Ventilatory and arousal responses of sleeping lambs to respiratory challenges: effect of prenatal maternal anemia

TIMOTHY J. MOSS AND RICHARD HARDING
Fetal and Neonatal Research Unit, Department of Physiology, Monash University, Clayton, Victoria 3168, Australia

Moss, Timothy J., and Richard Harding. Ventilatory and arousal responses of sleeping lambs to respiratory challenges: effect of prenatal maternal anemia. J. Appl. Physiol. 88: 641–648, 2000.—We have examined the effects of exposure to chronic maternal anemia, throughout the final one-third of gestation, on postnatal ventilatory and arousal responses to hypoxia, hypercapnia, and combined hypoxia–hypercapnia in sleeping lambs. While resting quietly awake, lambs from anemic ewes had higher arterial PCO2 levels than control animals during the first 2–3 postnatal wk, but pH, arterial PO2, and arterial O2 saturation were not different. During active and quiet sleep lambs from anemic ewes had higher end-tidal CO2 levels than control animals when breathing room air and at the time of spontaneous arousal or when aroused by progressive hypercapnia or by combined hypoxia–hypercapnia. Ventilation and arterial O2 saturation during uninterrupted sleep and ventilatory responsiveness to hypoxia (inspiratory O2 fraction, 10%), progressive hypercapnia, and combined hypoxia–hypercapnia were not significantly affected by exposure to maternal anemia. Our findings show that maternal anemia results in elevated PCO2 levels in the offspring. This effect may be due, at least in part, to altered pulmonary function. Hypoxia; hypercapnia; low birth weight; intrauterine growth retardation.

Accumulating evidence suggests that many victims of sudden infant death syndrome have experienced prenatal compromise, resulting in intrauterine growth restriction (16, 21). However, few investigations of ventilatory control in newborns after exposure to adverse intrauterine conditions have been conducted.

We have recently shown that low-birth-weight lambs, unlike normal lambs, do not increase their ventilatory responsiveness to progressive hypoxia during wakefulness as they age (18). Because sudden infant death syndrome is normally associated with sleep (31), we thought it important to extend these observations and examine ventilatory responses to respiratory stimuli during sleep in lambs that had experienced prenatal compromise.

We chose to induce chronic anemia in pregnant ewes as a means of causing prenatal compromise in fetuses that could be subsequently studied after birth. Maternal anemia during human pregnancy is associated with increased risks of prematurity and intrauterine growth restriction (1). Chronic maternal anemia in a sheep model results in fetal growth restriction (20). It was our aim to determine whether ventilatory and arousal responses of newborn lambs to respiratory stimuli experienced during sleep are affected by prenatal exposure to chronic maternal anemia.

METHODS

Seven control lambs and six lambs from chronically anemic ewes were studied repeatedly during the first 2–3 postnatal wk. Six date-mated sheep underwent aseptic surgery (halothane anesthesia) at 91 ± 1 days postmating for the implantation of a femoral artery and vein catheters to allow the induction, maintenance, and monitoring of chronic anemia. After surgery, arterial blood was sampled daily for measurement of arterial pH, PCO2 (PAco2), PO2 (PAo2), and O2 saturation (SAo2;ABL510, Radiometer). Hematocrit was measured in triplicate by using microcapsule tubes. Beginning on day 96 of pregnancy, “exchange transfusions” (during which 500 ml of blood were replaced with plasma) were commenced in six ewes to lower the maternal hematocrit to below 14% (20). On day 143 ± 1 of pregnancy, catheters were cut short and obstructed, and ewes were allowed to spontaneously deliver lambs at term. Control lambs were obtained from ewes that did not experience any intervention during pregnancy.

After delivery, lambs were weighed and body dimensions and ponderal index [body wt/(crown-rump length)3] were measured. Postnatal lambs (1–3 days of age) underwent aseptic surgery (halothane anesthesia) for the implantation of a femoral artery catheter (for blood sampling), an intrapleural balloon-tipped catheter, and pairs of stainless steel electrodes (AS632, Cooner) to monitor electrocorticogram (ECoG), electrooculogram (EOG), and nuchal electromyogram (EMG). Signals from these electrodes were used in the identification of sleep states and arousal.

