Ventilatory Responsiveness to Progressive Asphyxia during Sleep in Newborn Lambs: Effects of Maternal Anemia during Pregnancy

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Our aim was to determine the effects of intrauterine compromise, induced by maternal anemia, on ventilatory responsiveness of the sleeping newborn to progressive asphyxia. We induced anemia in 6 sheep for the final third of pregnancy and studied their offspring for 2-3 weeks after birth. Lambs from anemic ewes were growth-restricted at birth; they and 6 control lambs were chronically instrumented soon after birth and underwent studies during which we determined ventilatory and arousal responsiveness to a progressive asphyxic stimulus during sleep. During quiet wakefulness, active sleep and quiet sleep, lambs from anemic ewes had elevated end-tidal CO₂ levels (FtCO₂,%) compared to controls. Ventilatory responsiveness (i.e., gradient of relationship between minute ventilation and FtCO₂) was greater in quiet sleep than in active sleep for both groups of lambs but did not differ between the two groups in either active or quiet sleep. Lambs from anemic ewes had significantly higher FtCO₂ values than controls before arousing from either active or quiet sleep. Other indices of arousability (time to arousal, percent hemoglobin saturation at arousal) were not different between the two groups. Our results indicate that prenatal exposure to maternal anemia induces fetal growth restriction and elevates the CO₂ 'set-point' for normal ventilation. It does not, however, produce significant abnormalities in ventilatory responsiveness to progressive asphyxia during sleep.

CURRENT CLAIM: Prolonged prenatal exposure to maternal anemia, sufficient to restrict intrauterine growth, causes an elevation in CO₂ levels in lambs during quiet wakefulness, active sleep and quiet sleep.

An increasing body of evidence suggests that victims of the Sudden Infant Death Syndrome (SIDS) may have been exposed to adverse intrauterine conditions (Filiano and Kinney, 1994). SIDS victims are often growth-restricted at birth (Beal, 1989; Øyen et al., 1995), and factors resulting in intrauterine growth restriction, such as maternal smoking (Mitchell et al., 1992) and maternal anemia (Bulterys et al., 1990), increase the risk of SIDS. In this study we have used an animal model of maternal anemia (Mostello et al., 1991) to examine respiratory function following intrauterine compromise. Our aim was to determine the effects of prenatal exposure to maternal anemia on early postnatal ventilatory and arousal responsiveness to an asphyxic rebreathing stimulus during sleep. Little is known about the influence of prenatal compromise on ventilatory control in the affected newborn. We previously demonstrated that the normal postnatal development of ventilatory responsiveness to progressive hypoxia is impaired in awake, low birthweight lambs (Moss et al., 1996). We wished to extend these observations to examine ventilatory responses during sleep because sleep states have been shown to affect ventilatory responsiveness to various respiratory stimuli (Phillipson and Bowes, 1986). We also wished to employ a combined hypoxic/hypercapnic (i.e., asphyxic) stimulus to more closely mimic changes in blood gas tensions that may occur in infants during cessation of respiratory airflow, or during rebreathing of exhaled gases.

METHODS

We implanted arterial and venous catheters in six ewes at 91±1 days of pregnancy (term ~147 days). We aimed to halve maternal hematocrit by regular (approximately daily) removal of 500 ml of arterial blood while replacing it with an equal volume of the ewe’s plasma and saline as necessary. To examine respiratory function following intrauterine compromise. Our aim was to determine the effects of prenatal exposure to maternal anemia on early postnatal ventilatory and arousal responsiveness to an asphyxic rebreathing stimulus during sleep.

Soon after spontaneous delivery, six lambs from anemic ewes and six lambs from non-anemic ewes (controls) underwent surgery (2-3% halothane anaesthesia) for the implantation of electrodes to monitor the electrocorticogram (ECoG), electrooculogram (EOG) and dorsal nuchal electromyogram (EMG); an aortic catheter was also implanted. Lambs were allowed one day of recovery before sleep studies commenced. All lambs underwent 2-3 studies per week during the first 2-3 postnatal weeks, beginning 1-3 days after birth. During studies, lambs lay in a sling for 2-4 hours while digitised signals, sampled at 200 Hz (MacLab 8, AD Instruments,
in each sleep state during each study. During alternating studies, asphyxic rebreathing tests were not performed and lambs were allowed to sleep without interruption until they aroused spontaneously.

