Maturation of respiratory control and the propensity for breathing instability in a sheep model


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Edwards BA, Sands SA, Skuza EM, Brodecky V, Stockx EM, Wilkinson MH, Berger PJ. Maturation of respiratory control and the propensity for breathing instability in a sheep model. J Appl Physiol 107: 1463–1471, 2009. First published September 10, 2009; doi:10.1152/japplphysiol.00587.2009.—Limited evidence suggests that the ventilatory interaction between O2 and CO2 is additive after birth and becomes multiplicative with postnatal development. Such a switch may be linked to the propensity for periodic breathing (PB) in infancy. To test this idea, we characterized the maturation of the respiratory controller and its effect on breathing stability in ~10-day-old lambs and 6- to 8-month-old sheep. We measured 1) carotid body sensitivity via dynamic ventilatory responses to step changes in O2 and CO2, 2) steady-state ventilatory sensitivity to CO2 under hypoxic and hyperoxic conditions, 3) the dependence of the apneic threshold on arterial PO2, and 4) the effect of hypoxic or hypercapnic gas inhalation during induced PB. Stability of the system was assessed using surrogate measures of loop gain. Peripheral sensitivity to O2 was higher in newborn than in older animals (P < 0.05), but peripheral CO2 sensitivity was unchanged. Central CO2 sensitivity was reduced with age, but the slopes of the ventilatory responses to CO2 were the same in hypoxia and hyperoxia. Reduced arterial PO2 caused a leftward shift in the apneic threshold at both ages. Inspiration of hypoxic gas during PB immediately halted PB, whereas hypercapnia stopped PB only after one or two further PB cycles. We conclude that the controller in the sheep remains additive over the first 6 mo of life. Our results also show that the loop gain of the respiratory control system is reduced with age, possibly as a result of a reduction of peripheral O2 sensitivity.

control of breathing; maturation; periodic breathing; loop gain

AT THE TRANSITION from fetal to newborn life, a rapid increase in arterial PO2 (PaO2) is followed by a resetting of the peripheral chemoreceptor sensitivity to O2, which appears as an increase in the slope of the hypoxic ventilatory response, and is complete after the first few postnatal weeks (6, 25, 51). Most studies of steady-state CO2 sensitivity, which is a measure of the combined output from the peripheral and central chemoreceptors, report that CO2 sensitivity increases over the first 5 wk in preterm infants born at 32 wk (24). Although a similar increase is seen in newborn rats (17), studies of full-term human infants (27, 48) and piglets (49) report no evidence of postnatal development. When the contribution of the peripheral chemoreceptors is tested via the dynamic responses to CO2, there is no change with age in the lamb (10, 11), but the speed of the response has been reported to increase in full-term infants (50). Available evidence favors the view that overall CO2 sensitivity is at a peak early in life, since it is lower in adults than in young children (26, 38).

The limited available evidence suggests that in most species the net ventilatory response to a combined O2 and CO2 stimulus is a simple addition of the individual responses immediately after birth, whereas with postnatal development the ventilatory output is more than additive and is termed multiplicative (14, 36, 42, 50). In a multiplicative controller, the response to CO2 is augmented by hypoxia and attenuated by hyperoxia; crucially, the combined response to a hypoxic and hypercapnic stimulus is greater than the sum of each response. In an additive controller, when a hypoxic stimulus is added to a hypercapnic stimulus, the CO2 response shifts to the left but does not change in slope, whereas hyperoxia simply shifts the curve to the right. Available evidence suggests that the multiplicative interaction originates at the carotid bodies (35) and is clearly evident by 8 wk of age in cats (14) and infants (50). However, the switch to a multiplicative controller appears not to be a universal phenomenon, inasmuch as an additive interaction is reported in adult goats (39).

Important functional differences between additive and multiplicative controllers might provide a rationale for a shift from one to the other in early development. If additive, the apneic threshold (PAT, i.e., the PCO2 level at which breathing ceases) is O2 sensitive, so that, in the presence of hypoxemia, apnea is terminated at a lower arterial PCO2 (PaCO2) than if the controller was multiplicative (13, 18). Thus an additive controller limits the scope for development of dangerous hypoxemia during apnea; such a design feature may be advantageous in a newborn infant, whose arterial O2 saturation (SaO2) can fall extremely rapidly during apnea because of high metabolic rate (31) and low lung volume (44). By contrast, with a multiplicative controller, the PAT is at an essentially fixed PaCO2 that is only weakly affected by changes in PaO2 (53, 58). Crucially, in this form of controller, a hypocapnic apnea can be terminated only after PaCO2 rises to the PAT, even though, by this time, PaO2 may have reached a very low level. A potential advantage of a multiplicative compared with an additive controller is that, with a multiplicative controller, the ventilatory response to changes in PaCO2 is steepened by hypoxia, so falling PaO2 and rising PaCO2 produce a ventilatory response that is larger than the response produced by an additive controller. This enhanced response, however, may also destabilize the respiratory controller and predispose to unstable ventilatory patterns, such as periodic breathing (PB). The switch from an additive to a multiplicative controller could therefore explain why PB is rarely seen in the first few days of life in human infants but is
common at 2 mo of age, when a multiplicative interaction between O₂ and CO₂ becomes evident (50).