Studies of ventilation during sleep were performed daily beginning on the day after surgery until 13 ± 2 days of age; after this point, lambs were unlikely to sleep spontaneously in the laboratory. During studies, lambs were placed prone in a sling within a soundproof room. Rectal temperature was continuously monitored. While lambs rested quietly awake, an arterial blood sample was collected for determination of pH, PAco2, PAo2, and SAo2 (ABL510, Radiometer). A pulse oximeter (N-200, Nellcor) was attached to the lamb’s shaved tail to continuously measure transcutaneous O2 saturation (SO2).

Lambs were fitted with a flexible face mask (Kruus) to allow measurement of ventilation. Before the face mask was fitted, the snout was shaved and dental impression material (President, Coltène, Switzerland) was applied to the inside rim of the mask to ensure an airtight seal. The mask was
easily and painlessly removed at the end of each experiment. Gas was continually drawn from the face mask to record the inspired O\textsubscript{2} (F\textsubscript{IO2}) and end-tidal CO\textsubscript{2} fractions (F\textsubscript{ETCO2}; Eliza Duo, Enström, Sweden). A pneumotachometer (model 4500A, Hans Rudolph) was inserted into the outlet of the face mask to measure ventilation; it was attached to a differential pressure transducer (model PT5A, Grass Instruments), and the amplified signal was integrated (model PT10, Grass Instruments) to yield a measurement of volume.

Physiological data were sampled at 200 Hz by an analog-to-digital converter (MacLab, AD Instruments) and recorded on a computer (PowerMac 7200, Apple). "Chart" software (AD Instruments) was used to record the integrated pneumotachometer signal and to calculate the volume of each breath (tidal volume; V\textsubscript{T}). Breathing frequency (f) was determined from the integrated pneumotachometer record. Electrode signals were conditioned and amplified (Grass Instruments) before being sampled.

During each study, one of three different ventilatory tests was performed (hypercapnia, combined hypoxia-hypercapnia, or hypoxia), or lambs were allowed to sleep without interruption. Manipulation of respired gases was performed remotely from the airflow transducer. During hypoxic and combined hypoxia-hypercapnic tests, the direction of airflow through the Y piece was switched so that the breath directed into and from the rubber bag. The bag contained 200 ml/kg body weight of gas consisting of either 5% CO\textsubscript{2}-50% O\textsubscript{2}-balance N\textsubscript{2} (for hypercapnic tests) or 5% CO\textsubscript{2}-21% O\textsubscript{2}-balance N\textsubscript{2} (for combined hypoxic-hypercapnic tests).

During studies when hypoxic tests were performed, the pneumotachometer was attached to the side port in a tube through which gas mixtures flowed. Between hypoxic tests, 21% O\textsubscript{2} in N\textsubscript{2} flowed through the tube at 5–6 l/min. To perform hypercapnic and combined hypoxic-hypercapnic tests, the direction of airflow through the Y piece was switched so that the lamb breathed to and from the rubber bag. The bag contained 200 ml/kg body weight of gas consisting of either 5% CO\textsubscript{2}-50% O\textsubscript{2}-balance N\textsubscript{2} (for hypercapnic tests) or 5% CO\textsubscript{2}-21% O\textsubscript{2}-balance N\textsubscript{2} (for combined hypoxic-hypercapnic tests).

Ventilatory tests were performed during identified episodes of active sleep (AS; low-voltage ECoG, active EOG, inactive EMG) and quiet sleep (QS; high-voltage ECoG, inactive EOG, tonically active EMG). Ventilatory tests were discontinued when lambs aroused from sleep. Arousal from AS was defined as the appearance of nuchal EMG activity, and arousal from QS was defined as the switching from high- to low-voltage ECoG activity. Observation of the lambs by using a video camera aided in the identification of sleep states and arousal. Up to seven ventilatory tests or episodes of uninterrupted sleep were recorded in each sleep state during each study.