To quantify ventilatory responsiveness to asphyxia the period of rebreathing was separated into 5-second periods. For each of these periods, minute ventilation (determined from the product of tidal volume and breathing frequency, from the Chart record) was plotted against the mean FeCO₂ (yielding a significant linear relationship between these variables); the gradient of this relationship was used as an index of ventilatory responsiveness.

To determine arousal responsiveness during normal sleep or rebreathing tests, the duration of each period of sleep was calculated. Tidal volume (VT, ml/kg), breathing frequency (f), FeCO₂ (%) and SpO₂ (%) for each of the three breaths immediately preceding arousal were obtained and the mean of these three values was used to determine the level of ventilation and the degree of hypercapnia and hypoxia prevailing prior to arousal.

Data are presented as mean ± SEM. Statistical comparisons were made using unpaired t-tests, or repeated measures analysis of variance (ANOVA, Statistical Analysis Systems, USA) as appropriate. Where effects of treatment or sleep state were identified by ANOVA, the ‘least significant difference’ post-hoc test was employed to identify differences. Statistical significance was accepted where p<0.05. Only significant differences are reported.

RESULTS

Lambs from anemic ewes weighed less at birth (3.9±0.4 kg) than control lambs (5.1±0.2 kg). Gestation length was the same in each group (147±1 days). At rest (awake) lambs from anemic ewes had significantly higher PaCO₂ values (42.4±0.7 mmHg) than controls (39.5±0.6 mmHg). Arterial pH, PaO₂, SaO₂ and hematocrit were not different between groups.

While sleeping undisturbed, lambs from both groups had higher FeCO₂ levels during AS than during QS. Lambs from anemic ewes had higher FeCO₂ levels than controls during both AS (anemic, 6.2±0.1%; control, 5.5±0.1%) and QS (anemic, 5.5±0.1%; control, 4.9±0.1%). In both groups, minute ventilation during uninterrupted AS was lower than during uninterrupted QS and there were no differences in ventilation between the two groups in either sleep state (AS, 484.0±13.9 ml/min/kg; QS, 648.9±18.4 ml/min/kg). Breathing frequency and VT were not different between the two groups of lambs in either sleep state; however, VT and f were greater during QS (VT, 11.1±0.6 ml/kg; f, 60.3±3.7 breaths/min) than AS (VT, 9.6±0.5 ml/kg; f, 51.4±2.4 breaths/min).

The duration of uninterrupted AS episodes was longer than the duration of uninterrupted QS episodes in both groups of lambs, with no differences between the two groups (AS, 217.5±22.8 sec; QS, 104.2±7.7 sec). In both sleep states, all lambs aroused more quickly when made asphyxic (AS, 107.1±14.4 sec; QS, 55.9±7.8 sec) than when no rebreathing tests were performed. FeCO₂ levels at arousal from AS and QS...
Figure 2. End-tidal carbon dioxide levels of control lambs (light columns) and lambs from anemic ewes (dark columns) at arousal from uninterrupted active and quiet sleep (plain columns) and when asphyxic rebreathing tests were performed (patterned columns). End-tidal CO₂ at arousal was significantly elevated by asphyxic rebreathing tests for both groups, in each sleep state. Asterisks indicate significant differences in end-tidal CO₂ between groups (short bars) and between sleep states (long bar).

Figure 3. Ventilatory responsiveness to asphyxia of control lambs (light columns) and lambs from anemic ewes (dark columns) during active and quiet sleep. The asterisk indicates a significant reduction in responsiveness in both groups during active sleep when compared with quiet sleep. Ventilatory responsiveness during both sleep states was not significantly different between the two groups of lambs (p = 0.1).
were significantly greater in lambs from anemic ewes than controls (Fig. 2). For both groups of lambs, FeCO₂ was higher prior to arousal from AS than from QS (Fig. 2). In both groups of lambs, SpO₂ at arousal from asphyxia during AS (73.3±3.3%) was lower than when lambs aroused spontaneously (94.4±0.7%).

Ventilatory responsiveness to asphyxia was lower during AS than during QS for both groups of lambs. There was no significant difference in ventilatory responsiveness between lambs from anemic ewes and controls, although responsiveness tended to be lower in lambs from anemic ewes in both sleep states (Fig. 3).

**DISCUSSION**

Our results show that prolonged prenatal exposure to maternal anemia, sufficient to restrict intrauterine growth, causes an elevation in CO₂ levels in lambs during quiet wakefulness, active sleep and quiet sleep. This elevation of the 'set-point' for PaCO₂ occurs despite apparently normal ventilatory and arousal responsiveness during sleep.

