Paradoxical effect of oxygen administration on breathing stability following post-hyperventilation apnoea in lambs

M. H. Wilkinson, P. J. Berger, N. Blanch, V. Brodecky and C. A. Jones

Institute of Reproduction and Development, Monash Medical Centre, 246 Clayton Road, Clayton, Victoria 3168, Australia

1. Oxygen administration is thought to suppress periodic breathing (PB) by reducing carotid body activity, and yet earlier experiments in neonates have shown that PB incidence may be increased following the application of hyperoxia. To clarify this paradox, we studied the changes in the pattern of PB that occur following administration of oxygen in a lamb model of PB.

2. PB was induced in eleven of seventeen anaesthetized lambs following passive hyperventilation with air. When oxygen was administered during PB, the pattern was first enhanced, as evidenced by a sudden decrease in the ratio of the ventilatory duration to the apnoeic pause duration, and then suppressed, as evidenced by a progressive return to stable breathing which was associated with an increase in minute ventilation.

3. Five of the six lambs that did not show PB following passive hyperventilation with air could be made to do so if oxygen was substituted for air as the inspired gas following passive hyperventilation.

4. Five of the eleven lambs that showed PB following hyperventilation with air responded to the application of oxygen during PB by switching to a gross form of episodic breathing consisting of long apnoeic pauses followed by equally long periods of breathing during which minute ventilation fell progressively with time.

5. We conclude that when applied against a background of arterial hypoxaemia, oxygen has a destabilizing influence on ventilation in that (a) it accentuates the unstable breathing that occurs during PB, (b) it induces PB in lambs that exhibited stable breathing in air, and (c) it may precipitate episodic breathing.

The mechanism underlying periodic breathing (PB) in adults has long been considered to be an instability of the respiratory control system which is stimulated by hypoxia and mediated by the peripheral chemoreceptors (Cherniack & Longobardo, 1986). Evidence consistent with a similar mechanism for the generation of PB in the newborn is the association of PB with raised ventilatory sensitivity to hypoxia in the lamb (Canet, Carroll & Bureau, 1989; Wilkinson, Berger, Blanch, Brodecky & Jones, 1996). We have postulated that increased sensitivity to hypoxia destabilizes breathing in the lamb by enhancing the loop gain of the peripheral chemoreceptor control loop. Such an effect has been proposed on theoretical grounds (Khoo, Kronauer, Strohl & Slutsky, 1982) and it has been confirmed in adult humans during sleep at high altitude (Lahiri, Maret & Sherpa, 1983). Consequently, it is reasonable to hypothesize that breathing hyperoxic gas mixtures during PB would prevent or terminate PB by suppressing peripheral chemoreceptor activity. This hypothesis is consistent with reports of the suppression of PB when hyperoxia is applied during PB in adult cats (Lahiri, Hsiao, Zhang, Mokashi & Nishino, 1985).

However, two studies in the human infant have produced evidence that is difficult to reconcile with this hypothesis. In the first, Cross & Oppe (1952) showed that while PB could be produced in a proportion of premature infants during exposure to hypoxia, the incidence of PB increased still further in the first minute following a shift to hyperoxia. In a second, similar study, Rigatto & Brady (1972) showed that PB, associated with prolonged apnoeic pauses, was more frequent in the first minute following the return to room air than during the previous hypoxic exposure. The shift from hypoxia either to hyperoxia or to room air in these two studies would be expected to reduce carotid body discharge, and yet in both cases PB became more pronounced.

During the course of pilot studies in the lamb, we observed that hyperoxic gas had a paradoxical destabilizing effect on breathing similar to that described in the earlier studies of the human infant (Cross & Oppe, 1952; Rigatto & Brady, 1972). Accordingly, we set out to examine the mechanisms by which oxygen administration affects the stability of breathing in lambs and to develop a conceptual model to account for this apparent paradox.

METHODS

Animal preparation

Seventeen lambs, 9–23 days of age and weighing 7·5 ± 0·4 kg (mean ± S.E.M.), were anaesthetized with α-chloralose (80 mg kg^{-1} as a starting bolus followed by 20 mg kg^{-1} h^{-1} as a continuous infusion), intubated and ventilated with an infant ventilator (model BP-200, Bournes Medical Systems Inc., Riverside, CA, USA). This rate of chloralose infusion was carefully controlled to maintain an effective but light level of anaesthesia, based on continuous blood pressure and heart rate monitoring and regular tests for a response to stimulation of the inner canthus of the eye. The ventilator circuit allowed for the delivery of hypoxic, normoxic or hyperoxic gas mixtures as required via a flow-through system. A very high gas flow rate of 30 l min^{-1} was used enabling changes in inspired gas concentrations to be established within a second. When the ventilator was turned off, lambs were able to breathe freely via the ventilator circuit.

A catheter was placed in the carotid artery for blood gas sampling and for measurement of blood pressure via a transducer (HP-1280 blood pressure transducer, Hewlett-Packard, Waltham, MA, USA) and a DC amplifier (Hewlett-Packard 8805B carrier amplifier). Heart rate was derived from the blood pressure signal (Neotrace NT 122, Neomedix, Sydney, Australia). A second catheter was placed in the jugular vein for blood gas sampling and infusion of glucose–saline (5% glucose in 0·9% saline at a rate of 4·0 ml kg^{-1} h^{-1}). A rectal temperature probe was used to monitor body temperature which was controlled within the range 40·0 ± 1·0 °C using an overhead radiant heater.

Respiratory flow was measured using a Fleisch no. 0 pneumotachograph (Hans Rudolph, Kansas City, MO, USA), which was linear up to 25 l min^{-1}, in conjunction with a differential pressure transducer (model 8T-2, Gaeltec, Dunvegan, Isle of Sky, UK). Tidal volume was determined using an electronic integrator (HP-8815A, Hewlett-Packard). End-expired gases were measured using a CO_2 analyser (model 901 MK2, P. K. Morgan Ltd, Rainham, Kent, UK; P_{ET,CO_2}) and an O_2 analyser (model S-3A, Amatek, Pittsburgh, PA, USA; P_{ET,O_2}). To continuously record arterial oxygen saturation, a pulse oximeter (model N-200, Nellcor, Hayward, CA, USA) was placed in the trans-mandibular position in such a way that the optical path included the soft tissues of the jaw, but excluded the tongue. At the end of the protocols (see below) the lambs were killed with an overdose of anaesthetic (Lethabarb, 150 mg kg^{-1}; Virbac, Sydney, Australia).

Pulse oximeter calibration

The reading from the Nellcor N-200 pulse oximeter (S_{p,O_2}) was calibrated by directly measuring haemoglobin oxygen saturation (OSM-2 hemoximeter, Radiometer, Copenhagen, Denmark) in samples of carotid artery blood (S_{a,O_2}) taken while the animal breathed progressively more hypoxic gas mixtures. This calibration was performed in steps over a range of S_{p,O_2} from 100 to 40%. A calibration curve was constructed relating indicated saturation (Nellcor) to the true (Radiometer) value and all saturation values reported in this paper were corrected by using this curve; note, however, that Figs 1, 2 and 4 show uncorrected S_{p,O_2} values. We have previously demonstrated (Wilkinson, Berger, Blanch & Brodecky, 1995) that the Nellcor N-200 oximeter is capable of tracking changes in S_{a,O_2} as rapid as 5% s^{-1}.

