 8*A* shows the P_{ET,CO_2} at which the onset of spontaneous breathing occurred during each rebreathing run performed in wakefulness and non-REM sleep in each dog. An unpaired analysis of these data showed that the mean P_{ET,CO_2} at the onset of spontaneous breathing was not significantly different between wakefulness and non-REM sleep in any dog ($P = 0{\cdot}08$, $0{\cdot}45$ and $0{\cdot}68$ in the three dogs respectively, unpaired t tests). Calculation of the 95 % CI for the difference in the mean CO_2 threshold for the onset of spontaneous breathing showed that in each dog the 95 % CI incorporated a value of zero difference between wakefulness and non-REM sleep (Fig. 8*B*).

Paired analysis

Figure 9*A* shows the P_{ET,CO_2} at which the onset of spontaneous breathing occurred in wakefulness and non-REM sleep using data obtained from pairs of rebreathing runs that were performed closest in time to each other on the same day. Analysis of these data confirmed the results of the unpaired analysis (see above) and showed that the P_{ET,CO_2} at which the onset of spontaneous breathing occurred was not significantly different between wakefulness and non-REM sleep (mean difference = $0{\cdot}1$ mmHg, $P = 0{\cdot}898$, paired t test). The 95 % CI for the mean difference in the CO_2 threshold for the onset of spontaneous breathing incorporated a value of zero difference between wakefulness and non-REM sleep (95 % CI = $-1{\cdot}0$ to $+1{\cdot}1$ mmHg; see Fig. 9*B*). For these paired data, the mean time interval between the measurement of the CO_2 threshold for the onset of spontaneous breathing in sleep and wakefulness was 665 s (range 330–1275 s). This time interval ensured that the P_{CO_2} had sufficient time to return to the control steady-state level before the next rebreathing run was performed (see Methods).

DISCUSSION

The results of this study demonstrate that, during apnoea produced by constant mechanical hyperventilation, the level of tonic expiratory muscle activity observed during

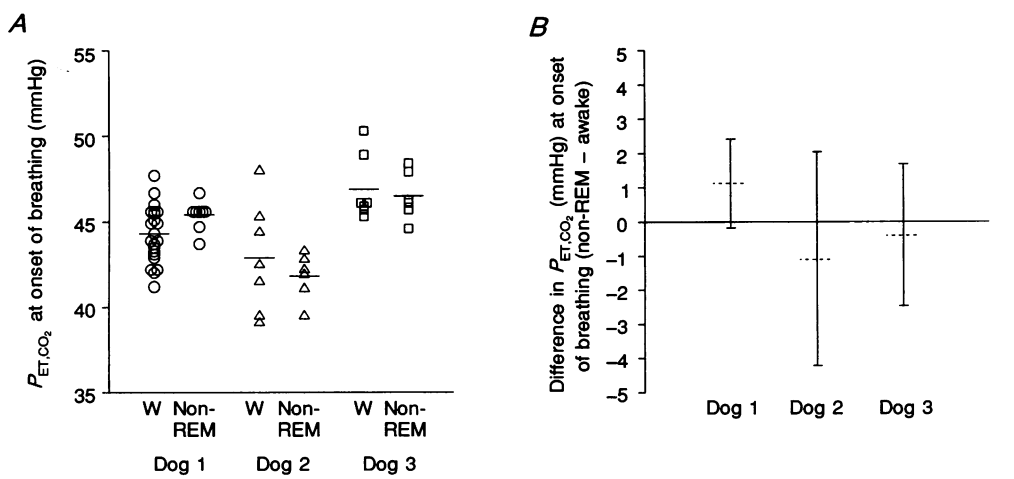

Figure 8.
A shows the P_{ET,CO_2} at which the onset of spontaneous breathing occurred during each rebreathing run performed in wakefulness and non-REM sleep in each dog. *B* shows, for each dog, the 95 % confidence interval for the difference in the mean CO_2 threshold for the onset of spontaneous breathing in wakefulness and non-REM sleep. These data show that in each dog the mean CO_2 threshold for the onset of spontaneous breathing was not significantly different between wakefulness and non-REM sleep.

wakefulness is reduced during sleep. In addition, the different sleep–wake states were associated with characteristically different levels and patterns of tonic expiratory muscle activity. Since these differences in tonic expiratory muscle activity were observed in the presence of apnoea produced by constant mechanical ventilation, with constant chemoreceptor input and without any spontaneous, rhythmic respiratory activity, the results suggest that changes in sleep–wake state *per se* can affect the tonic output to respiratory muscle independently of an effect on the magnitude of the phasic rhythmic output. In another set of experiments, also performed during constant mechanical hyperventilation, it was observed that sleep did not change the threshold for the generation of spontaneous, rhythmic respiratory activity in response to progressive increases in arterial P_{CO_2} generated by rebreathing. This result suggests that sleep does not increase the level of chemical respiratory stimulation required to generate spontaneous breathing. Taken together these observations suggest that a principal mechanism by which changes in sleep–wake state can produce changes in the magnitude of respiratory output is by an effect on the tonic drives to the respiratory motoneurones, rather than by an effect on the threshold for the generation of respiratory rhythm.