Just as the human infant starts life with an additive controller (50), we previously showed that the controller of the 10- to 20-day-old lamb is also additive (55). A single study has reported that by 1 mo of age the sheep carotid body discharge shows clear evidence of a multiplicative interaction (10). If this is so, the sheep would be an ideal model for studying the physiological consequences of the switch from an additive to a multiplicative controller. The present study aims to test whether the controller changes in the sheep with postnatal age and to characterize functional consequences of controller type on the PAT and the propensity for ventilatory instability.

MATERIALS AND METHODS

Experimental Animals and Design

Fifteen spontaneously delivered full-term lambs that were kept with their ewes and 15 mature sheep were housed in the animal house of Monash Medical Centre until the day of the experiment. Studies were carried out on 2-wk-old lambs and 4- to 6-mo-old mature sheep. Animals were instrumented for measurement of cardiovascular and respiratory variables, as previously described (23, 55). Briefly, a nonocclusive catheter (19-gauge Intracath, Becton Dickinson) was inserted percutaneously into the left jugular vein for delivery of a loading dose of ketamine hydrochloride (5 mg/kg at 100 mg/ml; Ketamil, ILEUM Veterinary Products) followed by α-chloralose (80 mg/kg starting bolus followed by continuous infusion at 20 mg·kg⁻¹·h⁻¹). To maintain adequate anesthesia, a response to regular stimulation of the inner and outer canthus of the eye was checked and heart rate and blood pressure were monitored. Supplemental doses of α-chloralose were delivered as needed. A glucose-saline drip connected to the same catheter was used to maintain fluid levels (5% glucose in 0.9% saline at 4.0 ml·kg⁻¹·h⁻¹).

An endotracheal tube was inserted through a midcervical tracheostomy, and the animal was connected to a dual intermittent, high-frequency oscillation ventilator (Humming2; BMO-20H) via a four-way valve (model 2420A/2510S, Hans Rudolph, Kansas City, MO). A flow-through ventilatory circuit, with a gas flow of 45 l/min, facilitated rapid changes between hypoxic, hyperoxic, and hypocapnic gas mixtures within 1 s and ensured that the animal did not rebreathe gas. When the ventilator was turned off, the valve allowed us to choose whether the lamb breathed gas from the ventilator circuit or from the room. Respiratory flow, tidal volume (VT), and end-expired gases were measured: [end-tidal Po₂ and PCO₂ (PETCO₂)] were measured: respiratory flow and PETCO₂ via a noninvasive cardiac output cardiopulmonary management system (model 7300, Respironics Novametrics, Wallingford, CT) and PETCO₂ via an O₂ analyzer (model S-3A1, Ametek Process Instruments, Pittsburgh, PA). Nonocclusive catheters, made from single-lumen polyvinylchloride tubing (Datamasters, Victoria, Australia; 1.52 mm OD, 0.86 mm ID), were secured in the carotid artery and jugular vein. Both catheters were used for blood gas sampling, and the arterial catheter was also connected to a pressure transducer [blood pressure transducer (model MP-15, Micron) connected to a carrier amplifier (model 8805B, Hewlett-Packard)] for continuous measurement of blood pressure. A pulse oximeter probe (model N-200E, Nellcor) was placed over the animal’s jaw, in such a way that the optical path included the soft tissues of the jaw but excluded the tongue. A plot of the O₂ saturations from the pulse oximeter and SaO₂, measured in blood samples drawn from the carotid artery (OSM-2 hemoximeter, Radiometer) was used to correct all saturation values. All physiological data were sampled at 400 Hz after analog-to-digital conversion (Powerlab, ADInstruments, Sydney, Australia) and recorded on a computer using LabChart 6 acquisition software (ADInstruments, Sydney, Australia). LabChart 6 software was used to determine VT and to calculate breathing frequency (f). Ventilation (Ve) was calculated breath-by-breath using the breath duration determined by the flow trace and the VT from the integrator. Rectal temperature was monitored throughout each experiment and maintained at 40.0 ± 1.0°C using an overhead radiant heater.