Experimental protocols

Four protocols were used to explore the impact of changing inspired oxygen levels on breathing stability. Each protocol incorporated three periods: hyperventilation at a tidal volume and frequency sufficient to achieve a desired level of arterial hypocapnia, followed by post-hyperventilation apnoea (PHA) and finally a period of spontaneous breathing. The protocols differ in the composition of the gas used for hyperventilation and in the composition and presentation of the gas provided for inspiration in the post-hyperventilation period.

Protocol 1. Hyperoxic hyperventilation. The lambs were hyperventilated with hyperoxic inspired gas (inspired O_2 fraction, $F_{I,O_2} = 0·8$) for a period of 5 min after which the ventilator was turned off. At the instant breathing recommenced following PHA, we measured S_{a,O_2} together with the P_{CO_2} of the jugular venous blood (P_{jv,CO_2}), which for reasons considered in the Discussion we equate to the central CO_2 ventilatory threshold ($P_{v,th}$). This procedure was repeated at least three times at different levels of hyperventilation as measured using end-tidal CO_2. The reported $P_{v,th}$ value for each animal (Fig. 7) represents the mean of these trials. We also measured the minute ventilation associated with the first five breaths following PHA. We refer to this measure as $\dot{V}_{jump}$ (Wilkinson *et al.* 1996).

Protocol 2. Hyperventilation with air. By adjusting the tidal volume and respiratory frequency during the hyperventilation period, it was possible to produce a PHA that was terminated at an S_{a,O_2} of approximately 60%. Our preliminary experiments had shown that this level of hypoxaemia would produce PB in the majority of animals. The inspired oxygen concentration during apnoea and prior to the onset of breathing was not changed from air.

When breathing recommenced it was categorized as either stable (no PB) or unstable (PB). The duration of PB (epoch length), measured from the first breath following PHA to the end of the last apnoeic pause, and the cycle duration, defined as the time separating successive peaks in ventilation, were measured for each epoch observed. For our purposes, the epoch had to have at least two apnoeic pauses separated by periods of breathing, so that periodic modulation of ventilation without apnoeic pauses was not included in our definition of PB. An apnoeic pause was said to have occurred when the expiratory pause duration exceeded that of the previous breath by more than a factor of 2. Ventilatory duration during a cycle of PB was measured from the start of the first inspiration to the start of the last expiration during a cluster of breaths.

For the first five cycles of PB we measured the mean V/A ratio, which was defined as the ratio of the ventilatory duration in a cycle of PB to the apnoeic duration in the same cycle.

Protocol 3. Effect of hyperoxia during PB. Those animals that exhibited PB on air in protocol 2 were switched to hyperoxic inspired gas ($F_{I,O_2} = 0·8$) after five cycles of PB. The cycle time, V/A ratio, apnoea duration, minute ventilation, respiratory rate, tidal volume and end-tidal CO_2 were measured for each of five cycles before and after the application of hyperoxia. We also measured the total epoch length of PB from the commencement of breathing following PHA to the end of the last apnoeic pause of PB.

Protocol 4. Effect of hyperoxia during PHA. The animals that had exhibited stable breathing in air following PHA in protocol 2 were switched to hyperoxic gas during PHA in a second test run. Immediately after cessation of mechanical ventilation, inspired F_{I,O_2} was increased to 0·8 so that when breathing commenced the lamb breathed a hyperoxic gas mixture against a background of arterial hypoxaemia which developed during PHA. If PB was induced, the epoch length was measured together with the cycle time and V/A ratio over the first five cycles of PB.

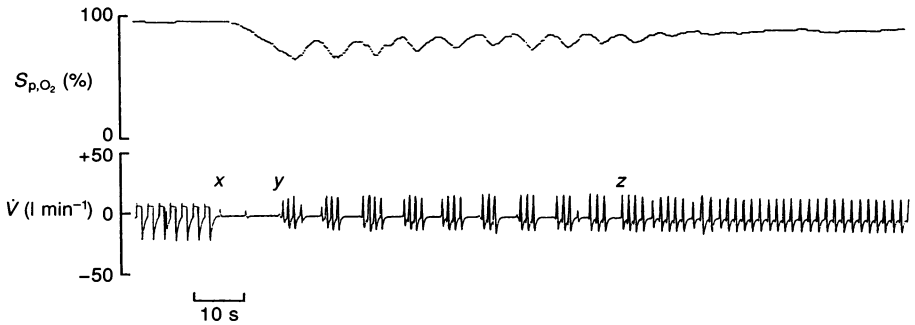

Figure 1. PB in a lamb following post-hyperventilation apnoea

Typical record of periodic breathing (PB) produced in a 16-day-old lamb. Hyperventilation with air ended at x in the lower trace and was followed by post-hyperventilation apnoea which ended at point y. PB continued until point z. $\dot{V}$ is the respiratory flow rate, S_{p,O_2} is the uncorrected oxyhaemoglobin saturation taken directly from the pulse oximeter.

Statistics

All data quoted are means ± S.E.M. Differences between means were tested using a Student's t test (paired or unpaired as required) with $P < 0{\cdot}05$ being taken as the critical level.

RESULTS

Protocol 1. Hyperoxic hyperventilation

Sixteen of seventeen animals showed stable breathing patterns after the post-hyperventilation apnoea (PHA) induced by hyperventilation with a hyperoxic gas mixture. The S_{a,O_2} at the end of PHA was 99·8 ± 0·1% ($n = 17$). The remaining animal showed a highly unstable pattern of breathing. This pattern, which we refer to as episodic breathing (see later), consists of periods of breathing lasting 40–90 s alternating with apnoeas of similar duration and may be analogous to the qualitatively similar pattern known as Biot's breathing (Plum & Brown, 1963; Younes, 1989).

Protocol 2. Hyperventilation with air

Eleven lambs exhibited PB following PHA (Fig. 1) with an epoch duration of 80·3 ± 12·0 s, a cycle duration of 9·5 ± 0·5 s and a ratio of ventilatory duration to apnoea duration (V/A ratio) of 1·28 ± 0·27. The remaining six lambs did not exhibit PB following PHA. There was no