Electromyography and rationale for choice of muscles

In these studies, the triangularis sterni was used as the measure of expiratory muscle activation because this muscle is considered to be a primary respiratory muscle (De Troyer & Ninane, 1986; De Troyer, Ninane, Gilmartin, Lemerre & Estenne, 1987). In addition, this muscle was consistently active during vagal block, unlike the expiratory abdominal muscles which often are not active in this condition (Smith *et al.* 1990; Yasuma, Kimoff, Kozar, England, Bradley & Phillipson, 1993). In this respect, these expiratory muscles differ from each other in their response to vagal afferent stimuli; such stimuli are thought to be inhibitory to the triangularis sterni (Smith *et al.* 1990) but facilitatory to the abdominal muscles (De Troyer & Ninane, 1987; Smith *et al.* 1990; Yasuma *et al.* 1993). The removal of an inhibitory vagal input to the triangularis sterni by vagal block would explain the increased EMG activity observed in this condition (Fig. 2) and the absence of any changes in activity resulting from the lung inflation produced by mechanical ventilation (Fig. 3). The former effect of vagal block proved advantageous in this study, because the presence of expiratory muscle activity during apnoea allowed the effects of sleep to be studied without the complication of changes in spontaneous breathing (see below); the latter effect of vagal block simplified data interpretation, because any changes in tonic EMG activity could be observed without interference in the signal related to the act of mechanical ventilation.

Rationale for methods

The present studies were performed under conditions of constant mechanical hyperventilation, via an endotracheal tube, in the presence of bilateral cold block of the cervical vagus nerves. The studies were performed under these controlled conditions in order to avoid an effect on respiratory muscle activity of sleep-induced changes in the pattern of breathing, blood gases, upper airway resistance and afferent vagal inputs. In addition, the complicating effects of changes in body posture were also avoided, because the levels of tonic respiratory muscle activity during relaxed wakefulness were compared with those during subsequent periods of sleep; comparisons were made in this direction because the transition into sleep was not associated with a change in body posture, whereas slight shifts in posture were sometimes observed upon arousal from

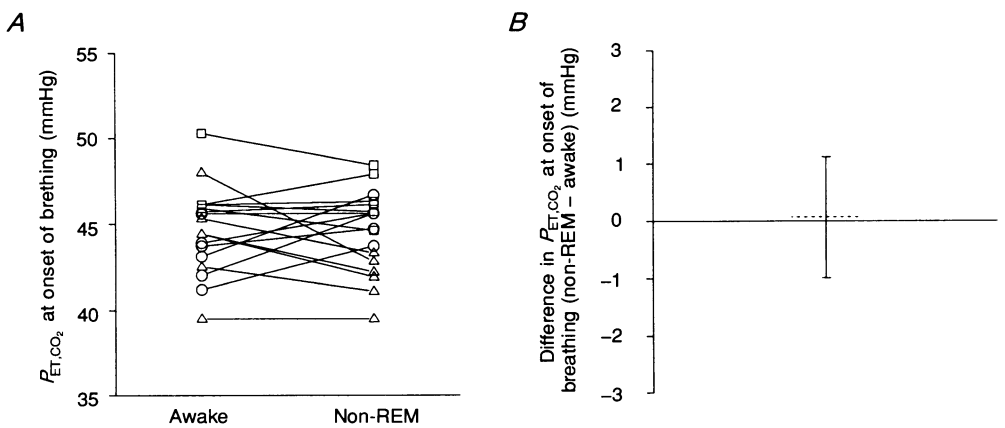

Figure 9.
A shows the P_{ET,CO_2} at which the onset of spontaneous breathing occurred in wakefulness and non-REM sleep using the data obtained from paired observations; each dog is represented by a different symbol. *B* shows the 95 % confidence interval for the difference in the CO_2 threshold for spontaneous breathing in wakefulness and non-REM sleep (from analysis of the pooled data); the difference in the CO_2 threshold for breathing in wakefulness and non-REM sleep was not significantly different from zero.

sleep. For these reasons, interpretation of the mechanisms underlying changes in respiratory muscle activity was greatly simplified, i.e. the observed changes are likely to have been due to the change in sleep–wake state *per se*.