Experimental Protocols

Resting ventilatory and hemodynamic parameters were measured at the beginning and end of each study. Six experimental protocols were carried out to characterize the respiratory controller and its impact on breathing stability. Each protocol was performed in each animal three times, and the results of each animal were averaged to produce one value per animal. All surgical and experimental protocols were performed in accordance with the guidelines established by the National Health and Medical Research Council of Australia and were approved by the Standing Committee in Ethics in Animal Experimentation of Monash University. At the completion of studies, animals were killed with a dose of pentobarbitone sodium (150 mg/kg; Virbac, Sydney, Australia).

Protocol 1: peripheral ventilatory sensitivity to isocapnic hypoxia.

To determine the ventilatory sensitivity to PaO₂, we measured ventilation in response to a 1-min challenge with inspiratory O₂ fraction (FIo₂) of 0.10–1.0 presented in a randomized order; inspired CO₂ (FiCO₂) was adjusted to maintain isocapnic conditions at each step. After a steady-state response in ventilation was reached, an arterial blood sample was taken and analyzed. From these data, we derived the ventilatory response to isocapnic hypoxia mediated by the peripheral chemoreceptors (VP) by subtracting the ventilation at FiO₂ = 1.0 from that at each PaO₂ level; the basis for this approach is that peripheral chemoreceptor drive to breathing can be assumed to be zero at FiO₂ = 1.0 (23). We fitted the data with an exponential function of the following form: \( V' = G_p e^{-kPaO_2} \), where \( G_p \) represents the y-intercept of the curve and \( k \) represents the exponent, which is a descriptor of the steepness of the ventilatory relationship to O₂. Slopes of the exponential curve for each animal were calculated during normoxia and at PaO₂ = 40 Torr as estimates of controller gain; 40 Torr was chosen for slope calculation, because it approximates the PaO₂ level at which breathing resumed at the end of posthyperventilation apnea.

Protocol 2: peripheral ventilatory sensitivity to CO₂. We used an established technique (11) to measure the dynamic sensitivity of the peripheral chemoreceptors to CO₂. After a period of stable baseline breathing, the inspired gas was switched to a hypercapnic mixture (14% CO₂-21% O₂-balance N₂) for three breaths before it was returned to room air. This procedure was repeated three times at both ages, with a 5-min recovery between tests. The peak ventilatory response was measured during the 10–15 s after the stimulus and expressed as the change from baseline per unit change in PETCO₂ (AV/ΔPETCO₂). We also measured the time to peak ventilatory response, with time 0 taken as the start of the first inspiration after the switch to the CO₂ stimulus.

Protocol 3: ontogeny of controller topology. To assess whether the controller characteristics or topology changes with age from an additive to a multiplicative form, we characterized how the slope of the Ve-venous PCO₂ (PVCO₂) relationship is affected by the level of O₂ and how the PaO₂ is altered by changes in the level of O₂. A constant slope and a leftward shift of PaT from hyperoxia to hypoxia indicate an additive controller, whereas a change in slope and no or little change in PaT indicates a multiplicative controller (Fig. 1). For measurement of the central CO₂ sensitivity, the ventilatory response to FiCO₂ = 0.07 was measured against a background of hypoxia or hyperoxia. To measure the complete ventilatory response to CO₂, including the response below eupcapnia, we adopted a previously described approach (18) in which the animal was hyperventilated below its PaT, the ventilator was turned off...
During the posthyperventilation apnea. When breathing restarted, human adult has small

was taken to confirm the constancy of $\text{PaO}_2$ during the run. In the

obtain a value at each time point. After 4 min, an additional sample

was held constant at 0.8.

To assess the interaction between $\text{O}_2$ and the $\text{PAT}$, we repeated the

Protocol 4: effect of age on propensity for PB. In a multiplicative model, inspiration of hypoxic gas during PB is known to exacerbate PB, whereas inspiration of hypercapnic gas immediately stabilizes breathing (7). By contrast, inspiration of hypoxic gas immediately suppresses PB in an additive model, whereas inspiration of hypercapnic gas stabilizes breathing, but only after a further one or two cycles of PB (55). Accordingly, when PB occurred after posthyperventilation apnea in the mature animals, we allowed two cycles of PB to occur before applying hypoxic (12%) or hypercapnic (5%) inspired gas for 1 min. At the end of this period, the inspired gas was switched back to hypoxemia.

Protocol 5: effect of $\text{O}_2$ and $\text{CO}_2$ on PB. In a multiplicative model, inspiration of hypoxic gas during PB is known to exacerbate PB, whereas inspiration of hypercapnic gas immediately stabilizes breathing (7). By contrast, inspiration of hypoxic gas immediately suppresses PB in an additive model, whereas inspiration of hypercapnic gas stabilizes breathing, but only after a further one or two cycles of PB (55). Accordingly, when PB occurred after posthyperventilation apnea in the mature animals, we allowed two cycles of PB to occur before applying hypoxic (12%) or hypercapnic (5%) inspired gas for 1 min. At the end of this period, the inspired gas was switched back to hypoxemia.