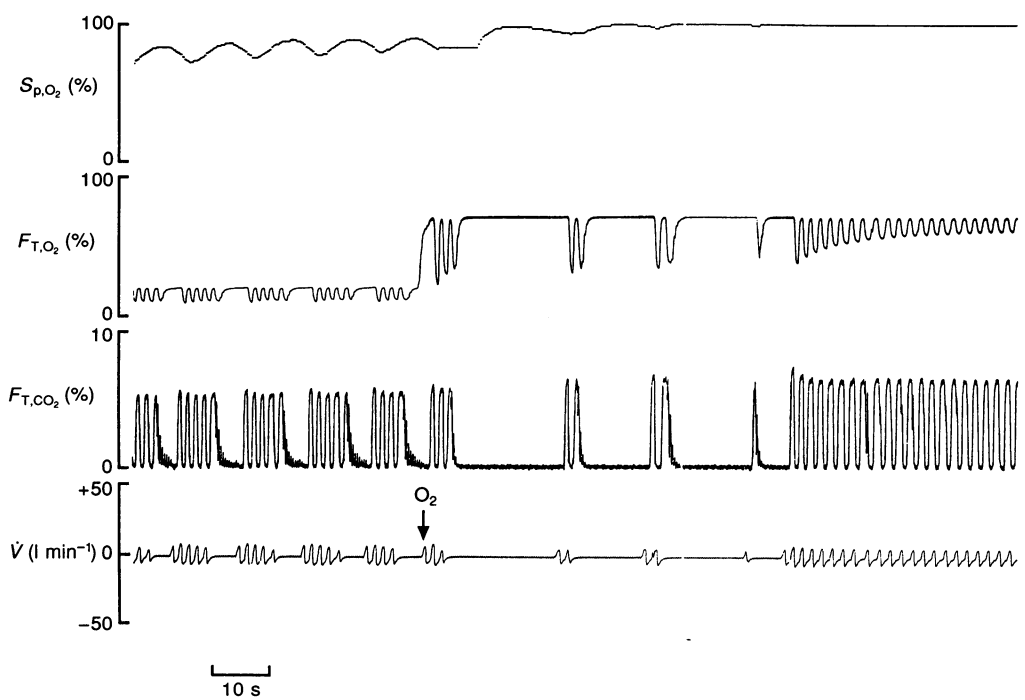

Figure 2. Effect of hyperoxia during PB in a lamb

Response to increasing the inspired oxygen from 21 to 80% during PB. Notice the immediate prolongation of cycle time and apnoeic duration and the reduced V/A ratio and the resulting hypoventilation which results in an increase in end-tidal CO_2. F_{T,O_2} (%) and F_{T,CO_2} (%) are the tracheal fractional oxygen and carbon dioxide concentrations, respectively.

difference in the age (16·4 days *vs.* 18·0 days) or weight (7·5 ± 0·5 kg *vs.* 7·6 ± 0·5 kg) of lambs that exhibited PB and those that did not. Furthermore there was no significant difference in the rectal temperature (40·3 ± 0·2 *versus* 40·5 ± 0·3 °C) between those exhibiting PB and those that did not. Although these temperatures are slighly above those seen in normal awake lambs in our laboratory, which are typically between 39·5 and 39·8 °C, the average respiratory rate in our lambs was 33·7 breaths min^{-1}, a rate similar to that reported in sleeping 14-day-old lambs under thermoneutral conditions by other investigators (Andrews, Symonds & Johnson, 1991).

Protocol 3. Effect of hyperoxia during PB

Ten of the eleven unstable lambs were challenged with hyperoxia during PB. All ten lambs showed a response typified by Fig. 2. The cycle time was significantly increased during the first cycle following the application of hyperoxia (16·4 ± 1·8 s) compared with the cycle time just prior to hyperoxia (9·4 ± 0·4 s, Fig. 3*A*). By the fourth cycle following hyperoxia, the mean cycle time of 11·5 ± 1·4 s was not significantly greater than the pre-hyperoxia value. The *V*/*A* ratio fell abruptly from 1·5 ± 0·3 just prior to oxygen administration to 0·3 ± 0·05 during the first cycle following hyperoxia and remained significantly depressed with respect to the pre-hyperoxic *V*/*A* ratio for four cycles. Minute ventilation fell abruptly from 0·19 ± 0·03 l min^{-1} kg^{-1} just prior to oxygen administration to 0·07 ± 0·01 l min^{-1} kg^{-1} immediately after. This fall was mediated predominantly by a fall in respiratory rate from 19·2 ± 2·9 to 8·3 ± 1·0 breaths min^{-1} (Fig. 3*B*). The epoch duration was significantly increased by the application of hyperoxia from 80·3 ± 12·0 to 121·2 ± 9·2 s.

Surprisingly, in four lambs, following the application of hyperoxia PB was soon replaced by episodic breathing identical in form to that described earlier in one lamb following hyperoxic hyperventilation in protocol 1 (Fig. 4*A* and *B*). In episodic breathing, mean epoch duration was 403 ± 120 s and mean *V*/*A* ratio was 1·24 ± 0·28. In two lambs we sampled jugular venous blood at the beginning and end of each cluster of breaths. As discussed later, we consider the jugular venous P_{CO_2} at the beginning of each cluster represents the ventilatory threshold ($P_{v,th}$), and the jugular venous P_{CO_2} at the end of each cluster represents the apnoeic threshold ($P_{ap,th}$). The P_{CO_2} difference (ΔP_{CO_2}) between these samples was 7·6 and 7·2 mmHg, respectively, in these two animals. Each breathing cluster displayed characteristic changes of respiratory rate, tidal volume, end-tidal P_{CO_2} and minute ventilation with time (see Fig. 5). At

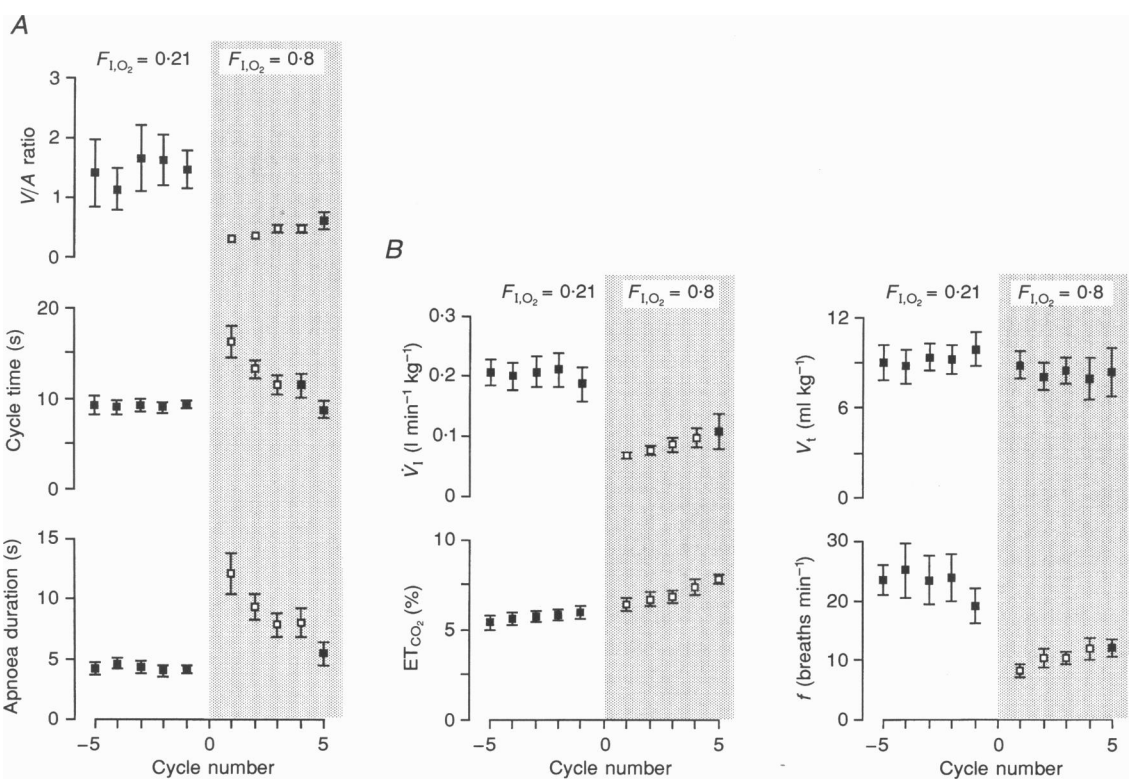

Figure 3. Effect of hyperoxia on periodic breathing

A, cycle by cycle changes in *V*/*A* ratio, cycle time and apnoea duration following an increase in F_{I,O_2} from 0·21 to 0·8 during PB. *B*, cycle by cycle changes in minute ventilation ($\dot{V}_I$), end-tidal CO_2 (ET_{CO_2}), tidal volume (V_t) and respiratory rate (f), following an increase in F_{I,O_2} from 0·21 to 0·8. Notice the dramatic fall in minute ventilation following hyperoxia, mediated mainly by a fall in respiratory rate. Open symbols indicate that the relevant variable is significantly different from the same variable one cycle prior to the application of hyperoxia.