Critique of preparation

The main advantage gained by performing these studies with constant mechanical ventilation and with control over the major inputs affecting respiratory muscle activation was simplified data interpretation (see above). However, these same factors also limit the extrapolation of these data to the normal spontaneously breathing condition. In this respect, although the present experiments were performed in a non-anaesthetized conscious preparation, the preparation is still somewhat reduced such that the results must be extrapolated with caution (Feldman *et al.* 1990). In addition, since the conditions of the experiments favoured the presence of tonic expiratory muscle activity during apnoea (see above subsection, 'Electromyography and rationale for choice of muscles') and since the experiments were performed with altered blood gases (i.e. hyperoxia and mild hypocapnia), the balance of tonic and phasic components within the respiratory control network may have been altered to some degree. However, to our knowledge, this is the first preparation that has allowed the effects of sleep on the tonic and phasic components of respiratory muscle activation to be studied independently in a controlled and systematic fashion in a non-anaesthetized preparation. The observation that expiratory muscle activity converted from rhythmic to tonic discharge when inspiratory muscle activity and spontaneous breathing movements were abolished by hypocapnia allowed these studies to be performed. The observation that vagal blockade favoured the presence of tonic TS EMG activity proved useful, since the TS motoneurones were above the threshold for the generation of a measurable motor output. This condition was advantageous because it allowed the observation to be made that sleep affects the tonic drive to respiratory motoneurones, a phenomenon that could not have been observed (except by more invasive neurophysiological procedures) if the motoneurones had been below the threshold for the generation of motor output. However, the concepts revealed in this study may be equally applicable to when the motoneurones are below the threshold for the generation of motor output. By analogy, during spontaneous breathing in anaesthetized animals there may be no spinal expiratory motoneurone discharge; nevertheless, the expiratory half of the central mechanism generating respiratory output is clearly in operation, as evidenced by the occurrence, even in deep anaesthesia, of expiratory bulbospinal neuronal activity and central respiratory drive potentials at the level of the spinal motoneurone (Sears, 1977). In addition to the influences of vagal blockade, all experiments were performed in the presence of hyperoxia. Hyperoxia was used to minimize the input from the peripheral chemoreceptors, in order to avoid the potential confounding influence of the tonic input from these chemoreceptors. However, hyperoxia may have had an effect on cerebral blood flow, and hence cerebral CO_2 and pH, but these effects would have been constant throughout the studies because the dogs were under constant (steady-state) mechanical ventilation. For this reason, the use of hyperoxia would not have been expected to detract from the results of this study.

Therefore, using this preparation, we have been able to perform two separate studies to investigate two hypotheses related to the impact of sleep on breathing. Given the experimental design, the changes observed in our studies are likely to have been due to the changes in sleep–wake state *per se*. The interpretation of the effects of sleep on respiratory output is more complicated in more intact preparations, due to the spontaneous changes in other variables during sleep that in turn can affect respiratory output (e.g. changes in breathing pattern, blood gases, upper airway resistance and afferent vagal inputs). For these reasons, we regard the results derived from this preparation as helpful in understanding the primary mechanism(s) by which sleep affects respiratory output; some of these mechanisms are discussed below.

Respiratory muscle activity during apnoea

In these studies, the effect of changes in sleep–wake state on the tonic output to respiratory muscle was investigated under conditions of constant chemoreceptor drive during apnoea. Under these conditions, the changes in tonic respiratory muscle activity are likely to be due to changes in tonic drives related to changes in sleep–wake state *per se* (see above). However, measurements of tonic respiratory muscle activity were confined to expiratory muscle, because in this preparation the inspiratory muscles were not active during apnoea. For this reason it was not possible to determine if changes in sleep–wake state had similar effects on the activity of inspiratory motoneurones, because only the expiratory motoneurones were above the threshold for discharge during apnoea. However, the observed effects of sleep on the level of tonic expiratory muscle activity were not dissimilar from the described effects on other motoneurone pools observed in other preparations (Glenn, Foutz & Dement, 1978; Orem, Osorio, Brooks & Dick, 1985; Orem, 1988; Kubin, Kimura, Tojima, Pack & Davies, 1992). Therefore, it seems likely that changes in sleep–wake state have qualitatively similar effects on tonic drives to expiratory, inspiratory and non-respiratory motoneurones, although the magnitude of these effects may differ among the different types of motoneurones (Glenn *et al.* 1978; Orem *et al.* 1985; Phillipson & Bowes, 1986; Orem, 1988; Monteau & Hilaire, 1991; Kubin *et al.* 1992).