Data analysis. Minute ventilation (V\textsubscript{E}; ml·min\textsuperscript{-1}·kg\textsuperscript{-1}); the product of VT and f), F\textsubscript{ETCO2}, and SO\textsubscript{2} measured during uninterrupted sleep were determined from averages of VT, f, F\textsubscript{ETCO2}, and SO\textsubscript{2} measured during entire episodes of uninterrupted AS and QS.

To quantitate ventilatory responses to hypercapnia and combined hypoxia-hypercapnia, each of the tests was divided into 5-s segments and mean VT and F\textsubscript{ETCO2} values for each of these segments were determined. We calculated the gradient of the relationship between V\textsubscript{E} and F\textsubscript{ETCO2} (from linear regression analysis; Fig. 1) to provide an index of ventilatory responsiveness (to hypercapnia or combined hypoxia/hypercapnia). Regression lines were also extrapolated to determine the F\textsubscript{ETCO2} level at which ventilation equaled zero. We used this measurement as an index of the F\textsubscript{ETCO2} level around which ventilatory responses were set.

To quantify ventilatory responsiveness to hypoxia, V\textsubscript{E} and SO\textsubscript{2} were averaged over 5 s, beginning 1 min after F\textsubscript{IO2} was reduced to 10%. As an index of hypoxic ventilatory responsiveness, we calculated the percent increase in ventilation above that measured immediately before the hypoxic test. To normalize ventilatory responsiveness, increases in ventilation in response to hypoxia were divided by the reduction in SO\textsubscript{2} that occurred during the test.

V\textsubscript{E}, VT, f, F\textsubscript{ETCO2}, SO\textsubscript{2}, and the inspiratory deflections in intrapleural pressure were measured from the three breaths immediately preceding arousal to provide indexes of ventilatory effort and the degree of hypoxia or hypercapnia experienced during normal sleep and when ventilatory tests were performed. The duration of each sleep episode was also measured.

Statistical analyses. Statistical analyses were performed by using SAS software (SAS). Initial analysis indicated that no variables changed with postnatal age (2–17 days) over the study period. Therefore, resting ventilation, arousal, and ventilatory responsiveness measurements and measurements of P\textsubscript{aCO2}, P\textsubscript{aO2}, S\textsubscript{aO2}, and pH were averaged for each day of study for each animal. Values were averaged to provide a single value for each animal.

Birth weights, crown-rump lengths, ponderal indexes, and measurements of rectal temperature, P\textsubscript{aCO2}, P\textsubscript{aO2}, S\textsubscript{aO2}, and pH were compared between groups by using unpaired t-tests. Responses to ventilatory tests and measurements made at arousal were compared by repeated-measures ANOVA with one between-groups factor (control vs. anemia) and one within-groups factor [either 1] AS vs. QS or 2] test type]. Post hoc comparisons were made by using the least significant difference test. Values of P < 0.05 were considered statistically significant, and only significant differences are reported unless otherwise indicated. All data are expressed as means ± SE.

RESULTS

Hematocrits of ewes that underwent exchange transfusions are shown in Fig. 2. Exchange transfusions initially were performed twice daily (over 3–5 days) until the ewe's hematocrit fell below 14%. After this time it was necessary to perform exchange transfusions at least twice every 3 days to maintain hematocrit at this reduced level. Maternal hematocrit was reduced from 33.5 ± 3.2% (before exchange transfusions were commenced) to 13.7 ± 0.2% throughout the anemic period.

Control and treated lambs were born spontaneously at full term (control, 147 ± 1 days; treated, 147 ± 1 days). Birth weights of treated lambs (3.9 ± 0.4 kg) were lower than those of control animals (5.2 ± 0.2 kg; Fig. 3). Treated lambs had lower ponderal indexes (2.53 ± 0.16 × 10\textsuperscript{-5} kg\textsuperscript{-1} cm\textsuperscript{-3}) than did control animals (3.15 ± 0.18 × 10\textsuperscript{-5} kg\textsuperscript{-1} cm\textsuperscript{-3}), resulting in treated lambs appearing thin and "wasted." Parity was not different between the two groups of lambs (control, 1.4 ± 0.2; treated, 1.2 ± 0.2). At all ages, treated lambs weighed less than control animals (Fig. 3). The mean daily growth rate for control lambs (290 ± 30 g/day) was not different from that of treated lambs (220 ± 30 g/day).