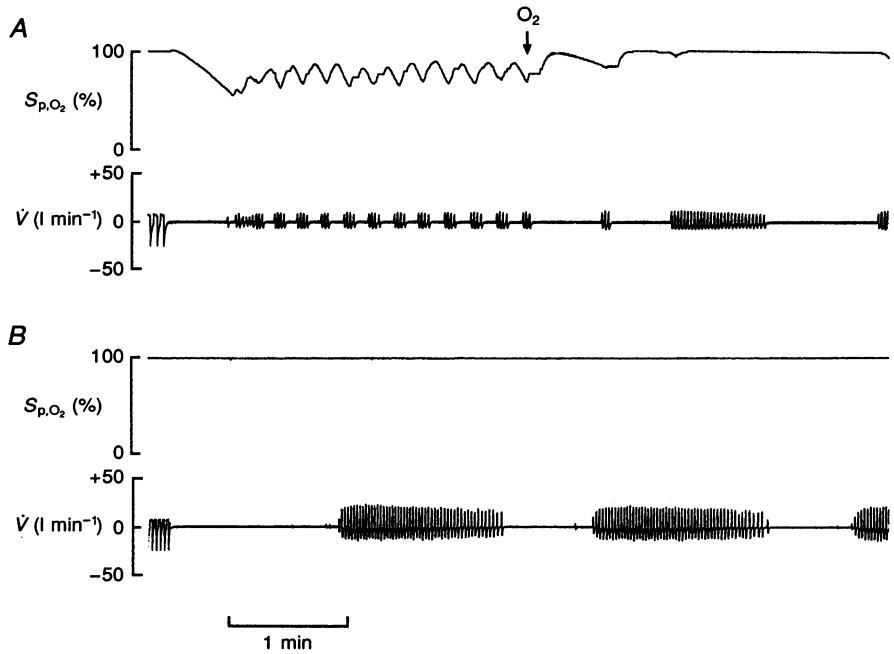

Figure 4. Episodic breathing in lambs

Two different circumstances under which episodic breathing was produced in lambs: by applying a hyperoxic gas mixture during the PB which followed passive hyperventilation with air (seen in 4 lambs) (*A*); and following passive hyperventilation with a hyperoxic gas mixture (seen in 1 lamb) (*B*).

the start of the breathing phase there was a sudden jump in ventilation ($\dot{V}_{jump}$) mediated predominantly by an increase in tidal volume; end-tidal P_{CO_2} decreased abruptly during this phase. Thereafter the minute ventilation, respiratory rate and tidal volume all declined progressively with time while end-tidal P_{CO_2} plateaued initially and then increased slowly until the breathing phase was suddenly terminated.

Protocol 4. Effect of hyperoxia during PHA

Five of the six lambs that exhibited stable breathing when the lamb breathed air at the end of PHA were found to exhibit PB when breathing began in hyperoxic gas (Fig. 6). The mean epoch length of 29·6 ± 7·8 s was significantly less than the epoch length manifested by lambs that were unstable in air (80·3 ± 12·0 s, $P < 0·01$). However, the *V*/*A*

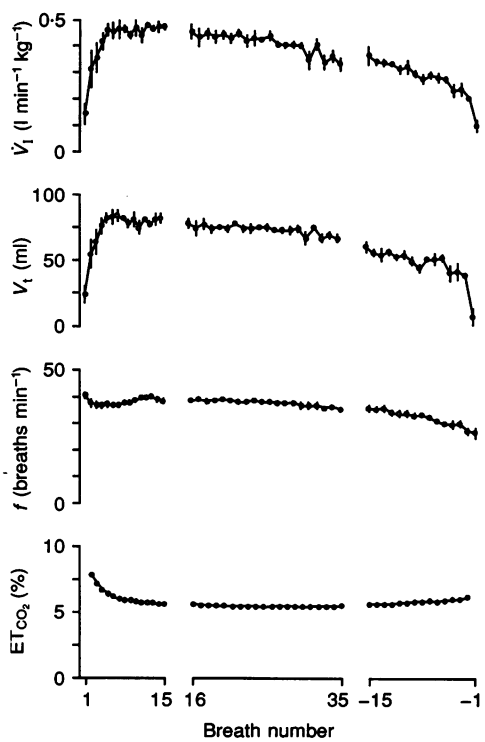

Figure 5. Mean ventilatory changes during the breathing phase of episodic breathing

Mean ± S.E.M. breath by breath ventilatory parameters measured over 4 breathing phases during episodic breathing in a lamb. Because each breathing phase contained a slightly different number of breaths, the averages are divided into three representative segments; breaths 1–15, breaths 16–35 and the last 15 breaths numbered from −15 to −1. End-tidal CO_2 measurements for the first and last breath are not shown because the expired volume for these breaths was too small to produce an alveolar plateau. Notice the sudden increase in minute ventilation at the start of the ventilatory phase and the equally sudden decrease near the end which was characteristic of episodic breathing. Notice also that minute ventilation continued to fall near the end of the ventilatory phase despite the rise in end-tidal CO_2. For definitions of abbreviations, see legend to Fig. 3.

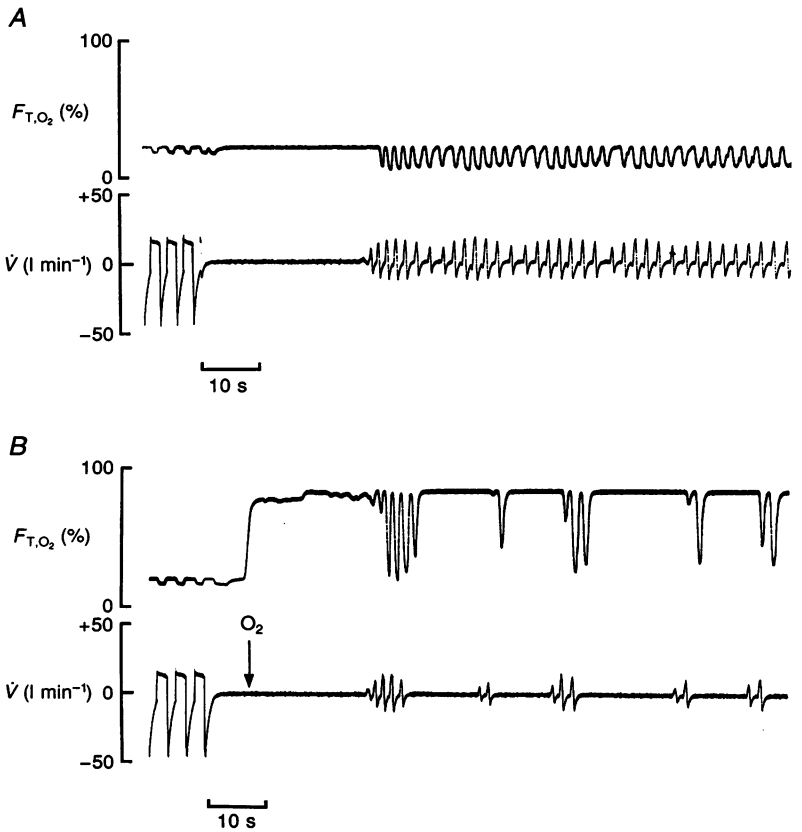

Figure 6. PB produced using hyperoxia following PHA

Example of a lamb that was not unstable after PHA when breathing restarted in air (*A*) but became unstable when breathing began in hyperoxic gas (*B*).

ratio of 0·95 ± 0·33 and cycle time of 9·7 ± 0·8 s were not significantly different from the unstable animals. Episodic breathing was not seen in any of these five lambs, all of which converted directly to a stable breathing pattern following PB.