Protocol 6: circulation delay. The circulation delay between the lung and peripheral chemoreceptors was measured during protocol 4 and was taken as the time from the start of the first inspiration of a cluster of breaths to the point where $\text{SaO}_2$ begins to rise. Our estimate assumes that the jaw of the sheep has approximately the same circulation delay as the carotid body (54); a comparable method has been used in the human based on the timing of carotid $\text{SaO}_2$ relative to the start of breathing following an apnea (37).

Statistics

Values are means ± SE. Differences between observed frequencies were tested using a $\chi^2$ test. Differences between means were tested using a Student’s $t$-test (unpaired). For all tests, $P < 0.05$ was taken as the critical level.

RESULTS

Respiratory variables in newborn lambs and mature sheep breathing air are presented in Table 1. $\text{PaCO}_2$ and venous $\text{PO}_2$ were significantly higher in mature sheep than in newborn lambs. $\text{Ve}$ was significantly less in mature sheep than in young lambs as a result of a significant reduction of $\text{f}$ but no change in $\text{Vt}$. There were no age-related changes in $\text{PvCO}_2$ and $\text{Pao}_2$, but a significant reduction in $\text{SaO}_2$ was observed in the older animals.
2.1 vs. 10.7
controller gain of the exponential curve at normoxia (14.9

Fig. 2. Effects of age on peripheral sensitivity to O2 and CO2 (means ± SE). Peripheral O2 sensitivity fell with age when measured at 40 Torr (A) or during normoxia (B). C: dynamic ventilatory responses to 14% CO2 in newborn lambs and mature sheep expressed as change in Ve per unit change in end-tidal PCO2 (ΔVe/ΔPETCO2). *P < 0.05.

<table>
<thead>
<tr>
<th>Control ventilatory variables</th>
<th>Newborn Lamb</th>
<th>Mature Sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, kg</td>
<td>8.9 ± 0.5</td>
<td>23.2 ± 1.1*</td>
</tr>
<tr>
<td>Ventilation, ml·min⁻¹·kg⁻¹</td>
<td>345.4 ± 20.1</td>
<td>213.6 ± 25.5*</td>
</tr>
<tr>
<td>VT, ml/kg</td>
<td>9.2 ± 0.6</td>
<td>8.8 ± 1.0</td>
</tr>
<tr>
<td>f, breaths/min</td>
<td>38 ± 2.1</td>
<td>24.6 ± 1.2*</td>
</tr>
<tr>
<td>Hb, g/dl</td>
<td>5.7 ± 0.5</td>
<td>9.8 ± 0.4*</td>
</tr>
<tr>
<td>pH</td>
<td>7.36 ± 0.010</td>
<td>7.378 ± 0.009</td>
</tr>
<tr>
<td>Arterial O₂ saturation, %</td>
<td>97.1 ± 0.5</td>
<td>94.5 ± 0.5*</td>
</tr>
<tr>
<td>Arterial PCO₂, Torr</td>
<td>93.5 ± 1.1</td>
<td>91.8 ± 1.7</td>
</tr>
<tr>
<td>Arterial PCO₂, Torr</td>
<td>42.8 ± 0.7</td>
<td>47.7 ± 1.6*</td>
</tr>
<tr>
<td>Venous PO₂, Torr</td>
<td>46.5 ± 1.8</td>
<td>61.8 ± 1.3*</td>
</tr>
<tr>
<td>Venous PCO₂, Torr</td>
<td>47.9 ± 0.7</td>
<td>50.3 ± 1.7</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 15). VT, tidal volume; f, respiratory rate. *Significantly different from newborn lamb (P < 0.001, by paired t-test).

Protocol 1: Peripheral Ventilatory Sensitivity to Isocapnic Hypoxia

The ventilatory response (Ve) to isocapnic hypoxia, which was performed at both ages, was expressed as the ventilation attributable to Ve. When the data for each animal were fitted by an exponential curve of the form Ve = Gp(1-PaO2/PaO2norm) and the parameter values were averaged, a significant increase in k (0.023 ± 0.001 vs. 0.028 ± 0.001) and in the slope or controller gain of the exponential curve at normoxia (14.9 ± 2.1 vs. 10.7 ± 1.5 ml·min⁻¹·kg⁻¹ · Torr⁻¹) and at PaO₂ = 40 Torr (4.1 ± 0.5 vs. 2.6 ± 0.4 ml·min⁻¹·kg⁻¹ · Torr⁻¹) was found in the mature sheep compared with the lamb (Fig. 2, A and B); however, Gp showed a directional trend toward reduction (1.7 ± 0.2 vs. 1.2 ± 0.2 l·min⁻¹·kg⁻¹) that just failed to reach significance (P = 0.06). The average r values of the exponential fits in newborn and adult animals were 0.97 ± 0.007 and 0.94 ± 0.010, respectively.