Arterial pH (control, 7.41 ± 0.01; treated, 7.40 ± 0.01), P\textsubscript{aO2} (control, 97.0 ± 2.1 Torr; treated, 94.3 ± 3.4
Torr), and SaO2 (control, 96.5 ± 0.6%; treated, 96.1 ± 1.6%) when lambs rested quietly awake breathing room air were not different between control and treated lambs. Mean PaCO2 of treated lambs (42.4 ± 0.7 Torr) was greater than in control animals (39.9 ± 0.6 Torr). PaCO2, pH, PAO2, and SaO2 did not change with age in either group. Rectal temperatures of control lambs (39.8 ± 0.01°C) were slightly higher than those of treated lambs (39.6 ± 0.03°C).

Measurements during sleep. VE during uninterrupted sleep was not different between groups but was lower in both groups during AS than during QS (Fig. 4). VT and f were not different between control and treated lambs but were both greater during QS than during AS (data not shown). In both groups, FETCO2 was higher during AS than during QS and was greater in treated than control lambs during both sleep states (Fig. 4). So2 was not different between groups but was lower during AS than during QS for both groups (Fig. 4).

Responses to combined hypoxia-hypercapnia were measured in all lambs, but responses to hypercapnia were not measured in one treated lamb. Ventilatory responses to hypercapnia and combined hypoxia-hypercapnia were not significantly different between control and treated lambs (P = 0.23), although there was a tendency for responses to be lower in the treated group (Fig. 5). In both groups, ventilatory responses to hypoxia and combined hypoxia-hypercapnia were greater during QS than during AS. During QS, ventilatory responses to combined hypoxia-hypercapnia were greater than responses to hypercapnia (Fig. 5). Responses to hypercapnia and combined hypoxia-hyper-
Capnia consisted of increases in both VT and f for both groups of lambs (Fig. 5). The value of $F_{ETCO_2}$ at which ventilation equals zero (by extrapolation of the regression equation) was not altered by sleep state and was not different between hypercapnic and combined hypoxic-hypercapnic tests. There were no significant differences in these estimates of CO2 set point between control ($3.9 \pm 0.7\% F_{ETCO_2}$) and treated lambs ($2.15 \pm 0.5\% F_{ETCO_2}$).

Responses to steady-state hypoxia ($F_{IO_2}$, 10%) were determined in six control lambs in AS and QS but could only be measured in four treated lambs during AS and in three during QS. Increases in $V_E$ were not different between groups. In both groups, ventilatory increases during hypoxia tended to be greater ($P = 0.09$) during QS than during AS. In both groups, decreases in $SO_2$ in response to hypoxia were greater during AS (control, $16.7 \pm 2.5\%$; treated, $16.1 \pm 3.3\%$) than during QS (control, $11.4 \pm 2.4\%$; treated, $11.8 \pm 4.2\%$). Normalized ventilatory responses to hypoxia (increase in $V_E$/decrease in $SO_2$) were not different between groups but were lower during AS than during QS (Fig. 5). Responses to hypoxia during AS and QS consisted of increases in VT and f for both groups of lambs.

Measurements at arousal. Measurements made at arousal from uninterrupted sleep, hypercapnic, combined hypoxic-hypercapnic, and hypoxic tests are presented in Table 1. Episodes of uninterrupted AS were significantly longer than episodes of uninterrupted QS for both groups of lambs and were not different between treatment groups for either sleep state. Similarly, lambs spent more time in AS than QS when ventilatory tests (hypercapnia, combined hypoxia-hypercapnia, or hypoxia) were performed. The duration of AS episodes was shortened by hypercapnic, combined hypoxic-hypercapnic, and hypoxic tests. QS episodes were shorter when hypercapnic and combined hypoxic-hypercapnic tests were performed but not when hypoxic tests were performed.