$\dot{V}_{jump}$ and $P_{v,th}$

The $\dot{V}_{jump}$ measured after PHA in protocol 1 was significantly greater ($P < 0·01$) in unstable (196 ± 22 ml min^{-1} kg^{-1}, $n = 11$) than in stable animals (70·9 ± 3·7 ml min^{-1} kg^{-1}, $n = 6$). The lambs that exhibited episodic breathing had a significantly higher $\dot{V}_{jump}$ (241 ± 26 ml min^{-1} kg^{-1}, $n = 5$) than those that did not (115 ± 19 ml min^{-1} kg^{-1}, $n = 12$, Fig. 7).

The ventilatory threshold, $P_{v,th}$, was not significantly greater in unstable lambs (61·7 ± 3·1 mmHg, $n = 11$) than in the stable group (54·9 ± 1·7 mmHg, $n = 6$). However, when the animals were divided into those with and without episodic breathing, the ventilatory threshold

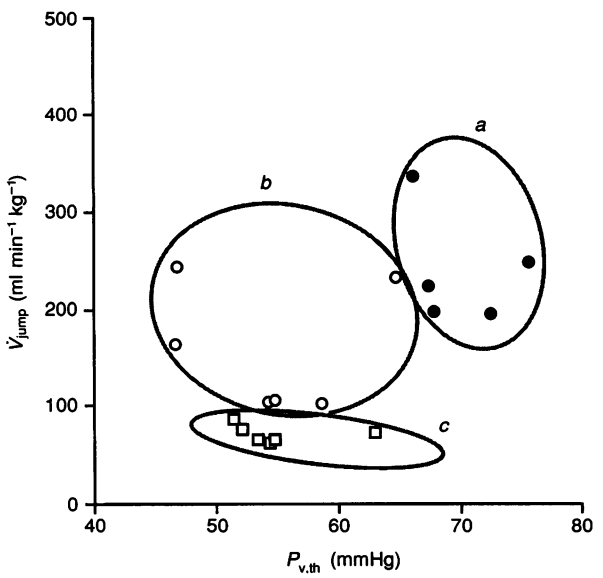

Figure 7. Ventilatory threshold and $\dot{V}_{jump}$ in lambs exhibiting stable, unstable and episodic breathing

Plot of $\dot{V}_{jump}$ against the ventilatory threshold ($P_{v,th}$). Lambs that exhibited episodic breathing (●, group *a*) had a $\dot{V}_{jump}$ and $P_{v,th}$ which were significantly larger than in the remaining lambs. The other groups contain those animals which exhibit PB following hyperventilation on air (○, group *b*), which have lower $P_{v,th}$ values but elevated $\dot{V}_{jump}$ and those that are stable following hyperventilation on air (□, group *c*), which have a lower $P_{v,th}$ and $\dot{V}_{jump}$.

was significantly higher in lambs that exhibited episodic breathing (69·9 ± 1·78 mmHg, $n = 5$) than in those that did not (54·6 ± 1·6 mmHg, $n = 12$, Fig. 7).

DISCUSSION

This study presents evidence which demonstrates that increasing inspired oxygen has a paradoxical destabilizing effect on breathing in the lamb. First, at the end of apnoea produced by hyperventilation with air, lambs which manifested a stable pattern of breathing when they initiated breathing in air were found to exhibit PB when they initiated breathing in hyperoxic gas. Second, when those lambs that were unstable in air after PHA were switched to hyperoxic gas during PB, there was an immediate transient increase in the cycle time of PB and a decrease in V/A ratio (prolongation of apnoea). As discussed later these two effects are consistent with an increase in the loop gain of the respiratory control system, and increasing loop gain tends to destabilize control systems. An unexpected finding in this study was the appearance of episodic breathing in four lambs when they had their inspired gas switched from air to hyperoxia during PB.

Periodic breathing in hyperoxia

The effect of hyperoxia in producing PB after PHA in lambs that were stable in air, and in reducing the V/A ratio during PB in lambs unstable in air, indicates that the respiratory controller is rendered unstable by hyperoxia. Paradoxically, it might have been predicted that no PB would be seen in hyperoxia, since carotid body activity has been shown to be suppressed under these conditions. This apparent paradox disappears when we examine the initial blood gas conditions at the end of PHA. In our study there were two distinct protocols in which the lambs breathed hyperoxic gas at the end of PHA. In one, mechanical hyperventilation was performed with hyperoxic gas; this gas was maintained during PHA and when the lambs began to breathe after apnoea. In this case, hypoxaemia did not develop during PHA and when breathing resumed it was immediately stable in all animals except one, which showed episodic breathing. In the second protocol, mechanical hyperventilation was performed with air and hypoxaemia developed during apnoea. The inspired gas was switched to a hyperoxic mixture during PHA and five of six lambs that had not exhibited PB in air did so in hyperoxia. The different outcomes of these two protocols demonstrate that inhalation of hyperoxic air *per se* does not prevent unstable breathing from developing. Instead, inhalation of hyperoxic air against a background of hypoxaemia promotes instability in the respiratory controller. Just how this occurs may be simply explained.

At the end of apnoea resulting from hyperventilation with air, central chemoreceptor drive is far below the apnoeic threshold. Breathing starts as rapidly developing hypoxaemia stimulates the carotid chemoreceptors. After a few breaths (the actual number of breaths is determined by the respiratory rate and the lung peripheral chemoreceptor circulation time), increased P_{a,O_2} reduces chemoreceptor output so that it is now insufficient to drive ventilation, and apnoea ensues. This cycle repeats until central CO_2 rises to a level in excess of the apnoeic threshold. The magnitude of the change in P_{a,O_2} that occurs when breathing recommences at the end of PHA will be proportional to the difference between inspired and alveolar P_{O_2}. To take an extreme case, if inspired and alveolar P_{O_2} are equal, no change in P_{a,O_2} will occur after the onset of ventilation. On the other hand, the more hyperoxic the inspired gas the greater the change (increase) that will be produced in alveolar P_{O_2} and P_{a,O_2} and consequently the greater the reduction in the hypoxic ventilatory drive produced by the peripheral chemoreceptors following the onset of breathing. If we conventionally define the loop gain as the ratio of the ventilatory reduction produced by the peripheral controller to the increase in ventilation that produced this reduction in the first place, it follows that the inspiration of progressively more hyperoxic gas has the effect of augmenting the loop gain of the peripheral controller, since the greater the inspired O_2 the greater the reduction in peripheral chemoreceptor drive that results from a given increase in ventilation. This increase in loop gain tends to render the respiratory control system unstable, and in our experiments led to five of our six stable animals becoming unstable in hyperoxic gas (see Fig. 6). It is interesting that this behaviour, which we have demonstrated experimentally, could have been predicted using the mathematical model of Khoo *et al.* (1982) in which the difference between inspired P_{O_2} and P_{a,O_2} appears as a multiplying factor in the peripheral chemoreceptor loop gain equation used to predict instability.