Protocol 2: Peripheral Ventilatory Sensitivity to CO₂

Dynamic peripheral chemoreceptor response to a step rise in CO₂, from room air to 14% CO₂, did not change significantly with age. As the step change occurred, Ve increased by 80.3 ± 21.5 and 52.8 ± 6.7 ml·min⁻¹·kg⁻¹ (no significant difference) in newborn lambs and mature sheep, respectively, in response to PETCO₂ increases of 15.5 ± 0.8 and 8.3 ± 0.8 Torr, respectively (P < 0.05). Figure 2C demonstrates no significant change in ventilatory sensitivity to CO₂ (ΔVe/ΔPETCO₂) between newborn and mature animals (5.4 ± 1.5 and 6.5 ± 0.6 ml·min⁻¹·kg⁻¹ · Torr⁻¹, respectively). However, time to reach peak ventilatory response to the CO₂ challenge was significantly longer for mature sheep than the newborn lamb (10.7 ± 0.8 vs. 6.4 ± 0.5 s).

Protocol 3: Ontogeny of Controller Topology

A ventilatory response to CO₂ in the newborn lamb is shown in Fig. 3A. For the group as a whole, the slope of the CO₂ response curve (0.031 ± 0.008 l·min⁻¹·Torr⁻¹·kg⁻¹) during hypoxia (PaO₂ = 49.9 ± 2.1 Torr) was not significantly different from the slope (0.028 ± 0.008 l·min⁻¹·Torr⁻¹·kg⁻¹) during hyperoxia (PaO₂ = 254.7 ± 29.6 Torr). The results from the CO₂ response for mature sheep (Fig. 3B) showed that the slope of the response curve (0.015 ± 0.004 l·min⁻¹·Torr⁻¹·kg⁻¹) during hypoxia (PaO₂ = 50.3 ± 1.8 Torr) was not significantly different from the slope (0.015 ± 0.005 l·min⁻¹·Torr⁻¹·kg⁻¹) during hyperoxia (PaO₂ = 258.3 ± 55.1 Torr). The slopes of the ventilatory responses to CO₂ were steeper during hypoxia and hyperoxia in the newborn lamb than in the mature sheep (P < 0.05).

The effect of O₂ on P₄T is illustrated for the newborn lamb in Fig. 4A, which shows P₄T derived from PVCO₂ and PAO₂ at the commencement of breathing plotted against SaO₂ at the commencement of breathing collected into 10% bins. There was no difference in PAO₂ and PVCO₂ samples at commencement of breathing; this result was expected, inasmuch as the arterial level rises rapidly to the venous level during apnea (32). Figure 4A shows a clear relationship between P₄T and SaO₂, with a rapid drop in P₄T between 100% and 90% SaO₂. Figure 4B shows a similar relationship in the mature animal. These data were fitted with a function of the following form: y = a – be⁻ᵏx. Statistical comparisons demonstrated that this relationship did not change significantly with age.

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CO2 to the inspired air also suppressed PB in all animals, but room air, PB reappeared in all animals. The addition of 5% when the animals were returned from hypoxic inspired gas restored continuous breathing in all animals (Fig. 5); also, moreceptors, measured as the difference between the start of a difference in SaO2 at which breathing began in newborn (3.3 ± 0.1%) and mature (3.6 ± 0.3%) animals, nor was there a difference in SaO2 at which breathing began in newborn (52.2 ± 2.4%) compared with mature animals (57.4 ± 4.7%). There was a significant increase in the duration of posthyperventilation apnea with age (26.0 ± 2.1 vs. 58.7 ± 2.8 s).

Protocol 5: Effect of O2 and CO2 on PB

In a subset of seven mature sheep, switching the inspired gas from air to 12% O2 after two cycles of PB immediately restored continuous breathing in all animals (Fig. 5); also, when the animals were returned from hypoxic inspired gas to room air, PB reappeared in all animals. The addition of 5% CO2 to the inspired air also suppressed PB in all animals, but this effect was delayed until after a further cycle of PB. When all animals were returned from 5% CO2 to room air, ventilation decreased but breathing remained stable. A similar effect of hypoxia and CO2 applied during PB has been reported for 11- to 20-day-old lambs (55).