$V_E$ at arousal was not different between sleep states but was significantly greater when hypercapnic and combined hypoxic-hypercapnic (but not hypoxic) ventilatory tests were performed than at arousal from...
uninterrupted sleep. Accordingly, inspiratory effort (i.e., inspiratory deflection in intrapleural pressure) was significantly greater immediately before arousal from hypercapnic and combined hypoxic-hypercapnic tests during QS than when no tests were performed in this state. In both groups, SO₂ at arousal from combined hypoxic-hypercapnic and hypoxic tests during AS, but not QS, was lower than when no ventilatory tests were performed. In both sleep states, FETCO₂ at arousal was significantly greater in treated lambs than in control lambs when hypercapnic, combined hypoxic-hypercapnic, or no ventilatory tests were performed. In both groups, FETCO₂ at arousal from AS was greater than at arousal from QS; this sleep-state-related difference was also present when hypercapnic and combined hypoxic-hypercapnic tests were performed. In both groups of lambs, FETCO₂ was greater at arousal from hypercapnic tests than at arousal from combined hypoxic-hypercapnic tests for both sleep states.

**DISCUSSION**

Exposure to maternal anemia throughout late gestation resulted in restricted fetal growth and chronic elevations in CO₂ levels throughout early postnatal life. Despite this relative hypercapnia, arterial pH, PAO₂, and ventilation were not significantly affected during sleep or wakefulness. Ventilatory responses to hypercapnia and combined hypoxic-hypercapnia tended to be reduced in treated lambs, whereas responses to hypoxia appeared to be unaffected.

We have extended the findings of Mostello et al. (20) by demonstrating that fetuses from chronically anemic ewes can survive this prenatal insult and pregnancies proceed to full-term. We have shown that lambs from anemic ewes are growth restricted at birth; their birth weights are low, but measurements of body dimensions are not affected, resulting in a thin, wasted appearance. In contrast, human neonates born to anemic mothers
have shorter body lengths as well as low birth weights (27). The type of growth restriction that we observed is typical of that caused by intrauterine compromise during late gestation (15), whereas the overall retardation of growth observed in humans may reflect an effect of intrauterine compromise from earlier in gestation. Like the low-birth-weight lambs that we have studied previously (18), lambs from anemic ewes grew at a rate similar to control lambs during the first few postnatal weeks, resulting in their body weights remaining below those of control animals throughout the study period. This observation is consistent with studies of postnatal growth in infants small for their gestational age who were born at or before full term (11, 29).

The intrauterine growth restriction we observed was presumably due to a reduced nutrient supply to fetuses of anemic ewes because chronic maternal anemia throughout the final one-third of gestation in this species has been shown to cause a failure of uterine artery blood flow to increase with gestational age (20). Despite this reduced uterine blood flow, fetuses from anemic ewes studied by Mostello et al. (20) were not chronically hypoglycemic or hypoxicemic, and hematocrit was not elevated. However, in our study, the presence of fetal hypoxemia and/or hypoglycemia cannot be excluded because the period of exposure to maternal anemia was ~2 wk greater than that of the fetuses studied by Mostello et al. Thus our lambs may have been more affected because of the increased duration of exposure to maternal anemia.

Mostello et al. (20) reported a tendency for \( \text{Pa}_\text{CO}_2 \) to be higher in fetuses from anemic ewes than in control animals (39.5 ± 0.5 vs. 36.2 ± 0.8 Torr, \( P = 0.08 \)), and this intrauterine hypercapnia (which may have been greater in our fetuses owing to the longer exposure to maternal anemia) may have altered the \( \text{CO}_2 \) ventilatory set point during prenatal development.

### Table 1. Measurements made at arousal from normal sleep and when combined hypoxic/hypercapnic, hypercapnic, and hypoxic ventilatory tests were performed in control and treated lambs