The foregoing discussion is also relevant to our experiments in which oxygen was administered during PB. This produced a sudden increase in apnoeic duration and cycle time, and an associated fall in V/A ratio (Fig. 2) and it has been demonstrated previously in adult animals that a decrease in the V/A ratio is associated with an increase in loop gain of the respiratory controller (Cherniack, von Euler, Homma & Kao, 1979). The changes in apnoeic duration and cycle time in response to hyperoxia were slowly reversed as end-tidal CO_2, and presumably central chemoreceptor CO_2, rose until breathing stabilized. During this time we would expect that the hypoxic respiratory drive would be progressively reduced and replaced by increased central CO_2 drive. Thus although oxygen administration initially destabilizes breathing, as evidenced by the decrease in V/A ratio, the consequent increase in central CO_2 drive eventually stabilizes breathing and resolves the cyclic arterial hypoxaemia. A critical point that follows from this discussion is that hyperoxia in essence stabilizes breathing only in so far as it reduces ventilation during PB, thereby allowing central CO_2 to rise more rapidly towards $P_{v,th}$ (Fig. 3*B*). Hyperoxia itself has only a destabilizing influence on ventilation, as evidenced by the fall in V/A ratio and the increased PB epoch duration which occurs following the administration of oxygen during PB.

The foregoing explanations may be of relevance to previous studies of PB in human infants. In both the studies of Cross & Oppe (1952) and Rigatto & Brady (1972), the incidence of PB was increased during exposure to hypoxic gas and increased still further in the first minute following a change to hyperoxia (Cross & Oppe, 1952) or on returning to room air (Rigatto & Brady, 1972). The observed fall in the V/A ratio, secondary to an increase in inspired oxygen (Rigatto & Brady, 1972), provides direct evidence for an increased respiratory controller gain. These results are similar to those from our experiments illustrated in Figs 2 and 3 and demonstrate the paradoxical destabilizing effect of oxygen applied against a background of arterial hypoxaemia.

Episodic breathing

Episodic breathing was observed in five lambs. In four, episodic breathing occurred following oxygen administration during PB that had been induced following PHA, while in the fifth it occurred immediately after PHA without any intervening PB. This pattern cannot be mediated by the peripheral chemoreceptors, since it occurs against a background of arterial hyperoxia (Fig. 4*A* and *B*) where the peripheral chemoreceptors are presumably silenced. Further supportive evidence for this view comes from the work of Webber (1981) who showed that episodic breathing in cats with bilateral pneumotaxic system lesions continues after carotid body denervation, although the apnoeic interval is lengthened.

Several clues to the genesis of episodic breathing come from our observations. First, the $\dot{V}_{jump}$ and ventilatory threshold for these animals were significantly higher than in the other animals (Fig. 7), and increases in either $\dot{V}_{jump}$ (Wilkinson *et al.* 1996) or the ventilatory threshold (Bulow, 1963) can destabilize breathing. Second, measurement of a P_{CO_2} difference between the ventilatory (on) threshold and the apnoeic (off) threshold during episodic breathing shows that the central P_{CO_2} at which breathing begins is 7–8 mmHg higher than the P_{CO_2} at which it is terminated. The relationship between ventilation and central P_{CO_2} therefore exhibits an apparent hysteresis because the apnoeic threshold is considerably below the ventilatory threshold. Interestingly, it has been shown recently in human adults that hyperventilation-induced apnoeas are commonly initiated at a P_{a,CO_2} which is significantly less than that present upon re-initiation of phasic respiration (Leevers, Simon, Xi & Dempsey, 1993). This observation is clearly compatible with our finding that the apnoeic and ventilatory thresholds are different. From their observation, Leevers *et al.* (1993) hypothesized that once the pattern generator is reduced below threshold via sensory inhibition of any type (including chemical), it requires a substantial increase in chemoreceptor stimuli above the apnoeic threshold to restore phasic respiration. They referred to this property of the central respiratory pattern generator as 'inertia' without elaborating a specific underlying mechanism. Whatever the cause, it manifests as a change in the CO_2 threshold, with the result that the apnoeic and ventilatory thresholds are different.

Accordingly, we propose a model to account for the genesis of episodic breathing in which we assume that the apnoeic and ventilatory thresholds are different by an amount equal to the difference in P_{jv,CO_2} we measure at the beginning and end of apnoea (Fig. 8). We propose that central P_{CO_2} rises

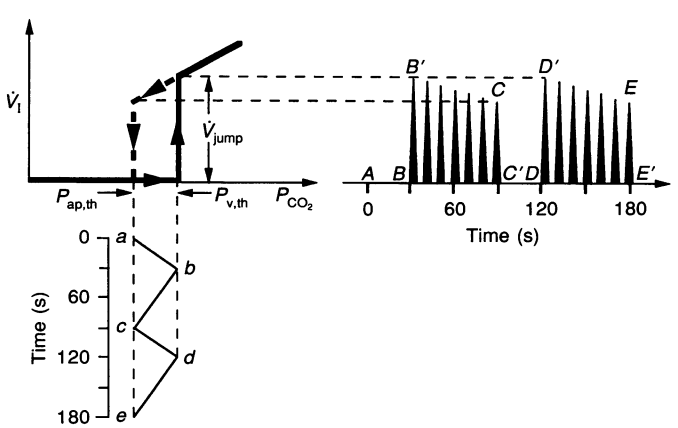

Figure 8. Proposed model of episodic breathing

Model showing how the respiratory controller operates at the apnoeic and ventilatory thresholds in episodic breathing. Central P_{CO_2} at each instant in time is indicated with a lower case letter and the ventilation at that instant with the same letter capitalized. Initially at *a*, central P_{CO_2} is below the ventilatory threshold ($P_{v,th}$) and breathing is suppressed (at *A*). During the apnoeic period, P_{CO_2} rises centrally until it reaches $P_{v,th}$ at *b*. Breathing then starts under the control of the central rhythm generator and jumps abruptly from *B* to *B*′. At the same time the threshold is reset to the apnoeic threshold ($P_{ap,th}$). $\dot{V}_I$ now falls progressively from *B*′ to *C* as central P_{CO_2} falls until the apnoeic threshold is reached at *c* when breathing turns off. At the same time the threshold is reset to $P_{v,th}$. During the apnoeic interval P_{CO_2} rises until breathing turns on again at *d*. The cycle then repeats, generating the pattern characteristic of episodic breathing.

and falls more or less linearly between the apnoeic threshold and the ventilatory threshold. Each time breathing changes state between on or off, the CO_2 threshold is reset (by approximately 7–8 mmHg) in such a direction as to 'lock in' that state – downwards (to the apnoeic threshold) if breathing turns on, or upwards (to the ventilatory threshold) if breathing turns off. Breathing (or apnoea) then follows for a period such that P_{jv,CO_2} reaches the new CO_2 threshold after which the existing state (breathing or apnoea) is terminated and the opposite state is temporarily 'locked in' in a cyclic fashion. Henceforth the cycle repeats indefinitely.