Protocol 6: Circulation Delay

The circulation delay from the lung to the peripheral chemoreceptors, measured as the difference between the start of the first inspiratory effort during PB and the point where SaO2 begins to rise, increased significantly with age (4.2 ± 0.2 and 10.0 + 0.9 s, P < 0.001). Figure 6 shows a significant relationship between circulation delay and body weight.

DISCUSSION

Our major finding is that hypercapnic and hypoxic ventilatory stimuli in the newborn lamb and mature sheep interact in an additive manner. The presence of an additive controller was confirmed by three major findings: 1) the response to CO2 has the same slope under hypoxic and hyperoxic conditions, 2) the PAT is highly dependent on the level of O2, shifting dramatically to the left under hypoxic conditions, and 3) inspiration of hypoxic gas eliminates PB in mature sheep just as it does in the newborn lamb (23, 55). In addition, we found that even though the peripheral sensitivity to CO2 was not altered, the peripheral sensitivity to O2 and the combined central and peripheral CO2 sensitivity decrease with age. Although the controller remained additive, we found that the duty ratio of induced PB increases with age, indicating a reduction in the loop gain of the respiratory control system during unstable breathing. Thus the respiratory control system becomes more stable with age in the sheep, probably as a consequence of the reduction in peripheral O2 sensitivity with age. This finding contrasts with data from humans showing that the controller is additive in the infant before a switch to a multiplicative controller becomes evident by ~2 mo of age (50). Our findings raise questions about the advantages and disadvantages of the two controller types and the mechanisms responsible for the two controller types.

The generalizability of results obtained in an anesthetized model for studying respiratory control maturation may be...
questioned. There are several reasons to conclude that our model behaves in a manner similar to that of unanesthetized animals. Baseline ventilation, respiratory rate, and resting blood gas values in our lambs and sheep are similar to those reported in awake lambs (6, 40) and awake mature sheep (40). The magnitude and speed of the ventilatory responses to O₂ and CO₂ we report are similar to those described in lambs that were awake or in quiet sleep (11, 29). The PAT in the anesthetized lamb is determined by CO₂ and O₂, just as it is in the unanesthetized lamb (13, 18). The cycle time of PB in our lambs was similar to that reported in studies of unanesthetized lambs (12). Finally, as we reported previously (55), PB is produced in our preparation after an apnea following a period of hyperventilation, just as it is in adult humans during quiet wakefulness (22).

Extrapolation of results from the newborn lamb to the human neonate must, however, be done with caution, inasmuch as they differ in several ways. The lamb is more mature at birth and does not exhibit spontaneous PB. The lack of PB in the lamb may result from the presence of an additive controller, low chemoreceptor sensitivity, or both; it is noteworthy that infants with low chemoreceptor gain, particularly in the first postnatal days, exhibit little or no PB (5). As the sensitivity of the chemoreceptors increases over the first 8 wk of life (6, 25, 51) and a multiplicative interaction between CO₂ and O₂ develops at the carotid body of the human (50), comparisons are not possible, inasmuch as our evidence clearly shows no multiplicative interaction in sheep, even at 6 mo of age. Nevertheless, lambs develop PB when a critical O₂-CO₂ combination is reached (12), so that PB in newborn lambs and human neonates is a chemoreceptor-driven event. Importantly, the fact that the lamb has a controller similar to the newborn infant makes it an effective model for the human infant. Our evidence shows that the sheep does not alter its controller, even at 6 mo of age, or well in excess of the 8 wk at which the multiplicative controller is altered in humans (50). Whether the sheep changes to a multiplicative controller beyond 6 mo of age is unknown. If it is assumed that the controller remains additive in the sheep, its use as a model is restricted to the period when the human infant has an additive controller.

Our data show that maturation in sheep is associated with a decrease in V˙E via a decrease in f, which is consistent with the resting ventilatory parameters in awake sheep (40) and humans (2). Previous estimates of circulation delay in newborn sheep were 3–5 s (11), similar to the values reported in the present

Table 2. Effect of age on the occurrence of PB following posthyperventilation apnea

<table>
<thead>
<tr>
<th></th>
<th>Newborn Lamb</th>
<th>Mature Sheep</th>
</tr>
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<tbody>
<tr>
<td>Animals displaying PB</td>
<td>15</td>
<td>111</td>
</tr>
<tr>
<td>Epoch duration of PB, s</td>
<td>35.7±4.6</td>
<td>101.8±33.2*</td>
</tr>
<tr>
<td>No. of cycles</td>
<td>4.0±0.5</td>
<td>5.0±1.5</td>
</tr>
<tr>
<td>Cycle duration, s</td>
<td>10.6±0.5</td>
<td>18.7±1.3‡</td>
</tr>
<tr>
<td>Duty ratio</td>
<td>0.54±0.02</td>
<td>0.70±0.05‡</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 15). PB, periodic breathing; duty ratio, ratio of duration of the ventilatory period during PB to duration of the PB cycle, defined as a cluster of breaths and the following period of apnea. *Significantly different from newborn lamb (P < 0.05, by unpaired t-test). †Significantly different from newborn lamb (P < 0.05, by χ² test). ‡Significantly different from newborn lamb (P < 0.001, by unpaired t-test).