The effect of sleep on tonic respiratory muscle activity during apnoea

In the presence of apnoea, the levels and patterns of tonic expiratory muscle activity were significantly different between periods of wakefulness, non-REM sleep and REM sleep (Figs 3, 5 and 6). These observations support the concept that the neural output to respiratory muscle can be affected by state changes alone and that different neural control mechanisms operate in the different sleep–wake states

(Phillipson & Bowes, 1986; Feldman, 1986; Orem, 1988). Furthermore, since the changes in tonic expiratory muscle activity were observed in the absence of any spontaneous rhythmic respiratory activity, the results also suggest that sleep can affect the tonic output to respiratory motoneurones independently of an effect on the phasic rhythmic output (Foutz, Boudinot, Morin-Surun, Champagnat, Gonsalves & Denavit-Saubie, 1987; Orem, 1988). The possible mechanisms by which the different sleep–wake states affected the level and pattern of tonic respiratory muscle activity are discussed below.

Effect of non-REM sleep on the tonic drive to respiratory motoneurones

Considerable evidence suggests that during non-REM sleep there is a decrease in the magnitude of tonic excitatory drive acting on the respiratory motoneurones of the brainstem and/or spinal cord (Kubota & Tanaka, 1966; Orem, Montplaisir & Dement, 1974; Lydic & Orem, 1979; Orem *et al.* 1985; Orem, 1988). This decrease can best be explained by withdrawal of a stimulatory effect of wakefulness on respiratory motoneurones (Phillipson & Bowes, 1986; Orem, 1988). Although this explanation is ultimately difficult to prove in an intact preparation, the reduction in tonic expiratory muscle activity demonstrated in the present study (Figs 3 and 5) strongly supports the notion that wakefulness *per se* exerts a tonic stimulatory influence on breathing, and that this influence is withdrawn in non-REM sleep (Orem *et al.* 1985; Phillipson & Bowes, 1986; Foutz *et al.* 1987; Orem, 1988).

Effect of REM sleep on the tonic drive to respiratory motoneurones

Compared to non-REM sleep, during REM sleep the level of tonic expiratory muscle activity was further decreased and was sometimes abolished. Furthermore, when TS EMG activity was present in REM sleep, this activity was observed to be highly variable (e.g. Fig. 3) and in some periods of P-REM sleep bursts of inspiratory EMG activity and active breathing movements were observed despite the hypocapnia (Fig. 3 and Table 1). These differences between non-REM and REM sleep were observed despite the maintenance of the same chemical and mechanical respiratory inputs during the two sleep states. Most evidence would suggest that the marked reduction in expiratory muscle activity during REM sleep was produced by active inhibition of the respiratory motoneurones (Glenn *et al.* 1978; Phillipson & Bowes, 1986; Orem, 1988; Monteau & Hilaire, 1991) and that the high variability in the level of respiratory muscle activity in REM sleep resulted from transient fluctuations in motoneurone excitability, similar to that observed in other motoneurone pools (Glenn *et al.* 1978; Orem, 1988). These same mechanisms are probably responsible for the irregular and erratic respiratory output typically observed during spontaneous breathing in REM sleep, a pattern that is also relatively independent of chemical and mechanical respiratory stimuli (Phillipson *et al.* 1976; Phillipson & Bowes, 1986; Shea *et al.* 1988; Orem, 1988).

The effect of sleep on the CO_2 threshold for the onset of spontaneous breathing

The observation that sleep decreases the level of tonic expiratory muscle discharge but has no effect on the CO_2 threshold for the generation of respiratory rhythm is in keeping with previous neurophysiological studies of the impact of sleep on medullary respiratory motoneurones (Orem *et al.* 1985; Foutz *et al.* 1987; Orem, 1988). In particular, such studies have demonstrated that respiratory motoneurones that are more tonically active during wakefulness show a decrease in discharge during non-REM sleep, whereas cells with a stronger respiratory-related (phasic) discharge, which is presumably tightly coupled to the respiratory rhythm generator, remain active during sleep (Foutz *et al.* 1987; Orem, 1988). Taken together, these observations suggest that sleep may reduce the overall tonic output to respiratory muscle without directly affecting the threshold for respiratory rhythm generation. In the intact animal, this conclusion is in keeping with the observation that sleep reduces the level of alveolar ventilation and increases arterial P_{CO_2} (Phillipson & Bowes, 1986). In this context, it should be noted that in the present study we measured only the CO_2 threshold for the generation of respiratory rhythm in the awake and asleep states, but did not measure the magnitude of the phasic respiratory output. These measurements were in keeping with the hypotheses under investigation. However, although we did not measure the magnitude of the phasic respiratory output, which may well have been reduced in sleep compared to wakefulness, the absence of a change in the CO_2 threshold for rhythm generation does not in any way conflict with the notion that respiratory output is reduced during sleep.