<table>
<thead>
<tr>
<th>Duration of sleep, s</th>
<th>Normal Sleep</th>
<th>Combined Hypoxia-Hypercapnia</th>
<th>Hypercapnia</th>
<th>Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AS</strong></td>
<td>Control</td>
<td>Anemia</td>
<td>Control</td>
<td>Anemia</td>
</tr>
<tr>
<td>Duration</td>
<td>230.4 ± 24.4</td>
<td>230.4 ± 24.4</td>
<td>105.9 ± 12.4</td>
<td>131.6 ± 12.7</td>
</tr>
<tr>
<td><strong>QS</strong></td>
<td>[101.7 ± 8.3]</td>
<td>[101.4 ± 14.4]</td>
<td>[68.1 ± 12.5]</td>
<td>[111.6 ± 22.0]</td>
</tr>
<tr>
<td><strong>V₆, ml·min⁻¹·kg⁻¹</strong></td>
<td>53.6 ± 3.3</td>
<td>53.6 ± 3.3</td>
<td>73.2 ± 3.4</td>
<td>60.3 ± 4.6</td>
</tr>
<tr>
<td><strong>FETCO₂, %</strong></td>
<td>5.4 ± 0.2‡</td>
<td>5.4 ± 0.1‡</td>
<td>8.6 ± 0.1‡</td>
<td>9.3 ± 0.3‡</td>
</tr>
<tr>
<td><strong>SO₂, %</strong></td>
<td>92.6 ± 1.9</td>
<td>94.7 ± 1.3</td>
<td>74.9 ± 4.6</td>
<td>94.4 ± 1.6</td>
</tr>
<tr>
<td><strong>IPPI, mmHg</strong></td>
<td>94.0 ± 2.1</td>
<td>97.3 ± 0.8</td>
<td>91.7 ± 1.5</td>
<td>98.4 ± 1.1</td>
</tr>
<tr>
<td><strong>AS</strong></td>
<td>Control</td>
<td>Anemia</td>
<td>Control</td>
<td>Anemia</td>
</tr>
<tr>
<td>Duration</td>
<td>230.1 ± 24.3</td>
<td>230.1 ± 24.3</td>
<td>105.9 ± 12.4</td>
<td>131.6 ± 12.7</td>
</tr>
<tr>
<td><strong>QS</strong></td>
<td>[101.7 ± 8.3]</td>
<td>[101.4 ± 14.4]</td>
<td>[68.1 ± 12.5]</td>
<td>[111.6 ± 22.0]</td>
</tr>
<tr>
<td><strong>V₆, ml·min⁻¹·kg⁻¹</strong></td>
<td>53.6 ± 3.3</td>
<td>53.6 ± 3.3</td>
<td>73.2 ± 3.4</td>
<td>60.3 ± 4.6</td>
</tr>
<tr>
<td><strong>FETCO₂, %</strong></td>
<td>5.4 ± 0.2‡</td>
<td>5.4 ± 0.1‡</td>
<td>8.6 ± 0.1‡</td>
<td>9.3 ± 0.3‡</td>
</tr>
<tr>
<td><strong>SO₂, %</strong></td>
<td>92.6 ± 1.9</td>
<td>94.7 ± 1.3</td>
<td>74.9 ± 4.6</td>
<td>94.4 ± 1.6</td>
</tr>
<tr>
<td><strong>IPPI, mmHg</strong></td>
<td>94.0 ± 2.1</td>
<td>97.3 ± 0.8</td>
<td>91.7 ± 1.5</td>
<td>98.4 ± 1.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. AS, active sleep; QS, quiet sleep; V₆, tidal volume; FETCO₂, end-tidal CO₂ fraction; SO₂, O₂ saturation; IPPI, inspiratory inspirapleural pressure. *Significantly different between the test condition and uninterrupted normal sleep (data from both treatment groups combined), P < 0.05. †Significant differences between sleep states (data from both treatments combined), P < 0.05. ‡Significant differences between control lambs (control) and lambs from anemic ewes (anemia), P < 0.05.
barrier) and function (increased chest wall and decreased lung compliance), persisting for at least 8 wk, in lambs that were growth restricted in utero; these lambs had elevated PaCO2 and reduced PaO2 levels (30). Thus it is possible that lambs from anemic ewes possess pulmonary abnormalities that restrict alveolar ventilation and hence elevate PaCO2. Impaired gas diffusion across the pulmonary membrane seems an unlikely explanation for our present observations because CO2 levels were affected without an effect on PO2 or SO2. A further possible explanation for the elevated CO2 levels observed in treated lambs is that CO2 production may have been increased. However, we collected no data relating to O2 consumption or CO2 production and are unaware of studies specifically examining the effects of intrauterine growth restriction on these variables.