Fig. 5. Effect of hypoxia and hypercapnia during periodic breathing (PB) in the mature sheep. A: generation of PB (using protocol 4) in a mature sheep. B: switch to hypoxia immediately suppressed PB; return to air destabilized breathing. C: effect of hypercapnia on PB; after 2 cycles, inspired air was switched to 5% CO₂, which suppressed PB, but with a delay of 1 PB cycle. FIO₂ and FICO₂ inspiratory O₂ and CO₂ fractions; V, airflow.

Fig. 6. Relationship between body weight and circulation delay measured in the lamb and mature sheep. Age-associated increase in body weight correlated strongly with measured circulation delay in sheep (R = 0.849, P < 0.001). Measures of circulation delay could not be obtained in 4 mature sheep because of poor signal quality of the oximeter. Dashed lines represent 95% confidence limits.
is well recognized that the ventilatory sensitivity to O2 is significantly lower in mature sheep than in newborn lambs. It reveals a significantly higher O2 consumption in the newborn metabolic rate that occur with age (38, 43). Our calculations have suggested that it may be due to changes in the resting metabolic rate that occur with age (38, 43). Our calculations reveal a significantly higher O2 consumption in the newborn lamb than in the mature sheep (18.0 ± 1.2 vs. 10.9 ± 1.3 ml·min⁻¹·kg⁻¹). A similar relationship is evident when O2 consumption is calculated from an earlier study of sheep at comparable ages (40).

We found that the dynamic hypercapnic sensitivity, which is a measure of the contribution of the peripheral chemoreceptors to the overall CO2 response (11), did not change postnatally in our sheep, extending the finding that the newborn lamb does not change its dynamic sensitivity over the 1st mo of life (10, 11). Importantly, the different maturation profiles of the peripheral response to O2 and CO2 confirm an earlier suggestion that there may be a different mode of chemotransduction at the carotid bodies for each stimulus (14). On the other hand, the present study demonstrated that the steady-state CO2 sensitivity in hypoxia and hyperoxia was less in the mature sheep than in the newborn lamb. Notably, a similar decrease in CO2 sensitivity has been reported in awake humans (26, 38). Comparisons during sleep in infants (16) and adults (8) show that sensitivity decreases and the response shifts to the right with age. This rightward shift has been attributed to the resetting of the CO2 sensitivity associated with the fall in metabolic rate and the increase in eupneic PCO2 with age (3, 15). Our results indicate that such resetting of the CO2 sensitivity is due to a reduction of the central chemosensitivity, inasmuch as peripheral CO2 sensitivity remains unchanged in the sheep.

Previous studies examining the interaction of ventilatory responses to O2 and CO2 have used the approach of Nielsen and Smith (41), who measured the steady-state change in ventilation with increasing levels of PaCO2 while controlling levels of O2. In adult humans, the hypercapnic ventilatory response is dramatically heightened by hypoxia (36), and it has been shown that the multiplicative interaction occurs at the peripheral chemoreceptors (59). In human infants, there is less certainty about how combined hypoxic and hypercapnic stimuli influence breathing. One study showed that O2 and CO2 have an additive action in preterm infants, at least over the hypoxic range (1); in another study, the interaction was found to be negative (46); however, it has been speculated that the results of the latter are an aberration caused by prolonged gas exposures (15). Recently, utilizing dynamic responses to O2 and CO2, as well as their interaction, in full-term infants, Sovik and Lossius (50) suggested that the controller is initially additive and becomes multiplicative by ~2 mo of age. Similarly, an isolated report measuring discharge from carotid body afferents in newborn lambs concluded that an interaction in the response to O2 and CO2 occurred by 1 mo of age (10). In other studies investigating the interaction of the ventilatory responses of newborn lambs (see Fig. 8 in Ref. 45), as well as the present study, an additive interaction up to 3 wk of life was reported. In contrast to the human and the findings of Calder et al. (10), our study demonstrates with three independent protocols that an additive interaction is still present 6 mo after birth in the sheep. Inasmuch as an additive interaction has also been found in adult goats (39), it is clear that the switch to a multiplicative controller does not occur in all species.