Our explanation of the genesis of episodic breathing, in which central CO_2 rises and then falls progressively with time between the apnoeic and ventilatory thresholds, may appear to be at variance with Fig. 5, which shows that during the breathing phase end-tidal CO_2 falls initially but thereafter plateaus, and even rises slightly, before breathing ceases. Bearing in mind that it is central chemoreceptor P_{CO_2} that is the dominant drive to breathing, and not P_{a,CO_2}, two pieces of evidence demonstrate that the rise in P_{a,CO_2} is unimportant during episodic breathing. First, we see clearly in Fig. 5 that ventilation continues to fall monotonically until apnoea intervenes. This evidence unambiguously supports the view that central chemoreceptor P_{CO_2} falls during the entire ventilatory phase. Second, regardless of the rise in P_{a,CO_2} near the end of the ventilatory phase, the arterio-venous P_{CO_2} difference remains so large that CO_2 must continue to be cleared from the head at a rate greater than it is being produced metabolically. This follows from the fact that under steady-state conditions an arterio-venous P_{CO_2} difference of approximately 8 mmHg is sufficient to clear all the metabolically produced CO_2 from the head; we estimate this difference at the end of the ventilatory phase of episodic breathing to be approximately 22 mmHg (this estimate is derived by subtracting the end-tidal CO_2 just before apnoea in Fig. 5 from the P_{jv,CO_2} at the apnoeic threshold, a value that was measured earlier in the experiment).

An important requirement of our model of episodic breathing is that once breathing commences following apnoea, the lamb must hyperventilate with respect to the apnoeic threshold so that the breathing phase can be terminated. The level of ventilation required to achieve this will be reduced when the apnoeic threshold (and thus the ventilatory threshold) is elevated, so that we would predict that those animals with both elevated ventilatory thresholds and $\dot{V}_{jump}$ would be predisposed to episodic breathing. This prediction is borne out by the results shown in Fig. 7. Moreover, since the apnoeic threshold has been shown to be significantly elevated during hyperoxia compared with normoxia (Delacourt, Canet & Bureau, 1996), this mechanism may also help explain why hyperoxia induces episodic breathing in some of our lambs. What is also clear from Fig. 7 is that the unstable group has a significantly higher $\dot{V}_{jump}$ than the stable group.

Use of P_{jv,CO_2} to estimate apnoeic and ventilatory thresholds

In the foregoing development we have assumed that measured P_{jv,CO_2} can be equated with central chemoreceptor P_{CO_2}; is this a reasonable assumption? Clearly jugular venous blood includes an extra-cerebral component of blood flow as well as that from the brain. Thus, while it reflects tissue P_{CO_2} in the head, it does not necessarily reflect the level at the central chemoreceptors in the hindbrain. To examine the extent to which changes in P_{jv,CO_2} do reflect what is occurring at the central chemoreceptors requires a careful examination of the balance between CO_2 production and clearance from the body and brain tissues during apnoea. Because elimination of CO_2 from the lungs ceases during apnoea, arterial P_{CO_2} comes rapidly into equilibrium with mixed venous P_{CO_2} and thereafter both rise linearly with time; this phenomenon represents the fundamental principle used by Read (1967) in his classic rebreathe method for quantifying the ventilatory response to CO_2. By utilizing a mathematical model similar to that of Read (1967), it can be shown that if the metabolic rate per unit tissue weight in each compartment of the brain is similar to that in the body, then after a brief transient phase, all tissue CO_2 levels within the brain rise linearly at the same rate, essentially independently of blood flow. In this case the change in P_{jv,CO_2} during apnoea would accurately reflect the change of P_{CO_2} in every compartment of the brain. On the other hand, if the metabolic rate per unit tissue weight in a single compartment were low, the transient phase would be prolonged and changes in P_{jv,CO_2} would overestimate changes in the tissue levels of CO_2 in that compartment. Lowering blood flow to that compartment now has a substantial effect and lengthens the transient phase even further. The net effect is analogous to filtering the rise and fall in P_{CO_2} that occurs during cycles of apnoea and ventilation. It follows that if the central CO_2 chemoreceptor area has a relatively low metabolic rate, and receives a low blood flow relative to the head as a whole, the change in P_{jv,CO_2} measured during apnoea would be greater than that actually occurring at the chemoreceptors. However, even in this case, our analysis shows that changes in the central chemoreceptor P_{CO_2} during apnoea will be directly related to changes in P_{jv,CO_2}. We contend, therefore, that P_{jv,CO_2} may be used, under the conditions of our experiments, to estimate the CO_2 level at the central chemoreceptors, recognizing, however, that it may overestimate the true difference between the apnoeic and ventilatory thresholds. Ultimately, resolution of the difference between the apnoeic and ventilatory thresholds may only be possible when the exact location of the central chemoreceptors is known, and a method for directly measuring the tissue P_{CO_2} surrounding the central chemoreceptors is available.

Unstable breathing in premature infants – periodic or episodic?

It has been suggested that the PB seen in premature infants may be of the episodic or cluster variety (Webber & Speck,

1981). Certainly, published recordings of PB in premature infants (Rigatto & Brady, 1972; Miller & DiFiore, 1995; Haider *et al.* 1995) show an abrupt onset of breathing with little change in tidal volume before apnoea develops; this is similar to the episodic breathing pattern illustrated in Fig. 4. Interestingly, the response of the human premature infant to inspired CO_2 during PB is characterized by an increased cycle duration but no change in the apnoeic pause (Fenner, Schalk, Hoenicke, Wendenburg & Roehling, 1973) until PB finally disappears when the inspired CO_2 reaches a level between 2 and 4%. This pattern of response is different from that seen when inspired CO_2 is increased during PB in adults, where cycle time and apnoeic pause duration are little affected although PB is eventually suppressed (Lahiri *et al.* 1983). The apparent differences between the response to CO_2 during PB in adults and during unstable breathing in premature infants would support the view that these breathing patterns are fundamentally different, as proposed previously (Webber & Speck, 1981; Younes, 1989).

Although we did not challenge the lambs by increasing inspired CO_2 during episodic breathing, we predict, using our suggested model illustrated in Fig. 8, that if inspired CO_2 were increased, P_{jv,CO_2} would fall more slowly during the breathing phase towards the apnoeic threshold and hence the breathing phase would be extended. If inspired CO_2 were sufficiently elevated, the apnoeic threshold would not be reached and no apnoeic pause would result, so that the periodic pattern would be suppressed in favour of continuous breathing. During the apnoeic phase, the rate of rise of P_{jv,CO_2} towards the ventilatory threshold would be affected only by the metabolic production rate of CO_2 in the brainstem, so we predict that the apnoeic interval would be unaffected by the previously inspired CO_2 level. These different effects of CO_2 on the timing of the apnoeic pause and the length of the breathing phase during episodic breathing are in substantial agreement with the observations of Fenner *et al.* (1973) in premature infants and lend further support to the idea that PB in premature infants may be of the episodic variety.