The present observations appear to be at variance with previous studies that have suggested that sleep increases the level of chemical respiratory stimulation required to generate rhythmic breathing (Skatrud & Dempsey, 1983; Prechter, Nelson & Hubmayr, 1990; Datta *et al.* 1991; Ingrassia *et al.* 1991; Simon, Dempsey, Landry & Skatrud, 1993). The basis for this apparent discrepancy is not clear, but may relate to the fact that the present study was performed with the vagus nerves blocked. In this respect, vagal blockade does not in itself change the CO_2 threshold for breathing (Phillipson *et al.* 1970), but does increase the magnitude of phasic inspiratory muscle discharge during spontaneous breathing (see Fig. 2). The presence of a larger inspiratory signal during spontaneous breathing thereby minimizes the chances of missing the onset of phasic respiratory activity at the end of apnoea. In contrast, the previous studies that showed that sleep increased the CO_2 threshold for the onset of respiratory rhythm were based largely on measurements of ventilation in human subjects, in whom the vagi were of course intact. Under these conditions, measurements of airflow or volume changes may not have been sufficiently sensitive to detect the onset of phasic respiratory activity during sleep. This may have been so if the magnitude of the phasic activity was reduced in sleep and/or if the phasic respiratory activity was superimposed on a reduced tonic drive such that the

phasic respiratory activity was subthreshold for the generation of motor output (Berger, 1979; Feldman & Smith, 1989); in either case, an apparent increase in the threshold for the generation of spontaneous breathing in sleep would have been observed.

Effects of mechanical ventilation on the CO_2 threshold for the onset of spontaneous breathing

It is conceivable that the mechanical ventilation used in the present study influenced the CO_2 threshold for the onset of breathing (Simon, Skatrud, Badr, Griffin, Iber & Dempsey, 1991; Simon *et al.* 1993). This technique was selected for the present study because the onset of spontaneous breathing during progressive hypercapnia was obvious and easy to identify and the P_{ET,CO_2} at this point could be accurately determined. In contrast, attempts to measure P_{ET,CO_2} at the termination of a hypocapnic-induced apnoea (generated by disconnecting the ventilator) were confounded by dilution of alveolar gas by the inspired air of the first breath. However, it has been suggested that the act of mechanical ventilation inhibits the onset of respiratory activity in response to increasing arterial CO_2 (Simon *et al.* 1991, 1993). Thus, it is of relevance to note that during wakefulness the mean P_{ET,CO_2} at the onset of spontaneous breathing during mechanical ventilation was 44, 43 and 47 mmHg in dogs 1, 2 and 3, respectively (see Fig. 8), whereas in the same dogs the P_{ET,CO_2} during spontaneous breathing was 32, 30 and 35 mmHg, respectively, and only slight reductions in P_{CO_2} abolished spontaneous inspiratory activity (Fig. 3). These observations are similar to those reported by others in humans, such that slight reductions in P_{CO_2} by mechanical ventilation are sufficient to abolish spontaneous respiratory activity (Prechter *et al.* 1990; Datta *et al.* 1991; Ingrassia *et al.* 1991; Simon *et al.* 1991, 1993) whereas the level of CO_2 required to re-establish breathing may be substantially higher (Simon *et al.* 1991, 1993). However, the mechanism by which mechanical ventilation inhibits the onset of respiratory activity in response to CO_2 is unknown (Simon *et al.* 1991, 1993). Moreover, with regard to the present observations, it is also not known whether this inhibitory effect of mechanical ventilation is different in wakefulness and sleep, but such a differential effect cannot be discounted (Simon *et al.* 1993).

Implications and conclusions

The results of the present study support the concept that sleep affects the magnitude of the tonic drive to the respiratory motoneurones (Orem, 1988) and that different neural control mechanisms are active in the different sleep–wake states (Phillipson & Bowes, 1986). In addition, the results suggest that sleep does not affect the point at which the respiratory rhythm generator produces a phasic respiratory output. Taken together, these results suggest that a principal mechanism by which changes in sleep–wake state produce changes in the magnitude of respiratory output is by an effect on the tonic drives to the respiratory motoneurones. This may be an important mechanism underlying changes in ventilation in sleep, in addition to the previously described changes in upper airway resistance and the magnitude of phasic respiratory output (Phillipson & Bowes, 1986; Henke *et al.* 1992).

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