In the absence of a mechanistic explanation for the O2-CO2 interaction at the carotid bodies, it is difficult to discuss how the interaction changes postnatally. In vitro and in vivo studies in the rat have focused on the mechanisms responsible for the maturation of hypoxic responsiveness and have shown a developmental increase in K⁺ channel density and intracellular Ca²⁺ concentration ([Ca²⁺]i) in response to hypoxia (4, 28, 52). Bamford et al. (4) examined the O2-CO2 interaction and found that the multiplicative interaction observed in carotid sinus nerve discharge did not result from a change in [Ca²⁺]i. They concluded that the interaction must mature at unidentified sites within the developing CB. The following question therefore arises: Where does the O2-CO2 interaction occur, if not at the type I cell? It has been speculated that the interaction results from maturational changes in the type or number of receptors for a number of transmitters in the early developmental period (20). It is known that hypoxia-induced secretion of catecholamine and other constituents of the dense-cored vesicles of glomus cells is enhanced with age (21). Also of interest is the postnatal reduction of the turnover of the major inhibitory transmitter dopamine (30), as well as the downregulation of specific transcription factors such as hypoxia-inducible factor-1α (47), which are crucial to postnatal responsiveness to hypoxia. Furthermore, on the postsynaptic side, the number of afferent synaptic sites increases four- to fivefold in newborn and mature sheep show a highly labile P_AT.
the postnatal period (34). Nonetheless, until the mechanisms underlying the O₂-CO₂ interaction within the carotid bodies are known, we cannot address why the sheep does not appear to develop a multiplicative interaction.

We report that the P_{AT} is highly dependent on the level of O₂ in the newborn lamb, consistent with earlier results using the same methodology in awake newborn lambs of similar age (18). Similarly, a study that utilized an extracorporeal CO₂ membrane exchanger to remove CO₂ from the venous blood until apnea occurred reported that the P_{AT} is highly dependent on the level of O₂ (13). Previous studies examining the O₂ sensitivity of P_{AT} with postnatal maturation (13, 18) have been limited to the first 2 wk in lambs. We are the first to show that such dependence is preserved even in the mature sheep. To highlight the differences between the additive controllers observed in the present study and a multiplicative controller, we used the mathematical model of Topor et al. (53) to determine how changes in the level of O₂ affect P_{AT} in the human adult; a multiplicative controller was found to exhibit little change in P_{AT} with decreasing levels of O₂ (Fig. 7), whereas a dramatic change occurs in sheep.

The finding that a multiplicative controller develops in most species over the first few months of life (14, 42, 50), whereas the sheep maintains an additive controller, raises the following question: Is there an advantage in making the switch? One possibility is that the emergence of a multiplicative interaction during infancy ensures a robust ventilatory and arousal response to a combined rise in P_{ACO₂} and decrease in PaO₂ resulting from upper airway obstruction. Balancing the potential advantage of a multiplicative interaction is the possibility that it contributes to the high prevalence of PB in newborn infants from 1–4 mo of age.

Our study shows an increase in duty ratio with age during PB. On the basis that an increase in the duty ratio signifies a fall in loop gain (23), we infer that the mature sheep is inherently more stable than the newborn lamb. Our finding of reduced peripheral O₂ sensitivity and central CO₂ sensitivity in the older sheep is consistent with mathematical predictions that a combined reduction in peripheral and central sensitivity causes a proportional reduction in loop gain (33); given our finding that inhaled hypoxic gas immediately terminates PB in the mature sheep, whereas inhaled CO₂ does not, we conclude that the O₂ feedback loop is responsible for the genesis of the unstable breathing pattern. Hence, we conclude that the reduced loop gain at 6 mo is attributable to the measured decline in peripheral O₂ sensitivity. In earlier work, we also used the epoch duration of PB as a surrogate measure of loop gain (23); the fact that epoch duration increased in the present study in the mature sheep would suggest an increased loop gain. However, a weakness of using epoch duration as an indicator of loop gain is that it merely reflects how long loop gain is >1, and not the degree to which it is elevated. Our explanation for the longer epoch duration is that loop gain falls more slowly as the epoch progresses in the mature sheep, perhaps as a result of the reduced sensitivity to CO₂ and O₂, causing a slower rise in ventilation and PaO₂. Similarly, the lower metabolic rate predisposes to a slower rise in central P_{CO₂}. In contrast to the epoch duration, the duty ratio is directly related to the degree to which loop gain is elevated above 1 (23) and is, thus, a superior measure of the stability of the system.

Conclusions

Our results establish that the controller of the sheep remains additive until at least 6 mo of age. We also found that loop gain and, thus, stability of the respiratory control system decline with age, possibly due to the observed reduction in peripheral O₂ sensitivity. Our findings demonstrate that the transformation from an additive to a multiplicative respiratory controller is not a universal developmental progression in mammals.

REFERENCES

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