Lambs from anemic ewes did not have ventilatory responses to hypercapnia or combined hypoxia-hypercapnia that were significantly different from those of control lambs. There was, however, a tendency for ventilatory responsiveness to hypercapnia and combined hypoxia-hypercapnia to be lower in treated lambs than in controls. Variability of ventilatory responses was observed during AS and QS in both groups of lambs, and thus, although the mean ventilatory response of lambs from anemic ewes to hypercapnia appears low, values in these lambs were within the range observed in control lambs. Variability of ventilatory responses to hypercapnia has been demonstrated in lambs during wakefulness (19) and in human infants during sleep (5). Unlike human infants, who display greater variability in ventilatory responses during AS than during QS (5), in our lambs variability in responses appeared similar during AS and QS.

Although we found no significant differences in ventilatory responses to hypoxia, hypercapnia, or combined hypoxia-hypercapnia between lambs from anemic ewes and control lambs, responses of both groups were affected by sleep state, in concurrence with previous studies (5, 8, 10, 13, 24). This effect of sleep state was also present for arousal responses. When hypoxic, hypercapnic, and combined hypoxic-hypercapnic ventilatory tests were performed, arousal from AS was delayed when compared with QS. During hypercapnic and combined hypoxic-hypercapnic tests, greater degrees of hypercapnia were experienced during AS, and, when combined hypoxia-hypercapnic and hypoxic tests were performed in AS, greater degrees of hypoxia were experienced. These findings demonstrate that, in lambs, AS is associated with reduced ventilatory and arousal responsiveness and hence increased vulnerability to respiratory challenges.

As in previous studies of developing animals (17, 26, 33), we found a positive interaction between hypoxia and hypercapnia in stimulating ventilation. The absence of this interaction during AS may be due to the weak association between respiratory stimuli and ventilation in this state (22). A positive interaction between hypoxia and hypercapnia in stimulating arousal from QS and AS was also present in both groups of lambs.

Ve immediately before arousal from combined hypercapnic-hypoxic and hypercapnic tests was not different and was not affected by sleep state despite the differences in ventilatory responses between these tests and between sleep states. Our data suggest that there is a ventilatory threshold for the initiation of arousal and add support to other studies demonstrating that arousal in response to respiratory stimuli occurs because of increased respiratory drive (3, 6, 7, 9, 12, 14). Reduced ventilatory responses during AS resulted in lambs rebreathing the hypercapnic or combined hypoxic-hypercapnic gas mixtures for longer periods of time; this resulted in FETCO2 levels increasing to a greater degree during AS before a level of ventilatory stimulation sufficient to cause arousal was attained. Our observation that arousal from QS did not occur significantly earlier when lambs were challenged with hypoxia than when ventilatory tests were not performed suggests that the level of hypoxia used did not cause a sufficient increase in respiratory drive to reach the threshold for arousal achieved by hypercapnic and combined hypoxic-hypercapnic stimulation. This is supported by the finding that ventilation at arousal from hypoxic tests was not different than at arousal from uninterrupted sleep.

In conclusion, exposure to chronic maternal anemia during late gestation results in fetal growth restriction and chronic hypercapnia during early postnatal life. The elevation in baseline CO2 levels in postnatal lambs was not associated with hypo- or hyperventilation, but ventilatory responses to CO2 tended to be reduced in lambs from anemic ewes. However, it appears likely that the chronic hypercapnia experienced by lambs from anemic mothers was due to altered pulmonary function.

These experiments were funded by the Sudden Infant Death Research Foundation of Australia and the National Health and Medical Research Council of Australia.

Address for reprint requests and other correspondence: T. J. Moss, Dept. of Obstetrics and Gynaecology, Lottery Commission Perinatal Research Laboratories, Large Animal Facility, Univ. of Western Australia, Hacket Drive, Nedlands, Western Australia 6009, Australia (E-mail: tmoss@cyllene.uwa.edu.au)

Received 11 March 1999; accepted in final form 4 October 1999.

REFERENCES