The restriction in prenatal growth of lambs from our anemic ewes concurs with observations made by Mostello et al. (1991). They used a similar protocol and made physiological measurements in fetuses from 113 to 138 days (~7 days prior to term) of gestation. These measurements showed that maternal anemia induced fetal growth restriction despite the absence of chronic alterations in fetal arterial pH, gas tensions and glucose levels. However, unlike normal ewes, uterine blood flow and uterine O₂ delivery failed to increase with advancing pregnancy in anemic ewes (Mostello et al., 1991) which may have contributed to the growth restriction. We have shown that pregnancies in chronically anemic ewes proceed to full-term and that lambs are delivered without complications in spite of their compromised prenatal environment. The only postnatal effect of prenatal exposure to maternal anemia that we observed, other than low birthweight, was the elevation in CO₂ levels.

The cause of the elevated CO₂ 'set-point' in lambs from anemic ewes is unknown. Further investigation, directed at addressing likely mechanisms, is therefore warranted. It is possible that slightly elevated fetal CO₂ levels may have been responsible for altering the CO₂ 'set-point' during prenatal development. Although Mostello et al. (1991) did not find significantly greater PaCO₂ values in fetuses from anemic ewes, there was a tendency for CO₂ levels to be higher in these animals than in controls. Prenatal development in a slightly hypercapnic environment could underlie the increased CO₂ 'tolerance' in lambs from anemic ewes.

We are surprised that the observed elevation in resting CO₂ levels was not accompanied by a reduced responsiveness to progressive asphyxia. Although ventilatory responsiveness to asphyxia tended to be lower in lambs from anemic ewes than in controls (Fig. 3), this difference failed to reach statistical significance.

We previously described a failure of ventilatory responsiveness to hypoxia during wakefulness to increase with advancing postnatal age in low birthweight lambs (Moss et al., 1996); however, ventilatory responsiveness to hypercapnia and arterial gas tensions and pH are normal in such animals. Lambs from anemic ewes are clearly different. The combined hypoxic/hypercapnic stimulus used in the present study is, of course, different from the purely hypoxic or hypercapnic stimuli that we previously used (Moss et al., 1996). In the present study, we chose to examine ventilatory responsiveness to asphyxia as it more accurately reproduces the situation that is believed to occur in sleeping infants during cessation of airflow as a result of central apnea, airway obstruction or rebreathing of exhaled gases.

In our previous study the difference in ventilatory responsiveness to isocapnic hypoxia between low birthweight and normally grown lambs was not evident until two weeks of age (Moss et al., 1996). As in another study on sleeping lambs (Harding et al., 1997), we found that lambs older than three weeks of age were less likely to sleep under laboratory conditions than younger lambs. It is possible that because our present study was conducted on lambs less than three weeks old, we were unable to measure differences in ventilatory responsiveness to asphyxia (a combination of hypoxia and hypercapnia). Alternatively the attenuation in ventilatory responsiveness observed previously (Moss et al., 1996) may be restricted to wakefulness.

Like Fewell and Baker (1989), we have shown that lambs arouse more quickly from QS than AS when presented with a respiratory challenge. Similarly, we have demonstrated increased ventilatory and arousal responsiveness during QS. Fewell and Baker (1989) suggested that differences between their findings and those in adult humans (in whom arousal occurs more quickly from AS than QS [Berthon-Jones and Sullivan, 1984]) were likely due to the absence of the influence of the upper airway in their tracheostomised lambs. Our present results and those from a recent study in our laboratory (Harding et al., 1997) clearly demonstrate that, with the upper airway in circuit, the differences in arousal responses between lambs and adult humans are still present. This observation may reflect a species difference. While it is possible that differences in the maturity of subjects may be responsible for the conflicting findings, this appears unlikely because sleep state-related differences in arousability are similar in human adults (Berthon-Jones and Sullivan, 1984) and infants (Campbell et al., 1995).

In conclusion, we have shown that ventilatory and arousal responsiveness to progressive asphyxia in newborn lambs is greater during quiet sleep than during active sleep but is not significantly affected by exposure to chronic maternal anemia throughout the final third of gestation. Despite apparently normal ventilatory responsiveness in lambs from anemic ewes, resting CO₂ levels in these lambs are elevated during sleep and wakefulness. The cause of the elevated CO₂ levels that occur as a result of prenatal exposure to maternal anemia is unknown and the potential mechanisms require investigation.

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REFERENCES