The episodic breathing pattern seen in seal pups (Castellini *et al.* 1994), hibernating mammals (Milsom, 1991) and some lower vertebrates (West, Smits & Burggren, 1989; Kinkead & Milsom, 1994) has baffled investigators for many years. The mechanism which controls breathing in each of these cases may be similar to the one we suggest underlies episodic breathing in the newborn lamb. Moreover, the pattern of episodic breathing seen in the clinical disorder known as Rett syndrome, which is characterized by alternating periods of hyperventilation and apnoea (Southall, Kerr, Tirosh, Amos, Lang & Stephenson, 1988), might be similarly explained.

In summary, the paradoxical destabilizing effect of oxygen documented in this work can be explained by recognizing that the administration of oxygen transiently increases the loop gain of the respiratory controller, producing PB which is mediated predominantly via the peripheral chemoreceptor loop. By contrast, the observation of episodic breathing following hyperoxia in some animals strongly suggests that this mode of breathing is mediated via the central chemoreceptors. The presence of hysteresis in the CO_2 threshold, and the destabilizing effect of the infinite gain segment characterized by Wilkinson *et al.* (1996), can explain the generation of this form of breathing and may account for the pattern of PB seen in premature infants.

Andrews, D. C., Symonds, M. E. & Johnson, P. (1991). Thermoregulation and the control of breathing during non-REM sleep in the developing lamb. *Journal of Developmental Physiology* **16**, 27–36.

Bulow, K. (1963). Respiration and wakefulness in man. *Acta Physiologica Scandinavica* **59**, suppl. 209, 1–110.

Canet, E., Carroll, J. L. & Bureau, M. A. (1989). Hypoxia-induced periodic breathing in newborn lambs. *Journal of Applied Physiology* **67**, 1226–1233.

Castellini, M. A., Milsom, W. K., Berger, R. J., Costa, D. P., Jones, D. R., Castellini, J. M., Rea, L. D., Bharma, S. & Harris, M. (1994). Patterns of respiration and heart rate during wakefulness and sleep in elephant seal pups. *American Journal of Physiology* **35**, R863–869.

Cherniack, N. S. & Longobardo, G. S. (1986). Abnormalities in respiratory rhythm. In *Handbook of Physiology*, section 3, *Respiration*, ed. Cherniack, N. S. & Widdicombe, J. G., pp. 729–749. American Physiological Society, Bethesda, MD, USA.

Cherniack, N. S., von Euler, C., Homma, I. & Kao, F. F. (1979). Experimentally induced Cheyne-Stokes breathing. *Respiration Physiology* **37**, 185–200.

Cross, K. W. & Oppe, T. E. (1952). The effect of inhalation of high and low concentrations of oxygen on the respiration of the premature infant. *Journal of Physiology* **117**, 38–55.

Delacourt, C., Canet, E. & Bureau, M. A. (1996). Predominant role for peripheral chemoreceptors in the termination of apnoea in maturing newborn lambs. *Journal of Applied Physiology* **80**, 892–898.

Fenner, A., Schalk, U., Hoenicke, H. Wendenburg, A. & Roehling, T. (1973). Periodic breathing in premature and neonatal babies: incidence, breathing pattern, respiratory gas tensions, response to changes in the composition of ambient air. *Pediatric Research* **7**, 174–183.

Haider, A. Z., Rehan, V., Al-Saedi, S., Alvaro, R., Kwiatkowski, K., Cates, D. & Rigatto, H. (1995). Effect of baseline oxygenation on the ventilatory response to inhaled 100% oxygen in preterm infants. *Journal of Applied Physiology* **79**, 2101–2105.

Khoo, M. C. K., Kronauer, R. E., Strohl, K. P. & Slutsky, A. S. (1982). Factors inducing periodic breathing in humans: a general model. *Journal of Applied Physiology* **53**, 644–659.

Kinkead, R. & Milsom, W. K. (1994). Chemoreceptors and control of episodic breathing in the bullfrog (*Rana catesbeiana*). *Respiration Physiology* **95**, 81–98.

Lahiri, S., Hsiao, C., Zhang, R., Mokashi, A. & Nishino, T. (1985). Peripheral chemoreceptors in respiratory oscillations. *Journal of Applied Physiology* **58**, 1901–1908.

LAHIRI, S., MARET, K. & SHERPA, M. G. (1983). Dependence of high altitude sleep apnoea on ventilatory sensitivity to hypoxia. *Respiration Physiology* **52**, 281–301.

LEEVERS, A. M., SIMON, P. M., XI, L. & DEMPSEY, J. A. (1993). Apnoea following normocapnic mechanical ventilation in awake mammals: a demonstration of control system inertia. *Journal of Physiology* **472**, 749–768.

MILLER, M. J. & DIFIORE, J. M. (1995). A comparison of swallowing during apnoea and periodic breathing in premature infants. *Pediatric Research* **37**, 796–799.

MILSOM, W. K. (1991). Intermittent breathing in vertebrates. *Annual Review of Physiology* **53**, 87–105.

PLUM, F. & BROWN, H. W. (1963). The effect on respiration of central nervous system disease. *Annals of the New York Academy of Sciences* **109**, 915–931.

READ, D. J. C. (1967). A clinical method for assessing the ventilatory response to carbon dioxide. *Australasian Annals of Medicine* **16**, 20–32.

RIGATTO, H. & BRADY, J. P. (1972). Periodic breathing and apnoea in preterm infants. II. Hypoxia as a primary event. *Pediatrics* **50**, 219–228.

SOUTHALL, D. P., KERR, A. M., TIROSH, E., AMOS, P., LANG, M. H. & STEPHENSON, J. B. (1988). Hyperventilation in the awake state: potentially treatable component of Rett syndrome. *Archives of Disease in Childhood* **63**, 1039–1048.

WEBBER, C. L. (1981). Modification of Biot periodic breathing pattern in cats by carotid sinus nerve section. *Federation Proceedings* **40**, 3.

WEBBER, C. L. & SPECK, D. F. (1981). Experimental Biot periodic breathing in cats: effects of changes in PI_{O_2} and PI_{CO_2}. *Respiration Physiology* **46**, 327–344.

WEST, N. H., SMITS, A. W. & BURGGREN, W. W. (1989). Factors terminating nonventilatory periods in the turtle, *Chelydra serpentina*. *Respiration Physiology* **77**, 337–350.

WILKINSON, M. H., BERGER, P. J., BLANCH, N. & BRODECKY, V. (1995). Effect of venous oxygenation on arterial desaturation rate during repetitive apnoeas in lambs. *Respiration Physiology* **101**, 321–331.

WILKINSON, M. H., BERGER, P. J., BLANCH, N., BRODECKY, V. & JONES, C. (1996). Source of respiratory drive during periodic breathing in lambs. *Respiration Physiology* **104**, 115–126.

YOUNES, M. (1989). The physiological basis of central apnoea and periodic breathing. *Current Pulmonology* **10**, 265–326.

Acknowledgements

We thank Professor Adrian Walker for a critical review of this manuscript. This study was supported by the National Sudden Infant Death Syndrome Council of Australia, the National Health and Medical Research Council of Australia, and the Windermere Foundation.

Author's email address

M. H. Wilkinson: callisto@vaxc.cc.monash.edu.au

Received 13 January 1997; accepted 25 June 1997.